Int J Pharma Res Health Sci. 2017; 5 (5): 1851-54
DOI:10.21276/ijprhs.2017.05.06
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CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
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International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Ion Chromatography Method for Determination of Dimethyl Sulphate Content in Lornoxicam Drug Substance with Suppressed Conductivity Detection

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ARTICLE INFO

Received: 03 Sep 2017
Accepted: 10 Oct 2017

ABSTRACT

A simple and sensitive ion chromatography method has been developed for the determination of dimethyl sulphate content in Lornoxicam drug substance. Efficient chromatographic separation was achieved on IonPac AS10, anion column 250 mm long with 4 mm i.d., 8.5 µ m particle diameter. Mobile phase consists of 2.7mM Na_2CO_3 + 0.3mM $NaHCO_3$. The mobile phase was delivered in an isocratic mode at a flow rate of 1.5 mL/min at ambient temperature conditions and the analyte was monitored by conductometric detector. The method was validated for specificity, precision, linearity, solution stability and accuracy. The limits of detection (LOD) and limits of quantification (LOQ) established for dimethyl sulphate are 1.60ppm and 3.85ppm respectively. The average recoveries for dimethyl sulphate are in the range of 103.4 % -105.3 % and the method can be successfully applied for the routine analysis of Lornoxicam Drug substance.

Keywords: Ion chromatography; Lornoxicam, Dimethyl sulphate.

1. INTRODUCTION

Chemically Lornoxicam is 6-chlor-4-hydroxy-2-metyl-N-2-pyridyl-2H-tieno[2,3-e]-1,2-tiazine-3-carboxamide 1,1-dioxide. Lornoxicam is a nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. Lornoxicam differs from other oxicam compounds in its potent inhibition of prostaglandin biosynthesis, a property that explains the particularly pronounced efficacy of the drug. The molecular formula is $C_{13}H_{10}ClN_3O_4S_2$ and the molecular weight is 371. [1]

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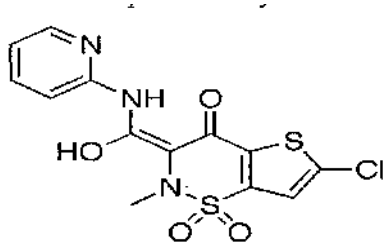

Fig 1: Chemical structure of Lornoxicam

Dimethyl sulphate (DMS) is classified as Category-2 carcinogen. Dimethyl sulphate is reported mutagen (may cause heritable genetic damage) [2]. Dimethyl sulphate is an alkylating reagent commonly used in organic syntheses and pharmaceutical manufacturing processes. In the synthesis process of Lornoxicam, dimethyl sulphate is used as a process reagent. Dimethyl sulphate is anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals [3-6]. Both the liquid and vapour forms of dialkyl sulphates are harmful to the skin, eyes and mucous membranes due to their potential carcinogenicity, the level of Dimethyl sulphate in the API process needs to be monitored and controlled with appropriate methods. In the available literature, many of the analytical procedures have been identified for the determination of Dimethyl sulphate. In 1980, J.C. Gilland and et al., developed and validated the analytical method in air samples using Gas chromatography technique and they reported, the minimum detectable concentrations (LOD) in the atmosphere for dimethyl sulphate, based on a 20 L air sample, are 0.04 ppm [7]. Low level detection of dimethyl sulphate 0.3 ppm was reported by using highphenated technique GC-MS in an aqueous soluble API intermediates in 2009 [8].

Subsequently, an ion chromatography (IC) method was developed and optimized to determine the contents of Dimethyl sulphate in Lornoxicam drug substance with sufficiently low levels of detection. To the best of our knowledge no report has been published on the analysis of dimethyl sulphate in Lornoxicam drug substance in literature.

2. MATERIALS AND METHODS

Chemicals, reagents and samples

The Analytical Standard Dimethyl sulphate was procured from Spectrochem, samples of Lornoxicam drug substance were procured from Apex Healthcare Pvt Ltd. Analytical reagents (AR grade) sodium carbonate and sodium bicarbonate were procured from Merck India. Highly purified water obtained from Millipore purification system was used.

A Dionex ICS-5000 chromatography system consisting of: Dual Pump (DP) Conductivity Detector (CDS) AS Autosampler (AS) Chromeleon® Chromatography Workstation with Chromeleon Chromatography Management Software was used.Mobile phase was 2.7mM $N_{a2}CO_3$ + 0.3mM $NaHCO_3$. The analysis was carried out on IonPac AS10, PN 043118 anion column 250 mm long with 4 mm i.d., 8.5 µ m particle diameter at 40 ˚Ccolumn temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.5 mL/min. The injection volume was 200 µ L and run time was 20 min. Milli Q water was used as diluent. The retention time of dimethyl sulphate peak is at about 10.0 minutes. Relative standard deviation for the peak areas of the six replicate injections for dimethyl sulphate standard peak is not more than 5.0%

Chromatographic Conditions

Columns	:	IonPac AS10 Analytical column, 4 × 250 mm (P/N 043118)
Eluent	:	2.7mM Na2CO3 + 0.3mM NaHCO3.
Flow Rate	:	1.5 mL/min
Column Temperature	:	40 ˚C
Injection	:	200 µL
Detection	:	Suppressed conductivity, ASRS ULTRA II (4 mm), recycle mode
Power Setting	:	22 [mA]
Run Time	:	20 min

Standard and sample solutions

Preparation of standard solutionAccurately weigh and transfer 100 mg of dimethyl sulphate into a 100 mL volumetric flask, add 70 mL of diluent mix well by shaking, and make up to volume with diluent. Diluted 2.5 mL this solution to 100 mL with diluent and further diluted 1.0 mL of this solution to 25 mL with diluent. Filter through the 0.2 µ porous membrane followed by Metrohm C18 PRP Cartridge. Before using the IC-RP cartridges, they have to be activated with 10 mL methanol and rinsed with 10 mL deionized water.

Sample solutionAccurately weigh and transfer 333 mg of sample into a 25 mL volumetric flask, add 10 mL of diluent and sonicate to dissolve, and make up to the volume with diluent. Filter through the 0.2 µ porous membrane followed by Metrohm C18 PRP Cartridge. Before using the IC-RP cartridges, they have to be activated with 10 mL methanol and rinsed with 10 mL deionized water.

3. RESULTS AND DISCUSSION

Method development and optimization

Dimethyl sulphate (DMS) is a process impurity during the synthesis of Lornoxicam drug substance. As there is no chromophore present in dimethyl sulphate, there was no possibility for UV or fluorescence detection. Method development for quantification of dimethyl sulphate content in Lornoxicam drug substance was initiated with Lornoxicam and dimethyl sulphate miscibility and Lornoxicam drug solubility studies, based on that water was chosen as diluent. Preliminary experiments were carried out

based on the retention of sulphate, which were discussed in many Metrohm and Dionex ion chromatography applications, using Metrosep A Supp 5, and Dionex Ion pac 10 columns. Elution of analytes were investigated using sodium carbonate, sodium bicarbonate mobile phase. Good reproducibility was achieved on, IonPac AS10, PN 043118 anion column 250 mm long with 4 mm i.d., 8.5 µ m particle diameter column, with Mobile phase composition of 2.7mM Na_2CO_3 + 0.3mM $NaHCO_3$ at a flow rate of 1.5 mL/min at 40 ˚C column temperature.

Method validation: In order to determine the content of dimethyl sulphate in Lornoxicam drug substance, the method was validated as per the ICH guidelines [9], individually in terms of specificity, limit of detection, limit of quantification, linearity, accuracy, precision and stability of sample solution.

Specificity: Specificity is the ability of the method to measure the analyte responses in the presence of all impurities. For specificity determination, the interference of diluent in the determination of dimethyl sulphate was studied. Prepared Blank, Standard solution, Dimethyl sulphate Identification solution, Test solution and Specificity mixture solution and injected separately into ion chromatograph and the chromatograms were recorded. It was observed that Blank & other Impurity peaks do not interfere with Dimethyl sulphate.

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ), of dimethyl sulphate were determined based on the residual standard deviation of a regression line and slope was plotted. Standard solution was injected into ion chromatograph from 0.05 ppm – 0.25 ppm. A plot of peak area (µS*min) versus concentration (ppm) was drawn and LOD/LOQ values were predicted by residual standard on deviation response (SD) and slope (S) method using the formula 3.3 x SD/S for LOD and 10 x SD/S for LOQ. The solutions of dimethyl sulphate for LOD and LOQ evaluation were prepared at predicted concentration levels and precised by analyzing six times. Chromatogram of Blank, LOQ and Standard solution are shown in fig. 1. The achieved precised values were shown in Table 1.

Table 1: Evaluation of LOD and LOQ, Linearity data for Dimethyl sulphate

Components	Dimethyl sulphate
Limit ppm	75 ppm
Limit of Detection ppm	1.60 ppm
Limit of Quantitation ppm	3.85 ppm
LOD (%RSD) a	10.50 %
LOQ (%RSD) a	6.50 %
Correlation Co-efficient	1.0000

a Average of n=6 determinations

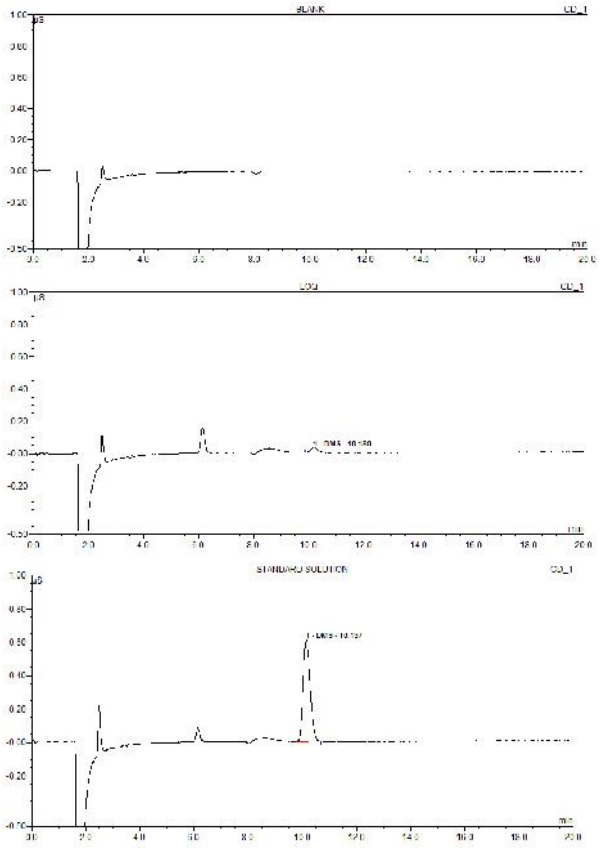

Fig 2: Chromatograms of (a) Blank, (b) LOQ, (c) Standard

Linearity: The linearity of the method was determined by taking the same data obtained in LOD and LOQ. The data was subjected to statistical analysis using a linear-regression model. The statistical parameters, residual standard on deviation response and correlation coefficient values are calculated and shown in Table 1.

Accuracy: Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries were determined by spiking dimethyl sulphate at four different levels ranging from LOQ to 150% (w.r.t 75 ppm limit level) into Lornoxicam drug substance. These samples were prepared as per the procedure, analyzed in triplicate and the percentage recoveries were calculated. The average recovery values were 103.4 % -105.3 %for dimethyl sulphate. The completely validated accuracy results are shown in Table 2.

Table 2: Accuracy data of Dimethyl sulphate

Component	Dimethyl sulphate			
Target Level (%)	LOQ	50	100	150
Spike Conc.(ppm) w.r.t Sample b	3.85	37.5	75.0	112.5
Percent Recovery b	104.8	103.4	103.8	105.3
Mean % Recovery	103.4 % -105.3 %			
b Average of n = 3 determinations				

Precision: The precision was the study of the method using repeatability and reproducibility (ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard

solution was analyzed six times for checking the performance of the ion chromatography system under the chromatographic conditions on the day tested (System precision). The relative standard deviation for peak area of dimethyl sulphate is 0.48 %. Repeatability was the intra-day variation (Method precision) and the relative standard deviation for the content of dimethyl sulphate is 0.98 %. The intermediate precision was the inter-day variation (Ruggedness) and the relative standard deviation for the content of dimethyl sulphate is0.94 %. The repeatability and reproducibility of the method was studied by analyzing six sample solutions separately by adding dimethyl sulphate at known concentration levels. The ruggedness of the method was defined as the degree of reproducibility obtained by the analysis of the same sample (which is used in the Method precision) under a variety of conditions using different series of column, with different analyst on different day by preparing new standards and new mobile phase. The experiment results of the precision (System precision, Method precision) are shown in Table 3.

Solution stability: Test samples were kept for 24 Hrs. at room temperature. At the end of 24 Hrs. this sample solution was analyzed against freshly prepared standard solution. From the data, change in Dimethyl sulphate content against initial value was calculated. The results indicate that the sample solution was stable for 24 hours at ambient temperature.

Table 3: Statistical data of Precision for Dimethyl sulphate

Repeatability (System precision) Area (µS*min)	
	Dimethylsulphate
1	0.1849
2	0.1853
3	0.1865
4	0.1862
5	0.1867
6	0.1874
Average	0.1862
SD	0.0009
%RSD	0.48
*Reproducibility(Methodprecision)*Content (ppm)	
1	82.7
2	81.8
3	82.1
4	83.2
5	83.8
6	83.6
Average	82.9
SD	0.8091
%RSD	0.98

Potential application of the method: The potential application of the method has been conducted for drug substances of the oxicam class for the determination of dimethyl sulphate content.

4. CONCLUSION

A simple and sensitive Ion chromatography method was developed and validated for the determination of dimethyl sulphate in Lornoxicam drug substance. The results of various validation parameters demonstrated that the method is specific, stability indicating, sensitive, linear, precise and accurate. The proposed method is sensitive, simple and user friendly, for the determination of dimethyl sulphate content in Lornoxicam drug substance.

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Conflict of Interest: None

Source of Funding: Nil