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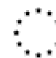
Botulinum Toxin Type B for Injection



General Notices

(Ph. Eur. monograph 2581)

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DEFINITION

Botulinum toxin type B for injection is a liquid preparation containing purified botulinum neurotoxin type B, which may be present in the form of a complex with haemagglutinins and non-toxic proteins. Botulinum neurotoxin type B 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 B. Suitable stabilisers may be added.

The toxin is present in its native form as a complex of neurotoxin and non-toxin proteins and haemagglutinins with a total relative molecular mass of approximately 700 000. The neurotoxin is synthesised by the bacterium as a single-chain polypeptide of approximately 150 000 relative molecular mass that is activated during the fermentation process via a proteolytic cleavage (nicking) by endogenous proteases. The nicked protein is a fully active double-chain polypeptide consisting of a heavy chain (100 000 relative molecular mass) and a light chain (50 000 relative molecular mass), connected by a disulfide bond.

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 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 B, and, if appropriate, associated non-toxic proteins.

BACTERIAL SEED LOTS

A highly toxigenic strain of *C. botulinum* of known toxin type B and confirmed absence of genes encoding other botulinum toxins (particularly botulinum toxin types A and F), 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

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Each seed lot is identified as containing pure cultures of *C. botulinum* type B 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 genomic location and 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 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 B 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 B 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 B toxin is confirmed by a suitable immunochemical method (*2.7.1*).

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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 LD_{50} assay and expressed in mouse LD_{50} units per milligram of protein. Specific activity must not be less than 1×10^8 mouse LD_{50} units per milligram of protein.

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 *Human albumin solution (0255)*.

FINAL LOT

The final bulk is distributed aseptically into sterile, tamper-evident 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 product is within ± 0.5 pH units of the limit approved for the particular product.

IDENTIFICATION

The presence of botulinum toxin type B 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.

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ASSAY

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The LD_{50} assay is associated with severe suffering of animals and manufacturers are strongly encouraged to develop and validate assays that will reduce the number of animals used, or refine or replace the test procedure with the goal of promoting animal welfare.

The potency of the product is determined by an LD_{50} assay in mice or by a method validated with respect to the LD_{50} assay. The potency is expressed in terms of the LD_{50} for mice or relative to the reference preparation.

For determination of the LD_{50}, graded doses of the product are injected intraperitoneally into groups of mice and the LD_{50} 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 LD_{50} assay (reference method), the product may also be assayed by other methods that are preferable in terms of animal welfare, for example mouse bioassays using paralysis as the end-point, *ex vivo* assays using mouse phrenic nerve diaphragm, endopeptidase assays *in vitro* and cell-based assays.

For alternative replacement methods the potency is calculated with respect to a suitable reference preparation calibrated in mouse LD_{50} 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 B.

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