
List of reagents, test solutions, and
volumetric solutions

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Attention is drawn to the notes at the head of the "List of reagents, test solutions, and volumetric solutions" published in Volume 2. These contain explanations of the various abbreviations used.

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Acetaldehyde R. Ethanal; C_2H_4O .

Description. A clear, colourless, flammable liquid.

Miscibility. Miscible with water and ethanol (~750 g/l) TS.

Refractive index. $n_D^{20} = 1.332$.

Relative density. $d_{20}^{20} = 0.788$.

Boiling point. About 21 °C.

Albendazole RS. International Chemical Reference Substance.

Alcuronium chloride RS. International Chemical Reference Substance.

Aluminium oxide R. Al_2O_3 .

A suitable grade for use in thin-layer chromatography.

7-[(2-Aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid RS (ciprofloxacin ethylenediamine compound). International Chemical Reference Substance.

Ammonium acetate TS

Procedure. Dissolve 150 g of ammonium acetate R in water, add 3 ml of glacial acetic acid R, and dilute with sufficient water to produce 1000 ml.

Note: Ammonium acetate TS must be used within 1 week of preparation.

Ammonium acetate (50 g/l) TS. A solution of ammonium acetate R containing about 50 g of $C_2H_7NO_2$ per litre.

Ammonium chloride (20 g/l) TS. A solution of ammonium chloride R containing about 20 g of NH_4Cl per litre.

Ammonium nitrate TS

Procedure. Dissolve 1.6 g of ammonium nitrate R in 30 ml of water, add 3.0 ml of ammonia (~260 g/l) TS, and dilute with sufficient water to produce 100 ml.

Ammonium thiocyanate (10 g/l) TS. A solution of ammonium thiocyanate R containing 10 g of NH_4SCN per litre.

Amoxicillin trihydrate RS. International Chemical Reference Substance.

Anisaldehyde R. 4-Methoxybenzaldehyde; $C_8H_8O_2$.

Description. A colourless to pale yellow, oily liquid.

Solubility. Very slightly soluble in water, miscible with ethanol (~750 g/l) TS and ether R.

Mass density. ρ_{20} = about 1.125 kg/l.

Boiling point. About 248 °C.

Anisaldehyde TS

Procedure. Mix in the following order 0.5 ml of anisaldehyde R, 10 ml of glacial acetic acid R, 85 ml of methanol R, and 5 ml of sulfuric acid (~1760 g/l) TS.

Anisaldehyde/methanol TS

Procedure. Slowly add 10 ml of glacial acetic acid R and 5 ml of sulfuric acid (~1760 g/l) TS to 55 ml of methanol R, and cool to room temperature. Separately add 0.5 ml of anisaldehyde R to 30 ml of methanol R. Mix the two solutions thoroughly.

Storage. Keep anisaldehyde/methanol TS protected from light.

Note: Anisaldehyde/methanol TS should be freshly prepared.

Anisaldehyde/sulfuric acid TS

Procedure. Add 5 ml of anisaldehyde R to 10 ml of sulfuric acid (~1760 g/l) TS.

Anthrone TS2

Procedure. Dissolve 200 mg of anthrone R in 100 ml of sulfuric acid (~1760 g/l) TS.

Aprotinin R. A polypeptide consisting of a chain of 58 amino acids.

A commercially available reagent of suitable grade.

Argon R. Ar. Contains not less than 99.995% of Ar.

Carbon monoxide. When used as described in the test for carbon monoxide in medicinal gases, after passage of 10 litres of argon at a flow rate of 4 litres per hour, not more than 0.05 ml of sodium thiosulfate (0.002 mol/l) VS is required for the titration (0.6 μ l/l).

Artemether RS. International Chemical Reference Substance.

Artemisinin RS. International Chemical Reference Substance.

Artemotil RS. International Chemical Reference Substance.

Artenimol RS. International Chemical Reference Substance.

Artesunate RS. International Chemical Reference Substance.

Atenolol RS. International Chemical Reference Substance.

Atenolol for column validation RS. International Chemical Reference Substance.

Barium hydroxide (0.15 mol/l) VS. Barium hydroxide R dissolved in carbon-dioxide-free water R to contain 25.7 g of Ba(OH)₂ in 1000 ml.

Benznidazole RS. International Chemical Reference Substance.

Benzophenone R. Diphenyl ketone; C₁₃H₁₀O.
A commercially available reagent of suitable grade.
Melting point. About 49 °C.

Benzoyl peroxide, hydrous R. C₁₄H₁₀O₄·xH₂O.
A commercially available reagent of suitable grade.
Description. A white, amorphous or granular powder.
Note: For safety reasons it should be kept moistened with about 23% w/w water.

Benzyl benzoate R. C₁₄H₁₂O₂.
Benzyl benzoate as described in the monograph in Vol. 2 (p. 46).
A commercially available reagent of suitable grade.
Description. A clear, colourless, oily liquid.

9,9'-Bisanthracene-10,10'(9H,9'H)-dione RS. International Chemical Reference Substance.

4,4'-Bis(dimethylamino)benzophenone R. Tetramethyldiaminobenzophenone; C₁₇H₂₀N₂O.
Other name. Michler's ketone.
Melting point. About 176 °C.

Bromophenol blue (1 g/l) TS. A solution of bromophenol blue R containing about 1.0 g of C₁₉H₁₀Br₄O₅S per litre.

Butylated hydroxyanisole R. Use C₁₁H₁₆O₂.
Butylated hydroxyanisole as described in the monograph in Vol. 4 (p. 140).

tert-Butyl methyl ether R. 1,1-Dimethylethyl methyl ether; C₅H₁₂O.
A commercially available reagent of suitable grade.
Description. A clear, colourless liquid; inflammable.
Refractive index. $n_D^{20} = 1.3756$.
Relative density. $d_4^{20} = 0.740-0.742$.

Caesium chloride R. CsCl.
A commercially available reagent of suitable grade.

Calcium chloride (3.7 g/l) TS. A solution of anhydrous calcium chloride R containing about 9 g of CaCl₂ per litre.

Captopril RS. International Chemical Reference Substance.

Captopril disulfide RS. International Chemical Reference Substance.

Carbon dioxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for hydrazine and crystal violet indicators. The minimum value indicated is 100 µl/l or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Carbon monoxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for di-iodine pentoxide, selenium dioxide and fuming sulfuric acid indicators. The minimum value indicated is 5 µl/l or less, with a relative standard deviation of at most ±15%. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Carbon monoxide R. CO.

A commercially available gas of suitable grade.

Cephalin TS

Procedure. Place a quantity between 0.5 and 1.0 g of acetone-dried ox brain R into a centrifuge tube, add 20 ml of acetone R, and allow to stand for 2 hours. Centrifuge for 2 minutes and decant the supernatant liquid. Dry the residue under reduced pressure, add to it 20 ml of chloroform R, and allow to stand for 2 hours, shaking frequently. Separate the solid material by filtration or centrifugation and evaporate the chloroform under reduced pressure. Suspend the residue in 5–10 ml of sodium chloride (9 g/l) TS. Solvents used to prepare cephalin TS should contain a suitable antioxidant, for example, a solution of 0.02 g/l of butylated hydroxyanisole R.

Storage. Store in a freezer or keep in a freeze-dried state.

Note: The reagent must be used within 3 months.

Chloramphenicol disodium disuccinate RS. International Chemical Reference Substance.

Chloramphenicol sodium succinate RS. International Chemical Reference Substance.

Chloride standard (5 µg/l) TS

Procedure. Weigh accurately 82.4 mg of sodium chloride R and dissolve in suf-

ficient water to produce 100 ml. Dilute 1.0 ml of this solution with water to 100 ml.

7-Chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazin-1-yl)quinoline-3-carboxylic acid RS (ciprofloxacin by-compound A). International Chemical Reference Substance.

1-Chloro-2,4 dinitrobenzene R. $C_6H_3ClN_2O_4$.
A commercially available reagent of suitable grade.
Melting point. About 144 °C.

1-Chloro-2,4 dinitrobenzene/ethanol TS
Procedure. Weigh 5 g of 1-Chloro-2,4 dinitrobenzene R and dissolve in sufficient ethanol (~750 g/l) TS to produce 100 ml.

Ciclosporin RS. International Chemical Reference Substance.

Ciclosporin U RS. International Chemical Reference Substance.

Ciprofloxacin RS. International Chemical Reference Substance.

Ciprofloxacin hydrochloride RS. International Chemical Reference Substance.

Clindamycin hydrochloride RS. International Chemical Reference Substance.

Clindamycin phosphate RS. International Chemical Reference Substance.

Colchicine RS. International Chemical Reference Substance.

1-Cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid RS (ciprofloxacin desfluoro-compound). International Chemical Reference Substance.

Dacarbazine RS. International Chemical Reference Substance.

Dacarbazine related compound A RS. International Chemical Reference Substance.

Dacarbazine related compound B RS. International Chemical Reference Substance.

Dantron R. 1,8-dihydroxyanthraquinone; $C_{14}H_8O_4$.
A commercially available reagent of suitable grade.

Description. An orange, microcrystalline powder.

Solubility. Practically insoluble in water; slightly soluble in ethanol (~750 g/l) TS and ether R.

Melting point. About 193 °C.

3,3'-Diaminobenzidine tetrahydrochloride R. $C_{12}H_{14}N_4, 4HCl, 2H_2O$.

A commercially available reagent of suitable grade.

Description. An almost white or slightly pink powder.

3,3'-Diaminobenzidine tetrahydrochloride (5 g/l) TS. A solution of 3,3'-diaminobenzidine tetrahydrochloride R containing 5 g of $C_{12}H_{14}N_4, 4HCl$ per litre.

Diethylphenylenediamine sulfate TS

Procedure. To 250 ml of water add about 2 ml of sulfuric acid (~1760 g/l) TS and 50 ml of disodium edetate (0.01 mol/l) VS. Dissolve 1.1 g of *N,N*-diethyl-*p*-phenylenediamine sulfate R into this solution and dilute with sufficient water to produce 1000 ml.

***N,N*-Diethyl-*p*-phenylenediamine sulfate R.** *N,N*-Diethyl-1,4-phenylenediamine sulfate; $C_{10}H_{16}N_2, H_2SO_4$.

A commercially available reagent of suitable grade.

Description. A white or slightly coloured powder.

Melting point. About 185 °C, with decomposition.

Storage. *N,N*-Diethyl-*p*-phenylenediamine sulfate R should be kept protected from light.

Diethyltoluamide RS. International Chemical Reference Substance.

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate RS. International Chemical Reference Substance.

Dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate RS. International Chemical Reference Substance.

***N,N*-Dimethyloctylamine R.** Octyldimethylamine; $C_{10}H_{23}N$.

A commercially available reagent of suitable grade.

Description. A colourless liquid.

Boiling point. About 195 °C.

Dinitrogen oxide R. N_2O .

A commercially available gas of suitable grade.

1,2-Diphenylethylammonium 3-mercapto-2-methylpropanoate RS. International Chemical Reference Substance.

Dissolution media

- **Buffer pH = 1.3**

Dissolve 2 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 1.3 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Buffer pH = 2.5**

Dissolve 2 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 2.5 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Buffer pH = 3.5**

Dissolve 7.507 g of glycine R and 5.844 g of sodium chloride R in 800 ml of deionized water, adjust the pH to 3.5 with hydrochloric acid (~70 g/l) TS, and dilute to 1000 ml with water.

- **Buffer pH = 4.5**

Dissolve 6.8 g of potassium dihydrogen phosphate R in 900 ml of deionized water, adjust the pH to 4.5 either with hydrochloric acid (~70 g/l) TS or sodium hydroxide (~80 g/l) TS, and dilute to 1000 ml with water.

- **Buffer pH = 6.8**

Dissolve 5.53 g of disodium hydrogen phosphate R in 800 ml of deionized water, adjust the pH to 6.8 with sodium hydroxide (~80 g/l) TS, and dilute to 1000 ml with water.

- **Buffer pH = 7.2**

Dissolve 9.075 g of potassium dihydrogen phosphate R in deionized water to produce 1000 ml (solution A). Dissolve 11.87 g of disodium hydrogen phosphate R in sufficient water to produce 1000 ml (solution B). Mix 300 ml of solution A with 700 ml of solution B.

- **Gastric fluid, simulated, TS**

Dissolve 2.0 g of sodium chloride R and 3.2 g of pepsin R in 7.0 ml of hydrochloric acid (~420 g/l) TS and sufficient water to produce 1000 ml. This test solution has a pH of about 1.2.

- **Intestinal fluid, simulated, TS**

Dissolve 6.8 g of potassium dihydrogen phosphate R in 250 ml of water, mix, and add 190 ml of sodium hydroxide (0.2 mol/l) VS and 400 ml of water. Add 10.0 g of pancreatin R, mix, and adjust the resulting solution with sodium hydroxide (0.2 mol/l) VS to a pH of 7.5 ± 0.1 . Dilute with sufficient water to produce 1000 ml.

5,5'-Dithiobis(2-nitrobenzoic acid) R. 3-Carboxy-4-nitrophenyl disulfide;
 $C_{14}H_8N_2O_8S_2$.

A commercially available reagent of suitable grade.

5,5'-Dithiobis-2-nitrobenzoic acid/methanol TS

Procedure. Dissolve 0.198 g of 5,5'-Dithiobis(2-nitrobenzoic acid) R in sufficient methanol R to produce 500 ml.

Storage. Keep under refrigeration, and warm to room temperature before use.

Dithranol RS. International Chemical Reference Substance.

Doxycycline hyclate RS. International Chemical Reference Substance.

Econazole nitrate RS. International Chemical Reference Substance.

Endotoxin RS. Second WHO International Standard for Endotoxin as established in 1996, containing 10 000 IU per ampoule, approximately 1 µg of freeze-dried endotoxin from *Escherichia coli*, with 1 mg of PEG and 10 mg of lactose (distributed by the National Institute for Biological Standards and Control (NIBSC), P.O. Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts. EN6 3QH, England), or another suitable preparation, the activity of which has been determined in relation to the WHO International Standard using the gelation test.

6-Epidoxycycline hydrochloride RS. International Chemical Reference Substance.

Erythromycin lactobionate RS. International Chemical Reference Substance.

Ethylene oxide R. C₂H₄O.

A commercially available gas of suitable grade.

Ethylene oxide TS

Procedure. Weigh 1.0 g of cold ethylene oxide stock solution R (equivalent to 2.5 mg of ethylene oxide) into a cold flask containing 40 g of cold macrogol 200 TS. Mix and determine the exact mass, and dilute to a calculated mass to obtain a solution containing 50 µg of ethylene oxide per 1.0 g of solution. Weigh 10.0 g into a flask and dilute with sufficient water to produce 50 ml (10 µg/ml of ethylene oxide). Dilute 10 ml of this solution to 50 ml with water (2 µg/ml of ethylene oxide).

Note: Ethylene oxide TS should be prepared immediately before use.

Ethylene oxide stock solution R

Note: All operations should be carried out in a fume-hood. The operator must protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask.

Procedure. Into a dry, clean test-tube, cooled in a mixture of 1 part of sodium chloride R and 3 parts of crushed ice, introduce a slow current of ethylene oxide R gas, allowing condensation onto the inner wall of the test-tube.

Using a glass syringe, previously cooled to -10°C , inject about $300\ \mu\text{l}$ (corresponding to about $0.25\ \text{g}$) of liquid ethylene oxide R into $50\ \text{ml}$ of macrogol 200 TS. Determine the absorbed quantity of ethylene oxide by weighing before and after absorption. Dilute to $100\ \text{ml}$ with macrogol 200 TS. Mix well before use.

Assay. To $10\ \text{ml}$ of a $500\ \text{g/l}$ suspension of magnesium chloride R in dehydrated ethanol R add $20\ \text{ml}$ of hydrochloric acid/ethanol ($0.1\ \text{mol/l}$) VS, stopper the flask, shake to obtain a saturated solution, and allow to stand overnight to equilibrate. Weigh $5\ \text{g}$ of the prepared ethylene oxide stock solution R (containing about $2.5\ \text{g/l}$) into the flask and allow to stand for 30 minutes. Titrate with potassium hydroxide/ethanol ($0.1\ \text{mol/l}$) VS, determining the end-point potentiometrically. Carry out a blank titration, replacing the substance to be examined with the same quantity of macrogol 200 TS. Calculate the content of ethylene oxide in mg/g .

Storage. Keep in a tightly closed container in a refrigerator at 4°C .

1-Ethylquinaldinium iodide R. 1-Ethyl-2-methylquinolinium iodide; $\text{C}_{12}\text{H}_{14}\text{IN}$.

A commercially available reagent of suitable grade.

Description. A yellow-green solid.

Solubility. Sparingly soluble in water.

1-Ethylquinaldinium iodide (15 g/l) TS. A solution containing about $15\ \text{g}$ of 1-ethylquinaldinium iodide R per litre.

Etoposide RS. International Chemical Reference Substance.

Ferrous sulfate/hydrochloric acid TS

Procedure. Dissolve $0.45\ \text{g}$ of ferrous sulfate R in $50\ \text{ml}$ of hydrochloric acid ($0.1\ \text{mol/l}$) VS and dilute with sufficient carbon-dioxide-free water R to produce $100\ \text{ml}$.

Note: Ferrous sulfate/hydrochloric acid TS should be prepared immediately before use.

Fluoroquinolonic acid RS. International Chemical Reference Substance.

Glycine R. Aminoacetic acid; $\text{C}_2\text{H}_5\text{NO}_2$.

Description. A white, crystalline powder.

Solubility. Very soluble in water; slightly soluble in ethanol ($\sim 750\ \text{g/l}$) TS.

Assay. Determine the nitrogen by the Kjeldahl method in the test substance previously dried at 105°C for 2 hours; between 18.4 and 18.8% of N is found, corresponding to not less than 98.6% and not more than the equivalent of 100.8% of $\text{C}_2\text{H}_5\text{NO}_2$.

Insoluble matter. $10\ \text{g}$ shows not more than $1.0\ \text{mg}$ of insoluble matter ($0.1\ \text{mg/g}$).

Sulfated ash. Not more than $0.5\ \text{mg/g}$.

Chlorides. Not more than $0.1\ \text{mg}$ of Cl/g .

Sulfates. Not more than 0.05 mg of SO_4 /g.

Heavy metals. Not more than 0.02 mg/g.

Iron. Not more than 0.01 mg of Fe/g, 3 ml of hydrochloric acid (~420 g/l) TS being used to facilitate solution.

Heparin RS. World Health Organization International Reference Material. Heparin, porcine, mucosal. 5th International Standard 1998. (Ampoules containing 2031 IU (distributed by the National Institute for Biological Standards and Control (NIBSC), P.O. Box 1193, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QH, England.))

Hexylamine R. Hexaneamine; $\text{C}_6\text{H}_{15}\text{N}$.

A commercially available reagent of suitable grade.

Description. A colourless liquid.

Boiling point. 127–131 °C.

Refractive index. n_D^{20} = about 1.418.

Mass density. ρ_{20} = about 0.766 kg/l.

Hydrochloric acid/ethanol (1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with dehydrated ethanol R to contain 36.47 g of HCl in 1000 ml of dehydrated ethanol R.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS (Vol. 1, p. 184).

Hydrochloric acid/ethanol (0.1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with dehydrated ethanol R to contain 3.647 g of HCl in 1000 ml of dehydrated ethanol R.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS (Vol. 1, p. 184).

Hydrochloric acid (0.1 mol/l) LAL. Prepare from hydrochloric acid (~420 g/l) TS and water LAL. It is suitable if, after adjustment to pH 6.5–7.5, it gives a negative result under the conditions prescribed in the “Test for bacterial endotoxins” (p. 30).

1-Hydroxy-9-anthrone RS. International Chemical Reference Substance.

Hydroxylamine hydrochloride TS2

Procedure. Dissolve 3.5 g of hydroxylamine hydrochloride R in 95 ml of ethanol (~535 g/l) TS, add 0.5 ml of bromophenol blue (1 g/l) TS and sufficient potassium hydroxide/ethanol (0.5 mol/l) TS until a greenish tint is developed. Dilute the solution to 100 ml with ethanol (~535 g/l) TS.

Idoxuridine RS. International Chemical Reference Substance.

Iodine pentoxide R. Iodic anhydride; I_2O_5 .
A commercially available reagent of suitable grade.

Kaolin, light, R. Kaolin as described in the monograph in Vol. 4 (p. 186).

Kaolin suspension TS

Procedure. Immediately before use mix equal volumes of cephalin TS and a suspension containing 4 g of kaolin R in 1000 ml of sodium chloride (9 g/l) TS.

Ketoconazole RS. International Chemical Reference Substance.

Lactobionic acid R. 4-O- β -D-Galactopyranosyl-D-gluconic acid; $C_{12}H_{22}O_{12}$.
A commercially available reagent of suitable grade.

Levamisole hydrochloride RS. International Chemical Reference Substance.

Limulus amoebocyte lysate. Reconstitute the lysate as stated on the label.
For each batch, confirm the stated sensitivity as prescribed under "Sensitivity of the lysate" (page 31). The sensitivity of the lysate is defined as the lowest concentration of endotoxin which yields a firm gel under test conditions and is expressed in endotoxin units per millilitre.

Lincomycin hydrochloride RS. International Chemical Reference Substance.

Macrogol 200 R

Description. A clear, colourless or almost colourless viscous liquid.

Solubility. Very soluble in acetone R and in ethanol (~750 g/l) TS; practically insoluble in ether R and in fatty oils.

Macrogol 200 TS

Procedure. Pour 500 ml of macrogol 200 R into a 1000-ml, round-bottom flask. Evaporate any volatile components using a rotation evaporator. Heat to 60 °C and apply a vacuum with a pressure of 1.5–2.5 kPa for 6 hours.

Mefloquine hydrochloride RS. International Chemical Reference Substance.

Metacycline hydrochloride RS. International Chemical Reference Substance.

DL-Methionine RS. International Chemical Reference Substance.

Methyl violet 2B R. CI 42535; C.I. basic violet.
A commercially available reagent of suitable grade.
Melting point. About 137 °C, with decomposition.

Metronidazole benzoate RS. International Chemical Reference Substance.

Monoethanolamine (0.1 mol/l) VS. A solution of monoethanolamine R in water to contain 6.108 g of C_2H_7NO in 1000 ml.

Nifedipine RS. International Chemical Reference Substance.

Nitric acid (0.05 mol/l) VS. Nitric acid (~1000 g/l) TS, diluted with water to contain 3.151 g of HNO_3 in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution by following the method described under nitric acid (1 mol/l) VS.

Nitrogen monoxide R. NO .

Nitric oxide, washed with water.

A commercially available gas of suitable grade.

Nitrogen monoxide and nitrogen dioxide detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for an oxidizing layer Cr(VI) salt and the diphenyl-benzidine indicator. The minimum value indicated is $5 \mu\text{l/l}$ or less, with a relative standard deviation of at most $\pm 15\%$. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Nonoxinol 9 RS. International Chemical Reference Substance.

Oil detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for the sulfuric acid indicator. The minimum value indicated is 0.1 mg/m^3 , with a relative standard deviation of at most $\pm 30\%$. Tubes can be verified with a calibration gas containing the appropriate impurity, if a negative result is obtained.

Olive oil R

A commercially available reagent of suitable grade.

Ox brain, acetone-dried, R

Procedure. Cut into small pieces a fresh ox brain previously freed from vascular and connective tissue. Place in acetone R for preliminary dehydration. Complete the dehydration by pounding in a mortar 30 g of the material with successive quantities, each of 75 ml of acetone R, until a dry powder is obtained after filtration. Dry at 37°C for 2 hours or until the odour of acetone is no longer perceptible.

n-Pentane R. C_5H_{12} .

A commercially available reagent of suitable grade.

Description. A colourless, volatile liquid.

Boiling point. About 36°C .

Relative density. d_D^{20} = about 1.359.

Transmittance. Not less than 20% at 200 nm, 50% at 210 nm, 85% at 220 nm, 93% at 230 nm, and 98% at 240 nm, determined using water in the reference cell.

Perchloric acid TS

Procedure. Dilute 82 ml of perchloric acid (~1170 g/l) TS with sufficient water to produce 1000 ml (approximately 1 mol/l).

Phenobarbital RS. International Chemical Reference Substance.

Phosphate buffer, pH 7.2, TS

Procedure. Dissolve 6.80 g of potassium dihydrogen phosphate R and 1.40 g of sodium hydroxide R in sufficient water to produce 1000 ml.

Phosphoric acid (~20 g/l) TS

Procedure. To 23 g of phosphoric acid (~1440 g/l) TS add 987 g of water and mix.

Phosphoric acid (~2.8 g/l) TS

Procedure. Dilute 2 ml of phosphoric acid (~1440 g/l) TS with sufficient water to produce 100 ml.

Plasma substrate R

Note: Use water-repellent equipment (made from materials such as suitable plastics or suitably silicone-treated glass) for taking and handling blood.

Procedure. Collect a sufficient volume of blood from each of at least 5 sheep. A 285-ml volume of blood collected into 15 ml of anticoagulant solution is recommended but smaller volumes may be collected. The blood should be taken either from a live animal or at the time of slaughter, using a needle attached to a cannula that is long enough to reach the bottom of the collecting flask. Discard the first few ml and collect only free-flowing blood. Collect the blood in a sufficient quantity of an anticoagulant solution containing 8.7 g of sodium citrate R and 4 mg of aprotinin R in 100 ml of water to give a final ratio of blood to anticoagulant solution of 19 to 1. During and immediately after collection, swirl the flask gently to ensure mixing but do not allow frothing to occur. When collection is complete, close the flasks and cool to a temperature between 10 and 15 °C. Then pool the contents of all the flasks, with the exception of any that shows obvious haemolysis or clots, and keep the pooled blood at 10–15 °C. Within 4 hours of collection, centrifuge the pooled and cooled blood at a speed of 1000–2000 g^1 for 30 minutes. Separate the supernatant liquid and centrifuge again at 5000 g for 30 minutes (*Note:* Faster centrifugation (20 000 g) may be necessary to clarify the plasma, but filtration procedures should not be used.) Separate the super-

¹ Acceleration due to gravity = 9.81 m/s².

nant liquid and immediately mix thoroughly and distribute the plasma substrate R into small stoppered containers in portions sufficient for a complete heparin assay (10–30 ml). Without delay, rapidly cool to a temperature below -70°C by immersing the containers in liquid nitrogen and store at a temperature below -30°C . The plasma is suitable for use as plasma substrate R in the assay for heparin if, under the conditions of the assay, it gives a clotting time appropriate to the method of detection used, and if it provides reproducible, steep, log dose–response curves. Just before use thaw the quantity of plasma substrate R required in a water-bath at 37°C and gently swirl until thawing is complete; once thawed it should be kept between 10 and 20°C and used without delay. The thawed plasma substrate R may be slightly centrifuged if necessary, but do not use any filtration procedures.

Polydimethylsiloxane R

A commercially available reagent of suitable grade for use in gas chromatography.

Potassium hydroxide/ethanol (0.1 mol/l) VS. Potassium hydroxide R, dissolved in ethanol (~ 710 g/l) TS to contain 5.610 g of KOH in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under potassium hydroxide (1 mol/l) VS (Vol. 1, p. 199).

Potassium hydroxide (~ 560 g/l) TS. A solution of potassium hydroxide R containing about 560 g of KOH per litre.

Potassium iodide (160 g/l) TS. A solution of potassium iodide R containing about 160 g of KI per litre.

Potassium iodobismuthate/acetic acid TS

Procedure. Dissolve 8 g of potassium iodide R in 20 ml of water and add to it a solution composed of 0.85 g of bismuth oxynitrate R dissolved in 40 ml of water and 10 ml of glacial acetic acid R.

Potassium permanganate (1 g/l) TS. A solution of potassium permanganate R containing about 1 g of KMnO_4 per litre.

Progupil hydrochloride RS. International Chemical Reference Substance.

Retinol acetate RS. International Chemical Reference Substance.

Retinol palmitate RS. International Chemical Reference Substance.

Retinol propionate RS. International Chemical Reference Substance.

Silica gel for chromatography R

A very finely divided (3–10 μm) silica gel. The particle size is indicated after the name of the reagent in the tests where it is used.

Description. A fine, white, homogeneous powder.

Solubility. Practically insoluble in water and ethanol (~750 g/l) TS.

Sodium chloride (300 g/l) TS. A solution of sodium chloride R containing about 300 g of NaCl per litre.

Sodium chloride (9 g/l) TS. A solution of sodium chloride R containing about 9 g of NaCl per litre.

Sodium dithionite R. Sodium hydrosulfite, sodium sulfoxylate; $\text{Na}_2\text{O}_4\text{S}_2$.

Description. A white or greyish white, crystalline powder.

Solubility. Very soluble in water, slightly soluble in ethanol (~750 g/l) TS.

Note: Sodium dithionite R oxidizes in air.

Sodium dithionite (200 g/l) TS. A solution of sodium dithionite R containing about 200 g of $\text{Na}_2\text{O}_4\text{S}_2$ per litre.

Sodium hexanesulfonate R. $\text{C}_6\text{H}_{13}\text{NaO}_3\text{S}$.

A commercially available reagent of suitable grade.

Sodium hydroxide (0.1 mol/l) LAL. Prepare from sodium hydroxide R and water LAL. It is suitable if, after adjustment to pH 6.5–7.5, it gives a negative result under the conditions prescribed in the “Test for bacterial endotoxins” (p. 30).

Sodium hypobromite TS

Procedure. Dissolve 2.5 g of sodium hydroxide R in 7.5 ml of water, add 0.5 ml of bromine R and a sufficient quantity of water to produce 10 ml.

Note: Sodium hypobromite TS must be freshly prepared.

Sodium nitrate R. NaNO_3 .

A commercially available reagent of suitable grade.

Sodium octanesulfonate R. $\text{C}_8\text{H}_{17}\text{NaO}_3\text{S}$.

Contains not less than 98.0% of $\text{C}_8\text{H}_{17}\text{NaO}_3\text{S}$.

A commercially available reagent of suitable grade.

Absorbance. A 0.05 g/ml solution of a 1-cm layer measured at a wavelength of about 250 nm has an absorbance of not greater than 0.01.

Sodium standard (200 μg Na/ml) TS

Procedure. Dissolve 0.5084 g of sodium chloride R, previously dried at 100–105 °C for 3 hours, in sufficient water to produce 1000 ml.

Sodium thiosulfate (0.002 mol/l) VS. Sodium thiosulfate R, dissolved in water to contain 0.316 g of $\text{Na}_2\text{S}_2\text{O}_3$ in 1000 ml.

Method of standardization. Ascertain the exact concentration of the solution following the method described under sodium thiosulfate (0.1 mol/l) VS (Vol. 1, p. 207, and Vol. 2, p. 319).

Sulfadiazine RS. International Chemical Reference Substance.

Supports for high-performance liquid chromatography

- **Stationary phase A.** Particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups.
- **Stationary phase B.** Particles of silica gel, the surface of which has been modified with chemically bonded octylsilyl groups.
- **Stationary phase C.** Particles of porous silica.
- **Stationary phase D.** Particles of styrene-divinylbenzene copolymer.

Tamoxifen citrate E-isomer RS. International Chemical Reference Substance.

Tetrabutylammonium hydrogen sulfate R. TBAHS; $\text{C}_{16}\text{H}_{37}\text{NO}_4\text{S}$.

A commercially available reagent of suitable grade.

Description. Colourless crystals or a white, crystalline powder.

Solubility. Freely soluble in water and methanol R; soluble in ethanol (~750 g/l) TS producing a slightly hazy, colourless solution.

Absorbance. A 0.05 g/ml solution of a 1-cm layer measured at a wavelength between 240 nm and 300 nm has an absorbance of not greater than 0.05.

Melting range. 169–173 °C.

Thiopental RS. International Chemical Reference Substance.

Triketohydrindene/butanol/acetic acid TS

Procedure. Prepare a 20 mg/ml solution of triketohydrindene hydrate R in a mixture of 95 volumes of 1-butanol R and 5 volumes of acetic acid (~120 g/l) TS.

Trinitrophenol/ethanol TS

Procedure. Dissolve 33 g of trinitrophenol R in sufficient ethanol (~750 g/l) TS to produce 1000 ml.

Tropicamide RS. International Chemical Reference Substance.

Vanillin/sulfuric acid TS1

Procedure. Dissolve 5 g of vanillin R in 100 ml of sulfuric acid (~1760 g/l) TS.

Note: Vanillin/sulfuric acid TS1 should be freshly prepared.

Vanillin/sulfuric acid TS2

Procedure. Dissolve 1 g of vanillin R in sufficient ethanol (~750 g/l) TS to produce 100 ml. Carefully add, drop by drop, 2 ml of sulfuric acid (~1760 g/l) TS.

Note: Vanillin/sulfuric acid TS2 must be used within 48 hours.

Water for injections R. Water for injections as described in Vol. 4, p. 132.

Water LAL. Water is suitable if it gives a negative result under the conditions prescribed in the "Test for bacterial endotoxins" (p. 30). It may be prepared by distilling water three times in an apparatus fitted with an effective device to prevent the entrainment of droplets, or by other means which give water of the requisite quality.

Water vapour detector tube. A cylindrical, sealed glass tube containing adsorbent filters and suitable supports for the magnesium perchlorate indicator. The minimum value indicated is $60 \mu\text{l/l}$ or less, with a relative standard deviation of at most $\pm 20\%$.

