University of Szeged

Laboratory Investigations in Pharmaceutical Chemsitry

Part 2

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Contents

1. GENERAL NOTICES

1.1. General statements

The official texts of the European Pharmacopoeia are published in English and French. Translations in other languages may be prepared by the signatory States of the European Pharmacopoeia Convention. In case of doubt or dispute, the English and French versions are alone authoritative.

A preparation must comply throughout its period of validity; a distinct period of validity and/or specifications for opened or broached containers may be decided by the competent authority. The subject of any other monograph must comply throughout its period of use. The period of validity that is assigned to any given article and the time from which that period is to be calculated are decided by the competent authority in light of experimental results of stability studies.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. General chapters become mandatory when referred to in a monograph, unless such reference is made in a way that indicates that it is not the intention to make the text referred to mandatory but rather to cite it for information.

The active substances, excipients, pharmaceutical preparations and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses). The quality standards represented by monographs are valid only where the articles in question are produced within the framework of a suitable quality system. The quality system must assure that the articles consistently meet the requirements of the Pharmacopoeia.

The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. With the agreement of the competent authority, alternative methods of analysis may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative.

An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and data derived, for example, from validation studies of the manufacturing process.

Certain materials that are the subject of a pharmacopoeial monograph may exist in different grades suitable for different purposes. Unless otherwise indicated in the monograph, the requirements apply to all grades of the material. In some monographs, particularly those on excipients, a list of functionality-related characteristics that are relevant to the use of the substance may be appended to the monograph for information. Test methods for determination of one or more of these characteristics may be given, also for information.

1.2. Other provisions applying to general chapters and monographs

Quantities. In tests with numerical limits and assays, the quantity stated to be taken for examination is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result is calculated from this exact quantity. In tests where the limit is not numerical, but usually depends upon comparison with the behaviour of a reference substance in the same conditions, the stated quantity is taken for examination. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision corresponds to plus or minus 5 units after the last figure stated (for example, 0.25 g is to be interpreted as 0.245 g to 0.255 g). For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero (for example, 10.0 ml or 0.50 ml), the volume is measured

using a pipette, a volumetric flask or a burette, as appropriate; otherwise, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

It is recognised, however, that in certain cases the precision with which quantities are stated does not correspond to the number of significant figures stated in a specified numerical limit. The weighings and measurements are then carried out with a sufficiently improved accuracy.

Apparatus and procedures. Volumetric glassware complies with Class A requirements of the appropriate International Standard issued by the International Organisation for Standardisation.

Unless otherwise prescribed, analytical procedures are carried out at a temperature between 15 °C and 25 °C.

Unless otherwise prescribed, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base; the volumes of liquid prescribed are for use with tubes having an internal diameter of 16 mm, but tubes with a larger internal diameter may be used provided the volume of liquid used is adjusted (2.1.5). Equal volumes of the liquids to be compared are examined down the vertical axis of the tubes against a white background, or if necessary against a black background. The examination is carried out in diffuse light.

Any solvent required in a test or assay in which an indicator is to be used is previously neutralised to the indicator, unless a blank test is prescribed.

Water-bath. The term ‘water-bath’ means a bath of boiling water unless water at another temperature is indicated. Other methods of heating may be substituted provided the temperature is near to but not higher than 100 °C or the indicated temperature.

Drying and ignition to constant mass. The terms ‘dried to constant mass’ and ‘ignited to constant mass’ mean that 2 consecutive weighings do not differ by more than 0.5 mg, the 2nd weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Where drying is prescribed using one of the expressions ‘in a desiccator’ or ‘*in vacuo*’, it is carried out using the conditions described in chapter *2.2.32. Loss on drying*.

Reagents. The proper conduct of the analytical procedures described in the Pharmacopoeia and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents are described in general chapter 4. It is assumed that reagents of analytical grade are used; for some reagents, tests to determine suitability are included in the specifications.

Solvents. Where the name of the solvent is not stated, the term ‘solution’ implies a solution in water.

Where the use of water is specified or implied in the analytical procedures described in the Pharmacopoeia or for the preparation of reagents, water complying with the requirements of the monograph Purified water (0008) is used, except that for many purposes the requirements for bacterial endotoxins (*Purified water in bulk*) and microbial contamination (*Purified water in containers)* are not relevant. The term ‘distilled water’ indicates purified water prepared by distillation.

The term ‘ethanol’ without qualification means anhydrous ethanol. The term ‘alcohol’ without qualification means ethanol (96 per cent). Other dilutions of ethanol are indicated by the term ‘ethanol’ or ‘alcohol’ followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Expression of content. In defining content, the expression ‘per cent’ is used according to circumstances with one of 2 meanings:

– per cent *m/m* (percentage, mass in mass) expresses the number of grams of substance in 100 g of final product;

– per cent *V/V* (percentage, volume in volume) expresses the number of millilitres of substance in 100 ml of final product.

The expression ‘parts per million’ (or ppm) refers to mass in mass, unless otherwise specified.

Temperature. Where an analytical procedure describes temperature without a figure, the general terms used have the following meaning:

– in a deep-freeze: below − 15 °C;

– in a refrigerator: 2 °C to 8 °C;
– cold or cool: 8 °C to 15 °C;
– room temperature: 15 °C to 25 °C

1.4. Monographs

Titles

Monograph titles are in English and French in the respective versions and there is a Latin subtitle.

Relative Atomic and Molecular Masses

The relative atomic mass (A_r) or the relative molecular mass (M_r) is shown, as and where appropriate, at the beginning of each monograph. The relative atomic and molecular masses and the molecular and graphic formulae do not constitute analytical standards for the substances described.

Definition

Statements under the heading Definition constitute an official definition of the substance, preparation or other article that is the subject of the monograph.

Limits of content.

Where limits of content are prescribed, they are those determined by the method described under Assay.

Characters.

The statements under the heading Characters are not to be interpreted in a strict sense and are not requirements.

Solubility.

In statements of solubility in the Characters section, the terms used have the following significance referred to a temperature between 15 °C and 25 °C.

Descriptive term	Approximate volume of solvent in millilitres per gram of solute			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1000
Very slightly soluble	from	1000	to	10 000
Practically insoluble	more than			10 000

The term “partly soluble” is used to describe a mixture where only some of the components dissolve. The term “miscible” is used to describe a liquid that is miscible in all proportions with the stated solvent.

Identification

The tests given in the Identification section are not designed to give a full confirmation of the chemical structure or composition of the product; they are intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label.

Tests and Assays

Scope.

The requirements are not framed to take account of all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated if common sense and good pharmaceutical practice require that it be absent.

Limits.

The limits prescribed are based on data obtained in normal analytical practice; they take account of normal analytical errors, of acceptable variations in manufacture and compounding and of deterioration to an extent considered acceptable. No further tolerances are to be applied to the limits prescribed to determine whether the article being examined complies with the requirements of the monograph.

In determining compliance with a numerical limit, the calculated result of a test or assay is first rounded to the number of significant figures stated, unless otherwise prescribed. The limits, regardless of whether the valuesare expressed as percentages or as absolute values, are considered significant to the last digit shown (for example 140 indicates 3 significant figures). The last figure is increased by one when the part rejected is equal to or exceeds one half-unit, whereas it is not modified when the part rejected is less than a half-unit.

Indication of permitted limit of impurities.

For comparative tests, the approximate content of impurity tolerated, or the sum of impurities, may be indicated in brackets for information only. Acceptance or rejection is determined on the basis of compliance or non-compliance with the stated test. If the use of a reference substance for the named impurity is not prescribed, this content may be expressed as a nominal concentration of the substance used to prepare the reference solution specified in the monograph, unless otherwise described.

Equivalents.

Where an equivalent is given, for the purposes of the Pharmacopoeia only the figures shown are to be used in applying the requirements of the monograph.

The equivalent amount given in the assay parts is the amount of sample (in mg) which reacts with 1 ml of the measuring reagent. The stoichiometry of the reaction and the concentration of the volumetric solution are taken into account in the equivalent amount, which is denoted E (mg/ml). This information is therefore not necessary to calculate the result of the assay.

Storage.

The information and recommendations given under the heading Storage do not constitute a pharmacopoeial requirement but the competent authority may specify particular storage conditions that must be met.

The articles described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Where special conditions of storage are recommended, including the type of container (see section 1.3. General chapters) and limits of temperature, they are stated in the monograph.

The following expressions are used in monographs under Storage with the meaning shown.

In an airtight container means that the product is stored in an airtight container (*3.2*). Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

Protected from light means that the product is stored either in a container made of a material that absorbs actinic light sufficiently to protect the contents from change induced by such light, or in a container enclosed in an outer cover that provides such protection, or is stored in a place from which all such light is excluded.

Warnings.

Materials described in monographs and reagents specified for use in the Pharmacopoeia may be injurious to health unless adequate precautions are taken. The principles of good quality control laboratory

practice and the provisions of any appropriate regulations are to be observed at all times. Attention is drawn to particular hazards in certain monographs by means of a warning statement; absence of such a statement is not to be taken to mean that no hazard exists.

1.5. Abbreviations and Symbols

A_r	Relative atomic mass
$\lfloor\alpha_D^{20}\rfloor$	Specific optical rotation
bp	Boiling point
d_{20}^{20}	Relative density
IU	International Unit
λ	Wavelength
M	Molarity
M_r	Relative molecular mass
mp	Melting point
$\lfloor n_D^{20}\rfloor$	Refractive index
ppm	Parts per million
R	Substance or solution defined under *Reagents*
RV	Substance used as a primary standard in volumetric analysis

2. METHODS OF ANALYSIS

2.1. Apparatus

2.1.1. Droppers

The term ‘drops’ means standard drops delivered from a standard dropper as described below.

Standard droppers (Figure 2.1.1-1) are constructed of practically colourless glass. The lower extremity has a circular orifice in a flat surface at right angles to the axis.

Other droppers may be used provided they comply with the following test.

20 drops of *water R* at 20 ± 1 °C flowing freely from the dropper held in the vertical position at a constant rate of 1 drop per second weighs 1000 ± 50 mg.

The dropper must be carefully cleaned before use. Carry out 3 determinations on any given dropper. No result may deviate by more than 5 per cent from the mean of the 3 determinations.

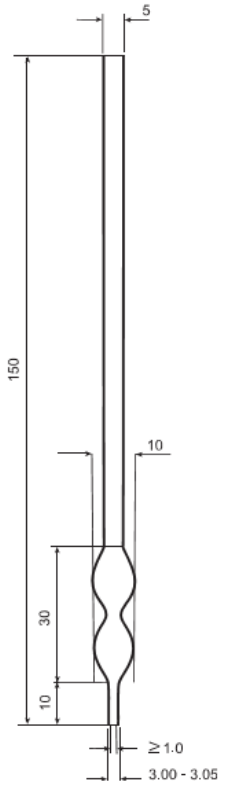


Figure 2.1.1.-1. – *Standard dropper*
Dimensions in millimetres

2.1.5. Tubes for Comparative Tests

Tubes used for comparative tests are matched tubes of colourless glass with a uniform internal diameter. The base is transparent and flat.

A column of the liquid is examined down the vertical axis of the tube against a white background, or if necessary, against a black background. The examination is carried out in diffused light.

It is assumed that tubes with an internal diameter of 16 mm will be used. Tubes with a larger internal diameter may be used instead but the volume of liquid examined must then be increased so that the depth of liquid in the tubes is not less than where the prescribed volume of liquid and tubes 16 mm in internal diameter are used.

2.2. Physical and Physicochemical Methods

2.2.1. Clarity and Degree of Opalescence of Liquids

Visual Method

Using identical test-tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15-25 mm, compare the liquid to be examined with a reference suspension freshly prepared as described below, the depth of the layer being 40 mm. Compare the solutions in diffused daylight 5 min after preparation of the reference suspension, viewing vertically against a black background. The diffusion of light must be such that reference suspension I can readily be distinguished from *water R*, and that reference suspension II can readily be distinguished from reference suspension I.

A liquid is considered *clear* if its clarity is the same as that of *water R* or of the solvent used when examined under the conditions described above, or if its opalescence is not more pronounced than that of reference suspension I.

Hydrazine sulfate solution. Dissolve 1.0 g of *hydrazine sulfate R* in *water R* and dilute to 100.0 ml with the same solvent. Allow to stand for 4-6 h.

Hexamethylenetetramine solution. In a 100 ml ground-glass-stoppered flask, dissolve 2.5 g of *hexamethylenetetramine R* in 25.0 ml of *water R*.

Primary opalescent suspension (formazin suspension). To the hexamethylenetetramine solution in the flask add 25.0 ml of the hydrazine sulfate solution. Mix and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

Standard of opalescence. Dilute 15.0 ml of the primary opalescent suspension to 1000.0 ml with *water R*. This suspension is freshly prepared and may be stored for up to 24 h.

Reference suspensions. Prepare the reference suspensions according to Table 2.2.1.-1. Mix and shake before use.

Table 2.2.1.-1

	I	II	III	IV
Standard of opalescence	5.0 ml	10.0 ml	30.0 ml	50.0 ml
Water R	95.0 ml	90.0 ml	70.0 ml	50.0 ml

Turbidity standard. The formazin suspension prepared by mixing equal volumes of the hydrazine sulfate solution and the hexamethylenetetramine solution is defined as a 4000 NTU (nephelometric turbidity units) primary reference standard. Reference suspensions I, II, III and IV have values of 3 NTU, 6 NTU, 18 NTU and 30 NTU respectively. Stabilised formazin suspensions that can be used to prepare stable, diluted turbidity standards are available commercially and may be used after comparison with the standards prepared as described.

Formazin has several desirable characteristics that make it an excellent turbidity standard. It can be reproducibly prepared from assayed raw materials. The physical characteristics make it a desirable light-scatter calibration standard. The formazin polymer consists of chains of different lengths, which fold into random configurations. This results in a wide assay of particle shapes and sizes, which analytically fits the possibility of different particle sizes and shapes that are found in the real samples. Due to formazin's reproducibility, scattering characteristics and traceability, instrument calibration algorithms and performance criteria are mostly based on this standard.

2.2.2. Degree of Coloration of Liquids

The examination of the degree of coloration of liquids in the range brown-yellow-red is carried out by one of the 2 methods below, as prescribed in the monograph.

A solution is *colourless* if it has the appearance of *water R* or the solvent or is not more intensely coloured than reference solution B_9.

Method I

Using identical tubes of colourless, transparent, neutral glass of 12 mm external diameter, compare 2.0 ml of the liquid to be examined with 2.0 ml of *water R* or of the solvent or of the reference solution (see Tables of reference solutions) prescribed in the monograph. Compare the colours in diffused daylight, viewing horizontally against a white background.

Method II

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare the liquid to be examined with *water R* or the solvent or the reference solution (see Tables of reference solutions) prescribed in the monograph, the depth of the layer being 40 mm. Compare the colours in diffused daylight, viewing vertically against a white background.

Reagents

Primary solutions

Yellow solution. Dissolve 46 g of *ferric chloride R* in about 900 ml of a mixture of 25 ml of *hydrochloric acid R* and 975 ml of *water R* and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 45.0 mg of $FeCl_3.6H_2O$ per millilitre by adding the same acidic mixture. Protect the solution from light.

Titration. Place in a 250 ml conical flask fitted with a ground-glass stopper, 10.0 ml of the solution, 15 ml of *water R*, 5 ml of *hydrochloric acid R* and 4 g of *potassium iodide R*, close the flask, allow to stand in the dark for 15 min and add 100 ml of *water R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 27.03 mg of $FeCl_3.6H_2O$.

The concentration of the yellow primary solution is measured by an iodometric method based on the oxidation of iodides by Fe^{3+}. The iodine formed is titrated with thiosulfate (for equations, see 'Assay' in the *Ferric chloride hexahydrate* monograph).

$$FeCl_3.6H_2O \text{ content (mg/ml)} = \frac{V_{Na_2S_2O_3}(ml) \cdot f_{Na_2S_2O_3} \cdot E\ (mg/ml)}{V_P\ (ml)}$$

where V_p is the volume of the solution pipetted out for the titration.

Red solution. Dissolve 60 g of *cobalt chloride R* in about 900 ml of a mixture of 25 ml of *hydrochloric acid R* and 975 ml of *water R* and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 59.5 mg of $CoCl_2.6H_2O$ per millilitre by adding the same acidic mixture.

Titration. Place in a 250 ml conical flask fitted with a ground-glass stopper, 5.0 ml of the solution, 5 ml of *dilute hydrogen peroxide solution R* and 10 ml of a 300 g/l solution of *sodium hydroxide R*. Boil gently for 10 min, allow to cool and add 60 ml of *dilute sulfuric acid R* and 2 g of *potassium iodide R*. Close the flask and dissolve the precipitate by shaking gently. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution turns pink.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 23.79 mg of $CoCl_2.6H_2O$.

Concentration of the red primary solution is measured by a iodometric method based ont the oxidation of iodides by cobalt(III) hydroxide. The latter dark brown compound is formed by oxidation of the pink cobalt(II) hydroxide precipitate by hydrogen peroxide. The excess of hydrogen peroxide is decomposed prior to addition of iodide by a gentle boiling of the mixture. The formed iodine is titrated by thiosulfate.

$$Co^{2+} + 2\ OH^- \rightarrow Co(OH)_2$$

$$2\ Co(OH)_2 + H_2O_2 \rightarrow 2\ Co(OH)_3$$

$$2\ H_2O_2 \rightarrow O_2 + H_2O$$

$$2\ Co(OH)_3 + 2\ I^- + 6\ H^+ \rightarrow 2\ Co^{2+} + I_2 + 6\ H_2O$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$CoCl_2.6H_2O \text{ content (mg/ml)} = \frac{V_{Na_2S_2O_3}(ml) \cdot f_{Na_2S_2O_3} \cdot E\ (mg/ml)}{V_P\ (ml)}$$

where V_p is the volume of the solution pipetted out for the titration.

Blue primary solution. Dissolve 63 g of *copper sulfate R* in about 900 ml of a mixture of 25 ml of *hydrochloric acid R* and 975 ml of *water R* and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 62.4 mg of $CuSO_4.5H_2O$ per millilitre by adding the same acidic mixture.

Titration. Place in a 250 ml conical flask fitted with a ground-glass stopper, 10.0 ml of the solution, 50 ml of *water R*, 12 ml of *dilute acetic acid R* and 3 g of *potassium iodide R*. Titrate the liberated iodine

with *0.1 M sodium thiosulfate*, using 0.5 ml of *starch solution R*, added towards the end of the titration, as indicator. The end-point is reached when the solution shows a slight pale brown colour.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 24.97 mg of $CuSO_4.5H_2O$.

The concentration of the blue primary solution is measured by an iodometric method based on the oxidation of iodide by Cu^{2+}. The iodine formed is titrated with thiosulfate (for equations, see 'Assay' in the *Copper sulfate pentahydrate* monograph).

$$CuSO_4.5H_2O \text{ content (mg/ml)} = \frac{V_{Na_2S_2O_3}(ml) \cdot f_{Na_2S_2O_3} \cdot E\ (mg/ml)}{V_P\ (ml)}$$

where V_p is the volume of the solution pipetted out for the titration.

Standard solutions

Using the 3 primary solutions, prepare the 5 standard solutions as follows (Table 2.2.2.-1):

Table 2.2.2.-1

Standard solution	Volume in millilitres			
	Primary solution			Hydrochloric acid (10 g/l HCl)
	Yellow	Red	Blue	
B (brown)	3.0	3.0	2.4	1.6
BY (brownish-yellow)	2.4	1.0	0.4	6.2
Y (yellow)	2.4	0.6	0.0	7.0
GY (greenish-yellow)	9.6	0.2	0.2	0.0
R (red)	1.0	2.0	0.0	7.0

The reference solutions applied in the tests for 'Appearance of Solution' are prepared from coloured inorganic compounds. Standard and reference solutions are distinguished according to the starting letter(s) of their colour (B, BY, Y, GY or R). Reference solutions 1-7 or 1-9 are prepared by diluting the standard solutions with 1% (m/v) dilute hydrochloric acid. Index numbers (1-7 or 1-9) at bottom right refer to the intensity of the colour – higher numbers mean weaker colours. Solutions 9 (B) or 7 (BY, Y, GY or R) are the palest.

Reference solutions for Methods I and II

Using the 5 standard solutions, prepare the following reference solutions.

Table 2.2.2.-2. - *Reference solutions B*

Reference solution	Volumes in millilitres	
	Standard solution B	Hydrochloric acid (10 g/l HCl)
B_1	75.0	25.0
B_2	50.0	50.0
B_3	37.5	62.5
B_4	25.0	75.0
B_5	12.5	87.5
B_6	5.0	95.0
B_7	2.5	97.5
B_8	1.5	98.5
B_9	1.0	99.0

Table 2.2.2.-3. - *Reference solutions BY*

Reference solution	Volumes in millilitres	
	Standard solution BY	Hydrochloric acid (10 g/l HCl)
BY_1	100.0	0.0
BY_2	75.0	25.0
BY_3	50.0	50.0
BY_4	25.0	75.0
BY_5	12.5	87.5
BY_6	5.0	95.0
BY_7	2.5	97.5

Table 2.2.2.-4. - *Reference solutions Y*

Reference solution	Volumes in millilitres	
	Standard solution Y	Hydrochloric acid (10 g/l HCl)
Y_1	100.0	0.0
Y_2	75.0	25.0
Y_3	50.0	50.0
Y_4	25.0	75.0
Y_5	12.5	87.5
Y_6	5.0	95.0
Y_7	2.5	97.5

Table 2.2.2.-5. - *Reference solutions GY*

Reference solution	Volumes in millilitres	
	Standard solution GY	Hydrochloric acid (10 g/l HCl)
GY_1	25.0	75.0
GY_2	15.0	85.0
GY_3	8.5	91.5
GY_4	5.0	95.0
GY_5	3.0	97.0
GY_6	1.5	98.5
GY_7	0.75	99.25

Table 2.2.2.-6. - *Reference solutions R*

Reference solution	Volumes in millilitres	
	Standard solution R	Hydrochloric acid (10 g/l HCl)
R_1	100.0	0.0
R_2	75.0	25.0
R_3	50.0	50.0
R_4	25.0	75.0
R_5	12.5	87.5
R_6	5.0	95.0
R_7	2.5	97.5

Storage

For Method I, the reference solutions may be stored in sealed tubes of colourless, transparent, neutral glass of 12 mm external diameter, protected from light.

For Method II, prepare the reference solutions immediately before use from the standard solutions.

2.2.3. Potentiometric Determination of pH

The pH is a number which represents conventionally the hydrogen ion concentration of an aqueous solution. For practical purposes, its definition is an experimental one. The pH of a solution to be examined is related to that of a reference solution (pH_s) by the following equation:

$$pH = pH_s - \frac{E - E_S}{k},$$

in which

E is the potential, expressed in volts, of the cell containing the solution to be examined and

E_S is the potential, expressed in volts, of the cell containing the solution of known pH (pH_s),

k is the change in potential per unit change in pH expressed in volts, and calculated from the Nernst equation (Table 2.2.3.- 1).

The potentiometric determination of pH is made by measuring the potential difference between 2 appropriate electrodes immersed in the solution to be examined: 1 of these electrodes is sensitive to hydrogen ions (usually a glass electrode) and the other is the reference electrode (for example, a saturated calomel electrode).

Apparatus. The measuring apparatus is a voltmeter with an input resistance at least 100 times that of the electrodes used. It is normally graduated in pH units and has a sensitivity such that discrimination of at least 0.05 pH unit or at least 0.003 V may be achieved.

Table 2.2.3.-1. – *Values of k at different temperatures*

Temperature (°C)	*k* (V)
15	0.0572
20	0.0582
25	0.0592
30	0.0601
35	0.0611

Method. Unless otherwise prescribed in the monograph, all measurements are made at the same temperature (20-25 °C). Table 2.2.3.-2 shows the variation of pH with respect to temperature of a number of reference buffer solutions used for calibration. For the temperature correction, when necessary, follow the manufacturer's instructions. The apparatus is calibrated with the buffer solution of potassium hydrogen phthalate (primary standard) and 1 other buffer solution of different pH (preferably one shown in Table 2.2.3.-2). The pH of a third buffer solution of intermediate pH read off on the scale must not differ by more than 0.05 pH unit from the value corresponding to this solution. Immerse the electrodes in the solution to be examined and take the reading in the same conditions as for the buffer solutions.

When the apparatus is in frequent use, checks must be carried out regularly. If not, such checks should be carried out before each measurement.

All solutions to be examined and the reference buffer solutions must be prepared using *carbon dioxide-free water R*.

Table 2.2.3.-2. – *pH of reference buffer solutions at various temperatures*

Temperature (°C)	**Potassium tetraoxalate 0.05 M**	**Potassium hydrogen tartrate saturated at 25 °C**	**Potassium dihydrogen citrate 0.05 M**	**Potassium hydrogen phthalate 0.05 M**	**Potassium dihydrogen phosphate 0.025 M + disodium hydrogen phosphate 0.025 M**	**Potassium dihydrogen phosphate 0.0087 M + disodium hydrogen phosphate 0.0303 M**	**Disodium tetraborate 0.01 M**	**Sodium carbonate 0.025 M + sodium bicarbonate 0.025 M**	**Calcium hydroxide, saturated at 25°C**
	$C_4H_3KO_8.2H_2O$	$C_4H_5KO_6$	$C_6H_7O_7$	$C_8H_5KO_4$	KH_2PO_4, + Na_2HPO_4	KH_2PO_4, + Na_2HPO_4	$Na_2B_4O_7.10H_2O$	Na_2CO_3, + $NaHCO_3$	$Ca(OH)_2$
15	1.67		3.80	4.00	6.90	7.45	9.28	10.12	12.81
20	1.68		3.79	4.00	6.88	7.43	9.23	10.06	12.63
25	1.68	3.56	3.78	4.01	6.87	7.41	9.18	10.01	12.45
30	1.68	3.55	3.77	4.02	6.85	7.40	9.14	9.97	12.29
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.93	12.13
$\frac{\Delta pH^*}{\Delta t}$	+0.001	-0.0014	-0.0022	+0.0012	-0.0028	-0.0028	-0.0082	-0.0096	-0.034

*pH variation per degree Celsius.

2.2.4. Relationship between Reaction of Solution, Approximate pH and Colour of Certain Indicators

To 10 ml of the solution to be examined, add 0.1 ml of the indicator solution, unless otherwise prescribed in Table 2.2.4.-1.

Table 2.2.4.-1

Reaction	pH	Indicator	Colour
Alkaline	> 8	*Red litmus paper R*	Blue
		Thymol blue solution R (0.05 ml)	Grey or violet-blue
Slightly alkaline	8.0 – 10.0	*Phenolphthalein solution R* (0.05 ml)	Colourless or pink
		Thymol blue solution R (0.05 ml)	Grey
Strongly alkaline	> 10	*Phenolphthalein paper R*	Red
		Thymol blue solution R (0.05 ml)	Violet-blue
Neutral	6.0 – 8.0	*Methyl red solution R*	Yellow
		Phenol red solution R (0.05 m1)	
Neutral to methyl red	4.5 – 6.0	*Methyl red solution R*	Orange-red
Neutral to phenol-phthalein	< 8.0	*Phenolphthalein solution R (0.05 ml)*	Colourless; pink or red after adding 0.05 ml of 0.1 M base
Acid	< 6	*Methyl red solution R*	Orange or red
		Bromothymol blue solution R1	Yellow
Faintly acid	4.0 – 6.0	*Methyl red solution R*	Orange
		Bromocresol green solution R	Green or blue
Strongly acid	< 4	*Congo red paper R*	Green or blue

2.2.5. Relative Density

The relative density $d_{t_2}^{t_1}$ of a substance is the ratio of the mass of a certain volume of a substance at temperature t_1 to the mass of an equal volume of water at temperature t_2.

Unless otherwise indicated, the relative density d_{20}^{20} is used. Relative density is also commonly expressed as d_4^{20}. Density ρ_{20}, defined as the mass of a unit volume of the substance at 20 °C may also be used, expressed in kilograms per cubic metre or grams per cubic centimetre (1 kg . m^{-3} = 10^{-3} g . cm^{-3}). These quantities are related by the following equations where density is expressed in grams per cubic centimetre:

$$\rho_{20} = 0.998203 \times d_{20}^{20} \quad \text{or} \quad d_{20}^{20} = 1.00180 \times \rho_{20}$$

$$\rho_{20} = 0.999972 \times d_4^{20} \quad \text{or} \quad d_4^{20} = 1.00003 \times \rho_{20}$$

$$d_4^{20} = 0.998230 \times d_{20}^{20}$$

2.2.6. Refractive Index

The refractive index of a medium with reference to air is equal to the ratio of the sine of the angle of incidence of a beam of light in air to the sine of the angle of refraction of the refracted beam in the given medium.

Unless otherwise prescribed, the refractive index is measured at 20 ± 0.5 °C, with reference to the wavelength of the D-line of sodium (λ = 589.3 nm); the symbol is then n_D^{20}.

Refractometers normally determine the critical angle. In such apparatus the essential part is a prism of known refractive index in contact with the liquid to be examined.

Calibrate the apparatus using certified reference materials.

When white light is used, the refractometer is provided with a compensating system. The apparatus gives readings accurate to at least the third decimal place and is provided with a means of operation at the temperature prescribed. The thermometer is graduated at intervals of 0.5 °C or less.

2.2.7. Optical Rotation

Optical rotation is the property displayed by chiral substances of rotating the plane of polarisation of polarised light.

Optical rotation is considered to be positive (+) for dextrorotatory substances (i.e. those that rotate the plane of polarisation in a clockwise direction) and negative (–) for laevorotatory substances.

The specific optical rotation $[\alpha_m]_\lambda^t$ is the rotation, expressed in radians (rad), measured at the temperature *t* and at the wavelength λ given by a 1 m thickness of liquid or a solution containing 1 kg/m^3 of optically active substance. For practical reasons the specific optical rotation $[\alpha_m]_\lambda^t$ is normally expressed in milliradians metre squared per kilogram (mrad . m^2 . kg^{-1}).

The Pharmacopoeia adopts the following conventional definitions.

The *angle of optical rotation* of a neat liquid is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C using a layer of 1 dm; for a solution, the method of preparation is prescribed in the monograph.

The *specific optical rotation* $[\alpha]_D^{20}$ of a liquid is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C in the liquid substance to be examined, calculated with reference to a layer of 1 dm and divided by the density expressed in grams per cubic centimetre.

The *specific optical rotation* $[\alpha]_D^{20}$ of a substance in solution is the angle of rotation α, expressed in degrees (°), of the plane of polarisation at the wavelength of the D-line of sodium (λ = 589.3 nm) measured at 20 °C in a solution of the substance to be examined and calculated with reference to a layer of 1 dm containing 1 g/ml of the substance. The specific optical rotation of a substance in solution is always expressed with reference to a given solvent and concentration.

In the conventional system adopted by the Pharmacopoeia the specific optical rotation is expressed by its value without units; the actual units, degree millilitres per decimetre gram [(°)·ml·dm^{-1}·g^{-1}] are understood.

The conversion factor from the International System to the Pharmacopoeia system is the following:

$$[\alpha_m]_\lambda^t = [\alpha]_\lambda^t \,.\, 0.1745$$

In certain cases specified in the monograph the angle of rotation may be measured at temperatures other than 20 °C and at other wavelengths.

The polarimeter must be capable of giving readings to the nearest 0.01°. The scale is usually checked by means of certified quartz plates. The linearity of the scale may be checked by means of sucrose solutions.

Method. Determine the zero of the polarimeter and the angle of rotation of polarised light at the wavelength of the D-line of sodium (λ = 589.3 nm) at 20 ± 0.5 °C. Measurements may be carried out at other temperatures only where the monograph indicates the temperature correction to be made to the measured optical rotation. Determine the zero of the apparatus with the tube closed; for liquids the zero is determined with the tube empty and for solids filled with the prescribed solvent.

Calculate the specific optical rotation using the following formulae.

For neat liquids:

$$[\alpha]_D^{20} = \frac{\alpha}{l \,.\, \rho_{20}}$$

For substances in solution:

$$[\alpha]_D^{20} = \frac{1000 \cdot \alpha}{l \cdot c}$$

where c is the concentration of the solution in grams per litre.

Calculate the content c in grams per litre or the content c' in per cent *m/m* of a dissolved substance using the following formulae:

$$c = \frac{1000 \cdot \alpha}{l \cdot [\alpha]_D^{20}} \qquad c' = \frac{100 \cdot \alpha}{l \cdot [\alpha]_D^{20} \cdot \rho_{20}}$$

α = angle of rotation in degrees read at 20 ± 0.5 °C,

l = length in decimetres of the polarimeter tube,

ρ_{20} = density at 20 °C in grams per cubic centimetre. For the purposes of the Pharmacopoeia, density is replaced by relative density (2.2.5).

2.2.14. Melting Point - Capillary Method

The melting point determined by the capillary method is the temperature at which the last solid particle of a compact column of a substance in a tube passes into the liquid phase. When prescribed in the monograph, the same apparatus and method are used for the determination of other factors, such as meniscus formation or melting range, that characterise the melting behaviour of a substance.

Apparatus. The apparatus consists of:

- a suitable glass vessel containing a liquid bath (for example, water, liquid paraffin or silicone oil) and fitted with a suitable means of heating,
- a suitable means of stirring, ensuring uniformity of temperature within the bath,
- a suitable thermometer with graduation at not more than 0.5 °C intervals and provided with an immersion mark. The range of the thermometer is not more than 100 °C,
- alkali-free hard-glass capillary tubes of internal diameter 0.9 mm to 1.1 mm with a wall 0.10 mm to 0.15 mm thick and sealed at one end.

Method. Unless otherwise prescribed, dry the finely powdered substance *in vacuo* and over *anhydrous silica gel R* for 24 h. Introduce a sufficient quantity into a capillary tube to give a compact column 4 mm to 6 mm in height. Raise the temperature of the bath to about 10 °C below the presumed melting point and then adjust the rate of heating to about 1 °C per minute. When the temperature is 5 °C below the presumed melting point, correctly introduce the capillary tube into the instrument. For the apparatus described above, immerse the capillary tube so that the closed end is near the centre of the bulb of the thermometer, the immersion mark of which is at the level of the surface of the liquid. Record the temperature at which the last particle passes into the liquid phase.

Calibration of the apparatus. The apparatus may be calibrated using melting point reference substances such as those of the World Health Organisation or other appropriate substances.

2.2.32. Loss on drying

Loss on drying is the loss of mass expressed as per cent *m/m*.

Method. Place the prescribed quantity of the substance to be examined in a weighing bottle previously dried under the conditions prescribed for the substance to be examined. Dry the substance to constant mass or for the prescribed time by one of the following procedures. Where the drying temperature is indicated by a single value rather than a range, drying is carried out at the prescribed temperature ± 2 °C.

a) “in a desiccator” : the drying is carried out over *diphosphorus pentoxide R* at atmospheric pressure and at room temperature;

b) “*in vacuo*”: the drying is carried out over *diphosphorus pentoxide R*, at a pressure of 1.5 kPa to 2.5 kPa at room temperature;

c) "*in vacuo* within a specified temperature range" : the drying is carried out over *diphosphorus pentoxide R*, at a pressure of 1.5 kPa to 2.5 kPa within the temperature range prescribed in the monograph;

d) "in an oven within a specified temperature range" : the drying is carried out in an oven within the temperature range prescribed in the monograph;

e) "under high vacuum": the drying is carried out over *diphosphorus pentoxide R* at a pressure not exceeding 0.1 kPa, at the temperature prescribed in the monograph.

If other conditions are prescribed, the procedure to be used is described in full in the monograph.

2.3. Identification

2.3.1. Identification Reactions of Ions and Functional Groups

Acetates

a) Heat the substance to be examined with an equal quantity of *oxalic acid R*. Acid vapours with the characteristic odour of acetic acid are liberated, showing an acid reaction *(2.2.4)*.

Oxalic acid is a stronger and less volatile acid than acetic acid. Therefore, when oxalic acid is heated with an acetate, the liberation of acetic acid is observed.

$$CH_3COO^- + (COOH)_2 \rightarrow CH_3COOH + HOOC{-}COO^-$$

b) Dissolve about 30 mg of the substance to be examined in 3 ml of *water R* or use 3 ml of the prescribed solution. Add successively 0.25 ml of *lanthanum nitrate solution R*, 0.1 ml of *0.05 M iodine* and 0.05 ml of *dilute ammonia R2*. Heat carefully to boiling. Within a few minutes a blue precipitate is formed or a dark blue colour develops.

In the presence of ammonia, basic lanthanum(III) acetate is formed.

$$2\ CH_3COO^- + La^{3+} + NH_3 + H_2O \rightarrow LaOH(CH_3COO)_2 + NH_4^+$$

Iodine is adsorbed on the surface of a basic lanthanum(III) acetate precipitate, resulting in a blue colour. Similarly to the blue iodine–starch, a basic lanthanum(III) acetate–iodine complex can be formed. A slightly alkaline pH (pH 9-11) is very important. At when pH<9, basic lanthanum(III) acetate is not formed, and under strong alkaline conditions (pH>11) iodide and hypoiodite are formed by the disproportionation of iodine. Anions forming a precipitate (*e.g.* BO_3^{3-}, PO_4^{3-}, SO_4^{2-} and F^-) or a complex (*e.g.* citrate and tartrate) with La^{3+}, and also compounds that reduce iodine (*e.g.* $S_2O_3^{2-}$ and SO_3^{2-}) can interfere.

Acetyl

In a test-tube about 180 mm long and 18 mm in external diameter, place about 15 mg of the substance to be examined, or the prescribed quantity, and 0.15 ml of *phosphoric acid R*. Close the tube with a stopper through which passes a small test-tube about 100 mm long and 10 mm in external diameter containing *water R* to act as a condenser. On the outside of the smaller tube, hang a drop of *lanthanum nitrate solution R*. Except for substances hydrolysable only with difficulty, place the apparatus in a water-bath for 5 min, then take out the smaller tube. Remove the drop and mix it with 0.05 ml of *0.01 M iodine* on a tile. Add at the edge 0.05 ml of *dilute ammonia R2*. After 1 min to 2 min, a blue colour develops at the junction of the two drops; the colour intensifies and persists for a short time.

For *substances hydrolysable only with difficulty* heat the mixture slowly to boiling over an open flame and then proceed as prescribed above.

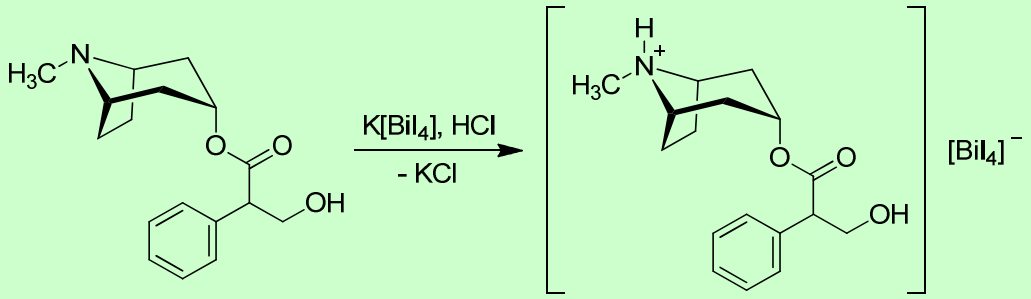
Hydrolysis of compounds containing an acetyl functional group with phosphoric acid results in acetic acid, which can be determined by the identification reaction **b)** of acetates, as basic lanthanum(III) acetate.

$$R{-}O{-}CO{-}CH_3 + H_2O \rightarrow CH_3COOH + R{-}OH$$

Alkaloids

Dissolve a few milligrams of the substance to be examined, or the prescribed quantity, in 5 ml of *water R*, add *dilute hydrochloric acid R* until an acid reaction occurs *(2.2.4)*, then 1 ml of *potassium iodobismuthate solution R*. An orange or orange-red precipitate is formed immediately.

Alkaloids form water-insoluble tetraiodobismuthate salts with potassium tetraiodobismuthate (**DRAGENDORFF** reagent).



atropine **atropinium tetraiodobismuthate**

Aluminium

Dissolve about 15 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add about 0.5 ml of *dilute hydrochloric acid R* and about 0.5 ml of *thioacetamide reagent R*. No precipitate is formed. Add dropwise *dilute sodium hydroxide solution R*. A gelatinous white precipitate is formed which dissolves on further addition of *dilute sodium hydroxide solution R*. Gradually add *ammonium chloride solution R*. The gelatinous white precipitate is re-formed.

In the first part of the identification, those ions (*e.g.* Hg^{2+}, Bi^{3+}, Pb^{2+}, Sn^{2+} and Sb^{3+}) are excluded which form precipitates with sodium hydroxide, but also form precipitates with chloride ($PbCl_2$) or (in weakly acidic medium) sulfide (*e.g.* Bi_2S_3 and SnS). The white, gelatinous precipitate dissolves in an excess of sodium hydroxide, forming the tetrahydroxoaluminate complex. On decrease of the pH with acidic ammonium chloride, aluminium hydroxide precipitates again.

$$Al^{3+} + 3\ OH^- \rightarrow Al(OH)_3$$

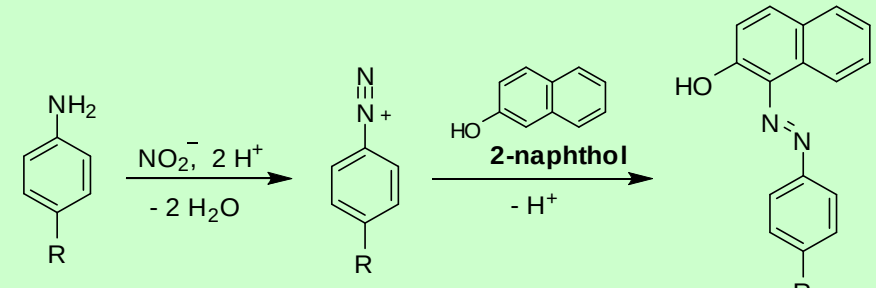
$$Al(OH)_3 + OH^- \rightarrow [Al(OH)_4]^-$$

$$[Al(OH)_4]^- + NH_4^+ \rightarrow Al(OH)_3 + NH_3 + H_2O$$

Amines, Primary Aromatic

Acidify the prescribed solution with *dilute hydrochloric acid R* and add 0.2 ml of *sodium nitrite solution R*. After 1 min to 2 min, add 1 ml of *β-naphthol solution R*. An intense orange or red colour and usually a precipitate of the same colour are produced.

In acidic solution, nitrite forms a diazonium salt with the primary amino group; this produces a red azo dye with 2-naphthol (β-naphthol) in an electrophilic substitution reaction.



primary aromatic amine **diazonium salt** **red azo dye**

Ammonium salts

To the prescribed solution add 0.2 g of *magnesium oxide R*. Pass a current of air through the mixture and direct the gas that escapes just beneath the surface of a mixture of 1 ml of *0.1 M hydrochloric acid* and 0.05 ml of *methyl red solution R*. The colour of the indicator changes to yellow. On addition of 1 ml of a freshly prepared 100 g/l *solution of sodium cobaltinitrite R* a yellow precipitate is formed.

Magnesium oxide liberates ammonia from its salts and the liberated ammonia makes the hydrochloric acid solution basic (the colour of the indicator changes to yellow). The pH interval of the colour change of the methyl red indicator is from 4.4 (red) to 6.0 (yellow). The resulting ammonium chloride gives a yellow precipitate of diammonium sodium cobaltinitrite with sodium cobaltinitrite (depending on the concentration of ammonia, triammonium or monoammonium salts can also be present).

$$MgO + 2\ NH_4^+ \rightarrow Mg^{2+} + 2\ NH_3 + H_2O$$

$$NH_3 + H^+ \rightarrow NH_4^+$$

$$2\ NH_4^+ + Na^+ + [Co(NO_2)_6]^{3-} \rightarrow (NH_4)_2Na[Co(NO_2)_6]$$

Ammonium Salts and Salts of Volatile Bases

Dissolve about 20 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 2 ml of *dilute sodium hydroxide solution R*. On heating, the solution gives off vapour that can be identified by its odour and by its alkaline reaction *(2.2.4)*.

Volatile ammonia or amines are liberated.

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

$$R\text{–}NH_3^+ + OH^- \rightleftharpoons R\text{–}NH_3OH \rightleftharpoons R\text{–}NH_2 + H_2O$$

Arsenic

Heat 5 ml of the prescribed solution on a water-bath with an equal volume of *hypophosphorous reagent R*. A brown precipitate is formed.

Hypophosphite reduces arsenite or arsenate compounds to elementary arsenic (**THIELE** test).

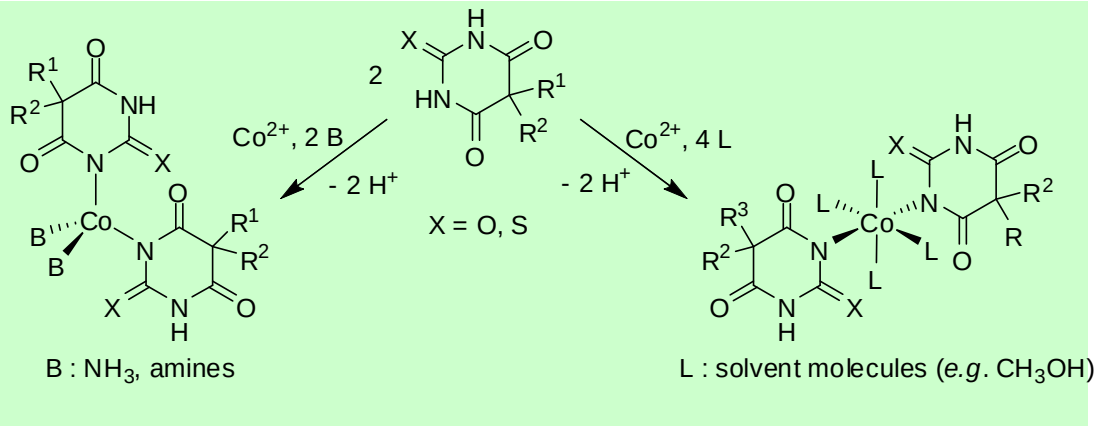
$$2\ AsO_3^{3-} + 3\ H_2PO_2^- + 9\ H^+ \rightarrow 2\ As + 3\ H_3PO_3 + 3\ H_2O$$

$$2\ AsO_4^{3-} + 5\ H_2PO_2^- + 11\ H^+ \rightarrow 2\ As + 5\ H_3PO_3 + 3\ H_2O$$

Barbiturates, Non-Nitrogen Substituted

Dissolve about 5 mg of the substance to be examined in 3 ml of *methanol R*, add 0.1 ml of a solution containing 100 g/l of *cobalt nitrate R* and 100 g/l of *calcium chloride R*. Mix and add, with shaking, 0.1 ml of *dilute sodium hydroxide solution R*. A violet-blue colour and precipitate are formed.

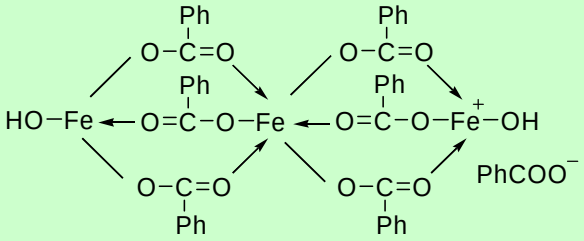
Barbitals and thiobarbitals form violet complexes with Co^{2+} in methanol (**PARRI-ZWIKKER** reaction). In the presence of a nitrogen-containing base, tetrahedral complexes are formed. Nitrogen-free bases form octahedral complexes with the solvent molecules. The reaction is not specific: hydantoins, some pyridine and piperidine derivatives, sulfonamides and purines also give a positive reaction. (Sodium hydroxide promotes the deprotonation of barbital; calcium chloride warrants the basic condition.)



Benzoates

a) To 1 ml of the prescribed solution add 0.5 ml of *ferric chloride solution R1*. A dull-yellow precipitate, soluble in *ether R,* is formed.

A three-centred iron(III) complex ($[Fe_3(PhCOO)_6(OH)_2]^+$ $PhCOO^-$) is formed.



dihydroxohexabenzoato triiron(III)monobenzoate

b) Place 0.2 g of the substance to be examined, treated if necessary as prescribed, in a test-tube. Moisten with 0.2 ml to 0.3 ml of *sulfuric acid R*. Gently warm the bottom of the tube. A white sublimate is deposited on the inner wall of the tube.

Sulfuric acid liberates benzoic acid, which readily sublimes on heating.

c) Dissolve 0.5 g of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.5 ml of *hydrochloric acid R*. The precipitate obtained, after crystallisation from warm *water R* and drying *in vacuo*, has a melting point *(2.2.14)* of 120 °C to 124 °C.

Hydrochloric acid liberates benzoic acid.

Bismuth

d) To 0.5 g of the substance to be examined add 10 ml of *dilute hydrochloric acid R* or use 10 ml of the prescribed solution. Heat to boiling for 1 min. Cool and filter if necessary. To 1 ml of the solution obtained add 20 ml of *water R*. A white or slightly yellow precipitate is formed which on addition of 0.05 ml to 0.1 ml of *sodium sulfide solution R* turns brown.

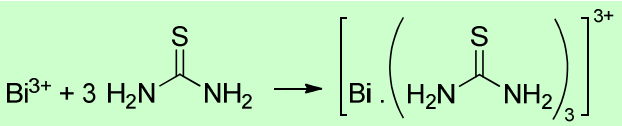
A basic salt of bismuth is formed, which gives a black precipitate of bismuth sulfide with sodium sulfide.

$$Bi^{3+} + Cl^- + H_2O \rightarrow BiOCl + 2\ H^+$$

$$2\ BiOCl + 3\ S^{2-} + 4\ H^+ \rightarrow Bi_2S_3 + 2\ Cl^- + 2\ H_2O$$

e) To about 45 mg of the substance to be examined add 10 ml of *dilute nitric acid R* or use 10 ml of the prescribed solution. Boil for 1 min. Allow to cool and filter if necessary. To 5 ml of the solution obtained add 2 ml of a 100 g/l *solution of thiourea R*. A yellowish-orange colour or an orange precipitate is formed. Add 4 ml of a 25 g/l solution of *sodium fluoride R*. The solution is not decolorised within 30 min.

In nitric acid solution, bismuth produces a stable orange complex with thiourea. If antimony(III) is present, a similar complex is formed, but the latter decomposes when sodium fluoride is added to the solution.



Bromides

a) Dissolve in 2 ml of *water R* a quantity of the substance to be examined equivalent to about 3 mg of bromide (Br^-) or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled, pale yellow precipitate is formed. Centrifuge and wash the precipitate with three quantities, each of 1 ml, of *water R*. Carry out this operation rapidly in subdued light disregarding the fact that the supernatant solution may not become perfectly clear.

Suspend the precipitate obtained in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate dissolves with difficulty.

A silver bromide precipitate is less soluble than silver chloride in ammonia.

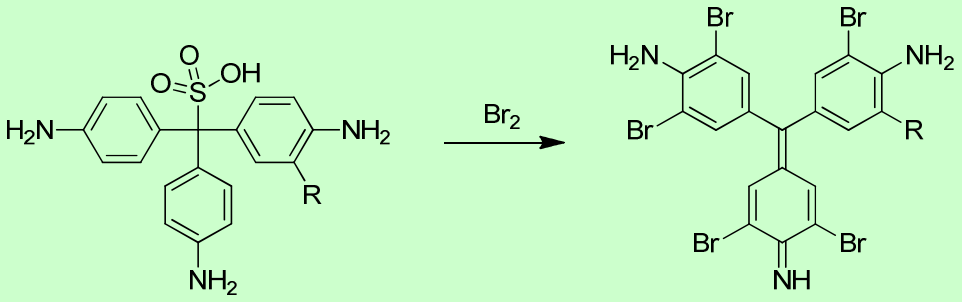
$$Br^- + Ag^+ \rightarrow AgBr$$

$$AgBr + 2\ NH_3 \rightarrow [Ag(NH_3)_2]^+ + Br^-$$

b) Introduce into a small test-tube a quantity of the substance to be examined equivalent to about 5 mg of bromide (Br^-) or the prescribed quantity. Add 0.25 ml of *water R*, about 75 mg of *lead dioxide R*, 0.25 ml of *acetic acid R* and shake gently. Dry the inside of the upper part of the test-tube with a piece of filter paper and allow to stand for 5 min. Prepare a strip of suitable filter paper of appropriate size. Impregnate it by capillarity, by dipping the tip in a drop of *decolorised fuchsin solution R* and introduce the impregnated part immediately into the tube. Starting from the tip, a violet colour appears within 10 s that is clearly distinguishable from the red colour of fuchsin, which may be visible on a small area at the top of the impregnated part of the paper strip.

In acidic medium, lead(IV) oxide oxidizes bromide to bromine. The liberated bromine reacts with the decolorized fuchsin (**SCHIFF** reagent), resulting in violet bromo-substituted products (hexabromopararosaniline and pentabromorosaniline).

$$2\ Br^- + PbO_2 + 4\ H^+ \rightarrow Br_2 + Pb^{2+} + 2\ H_2O$$



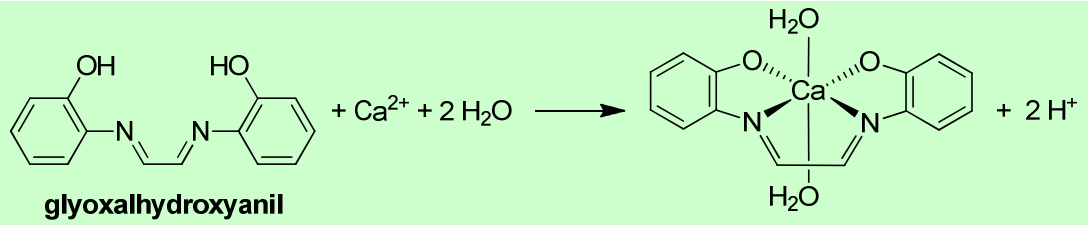
decolorized fuchsin (R = H, CH_3)

hexabromopararosaniline (R = Br)
pentabromorosaniline (R = CH_3)

Calcium

a) To 0.2 ml of a neutral solution containing a quantity of the substance to be examined equivalent to about 0.2 mg of calcium (Ca^{2+}) per millilitre or to 0.2 ml of the prescribed solution add 0.5 ml of a 2 g/l solution of *glyoxalhydroxyanil R* in *ethanol (96 per cent) R*, 0.2 ml of *dilute sodium hydroxide solution R* and 0.2 ml of *sodium carbonate solution R*. Shake with 1 ml to 2 ml of *chloroform R* and add 1 ml to 2 ml of *water R*. The chloroform layer is coloured red.

With Ca^{2+}, glyoxalhydroxyanil forms a red chelate complex, which dissolves in chloroform (Ba^{2+} and Sr^{2+} result in similar complexes, but these latter do not dissolve in chloroform). Sodium carbonate increases the sensitivity of the reaction by precipitating the disturbing Ba^{2+} and Sr^{2+} as carbonates.



b) Dissolve about 20 mg of the substance to be examined or the prescribed quantity in 5 ml of *acetic acid R*. Add 0.5 ml of *potassium ferrocyanide solution R*. The solution remains clear. Add about 50 mg of *ammonium chloride R*. A white, crystalline precipitate is formed.

In acetic acid solution, a precipitate of diammonium calcium hexacyanoferrate(II) is formed. Ba^{2+} and Mg^{2+} also give positive reactions.

$$Ca^{2+} + 2\ NH_4^+ + [Fe(CN)_6]^{4-} \rightarrow (NH_4)_2Ca[Fe(CN)_6]$$

Carbonates and bicarbonates

Introduce into a test-tube 0.1 g of the substance to be examined and suspend in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 3 ml of *dilute acetic acid R*. Close the tube immediately using a stopper fitted with a glass tube bent twice at right angles. The solution or the suspension becomes effervescent and gives off a colourless and odourless gas. Heat gently and collect the gas in 5 ml of *barium hydroxide solution R*. A white precipitate is formed that dissolves on addition of an excess of *hydrochloric acid R1*.

Acetic acid reacts with carbonates and hydrogencarbonates and the liberated carbon dioxide forms a precipitate of barium carbonate with barium hydroxide. The precipitate is soluble in hydrochloric acid solution.

$$CO_3^{2-} + 2\ H^+ \rightleftharpoons CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

$$Ba^{2+} + 2\ OH^- + CO_2 \rightarrow BaCO_3 + H_2O$$

$$BaCO_3 + 2\ H^+ \rightarrow Ba^{2+} + CO_2 + H_2O$$

Chlorides

a) Dissolve in 2 ml of *water R* a quantity of the substance to be examined equivalent to about 2 mg of chloride (Cl^-) or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled, white precipitate is formed. Centrifuge and wash the precipitate with three quantities, each of 1 ml, of *water R*. Carry out this operation rapidly in subdued light, disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate dissolves easily with the possible exception of a few large particles which dissolve slowly.

A curdled white silver chloride precipitate is produced, which dissolves in an excess of ammonia solution (the diamminesilver complex is formed).

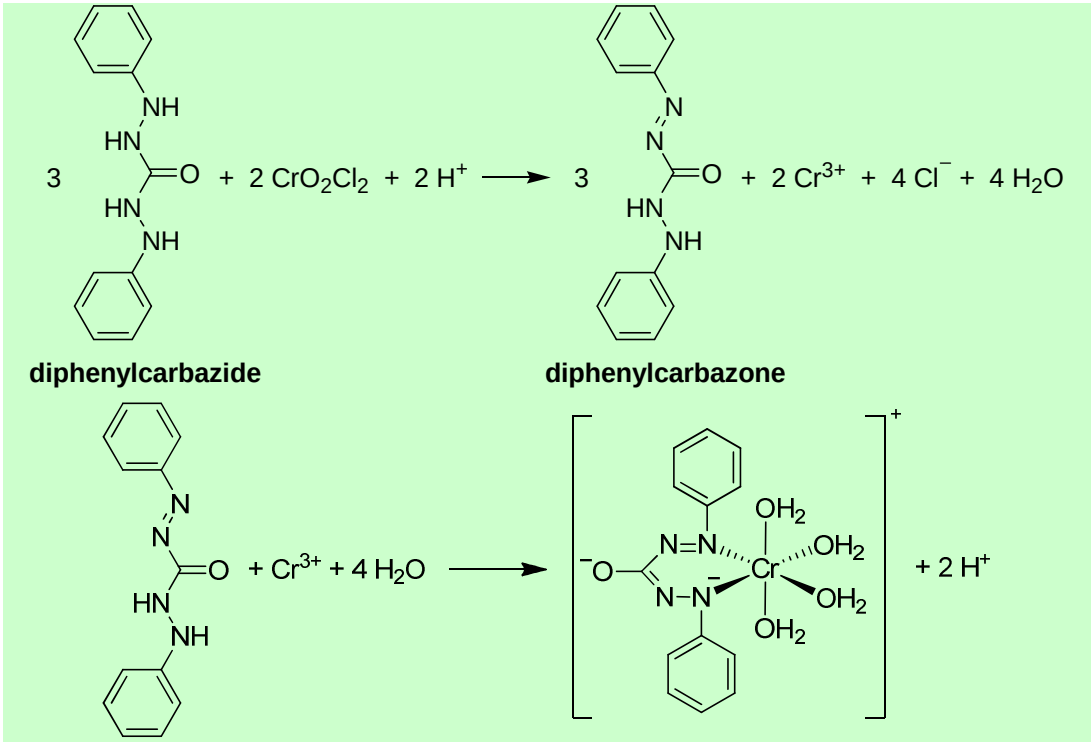
$$Cl^- + Ag^+ \rightarrow AgCl$$

$$AgCl + 2\ NH_3 \rightarrow [Ag(NH_3)_2]^+ + Cl^-$$

b) Introduce into a test-tube a quantity of the substance to be examined equivalent to about 15 mg of chloride (Cl^-) or the prescribed quantity. Add 0.2 g of *potassium dichromate R* and 1 ml of *sulfuric acid R*. Place a filter-paper strip impregnated with 0.1 ml of *diphenylcarbazide solution R* over the opening of the test-tube. The paper turns violet-red. The impregnated paper must not come into contact with the potassium dichromate.

Under acidic conditions, the reaction of chloride with dichromate produces volatile chromyl chloride, which oxidizes diphenylcarbazide to diphenylcarbazone. Diphenylcarbazone forms a violet-red complex with Cr^{3+}.

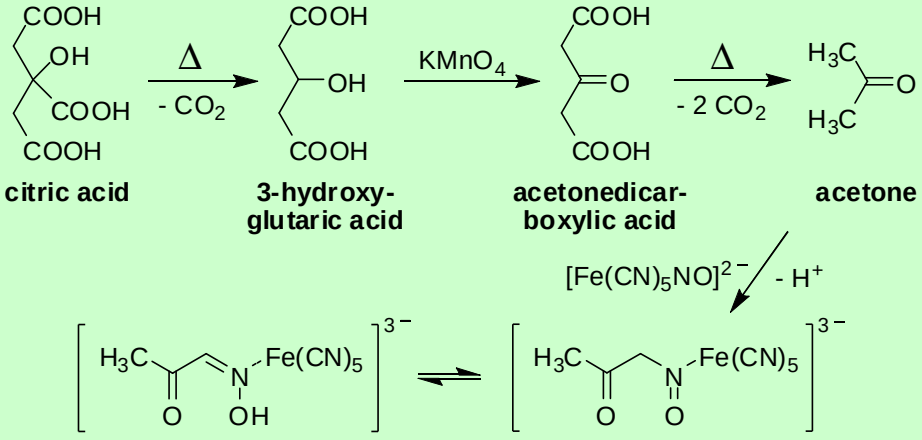
$$4\ Cl^- + Cr_2O_7^{2-} + 6\ H^+ \rightarrow 2\ CrO_2Cl_2 + 3\ H_2O$$



Citrates

Dissolve in 5 ml of *water R* a quantity of the substance to be examined equivalent to about 50 mg of citric acid or use 5 ml of the prescribed solution. Add 0.5 ml of *sulfuric acid R* and 1 ml of *potassium permanganate solution R.* Warm until the colour of the permanganate is discharged. Add 0.5 ml of a 100 g/l solution of *sodium nitroprusside R* in *dilute sulfuric acid R* and 4 g of *sulfamic acid R*. Make alkaline with *concentrated ammonia R*, added dropwise until all the sulfamic acid has dissolved. Addition of an excess of *concentrated ammonia R* produces a violet colour, turning to violet-blue.

Oxidation of citric acid with permanganate results in acetonedicarboxylic acid, which on heating is decarboxylated to acetone. Under basic conditions, the deprotonated acetone forms a violet complex with sodium nitroprusside (**LEGAL** reaction).



Sulfamic acid is used to eliminate the incidentally liberated nitrous gas or nitrous acid.

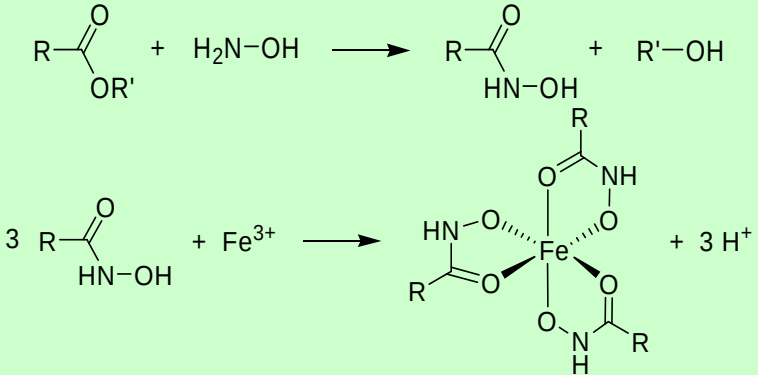
$$HOSO_2NH_2 + NO_2^- + H^+ \rightarrow H_2SO_4 + N_2 + H_2O$$

Esters

To about 30 mg of the substance to be examined or the prescribed quantity add 0.5 ml of a 70 g/l solution of *hydroxylamine hydrochloride R* in *methanol R* and 0.5 ml of a 100 g/l solution of *potassium*

hydroxide R in *ethanol (96 per cent) R*. Heat to boiling, cool, acidify with *dilute hydrochloric acid R* and add 0.2 ml of *ferric chloride solution R1* diluted ten times. A bluish-red or red colour is produced.

Esters react with hydroxylamine to result in hydroxamic acids, which form red, three-ligand complexes with Fe^{3+}. Cyclic esters (lactones), anhydrides and acid chlorides also give a positive reaction.



Iodides

a) Dissolve a quantity of the substance to be examined equivalent to about 4 mg of iodide (I^-) in 2 ml of *water R* or use 2 ml of the prescribed solution. Acidify with *dilute nitric acid R* and add 0.4 ml of *silver nitrate solution R1*. Shake and allow to stand. A curdled, pale-yellow precipitate is formed. Centrifuge and wash with three quantities, each of 1 ml, of *water R.* Carry out this operation rapidly in subdued light disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in 2 ml of *water R* and add 1.5 ml of *ammonia R*. The precipitate does not dissolve.

A yellow, photosensitive precipitate of silver iodide is produced, which does not dissolve in an excess of ammonia solution.

$$I^- + Ag^+ \rightarrow AgI$$

b) To 0.2 ml of a solution of the substance to be examined containing about 5 mg of iodide (I^-) per millilitre, or to 0.2 ml of the prescribed solution, add 0.5 ml of *dilute sulfuric acid R*, 0.1 ml of *potassium dichromate solution R*, 2 ml of *water R* and 2 ml of *chloroform R*. Shake for a few seconds and allow to stand. The chloroform layer is coloured violet or violet-red.

Dichromate oxidizes iodide to iodine, which dissolves in chloroform to give a violet colour.

$$6\ I^- + Cr_2O_7^{2-} + 14\ H^+ \rightarrow 3\ I_2 + 2\ Cr^{3+} + 7\ H_2O$$

Iron

a) Dissolve a quantity of the substance to be examined equivalent to about 10 mg of iron (Fe^{2+}) in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml of *potassium ferricyanide solution R*. A blue precipitate is formed that does not dissolve on addition of 5 ml of *dilute hydrochloric acid R*.

In the main reaction, Fe^{2+} forms a precipitate of **Turnbull blue** with hexacyanoferrate(III), but because of the redox side-reaction [resulting in Fe^{3+} and hexacyanoferrate(II)], a precipitate of Prussian blue is also formed.

$$3\ Fe^{2+} + 2\ [Fe(CN)_6]^{3-} \rightarrow Fe_3[Fe(CN)_6]_2$$

Turnbull blue

$$Fe^{2+} + [Fe(CN)_6]^{3-} \rightleftharpoons Fe^{3+} + [Fe(CN)_6]^{4-}$$

$$4\ Fe^{3+} + 3\ [Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

Prussian blue

b) Dissolve a quantity of the substance to be examined equivalent to about 1 mg of iron (Fe^{3+}) in 30 ml of *water R*. To 3 ml of this solution or to 3 ml of the prescribed solution, add 1 ml of *dilute hydrochloric acid R* and 1 ml of *potassium thiocyanate solution R*. The solution is coloured red. Take two portions, each of 1 ml, of the mixture. To one portion add 5 ml of *isoamyl alcohol R* or 5 ml of *ether R*. Shake

and allow to stand. The organic layer is coloured pink. To the other portion add 2 ml of *mercuric chloride solution R*. The red colour disappears.

The composition of the resulting thiocyanatoferrate(III) complex depends on the concentrations of Fe^{3+} and thiocyanate. When Hg^{2+} is added to the solution, a more stable tetrathiocyanatomercurate(II) is formed and the red colour disappears.

$$Fe^{3+} + n\ SCN^- \rightarrow [Fe(SCN)_n]^{3-n} \quad (n = 1\text{-}6)$$

$$4\ Fe(SCN)_3 + 3\ Hg^{2+} \rightarrow 3\ [Hg(SCN)_4]^{2-} + 4\ Fe^{3+}$$

c) Dissolve a quantity of the substance to be examined equivalent to not less than 1 mg of iron (Fe^{3+}) in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml of *potassium ferrocyanide solution R*. A blue precipitate is formed that does not dissolve on addition of 5 ml of *dilute hydrochloric acid R*.

Fe^{3+} forms a precipitate of **Prussian blue** with hexacyanoferrate(II).

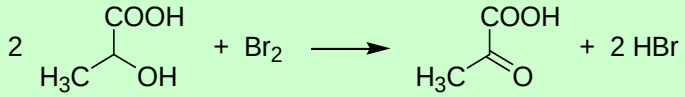
$$4\ Fe^{3+} + 3\ [Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

Prussian blue

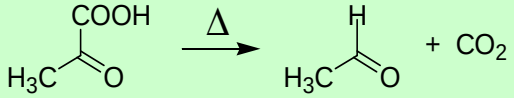
Lactates

Dissolve a quantity of the substance to be examined equivalent to about 5 mg of lactic acid in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 1 ml of *bromine water R* and 0.5 ml of *dilute sulfuric acid R*. Heat on a water-bath until the colour is discharged, stirring occasionally with a glass rod. Add 4 g of *ammonium sulfate R* and mix. Add dropwise and without mixing 0.2 ml of a 100 g/l solution of s*odium nitroprusside R* in *dilute sulfuric acid R*. Still without mixing add 1 ml of *concentrated ammonia R*. Allow to stand for 30 min. A dark green ring appears at the junction of the two liquids.

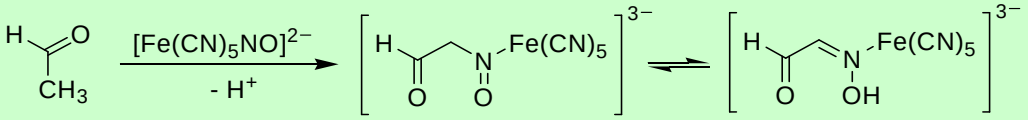
In acidic solution, lactates hydrolyse to lactic acid, which is oxidized to pyruvic acid by bromine. Pyruvic acid undergoes decarboxylation, resulting in acetaldehyde, which forms a green complex with sodium nitroprusside in alkaline solution (**LEGAL** reaction).



lactic acid **pyruvic acid**



acetaldehyde



Magnesium

Dissolve about 15 mg of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 1 ml of *dilute ammonia R1*. A white precipitate is formed that dissolves on addition of 1 ml of *ammonium chloride solution R*. Add 1 ml of *disodium hydrogen phosphate solution R*. A white crystalline precipitate is formed.

Ammonia precipitates white magnesium hydroxide. The precipitation is not complete, because the ammonium salt formed depresses the hydroxide ion concentration. For this reason, the precipitate is soluble in ammonium chloride. In the presence of ammonium chloride and ammonia, disodium hydrogenphosphate produces a precipitate of white crystalline magnesium ammonium phosphate.

$$Mg^{2+} + 2\ NH_3 + 2\ H_2O \rightleftharpoons Mg(OH)_2 + 2NH_4^+$$

$$Mg^{2+} + NH_4^+ + HPO_4^{2-} \rightarrow MgNH_4PO_4 + H^+$$

Mercury

a) Place about 0.1 ml of a solution of the substance to be examined on well-scraped copper foil. A dark-grey stain that becomes shiny on rubbing is formed. Dry the foil and heat in a test-tube. The spot disappears.

Hg_2^{2+} and Hg^{2+} can be reduced with metal copper, resulting in metallic mercury (mercury reacts with copper to form an amalgam) and Cu^{2+}. On heating, some of the mercury may vaporize. Because of the toxicity of mercury vapour, the reaction must be carried out under a well-ventilated hood.

$$Hg_2^{2+} + Cu \rightarrow 2\ Hg + Cu^{2+}$$

$$Hg^{2+} + Cu \rightarrow Hg + Cu^{2+}$$

b) To the prescribed solution add *dilute sodium hydroxide solution R* until strongly alkaline (*2.2.4*). A dense yellow precipitate is formed (mercuric salts).

Sodium hydroxide gives a yellow precipitate of mercury(II) oxide. In the case of Hg_2^{2+}, sodium hydroxide precipitates a black mercury(I) oxide, which decomposes to mercury (black) and mercury(II) oxide (yellow) by disproportionation.

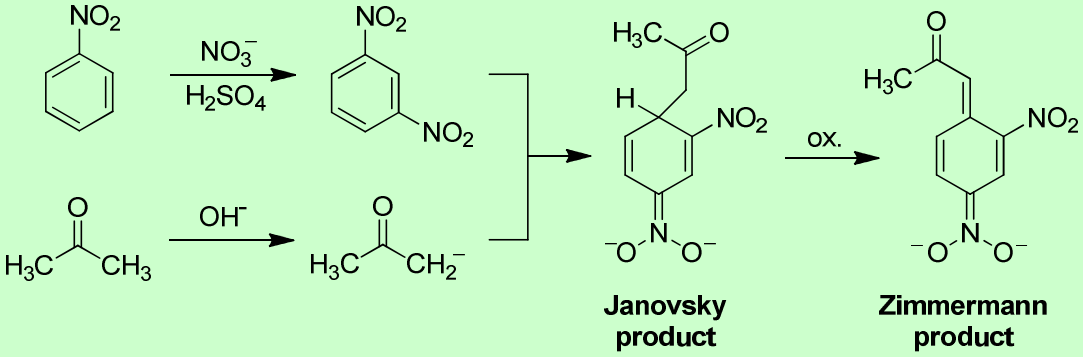
$$Hg^{2+} + 2\ OH^- \rightarrow HgO + H_2O$$

$$Hg_2^{2+} + 2\ OH^- \rightarrow HgO + Hg + H_2O$$

Nitrates

To a mixture of 0.1 ml of *nitrobenzene R* and 0.2 ml of *sulfuric acid R*, add a quantity of the powdered substance equivalent to about 1 mg of nitrate (NO_3^-) or the prescribed quantity. Allow to stand for 5 min. Cool in iced water and add slowly and with mixing 5 ml of *water R*, then 5 ml of strong *sodium hydroxide solution R*. Add 5 ml of *acetone R*. Shake and allow to stand. The upper layer is coloured deep violet.

The identification of nitrate ion (**PESEZ** reaction) is based on the **JANOVSKY-ZIMMERMANN** reaction, a selective and sensitive reaction for the identification of active methyl and methylene groups. The nitric acid liberated from nitrates by concentrated sulfuric acid causes the nitration of nitrobenzene in the *meta* position. *meta*-Dinitrobenzene forms a violet **MEISENHEIMER** complex (**JANOVSKY** product) with acetone, which is deprotonated in strongly alkaline medium. Oxidation of this product results in a brown compound (**ZIMMERMANN** product). The reaction is specific for nitrates (no reaction with nitrites).



Phosphates (Orthophosphates)

a) To 5 ml of the prescribed solution, neutralised if necessary, add 5 ml of *silver nitrate solution R1*. A yellow precipitate is formed whose colour is not changed by boiling and which dissolves on addition of *ammonia R*.

The resulting silver phosphate dissolves in ammonia as the diamminesilver complex.

$$PO_4^{3-} + 3\ Ag^+ \rightarrow Ag_3PO_4$$

$$Ag_3PO_4 + 6\ NH_3 \rightarrow 3\ [Ag(NH_3)_2]^+ + PO_4^{3-}$$

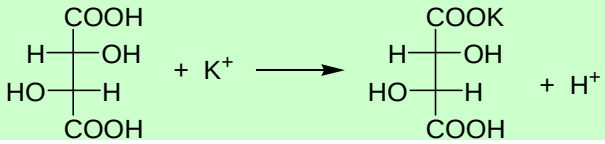
b) Mix 1 ml of the prescribed solution with 2 ml of *molybdovanadic reagent R*. A yellow colour develops.

With phosphate, the reagent containing $(NH_4)_2MoO_4$ (ammonium molybdate) and $(NH_4)_3VO_4$ (ammonium vanadate) forms a mixed divanadate-decamolybdatophosphate ($PV_2Mo_{10}O_{40}^{5-}$).

Potassium

a) Dissolve 0.1 g of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 1 ml of *sodium carbonate solution R* and heat. No precipitate is formed. Add to the hot solution 0.05 ml of *sodium sulfide solution R*. No precipitate is formed. Cool in iced water and add 2 ml of a 150 g/l solution of *tartaric acid R*. Allow to stand. A white crystalline precipitate is formed.

In the first part of the identification, ions that form precipitates with carbonate or sulfide (*e.g.* alkaline earth metals and heavy metals) are excluded. Tartaric acid precipitates white potassium hydrogentartrate (the dipotassium salt dissolves well in water). The tartaric acid used for this reaction is L-tartaric acid [(2*R*,3*R*)-2,3-dihydroxybutanedioic acid].



b) Dissolve about 40 mg of the substance to be examined in 1 ml of *water R* or use 1 ml of the prescribed solution. Add 1 ml of *dilute acetic acid R* and 1 ml of a freshly prepared 100 g/l solution of *sodium cobaltinitrite R*. A yellow or orange-yellow precipitate is formed immediately.

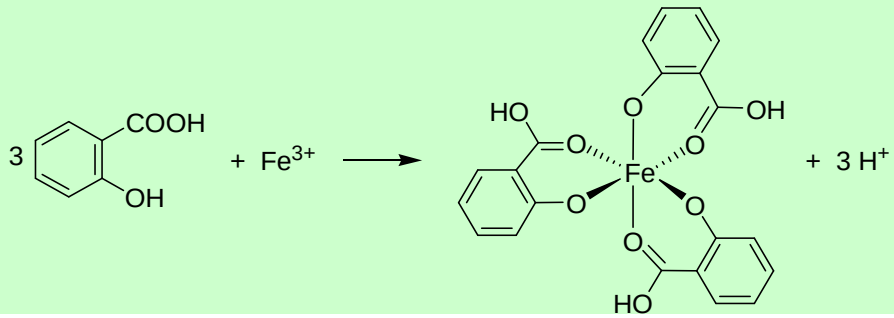
Dipotassium cobaltinitrite is formed (depending on the potassium concentration, a tripotassium or monopotassium salt may also be present). NH_4^+ ion can interfere.

$$2\ K^+ + Na^+ + [Co(NO_2)_6]^{3-} \rightarrow K_2Na[Co(NO_2)_6]$$

Salicylates

a) To 1 ml of the prescribed solution add 0.5 ml of *ferric chloride solution R1*. A violet colour is produced that persists after the addition of 0.1 ml of *acetic acid R*.

A stable, three-ligand chelate complex is formed.



b) Dissolve 0.5 g of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.5 ml of *hydrochloric add R*. The precipitate obtained, after recrystallisation from hot *water R* and drying *in vacuo*, has a melting point *(2.2.14)* of 156 °C to 161 °C.

Hydrochloric acid liberates salicylic acid from its salts.

Silicates

Mix the prescribed quantity of the substance to be examined in a lead or platinum crucible by means of a copper wire with about 10 mg of *sodium fluoride R* and a few drops of *sulfuric acid R* to give a thin slurry. Cover the crucible with a thin, transparent plate of plastic under which a drop of *water R* is suspended and warm gently. Within a short time a white ring is rapidly formed around the drop of water.

Hydrogen fluoride is formed, which reacts with silicates or silica to result in silicon tetrafluoride. The latter is hydrolysed by water and a white ring of silicic acid is formed around a drop of water.

$$2\ NaF + H_2SO_4 \rightarrow H_2F_2 + Na_2SO_4$$

$$SiO_2 + 2\ H_2F_2 \rightarrow SiF_4 + 2\ H_2O$$

$$3\ SiF_4 + (n+2)\ H_2O \rightarrow SiO_2,(H_2O)_n + 2\ H_2[SiF_6]$$

Silver

Dissolve about 10 mg of the substance to be examined in 10 ml of *water R* or use 10 ml of the prescribed solution. Add 0.3 ml of *hydrochloric acid R1*. A curdled, white precipitate is formed that dissolves on addition of 3 ml of *dilute ammonia R1*.

A curdy, white precipitate of silver chloride is produced, which dissolves in an excess of ammonia solution (the diamminesilver complex is formed).

$$Ag^+ + Cl^- \rightarrow AgCl$$

$$AgCl + 2\ NH_3 \rightarrow [Ag(NH_3)_2]^+ + Cl^-$$

Sodium

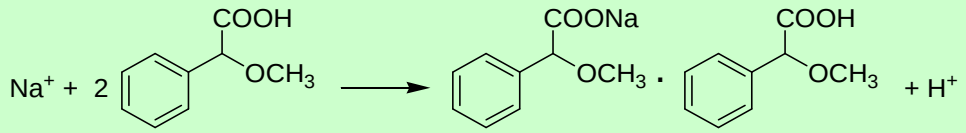
a) Dissolve 0.1 g of the substance to be examined in 2 ml of *water R* or use 2 ml of the prescribed solution. Add 2 ml of a 150 g/l solution of *potassium carbonate R* and heat to boiling. No precipitate is formed. Add 4 ml of *potassium pyroantimonate solution R* and heat to boiling. Allow to cool in iced water and if necessary rub the inside of the test-tube with a glass rod. A dense white precipitate is formed.

In the first part of the identification, ions are excluded that react with potassium pyroantimonate, but also form a precipitate with carbonate (*e.g.* Ca^{2+}, Mg^{2+} and Ba^{2+}).

$$Na^+ + [Sb(OH)_6]^- \rightarrow Na[Sb(OH)_6]$$

b) Dissolve a quantity of the substance to be examined equivalent to about 2 mg of sodium (Na^+) in 0.5 ml of *water R* or use 0.5 ml of the prescribed solution. Add 1.5 ml of *methoxyphenylacetic reagent R* and cool in ice-water for 30 min. A voluminous, white, crystalline precipitate is formed. Place in water at 20 °C and stir for 5 min. The precipitate does not disappear. Add 1 ml of *dilute ammonia R1*. The precipitate dissolves completely. Add 1 ml of a*mmonium carbonate solution R.* No precipitate is formed.

With racemic methoxyphenylacetic acid, Na^+ forms a precipitate in 1 : 2 ratio, which dissolves in ammonia solution. The reaction is specific for Na^+.



Sulfates

a) Dissolve about 45 mg of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 1 ml of *dilute hydrochloric acid R* and 1 ml of *barium chloride solution R1*. A white precipitate is formed.

Barium sulfate is formed.

$$SO_4^{2-} + Ba^{2+} \rightarrow BaSO_4$$

b) To the suspension obtained during reaction (a), add 0.1 ml of *0.05 M iodine*. The suspension remains yellow (distinction from sulfites and dithionites), but is decolorised by adding dropwise *stannous chloride solution R* (distinction from iodates). Boil the mixture. No coloured precipitate is formed (distinction from selenates and tungstates).

Sulfite and dithionate reduce iodine to iodide.

$$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2\ I^- + 2\ H^+$$

$$S_2O_4^{2-} + 3\ I_2 + 4\ H_2O \rightarrow 2\ SO_4^{2-} + 6\ I^- + 8\ H^+$$

Tin(II) chloride also reduces iodine to iodide. In this case, the decolorization of the solution excludes the presence of iodate, because iodate forms iodine with iodide. Selenate or tungstate produces red selenium or a blue W(V)-containing polyanion (tungsten blue).

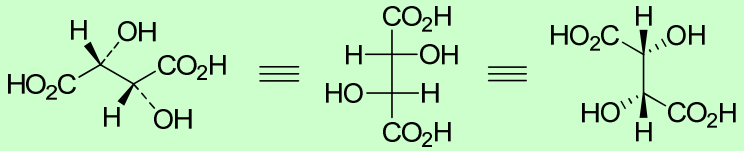
$$Sn^{2+} + I_2 \rightarrow Sn^{4+} + 2\ I^-$$

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 6\ H_2O$$

Tartrates

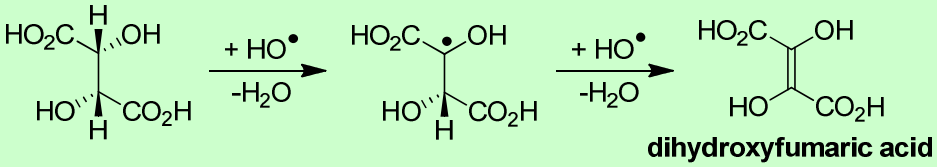
a) Dissolve about 15 mg of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 0.05 ml of a 10 g/l solution of *ferrous sulfate R* and 0.05 ml of *dilute hydrogen peroxide solution R*. A transient yellow colour is produced. After the colour has disappeared add *dilute sodium hydroxide solution R* dropwise. A violet or purple colour is produced.

In the presence of Fe^{2+}, hydrogen peroxide oxidizes tartrate to dihydroxyfumaric acid, which produces an intense bluish-violet complex with Fe^{3+}, formed in the oxidation process (**FENTON** reaction).



***R,R*-tartaric acid**

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$



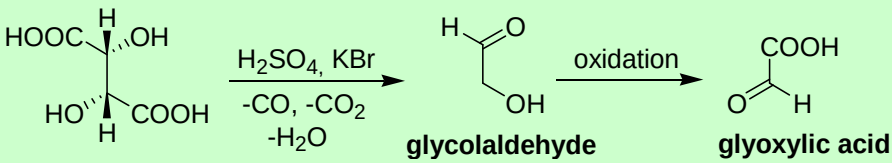
dihydroxyfumaric acid

Fe^{3+}

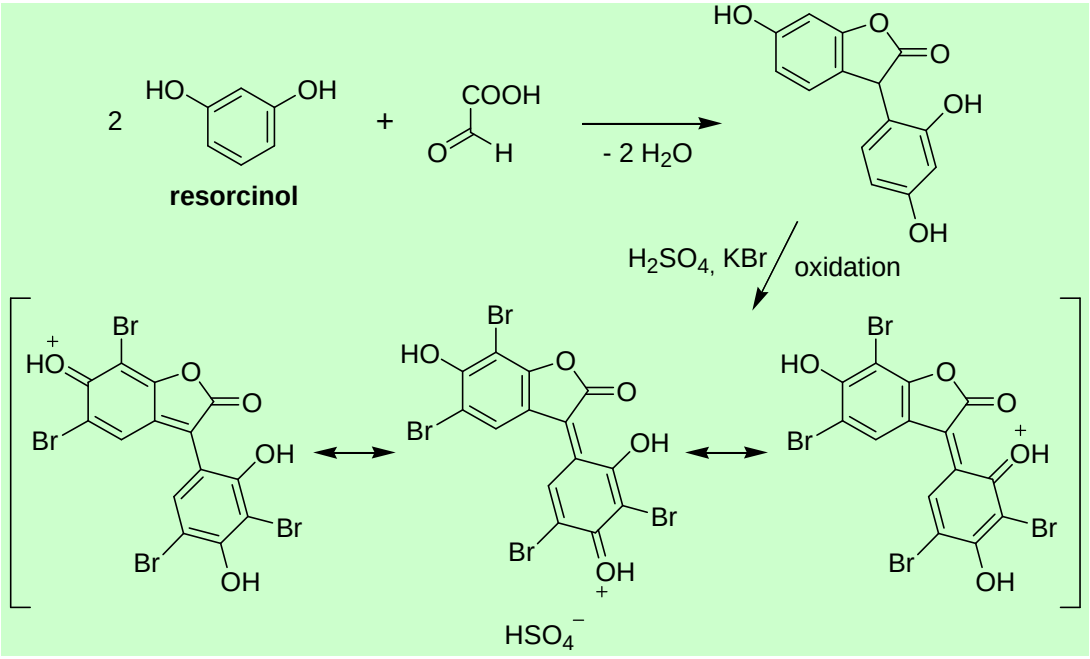
bluish-violet komplex

b) To 0.1 ml of a solution of the substance to be examined containing the equivalent of about 15 mg of tartaric acid per millilitre or to 0.1 ml of the prescribed solution add 0.1 ml of a 100 g/l solution of *potassium bromide R*, 0.1 ml of a 20 g/l solution of *resorcinol R* and 3 ml of *sulfuric acid R*. Heat on a water-bath for 5 min to 10 min. A dark-blue colour develops. Allow to cool and pour the solution into *water R*. The colour changes to red.

Tartaric acid is converted to glycolaldehyde by decarboxylation, decarbonylation, and finally water elimination. Glycolaldehyde is readily oxidized to glyoxylic acid, which undergoes condensation with 2 molecules of resorcinol to result in a diphenylmethane-type lactone derivative. The tetrabromo-substituted derivative of the above-mentioned lactone exists as a quinoidal-type blue oxonium salt in concentrated sulfuric acid.



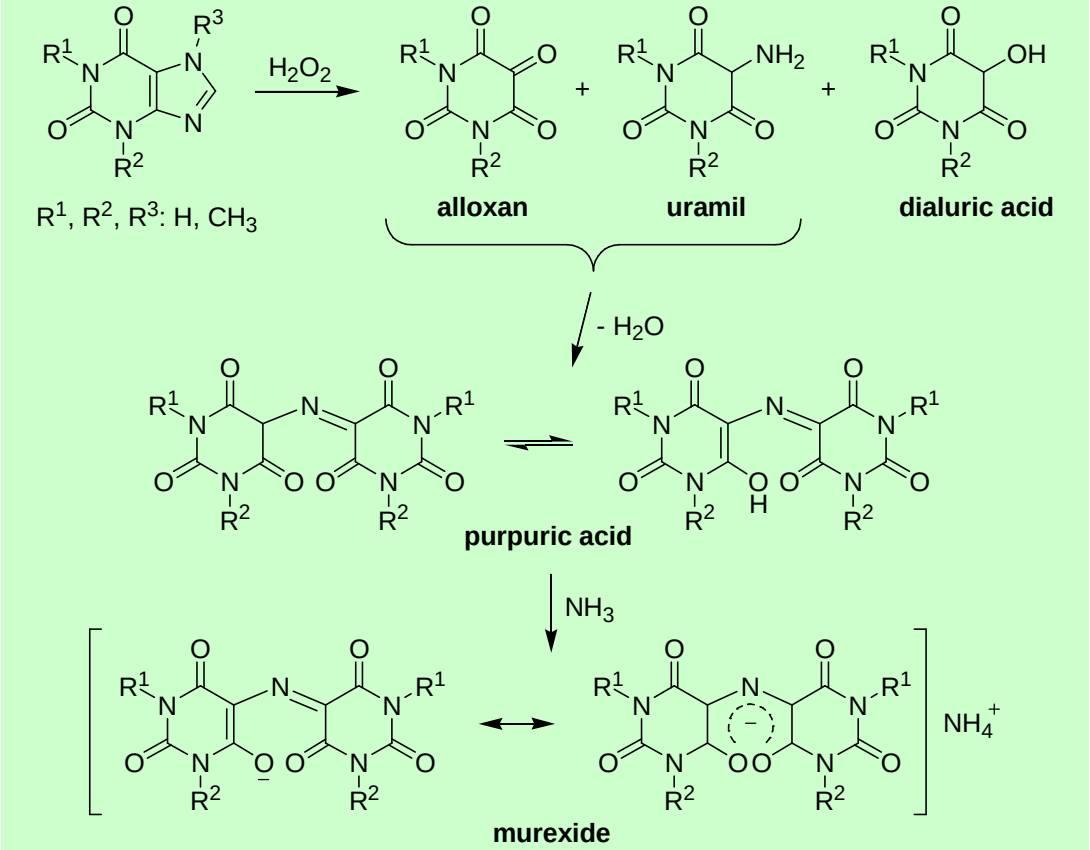
glycolaldehyde **glyoxylic acid**



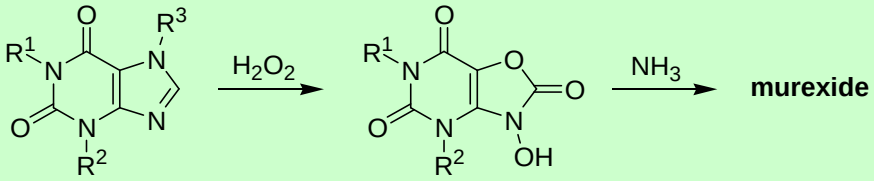
Xanthines

To a few milligrams of the substance to be examined or the prescribed quantity add 0.1 ml of *strong hydrogen peroxide solution R* and 0.3 ml of *dilute hydrochloric acid R*. Heat to dryness on a water-bath until a yellowish-red residue is obtained. Add 0.1 ml of *dilute ammonia R2*. The colour of the residue changes to violet-red.

Early studies explained the murexide group reaction of xanthine derivatives by the condensation of pyrimidine derivatives (alloxan, uramil and dialuric acid) formed oxidative/hydrolytic decomposition of xanthines to purpuric acid, which forms an ammonium salt, murexide.



Recent studies on the murexide reaction indicate that murexide is formed not from the above-mentioned pyrimidine derivatives, but from the oxazolo[4,5-*d*]pyrimidine-type oxidation intermediates.



The corresponding *N*-substituted murexides formed from the differently 1,3-substituted xanthine derivatives are all purple.

Zinc

Dissolve 0.1 g of the substance to be examined in 5 ml of *water R* or use 5 ml of the prescribed solution. Add 0.2 ml of *strong sodium hydroxide solution R*. A white precipitate is formed. Add a further 2 ml of *strong sodium hydroxide solution R*. The precipitate dissolves. Add 10 ml of *ammonium chloride solution R*. The solution remains clear. Add 0.1 ml of *sodium sulfide solution R*. A flocculent white precipitate is formed.

Sodium hydroxide gives a white precipitate of zinc hydroxide, which is soluble in an excess of the reagent. With ammonium chloride, zinc hydroxide can not be reprecipitated, even on boiling (difference from aluminium), while sulfide gives a precipitate of stable zinc sulfide.

$$Zn^{2+} + 2\ OH^- \rightarrow Zn(OH)_2$$

$$Zn(OH)_2 + 2\ OH^- \rightarrow [Zn(OH)_4]^{2-}$$

$$[Zn(OH)_4]^{2-} + 4\ NH_4^+ \rightarrow [Zn(NH_3)_4]^{2+} + 4\ H_2O$$

$$[Zn(OH)_4]^{2-} + S^{2-} \rightarrow ZnS + 4\ OH^-$$

2.3.4. Odour

On a watch-glass 6 cm to 8 cm in diameter, spread in a thin layer 0.5 g to 2.0 g of the substance to be examined. After 15 min, determine the odour or verify the absence of odour.

2.4. Limit Tests

2.4.1. Ammonium

Unless otherwise prescribed, use method A.

Method A

Introduce the prescribed solution into a test-tube or dissolve the prescribed quantity of the substance to be examined in 14 ml of *water R* in a test-tube. Make the solution alkaline if necessary by the addition of *dilute sodium hydroxide solution R*, dilute to 15 ml with *water R* and add 0.3 ml of *alkaline potassium tetraiodomercurate solution R*. Prepare a standard by mixing 10 ml of *ammonium standard solution* (*1 ppm NH_4*) R, 5 ml of *water R* and 0.3 ml of *alkaline potassium tetraiodomercurate solution R*. Stopper the test-tubes.

After 5 min, any yellow colour in the test solution is not more intense than that in the standard.

Sodium hydroxide liberates ammonia from solutions of ammonium salts. Depending on the quantity of ammonia, a yellowish-brown coloration or a brown precipitate of mercury(II) oxide–mercury(II) amidoiodide is formed with potassium tetraiodomercurate (**NESSLER** reagent).

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

$$NH_3 + 2\ [HgI_4]^{2-} + 3\ OH^- \rightarrow HgO.HgNH_2I + 7\ I^- + 2\ H_2O$$

Method B

In a 25 ml jar fitted with a cap, place the prescribed quantity of the finely powdered substance to be examined and dissolve or suspend in 1 ml of *water R*. Add 0.30 g of *heavy magnesium oxide R*. Close immediately after placing a piece of *silver manganese paper R* 5 mm square, wetted with a few drops of *water R*, under the polyethylene cap. Swirl, avoiding projections of liquid, and allow to stand at 40 °C for 30 min. If the silver manganese paper shows a grey colour, it is not more intense than that of a standard prepared at the same time and in the same manner using the prescribed volume of *ammonium standard solution* (*1 ppm NH_4*) R, 1 ml of *water R* and 0.30 g of *heavy magnesium oxide R*.

Magnesium oxide liberates ammonia from solutions of ammonium salts. The volatile gas ammonia dissolves in a wet filter paper impregnated with silver nitrate and manganese sulfate: brown manganese(IV) oxide and grey silver metal are precipitated.

$$MgO + 2\ NH_4^+ \rightarrow Mg^{2+} + 2\ NH_3 + H_2O$$

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

$$Mn^{2+} + 2\ Ag^+ + 4\ OH^- \rightarrow MnO_2 + 2\ Ag + 2\ H_2O$$

2.4.2. Arsenic

Method A

The apparatus (see Figure 2.4.2.-1) consists of a 100 ml conical flask closed with a ground-glass stopper through which passes a glass tube about 200 mm long and of internal diameter 5 mm. The lower part of the tube is drawn to an internal diameter of 1.0 mm, and 15 mm from its tip is a lateral orifice 2 mm to 3 mm in diameter. When the tube is in position in the stopper, the lateral orifice should be at least 3 mm below the lower surface of the stopper. The upper end of the tube has a perfectly flat, ground surface at right angles to the axis of the tube. A second glass tube of the same internal diameter and 30 mm long, with a similar flat ground surface, is placed in contact with the first, and is held in position by two spiral springs. Into the lower tube insert 50 mg to 60 mg of *lead acetate cotton R*, loosely packed, or a small plug of cotton and a rolled piece of *lead acetate paper R* weighing 50 mg to 60 mg. Between the flat surfaces of the tubes place a disc or a small square of *mercuric bromide paper R* large enough to cover the orifice of the tube (15 mm x 15 mm).

In the conical flask dissolve the prescribed quantity of the substance to be examined in 25 ml of *water R*, or in the case of a solution adjust the prescribed volume to 25 ml with *water R*. Add 15 ml of *hydrochloric acid R*, 0.1 ml of *stannous chloride solution R* and 5 ml of *potassium iodide solution R*, allow to stand for 15 min and introduce 5 g of *activated zinc R*. Assemble the two parts of the apparatus immediately and immerse the flask in a bath of water at a temperature such that a uniform evolution of gas is maintained. Prepare a standard in the same manner, using 1 ml of *arsenic standard solution* (*1 ppm As*) *R*, diluted to 25 ml with *water R*.

After not less than 2 h the stain produced on the mercuric bromide paper in the test is not more intense than that in the standard.

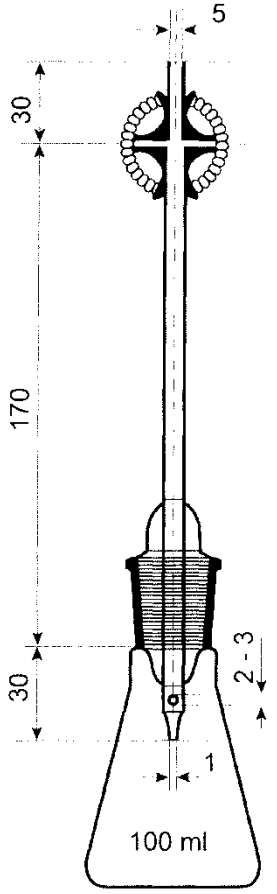


Figure 2.4.2.1. - Apparatus for limit test A for arsenic. Dimensions in millimetres

Under acidic conditions, zinc reduces arsenates or arsenites to volatile arsine, which reacts gradually with mercury(II) bromide. The yellow bromo-mercury(II) arsenide (Hg_3AsBr_3) is first formed, and then the brown mercury(II) arsenide (Hg_3As_2).

$$AsO_3^{3-} + 3\ Zn + 9\ H^+ \rightarrow AsH_3 + 3\ Zn^{2+} + 3\ H_2O$$

$$AsO_4^{3-} + 4\ Zn + 11\ H^+ \rightarrow AsH_3 + 4\ Zn^{2+} + 4\ H_2O$$

$$AsH_3 + 3\ HgBr_2 \rightarrow Hg_3AsBr_3 + 3\ HBr$$

$$AsH_3 + Hg_3AsBr_3 \rightarrow Hg_3As_2 + 3\ HBr$$

The role of the iodide is to reduce arsenate to arsenite.

$$AsO_4^{3-} + 2\ I^- + 2\ H^+ \rightleftharpoons AsO_3^{3-} + I_2 + H_2O$$

Lead acetate binds hydrogen sulfide (liberated from sulfide impurity in the acidic medium), which would disturb the test for arsenic.

$$H_2S + Pb^{2+} \rightarrow PbS + 2\ H^+$$

Method B

Introduce the prescribed quantity of the substance to be examined into a test-tube containing 4 ml of *hydrochloric acid R* and about 5 mg of *potassium iodide R* and add 3 ml of *hypophosphorous reagent R*. Heat the mixture on a water-bath for 15 min, shaking occasionally. Prepare a standard in the same manner, using 0.5 ml of *arsenic standard solution* (*10 ppm As*) *R*.

After heating on the water-bath, any colour in the test solution is not more intense than that in the standard.

Hypophosphite reduces arsenates or arsenites to arsenic (**THIELE** test).

$$2\ AsO_3^{3-} + 3\ H_2PO_2^- + 9\ H^+ \rightarrow 2\ As + 3\ H_3PO_3 + 3\ H_2O$$

$$2\ AsO_4^{3-} + 5\ H_2PO_2^- + 11\ H^+ \rightarrow 2\ As + 5\ H_3PO_3 + 3\ H_2O$$

The role of the iodide is to reduce arsenate to arsenite.

$$AsO_4^{3-} + 2\ I^- + 2\ H^+ \rightleftharpoons AsO_3^{3-} + I_2 + H_2O$$

2.4.3. Calcium

All solutions used for this test should be prepared with distilled water R.

To 0.2 ml of *alcoholic calcium standard solution* (*100 ppm Ca*) *R*, add 1 ml of *ammonium oxalate solution R*. After 1 min, add a mixture of 1 ml of *dilute acetic acid R* and 15 ml of a solution containing the prescribed quantity of the substance to be examined and shake. Prepare a standard in the same manner using a mixture of 10 ml of *aqueous calcium standard solution* (*10 ppm Ca*) *R*, 1 ml of *dilute acetic acid R* and 5 ml of *distilled water R*.

After 15 min, any opalescence in the test solution is not more intense than that in the standard.

A white precipitate of calcium oxalate is formed, which is insoluble in acetic acid.

$$Ca^{2+} + (COO)_2^{2-} \rightarrow Ca(COO)_2$$

2.4.4. Chlorides

To 15 ml of the prescribed solution add 1 ml of *dilute nitric acid R* and pour the mixture as a single addition into a test-tube containing 1 ml of *silver nitrate solution R2*. Prepare a standard in the same manner using 10 ml of *chloride standard solution* (*5 ppm Cl*) *R* and 5 ml of *water R*. Examine the tubes laterally against a black background.

After standing for 5 min protected from light, any opalescence in the test solution is not more intense than that in the standard.

A silver chloride precipitate is formed.

2.4.6. Magnesium

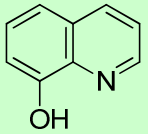
To 10 ml of the prescribed solution add 0.1 g of *disodium tetraborate R*. Adjust the solution, if necessary, to pH 8.8 to pH 9.2 using *dilute hydrochloric acid R* or *dilute sodium hydroxide solution R*. Shake with 2 quantities, each of 5 ml, of a 1 g/l solution of *hydroxyquinoline R* in *chloroform R*, for 1 min each time. Allow to stand. Separate and discard the organic layer. To the aqueous solution add 0.4 ml of *butylamine R* and 0.1 ml of *triethanolamine R*. Adjust the solution, if necessary, to pH 10.5 to pH 11.5. Add 4 ml of the *solution of hydroxyquinoline* in chloroform, shake for 1 min, allow to stand and separate. Use the lower

layer for comparison. Prepare a standard in the same manner using a mixture of 1 ml of *magnesium standard solution* (*10 ppm Mg*) *R* and 9 ml of *water R*.

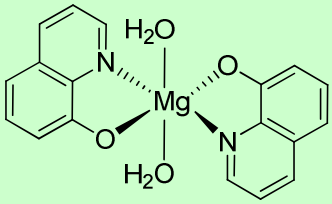
Any colour in the solution obtained from the substance to be examined is not more intense than that in the standard.

8-Hydroxyquinoline (oxine) forms slightly water-soluble complexes with several metal ions. Depending on the pH, these complexes are soluble in apolar solvents (*e.g.* chloroform). In the first part of the test (when the pH is 8.8–9.2), the complex $Mg(oxinate)_2.2H_2O$ is formed, which does not dissolve in chloroform. On the addition of butylamine to the mixture (the pH becomes 10.5–11.5), a pale-yellow water-soluble complex ($CH_3(CH_2)_3NH_3^+$ $[Mg(oxinate)_3]^-$) is formed.

The complexes of other metal ions with oxine are soluble in chloroform at pH 8.8–9.2 and can be eliminated by extraction.



oxine



$Mg(oxinate)_2 . 2 H_2O$

2.4.7. Magnesium and Alkaline-Earth Metals

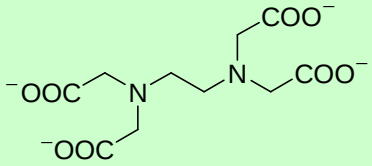
To 200 ml of *water R* add 0.1 g of *hydroxylamine hydrochloride R*, 10 ml of *ammonium chloride buffer solution pH 10.0 R*, 1 ml of *0.1 M zinc sulfate* and about 15 mg of *mordant black 11 triturate R*. Heat to about 40 °C. Titrate with *0.01 M sodium edetate* until the violet colour changes to full blue. To the solution add the prescribed quantity of the substance to be examined dissolved in 100 ml of *water R* or use the prescribed solution. If the colour of the solution changes to violet, titrate with *0.01 M sodium edetate* until the full blue colour is again obtained.

The volume of *0.01 M sodium edetate* used in the second titration does not exceed the prescribed quantity.

The contents of magnesium and alkaline-earth metal ions can be determined by complexometry in weakly basic medium. Metal ion–EDTA complexes are formed (M^{2+} = Zn^{2+}, Mg^{2+}, Ca^{2+}, *etc*.).

$$M^{2+} + H_2Y^{2-} \rightarrow MY^{2-} + 2H^+$$

Y^{4-}:



2.4.8. Heavy Metals

The methods described below require the use of *thioacetamide reagent R*. As an alternative, *sodium sulfide solution R1* (0.1 ml) is usually suitable. Since tests prescribed in monographs have been developed using *thioacetamide reagent R*, if *sodium sulfide solution R1* is used instead, it is necessary to include also for methods A, B and H a monitor solution, prepared from the quantity of the substance to be examined prescribed for the test, to which has been added the volume of lead standard solution prescribed for preparation of the reference solution. The test is invalid if the monitor solution is not at least as intense as the reference solution.

Method A

Test solution. 12 ml of the prescribed aqueous solution of the substance to be examined.

Reference solution (standard). Amixture of 10 ml of *lead standard solution (1 ppm Pb) R* or *lead standard solution (2 ppm Pb) R*, as prescribed, and 2 ml of the prescribed aqueous solution of the substance to be examined.

Blank solution. A mixture of 10 ml of *water R* and 2 ml of the prescribed aqueous solution of the substance to be examined.

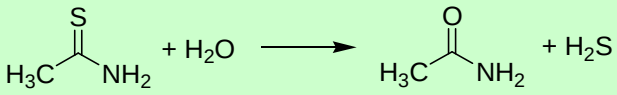
To each solution, add 2 ml of *buffer solution pH 3.5 R*. Mix and add to 1.2 ml of *thioacetamide reagent R*. Mix immediately. Examine the solutions after 2 min.

System suitability: the reference solution shows a slight brown colour compared to the blank solution.

Result: any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 0.45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions.

Thioacetamide is used as hydrogen sulfide source. Hydrogen sulfide gives black or brownish-black precipitates with heavy metals (*e.g.* Pb^{2+}, Hg^{2+}, Ni^{2+} and Fe^{2+}).



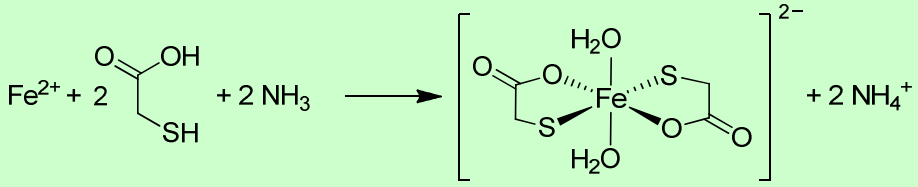
$$Pb^{2+} + H_2S \rightarrow PbS + 2\ H^+$$

2.4.9. Iron

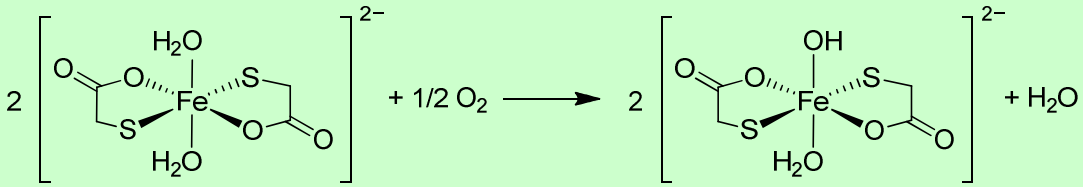
Dissolve the prescribed quantity of the substance to be examined in *water R* and dilute to 10 ml with the same solvent or use 10 ml of the prescribed solution. Add 2 ml of a 200 g/l solution of *citric acid R* and 0.1 ml of *thioglycollic acid R*. Mix, make alkaline with *ammonia R* and dilute to 20 ml with *water R*. Prepare a standard in the same manner, using 10 ml of *iron standard solution* (*1 ppm Fe*) *R*.

After 5 min, any pink colour in the test solution is not more intense than that in the standard.

Thioglycollic acid forms a colourless or pale-yellow complex with Fe^{2+}, which is oxidized to a red iron(III) complex in the air. Co^{2+} gives a similar reaction. Citric acid inhibits the precipitation of hydroxides of iron and other heavy metals.

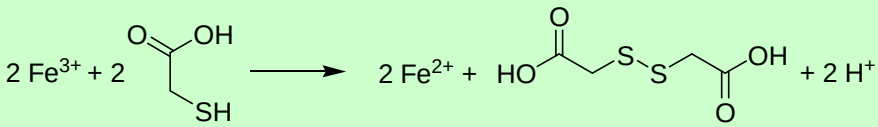


pale-yellow iron(II) complex



red iron(III) complex

Fe^{3+} also gives the reaction because it is reduced to Fe^{2+} by thioglycollic acid.



2.4.11. Phosphates

To 100 ml of the solution prepared and, if necessary, neutralised as prescribed add 4 ml of *sulfomolybdic reagent R3*. Shake and add 0.1 ml of *stannous chloride solution R1*. Prepare a standard in the same manner using 2 ml of *phosphate standard solution* (*5 ppm PO_4*) *R* and 98 ml of *water R*. After 10 min, compare the colours using 20 ml of each solution.

Any colour in the test solution is not more intense than that in the standard.

Phosphate forms phosphomolybdic acid ($H_3[PMo_{12}O_{40}]$) with the sulfomolybdic reagent. Phosphomolybdic acid can be reduced to molybdenum blue with stannous chloride.

2.4.12. Potassium

To 10 ml of the prescribed solution add 2 ml of a freshly prepared 10 g/l solution of *sodium tetraphenylborate R*. Prepare a standard in the same manner using a mixture of 5ml of *potassium standard solution* (*20 ppm K*) *R* and 5 ml of *water R*.

After 5 min, any opalescence in the test solution is not more intense than that in the standard.

Water-insoluble potassium tetraphenylborate is precipitated.

$$K^+ + [B(C_6H_5)_4]^- \rightarrow K[B(C_6H_5)_4]$$

2.4.13. Sulfates

All solutions used for this test should be prepared with distilled water R.

Add 3 ml of a 250 g/l solution of *barium chloride R* to 4.5 ml of *sulfate standard solution (10 ppm SO_4) R1*. Shake and allow to stand for 1 min. To 2.5 ml of this suspension add 15 ml of the prescribed solution and 0.5 ml of *acetic acid R*. Prepare a standard in the same manner using 15 ml of *sulfate standard solution (10 ppm SO_4) R* instead of the prescribed solution.

After 5 min, any opalescence in the test solution is not more intense than that in the standard.

Water-insoluble barium sulfate is precipitated.

2.5. Assays

2.5.11. Complexometric Titrations

Bismuth

Introduce the prescribed solution into a 500 ml conical flask. Dilute to 250 ml with *water R* and then, unless otherwise prescribed, add dropwise, with shaking, *concentrated ammonia R* until the mixture becomes cloudy. Add 0.5 ml of *nitric acid R*. Heat to about 70 °C until the cloudiness disappears completely. Add about 50 mg of *xylenol orange triturate R* and titrate with *0.1 M sodium edetate* until the colour changes from pinkish-violet to yellow.

1 ml of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

Calcium

Introduce the prescribed solution into a 500 ml conical flask, and dilute to 300 ml with *water R*. Add 6.0 ml of *strong sodium hydroxide solution R* and about 200 mg of *calconecarboxylic acid triturate R*. Titrate with 0.1 M sodium edetate until the colour changes from violet to full blue.

1 ml of *0.1 M sodium edetate* is equivalent to 4.008 mg of Ca.

Magnesium

Introduce the prescribed solution into a 500 ml conical flask and dilute to 300 ml with *water R*. Add 10 ml of *ammonium chloride buffer solution pH 10.0 R* and about 50 mg of *mordant black 11 triturate R*. Heat to about 40 °C then titrate at this temperature with *0.1 M sodium edetate* until the colour changes from violet to full blue.

1 ml of *0.1 M sodium edetate* is equivalent to 2.431 mg of Mg.

Zinc

Introduce the prescribed solution into a 500 ml conical flask and dilute to 200 ml with *water R*. Add about 50 mg of *xylenol orange triturate R* and *hexamethylenetetramine R* until the solution becomes violet-pink. Add 2 g of *hexamethylenetetramine R* in excess. Titrate with *0.1 M sodium edetate* until the violet-pink colour changes to yellow.

1 ml of *0.1 M sodium edetate* is equivalent to 6.54 mg of Zn.

2.5.12. Water: Semi-Micro Determination

The semi-micro determination of water is based upon the quantitative reaction of water with sulfur dioxide and iodine in a suitable anhydrous medium in the presence of a base with sufficient buffering capacity.

The fundamental principle behind this method (**KARL FISCHER** titration) is based on the reaction between iodine and sulfur dioxide requiring a stoichiometric amount of water. Therefore, the water content of the sample can be calculated on the basis of the the consumed iodine. The volumetric solution is a non-aqueous system containing iodine, sulfur dioxide, a primary alcohol (methanol) as the solvent, and a base (pyridine) as the buffering agent. This method for quantification of the water content can be used in both volumetric and coulometric titration systems.

$$SO_2 + I_2 + H_2O \rightarrow SO_3 + 2\ I^- + 2\ H^+$$

2.9. Pharmaceutical Technical Procedures

2.9.34. Bulk density or tapped density of powders

Bulk density

The bulk density of a powder is the ratio of the mass of an untapped powder sample to its volume, including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per millilitre despite the International Unit being kilogram per cubic metre (1 g/ml = 1000 kg/m^3), because the measurements are made using cylinders. It may also be expressed in grams per cubic centimetre.

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it has been handled. The particles can be packed to have a range of bulk densities and, moreover, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

The bulk density of a powder is determined either by measuring the volume of a known mass of powder sample, which may have been passed through a sieve, in a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or has been introduced into a measuring vessel (Method 3).

Methods 1 and 3 are favoured.

Method 1: Measurement in a graduated cylinder

Procedure. Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry, graduated, 250 ml cylinder (readable to 2 ml), gently introduce, without compacting, approximately 100 g (m) of the test sample weighed with 0.1 per cent accuracy. If necessary, carefully level the powder without compacting, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in grams per millilitre using the formula m/V_0. Generally, replicate determinations are desirable for the determination of this property.

If the powder density is too low or too high, such that the test sample has an untapped apparent volume of more than 250 ml or less than 150 ml, it is not possible to use 100 g of powder sample. In this case, a different amount of powder is selected as the test sample, such that its untapped apparent volume is between 150 ml and 250 ml (apparent volume greater than or equal to 60 per cent of the total volume of the cylinder); the mass of the test sample is specified in the expression of results.

For test samples having an apparent volume between 50 ml and 100 ml, a 100 ml cylinder readable to 1 ml can be used; the volume of the cylinder is specified in the expression of results.

4. REAGENTS

4.1. Reagents, Standard Solutions, Buffer Solutions

Where the name of substance or a solution is followed by the letter R (the whole in italics), this indicates a reagent included in the following list. The specifications given for reagents do not necessarily guarantee their quality for use in medicines.

> As far as a compound or a solution is official in different qualities or concentrations (*e.g.* ammonium nitrate or dilute hydrochloric acid), notations *R*, *R1*, *R2*, *etc.* are applied by the Pharmacopoeia to distinguish between them. Reagents (*e.g.* potassium bromate or sodium chloride) used for the preparation and checking of volumetric solutions bear the sign *RV*.

Within the description of each reagent there is a 7-digit reference code in italics (for example, 1002501). This number, which will remain unchanged for a given reagent during subsequent revisions of the list, is used for identification purposes by the Secretariat, and users of the Pharmacopoeia may also find it useful, for example in the management of reagent stocks. The description may also include a CAS number (Chemical Abstract Service Registry Number) recognisable by its typical format, for example 9002-93-1.

Some of the reagents included in the list are toxic and are to be handled in conformity with good quality control laboratory practice.

Reagents in aqueous solution are prepared using *water R*. Where a reagent solution is described using an expression such as “hydrochloric acid (10 g/l HCl)”, the solution is prepared by an appropriate dilution with *water R* of a more concentrated reagent solution specified in this chapter. Reagent solutions used in the limit tests for barium, calcium and sulfates are prepared using *distilled water R*. Where the name of the solvent is not stated, an aqueous solution is intended.

The reagents and reagent solutions are to be stored in well-closed containers. The labelling should comply with the relevant national legislation and international agreements.

4.2. Volumetric Analysis

4.2.2. Volumetric Solutions

Volumetric solutions are prepared according to the usual chemical analytical methods. The accuracy of the apparatus used is verified to ensure that it is appropriate for the intended use.

The concentration of volumetric solutions is indicated in terms of molarity. Molarity expresses, as the number of moles, the amount of substance dissolved in 1 litre of solution. A solution which contains *x* moles of substance per litre is said to be *x* M.

Volumetric solutions do not differ from the prescribed strength by more than 10 per cent. The molarity of the volumetric solutions is determined by an appropriate number of titrations. The repeatability does not exceed 0.2 per cent (relative standard deviation).

Volumetric solutions are standardised by the methods described below. When a volumetric solution is to be used in an assay in which the end-point is determined by an electrochemical process (for example, amperometry or potentiometry) the solution is standardised by the same method. The composition of the medium in which a volumetric solution is standardised should be the same as that in which it is to be used.

Solutions more dilute than those described are obtained by diluting with *carbon dioxide-free water R* of the least-concentrated solution that describes a standardisation. The correction factors of these solutions are the same as those from which the dilutions were prepared.

0.1 M Ammonium thiocyanate. *3000500.*

Dissolve 7.612 g of *ammonium thiocyanate R* in *water R* and dilute to 1000.0 ml with the same solvent.

Standardisation. To 20.0 ml of *0.1 M silver nitrate* add 25 ml of *water R*, 2 ml of *dilute nitric acid R* and 2 ml of *ferric ammonium sulfate solution R2*. Titrate with the ammonium thiocyanate solution until a reddish-yellow colour is obtained.

1 M Hydrochloric acid. *3001800.*

Dilute 103.0 g of *hydrochloric acid R* to 1000.0 ml with *water R*.

Standardisation. Dissolve 1.000 g of *sodium carbonate RV* in 50 ml of *water R*, add 0.1 ml of *methyl orange solution R* and titrate with the hydrochloric acid until the solution just becomes yellowish-red. Boil for 2 min. The solution reverts to yellow. Cool and continue the titration until a yellowish-red colour is obtained.

1 ml of *1 M hydrochloric acid* is equivalent to 53.00 mg of Na_2CO_3.

0.1 M Hydrochloric acid. *3002100.*

Dilute 100.0 ml of *1 M hydrochloric acid* to 1000.0 ml with *water R*.

Standardisation. Carry out the titration described for *1 M hydrochloric acid* using 0.100 g of *sodium carbonate RV* dissolved in 20 ml of *water R*.

1 ml of *0.1 M hydrochloric acid* is equivalent to 5.30 mg of Na_2CO_3.

0.05 M Iodine. *3002700.*

Dissolve 12.7 g of *iodine R* and 20 g of *potassium iodide R* in *water R* and dilute to 1000.0 ml with the same solvent.

Standardisation. To 20.0 ml of the iodine solution add 1 ml of *dilute acetic acid R* and 30 ml of *water R*. Titrate with *0.1 M sodium thiosulfate*, using *starch solution R* as indicator.

Storage: protected from light.

0.1 M Perchloric acid. *3003900.*

Place 8.5 ml of *perchloric acid R* in a volumetric flask containing about 900 ml of *glacial acetic acid R* and mix. Add 30 ml of *acetic anhydride R*, dilute to 1000.0 ml with *glacial acetic acid R*, mix and allow to stand for 24 h. Determine the water content *(2.5.12)* without addition of methanol and, if necessary, adjust the water content to between 0.1 per cent and 0.2 per cent by adding either *acetic anhydride R* or *water R*. Allow to stand for 24 h.

Standardisation. Dissolve 0.350 g of *potassium hydrogen phthalate RV* in 50 ml of *anhydrous acetic acid R*, warming gently if necessary. Allow to cool protected from the air, and titrate with the perchloric acid solution, using 0.05 ml of *crystal violet solution R* as indicator. Note the temperature of the perchloric acid solution at the time of the titration. If the temperature at which an assay is carried out is different from that at which the *perchloric acid R* has been standardised the volume used in the assay becomes:

$$V_c = V \cdot [1 + (t_1 - t_2) \cdot 0.0011],$$

t_1 = temperature during standardization,

t_2 = temperature during the assay,

V_c = corrected volume,

V = observed volume.

1 ml of *0.1 M perchloric acid* is equivalent to 20.42 mg of $C_8H_5KO_4$.

0.033 M Potassium bromate. *3004200.*

Dissolve 5.5670 g of *potassium bromate RV* in *water R* and dilute to 1000.0 ml with the same solvent.

0.0167 M Potassium bromate. *3004400.*

Prepare by diluting *0.033 M Potassium bromate*.

0.02 M Potassium permanganate. *3005300.*

Dissolve 3.2 g of *potassium permanganate R* in *water R* and dilute to 1000.0 ml with the same solvent. Heat the solution for 1 h on a water-bath, allow to cool and filter through a sintered-glass filter (2.1.2).

Standardisation. To 20.0 ml of the potassium permanganate solution, add 2 g of *potassium iodide R* and 10 ml of *dilute sulfuric acid R*. Titrate with *0.1 M sodium thiosulfate*, using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Standardise immediately before use.

Storage: protected from light.

0.1 M Sodium edetate. *3005900.*

Dissolve 37.5 g of *sodium edetate R* in 500 ml of *water R*, add 100 ml of *1 M sodium hydroxide* and dilute to 1000.0 ml with *water R*.

Standardisation. Dissolve 0.120 g of *zinc RV* in 4 ml of *hydrochloric acid R1* and add 0.1 ml *of bromine water R*. Drive off the excess of bromine by boiling, add *dilute sodium hydroxide solution R* until the solution is weakly acid or neutral and carry out the assay of zinc by complexometry (2.5.11).

1 ml of *0.1 M sodium edetate* is equivalent to 6.54 mg of Zn.

Storage: in a polyethylene container.

1 M Sodium hydroxide. *3006300.*

Dissolve 42 g of *sodium hydroxide R* in *carbon dioxide-free water R* and dilute to 1000.0 ml with the same solvent.

Standardisation. Titrate 20.0 ml of the sodium hydroxide solution with *1 M hydrochloric acid* using the indicator prescribed in the assay in which *1 M sodium hydroxide* is used.

0.1 M Sodium hydroxide. *3006600.*

Dilute 100.0 ml of *1 M sodium hydroxide* to 1000.0 ml with *carbon dioxide-free water R*.

Standardisation. Titrate 20.0 ml of the sodium hydroxide solution with *0.1 M hydrochloric acid*, using the end-point detection prescribed for the assay in which the *0.1 M sodium hydroxide* is used.

5. GENERAL TEXTS

5.9. Polymorphism

Polymorphism (or crystal polymorphism) is a phenomenon related to the solid state; it is the ability of a compound in the solid state to exist in different crystalline forms having the same chemical composition. Substances that exist in a non-crystalline solid state are said to be amorphous.

When this phenomenon is observed for a chemical element (for example, sulfur), the term allotropy is used instead of polymorphism.

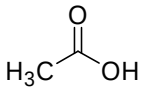
Where a monograph indicates that a substance shows polymorphism, this may be true crystal polymorphism, occurrence of solvates, allotropy or occurrence of the amorphous form.

5.12. European Pharmacopoeia chemical reference substances

Reference standard' is used in this chapter as a general term covering reference substances, reference preparations and reference spectra.
Reference standards are frequently necessary to achieve adequate quality control of medicinal products and their components.
Reference standards are established using suitable procedures and their continued suitability for use is monitored according to a predefined programme. Where a reference standard is needed, it is an integral part of the pharmacopoeial monograph or the manufacturer's specification. Where a European Pharmacopoeia reference standard is referred to in a monograph or general chapter, it represents the official standard that is alone authoritative in case of doubt or dispute.

ACETIC ACID, GLACIAL

Acidum aceticum glaciale



$C_2H_4O_2$ **M_r 60.1**

Definition

Content: 99.0 per cent *m/m* to 100.5 per cent *m/m*.

Characters

Appearance: crystalline mass or clear, colourless, volatile liquid.

Solubility: miscible with water, with ethanol (96 per cent) and with methylene chloride.

Concentrated acetic acid is applied as a solvent or reagent in pharmaceutical analysis. Its dilute solution is used as a disinfectant.

Identification

A. A 100 *g/l* solution is strongly acid *(2.2.4)*.

B. To 0.03 ml add 3 ml of *water R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives reaction (**b**) of acetates *(2.3.1)*.

Tests

Solution S. Dilute 20 ml to 100 ml with *distilled water R*.

Appearance. The substance to be examined is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Reducing substances. Dilute 2.0 ml to 10.0 ml with water R. Add 0.1 ml of 0.02 M potassium permanganate. Heat on a water-bath for 1 min, the colour remains pink.

Reducing substances reduce permanganate to manganese(II) and the purple-red solution becomes colourless.

Chlorides *(2.4.4)*: maximum 25 mg/l.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulfates *(2.4.13)*: maximum 50 mg/l, determined on 15 ml of solution.

Assay

Weigh accurately a conical flask with a ground-glass stopper containing 25 ml of *water R*. Add 1.0 ml of the substance to be examined and weigh again accurately. Add 0.5 ml of *phenolphthalein solution R* and titrate with *1 M sodium hydroxide*.

1 ml of *1 M sodium hydroxide* is equivalent to 60.1 mg of $C_2H_4O_2$.

Acetic acid is titrated via alkalimetry, resulting in sodium acetate. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

$$CH_3COOH + NaOH \rightarrow CH_3COONa + H_2O$$

$$C_2H_4O_2 \text{ content (\%)} = \frac{V_{NaOH}\text{ (ml)} \cdot f_{NaOH} \cdot E\text{ (mg/ml)}}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See the **Appearance** paragraph in the *Acetic acid, glacial* monograph.

2. See identification **A** in the *Acetic acid, glacial* monograph.

3. Add 2 drops of sample to a mixture of 1.0 ml of *water R*, 1.0 ml of *alcohol R* and 1.0 ml of *sulfuric acid R*. On boiling, the odour of ethyl acetate is evolved.

Esterification of acetic acid in the presence of sulfuric acid results in ethyl acetate.

$$CH_3COOH + CH_3CH_2OH \rightleftharpoons CH_3COOCH_2CH_3 + H_2O$$

4. See the **Assay** paragraph in the *Acetic acid, glacial* monograph.

ALUM

Alumen

$AlK(SO_4)_2,12H_2O$ **M_r 474.4**

Definition

Content: 99.0 per cent to 100.5 per cent of $AlK(S0_4)_2,12H_2O$.

Characters

Appearance: granular powder or colourless, transparent, crystalline masses.
Solubility: freely soluble in water, very soluble in boiling water, soluble in glycerol, practically insoluble in ethanol (96 per cent).

Because of its astringent action, it is used externally as a styptic.

Identification

A. Solution S (see Tests) gives the reactions of sulfates (*2.3.1*).

B. Solution S gives the reaction of aluminium (*2.3.1*).

C. Shake 10 ml of solution S with 0.5 g of *sodium hydrogencarbonate R* and filter. The filtrate gives reaction (a) of potassium (*2.3.1*).

Tests

Solution S. Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 3.0 to 3.5.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Ammonium (*2.4.1*): maximum 0.2 per cent.
To 1 ml of solution S add 4 ml of *water R*. Dilute 0.5 ml of this solution to 14 ml with *water R*.

Iron (*2.4.9*): maximum 100 ppm.
Dilute 2 ml of solution S to 10 ml with *water R*. Use in this test 0.3 ml of *thioglycollic acid R*.

Heavy metals (*2.4.8*): maximum 20 ppm.
12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. When gently heated, the compound melts and loses its water. It solidifies as its crystal water boils off. The residue is a white powder.
2. See the **Appearance of solution** test in the *Alum* monograph.
3. See identification **A** in the *Alum* monograph.
4. See identification **B** in the *Alum* monograph.
5. See identification **C** in the *Alum* monograph.

ALUMINIUM SULFATE

Aluminii sulfas

$Al_2(SO_4)_3$ **M_r 342.1**

Definition

Content: 51.0 per cent to 59.0 per cent of $Al_2(SO_4)_3$ (M_r 342.1).

It contains a variable quantity of water of crystallisation.

Characters

Appearance: colourless, lustrous crystals or crystalline masses.

Solubility: soluble in cold water, freely soluble in hot water, practically insoluble in ethanol (96 per cent).

Because of its astringent action, aluminium sulfate is used in different antisudoric dusting powders.

Identification

A. Solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

B. Solution S gives the reaction of aluminium (*2.3.1*).

Tests

Solution S. Dissolve 2.5 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension III (*2.2.1*) and is colourless (*2.2.2, Method II*).

pH (*2.2.3*): 2.5 to 4.0.

Dissolve 0.5 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Ammonium (*2.4.1*): maximum 500 ppm.

Dilute 0.4 ml of solution S diluted to 14 ml with *water R*.

Iron (*2.4.9*): maximum 100 ppm.

Dilute 2 ml of solution S to 10 ml with *water R*. Use 0.3 ml of *thioglycollic acid R* in this test.

Heavy metals (*2.4.8*): maximum 50 ppm.

Dilute 8 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. When heated, the compound melts. It solidifies as its crystal water boils off. The residue remains a white powder even if heated for a long time.
2. See the **Appearance of solution** test in the *Aluminium sulfate* monograph.
3. See identification **A** in the *Aluminium sulfate* monograph.
4. See identification **B** in the *Aluminium sulfate* monograph.

AMMONIUM BROMIDE

Ammonii bromidum

NH_4Br ***M_r* 97.9**

Definition

Content: 98.5 per cent to 100.5 (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent).

It becomes yellow when exposed to light or air.

It has a sedative effect.

Identification

A. It gives reaction (a) of bromides (*2.3.1*).

B. 10 ml of solution S (see Tests) give the reaction of ammonium salts (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2*, *Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The solution of ammonium bromide is weakly basic. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow). If a yellow colour appears after the addition of the indicator, hydrochloric acid is required to change the colour to red; when the original colour of the indicator is red, sodium hydroxide is required to change it to yellow. An intermediate orange colour may be seen.

Bromates. To 10 ml of solution S add 1ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium iodide R* and 0.25 ml of *0.5 M sulfuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Bromate oxidizes iodide to iodine via the formation of bromine under acidic conditions, and the methylene chloride phase turns violet.

$$BrO_3^- + 5\ Br^- + 6\ H^+ \rightarrow 3\ Br_2 + 3\ H_2O$$
$$Br_2 + 2\ I^- \rightarrow I_2 + 2\ Br^-$$

Iodides. To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless (*2.2.2, Method I*).

Fe^{3+} oxidizes iodide to iodine and the methylene chloride phase turns violet.

$$2\ I^- + 2\ Fe^{3+} \rightarrow I_2 + 2\ Fe^{2+}$$

Iron (*2.4.9*): maximum 20 ppm.

Diluted 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

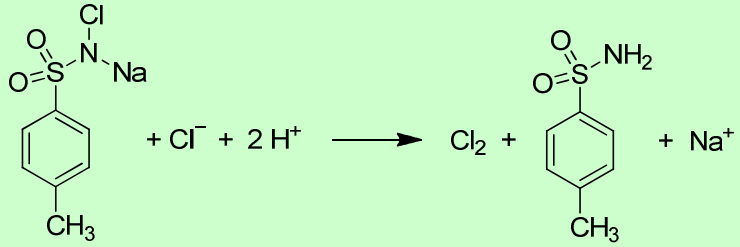
12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Informative test

1. See the **Appearance of solution** test in the *Ammonium bromide* monograph.

2. To 5 ml of solution S, add *hydrochloric acid R* and 2.0 ml of *chloroform R* and 2 drops of *chloramine solution*, and shake. The chloroform layer attains a yellow colour. When more *chloramine solution* is added, the colour of the chloroform layer turns orange-red.

Chloramine (*N*-chloro-4-methylbenzenesulfonamide sodium) is generally used as chlorine source; it forms chlorine with chlorides in acidic solution.



Chlorine liberates the brown bromine, which forms pale-yellow bromochloride with an excess of chlorine.

$$2\ Br^- + Cl_2 \rightarrow Br_2 + 2\ Cl^-$$

$$Br_2 + Cl_2 \rightarrow 2\ BrCl$$

3. When 5.0 ml of solution S is heated with 2.0 ml of *sodium hydroxide solution R*, the odour of ammonia is perceptible.

Sodium hydroxide liberates ammonia.

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

AMMONIUM CHLORIDE

Ammonii chloridum

NH_4Cl **M_r 53.49**

Definition

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water.

It has expectorant and diuretic effects.

Identification

A. It gives the reactions of chlorides (*2.3.1*).

B. 10 ml of solution S (see Tests) gives the reaction of ammonium salts (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2*, *Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.05 ml of *methyl red solution R*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

A solution of ammonium chloride is weakly acidic. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow). If a yellow colour appears after the addition of the indicator, hydrochloric acid is required to change the colour to red; when the original colour of the indicator is red, sodium hydroxide is required to change the colour of the indicator to yellow. An intermediate orange colour may also be observed.

Bromides and iodides. To 10 ml of solution S add 0.1 ml of *dilute hydrochloric acid R* and 0.05 ml of *chloramine solution R*. After 1 minute, add 2 ml of *chloroform R* and shake vigorously. The chloroform layer remains colourless (*2.2.2, Method I*).

Chloramine decomposes into chlorine in hydrochloric acid solution (see Informative test 2 in the *Ammonium bromide* monograph). Chlorine oxidizes bromide to bromine (orange-red colour in chloroform) and iodide to iodine (violet colour in chloroform).

$$2\ Br^- + Cl_2 \rightarrow Br_2 + 2\ Cl^-$$

$$2\ I^- + Cl_2 \rightarrow I_2 + 2\ Cl^-$$

Sulfates (*2.4.13*): maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Calcium (*2.4.3*): maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Assay

Dissolve 1.000 g in 20 ml of *water R* and add a mixture 5 ml of *formaldehyde solution R*, previously neutralised to *phenolphthalein solution R*, and 20 ml of *water R*. After 1-2 min, titrate slowly with *1 M sodium hydroxide*, using a further 0.2 ml of the same indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 53.49 mg of NH_4Cl.

Sodium hydroxide liberates ammonia, which forms hexamethylenetetramine with formaldehyde.

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

$$4\ NH_3 + 6\ CH_2O \rightarrow (CH_2)_6N_4 + 6\ H_2O$$

$$NH_4Cl\ \text{content}\ (\%) = \frac{V_{NaOH}\ (\text{ml}) \cdot f_{NaOH} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative test

1. See the **Appearance of solution** test in the *Ammonium chloride* monograph.

2. Dilute 1.0 ml of solution S with 5 ml of *water R* and 1.0 ml of *dilute nitric acid R*. Add 1.0 ml of *silver nitrate solution R1.* A curdled, white precipitate is formed, which dissolves in an excess of *ammonia solution R2*.

$$AgCl + 2\ NH_3 \rightarrow Ag(NH_3)_2^+ + Cl^-$$

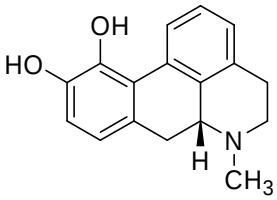
3. When 5.0 ml of solution S is heated with 2.0 ml of *sodium hydroxide solution R*, the odour of ammonia is perceptible.

Sodium hydroxide liberates ammonia.

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

APOMORPHINE HYDROCHLORIDE HEMIHYDRATE

Apomorphini hydrochloridum hemihydricum



, HCl, 1/2 H_2O

$C_{17}H_{18}ClNO_2$. ½ H_2O ***M*r 312.8**

Definition

(6a*R*)-6-Methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinoline-10,11-diol hydrochloride hemihydrate.
Content: 98.5 per cent to 101.5 per cent (dried substance).

Characters

Appearance: white or slightly yellowish-brown or green-tinged greyish, crystalline powder or crystals; on exposure to air and light, the green tinge becomes more pronounced.

Solubility: sparingly soluble in water and in ethanol (96 per cent), practically insoluble in toluene.

It is an emetic. It is also used for the treatment of Parkinson disease.

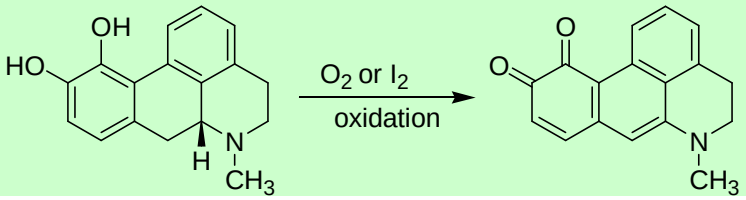
Identification

A. Ultraviolet and visible absorption spectrophotometry.

B. Infrared absorption spectrophotometry.

C. To 5 ml of solution S (see Tests) add a few millilitres of *sodium hydrogen carbonate solution R* until a permanent, white precipitate is formed. The precipitate slowly becomes greenish. Add 0.25 ml of *0.05 M iodine* and shake. The precipitate becomes greyish-green. Collect the precipitate. The precipitate dissolves in *methylene chloride R* giving a violet-blue solution and in *ethanol (96 per cent) R* giving a blue solution.

The pyrocatechin constituent part of the liberated apomorphine base is readily oxidized to green *ortho*-quinone in alkaline medium. The oxygen from the air initiates the oxidation and the process is completed by iodine (**PELLAGRI** reaction). The colour of the resulting *ortho*-quinone derivative depends on the interaction of the product with the solvent molecules (solvatochrome effect).



D. To 2 ml of solution S (see Tests) add 0.1 ml of *nitric acid R*. Mix and filter. The filtrate gives reaction (a) of chlorides *(2.3.1)*.

Tests

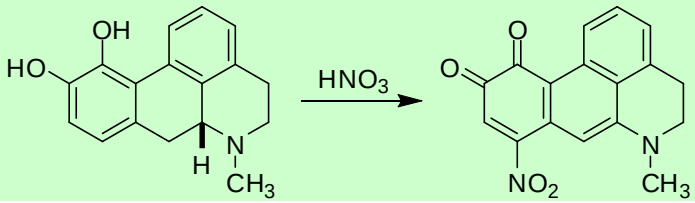
Solution S. Dissolve 0.25 g without heating in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_5 or GY_5 *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Apomorphine hydrochloride* monograph.
2. See identification **C** in the *Apomorphine hydrochloride* monograph (It is enough to check the solubility of the resulting precipitate in *ethanol (96 per cent) R*).
3. 10 mg turns red when 1 drop of *nitric acid R* is added.

   Nitric acid oxidizes and nitrates apomorphine to the *ortho*-quinone derivative of 8-nitroapomorphine (**HUSEMANN** reaction).



4. Dissolve 10 mg in 5.0 ml of *water R* acidified with a few drops of *dilute nitric acid*. When a few drops of *silver nitrate solution R1* are added to the solution, a white precipitate is formed.

   A silver chloride precipitate is formed.

ARSENICUM ALBUM FOR HOMOEOPATHIC PREPARATIONS

Arsenii trioxidum ad praeparationes homoeopathicas

As_2O_3 **M_r 197.8**

Definition

Content: 99.5 per cent to 100.5 per cent of As_2O_3.

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble to sparingly soluble in water. It dissolves in solutions of alkali hydroxides and carbonates.

In low doses, arsenic(III) has a roborant effect. It is also used in the therapy of anaemia.

Identification

A. Dissolve 20 mg in 1 ml of *dilute hydrochloric acid R*, add 4 ml of *water R* and 0.1 ml of *sodium sulfide solution R*. The resulting yellow precipitate is soluble in *dilute ammonia R1*.

Arsenic trioxide dissolves as arsenous acid in a weakly acidic medium, and with sodium sulfide forms yellow arsenic(III) sulfide, which dissolves as colourless arsenite and thioarsenite in ammonia.

$$As_2O_3 + 3\ H_2O \rightarrow 2\ H_3AsO_3$$

$$2\ H_3AsO_3 + 3\ S^{2-} + 6\ H^+ \rightarrow As_2S_3 + 6\ H_2O$$

$$As_2S_3 + 6\ OH^- \rightarrow AsO_3^{3-} + AsS_3^{3-} + 3\ H_2O$$

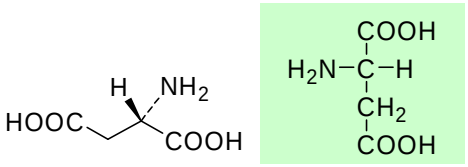
B. Dissolve 20 mg in 1 ml of *hydrochloric acid R1*, add 5 ml of *hypophosphorous reagent R* and heat for 15 min on a water-bath. A black precipitate develops.

Hypophosphite reduces arsenous acid to black arsenic (**THIELE** test)

$$2\ H_3AsO_3 + 3\ H_2PO_2^- + 3\ H^+ \rightarrow 2\ As + 3\ H_3PO_3 + 3\ H_2O$$

ASPARTIC ACID

Acidum asparticum



$C_4H_7NO_4$ **M_r 133.1**

Definition

Aspartic acid contains not less than 98.5 per cent and not more than the equivalent of 101.5 per cent of (2*S*)-2-aminobutanedioic acid, calculated with reference to the dried substance.

Characters

A white or almost white, crystalline powder or colourless crystals, slightly soluble in water, practically insoluble in alcohol. It dissolves in dilute mineral acids and in dilute solutions of alkali hydroxides.

It is an amino acid, essential for the building of peptides, and a component of nutrients.

Identification

A. Specific optical rotation (see Tests).

B. A suspension of 1 g in 10 ml of water R is strongly acid *(2.2.4)*.

C. Examine by infrared absorption spectrophotometry *(2.2.24).*

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances.

Tests

Appearance of solution. Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*. Dissolve 2.000 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is +24.0 to +26.0, calculated with reference to the dried substance.

Chlorides *(2.4.4)*. Dissolve 0.25 g in 3 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution, to which 1 ml of *water R* is added instead of *dilute nitric acid R*, complies with the limit test for chlorides (200 ppm).

Sulfates *(2.4.13)*. Dissolve 0.5 g in 4 ml of *hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulfates (300 ppm). Carry out the evaluation of the test after 30 min.

Ammonium.*(2.4.1)* 50 mg complies with limit test B (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4^+) R*.

Assay

Dissolve 0.100 g in 50 ml of *carbon dioxide-free water R*, with slight heating if necessary. Cool and add 0.1 ml of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide* until the colour changes from yellow to blue. 1 ml of *0.1 M sodium hydroxide* is equivalent to 13.31 mg of $C_4H_7NO_4$.

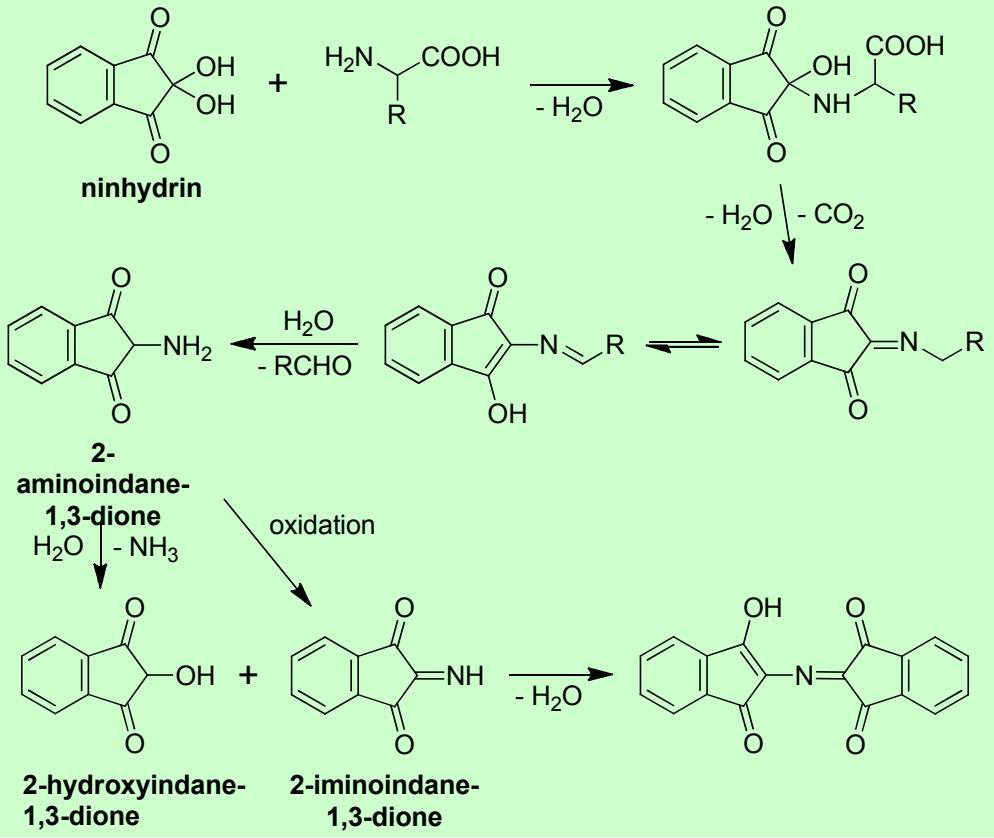
Aspartic acid can be determined as a monovalent acid by alkalimetry. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

$$C_4H_7NO_4 \text{ content (\%)} = \frac{V_{NaOH}\ (ml)\ .\ f_{NaOH}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative tests

1. See the **Appearance of solution** test in the *Aspartic acid* monograph.
2. Dissolve 20 mg in 2.0 ml of *water R*. On heating with several drops of *ninhydrin solution R1*, the mixture turns bluish-violet.

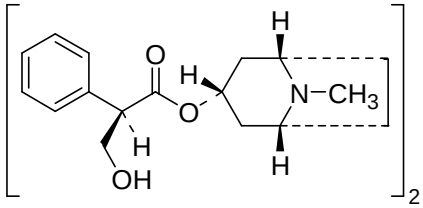
Ninhydrin : (2,2-dihydroxy-1,3-indanedione) is a very sensitive reagent of α-amino acids; it gives violet azomethine derivatives. In the first step a hemiaminal is formed, which turns to a **SCHIFF** base via carbon dioxide and water elimination followed by hydrolysis, resulting in 2-aminoindane-1,3-dione. 2-Aminoindane-1,3-dione is a strong reducing agent, which reduces ninhydrin to 2-hydroxyindane-1,3-dione, while 2-iminoindane-1,3-dione is produced. Violet azomethine, which indicates the presence of amino acid, is formed by the condensation of 2-iminoindane-1,3-dione and 2-hydroxyindane-1,3-dione. The reaction can be applied to identify primary or secondary amines or ammonia.



3. To a solution of 20 mg of sample in 2.0 ml of *water R*, add 2.5 ml of *chloramine solution R* and 1.0 ml of *sodium carbonate solution R*. In another test tube, add 1.0 ml of *sulfanilic acid solution R1* and 1.0 ml of a 10 g/l solution of *sodium nitrite R*. Shake the mixture and, after 5 min, pour it into the previous test tube. After 10–15 min, the solution turns orange-yellow.

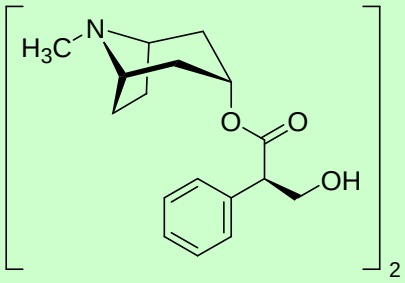
ATROPINE SULFATE

Atropini sulfas



, H_2SO_4, H_2O

and enantiomer



, H_2SO_4, H_2O

and enantiomer

$C_{34}H_{48}N_2O_{10}S$. H_2O **M_r 695**

Definition

Bis[(1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-3-hydroxy-2-phenylpropanoate] sulfate monohydrate.
Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.
Solubility: very soluble in water, freely soluble in ethanol (96 per cent).

It is an alkaloid of plants belonging in the *Solanaceae* family, with a parasympatholytic pharmacological effect. Atropine is a tropine ester of racemic tropic acid.

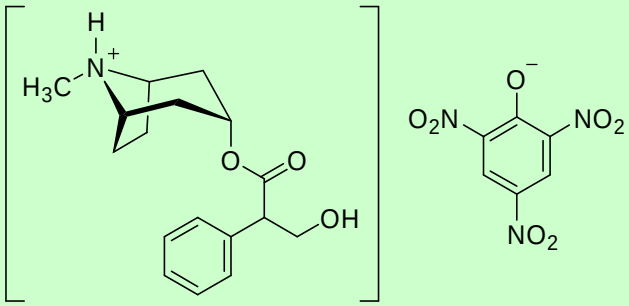
Identification

A. Optical rotation (see Tests).

B. Infrared absorption spectrophotometry.

C. Dissolve about 50 mg in 5 ml of *water R* and add 5 ml of *picric acid solution R*. The precipitate, washed with *water R* and dried at 100 °C to 105 °C for 2 h, melts (2.2.14) at 174 °C to 179 °C.

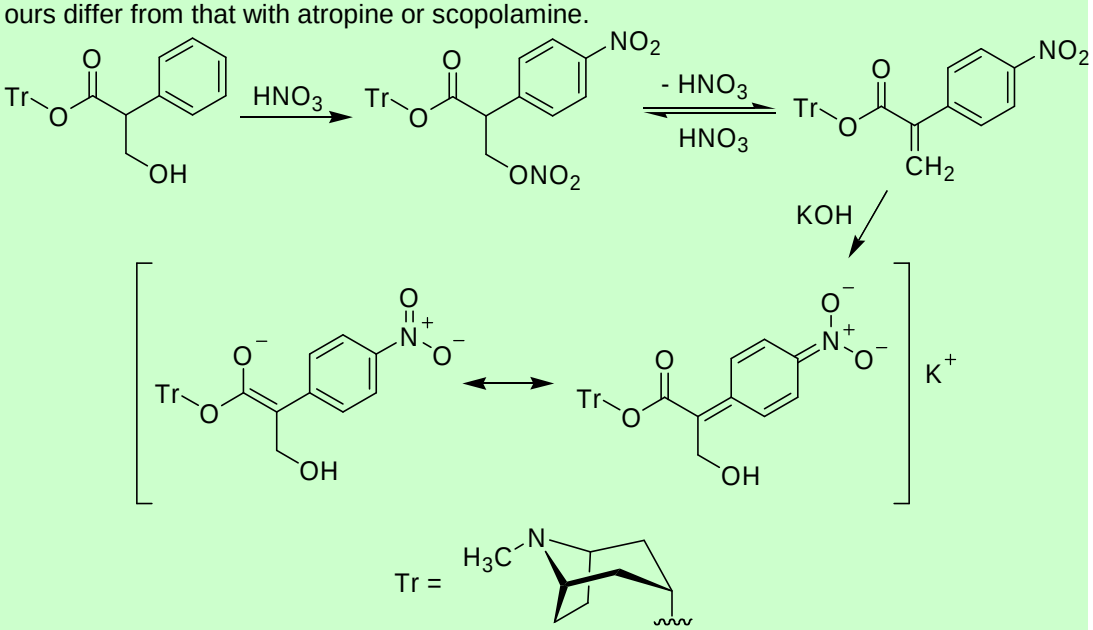
The picrate salt of atropine is formed.



D. To about 1 mg add 0.2 ml of *fuming nitric acid R* and evaporate to dryness in a water-bath. Dissolve the residue in 2 ml of *acetone R* and add 0.1 ml of a 30 g/l solution of *potassium hydroxide R* in *methanol R*. A violet colour develops.

In the **Vitali** reaction, nitric acid nitrates the phenyl group of tropic acid in the *para* position and esterifies the hydroxy group. Under acidic conditions, the unstable nitrate ester turns to *p*-nitroapoatropine via nitrous acid elimination, and finally, in alkaline solution, a violet anion stabilized by mesomerism is obtained. The **Vitali** reaction is a group reaction of tropic esters.

Other alkaloids (*e.g.* strychnine and apomorphine) also give a positive reaction, but the colours differ from that with atropine or scopolamine.



E. It gives the reactions of sulfates *(2.3.1)*.

F. It gives the reaction of alkaloids *(2.3.1)*.

Test

Optical rotation (2.2.7): − 0.50° to + 0.05° (measured in a 2 dm tube).
Dissolve 2.50 g in water R and dilute to 25.0 ml with the same solvent.

Informative tests

1. See identification **D** in the *Atropine sulfate* monograph.

2. See identification **E** in the *Atropine sulfate* monograph.

3. To 50 mg dissolved in 1.0 ml of *water R,* add 1–2 drops of dilute sodium hydroxide solution *R*; a white precipitate is obtained (distinction from scopolamine).

Base liberates the water-insoluble atropine base.

BARIUM SULFATE

Barii sulfas

$BaSO_4$ **M_r 233.4**

Characters

Appearance: fine, white or almost white powder, free from gritty particles.

Solubility: practically insoluble in water and in organic solvents. It is very slightly soluble in acids and in solutions of alkali hydroxides.

Barium sulfate is a radiopaque substance used in investigations of the gastrointestinal tract.

Identification

A. Boil a suspension of 0.2 g with 5 ml of a 500 g/l solution of *sodium carbonate R* for 5 min, add 10 ml of *water R*, filter and acidify a part of the filtrate with *dilute hydrochloric acid R*. The solution gives the reactions of sulfates (*2.3.1*).

As barium sulfate is insoluble in water, its identification is performed after boiling with sodium carbonate, when the barium sulfate is converted to a water-insoluble precipitate (barium carbonate, Identification B) and water-soluble sodium sulfate. On acidification, the sulfate can be detected in the filtrate with barium.

$$BaSO_4 + CO_3^{2-} \rightarrow BaCO_3 + SO_4^{2-}$$

B. Wash the residue collected in the preceding test with 3 successive small quantities of *water R*. To the residue add 5 ml of *dilute hydrochloric acid R*, filter and add to the filtrate 0.3 ml of *dilute sulfuric acid R*. A white precipitate is formed that is insoluble in *dilute sodium hydroxide solution R*.

After the precipitate obtained identification **A** has been washed sulfate-free, the barium carbonate is dissolved in acid and the barium is identified.

$$BaCO_3 + 2\ H^+ \rightarrow Ba^{2+} + CO_2 + H_2O$$

$$Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$$

Tests

Solution S. To 20.0 g add 40 ml of *distilled water R* and 60 ml of *dilute acetic acid R*. Boil for 5 min, filter and dilute the cooled filtrate to 100 ml with *distilled water R*.

Acidity or alkalinity. Heat 5.0 g with 20 ml of *carbon dioxide-free water R* on a water-bath for 5 min and filter. To 10 ml of the filtrate add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The shaken mixture of barium sulfate and water is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Oxidisable sulfur compounds. Shake 1.0 g with 5 ml of *water R* for 30 s and filter. To the filtrate add 0.1 ml of *starch solution R*, dissolve 0.1 g of *potassium iodide R* in the mixture, add 1.0 ml of a freshly prepared 3.6 mg/l solution of *potassium iodate R* and 1 ml of *1 M hydrochloric acid* and shake well. The colour of the solution is more intense than that of a standard prepared at the same time and in the same manner omitting the potassium iodate.

Under acidic conditions, iodate oxidizes iodide to iodine, which forms blue iodine–starch. When oxidizable sulfur compounds are present (*e.g.* sulfite or thiosulfate), iodine is reduced to iodide and blue iodine–starch is not formed.

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

$$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2\ I^- + 2\ H^+$$

$$2\ S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2\ I^-$$

Soluble barium salts: maximum 10 ppm.

To 2.5 ml of a 0.2 mg/l solution of *barium nitrate R* in a mixture of 30 volumes of *ethanol (96 per cent) R* and 70 ml of *water R*, add 10 ml of *dilute sulfuric acid R*. Shake and allow to stand for 5 min. To 1 ml of this solution, add 10 ml of solution S. Prepare a standard in the same manner using 10 ml of *barium standard solution (2 ppm Ba) R* instead of solution S.

After 10 min, any opalescence in the test solution is not more intense than that in the standard.

A barium sulfate precipitate is formed.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

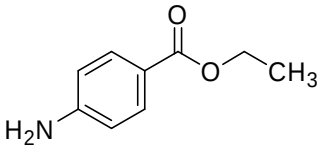
Informative test

1. See identification **A** in the *Barium sulfate* monograph.

2. See identification **B** in the *Barium sulfate* monograph.

BENZOCAINE

Benzocainum



$C_9H_{11}NO_2$ **M_r 165.2**

Definition

Ethyl 4-aminobenzoate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very slightly soluble in water, freely soluble in ethanol (96 per cent).

It is a local anaesthetic.

Identification

A. Infrared absorption spectrophotometry.

Informative tests

1. Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute the solution to 20 ml with the same solvent. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

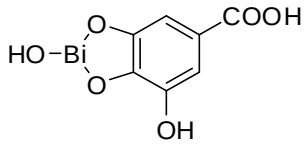
2. Dissolve about 50 mg in ethanol *(96 per cent) R* and dilute the solution to 100 ml with the same solvent. 2 ml of the solution gives the reaction of primary aromatic amines *(2.3.1)*.

3. Dissolve approx. 50 mg made wet with 1–2 drops of *dilute hydrochloric acid R* in 5.0 ml of *water R*. No change can be observed after the addition of 1–2 drops of *potassium tetraiodomercurate(II) solution R* (difference from procaine, tetracaine and cocaine).

With potassium tetraiodomercurate(II) (**MAYER** reagent), compounds bearing a basic tertiary nitrogen (*e.g.* procaine and tetracaine) form precipitates that are insoluble in dilute acids. Benzocaine has no tertiary nitrogen, and therefore a precipitate is not formed.

BISMUTH SUBGALLATE

Bismuthi subgallas



$C_7H_5BiO_6$ **M_r 394.1**

Definition

Complex of bismuth and gallic acid.

Content: 48.0 per cent to 51.0 per cent of Bi (A_r 209.0) (dried substance).

Characters

Appearance: yellow powder.

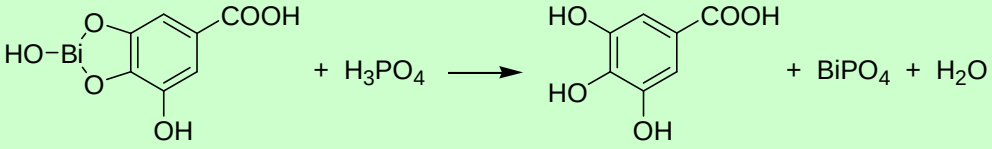
Solubility: practically insoluble in water and in ethanol (96 per cent) It dissolves in mineral acids with decomposition and in solutions of alkali hydroxides, producing a reddish-brown liquid.

Bismuth subgallate is used internally in cases of enteritis or intestinal catarrh. Because of its scarring, drying and antiseptic pharmacological effect, it is used externally as an astringent in dermatology.

Identification

A. Mix 0.1 g with 5 ml of *water R* and 0.1 ml of *phosphoric acid R*. Heat to boiling and maintain boiling for 2 min. Cool and filter. To the filtrate, add 1.5 ml of *ferric chloride solution R1*, a blackish-blue colour develops.

When it as boiled with phosphoric acid, the less soluble bismuth phosphate ($BiPO_4$) and gallic acid are formed. Gallic acid forms a blackish-blue complex with Fe^{3+}.



gallic acid

B. It gives reaction (b) of bismuth *(2.3.1)*.

Informative tests

1. See identification **A** in the *Bismuth subgallate* monograph.

2. See identification **B** in the *Bismuth subgallate* monograph.

BISMUTH SUBSALICYLATE

Bismuthi subsalicylas

$C_7H_5BiO_4$ **M_r 362.1**

Definition

Complex of bismuth and salicylic acid.

Content : 56.0 per cent to 59.4 per cent of Bi (A_r 209.0) (dried substance).

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble in water and in alcohol. It dissolves in mineral acids with decomposition.

It is used internally in cases of enteritis or intestinal catarrh. It is also used externally as an astringent in dermatology.

Identification

A. To 0.5 g add 10 ml of *hydrochloric acid R1*. Heat on a boiling water-bath for 5 min. Cool and filter. Retain the filtrate for identification test **B**. Wash the residue with *dilute hydrochloric acid R* and then with *water R.* Dissolve the residue in 0.5–1 ml of *dilute sodium hydroxide solution R*. Add 15 ml of *water R*. Neutralise with dilute *hydrochloric acid R*. The solution gives reaction (a) of salicylates *(2.3.1)*.

B. The filtrate obtained in identification test **A** gives reaction (b) of bismuth *(2.3.1)*.

Tests

Chlorides *(2.4.4)*: maximum 200 ppm.

Dissolve 0.250 g in a mixture of 2 ml of *nitric acid R*, 5 ml of *water R* and 8 ml of *methanol R*.

Assay

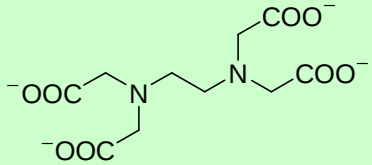
Dissolve with heating 0.300 g in 10 ml of a mixture of 2 volumes of *perchloric acid R* and 5 volumes of *water R* To the hot solution, add 200 ml of *water R* and 50 mg of *xylenol orange triturate R*. Titrate with *0.1 M sodium edetate* until a yellow colour is obtained.

1 ml of *0.1 M sodium edetate* is equivalent to 20.90 mg of Bi.

Following boiling with perchloric acid, bismuth can be measured directly in acidic medium through complexometric titration. Perchloric acid oxidizes the organic moiety of the compound, which could otherwise disturb the assay.

$$Bi^{3+} + H_2Y^{2-} \rightarrow BiY^{-} + 2\ H^{+}$$

Y^{4-}:



$$\text{Bi content (\%)} = \frac{V_{EDTA}\ (ml)\ .\ f_{EDTA}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative tests

1. Shake 0.10 g with 2.0 ml of *ferric chloride R2* and 4.0 ml of water *R*. A violet colour is produced.

A stable three-ligand chelate complex is formed (see general identification of ”Salicylates”).

2. See identification **B** in the *Bismuth subsalicylate* monograph.

BORAX

Borax

$Na_2B_4O_7,10H_2O$ **M_r 381.4**

Definition

Disodium tetraborate decahydrate.

Content: 99.0 per cent to 103.0 per cent of $Na_2B_4O_7,10H_2O$.

Characters

Appearance: white or almost white, crystalline powder, colourless crystals or crystalline masses, efflorescent.

Solubility: soluble in water, very soluble in boiling water, freely soluble in glycerol.

Because of its bacteriostatic and mycostatic activity, borax is used externally.

Identification

A. To 1 ml of solution S (see Tests) add 0.1 ml of *sulfuric acid R* and 5 ml of *methanol R* and ignite. The flame has a green border.

Via esterification, methyl borate is formed, which is volatile and colours the flame green.

$$H_3BO_3 + 3\ CH_3OH \rightarrow B(OCH_3)_3 + 3\ H_2O$$

B. To 5 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is red. On the addition of 5 ml of *glycerol* (*85 per cent*) *R* the colour disappears.

The aqueoussolution of alkali metal borates is weakly basic. Polyhydroxy compounds (*e.g.* glycerol, mannitol or invert sugars) dissolve the weakly acidic boric acid (formed by the hydrolysis of borax) to form a stronger acid (see Assay), which can be detected by the colour change of the phenolphthalein indicator.

C. Solution S gives the reactions of sodium (*2.3.1*).

Tests

Solution S. Dissolve 4.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2*, *Method II*).

pH (*2.2.3*): 9.0 to 9.6 for solution S.

Sulfates (*2.4.13*): maximum 50 ppm, determined on solution *S*.

Use in this test 1.0 ml of *acetic acid R*. Prepare the standard using a mixture of 3 ml of *sulfate standard solution* (*10 ppm SO_4^{2-}*) *R* and 12 ml of *distilled water R*.

Ammonium (*2.4.1*): maximum 10 ppm.

Dilute 6 ml of solution S to 14 ml with *water R*. Prepare the standard using a mixture of 2.5 ml of *ammonium standard solution* (*1 ppm NH_4^+*) *R* and 7.5 ml of *water R*.

Arsenic (*2.4.2, Method A*): maximum 5 ppm, determined on 5 ml of solution S.

Calcium (*2.4.3*): maximum 100 ppm, determined on solution S.

Prepare the standard using a mixture of 6 ml of *calcium standard solution* (*10 ppm Ca*) *R* and 9 ml of *distilled water R*.

Heavy metals (*2.4.8*): maximum 25 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Assay

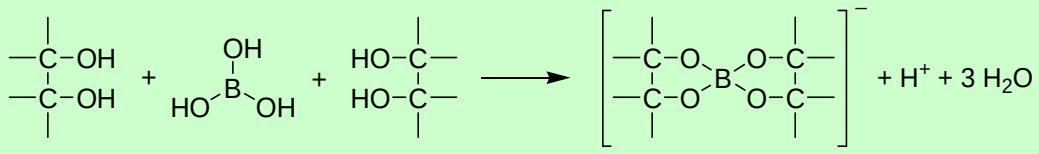
Dissolve 20 g of *mannitol R* in 100 ml of *water R*, heating if necessary, cool, and add 0.5 ml of *phenolphthalein solution R* and neutralise with *0.1 M sodium hydroxide* until a pink colour is obtained. Add 3.00

g of the substance to be examined, heat until dissolution is complete, cool, and titrate with *1 M sodium hydroxide* until the pink colour reappears.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1907 g of $Na_2B_4O_7,10H_2O$.

Polyhydroxy compounds (*e.g.* mannitol) dissolve the weakly acidic boric acid (formed by the hydrolysis of borax) to form a stronger acid, which can be titrated directly with sodium hydroxide in the presence of the phenolphthalein indicator.

$$Na_2B_4O_7 + 5\ H_2O \rightarrow 2\ NaH_2BO_3 + 2\ H_3BO_3$$



$$Na_2B_4O_7,10H_2O\ \text{content}\ (\%) = \frac{V_{NaOH}\ (ml)\ .\ f_{NaOH}\ .\ E\ (g/ml)}{\text{amount of substance}\ (g)}\ .\ 100$$

Informative test

1. When heated, sodium tetraborate swells to a spongy mass. On stronger heating, it melts to a transparent, colourless bead. When heated in a non-luminous flame, a persistent vivid-yellow colour is produced.
2. See the **Appearance of solution** test in the *Borax* monograph.
3. See identification **A** in the *Borax* monograph.

BORIC ACID

Acidum boricum

H_3BO_3 **M_r 61.8**

Definition

Content: 99.0 per cent to 100.5 per cent.

Characters

Appearance: white or almost white, crystalline powder, colourless, shiny plates greasy to the touch, or white or almost white crystals.

Solubility: soluble in water and in ethanol (96 per cent), freely soluble in boiling water and in glycerol (85 per cent).

Boric acid has bacterio- and fungiostatic activity. It is used externally.

Identification

A. Dissolve 0.1 g by gently heating in 5 ml of *methanol R*, add 0.1 ml of *sulfuric acid R* and ignite the solution. The flame has a green border.

On esterification, methyl borate is formed, which is volatile and colours the flame green.

$$H_3BO_3 + 3\ CH_3OH \rightarrow B(OCH_3)_3 + 3\ H_2O$$

B. Solution S (see Tests) is acid (*2.2.4*).

Tests

Solution S. Dissolve 3.3 g in 80 ml of boiling *distilled water R*, cool and dilute to 100 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.1, Method II*).

pH (*2.2.3*): 3.8 to 4.8 for solution S.

Solubility in ethanol (96 per cent). The solution is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Dissolve 1.0 g in 10 ml of boiling *ethanol (96 per cent) R*.

Organic matter. It does not darken on progressive heating to dull redness.

Sulfates (*2.4.13*): maximum 450 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Heavy metals (*2.4.8*): maximum 15 ppm.

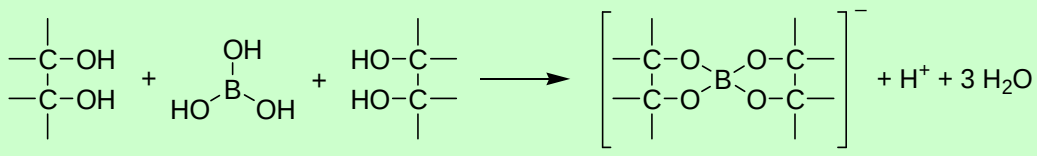
12 ml of solution S complies with test A. Prepare the reference solution using a mixture of 2.5 ml of *lead standard solution* (*2 ppm Pb*) *R* and 7.5 ml of *water R*.

Assay

Dissolve 1.000 g with heating in 100 ml of *water R* containing 15 g of *mannitol R*. Titrate with *1 M sodium hydroxide*, using 0.5 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *1 M sodium hydroxide* is equivalent to 61.8 mg of H_3BO_3.

Polyhydroxy compounds (*e.g.* mannitol) dissolve the weakly acidic boric acid to form a stronger, acetic acid-like acid, which can be titrated directly with sodium hydroxide in the presence of phenolphthalein as indicator.



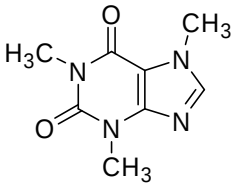
$$H_3BO_3 \text{ content } (\%) = \frac{V_{NaOH}\,(ml) \cdot f_{NaOH} \cdot E\,(mg/ml)}{\text{amount of substance } (mg)} \cdot 100$$

Informative test

1. See the **Appearance of solution** test in the *Boric acid* monograph.
2. See identification **A** in the *Boric acid* monograph.
3. See identification **B** in the *Boric acid* monograph.

CAFFEINE

Coffeinum



$C_8H_{10}N_4O_2$ **M_r 194,2**

Definition

1,3,7-Trimethyl-3,7-dihydro-1*H*-purine-2,6-dione.
Content: 98.5 per cent to 101.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or silky, white or almost white, crystals.
Solubility: sparingly soluble in water, freely soluble in boiling water, slightly soluble in ethanol (96 per cent). It dissolves in concentrated solutions of alkali benzoates or salicylates.
It sublimes readily

It is used as a CNS stimulant, a mild diuretic, and a respiratory stimulant.

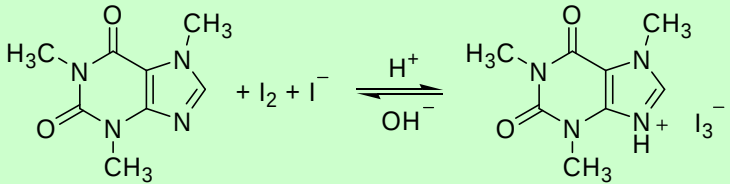
Identification

A. Melting point *(2.2.14):* 234 °C to 239 °C.

B. Infrared absorption spectrophotometry.

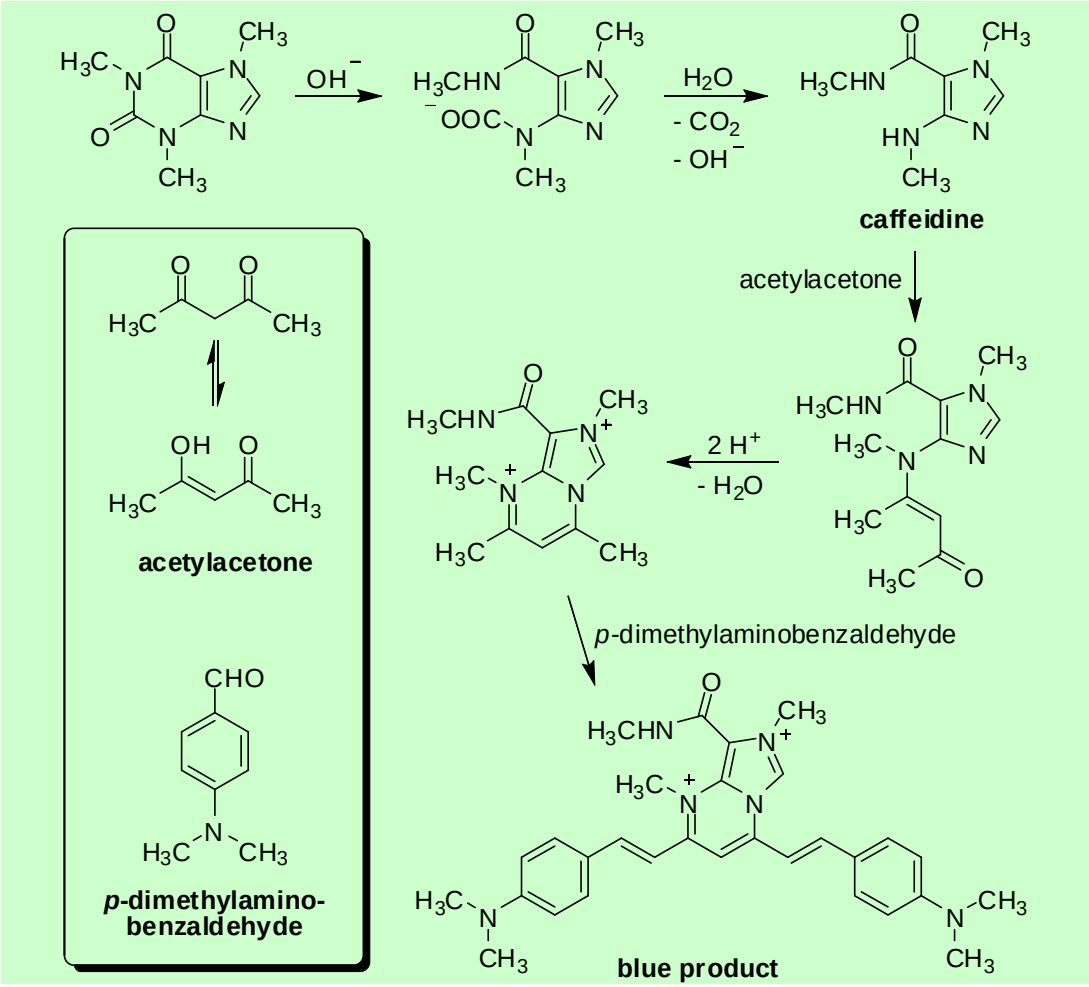
C. To 2 ml of a saturated solution add 0.05 ml of *iodinated potassium iodide solution R*. The solution remains clear. Add 0.1 ml of *dilute hydrochloric acid R.* A brown precipitate is formed. Neutralise with *dilute sodium hydroxide solution R*; the precipitate dissolves.

Under acidic conditions, the protonated form of caffeine forms a brown precipitate with triiodide ions. In alkaline medium, the precipitate dissolves. The reaction is not characteristic for caffeine; other purine alkaloids also give a positive reaction.



D. In a ground-glass-stoppered tube, dissolve about 10 mg in 0.25 ml of a mixture of 0.5 ml of *acetylacetone R* and 5 ml of *dilute sodium hydroxide solution R*. Heat in a water-bath at 80 °C for 7 min. Cool and add 0.5 ml of *dimethylaminobenzaldehyde solution R2*. Heat again in a water-bath at 80 °C for 7 min. Allow to cool and add 10 ml of *water R*; an intense blue colour develops.

In alkaline solution, caffeidine is formed by the ring opening of the pyrimidine ring of caffeine. Caffeidine reacts with acetylacetone to give an enamine-type compound, which turns to an imidazo[1,5-*a*]pyrimidinium salt in acidic medium through ring closure (the *dimethylaminobenzaldehyde solution R2* is strongly acidic). The latter compound forms a blue condensed product with *p*-dimethylaminobenzaldehyde.



E. Loss on drying (see Tests).

F. It gives the reaction of xanthines *(2.3.1)*.

Tests

Solution S. Dissolve 0.5 g with heating in 50 ml of *carbon dioxide-free water R* prepared from *distilled water R*, cool and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity. To 10 ml of solution S add 0.05 ml of *bromothymol blue solution R1*; the solution is green or yellow. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

The aqueous solution of caffeine is neutral. The pH interval of the colour change of bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Sulfates *(2.4.13)*. maximum 500 ppm, determined on 15 ml of solution S.

Prepare the standard using a mixture of 7.5 ml of *sulfate standard solution (10 ppm SO_4) R* and 7.5 ml of *distilled water R*.

Loss on drying *(2.2.32)*: maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 1 h.

Informative tests

1. See the **Appearance of solution** test in the *Caffeine* monograph.

2. See identification **F** in the *Caffeine* monograph.

3. A mixture of 5.0 ml of *water R,* 0.05 ml of *0,1 M sodium hydroxide* and 2 drops of *R phenolphthalein* and 0.1 g of substance is boiled. The red colour of the solution remains.

The **WINKLER** test, a suitable reaction for distinction between caffeine, theobromine and theophylline, is based on the different acidities and solubilities of the compounds. Theophylline and theobromine are weak acids. They therefore neutralize the sodium hydroxide solution and the red colour of phenolphthalein disappears (for theobromine, due to the low solubility of the substance at ambient temperature, the neutralization occurs only in the boiled solution).

CALCIUM CARBONATE

Calcii carbonas

$CaCO_3$ **M_r 100.1**

Definition

Content: 98.5 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble in water.

It is used in calcium therapy and as an antacid.

Identification

A. It gives the reaction of carbonates (*2.3.1*).

B. 0.2 ml of solution S (see Tests) gives the reactions of calcium (*2.3.1*).

Tests

Solution S. Dissolve 5.0 g in 80 ml of *dilute acetic acid R*. When the effervescence ceases, boil the solution for 2 min, allow to cool, dilute to 100 ml with *dilute acetic acid R* and filter, if necessary, through a sintered-glass filter.

Carbonates form carbon dioxide during dissolution in acids. Solution S contains calcium acetate in acetic acid solution.

Chlorides (*2.4.4*): maximum 330 ppm.

Dilute 3 ml of solution S to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum 0.25 per cent.

Dilute 1.2 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2 Method A*): maximum 4 ppm, determined on 5 ml of solution S.

Barium. To 10 ml of solution S add 10 ml of *calcium sulfate solution R*. After at least 15 min, any opalescence in the solution is not more intense than that in a mixture of 10 ml of solution S and 10 ml of *distilled water R*.

The barium impurity can be detected as a precipitate of barium sulfate.

Iron (*2.4.9*): maximum 200 ppm.

Dissolve 50 mg in 5 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Assay

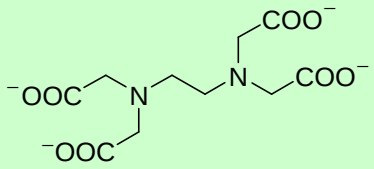
Dissolve 0.150 g in a mixture of 3 ml of *dilute hydrochloric acid R* and 20 ml of *water R*. Boil for 2 min, allow to cool and dilute to 50 ml with *water R*. Carry out the complexometric titration of calcium (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 10.01 mg of $CaCO_3$.

When heated with hydrochloric acid solution, calcium carbonate dissolves, carbon dioxide gas being liberated. The released calcium is determined by complexometric titration involving formation of the Ca^{2+}– EDTA complex.

$$CaCO_3 + 2\ H^+ \rightarrow Ca^{2+} + CO_2 + H_2O$$

$Ca^{2+} + H_2Y^{2-} \rightarrow CaY^{2-} + 2\ H^+\ Y^{4-}$:



$$CaCO_3 \text{ content (\%)} = \frac{V_{EDTA}\ (ml)\ .\ f_{EDTA}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative test

1. Heat a small sample, wetted with 1 drop of *dilute hydrochloric acid R*, in a non-luminous flame. A persistent, orange-red colour is produced in the flame.

2. To 50 mg of compound dissolved in 1 ml of *dilute acetic acid R*, add 1.0 ml of *water R* and a few drops of *ammonium oxalate R*. A white, microcrystalline precipitate is formed.

Calcium ions form an insoluble, white precipitate of calcium oxalate in dilute acetic acid.

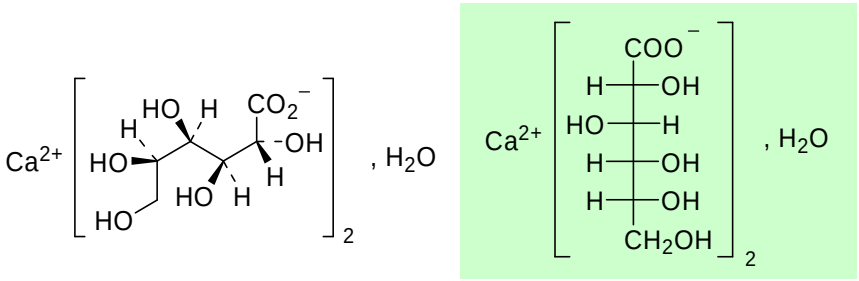
$$Ca^{2+} + (COO)_2^{2-} \rightarrow Ca(COO)_2$$

3. When 1.00 g of compound is dissolved in 12.0 ml of *dilute hydrochloric acid R*, a colourless gas is liberated.

Carbonate ions react with hydrochloric acid and carbon dioxide is liberated.

CALCIUM GLUCONATE

Calcii gluconas



$C_{12}H_{22}CaO_{14} . H_2O$ ***Mr* 448.4**

Definition

Calcium bis[(2*R*,3*S*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexanoate] monohydrate (calcium di(D-gluconate) monohydrate).

Content: 98.5 per cent to 102.0 per cent of $C_{12}H_{22}CaO_{14},H_2O$.

Characters

Appearance: white or almost white, crystalline or granular powder.

Solubility: sparingly soluble in water, freely soluble in boiling water.

It is used in calcium therapy. Because of its low solubility, it allows a retard pharmacological effect.

Identification

A. Thin-layer chromatography.

B. Solution S (see Tests) gives the reactions of calcium *(2.3.1)*.

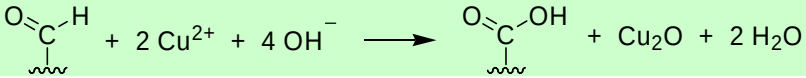
Tests

Solution S. Dissolve 1.0 g in *water R* heated to 60 °C and dilute to 50 ml with the same solvent.

Appearance of solution. At 60 °C, solution S is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*. After cooling, it is not more opalescent than reference suspension II *(2.2.1)*.

Sucrose and reducing sugars. Dissolve 0.5 g in a mixture of 2 ml of *hydrochloric acid R1* and 10 ml of *water R*. Boil for 5 min, allow to cool, add 10 ml of *sodium carbonate solution R* and allow to stand. Dilute to 25 ml with *water R* and filter. To 5 ml of the filtrate add 2 ml of *cupri-tartaric solution R* and boil for 1 min. Allow to stand for 2 min. No red precipitate is formed.

The purity test is based on the **FEHLING** reaction of reducing sugars. Reduction of Cu^{2+} results in red copper(I) oxide. On heating with hydrochloric acid, sucrose is hydrolysed to glucose and fructose (see *Saccharum* monograph, Identification **C**).



Chlorides *(2.4.4)*: maximum 200 ppm.

Dilute 12.5 ml of solution S to 15 ml with *water R*.

Sulfates *(2.4.13)*: maximum 100 ppm.

Dissolve 10.0 g with heating in a mixture of 10ml of *acetic acid R* and 90 ml of *distilled water R*.

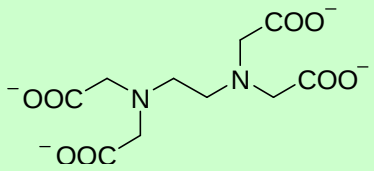
Assay

Dissolve 0.8000 g in 20 ml of hot *water R*, allow to cool and dilute to 300 ml with *water R.* Carry out the complexometric titration of calcium *(2.5.11).*

1 ml of *0.1 M sodium edetate* is equivalent to 44.84 mg of $C_{12}H_{22}CaO_{14},H_20$.

The content of calcium can be determined by complexometry.

$Ca^{2+} + H_2Y^{2-} \rightarrow CaY^{2-} + 2\ H^+$ Y^{4-}:



$$C_{12}H_{22}CaO_{14}.H_2O \text{ content (\%)} = \frac{V_{EDTA}\ (ml)\ .\ f_{EDTA}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative tests

1. On gentle heating, the sample emits a burnt sugar smell during carbonization. When the residue wetted with *diluted hydrochloric acid R* is heated, a red colour must be produced in the flame.

Ca^{2+} produces a brick-red colour in the flame.

2. Dissolve 1.00 g in 20.0 ml of hot *water R*. Dilute 1.0 ml of the hot solution with 5.0 ml of *water R*, add a few drops of *diluted acetic acid R* and 1.0 ml of *ammonium oxalate solution R*. A white, crystalline precipitate is formed.

Calcium ions form an insoluble, white precipitate of calcium oxalate in dilute acetic acid.

$$Ca^{2+} + (COO)_2{}^{2-} \rightarrow Ca(COO)_2$$

3. Mix 1.0 ml of the solution prepared in **Informative test 2** with 3.0 ml of *water R* and 1 drop of *iron(II) chloride solution R2*. The colourless solution turns yellow.

CALCIUM SULFATE DIHYDRATE

Calcii sulfas dihydricus

$CaSO_4,2H_2O$ **M_r 172.2**

Definition

Content: 98.0 per cent to 102.0 per cent of $CaSO_4,2H_2O$.

Characters

Appearance: white or almost white fine powder.

Solubility: very slightly soluble in water, practically insoluble in ethanol (96 per cent).

It is used for the preparation of anhydrous calcium sulfate (heating at 130 °C), a compound applied during the fixing of broken bones.

Identification

A. Loss on ignition (see Tests).

B. Solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

C. Solution S gives reaction (a) of calcium (*2.3.1*).

Tests

Solution S. Dissolve 1.0 g in 50 ml of a 10 per cent V/V solution of *hydrochloric acid R* by heating at 50 °C for 5 min. Allow to cool.

Acidity or alkalinity. Shake 1.5 g with 15 ml of *carbon dioxide-free water R* for 5 min. Allow to stand for 5 min and filter. To 10 ml of the filtrate, add 0.1 ml of *phenolphthalein solution R* and 0.25 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.30 ml of *0.01 M hydrochloric acid*. The solution is colourless. Add 0.2 ml of *methyl red solution R*. The solution is reddish-orange.

The shaken mixture of calcium sulfate with water is nearly neutral. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red), while thatof the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

Chlorides (*2.4.4*): maximum 300 ppm.

Shake 0.5 g with 15 ml of *water R* for 5 min. Allow to stand for 15 min and filter. Dilute 5 ml of the filtrate to 15 ml with *water R*.

Arsenic (*2.4.2*): maximum 10 ppm, determined on 5 ml of solution S.

Iron (*2.4.9*): maximum 100 ppm.

To 0.25 g add a mixture of 5 ml of *hydrochloric acid R* and 20 ml of *water R*. Heat to boiling, cool and filter.

Heavy metals (*2.4.8*): maximum 20 ppm.

To 2.5 g add a mixture of 2 ml of *hydrochloric acid R* and 15 ml of *water R*. Heat to boiling. Cool and then add 0.5 ml of *phenolphthalein solution R*. Cautiously add *concentrated ammonia R* until the colour changes to pink. Add 0.5 ml of *glacial acetic acid R* and dilute to 25 ml with *water R*. Filter. 12 ml of the filtrate complies with test A. Prepare the reference solution using *lead standard solution* (*2 ppm Pb*) *R*.

Loss on ignition: 18.0 per cent to 22.0 per cent, determined on 1.000 g by ignition to constant mass at 800 ± 50 °C.

CHARCOAL, ACTIVATED

Carbo activatus

Definition

Obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorption power.

Characters

Appearance: black, light powder free from grittiness.

Solubility: practically insoluble in all usual solvents.

Activated charcoal adsorbs poisonous substances (*e.g.* toxins) from the intestines, and also gases or bacteria.

Identification

D. When heated to redness it burns slowly without a flame.

E. Adsorption power (see Tests).

Tests

Acidity or alkalinity. To 2.0 g add 40 ml of *water R* and boil for 5 min. Cool, restore to the original mass with *carbon dioxide-free water R* and filter. Reject the first 20 ml of the filtrate. To 10 ml of the filtrate add 0.25 ml of *bromothymol blue solution R1* and 0.25 ml of *0.02 M sodium hydroxide*. The solution is blue. Not more than 0.75 ml of *0.02 M hydrochloric acid* is required to change the colour of the indicator to yellow.

The shaken mixture of activated charcoal and water is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Alkali-soluble coloured substances. To 0.25 g add 10 ml of *dilute sodium hydroxide solution R* and boil for 1 min. Cool, filter and dilute the filtrate to 10 ml with *water R*. The solution is not more intensely coloured than reference solution GY_4 (*2.2.2, Method II*.)

Sulfides. To 1.0 g in a conical flask add 5 ml of *hydrochloric acid R1* and 20 ml of *water R*. Heat to boiling. The fumes released do not turn *lead acetate paper R* brown.

When heated with hydrochloric acid, sulfides form hydrogen sulfide, which can be detected as a black or brown precipitate of lead sulfide (a small amount of PbS causes a brown colour).

$$S^{2-} + 2\ H^+ \rightarrow H_2S$$

$$H_2S + Pb^{2+} \rightarrow PbS + 2\ H^+$$

Adsorption power. To 0.300 g in a 100 ml ground-glass-stoppered conical flask add 25.0 ml of a freshly prepared solution of 0.5 g of *phenazone R* in 50 ml of *water R*. Shake thoroughly for 15 min. Filter and reject the first 5 ml of filtrate. To 10.0 ml of the filtrate add 1.0 g of *potassium bromide R* and 20 ml of *dilute hydrochloric acid R*. Using 0.1 ml of *methyl red solution R* as indicator, titrate with *0.0167 M potassium bromate* until the red colour is discharged. Titrate slowly (1 drop every 15 s) towards the end of the titration. Carry out a blank titration using 10.0 ml of the phenazone solution.

Calculate the quantity of phenazone adsorbed per 100 g of activated charcoal from the expression:

$$\frac{2.353\ .\ (a-b)}{m}$$

a = number of millilitres of *0.0167 M potassium bromate* used for the blank,

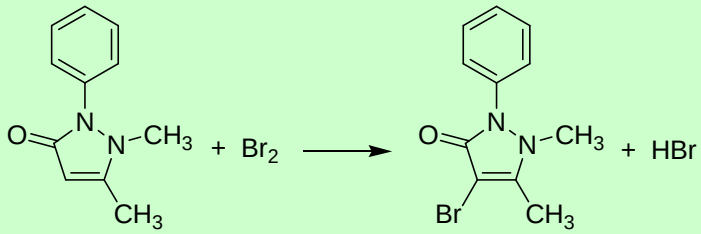
b = number of millilitres of *0.0167 M potassium bromate* used for the test,

m = mass in grams of the substance to be examined.

Minimum 40 g of phenazone is adsorbed per 100 g of activated charcoal, calculated with reference to the dried substance.

The quantity of non-adsorbed phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) is titrated by bromatometry. Electrophilic substitution occurs at position 4 of phenazone. At the end of the titration, the methyl red indicator is oxidized too by bromine. Therefore, to use more indicator than prescribed is not recommended.

$$BrO_3^- + 5\ Br^- + 6\ H^+ \rightarrow 3\ Br_2 + 3\ H_2O$$

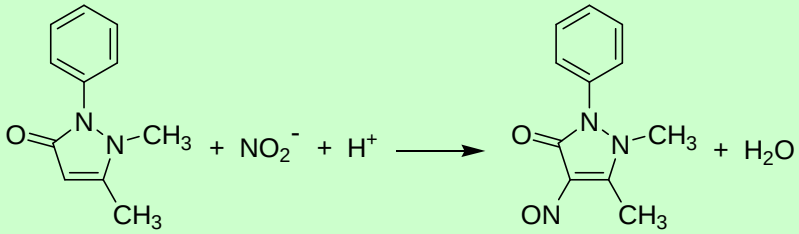


Informative test

1. See identification **A** in the *Activated charcoal* monograph.

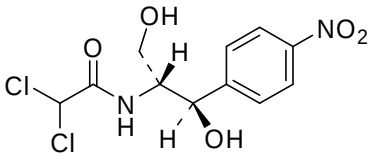
2. Add 10.0 ml of *phenazone R* (1 g/100 ml) to 0.10 g of charcoal in a tube and shake the mixture for 3 min. Filter it through a paper filter (make it wet with *water R* before filtration). Repeat the above-mentioned procedure with 0.10 g of charcoal twice. On shaking a few drops of the final solution with 1-2 drops of *dilute sulfuric acid R* and 2 drops of *sodium nitrite solution R* (10 g/l), the colour of the solution should not be green.

The full amount of phenazone must be adsorbed; otherwise, phenazone produces green 4-nitrosophenazone with sodium nitrite under acidic conditions.



CHLORAMPHENICOL

Chloramphenicolum



$C_{11}H_{12}Cl_2N_2O_5$ **M_r 323.1**

Definition

Chloramphenicol is 2,2-dichloro-*N*-[(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]acetamide, produced by the growth of certain strains of *Streptomyces venezuelae* in a suitable medium. It is normally prepared by synthesis. It contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $C_{11}H_{12}C_{12}N_2O_5$, calculated with reference to the dried substance.

Characters

A white, greyish-white or yellowish-white, fine, crystalline powder or fine crystals, needles or elongated plates, slightly soluble in water, freely soluble in alcohol and in propylene glycol.

A solution in ethanol is dextrorotatory and a solution in ethyl acetate is laevorotatory.

Chloramphenicol is a broad-spectrum antibiotic.

Identification

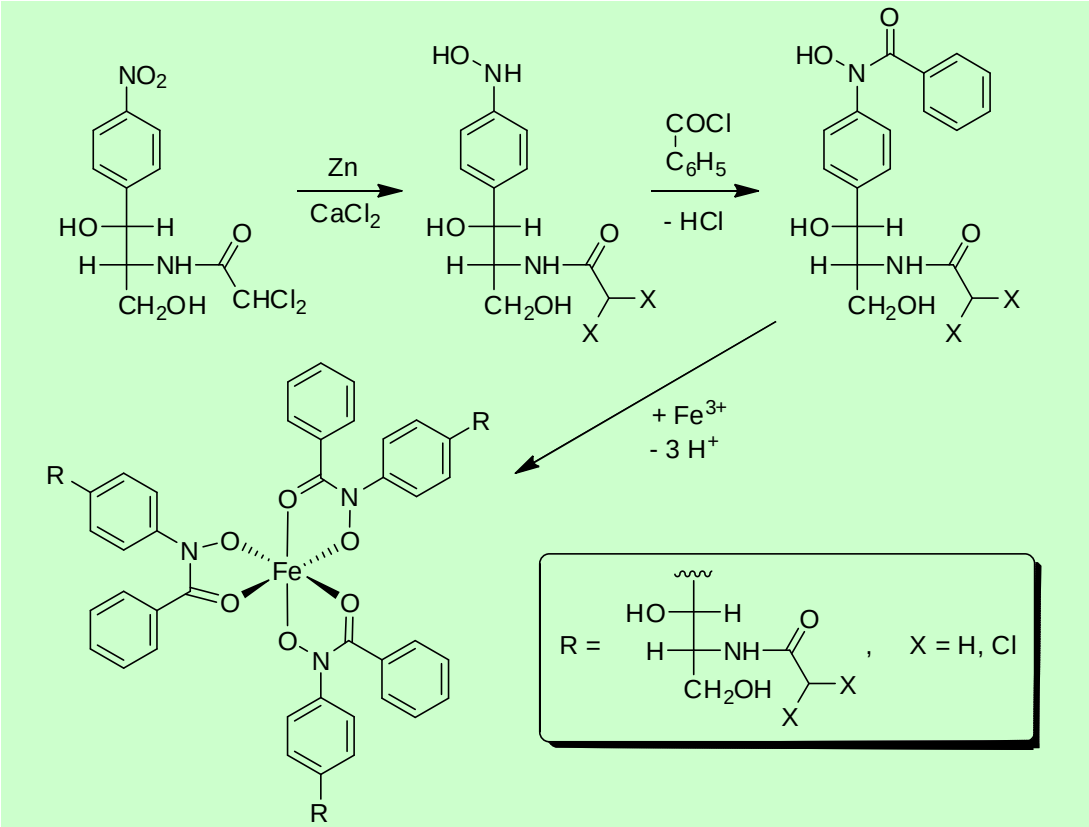
A. Melting point *(2.2.14)*: 149 °C to 153 °C.

B. Infrared absorption spectrophotometry.

C. Thin-layer chromatography.

D. Dissolve about 10 mg in 1 ml of *alcohol (50 per cent V/V) R*, add 3 ml of a 10 *g/l* solution of *calcium chloride R* and 50 mg of *zinc powder R* and heat on a water-bath for 10 min. Filter the hot solution and allow to cool. Add 0.1 ml of *benzoyl chloride R* and shake for 1 min. Add 0.5 ml of *ferric chloride solution R1* and 2 ml of *chloroform R* and shake. The aqueous layer is coloured light violet-red to purple.

In neutral solution, the nitro group of chloramphenicol is reduced to hydroxylamine by zinc powder (under the reaction conditions, the covalent chlorine is ionized to chloride via reductive dehalogenation). *N*-Arylhydroxamic acid, formed by the *N*-benzoylation of hydroxylamine, forms a violet complex with Fe^{3+}.



E. To 50 mg in a porcelain crucible add 0.5 g of *anhydrous sodium carbonate R*. Heat over an open flame for 10 min. Allow to cool. Take up the residue with 5 ml of *dilute nitric acid R* and filter. To 1 ml of the filtrate add 1 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1).*

When chloramphenicol is heated in the presence of sodium carbonate, the covalent chlorine is ionized to chloride.

Tests

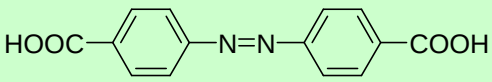
Acidity or alkalinity. To 0.1 g add 20 ml of *carbon dioxide-free water R*, shake and add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.1 ml of *0.02 M hydrochloric acid* or *0.02 M sodium hydroxide* is required to change the colour of the indicator.

The shaken mixture of chloramphenicol and water is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Informative test

1. To 0.10 g, add 5.0 ml of *dilute sodium hydroxide solution R* and heat the mixture. It acquires a yellow colour. When the solution is boiled, the colour changes to orange-red.

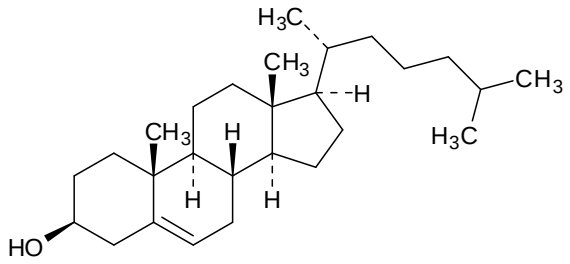
In consequence of hydrolysis, chloramphenicol decomposes among others to yellow azobenzene-4,4'-dicarboxylic acid and azophenol.



azobenzene-4,4'-dicarboxylic acid

CHOLESTEROL

Cholesterolum



$C_{27}H_{46}O$ **M_r 386.7**

Definition

Cholest-5-en-3β-ol.

Content:

- *cholesterol*: minimum 95.0 per cent (dried substance);
- *total sterols*: 97.0 per cent to 103.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in acetone and in ethanol (96 per cent).

It is sensitive to light.

It is often used as an auxiliary component for the preparation of w/o ointments.

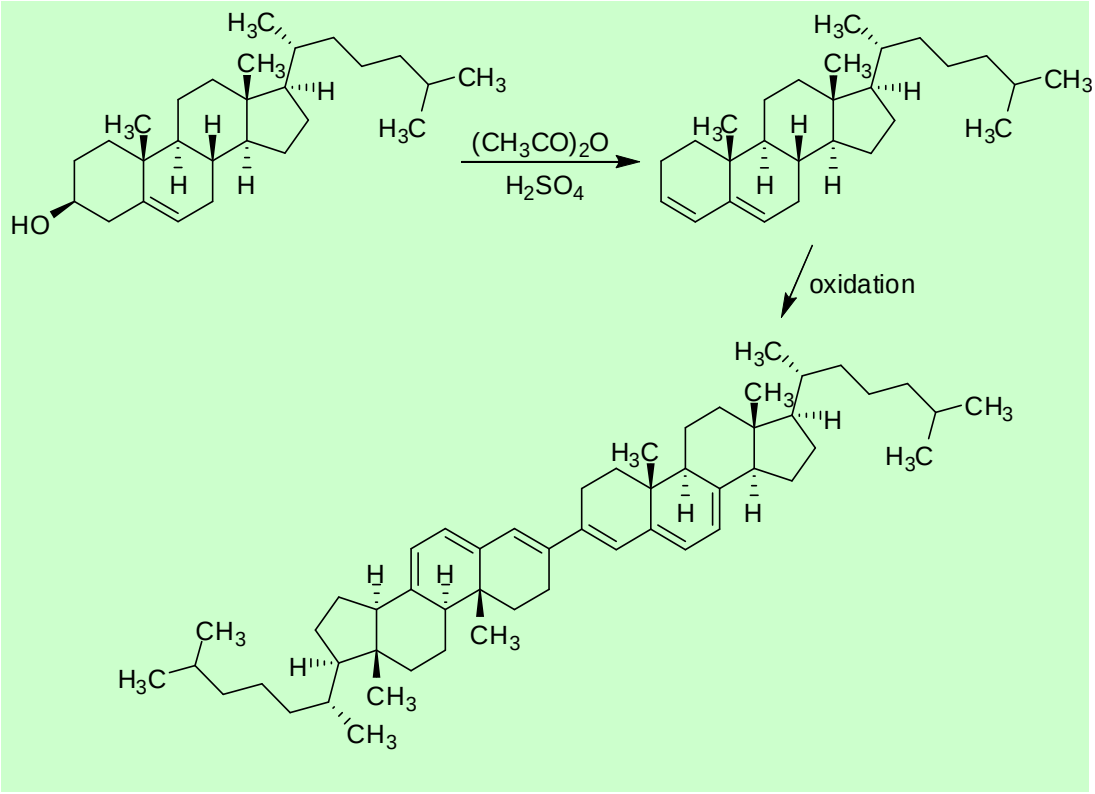
Identification

A. Melting point *(2.2.14)*: 147 °C to 150 °C.

B. Thin-layer chromatography.

C. Dissolve about 5 mg in 2 ml of *methylene chloride R*. Add 1 ml of *acetic anhydride R*, 0.01 ml of *sulfuric acid R* and shake. A pink colour is produced which rapidly changes to red, then to blue and finally to brilliant green.

Acetic anhydride forms an *O*-acetyl derivative with cholesterol. Elimination of acetic acid results in cholesta-3,5-diene, which is oxidized to a green dimer of cholesta-3,5,7-triene (**LIEBERMANN-BURCHARD** reaction).



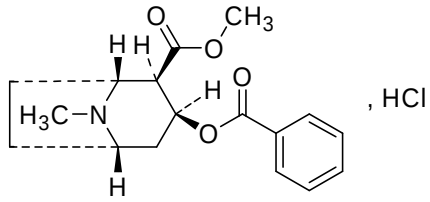
Informative test

1. Dissolve about 10 mg in 1.0 ml of *chloroform R*, add 1.0 ml of *sulfuric acid R* to the solution and shake it. The separated organic phase is red, while the sulfuric acid layer exhibits a brownish-green fluorescence.

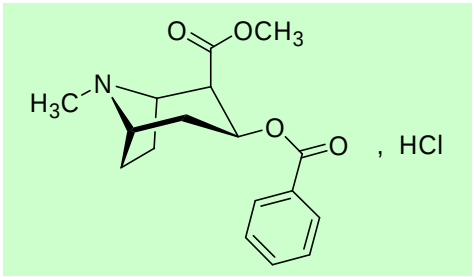
Dehydration of cholesterol results in fluorescent olefin derivatives.

COCAINE HYDROCHLORIDE

Cocaini hydrochloridum



, HCl



, HCl

$C_{17}H_{22}ClNO_4$ **M_r 339.8**

Definition

Methyl (1*R*,2*R*,3*S*,5*S*)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very soluble in water, freely soluble in alcohol, slightly soluble in methylene chloride.

mp: about 197 °C, with decomposition.

Cocaine is applied as a local anaesthetic agent in therapy. It is also used as an illegal drug (psychostimulant).

Identification

A. Ultraviolet and visible absorption spectrophotometry.

B. Infrared absorption spectrophotometry.

C. Dissolve 0.1 g in 5 ml of *water R* and add 1 ml of *dilute ammonia R2*. A white precipitate is formed. Initiate crystallisation by scratching the wall of the tube with a glass rod. The crystals, washed with *water R* and dried in vacuo, melt *(2.2.14)* at 96 °C to 99 °C.

Free, crystalline cocaine base is liberated.

D. It gives reaction (a) of chlorides *(2.3.1)*.

E. It gives the reaction of alkaloids *(2.3.1).*

Tests

Solution S. Dissolve 0.5 g in *water R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Cocaini hydrochloridum* monograph.

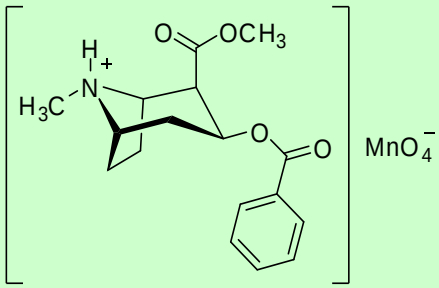
2. See identification **E** in *Cocaini hydrochloridum* monograph.

3. Dissolve 20 mg of the compound in the 5.0 ml of *distilled water*. To 2.0 ml of this solution, add 2 drops of *diluted nitric acid R* and a few drops of *silver nitrate solution R1*. A white curdled precipitate is formed.

A silver chloride precipitate is formed.

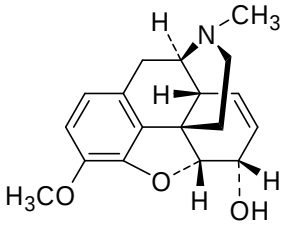
4. Dissolve 10 mg anyagot 2 dropps of *distilled water*. To this solution add 1.0 ml of a 5 g/l solution of *potassium permanganate R.* Violet, lamelled crystalline precipitate is formed (difference from procaine).

A violet cocaine permanganate precipitate is formed.

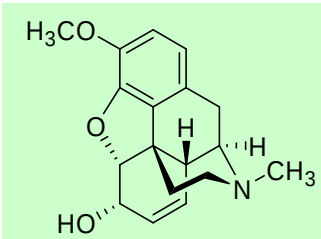


CODEINE HYDROCHLORIDE DIHYDRATE

Codeini hydrochloridum dihydricum



, HCl, 2 H_2O



, HCl, 2 H_2O

$C_{18}H_{22}ClNO_3$. 2 H_2O ***M*r 371.9**

Definition

7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol hydrochloride dihydrate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder or small, colourless crystals.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in cyclohexane.

It is an analgetic and contratussive compound.

Identification

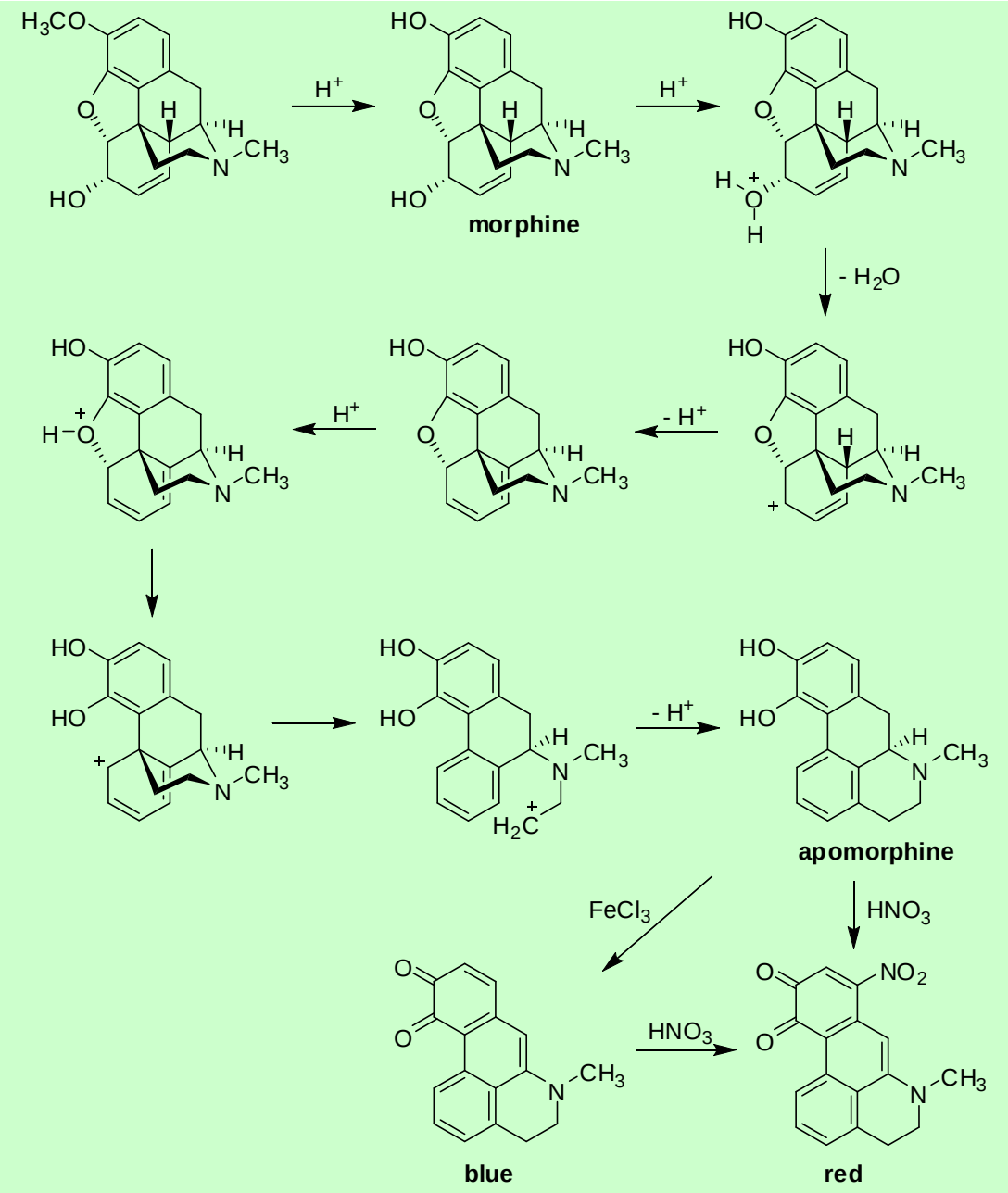
A. Infrared absorption spectrophotometry.

B. To 5 ml of solution S (see Tests) add 1 ml of a mixture of equal volumes of *strong sodium hydroxide solution R* and *water R* and initiate crystallisation, if necessary, by scratching the wall of the tube with a glass rod and cooling in iced water. Wash the precipitate with *water R* and dried at 100-105 °C. It melts *(2.2.15)* at 155 °C to 159 °C.

Bases liberate crystalline codeine base.

C. To about 10 mg add 1 ml of *sulfuric acid R* and 0.05 ml of *ferric chloride solution R2* and heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour changes to red.

Through acidic phenol ether cleavage, codeine is hydrolysed to morphine, which rearranges to apomorphine. Apomorphine is oxidized by Fe^{3+} to a blue *ortho*-quinone derivative, which turns to a red nitro derivative in the presence of nitric acid (**CALMBERG-HUSEMANN** reaction).



D. Solution S gives reaction (a) of chlorides *(2.3.1).*

E. It gives the reaction of alkaloids *(2.3.1)*.

Tests

Solution S. Dissolve 2.00 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Codeine hydrochloride dihydrate* monograph.
2. See identification **C** in the *Codeine hydrochloride dihydrate* monograph.
3. Add a few drops of *dilute nitric acid R* and a few drops of *silver nitrate solution* to 1.0 ml of solution S. A white precipitate is formed.

A silver chloride precipitate is formed.

4. Add 5 drops of *dilute sodium hydroxide solution R* to 5.0 ml of solution S. On shaking, separation of a crystalline precipitate can not be observed (a distinction from ethylmorphine).

Codeine base dissolves better than ethylmorphine base in water. Under the prescribed conditions, therefore, it does not precipitate from solution.

COPPER SULFATE PENTAHYDRATE

Cupri sulfas pentahydricus

$CuSO_4,5H_2O$ **M_r 249.7**

Definition

Content: 99.0 per cent to 101.0 per cent.

Characters

Appearance: blue, crystalline powder or transparent, blue crystals.

Solubility: freely soluble in water, soluble in methanol, practically insoluble in ethanol (96 per cent).

Copper sulfate can be used as an emetic. Because of its fungicidal effect, Cu^{2+} is used as a plant-protecting agents in agriculture.

Identification

A. Add several drops of *dilute ammonia R2* to 1 ml of solution S (see Tests). A blue precipitate is formed. On further addition of *dilute ammonia R2*, the precipate dissolves and a dark blue colour is produced.

Ammonia precipitates pale-blue copper(II) hydroxide. This dissolves in an excess of the reagent to give the tetraamminecopper(II) complex, which has an intense blue colour.

$$Cu^{2+} + 2\ OH^- \rightarrow Cu(OH)_2$$

$$Cu(OH)_2 + 4\ NH_3 \rightarrow [Cu(NH_3)_4]^{2+} + 2\ OH^-$$

B. Loss on drying (see Tests).

C. Dilute 1 ml of solution S to 5 ml with *water R*. The solution gives reaction (a) of sulfates (*2.3.1*).

Tests

Solution S. Dissolve 5 g in *water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*).

Chlorides (*2.4.4*): maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Loss on drying (*2.2.32*): 35.0 per cent to 36.5 per cent, determined on 0.500 g by drying in an oven at 250 ± 10 °C.

Assay

Dissolve 0.200 g in 50 ml of *water R*. Add 2 ml of *sulfuric acid R* and 3 g of *potassium iodide R*. Titrate with *0.1 M sodium thiosulfate*, adding 1 ml of *starch solution R* towards the end of the titration.

1 ml *0.1 M sodium thiosulfate* is equivalent to 24.97 mg of $CuSO_4,5H_2O$.

Copper(II) oxidizes iodide to iodine, which is titrated with thiosulfate. The addition of starch to the assay is recommended just before the end-point of the titration, because the blue starch–iodine complex is less soluble in water, and therefore reacts with thiosulfate slowly.

$$2\ Cu^{2+} + 4\ I^- \rightarrow 2\ CuI + I_2$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$CuSO_4,5H_2O\ \text{content}\ (\%) = \frac{V_{Na_2S_2O_3}\ (\text{ml}) \cdot f_{Na_2S_2O_3} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative test

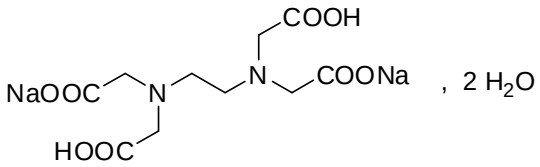
1. See the **Appearance of solution** test in the *Copper sulfate pentahydrate* monograph.

2. See identification **A** in the *Copper sulfate pentahydrate* monograph.

3. See identification **C** in the *Copper sulfate pentahydrate* monograph.

DISODIUM EDETATE

Dinatrii edetas



$C_{10}H_{14}N_2Na_2O_8$, 2 H_2O **M_r 372.2**

Definition

Disodium dihydrogen (ethylenedinitrilo)tetraacetate dihydrate.

Content: 98.5 per cent to 101.0 per cent.

Characters

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water, practically insoluble in ethanol (96 per cent) *R*.

The calcium complex of edetate is used in heavy metal poisoning.

Identification

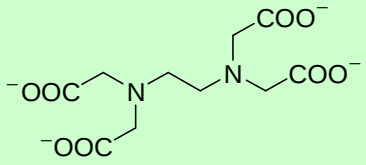
A. Infrared absorption spectrophotometry.

B. Dissolve 2 g in 25 ml of *water R*, add 6 ml of *lead nitrate solution R*, shake and add 3 ml of *potassium iodide solution R*. No yellow precipitate is formed. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 ml of *ammonium oxalate solution R*. No precipitate is formed.

Disodium edetate (Na_2H_2Y) forms a stable complex with Pb^{2+}, so that the formation of lead iodide or lead oxalate is inhibited.

$$Pb^{2+} + H_2Y^{2-} \rightarrow PbY^{2-} + 2\ H^+$$

Y^{4-}:



$$Pb^{2+} + 2\ I^- \rightarrow PbI_2$$

$$Pb^{2+} + (COO)_2^{2-} \rightarrow Pb(COO)_2$$

C. Dissolve 0.5 g in 10 ml of *water R* and add 0.5 ml of calcium *chloride solution R*. Make alkaline to *red litmus paper R* by the addition of *dilute ammonia R2* and add 3 ml of *ammoniurn oxalate solution R*. No precipitate is formed.

Similarly to test **B**, the formation of a stable calcium complex prevents the precipitation of white calcium oxalate.

$$Ca^{2+} + (COO)_2^{2-} \rightarrow Ca(COO)_2$$

D. It gives the reactions of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*: 4.0 to 5.5 for solution S.

Iron *(2.4.9)*: maximum 80 ppm.

Dilute 2.5 ml of solution S to 10 ml with *water R*. Add 0.25 g of *calcium chloride R* to the test solution and the standard before the addition of the *thioglycollic acid R*.

Assay

Dissolve 0.300 g in *water R* and dilute to 300 ml with the same solvent. Add 2 g of *hexamethylenetetramine R* and 2 ml of dilute *hydrochloric acid R*. Titrate with *0.1 M lead nitrate*, using about 50 mg of *xylenol orange triturate R* as indicator.

1 ml of 0.1 M *lead nitrate* is equivalent to 37.22 mg of $C_{10}H_{14}N_2Na_2O_8,2H_2O$.

The Pb-EDTA complex is formed. Methenamine (hexamethylenetetramine) binds protons liberated during complex formation.

$$C_{10}H_{14}N_2Na_2O_8.2H_2O \text{ cont. } (\%) = \frac{V_{Pb(NO_3)_2}(ml) \cdot f_{Pb(NO_3)_2} \cdot E\ (mg/ml)}{\text{amount of substance } (mg)} \cdot 100$$

Informative tests

1. See the **Appearance of solution** test in the *Disodium edetate* monograph.

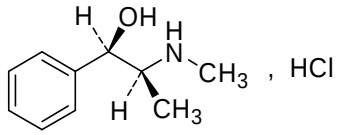
2. See identification **C** in the *Disodium edetate* monograph.

3. The sample burns with a sooty flame; the residue imparts a persistent, vivid-yellow colour to the flame.

The colour of the flame given by the residue of the incinerated sample is characteristic of sodium.

EPHEDRINE HYDROCHLORIDE

Ephedrini hydrochloridum



$C_{10}H_{16}ClNO$ **M_r 201.7**

Definition

(1*R*,2*S*)-2-(Methylamino)-1-phenylpropan-1-ol hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, soluble in ethanol (96 per cent).

mp: about 219 °C.

It is a sympathomimetic and psychomotor stimulant agent. It is used to relieve nasal and sinus congestion and to dilate the bronchi.

Identification

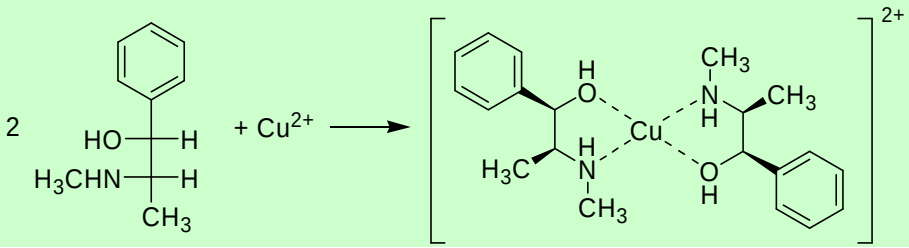
A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry.

C. Thin-layer chromatography.

D. To 0.1 ml of solution S (see Tests) add 1 ml of *water R*, 0.2 ml of *copper sulfate solution R* and 1 ml of *strong sodium hydroxide solution R*. A violet colour is produced. Add 2 ml of *methylene chloride R* and shake. The lower (organic) layer is dark grey and the upper (aqueous) layer is blue.

In the **CHEN-KAO** reaction, a two-ligand chelate complex is formed which is soluble in organic solvents (*e.g.* ether, methylene chloride or *n*-butanol).



E. To 5 ml of solution S add 5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

Tests

Solution S. Dissolve 5.00 g in *distilled water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*. −33.5 to −35.5 (dried substance).

Dilute 12.5 ml of solution S to 25.0 ml with *water R*.

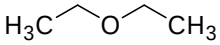
Informative tests

1. See the **Appearance of solution** test in the *Ephedrine hydrochloride* monograph.
2. See identification **D** in the *Ephedrine hydrochloride* monograph.
3. Add a few drops of *dilute nitric acid R* and 1.0 ml of *silver nitrate solution R1* to 1.0 ml of solution S. A white precipitate is formed which is soluble in an excess of *dilute ammonia R2*.

   A silver chloride precipitate is formed, which dissolves in an excess of ammonia solution (the diamminesilver complex is formed).

ETHER

Aether



$C_4H_{10}O$ **M_r 74.1**

Definition

Diethyl ether.

It may contain a suitable non-volatile antioxidant at a suitable concentration.

Characters

Appearance: clear, colourless liquid, volatile.

Solubility: soluble in water, miscible with ethanol (96 per cent), with methylene chloride and with fatty oils.

It is highly flammable.

It is a general anaesthetic. Nowadays it is less frequently applied because of its flammability and unwanted side-effects. In pharmaceutical analysis, ether is used as an organic solvent.

Tests

Acidity. To 20 ml of *ethanol (96 per cent) R* add 0.25 ml of *bromothymol blue solution R1* and, dropwise, *0.02 M sodium hydroxide* until a blue colour persists for 30 s. Add 25 ml of the substance to be examined, shake and add, dropwise, *0.02 M sodium hydroxide* until the blue colour reappears and persists for 30 s. Not more than 0.4 ml of *0.02 M sodium hydroxide* is required.

The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). When the mildly alkaline solution is shaken with ether, the pH should not decrease notably.

Substances with a foreign odour. Moisten a disc of filter paper 80 mm in diameter with 5 ml of the substance to be examined and allow to evaporate. No foreign odour is perceptible immediately after the evaporation.

Aldehydes. To 10.0 ml in a ground-glass-stoppered cylinder add 1 ml of *alkaline potassium tetraiodomercurate solution R* and shake for 10 s. Allow to stand for 5 min, protected from light. The lower layer may show yellow or reddish-brown opalescence but not grey or black opalescence.

Acetaldehyde (formed by the oxidation of ether, see the paragraph “Peroxides”) precipitates mercury metal from a mercury(II) complex.

$$[HgI_4]^{2-} + CH_3CHO + H_2O \rightarrow Hg + CH_3COOH + 4\ I^- + 2\ H^+$$

Peroxides. Place 8 ml of *potassium iodide and starch solution R* in a 12 ml ground-glass-stoppered cylinder about 15 mm in diameter. Fill completely with the substance to be examined, mix and allow to stand protected from light for 5 min. No colour develops.

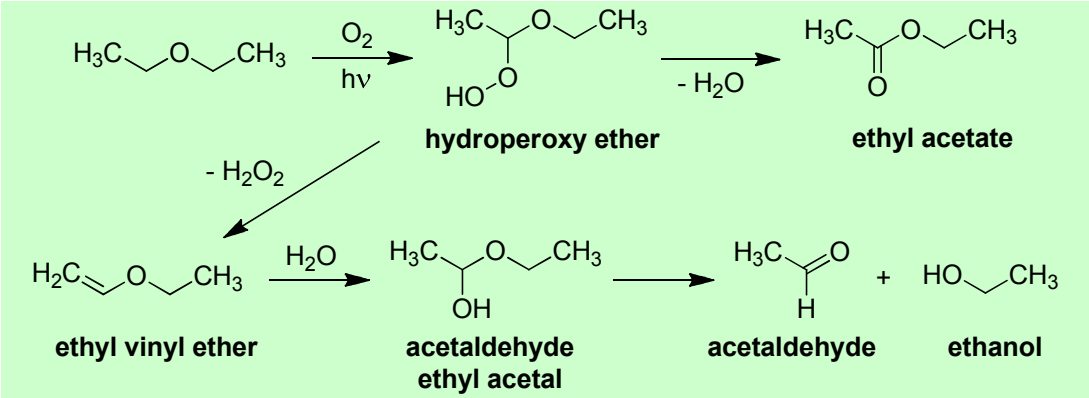
Peroxide impurities oxidize iodide to iodine, which can be detected as the blue iodine–starch complex.

$$R^1{-}O{-}O{-}R^2 + 2\ I^- + 2\ H^+ \rightarrow I_2 + R^1{-}OH + R^2{-}OH$$

$$R{-}O{-}O{-}H + 2\ I^- + 2\ H^+ \rightarrow I_2 + R{-}OH + H_2O$$

Distillation of ether is permitted only if the presence of peroxides is excluded !

The air oxidizes ether to explosive peroxides. The reaction is catalysed by sunlight, Fe^{3+} or organic impurities.

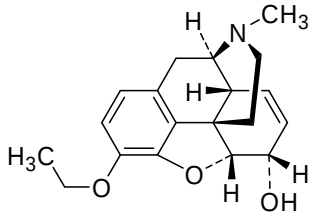


Informative test

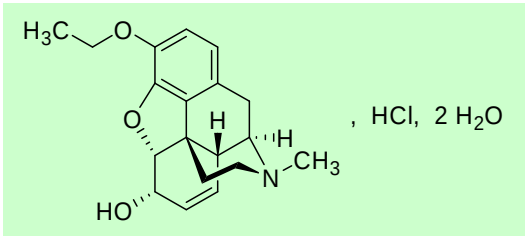
1. See the "**Substances with a foreign odour**" test in the *Ether* monograph.

ETHYLMORPHINE HYDROCHLORIDE

Ethylmorphini hydrochloridum



, HCl, 2 H_2O



, HCl, 2 H_2O

$C_{19}H_{24}ClNO_3$. 2 H_2O ***M*r 385.9**

Definition

7,8-Didehydro-4,5α-epoxy-3-ethoxy-17-methylmorphinan-6α-ol hydrochloride dihydrate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: soluble in water and in alcohol, insoluble in cyclohexane.

It is an analgetic and contratussive compound.

Identification

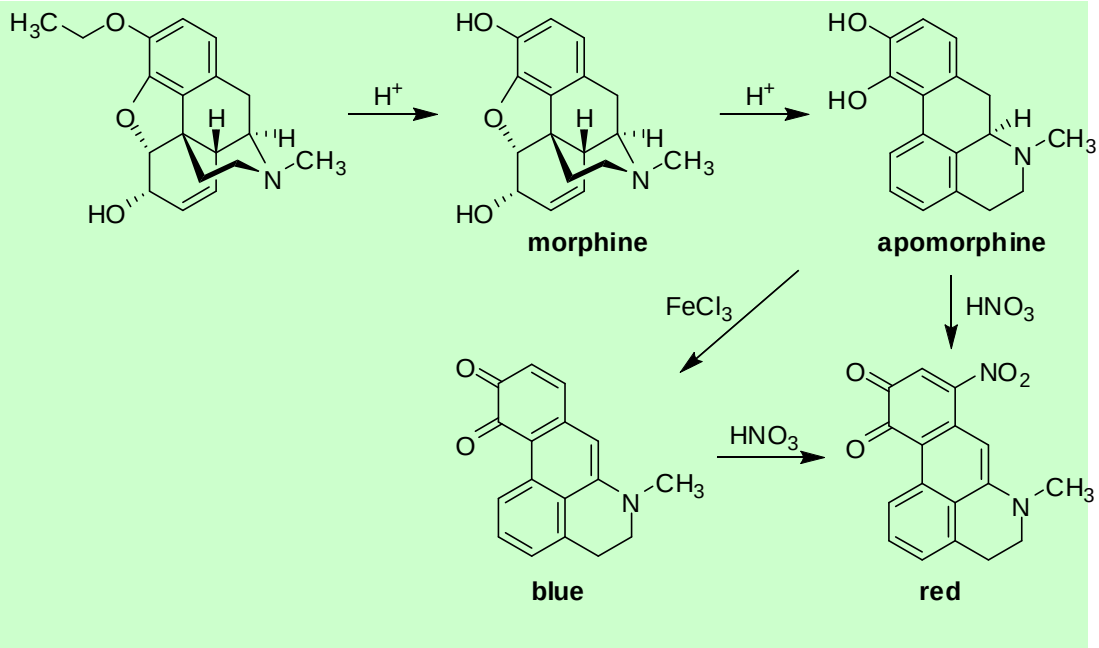
A. Infrared absorption spectrophotometry.

B. In a test-tube, dissolve 0.5 g in 6 ml of *water R* and add 15 ml of *0.1 M sodium hydroxide*. Scratch the wall of the tube with a glass rod. A white, crystalline precipitate is formed. Collect the precipitate, wash and dissolve in 20 ml of *water R* heated to 80 °C. Filter and cool in iced water. The crystals, after drying in vacuo for 12 h, melt *(2.2.14)* at 85 °C to 89 °C.

Bases liberate crystalline ethylmorphine base.

C. To about 10 mg add 1 ml of *sulfuric acid R* and 0.05 ml of *ferric chloride solution R2*. Heat on a water-bath. A blue colour develops. Add 0.05 ml of *nitric acid R*. The colour becomes red.

On acidic phenol ether cleavage, codeine is hydrolysed to morphine, which rearranges to apomorphine. Apomorphine is oxidized by Fe^{3+} to a blue *ortho*-quinone derivative, which turns to a red nitro derivative in the presence of nitric acid (**CALMBERG-HUSEMANN** reaction, see the *Codeine hydrochloride* monograph, Identification **C**).



D. Solution S (see Tests) gives reaction (a) of chlorides *(2.3.1)*.

Tests

Solution S. Dissolve 0.500 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Ethylmorphine hydrochloride* monograph.
2. See identification **C** in the *Ethylmorphine hydrochloride* monograph.
3. Add a few drops of *dilute nitric acid R* and a few drops of *silver nitrate solution* to 1.0 ml of solution S. A white precipitate is formed.

   A silver chloride precipitate is formed.
4. Add 5 drops of *dilute sodium hydroxide solution R* to 5.0 ml of solution S. On shaking, a crystalline precipitate slowly separates (a distinction from codeine).

   Ethylmorphine base is not so soluble as codeine base in water, and under the prescribed conditions, therefore a crystalline precipitate is formed (see the *Codeine hydrochloride* monograph, Informative test **4**).

FERRIC CHLORIDE HEXAHYDRATE

Ferri chloridum hexahydricum

$FeCl_3,6H_2O$ **M_r 270.3**

Definition

Content: 98.0 per cent to 102.0 per cent of.

Characters

Appearance: crystalline mass or orange-yellow or brownish-yellow crystals, very hygroscopic.

Solubility: very soluble in water and in ethanol (96 per cent), freely soluble in glycerol.

Ferric chloride has an astringent effect. It is also used as an analytical reagent.

Identification

A. It gives reaction (a) of chlorides (*2.3.1*).

B. It gives reaction (c) of iron (*2.3.1*).

Tests

Solution S. Dissolve 10 g in distilled *water R* and dilute to 100 ml with the same solvent.

Free chlorine. Heat 5 ml of solution S. The vapour does not turn *starch iodide paper R* blue.

Free chlorine oxidizes iodide to iodine, which can be detected as blue starch–iodine.

$$Cl_2 + 2\ I^- \rightarrow I_2 + 2\ Cl^-$$

Ferrous ions. maximum 50 ppm.

To 10 ml of solution S, add 1 ml of *water R*, 0.05 ml of *potassium ferricyanide solution R* followed by 4 ml of *phosphoric acid R*. After 10 min, any blue colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *water R* and 1 ml of a freshly prepared 0.250 g/l solution of *ferrous sulfate R*.

Fe^{2+} reacts with potassium ferricyanide to form a dark-blue precipitate (**TURNBULL** blue reaction).

$$3\ Fe^{2+} + 2\ [Fe(CN)_6]^{3-} \rightarrow Fe_3[Fe(CN)_6]_2$$

Fe^{3+} is bound in a colourless complex with phosphoric acid.

Assay

In a conical flask with a ground-glass stopper, dissolve 0.200 g in 20 ml of *water R*. Add 10 ml of *dilute hydrochloric acid R* and 2 g of *potassium iodide R*. Allow the stoppered flask to stand for 1 h protected from light. Titrate with *0.1 M sodium thiosulfate*, using 5 ml of *starch solution R* as indicator, added towards the end of the titration.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 27.03 mg of $FeCl_3,6H_2O$.

Fe^{3+} oxidizes iodide to iodine, which can be titrated with thiosulfate.

$$2\ Fe^{3+} + 2\ I^- \rightarrow 2\ Fe^{2+} + I_2$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$FeCl_3,6H_2O\ \text{content}\ (\%) = \frac{V_{Na_2S_2O_3}\,(\text{ml}) \cdot f_{Na_2S_2O_3} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative test

1. Dissolve 50 mg of compound in 5.0 ml of *distilled water*. To this solution, add 1.0 ml of *diluted nitric acid R* and 2.0 ml of *silver nitrate solution R1*. A white curdled precipitate is formed.

A silver chloride precipitate is formed.

2. See identification **B** in the *Ferric chloride hexahydrate* monograph.

FERROUS SULFATE HEPTAHYDRATE

Ferrosi sulfas heptahydricus

$FeSO_4,7H_2O$ **M_r 278.0**

Definition

Content: 98.0 per cent to 105.0 per cent.

Characters

Appearance: light green, crystalline powder or bluish-green crystals, efflorescent in air.

Solubility: freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96%) *R*.

Ferrous sulfate heptahydrate is oxidised in moist air, becoming brown.

It has haemopoietic action.

Identification

A. It gives the reaction of sulfates (*2.3.1*).

B. It gives reaction (a) of iron (*2.3.1*).

C. It complies with the limits of the assay.

Tests

Solution S. Dissolve 4.0 g in a 5 per cent *V/V* solution of lead-free nitric acid R and dilute to 100.0 ml with the same solution.

pH (*2.2.3*): 3.0 to 4.0.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 5 ml of solution S to 10 ml with *water R* and add 5 ml of *dilute nitric acid R*. Prepare the standard with a mixture of 2 ml of water R, 5 ml of dilute nitric acid R and 8 ml of *chloride standard solution* (*5 ppm Cl*) *R.* Use 0.15 ml of *silver nitrate solution R2* in this test.

Informative test

1. See identification **A** in the *Ferrous sulfate* monograph.

2. See identification **B** in the *Ferrous sulfate* monograph.

Formaldehyde solution (35 per cent)

Formaldehydi solutio (35 per centum)

Definition

Content: 34.5 per cent *m/m* to 38.0 per cent *m/m* of formaldehyde (CH_2O; M_r 30.03).

It contains methanol as stabiliser.

Characters

Appearance: clear, colourless liquid.

Solubility: miscible with water and with ethanol (96 per cent).

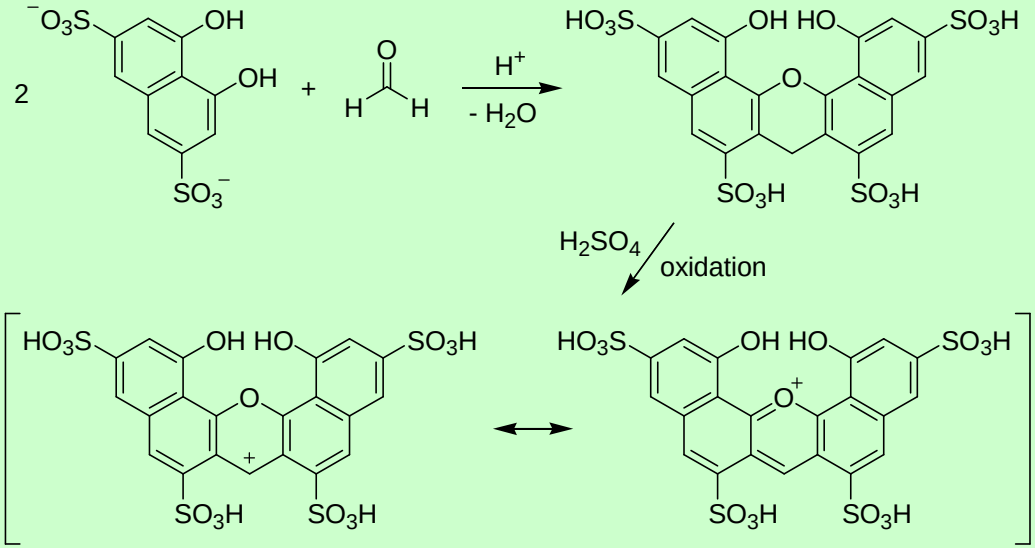
It may be cloudy after storage.

It is a disinfectant.

Identification

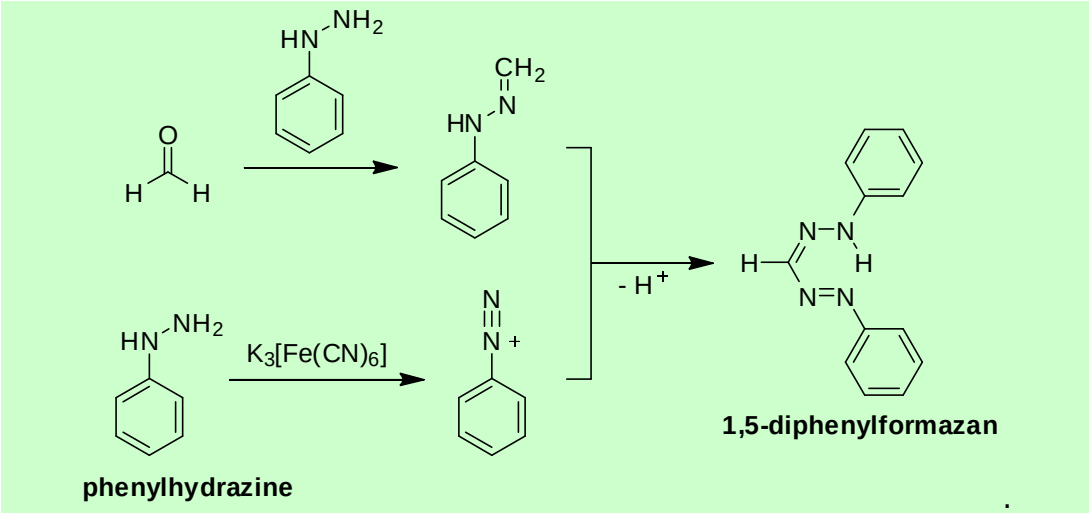
A. Dilute 1 ml of solution S (see Tests) to 10 ml with *water R*. To 0.05 ml of the solution add 1 ml of a 15 *g/l* solution of *chromotropic acid, sodium salt R*, 2 ml of *water R* and 8 ml of *sulfuric acid R*. A violet-blue or violet-red colour develops within 5 min.

Formaldehyde forms a violet dibenzoxanthene derivative with chromotropic acid. Sulfuric acid oxidizes the xanthene derivative to the violet-red, mesomerism stabilized dibenzoxanthenium cation.



B. To 0.1 ml of solution S add 10 ml of *water R*. Add 2 ml of a 10 *g/l* solution of *phenylhydrazine hydrochloride R*, prepared immediately before use, 1 ml of *potassium ferricyanide solution R* and 5 ml of *hydrochloric acid R*. An intense red colour is formed.

The hydrazone formed by the reaction of formaldehyde and phenylhydrazine reacts with the phenyldiazonium salt formed by the oxidation of phenylhydrazine with potassium ferricyanide to result in red 1,5-diphenylformazan.



C. Mix 0.5 ml with 2 ml of *water R* and 2 ml of *silver nitrate solution R2* in a test-tube. Add *dilute ammonia R2* until slightly alkaline. Heat on a water-bath. A grey precipitate or a silver mirror is formed.

Formaldehyde reduces diamminesilver to metallic silver.

$$2\ Ag^+ + 2\ OH^- \rightarrow Ag_2O + H_2O$$

$$Ag_2O + 2\ NH_4^+ + 2\ NH_3 \rightarrow 2\ [Ag(NH_3)_2]^+ + H_2O$$

$$2\ [Ag(NH_3)_2]^+ + CH_2O + 2OH^- \rightarrow 2\ Ag + HCOO^- + 3\ NH_3 + NH_4^+ + H_2O$$

D. It complies with the limits of the assay.

Tests

Solution S. Dilute 10 ml, filtered if necessary, to 50 ml with *carbon dioxide-free water R*.

Appearance of solution. Solution S is colourless *(2.2.2, Method II)*.

Acidity. To 10 ml of solution S add 1 ml of *phenolphthalein solution R*. Not more than 0.4 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

The 35% aqueous solution of formaldehyde is neutral or weakly acidic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

Assay

Into a 100 ml volumetric flask containing 2.5 ml of *water R* and 1 ml of *dilute sodium hydroxide solution R*, introduce 1.000 g of the solution to be examined, shake and dilute to 100.0 ml with *water R*. To 10.0 ml of the solution add 30.0 ml of *0.05 M iodine.* Mix and add 10 ml of *dilute sodium hydroxide solution R*. After 15 min, add 25 ml of *dilute sulfuric acid R* and 2 ml of *starch solution R*. Titrate with *0.1 M sodium thiosulfate*.

1 ml of *0.05 M iodine* is equivalent to 1.501 mg of CH_2O.

The basis of the **ROMIJN** assay is that hypoiodite, formed under basic conditions, oxidizes formaldehyde to formate.

$$I_2 + 2\ OH^- \rightarrow I^- + IO^- + H_2O$$

$$CH_2O + IO^- + OH^- \rightarrow HCOO^- + I^- + H_2O$$

After acidification, the excess of hypoiodite and also the iodate (formed during standing) turns to iodine, which can be back-titrated with thiosulfate.

$$3\ IO^- \rightarrow IO_3^- + 2\ I^-$$

$$IO^- + I^- + 2\ H^+ \rightarrow I_2 + H_2O$$

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$CH_2O\ \text{cont.}\ (\%) = \frac{[V_{I_2}(\text{ml}) \cdot f_{I_2} - V_{Na_2S_2O_3}(\text{ml}) \cdot f_{Na_2S_2O_3}] \cdot E\ (\text{mg/ml}) \cdot V_{bulb}}{\text{amount of substance (mg)} \cdot V_P} \cdot 100$$

where V_{bulb} is the volume of the stock solution and $V_{pipetted}$ is the volume of stock solution pipetted out for the assay.

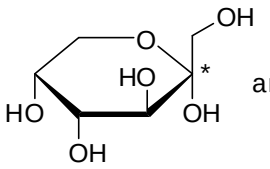
Informative tests

1. See the **Appearance of solution** test in the *Formaldehyde solution (35 per cent)* monograph.

2. See identification **C** in the *Formaldehyde solution (35 per cent)* monograph.

FRUCTOSE

Fructosum



and C* epimer

$C_6H_{12}O_6$ **M_r 180.2**

Definition

(-)-D-*Arabino*-hex-2-ulopyranose.

The substance described in this monograph is not necessarily suitable for parenteral use.

Characters

Appearance: white or almost white, crystalline powder.

It has a very sweet taste.

Solubility: very soluble in water, soluble in ethanol (96 per cent).

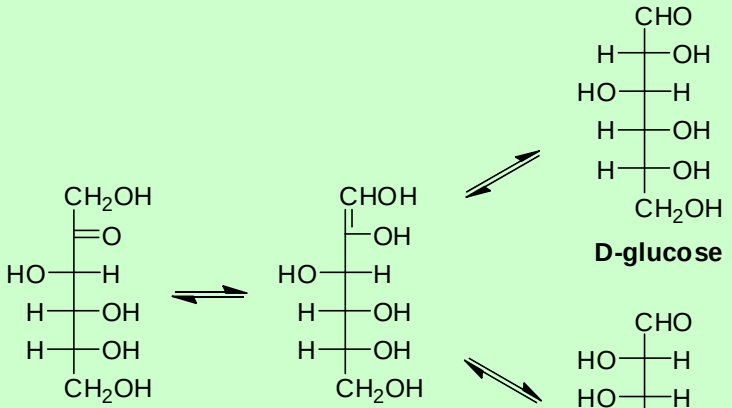
It is the sweetest sugar, and is used as a sweetener in diabetic foods.

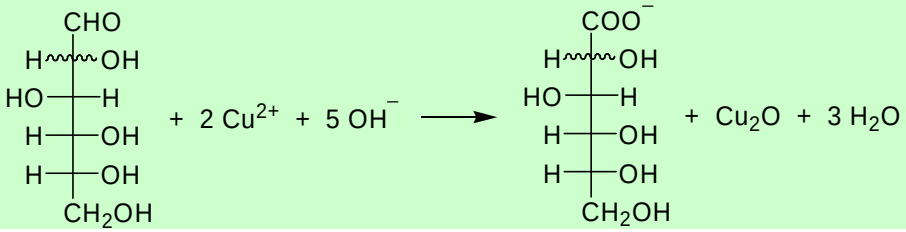
Identification

A. Thin-layer chromatography.

B. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

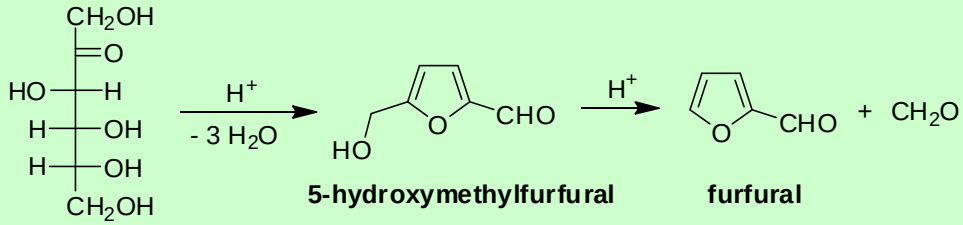
In an alkaline solution of copper(II) tartrate (**FEHLING** solution), fructose reduces Cu^{2+} to brick-red copper(I) oxide (**FEHLING** reaction). D-Fructose is converted to D-glucose and D-mannose via enol-oxo tautomerism; the latter form aldonic acids during the reaction.





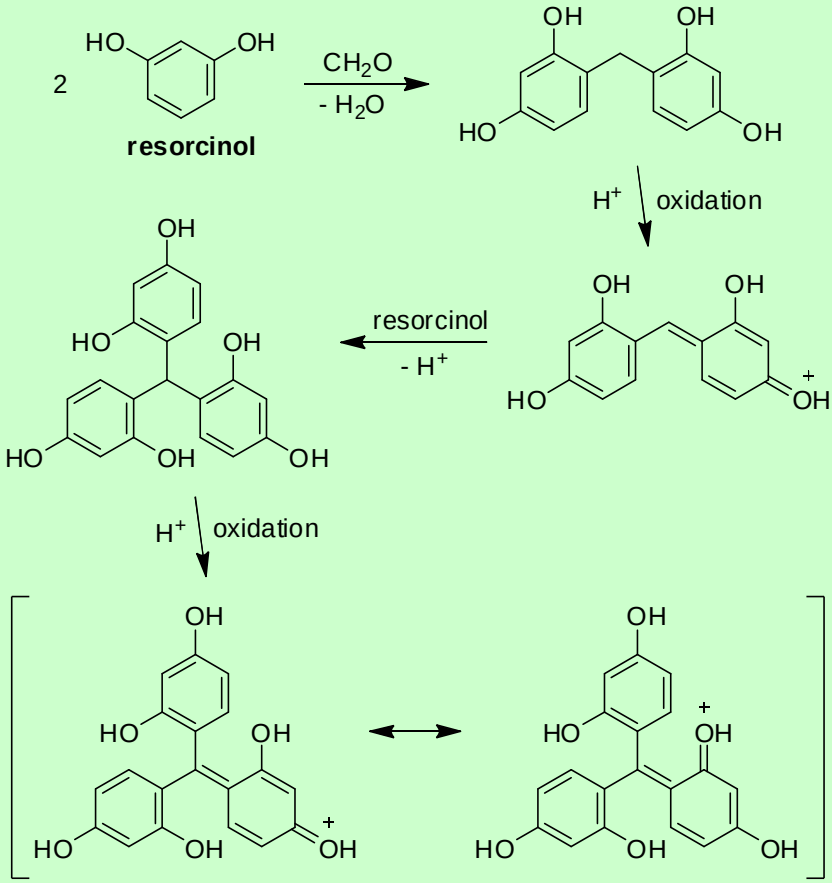
C. To 1 ml of solution S (see Tests) add 9 ml of *water R*. To 1 ml of the solution add 5 ml of *hydrochloric acid R* and heat to 70 °C. A brown colour develops.

When heated with acids, hexoses decompose to 5-hydroxymethylfurfural, and then to furfural and formaldehyde. The decomposition of ketoses is much faster than that of aldoses. The oxidation products of 5-hydroxymethylfurfural and furfural are brown.



D. Dissolve 5 g in *water R* and dilute to 10 ml with the same solvent. To 0.5 ml of the solution add 0.2 g of *resorcinol R* and 9 ml of *dilute hydrochloric acid R* and heat on a water-bath for 2 min. A red colour develops.

Formaldehyde resulting from the acidic decomposition of fructose forms a triarylmethane-type red dye with resorcinol (**SELIVANOV** reaction). The reaction is suitable for the distinction between ketoses and aldoses (the latter give the reaction much more slowly). Sucrose also gives a fast reaction.



Tests

Solution S. Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Dissolve 5.0 g in *water R* and dilute to 10 ml with the same solvent. The solution is clear *(2.2.1).* Add 10 ml of *water R*. The solution is colourless *(2.2.2, Method II)*.

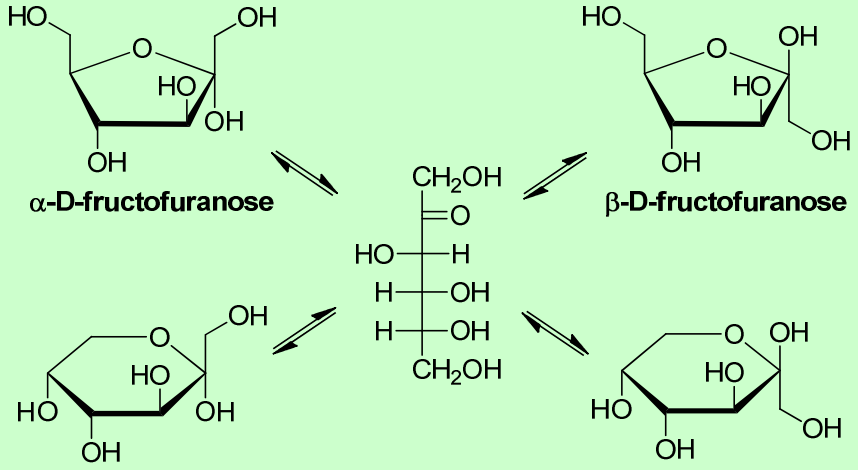
Acidity or alkalinity. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

The aqueous solution of fructose is neutral. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

Specific optical rotation *(2.2.7)*: -91.0 to -93.5 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Because of the ring-chain tautomerism of fructose, the specific optical rotation of fructose changes after the preparation of the solution until a constant value is achieved (**mutarotation**; for an explanation see the *Glucose, anhydrous* monograph "**Specific optical rotation**" test). The aqueous solution of fructose contains 6.5% α-D-fructofuranose, 25.2% β-D-fructofuranose, 2.6% α-D-fructopyranose and 64.8% β-D-fructopyranose besides 0.8% of the open chain form at 31 ºC.



Foreign sugars. Dissolve 5.0 g in *water R* and dilute to 10 ml with the same solvent. To 1 ml of the solution add 9 ml of *ethanol (96 per cent) R*. Any opalescence in the solution is not more intense than that in a mixture of 1 ml of the initial solution and 9 ml of *water R*.

Sugars (*e.g.* lactose and sucrose) are not soluble in 90% ethanol and cause opalescence.

Barium. To 10 ml of solution S add 1 ml of *dilute sulfuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

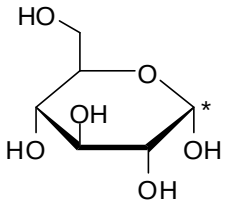
A barium sulfate precipitate is formed.

Informative tests

1. See the **Appearance of solution** test in the *Fructose* monograph.
2. See identification **B** in the *Fructose* monograph.
3. See identification **D** in the *Fructose* monograph.

GLUCOSE, ANHYDROUS

Glucosum anhydricum



and C* epimer

$C_6H_{12}O_6$ **M_r 180.2**

Definition

D-Glucopyranose.

Characters

Appearance: white or almost white, crystalline powder.

It has a sweet taste.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent).

It is used in parenteral nutrition for the treatment of hypoglycaemia.

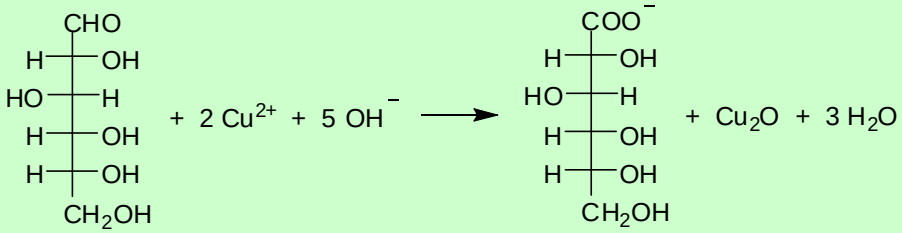
Identification

A. Specific optical rotation (see Tests).

B. Thin-layer chromatography.

C. Dissolve 0.1 g in 10 ml of *water R*. Add 3 ml of *cupri-tartaric solution R* and heat. A red precipitate is formed.

In an alkaline solution of copper(II) tartrate (**FEHLING** solution), fructose reduces Cu^{2+} to brick-red copper(I) oxide (**FEHLING** reaction). Glucose forms aldonic acids during the reaction. **FEHLING** solution is freshly prepared from copper(II) sulfate solution and alkaline potassium sodium tartrate solution. The reaction is suitable for the identification of sugars bearing a free hemiacetal hydroxy group (reducing sugars).



gluconate

Tests

Solution S. Dissolve 10.0 g in distilled *water R* and dilute to 100 *ml* with the same solvent.

Appearance of solution. The solution is clear *(2.2.1),* odourless, and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 10.0 g in 15 ml of *water R*.

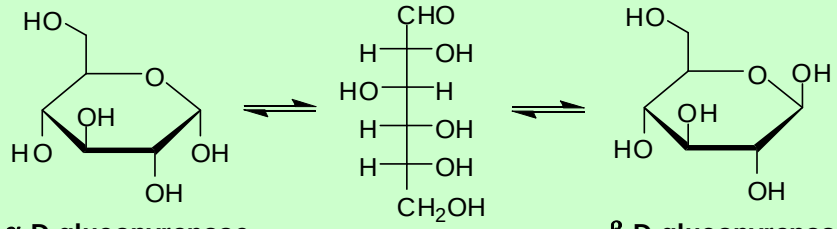
Acidity or alkalinity. Dissolve 6.0 g in 25 ml of *carbon dioxide-free water R* and add 0.3 *ml* of *phenolphthalein solution R.* The solution is colourless. Not more than 0.15 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

The aqueous solution of glucose is neutral. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

Specific optical rotation *(2.2.7)*. +52.5 to +53.3 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, add 0.2 ml of *dilute ammonia R1*, allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Because of the ring-chain tautomerism of glucose, the specific optical rotation of glucose changes after the preparation of the solution until a constant value (approx. +53) is achieved. Crystalline glucose exists as α-D-glucopyranose or β-D-glucopyranose (depending on the crystallization process), but in aqueous solution epimeric α and β ring forms can be interconverted through an opened hydroxyaldehyde form. The ratio of the epimers is a constant at a defined temperature and solvent (in aqueous solution, glucose contains approx. 36% α-D-glucopyranose and very little opened form). Since the specific optical rotations of the epimers are different, the sum of the rotation changes until tautomeric equilibrium is achieved. The duration of this period can be reduced by adding acidic or basic catalysts (*e.g*. ammonia).



α-D-glucopyranose **β-D-glucopyranose**

Foreign sugars, soluble starch, dextrins. Dissolve 1.0 g by boiling in 30 ml of *ethanol (90 per cent V/V) R.* Cool; the appearance of the solution shows no change.

Sugars (*e.g.* lactose and sucrose) are not soluble in 90% ethanol and cause opalescence.

Chlorides *(2.4.4): maximum 125 ppm.*

Dilute 4 ml of solution S to 15 ml with *water R*.

Sulfates *(2.4.13)*: maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Arsenic *(2.4.2)*: maximum 1 ppm, determined on 1.0 g.

Barium. To 10 ml of solution S add 1 ml of *dilute sulfuric acid R*. When examined immediately and after 1 h, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *distilled water R* and 10 ml of solution S.

A barium sulfate precipitate is formed.

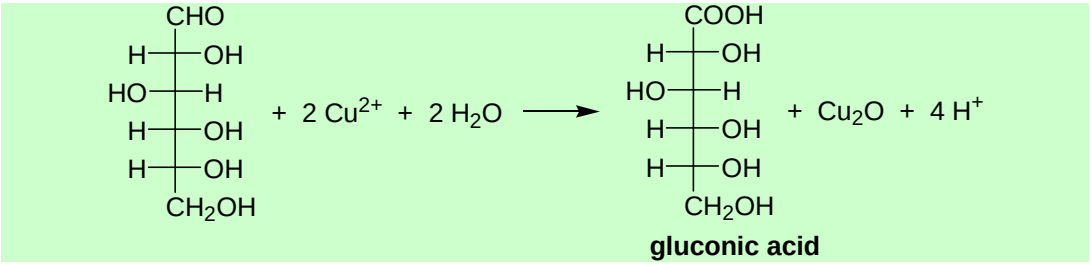
Calcium *(2.4.3)*: maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Informative tests

1. See the **Appearance of solution** test in the *Glucose anhydous* monograph.
2. Add 1 drop of solution to 1.0 ml of a *50 g/l solution of copper(II) acetate R* (preparation of reagent: dissolve 5.0 g of *copper(II) acetate R* in 5.0 ml of *dilute acetic acid R* and make the volume up to 100.0 ml with *water R*) and heat the solution gentle. A fine, red precipitate is formed.

   Glucose can reduce not only alkaline copper(II) tartrate (**FEHLING** reaction), but also acidic (acetic acid) copper(II) acetate to copper(I) oxide (**BARFOED** reaction). Fructose also gives a positive **BARFOED** reaction, while in the case of lactose only a green colour is observed.



gluconic acid

GLYCEROL (85 PER CENT)

Glycerolum (85 per centum)

Definition

Aqueous solution of propane-1,2,3-triol.

Content: 83.5 per cent *m/m* to 88.5 per cent *m/m* of propane-1,2,3-triol ($C_3H_8O_3$; M_r 92.1).

Characters

Aspect: syrupy liquid, unctuous to the touch, colourless or almost colourless, clear, very hygroscopic.

Solubility: miscible with water and with ethanol (96 per cent), slightly soluble in acetone, practically insoluble in fatty oils and in essential oils.

It is used as an osmotic dehydration agent to decrease pressure in the eye and the cranium. As a suppository, it has a laxative effect. It is a component of rehydrating ointments. It is very often used in pharmaceutical technology (as a solvent, vehicle, preservative and sweetener).

Identification

A. Refractive index.

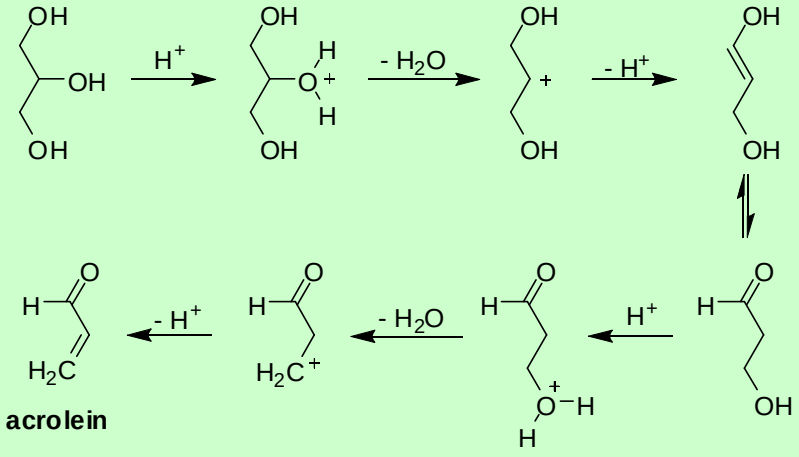
B. Infrared absorption spectrophotometry.

C. Mix 1 ml with 0.5 ml of *nitric acid R*. Superimpose 0.5 ml of *potassium dichromate solution R*. A blue ring develops at the interface of the liquids. Within 10 min, the blue colour does not diffuse into the lower layer.

Primary and secondary alcohols can be oxidized to oxo compounds with dichromate in acidic solution, during which greenish-blue Cr^{3+} is formed.

D. Heat 1 ml with 2 g of *potassium hydrogensulfate R* in an evaporating dish. Vapours (acrolein) are evolved which blacken filter paper impregnated with *alkaline potassium tetraiodomercurate solution R*.

When it is heated with potassium hydrogensulfate, the pungent vapour of acrolein (acrylaldehyde; 2-propenal) is evolved, which reduces potassium tetraiodomercurate(II) to mercury metal.



acrolein

$$CH_2{=}CH{-}CHO + [HgI_4]^{2-} + 2\ OH^- \rightarrow Hg + CH_2{=}CH{-}COOH + 4\ I^- + H_2O$$

acrylic acid

Tests

Solution S. Dilute 117.6 g to 200.0 ml with *carbon dioxide-free water R*.

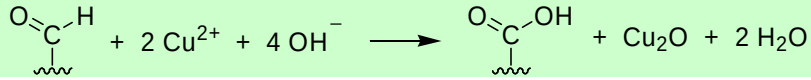
Appearance of solution. Solution S is clear *(2.2.1).* Dilute 10 ml of solution S to 25 ml with *water R*. The solution is colourless *(2.2.2, Method II)*.

Acidity or alkalinity. To 50 ml of solution S add 0.5 ml of *phenolphthalein solution R*. The solution is colourless. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to pink.

The aqueous solution of glycerol is neutral. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

Sugars. To 10 ml of solution S add 1 ml of *dilute sulfuric acid R* and heat on a water-bath for 5 min. Add 3 ml of *carbonate-free dilute sodium hydroxide solution R* (prepared by the method described for carbonate-free *1 M sodium hydroxide*, mix and add dropwise 1 ml of freshly prepared *copper sulfate solution R*. The solution is clear and blue. Continue heating on the water-bath for 5 min. The solution remains blue and no precipitate is formed.

The test, which serves to exclude syrups, is based on the **FEHLING** reaction of reducing sugars. Reducing sugars reduce Cu^{2+} to brick-red copper(I) oxide. Heating with hydrochloric acid is necessary to hydrolyse the oligo- and polysaccharides to reducing monosaccharides.



Chlorides *(2.4.4):* maximum 10 ppm.

Dilute 1 ml of solution S to 15 ml with *water R*. Prepare the standard using 1 ml of *chloride standard solution (5 ppm Cl) R* diluted to 15 ml with *water R.*

Heavy metals *(2.4.8)*: maximum 5 ppm.

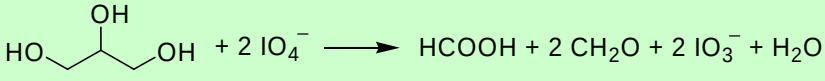
Dilute 8 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

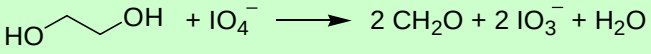
Assay

Thoroughly mix 0.075 g with 45 ml of *water R*. Add 25.0 ml of a mixture of 1 volume of *0.1 M sulfuric acid* and 20 volumes of *0.1 M sodium periodate*. Allow to stand protected from light for 15 min. Add 5.0 ml of a 500 g/l solution of *ethylene glycol R* and allow to stand protected from light for 20 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide*. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 9.21 mg of $C_3H_8O_3$.

Sodium periodate oxidizes glycerol to 2 mol of formaldehyde and 1 mol of formic acid. After decomposition of the excess periodate with ethylene glycol, the remaining formic acid is titrated by alkalimetry. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).





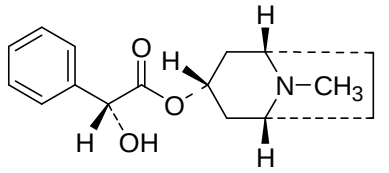
ethylene glycol

$$HCOOH + NaOH \rightarrow HCOONa + H_2O$$

$$C_3H_8O_3 \text{ content (\%)} = \frac{[V_{NaOH}\,(ml) - V_{NaOH}^{empty}(ml)] \cdot f_{NaOH} \cdot E\,(mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

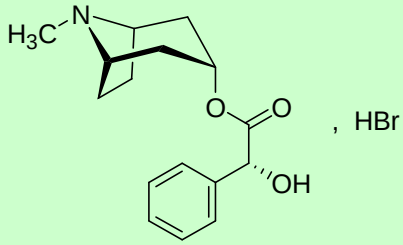
HOMATROPINE HYDROBROMIDE

Homatropini hydrobromidum



, HBr

and enantiomer



, HBr

and enantiomer

$C_{16}H_{22}BrNO_3$ **M_r 356.3**

Definition

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl-(2*RS*)-2-hydroxy-2-phenylacetate hydrobromide.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, sparingly soluble in alcohol.

mp: about 215 °C, with decomposition.

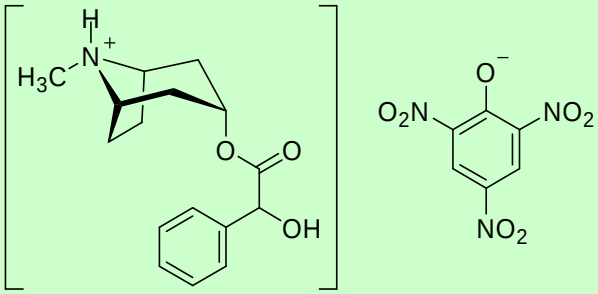
It is a parasympatholytic compound which is used as a mydriatic compound in ophthalmology and as an anticonvulsive agent in gastroenterology. Homatropine is a tropine ester of racemic mandelic acid.

Identification

A. Infrared absorption spectrophotometry.

B. Dissolve 50 mg in 1 ml of *water R* and add 2 ml of *dilute acetic acid R*. Heat and add 4 ml of *picric acid solution R*. Allow to cool, shaking occasionally. Collect the crystals, wash with two quantities, each of 3 ml, of iced *water R* and dry at 100 °C to 105 °C. The crystals melt *(2.2.14)* at 182 °C to 186 °C.

The picric salt of homatropine is separated off.



C. It gives reaction (a) of bromides *(2.3.1)*.

Tests

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Homatropine hydrobromide* monograph.

HYDROGEN PEROXIDE SOLUTION (3 PER CENT)

Hydrogenii peroxidum 3 per centum

Definition

Content: 2.5 per cent *m/m* to 3.5 per cent *m/m* of H_2O_2 (M_r 34.01). 1 volume of hydrogen peroxide solution (3 per cent) corresponds to about 10 times its volume of oxygen. A suitable stabiliser may be added.

Characters

Appearance: colourless, clear liquid.

In 3% solution, hydrogen peroxide is used to purify and deodorize wounds.

Identification

A. To 2 ml, add 0.2 ml of *dilute sulfuric acid R* and 0.2 ml of *0.02 M potassium permanganate*. The solution becomes colourless or slightly pink within 2 min.

Permanganate oxidizes peroxide, during which the purple solution becomes colourless and the evolution of oxygen gas is observed.

$$5\ H_2O_2 + 2\ MnO_4^- + 6\ H^+ \rightarrow 2\ Mn^{2+} + 5\ O_2 + 8\ H_2O$$

B. To 1 mL, add 0.1 ml of dilute *hydrochloric acid R* and 0.1 ml of *potassium iodide solution R*. A brown colour appears. Black particles may be formed.

Hydrogen peroxide oxidizes iodide to iodine.

$$H_2O_2 + 2\ I^- + 2\ H^+ \rightarrow I_2 + 2\ H_2O$$

C. It complies with the requirement for the content of H_2O_2.

Tests

Acidity. To 10 ml add 20 ml of *water R* and 0.25 ml of *methyl red solution R*. Not less than 0.05 ml and not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of hydrogen peroxide is weakly acidic. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

Assay

Dilute 10.0 g to 100.0 ml with *water R*. To 10.0 ml of this solution add 20 ml of *dilute sulfuric acid R*. Titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

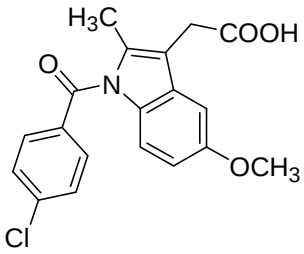
1 ml of 0.02 M potassium permanganate is equivalent to 1.701 mg of H_2O_2 or 0.56 ml of oxygen.

The end-point of the permanganometric titration (equation: see the explanation of **Identification A**) is indicated by the permanent pink colour of potassium permanganate.

$$H_2O_2\ \text{cont.}\ (\%) = \frac{V_{KMnO_4}(\text{ml}).\ f_{KMnO_4}.\ E\ (\text{mg/ml})}{\text{amount of substance (mg)}}.\ 100$$

INDOMETACIN

Indometacinum



$C_{19}H_{26}ClNO_4$ **M_r 357.8**

Definition

[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid.
Content: 98.5 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or yellow, crystalline powder.
Solubility: practically insoluble in water, sparingly soluble in ethanol (96 per cent).
It shows polymorphism (*5.9*)

It is a non-steroidal anti-inflammatory drug.

Identification

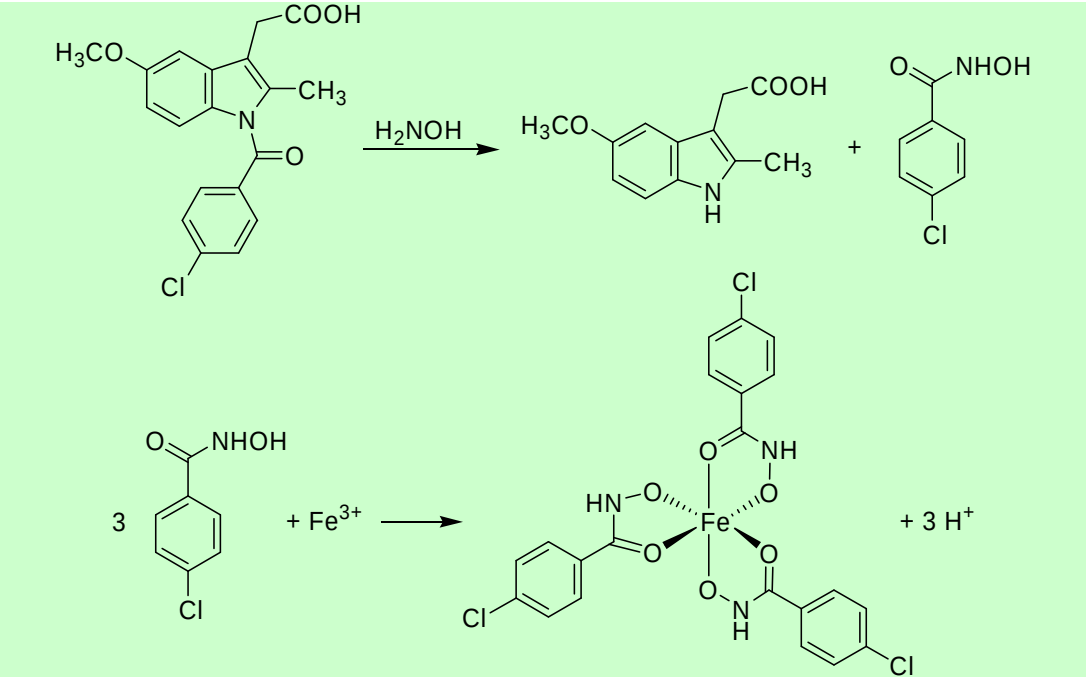
A. Melting point *(2.2.14)*: 158 °C to 162 °C.

B. Ultraviolet and visible absorption spectrophotometry.

C. Infrared absorption spectrophotometry.

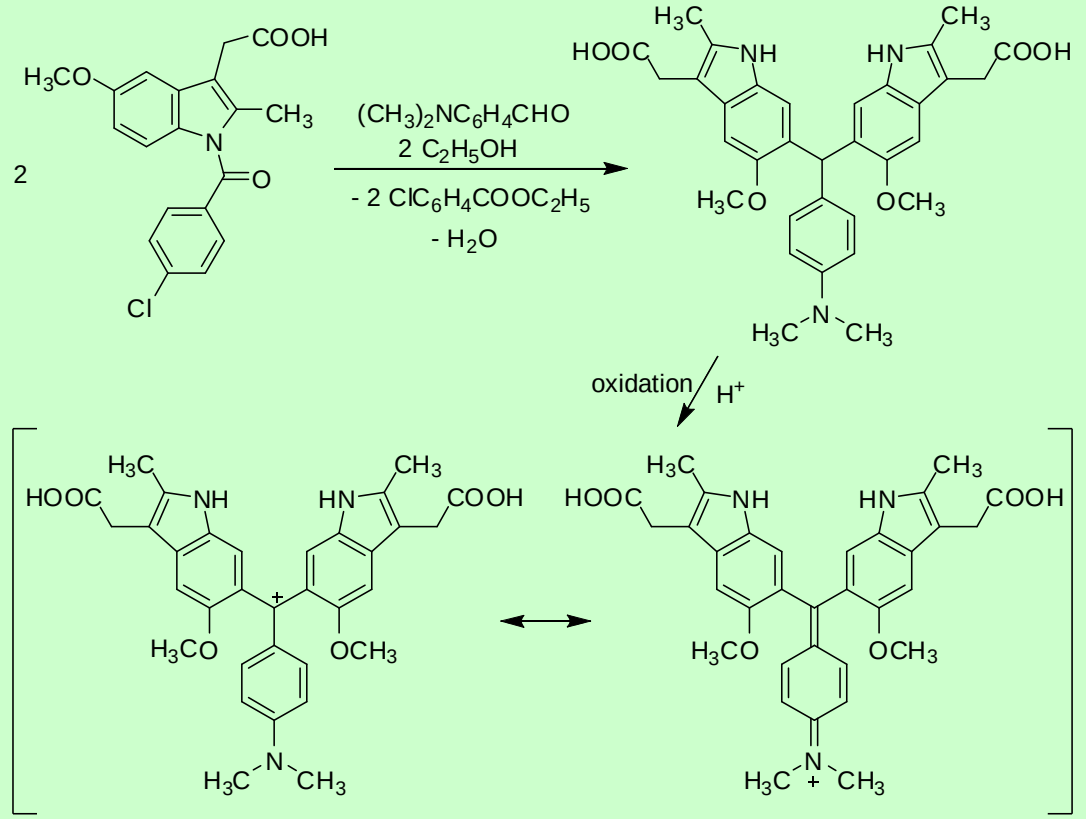
D. Dissolve 0.1 g in 10 ml of *ethanol (96 per cent) R*, heating slightly if necessary. To 0.1 ml of the solution add 2 ml of a freshly prepared mixture of 1 volume of a 250 g/l solution of *hydroxylamine hydrochloride R* and 3 volumes of *dilute sodium hydroxide solution R*. Add 2 ml of *dilute hydrochloric acid R* and 1 ml of *ferric chloride solution R2* and mix. A violet-pink colour develops.

The amide bond of indometacin is split by hydroxylamine, resulting in (5-methoxy-2-methyl-indol-3-yl)acetic acid and *p*-chlorophenylhydroxamic acid. The latter forms a violet complex with Fe^{3+}.



E. To 0.5 ml of the solution in ethanol (96 per cent) prepared in identification test **D**, add 0.5 ml of *dimethylaminobenzaldehyde solution R2*. A precipitate is formed that dissolves on shaking. Heat on a water-bath. A bluish-green colour is produced. Continue to heat for 5 min and cool in iced water for 2 min. A precipitate is formed and the colour changes to light greyish-green. Add 3 ml of *ethanol (96 per cent) R* The solution is clear and violet-pink in colour.

Indometacin forms a triarylmethane-type compound with *p*-dimethylaminobenzaldehyde, which is oxidized by air to a mesomerically stabilized coloured triarylcarbonium derivative.



Informative test

1. See identification **D** in the *Indometacin* monograph.

IODINE

Iodum

I_2 M_r 253.8

Definition

Content: 99.5 per cent to 100.5 per cent of I

Characters

Appearance: greyish-violet, brittle plates or fine crystals with a metallic sheen.

Solubility: very slightly soluble in water, very soluble in concentrated solutions of iodides, soluble in ethanol (96 per cent), slightly soluble in glycerol.

It volatilises slowly at room temperature.

In alcoholic solution, it is used as an external disinfectant.

Identification

A. Heat a few fragments in a test-tube. Violet vapour is evolved and a bluish-black crystalline sublimate is formed.

B. To a saturated solution add *starch solution R*. A blue colour is produced. Heat until decolourised. On cooling, the colour reappears.

Iodine produces a blue addition product with starch, while the pentaiodine chain is linked to the hydroxy groups of the glucose moieties of the helical starch. On heating, the spiral straightens out, the polyiodine chain splits and the colour disappears, but both the structure and the colour reappear on cooling.

Tests

Solution S. Triturate 3.0 g with 20 ml of *water R*, filter, wash the filter with *water R* and dilute the filtrate to 30 ml with the same solvent. To the solution add 1 g of *zinc powder R*. When the solution is decolourised, filter, wash the filter with *water R* and dilute to 40 ml with the same solvent.

Zinc reduces iodine to iodide. Solution S is a zinc iodide solution containing all the water-soluble impurities of the iodine.

$$I_2 + Zn \rightarrow 2\ I^- + Zn^{2+}$$

Bromides and chlorides: maximum 250 ppm.

To 10 ml of solution S add 3 ml of *ammonia R* and 6 ml of *silver nitrate solution R2*. Filter, wash the filter with *water R* and dilute the filtrate to 20 ml with the same solvent. To 10 ml of the solution add 1.5 ml of *nitric acid R*. After 1 min, any opalescence in the solution is not more intense than that in a standard prepared at the same time by mixing 10.75 ml of *water R*, 0.25 ml of *0.01 M hydrochloric acid*, 0.2 ml of *dilute nitric acid R* and 0.3 ml of *silver nitrate solution R2*.

Bromide and chloride form silver chloride or silver bromide precipitates, which are soluble in an excess of ammonia. Silver iodide is insoluble in ammonia; hence, the filtrate contains only the bromide and chloride, as diamminesilver complexes. From these, silver bromide and chloride are reprecipitated with acid.

FERRUM METALLICUM FOR HOMOEOPATHIC PREPARATIONS

Ferrum ad praeparationes homoeopathicas

Fe **A_r 55.85**

Definition

Iron obtained by reduction or sublimation as a fine blackish-grey powder.

Content: 97.5 per cent to 101.0 per cent.

Characters

Appearance: fine, blackish-grey powder, without metallic lustre.

Solubility: practically insoluble in water and in ethanol (96 per cent). It dissolves with heating in dilute mineral acids.

Identification

C. Dissolve 50 mg in 2 ml of *dilute sulfuric acid R* and dilute to 10 ml with *water R*. The solution gives reaction (a) of iron (*2.3.1*).

Iron dissolves in acids with the liberation of hydrogen gas.

$$Fe + 2\ H^+ \rightarrow Fe^{2+} + H_2$$

Tests

Solution S. To 10.0 g add 40 ml of *water R*. Boil for 1 min. Cool, filter and dilute to 50.0 ml with *water R*.

Alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow.

The shaken mixture of iron and water is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Chlorides *(2.4.4)*: maximum 50 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulfide and phosphide. In a 100 ml conical flask carefully mix 1.0 g with 10 ml of *dilute hydrochloric acid R*. Within 30 s *lead acetate paper R* moistened with *water R* and placed over the mouth of the flask is not coloured more intensely than light brown by the resulting fumes.

In acidic medium, sulfide and phosphide impurities form hydrogen sulfide or hydrogen phosphide (phosphine), which precipitate black lead sulfide or lead phosphide with Pb^{2+}.

$$FeS + 2\ H^+ \rightarrow Fe^{2+} + H_2S$$

$$Fe_3P_2 + 6\ H^+ \rightarrow 3\ Fe^{2+} + 2\ H_3P$$

$$H_2S + Pb^{2+} \rightarrow PbS + 2\ H^+$$

$$2\ H_3P + 3\ Pb^{2+} \rightarrow Pb_3P_2 + 6\ H^+$$

Arsenic (*2.4.2*): maximum 5 ppm.

Boil 0.2 g in 25 ml of *dilute hydrochloric acid R* until completely dissolved. The solution complies with limit test A.

Assay

Stir for 10 min 0.100 g in a hot solution of 1.25 g of *copper sulfate R* in 20 ml of *water R* in a 100 ml conical flask with a ground-glass stopper. Filter rapidly and wash the filter. Combine the filtrate and the washings, acidify with *dilute sulfuric acid R* and titrate with *0.02 M potassium permanganate* until a pink colour is obtained.

1 ml of *0.02 M potassium permanganate* is equivalent to 5.585 mg of Fe.

Because of the standard potentials of iron and copper, iron dissolves in copper sulfate solution with the separation of copper. Iron(II) is very sensitive to air (oxidizable with oxygen),

and it is therefore important to perform the dissolution for exactly 10 min and to filter rapidly. After dissolution of the iron, Fe^{2+} is determined by permanganometric titration. The end-point is indicated by the permanent pink colour of potassium permanganate.

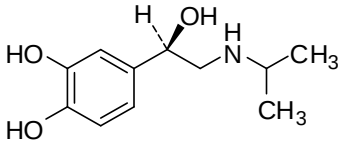
$$Fe + Cu^{2+} \rightarrow Fe^{2+} + Cu$$

$$5\ Fe^{2+} + MnO_4^- + 8\ H^+ \rightarrow 5\ Fe^{3+} + Mn^{2+} + 4\ H_2O$$

$$\text{Fe content (\%)} = \frac{V_{KMnO_4(ml)} \cdot f_{KMnO_4} \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

ISOPRENALINE HYDROCHLORIDE

Isoprenalini hydrochloridum



and enantiomer, HCl

$C_{11}H_{18}ClNO_3$ **M_r 247.7**

Definition

(1*RS*)-1-(3,4-Dihydroxyphenyl)-2-[(1-methylethyl)amino]ethanol hydrochloride.

Content: 98.0 per cent to 101.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in methylene chloride.

Isoprenaline is a relatively selective β_2-adrenergic bronchodilator.

Identification

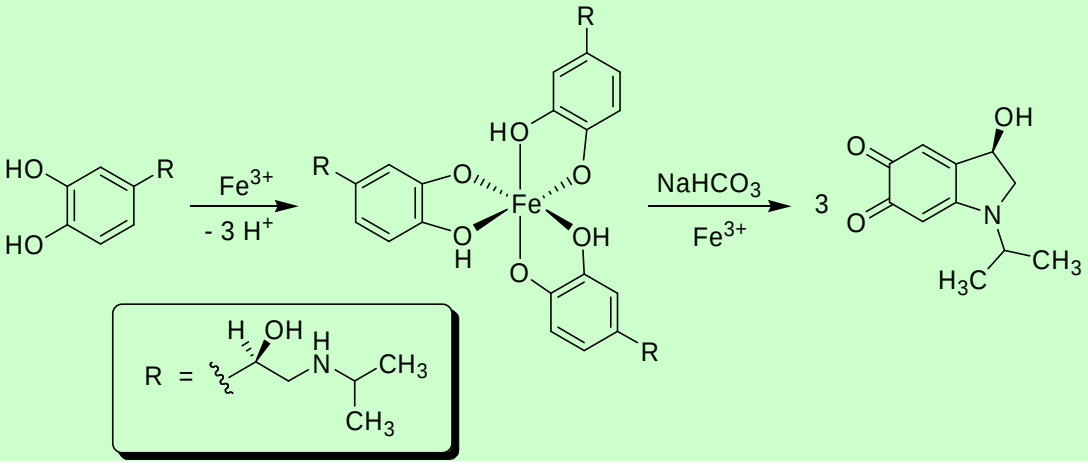
A. Melting point *(2.2.14)*: 165 °C to 170 °C, with decomposition.

B. Infrared absorption spectrophotometry.

C. Optical rotation (see Tests).

D. To 0.1 ml of solution S (see Tests) add 0.05 ml of *ferric chloride solution R1* and 0.9 ml of *water R*. A green colour is produced. Add dropwise *sodium hydrogen carbonate solution R*. The colour becomes blue then red.

With Fe^{3+}, the phenolic hydroxy groups of isoprenaline form a three-ligand chelate complex. In alkaline solution (adding sodium hydrogencarbonate solution), the complex is decomposed and oxidized to the *ortho*-quinoidal, reddish-violet *N*-isopropylnoradrenochrome by Fe^{3+}.



E. To 0.5 ml of solution S add 1.5 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

Tests

Prepare the solutions immediately before use.

The catechol moiety of isoprenaline is easily oxidizable in solution (especially in alkaline medium). This can be avoided by the preparation of the solution immediately before use.

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_7 or BY_7 *(2.2.2, Method II)*.

pH *(2.2.3)*: 4.3 to 5.5.

Mix 5 ml of solution S and 5 ml of *carbon dioxide-free water R*.

Optical rotation *(2.2.7)*: -0.10 to +0.10, determined on solution S.

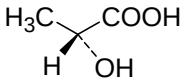
Informative tests

1. See the **Appearance of solution** test in the *Isoprenaline hydrochloride* monograph.
2. See identification **D** in the *Isoprenaline hydrochloride* monograph.
3. To 4 drops of solution S, add 3.0 ml of *water R*, a few drops of *dilute nitric acid R* and 2.0 ml of *silver nitrate solution R1*. A white precipitate is formed.

   A silver chloride precipitate is formed.

LACTIC ACID

Acidum lacticum



and enantiomer

$C_3H_6O_3$ **M_r 90.1**

Definition

Mixture of 2-hydroxypropanoic acid, its condensation products, such as lactoyl-lactic acid and polylactic acids, and water. The equilibrium between lactic acid and polylactic acids depends on the concentration and temperature. It is usually the racemate ((*RS*)-lactic acid).

Content: 88.0 per cent *m/m* to 92.0 per cent *m/m* of $C_3H_6O_3$.

Characters

Appearance: colourless or slightly yellow, syrupy liquid.

Solubility: miscible with water and with ethanol (96 per cent).

A dilute aqueous solution of lactic acid is used as a disinfectant.

Identification

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly acidic *(2.2.4)*.

B. Relative density *(2.2.5)*: 1.20 to 1.21.

C. It gives the reaction of lactates *(2.3.1)*.

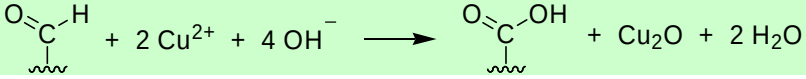
Tests

Solution S. Dissolve 5.0 g in 42 ml of *1 M sodium hydroxide* and dilute to 50 ml with *distilled water R*.

Appearance. The substance to be examined is not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Sugars and other reducing substances. To 1 ml of solution S add 1 ml of *1 M hydrochloric acid,* heat to boiling, allow to cool and add 1.5 ml of *1 M sodium hydroxide* and 2 ml of *cupri-tartaric solution R*. Heat to boiling. No red or greenish precipitate is formed.

The test, which is also suitable for the exclusion of syrups, is based on the **FEHLING** reaction of reducing sugars. Brick-red copper(I) oxide is formed. Gum impurities give a green colour with Cu^{2+}.



Citric, oxalic and phosphoric acids. To 5 ml of solution S add *dilute ammonia R1* until slightly alkaline *(2.2.4)*. Add 1 ml of *calcium chloride solution R*. Heat on a water-bath for 5 min. Both before and after heating, any opalescence in the solution is not more intense than that in a mixture of 1 ml of *water R* and 5 ml of solution S.

Calcium oxalate, citrate or phosphate is formed. The reaction is suitable for distinguishing between lactic acid and phosphoric acid.

Sulfates *(2.4.13)*: maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R*.

Calcium *(2.4.3):* maximum 200 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Heavy metals *(2.4.8):* maximum 10 ppm.

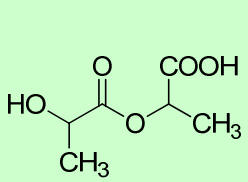
12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Assay

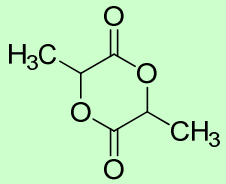
Place 1.000 g in a ground-glass-stoppered flask and add 10 ml of *water R* and 20.0 ml of *1 M sodium hydroxide.* Close the flask and allow to stand for 30 min. Using 0.5 ml of *phenolphthalein solution R* as indicator, titrate with *1 M hydrochloric acid* until the pink colour is discharged.

1 ml of *1 M sodium hydroxide* is equivalent to 90.1 mg of $C_3H_6O_3$.

Because of the intermolecular esterification of hydroxyacids, the substance always contains some ester derivatives besides the free lactic acid (*O*-lactoyllactic acid and other oligomers).

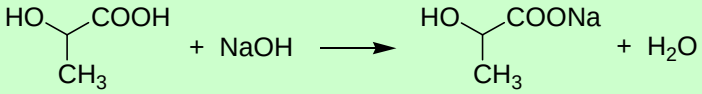


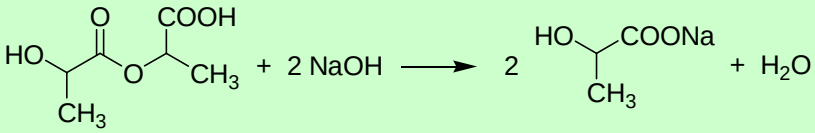
***O*-lactoyllactic acid**

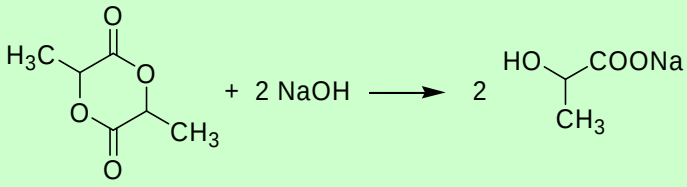


dilactide

Direct alkalimetric assay is not successful, sodium hydroxide is therefore applied in excess to hydrolyse all the ester derivatives. Finally, the excess of base is back-titrated with hydrochloric acid. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).





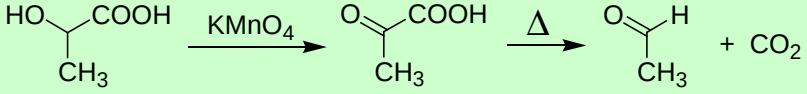


$$C_3H_6O_3 \text{ cont. (\%)} = \frac{[V_{NaOH}\text{ (ml)} \cdot f_{NaOH} - V_{HCl}\text{ (ml)} \cdot f_{HCl}] \cdot E\text{ (mg/ml)}}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See the **Appearance of solution** test in the *Lactic acid* monograph.
2. See identification **A** in the *Lactic acid* monograph.
3. Heat a mixture of 5.0 ml of *potassium permanganate solution R* and 5 drops of the sample to boiling. The solution decolorizes and a smell of acetaldehyde is evolved.

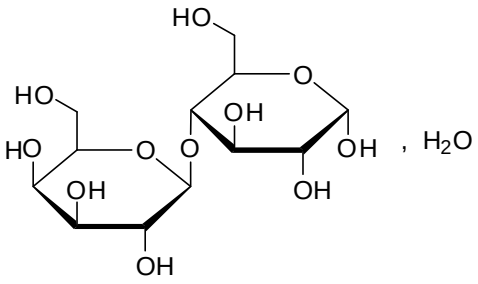
The oxidation of lactic acid leads via pyruvic acid to acetaldehyde and carbon dioxide.



pyruvic acid **acetaldehyde**

LACTOSE MONOHYDRATE

Lactosum monohydricum



$C_{12}H_{22}O_{11}$. H_2O **M_r 360.3**

Definition

O-β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate.

Characters

Appearance: white or almost white, crystalline powder.

Solubility: freely but slowly soluble in water, practically insoluble in ethanol (96 per cent).

Lactose is used in pharmacy as an additive.

Identification

A. Infrared absorption spectrophotometry.

B. Thin-layer chromatography.

C. Dissolve 0.25 g in 5 ml of *water R*. Add 5 ml of *ammonia R* and heat in a water-bath at 80 °C for 10 min. A red colour develops.

When heated with ammonia, lactose (and also maltose) gives a red colour, while in the case of glucose or fructose a yellowish-brown colour is observed (**WÖHLK** reaction).

D. Water (see Tests).

Tests

Appearance of solution. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Dissolve 1.0 g in boiling *water R*, dilute to 10 ml with the same solvent.

Specific optical rotation *(2.2.7)*: +54.4 to +55.9 (anhydrous substance).

Dissolve 10.0 g in 80 ml of *water R*, heating to 50 °C. Allow to cool and add 0.2 ml of *dilute ammonia R1*. Allow to stand for 30 min and dilute to 100.0 ml with *water R*.

Because of the ring-chain tautomerism of lactose, the specific optical rotation of fructose changes after the preparation of the solution until a constant value (approx. +55) is achieved (**mutarotation**; for anexplanation, see the *Glucose anhydrous* monograph "Specific optical rotation" test).

Water *(2.5.12):* 4.5 per cent to 5.5 per cent, determined on 0.50 g, using a mixture of 1 volume of *formamide R* and 2 volumes of *methanol R* as the solvent.

Informative tests

1. See the **Appearance of solution** test in the *Lactose monohydrate* monograph.

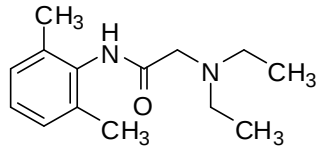
2. Dilute 1 drop of solution S with 5.0 ml of *water R,* add 1 ml of *50 g/l solution of copper(II) acetate R* and heat the light-blue liquid for 10 min in a hot water bath. Not more than a greenish tint should be

produced, but no finely-dispersed red precipitate must appear. Add 2 ml of *dilute sodium hydroxide solution R* to the liquid: a voluminous brick-red precipitate is produced.

Lactose is a weaker reducing agent than glucose or fructose, and in the **BARFOED** reaction it therefore reduces Cu(II) only in strongly alkaline solution.

LIDOCAINE

Lidocainum



$C_{14}H_{22}N_2O$ **M_r 234.3**

Definition

2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, very soluble in ethanol (96 per cent) and in methylene chloride.

It is a local anaesthetic and antiarrhythmic drug.

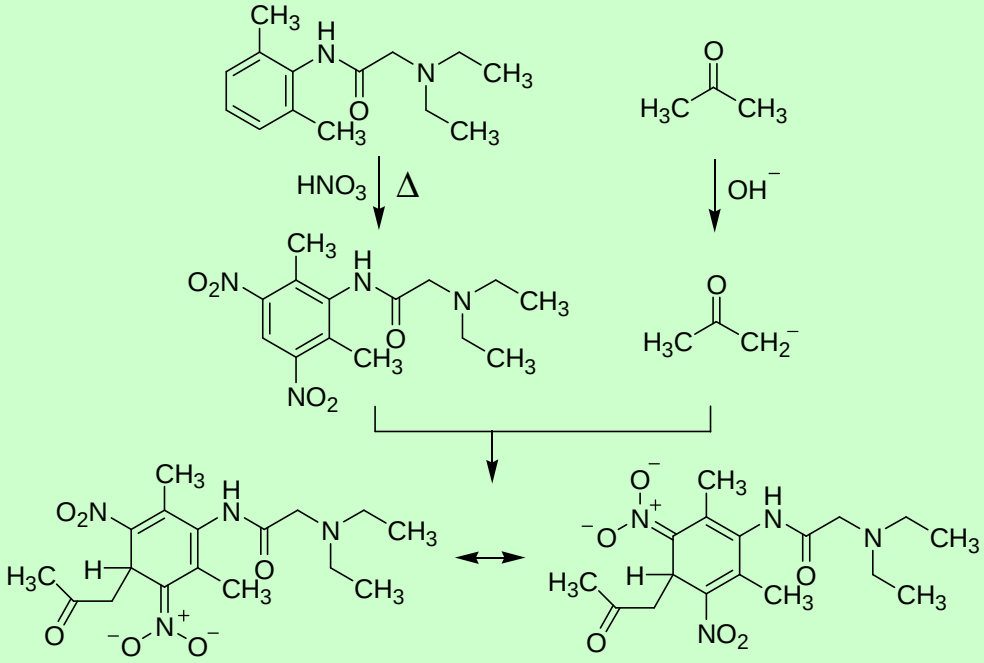
Identification

A. Infrared absorption spectrophotometry.

B. Melting point *(2.2.14)*: 66 °C to 70 °C, determined without previous drying.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R.* Evaporate to dryness on a water-bath, cool and dissolve the residue in 5 ml of *acetone R*. Add 0.2 ml of *alcoholic potassium hydroxide solution R*. A green colour develops.

Lidocaine reacts with nitric acid to result in 3,5-dinitrolidocaine, which gives a green **MEISENHEIMER** complex (**VITALI-MORIN** reaction) with acetone in alkaline solution. The reaction is appropriate for distinguishing procaine, tetracaine and lidocaine (procaine: reddish-brown; tetracaine: violet).



green Meisenheimer complex

Informative tests

2. Dissolve 0.10 g in 1.0 ml of *dilute nitric acid R* and add 6.0 ml of *mercury(II) nitrate solution R*. Heat the mixture to boiling: a yellowish-green colour appears.

MAGNESIUM CARBONATE, LIGHT

Magnesii subcarbonas levis

Definition

Hydrated basic magnesium carbonate.

Content: 40.0 per cent to 45.0 per cent, calculated as MgO (*M*r 40.30).

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble in water. It dissolves in dilute acids with effervescence.

It is used as an antacid owing to its ability to react with the hydrochloric acid of the stomach.

Identification

A. Bulk density (2.9.34): maximum 0.15 g/ml.

B. It gives the reaction of carbonates (*2.3.1*).

C. Dissolve about 15 mg in 2 ml of *dilute nitric acid R* and neutralise with *dilute sodium hydroxide solution R*. The solution gives the reaction of magnesium (*2.3.1*).

Magnesium nitrate and carbon dioxide are formed.

Tests

Solution S. Dissolve 5.0 g in 100 ml of *dilute acetic acid R*. When the effervescence has ceased, boil for 2 min, cool and dilute to 100 ml with *dilute acetic acid R*. Filter, if necessary, through a previously ignited and tared porcelain or silica filter crucible of suitable porosity to give a clear filtrate.

Magnesium acetate and carbon dioxide are formed.

Appearance of solution. Solution S is not more intensely coloured than reference solution B_4 (*2.2.2, Method II*).

Chlorides (*2.4.4*): maximum 700 ppm.

Dilute1.5 ml of solution S to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum 0.3 per cent.

Dilute 1 ml of solution S to 15 ml with *distilled water R*.

Arsenic (*2.4.2*): maximum 2 ppm, determined on 10 ml of solution.

Calcium (2.4.3): maximum 0.75 per cent.

Dilute 2.6 ml of solution S to 150 ml with *distilled water R*. 15 ml of the solution complies with the test.

Iron (*2.4.9*): maximum 400 ppm.

Dissolve 0.1 g in 3 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Dilute 2.5 ml of the solution to 10 ml with *water R*.

Informative test

1. To 0.50 g, add 5.0 ml of *water R* and 15.0 ml of *dilute acetic acid R*. The substance dissolves with the liberation of an odourless gas.

2. See identification **C** in the *Magnesium carbonate, light* monograph.

MAGNESIUM SULFATE HEPTAHYDRATE

Magnesii sulfas heptahydricus

$MgSO_4,7H_2O$ **M_r 246.5**

Definition

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or brilliant, colourless crystals.

Solubility: freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

Magnesium sulfate is used as an osmotic laxative. Because of its bitter taste, it is also called bitter salt.

Identification

A. It gives the reactions of sulfates (*2.3.1*).

B. It gives the reaction of magnesium (*2.3.1*).

Tests

Solution S. Dissolve 5.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.05 ml of *phenol red solution R*. Not more than 0.2 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of tie indicator.

The aqueous solution of magnesium sulfate is neutral. The pH interval of the colour change of the phenol red indicator is between 6.8 (yellow) and 8.4 (reddish-violet). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to reddish-violet; when the original colour of the indicator is reddish-violet, hydrochloric acid is required to change it to yellow. An intermediate orange colour may be observed.

Chlorides (*2.4.4*): maximum 300 ppm.

Dilute 1.7 ml of solution S to 15 ml with *water R*).

Arsenic (*2.4.2 Method A*): maximum 2 ppm, determined on 0.5 g.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Informative test

1. See the **Appearance of solution** test in the *Magnesium sulfate heptahydrate* monograph.

2. See identification **A** in the *Magnesium sulfate heptahydrate* monograph.

3. See identification **B** in the *Magnesium sulfate heptahydrate* monograph.

MAGNESIUM TRISILICATE

Magnesii trisilicas

Definition

It has a variable composition corresponding approximately to $Mg_2Si_3O_8,xH_2O$.

Content:

– *magnesium oxide* (MgO; *M*r 40.30) : minimum 29.0 per cent (ignited substance),

– *silicon dioxide* (SiO_2 ; *M*r 60.1) : minimum 65.0 per cent (ignited substance).

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble in water and in ethanol (96 per cent).

It is used as an antacid owing to its ability to react with the hydrochloric acid of the stomach.

Identification

A. 0.25 g gives the reaction of silicates (*2.3.1*).

B. 1 ml of solution S (see Tests) neutralised with *dilute sodium hydroxide solution R* gives the reaction of magnesium (*2.3.1*).

Tests

Solution S. To 2.0 g add a mixture of 4 ml of *nitric acid R* and 4 ml of *distilled water R*. Heat to boiling with frequent shaking. Add 12 ml of *distilled water R* and allow to cool. Filter or centrifuge to obtain a clear solution and dilute to 20 ml with *distilled water R*.

On acidification, magnesium trisilicate decomposes, resulting in water-insoluble silicic acid (filtered off) and magnesium nitrate (and also water-soluble impurities).

$$Mg_2Si_3O_8 + 4\ H^+ + H_2O \rightarrow 2\ Mg^{2+} + 3\ H_2SiO_3$$

Informative test

1. Shake 0.50 g with 10 ml of *dilute hydrochloric acid R* for 1 min, and filter the turbid liquid. Add to the filtrate first 10.0 ml of *dilute ammonia solution R1*, and then 0.40 g *diammonium hydrogenphosphate R*. A white jelly-like precipitate is produced, which soon becomes crystalline on shaking.

$$Mg^{2+} + NH_4^+ + PO_4^{3-} \rightarrow MgNH_4PO_4$$

2. Boil 0.5 g with 10.0 ml of *sodium carbonate solution R* for 3 min. Filter the cooled mixture. Add *concentrated hydrochloric acid R* dropwise, in small portions, to the filtrate until the gas evolution ceases. Add another 1 ml of *concentrated hydrochloric acid R,* and heat the liquid to boiling. A colourless, jelly-like precipitate is formed.

On boiling with sodium carbonate, water-soluble sodium silicate is formed. After acidification, silicic acid separates out.

$$Mg_2Si_3O_8 + 2\ Na_2CO_3 \rightarrow Na_4Si_3O_8 + 2\ MgCO_3$$

$$Na_4Si_3O_8 + 4\ HCl + H_2O \rightarrow 3\ H_2SiO_3 + 4\ NaCl$$

3. When some of the powder is sprinkled onto the surface of *water R*, fast and complete sedimentation must be observed.

MANGANESE SULFATE MONOHYDRATE

Mangani sulfas monohydricum

$MnSO_4,H_2O$ **M_r 169.0**

Definition

Content: 99.0 per cent to 101.0 per cent (ignited substance).

Characters

Appearance: pale pink crystalline powder, slightly hygroscopic.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent).

Manganese is an essential trace element. Manganese compounds are used as roborants and haemopoietic agents.

Identification

A. Solution S (see Tests) gives reaction (a) of sulfates (*2.3.1*).

B. Dissolve 50 mg in 5 ml of *water R*. Add 0.5 ml of *ammonium sulfide solution R*. A pale pink precipitate is formed which dissolves on the addition of 1 ml of *anhydrous acetic acid R*.

In neutral or slightly alkaline solution, Mn^{2+} forms a precipitate of manganese sulfide (cation group III, group reaction), which is soluble in acids.

$$Mn^{2+} + (NH_4)_2S \rightarrow MnS + 2NH_4^+$$

$$MnS + 2\ H^+ \rightarrow H_2S + Mn^{2+}$$

Tests

Solution S. Dissolve 10.0 g in *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension II (*2.2.1*).

Chlorides (*2.4.4*): maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Iron (*2.4.9*): maximum 10 ppm, determined on solution S.

Zinc: maximum 50 ppm.

To 10 ml of solution S add 1 ml of *sulfuric acid R* and 0.1 ml of *potassium ferrocyanide solution R*. After 30 s, any opalescence in the solution is not more intense than that in a mixture of 5 ml of *zinc standard solution* (*10 ppm Zn*) *R*, 5 ml of *water R*, 1 ml of *sulfuric acid R* and 0.1 ml of *potassium ferrocyanide solution R*.

Hexacyanoferrate(II) precipitates white zinc(II) hexacyanoferrate(II).

$$2\ Zn^{2+} + [Fe(CN)_6]^{4-} \rightarrow Zn_2[Fe(CN)_6]$$

Heavy metals (*2.4.8*). maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*2 ppm Pb*) *R*.

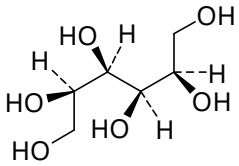
Informative test

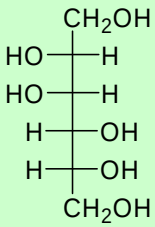
1. See identification **A** in the *Manganese sulfate monohydrate* monograph.

2. See identification **B** in the *Manganese sulfate monohydrate* monograph.

MANNITOL

Mannitolum





$C_6H_{14}O_6$ ***M*r 182,2**

Other name: Mannitum (Ph. Hg. VII.)

Definition

D-Mannitol.

Content: 98.0 per cent to 102.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalls or powder.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

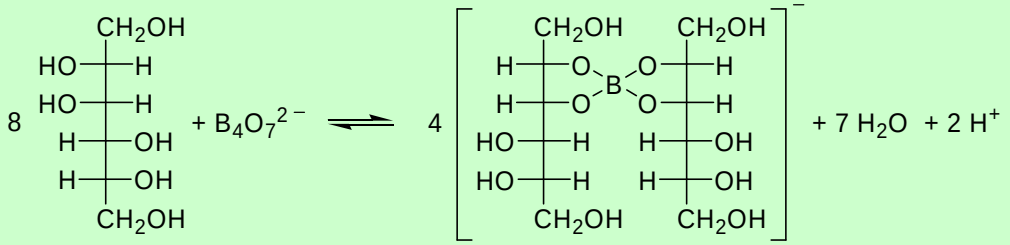
It is an osmotic diuretic and laxative.

Identification

A. Specific optical rotation *(2.2.7)*: +23 to +25 (dried substance).

Dissolve 2.00 g of the substance to be examined and 2.6 g of *disodium tetraborate R* in about 20 ml of *water R* at a temperature of 30 °C; shake continuously for 15-30 min without further heating. Dilute the resulting clear solution to 25.0 ml with *water R*.

The specific optical rotation of D-mannitol in water is very low (aprox. −0.2). With borax, mannitol forms a higher specific optical rotation complex with reversal of the direction (approx. +24).



B. Melting point *(2.2.14)*: 165 °C to 170 °C.

C. Infrared absorption spectrophotometry *(2.2.24)*.

D. Thin-layer chromatography.

Tests

Informative test

1. 0.25 g of the substance is dissolved in 5.0 ml of *watrer R,* and 5.0 ml of *ferric chloride R2 solution* and 2.5 ml of *diluted sodium hydroxide R* are added. The solution turns yellowish-brown, but it remains clear.

Mannitol reacts with Fe^{3+} to give a water-soluble, stable chelate complex. When sodium hydroxide solution is added to this, a reddish-brown precipitate of $Fe(OH)_3$ is not formed.

MERCURIC CHLORIDE

Hydrargyri dichloridum

$HgCl_2$ **M_r 271.5**

Definition

Content: 99.5 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless or white or almost white crystals or heavy crystalline masses.

Solubility: soluble in water and in glycerol, freely soluble in ethanol (96 per cent).

In very dilute solution, mercuric chloride has been used as a disinfectant. It is a violent poison.

Identification

A. It gives the reactions of chlorides (*2.3.1*).

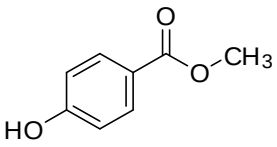
B. Solution S (see Tests) give the reactions of mercury (*2.3.1*).

Tests

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

METHYL PARAHYDROXYBENZOATE

Methylis parahydroxybenzoas



$C_8H_8O_3$ **M_r 152.1**

Definition

Methyl 4-hydroxybenzoate.

Content: 98.0 per cent to 102.0 per cent.

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very slightly soluble in water, freely soluble in ethanol (96 per cent) and in methanol.

It is a microbiological preservative.

Identification

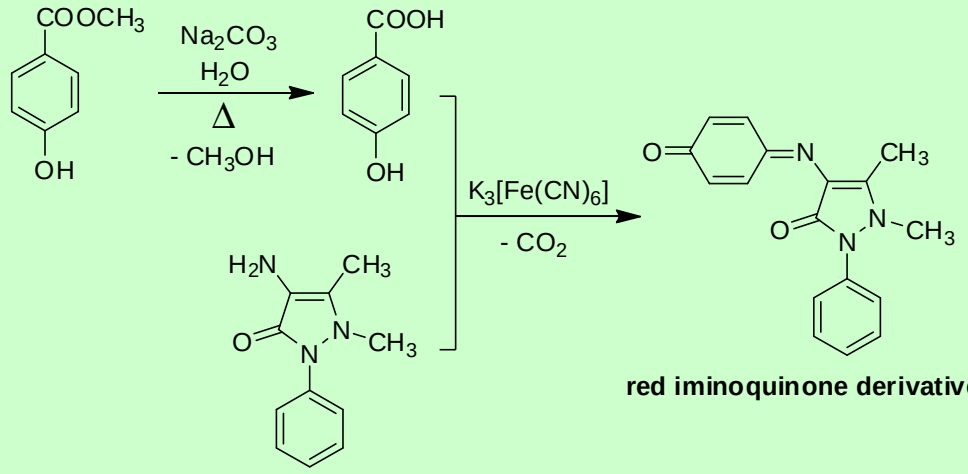
A. Melting point *(2.2.14)*: 125 °C to 128 °C.

B. Infrared absorption spectrophotometry

C. Thin-layer chromatography.

D. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution R*, boil for 30 s and cool (solution A). To a further 10 mg in a similar test-tube add 1 ml of *sodium carbonate solution R*; the substance partly dissolves (solution B). Add at the same time to solution A and solution B 5 ml of *aminopyrazolone solution R* and 1 ml of *potassium ferricyanide solution R* and mix. Solution B is yellow to orange-brown. Solution A is orange to red, the colour being clearly more intense than any similar colour which may be obtained with solution B.

When heated with sodium carbonate solution, methyl *p*-hydroxybenzoate is hydrolysed to *p*-hydroxybenzoic acid (solution A), which reacts with aminopyrazolone in the presence of an oxidizing agent (potassium hexacyanoferrate(III)) to give a *p*-iminoquinone derivative via decarboxylation (**EMERSON** reaction). Without heating, in solution B methyl *p*-hydroxybenzoate is hydrolysed only partially, and iminoquinone is therefore not formed. The **EMERSON** reaction is a suitable reaction for the identification of 4-unsubstituted phenols.



Tests

Solution S. Dissolve 1.0 g in *ethanol (96 per cent) R* and dilute to 10 ml with the same solvent.

Appearance of solution. Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

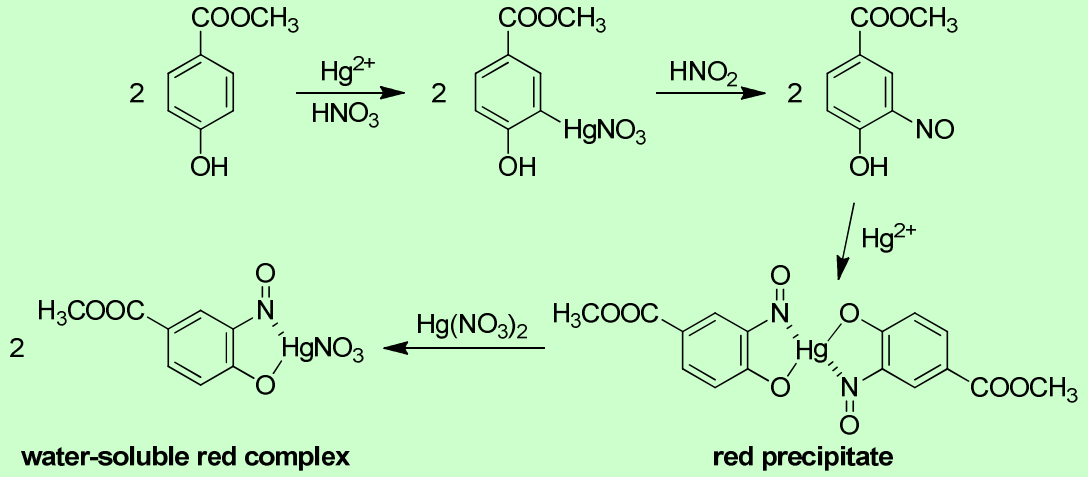
Acidity. To 2 ml of solution S add 3 ml of *ethanol (96 per cent) R*, 5 ml of *carbon dioxide-free water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to blue.

An aqueous alcoholic solution of methyl para-hydroxybenzoate is slightly acidic. The pH interval of the colour change of the bromocresol green indicator is between 3.6 (yellow) and 5.2 (blue).

Informative tests

1. See the **Appearance of solution** test in the *Methyl parahydroxybenzoate* monograph.
2. To about 20 mg, add a few drops of *mercury nitrate solution R* and heat the mixture. A dark-red colour is produced.

The mercury nitrate reagent (**MILLON** reagent) is prepared by dissolving metallic mercury in concentrated nitric acid: it is a nitric acid solution of mercury(I) and mercury(II) which also contains nitrite. In the reaction, methyl para-hydroxybenzoate is mercurated; an *o*-nitrosophenol derivative is then formed, which gives a red complex with Hg^{2+}.



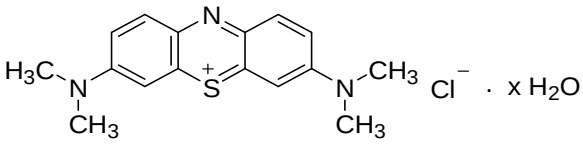
water-soluble red complex **red precipitate**

3. Put about 20 mg of sample into a dry tube and heat it on a boiling water bath. The crystals do not melt at this temperature (a distinction from propyl para-hydroxybenzoate).

The melting point of propyl para-hydroxybenzoate is 96–99 ºC, and it therefore melts at the temperature of boiling water, while methyl para-hydroxybenzoate has a higher melting point (125–128 ºC).

METHYLTHIONINIUM CHLORIDE

Methylthioninii chloridum



$C_{16}H_{18}ClN_3S$, *x* H_2O **M_r 319.9** (anhydrous substance)

Other name: methylene blue

Definition

3,7-Bis(dimethylamino)phenothiazin-5-ylium chloride (methylene blue).

Content: 95.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: dark blue, crystalline powder with a copper-coloured sheen, or green crystals with a bronze-coloured sheen.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent).

Methylthioninium chloride is used in the treatment of methaemoglobinaemia (*e.g.* in nitrite poisoning). It has a mildly antiseptic action. It is also used as a bacteriological stain and as a dye in diagnostic procedures (*e.g.* chromoendoscopy).

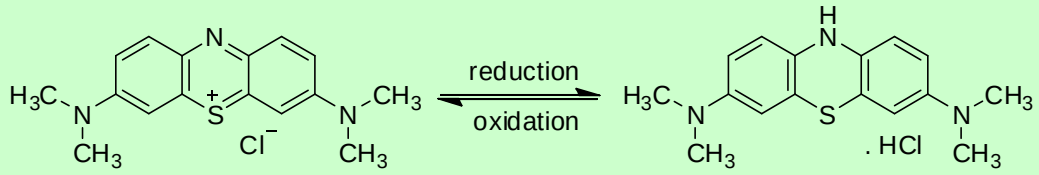
Identification

A. Ultraviolet and visible absorption spectrophotometry.

B. Thin-layer chromatography.

C. Dissolve about 1 mg in 10 ml of *water R*. Add 1 ml of *glacial acetic acid R* and 0.1 g of *zinc powder R*. Heat to boiling. The solution becomes colourless. Filter and shake the filtrate. It becomes blue in contact with air.

An aqueous solution of methylthioninium chloride is blue in an oxidizing environment, but its colour disappears on treatment with a reducing agent.



methylene blue **leucomethylene blue**

D. Ignite 50 mg with 0.5 g of *anhydrous sodium carbonate R.* Cool and dissolve the residue in 10 ml of *dilute nitric acid R*. Filter. The filtrate, without further addition of *dilute nitric acid R*, gives reaction (a) of chlorides*(2.3.1)*.

On ignition with sodium carbonate, methylene blue decomposes and sodium chloride is formed.

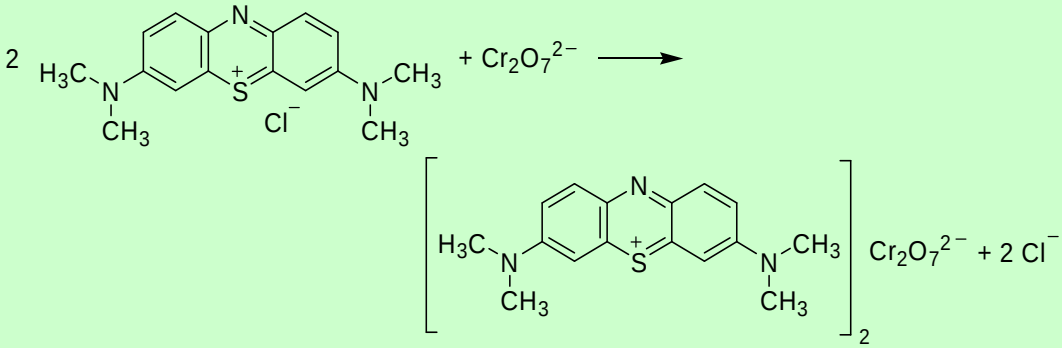
Assay

Dissolve 0.300 g in 30 ml of *water R* with heating. Cool, add 50.0 ml of *potassium dichromate solution R1* and dilute to 100.0 ml with *water R*. Allow to stand for 10 min. Filter and discard the first 20 ml of filtrate. Introduce 50.0 ml of the filtrate into a flask with a ground-glass neck, add 50 ml of *dilute sulfuric acid R* and 8.0 ml of *potassium iodide solution R*. Allow to stand protected from light for 5 min, then add 80 ml of

water R. Titrate with *0.1 M sodium thiosulfate* using 2 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 10.66 mg of $C_{16}H_{18}ClN_3S$.

Methylthioninium chloride gives a reddish-brown precipitate with dichromate. After the precipitate has been filtered off, the excess of dichromate is determined via iodometry.



$$Cr_2O_7^{2-} + 6\ I^- + 14\ H^+ \rightarrow 2\ Cr^{3+} + 3\ I_2 + 7\ H_2O$$

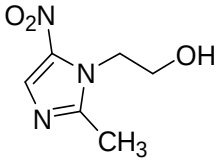
$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$C_{16}H_{18}ClN_3S\ (\%) = \frac{[V^{empty}_{Na_2S_2O_3}(ml) - V_{Na_2S_2O_3}(ml)] \cdot f_{Na_2S_2O_3} \cdot E\ (mg/ml) \cdot V_{bulb}\ (ml)}{\text{amount of substance (mg)} \cdot V_p\ (ml)} \cdot 100$$

where V_{bulb} is the volume of the stock solution and V_p is the volume of stock solution pipetted out for the assay.

METRONIDAZOLE

Metronidazolum



$C_6H_9N_3O_3$ **M_r 171.2**

Definition

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethanol.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or yellowish, crystalline powder.

Solubility: slightly soluble in water, in acetone, in alcohol and in methylene chloride.

Metronidazole, a synthetic antibacterial and antiprotozoal agent of the nitroimidazole class, is used against protozoa such as *Trichomonas vaginalis*, amoebiasis and giardiasis.

Identification

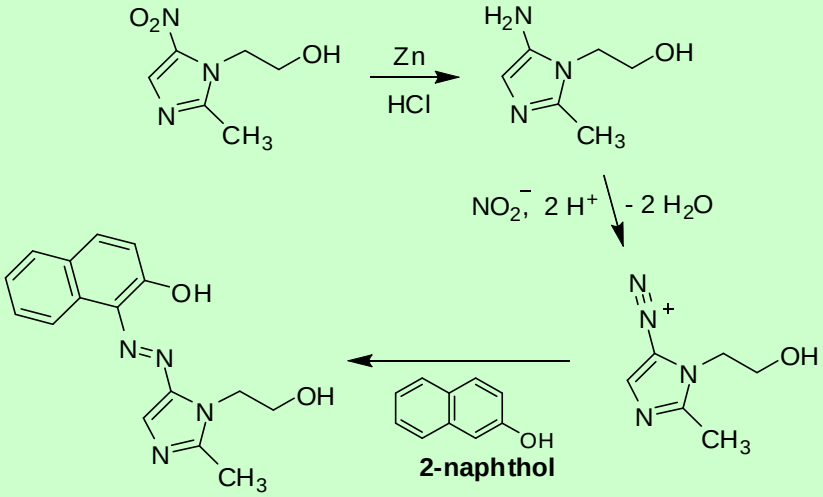
A. Melting point *(2.2.14)*: 159 °C to 163 °C.

B. Ultraviolet and visible absorption spectrophotometry

C. Infrared absorption spectrophotometr.

D. To about 10 mg add about 10 mg of *zinc powder R*, 1 ml of *water R* and 0.25 ml of *dilute hydrochloric acid R*. Heat on a water-bath for 5 min. Cool. The solution gives the reaction of primary aromatic amines *(2.3.1)*.

The nitro group of metronidazole is reduced with zinc–hydrochloric acid to a primary amino group, which is identified by the red azo dye reaction.

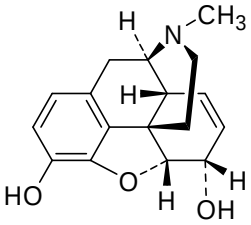


Informative test

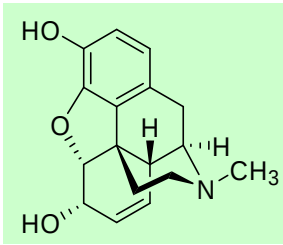
1. See identification **D** in the *Metronidazole* monograph.

MORPHINE HYDROCHLORIDE

Morphini hydrochloridum



, HCl, 3 H_2O



, HCl, 3 H_2O

$C_{17}H_{20}ClNO_3$. 3 H_2O **M_r 375.8**

Definition

7,8-Didehydro-4,5α-epoxy-17-methylmorphinane-3,6α-diol hydrochloride trihydrate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder or colourless, silky needles or cubical masses, efflorescent in a dry atmosphere.

Solubility: soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in toluene.

It is a narcotic analgesic.

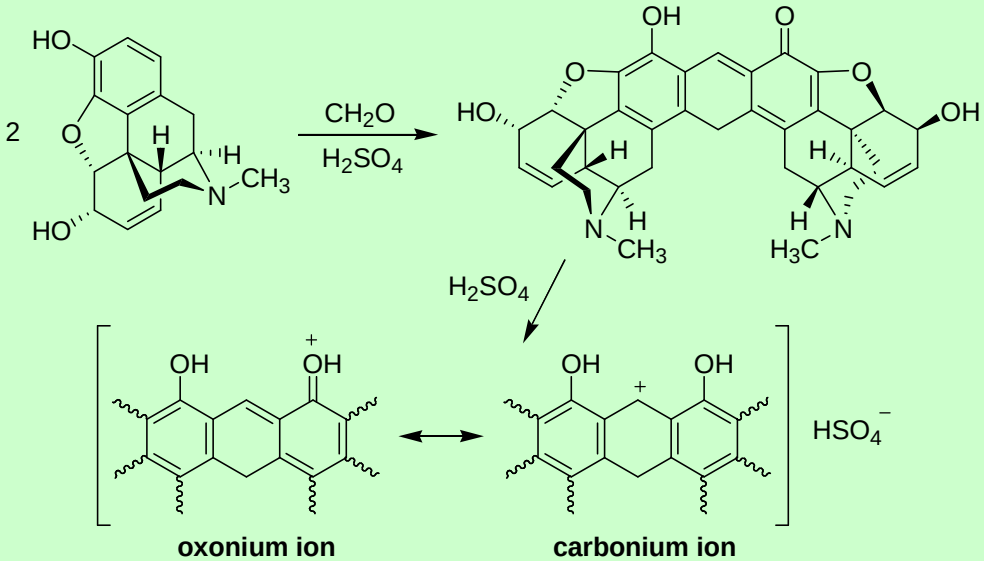
Identification

A. Infrared absorption spectrophotometry.

B. Ultraviolet and visible absorption spectrophotometry.

C. To about 1 mg of powdered substance in a porcelain dish add 0.5ml of sulf*uric acid-formaldehyde reagent R*. A purple colour develops and becomes violet.

Two equivalents of morphine react with two equivalents of formaldehyde. The polycyclic compound produced is a purple dinaphthoanthracene derivative, which with sulfuric acid forms a violet mesomerism-stabilized oxonium-carbonium ion (**MARQUIS** reaction). The reaction is suitable for distinguishing between morphine and codeine, because codeine gives a violet coloration immediately.



D. It gives the reaction of alkaloids *(2.3.1)*.

E. It gives reaction (a) of chlorides *(2.3.1)*.

Tests

Solution S. Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 or BY_6 *(2.2.2, Method II)*.

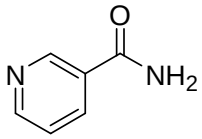
Informative tests

1. See the **Appearance of solution** test in the *Morphine hydrochloride* monograph.
2. See identification **C** in the *Morphine hydrochloride* monograph.
3. See identification **D** in the *Morphine hydrochloride* monograph.
4. Dissolve 10 mg of sample in 5.0 ml of *water R* made acidic with a few drops of *dilute nitric acid R*. Add a few drops of *silver nitrate solution R1* to the solution. A white precipitate is formed.

   A silver chloride precipitate is formed.

NICOTINAMIDE

Nicotinamidum



$C_6H_6N_2O$ **M_r 122.1**

Definition

Nicotinamide contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of pyridine-3-carboxamide, calculated with reference to the dried substance.

Characters

A white or almost white, crystalline powder or colourless crystals, freely soluble in water and in ethanol.

Nicotinamide is one of the two principal forms of the B-complex vitamin B_3.

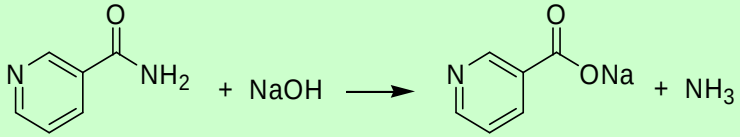
Identification

A. Melting point *(2.2.14):* 128 °C to 131 °C.

B. Infrared absorption spectrophotometry.

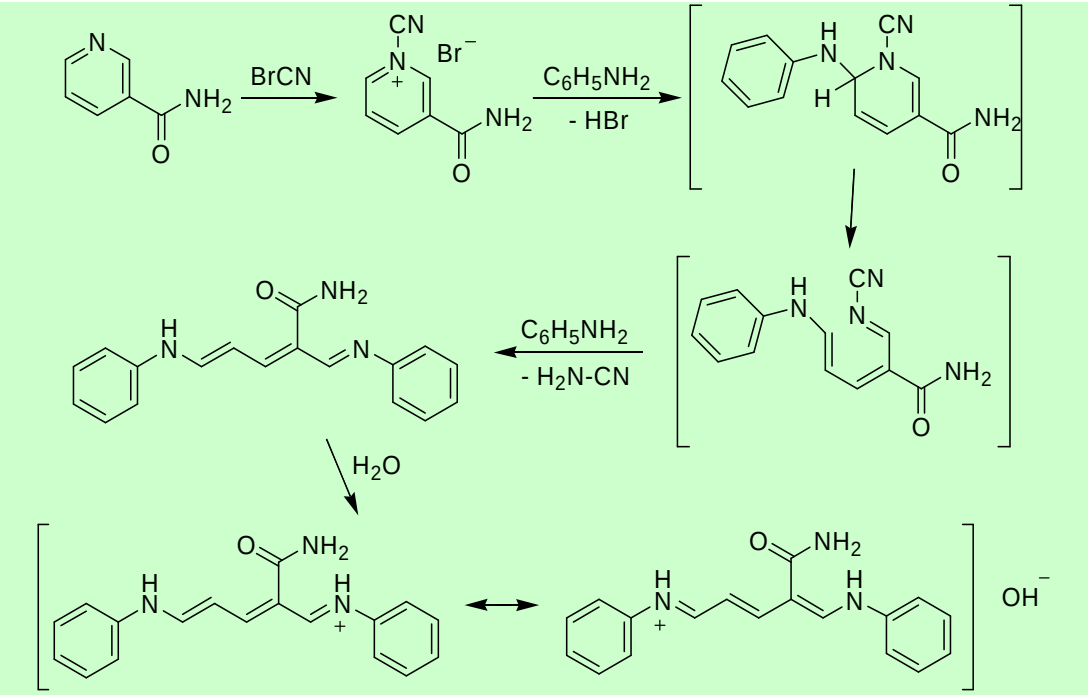
C. Boil 0.1 g with 1 ml of *dilute sodium hydroxide solution R*. Ammonia is evolved which is recognisable by its odour.

Alkaline hydrolysis of nicotinamide results in sodium nicotinate and ammonia.



D. Dilute 2 ml of solution S (see Tests) to 100 ml with *water R*. To 2 ml of the solution, add 2 ml of *cyanogen bromide solution R* and 3 ml of a 25 g/l solution of *aniline R* and shake. A yellow colour develops.

With cyanogen bromide, nicotinic acid forms 1-cyano-3-carboxypyridinium bromide. The electron-deficient carbon at position 2 of the latter pyridinium salt forms a phenylimino adduct with aniline, followed by ring opening of the pyridine ring. The next step is the transimination of the cyanoimine group with aniline, resulting in a yellow polymethine compound. The method is suitable for the identification 2-unsubstituted pyridine derivatives (**KÖNIG** reaction).



Tests

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

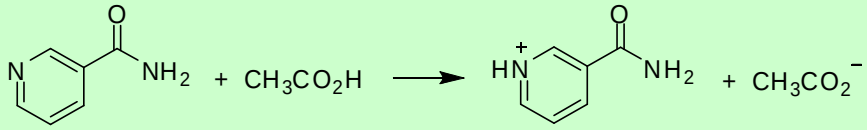
Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_7 *(2.2.2, Method II)*.

Assay

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R*, heating slightly if necessary, and add 5 ml of *acetic anhydride R.* Titrate with *0.1 M perchloric acid,* using *crystal violet solution R* as indicator until the colour changes to greenish-blue.

1 ml of *0.1 M perchloric acid* is equivalent to 12.21 mg of $C_6H_6N_2O$.

The slightly basic nicotinamide is determined in non-aqueous medium with perchloric acid.



$$HClO_4 + CH_3COOH \rightarrow ClO_4^- + CH_3COOH_2^+$$

$$CH_3COO^- + CH_3COOH_2^+ \rightarrow 2\ CH_3COOH$$

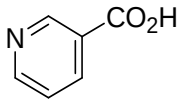
$$C_6H_6N_2O\ \text{content}\ (\%) = \frac{V_{HClO_4}(\text{ml}) \cdot f_{HClO_4} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative test

1. See the **Appearance of solution** test in the *Nicotinamide* monograph.
2. See identification **C** in the *Nicotinamide* monograph.

NICOTINIC ACID

Acidum nicotinicum



$C_6H_5NO_2$ ***M*r 123.1**

Definition

Pyridine-3-carboxylic acid.

Content: 99.5 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, soluble in boiling water and in boiling ethanol (96 per cent). It dissolves in dilute solutions of the alkali hydroxides and carbonates.

It is an antihyperlipidaemic and vasodilator agent. It is one of the two principal forms of the B-complex vitamin B_3.

Identification

A. Melting point *(2.2.14)*: 234 °C to 240 °C.

B. Infrared absorption spectrophotometry.

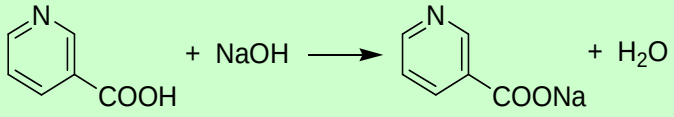
C. Ultraviolet and visible absorption spectrophotometry.

Assay

Dissolve 0.250 g in 50 ml of *water R*. Add 0.25 ml of *phenolphthalein solution R*. Titrate with *0.1 M sodium hydroxide* until a pink colour is obtained. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 12.31 mg of $C_6H_5NO_2$.

Nicotinic acid is determined as a monovalent acid via alkalimetry. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).



$$C_6H_5NO_2 \text{ content (\%)} = \frac{[V_{NaOH}\ (ml) - V_{NaOH}^{empty}\ (ml)] \cdot f_{NaOH} \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

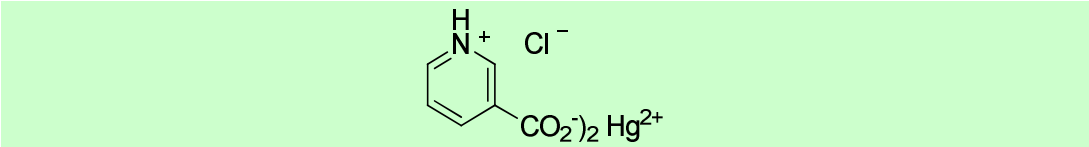
Informative tests

1. Dissolve 0.10 g in 10.0 ml of *carbon dioxide-free water R*. The solution changes the colour of *blue litmus paper R* to red.

The solution of nicotinic acid is acidic. The pH interval of the colour change of litmus paper is between 5 (red) and 8 (blue).

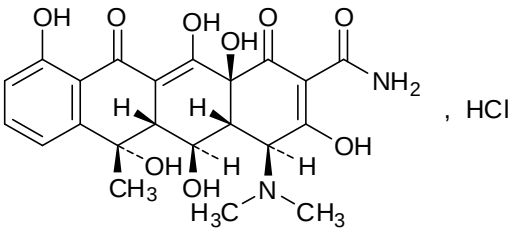
2. To 5.0 ml of the solution prepared for „Informative test 1”, add 2.5 ml of *mercury(II) chloride solution R*. A white precipitate separates out slowly.

A water-insoluble mercury(II) salt of nicotinic acid is formed.


H
N +
Cl −
CO2-)2 Hg2+

OXYTETRACYCLINE HYDROCHLORIDE

Oxytetracyclini hydrochloridum



, HCl

$C_{22}H_{25}ClN_2O_9$ ***M*r 496.9**

Other name: Oxytetracyclinium chloratum (Ph. Hg. VII.)

Definition

(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(Dimethylamino)-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide hydrochloride.

Substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

Characters

Appearance: yellow, crystalline powder, hygroscopic.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent). Solutions in water become turbid on standing, owing to the precipitation of oxytetracycline.

It is a broad-spectrum antibiotic.

Identification

A. Thin-layer chromatography.

B. To about 2 mg add 5 ml of *sulfuric acid R*. A deep red colour develops. Add the solution to 2.5 ml of *water R.* The colour becomes yellow.

Oxytetracycline gives a typical colour reaction with concentrated sulfuric acid, followed by dilution of the reaction mixture with water, which is suitable for distinction from tetracycline and chlorotetracycline (tetracycline gives a violet and then a yellow colour, while chlorotetracycline gives a dark-blue/olive-green and then a brown colour).

C. It gives reaction (a) of chlorides *(2.3.1)*.

Informative tests

1. See identification **B** in the *Oxytetracycline hydrochloride* monograph.

2. Dissolve 20 mg in 5.0 ml of *water R*. When 2-3 drops of *ferric chloride solution R2* are added to 1.0 ml of sample solution, a brown colour is observed.

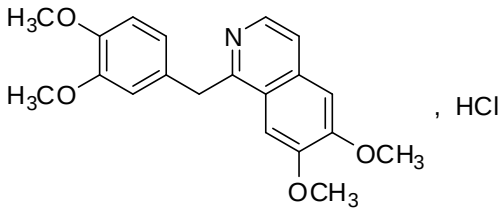
Because of its phenolic hydroxy group, oxytetracycline gives a brown complex with Fe^{3+}.

3. To a further 4.0 ml of the sample solution, add 2–3 drops of *dilute nitric acid R* and 4-5 drops of *silver nitrate solution R1*. A white precipitate is formed.

A silver chloride precipitate is formed.

PAPAVERINE HYDROCHLORIDE

Papaverini hydrochloridum



, HCl

$C_{20}H_{22}ClNO_4$ **M_r 375.9**

Definition

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride.

Content : 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or white or almost white crystals.

Solubility: sparingly soluble in water, slightly soluble in ethanol (96 per cent).

It is a spasmolytic agent. It has a direct vasodilating action on cardiac and smooth muscles and cerebral blood vessels.

Identification

A. Infrared absorption spectrophotometry.

B. Thin-layer chromatography.

C. To 10 ml of solution S (see Tests) add 5 ml of *ammonia R* dropwise and allow to stand for 10 min. The precipitate, washed and dried, melts *(2.2.14)* at 146 °C to 149 °C.

Ammonia liberates papaverine base.

D. It gives reaction (a) of chlorides *(2.3.1)*.

Tests

Solution S. Dissolve 0.4 g in *carbon dioxide-free water R*, heating gently if necessary, and dilute to 20 ml with the same solvent.

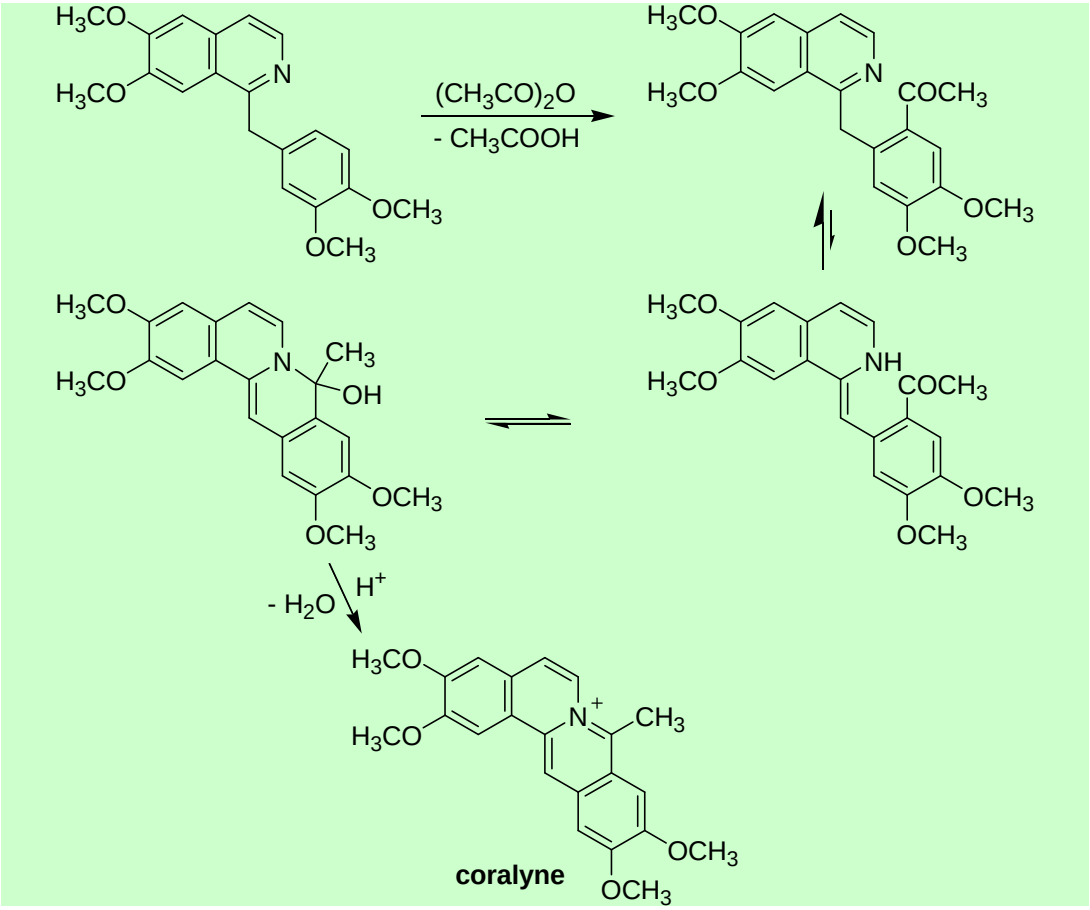
Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution BY_6 *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Papaverine hydrochloride* monograph.

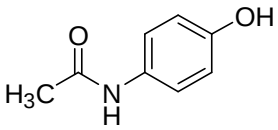
2. Dissolve 5 mg in 3.0 ml of *acetic anhydride R*. Heat the solution on a water bath for 3–4 min and add 5 drops of *sulfuric acid R* to the solution. An intense yellowish-green fluorescence appears, which remains if, after cooling, the mixture is carefully diluted with *alcohol R*.

Acetic anhydride acylates the 3,4-dimethoxyphenyl group of papaverine at position 6. An aminal-type derivative is formed by the tautomeric rearrangement of the C=N double bond, followed by attack of nitrogen on the carbonyl carbon of the acetyl substituent. The resulting aminal is converted to condensed tetracyclic fluorescent coralyne by water elimination (coralyne reaction).


H_3CO
H_3CO
N
OCH_3
OCH_3
$(CH_3CO)_2O$
$- CH_3COOH$
$COCH_3$
NH
CH_3
OH
$- H_2O$
H^+
N^+
coralyne

PARACETAMOL

Paracetamolum



$C_8H_9NO_2$ **M_r 151.2**

Definition

N-(4-Hydroxyphenyl)acetamide.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, freely soluble in alcohol, very slightly soluble in methylene chloride.

It is an anti-inflammatory and antipyretic agent.

Identification

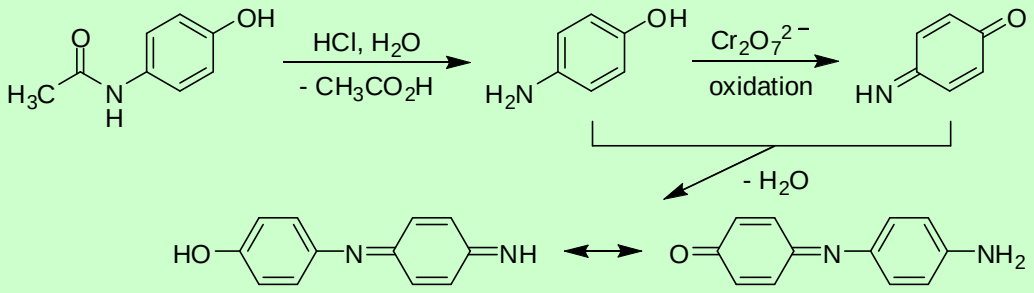
A. Melting point *(2.2.14)*: 168 °C to 172 °C.

B. Ultraviolet and visible absorption spectrophotometry.

C. Infrared absorption spectrophotometry.

D. To 0.1 g add 1 ml of *hydrochloric acid R*, heat to boiling for 3 min, add 1 ml of *water R* and cool in an ice bath. No precipitate is formed. Add 0.05 ml of a 4.9 g/l solution of *potassium dichromate R*. A violet colour develops which does not change to red.

Acidic hydrolysis of paracetamol results in 4-aminophenol, which is oxidized to 1,4-benzoquinone monoamine by dichromate. Quinonimine forms violet indaniline with 4-aminophenol.



indaniline

E. It gives the reaction of acetyl *(2.3.1)*. Heat over a naked flame.

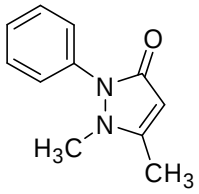
Informative tests

1. See identification **D** in the *Paracetamol* monograph.

2. Sparingly soluble in water, freely soluble in alcohol.

PHENAZONE

Phenazonum



$C_{11}H_{12}N_2O$ **M_r 182.2**

Definition

1,5-Dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very soluble in water, in ethanol (96 per cent) and in methylene chloride.

It is a non-steroidal anti-inflammatory agent, rarely used in human therapy. It is also used as an analytical reagent.

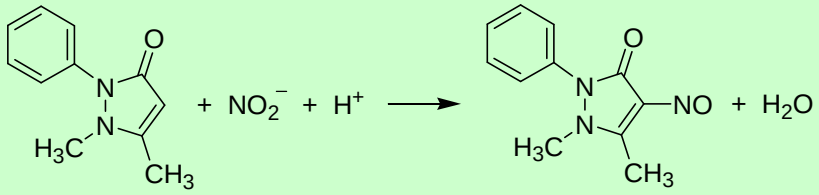
Identification

A. Melting point *(2.2.14)*: 109 °C to 113 °C.

B. Infrared absorption spectrophotometry.

C. To 1 ml of solution S (see Tests) add 4 ml of *water R* and 0.25 ml of *dilute sulfuric acid R*. Add 1 ml of *sodium nitrite solution R*. A green colour develops.

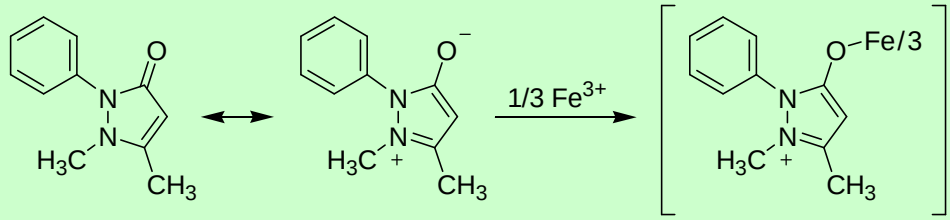
In acidic solution, green 4-nitrosophenazone is formed.



4-nitrosophenazone

D. To 1 ml of solution S add 4 ml of *water R* and 0.5 ml of *ferric chloride solution R2*. A red colour develops which is discharged on the addition of dilute *sulfuric acid R*.

The zwitterion enolate form of phenazone forms a red iron(III)–(phenazone)$_3$ complex with Fe^{3+}, which is decomposed by sulfuric acid.



red complex

Tests

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide;* the solution is red. Add 0.25 ml of *methyl red solution R* and 0.4 ml of *0.01 M hydrochloric acid*; the solution is red or yellowish-red.

An aqueous solution of phenazone is neutral or slightly acidic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red), while that of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

Chlorides *(2.4.4):* maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulfates *(2.4.13)*: maximum 100 ppm.

Dissolve 1.5 g in *distilled water R* and dilute to 15 ml with the same solvent.

Heavy metals *(2.4.8)*: maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

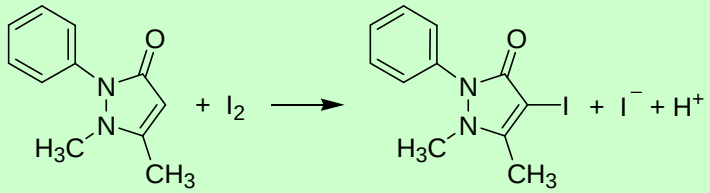
Assay

Dissolve 0.150 g in 20 ml of *water R*. Add 2 g of *sodium acetate R* and 25.0 ml of *0.05 M iodine*. Allow to stand protected from light for 30 min. Add 25 ml of *methylene chloride R* and shake until the precipitate dissolves. Titrate with *0.1 M sodium thiosulfate*, using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 9.41 mg of $C_{11}H_{12}N_2O$.

In slightly alkaline solution (buffered with sodium acetate), phenazone reacts with iodine used in excess, resulting in the less water-soluble 4-iodophenazone. 4-Iodophenazone is eliminated by dichloromethane extraction and the excess of iodine is determined with thiosulfate. Starch must be added nearly at the end of titration (pale-yellow colour).

1 ml of *0.1 M sodium thiosulfate* is equivalent to 9.41 mg of $C_{11}H_{12}N_2O$.



4-iodophenazone

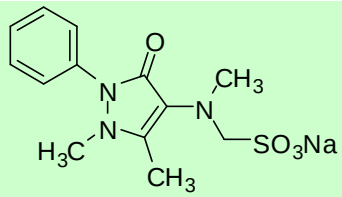
$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$C_{11}H_{12}N_2O\text{-cont. (\%)} = \frac{[V^{empty}_{Na_2S_2O_3}(ml) - V_{Na_2S_2O_3}(ml)]\ .\ f_{Na_2S_2O_3}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative tests

1. See the **Appearance of solution** test in the *Phenazone* monograph.
2. See identification **C** in the *Phenazone* monograph.
3. See identification **D** in the *Phenazone* monograph.
4. On the addition of 1.0 ml of *nitric acid R* to 0.5 ml of solution S, the solution must be colourless (a distinction from metamisol sodium).

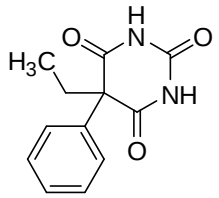
Metamisol sodium gives a transient blue colour with concentrated nitric acid.



metamisol sodium

PHENOBARBITAL

Phenobarbitalum



$C_{12}H_{12}N_2O_3$ ***M*r 232.2**

Definition

5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very slightly soluble in water, freely soluble in ethanol (96 per cent). It forms water-soluble compounds with alkali hydroxides, carbonates and ammonia.

It is a sedative, hypnotic and anticonvulsant drug.

Identification

A. Determine the melting point *(2.2.14)* of the substance to be examined. Mix equal parts of the substance to be examined and *phenobarbital CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 176 °C) is not greater than 2 °C.

B. Infrared absorption spectrophotometry.

C. Thin-layer chromatography.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

Tests

Appearance of solution. The solution is clear (2.2.1) and not intensively coloured than reference solution Y_6 (2.2.2, Method II)

Dissolve 1.0 g in a mixture of 4 ml of *dilute sodium hydroxide solution R* and 6 ml of *water R*.

Acidity. Boil 1.0 g with 50 ml of *water R* for 2 min, allow to cool and filter. To 10 ml of the filtrate add 0.15 ml of *methyl red solution R*. The solution is orange-yellow. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to produce a pure yellow colour.

The solution of phenobarbital prepared by boiling in water, followed by cooling and filtration, is weakly acidic. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

Informative tests

1. See the **Appearance of solution** test in the *Phenobarbital* monograph.

2. See **Acidity** test in the *Phenobarbital* monograph.

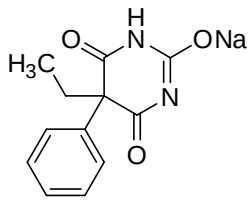
3. See identification **D** in the *Phenobarbital* monograph.

4. Dissolve 0.10 g in a mixture of 5.0 ml of *water R* and 8–10 drops of *dilute sodium hydroxide solution R* and add 1.0 ml of a 100 g/l solution of *citric acid monohydrate R*. An opulent, white precipitate is formed (a difference from *Barbital*).

Phenobarbital is less soluble than barbital in water, and it therefore precipitates from a solution acidified with citric acid.

PHENOBARBITAL SODIUM

Phenobarbitalum natricum



$C_{12}H_{11}N_2NaO_3$ M_r 254.2

Definition

Phenobarbital sodium contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of the sodium derivative of 5-ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, calculated with reference to the dried substance.

Characters

A white or almost white, crystalline powder, hygroscopic, freely soluble in carbon dioxide-free water (a small fraction may be insoluble), soluble in alcohol, practically insoluble in methylene chloride.

It is a sedative, hypnotic and anticonvulsant drug.

Identification

A. Acidify 10 ml of solution S (see Tests) with *dilute hydrochloric acid R* and shake with 20 ml of *ether R*. Separate the ether layer, wash with 10 ml of *water R*, dry over anhydrous *sodium sulfate R* and filter. Evaporate the filtrate to dryness and dry the residue at 100 °C to 105 °C. Determine the melting point *(2.2.14)* of the test residue. Mix equal parts of the residue and of *phenobarbital CRS* and determine the melting point of the mixture. The difference between the two melting points (which are about 176 °C) is not greater than 2 °C.

Phenobarbital is liberated from the acidified solution. After extraction with ether, phenobarbital is identified by melting point determination.

B. Infrared absorption spectrophotometry.

C. Thin-layer chromatography.

D. It gives the reaction of non-nitrogen substituted barbiturates *(2.3.1)*.

E. It gives reaction (a) of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 5.0 g in *alcohol (50 per cent V/V) R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

pH *(2.2.3)*. Dissolve 5.0 g as completely as possible in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent. The pH of the solution is not greater than 10.2.

Informative tests

1. The sample burns with a sooty flame; the residue imparts a persistent, vivid-yellow colour to the flame.

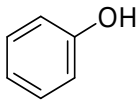
The colour of the flame of the residue of the incinerated sample is characteristic of sodium.

2. See the **Appearance of solution** test in the *Phenobarbital sodium* monograph.

3. See identification **D** in the *Phenobarbital sodium* monograph.

PHENOL

Phenolum



C_6H_6O **M_r 94.1**

Definition

Content: 99.0 per cent to 100.5 per cent.

Characters

Appearance: colourless or faintly pink or faintly yellowish, crystals or crystalline masses, deliquescent.

Solubility: soluble in water, very soluble in ethanol (96 per cent), in glycerol and in methylene chloride.

It is a disinfectant, previously known as carbolic acid.

Identification

A. Dissolve 0.5 g in 2 ml of *concentrated ammonia R*. The substance dissolves completely. Dilute to about 100 ml with *water R*. To 2 ml of the dilute solution add 0.05 ml of *strong sodium hypochlorite solution R*. A blue colour develops and becomes progressively more intense.

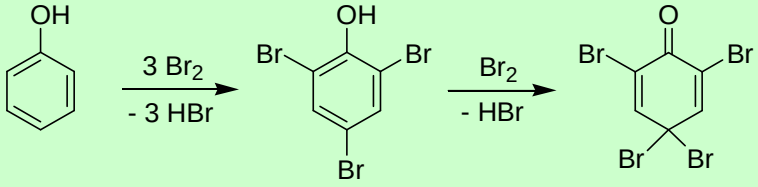
Hypochlorite oxidizes phenol to coloured quinone-imine-type compounds in the presence of ammonia.

B. To 1 ml of solution S (see Tests) add 10 ml of *water R* and 0.1 ml of *ferric chloride solution R1*. A violet colour is produced which disappears on addition of 5 ml of *2-propanol R*.

The violet ferrihexaphenolate complex ($[Fe(OC_6H_5)_6]^{3-}$) is formed, which decomposes on addition of 2-propanol.

C. To 1 ml of solution S add 10 ml of *water R* and 1 ml of *bromine water R*. A white precipitate is formed.

Bromination of phenol results in the water-insoluble, colourless 2,4,6-tribromophenol and yellow 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one.



colourless **yellow**

Tests

Solution S. Dissolve 1.0 g in *water R* and dilute to 15 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Acidity. To 2 ml of solution S add 0.05 ml of *methyl orange solution R*. The solution is yellow.

The aqueous solution of phenol is slightly acidic. The pH interval of the colour change of the methyl orange indicator is between 3.0 (red) and 4.4 (yellow).

Assay

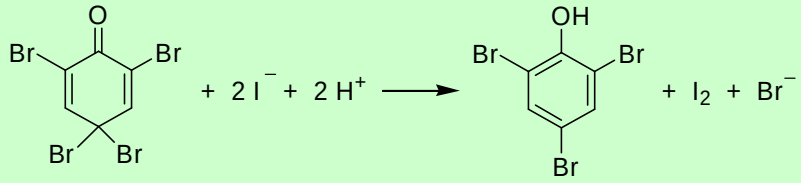
Dissolve 2.000 g in *water R* and dilute to 1000.0 ml with the same solvent. Transfer 25.0 ml of the solution to a ground-glass-stoppered flask and add 50.0 ml of *0.0167 M bromide-bromate* and 5 ml of *hydrochloric acid R*, close the flask, allow to stand with occasional swirling for 30 min. Then allow to stand for a further

15 min. Add 5 ml of a 200 *g/l* solution of *potassium iodide R*, shake and titrate with *0.1 M sodium thiosulfate* until a faint yellow colour remains. Add 0.5 ml of *starch solution R* and 10 ml of *chloroform R* and continue the titration with vigorous shaking. Carry out a blank titration.

1 ml of *0.0167 M bromide-bromate* is equivalent to 1.569 mg of C_6H_6O.

Bromination of phenol results in the water-insoluble, colourless 2,4,6-tribromophenol and yellow 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one via electrophilic substitution followed by oxidation (see test **C** in the monograph "Phenol"). The excess of bromine and the resulting 2,4,4,6-tetrabromo-2,5-cyclohexadien-1-one oxidize iodide to iodine, which can be titrated with thiosulfate in the presence of starch indicator (**KOPPESCHAAR** phenol assay). Chloroform is necessary to extract the water-insoluble 2,4,6-tribromophenol.

1 ml of 0.1 M sodium thiosulfate is equivalent to 1.569 mg of C_6H_6O.



$$BrO_3^- + 5\ Br^- + 6\ H^+ \rightarrow 3\ Br_2 + 3\ H_2O$$

$$Br_2 + 2\ I^- \rightarrow I_2 + 2\ Br^-$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$C_6H_6O\ \text{cont.}\ (\%) = \frac{[V^{\text{empty}}_{Na_2S_2O_3}(\text{ml}) - V_{Na_2S_2O_3}(\text{ml})]\ .\ f_{Na_2S_2O_3}\ .\ E\ (\text{mg/ml})\ .\ V_{bulb}}{\text{amount of substance (mg)}\ .\ V_{pipetted}}\ .\ 100$$

where V_{bulb} is the volume of the stock solution and $V_{pipetted}$ is the volume of stock solution pipetted out for the assay.

Informative tests

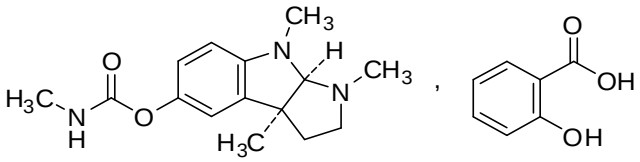
1. See the **Appearance of solution** test in the *Phenol* monograph.

2. See identification **A** in the *Phenol* monograph.

PHYSOSTIGMINE SALICYLATE

Physostigmini salicylas

Eserini salicylas



$C_{22}H_{27}N_3O_5$ **M_r 413.5**

Definition

Physostigmine salicylate contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of (3a*S*,8a*R*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-yl methylcarbamate salicylate, calculated with reference to the dried substance.

Characters

Colourless or almost colourless crystals, sparingly soluble in water, soluble in alcohol, very slightly soluble in ether. The crystals gradually become red when exposed to air and light; the colour develops more quickly when the crystals are also exposed to moisture. Aqueous solutions are unstable.

It melts at about 182 °C, with decomposition.

Physostigmine is a parasympathomimetic: specifically, a reversible cholinesterase inhibitor. It is used in the treatment of glaucoma.

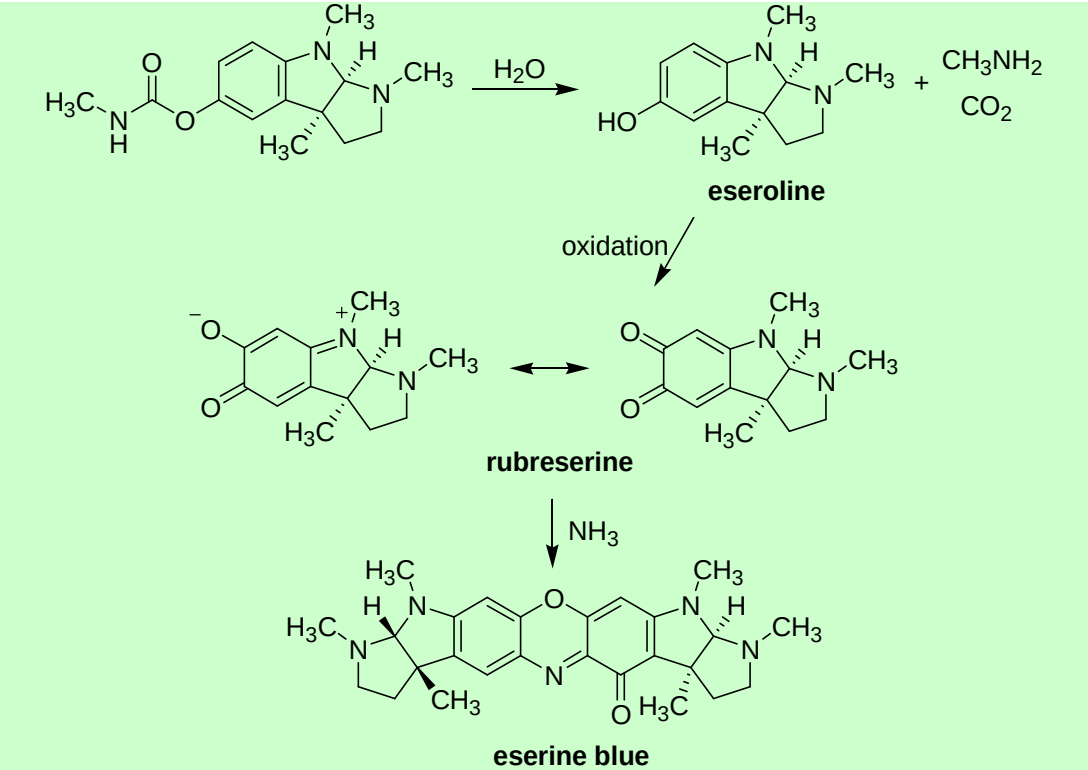
Identification

A. Infrared absorption spectrophotometry.

B. Thin-layer chromatography.

C. Heat about 10 mg in a porcelain dish with a few drops of *dilute ammonia R1*. An orange colour develops. Evaporate the solution to dryness. The residue dissolves in *alcohol R* giving a blue solution. Add 0.1 ml of *glacial acetic acid R*. The colour becomes violet. Dilute with *water R*. An intense red fluorescence appears.

Sodium hydroxide hydrolyses physostigmine to eseroline, which is oxidized to red rubreserine by the air. With ammonia, rubreserine forms eserine blue, which dissolves in acetic acid to give a violet colour.



D. Solution S (see Tests) gives reaction (a) of salicylates *(2.3.1)*.

Tests

Solution S. Dissolve 0.900 g, without heating, in 95 ml of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent. Prepare immediately before use.

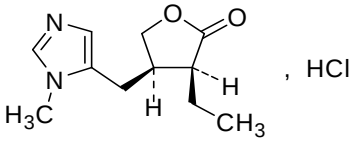
Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Physostigmine salicylate* monograph.
2. See identification **C** in the *Physostigmine salicylate* monograph.
3. See identification **D** in the *Physostigmine salicylate* monograph.

PILOCARPINE HYDROCHLORIDE

Pilocarpini hydrochloridum



, HCl

$C_{11}H_{17}ClN_2O_2$ **M_r 244.7**

Definition

(3*S*,4*R*)-3-Ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appoearance: white or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility: very soluble in water and freely soluble in ethanol (96 per cent).

mp: about 203 °C.

Pilocarpine is a cholinergic parasympathomimetic, used to treat glaucoma and dry eye syndrome.

Identification

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry.

C. Thin-layer chromatography.

D. It gives reaction (a) of chlorides *(2.3.1)*.

Tests

Solution S. Dissolve 2.50 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_7 *(2.2.2, Method II)*.

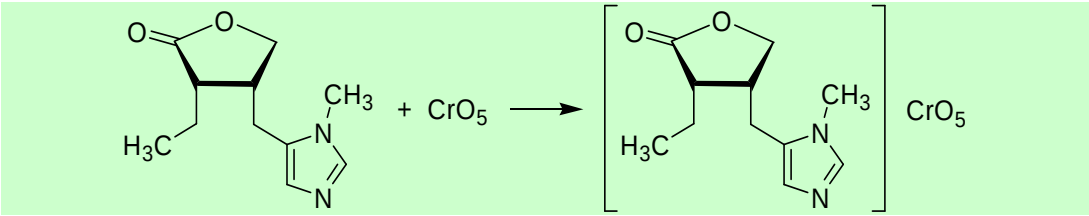
Specific optical rotation *(2.2.7)*: +89 to +93 (dried substance), determined on solution S.

Informative tests

1. See the **Appearance of solution** test in the *Pilocarpine hydrochloride* monograph.
2. Dilute 0.2 ml of solution S (see Tests) to 2 ml with *water R*. Add 0.05 ml of a 50 g/l solution of *potassium dichromate R*, 1 ml of *dilute hydrogen peroxide solution R* and 2 ml of *methylene chloride R* and shake. A violet colour develops in the organic layer.

Dichromate forms chromium(VI) oxide peroxide in acidified hydrogen peroxide solution. With pilocarpine, chromium(VI) oxide peroxide forms an adduct, which is soluble in dichloromethane. All nitrogen-containing organic compounds which are soluble both in water and in organic solvents and are non-oxidizable by hydrogen peroxide give this test (**HELCH** reaction).

$$Cr_2O_7^{2-} + 4\ H_2O_2 + 2\ H^+ \rightarrow 2\ CrO_5 + 5\ H_2O$$



3. Add a few drops of *dilute nitric acid R* and 2.0 ml of *silver nitrate solution R1* to 1.0 ml of solution S. A white precipitate is formed.

A silver chloride precipitate is formed.

POTASSIUM BROMIDE

Kalii bromidum

KBr M_r **119.0**

Definition

Content: 98.5 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water and in glycerol, slightly soluble in ethanol (96 per cent).

It has a sedative effect. An overdosage of bromide can cause the symptoms of brominism.

Identification

A. It gives reaction (a) of bromides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of potassium bromide is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Bromates. To 10 ml of solution S add 1 ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium iodide R* and 0.25 ml of *0.5 M sulfuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

Bromate oxidizes iodide to iodine via bromine formation under acidic conditions (see the test for bromates in the *Ammonium bromide* monograph), and the iodine can be detected as the blue iodine–starch complex.

Iodides. To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml *methylen chloride R*. Shake and allow to separate. The chloroform layer is colourless (*2.2.2, Method I*).

Fe^{3+} oxidizes iodide to iodine and the methylene chloride phase becomes violet.

$$2\ I^- + 2\ Fe^{3+} \rightarrow I_2 + 2\ Fe^{2+}$$

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. See the **Appearance of solution** test in the *Potassium bromide* monograph.

2. Add 0.10 g of *sodium acetate R* and 0.10 g of *tartaric acid R* to 2.0 ml of solution S. A white, crystalline precipitate is formed.

Tartaric acid precipitates white potassium hydrogentartrate. See 2nd "Informative test" in the *Potassium chloride* monograph.

3. To 1.0 ml of solution S, add 4-5 drops of *hydrochloric acid R*, 2.0 ml of *chloroform R* and 2 drops of *chloramine solution*, and shake the mixture. The chloroform layer attains a yellow colour; when more *chloramine solution* is added, the chloroform layer turns orange-red.

See 2nd "Informative test" in the *Ammonium bromide* monograph.

POTASSIUM CARBONATE

Kalii carbonas

K_2CO_3 **M_r 138.2**

Definition

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white granular powder, hygroscopic.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent).

It is an analytical reagent, and it is also used for the preparation of medicines applied for rehydration and alkalinization.

Identification

A. Dissolve 1 g in 10 ml of *water R*. The solution is strongly alkaline (*2.2.4*).

B. 2 ml of the solution prepared for identification test A gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 ml of the solution prepared for identification test A gives reaction (b) of potassium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in 25 ml of distilled *water R*. Slowly add 14 ml of *hydrochloric acid R*. When the effervescence has ceased, boil for a few minutes. Allow to cool and dilute to 50 ml with *distilled water R*.

Carbon dioxide is formed. Solution S contains hydrochloric acid and potassium chloride.

Appearance of solution. Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Chlorides (*2.4.4*): maximum 100 ppm.

Dissolve 0.50 g in 10 ml of *water R*. Carefully add dropwise 1 ml of *nitric acid R*. Boil. Cool, add 5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum100 ppm.

Dilute 7.50 ml of solution S to 15 ml with *distilled water R*.

Calcium (*2.4.3*): maximum 100 ppm.

To 5 ml of solution S add 1 ml of *concentrated ammonia R*. Boil. Cool. Dilute to 15 ml with *distilled water R*.

Iron (*2.4.9*): maximum 10 ppm.

Dilute 5 ml of solution S to 10 ml with *water R.*

Heavy metals (*2.4.8*): maximum 20 ppm.

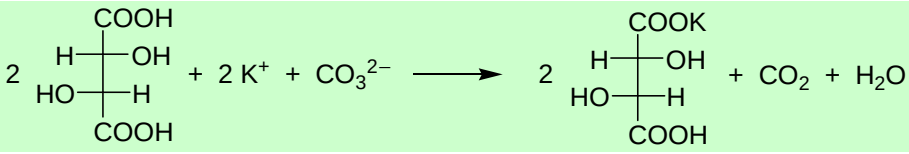
Dilute 10 ml of solution S to 20 ml with *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution* (*2 ppm Pb*) *R*.

Informative test

1. See identification **A** in the *Potassium carbonate* monograph.

2. Add 0.20 g of *tartaric acid R* to 1.0 ml of solution S. Besides the evolution of a colourless gas, a white, crystalline precipitate is produced.

Tartaric acid precipitates white potassium hydrogentartrate. Because of the basicity of potassium carbonate solution, it is not necessary to add sodium acetate to the solution.



3. Add 2.0 ml of *water R* and 1 ml of the solution of *magnesium sulfate R* (123 g/l) to 2.0 ml of solution S. A white precipitate is formed.

Light magnesium carbonate is formed.

$$4\ CO_3^{2-} + 4\ Mg^{2+} + H_2O \rightarrow Mg(OH)_2.3MgCO_3 + CO_2$$

POTASSIUM CHLORIDE

Kalii chloridum

KCl M_r **74.6**

Definition

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, practically insoluble in anhydrous *ethanol (96 per cent)*.

It is used for the treatment of hypopotassaemia (*e.g.* loss of potassium in the case of vomiting and diarrhoea). Hyperpotassaemia can cause arrhythmia.

Identification

A. It gives the reactions of chlorides (2.3.1).

B. Solution S (see Tests) gives the reactions of potassium (2.3.1).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 50 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of potassium chloride is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Iodides. Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 ml of *sodium nitrite solution R*, 2 ml of *0.5 M sulfuric acid*, 25 ml of *iodide-free starch solution R* and 25 ml of *water R*. After 5 min, examine in daylight. The substance shows no blue colour.

Nitrite oxidizes iodide to iodine under acidic conditions, and the iodine can be detected as the blue iodine–starch complex.

$$2\ I^- + 2\ NO_2^- + 4\ H^+ \rightarrow I_2 + 2\ NO + 2\ H_2O$$

Sulfates (*2.4.13*): maximum 300 ppm.

Dilute 5 ml of solution S to 15 ml with *distilled water R*.

Barium. To 5 ml of solution S add 5 ml of *distilled water R* and 1 ml of *dilute sulfuric acid R*. After 15 min, any opalescence in the solution is not more intense than that in a mixture of 5 ml of solution S and 6 ml of *distilled water R*.

A precipitate of barium sulfate is formed.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

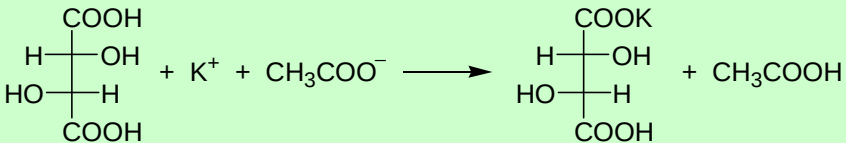
Magnesium and alkaline-earth metals (*2.4.7*): maximum 200 ppm, calculated as Ca, determined on 10.0 g using 0.15 g of mordant black 11 triturate R. The volume of 0.01 M sodium edentate used does not exceed 5.0 ml..

Informative test

1. See the **Appearance of solution** test in the *Potassium chloride* monograph.

2. Add 0.10 g of *sodium acetate R* and 0.10 g of *tartaric acid R* to 2.0 ml of solution S. A white, crystalline precipitate is formed.

Tartaric acid precipitates white potassium hydrogentartrate. As potassium hydrogentartrate is soluble in strong acids, sodium acetate buffer is used (weakly acidic acetic acid is liberated).



3. Dilute 1.0 ml of solution S with 4.0 ml of *water R* and 1.0 ml of *dilute nitric acid R*. Add 4.0 ml of *silver nitrate solution R.* A curdled, white precipitate is formed, which dissolves in an excess of *ammonia solution R2*.

A silver chloride precipitate is formed.

POTASSIUM HYDROGEN CARBONATE

Kalii hydrogenocarbonas

$KHCO_3$ **M_r 100.1**

Definition

Content: 99.0 per cent to 101.0 per cent.

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent).

When heated in the dry state or in solution, it is gradually converted to potassium carbonate.

It is used for the treatment of metabolic acidosis.

Identification

A. To 5 ml of solution S (see Tests) add 0.1 ml of *phenolphthalein solution R*. A pale pink colour is produced. Heat; gas is evolved and the colour becomes red.

The aqueous solutions of alkali metal hydrogencarbonates are weakly basic (pH ~9); the colour of phenolphthalein is pale-pink. Boiling causes the loss of carbon dioxide; hence, the carbonate concentration increases (pH >10) and the solution becomes red.

$$2\ HCO_3^- \rightarrow CO_3^{2-} + CO_2 + 3\ H_2O$$

B. It gives the reaction of carbonates and bicarbonates (*2.3.1*).

C. 1 ml of solution S gives reaction (b) of potassium (*2.3. 1*).

Tests

Solution S. Dissolve 5.0 g in 90 ml of *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Carbonates. The pH (*2.2.3*) of freshly prepared solution S is not greater than 8.6.

Chlorides (*2.4.4*): maximum 150 ppm.

Dilute 7 ml of solution S to 15 ml with *dilute nitric acid R*.

As carbonate and hydrogencarbonate give a precipitate with silver nitrate, they must be eliminated with nitric acid.

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

Sulfates (*2.4.13*): maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *acetic acid R*. Prepare the standard using a mixture of 7.5 ml of *sulfate standard solution* (*10 ppm SO_4*) *R* and 7.5 ml of *distilled water R*.

Ammonium (*2.4.1*): maximum 20 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Calcium (*2.4.3*): maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *acetic acid R*. Prepare the standard using 5 ml of *calcium standard solution* (*10 ppm Ca*) *R* and 10 ml of *distilled water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

Dissolve 2.0 g in a mixture of 2 ml of *hydrochloric acid R* and 18 ml of *water R*. 12 ml of the solution complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Iron (*2.4.9*): maximum 20 ppm, determined on solution S.

Informative test

1. See the **Appearance of solution** test in the *Potassium hydrogen carbonate* monograph.

2. See identification **A** in the *Potassium hydrogen carbonate* monograph.

3. See identification **B** in the *Potassium hydrogen carbonate* monograph.

POTASSIUM IODIDE

Kalii iodidum

KI M_r **166.0**

Definition

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white powder or colourless crystals.

Solubility: very soluble in water, freely soluble in glycerol, soluble in ethanol (96 per cent).

Iodide used internally decreases arteriosclerosis and has a secretion-relieving effect. Iodide is essential for the biosynthesis of thyroxine (thyroid gland hormone).

Identification

A. Solution S (see Tests) gives the reactions of iodides (*2.3.1*).

B. Solution S gives the reactions of potassium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity. To 12.5 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

The aqueous solution of potassium iodide is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Iodates. To 10 ml of solution S add 0.25 ml of *iodide-free starch solution R* and 0.2 ml of *dilute sulfuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Iodate reacts with iodide to form iodine, which can be detected as the blue iodine–starch complex.

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

The above reaction is also appropriate to determine impurities (*e.g.* Fe^{3+}, Cu^{2+} or NO^{2-}) which oxidize iodide to iodine under acidic conditions.

Sulfates (*2.4.13*):maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Thiosulfates. To 10 ml of solution S add 0.1 ml of *starch solution R* and 0.1 ml of *0.005 M iodine*. A blue colour is produced.

The formation of the blue iodine–starch complex fails because thiosulfate reduces iodine to iodide.

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Assay

Dissolve 1.500 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 40 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to

yellow. Add 5 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolourised.

1 ml of *0.05 M potassium iodate* is equivalent to 16.60 mg of KI.

In acidic medium, iodate oxidizes iodide in two steps: first to iodine, which dissolves in chloroform to give a violet colour, and then to almost colourless iodochloride, in which iodine has oxidation state +1. Strong shaking is necessary, because iodine dissolves better in chloroform than in water.

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

$$IO_3^- + 2\ I_2 + 5\ Cl^- + 6\ H^+ \rightarrow 5\ ICl + 3\ H_2O$$

Combining the above equations:

$$IO_3^- + 2\ I^- + 3\ Cl^- + 6\ H^+ \rightarrow 3\ ICl + 3\ H_2O$$

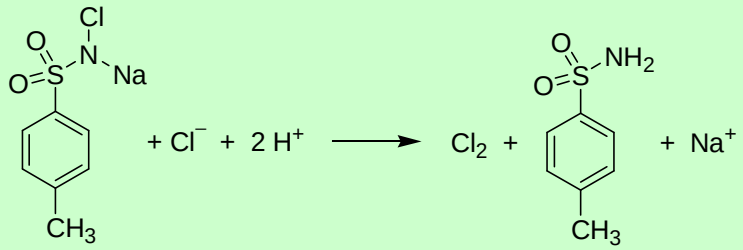
$$\text{KI content (\%)} = \frac{V_{KIO_3}\ (ml)\ .\ f_{KIO_3}\ .\ E\ (mg/ml)\ .\ V_{bulb}\ (ml)}{\text{amount of compound (mg)}\ .\ V_{pipetted}\ (ml)}\ .\ 100$$

where V_{bulb} is the volume of the stock solution and $V_{pipetted}$ is the volume of the stock solution pipetted out for the assay.

Informative test

1. See the **Appearance of solution** test in the *Potassium iodide* monograph.
2. See identification **B** in the *Potassium iodide* monograph.
3. To 1.0 ml of solution S, add 4-5 drops of *hydrochloric acid R,* 2.0 ml of *chloroform R* and 2 drops of *chloramine solution*, and shake. The chloroform layer attains a violet colour; when more *chloramine solution* is added, the chloroform layer becomes colourless.

Chloramine (*N*-chloro-4-methylbenzenesulfonamide sodium) is generally used as chlorine source; it forms chlorine with chlorides in acidic solution.



Chlorine liberates violet iodine (in chloroform), which forms colourless iodate with an excess of chlorine.

$$2\ I^- + Cl_2 \rightarrow I_2 + 2\ Cl^-$$

$$I_2 + 5\ Cl_2 + 6\ H_2O \rightarrow 2\ IO_3^- + 10\ Cl^- + 12\ H^+$$

POTASSIUM NITRATE

Kalii nitras

KNO_3 **M_r 101.1**

Definition

Content: 99.0 to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, very soluble in boiling water, practically insoluble in ethanol (96 per cent).

It is used for the preservation of foods and medicines.

Identification

A. It gives the reaction of nitrates (*2.3.1*).

B. Solution S (see Tests) gives the reactions of potassium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.05 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of potassium nitrate is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Reducible substances. To 10 ml of solution S, add 0.5 ml of *dilute sulfuric acid R* and 2 ml of *zinc iodide and starch solution R*. The solution does not become blue within 2 min.

Reducible substances (*e.g.* Fe^{3+} or Cu^{2+}) reduce iodide to iodine, which can be detected as the blue iodine–starch complex.

Chlorides (*2.4.4*): maximum 20 ppm, if intended for ophthalmic use.

Dissolve 2.5 g in *water R* and dilute to 15 ml with the same solvent.

Sulfates (*2.4.13*): maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Ammonium (*2.4.1*): maximum 100 ppm, determined in 1 ml of solution S, maximum 50 ppm if intended for ophthalmic use.

Calcium (*2.4.3*): maximum 100 ppm; maximum 50 ppm if intended for ophthalmic use.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Iron (*2.4.9*): maximum 20 ppm; maximum 10 ppm if intended for ophthalmic use.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. See the **Appearance of solution** test in the *Potassium nitrate* monograph.

2. Add 0.10 g of *sodium acetate R* and 0.10 g of *tartaric acid R* to 2.0 ml of solution S. A white, crystalline precipitate is formed.

See the explanation of the 2nd "Informative test" in the *Potassium chloride* monograph.

3. To a sample of *ca.* 50 mg, add 5 drops of *dilute sulfuric acid R,* and then dissolve it in 2.0 ml of *sulfuric acid R*. Cautiously introduce 3.0 ml of *iron(II) sulfate solution R2* over the cooled solution to form an upper layer. A dark-brown ring is produced at the junction of the two liquids.

See the explanation of the 3rd "Informative test" in the *Silver nitrate* monograph (brown ring test).

POTASSIUM PERCHLORATE

Kalii perchloras

$KClO_4$ **M_r 138,6**

Definition

Content: 99.0 per cent to 102.0 per cent.

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

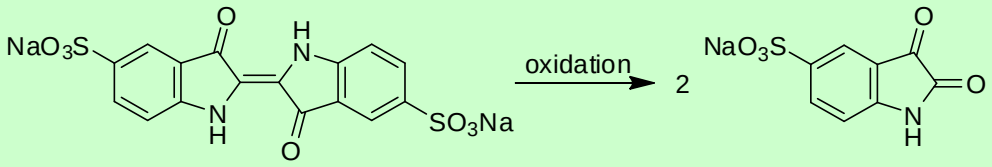
Solubility: sparingly soluble in water, practically insoluble in ethanol (96 per cent).

Perchlorate inhibits the accumulation of iodide in the thyroid gland. It is therefore used in cases of a hyperfunction (in hyperthyroidism).

Identification

A. Dissolve 0.1 g in 5 ml of *water R*. Add 5 ml of *indigo carmine solution R* and heat to boiling. The colour of the solution does not disappear.

Some oxidative agents (*e.g.* nitrate, chlorate or hypochlorite) oxidize indigo carmine to a colourless isatin derivative. Perchlorate salts do not oxidize indigo carmine; the blue colour of the solution therefore remains.



indigo carmine sodium isatin-5-sulfonate

B. Chlorates and chlorides (see Tests).

C. Heat 10 mg over a flame for 2 min. Dissolve the residue in 2 ml of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).

Perchlorate decomposes to chloride and oxygen on heating.

$$KClO_4 \rightarrow KCl + 2\ O_2$$

D. Dissolve 50 mg with heating in 5 ml of *water R*. Allow to cool to room temperature. The solution gives reaction (a) of potassium (*2.3.1*).

Tests

Solution S. Suspend 5.0 g in 90 ml of distilled *water R* and heat to boiling. Allow to cool. Filter. Dilute the filtrate to 100 ml with *carbon dioxide-free water R*.

Appearance of solution. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.20 g in water R and dilute to 20 ml with the same solvent.

Acidity or alkalinity. To 5 ml of solution S add 5 ml of *water R* and 0.1 ml of *phenolphthalein solution R*. Not more than 0.25 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator. To 5 ml of solution S, add 5 ml of *water R* and 0.1 ml of *bromocresol green solution R*. Not more than 0.25 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

The aqueous solution of potassium perchlorate is neutral. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red). The pH interval of the colour change of the bromocresol green indicator is between 3.6 (yellow) and 5.2 (blue).

Chlorates and chlorides (*2.4.4*): maximum 100 ppm (calculated as chlorides).

To 5 ml of solution S, add 5 ml of *water R* and heat to boiling. Add 1 ml of *nitric acid R* and 0.1 g of *sodium nitrite R*. Allow to cool to room temperature. Dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides. Prepare the standard using 5 ml of *chloride standard solution* (*5 ppm Cl*) *R* and 10 ml of *water R*, and adding only 1 ml of *dilute nitric acid R*.

Sulfates (*2.4.13*): maximum 100 ppm.

15 ml of solution S complies with the limit test for sulfates. Prepare the standard using a mixture of 7.5 ml of *sulfate standard solution* (*10 ppm* SO_4^{2-}) *R* and 7.5 ml of *water R*.

Calcium (*2.4.3*): maximum 100 ppm, determined on solution S.

Prepare the standard using a mixture of 7.5 ml of *calcium standard solution* (*10 ppm Ca*) *R*, 1 ml of *dilute acetic acid R* and 7.5 ml of *distilled water R*.

Heavy metals (*2.4.8*): maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. See identification **D** in the *Potassium perchlorate* monograph.

2. To 0.10 g sample, add 1-2 drops of *sulfuric acid R*. The mixture must remain colourless, and no gas with an odour of hydrochloric acid or chlorine develops from it.

Sulfuric acid liberates hydrochloric acid or chlorous acid from chloride or chlorate salts; from the latter, the yellowish-brown chlorine dioxide is formed by disproportionation. Chlorine dioxide dissolves in sulfuric acid to give an orange colour.

$$2\ KCl + H_2SO_4 \rightarrow 2\ HCl + K_2SO_4$$
$$2\ KClO_3 + H_2SO_4 \rightarrow 2\ HClO_3 + K_2SO_4$$
$$3\ HClO_3 \rightarrow HClO_4 + 2\ ClO_2 + H_2O$$

3. Heat 0.10 g of sample on a porcelain test plate for few minutes until it melts. Dissolve the cooled melt in 10.0 ml of *water R*, and add 1.0 ml of *dilute nitric acid R* and 2.0 ml *silver nitrate solution R1* to the solution. A white, curdled precipitate is formed, which dissolves in the excess of *dilute ammonia solution R2*.

On heating, perchlorate decomposes to chloride, which can be identified as silver chloride.

POTASSIUM PERMANGANATE

Kalii permanganas

$KMnO_4$ **M_r 158.0**

Definition

Content: 99.0 per cent to 100.5 per cent.

Characters

Appearance: dark purple or brownish-black, granular powder or dark purple or almost black crystals, usually having a metallic lustre.

Solubility: soluble in cold water, freely soluble in boiling water.

It decomposes on contact with certain organic substances.

It is an oxidizing disinfectant.

Identification

A. Dissolve about 50 mg in 5 ml of *water R* and add 1 ml of *ethanol (96 per cent) R* and 0.3 ml of *dilute sodium hydroxide solution R*. A green colour develops. Heat to boiling. A dark brown precipitate is formed.

In alkaline solution, alcohol is oxidized to acetaldehyde with a characteristic odour, while the violet permanganate is reduced through green manganate to brown manganese(IV) oxide.

$$2\ MnO_4^- + CH_3CH_2OH + 2\ OH^- \rightarrow 2\ MnO_4^{2-} + CH_3CHO + 2\ H_2O$$

$$MnO_4^{2-} + CH_3CH_2OH \rightarrow MnO_2 + CH_3CHO + 2\ OH^-$$

B. Filter the mixture obtained in identification test A. The filtrate gives reaction (b) of potassium (*2.3.1*).

Tests

Solution S. Dissolve 0.75 g in 25 ml of *distilled water R*, add 3 ml of *ethanol (96 per cent) R* and boil for 2-3 min. Cool, dilute to 30 ml with *distilled water R* and filter.

Under neutral conditions, permanganate oxidizes ethanol to acetaldehyde, while manganese(IV) oxide is produced. The filtrate is practically a solution of potassium hydroxide containing the water-soluble impurities of permanganate.

Appearance of solution. Solution S is colourless (*2.2.2, Method II*).

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum 500 ppm.

Dilute12 ml of solution S to 15 ml with *distilled water R*.

Assay

Dissolve 0.300 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 20 ml of *water R*, 1 g of *potassium iodide R* and 10 ml of *dilute hydrochloric acid R*. Titrate the liberated iodine with *0.1 M sodium thiosulfate*, using 1 ml of *starch solution R* as indicator.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 3.160 mg of $KMnO_4$.

Under acidic conditions, permanganate oxidizes iodide to iodine, which can be titrated with thiosulfate.

$$2\ MnO_4^- + 10\ I^- + 16\ H^+ \rightarrow 2\ Mn^{2+} + 5\ I_2 + 8\ H_2O$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$KMnO_4\ \text{content}\ (\%) = \frac{V_{Na_2S_2O_3}\ (\text{ml}) \cdot f_{Na_2S_2O_3} \cdot E\ (\text{mg/ml}) \cdot V_{bulb}}{\text{amount of substance (mg)} \cdot V_{pipetted}} \cdot 100$$

where V_{bulb} is the volume of the stock solution and $V_{pipetted}$ is the volume of stock solution pipetted out for the assay.

Informative test

1. See identification **A** in the *Potassium permanganate* monograph.

2. See identification **B** in the *Potassium permanganate* monograph.

POTASSIUM SULFATE

Kalii sulfas

K_2SO_4 **M_r 174.3**

Definition

Content: 98.5 per cent to 101.0 per cent of K_2SO_4 (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: soluble in water, practically insoluble in ethanol.

Potassium sulfate has a laxative effect (osmotic laxative).

Identification

A. It gives the reactions of sulfates (*2.3.1*).

B. It gives the reactions of potassium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in 90 ml of *carbon dioxide-free water R* prepared from *distilled water R*, heating gently. Allow to cool and dilute to 100 ml with *carbon dioxide-free water R* prepared from *distilled water R*.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of potassium sulfate is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Chlorides (*2.4.4*): maximum 40 ppm.

Dilute 12.5 ml of solution S to 15 ml with *water R*.

Calcium (*2.4.3*): maximum 200 ppm.

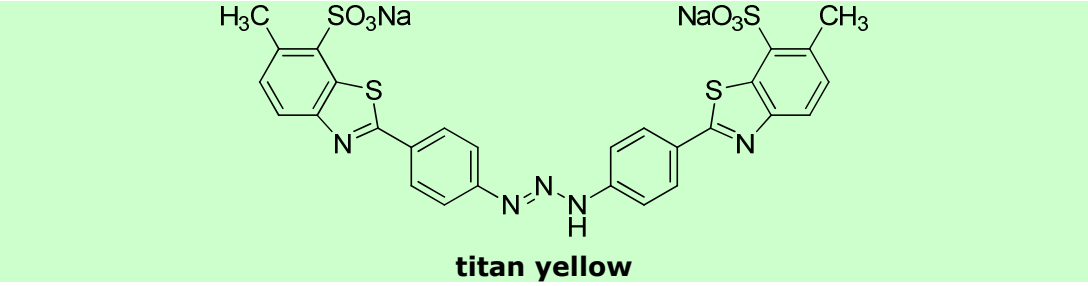
Dilute 5 ml of solution S to 15 ml with *distilled water R.*

Iron (*2.4.9*): maximum 10 ppm, determined on 10 ml of solution S.

Magnesium: maximum 20 ppm.

To 5 ml of solution S add 5 ml of *water R*, 1 ml of *glycerol (85 per cent) R*, 0.15 ml of *titan yellow solution R*, 0.25 ml of *ammonium oxalate solution R* and 5 ml of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 1 ml of *magnesium standard solution* (*10 ppm Mg*) *R* and 9 ml of *water R*.

Under basic conditions, titan yellow forms a pale-red dye with magnesium. In the presence of Ca^{2+}, this colour deepens; this can be avoided by trapping the Ca^{2+} possibly present with oxalate. Glycerol can inhibit the precipitation of $Mg(OH)_2$ (basic conditions).



Heavy metals (*2.4.8*): maximum 20 ppm.

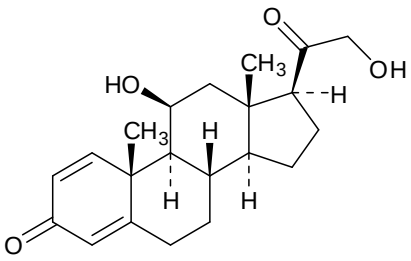
12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution* (*2 ppm Pb*) *R*.

Informative test

1. See the **Appearance of solution** test in the *Potassium sulfate* monograph.
2. See identification **A** in the *Potassium sulfate* monograph.
3. See identification **B** in the *Potassium sulfate* monograph.

PREDNISOLONE

Prednisolonum



$C_{21}H_{28}O_5$ ***M_r*** **360.4**

Definition

11β,17,21-Trihydroxypregna-1,4-diene-3,20-dione.

Content: 96.5 per cent to 102.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline, hygroscopic powder.

Solubility: very slightly soluble in water, soluble in ethanol (96 per cent) and in methanol, sparingly soluble in acetone, slightly soluble in methylene chloride.

It shows polymorphism (*5.9*).

It is a steroidal anti-inflammatory agent.

Identification

A. Infrared absorption spectrophotometry

B. Liquid chromatography.

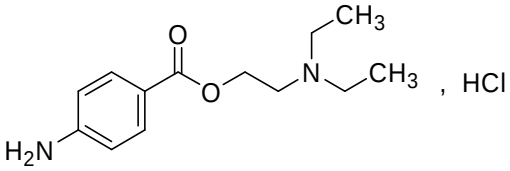
Informative test

1. Dissolve about 2 mg in 2.0 ml of *sulfuric acid R*. The red solution turns wine-red within 5 min. Cautiously dilute the solution with 10.0 ml of *water R*. The solution becomes colourless and a grey, flocculent precipitate is formed.

Some steroid derivatives give a typical colour reaction with concentrated sulfuric acid. The above reaction of prednisolone is suitable for distinction from hydrocortisone. (Hydrocortisone dissolves in concentrated sulfuric acid to give a yellowish-brown colour and a greenish fluorescence, which turns reddish-brown in 5 min. When the mixture is diluted with water, the solution turns brownish-yellow, the greenish fluorescence remaining unchanged.)

PROCAINE HYDROCHLORIDE

Procaini hydrochloridum



$C_{13}H_{21}ClN_2O_2$ **M_r 272.8**

Definition

Procaine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-(diethylamino)ethyl 4-aminobenzoate hydrochloride, calculated with reference to the dried substance.

Characters

A white or almost white, crystalline powder or colourless crystals, very soluble in water, soluble in ethanol (96 per cent).

It is alocal anaesthetic.

Identification

A. Melting point *(2.2.14)*: 154 °C to 158 °C.

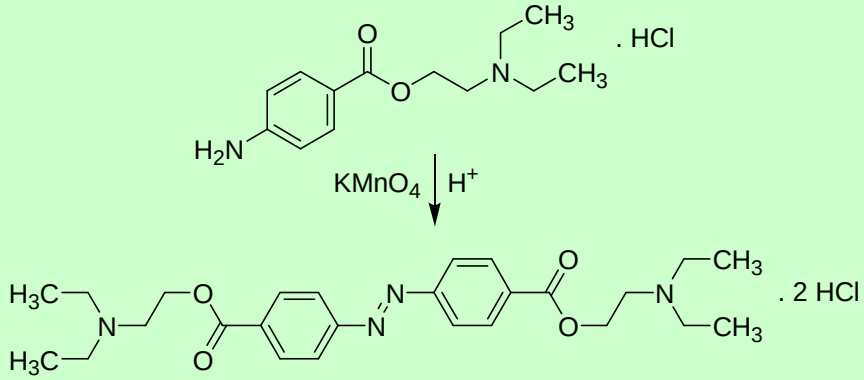
B. Examine by infrared absorption spectrophotometry *(2.2.24)*, comparing with the spectrum obtained with *procaine hydrochloride CRS*.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 ml of *acetone R*. Add 1 ml of *0.1 M alcoholic potassium hydroxide*. Only a brownish-red colour develops.

The **VITALI-MORIN** reaction is suitable for to distinguishing procaine, tetracaine and lidocaine (lidocaine: a green; tetracaine: a violet **MEISENHEIMER** complex is produced).

D. To 0.2 ml of solution S (see Tests) add 2 ml of *water R* and 0.5 ml of dilute *sulfuric acid R* and shake. Add 1 ml of a 1 g/l solution of *potassium permanganate R*. The colour is immediately discharged.

In acidic solution, permanganate oxidizes procaine to a yellow azo dye.



yellow azo dye

E. It gives reaction (a) of chlorides *(2.3.1)*.

F. Dilute 1 ml of solution S to 100 ml with *water R*. 2 ml of this solution gives the reaction of primary aromatic amines *(2.3.1)*.

Tests

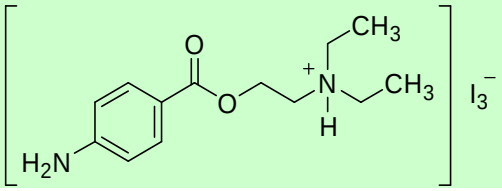
Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Informative tests

1. See the **Appearance of solution** test in the *Procaine hydrochloride* monograph.
2. See identification **F** in *Procaine hydrochloride* monograph.
3. Add 1 drop of *dilute hydrochloric acid R* and a few drops of *iodine solution R4* to 1.0 ml of solution S. A brown precipitate is formed.

   Procaine hydrochloride forms a brown, water-insoluble salt with triiodide ion. The reaction is characteristic of protonated tertiary amines.



4. Dilute 1.0 ml of solution S with 4.0 ml of *water R* and add 1.5 ml of *potassium thiocyanate solution R* to it. The reaction mixture must not change (distinction from tetracaine).

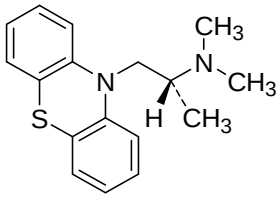
   In contrast with tetracaine, procaine does not form a water-insoluble salt with thiocyanate.

5. Mix 1.0 ml of solution S with 2 drops of *dilute nitric acid R* and 8–10 drops of *silver nitrate solution R1*. A white precipitate is formed.

   A silver chloride precipitate is formed.

PROMETHAZINE HYDROCHLORIDE

Promethazini hydrochloridum



and enantiomer . HCl

$C_{17}H_{21}ClN_2S$ ***M*r 320.9**

Definition

(2*RS*)-*N*,*N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-amine hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or faintly yellowish, crystalline powder.

Solubility: very soluble in water, freely soluble in ethanol (96 per cent) and in methylene chloride.

mp: about 222 °C, with decomposition.

Promethazine, a phenothiazine, is an H_1-antagonist with anticholinergic, sedative and antiemetic effects and some local anaesthetic properties. Promethazine is used as an antiemetic or to prevent motion sickness.

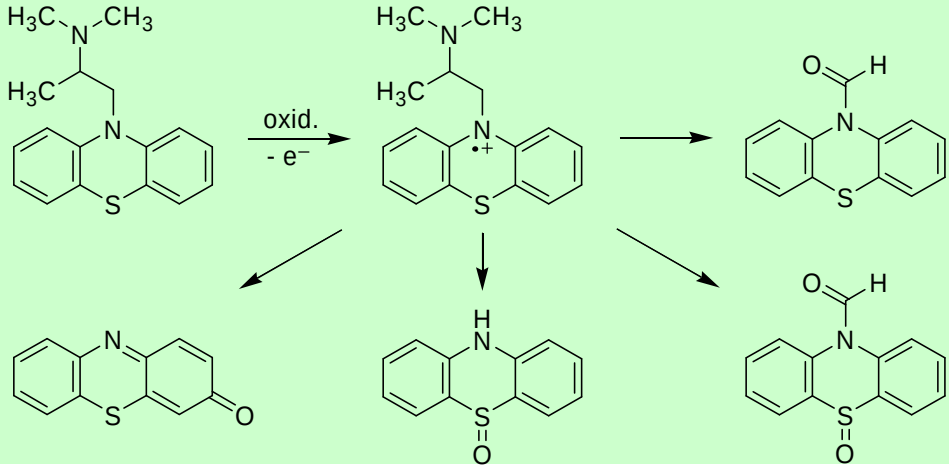
Identification

A. Infrared absorption spectrophotometry

B. Thin-layer chromatography.

C. Dissolve 0.1 g in 3 ml of *water R*. Add dropwise 1 ml of *nitric acid R*. A precipitate is formed which rapidly dissolves to give a red solution, becoming orange and then yellow. Heat to boiling. The solution becomes orange and an orange-red precipitate is formed.

Oxidizing agents transform phenothiazine-type compounds to coloured derivatives. In the first step, nitric acid oxidizes promethazine to a red radical cation. In the next steps, oxidation of the alkyl side-chain or/and phenothiazine ring takes place and further oxidized products (*e.g*. 10-formylphenothiazine, phenothiazine sulfoxide and quinoidal phenothiazone) are formed.



D. It gives reaction (b) of chlorides *(2.3.1).*

Informative tests

1. See identification **C** in the *Promethazine hydrochloride* monograph.

2. To 50 mg dissolved in 3.0 ml of *water R*, add 2.0 ml of *silver nitrate solution R1*; a white, curdy precipitate is formed.

A silver chloride precipitate is formed.

QUINIDINE SULFATE

Chinidini sulfas

$C_{40}H_{50}N_4O_8S \cdot 2\ H_2O$ M_r 783

Definition

Content: 99.0 per cent to 101.0 per cent of alkaloid monosulfates, calculated as bis[(*S*)-[(2*R*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol] sulfate (dried substance).

Characters

Appearance: white or almost white, crystalline powder or silky, colourless needles.

Solubility: slightly soluble in water, soluble in boiling water and in ethanol (96 per cent), practically insoluble in acetone.

It is an anti-arrhythmic and antimalarial agent.

Identification

A. Thin-layer chromatography.

B. Dissolve about 5 mg in 5 ml of *water R.* Add 0.2 ml of *bromine water R* and 1 ml of *dilute ammonia R2.* A green colour develops.

For the explanation of the thalleiochin reaction, see identification **B** of the *Quinine sulfate* monograph.

C. Dissolve 0.1 g in 3 ml of *dilute sulfuric acid R* and dilute to 100 ml with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on addition of 1 ml of *hydrochloric acid R*.

Dilute solutions of 6-methoxy-substituted cinchona alkaloids with oxygen-containing acids (*e.g.* sulfuric acid or acetic acid) fluoresce. Halogenides extinguish the fluorescence.

D. Dissolve about 50 mg in 5 ml of hot *water R*, cool, add 1 ml of *silver nitrate solution R1* and stir with a glass rod. After a few minutes, a white precipitate is formed that dissolves on the addition of *dilute nitric acid R*.

Quinidine sulfate forms a water-insoluble 1:1 addition molecule with silver nitrate. This reaction is suitable for distinction from quinine sulfate, which gives a water-soluble addition product.

E. It gives reaction (a) of sulfates *(2.3.1)*.

F. pH (see Tests).

Tests

pH *(2.2.3):* 6.0 to 6.8.

Dissolve 0.10 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Assay

Dissolve 0.200 g in 20 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, using 0.15 ml of *naphtholbenzein solution R* as indicator.

1 ml of *0.1 M perchloric acid* is equivalent to 24.90 mg of $C_{40}H_{50}N_4O_8S$.

In non-aqueous medium, quinidine sulfate reacts with three equivalents of perchloric acid (protonation of the less basic quinoline nitrogens and conversion of the sulfate to hydrogensulfate).

$$C_{40}H_{50}N_4O_8S \text{ content } (\%) = \frac{V_{HClO_4}(ml) \cdot f_{HClO_4} \cdot E\ (mg/ml)}{\text{amount of substance } (mg)} \cdot 100$$

Informative tests

1. A solution of 0.10 g in 10.0 ml of warmed *water R* has a weak blue fluorescence, which becomes more intensive when a small amount of *dilute sulfuric acid R* is added to the solution.

See identification **C** of the *Quinidine sulfate* monograph.

2. See identification **B** of the *Quinidine sulfate* monograph.

3. To 1.0 ml of the solution prepared in ”Informative test 1”, add a few drops of *dilute hydrochloric acid R* and 1.0 ml of *barium chloride solution R1.* A white precipitate is formed.

A barium sulfate precipitate is formed.

4. Divide the remaining solution from test 1 into two tubes. To the first solution, add 0.10 g of potassium iodide R; a white precipitate is formed. To the other solution, add 0.50 g of *potassium sodium tartrate R*; the liquid must not change.

The reaction is suitable for distinguishing between quinidine and quinine. Quinidinium iodide is less water-soluble than quinidinium tartrate (in the case of the quinine salts, the solubilities are the opposite).

QUININE SULFATE

Chinini sulfas

$C_{40}H_{50}N_4O_8S \cdot 2\ H_2O$ M_r 783

Definition

Content: 99.0 per cent to of 101.0 per cent of alkaloid monosulfates, calculated as bis[(*R*)-[(2*S*,4*S*,5*R*)-5-ethenyl-1-azabicyclo[2.2.2]oct-2-yl](6-methoxyquinolin-4-yl)methanol] sulfate (dried substance).

Characters

Appearance: white or almost white, crystalline powder or fine, colourless needles.

Solubility: slightly soluble in water, sparingly soluble in boiling water and in ethanol (96 per cent).

It is an antimalarial agent.

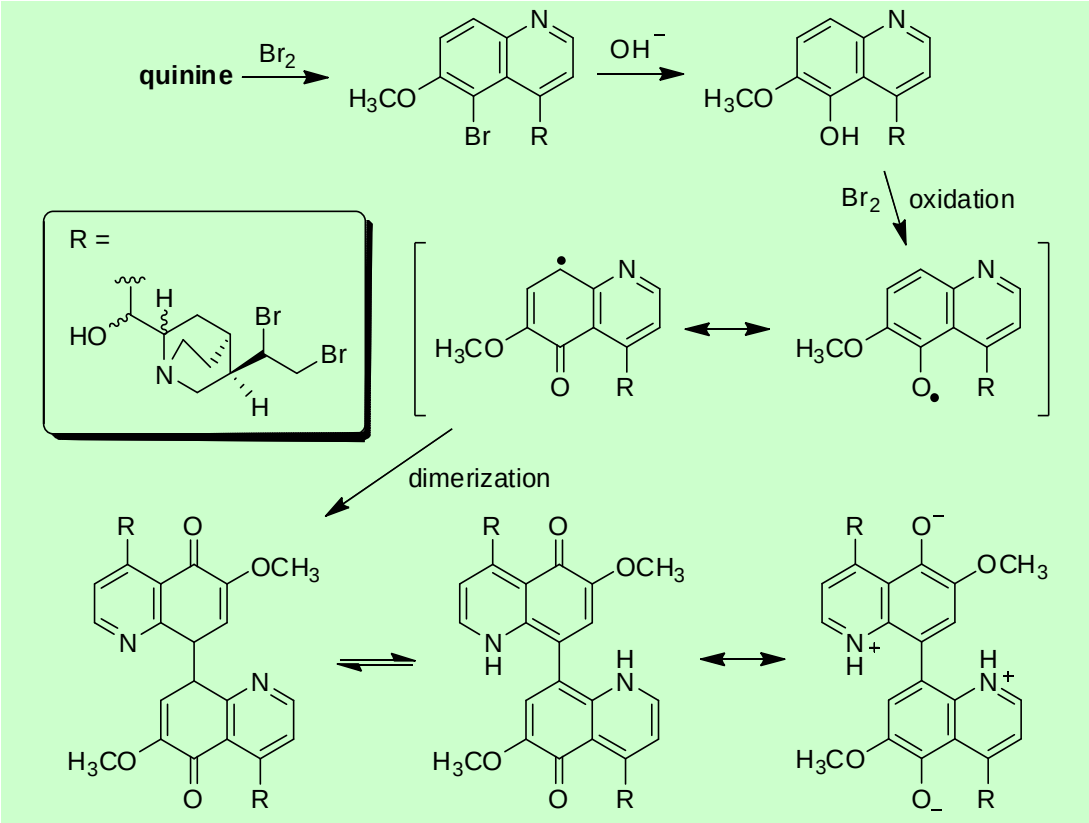
Identification

A. Thin-layer chromatography.

B. Dissolve about 5 mg in 5 ml of *water R*. Add 0.2 ml of *bromine water R* and 1 ml of *dilute ammonia R2*. A green colour develops.

With bromine water, bromine is added to the vinyl group, and the quinoline ring is bromo-substituted at position 5, followed by hydroxy–bromine exchange, resulting in a 5-hydroxy-substituted derivative. The final step is the oxidation of the phenolic hydroxy group with bromine, followed by dimerization, resulting in a red *bis*-quinoline dimer.

The emerald coloured reaction [*thallein* (Greek) = to turn green] is characteristic for quinoline derivatives bearing an oxygen-containing functional group at position 6.



C. Dissolve 0.1 g in 3 ml of *dilute sulfuric acid R* and dilute to 100 ml with *water R*. When examined in ultraviolet light at 366 nm, an intense blue fluorescence appears which disappears almost completely on the addition of 1 ml of *hydrochloric acid R*.

See identification **C** of the *Quinidine sulfate* monograph.

D. Dissolve about 45 mg in 5 ml of *dilute hydrochloric acid R*. The solution gives reaction (a) of sulfates *(2.3.1).*

E. pH (see Tests).

Tests

pH *(2.2.3)*: 5.7 to 6.6 for a 10 *g/l* suspension in *water R.*

Informative tests

1. Solution of 0.10 g in 10.0 ml of warmed *water R* has weak blue fluorescency which becomes more intensive adding small amount of *dilute sulfuric acid R* to the solution.

See identification **C** of the *Quinidine sulfate* monograph.

2. See identification **B** of the *Quinine sulfate* monograph.

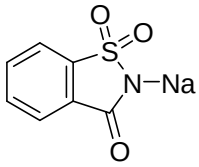
3. To 4.0 ml of solution prepared in „Informative test 1”, add 0.5 g of *potassium sodium tartrate R*. White precipitate is formed.

4. To 4.0 ml of solution prepared in „Informative test 1”, add 0.1 g of *potassium iodide R*. The liquid must not change.

Reactions 3 and 4 are suitable for distinguishing between quinidine and quinine. Quininium tartrate is less water-soluble than quininium iodide (in the case of the quinidine salts, the solubilities are the opposite).

SACCHARIN SODIUM

Saccharinum natricum



$C_7H_4NNaO_3S$ ***M*r 205.2**

Definition

2-Sodio-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

It may contain a variable quantity of water.

Characters

Appearance: white or almost white, crystalline powder or colourless crystals, efflorescent in dry air.

Solubility: freely soluble in water, sparingly soluble in ethanol (96 per cent).

It is an artificial sweetener.

Identification

A. Melting point (2.2.14): 226 °C to 230 °C.

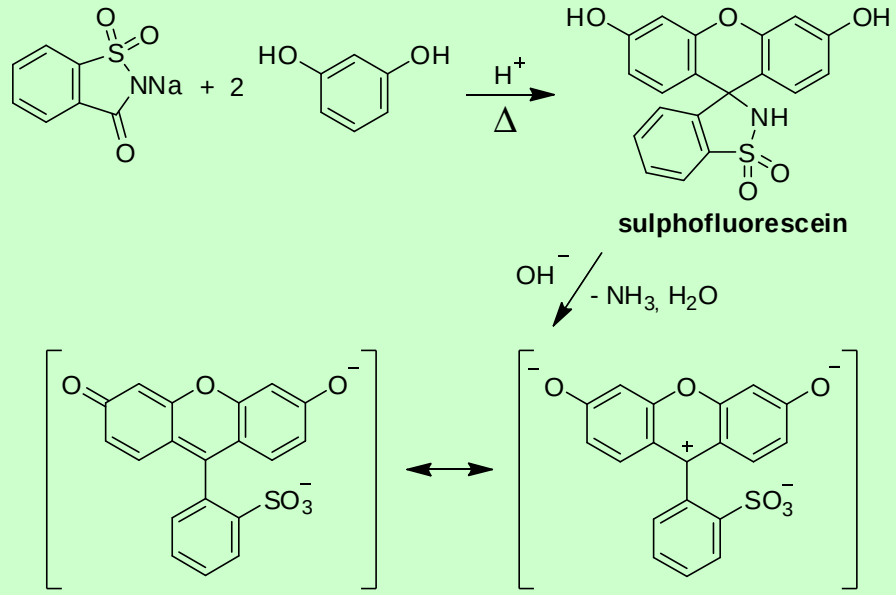
To 5 ml of solution S (see Tests) add 3 ml of *dilute hydrochloric acid R*. A white precipitate is formed. Filter and wash with *water R*. Dry the precipitate at 100-105 °C.

The liberated saccharin precipitates from the solution.

B. Infrared absorption spectrophotometry *(2.2.24)*.

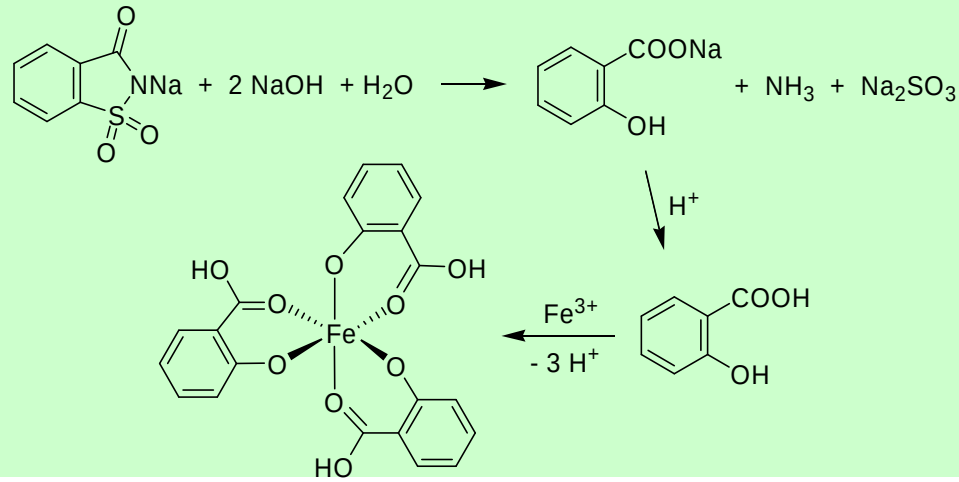
C. Mix about 10 mg with about 10 mg of *resorcinol R*, add 0.25 ml of *sulfuric acid R* and carefully heat the mixture over a naked flame until a dark green colour is produced. Allow to cool, add 10 ml of *water R* and *dilute sodium hydroxide solution R* until an alkaline reaction produced. An intense green fluorescence develops.

When heated with resorcinol in the presence of sulfuric acid, saccharin sodium is converted to sulfofluorescein, which has an intense green fluorescence.



D. To 0.2 g add 1.5 ml of *dilute sodium hydroxide solution R*, to dryness and heat the residue carefully until it melts, avoiding carbonisation. Allow to cool, dissolve the mass in about 5 ml of *water R*, add *dilute hydrochloric acid R* until a weak acid reaction is produced and filter, if necessary. To the filtrate add 0.2 ml of *ferric chloride solution R2*. A violet colour develops.

When heated with sodium hydroxide, saccharin sodium is converted to sodium salicylate. In acidic solution, salicylic acid can be determined with ferric chloride (a violet three-ligand chelate complex is formed).



E. 0.5 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50.0 ml with the same solvent.

Appearance of solution. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 5.0 g in 25 ml of *carbon dioxide-free water R*.

Informative tests

1. When the sample is heated in a flame, it burns, but the burning stops when the sample is removed from the flame. The burning sample imparts a persistent, vivid-yellow colour to the flame

The colour of the flame of the residue of the sample is characteristic of sodium.

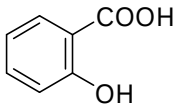
2. See the **Appearance of solution** test in the *Saccharin sodium* monograph.

3. See identification **C** in the *Saccharin sodium* monograph.

4. See identification **D** in the *Saccharin sodium* monograph.

SALICYLIC ACID

Acidum salicylicum



$C_7H_6O_3$ M_r 138.1

Definition

2-Hydroxybenzenecarboxylic acid.

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or white or colourless, acicular crystals.

Solubility: slightly soluble in water, freely soluble in ethanol (96 per cent), sparingly soluble in methylene chloride.

It is used as a ceratolytic and antimycotic agent in dermatology.

Identification

A. Melting point *(2.2.14)*: 158 °C to 161 °C.

B. Infrared absorption spectrophotometry.

C. Dissolve about 30 mg in 5 ml of *0.05 M sodium hydroxide*, neutralise if necessary and dilute to 20 ml with *water R*. 1 ml of the solution gives reaction (a) of salicylates *(2.3.1)*.

Tests

Solution S. Dissolve 2.5 g in 50 ml of boiling *distilled water R*, cool and filter.

Appearance of solution. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 1 g in 10 ml of *ethanol (96 per cent) R*.

Chlorides *(2.4.4)*: maximum 100 ppm.

Dilute 10 ml of solution S to 15 ml with *water R*.

Sulfates: maximum 200 ppm.

Dissolve 1.0 g in 5 ml of *dimethylformamide R* and add 4 ml of *water R.* Mix thoroughly. Add 0.2 ml of *dilute hydrochloric acid R* and 0.5 ml of a 25 per cent *m/m* solution of *barium chloride R*. After 15 min any opalescence in the solution is not more intense than that in a standard prepared as follows: to 2 ml of *sulfate standard solution (100 ppm SO_4) R* add 0.2 ml of *dilute hydrochloric acid R*, 0.5 ml of a 25 per cent *m/m* solution of *barium chloride R*, 3 ml of *water R* and 5 ml of *dimethylformamide R*.

A barium sulfate precipitate is formed.

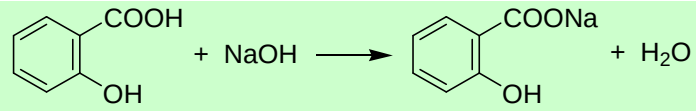
$$SO_4^{2-} + Ba^{2+} \rightarrow BaSO_4$$

Assay

Dissolve 0.120 g in 30 ml of *ethanol (96 per cent) R* and add 20 ml of *water R*. Titrate with *0.1 M sodium hydroxide*, using 0.1 ml of *phenol red solution R* as indicator.

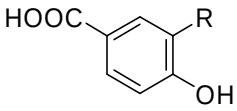
1 ml of *0.1 M sodium hydroxide* is equivalent to 13.81 mg of $C_7H_6O_3$.

Salicylic acid is titrated as a monovalent acid via alkalimetry, with phenol red as indicator. The pH interval of the colour change of the phenol red indicator is between 6.8 (yellow) and 8.4 (reddish-violet).



$$C_7H_6O_3 \text{ content (\%)} = \frac{V_{NaOH}\ (ml)\ .\ f_{NaOH}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Impurities



A. R = H: 4-hydroxybenzoic acid,

B. R = CO_2H: 4-hydroxyisophthalic acid,

C. phenol.

Informative tests

1. See the **Appearance of solution** test in the *Salicylic acid* monograph.

2. Dissolve about 10 mg in 5.0 ml of *water R* by gentle warming. The solution changes the colour of *blue litmus paper R* to red.

The solution of salicylic acid is acidic. The pH interval of the colour change of blue litmus paper is between 5 (red) and 8 (blue).

3. Add a few drops of *ferric chloride solution R2* to the cooled solution; a dark-violet colour is produced.

A three-ligand chelate complex is formed with Fe^{3+} (see general test of salicylates).

SILICA, COLLOIDAL HYDRATED

Silica colloidalis hydrica

Definition

Colloidal hydrated silica produced by precipitation or gelation process.
Content: 98.0 per cent to 100.5 per cent of SiO_2 (M_r 60.1) (ignited substance).

Characters

Appearance: white or almost white, light, fine, amorphous powder.
Solubility: practically insoluble in water and in mineral acids, with the exception of hydrofluoric acid. It dissolves in hot solutions of alkali hydroxides.

It is used as an additive in pharmaceutical technology (*e.g.* suppositories and tablets)

Identification

A. About 20 mg gives the reaction of silicates (*2.3.1*).

Informative test

1. A 0.20 g sample dissolves in 5.0 ml of *dilute sodium hydroxide solution R*, when boiled.

Water-soluble sodium silicate is formed.

$$SiO_2 + 2\ OH^- \rightarrow SiO_3^{2-} + H_2O$$

2. Shake the above-mentioned solution with 6.0 ml of *dilute hydrochloric acid R* and with 8.0 ml of *dilute ammonia solution R*. A white, gelatinous precipitate is formed.

Water-insoluble silicic acid is precipitated.

$$SiO_3^{2-} + 2\ H^+ \rightarrow H_2SiO_3$$

3. When 0.10 g of sample is sprinkled onto the surface of *water R*, a homogeneous suspension is formed after some minutes.

SILVER NITRATE

Argenti nitras

$AgNO_3$ **M_r 169.9**

Definition

Content: 99.0 per cent to100.5 per cent.

Characters

Appearance: white or almost white, crystalline powder or transparent, colourless crystals.

Solubility: very soluble in water, soluble in ethanol (96 per cent).

In solid stick form, it has been used to treat ulcerous wounds. For external use, in solutions incorporated in ointments, it is often used for the treatment of wounds. In very low concentration, it inhibits the multiplication of microroganisms.

Identification

A. 10 mg gives the reaction of nitrates (*2.3.1*).

B. 10 mg gives the reaction of silver (*2.3.1*).

Tests

Solution S. Dissolve 2.0 g in *water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 2 ml of solution S add 0.1 ml of *bromocresol green solution R*. The solution is blue. To 2 ml of solution S add 0.1 ml of *phenol red solution R*. The solution is yellow.

A blue colour indicates the pH of solution S between 5.2 and 6.8. The pH interval of the colour change of the phenol red indicator is between 6.8 (yellow) and 8.4 (reddish-violet). The pH interval of the colour change of the bromocresol green indicator is between 3.6 (yellow) and 5.2 (blue).

Aluminium, lead, copper and bismuth. Dissolve 1.0 g in a mixture of 4 ml of *concentrated ammonia R* and 6 ml of *water R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Al^{3+}, Pb^{2+} or Bi^{3+} forms a white precipitate of $Al(OH)_3$, $Pb(OH)_2$ or $Bi(OH)_3$ with ammonium hydroxide, which does not dissolve in an excess of the reagent

The light-blue $Cu(OH)_2$ precipitate formed in the presence of Cu^{2+} dissolves in the excess of the reagent, to give an intense blue colour (the tetraamminecopper(II) complex is formed).

$$Cu(OH)_2 + 4\ NH_3 \rightarrow [Cu(NH_3)_4]^{2+} + 2\ OH^-$$

Informative test

1. See the **Appearance of solution** test in the *Silver nitrate* monograph.

2. See identification **B** in the *Sliver nitrate* monograph.

3. To *ca.* 50 mg of sample, add 2 drops of *water R,* and then dissolve it in 3.0 ml of *sulfuric acid R*. Cautiously introduce 3.0 ml of *iron(II) sulfate solution R2* over the cooled solution to form an upper layer. A dark-brown ring is produced at the junction of the two liquids (brown ring test).

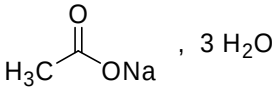
In concentrated sulfuric acid solution, Fe^{2+} reduces nitrate and the nitrogen monoxide formed produces a brown nitroso-iron(II) complex.

$$NO_3^- + 3\ Fe^{2+} + 4\ H^+ \rightarrow NO + 3\ Fe^{3+} + 2\ H_2O$$

$$Fe^{2+} + NO \rightarrow [Fe(NO)]^{2+}$$

SODIUM ACETATE TRIHIDRATE

Natrii acetas trihydricus



$C_2H_3NaO_2$. 3 H_2O M_r **136.1**

Definition

Sodium ethanoate trihydrate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: colourless crystals.

Solubility: very soluble in water, soluble in ethanol (96 per cent).

It is used to buffer infusions and solutions used for dialysis. It is an analytical reagent.

Identification

A. 1 ml of solution S (see Tests) gives reaction (b) of acetates *(2.3 1).*

B. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*: 7.5 to 9.0.

Dilute 5 ml of solution S to 10 ml with *carbon dioxide free water R*.

Reducing substances. Dissolve 5.0 g in 50 ml of *water R*, then add 5 ml of *dilute sulfuric acid R* and 0.5 ml of *0.002 M potassium permanganate*. The pink colour persists for at least 1 h. Prepare a blank in the same manner but without the substance to be examined.

In acidic solution, reducing impurities reduce permanganate to manganese(II) and the purple colour of permanganate disappears.

Chlorides *(2.4.4)*: maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulfates *(2.4.13)*: maximum 200 ppm.

Dilute 7.5 ml of solution S to 15 ml with *distilled water R.*

Arsenic (*2.4.2, Method A*) : maximum 2 ppm, determined on 0.5 g.

Heavy metals *(2.4.8)*: maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Iron *(2.4.9)*: maximum 10 ppm, determined on 10 ml of solution S.

Assay

Dissolve 0.250 g in 50 ml of *anhydrous acetic acid R*, add 5 ml of *acetic anhydride R*, mix and allow to stand for 30 min. Using 0.3 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 8.20 mg of $C_2H_3NaO_2$.

Sodium acetate is determined in non-aqueous medium with perchloric acid. Acetic anhydride eliminates the disturbing crystal water, resulting in acetic acid.

$$CH_3COONa \cdot 3\ H_2O + 3\ (CH_3CO)_2O \rightarrow CH_3COO^- + Na^+ + 6\ CH_3COOH$$

$$HClO_4 + CH_3COOH \rightarrow ClO_4^- + CH_3COOH_2^+$$

$$CH_3COO^- + CH_3COOH_2^+ \rightarrow 2\ CH_3COOH$$

$$C_2H_3NaO_2 \text{ content (\%)} = \frac{V_{HClO_4}(ml) \cdot f_{HClO_4} \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See the **Appearance of solution** test in the *Sodium acetate* monograph.

2. When gently heated, the compound melts and loses its water and solidifies by boiling off its crystal water. The residue is a white powder, which makes bubbles with hydrochloric acid and produces a vivid-yellow colour in the flame.

The vivid-yellow colour in the flame is a sensitive and characteristic identification reaction of sodium.

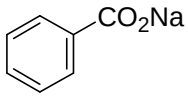
3. Heat 0.10 g of sample carefully with 1.0 ml of *sulfuric acid R*. The odour of acetic acid is observed.

The stronger and less volatile sulfuric acid liberates volatile acetic acid from sodium acetate.

$$2\ CH_3COONa + H_2SO_4 \rightarrow 2\ CH_3COOH + Na_2SO_4$$

SODIUM BENZOATE

Natrii benzoas



$C_7H_5NaO_2$ ***M*r 144.1**

Definition

Sodium benzenecarboxylate.

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline or granular powder or flakes, slightly hygroscopic.

Solubility: freely soluble in water, sparingly soluble in ethanol (90 per cent *V/V*).

It is used as a microbiological preservative of foods and drugs.

Identification

A. It gives reactions (b) and (c) of benzoates *(2.3.1)*.

B. It gives reaction (a) of sodium *(2.3.1).*

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

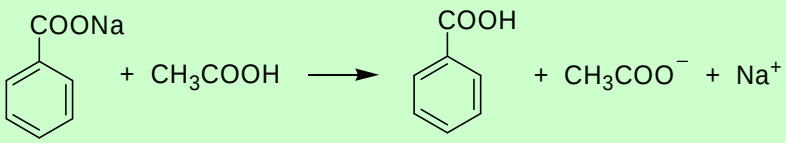
Acidity or alkalinity. To 10 ml of solution S add 10 ml of *carbon dioxide-free water R* and 0.2 ml of *phenolphthalein solution R.* Not more than 0.2 ml of *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the indicator.

The aqueous solution of sodium benzoate is weakly alkaline. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red). If a pink colour appears after the addition of the indicator, hydrochloric acid is required to change the colour to colourless; when the original colour of the indicator is colourless, sodium hydroxide is required to change it to pink.

Assay

Dissolve 0.250 g in 20 ml of *anhydrous acetic acid R*, heating to 50 °C if necessary. Cool. Using 0.05 ml of *naphtholbenzein solution R* as indicator, titrate with *0.1 M perchloric acid* until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 14.41 mg of $C_7H_5NaO_2$.



$$HClO_4 + CH_3COOH \rightarrow ClO_4^- + CH_3COOH_2^+$$

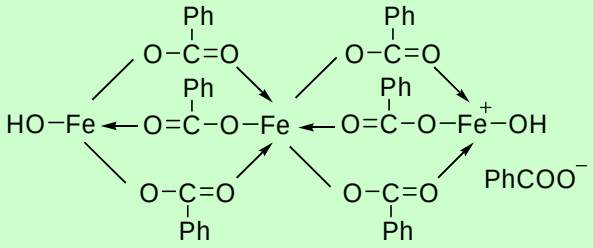
$$CH_3COO^- + CH_3COOH_2^+ \rightarrow 2\ CH_3COOH$$

$$C_7H_5NaO_2\ \text{content (\%)} = \frac{V_{HClO_4}(\text{ml}) \cdot f_{HClO_4} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See the **Appearance of solution** test in the *Sodium benzoate* monograph.
2. When a small sample is heated, it turns brown, melts and carbonizes and a smell of benzene is evolved. The sample burns with a sooty flame; the residue imparts a persistent, vivid-yellow colour to the flame.
3. Dissolve 0.10 g in 2.0 ml of *water R*. Dilute 5 drops of the solution with 2.0 ml of *water R* and add 5–10 drops of ferric chloride solution *R2.* A flesh-coloured precipitate is formed, which dissolves in a few drops of *dilute hydrochloric acid R*.

The three-centred dihydroxyhexabenzoateiron(III) monobenzoate which precipitates out ($[Fe_3(C_6H_5COO)_6(OH)_2]^+$ $C_6H_5COO^-$) decomposes when acid is added to the solution.



dihydroxyhexabenzoateiron(III) monobenzoate

SODIUM BROMIDE

Natrii bromidum

NaBr M_r **102.9**

Definition

Content: 98.5 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, granular powder or small, colourless, transparent or opaque crystals, slightly hygroscopic.

Solubility: freely soluble in water, soluble in ethanol (96 per cent).

It has sedative and anticonvulsive effects.

Identification

A. It gives reaction (a) of bromides (*2.3.1*).

B. Solution S (see Tests) gives the reactions of sodium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of sodium bromide is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Bromates. To 10 ml of solution S add 1 ml of *starch solution R*, 0.1 ml of a 100 g/l solution of *potassium iodide R* and 0.25 ml of *0.5 M sulfuric acid* and allow to stand protected from light for 5 min. No blue or violet colour develops.

See the “Bromates” test in the *Ammonium bromide* monograph. Bromate oxidizes iodide to iodine via bromine formation under acidic conditions, and the methylene chloride phase turns violet.

$$b\ (\%) = \text{chloride content}\ (\%) = \frac{V_{AgNO_3}\ (\text{ml}) \cdot f_{AgNO_3} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Iodides. To 5 ml of solution S add 0.15 ml of *ferric chloride solution R1* and 2 ml of *methylene chloride R*. Shake and allow to separate. The lower layer is colourless (*2.2.2, Method I*).

Fe^{3+} oxidizes iodide to iodine and the methylene chloride phase turns violet.

$$2\ I^- + 2\ Fe^{3+} \rightarrow I_2 + 2\ Fe^{2+}$$

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. Heat a small powdered and moistened sample in a non-luminous flame. A persistent, vivid-yellow colour must be produced in the flame.
2. See the **Appearance of solution** test in the *Sodium bromide* monograph.
3. To 1.0 ml of solution S, add 4-5 drops of *hydrochloric acid R*, 2.0 ml of *chloroform R* and 2 drops of *chloramine solution*, and shake. The chloroform layer attains a yellow colour; when more *chloramine solution* is added, the chloroform layer turns orange-red.

   See the 2nd "Informative test" in the *Ammonium bromide* monograph. Chloramine (*N*-chloro-4-methylbenzenesulfonamide sodium) is generally used as a chlorine source; it forms chlorine with chlorides in acidic solution. Chlorine liberates the brown bromine, which forms pale-yellow bromochloride with an excess of chlorine.

SODIUM CHLORIDE

Natrii chloridum

NaCl M_r **58.44**

Definition

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals or white or almost white pearls.

Solubility: freely soluble in water, practically insoluble in anhydrous ethanol.

Its 0.9% solution is isotonic. It is used for the preparation of isotonic, isoionic and isohydric solutions with some other salts.

Identification

A. It gives the reaction (a) of chlorides (*2.3.1*).

B. It gives the reactions of sodium (*2.3.1*).

Tests

If the substance is in the form of pearls crush before use.

Solution S. Dissolve 20.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 20 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of sodium chloride is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Ferrocyanides. Dissolve 2.0 g in 6 ml of *water R*. Add 0.5 ml of a mixture of 5 ml of a 10 g/l solution of *ferric ammonium sulfate R* in a 2.5 g/l solution of *sulfuric acid R* and 95 ml of a 10 g/l solution of *ferrous sulfate R*. No blue colour develops within 10 min.

In the presence of cyanides, hexacyanoferrate(II) is formed, which gives a blue precipitate (**Prussian blue**) with Fe^{3+}.

$$4\ Fe^{3+} + 3\ [Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

Iodides. Moisten 5 g by the dropwise addition of a freshly prepared mixture of 0.15 ml of *sodium nitrite solution R*, 2 ml of *0.5 M sulfuric acid*, 25 ml of *iodide-free starch solution R* and 25 ml of *water R*. After 5 min, examine in daylight. The substance shows no blue colour.

Nitrite oxidizes iodide to iodine under acidic conditions, and the iodine can be detected as the blue iodine–starch complex.

$$2\ I^- + 2\ NO_2^- + 4\ H^+ \rightarrow I_2 + 2\ NO + 2\ H_2O$$

Phosphates (*2.4.11*): maximum 25 ppm.

Dilute 2 ml of solution S to 100 ml with *water R*.

Sulfates (*2.4.13*): maximum 200 ppm.

Dilute 7.5 ml of solution S to 30 ml with *distilled water R*.

Arsenic (*2.4.2. Method A*): maximum 1 ppm, determined on 5 ml of solution S.

Barium. To 5 ml of solution S add 5 ml of *distilled water R* and 2 ml of *dilute sulfuric add R*. After 2 h, any opalescence in the solution is not more intense than that in a mixture of 5 ml of solution S and 7 ml of *distilled water R*.

A white precipitate of barium sulfate is formed.

Iron (*2.4.9*): maximum 2 ppm, determined on solution S.

Prepare the standard using a mixture of 4 ml of *iron standard solution* (*1 ppm Fe*) *R* and 6 ml of *water R*.

Magnesium and alkaline-earth metals (*2.4.7*): maximum 100 ppm, calculated as Ca and determined on 10.0 g.

Use 0.15 g of *mordant black 11 triturate R*. The volume of *0.01 M sodium edetate* used is not more than 2.5 ml.

Heavy metals (*2.4.8*): maximum 5 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

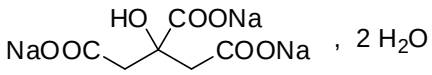
Informative test

1. See the **Appearance of solution** test in the *Sodium chloride* monograph.
2. Heat a small powdered and moistened sample in a non-luminous flame. A persistent, vivid-yellow colour must be produced in the flame. When heated for a long time, sodium chloride melts.
3. Add a few drops of *dilute nitric acid R* and 2.0 ml of *silver nitrate solution R* to 1.0 ml of solution S. A curdled, white precipitate is formed, which dissolves in an excess of *ammonia solution R2*.

   A silver chloride precipitate is formed.

SODIUM CITRATE

Natrii citras



$C_6H_5Na_3O_7 . 2 H_2O$ **M_r 294.1**

Definition

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder or white or almost white, granular crystals, slightly deliquescent in moist air.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent).

It is used to buffer the pH of infusions and dialysis solutions. It is also used as an *in vitro* anticoagulant agent.

Identification

A. To 1 ml of solution S (see Tests) add 4 ml of *water R.* The solution gives the reaction of citrates *(2.3.1)*.

B. 1 ml of solution S gives reaction (a) of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *phenolphthalein solution R*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the indicator.

The solution of sodium citrate is weakly basic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

Chlorides *(2.4.4)*: maximum 50 ppm.

Dilute 10 ml of solution S to 15 ml with *water R.*

Oxalates: maximum 300 ppm.

Dissolve 0.50 g in 4 ml of *water R,* add 3 ml of *hydrochloric acid R* and 1 g of *zinc R* in granules and heat on a water-bath for 1 min. Allow to stand for 2 min, decant the liquid into a test-tube containing 0.25 ml of a 10 g/l solution of *phenylhydrazine hydrochloride R* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid R* and 0.25 ml of *potassium ferricyanide solution R.* Shake and allow to stand for 30 min. Any pink colour in the solution is not more intense than that in a standard prepared at the same time in the same manner using 4 ml of a 50 mg/l solution of *oxalic acid*.

Nascent hydrogen, liberated from the reaction of zinc and hydrochloric acid, reduces oxalates to glyoxylic acid. Glyoxylic acid forms a hydrazone derivative, which reacts with a phenylhydrazonium salt (formed by the oxidation of phenylhydrazine with ferricyanide), resulting in red 1,5-diphenylformazan (see the same reaction at the “Oxalic acid” test of the “Citric acid monohydrate“ monograph.

Sulfates *(2.4.13)*: maximum 150 ppm.

To 10 ml of solution S add 2 ml of *hydrochloric acid R1* and dilute to 15 ml with *distilled water R.*

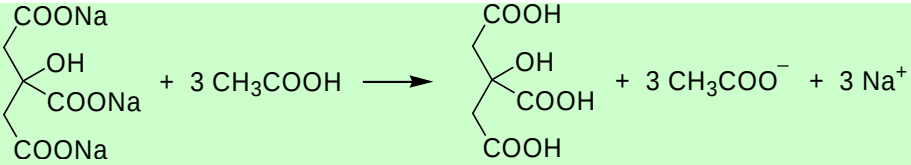
Heavy metals *(2.4.8)*: maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R.*

Assay

Dissolve 0.150 g in 20 ml of *anhydrous acetic acid R*, heating to about 50 °C. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *naphtholbenzein solution R* as indicator until a green colour is obtained.

1 ml of *0.1 M perchloric acid* is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.



$$HClO_4 + CH_3COOH \rightarrow ClO_4^- + CH_3COOH_2^+$$

$$CH_3COO^- + CH_3COOH_2^+ \rightarrow 2\ CH_3COOH$$

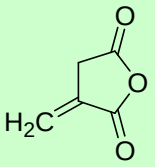
$$C_6H_5Na_3O_7\ \text{content (\%)} = \frac{V_{HClO_4}(\text{ml}) \cdot f_{HClO_4} \cdot E\ (\text{mg/ml})}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

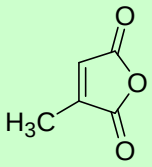
1. See the **Appearance of solution** test in the *Sodium citrate* monograph.

2. The sample melts when heated, and changes to yellow; on futher heating, it chars and emits a penetrating odour. It takes fire and burns with a luminous flame.

On heating, itaconic anhydride and citraconic anhydride are formed. The shaken mixture of zinc oxide with *water R* is weakly basic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).



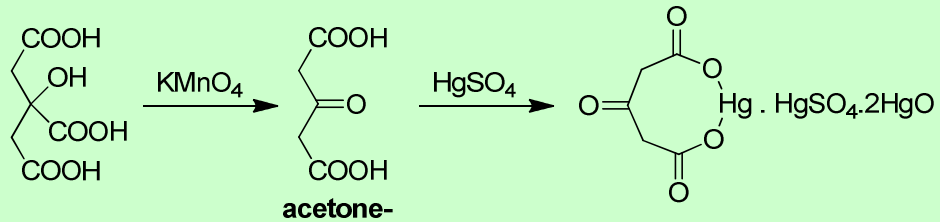
itaconic anhydride



citraconic anhydride

3. To 1.0 ml of solution S add 4.0 ml of *water R* and 2.0 ml of *mercury(II) sulfate solution R* and boil. Add few drops of *potassium permanganate solution R* to the hot solution. The solution decolourized and a white precipitate forms.

With permanganate, citric acid can be oxidized to acetonedicarboxylic acid, which forms a white basic salt with mercury(II) sulfate (**DENIGÉS** reagent) (see general identification of citrates).



acetone-dicarboxylic acid

SODIUM DIHYDROGEN PHOSPHATE DIHYDRATE

Natrii dihydrogenophosphas dihydricus

$NaH_2PO_4,2H_2O$ **M_r 156.0**

Definition

Content: 98.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white powder or colourless crystals.

Solubility: very soluble in water, very slightly soluble in ethanol (96 per cent).

Dihydrogenphosphate acidifies the urine. It is also used as a tonic.

Identification

A. Solution S (see Tests) is slightly acid (*2.2.4*).

B. Solution S gives the reactions of phosphates (*2.3.1*).

C. Solution S previously neutralised using a 100 g/l solution of *potassium hydroxide R* gives reaction (a) of sodium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 4.2 to 4.5.

To 5 ml of solution S add 5 ml of *carbon dioxide-free water R*.

Reducing substances. To 5 ml of solution S add 0.25 ml of *0.02 M potassium permanganate* and 5 ml of *dilute sulfuric acid R* and heat in a water-bath for 5 min. The colour of the permanganate is not completely discharged.

Reducing substances reduce permanganate to manganese(II) and the red solution becomes colourless.

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 2.5 ml of solution S to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum 300 ppm.

To 5 ml of solution S add 0.5 ml of *hydrochloric acid R* and dilute to 15 ml with *distilled water R*.

Arsenic (*2.4.2*): maximum 2 ppm,determined on 0.5 g.

Iron (*2.4.9*): maximum 10 ppm, determined on solution S.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Informative test

1. See the **Appearance of solution** test in the *Sodium dihydrogen phosphate dihydrate* monograph.

2. When heated, s*odium dihydrogen phosphate dihydrate* melts and swells to a spongy mass. When it is heated in a non-luminous flame, a persistent vivid-yellow colour is produced.

3. Add 5 drops of methyl red indicator to 2.0 ml of S solution. The solution turns red. When the red solution is shaken with 5.0 ml of *silver nitrate R1,* a yellow precipitate is formed, while the supernatant remains red. The precipitate dissolves in an excess of *dilute nitric acid*.

The solution of sodium dihydrogenphosphate is acidic (red colour of the methyl red indicator). With silver nitrate, yellow silver phosphate precipitates and the solution remains acidic. This

reaction is appropriate to distinguish between dihydrogenphosphate and hydrogenphosphate (see the 3rd test in the "Informative test" part in the *Disodium phosphate decahydrate* monograph).

$$HPO_4^{2-} + 3\ Ag^+ \rightleftharpoons Ag_3PO_4 + H^+$$

When dilute acid is added in excess, the equilibrium of the reaction is shifted in the direction of dissolution of the precipitate.

SODIUM FLUORIDE

Natrii fluoridum

NaF M_r **41.99**

Definition

Content: 98.5 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white powder or colourless crystals.

Solubility: soluble in water, practically insoluble in ethanol (96 per cent).

Sodium fluoride is used for the prevention of caries.

Identification

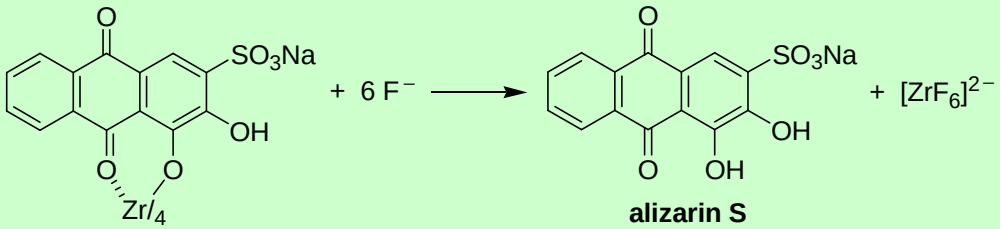
A. To 2 ml of solution S (see Tests) add 0.5 ml of *calcium chloride solution R*. A gelatinous white precipitate is formed that dissolves on adding 5 ml of *ferric chloride solution R1*.

A water-insoluble calcium fluoride precipitate is produced, which dissolves in iron(III) chloride as colourless hexafluoroferrate(III) ($[FeF_6]^{3-}$).

$$2\ F^- + Ca^{2+} \rightarrow CaF_2$$

B. To about 4 mg add a mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R* and mix. The colour changes from red to yellow.

Alizarin-S (sodium 3,4-dihydroxy-9,10-dioxo-9,10-dihydro-2-anthracenesulfonate) forms a red zirconium(IV)–alizarin-S complex with zirconyl nitrate. In the presence of fluoride ions, the zirconium(IV)–alizarin-S complex decomposes to yellow hexafluorozirconate(IV) and yellow alizarin-S.



alizarin S

C. Solution S gives reaction (a) of sodium (*2.3.1*).

Tests

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* without heating and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Informative test

1. Heat a small powdered and moistened sample in a non-luminous flame. A persistent, vivid-yellow colour must be produced in the flame.
2. See the **Appearance of solution** test in the *Sodium fluoride* monograph.
3. See identification **A** in the *Sodium fluoride* monograph.

SODIUM IODIDE

Natrii iodidum

NaI M_r **149.9**

Definition

Content: 99.0 per cent to 100.5 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals, hygroscopic.

Solubility: very soluble in water, freely soluble in ethanol (96 per cent).

Iodide is essential for the biosynthesis of thyroxine (thyroid gland hormone).

Identification

A. Solution S (see Tests) gives the reactions of iodides (*2.3.1*).

B. Solution S gives the reactions of sodium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in carbon *dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Alkalinity. To 12.5 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.7 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator.

The aqueous solution of sodium iodide is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Iodates. To 10 ml of solution S add 0.25 ml of *iodide-free starch solution R* and 0.2 ml of *dilute sulfuric acid R* and allow to stand protected from light for 2 min. No blue colour develops.

Iodate reacts with iodide to form iodine, which can be detected as the blue iodine–starch complex.

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

Sulfates (*2.4.13*): maximum 150 ppm.

Dilute 10 ml of solution S to 15 ml with *distilled water R*.

Thiosulfates. To 10 ml of solution S add 0.1 ml of *starch solution R* and 0.1 ml of *0.005 M iodine*. A blue colour is produced.

Formation of the blue iodine–starch complex fails because thiosulfate reduces iodine to iodide.

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 ml of solution S to 10 ml with *water R*.

Heavy metals (*2.4.8*): maximum 10 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

Assay

Dissolve 1.300 g in *water R* and dilute to 100.0 ml with the same solvent. To 20.0 ml of the solution add 40 ml of *hydrochloric acid R* and titrate with *0.05 M potassium iodate* until the colour changes from red to yellow. Add 5 ml of *chloroform R* and continue the titration, shaking vigorously, until the chloroform layer is decolorised.

1 ml of *0.05 M potassium iodate* is equivalent to 14.99 mg of NaI.

In acidic medium, iodate oxidizes iodide in two steps: first to iodine, which dissolves in chloroform to give a violet colour, and then to almost colourless iodochloride, in which iodine has oxidation state +1. Strong shaking is necessary, because iodine dissolves better in chloroform than in water.

$$IO_3^- + 5\ I^- + 6\ H^+ \rightarrow 3\ I_2 + 3\ H_2O$$

$$IO_3^- + 2\ I_2 + 5\ Cl^- + 6\ H^+ \rightarrow 5\ ICl + 3\ H_2O$$

Combining the above equations:

$$IO_3^- + 2\ I^- + 3\ Cl^- + 6\ H^+ \rightarrow 3\ ICl + 3\ H_2O$$

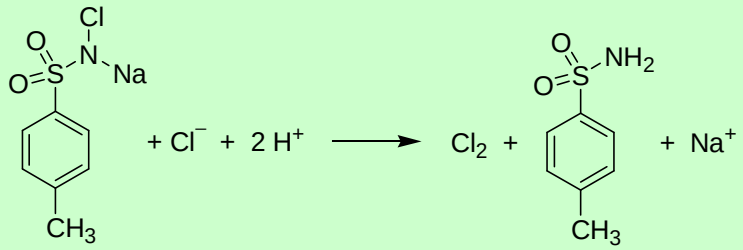
$$\text{NaI content (\%)} = \frac{V_{KIO_3}\ (ml)\ .\ f_{KIO_3}\ .\ E\ (mg/ml)\ .\ V_{bulb}\ (ml)}{\text{amount of compound (mg)}\ .\ V_{pipetted}\ (ml)}\ .\ 100$$

where V_{bulb} is the volume of the stock solution and $V_{pipetted}$ is the volume of stock solution pipetted out for the assay.

Informative test

1. Heat a small powdered sample in a non-luminous flame. The sample melts and a persistent, vivid-yellow colour is produced in the flame.
2. See the **Appearance of solution** test in the *Sodium iodide* monograph.
3. To 1.0 ml of solution S, add 4-5 drops of *hydrochloric acid R,* 2.0 ml of *chloroform R* and 2 drops of *chloramine solution*, and shake the mixture. The chloroform layer attains a violet colour; when more *chloramine solution* is added, the chloroform layer becomes colourless.

Chloramine (*N*-chloro-4-methylbenzenesulfonamide sodium) is generally used as chlorine source; it forms chlorine with chlorides in acidic solution.



Chlorine liberates violet iodine (in chloroform), which forms colourless iodate with an excess of chlorine.

$$2\ I^- + Cl_2 \rightarrow I_2 + 2\ Cl^-$$

$$I_2 + 5\ Cl_2 + 6\ H_2O \rightarrow 2\ IO_3^- + 10\ Cl^- + 12\ H^+$$

SODIUM METABISULFITE

Natrii metabisulfis

$Na_2S_2O_5$ **M_r 190.1**

Definition

Sodium metabisulfite also called sodium disulfite.

Content: 95.0 per cent to 100.5 per cent.

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: freely soluble in water, slightly soluble in ethanol (96 per cent).

It is used as an antioxidant and preservative.

Identification

A. pH (see Tests).

B. To 0.4 ml of *iodinated potassium iodide solution R* add 8 ml of *distilled water R* and 1 ml of solution S diluted 1 to 10 in *distilled water R*. The solution is colourless and gives reaction (a) of sulfates (*2.3.1*).

Sodium metabisulfite decolorizes a solution of iodine and sulfate ion is produced.

$$S_2O_5^{2-} + H_2O \rightarrow 2\ HSO_3^-$$

$$HSO_3^- + I_2 + H_2O \rightarrow SO_4^{2-} + 2\ I^- + 3\ H^+$$

C. Solution S gives reaction (a) of sodium (*2.3.1*).

Tests

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*). 3.5 to 5.0 for solution S.

Thiosulfates. To 5 ml of solution S add 5 ml of *dilute hydrochloric acid R*. The solution remains clear (*2.2.1*) for at least 15 min.

When a solution of sodium metabisulfite is acidified, sulfur dioxide is liberated. If thiosulfate impurity is present, it reacts with hydrochloric acid and forms not only sulfur dioxide, but also elementary sulfur (the solution becomes opalescent).

$$S_2O_5^{2-} + 2\ H^+ \rightarrow 2\ SO_2 + H_2O$$

$$S_2O_3^{2-} + 2\ H^+ \rightarrow S + SO_2 + H_2O$$

Iron (*2.4.9*): maximum 20 ppm, determined on solution S.

Assay

Dissolve 0.200 g in 50.0 ml of *0.05 M iodine*, and add 5 ml of *dilute hydrochloric acid R*. Titrate the excess of iodine with *0.1 M sodium thiosulfate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 4.753 mg of $Na_2S_2O_5$.

The metabisulfite content is determined by iodometry (see the equations in "Identification **B**"). The excess of iodine is back-titrated with thiosulfate.

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$Na_2S_2O_5 \text{ content. (\%)} = \frac{[V_{I_2}\,(ml) \cdot f_{I_2} - V_{Na_2S_2O_3}\,(ml) \cdot f_{Na_2S_2O_3}] \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

Informative test

1. Heat a small powdered sample in a non-luminous flame for a long time. The sample turns orange and a persistent, vivid-yellow colour is produced in the flame. After heating for several minutes, the cooled residue is a white mass.
2. See the **Appearance of solution** test in the *Sodium metabisulfite* monograph.
3. See identification **B** in the *Sodium metabisulfite* monograph.

SODIUM SULFATE DECAHYDRATE

Natrii sulfas decahydricus

$Na_2SO_4,10H_2O$ **M_r 322.2**

Definition

Content: 98.5 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless, transparent crystals.

Solubility: freely soluble in water, practically insoluble in ethanol (96 per cent). It partly dissolves in its own water of crystallisation at about 33 °C.

Sodium sulfate is used as an osmotic laxative. It is also called "Glauber salt".

Identification

A. It gives the reactions of sulfates (*2.3.1*).

B. It gives the reactions of sodium (*2.3.1*).

Tests

Solution S. Dissolve 5.0 g in carbon dioxide-free water R prepared from distilled *water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Not more than 0.5 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

The aqueous solution of sodium sulfate is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue). If a yellow colour appears after the addition of the indicator, sodium hydroxide is required to change the colour to blue; when the original colour of the indicator is blue, hydrochloric acid is required to change it to yellow. An intermediate green colour may be observed.

Chlorides (*2.4.4*). maximum 200 ppm.
5 ml of solution S diluted to 15 ml with *water R*.

Calcium (*2.4.3*). maximum 200 ppm, if intended for use in the manufacture of parenteral preparations.

Dilute 10 ml of solution S to 15 ml with *distilled water R.*

Heavy metals (*2.4.8*). maximum 20 ppm.

12 ml of solution S complies with limit test A. Prepare the reference solution using *lead standard solution* (*1 ppm Pb*) *R*.

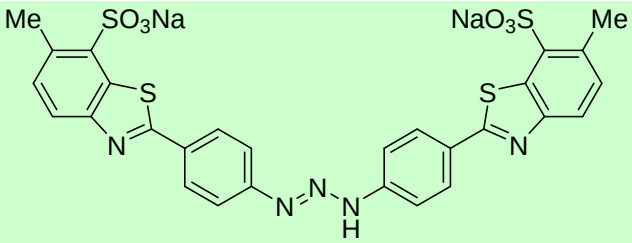
Iron (*2.4.9*). maximum 40 ppm, if intended for use in the manufacture of parenteral dosage forms.

5 ml of solution S diluted to 10 ml with *water R* complies with the limit test for iron.

Magnesium. maximum 100 ppm, if intended for use in the manufacture of parenteral preparations.

To 10 ml of solution S add 1 ml of *glycerol (85 per cent) R*, 0.15 ml of *titan yellow solution R*, 0.25 ml of *ammonium oxalate solution R* and 5 ml of *dilute sodium hydroxide solution R* and shake. Any pink colour in the test solution is not more intense than that in a standard prepared at the same time in the same manner using a mixture of 5 ml of *magnesium standard solution* (*10 ppm Mg*) *R* and 5 ml of *water R*.

Under basic conditions, titan yellow forms a pale-red dye with Mg^{2+}. In the presence of Ca^{2+}, the colour becomes deeper; this can be avoided by trapping the possible Ca^{2+} present with oxalate. Glycerol can inhibit the precipitation of $Mg(OH)_2$ (basic conditions).



titan yellow

Informative test

1. When gently heated, the compound melts and loses its water and solidifies as its crystal water is boiled off. The residue is a white powder, which produces a vivid-yellow colour in the flame.
2. See the **Appearance of solution** test in the *Sodium sulfate decahydrate* monograph.
3. Add a few drops of *dilute hydrochloric acid R* and *barium chloride solution R1* to 2.0 ml of solution S. A white precipitate is formed.

   A barium sulfate is precipitated.

SODIUM SULFITE, ANHYDROUS

Natrii sulfis anhydrous

Na_2SO_3 **M_r 126.0**

Definition

Content: 95.0 per cent to 100.5 per cent of Na_2SO_3.

Characters

Appearance: white or almost white powder.

Solubility: freely soluble in water, very slightly soluble in ethanol (96 per cent).

It is an antioxidant.

Identification

A. Solution S (see Tests) is slightly alkaline (*2.2.4*).

B. To 5 ml of solution S, add 0.5 ml of *0.05 M iodine*. The solution is colourless and gives reaction (a) of sulfates (*2.3.1*).

Sulfite decolorizes iodine and the resulting sulfate can be detected with barium (see *Sodium sulfate decahydrate* monograph).

$$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2\ I^- + 2\ H^+$$

C. Solution S gives reaction (a) of sodium (*2.3.1*).

D. It complies with the limits of the assay.

Tests

Solution S. Dissolve 5 g in *water R* and dilute to 100 ml with the same solvent.

Solution S1. To 10.0 g add 25 ml of *water R*. Shake until mostly dissolved, carefully and progressively add 15 ml of *hydrochloric acid R*. Heat to boiling. Cool and dilute to 100.0 ml with *water R*.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method I*).

Thiosulfates: maximum 0.1 per cent.

To 2.00 g add 100 ml of *water R*. Shake, add 10 ml of *formaldehyde solution R* and 10 ml of *acetic acid R*. Allow to stand for 5 min. Add 0.5 ml of *starch solution R* and titrate with *0.05 M iodine*. Carry out a blank titration. The difference between the volumes used in the titrations is not more than 0.15 ml.

Sulfite forms additional products with aldehydes and ketones in acidic media:

$$SO_3^{2-} + CH_2O + H^+ \rightarrow HOCH_2SO_3^-$$

These stable compounds can not be oxidized directly with iodine; iodine reacts only with the thiosulfate impurity.

$$2\ S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2\ I^-$$

Iron (*2.4.9*): maximum 10 ppm, determined on solution S1.

Selenium: maximum 10 ppm.

To 3.0 g add 10 ml of *formaldehyde solution R*, carefully and progressively add 2 ml of *hydrochloric acid R*. Heat on a water-bath for 20 min. Any pink colour in the solution is not more intense than that of a standard prepared at the same time and in the same manner using 1.0 g of the substance to be examined to which 0.2 ml of *selenium standard solution* (*100 ppm Se*) *R* has been added.

The selenite ion is reduced by formaldehyde to elementary selenium, which forms a red colloidal solution on standing.

$$SeO_3^{2-} + 2\ CH_2O + 2\ H^+ \rightarrow Se + 2\ HCOOH + H_2O$$

Heavy metals (*2.4.8*): maximum 10 ppm.

Evaporate 20 ml of solution S1 almost to dryness. Add 10 ml of *water R*, neutralise with *concentrated ammonia R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with test A. Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

Assay

Introduce 0.250 g into a 500 ml conical flask containing 50.0 ml of *0.05 M iodine*. Shake until completely dissolved. Add 1 ml of *starch solution R* and titrate the excess of iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 6.30 mg of Na_2SO_3.

The sulfite content is determined by iodometric titration, back-titrating the excess of iodine with thiosulfate.

$$Na_2SO_3 \text{ content (\%)} = \frac{[V^{empty}_{Na_2S_2O_3}(ml) - V_{Na_2S_2O_3}(ml)] \cdot f_{Na_2S_2O_3} \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

SODIUM THIOSULFATE

Natrii thiosulfas

$Na_2S_2O_3,5H_2O$ **M_r 248.2**

Definition

Content: 99.0 per cent to 101.0 per cent of $Na_2S_2O_3,5H_2O$.

Characters

Appearance: transparent, colourless crystals, efflorescent in dry air.

Solubility: very soluble in water, practically insoluble in ethanol 96 per cent. It dissolves in its water of crystallisation at about 49 °C.

It is used as an antidote in cases of poisoning (cyanide). It has an antimycotic effect too.

Identification

A. It decolourises *iodinated potassium iodide solution R*.

Tetrathionate is formed in a fast reaction.

$$2\ S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2\ I^-$$

B. To 0.5 ml of solution S (see Tests) add 0.5 ml of *water R* and 2 ml of *silver nitrate solution R*. A white precipitate is formed which rapidly becomes yellowish and then black.

With the excess of silver ions, a white precipitate (silver thiosulfate) is produced; this turns yellow, then brown and black. Silver sulfide is formed.

$$S_2O_3^{2-} + 2\ Ag^+ \rightarrow Ag_2S_2O_3$$

$$Ag_2S_2O_3 + H_2O \rightarrow Ag_2S + SO_4^{2-} + 2\ H^+$$

C. To 2.5 ml of solution S add 2.5 ml of *water R* and 1 ml of *hydrochloric acid R*. A precipitate of sulfur is formed and gas is evolved which gives a blue colour to *starch iodate paper R*.

On the action of acid, sulfur is precipitated and sulfur dioxide is liberated. Sulfur dioxide reduces iodate to iodine.

$$S_2O_3^{2-} + 2\ H^+ \rightarrow S + SO_2 + H_2O$$

$$5\ SO_2 + 2\ IO_3^- + 4\ H_2O \rightarrow 5\ SO_4^{2-} + I_2 + 8\ H^+$$

D. 1 ml of solution S gives reaction (a) of sodium (*2.3.1*).

Tests

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. The freshly prepared solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*). Dissolve 10.0 g in 50 ml of distilled water R, add 1 ml of 0.1 M sodium hydroxide and dilute to 100 ml with the same solvent.

pH (*2.2.3*). 6.0 to 8.4 for the freshly prepared solution S.

Sulfates and sulfites: maximum 0.2 per cent.

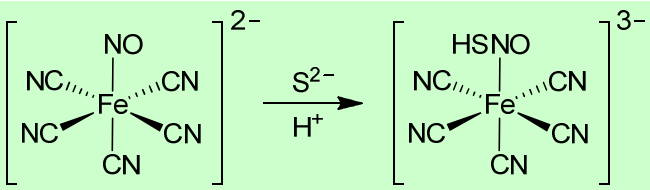
Dilute 2.5 ml of freshly prepared solution S to 10 ml with *distilled water R*. To 3 ml of this solution first add 2 ml of *iodinated potassium iodide solution R* and continue the addition dropwise until a very faint persistent yellow colour appears. Dilute to 15 ml with *distilled water R*.

Sulfite impurity can be oxidized to sulfate, which can be detected with barium.

$$SO_3^{2-} + I_2 + H_2O \rightarrow SO_4^{2-} + 2\ I^- + 2\ H^+$$

Sulfides. To 10 ml of solution S, add 0.05 ml of a freshly prepared 50 g/l solution of *sodium nitroprusside R*. The solution does not become violet.

A violet adduct is formed.



Heavy metals maximum 10 ppm.

To 10 ml of solution S add 0.05 ml of *sodium sulfide solution R*. After 2 min, any brown colour in the solution is not more intense than that in a reference solution prepared at the same time and in the same manner using 10 ml of *lead standard solution* (*1 ppm Pb*) *R* (10 ppm).

Assay

Dissolve 0.500 g in 20 ml of *water R* and titrate with *0.05 M iodine*, using 1 ml of *starch solution R*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 24.82 mg of $Na_2S_2O_3,5H_2O$.

The sulfite content is determined by iodometric titration (see "Identification **A**"). At the end-point, the excess iodine forms the blue iodine–starch complex.

$$Na_2S_2O_3,5H_2O \text{ content (\%)} = \frac{V_{I_2}\,(ml) \,.\, f_{I_2} \,.\, E\,(mg/ml)}{\text{amount of substance (mg)}} \,.\, 100$$

Informative test

1. When carefully heated, the sample melts and becomes dry. The continuously heated sample turns reddish-brown and burns with a blue flame. The ignited residue imparts a vivid-yellow colour to the flame. When the cooled residue is treated with a few drops of *dilute hydrochloric acid R,* the odour of hydrogen sulfide appears.

Sodium thiosulfate decomposes to sodium sulfate and sodium pentasulfide, which melts and decomposes to sodium sulfide (hydrogen sulfide liberation with hydrochloric acid) and sulfur (burns to sulfur dioxide in the flame).

$$4\ Na_2S_2O_3 \rightarrow 3\ Na_2SO_4 + Na_2S_5$$

$$Na_2S_5 \rightarrow Na_2S + 4\ S$$

$$S + O_2 \rightarrow SO_2$$

$$Na_2S + 2\ HCl \rightarrow H_2S + 2\ NaCl$$

2. See the **Appearance of solution** test in the *Sodium thiosulfate* monograph.

3. To 1.0 ml of solution S, add 1 ml of *silver nitrate solution R1*. A white precipitate is produced, which quickly turns yellow, then brown and black.

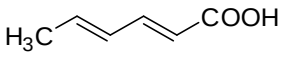
With an excess of silver ions, a white precipitate (silver thiosulfate) is produced; this turns yellow, then brown and black. Silver sulfide is formed.

$$S_2O_3^{2-} + 2\ Ag^+ \rightarrow Ag_2S_2O_3$$

$$Ag_2S_2O_3 + H_2O \rightarrow Ag_2S + SO_4^{2-} + 2\ H^+$$

SORBIC ACID

Acidum sorbicum



$C_6H_8O_2$ **M_r 112.1**

Other name: Acidum sorbinicum (Ph. Hg. VII.)

Definition

(*E*,*E*)-Hexa-2,4-dienoic acid.

Content: 99.0 per cent to 101.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, freely soluble in ethanol (96 per cent).

It is an antifungal microbiological preservative.

Identification

A. Melting point *(2.2.14):* 132 °C to 136 °C.

B. Ultraviolet and visible absorption spectrophotometry.

C. Infrared absorption spectrophotometry.

D. Dissolve 0.2 g in 2 ml of *ethanol (96 per cent) R* and add 0.2 ml of *bromine water R*. The solution is decolorised.

Bromine addition to the double bond of sorbic acid takes place.

Tests

Solution S. Dissolve 1.25 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Informative tests

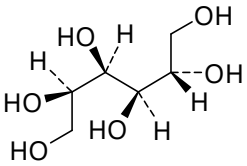
1. See the **Appearance of solution** test in the *Sorbic acid* monograph.

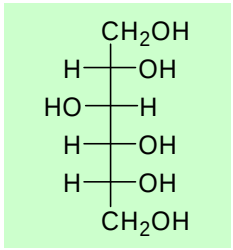
2. The saturated solution of the sample is acidic.

3. See identification **D** in the *Sorbic acid* monograph.

SORBITOL

Sorbitolum





$C_6H_{14}O_6$ **M_r 182.2**

Definition

D-Glucitol (D-sorbitol).

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: very soluble in water, practically insoluble in ethanol (96 per cent).

It shows polymorphism *(5.9)*.

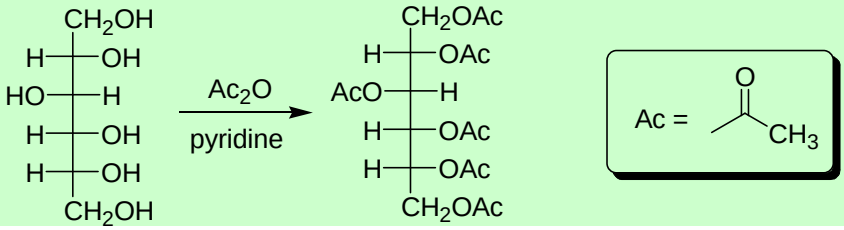
It is an osmotic laxative and diuretic. It is also used as an artifical sweetener.

Identification

A. Examine the chromatograms obtained in the assay.

B. Dissolve 0.5 g with heating in a mixture of 0.5 ml of *pyridine R* and 5 ml of *acetic anhydride R*. After 10 min, pour the solution into 25 ml of *water R* and allow to stand in iced water for 2 h. The precipitate, recrystallised from a small volume of *ethanol (96 per cent) R* and dried in vacuo, melts *(2.2.14)* at 98 °C to 104 °C.

The hexaacetyl derivative of sorbitol is formed. The melting point of the similar derivative of mannitol is 121-124 ºC.



C. Thin-layer chromatography.

D. Specific optical rotation *(2.2.7)*: +4.0 to +7.0 (anhydrous substance).

Dissolve 5.00 g of the substance to be examined and 6.4 g of *disodium tetraborate R* in 40 ml of *water R*. Allow to stand for 1 h, shaking occasionally, and dilute to 50.0 ml with *water R*. Filter if necessary.

The aqueous solution of D-sorbitol presents very weak optical rotation (approx. −2). The borate complex of sorbitol has somewhat greater optical rotation int he opposite direction(approx. +6).

Tests

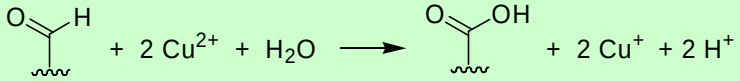
Appearance of solution. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dissolve 5 g in *water R* and dilute to 50 ml with the same solvent.

Reducing sugars: maximum 0.2 per cent, expressed as glucose equivalent.

Dissolve 5.0 g in 6 ml of *water R* with the aid of gentle heat. Cool and add 20 ml of *cupri-citric solution R* and a few glass beads. Heat so that boiling begins after 4 min and maintain boiling for 3 min. Cool rapidly and add 100 ml of a 2.4 per cent *V/V* solution of *glacial acetic acid R* and 20.0 ml of *0.025 M iodine*. With continuous shaking, add 25 ml of a mixture of 6 volumes of *hydrochloric acid R* and 94 volumes of *water R* and, when the precipitate has dissolved, titrate the excess of iodine with *0.05 M sodium thiosulfate* using 1 ml of *starch solution R*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulfate* is required.

Reducing mono- and disaccharides reduce Cu^{2+} to Cu^{+}. Iodine oxidizes Cu^{+} back to Cu^{2+} and the excess of iodine can be determined with thiosulfate.



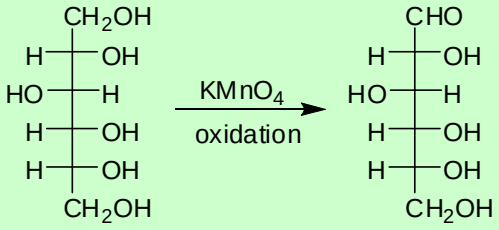
$$2\ Cu^{+} + I_2 \rightarrow 2\ Cu^{2+} + 2\ I^{-}$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^{-} + S_4O_6^{2-}$$

Informative tests

1. See the **Appearance of solution** test in the *Sorbitolum* monograph.
2. Dissolve 0.70 g in 10.0 ml of *water R*. To 2.0 ml of the solution, add 2.0 ml of *cupri-tartaric solution R*. Even on heating the solution to boiling, change is not observed. To another 2.0 ml of the solution, add 1 drop of *dilute sodium hydroxide solution R* and 7 drops of *0.02 M potassium permanganate solution*, with shaking. The boiled solution turns colourless. Add 2.0 ml of *cupri-tartaric solution R* to the solution and boil the dark-blue solution, when a brick-red precipitate is produced.

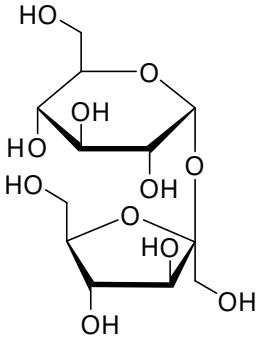
Sorbitol does not reduce Cu^{2+}, while D-glucose, produced by the oxidation of sorbitol with permanganate, gives a positive **FEHLING** reaction.



D-glucose

SUCROSE

Saccharum



$C_{12}H_{22}O_{11}$ **M_r 342.3**

Definition

β-D-Fructofuranosyl α-D-glucopyranoside.

It contains no additives.

Characters

Appearance: white or almost white, crystalline powder or lustrous, dry, colourless white or almost white crystals.

Solubility: very soluble in water, slightly soluble in ethanol (96 per cent), practically insoluble in anhydrous ethanol.

It is an important foodstuff, and is also used to prepare medicalments containing syrup.

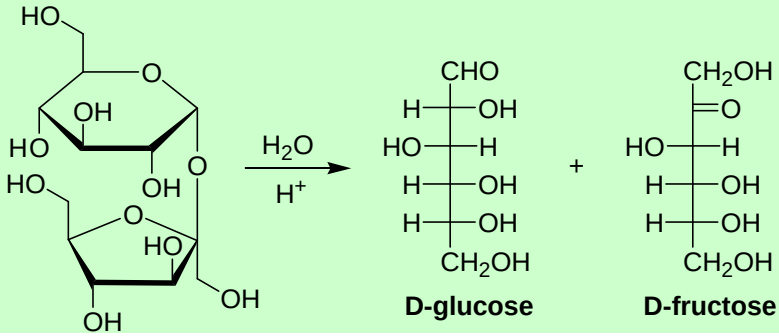
Identification

A. Infrared absorption spectrophotometry.

B. Thin-layer chromatography *(2.2.27)*.

C. Dilute 1 ml of solution S (see Tests) to 100 ml with *water R*. To 5 ml of the solution add 015 ml of freshly prepared *copper sulfate solution R* and 2 ml of freshly prepared *dilute sodium hydroxide solution R*. The solution Is blue and clear and remains se after boiling. To the hot solution add 4 ml of *dilute hydrochloric acid R* and boil for 1 min. Add 4 ml of *dilute sodium hydroxide solution R*. An orange precipitate Is formed immediately.

Sucrose is a non-reducing sugar (it has no free hemiacetal hydroxy groups), and it therefore does not give a positive **FEHLING** reaction, except after acidic hydrolysis to glucose and fructose (see the equations of the identification test **C** of the *Glucose, anhydrous* monograph and test **B** of the *Fructose* monograph).



Tests

Solution S. Dissolve 50.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)*.

Specific optical rotation *(2.2.7)*: +66.3 to +67.0.

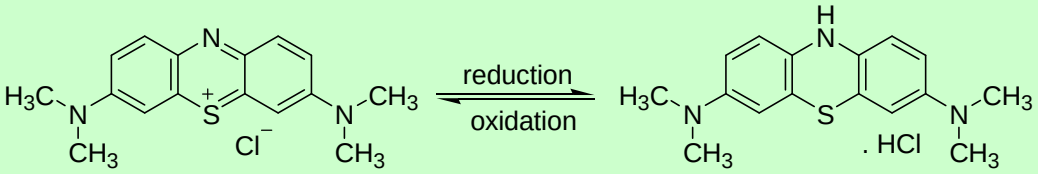
Dissolve 26.0 g in *water R* and dilute to 100.0 ml with the same solvent.

Dextrins. If intended for use in the preparation of large-volume infusions, it complies with the test for dextrins. To 2 ml of solution S add 8 ml of *water R*, 0.05 ml of dilute *hydrochloric acid R* and 0.05 ml of *0.05 M iodine*. The solution remains yellow.

Dextrins arise from the enzymatic degradation of starch. They vary in molecular weight in the following decreasing sequence: amylodextrin, erythrodextrin and archrodextrin. Lack of homogeneity precludes the assignment of a definite molecular weight. With decrease in molecular weight, the colour produced with iodine changes from blue to red and to colourless.

Reducing sugars. To 5 ml of solution S in a test-tube about 150 mm long and 16 mm in diameter add 5 ml of *water R*, 1.0 ml of *1 M sodium hydroxide* and 1.0 ml of a 1 g/l solution of *methylene blue R*. Mix and place in a water-bath. After exactly 2 min, take the tube out of the bath and examine the solution immediately. The blue colour does not disappear completely. Ignore any blue colour at the air/solution interface.

Glucose and invert sugar (the hydrolysed product of sucrose) are reducing sugars and transform methylene blue into a colourless leuko derivative.



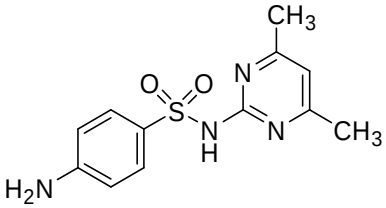
methylene blue **leukomethylene blue**

Informative tests

1. See the **Appearance of solution** test in the *Sucrose* monograph.
2. See identification **C** in the *Sucrose* monograph.

SULFADIMIDINE

Sulfadimidinum



$C_{12}H_{14}N_4O_2S$ **M_r 278.3**

Definition

Sulfadimidine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide, calculated with reference to the dried substance.

Characters

White or almost white powder or crystals, very slightly soluble in water, soluble in acetone, slightly soluble in alcohol. It dissolves in solutions of alkali hydroxides and in dilute mineral acids.

It melts at about 197 °C, with decomposition.

It is an antibacterial sulfonamide derivative.

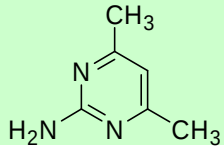
Identification

A. Infrared absorption spectrophotometry.

B. Chromatograms obtained in the test for related substances

C. Place 3 g in a dry tube. Immerse the lower part of the tube, inclined at 45°, in a silicone oil bath and heat to about 270 °C. The substance to be examined decomposes and a white or yellowish-white sublimate is formed which, after recrystallisation from *toluene R* and drying at 100 °C, melts (2.2.14) at 150 °C to 154 °C.

On heating, 2-amino-4,6-dimethylpyrimidine is formed, which sublimes onto the cooler wall of the tube.



D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid.* Dilute 1 ml of the solution to 10 ml with *water R*. The solution, without further acidification, gives the reaction of primary aromatic amines *(2.3.1).*

Tests

Appearance of solution. Dissolve 0.5 g in a mixture of 5 ml of *dilute sodium hydroxide solution R* and 5 ml of *water R*. The solution is not more intensely coloured than reference solution Y_5, BY_5 or GY_5 *(2.2.2, Method II)*.

Informative tests

1. When heated in a dry tube, the sample melts to form a yellow liquid, which turns brown and then black.

2. See the **Appearance of solution** test in the *Sulfadimidine* monograph.

3. See identification **D** in the *Sulfadimidine* monograph.

SULFUR FOR EXTERNAL USE

Sulfur ad usum externum

S A_r **32.07**

Definition

Content. 99.0 per cent to 101.0 per cent.

Characters

Appearance: yellow powder.

Solubility: practically insoluble in water, soluble in carbon disulfide, slightly soluble in vegetable oils.

mp: about 120 °C.

The size of most of the particles is not greater than 20 µm and that of almost all the particles is not greater than 40 µm.

Sulfur is used as a keratolytic, antiseptic and antimycotic agent in dermatology.

Identification

A. Heated in the presence of air, it burns with a blue flame, emitting sulfur dioxide which changes the colour of moistened *blue litmus paper R* to red.

Sulfur burns to sulfur dioxide in oxygen. Sulfur dioxide dissolves in water to give sulfurous acid (acidic medium). In acidic medium, the colour of moistened blue litmus paper changes to red. The pH interval of the colour change of litmus paper is between 5 (red) and 8 (blue).

$$S + O_2 \rightarrow SO_2$$

$$SO_2 + H_2O \rightleftharpoons H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \rightleftharpoons 2\ H^+ + SO_3^{2-}$$

B. Heat 0.1 g with 0.5 ml of *bromine water R* until decolourised. Add 5 ml of *water R* and filter. The solution gives reaction (a) of sulfates (*2.3.1*).

Bromine oxidizes sulfur to sulfate.

$$S + 3\ Br_2 + 4\ H_2O \rightarrow SO_4^{2-} + 6\ Br^- + 8\ H^+$$

TESTS

Solution S. To 5 g add 50 ml of *carbon dioxide-free water R* prepared from *distilled water R*. Allow to stand for 30 min with frequent shaking and filter.

Appearance of solution. Solution S is colourless (*2.2.2, Method II*).

Odour (*2.3.4*). It has no perceptible odour of hydrogen sulfide.

Acidity or alkalinity. To 5 ml of solution S add 0.1 ml of *phenolphthalein solution R1*. The solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*. The solution is red. Add 0.3 ml of 0*.01 M hydrochloric acid*. The solution is colourless. Add 0.15 ml of *methyl red solution R*. The solution is orange-red.

The shaken mixture of sulfur with water is neutral or weakly acidic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red) and that of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

Chlorides (*2.4.4*): maximum 100 ppm.

Dilute 5 ml of solution S to 15 ml with *water R*.

Sulfates (*2.4.13*): maximum 100 ppm, determined on solution S.

Sulfides. To 10 ml of solution S add 2 ml of *buffer solution pH 3.5 R* and 1 ml of a freshly prepared 1.6 g/l solution of *lead nitrate R* in *carbon dioxide-free water R*. Shake. After 1 min any colour in the solution is not more intense than that in a reference solution prepared at the same time using 1 ml of *lead standard solution* (*10 ppm Pb*) *R*, 9 ml of *carbon dioxide-free water R*, 2 ml of *buffer solution pH 3.5 R* and 1.2 ml of *thioacetamide reagent R*.

Sulfide impurity can be detected as lead sulfide. The sulfide ions of the reference test solution are supplied by the hydrolysis of thioacetamide to hydrogen sulfide.

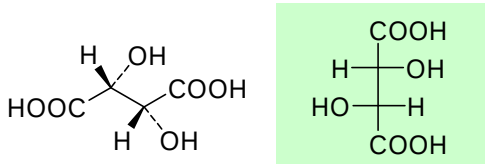
$$Pb^{2+} + S^{2-} \rightarrow PbS$$

Informative test

1. See identification **A** in the *Sulfur for external use* monograph.

TARTARIC ACID

Acidum tartaricum



$C_4H_6O_6$ **M_r 150.1**

Definition

(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid.

Content: 99.5 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: very soluble in water, freely soluble in ethanol (96 per cent).

It is an antioxidant. It is also used as a food additive to make foods tart, or to adjust the acidity, or to mix with bicarbonates to generate carbon dioxide gas (degradation subservient component of effervescent tablets and granules). Tartaric acid is also used as an analytical reagent.

Identification

A. Solution S (see Tests) is strongly acid *(2.2.4)*.

B. It gives the reactions of tartrates *(2.3.1)*.

Tests

Solution S. Dissolve 5.0 g in *distilled water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and not more intensely coloured than reference solution Y_6 *(2.2.2, Method II)*.

Specific optical rotation *(2.2.7)*: +12.0 to +12.8 (dried substance).

Dissolve 5.00 g in *water R* and dilute to 25.0 ml with the same solvent.

Assay

Dissolve 0.650 g in 25 ml of *water R*. Titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution R* as indicator, until a pink colour is obtained.

1 ml of *1 M sodium hydroxide* is equivalent to 75.05 mg of $C_4H_6O_6$.

Tartaric acid is determined as a bivalent acid by alkalimetry. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

$$C_4H_6O_6 \text{ content (\%)} = \frac{V_{NaOH}\text{ (ml)} \cdot f_{NaOH} \cdot E\text{ (mg/ml)}}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

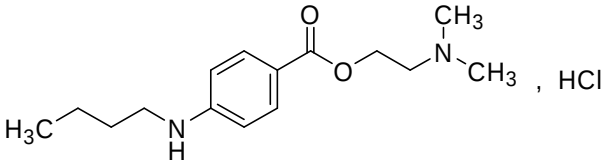
1. See the **Appearance of solution** test in the *Tartaric acid* monograph.

2. See identification **A** in the *Tartaric acid* monograph.

3. See identification **B** in the *Tartaric acid* monograph.

TETRACAINE HYDROCHLORIDE

Tetracaini hydrochloridum



$C_{15}H_{25}ClN_2O_2$ **M_r 300.8**

Definition

2-(Dimethylamino)ethyl 4-(butylamino)benzoate hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, slightly hygroscopic, crystalline powder.

Solubility: freely soluble in water, soluble in ethanol (96 per cent).

It melts at about 148 °C or it may occur in either of 2 other crystalline forms which melt respectively at about 134 °C and 139 °C. Mixtures of these forms melt within the range 134 °C to 147 °C.

It is a local anaesthetic.

Identification

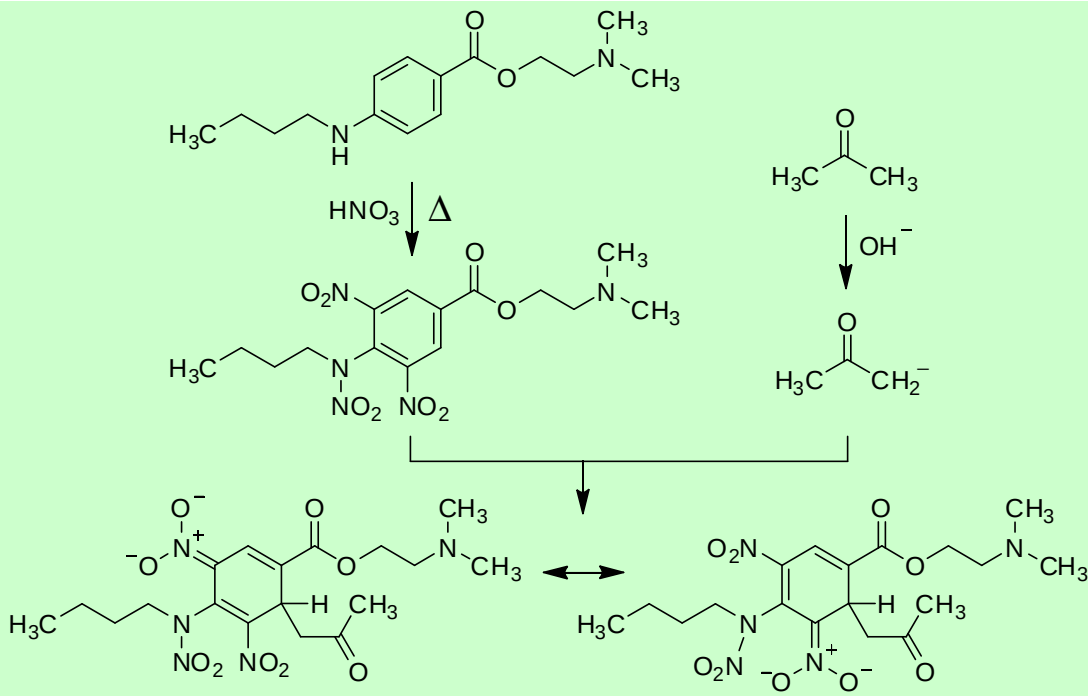
A. Infrared absorption spectrophotometry.

B. To 10 ml of solution S (see Tests) add 1 ml of ammonium thiocyanate solution R. A white, crystalline precipitate is formed which, after recrystallisation from water R and drying at 80 °C for 2 h, melts (2.2.14) at about 131 °C.

Tetracaine forms a water-insoluble salt with thiocyanate.

C. To about 5 mg add 0.5 ml of fuming nitric acid R. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 ml of acetone R. Add 1 ml of 0.1 M alcoholic potassium hydroxide. A violet colour develops.

Tetracaine reacts with nitric acid to form a 3,5-dinitronitramine derivative, which gives a violet **MEISENHEIMER** complex (**VITALI-MORIN** reaction) with acetone in alkaline solution. The reaction is appropriate to distinguish procaine, tetracaine and lidocaine (procaine: reddish-brown; lidocaine: green).



D. Solution S gives reaction (a) of chlorides (2.3.1).

Tests

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. The solution is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Dilute 2 ml of solution S to 10 ml with *water R*.

Informative tests

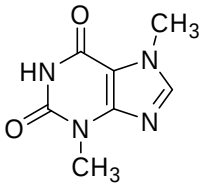
1. See the **Appearance of solution** test in the *Tetracaine hydrochloride* monograph.
2. Dilute 1.0 ml of solution S with 4.0 ml of *water R* and add 1.5 ml of *potassium thiocyanate solution R* to it. A white crystalline compound precipitates, which dissolves when the mixture is heated, but on cooling it reseparates as needles.

   Among the local anaesthetics official in the Pharmacopoeia, only tetracaine forms a water-insoluble salt with thiocyanate.
3. Mix 1.0 ml of solution S with 4–5 drops of *dilute nitric acid R* and 1.0 ml of *silver nitrate solution R1*. A white precipitate is formed.

   A silver chloride precipitate is formed.

THEOBROMINE

Theobrominum



$C_7H_8N_4O_2$ **M_r 180.2**

Definition

Theobromine contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, calculated with reference to the dried substance.

It is used as a CNS stimulant, and a mild diuretic agent.

Characters

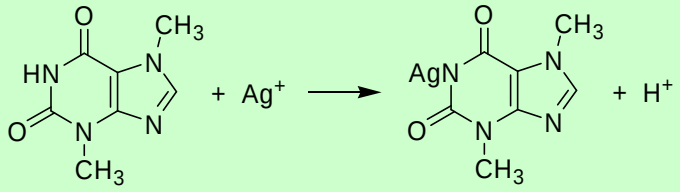
A white or almost white powder, very slightly soluble in water and in anhydrous ethanol, slightly soluble in ammonia. It dissolves in dilute solutions of alkali hydroxides and in mineral acids.

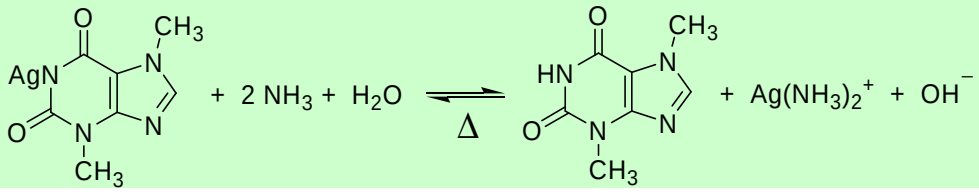
Identification

A. Infrared absorption spectrophotometry.

B. Dissolve about 20 mg in 2 ml of *dilute ammonia R1*, warming slightly, and cool. Add 2 ml of *silver nitrate solution R2*. The solution remains clear. Boil the solution for a few minutes. A white, crystalline precipitate is formed.

This reaction is suitable for distinguishing between xanthine derivatives. Caffeine has no acidic proton and it is, therefore poorly soluble in ammonia and does not react with silver ions. The silver salt of theophylline dissolves neither in water nor in ammonia solution. The silver salt of theobromine is not soluble in water, but dissolves in ammonia with the formation of the diamminesilver complex. When heated, the silver salt of theobromine is reprecipitated.





C. It gives the reaction of xanthines *(2.3.1)*.

Tests

Acidity. To 0.4 g add 20 ml of boiling *water R* and boil for 1 min. Allow to cool and filter. Add 0.05 ml of *bromothymol blue solution R1*. The solution is yellow or yellowish-green. Not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

The shaken mixture of theobromine is neutral. The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Assay

Dissolve, with stirring, 0.150 g in 125 ml of boiling *water R*, cool to 50 °–60 °C and add 25 ml of *0.1 M silver nitrate*. Using 1 ml of *phenolphthalein solution R* as indicator, titrate dropwise with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

Protons liberated during the precipitation of the silver salt of theobromine (see identification **A** of the *Theobromine* monograph) can be determined via alkalimetry. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).

$$C_7H_8N_4O_2 \text{ content (\%)} = \frac{V_{NaOH}\ (ml) \cdot f_{NaOH} \cdot E\ (mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See identification **B** in the *Theobromine* monograph.

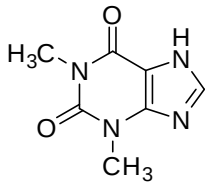
2. See identification **C** in the *Theobromine* monograph.

3. Add 0.1 g to 5.0 ml of *water R,* 0.05 ml of *0.1 M sodium hydroxide* and 2 drops of *R phenolphthalein.* On boiling, the red colour disappears, but it appears again on cooling (a distinction from caffeine and theophylline).

The **WINKLER** test, a suitable reaction for distinction between caffeine, theobromine and theophylline, is based on the different acidities and solubilities of the compounds. Theophylline and theobromine are weak acids. They therefore neutralize sodium hydroxide solution and the red colour of phenolphthalein disappears (for theobromine, due to the low solubility of the substance at ambient temperature, the neutralization occurs only in the boiled solution).

THEOPHYLLINE

Theophyllinum



$C_7H_8N_4O_2$ **M_r 180.2**

Definition

1,3-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Characters

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides, in ammonia and in mineral acids.

It is used to treat asthma and bronchospasm, because it relaxes the smooth muscle of the bronchial airways and pulmonary blood vessels.

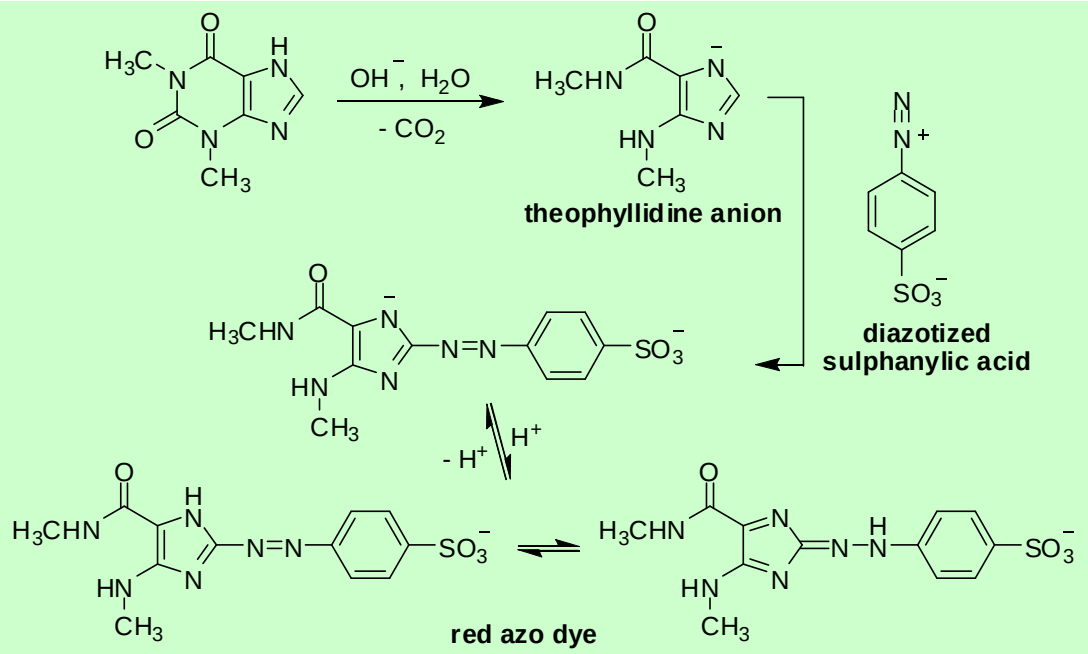
Identification

A. Melting point *(2.2.14)*: 270 °C to 274 °C, determined after drying at 100–105 °C.

B. Infrared absorption spectrophotometry.

C. Heat 10 mg with 1.0 ml of a 360 g/l solution of *potassium hydroxide R* in a water-bath at 90 °C for 3 min, then add 1.0 ml of *diazotized sulfanilic acid solution R*. A red colour slowly develops. Carry out a blank test.

In alkaline solution, theophyllidine is formed by the ring opening of the pyrimidine ring of theophylline. Theophyllidine forms a red azo dye with diazotized sulfanilic acid (theophyllidine reaction). The diazonium salt attacks at position 2 of the imidazole ring. Caffeine and theobromine give similar reactions only after prolonged heating.



D. Loss on drying (see Tests).

E. It gives the reaction of xanthines *(2.3.1).*

Tests

Solution S. Dissolve 0.5 g with heating in *carbon dioxide-free water R*, cool and dilute to 75 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

Acidity. To 50 ml of solution S add 0.1 ml of *methyl red solution R*. The solution is red. Not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

The aqueous solution of theophylline is weakly acidic. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

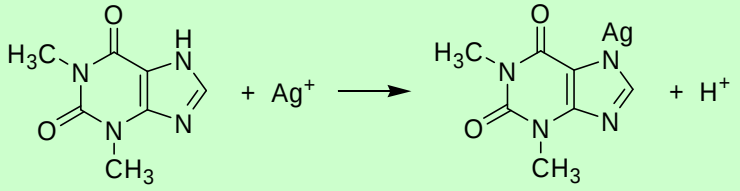
Loss on drying *(2.2.32)*: maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Assay

Dissolve 0.150 g in 100 ml of *water R*, add 20 ml of *0.1 M silver nitrate* and shake. Add 1 ml of *bromothymol blue solution R1*. Titrate with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 18.02 mg of $C_7H_8N_4O_2$.

Protons liberated during the precipitation of the silver salt of theophylline can be determined via alkalimetry. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red).



$$C_7H_8N_4O_2 \text{ content (\%)} = \frac{V_{NaOH}\ (ml)\ .\ f_{NaOH}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative tests

1. See the **Appearance of solution** test in the *Theophylline* monograph.

2. See identification **E** in the *Theophylline* monograph.

3. A mixture of 5.0 ml of *water R,* 0.05 ml of *0,1 M sodium hydroxide,* 2 drops of *R phenolphthalein* and 0.1 g of substance is boiled. After shaking, the mixture must be colourless (a distinction from caffeine and theobromine).

The **WINKLER** test, a suitable reaction for distinction between caffeine, theobromine and theophylline, is based on the different acidities and solubilities of the compounds. Theophylline and theobromine are weak acids. They therefore neutralize sodium hydroxide solution and the red colour of phenolphthalein disappears (for theobromine, due to the low solubility of the substance at ambient temperature, neutralization occurs only in the boiled solution).

TITANIUM DIOXIDE

Titanii dioxidum

TiO_2 **M_r 79.9**

Definition

Content: 98.0 per cent to 100.5 per cent.

Characters

Appearance: white or almost white powder.

Solubility: practically insoluble in water. It does not dissolve in dilute mineral acids but dissolves slowly in hot concentrated sulfuric acid.

It is used as a UV-protective substance in ointments and creams. It is also used as a whitening component of cosmetics and as a food colour additive.

Identification

A. When strongly heated, it becomes pale yellow; the colour disappears on cooling.

B. To 5 ml of solution S2 (see Tests) add 0.1 ml of *strong hydrogen peroxide solution R*. An orange-red colour appears.

When dissolved in sulfuric acid, TiO^{2+} gives an orange peroxytitanium(IV) complex with hydrogen peroxide.

$$TiO^{2+} + H_2O_2 + H_2O \rightarrow H_2Ti(O_2)O_2 + 2\ H^+$$

C. To 5 ml of solution S2 add 0.5 g of *zinc R* in granules. After 45 min, the mixture has a violet-blue colour.

Titanyl ion is reduced by zinc to Ti^{3+}, which forms a violet hexaaqua complex.

$$2\ TiO^{2+} + Zn + 4\ H^+ + 10\ H_2O \rightarrow 2\ [Ti(H_2O)_6]^{3+} + Zn^{2+}$$

Tests

Solution S2. Mix 0.500 g (*m* g) with 5 g of *anhydrous sodium sulfate R* in a 300 ml long-necked combustion flask. Add 10 ml of *water R* and mix. Add 10 ml of sulf*uric acid R* and boil vigorously, with the usual precautions, until a clear solution is obtained. Cool, add slowly a cooled mixture of 30 ml of *water R* and 10 ml of *sulfuric acid R*, cool again and dilute to 100.0 ml with *water R*.

When heated with concentrated hydrochloric acid or concentrated sulfuric acid, titanium dioxide dissolves to form titanyl ions.

$$TiO_2 + 2\ H^+ \rightarrow TiO^{2+} + H_2O$$

Informative test

1. See identification **A** in the *Titanium dioxide* monograph.

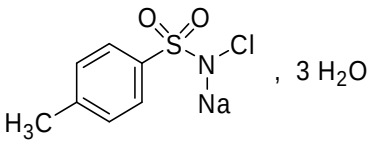
2. Add 3.0 ml of concentrated sulfuric acid to 0.20 g of sample in a porcelain tube, and heat the mixture until fumes of sulfuric acid appear. Dilute the cooled suspension carefully with 10 ml of *water R*. Filter the suspension and add 1-2 drops of *concentrated hydrogen peroxide R* to a 5 ml portion of the filtrate. The mixture turns orange.

See "Identification **B**".

3. 0.10 g of sample does not dissolve in 2.0 ml of *dilute hydrochloric acid R* (a distinction from zinc oxide).

TOSYLCHLORAMIDE SODIUM

Tosylchloramidum natricum



$C_7H_7ClNNaO_2S$. 3 H_2O **M_r 281.7**

Other name: Chloramine-T

Definition

Sodium *N*-chloro-4-methylbenzene-sulfonimidate trihydrate.

Content: 98.0 per cent to 103.0 per cent of $C_7H_7ClNNaO_2S$, 3 H_2O.

Characters

Appearance: white or slightly yellow, crystalline powder.

Solubility: freely soluble in water, soluble in ethanol (96 per cent).

It is a disinfectant. It is also used in analytical reactions as a substitute for chlorine water. Since the aqueous solution of tosylchloramide sodium is unstable, it must be prepared fresh.

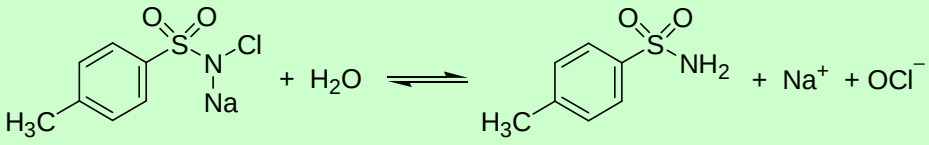
Identification

A. Solution S (see Tests) turns *red litmus paper R* blue and then bleaches it.

The aqueous solutions of chloramines are alkaline; the colour of moistened red litmus paper therefore changes to blue. The pH interval of the colour change of litmus is between 5 (red) and 8 (blue). Tosylchloramide oxidizes the indigo-type blue dye of litmus and the colour disappears.

B. To 10 ml of solution S add 10 ml of *dilute hydrogen peroxide solution R*. A white precipitate is formed which dissolves on heating. Filter the hot solution and allow to cool. White crystals are formed which, when washed and dried at 100 °C to 105 °C, melt *(2.2.14)* at 137 °C to 140 °C.

Tosylchloramide sodium is hydrolysed to *p*-toluenesulfonamide, which is identified by its melting point. The equilibrium of the hydrolysis of tosylchloramide can be shifted to the right-hand side by elimination of the product hypochlorite with hydrogen peroxide.



***p*-toluenesulfonamide**

$$OCl^- + H_2O_2 \rightarrow O_2 + Cl^- + H_2O$$

C. Ignite cautiously 1 g, because of the risk of deflagration. Dissolve the residue in 10 ml of *water R*. The solution gives reaction (a) of chlorides *(2.3.1)*.

Tosylchloramide decomposes to chloride and sulfate on heating.

D. The solution prepared for identification test **C** gives reaction (a) of sulfates *(2.3.1)*.

E. The solution prepared for identification test **C** gives reaction (b) of sodium *(2.3.1)*.

Tests

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension II *(2.2.1)* and is colourless *(2.2.2, Method II)*.

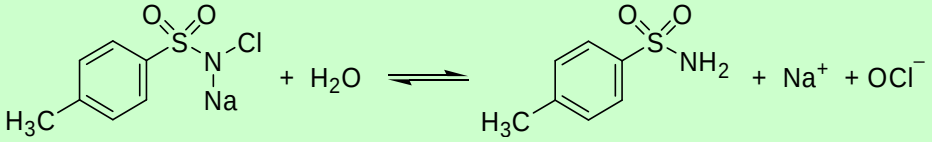
pH *(2.2.3)*: 8.0 to 10.0 for solution S.

Assay

Dissolve 0.125 g in 100 ml of *water R* in a ground-glass-stoppered flask. Add 1 g of *potassium iodide R* and 5 ml of *dilute sulfuric acid R*. Allow to stand for 3 min. Titrate with *0.1 M sodium thiosulfate*, using 1 ml of *starch solution R* as indicator.

1 ml of *0.1 M sodium thiosulfate* is equivalent to 14.08 mg of $C_7H_7ClNNaO_2S,3H_2O$.

In hydrochloric acid solution, tosylchloramide decomposes to hypochlorite, which oxidizes iodide to iodine. Iodine is determined with thiosulfate. The oxidation process must be performed in a well-closed flask to avoid the escape of the volatile chlorine formed by the reaction of hypochlorite with acid.



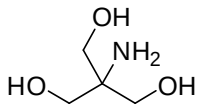
$$OCl^- + 2\ I^- + 2\ H^+ \rightarrow I_2 + Cl^- + H_2O$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6^{2-}$$

$$C_7H_7ClNNaO_2S.3H_2O\ \text{content}\ (\%) = \frac{V_{Na_2S_2O_3}(ml)\ .\ f_{Na_2S_2O_3}.\ E\ (mg/ml)}{\text{amount of substance}\ (mg)}\ .\ 100$$

TROMETAMOL

Trometamolum



$C_4H_{11}NO_3$ *M*r 121.1

Definition

Trometamol contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of aminomethylidynetri(methanol), calculated with reference to the dried substance.

Characters

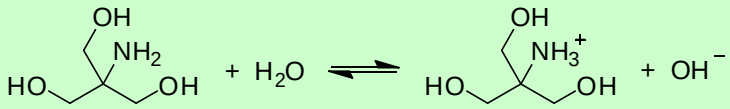
A white or almost white, crystalline powder, or colourless crystals, freely soluble in water, sparingly soluble in alcohol, very slightly soluble in ethyl acetate.

It is used in the treatment of metabolic acidosis and as osmotic diuretic. It is also used as a buffer to regulate the pH of drug solutions (tris buffer).

Identification

A. Solution S (see Tests) is strongly alkaline *(2.2.4)*.

Because of its amino group, the aqueous solution of trometamol is strongly alkaline.



B. Melting point *(2.2.14)*: 168 °C to 174 °C.

C. Infrared absorption spectrophotometry.

D. Thin-layer chromatography.

Tests

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear *(2.2.1)* and colourless *(2.2.2, Method II)*.

pH *(2.2.3)*. The pH of freshly prepared solution S is 10.0 to 11.5.

Chlorides *(2.4.4)*. To 10 ml of solution S add 2.5 ml of *dilute nitric acid R* and dilute to 15 ml with *water R*. The solution complies with the limit test for chlorides (100 ppm).

Heavy metals *(2.4.8).* Dissolve 2.0 g in 10 ml of *water R*. Neutralise the solution with *hydrochloric acid R1* and dilute to 20 ml with *water R*. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the reference solution using *lead standard solution (1 ppm Pb) R*.

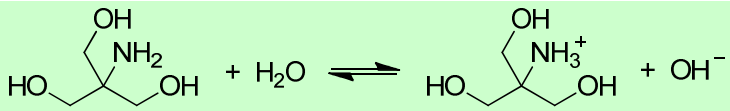
Iron *(2.4.9)*. Dissolve 1.0 g in *water R* and dilute to 10 ml with the same solvent. The solution complies with the limit test for iron (10 ppm).

Assay

Dissolve 0.100 g in 20 ml of *water R*. Add 0.2 ml of *methyl red solution R*. Titrate with *0.1 M hydrochloric acid* until the colour changes from yellow to red.

1 ml of *0.1 M hydrochloric acid* is equivalent to 12.11 mg of $C_4H_{11}NO_3$.

Trometamol is titrated with hydrochloric acid as an monovalent base. The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).



$$C_4H_{11}NO_3 \text{ content (\%)} = \frac{V_{HCl}\,(ml) \cdot f_{HCl} \cdot E\,(mg/ml)}{\text{amount of substance (mg)}} \cdot 100$$

Informative tests

1. See the **Appearance of solution** test in the *Trometamol* monograph.

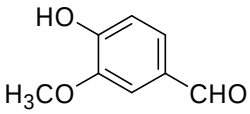
2. See identification **A** in the *Trometamol* monograph.

3. Boil the mixture of 2.0 ml of solution S, 2.0 ml of *dilute sulfuric acid R* and 2 ml of *sodium periodate solution R*. The odour of formaldehyde is observed. After cooling and the addition of 2.0 ml of *dilute sodium hydroxide solution R* to the mixture, reheating results in ammonia gas liberation.

Periodate oxidizes trometamol to formaldehyde and ammonia.

VANILLIN

Vanillinum



$C_8H_8O_3$ **M_r 152.1**

Definition

Vanillin contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-hydroxy-3-methoxybenzaldehyde, calculated with reference to the dried substance.

Characters

White or slightly yellowish, crystalline powder or needles, slightly soluble in water, freely soluble in alcohol and in methanol. It dissolves in dilute solutions of alkali hydroxides.

Vanillin is used as an analytical reagent and as a flavouring in medicines and food products.

Identification

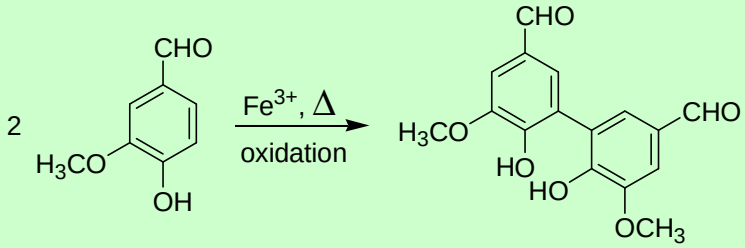
A. Melting point (2.2.14): 81 °C to 84 °C.

B. Infrared absorption spectrophotometry.

C. Chromatograms obtained in the test for related substances.

D. To 5 ml of a saturated solution of the substance to be examined add 0.2 ml of *ferric chloride solution R1*. A blue colour is produced. Heat to 80 °C. The solution becomes brown. On cooling, a white precipitate is formed.

Vanillin (like most phenol derivatives) forms a coloured complex with Fe^{3+}. When heated, this complex decomposes and the iron(III) oxidizes vanillin to dehydrodivanillin (mp: 302-305 ºC), which precipitates as a white crystalline product on cooling.



dehydrodivanillin

Tests

Appearance of solution. Dissolve 1.0 g in *alcohol R* and dilute to 20 ml with the same solvent. The solution is clear *(2.2.1)* and not more intensely coloured than reference solution B_6 *(2.2.2, Method II)*.

Informative test

1. See the **Appearance of solution** test in the *Vanillin* monograph.

2. See identification **D** in the *Vanillin* monograph.

WATER, PURIFIED

Aqua purificata

H_2O **M_r 18.02**

Definition

Water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorised.

Purified water in bulk

Production

Purified water in bulk is prepared by distillation, by ion exchange, by reverse osmosis or by any other suitable method from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

Purified water in bulk is stored and distributed in conditions designed to prevent growth of micro-organisms and to avoid any other contamination.

During production and subsequent storage, appropriate measures are taken to ensure that the microbial count is adequately controlled and monitored. Appropriate alert and action levels are set so as to detect adverse trends.

Characters

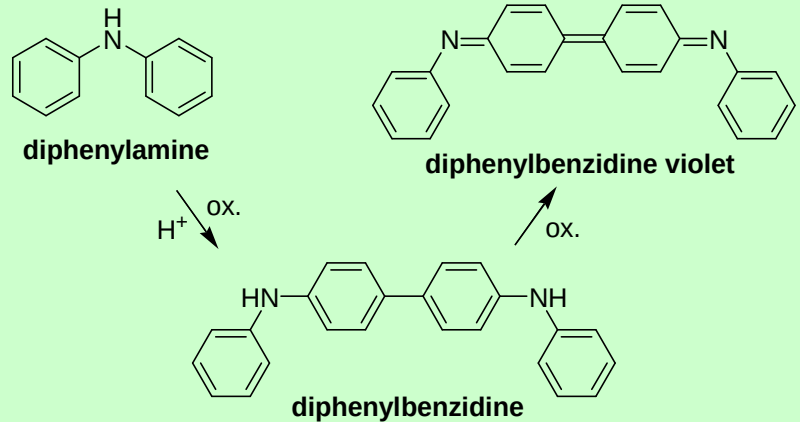
Appearance: clear and colourless liquid.

Tests

Nitrates: maximum 0.2 ppm.

Place 5 ml in a test-tube immersed in iced water, add 0.4 ml of a 100 g/l solution of *potassium chloride R*, 0.1 ml of *diphenylamine solution R* and, dropwise with shaking, 5 ml of *nitrogen-free sulfuric acid R*. Transfer the tube to a water-bath at 50 °C. After 15 min, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of *nitrate-free water R* and 0.5 ml of *nitrate standard solution* (*2 ppm NO_3^-*) *R* (0.2 ppm).

In strongly acidic medium, nitrate oxidizes diphenylamine to diphenylbenzidine, and then to diphenylbenzidine violet dye (diphenylamine blue). The reaction is highly sensitive, but not selective. The presence of chloride ions increases the sensitivity of the reaction.



Purified water in containers

Purified water in bulk that has been filled and stored in conditions designed to assure the required micro-biological quality. It is free from any added substances.

Characters

Appearance: clear and colourless liquid.

Tests

It complies with the tests prescribed in the section on *Purified water in bulk* and with the following additional tests.

Acidity or alkalinity. To 10 ml, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution R*. The solution is not coloured red.

The pH interval of the colour change of the methyl red indicator is between 4.4 (red) and 6.0 (yellow).

To 10 ml add 0.1 ml of *bromothymol blue solution R1*. The solution is not coloured blue.

The pH interval of the colour change of the bromothymol blue indicator is between 5.8 (yellow) and 7.4 (blue).

Oxidisable substances. To 100 ml add 10 ml of *dilute sulfuric acid R* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 min. The solution remain faintly pink.

Oxidizable substances decolorize permanganate solution.

Chlorides. To 10 ml add 1 ml of *dilute nitric acid R* and 0.2 ml of *silver nitrate solution R2*. The solution shows no change in appearance for at least 15 min.

A silver chloride precipitate is formed.

Sulfates. To 10 ml add 0.1 ml of *dilute hydrochloric acid R* and 0.1 ml of *barium chloride solution R1*. The solution shows no change in appearance for at least 1 h.

A barium sulfate precipitate is formed.

Ammonium: maximum 0.2 ppm.

To 20 ml add 1 ml of *alkaline potassium tetraiodomercurate solution R*. After 5 min, examine the solution down the vertical axis of the tube. The solution is not more intensely coloured than a standard prepared at the same time by adding 1 ml of *alkaline potassium tetraiodomercurate solution R* to a mixture of 4 ml of *ammonium standard solution* (*1 ppm* NH_4^+) *R* and 16 ml of *ammonium-free water R* (0.2 ppm).

Depending on the quantity of ammonia, a brown coloration or a brown precipitate of mercury(II) oxide–mercury(II) amidoiodide is formed (see limit test 2.4.1/A).

Calcium and magnesium. To 100 ml add 2 ml of *ammonium chloride buffer solution pH 10.0 R*, 50 mg of *mordant black 11 triturate R* and 0.5 ml of *0.01 M sodium edetate*. A pure blue colour is produced.

The colour of the *mordant black 11* indicator is blue at pH 8-12, but in the presence of Ca^{2+} or Mg^{2+} the colour changes to red.

Informative test

1. Pour 200 ml into a 200 ml colourless beaker. When the liquid is examined down the vertical axis of the beaker against a white, and then a black background, it is colourless and clear.
2. See the **Acidity or alkalinity** test in the *Water, Purified* monograph.
3. See the **Oxidizable substances** in the *Water, Purified* monograph.

ZINC OXIDE

Zinci oxidum

ZnO M_r **81.4**

Definition

Content: 99.0 per cent to 100.5 per cent (ignited substance).

Characters

Appearance: soft, white or faintly yellowish-white, amorphous powder, free from gritty particles.

Solubility: practically insoluble in water and in ethanol (96 per cent). It dissolves in dilute mineral acids.

Zinc oxide has astringent and disinfectant properties and is therefore used in dusting powders and in ointments.

Identification

A. It becomes yellow when strongly heated; the yellow colour disappears on cooling.

B. Dissolve 0.1 g in 1.5 ml of *dilute hydrochloric acid R* and dilute to 5 ml with *water R*. The solution gives the reaction of zinc (*2.3.1*).

Zinc oxide dissolves in acids.

$$ZnO + 2\ H^+ \rightarrow Zn^{2+} + H_2O$$

Tests

Alkalinity. Shake 1.0 g with 10 ml of boiling *water R*. Add 0.1 ml of *phenolphthalein solution R* and filter. If the filtrate is red, not more than 0.3 ml of *0.1 M hydrochloric acid* is required to change the colour of the indicator.

The shaken mixture of zinc oxide with *water R* is weakly basic. The pH interval of the colour change of the phenolphthalein indicator is between 8.2 (colourless) and 10.0 (red)

Carbonates and substances insoluble in acids. Dissolve 1.0 g in 15 ml of *dilute hydrochloric acid R*. It dissolves without effervescence and the solution is not more opalescent than reference suspension II (*2.2.1*) and is colourless (*2.2.2, Method II*).

Arsenic (*2.4.2*): maximum 5 ppm, determined on 0.2 g.

Iron (*2.4.9*): maximum 200 ppm.

Dissolve 50 mg in 1 ml of *dilute hydrochloric acid R* and dilute to 10 ml with *water R*. Use in this test 0.5 ml of *thioglycollic acid R*.

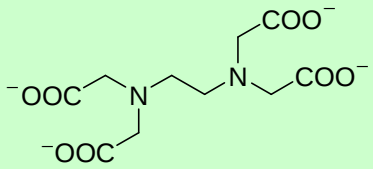
Assay

Dissolve 0.150 g in 10 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 8.14 mg of ZnO.

Zn^{2+} is determined by complexometric titration, when the Zn^{2+}–EDTA complex is formed.

$$Zn^{2+} + H_2Y^{2-} \rightarrow ZnY^{2-} + 2\ H^+$$ Y^{4-}:



$$\text{ZnO content (\%)} = \frac{V_{EDTA}\ (ml)\ .\ f_{EDTA}\ .\ E\ (mg/ml)}{\text{amount of substance (mg)}}\ .\ 100$$

Informative test

1. See identification **A** in the *Zinc oxide* monograph.

2. See identification **B** in *Zinc oxide* monograph.

ZINC SULFATE HEPTAHYDRATE

Zinci sulfas heptahydricus

$ZnSO_4,7H_2O$ **M_r 287.5**

Definition

Content: 99.0 per cent to 104.0 per cent.

Characters

Appearance: white or almost white, crystalline powder or colourless, transparent crystals, efflorescent.

Solubility: very soluble in water, practically insoluble in ethanol (96%).

As an astringent and antiseptic substance, it is used in ophthalmic drops for the treatment of conjunctivitis.

Identification

A. Solution S (see Tests) gives the reactions of sulfates (*2.3.1*).

B. Solution S gives the reaction of zinc (*2.3.1*).

C. It complies with the limits of the assay.

Tests

Solution S. Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Assay

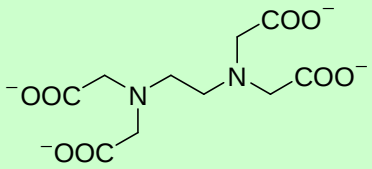
Dissolve 0.200 g in 5 ml of *dilute acetic acid R*. Carry out the complexometric titration of zinc (*2.5.11*).

1 ml of *0.1 M sodium edetate* is equivalent to 28.75 mg of $ZnSO_4,7H_2O$.

Zinc is determined by complexometric titration, when the Zn^{2+}–EDTA complex is formed.

$$Zn^{2+} + H_2Y^{2-} \rightarrow ZnY^{2-} + 2\ H^+$$

Y^{4-}:



$$ZnSO_4,7H_2O\ \text{content}\ (\%) = \frac{V_{EDTA}\ (ml)\ .\ f_{EDTA}\ .\ E\ (mg/ml)}{\text{amount of substance}\ (mg)}\ .\ 100$$

Informative test

1. See the **Appearance of solution** test in the *Zinc sulfate* monograph.

2. See identification **A** in the *Zinc sulfate* monograph.

3. See identification **B** in the *Zinc sulfate* monograph.

MONITORING QUESTIONS

What kind of water can be applied in the analytical procedures described in the Pharmacopoeia or for the preparation of reagents?

How do the quantitative conditions influence the dissolution of a silver chloride precipitate in ammonia solution?

What volatile product is formed in the reaction of chloride with the dichromate/diphenylcarbazide system based on the chloride identification reaction of the Pharmacopoeia? Which step is the oxidoreduction during the formation of the diphenylcarbazone complex?

Why is it important to work with the same volume of the liquid examined and the reference solution for comparative tests? Why do we compare the contents of the tubes vertically and not horizontally? What kind of background do we have to apply for the comparison of the clarity and degree of opalescense or coloration of liquids examined?

What does the definition “clear and colourless” mean in the Pharmacopoeia?

Why is it possible to apply a standard solution of either sodium hydroxide or hydrochloric acid when we observe a green colour on adding bromothymol blue indicator to a solution of sodium chloride?

What does “limit” mean in limit test reactions? Why is it necessary to prepare and compare standards?

Why do we apply lead acetate cotton in the limit test of arsen is? Why is it essential?

Expain the advantages of the application of sodium sulfide solution instead of thioacetamide reagent in the “heavy metals” limit test reaction. Why is it essential to prepare the thioacetamide reagent fresh before the test reaction?

For what purpose do we apply chlorine in analytical chemistry? How can we generate it in laboratory practice?

What is the fundamental reaction of Karl Fischer water content determination?

How can we identify sulfur dioxide gas?

How can we make a distinction between sulfite and thiosulfate salts?

What happens if we add hydrochloric acid to an aqueous solution of sodium thiosulfate?

What is the pH of an aqueous solution of sodium iodide, ammonium chloride or sodium sulfite? Why?

How can we make a distinction between primary and secondary phosphate ions?

How can we explain the temperature-dependent colour change of the iodine–starch complex?

What arsenic test reactions can be found in the Pharmacopoeia?

What chemical reaction can be used for the identification of carbon dioxide liberated in the reactions of carbonates with acids?

What reaction takes place in the flame test for borates?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between sodium bromide and ammonium bromide?

What are the oxidation number and oxidation state of the element Mn in the compounds of manganese? What colour do they have?

How do we determine the end-point of the titration in permanaganometry?

How can we distinguish sodium carbonate from sodium hydrogencarbonate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between lithium carbonate and calcium carbonate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between potassium chloride and potassium perchlorate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between potassium bromide and potassium iodide?

How can the disappearance of the colour of phenolphthalein indicator be explained when glycerol is added to an aqueous solution of borax?

Why is the fast preparation and filtration of the solution prepared for the quantitative analysis of *Ferrum metallicum for homeopathic preparations* important?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between aluminium sulfate and zinc sulfate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between zinc oxide and magnesium oxide light?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between aluminium sulfate, zinc sulfate and magnesium sulfate heptahydrate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between silver nitrate, bishmuth subnitrate heavy and potassium nitrate?

What identification and informative test reactions (based on the European Pharmacopoeia) can we apply to make a distinction between magnesium sulfate heptahydrate, magnesium carbonate light, magnesium trisilicate and magnesium oxide light?

How can we dissolve barium sulfate? Why is it essential to acidify the filtered alkaline solution before determination of sulfate ion in the identification of barium sulfate official in Pharmacopoeia?

What oxidation state changes occur in theidentification of potassium permanganate with ethanol?

What reagents are applied for the preparation of azo dye from primary aromatic amines?

What is the principal reaction for the detection of the peroxide impurities in diethyl ether?

What properties are used to distinguish between barbital and phenobarbital in informative tests?

What is the oxidation number of chlorine in tosylchloramide sodium?

What functional groups can be determined with the idoform test?

What chemical reactions can be applied to distinguish between morphine hydrochloride and codeine hydrochloride on the basis of the European Pharmacopoeia?

What identification reaction can be applied to distinguish between benzocaine and lidocaine?

What is the König reaction? What compounds can be identified with this reaction?

What is the Helch reaction? What compounds can be identified with this reaction?

How can we distinguish between theobromine, theophylline and caffeine with the Winkler test?

What compounds can be identified with the thalleiochin reaction?

What reactions can we used to distinguish quinine sulfate from quinidine sulfate?

What chemical reactions can be applied to distinguish between glucose, fructose, lactose and sucrose on the basis of the European Pharmacopoeia?

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