**Citric acid.** To 20.0 ml add 0.1 ml of phenolphthalein solution R1 and titrate with 0.2 M sodium hydroxide.

Calculate the content of citric acid monohydrate (C), or anhydrous citric acid (C′), in grams per litre from the equations:

\[
C = 0.7005n - 0.4490P
\]

\[
C' = 0.8404n - 0.4105P
\]

\(n\) = number of millilitres of 0.2 M sodium hydroxide used in the titration,

\(P\) = content of sodium dihydrogen phosphate dihydrate in grams per litre determined as prescribed above.

**Sodium citrate.** Prepare a chromatography column 0.10 m long and 10 mm in internal diameter and filled with strongly acidic ion-exchange resin R (300 µm to 840 µm). Maintain a 1 cm layer of liquid above the resin at all times. Wash the column with 50 ml of de-ionised water R at a flow rate of 12-14 ml/min.

Dilute 10.0 ml of the solution to be examined to about 40 ml with de-ionised water R in a beaker and transfer to the column reservoir, washing the beaker 3 times with a few millilitres of de-ionised water R. Allow the solution to run through the column at a flow rate of 12-14 ml/min and collect the eluate. Wash the column with 2 quantities, each of 30 ml, and with one quantity of 50 ml, of de-ionised water R. The column can be used for 3 successive determinations before regeneration with 3 times its volume of dilute hydrochloric acid R. Titrate the combined eluate and washings (about 150 ml) with 0.2 M sodium hydroxide, using 0.1 ml of phenolphthalein solution R1 as indicator.

Calculate the content of sodium citrate in grams per litre from the following expressions:

\[
1.961n - 1.257P - 1.411C
\]

\[
1.961n - 1.257P - 1.53C'
\]

\(n\) = number of millilitres of 0.2 M sodium hydroxide used in the titration,

\(P\) = content of sodium dihydrogen phosphate dihydrate in grams per litre determined as prescribed above,

\(C\) = content of citric acid monohydrate in grams per litre determined as prescribed above,

\(C'\) = content of anhydrous citric acid in grams per litre determined as prescribed above.

**Reducing sugars.** Dilute 5.0 ml to 100.0 ml with water R. Introduce 25.0 ml of the solution into a 250 ml conical flask with ground-glass neck and add 25.0 ml of cupric-citric solution R1. Add a few pieces of porous material, attach a reflux condenser, heat so that boiling begins within 2 min and boil for exactly 10 min. Cool and add 3 g of potassium iodide R dissolved in 3 ml of water R. Add 25 ml of a 25 per cent m/m solution of sulphuric acid R with caution and in small quantities. Titrate with 0.1 M sodium thiosulphate using 0.5 ml of starch solution R, added towards the end of the titration, as indicator (\(n_1\) ml). Carry out a blank titration using 25.0 ml of water R (\(n_2\) ml).

Calculate the content of reducing sugars as anhydrous glucose or as glucose monohydrate, as appropriate, from the Table 0209-1.

**STORAGE**

Store in an airtight, tamper-proof container, protected from light.

**LABELLING**

The label states:

- the composition and volume of the solution,
- the maximum amount of blood to be collected in the container.

**01/2005:1928**

**ANTI-T LYMPHOCYTE IMMUNOGLOBULIN FOR HUMAN USE, ANIMAL**

Immunoglobulinum anti-T lymphocytorum ex animale ad usum humanum

**DEFINITION**

Anti-T lymphocyte animal immunoglobulin for human use is a liquid or freeze-dried preparation containing immunoglobulins, obtained from serum or plasma of animals, mainly rabbits or horses, immunised with human lymphocytic antigens.

The immunoglobulin has the property of diminishing the number and function of immunocompetent cells, in particular T lymphocytes. The preparation contains principally immunoglobulin G. It may contain antibodies against other lymphocyte subpopulations and against other cells. The preparation is intended for intravenous administration, after dilution with a suitable diluent where applicable.

Applicable provisions of the monograph on Immunosera for human use, animal (0084) are stated below.

**PRODUCTION**

**GENERAL PROVISIONS**

The production method has been shown to yield consistently immunoglobulins of acceptable safety, potency in man and stability.

Any reagent of biological origin used in production shall be free of contamination with bacteria, fungi and viruses. The method of preparation includes a step or steps that have been shown to remove or inactivate known agents of infection.

During development studies, it shall be demonstrated that the production method yields a product that:

- does not transmit infectious agents,
- is characterised by a defined pattern of immunological activity, notably: antigen binding, complement-dependent and independent cytotoxicity, cytokine release, induction of T-cell activation, cell death,
- does not contain antibodies that react with human tissues to a degree that would impair clinical safety,
- has a defined maximum content of anti-thromocyte antibody activity,
- has a defined maximum content of haemoglobin.

The product has been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated.

**Reference preparation.** A batch shown to be suitable for checking the validity of the assay and whose efficacy has been demonstrated in clinical trials, or a batch representative thereof.
ANIMALS
The animals used are of a species approved by the competent authority, are healthy and exclusively reserved for production of anti-T lymphocyte immunoglobulin. They are tested and shown to be free from a defined list of infectious agents. The introduction of animals into a closed herd follows specified procedures, including definition of quarantine measures. Where appropriate, tests for additional specific agents are considered depending on the geographical localisation of the establishment used for the breeding and production of the animals. The feed originates from a controlled source and no animal proteins are added. The suppliers of animals are certified by the competent authority.

If the animals are treated with antibiotics, a suitable withdrawal period is allowed before collection of blood or plasma. The animals are not treated with penicillin antibiotics. If a live vaccine is administered, a suitable waiting period is imposed between vaccination and collection of serum or plasma for immunoglobulin production.

The species, origin and identification number of the animals are specified.

IMMUNISATION
The antigens used are identified and characterised, where appropriate. They are identified by their names and a batch number; information on the source and preparation are recorded.

The selected animals are isolated for at least 1 week before being immunised according to a defined schedule with booster injections at suitable intervals. Adjuvants may be used.

Animals are kept under general health surveillance and specific antibody production is controlled at each cycle of immunisation.

Animals are thoroughly examined before collection of blood or plasma. If an animal shows any pathological lesion not related to the immunisation process, it is not used, nor are any other of the animals in the group concerned, unless it is evident that their use will not impair the safety of the product.

Human antigens such as continuously growing T-lymphocyte cell lines or thymocytes are used to immunise the animals. Cells may be subjected to a sorting procedure. The immunising antigens are shown to be free from infectious agents by validated methods for relevant blood-borne pathogens, notably hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and other relevant adventitious agents originating from the preparation of the antigen. The cells used comply with defined requirements for purity of the cell population and freedom from adventitious agents.

COLLECTION OF BLOOD OR PLASMA
Collection of blood is made by venepuncture or plasmapheresis. The puncture area is shaved, cleaned and disinfected. The animals may be anaesthetised under conditions that do not influence the quality of the product.

No antimicrobial preservative is added to the plasma and serum samples. The blood or plasma is collected in such a manner as to maintain sterility of the product. The blood or plasma collection is conducted at a site separate from the area where the animals are kept or bred and the area where the immunoglobulin is purified. If the serum or plasma is stored before further processing, precautions are taken to avoid microbial contamination.

Several single plasma or serum samples may be pooled before purification. The single or pooled samples are tested before purification for the following tests.

Tests for contaminating viruses. Each pool is tested for contaminating viruses by suitable in vitro tests including inoculation to cell cultures capable of detecting a wide range of viruses relevant for the particular product. Where applicable, in vitro tests for contaminating viruses are carried out on the adsorbed pool, after the last purification stage that may introduce viral contaminants.

PURIFICATION AND VIRAL INACTIVATION
The immunoglobulins are concentrated and purified by fractional precipitation, chromatography or other suitable methods. The methods are selected and validated to avoid contamination at all steps of processing and to avoid formation of protein aggregates that effect immunobiological characteristics of the product.

Unless otherwise justified and authorised, validated procedures are applied for removal and/or inactivation of viruses.

After purification and treatment for removal and/or inactivation of viruses, a stabiliser may be added to the intermediate product, which may be stored for a period defined in the light of stability data.

Only an intermediate product that complies with the following requirements may be used in the preparation of the final bulk.

If the method of preparation includes a step for adsorption of cross-reacting anti-human antibodies using material from human tissues and/or red blood cells, the human materials are subjected to a validated procedure for inactivation of infectious agents, unless otherwise justified and authorised.

If erythrocytes are used for adsorption, the donors for such materials comply with the requirements for donors of blood and plasma of the monograph on Human plasma for fractionation (0853). If other human material is used, it is shown by validated methods to be free from relevant blood-borne pathogens, notably HBV, HCV and HIV. If substances are used for inactivation or removal of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the anti-T lymphocyte immunoglobulin.

FINAL BULK
The final bulk is prepared from a single intermediate product or from a pool of intermediate products obtained from animals of the same species. A stabiliser may be added. No antimicrobial preservative is added either during the manufacturing procedure or for preparation of the final bulk solution. During manufacturing, the solution is passed through a bacteria-retentive filter.

FINAL LOT
The final bulk of anti-T-lymphocyte immunoglobulin is distributed aseptically into sterile, tamper-proof containers. The containers are closed as to prevent contamination. Only a final lot that complies with the requirements prescribed below under Identification, Tests and Assay may be released for use.

CHARACTERS
The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a white or slightly yellow powder or solid friable mass, which after reconstitution gives a liquid preparation corresponding to the description above.

IDENTIFICATION
A. Using a suitable range of species-specific antisera, carry out precipitation tests on the preparation to be examined. It is recommended that the test be carried...
out using antisera specific to the plasma proteins of each species of domestic animal commonly used in the preparation of materials of biological origin in the country concerned and antisera specific to human plasma proteins. The preparation is shown to contain proteins originating from the animal used for the anti-T lymphocyte immunoglobulin production.

B. Examine by a suitable immunoelectrophoresis technique. Using antisera to normal serum of the animal used for production, compare this serum and the preparation to be examined, both diluted to a concentration that will allow a clear gammaglobulin precipitation arc to be obtained on the gel. The main component of the preparation to be examined corresponds to the IgG component of normal serum of the animal used for production.

C. The preparation complies with the assay.

TESTS

Solubility. To a container of the preparation to be examined, add the volume of the liquid for reconstitution stated on the label. The preparation dissolves completely within the time stated on the label.

Extractable volume (2.9.17). It complies with the requirement for extractable volume.

pH (2.2.3). The pH is within the limits approved for the particular product.

Osmolality (2.2.35): minimum 240 mosmol/kg after dilution, where applicable.

Proteins (2.5.33): 90 per cent to 110 per cent of the amount stated on the label.

Stabiliser. Determine the amount of stabiliser by a suitable physico-chemical method. The preparation contains not less than 80 per cent and not more than 120 per cent of the quantity stated on the label.

Distribution of molecular size. Size exclusion chromatography (2.2.30).

Test solution. Dilute the preparation to be examined with a 9 g/l solution of sodium chloride R to a concentration suitable for the chromatographic system used. A concentration in the range 2-20 g/l is usually suitable.

Reference solution. Dilute human immunoglobulin BRP with a 9 g/l solution of sodium chloride R to the same protein concentration as the test solution.

Column:
- size: l = 0.6 m, Ø = 7.5 mm,
- stationary phase: silica gel for size-exclusion chromatography R, a grade suitable for fractionation of globular proteins in the molecular mass range of 20 000 to 200 000.

Mobile phase: dissolve 4.873 g of disodium hydrogen phosphate dihydrate R, 1.741 g of sodium dihydrogen phosphate monohydrate R and 11.688 g of sodium chloride R in 1 litre of water R.

Flow rate: 0.5 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 50-600 µg of protein.

Retention time: identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of dimer corresponds to polymers and aggregates.

System suitability:
- reference solution: the principal peak corresponds to IgG monomer and there is a peak corresponding to dimer with a retention time relative to monomer of 0.85 ± 0.05,
- test solution: the relative retentions of monomer and dimer are 1 ± 0.05 with reference to the corresponding peaks in the chromatogram obtained with the reference solution.

Limits:
- total monomer and dimer: at least 95 per cent of the total area of the peaks,
- total polymers and aggregates: maximum 5 per cent of the total area of the peaks.

Purity. Polyacrylamide gel electrophoresis (2.2.31), under non-reducing and reducing conditions.

Resolving gel. Non-reducing conditions: 8 per cent acrylamide; reducing conditions: 12 per cent acrylamide.

Test solution. Dilute the preparation to be examined to a protein concentration of 0.5-2 mg/ml.

Reference solution. Dilute the reference preparation to the same protein concentration as the test solution.

Application: 10 µl.

Detection: Coomassie staining.

Results: compared with the electropherogram of the reference solution, no additional bands are found in the electropherogram of the test solution.

Anti-A and anti-B haemagglutinins (2.6.20). The 1 to 64 dilution does not show agglutination.

Where applicable, dilute the preparation to be examined as prescribed before preparing the dilutions for the test.

Haemolysins. Prepare a 1 to 64 dilution of the preparation to be examined, diluted if necessary as stated on the label. Take 6 aliquots of the 1 to 64 dilution. To 1 volume of 3 of the aliquots, add 1 volume of a 10 per cent V/V suspension of group A1, group B and group O erythrocytes in a 9 g/l solution of sodium chloride R, respectively. To 1 volume of the remaining 3 aliquots, add 1 volume of a 10 per cent V/V suspension of group A1, group B and group O erythrocytes in a 9 g/l solution of sodium chloride R, respectively, and to each aliquot 1 volume of fresh group AB serum (as a source of complement). Mix and incubate at 37 °C for 1 h. Examine the supernatant liquids for haemolysis. No signs of haemolysis are present.

Thrombocyte antibodies. Examined by a suitable method, the level of thrombocyte antibodies is shown to be below that approved for the specific product.

Water (2.5.12): maximum 3 per cent.

Sterility (2.6.7). It complies with the test for sterility.

Pyrogens (2.6.8). Unless otherwise justified and authorised, it complies with the test for pyrogens. Unless otherwise prescribed, inject 1 ml per kilogram of the rabbit’s body mass.

ASSAY

The biological activity is determined by measuring the complement-dependent cytotoxicity on target cells. Flow cytometry is performed with read-out of dead cells stained using propidium iodide. The activity is expressed as the concentration of anti-T lymphocyte immunoglobulin in milligrams per millilitre which mediates 50 per cent cytotoxicity.

Lymphocyte separation medium. Commercial separation media with low viscosity and a density of 1.077 g/ml. Complement. Commercial complement is suitable.
Buffered salt solution pH 7.2. Dissolve 8.0 g of sodium chloride R, 0.2 g of potassium chloride R, 3.18 g of disodium hydrogen phosphate R and 0.2 g of potassium dihydrogen phosphate R in water R and dilute to 1000.0 ml with the same solvent.

Buffer solution for flow cytometry. Add 40 ml of 0.1 per cent V/V sodium azide R and 10 ml of foetal calf serum to 440 ml of buffered salt solution pH 7.2. The foetal calf serum is inactivated at 56 °C for 30 min prior to use. Store at 4 °C.

Propidium iodide solution. Dissolve propidium iodide R in buffered salt solution pH 7.2, to a concentration of 1 mg/ ml. Store this stock solution at 2-8 °C and use within 1 month.

If necessary, the first PBMC pellet may be resuspended in buffered salt solution pH 7.2 containing 20 per cent foetal calf serum and stored overnight at 2 °C. Centrifuge at 400 g at 2-8 °C for 10 min. Discard the supernatant. Suspend the cell pellet in buffer solution for flow cytometry. Repeat the centrifugation and resuspension procedure of the cells twice. After the third centrifugation, resuspend the cell pellet in 1 ml of buffer solution for flow cytometry. Determine the number and vitality of the cells using a haemocytometer. Cell viability of at least 90 per cent is required. Adjust the cell number to 7 × 10⁶/ml by adding buffer solution for flow cytometry. Store the cell suspension at 4 °C and use within 12 h.

Buffer solution for freezing. To 20 ml of cell culture medium, add 25 ml of foetal calf serum and 5 ml of dimethyl sulfoxide (DMSO). Store this solution at 2-8 °C and use within 3 h.

20 × 10⁶ cells per ampoule are frozen. These ampoules are stored in liquid nitrogen.

Buffer solution for thawing. To 450 ml of cell culture medium, add 50 ml of foetal calf serum. Store this solution at 2-8 °C and use within 3 h.

Each ampoule is thawed in a water-bath at 37 °C with shaking. Cell suspension is repeated in a buffer solution for thawing. Centrifuge at 200 g at 2-8 °C for 10 min. Discard the supernatant. Suspend the cell pellet in buffer solution for flow cytometry. Repeat the procedure for centrifugation and resuspension of cells once. After the second centrifugation, resuspend the cells pellet in 1 ml of buffer solution for flow cytometry. Determine the number and vitality of the cells using a haemocytometer. Cell viability of at least 90 per cent is required. Adjust the cell number to 7 × 10⁷/ml by adding buffer solution for flow cytometry. Store the cell suspension at 4 °C and use within 3 h.

Test solutions. For freeze-dried preparations, reconstitute as stated on the label. Prepare 3 independent series of not fewer than 7 dilutions using buffer solution for flow cytometry as diluent.

Reference solutions. For freeze-dried preparations, reconstitute according to the instructions for use. Prepare 3 independent dilution series of not fewer than 7 dilutions using buffer solution for flow cytometry as diluent.

Distribute 75 µl of each of the dilutions of the test solution or reference solution to each of a series of wells of a microtitre plate. Add 25 µl of the cell suspension of PBMC into each well. Add 25 µl of rabbit complement to each of the wells. Incubate at 37 °C for 30 min.

Centrifuge the plates at 200 g at 4 °C for 8 min, discard the supernatant and keep the plate on ice. Preparation for flow cytometry measurement is done step-wise by using a certain number of wells in order to allow labelling with propidium iodide R solution and measurement within a defined time period. Resuspend carefully the cell pellet of a certain number of wells with 200 µl of propidium iodide solution. Transfer the suspension into tubes. Incubate at 25 °C for 10 min then place immediately on ice.

Proceed with fluorescence measurement in a flow cytometer. Define a region including all propidium iodide-positive cells on the basis of Forward-Scattered, light (FSC) and fluorescence (FL2 or FL3 for propidium iodide). Measure the percentage of propidium iodide-positive cells, without gating but excluding debris. Analyse at least 3000 cells for each of the test and reference solutions.

Use the percentages of dead cells to estimate the potency as the concentration in milligrams per millilitre of the preparation to be examined necessary to induce 50 per cent of cytotoxicity by fitting a sigmoidal dose response curve to the data obtained with the test and the reference preparations and by using a 4-parameter logistic model (see, for example, chapter 5.3) and suitable software. The test is not valid unless the percentage of propidium iodide-positive cells at the lower asymptote of the curve is less then 15 per cent and the percentage of propidium iodide-positive cells at the upper asymptote of the curve is at least 80 per cent.

The estimated activity is 70 per cent to 130 per cent of the activity approved for the particular product. The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

STORAGE

Protected from light at the temperature stated on the label. Expiry date. The expiry date is calculated from the beginning of the assay.

LABELLING

The label states:

- for liquid preparations, the volume of the preparation in the container and the protein content,
- for freeze-dried preparations:
  - the name and the volume of the reconstitution liquid to be added,
  - the quantity of protein in the container,
  - that the immunoserum is to be used immediately after reconstitution,
  - the time required for complete dissolution,
  - the animal species of origin,
APOMORPHINE HYDROCHLORIDE

Apomorphini hydrochloridum

\[
\text{C}_{17}\text{H}_{19}\text{ClNO}_{2}\cdot\frac{1}{2}\text{H}_{2}\text{O}
\]

M, 312.8

DEFINITION

(6aR)-6-Methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de, g]quinoline-10,11-diol hydrochloride hemihydrate.

Content: 99.0 per cent to 101.0 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 alcohol, practically insoluble in toluene.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Dissolve 10.0 mg in 0.1 M hydrochloric acid and dilute to 100.0 ml with the same acid. Dilute 10.0 ml of the solution to 100.0 ml with 0.1 M hydrochloric acid.

Examined between 230 nm and 350 nm (0.02 per cent), the absorption maximum at 273 nm and a minimum 20 ppm.


B. Infrared absorption spectrophotometry (2.2.24).


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 ether R giving a purple solution, in methylene chloride R giving a violet-blue solution and in alcohol R giving a blue solution.

D. To 2 ml of solution S 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 BY₅ or GY₅ (2.2.2, Method II).

pH (2.2.3): 4.0 to 5.0 for solution S.

Specific optical rotation (2.2.7): -48 to -52 (dried substance).

Dissolve 0.25 g in 0.02 M hydrochloric acid and dilute to 25.0 ml with the same acid.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 0.25 g of the substance to be examined in a 1 per cent V/V solution of glacial acetic acid R and dilute to 100.0 ml with the same solution.

Reference solution (a). Dilute 1.0 ml of the test solution to 10.0 ml with a 1 per cent V/V solution of glacial acetic acid R. Dilute 1.0 ml to 100.0 ml with a 1 per cent V/V solution of glacial acetic acid R.

Reference solution (b). Dissolve 25 mg of boldine R in a 1 per cent V/V solution of glacial acetic acid R and dilute to 10.0 ml with the same solvent. To 1 ml of this solution, add 1 ml of the test solution and dilute to 10.0 ml with a 1 per cent V/V solution of glacial acetic acid R.

Column:

- size: l = 0.15 m, Ø = 4.6 mm,
- stationary phase: octadecylsilyl silica gel for chromatography R (5 µm),
- temperature: 35 °C.

Mobile phase:

- mobile phase A: 1.1 g/l solution of sodium octanesulphonate R, adjusted to pH 2.2 using a 50 per cent m/m solution of phosphoric acid R,
- mobile phase B: acetonitrile R,

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 30</td>
<td>85 → 68</td>
<td>15 → 32</td>
</tr>
<tr>
<td>30 - 35</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>35 - 45</td>
<td>68 → 85</td>
<td>32 → 45</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 10 µl.

System suitability: reference solution (b):

- resolution: minimum 2.5 between the peaks due to boldine and apomorphine.

Limits:

- any impurity: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent),
- total: not more than 8 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.8 per cent),
- disregard limit: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals (2.4.8): maximum 20 ppm.

1.0 g complies with limit test C. Prepare the standard using 2 ml of lead standard solution (10 ppm Pb) R.

Loss on drying (2.2.32): 2.5 per cent to 4.2 per cent, determined on 1.000 g by drying in an oven at 100-105 °C.

Sulphated ash (2.4.14): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 ml of 0.01 M hydrochloric acid and 50 ml of alcohol R. Carry out a potentiometric titration (2.2.20), using 0.1 M sodium hydroxide. Read the volume added between the first 2 points of inflexion.