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RP-HPLC Method Development and Validation for the Estimation of Fidaxomicin from Bulk and Tablet Dosage Form

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Abstract:
Background: Fidaxomicin is a vital antibiotic for pharmaceutical formulations and its precise estimation in bulk and dosage forms is crucial for quality control and regulatory purposes. A validated analytical method is used to generate trustworthy data for fidaxomicin determination.
Objective: The purpose of this study was to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of fidaxomicin in bulk and pharmaceutical dosage form with respect to accuracy, precision, and robustness in based on ICH guidelines.
Method: The method was undertaken on a Symmetry C18 Inertsil ODS-3V column (4.6x250mm, 5µm). The mobile phase consisted of 0.1% ortho-phosphoric acid (OPA) and acetonitrile (05:95), with a flow rate of 1.0 mL/min. Detection was performed with a UV detector at 228 nm. The proposed method was validated as per ICH guidelines.
Result: The validation parameters are met by Specificity, linearity, precision, accuracy, robustness, and system suitability. The retention time for fidaxomicin was 6.5 minutes, and the technique showed linearity within a concentration range of 10 to 150 µg/mL. This method's limits of detection (LOD) and quantification (LOQ) were set at 6.67 µg/mL and 20.22 µg/mL, respectively.
Conclusion: Fidaxomicin determined in bulk and pharmaceutical dosage forms using the established RP-HPLC technique, which is robust, specific, linear, precise, and accurate. This validated method can be reliably used for routine quality control analysis and regulatory compliance in pharmaceutical industries.

Keywords: Fidaxomicin, RP-HPLC, Analytical Method Development, Validation, ICH Guidelines

1. Introduction:

The Food and Drug Administration (FDA) gave fidaxomicin its first approval in May 2011. It was particularly approved for use in treating adult patients aged 18 and up who have diarrhea linked to *Clostridium difficile*. The European Medicines Agency subsequently approved the same indication in December 2011. Health Canada's approval of fidaxomicin for clinical usage in June 2012 added even more validation. In January 2020, the FDA made a major advancement by adding paediatric patients 6 months of age and older to the approved treatment population by expanding the approved indication for fidaxomicin. This extension shows a growing comprehension of the drug's suitability for use in a variety of age groups. [1-3]

Fidaxomicin (Dificid) (Fig.1) is the first member of a class of narrow spectrum macrocyclic antibiotic drugs known as tiacumicins. [4] The agent demonstrates limited efficacy specifically targeting Gram-positive anaerobes, while exhibiting bactericidal properties towards *Clostridium difficile.* [5] Structurally, it features an 18-membered lactone ring derived from fermentation and contains two carbohydrate units. [6] Fidaxomicin was the first antibiotic reported to exhibit activity against Gram positive anaerobes. The antibacterial effects are exerted through the inhibition of bacterial RNA polymerase at the stage of transcription initiation. [7]

Limited literature is available on method validation for quantification in bulk and tablets for Fidaxomicin. Therefore, there was a need to establish a new and reliable method using the HPLC technique. Validation of the developed methodology was conducted in compliance with the guidelines established by the International Council for Harmonization Q2. [8]

2. Materials and Methods

2.1 Chemicals

The Fidaxomicin was gifted from Aizant Drug Research Solutions, Hyderabad. Distilled water, methanol and acetonitrile (ACN) of HPLC grade were used in the study. Dificid tablets, containing 200mg of fidaxomicin, were purchased from a local pharmacy & manufactured by Merck & Co., Inc.

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2.2 Chromatographic Conditions
A Water Alliance e2695 system with a UV detector was used to conduct the HPLC analysis. Empower software was used to collect the data. An isocratic elution including utilized 0.1% OPA and acetonitrile (05:95) with an Inertsil ODS-3V C18 column (4.6x250mm, 5µm) at a temperature of 25°C and a flow rate of 1.0 mL/min. Analytes were measured at a wavelength of 228 nm, and the injection volume was set at 20 µL.

2.3 Preparation of Solutions
2.3.1 Preparation of 0.1% OPA
Accurately measure 1 mL of orthophosphoric acid (OPA) and dissolve it in 1000 mL of distilled water. Mix thoroughly and sonicate for 10 minutes to ensure complete dissolution.

2.3.2 Preparation of Mobile Phase
Combine 0.1% OPA and ACN in a 05:95% v/v ratio. Filter the mixture through a 0.45 µm membrane filter and sonicate to degas.

2.3.3 Standard solution preparation
Accurately weigh 50mg of Fidaxomicin and dissolve it in 50 mL of HPLC grade methanol to prepare a stock solution of 1000 µg/mL. From this stock solution, transfer 5 mL to a 50 mL volumetric flask and dilute with methanol to obtain a concentration 100 µg/mL. From this secondary stock, aliquots of 1 to 15 mL were taken and diluted to 10 mL with methanol to achieve final concentrations of 10, 20, 30, 40, 50, 60, 80, 100, 150 µg/mL, respectively. All standards solutions were injected into the column in triplicate.

3. Method Validation
3.1 System suitability study
The injection of six replicates of the Fidaxomicin solution (100 µg/mL) was tested in the HPLC system. An injection volume of 20 µL was chosen. The area, theoretical plates, retention time and tailing factor were observed and % relative standard deviation (RSD) was calculated.

3.2 Specificity
In this assessment, a placebo, standard, and sample solution were injected, and excipient and analyte interference were examined.

3.3 Linearity
It was performed using least square regression analysis of the calibration curve. Each solution (stock) of Fidaxomicin has aliquots (1, 2, 3, 4, 5, and 6, 8, 10, 15 mL) in respective volumes and is filled in 10 mL volumetric flasks separately. The level was made up with methanol. Concentration final range: 10 to 150 µg/mL. The calibration curve was obtained from the annotation of concentration (x-axis) and mean peak area (y-axis), and thus R^2 and $y = mx + c$ were calculated.

3.4 Precision
Repeatability, intraday and interday precision were assessed. Repeatability was tested using the solution of Fidaxomicin (60 µg/mL). The analyzed solution was performed six times and % RSD was calculated. Intermediate precision was carried out on different times within each day (intraday) and on three different days (interday). The % RSD was determined for every analysis.

3.5 Accuracy
The placebo recovery method was used for accuracy. The API was spiked at 80, 100 and 120% of the label claims in placebo. Fidaxomicin standard was spiked into placebo. There was made suitable dilutions for that so that final concentration should remain within linearity. The recovery test was conducted in triplicate.

3.6 Limit of detection and limit of quantitation (LOD & LOQ)
They were calculated according to ICH recommendations for Fidaxomicin, replacing the Y-intercept (standard deviation) and mean slope in the equation.

3.7 Robustness
The measured parameter was achieved by varying the flow rate (± 0.1 mL/min), wavelength (± 2nm) and temperature (± 5°C). Fidaxomicin (100 µg/mL) was used in three tests. The outcomes were computed.

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4. Result and Discussion

4.1 Method Development

The chromatographic parameters such as preparation of eluent, column, estimation wavelength, flow rate and column temperature were optimized in the technique development to improve the efficiency of the chromatographic system. Literature review and physicochemical properties of the drugs also guided the development trials. Different columns like YMC ODS C18 (4.6×250mm, 3.5µm), Sunfire C18 (4.6×250mm, 5µm), Inertsil ODS-3V C18 (4.6x250mm, 5µm) were tried. Column Inertsil ODS-3V C18 (4.6x250mm, 5µm) during adaptation methods, retention time, tailing factor, theoretical plates and peak type after selection of the column which gave the best results among others. Different ratio of solvents like water, acetonitrile, methanol and OPA were screened for the considerable retention time. Catch the simulation of the eluents at 228 nm for quantification with a UV-Detector (Fig. 2). Following multiple attempts, an optimized chromatographic method was established using a mobile phase composed of 0.1% OPA in water and acetonitrile in the ratio of 05:95% v/v ratio. The method was performed at a flow rate of 1.0 mL/min and a temperature of 25°C. Fidaxomicin showed a sharp peak in mobile phase 0.1% OPA: Acetonitrile (05:95) with retention time 6.5 minutes, as shown in Fig. 3.

4.2 Method Validation

4.2.1 System suitability

The reliability of the chromatographic system, an essential aspect of the analytical process, was assessed through system suitability and repeatability parameters (Table 1). All predefined criteria, including theoretical plates (> 2000), tailing factor (< 2), were successfully met, with results falling within acceptable limits.

4.2.2 Specificity

No additional peaks were detected at the retention times of the target drugs. This confirms that the developed method is highly specific for the simultaneous quantification of both drugs in a laboratory-prepared mixture.

4.2.3 Linearity

The method exhibited excellent linearity across the concentration range of 10 to 150 µg/mL with a correlation coefficient (R^2) of 0.9991 for Fidaxomicin. The results are summarized in Table 2.

4.2.4 Precision

Six replicates of standard solution were prepared for system precision and method precision and %RSD was calculated as shown in Table 3 & 4.

4.2.5 Accuracy

The evaluation of the recovery performance associated with the employed methodology serves as a reflection of its correctness. Specifically, three distinct levels of known drug concentrations – 80%, 100%, and 120% were utilized to spike a placebo, thereby confirming the accuracy of the existing methodology. The data pertaining to accuracy are documented in Table 5 and Fig. 4-6.

4.2.6 LOD and LOQ

The determined values for the limit of detection (LOD) and limit of quantification (LOQ) were established at 6.67 and 20.22, respectively. These metrics are critical for understanding the sensitivity and reliability of the analytical method employed.

4.2.7 Robustness

An evaluation of the robustness of the chromatographic method was conducted through the alteration of several conditions, specifically flow rate (± 0.1 mL/min), wavelength (± 2nm) and temperature (± 5°C). The resulting percent relative standard deviation (% RSD) values were calculated, revealing that the % RSD for peak area remained below 2%. This outcome indicates a high degree of robustness in the proposed method, as documented in Table 6.

Conclusion

This research articulates a linear and accurate RP-HPLC method for the simultaneous measurement of fidaxomicin in a mixture generated in the lab is described in this study. Favorable regression statistics, low percentage relative standard deviations (% RSD), and minimal standard deviations all support the method's high degree of reliability. These statistical evaluations support the method's capacity to accurately and consistently estimate fidaxomicin in synthetic combinations, expanding its use in pharmaceutical analysis. To achieve the best chromatographic results, important parameters such as flow rate, wavelength detection, column temperature, and mobile phase composition were carefully analyzed and modified. As a result, the method's reliability and conformity to validation requirements make it a trustworthy tool for regular quality control and other uses in the field of pharmaceutical analysis. The approach was

systematically optimized and validated in compliance with the ICH recommendations. This made guaranteed that under a range of experimental circumstances, its performance would always be dependable and constant.

Acknowledgments:

We would like to express our gratitude to the individuals and organizations that helped us finish this research. Special thanks are owed to mentors and coworkers, whose unwavering support, helpful criticism, and important advice have greatly impacted how this study was conducted. Also acknowledged are family members and friends, whose constant support and comprehension during the course of this effort have been necessary.

References:

1. Crawford, T., Huesgen, E., & Danziger, L. (2012). Fidaxomicin: A novel macrocyclic antibiotic for the treatment of Clostridium difficile infection. American Journal of Health-System Pharmacy, 69(11), 933–943.
2. Hostler, C. J., & Chen, L. F. (2013). Fidaxomicin for treatment of clostridium difficile-associated diarrhea and its potential role for prophylaxis. Expert Opinion on Pharmacotherapy, 14(11), 1529–1536.
3. Golan, Y., & Epstein, L. (2012). Safety and efficacy of fidaxomicin in the treatment of Clostridium difficile-associated diarrhea. Therapeutic Advances in Gastroenterology, 5(6), 395–402.
4. Revill P, Serradell N, Bolos J (2006). "Tiacumicin B". *Drugs of the Future*. **31** (6): 494.
5. Crawford, T., Huesgen, E., & Danziger, L. (2012). *Fidaxomicin: A novel macrocyclic antibiotic for the treatment of Clostridium difficile infection. American Journal of Health-System Pharmacy, 69(11), 933–943.*
6. Whitman, C. B., & Czosnowski, Q. A. (2012). *Fidaxomicin for the Treatment of Clostridium difficile Infections. Annals of Pharmacotherapy, 46(2), 219–228.*
7. Golan, Y., & Epstein, L. (2012). *Safety and efficacy of fidaxomicin in the treatment of Clostridium difficile-associated diarrhea. Therapeutic Advances in Gastroenterology, 5(6), 395–402.*
8. International Conference on Harmonization (ICH) (2005) *Validation of Analytical Procedures: Text and Methodology, Q2(R1)* https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf

Tables:

Table 1: Result of System suitability parameters

Analyte	Retention Time (mins)	Tailing factor(T)	Theoretical Plates(N)
Fidaxomicin	6.5	0.98	4401
Required limits	-	T < 2	N > 2000

Table 2: Linearity Data for HPLC

Sr. No.	Concentration (µg/mL)	Area at 228 nm
1.	10	419899
2.	20	845501
3.	30	1268657
4.	40	1655505
5.	50	2056720
6.	60	2476330
7.	80	3398645
8.	100	4369978
9.	150	6598456
Correlation coefficient (r^2)	0.9991	
Y-intercept	y = 44236x – 88637	

Table 3: Precision data (System) of Fidaxomicin by HPLC Method

Standarad number	Concentration (µg/ml)	Fidaxomicin (Area)	Calculated Conc.(µg/ml)
1	60	2504698	58.63
2	60	2456602	57.56
3	60	2475463	58.00
4	60	2496533	58.45
5	60	2498885	58.51
6	60	2455899	57.54
Mean		2511325	58.11
SD		49875.26	0.445
% RSD		1.9	0.76

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Table 4: Precision data (Method) of Fidaxomicin by HPLC Method

Standarad number	Concentration (µg/ml)	Fidaxomicin (Area)	Calculated Conc. (µg/ml)
1	60	2496533	58.45
2	60	2612352	61.06
3	60	2475463	58.00
4	60	2504698	58.63
5	60	2498885	59.51
6	60	2456602	59.56
Mean		2511325	58.70
± SD		49875.26	1.222
% RSD		1.9	2.08

Table 5: Accuracy of Fidaxomicin at 228nm

Level (%)	Sample conc. (µg/mL)	Standard conc. (µg/mL)	Total conc. (µg/mL)	Peak area	Calculated Conc. (µg/mL)	% Mean Recovery	Mean	SD	% RSD
80	30	24	54	2233212	54.5	100.70	54.5	0.7	1.28
				2243635	53.8				
				2253624	55.2				
100	30	30	60	2481347	59.3	98.91	60.27	0.88	1.46
				2496533	60.5				
				2475463	61.02				
120	30	36	66	2729498	67.6	101.83	66.56	0.90	1.36
				2739353	66.2				
				2741346	65.9				

Table 6: Robustness study for Fidaxomicin

Parameter	Variation		1	2	3	Mean	SD	% RSD
Flow rate (1± 0.1 mL/min)	0.9 ml/min	AREA	2446231	2476730	2434334	2452431	37176.5	1.54
		Rt	6.817	6.799	6.829	6.82	0.01	0.14
		NTP	9504	9554	9353	9470	104.64	1.10
	1.1 ml/min	AREA	2473124	2474330	2475312	2474255	1093.4	0.04
		Rt	6.364	6.321	6.295	6.36	0.02	0.31
		NTP	18213	18106	17715	18011	262.15	1.46
Temperature (25 ± 5°C)	20°C	AREA	2456533	2436330	2446320	2446394	10100.1	0.41
		Rt	6.523	6.541	6.589	6.55	0.03	0.45
		NTP	9514	9533	9545	9530	15.63	0.16
	30°C	AREA	2474642	2472339	2476750	2474577	2206.7	0.08
		Rt	6.578	6.536	6.595	6.56	0.03	0.45
		NTP	9949	9945	9950	9948	2.64	0.02
Wavelength (228± 2nm)	226	AREA	2426520	2486535	2446730	2453261	30538.3	1.25
		Rt	6.453	6.384	6.491	6.47	0.05	0.77
		NTP	9685	9690	9680	9685	5	0.05
	230	AREA	2466332	2473736	2486360	2475476	21877	0.88
		Rt	6.582	6.558	6.582	6.57	0.01	0.15
		NTP	9514	9533	9545	9530.66	15.63	0.164

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Figures:

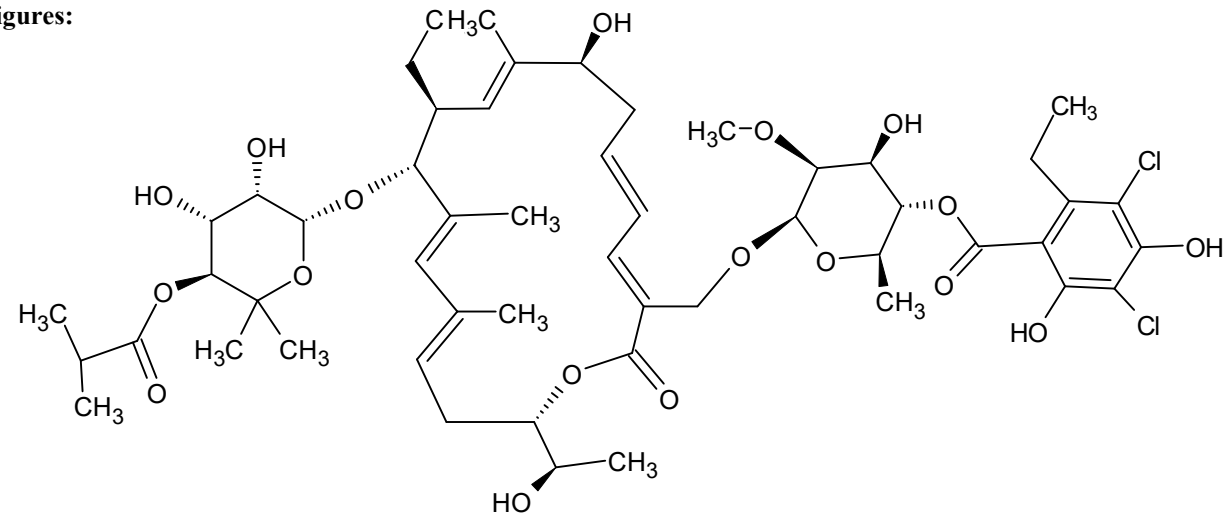

Figure 1: Molecular structure of Fidaxomicin

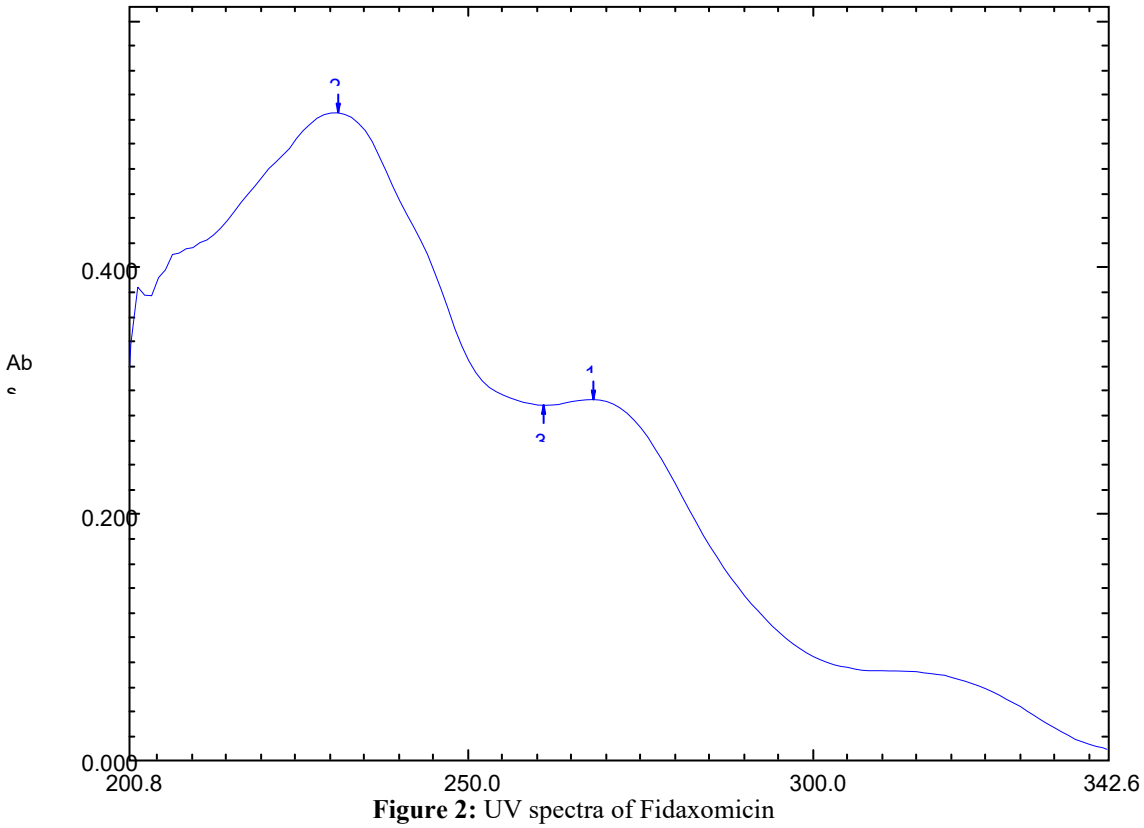

Figure 2: UV spectra of Fidaxomicin

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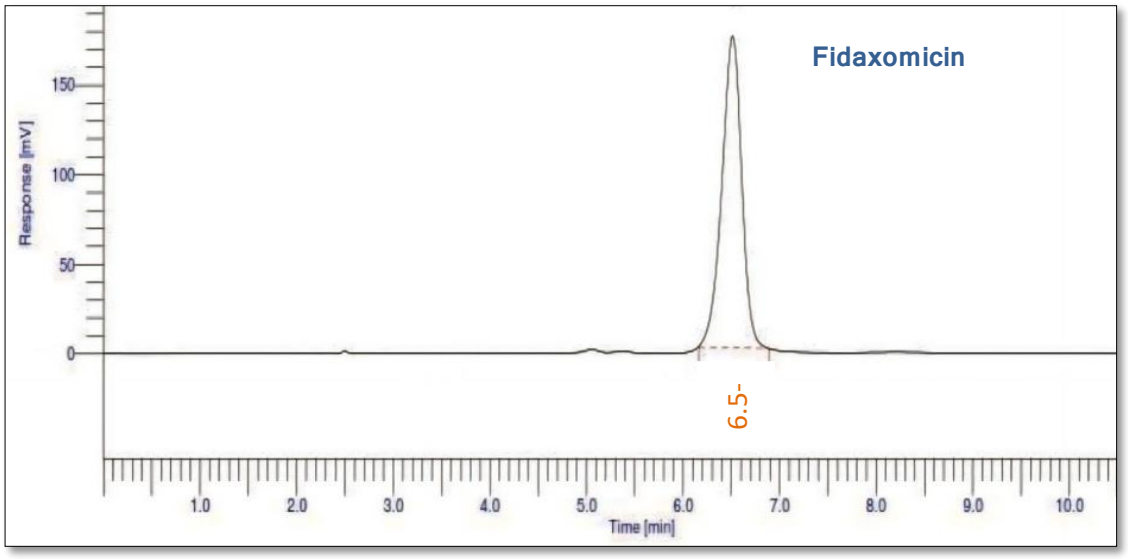

Figure 3: Chromatogram for Fidaxomicin Standard 100 µg/mL

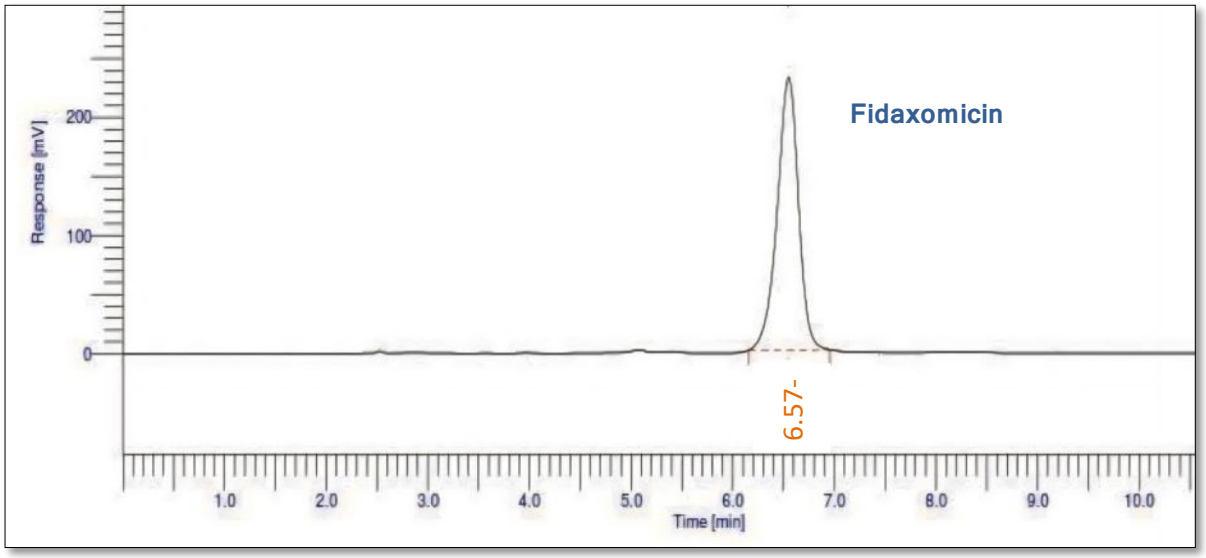

Figure 4: Chromatogram of Accuracy at 80% level for Fidaxomicin

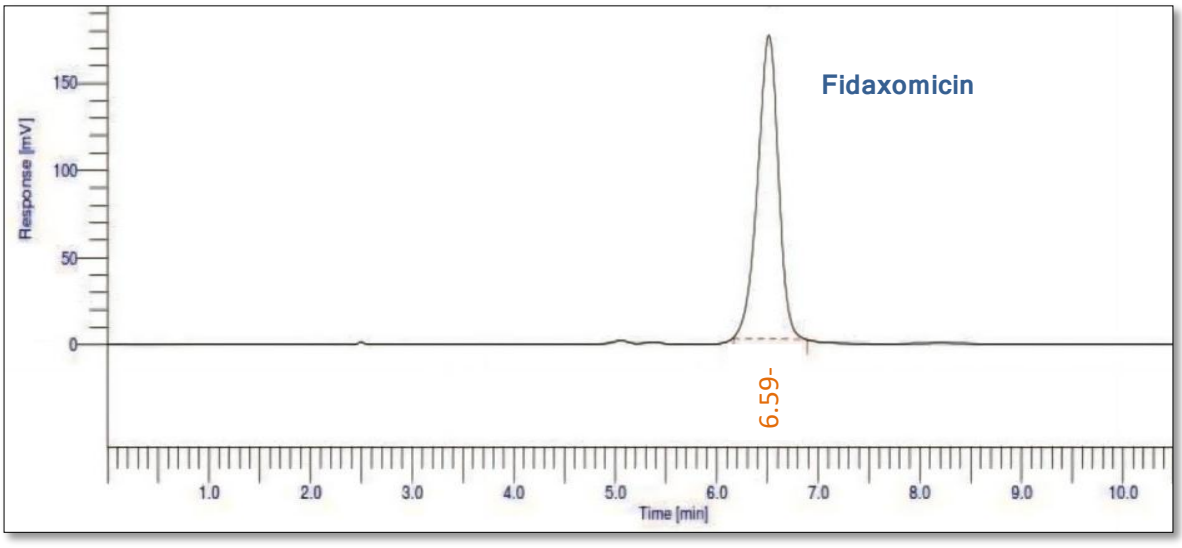

Figure 5: Chromatogram of Accuracy at 100 % level for Fidaxomicin

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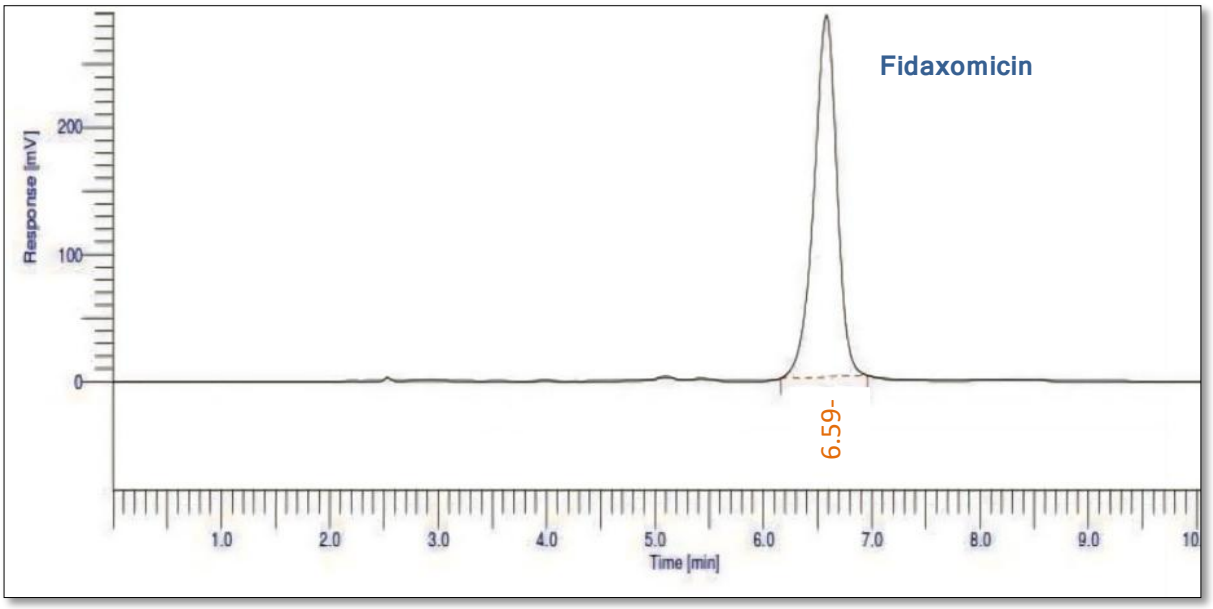

Figure 6: Chromatogram of Accuracy at 120% level for Fidaxomicin