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The Estimation of Fidaxomicin in tablet dosage forms by RP-HPLC

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Abstract

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Fidaxomicin in tablet dosage form. An Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.3M Potassium Dihydrogenphosphate in water and adjusted to pH 3.0 by *ortho*-phosphoric acid and acetonitrile in the gradient program was used. The flow rate was 1.0 mL/min and the effluents were monitored at 222 nm. The retention time was 13.1 min. The detector response was linear in the concentration of 5-50 mcg/mL. The respective linear regression equation being y=2228.4x-2227.3. The limit of detection and limit of quantification was 0.01mcg/mL and 0.03mcg/mL respectively. The percentage assay of Fidaxomicin was 99.3 %. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Fidaxomicin in bulk drug and in its pharmaceutical tablet dosage form.

Keywords: Fidaxomicin, RP-HPLC and Tablets.

Contents

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1. Introduction

Fidaxomicin is a macrolide antibacterial drug for oral administration. It is used to reduce the development of drug-resistant bacteria and maintain the effectiveness of fidaxomicin and other antibacterial drugs[1,2]. Fidaxomicin should be used only to treat infections that are proven or strongly suspected to be caused by Clostridium difficile. Fidaxomicin is the first macrolide antibacterial drug indicated for *Clostridium difficile*-associated diarrhea (CDAD)

to be approved in over 25 years in the U.S. It is indicated in the U.S. for the treatment of CDAD in adults 18 years of age or older[3,4]. Fidaxomicin is administered in 200 milligram tablets given orally, twice daily. Chemically, fidaxomicin is 3-(((6- Deoxy-4-O- (3,5-dichloro-2-ethyl-4,6- dihydroxy benzoyl)-2-O-methyl-β-D-manno pyranosyl)oxy)-methyl)-12(R)-[(6-deoxy-5-C-methyl-4-O-(2-methyl-1-oxopropyl)-β-D-lyxo-hexopyranosyl) oxy]-11(S)- ethyl-8 (S)- hydroxy- 18(S)- (1(R)-hydroxy ethyl)-9,13,15-trimethyl oxacyclooctadeca-3,5,9,13,15-pentaene-2-one. The empirical formula is $C_{52}H_{74}Cl_2O_8$, with a molecular weight of 1058.04 g/mol. The solubility of Fidaxomicin, as the macrolide, Freely soluble in tetrahydrofuran, dimethyl sulfoxide and methanol and soluble in acetone and sparingly soluble in ethyl acetate, ethanol (200 proof), dichloromethane and acetonitrile. It is slightly soluble in isopropanol and practically insoluble in water. Its pKa is 9.31 at room temperature and partition coefficient (Log P) is 3.7 (n-octanol-water system). Literature survey reveals a no chromatographic methods have appeared in the literature for the estimation of Fidaxomicin from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Fidaxomicin in pharmaceutical formulations. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines.

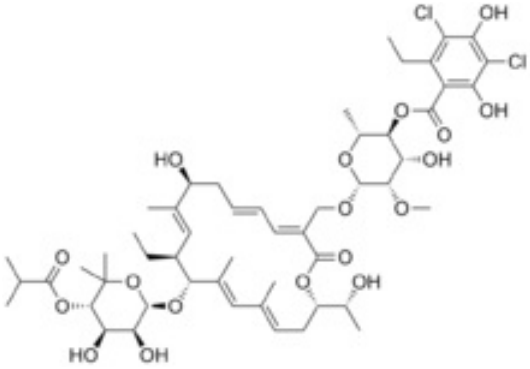

Figure 1. Structure of Fidaxomicin

2. Experimental

Materials and Methods:

Fidaxomicin was obtained as a gift sample from M/s. Vishnu Chemicals Ltd, Hyderabad. Acetonitrile, Potassium Dihyrogenphosphate and ortho-phosphoric acid and water used were of HPLC grade (Qualigens). Commercially available Fidaxomicin Tablets 200mg (Dificid® 200 mg, Cubist Pharmaceuticals Inc, USA) were procured from local market.

Instrument:

Quantitative HPLC was performed on liquid chromatography, Waters Alliance system with equipped with Diode Array Detector and automatic injector with injection volume 20 µl. The HPLC data was analyzed with Empower-2 Software.

HPLC Conditions:

The contents of the mobile phase were mixture of buffer 0.3M Potassium Dihydrogenphosphate in water and adjusted to pH 3.0 by ortho-phosphoric acid and acetonitrile in the gradient program was used (shown in table-IV). They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 25.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 25 min with the mobile phase flowing through the system. The eluents were monitored at 222 nm.

Preparation of Standard Stock solution:

A standard stock solution of the drug was prepared by dissolving 50 mg of Fidaxomicin in 100 mL volumetric flask and dissolved in diluent (Acetonitrile and Water:50:50), sonicated for about 15 min and then made up to 100 mL with diluent get 500 mcg/mL standard stock solution.

Working Standard solution:

1.0 mL of the above stock solution was taken with micropipette in 10 mL volumetric flask and thereafter made up to 10 mL with diluent (Acetonitrile and Water: 50:50) to get a concentration of 50mcg/mL.

Preparation of Sample solution:

Twenty tablets (Dificid® 200 mg, Cubist Pharmaceuticals Inc, USA) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 50mg of the active ingredient, was mixed with 30 mL of diluent in 100 mL volumetric flask. The mixture was allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding diluent up to 100 mL

to obtain a stock solution of 500mcg/mL. 1 mL of the above solution was taken and further diluted with diluent up to 10 mL to get working sample solution of 50 mcg/mL.

Linearity:

Aliquots of standard Fidaxomicin stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of Fidaxomicin are in the range of 5-50 µg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with Diode Array detector at 222 nm and a Calibration graph was obtained by plotting peak area versus concentration of Fidaxomicin (Fig 3). The plot of peak area of each sample against respective concentration of Fidaxomicin was found to be linear in the range of 5–50 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being y=2228.4x-2227.3. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in table I.

Assay:

20 µL of sample solution was injected into the injector of liquid chromatography. The retention time was found to be 13.1 minutes. The amount of drug present per parentaral was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in table II.

Recovery Studies:

Accuracy was determined by recovery studies of Fidaxomicin, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in table II. The study was done at three different concentration levels.

Table 1. Linear Regression Data for Calibration curves

Drug	Fidaxomicin
Concentration range (mcg/mL)	5-50
Slope (m)	2228.4
Intercept (b)	-2227.3
Correlation coefficient	0.9999
% RSD	0.24

Table 2. Results of HPLC Assay and Recovery studies

Sample	Amount claim (mg/Tablet)	% found by the proposed method	% Recovery*
1.	200	99.2	99.1
2.	200	99.2	99.3
3.	200	99.4	98.9

*Average of three different concentration levels

Table 3. Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	16753
Tailing factor	1.22
Retention time in minutes	13.1
% Area	99.71
LOD (mcg/mL)	0.01
LOQ (mcg/mL)	0.03

Table 4. Gradient Program in HPLC method

Time in minutes	Buffer	Acetonitrile
0.01	70	30
4	70	30
8	20	80
18	20	80
20	70	30
25	70	30

3. Results and Discussion

The system suitability tests were carried out on freshly prepared standard stock solution of Fidaxomicin. The parameters studied to evaluate the suitability of the system are given in table III.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Fidaxomicin were found to be 0.01 mcg/mL and 0.03 mcg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of Fidaxomicin as shown in fig 2, it was found that the retention time was 13.1 min. A mixture of buffer 0.3M Potassium Dihydrogenphosphate in water and adjusted to pH 3.0 by ortho-phosphoric acid and acetonitrile in the gradient program was used (shown in table-IV) was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r2=0.9999) was observed between the concentration range of 5-50 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Fidaxomicin tablets was found to be 99.3 %. From the recovery studies it was found that about 99.1% of Fidaxomicin was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of parental dosage forms of Fidaxomicin within a short analysis time.

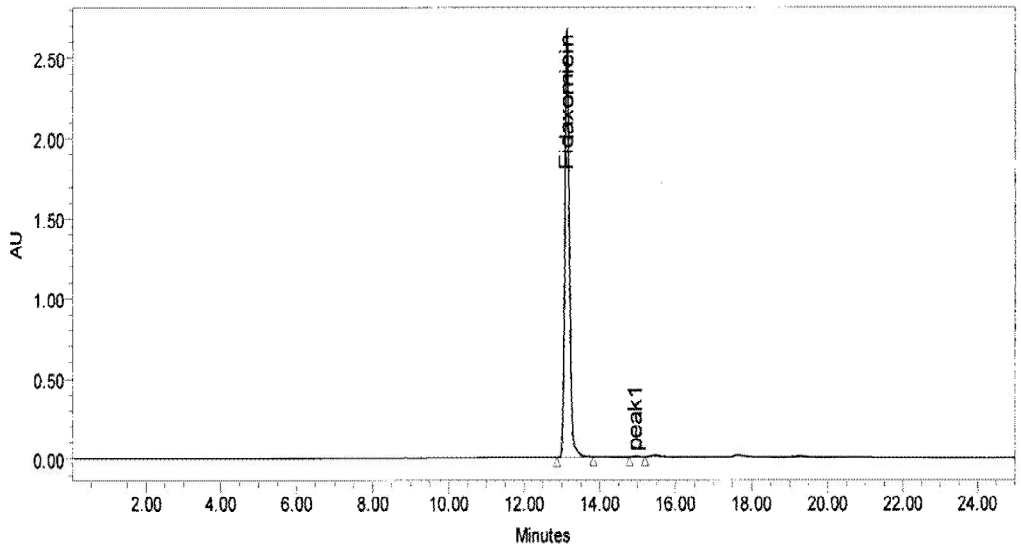

Figure 2. Typical Chromatogram of Fidaxomicin by HPLC

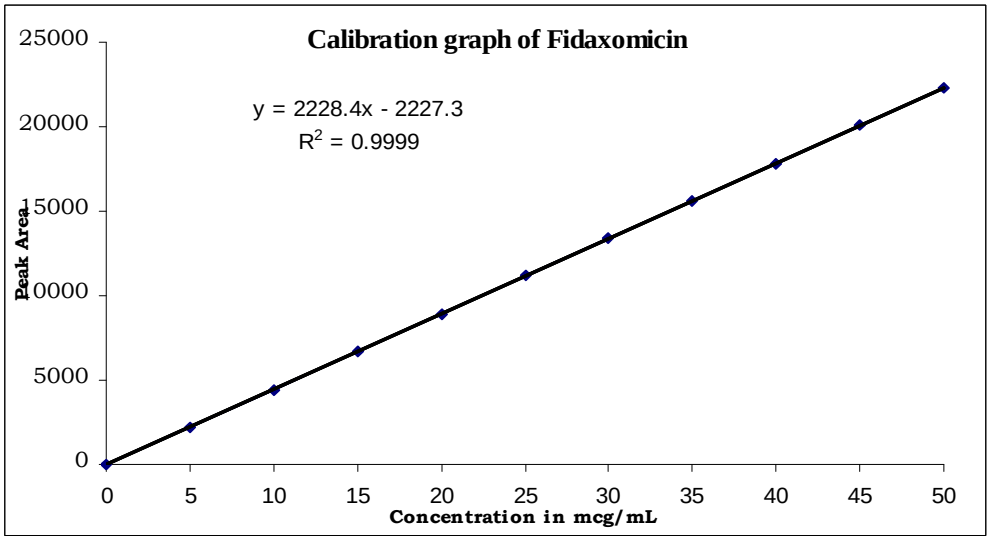

Figure 3. Calibration curve of the Fidaxomicin by RP-HPLC

4. Acknowledgement

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