Content of Botulinum Neurotoxin in Botox®, Vistabel®, Dysport®, Azzalure®, and Xeomin®/Bocouture®

Jürgen Frevert
Merz Pharmaceuticals GmbH, Frankfurt, Germany

Abstract

Background: Botulinum neurotoxin type A (BoNT/A) is the active substance in preparations used for the highly effective treatment of neurologic disorders such as cervical dystonia, blepharospasm, or spasticity, as well as other indications such as axillary and palmar hyperhidrosis, and urologic disorders.

Objective: To determine the amount of BoNT/A protein present in pharmaceutical preparations of Botox®, Dysport®, and Xeomin®, which are identical with Vistabel®, Azzalure®, and Bocouture®, respectively.

Methods: Rabbit and guinea pig antibodies raised against the 150 kD BoNT/A neurotoxin purified from Clostridium botulinum type A, strain ATCC 3502 (‘Hall strain’), were used in a sensitive sandwich ELISA to determine the overall mean concentration of the 150 kD neurotoxin present in four batches of Botox® (C2344C3, C2384C3, C2419, and C2385), two batches of Dysport® (678F and 689X) and three batches of Xeomin® (61111, 70604, and 81208). The specific neurotoxin potency, defined as the potency or biologic activity (units) per mass of neurotoxin protein (ng), was calculated based on the overall mean concentration of BoNT/A neurotoxin.

Results: Overall, the mean concentration of BoNT/A neurotoxin in Botox® was 0.73 ng per 100 unit vial (coefficient of variation [CV] = 3.5%), 3.24 ng per 500 unit vial of Dysport®, corresponding to 0.65 ng in 100 units (CV = 11.4%), and 0.44 ng per 100 unit vial of Xeomin® (CV = 1.9%). The specific potency of the 150 kD BoNT/A neurotoxin was calculated as 137 units/ng for Botox®, 154 units/ng Dysport®, and 227 units/ng Xeomin®.

Conclusions: The current study has shown that of the three products, Xeomin® contains the highest specific neurotoxin activity, followed by Dysport®, with Botox® having the lowest specific activity. This result suggests that Xeomin® contains only active neurotoxin in contrast with Botox®, which is likely to contain additional denatured/inactive neurotoxin.

Background

Botulinum neurotoxin type A (BoNT/A) is the active substance in preparations used for the highly effective treatment of neurologic disorders such as cervical dystonia,[1] blepharospasm, or spasticity, as well as other indications such as axillary and palmar hyperhidrosis,[2] and
urologic disorders. BoNT/A containing therapies are also used in aesthetic dermatology to treat facial wrinkles (e.g. glabella lines).

There are several BoNT/A containing products on the market: Botox®/Vistabel® (Allergan Inc., Irvine, CA, USA), Dysport®/Azzalure® (Ipsen, Slough, UK/Galderma, Paris, France), and Xeomin®/Bocouture® (NT 201; Merz Pharmaceuticals GmbH, Frankfurt, Germany). In all three products, the neurotoxin is derived from the identical Hall strain of Clostridium botulinum type A. For the following discussion, these products will be referred to as Botox®, Dysport®, and Xeomin®, regardless of their clinical indication because the product compositions do not differ between indications.

The BoNT/A protein has a molecular weight of 150 kD, consisting of a light chain (50 kD) and a heavy chain (100 kD); both chains have different functions in the neurotoxin’s mechanism of action. Whereas Xeomin® contains only the 150 kD neurotoxin, the 150 kD neurotoxin in Botox® and Dysport® is part of a complex with other proteins (complexing proteins), which play no role in the mechanism of action. Botox® is composed of a 900 kD complex, with one molecule of the 150 kD neurotoxin per complex. The biochemical composition of Dysport® is unknown. In an early publication, it was reported that a similar purification process to that used for the drug substance in Dysport® produces a mixture composed of the L complex (600 kD) and M complex (300 kD). These findings were later supplemented by protein composition analyses of the drug substance in Dysport®.

The biologic activity of all BoNT/A containing products is given in units relating to the dose that is lethal to 50% of mice tested (LD₅₀); however, the assay procedure used to determine biologic activity can differ between companies. Indeed, the most informative comparisons of BoNT/A containing products have been made in clinical studies. In a 16-week, randomized, double-blind, non-inferiority trial in 463 patients with cervical dystonia, Xeomin® was shown to be as effective as Botox® with a comparable adverse event profile when a clinical conversion ratio of 1 U : 1 U was used. Moreover, in a double-blind, non-inferiority trial in 300 patients with blepharospasm, treatment with Xeomin® was as effective as and as safe as Botox® (using a 1 U : 1 U conversion ratio) when assessed by the Jankovic Rating Scale. This equipotency of Botox® and Xeomin®, when used at a clinical conversion ratio of 1 U : 1 U, was also confirmed in a comparative trial in the treatment of glabellar frown lines in 381 patients. The 1 U : 1 U ratio was not unexpected because both products have demonstrated the same activity in a potency assay, which showed that five different batches of Botox® contained a mean of 101.7 ± 6.2 LD₅₀ units per vial and five different batches of Xeomin® contained a mean of 103.0 ± 5.7 LD₅₀ units per vial. Thus, both clinical and preclinical analyses have demonstrated a 1 : 1 ratio between Botox® and Xeomin®.

In contrast, the conversion ratio between Dysport® and Botox® or Xeomin® is not yet clear. Conversion ratios of up to 6 : 1 (Dysport® : Botox®) have been suggested.

The amount of clostridial protein in Botox® and Dysport® is known – one vial of Botox® contains 5 ng of clostridial protein per 100 unit vial, whereas one vial of Dysport® contains 4.35 ng of clostridial protein per 500 unit vial, although earlier publications from the manufacturer stated there were 12.5 ng per vial (500 units). Xeomin® is reported to contain approximately 0.6 ng of clostridial protein per 100 unit vial.

The amount of the 150 kD neurotoxin (i.e. the active substance) in Botox® and Dysport® is not published. There are two important reasons to know both the amount of clostridial protein and the amount of the 150 kD neurotoxin in these preparations. Firstly, accompanying proteins or impurities may stimulate the immune response against the therapeutic agent, and secondly, the dose of the antigen (i.e. the injected amount of neurotoxin protein itself), as well as the injected amount of denatured/inactive neurotoxin, determines the immune response. Therefore, the aim of this study is to determine the concentration of the 150 kD neurotoxin in Botox®, Dysport®, and Xeomin® using a sensitive sandwich ELISA.

A similar study was carried out with Botox® and Dysport® but the results were reported anonymously.
Materials and Methods

Materials

The incubation buffer solutions that were used were phosphate buffered saline (PBS) + 0.1% bovine serum albumin (BSA) [buffer solution 1], and PBS + 6% gelafusal [buffer solution 2] (Merck, Darmstadt, Germany, or Riedel-de-Haen, Germany). Gelafusal was provided by Serumwerke Bernburg, Germany, and o-phenylenediamine dihydrochloride by Sigma, St Louis, MO, USA. A horse antisera against botulinum toxin type A reacting with the neurotoxin complex was provided by the UK National Institute for Biological Standards and Control (NIBSC).

The 150 kD neurotoxin protein was purified from C. botulinum type A, strain ATCC 3502 ('Hall strain'), according to a modified protocol of DasGupta and Sathyamoorthy.[20] It was demonstrated by Western Blot that no complexing proteins were present using an antiserum provided by the NIBSC. Antibodies to the 150 kD proteins were raised in rabbits and guinea pigs provided by the NIBSC. Antibodies to the 150 kD neurotoxin were raised in rabbits and guinea pigs (Biogenes, Berlin, Germany) according to a standard protocol. The neurotoxin was detoxified by treatment with formaldehyde (0.4%, 15 days at 37°C).

Complexing proteins of botulinum toxin type A free from neurotoxin were prepared from the botulinum toxin complex produced according to the methods of Schantz and Johnson.[6] The botulinum toxin complex was dialysed against 50 mM TRIS (tris(hydroxymethyl) aminomethane)/HCl pH = 7.9 and purified by column chromatography on Q sepharose (GE Healthcare, Freiburg, Germany) by elution of the bound complexing proteins with a NaCl gradient. Traces of BoNT/A were removed by affinity chromatography. The chromatography matrix was prepared by immobilizing rabbit antibodies against BoNT/A on a CNBr sepharose (GE Healthcare) according to the instructions of the supplier. During the affinity chromatography step, BoNT/A was bound to the affinity column whereas all complexing proteins were eluted in the flow through. The composition of the complexing proteins was not changed after chromatography.

ELISA Procedure

A sensitive sandwich ELISA, which employed rabbit and guinea pig antisera raised against the 150 kD neurotoxin, was used to measure the amount of BoNT/A in pharmaceutical preparations of Botox®, Dysport®, and Xeomin®.

Microtiter plates (Nunc 4 39 454) were coated overnight at 2–8°C with anti-150 kD neurotoxin rabbit antisera diluted 1 : 1000 in 100 mmol/L sodium carbonate, pH = 9.5. After washing five times with buffer solution 1, unspecific binding was blocked by incubation for 1 hour with PBS + 2% BSA. Vials of Botox® (100 units), Dysport® (500 units), or Xeomin® (100 units) were reconstituted with 1 mL of buffer solution 1, and 100 μL (10 units of Botox® or Xeomin®, or 30 μL of the Dysport® (supplemented to a total volume of 100 μL with buffer solution 1) solution was pipetted into a Microtiter plate well coated with the anti-150 kD neurotoxin rabbit antisera and incubated for 1 hour at 37°C. Each vial was analyzed in triplicate.

Wells were washed five times with buffer solution 1, incubated with the anti-150 kD neurotoxin guinea pig antisera diluted 1 : 2000 in buffer solution 2 for 1 hour at 37°C, then washed five times with buffer solution 1 to remove unbound antibodies. Bound 150 kD neurotoxin antibodies were detected by incubation for 1 hour at 37°C with an anti-guinea pig IgG peroxidase conjugate (Sigma, A 7289) diluted 1 : 5000 in 100 mmol/L citrate buffer (pH = 5.0) containing 0.06% H2O2. The color reaction was stopped by the addition of 1 mol/L sulphuric acid.

The optical density of each well was measured at 490 nm on a Microtiter plate reader SpectraMax Plus (Molecular Devices, Sunnyvale, CA, USA) operated via the ‘SoftMax Pro GxP’ program. To quantify the concentration of the 150 kD neurotoxin, a standard curve was generated between 0.2 ng/mL and 1.6 ng/mL of the neurotoxin in steps of 0.2 ng/mL. Figure 1 depicts a representative standard curve covering a concentration range between 0.2 and 1.6 ng/mL. On each Microtiter
plate, a control sample was analyzed to assess the validity of each assay. The ELISA was validated according to the International Conference on Harmonisation (ICH)[21] guidelines for linearity of the standard curve, specificity, precision, accuracy, and robustness. The specificity of the ELISA was determined by analyzing purified C. botulinum toxins type B (strain Okra), type C (provided by Wako Ltd, Osaka, Japan) and type E (strain ATCC 17854).

The amount of the 150 kD neurotoxin in Botox® and Xeomin® was determined by ELISA on two different days. On day 1, two vials each of two Botox® batches (C2344C3 and C2384C3) and two vials each of two Xeomin® batches (61111 and 70604) were analyzed in parallel on the same Microtiter plate. On day 2, two batches of Botox® (four vials of C2419 and six vials of C2385) and five vials each of two Xeomin® batches (61111 and 81208) were analyzed. For the comparison of Dysport® with Xeomin®, five vials each of two Dysport® batches (678F and 689X) and two vials of one Xeomin® batch (61111) were analyzed.

Statistical Analysis

The concentration of BoNT/A in Botox®, Dysport®, and Xeomin® was reported using descriptive statistics. The coefficient of variation (CV) was calculated by dividing the standard deviation by the mean of all values, and is expressed as a percentage.

The specific neurotoxin potency, defined as the potency or biologic activity (units) per mass of neurotoxin protein (ng) for Botox®, Dysport®, and Xeomin® was calculated based on the overall mean concentration of the BoNT/A.

Results

ELISA Specificity

Purified C. botulinum toxins type B, type C, and type E showed no signal at a maximum concentration of 100 ng/mL (data not shown), confirming the specificity of the ELISA assay for BoNT/A.

None of the product’s excipients (sucrose and lactose) interfered with the ELISA assay (data not shown). Sodium chloride and human serum albumin were present in the same concentration in the samples. There was no reactivity when ≤10 ng/mL of complexing proteins, purified from the botulinum toxin complexes by affinity chromatography with immobilized neurotoxin, was tested in the ELISA (data not shown). A recovery of 97.3–100% was achieved by the ELISA when 0.9 ng/mL of the 150 kD neurotoxin protein was added to 10 ng/mL of complexing proteins. The ELISA detected denatured neurotoxin after heat treatment (5 days after 80°C).[22]

Botulinum Neurotoxin Type A Concentration of Botox®, Dysport®, and Xeomin®

The mean concentration of BoNT/A in the two Botox® batches, as determined by ELISA on day 1, was 0.74 ng/vial (C2344C3) and 0.73 ng/vial (C2384C3). For Xeomin®, this was 0.45 ng/vial for batch number 61111 and 0.43 ng/vial for batch number 70604 (table I).

| Table I. Mean concentration of botulinum neurotoxin type A (BoNT/A) in Botox® and Xeomin® analyzed by a sensitive sandwich ELISA on day 1 |
|-----------------|-----------------|-----------------|-----------------|
| BoNT/A per vial [ng] (CV [%]) | (BoNT/A) in Botox® | C2344C3 | 0.74 (6.4) |
| | | C2384C3 | 0.73 (2.1) |
| | | 61111 | 0.45 (2.5) |
| | | 70604 | 0.43 (5.7) |

CV = coefficient of variation.
The repeat analysis on day 2 (table II) showed a mean BoNT/A concentration of 0.76 ng/vial (C2419) and 0.70 ng/vial (C2385) for the two Botox® batches. Both Xeomin® batches (61111 and 81208) contained 0.44 ng/vial of BoNT/A; therefore, the repeat analysis of batch number 61111 showed a comparable amount of BoNT/A on days 1 and 2.

Analysis of the two Dysport® batches showed a mean concentration of BoNT/A of 3.5 ng/vial (678F) and 2.97 ng/vial (689X) (table III). This corresponded to a mean concentration of 3.24 ng/vial of 500 units. Thus, 100 units of Dysport® contained 0.65 ng of the 150 kD BoNT/A (CV = 11.4%).

Specific Neurotoxin Potency of Botox®, Dysport®, and Xeomin®

The specific potency of the 150 kD BoNT/A was calculated as 137 units/ng for Botox®, 154 units/ng for Dysport®, and 227 units/ng Xeomin®.

Discussion

This study has shown that 100 units of Botox®, Dysport®, and Xeomin® contain 0.73 ng, 0.65 ng and 0.44 ng of BoNT/A, respectively. From these results, we have been able to calculate the specific neurotoxin potency for each preparation. The highest specific neurotoxin activity was found in Xeomin® (227 units/ng), Dysport® had 154 units/ng, and Botox® had the lowest biologic activity with 137 units/ng.

Interestingly, the 0.44 ng of neurotoxin protein in Xeomin® found in the current study is lower than previously reported.[14] However, the higher sensitivity and precision of the ELISA assay used in the present investigation may have contributed to this discrepancy.

As mentioned in the introduction, the 150 kD neurotoxin is the only active pharmaceutical ingredient in Xeomin®. Like Xeomin®, the neurotoxin in Botox® and Dysport® (as part of the toxic complex) is derived from the Hall strain of C. botulinum type A and has an identical amino acid sequence. However, it cannot be excluded that there may be differences in the epitope structure of the neurotoxin in Botox® and Dysport® compared with Xeomin® because they are prepared according to different procedures,[6,9] which may affect their relative reactivity to the antibody. Nevertheless, it may be speculated that the differing epitopes (by structure, if there were any) were not recognized by the applied antibodies that are highly specific for the neurotoxin in Xeomin®. If that were the case, the binding of the used antibodies to the neurotoxin in Botox® and Dysport® would be lower in the ELISA. Because the quantification of the ELISA depends on the binding of the antibodies to the substance to be analyzed, the relative concentration of the neurotoxin determined in the assay would be lower and the neurotoxin content in Botox® and Dysport® would be even higher than that described in this study. For a similar reason it would be problematical to apply an immunoassay based on monoclonal antibodies, because it has to be demonstrated that these antibodies

| Table II. Mean concentration of botulinum neurotoxin type A (BoNT/A) in Botox® and Xeomin® analyzed by a sensitive sandwich ELISA on day 2 |
|---------------------------------------------------------------|
| BoNT/A per vial [ng] (CV [%])                        |
| Botox®        | C2419 | 0.76 (4.7) |
|               | C2385 | 0.70 (4.3) |
| Xeomin®       | 61111 | 0.44 (6.5) |
|               | 81208 | 0.44 (3.5) |
| CV = coefficient of variation.                        |

| Table III. Mean concentration of botulinum neurotoxin type A (BoNT/A) in Dysport® and Xeomin® analyzed by a sensitive sandwich ELISA |
|--------------------------------------------------------------------------------|
| BoNT/A per vial [ng] (CV [%])                        |
| Dysport®      | 678F  | 3.50 (3.6) |
|               | 689X  | 2.97 (1.9) |
| Xeomin®       | 61111 | 0.43 (4.8) |
| CV = coefficient of variation.                        |

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react with respective epitopes of active as well as inactive BoNT/A in all products.

Despite differences in specific neurotoxin activity (defined as the biologic activity in units per mass of neurotoxin protein), clinical studies have shown that the efficacy of Xeomin® and Botox® is comparable.[10-12] Dressler and colleagues[13] also showed an equivalent potency of Botox® and Xeomin® in a mouse LD₅₀ bioassay. Together, these results demonstrate that the amount of neurotoxin in Botox® and Xeomin® exhibits the same activity in the clinical environment as in a standard mouse in vivo assay.[14]

From the results of the current study, one can propose that the 0.44 ng neurotoxin protein in 100 units of Xeomin® is as potent as the 0.73 ng neurotoxin protein in 100 units of Botox®. This suggests that Botox® contains a considerable amount of inactivated or partly denatured/inactivated neurotoxin – a suggestion that is supported by a previous study that showed that the 900 kD complex had a specific activity of 3±20%×10⁷ LD₅₀ units per mg of protein.[6] Thus, 100 units would correspond to 2.4–3.6 ng clostridial protein, which differs markedly from the 5 ng reported for Botox® in its prescribing information.[16] Of note, the presence of residual denatured/inactive neurotoxin in Botox® or Dysport® (i.e. a higher dose of antigen) can, amongst other factors, be associated with the stimulation of neurotoxin neutralizing antibodies in some patients.

The amount of neurotoxin in Botox® can also be estimated from the proportion of neurotoxin in the complex: the 150 kD neurotoxin should be one-sixth of the 900 kD complex (i.e. 5 ng/6=0.83 ng), which is similar to the 0.73 ng demonstrated in the present investigation. Our calculation is supported by a patent application filed by Allergan, which reported that the average specific potency of the purified complex before formulation was 28 pg protein/unit or 2.8 ng per 100 units.[23] Since Botox® contains 5 ng and not 2.8 ng clostridial protein, is it likely that part of the neurotoxin may be denatured,[24] possibly due to the use of sodium chloride in the formulation, which seems to be detrimental for activity during the drying process.[25]

The current study found 3.24 ng per vial (500 units) of neurotoxin in Dysport®, corresponding to 0.65 ng per 100 units. Considering there is 4.35 ng of clostridial protein in a 500 unit vial of Dysport®, the proportion of the neurotoxin is approximately 70%, with around 30% made up of complexing proteins, and other clostridial proteins.[9] This calculation is consistent with a rough estimation of the proportion of the neurotoxin from the electrophoresis of three batches of Dysport®.[9] Although all complexing proteins were present in the drug substance,[9] the amount of these proteins in the preparation seems to be too low to form a high molecular weight complex with all neurotoxin molecules, hence, a part of the neurotoxin in Dysport® seems to be present as a free molecule.

In this study, a 100 unit vial of Xeomin® contained a mean concentration of BoNT/A neurotoxin of 0.44 ng. The high specific potency corresponds to high specific potency values in the literature for the purified BoNT/A.[26] The reason for the high specific activity in Xeomin® might be that no inactivation of the neurotoxin takes place during the manufacturing process of the drug product. As a result, the final Xeomin® product contains only the active neurotoxin. This is corroborated by the finding that the active substance before formulation and lyophilizing has the same specific potency as the final product.[27]

Thus, although the amount of units of neurotoxin (specific biologic activity) is the same when Botox® or Xeomin® is administered to the patient, the amount of neurotoxin protein administered is different. Patients treated with Botox® receive about 60% more of neurotoxin protein, which represents denatured/inactive neurotoxin. This means that the dose of antigen is higher, which may be responsible for the formation of neutralizing antibodies leading to therapeutic failure. As a consequence, this could exclude, for instance, patients with dystonia or spasticity from receiving adequate treatment.

Conclusions

The current study has shown that of the three products investigated, Xeomin® contains the highest...
specific neurotoxin activity, followed by Dysport®, with Botox® having the lowest biologic activity. This result suggests that Xeomin® contains only active neurotoxin, whereas Botox® is likely to contain denatured/inactive neurotoxin.

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References

1. Jankovic J, Albanese A, Attassi MZ, et al. Botulinum toxin-therapeutic clinical practice and science. Philadelphia (PA): Saunders Elsevier, 2009
2. Bhidayasiri R, Truong DD. Evidence for effectiveness of botulinum toxin for hyperhidrosis. J Neurol Transm 2008; 115 (4): 641-5
3. Smith CP. Botulinum toxin in the treatment of OAB, BPH, and IC. Toxicol 2009 Oct; 54 (5): 639-46
4. Fagien S, Carruthers JD. A comprehensive review of patient-reported satisfaction with botulinum toxin type A for aesthetic procedures. Plast Reconstr Surg 2008 Dec; 122 (6): 1915-25
5. Rossetto O, Morbliato L, Caccin P, et al. Presynaptic enzymatic neurotoxins. J Neurochem 2006 Jun; 97 (6): 1534-45
6. Schantz EJ, Johnson EA. Properties and use of botulinum toxin and other microbial neurotoxins in medicine. Microb Rev 1992 Mar; 56 (1): 80-99
7. Lietzow MA, Gielow ET, Le D, et al. Subunit stoichiometry of the Clostridium botulinum type A neurotoxin complex determined using denaturing capillary electrophoresis. Protein J 2008 Dec; 27 (7-8): 420-5
8. Hambleton P. Clostridium botulinum toxins: a general review of involvement in disease, structure, mode of action and preparation for clinical use. J Neurol 1992 Jan; 239 (1): 16-20
9. Panjwani N, O’Keeffe R, Pickett A. Biochemical, functional and potency characteristics of type A botulinum toxin in clinical use. Botulinum J 2008; 1 (1): 153-66
10. Benecke R, Jost WH, Kanovsky P, et al. A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. Neurology 2005 Jun 14; 64 (11): 1949-51
11. Roggenkamper P, Jost WH, Bihari K, et al. Efficacy and safety of a new botulinum toxin type A free of complexing proteins in the treatment of blepharospasm. J Neural Transm 2006 Mar; 113 (3): 303-12
12. Sattler G. Comparison of the efficacy and safety of NT 201, free of complexing proteins, with a licensed botulinum toxin type A in the treatment of glabellar frown lines. IMCAS conference; 2010 Jan 8-11; Paris
13. Dressler D, Mander G, Fink K. Equivalent potency of Xeomin® and Botox® [abstract]. Movement Disorder Society’s Twelfth International Congress of Parkinson’s Disease and Movement Disorders. Mov Disord 2008; 23 (S1): S20-1
14. Jost WH, Blumel J, Grafe S. Botulinum neurotoxin type A free of complexing proteins (XEOMIN) in focal dystonia. Drugs 2007; 67 (5): 669-83
15. Marchetti A, Magar R, Findley L, et al. Retrospective evaluation of the dose of Dysport and BOTOX in the management of cervical dystonia and blepharospasm: the REAL DOSE study. Mov Disord 2005 Aug; 20 (8): 937-44
16. Allergan Inc. BOTOX (Botulinum toxin type A) purified neurotoxin complex: prescribing information revised March 2010 [online]. Available from URL: http://www.allergan.com/assets/pdf/botox_pi.pdf [Accessed 2010 Jul 26]
17. Speywood Pharmaceuticals Limited. Dysport Clostridium botulinum type A toxin-haemagglutinin complex: prescribing information revised 1994. Speywood Pharmaceuticals Limited: Maidenhead (now Ipsen, Slough, UK), 1994
18. Schellekens H. How to predict and prevent the immunogenicity of therapeutic proteins. Biotechnol Annu Rev 2008; 14: 191-202
19. Ekong TA, McLellan D, Sesardic D. Immunological detection of Clostridium botulinum toxin type A in therapeutic preparations. J Immunol Methods 1995 27; 180 (2): 181-91
20. DasGupta BR, Sathyamoorthy V. Purification and amino acid composition of type A botulinum neurotoxin. Toxicon 1984; 22 (3): 415-24
21. International Conference on Harmonization Guideline Q2B validation of analytical procedures: methodology. US FDA, 1997
22. Grein S, Mander GJ, d Harold V, et al. Xeomin® is stable without refrigeration: complexing proteins are not required for stability of botulinum neurotoxin type A preparations [abstract and poster]. Toxins conference; 2008 Jun 12-14, Baveno. Toxicon 2008; 51 Suppl. 1: 13
23. Hunt TJ. Improved botulinum toxin composition. US Patent application 20070025019 [online]. Available from URL: http://www.wipo.int/pctdb/en/wo.jsp?wo=2007016018&IA=US2006028603&DISPLAY=DESC, [Accessed 2010 Jul 23]
24. Bigalke H. Properties of pharmaceutical products of botulinum neurotoxins (chapter 32). In: Jankovic J, Albanese A, Attassi MZ, et al., editors. Botulinum toxin-therapeutic clinical practice and science. Philadelphia (PA): Saunders Elsevier, 2009
25. Goodnough MC, Johnson EA. Stabilization of botulinum toxin type A during lyophilization. Appl Environ Microbiol 1992 Oct; 58 (10): 3426-8
26. Shone CC, Tranter HS. Growth of clostridia and preparations. J Immunol Methods 1995; 195: 143-60
27. Data on file. Merz Pharmaceuticals GmbH, 2004

Correspondence: Dr Jürgen Frevert, Merz Pharmaceuticals GmbH, Hermannsweder Haus 15, 14473 Potsdam, Germany.
E-mail: Juergen.Frevert@merz.de