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Research Article

A SENSITIVE ANALYTICAL LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE ESTIMATION OF FINGOLIMOD HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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Abstract

Considering, the use of Fingolimod hydrochloride for the treatment of multiple sclerosis and its prophylaxis. The objective of the current study is to establish a simple, precise and rapid liquid chromatography tandem mass spectrometer method using electrospray ionization for the quantitative determination of the immunosuppressive drug Fingolimod hydrochloride in its formulation. In this study, a Zorbax C_{18} reversed (50 mm x 4.6 mm x 5 µm) column was used for the separation and detection using the 0.1% formic acid: acetonitrile (80: 20, v/v) as the mobile phase with a flow rate of 0.6 ml/min was used. The retention time of Fingolimod hydrochloride was 0.68 min with the run time of 1.5 min. The correlation coefficient of Fingolimod hydrochloride was found to be 0.9995 over the concentration ranging from 1.5 to 40 ng/ml. The method was validated as per the international conference on harmonisation guidelines. The detection and quantification limit was found to be 0.4 and 0.8 ng/ml, respectively. The recovery of the method was found to be 92.91 % to 98.31 % at different concentration levels of Fingolimod hydrochloride. Further, for the quantification of Fingolimod hydrochloride in bulk and marketed formulation the developed method was found to be simple, accurate and precise.

Keywords: Fingolimod hydrochloride, Formulation, LC-MS/MS, Validation

Introduction

Fingolimod hydrochloride, chemically known as 2-amino-2-(2-(4-octylphenyl) ethyl)-1, 3-propanediol hydrochloride is an immunosuppressant drug having a molecular formula $C_{19}H_{34}ClNO_2$ and has a molecular

weight of 343.93 g/mol [1]. It is approved for the treatment of multiple sclerosis and prophylactics. Fingolimod-phosphate, which is a sphingosine 1-phosphate receptor modulator, is an active metabolite of Fingolimod hydrochloride that is metabolized by the enzyme sphingosine kinase.

As per the literature review few analytical methods are reported for the determination of Fingolimod hydrochloride by RP-HPLC method[2-4] and to best of our knowledge a liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been reported[5]. From the literature survey, it was also observed that the existing methods for the estimation of Fingolimod hydrochloride are less sensitive and requires long run time to determine Fingolimod hydrochloride. Hence, our aim of the present study was to develop a highly sensitive, specific, precise, and rapid LC-MS/MS method for quantitative determination of Fingolimod hydrochloride in its formulation.

All the chemicals and reagents used in the present study were procured from Sigma Aldrich. The milli- Q water was from milli- Q- purification system. The gift sample of Fingolimod hydrochloride was from Indian pharmacopoeia commission (IPC), New Delhi. An LC-MS/MS system was equipped with an electrospray ionization (ESI) interface and triple quadrupole mass analyser. The LC parameters are CBM - 20 alite controller, LC - 20AD pump, and SIL - 20AC auto sampler. The system was controlled by lab solution data station. The separation was achieved by using stationary phase Zorbax C_{18} column reversed (50mm × 4.6 mm × 5µm) using the mobile phase consist of acetonitrile and 0.1% formic acid at the ratio of 80:20, v/v at a flow rate of 0.6 ml/min and injection volume of 10 µl.

The stock solution of standard 10 mg of Fingolimod hydrochloride was prepared by dissolving 10 ml of organic solvent (ethanol) to obtain 10 mg/ml. From the 10mg/ml solution, 1 µg/ml working concentrations was prepared by further dilutions of stock. The standard calibration ranges from 1.5 – 40 ng/ml was also prepared. The solution were stored at 2-8˚C until analysis. The sample solution was prepared by accurately weighing ten capsules with the amount equivalent to 10 mg of Fingolimod hydrochloride and transferred to 10 ml volumetric flask. About 5 ml of ethanol was added and the solution was sonicated for about 30 min and then the volume was made up to 10 ml using ethanol. Further, the solution was thoroughly shaken and filtered through a syringe filter and the quality control (QC) samples were prepared.

The mass range was selected by injecting 1000 ng/ml of Fingolimod hydrochloride solution. The multiple reaction monitoring (MRM) transitions were obtained at 308.10 → 255.15 (CE −20.0), 308.10 → 57.10

(CE: -31.0) and 308.10→18.0 (CE: -18.0) and these transitions were used for the determination Fingolimod hydrochloride (Figure 1).

Further, in order to determine the suitability of the method, the method was validated as per the ICH guidelines for specificity, precision, linearity, accuracy, detection limit (LOD), quantification limit (LOQ), robustness and system suitability[6]. The specificity of the method was determined by analysing the QC samples for any interference in retention of Fingolimod hydrochloride. The precision (intra- and inter-day) studies were determined at three QC concentrations (2.4, 15 and 35 ng/ml) of low quality control (LQC), middle quality control (MQC) and high quality control (HQC). The results were represented as percentage relative standard deviation (% RSD). The accuracy of the method was represented in terms of recovery. The linearity of the method was determined at six concentration levels ranging between 1.5 to 40 ng/ml. The linearity was evaluated by calculating the coefficient correlation, slope and the intercept values. The sensitivity of the method was determined based on the signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. Further, the method robustness and system suitability of the method was studied.

The results obtained from the validation parameters are as follows: From the Figure 2a. It is observed that no peaks were found to elute alongside the retention time of Fingolimod hydrochloride indicating that the additives utilized are not meddling with the fundamental compound peak. Consequently, the results demonstrated that the method was specific to Fingolimod hydrochloride formulation. Calibration curve was straight, with mean regression equation ($y= 12063x + 297.18$ $R^2 = 0.9995$) where y signifies the analyte peak area for Fingolimod hydrochloride and x signifies analyte concentration in ng/ml. The accuracy results were found to be 95.83 to 98.57 % (Table 1). Application of the method was intend to study the estimate the Fingolimod hydrochloride in its formulation and the results are described in Table 2 and Figure 2b. The precision results were found to be with the limits with % RSD over the range of 2.17 to 1.73 %. Based on a signal-to-noise ratio of 3:1, the lowest limit detected for Fingolimod hydrochloride formulation was found to be 0.4 ng/ml and quantification limit of 0.8 ng/ml. Robustness of the developed method for Fingolimod hydrochloride was established by changing the conditions of chromatography and confirming that the results are within the limits. The parameters that were studied in the robustness studies include mobile phase concentration (formic acid) percentage (70, 80, and 90 %, v/v) and the flow rate of mobile phase (0.3, 0.6 and 0.9 ml). The % RSD was found to be in the acceptable limits of 2.0 %.The system suitability parameters

studied are summarized in Table 3 the results obtained signifies the developed method is suitable for its intended purpose.

In conclusion, the developed method for the estimation of Fingolimod hydrochloride, using liquid chromatography tandem mass spectrometry is simple, novel, accurate and precise. Therefore, it can be successfully utilised for the estimation of marketed formulations and APIs of Fingolimod hydrochloride. All the results obtained were observed to be within the acceptable limits as per the validation protocol.

Table-1: Accuracy and Precision Results of Fingolimod.

Sample (ng/ml)	Amount found (ng/ml) ± SD	Intra-day		Inter-day	
		Accuracy (% N)	Precision (% RSD)	Accuracy (% N)	Precision (% RSD)
2.4	2.3 ± 0.05	95.83	2.17	90.85	3.01
15	14.6 ± 0.21	97.33	1.43	95.77	2.14
35	34.5 ± 0.60	98.57	1.73	96.65	1.85

SD: Standard deviation; RSD: Relative standard deviation.

Table-2: Recovery Results for fingolimod in the Formulation.

Formulation	Label claim	Amount taken for assay (ng/ml)	Amount found ± SD*	% Recovery
Fingolimod	0.5 mg	2.4	2.23 ± 0.05	92.91
		15	14.50 ± 0.15	96.67
		35	34.41 ± 0.20	98.31

* The data represents mean ± standard deviation (SD)

Table-3: System Suitability Parameter.

S.No	Parameters	Fingolimod hydrochloride
1	Limit of Detection	0.4 ng/ml
2	Limit of Quantitation	0.8 ng/ml
3	Theoretical Plates	3293
4	Tailing Factors	1.1
5	Linearity Range	1.5 – 40 ng/ml
6	Correlation Coefficient (r^2)	0.9995

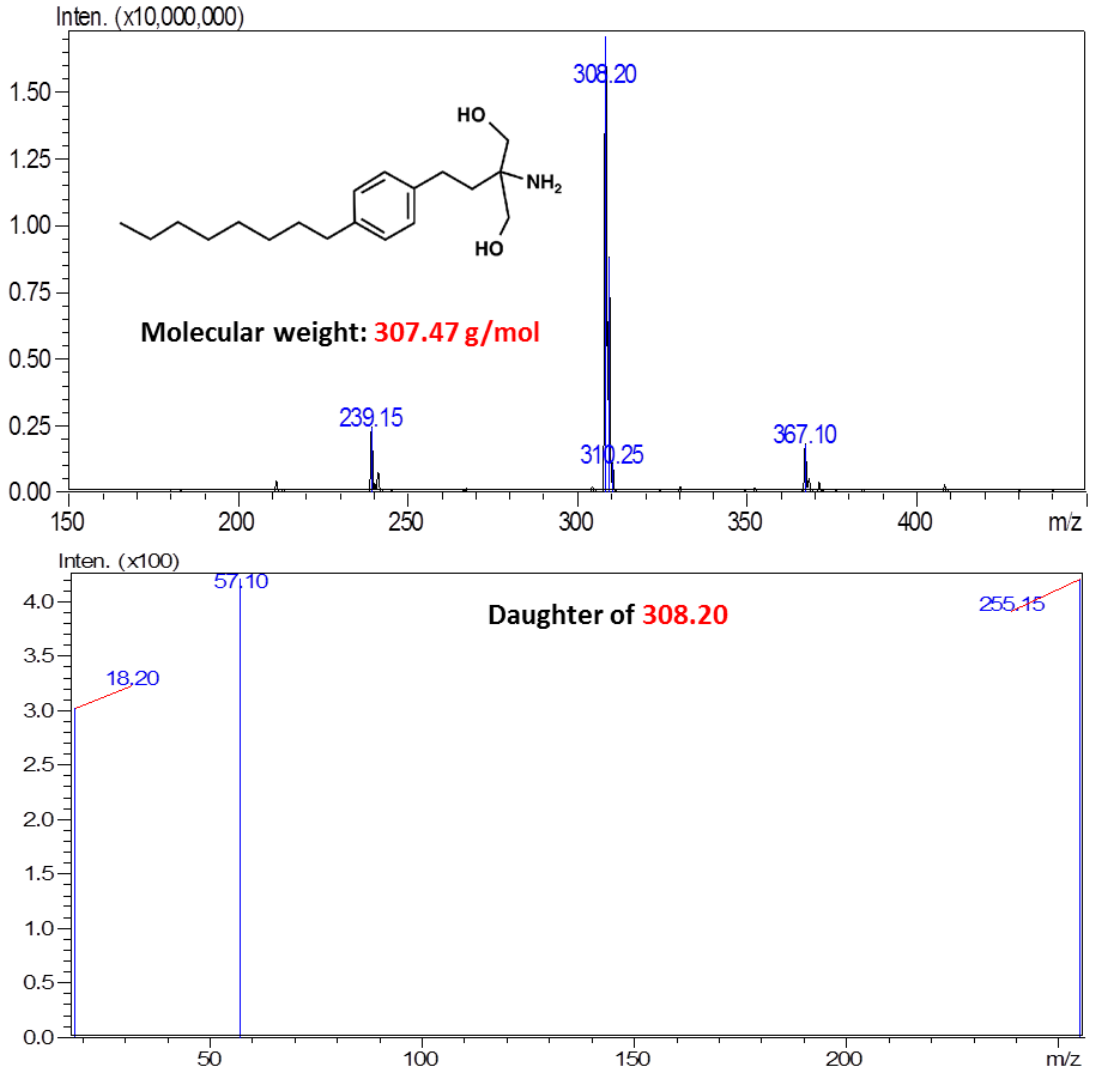

Figure 1: Mass Scan and MRM spectra of Fingolimod.

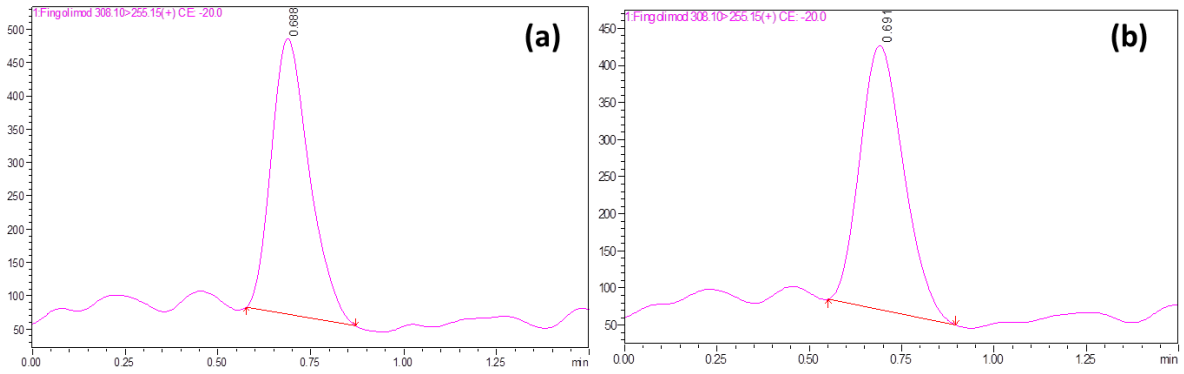

Figure 2: (a) standard chromatogram and (b) sample chromatogram of Fingolimod.

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Conflict of interest

The authors declare that there is no conflict of interest.

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