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Divya Uthaman
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

Smita
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

Pranshu Kapoor
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

Rakhi Singh
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

ML Aggarwal
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

KM Chacko
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

Corresponding Author:
Divya Uthaman
Shriram Institute for Industrial Research, 19 University Road, Delhi, India

Method validation of residual cyanide determination in lamotrigine API using sensitive spectrophotometric method

Divya Uthaman, Smita, Pranshu Kapoor, Rakhi Singh, ML Aggarwal and KM Chacko

Abstract
Lamotrigine is known as an anticonvulsant or antiepilectic drug. Trace level of cyanide may remain in the product in cyanation step during manufacturing process. Based on the principle of sensitive colorimetric procedure, converting the product to cyanogen chloride, CNCl by reaction with chloramine-T and subsequently the formation of colored complex Quinquevalent Cyanide with pyridine-barbituric acid reagent, the residual cyanide content was determined. The method was validated for various parameters such as System suitability, Linearity, Method Precision, Accuracy and Intermediate precision by employing UV-Spectrophotometer. A wide linear range concentration of 0.015-0.1 mg/Kg was observed with r^2 values ≥ 0.99. The sample was spiked at concentration level of 0.025; 0.05; 0.075 and 0.1 mg/Kg respectively and better recoveries between 80-120% with the acceptable relative standard deviation (RSD) i.e. <20% were obtained. The proposed technique efficiently screens residual cyanide in Lamotrigine API as residual Cyanide.

Keywords: Spectrophotometry, lamotrigine, validation, cyanide

1. Introduction
Lamotrigine (3, 5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine), a phenyl-traizine derivative was first disclosed in the early 1980s by researchers at the Wellcome Foundation [1, 2]. Lamotrigine continues to be a front-line anti-convulsant medicine for the treatment of epilepsy, bipolar disorder, and other conditions [3]. As per literature reported, it is chemically unrelated with other antiepileptic drugs in current use, differing in structure and pharmacology and has a broad spectrum in antiepileptic activity [4, 5].
Lamotrigine is completely absorbed after oral administration. It exhibits excellent oral bioavailability with first-order linear pharmacokinetics and has a mean plasma half-life of approximately 24 h [6]. Lamotrigine (Figure 1) is a lipophilic weak base with plasma protein binding of 55%. It gets extensively metabolized in human liver via hepatic glucuronidation by uridine 50-diphosphate-glucuronosyl transferase (UGT1A4) [7].
In the intervening years, vast majority of syntheses of Lamotrigine have been reported following the general synthetic route [8-13] with 2,3- Dichlorobenzoic acid (2,3-DCBA) as the key starting material (main moiety) as shown in Scheme 1. Therefore, it can be stated that the cyanation of 2,3-DCBA is the route for the synthesis of lamotrigine.
Among these routes, the reported conditions for cyanation of the acid chloride intermediate to give the acyl cyanide are the most varied [14-20]. Known systems differ in cyanide source, solvent, reaction temperature and additive/catalyst. Classic methods for the cyanation of acyl chlorides involve high-temperature (>150°C) treatment with CuCN, often conducted neat. Finally, combining alternative cyanide sources such as NaCN, $Zn(CN)^2$, or $K4Fe(CN)^6$ with various catalysts has also been reported previously for cyanation of aroyl chlorides [20-22]. Condensation of the 2,3-dichlorobenzoyl chloride with copper (I) cyanide was useful for the preparation of 2,3-dichlorobenzoyl cyanide.
The presence of cyanide in the body leads to inhibition of cell respiration, as a result of the merger of cyanide ion with trivalent iron cation of cytochrome a3, an integral component of cytochrome oxidase, located in the mitochondria of the liver. Hypoxia results in disorder in functioning of all cells; however, the most sensitive to the toxic effects of cyanide are those tissues with the fastest oxygen metabolism like cardiac muscle and the brain [23, 24].

Hence, there exists a need to evaluate any traces of cyanide left unreacted in the produced drug. Therefore, the analysis of cyanide in samples with different matrix compositions becomes a very important issue.

Several instrumental methods have been established for cyanide detection [25], including UV-Vis spectroscopy, Raman spectroscopy, voltammetry, chromatography, potentiometry with cyanide-selective electrodes, flow injection (FI) - amperometry and atomic absorption spectroscopy. Measurement of cyanide in different matrices including water, soil, air, exhaled breath, food and biological fluids (blood, urine, saliva, etc.) have also been reported. Typically, when analyzing concentrations of a target analyte i.e. cyanide in the sample matrices, the extraction method is as important as the analytical technique. With the growing interest in cyanide determination; almost all aspects of modern instrumental analysis and related publications have appeared during the last five years. Recently, Xu *et al*. and Zelder and Männel-Croisé have respectively reviewed optical sensors and colorimetric measurement of cyanide [26].

A review of the literature indicates that colorimetric methods are superior to the others [27]. Spectrophotometry has widely been used to determine cyanide [28]. These methods have mainly been based on measuring the absorbance of complexing agents due to complexation with cyanide. In the sensitive colorimetric measurement, the cyanide is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at a pH less than 8 without hydrolyzing to the cyanate.On completion of reaction, color is formed on addition of pyridine-barbituric acid reagent. This colored compound is a complex of Quinquevalent Cyanide and is determined spectrophotometrically [29]. The sensitivity of this method surpasses the other reported spectrophotometric methods.

The aim of the present scope of the work is to optimize a sensitive spectrophotometric method for the determination of residual cyanide in Lamotrigine drug involving validation parameters.

2. Materials and Methods

2.1 Chemicals and reagents

Potassium Cyanide Standard Solution (100g/L) was procured from Chem-Lab. Chloramine-T Solution AR grade from Merk was used. Pyridine and Hydrochloric Acid of LR grade were purchased from Thomas Baker. Barbituric acid, AR grade from Loba Chemical was used in the present study. The aqueous solutions were prepared with deionized water (Milli Q Millipore). All absorbance measurements were carried out on UV-Visible spectrophotometer, Shimadzu UV1800 (A11635101719), equipped with 1.0 cm quartz cell.

2.2 Preparation of reagents and standard solutions

2.2.1 Preparation of Chloramine-T solution

About 1.0 g of Chloramine-T was weighed and dissolved in 100mL of Milli-Q water. This solution was prepared freshly.

2.2 Preparation of Pyridine-Barbituric acid Reagent solution

Barbituric acid of about 6.0-7.0 g was taken into 100mL of volumetric flask containing 10mL of water followed by slow addition of 30mL Pyridine (portion wise 10mL) and cooled the mixture in ice bath. Then to this mixture, slowly added portion wise of 6-7 mL concentrated hydrochloric acid (Conc. HCl) in ice bath with proper shaking. The volume was made up to the mark with water.

2.2.3 Preparation of Standard solution stock (1000 mg/kg Cyanide solution)

Cyanide standard stock solution of 1000 mg/Kg concentration was prepared by taking accurately 0.25 mL standard solution of Potassium cyanide (100g/L ≈ 10%) into 10.0 mL volumetric flask. Dissolved in 5 mL of water and made upto the mark with same, mixed well and vortexed.

2.2.4 Preparation of working standards

Working standard solutions were prepared by serial dilution of the standard stock solution into 10 mL volumetric flasks containing 0.2mL of Chloramine-T solution and 2.5mL of Pyridine-Barbituric acid to achieve the desired linearity range concentration of0.015-0.1 mg/Kg of Cyanide. After shaking for 5 min, final volume was made up to the mark with chilled water.

2.2.5 Preparation of Sample and Blank solution

About 0.05 g of Lamotrigine sample was weighed accurately into 10 mL volumetric flask. To this added 5 mL chilled water and mixed well. Followed by addition of 0.2 mL of Chloramine-T solution and slowly addition of 2.5 mL of Pyridine-Barbituric acid Reagent solution. This was then shaken for 5 min and made upto the mark with chilled water. Blank solution was prepared by the same procedure without taking cyanide sample.

2.3 Instrumentation and conditions

Instruments: UV –Spectrophotometer

Wavelength: 578 nm

3. Results and Discussion

Method validation

As per the ICH guidelines Validation of the analytical method was performed by carrying out Linearity, Precision, Accuracy, Intermediate precision and limit of quantification (LOQ).

3.1 Linearity

The linearity regression analysis was demonstrated to check the acceptability of the method for quantitative determination range of LOQ to 150% of the specification limit. The regression coefficient r^2 = 0.99 is also well within limit. In this study, five concentration levels of Cyanide standard solutions ranging from 0.015 to 0.1mg/kg were analyzed to evaluate the linearity of the calibration curves by plotting the peak areas which were used as the analytical signal response versus concentration. The linearity data for both the methods is shown in Table 1 whereas Linearity graph is shown in Figure 2.The calibration curves obtained were linear within the range and showed good regressions (correlation coefficients (r^2) > 0.99).

Table 1: Linearity regression analysis

Concentration (mg/Kg)	Absorbance (Reading)	Rf
0.015	0.022	681.8182
0.025	0.040	625.0000
0.05	0.080	625.0000
0.075	0.117	641.0256
0.1	0.158	632.9114

3.2 Limit of Quantification (LOQ)

The limit of quantitation (LOQ) was determined by gradually decreasing the concentrations of standard solution of cyanide

and reading the absorbance. The limits of quantification found was 0.015mg/kg.

3.3 System suitability

System suitability was performed using0.05 mg/kg concentration of standard solution and calculating the relative standard deviation of six replicate absorbance readings of the same. The relative standard deviation was less than 2.

Table 2: Precision of Standard Solution (0.05 mg/Kg)

Standard solution	Absorbance	% RSD
Solution 1	0.083	1.62
Solution 2	0.083	
Solution 3	0.083	
Solution 4	0.080	
Solution 5	0.081	
Solution 6	0.081	

Method Precision

Method precision was determined by repeatability (intra-day) and intermediate precision (inter-day). Precision were evaluated with six replicates of sample fortified at 0.015 mg/Kg. The intra- and inter-day precision results are shown in Table 3 and 4. The intra- and inter-day precision of the fortified samples were satisfactory with RSD less than 2%.

Table 3: Precision data of Intra-day fortified at 0.015 mg/Kg

S. No	Sample wt. (g)	Sample Abs	% RSD
1	0.0498	0.018	1.39
2	0.0499	0.018	
3	0.0535	0.020	
4	0.0545	0.020	
5	0.0551	0.020	
6	0.0542	0.020	

Table 4: Precision data of Inter-day fortified at 0.015 mg/Kg

S. No	Sample wt. (g)	Sample Abs	% RSD
1	0.0532	0.017	1.54
2	0.0541	0.017	
3	0.0535	0.017	
4	0.0545	0.017	
5	0.0521	0.016	
6	0.0485	0.015	

3.5 Accuracy

Typically, accuracy is represented and determined by recovery studies. The accuracy of the method was evaluated by spiking the samples at four levels i.e. 30, 50, 100 and 150% of the specification level i.e. at concentration level of 0.025; 0.05; 0.075 and 0.1 mg/Kg respectively. The recovery data for the methods is as shown in Table 5. Three absorbance reading were taken at each level. The relative standard deviation (% RSD) for all spiked levels were found lower than 20% and the recovery obtained were between 92.4% to 96.9%.

Table 5: Recovery data for cyanide

Specification Level %	Concentration level (mg/Kg)	% Recovery
30	0.025	92.4
50	0.05	93.0
100	0.075	96.9
150	0.1	95.8

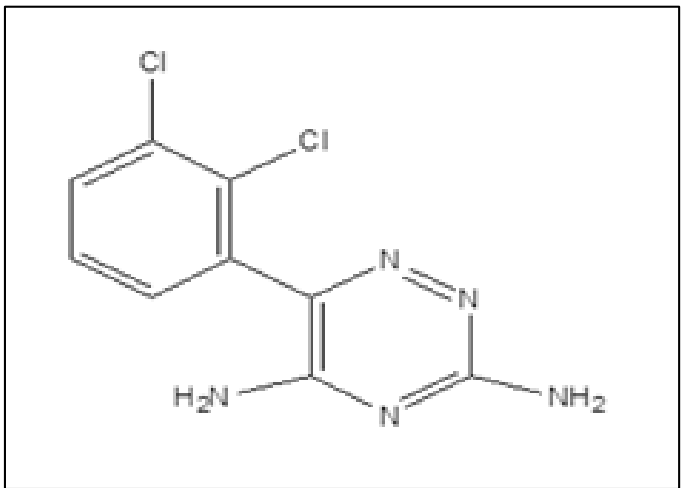
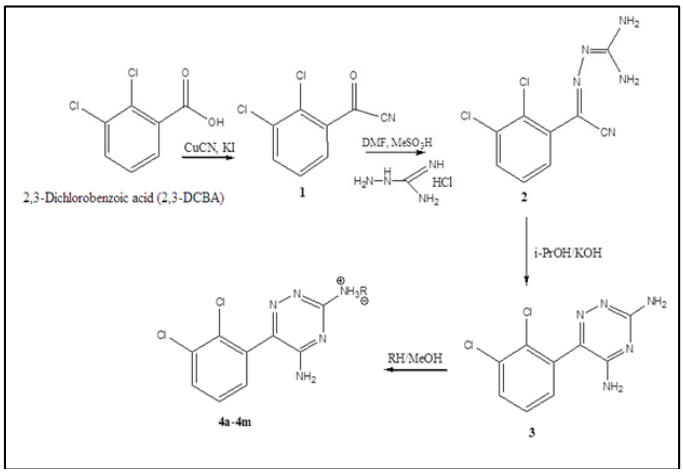


Fig 1: Chemical structure of Lamotrigine



Scheme 1: Synthetic route of Lamotrigine

4. Conclusion

The simple, sensitive analytical method has been validated for the residual content of Cyanide in Lamotrigine. No trace amount of cyanide was detected in Lamotrigine samples. The data of the spiked samples showed relatively high recoveries. Quantitative calibrations performed in a standard matrix showed excellent linearity and precision. The validation data showed that this method has good accuracy and sensitivity. The method is simple, reproducible and accurate for determination of residual cyanide in Lamotrigine API. The developed analytical method is useful as a routine alternative choice for the trace determination of cyanide. The applicability of the proposed spectrophotometric procedure for the determination of residual cyanide could be applied in other API samples. The method well suits for the intended purpose.

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6. References

1. Santosh Ghatol, Vatsal Vithlani, Sanjay Gurule, Arshad Khuroo, Tausif Monif, Pankaj Partani. Liquid chromatography tandem mass spectrometry method for the estimation of lamotrigine in human plasma: Application to a pharmacokinetic study. Journal of Pharmaceutical Analysis 2013; 3(2):75-83.

2. David C Leitch, Matthew P John, Paul A Slavin, Andrew D Searle. An Evaluation of Multiple Catalytic Systems for the Cyanation of 2,3-Dichlorobenzoyl Chloride: Application to the Synthesis of Lamotrigine. Org. Process Res. Dev, 2017, DOI: 10.1021/acs.oprd.7b00262.
3. Miller AA, Wheatley P, Sawyer DA *et al*. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: I. Anticonvulsant profile in mice and rats. Epilepsia. 1986; 27:483-489.
4. Ben-Menachem E. New antiepileptic drugs and non-pharmacological treatments. Current Opinion in Neurology, 2000; 13(2):165-170,
5. Cheng CL, Chou CH, Hu OYP. Determination of lamotrigine in small volumes of plasma by high performance liquid chromatography. Journal of Chromatography B. 2005; 817(2):199-206.
6. Cohen AF, Land GS, Breimer DD, *et al*. Lamotrigine, a new anticonvulsant: pharmacokinetics in normal humans. Clin. Pharmacol. Ther. 1987; 42:535-541.
7. Magda T Martins, Cl´esio S Paim, Martin Steppe LC, UV methods for Lamotrigine determination in Pharmaceutical formulation. Chromatography Research International, 2011, Article ID 860168, 8 pages.
8. Krishnaiah Ch, Khagga Bhavya Sri. A validated LC method for determination of 2,3-Dichlorobenzoic acid and its associated regio isomers. Journal of Chromatographic Science 2012; 50:440-445
9. Krishnaiah CH, Vishnu Murthy M, Raghavacharyulu KSVD, Durga Prasad BJ, Bangaru Babu R, Mukkanti K *et al*. An LC method for quantification of 2,3-dichlorobenzoyl cyanide and its related regio isomers. Chromatographia. 2009; 69:1119-1122.
10. Carrier DJ, Eckers C, Wolff JC. In-source fragmentation of an isobaric impurity of lamotrigine for its measurement by liquid chromatography tandem mass spectrometry after pre-concentration using solid phase extraction. Journal of Pharmaceutical and Biomedical Analysis. 2008; 47:731-737.
11. Reddy VV, Govardan G, Srinivasulu K, Reddy GM, Himabindu VR An impurity profile study of Lamotrigine. Journal of Chemistry, 2008; 1:301-305.
12. Youssef NF, Taha EA. Development and validation of spectrophotometric, TLC and HPLC methods for the determination of lamotrigine in presence of its impurity. Chemical and Pharmaceutical Bulletin. 2007; 55:541-545.
13. Ashton DS, Ray AD, Valko K. Detection of the principal synthetic route indicative impurity in Lamotrigine. International Journal of Pharmacy. 1999; 189:241-248.
14. Zhang C, Wang H, Chen Y, Zhang D, Cheng L, Zhang C, *et al*. Process for Preparation of Acyl Nitrile Compounds. CN 104387292, 2014.
15. Joyce PJ, Bielski R, Halpern M. Process for making organic products and improving the quality of non-product streams using Phase-Transfer Catalysis. US 20030158435, 2003.
16. Bielski R, Joyce PJ. Conversion of pollutants in dilute aqueous waste streams to useful products: A potential method based on Phase –Transfer catalysis. Org. Process Res. Dev. 2003; 7:551-552.
17. Hoffmann HMR, Haase K, Ismail ZM, Preftitsi S, Weber A. Synthesis of α,β-unsaturated and other reactive acyl cyanides. Chem. Ber. 1982; 115:3880-3885.
18. Koenig KE, Weber WP. Synthesis of benzoyl cyanides by Phase-Transfer catalysis. Tetrahedron Lett. 1974; 15:2275-2278.
19. Li Z, Shi S, Yang J. AgI-PEG400-KI catalyzed environmentally benign synthesis of Aroyl cyanides using potassium hexacyanoferrate (II) as the cyanating agent. Synlett 2006, 2495-2497.
20. Cao YQ, Du YF, Chen BH, Li JT. A Novel heterogenous synthesis of acyl cyanides catalyzed by PEG 400 and zinc iodide. Synth. Commun. 2004; 34(16):2951-2957.
21. Job A, Schlummer B. Process for preparing substituted benzoyl cyanides. US 20060281948, December 14, 2006.
22. Roduit JP, Djojo F. A process for the preparation of Lamotrigine. WO 2008019798, February 21, 2008.
23. Ewa Jaszczak, Marek Ruman, Sylwia Narkowicz, Jacek NamieVnik, Zaneta Polkowska. Development of an analytical protocol for determination of Cyanide in human biological samples based on application of Ion Chromatography with Pulsed Amperometric Detection. Journal of Analytical Methods in Chemistry, 2017, 7. Article ID 7157953,
24. Ballantyne B. The influence of exposure route and species on the acute lethal toxicity and tissue concentrations of cyanide. Developments in Toxicology and Environmental Science, 1989; 11:583-586.
25. Sujin Lee, Yun-Sik Nam, Sung-Hee Choi, Yeonhee Lee, Kang-Bong Lee. Highly sensitive photometric determination of cyanide based on selective etching of gold nanorods. Microchim Acta, 2016; 183:3035-3041.
26. Jian Ma, Purnendu K. Dasgupta. Recent developments in cyanide detection: A review. Anal Chim Acta. 2010; 673(2):117-125. doi:10.1016/j.aca.2010.05.042.
27. Lara R, Cerutti S, Salonia JA, Olsina RA, Martinez LD. Trace element determination of Argentine wines using ETAAS and USN-ICP-OES. Food Chem. Toxicol., 2005; 43(2):293-297
28. Romteera Chueachot and Saksit Chanthai. Spectrophotometric Determination of Trace Cyanide in Fruit Wines by the Catalytic Reaction of Ninhydrin following Micro-distillation. Oriental Journal of Chemistry, 2014; 30(1):119-131, doi:10.13005/ojc/300115.
29. EPA METHOD: 335.2, Total Cyanide, Approved for NPDES (Technical Revision 1980).