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Review Article

The ICH guidelines in practices: Stress degradation studies on botulinum toxin and validation of developed stability-indicating HPLC method

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ABSTRACT

Introduction: A fast, simple, reliable and accurate RPHPLC analytical method was developed for the evaluation of botulinum toxin, and the developed method was subsequently validated according to ICH guidelines in sterile dosage form for stability studies. A C18 column with a flow rate of 2 ml/min was selected. The mobile phase chosen consisted of sodium phosphate buffer (0.05 M) at pH 2.8 and acetonitrile in a ratio of 30:70 respectively at 214 nm. Measured at an Rt of 2.1 min. 10 minutes running time. Linearity and range were observed at concentrations from 1 μg/ml to 10 μg/ml. The method developed was linear with a correlation coefficient of 0.99.
Conclusions: Validation of the method was performed according to ICH guidelines for assay, linearity and range, precision, limit of detection, limit of quantitation, and forced degradation test.

1. Introduction

In 2010, the Food and Drug Administration (FDA) approved Botox as a prescription drug treatment for chronic migraine sufferers. Using Botox to treat migraines has been shown to be beneficial for patients who experience more than 15 migraines per month, but using Botox is not without risks. Read more in this overview, including the benefits and risks of using Botox to treat migraines.

Botox is a botulinum neurotoxin, a neurotoxin made by a bacterium called Clostridium botulinum. This is the same type of bacteria that causes botulism. Botulism is a progressive, potentially fatal infection that can cause symptoms such as muscle paralysis, slurred speech, and drooping eyelids. But when this neurotoxin is administered by injection, its effects are concentrated and not dangerous. [1]

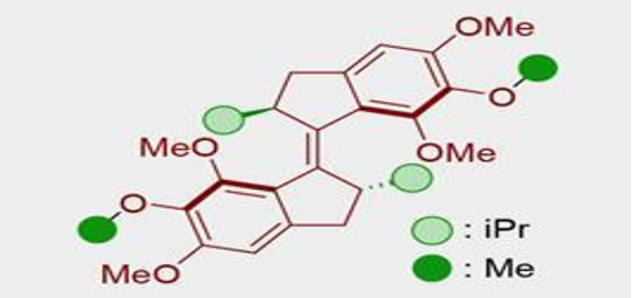

Fig. 1: Structural formula of botulinum toxin

2. Materials and Methods

2.1. Chemical and reagent

Sodium phosphate buffer and acetonitrile were ordered by Merck Private Ltd, Mumbai, India. API of Botulinum toxin as bulk drug were purchased from Teva API India Ltd (2927057), Bijnor, Uttar Pradesh India.

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2.2. Instrumentation

HPLC model SHIMATZU with pump SPD-20AD (LC-20A) having variable wavelength, UV-Visible detector and Rheodyne injector (20 μl). Column- The analytical column was Phenomenex 100-5C-18, 5 μm 100 A °, 250 mm × 4.6 mm.

2.3. Stock solution and standard solution preparation

2.3.1. Standard solutions and its preparations

Weighed accurately 10 mg of Botulinum toxin and transferred to volumetric flask(100 ml). Then add 70 ml HPLC grade water and dissolve it, sonication was done for arround15 min and made the final volume with HPLC grade water. Take 10 ml of stock solution and dissolve in 100ml volumetric flask with HPLC grade water. Filter the stock solution with suction pump assembly.

2.3.2. Preparation of sample solution

Add 1 ml of WFI (water for injection), and shake well. Take out 0.1 ml of sample solution and dissolve in 100 ml HPLC grade water.

2.4. Validation of method

HPLC assay method was validated following ICH guidelines Q2R1for linearity and range, accuracy, detection limit, quantitation limit, stability study, etc.[2]

3. Results

3.1. Development of method and its optimization

The development of RP-HPLC analytical method for Botulinum toxin was done using C8 column after optimization of chromatographic conditions for specificity, retention time and resolution. The suitable mobile phase was sodium phosphate buffer and acetonitrile in the ration of 30:70 at 214 nm. 2.1 min was the retention time at the flow rate of 2ml/min. When the flow was reduced, the resultant chromatogram showed increase in retention time with tailing effect.

Table 1: HPLC method development parameters for botulinum toxin

Sr. No.	Parameter	HPLC method
1	Column	C8
2	Flow rate	2ml/min
3	λ max	214 nm
4	Run time	20 min
5	Injection volume	6 μl
6	Mobile phase	Sodium phosphate buffer: Acetonitrile

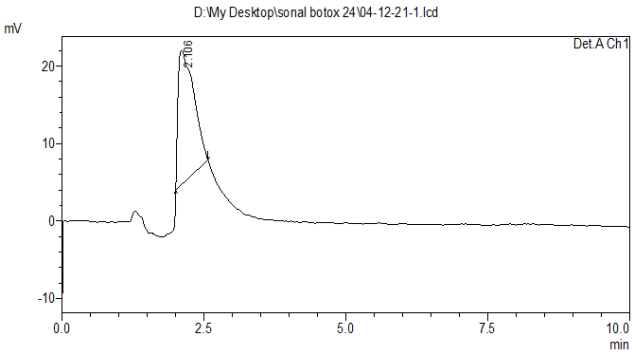

Fig. 2: Chromatogram of botulinum toxin at 214 nm.

3.2. Validation method

The validation of HPLC assay were carried out following ICH guidelines (Q2 R1).[3]

3.3. System suitability studies

The six replication of standard solution was inserted into the HPLC and observed that parameters for system suitability are within the limit

Table 2: System suitability parameters

Sr. No.	Parameters	Result
1	Peak Area	27572
2	Theoretical plates	5232
3	Tailing factor	1.8
4	Resolution	0.09

3.3.1. Linearity and range

The standard solutions were analysed for concentration against peak area in different concentrations in the range of 2, 4, 6, 8, 10 μg/ml. The regretion equation was found to be y=28351x+10591 and correlation coefficient was 0.9947.

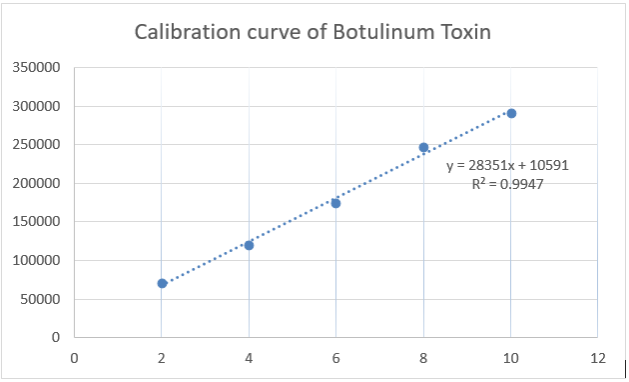

Fig. 3: Calibration curve of Botulinum toxin

Table 3: Result of linearity and range

S. No	Con. (μg/ml)	Peak Area
I	2	70604
II	4	120025
III	6	175072
IV	8	247324
V	10	290467

3.3.2. Accuracy

Recovery study was carried out to find out accuracy was with 3 different concentration levels (80%, 100%, 120%). Botulinum toxin standard of concentration 8μg, 10 μg, 12μg were prepared and recovery study was done. The values of recovery study were observed in the specific limit of 98-102%.

Table 4: Result of accuracy

Sr. No.	% Level	% Recovery	Average
1		100.4	
2	80 %	100.1	100.1
3		99.8	
4		100.2	
5	100 %	100.1	100
6		99.7	
7		100.0	
8	120%	99.6	99.6
9		99.3	

4. Precision

Precision of the developed method was carried out by repeatability, intermediate precision.

4.1. Repeatability-6 replicates of the standard stock were analysed for SD and RSD

Table 5: Result of repeatability

Analyte	Concentration	% RSD
Botulinum toxin	5 μg/ml	0.43

4.2. Intermediate precision-6 replicates of the standard stock was analysed on interday, intraday, different analyst

Table 6: Resultof intermediate precision

Analyte	Concentration	% RSD
Botulinum toxin	5 μg/ml	0.32

5. Detection Limit and Quantification Limit

The detection limit concentration of Botulinum toxin was 1μg/ml and quantitation limit concentration was 3.3 μg/ml.[4]

5.1. Stability

5.1.1. Degradation effect

Botulinum toxin sample was subjected to various degradation studies. Stress degradation parameters were carried out to find the developed HPLC assay is liable for decomposed materials. This study provides results about the conditions wherein the drug is unstable.[5]

Table 7: Resultsof degradation study

Degradation parameters	% degradation
Alkali Degradation	64.2%
Acid Degradation	57.3%
Unstressed Degradation	100 %
Photolytic Degradation	58.2%
Neutral Degradation	90.3%
Peroxide Degradation	87.6%
Thermal Degradation	37%

6. Discussion

Research studies have used reversed-phase HPLC for the elution of botulinum toxin. We chose his C18 column with a flow rate of 2 mL/min. The mobile phase chosen consisted of sodium phosphate buffer (0.05 M) at pH 2.8 and acetonitrile in a ratio of 30:70 respectively at 214 nm. It is within ICH and FDA limits. In addition, analysis of a commercial formulation of botulinum toxin showed that the drug elutes without interfering peaks generated by excipients in the commercial product.[6–11] Therefore, we found the method results to be stable for different parameters.

7. Conclusion

In the current study, a fast, simple, accurate, well-defined, and linear HPLC method for demonstrating botulinum toxin stability was developed and validated and can be rented for routine quality control analysis. The analytical technique and mobile phase solvent conditions provided good separation of botulinum toxin. In addition, the main features of the developed method are short run time and retention time of about 3.1 min. The method has been validated according to ICH guidelines. The method is robust enough to reproduce accurate and precise results under a variety of chromatographic conditions.

8. List of Abbreviation

Sr. No	Abbreviation	Name of Abbreviation
1	BTX	Botulinum Toxin
2	RPHPLC	Reversed Phase High Performance Liquid Chromatography
3	ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
4	SD	Standard Deviation
5	RSD	Relative Standard Deviation

9. Source of Funding

None.

10. Conflict of Interest

None.

References

1. Xavier B, Perobelli RF, Walter ME, da Silva FS. Content/Potency Assessment of Botulinum Neurotoxin Type-A by Validated Liquid Chromatography Methods and Bioassays. *Toxins (Basel)*. 2019;11(1):35. doi:10.3390/toxins11010035.
2. Pharmacopoeia of India : (the Indian pharmacopoeia); 1955. Available from: http://www.ipc.gov.in/.
3. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite guideline validation of analytical procedures: text and methodology q2(r1) current step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005);.
4. Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for; 2015. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/analytical-procedures-and-methods-validation-drugs-and-biologics.
5. for Industry and FG. Stability Testing of Drug Substances and Drug Products; 1998. Available from: https://www.fda.gov/files/drugs/published/ANDAs--Stability-Testing-of-Drug-Substances-and-Products--Questions-and-Answers.pdf.
6. FDA. Expiration Dating and Stability Testing for Human Drug Products; 2014. Available from: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/expiration-dating-and-stability-testing-human-drug-products.
7. De Freitas G, Perobelli RF, Maldaner FPS, Xavier B, Dalmora DA, Schramm VG. Evaluation of botulinum toxin type A by bioassays and a validated reversed-phase liquid chromatography method. *J Analytical Methods*. 2016;8(3):587–92.
8. Ji M, Lee HS, Kim Y. Method development for acetyl octapeptide-3 analysis by liquid chromatography-tandem mass spectrometry. *J Anal Sci Technol*. 2020;11:34. Available from: https://jast-journal.springeropen.com/counter/pdf/10.1186/s40543-020-00232-8.pdf.
9. Poras H, Ouimet T, Orng SV, Fournié-Zaluski MC, Popoff MR. Detection and quantification of botulinum neurotoxin type a by a novel rapid in vitro fluorimetric assay. *Appl Environ Microbiol*. 2009;75(13):4382–90.
10. Kirkwood M, CLand, David L. Technologies for detecting Botulinum Neurotoxins in Biological and Environmental Matrices written by Luisa W. Cheng. and others, editor; 2016. p. 314.
11. Chan NWC, Tang T, Lee WE. Liquid chromatography - mass spectrometric analysis of botulinum neurotoxin serotype A light chain assay mixtures ; 2010. Available from: https://cradpdf.drdc-rddc.gc.ca/PDFS/unc253/p804741_A1b.pdf.

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