

IDENTIFICATION

- A. To 1 ml of solution S (see Tests) add 0.1 ml of *sulphuric acid R* and 5 ml of *methanol R* and ignite. The flame has a green border.
- 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.
- 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). The pH of solution S is 9.0 to 9.6.

Sulphates (2.4.13). 15 ml of solution S complies with the limit test for sulphates (50 ppm). Use 1.0 ml of *acetic acid R* instead of the 0.5 ml prescribed. Prepare the standard using a mixture of 3 ml of *sulphate standard solution (10 ppm SO₄) R* and 12 ml of *distilled water R*.

Ammonium (2.4.1). 6 ml of solution S diluted to 14 ml with *water R* complies with the limit test for ammonium (10 ppm). Prepare the standard using a mixture of 2.5 ml of *ammonium standard solution (1 ppm NH₄) R* and 7.5 ml of *water R*.

Arsenic (2.4.2). 5 ml of solution S complies with limit test A for arsenic (5 ppm).

Calcium (2.4.3). 15 ml of solution S complies with the limit test for calcium (100 ppm). 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). 12 ml of solution S complies with limit test A for heavy metals (25 ppm). Prepare the standard 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, add 0.5 ml of *phenolphthalein solution R* and neutralise with 0.1 M *sodium hydroxide* until a pink colour is obtained. To this solution 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 Na₂B₄O₇·10H₂O.

01/2005:0001

BORIC ACID

Acidum boricum

H₃BO₃M_r 61.8

DEFINITION

Boric acid contains not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of H₃BO₃.

CHARACTERS

A white, crystalline powder, colourless, shiny plates greasy to the touch, or white crystals, soluble in water and in alcohol, freely soluble in boiling water and in glycerol (85 per cent).

IDENTIFICATION

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

- 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 (2.2.2, *Method II*).

pH (2.2.3). The pH of solution S is 3.8 to 4.8.

Solubility in alcohol. Dissolve 1.0 g in 10 ml of boiling *alcohol R*. The solution is not more opalescent than reference suspension II (2.2.1) and is colourless (2.2.2, *Method II*).

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

Sulphates (2.4.13). 10 ml of solution S diluted to 15 ml with *distilled water R* complies with the limit test for sulphates (450 ppm).

Heavy metals (2.4.8). 12 ml of solution S complies with limit test A for heavy metals (15 ppm). Prepare the standard 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 H₃BO₃.

01/2005:2113

BOTULINUM TOXIN TYPE A
FOR INJECTION

Toxinum botulinicum typum A ad iniectionabile

DEFINITION

Botulinum toxin type A for injection is a dried preparation containing purified botulinum neurotoxin type A which may be present in the form of a complex with haemagglutinins and non-toxic proteins. Botulinum neurotoxin type A or its haemagglutinin complex is prepared by a suitable purification process of the liquid supernatant from a broth-culture of a suitable strain of *Clostridium botulinum* type A.

The purified complexes consist of several proteins and can be of various sizes. The largest complex (relative molecular mass of about 900 000) consists of a 150 000 relative molecular mass neurotoxin, a 130 000 relative molecular mass non-toxic protein and various haemagglutinins ranging between relative molecular mass 14 000 and 43 000.

The purified toxin moiety is composed of only the same 150 000 relative molecular mass neurotoxin as is found in the 900 000 relative molecular mass neurotoxin complex, which is initially produced as a single chain and further cleaved (nicked) by endogenous proteases into a fully active, disulphide-linked, 54 000 relative molecular mass light chain and a 97 000 relative molecular mass heavy chain.

The preparation is reconstituted before use, as stated on the label.

PRODUCTION

GENERAL PROVISIONS

Production of the toxin is based on seed cultures, managed in a defined seed-lot system in which the ability to produce toxin is conserved. The production method must be shown to yield consistently product of activity and profile comparable to that of lots shown in clinical studies to be of adequate safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the general test of abnormal toxicity (2.6.9) using not less than the maximum human clinical dose, in the presence of a suitable amount of specific botulinum type A antitoxin used for neutralisation.

The production method and stability of the finished product and relevant intermediates are evaluated using the tests below. Such tests include the specific toxin activity per milligram of protein of purified toxin in an appropriate functional model of toxin activity and may be supported by tests confirming the presence of botulinum toxin type A, and, if appropriate, associated non-toxic proteins.

BACTERIAL SEED LOTS

A highly toxigenic strain of *C. botulinum* of known toxin type A and confirmed absence of genes encoding other botulinum toxins (particularly botulinum toxin type B), with known origin and history, is grown using suitable media. The bacterial strain, used for the master seed lot, shall be identified by historical records that include information on its origin and the tests used to characterise the strain. These will include morphological, cultural, biochemical, genetic and serological properties of the strain. The master seed lot and the working seed lot, where applicable, must be demonstrated to have identical profiles. Only a seed lot that complies with the following requirements may be used.

Identification. Each seed lot is identified as containing pure cultures of *C. botulinum* type A bacteria with no extraneous bacterial or fungal contamination.

Microbial purity. Each seed lot complies with the requirements for absence of contaminating micro-organisms. The purity of bacterial cultures is verified by methods of suitable sensitivity. These may include inoculation into suitable media and examination of colony morphology.

Phenotypic parameters. Each seed lot must have a known fatty acid profile, sugar fermentation profile (glucose, lactose, mannose, etc.) and proteolytic activity and must demonstrate relevant lipase, lecithinase and gelatinase activity.

Genetic purity. Each seed lot must have information on the toxin gene sequence and comply with requirements for the absence of other genes encoding other toxin serotypes.

Production of active toxin. A bacterial strain producing a high yield of active toxin, as determined by an acute toxicity assay, is suitable. Seed lots should demonstrate a capability of producing at least a minimum toxicity level appropriate for the manufacturing process and scale.

MANUFACTURER'S REFERENCE PREPARATIONS

During development, reference preparations are established for subsequent verification of batch consistency during production and for control of the bulk purified toxin and finished product. They are derived from representative batches of botulinum toxin type A that are characterised as described under Bulk Purified Toxin.

The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their suitability.

BULK PURIFIED TOXIN

C. botulinum type A strain is grown anaerobically, in suitable media, from which cultures are selected for step-up incubations under a suitably controlled anaerobic atmosphere through the seed culture and bulk fermentation stages to allow maximum production of toxin. The toxin is purified by suitable methods to remove nucleic acids and components likely to cause adverse reactions.

Only a purified toxin that complies with the following requirements may be used in the preparation of the final bulk. For each test and for each product, limits of acceptance are established and each new purified toxin must comply with these limits.

Residual reagents. Removal of residual reagents used in purification steps is confirmed by suitable limit tests or by validation of the process.

Nucleic acids. Removal of nucleic acids is confirmed by suitable limit tests or by validation of the process.

Immunological identity. The presence of specific type A toxin is confirmed by a suitable immunochemical method (2.7.1).

Specific activity. The specific activity is confirmed in a mouse model of toxicity or by *in vivo/ex vivo* methods validated with respect to the LD50 assay and expressed in mouse LD50 units per milligram of protein. Specific activity must not be less than 1×10^8 mouse LD50 units per milligram of protein for the 150 000 relative molecular mass neurotoxin and must not be less than 1×10^7 mouse LD50 units per milligram of protein for the 900 000 relative molecular mass neurotoxin complex.

Protein. The total protein concentration is determined by a suitable method. An acceptable value is established for the product and each batch must be shown to comply with the limits.

Protein profile. Identity and protein composition are determined by polyacrylamide gel electrophoresis (2.2.31) under reducing or non-reducing conditions or by other suitable physicochemical methods such as size-exclusion chromatography (2.2.30), comparing with suitable reference standards.

Total viable count. It complies with the limits approved for the particular product.

FINAL BULK

The final bulk is prepared by adding approved excipients to the bulk purified toxin. The solution is filtered through a bacteria-retentive filter. If human albumin is added, it complies with the monograph on *Human albumin solution* (0255).

FINAL LOT

The final bulk is distributed aseptically into sterile, tamper-proof containers. Uniformity of fill is verified during filling and the test for uniformity of content (2.9.6) is not required. The containers are closed so as to prevent contamination.

Only a final lot that is within the limits approved for the particular product and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

pH (2.2.3). The pH of the reconstituted product is within ± 0.5 pH units of the limit approved for the particular product.

Water: not more than the limit approved for the particular product.

IDENTIFICATION

The presence of botulinum toxin type A is confirmed by a suitable immunochemical method (2.7.1).

TESTS

Sterility (2.6.1). It complies with the test for sterility.

Bacterial endotoxins (2.6.14): less than 10 IU per vial.

ASSAY

The potency of the reconstituted product is determined by an LD50 assay in mice or by a method validated with respect to the LD50 assay. The potency is expressed in terms of the LD50 for mice or relative to the reference preparation.

For determination of the LD50, graded doses of the product are injected intraperitoneally into groups of mice and the LD50 is calculated by the usual statistical methods (5.3) from the mouse lethality in each group. A suitable reference preparation is assayed in parallel; the potency of the toxin is expressed relative to the reference or the value found for the reference is within suitable limits defined in terms of the assigned potency.

After validation with respect to the LD50 assay (reference method), the product may also be assayed by other methods that are preferable in terms of animal welfare, including 1 of the following:

1. endopeptidase assay *in vitro*;
2. *ex vivo* assay using the mouse phrenic nerve diaphragm;
3. mouse bioassay using paralysis as the end-point.

For these other methods, the potency is calculated with respect to a suitable reference preparation calibrated in mouse LD50 units.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 80 per cent and not more than 125 per cent of the estimated potency.

The test may be repeated but when more than 1 test is performed, the results of all valid tests must be combined in the estimate of potency.

LABELLING

The label states:

- the number of units of toxin per vial with a statement that units are product specific and not applicable to other preparations containing botulinum toxin type A,
- the name and the volume of the diluent to be added for reconstitution of a dried product.

01/2005:0879

BROMAZEPAM

Bromazepamum

C₁₄H₁₀BrN₃OM_r 316.2

DEFINITION

Bromazepam contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 7-bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one, calculated with reference to the dried substance.

CHARACTERS

A white or yellowish, crystalline powder, practically insoluble in water, sparingly soluble in alcohol and in methylene chloride.

IDENTIFICATION

First identification: B.

Second identification: A, C, D, E.

- A. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of the solution to 100.0 ml with *methanol R*. Examined between 220 nm and 350 nm (2.2.25), the solution shows an absorption maximum at 233 nm and a shoulder at about 260 nm and may show a broad absorption maximum at about 325 nm. The specific absorbance at the maximum at 233 nm is 980 to 1080.
- B. Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with *bromazepam CRS*. Examine the substances prepared as discs.
- C. Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution. Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (a). Dissolve 10 mg of *bromazepam CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dissolve 10 mg of *bromazepam CRS* and 10 mg of *temazepam CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 30 volumes of *diethylamine R* and 70 volumes of *ether R*. Dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

- D. Dissolve about 20 mg in 5 ml of *methanol R*. Add 5 ml of *water R* and 1 ml of a 10 g/l solution of *ferrous ammonium sulphate R*. A violet colour develops.
- E. To 0.15 g 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 in 10 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 bromides (2.3.1).