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Research article

Development of analytical method validation procedure for Fidaxomicin tablet for oral suspension

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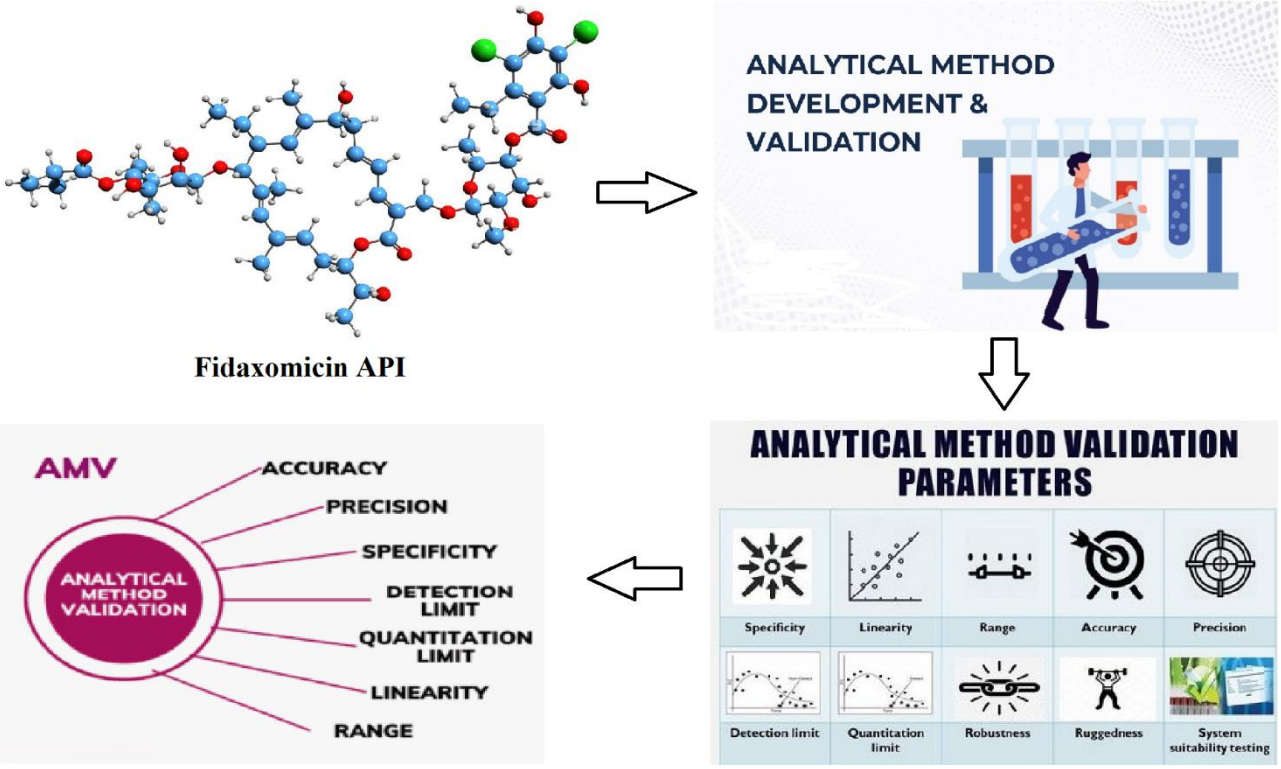
ABSTRACT

Analytical procedures for oral solid dosage forms are necessary to comply various validation criteria which mainly are the accuracy, precision, specificity, robustness, and limit of detection. For the Fidaxomicin tablets for oral suspension, we have checked all these parameters to ensure the efficacy of the formulation. In this research article, all above parameters are tested in line with The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), and United States Food and Drug Administration (USFDA) guidelines and results are reported in a scientific manner.



Keywords: Analytical procedures, Method validation, Fidaxomicin, Accuracy, Precision.

INTRODUCTION

The analytical procedures have been demonstrated to be suitable for testing identity (identification), purity, strength, quality, and consistency of Fidaxomicin tablets. Validation of the analytical procedures were performed in accordance with ICH Q2 (R1) and FDA Guidance for Industry "Analytical Procedures and Methods Validation for Drug and Biologics" (2015). The validation status for each analytical procedure is presented for noncompendial analytical procedures and for compendia analytical procedures [1].

A HPLC analytical procedure was validated for the determination of identification, assay and degradation products in Fidaxomicin tablet, 200 mg. All validation results met the acceptance criteria established for validation of the method and demonstrated that the HPLC method is stability-indicating and suitable for its intended purpose. The assay and degradation products by HPLC were validated for Fidaxomicin 200 mg [2].

System suitability, identification, specificity, linearity and range, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), solution stability, and robustness have been demonstrated for the method. In addition, the method suitability for identification is appropriate, as Fidaxomicin is identified by comparison of the retention time and Photo Diode Array (PDA)-UV spectra of the sample to those of the Fidaxomicin reference standard. Validation parameters for degradation products were assessed using low level impurity markers (Fidaxomicin Impurity A, Fidaxomicin-D7, Fidaxomicin Metabolite-OP-1118, and Di-Methylated Fidaxomicin) as surrogates for degradation products [3]. The stability-indicating characteristics of the method were evaluated and demonstrated through forced degradation studies under light, heat, acidic, basic, and oxidative conditions [4].

MATERIAL AND METHOD

Materials

Fidaxomicin API, and excipients Microcrystalline Cellulose, Croscarmellose Sodium, Colloidal Silicon Dioxide, Magnesium Stearate has been procured from the Montage Laboratories Pvt. Ltd., Himatnagar, Gujarat. While Sodium Lauryl Sulfate, Opadry II Yellow and artificial grapefruit flavour has been acquired by Kentreck Laboratories Pvt. Ltd., Ahmedabad.

Methods

Analytical procedures used to control the quality of the Fidaxomicin tablet, 200 mg, are as follows:

Noncompendial Analytical Procedures

Appearance: Visual inspection

Identification of Fidaxomicin: HPLC-UV spectrum, HPLC-retention time

Assay: high performance liquid chromatography (HPLC)

Degradation Products: HPLC

Compendial Analytical Procedures

Uniformity of Dosage Units: USP <905>, weight variation

Water Content: USP <921>, Karl Fischer Titration

Microbial Limit: Microbial Enumeration: USP <61>, Ph. Eur. 2.6.12

Microbial Limit (*E. Coli)*: USP <62>, Ph. Eur. 2.6.13

Appearance

The appearance of Fidaxomicin tablet, 200 mg, was assessed by visual inspection of the tablets under bright light for color, and shape, and compared with the specification.

Identification, Assay and Degradation Products

The identification, assay, and degradation products for Fidaxomicin tablet, 200 mg are determined by high performance liquid chromatography (HPLC). Identification is performed by comparison of the retention time and the UV spectrum of the sample to the Fidaxomicin reference standard. Assay is expressed as percent label claim (% LC) and determined as the ratio of the calculated sample concentration to the theoretical concentration. The calculated sample concentration is determined by comparing the sample peak area response with an external Fidaxomicin reference standard. Quantitation of unspecified degradation products is reported on a weight percent basis which is determined based on the area percentage with an assumption of relative response factor of 1.00 for each potential degradation product [5].

Preparation of Diluent and Solutions

The solutions described below were prepared proportionally. Mobile Phase A (0.02% Trifluoroacetic Acid (TFA) in water): For every litter of Mobile Phase A, add 200 µL of TFA in 1000 mL of water. Mixed before use.

Mobile Phase B (0.02% TFA in Acetonitrile (ACN)): For every litter of Mobile Phase B, add 200 µL of TFA in 1000 mL of acetonitrile (ACN). Mixed before use.

Diluent (60% Methanol / 40% Water): Methanol and water are mixed at a ratio of 6:4 (v/v) ratio.

Preparation of Working Standard and Check Standard Solutions (approximately 500 µg/mL each): Both Working Standard and Check Standard Solutions follow the same procedures. Fidaxomicin reference standard was accurately weighed and transferred approximately 25.00 mg of into a 50 mL volumetric flask. Approximately 40 mL of diluent was added and sonicated until dissolved. After sonication, the solution was allowed to cool to ambient condition. Volume has been diluted with diluent and mixed well.

Preparation of Sensitivity Solution (approximately 0.25 µg/mL): Working Standard Solution has been diluted with the diluent in an appropriate volumetric flask to obtain a concentration of approximately 0.25 µg/mL. Serial dilutions using the diluent has been applied.

Resolution Solution (approximately 1.0 µg/mL Fidaxomicin Impurity A Stock and 500 µg/mL Fidaxomicin): Fidaxomicin Impurity

A Stock Solution (approximately 100 µg/mL): Fidaxomicin Impurity A Stock Solution (approximately 100 µg/mL) has been prepared by dissolving appropriate amount of Fidaxomicin Impurity A reference standard with the diluent in a volumetric flask.

Resolution Solution: Working Standard Solution and the Fidaxomicin Impurity A Stock Solution was mixed at 100:1 (v/v) ratio in a suitable container.

Preparation of Sample Solution (approximately 500 µg/mL): 10 tablets were weighed and grinded into fine powder using a mortar and a pestle and mixed well. In duplicate, sample powder weighed equivalent to 1.5 times of the average tablet weight of 10 tablets and added to a 500 mL volumetric flask. About 450 mL diluent was added to the flask. Powder was allowed to be soaked in the diluent, and then sonicated to mix the powder with the diluent. Diluent was added to the volume and mixed well. A portion of the mixture was filtered through a syringe filter with a 0.45 µm nylon membrane. Initial 5mL filtrate was then discarded and after that filtrate was collected. One volume of the filtrate was diluted in a volumetric flask (in a flask size of three times the volume of the filtrate) and mixed well.

Equipment and Chromatographic Conditions

Column: Waters Cortecs C18, 2.7 µm, 4.6 mm × 150 mm, or equivalent

Detection: UV at 275 nm, collect spectrum (200 to 400 nm) for identification test

Column temperature: 40 °C

Injection volume: 5 µL

Flow rate: 1.0 mL/min

Run time: 31 minutes

Mobile Phase A: 0.02% TFA in water

Mobile Phase B: 0.02% TFA in CAN

Gradient: Time (Minute):0.0, 18.0, 27.0, 27.5, 31.0

Mobile Phase A (%): 80, 40, 10, 80, 80

Mobile Phase B (%): 20, 60, 90, 20, 20

Sample Analysis

Two injections of diluent for equilibration have been prepared. One injection of diluent for specificity has been made. One injection of Sensitivity Solution (not required for Identification testing) has been prepared. One injection for Resolution Solution has been made. Five consecutive injections of the working standard solution have been prepared [6]. One injection to check standard solution (not required for Identification testing) is prepared. One injection of each sample solution has been prepared. One injection of the working standard solution after every six sample injections and at the end of the sequence run has been prepared. System suitability before sample injections has been demonstrated [7].

System Suitability Criteria

Interference: No interfering peak area ≥ 0.05% of the average Fidaxomicin peak area from the first five injections of the working standard solution must be observed at the retention times of Fidaxomicin and all known impurities in the last diluent injection.

Sensitivity: The signal-to-noise ratio (s/n) for the Fidaxomicin peak in sensitivity solution injection must be ≥ 10.

Resolution: The USP Resolution between Fidaxomicin and Fidaxomicin Impurity A peak in the Resolution Solution must be ≥ 3.0.

Injection Reproducibility: The percent RSD of the Fidaxomicin peak areas and retention time from the first five consecutive injections of the Working Standard Solution must be ≤ 2.0%.

Tailing Factor: The USP tailing factor of the Fidaxomicin peak in the first injection of the Working Standard solution must be ≤ 2.0.

Check Standard Recovery: The percent recovery of Fidaxomicin in the Check Standard Solution must be within 98.0% to 102.0%.

System Drift: The percent recovery of Fidaxomicin throughout the sequence run must be within 98.0% to 102.0%.

Calculations

Identification

Identification of fidaxomicin by Retention Time (RT)

$$\% \text{ Agreement} = ((RT_{spl} - RT_{ws}) / RT_{ws}) \times 100$$

Where, RT_{spl} = Retention time of fidaxomicin peak in the sample

RT_{ws} = Average Retention time of fidaxomicin peak in the first five injections of the Working Standard Solution

Identification of fidaxomicin by UV Spectrum

The diode array spectra were extracted from 240 to 400 nm at the apex of the fidaxomicin peak from the chromatograms of the samples and the first injection of the reference standard. Visual comparison of the spectra has been carried out [8].

Assay

Calculation percent label claim (%LC) of Fidaxomicin:

$$\% LC = \frac{Aspl}{Astd} \times W_{std} \times Pstd \times \frac{Wave}{Wspl} \times \frac{DFspl}{DFstd} \times \frac{1}{LC} \times 100\%$$

Where,

W_{std} = Weight of Fidaxomicin reference standard used in Working Standard Solution preparation (mg)

P_{std} = Absolute purity of reference standard (%, w/w)

A_{std} = Average peak area of Fidaxomicin

A_{spl} = Peak area of Fidaxomicin from sample solution

DF_{spl} = Dilution factor for sample solution

DF_{std} = Dilution factor for Working Standard Solution

LC = Label claim of Fidaxomicin tablet

W_{spl} = Weight of sample powder

W_{ave} = Average weight of 10 tablets

Degradation Products

For degradation products determination, peaks observed in the blank, excipient blank and drug substance impurities are not integrated. Individual unspecified degradation products ≥ 0.05% (area %) are calculated and reported as weight % with the adjustment of a

relative response factor of 1.00. Total degradation products have been calculated by adding all degradation products ≥ 0.05%.

RESULTS

The assay and degradation products by high performance liquid chromatography (HPLC), dissolution, and water content procedures were validated for Fidaxomicin tablets, 200 mg. A summary of the validation results is presented in Table 1 through Table 9.vh

Table 1: System Suitability for the Validation of Identification, Assay and Degradation Products

Parameter	Test	Acceptance Criteria	Results
System Suitability	Specificity	The Diluent Blank injection must not exhibit any peaks ≥ 0.05% of the average peak area of Fidaxomicin in the first 5 Working Standard injections at the retention time of Fidaxomicin or any known impurities.	No interfering peak found in diluent. Pass
	Sensitivity	USP Signal-to-noise ratio (s/n) of the Fidaxomicin peak in the sensitivity standard chromatogram ≥ 10	All Pass
	Resolution	Resolution between Fidaxomicin and Fidaxomicin Impurity A in the resolution solution chromatograms ≥ 3.0. Resolution between any two adjacent specified compound impurity peaks in the resolution solution chromatograms ≥ 1.0	All Pass
	Repeatability	The %RSD of the peak area response of Fidaxomicin in the first 5 Working Standard injections: ≤ 2.0%	All Pass
		The %RSD of the peak area response of Fidaxomicin from all Working Standard injections: ≤ 3.0%	All Pass
		The %RSD of the retention time of the Fidaxomicin peak in the first 5 Working Standard injections ≤ 2.0%	All Pass
	Accuracy of the standard	%Recovery of Fidaxomicin in the check standard versus the first 5 Working Standard chromatograms 98.0% to 102.0%	All Pass
	Column performance	USP tailing factor for the Fidaxomicin peak in the first Working Standard chromatogram ≤ 2.0	All Pass
	System drift	%Recovery of Fidaxomicin from each bracketing standard injection versus the first 5 Working Standard chromatograms is 98.0% to 102.0%	All Pass

Table 2: Results of Identification

Parameter	Test	Acceptance Criteria	Results
Identification	Compare the retention time of the main peak in the sample chromatograms with the average retention time of the Fidaxomicin peak in the standard chromatograms.	Retention time of the Fidaxomicin peak in sample is 100% ± 3% of the average retention time of the Fidaxomicin standard peak.	100%
	Extract the diode array spectra from 240 nm to 400 nm at the apex of the Fidaxomicin peak from the chromatograms sample and the first injection of the standard. Visually compare the spectra.	Fidaxomicin PDA-UV spectra from the apex of the Fidaxomicin peak from the sample and 1st reference standard chromatograms show the same spectral pattern.	Conformed

Table 3: Results of Specificity

Parameter	Test	Acceptance Criteria	Results
Specificity	Placebo blank: • No interfering peak at the retention time (RT) of Unknown Fidaxomicin Impurity • No peak at the RT of a known impurity	• A peak is considered noninterfering if it has a peak area ≤ 0.1% of the average peak area of the Unknown Fidaxomicin Impurity reference standard peak. • Not Detected (ND)	• Fidaxomicin: ND • Impurity: Fidaxomicin-D7: ND Di-methylated Fidaxomicin: ND Fidaxomicin metabolite-OP-1118: ND Fidaxomicin Impurity A: ND
Specificity, forced degradation	Peak purity of the Fidaxomicin peak in stressed and unstressed samples	Peak purity angle is less than the threshold	Conformed

Table 4: Results of Linearity and range

Parameter	Test	Acceptance Criteria	Results
High Concentration Linearity (assay)	Assess linearity of Fidaxomicin from 0.35 to 0.65 mg/mL (70% to 130% of nominal 0.5 mg/mL Fidaxomicin assay concentration). Perform linear regression. Calculate y-intercept, slope, and correlation coefficient (r) for each of the replicate preparations separately (n=5 level, triplicate per level) and together (n=15)	• r ≥ 0.995 • Report the y-intercept as % of the nominal level (recommended value not more than 3%)	Preparation 1 (n=5): r=1.000 Y-intercept=136364 Slope=11181998 Y-Intercept as % of 100%=2
			Preparation 2 (n=5): r=1.000 Y-intercept=159288 Slope=11068024 Y-Intercept as % of 100%=3
			Preparation 3 (n=5): R=1.000 Y-intercept=176460 Slope=11128858 Y-Intercept as % of 100%=3

			Over all (n=15) r=1.000 Y-intercept=156915 Slope=11127380 Y-Intercept as % of 100%=3
Low Concentration Linearity (impurity)	Assess linearity of Fidaxomicin from 0.05% to 1.5% of nominal 0.5 mg/mL Fidaxomicin assay concentration. Perform linear regression. Calculate y-intercept, slope, and correlation coefficient (r) for each of the replicate preparations separately (n=5 level, triplicate per level) and together (n=15)	• $r \geq 0.99$ • Report the y-intercept as % of the 100% specification level (Recommended value not more than 15%)	Preparation 1 (n=5): r=1.00 Y-intercept=320 Slope=11554001 Y-Intercept as % of 100%=1
			Preparation 2 (n=5): r=1.00 Y-intercept=337 Slope=11494147 Y-Intercept as % of 100%=1
			Preparation 3 (n=5): r=1.00 Y-intercept=194 Slope=11597824 Y-Intercept as % of 100%=0
			Overall (n=15) r=1.00 Y-intercept=283 Slope=11548732 Y-Intercept as % of 100%=0
Low Concentration Linearity • Fidaxomicin metabolite-OP-1118 • Fidaxomicin Impurity A • Di-methylated Fidaxomicin • Fidaxomicin-D7	Assess linearity of known impurity from 0.05% (LOQ) to 1.5% of nominal 0.5 mg/mL Fidaxomicin assay concentration. Perform linear regression. Calculate y-intercept, slope, and correlation coefficient (r) for each of the replicate preparations separately (n=5 level, one set of preparation)	Report the y-intercept as % of the 100% specification level (Recommended value not more than 15%)	Fidaxomicin metabolite-OP-1118 (n=5): r=1.00 Y-intercept=79 Slope=5085939 Y-Intercept as % of 100%=0
			Fidaxomicin Impurity A (n=5): r=1.00 Y-intercept=47 Slope=7846681 Y-Intercept as % of 100%=0
			Di-methylated Fidaxomicin (n=5): r=1.00 Y-intercept= -170 Slope=22574480 Y-Intercept as % of 100%=0
			Fidaxomicin-D7 (n=5): r=1.00 Y-intercept=6 Slope=6672897 Y-Intercept as % of 100%=0

Table 5: Results of Accuracy and Range

Parameter	Test	Acceptance Criteria	Results
High Concentration Assay Accuracy (recovery) and Range	Assess accuracy (recovery) and range for triplicate of Fidaxomicin tablet Formulation 2 samples at each level: 70%, 100%, and 130% of 0.5 mg/mL (nominal) Fidaxomicin assay concentration. Calculate Individual % recovery and %RSD	• Each level: individual % recovery: 97.0% to 103.0% • Each level: %RSD ≤ 3.0% (n=3)	70%: Recovery 100.0% to 101.1% RSD 0.5%
			100%: Recovery 100.0% to 100.5% RSD 0.3%
			130%: Recovery 98.8% to 99.8% RSD 0.5%.
Low Concentration Accuracy (recovery) and Range • Fidaxomicin metabolite-OP-1118 • Fidaxomicin Impurity A • Di-methylated Fidaxomicin • Fidaxomicin-D7	Assess accuracy (recovery) and range for triplicate of: Fidaxomicin Impurity A, Di-methylated Fidaxomicin, Fidaxomicin-D7 and Fidaxomicin metabolite-OP-1118 samples in Fidaxomicin tablet Formulation 2 at each level: 1.5%, 1.0%, 0.50% and 0.05% of the nominal concentration of 0.5 mg/mL Fidaxomicin	<u>0.5% and 1.50% levels:</u> • Individual %recovery: 80% to 120% for each level • %RSD ≤ 15.0% for each level <u>0.05% level:</u> • Individual %recovery: 70% to 130% for each level • %RSD ≤ 20.0% for each level	Fidaxomicin-D7: 1.5%: Individual %Recovery: 84% %RSD: 0.2% 1.0% Individual %Recovery: 84% %RSD: 0.2% 0.5% Individual %Recovery: 83% to 85% RSD: 0.7% 0.05% Individual %Recovery: 82% to 85% %RSD: 1.7%

			Di-methylated Fidaxomicin: 1.5%: Individual %Recovery: 98% to 99% %RSD: 0.3% 1.0% Individual %Recovery: 98% to 99% %RSD: 0.3% 0.5% Individual %Recovery: 98% to 99% RSD: 0.6% 0.05% Individual %Recovery: 98% %RSD: 0.2%
			Fidaxomicin metabolite-OP-1118: 1.5%: Individual %Recovery: 84% %RSD: 0.2% 1.0% Individual %Recovery: 84% %RSD: 0.2% 0.5% Individual %Recovery: 84% RSD: 0.1% 0.05% Individual %Recovery: 81% to 92% %RSD: 7.3%
			Fidaxomicin Impurity A: 1.5%: Individual %Recovery: 97% to 98% %RSD: 0.4% 1.0% Individual %Recovery: 98% %RSD: 0.3% 0.5% Individual %Recovery: 98% to 99% RSD: 0.5% 0.05% Individual %Recovery: 95% to 97% %RSD: 1.3%

Table 6: Results of Precision

Parameter	Test	Acceptance Criteria	Results
Assay Precision – Repeatability (same lab)	For analyst 1 and analyst 2, individually analyze 6 Fidaxomicin tablet, 200 mg samples. Calculate individual and mean assay results and %RSD.	%RSD ≤ 5.0% (n=6) for each analyst	Analyst 1: (n=6) Mean: 102.7% %RSD: 1.2% Analyst 2: (n=6) Mean: 102.8% %RSD: 1.3%
Assay – Intermediate Precision (same lab)	Calculate %RSD of assay results for all twelve samples for each dose strength from both analyst 1 and analyst 2	%RSD ≤ 5.0% for analyst 1 and analyst 2 (n=12)	%RSD=1.2% (n=12)
Assay Precision – Reproducibility (2 different labs)[a]	For analyst 1 (lab 1) and analyst 2 (lab 2), individually analyze 6 replicates samples (Fidaxomicin tablet, 200 mg). Calculate individual and mean assay results and %RSD	%RSD ≤ 3.0%(n=6) for each analyst	Analyst 1: (n=6) Mean: 99.9% %RSD: 0.5%
			Analyst 2: (n=6) Mean: 101.2% %RSD: 0.3%
	Calculate absolute difference between mean assay results for analyst 1 (lab 1) and analyst 2 (lab 2)	Absolute difference ≤ 3.0%	Absolute difference: 1.3%
Degradation Products –	Calculate mean results and %RSD from both analyst 1 and analyst 2	Individual Fortified Impurities: %RSD ≤ 10.0% for	Analyst 1 and Analyst 2: (n=12) Fidaxomicin-D7: %RSD: 7.2%

Intermediate Precision (same lab)		analyst 1 and analyst 2 (n=12)	DI-methylated fidaxomicin: %RSD: 1.2% Fidaxomicin metabolite-OP-1118: %RSD: 8.8% Fidaxomicin Impurity A: %RSD: 1.8%
Degradation Products Precision – Reproducibility (2 different labs) [a]	For analyst 1 (lab 1) and analyst 2 (lab 2), individually analyze 6 replicates samples (Fidaxomicin tablet, 200 mg). Calculate individual and mean degradation product results and %RSD.	Individual Degradation Products: • ≥ 0.10% to < 0.50%: %RSD ≤ 20.0% (n=6) for each analyst • ≥ 0.50: %RSD ≤ 10.0% (n=6) for each analyst	Analyst 1: (n=6) Individual Impurities: Fidaxomicin Impurity A: Mean: 0.07% %RSD: N/A
			Analyst 2: (n=6) Individual Impurities: Fidaxomicin Impurity A: Mean: 0.06% %RSD: N/A
	Calculate absolute difference between mean degradation product results for analyst 1 (lab 1) and analyst 2 (lab 2)	Individual Degradation Products: • ≥ 0.10% to < 0.50%: absolute difference between individual degradation product ≤ 0.10% • ≥ 0.50%: % difference between individual degradation product ≤ 20.0% • ≥ 0.05% to < 0.10%: lcomparable from both labs	Individual Degradation Products: Fidaxomicin Impurity A: • ≥ 0.10%: No degradation product ≥ 0.10% was detected • ≥ 0.05% to < 0.10%: Absolute difference: 0.01% comparable • No other degradation product ≥ 0.05% (QL) was detected from both labs.

[a] Ten (10) composite tablets were used for sample preparation**.**

Table 7: Results of Robustness

Parameter	Test	Acceptance Criteria	Results
Robustness	Perform system suitability tests and Fidaxomicin samples under the following conditions: 1. Column temp: 39 °C 2. Column temp: 43 °C (nominal is 40 °C) 3. Flow rate: 1.90 mL/min 4. Flow rate: 1.10 mL/min (nominal is 1.00mL/min) 5. Detector Wavelength: 273 nm 6. Detector Wavelength: 277 nm (nominal is 275 nm)	• System Suitability tests meet the requirements. • The % recovery of Fidaxomicin compared to the nominal values as calculated for the method for any variation condition is 98.0% to 102.0%. • The % recovery of each fortified impurity compared to the nominal values are calculated by the method for any variation condition is 80% to 120%.	• All system suitability acceptance criteria were met for all robustness studies. • % recovery compared to the nominal values: Fidaxomicin: 99.7% to 100.5% Fidaxomicin-D7: 92% to 106% Di-methylated Fidaxomicin: 98% to 102% Fidaxomicin metabolite-OP-1118: 90% to 107% Fidaxomicin Impurity A: 93% to 105%

Table 8: Results of Solution Stability and Filter Study

Parameter	Test	Acceptance Criteria	Results
Solution Stability – Reference Standard	Assess the stability of Fidaxomicin working standard at 2 to 8 °C and Room Temp (RT) over 14 days. Evaluate % recovery compared to time 0 at days 1, 3, 8 and 14.	% Recovery within 98.0% to 102.0% of day 0 value	2 to 8 °C day 14: 100.8% of day 0 RT day 14: 100.0% of day 0
Solution Stability – Samples	Assess the stability of Fidaxomicin tablet 200 mg F2 samples at 2 to 8 °C and Room Temp (RT) over 6 days. Evaluate % recovery compared to time 0 at days 1, 2, and 6.	Assay: within 98.0% to 102.0% of day 0 value Individual Degradation Product: • 0.10% to < 0.50%: absolute difference of day 0 and each time point: ≤ 0.10% • ≥ 0.50%: % difference of day 0 and each time point: ≤ 20.0% • No new degradation product ≥ 0.05% (LOQ) should be observed.	Assay Results 2 to 8 °C day 6: 99.2% of day 0 RT day 6: 98.8% of day 0 Individual Degradation Product: Fidaxomicin Impurity A: day 0: 0.06% 2 to 8°C day 6: 0.07% RT day 6: 0.06% Fidaxomicin-D7: day 0: 0.00% 2 to 8 °C day 6: 0.01% RT day 6: 0.04% No other related substance ≥ 0.05% (LOQ) was observed from both labs.
Filter Study	• The % difference of Fidaxomicin peak areas between the filtered and unfiltered solutions for each of the 3 Sample • The % difference of fortified impurity peak areas between the filtered and unfiltered solutions for each of the 3 Sample	• Fidaxomicin: ≤ 2% • Individual impurity: ≤ 10%	Fidaxomicin: 1% to 2% Individual impurity Fidaxomicin-D7: 1% to 2% Di-methylated Fidaxomicin: 0% to 2% Fidaxomicin metabolite-OP-1118: 2% Fidaxomicin Impurity A: 1%

Table 9: Results of Limit of Quantitation and Limit of Detection

Parameter	Test	Acceptance Criteria	Results
Limit of	Analyze Fidaxomicin at	S/N ≥ 10%	S/N ratio: 58 to 103

Quantitation (LOQ)	0.25 µg/mL (0.05% of the nominal Fidaxomicin drug product concentration) 6 replicates injections.	RSD ≤ 10.0%	Mean S/N ratio: 81 %RSD: 1% LOQ: 0.05%
Limit of Detection (LOD)	Analyze Fidaxomicin at ~0.125 µg/mL (~0.025% of the nominal Fidaxomicin tablet concentration).	S/N ≥ 3	S/N: 41 LOD: 0.025%

Chromatography

Chromatography is a highly sensitive method that can detect drugs and their metabolites at very low concentrations, making it an essential tool for drug testing [9]. Additionally, chromatography can identify specific drugs and their metabolites [10]. The corresponding UV spectra are shown in Figure 1. Chromatograms are provided for the diluent blank in Figure 2, and resolution solution with all known impurities are shown in Figure 3.

Figure 1: UV Spectra for Reference Standard (Upper Panel) and Sample (Lower Panel)

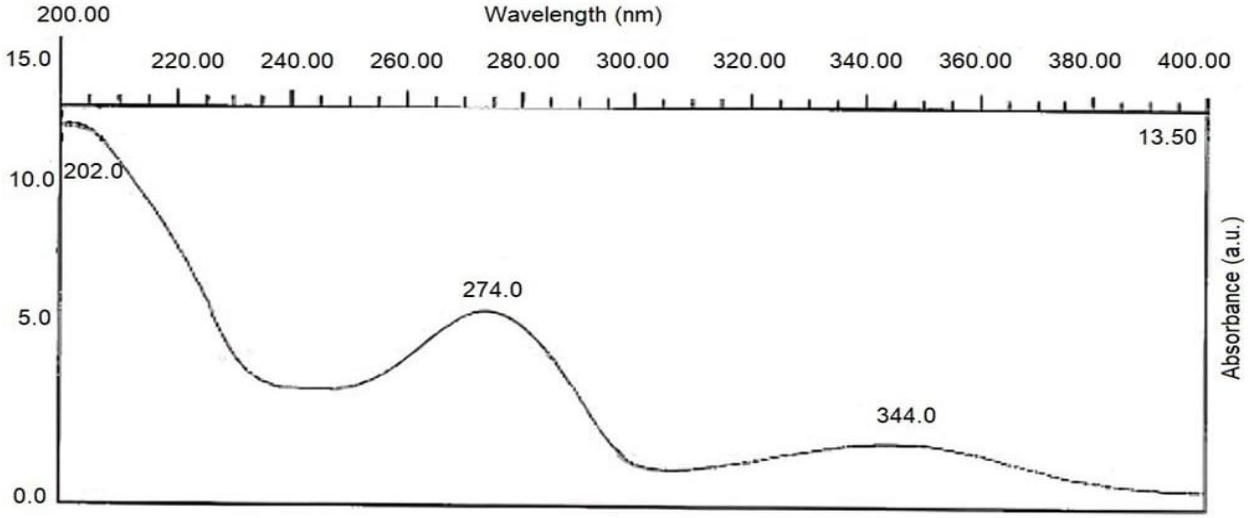


Figure 2: Diluent Blank Chromatogram

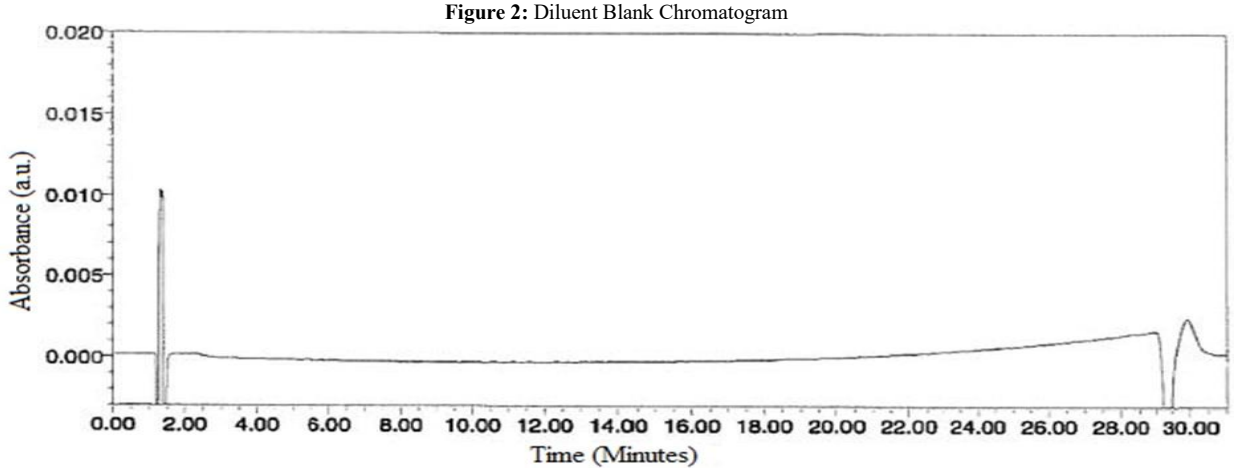
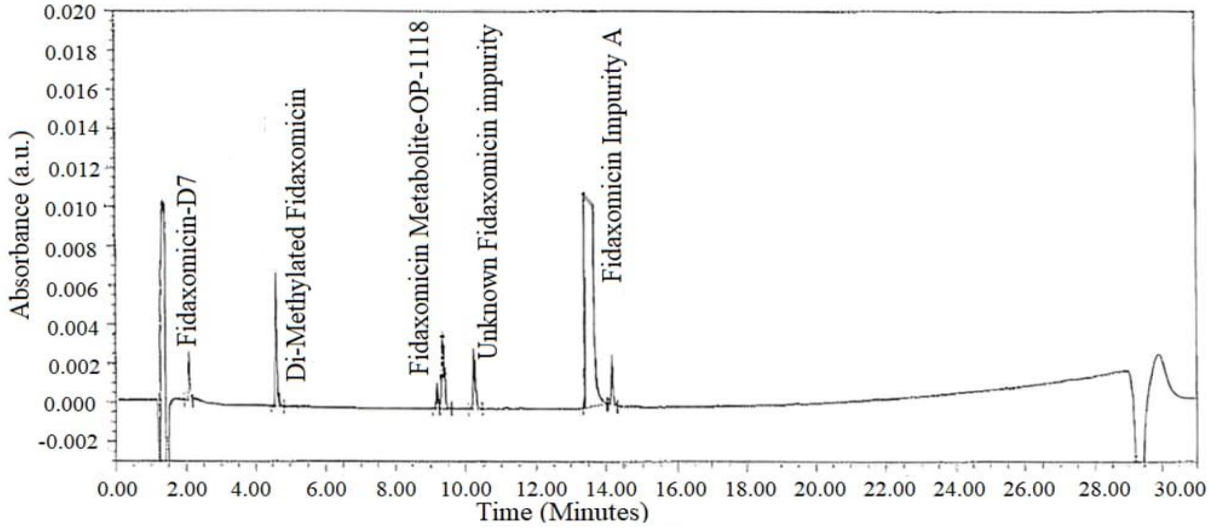


Figure 3: Resolution Solution Chromatogram



CONCLUSION

Fidaxomicin identity is confirmed as both of the following criteria are met. The retention time of the fidaxomicin peak in the sample is within 100% ± 3% of the average retention time of the fidaxomicin peak in the first five reference standard injections and fidaxomicin PDA-UV spectra from the apex of the fidaxomicin peaks from the sample and the first reference standard injection show the same spectral pattern. The absolute difference between the duplicate assay results is ≤ 3.0%. Individual degradation products ≥ 0.05%. The uniformity of dosage units is demonstrated using the weight variation approach as described in USP <905>. The water content of fidaxomicin tablet is determined by volumetric Karl Fischer (KF) analysis based on USP <921> Method 1a (direct titration). Sample analysis was performed by lightly crushing one fidaxomicin tablet using a homogenizer and obtained the weight of the tablet. Entire tablet content is titrated with to the endpoint of KF reagent Aqualine 5 for volumetric titration of aldehydes. Duplicate sample analysis is performed. System suitability was performed. The % difference of the bracketing standard compared to the certified Hydranal Water Standard value must be ≤ 2.0%. The microbial limit test is performed per USP <61>, USP <62> and harmonized Ph. Eur. 2.6.12 and Ph. Eur. 2.6.13. So, based on the results of all acceptance criteria established for the validation of the HPLC assay and degradation products method, we can conclude that all parameters are within the criteria. The HPLC method has been validated for the determination of fidaxomicin tablets, identification, assay, and degradation products, and is suitable for its intended purpose.

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