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Article

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Validated Stability Indicating HPLC Method for the Quantification of Process Related Impurities of Ubrogepant in Pharmaceutical Formulations

Ubrogepant is a medical drug prescribed for the treatment of migraine in adults. Literature analysis has shown, that no suitable analytical method has been published to date for the quantification of impurities of Ubrogepant. Therefore, this study aims to develop a simple and sensitive stability indicating HPLC method for quantifying Ubrogepant and its impurities 1 and 2. The optimized and best separation was achieved using ProntoSIL ODS C18 (250×4.6 mm; 5 µ id) column as stationary phase, phosphate buffer (pH 4.5) and methanol in 65:35 (v/v) at 1.0 mL/min as mobile phase and 246 nm as detector wavelength. The method reports 0.015 µg/mL and 0.05 µg/mL as limit of detection *(*LOD*)* and limit of quantitation (LOQ) for both impurities. This proves that the method has sufficient levels of sensitivity to detect impurities. The method passes all validation parameters as recommended, confirming that the method is valid. The method can show very less % degradation in various stress tests such as acid, base, peroxide, thermal and UV light conditions, and can efficiently resolve different compounds generation during stress exposure, as well as its known impurities prove the stability indicating nature of the method. Based on the experimental findings, it was shown that the method is significantly useful for the routine analysis of Ubrogepant and its impurities 1 and 2.

Keywords: Ubrogepant, HPLC impurity analysis, impurity A, impurity B, Method Development, Method Validation, Formulation analysis, Stress studies.

Introduction

Ubrogepant is the first approved Calcitonin gene-related peptide antagonist prescribed for the immediate treatment of migraine in adults [1]. It was not indicated for the preventive treatment of migraine [2]. Dry mouth, tiredness and nausea are the possible side effects occurred while using the Ubrogepant [3]. It has the molecular formula of $C_{29}H_{26}F_3N_5O_3$ with molecular mass of 549.55 g/mol and IUPAC name of *(6S)-N-[(3S,5S,6R)-6-Methyl-2-oxo-5-phenyl-1-(2,2,2-trifluoroethyl)-3-piperidinyl]-2'-oxo-1',2',5,7-tetrahydrospiro-[cyclopenta[b]pyridine-6,3'-pyrrolo[2,3-b]pyridine]-3-carboxamide*. Molecular structure of Ubrogepant is depicted in Figure 1.

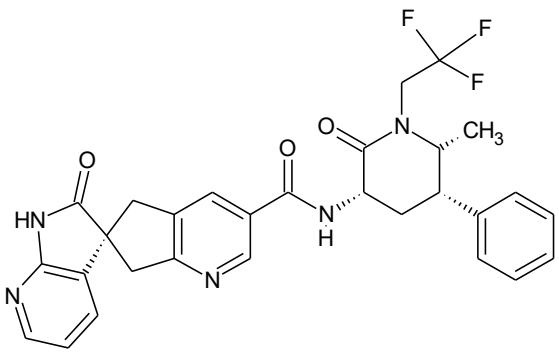
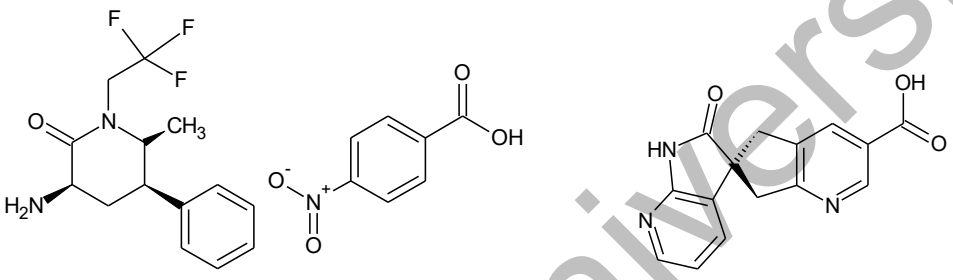


Figure 1. Molecular structure of Ubrogepant

Impurities in a pharmaceutical product are the unwanted chemical compound / substance that remain along with active pharmaceutical ingredients (APIs). These were arise from source as starting material or may be during the synthesis of API like reagents, solvents, catalysts, intermediates and reaction by-products. The ultimate safety of a drug product depends on the quantity of various impurities present on it, and therefore the need to identify, quantify and control impurities plays an important role in drug development. High performance liquid chromatography (HPLC) is a versatile analytical tool that has the ability to identify and quantify a wide range of pharmaceutical impurities.

The extensive review of the available analytical methods for the evaluating of Ubrogepant confirms that there is no analytical method reported / published on open access for the separation and quantification of impurities of Ubrogepant. Therefore, the present study aimed to develop a simple and economical HPLC method for the separation and simultaneous quantification of Ubrogepant impurities. Based on the availability, the impurity 1 (4-Nitro benzoate salt of amine intermediate) with IUPAC name *(3R,5R,6S)-3-amino-6-methyl-5-phenyl-1-(2,2,2-trifluoroethyl)piperidin-2-one, 4-nitrobenzoic acid* and impurity 2 (acid intermediate) with IUPAC name *2'-Oxo-1',2',5,7-tetrahydrospiro[cyclopenta[b]pyridine-6,3'-pyrrolo[2,3-b]pyridine]-3-carboxylic acid* were selected for the study. The molecular structure of impurities is shown in Figure 2.



Impurity 1 (4-Nitro benzoate salt of amine intermediate)

Impurity 2 (Acid intermediate)

Figure 2. Molecular structure of Ubrogepant impurities under study

The synthesis mechanism given by Chi *et al*. (2018) in patent owned by Merck Sharp & Dohme Corp., Rahway, NJ, USA [4], confirms that possible route of formation of impurities in the final product. The impurity 1 was the intermediate product whereas the impurity 2 was used as intermediate reactant in the synthesis of Ubrogepant. The unreacted and leftover of these compounds will remain in the final product of Ubrogepant. Therefore, there is a need to quantify these impurities in the Ubrogepant final product in order to obtain high quality and pure APi of Ubrogepant.

Experimental

Chemicals and reagents:

The 98.26 % pure Ubrogepant API with its two studied impurities, and its 100 mg tablet form under the brand Ubrelvy® were purchased from Allergan India Private Ltd, Bangalore. The ultra-pure (Milli-Q®) water and other HPLC grade solvents used in the study were purchased from Merck chemicals, Mumbai. The analytical reagent grade chemicals used in the study such as hydrogen peroxide, sodium hydroxide, hydrochloric acid and buffer chemicals were also purchased from Merck chemicals, Mumbai.

Instrumental conditions:

The study was carried out on an Agilent (USA) 1100HPLC instrument, which includes a G1311 Aquaternary pump for delivery of solvents, a 0.1–1500 µL volume injectable auto-sampler with thermostat and UV detector (G 1314 A). Various configurations of stationary phases were used for the method development studies and the column eluents were integrated using Agilent chem-station software.

Sample preparation:

Standard solution: An accurately weighed 50 mg of Ubrogepant, impurity 1 and 2 were dissolved separately in a 50 mL clean and dry volumetric flask. Then, 25 mL of methanol were added separately to each flask, and the flasks were sonicated for 2 min to completely dissolve the analytes in the solvent. Then the content was filtered through 0.2 µ membrane filter in a separate clean and dry flask and the final volume was made up to the mark with the same solvent. The standard Ubrogepant, impurity 1 and 2 solution at a concentration of 1000 µg/mL was obtained separately. The combined standard solutions were prepared by accurate-

ly mixing equal volumes of individual known standard stock solutions in a separate flask and were used to develop and validate the method.

Sample solution: Ubrelvy® 100 mg tablets were made in to a fine and uniform powder using a clean and dry mortar and pestle. The tablet powder, weighed so that the powder sample contained 10 mg of Ubrogepant equivalent, was taken into a dry 100 mL volumetric flask. Then diluent (50 mL) was added and sonicated at room temperature for 10 min. Then the content was filtered through a 0.2 µm membrane filter and the solution was brought to the mark with the same diluent. The standard formulation solution at a concentration of 1000 µg/mL was obtained and was further diluted to the required concentration using the same diluents, and the selected concentration solution was used for the quantification of Ubrogepant and its impurities in the formulation sample.

Method development:

The systematic method development strategies were applied to develop a method for the analysis of Ubrogepant and its impurities. While developing the analytical method, the maximum absorbing wavelength for the detection of analytes was assessed using spectrophotometer. The iso-absorption wavelength of impurities 1, 2 and Ubrogepant was determined using a spectrophotometer, and the iso-absorption wavelength was fixed as the detection wavelength during the development of HPLC method. During the initial method development steps, the mobile phase flow rate was fixed as 1.0 mL/min, and after the completion of the development the flow was further optimized in the range of 0.5 mL/min to 1.5 mL/min. The analytes in the study were polar in nature, also non-polar columns were used as stationary phases in the method development. The high non-polar c18 columns of various brands and configurations were studied as stationary phase in the development study. The solvent ratios and its pH was finalized by change in various ratios of the mobile phase with different pH ranges was studied.

In all the method development studied conditions, the standard solution was injected at a concentration of 100 µg/mL and the chromatographic response was recorded. The peak area response, peak intensity, peak shape, and the system suitability were summarized in all the studied conditions. The method conditions providing the best system suitability with high peak intensity and significant absence of noise were considered as suitable conditions for the separation and analysis of Ubrogepant and its impurities [5–11]. These developed method conditions were further studied for method validation study.

Method Validation:

The Ubrogepant standard solution spiked with 0.1 % of both the impurities was analyzed by the optimized method, and the chromatographic response of the obtained chromatograms was summarized to evaluate the system suitability. The Ubrogepant standard solution without impurities, blank (only solvent), placebo solution prepared with commonly used formulation excipients, was analyzed in the developed method to evaluate method specificity.

A series of dilution of Ubrogepant standard solution spiked with 0.1 % of both the impurities was prepared in various concentration levels. The prepared dilutions were analyzed in the developed method and the peak area response of standard and both the impurities were tabulated separately. The calibration curve was plotted for Ubrogepant and its impurities separately by taking the peak area response of analyte in y-axis and its concentration on x-axis. The correlation coefficient and the regression equation of standard Ubrogepant and its impurities were obtained from its corresponding calibration curves.

The method accuracy was evaluated by conducting the spiked recovery study and was performed at 50 %, 100 % and 150 % spiked levels. The spiked level solution of Ubrogepant containing 0.1 % of Ubrogepant solution was spiked to a 100 % formulation solution and the recovery solution was analyzed in the optimized method. The peak area response of the recovery solution was compared with the calibration curve results in the same level and the % recovery of each analysis results and in each spiked level the % relative standard deviation (% RSD) was calculated. The % recovery of 98-102 and %RSD of < 2 was considered as acceptable.

The reproducibility of the method was evaluated in terms of precision and was carried as intraday and interday precision. At the same time, the standard Ubrogepant solution containing 0.1 % of studied impurities was spiked, and the spiked solution was evaluated six times in one day for intraday precision and 6 times in three consecutive days for interday precision. The peak area response of standard and both impurities was tabulated and the %RSD of the peak area response was calculated. The %RSD of less than 2 in both the precision studies for all the analytes was considered as the method was precise and repeatable.

The efficiency of the developed method that remains unaffected when there is a small change in the established method conditions as well as the change in analyte was assessed in ruggedness and robustness

study. In ruggedness, the solution at precision level was prepared and analyzed by three different analysts and the peak area values were tabulated and %RSD of < 2 was acceptable. In robustness study, both positive and negative minor variations in the established method conditions made intentionally and the standard solution at precision level was analyzed in each changed condition. The % change in peak area of each analyte in each changed condition was determined and a value of < 2 was acceptable.

The smallest analyte concentration that can detect and quantify in the established method was considered as limit of detection and quantification respectively. This information of the method confirms its sensitivity. The signal (s) to noise (n) ratio method was adopted for the evaluation of sensitivity.

The stability indicating nature of the method was assessed by performing stress degradation studies. Stress studies such as acid, base, peroxide, thermal and UV light degradation was performed for the standard drug. An accurately weighed 50 mg of standard Ubrogepant was mixed separately with 50 mL of hydrochloric acid (0.1 N), sodium hydroxide (0.1 N) and hydrogen peroxide (3 %) in acid, base and peroxide degradation studies respectively. The solutions were incubated for 24 h in dark, neutralized and then bring it to standard concentration prior to the analysis. The standard Ubrogepant was exposed to 60 ^{0}C for 24 h in an air oven and UV light at 254 nm for 24 h in thermal and UV light degradation studies, respectively. Both these standard drugs after stress exposure were diluted to standard concentration before analysis. All the stress exposed Ubrogepant dilute solutions were evaluated by the established method, and the chromatograms observed in each analysis were used to confirm the acceptability of the method. The resultant chromatograms provides the number of stress degradation compounds generated as a results of stress exposure, and the method applicability for the separation of stress degradation compounds was assessed. The peak area in each stress study was used for calculating the % degradation of Ubrogepant by comparing with unstressed peak area response of Ubrogepant in the developed method [4–10].

The developed method was applied for the separation, detection and quantification of Ubrogepant and its impurities in formulation. The formulation sample solution prepared from 100 mg tablets of Ubrogepant (Ubrelvy®) was assessed in the developed method. The peak area response was used to calculate the % content in the sample by comparison with the corresponding standard calibration curve results.

Results and Discussions

The wavelength overlay scan spectrum of the individual wavelength scans of Ubrogepant and its impurities confirms that 246 nm is the appropriate wavelength for the detection of analytes. Therefore, 246 nm was initially confirmed as a suitable wavelength and was fixed as detector wavelength in the method development study.

Initially, Kromasil C18 (250×4.6 mm, 5 μm) column was selected as stationary phase, pH 5.2 sodium acetate buffer and methanol in equal volumes were selected as mobile phase. The chromatogram identified in this condition (Fig. 3A) does not show a clear separation of the analytes. A single asymmetric split peak was detected on the chromatogram, confirming that the used column could not separate the analytes and therefore was not suitable for further study.

The column was replaced with Phenomenex Luna C18 (250×4.6 mm, 5 μm) and the same mobile phase was continued in sample 2. In this case, the peaks correspond to Ubrogepant and its impurities were identified, but no clear separation of analytes was observed. Baseline on the chromatogram was unstable and retention time of identified compounds was very high (Fig. 3B). The peak symmetry was also observed to be asymmetric, and hence the conditions were not suitable for the separation of analytes.

The method development was continued with the same column with change in buffer. In this case, pH 5.5 phosphate buffer and acetonitrile in the ratio of 75:25 (v/v) were used as mobile phase. The obtained chromatogram in this case (Fig. 3C) doesn't show a peak for Ubrogepant, whereas the peaks for both impurities were detected and well resolved. This proved that the column in the study could not separate the analytes, and therefore further study was proposed with change in the column as well as the mobile phase composition.

Further method development was continued with ProntoSIL ODS C18 (250×4.6 mm; 5 μm) column as stationary phase, and pH 4.5 phosphate buffer and methanol in 20:80 (v/v) were selected as mobile phase. The chromatogram observed under these conditions (Fig. 3D) shows clear separation of the peaks corresponding to Ubrogepant and its impurities. The baseline throughout the run time was noticed to be little fluctuated and the identified peaks were slightly broad with high tail factors. The peak area response corresponding to impurity 2 was smaller compared with the other analytes in the study. Therefore, the conditions were not suitable for the analysis. Figure 3 represents the chromatograms observed in the method development study.

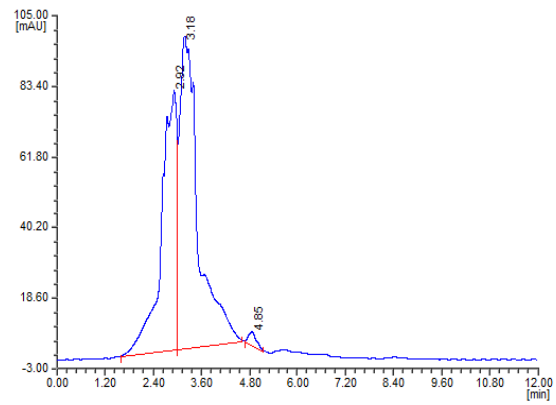


Fig. 3A

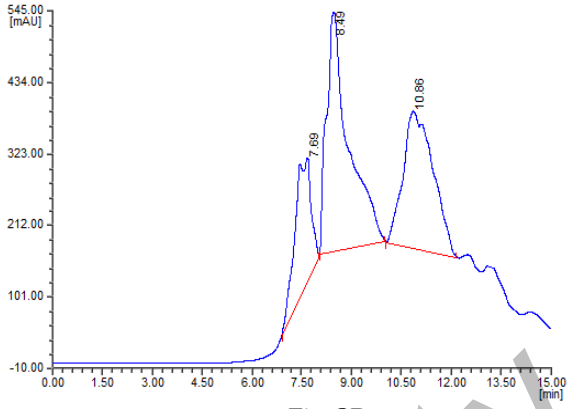


Fig. 3B

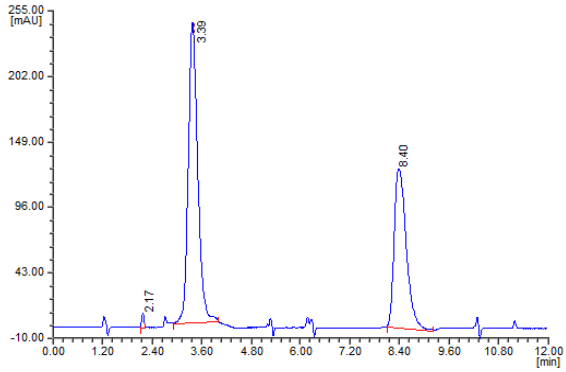


Fig. 3C

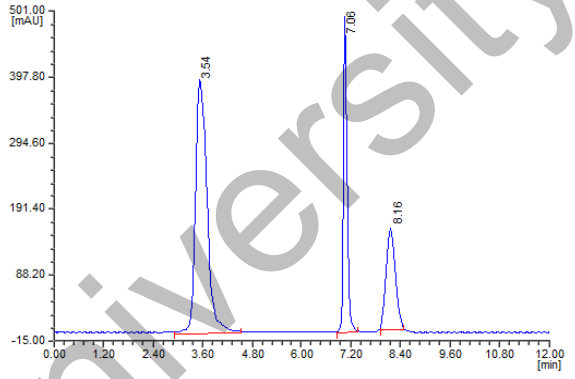


Fig. 3D

Fig. 3A & 3B: No clear separation of analytes was observed in this trail condition;
Fig. 3C: Ubrogepant peak was not identified and the peaks corresponding the impurities were detected in this trail;
Fig. 3D: Peaks corresponds to standard and impurities were identified but it doesn't pass system suitability and base line fluctuations identified throughout the run time

Figure 3. Chromatograms observed during method development

The method development study was continued with increase and decrease in the composition of buffer in the mobile phase, flow rate of mobile phase and detector wavelength for achieving the best chromatographic separation with acceptable system suitability. The separation was achieved on the ProntoSIL ODS C18 (250×4.6 mm; 5 µm) as stationary phase, pH 4.5 phosphate buffer and methanol in 65:35 (v/v) as mobile phase at 1.0 mL/min and UV detection at 246 nm. Under these optimized chromatographic conditions, a clear separation of Ubrogepant and its impurities was achieved without additional detection of impurities or other co-eluting compounds. The analytes were identified at a retention time of 7.05 min, 3.54 min and 8.14 min respectively for Ubrogepant, impurity 1 and 2, whereas the blank chromatogram does not show any chromatographic detection throughout the run time. This confirms the specificity of the established method for the detection of Ubrogepant and its impurities in the study. The chromatogram of the blank and standard observed under the developed method condition is shown in Figures 4A and 4B, respectively.

The signal-to-noise (S/N) approach was adopted for the evaluation of LOD and LOQ of method optimized for analyzing Ubrogepant with its impurities 1 and 2. The LOD was determined as 1.5 µg/mL and 0.015 µg/mL respectively for Ubrogepant and impurities. Based on LOD, the LOQ was calculated as 5 µg/mL and 0.05 µg/mL respectively for Ubrogepant and impurities. This confirms that the method can effectively detect the impurities down to a very low concentration of 0.015 µg/mL and can quantify down to 0.05 µg/mL. The sensitivity levels of analytes were taken into account when preparing the standard dilution of Ubrogepant and impurities. The standard calibration curve solutions of Ubrogepant containing 0.1 % of each impurity was prepared and analyzed by the optimized method. The high correlated calibration curve was obtained in the analyte range of 50–300 µg/mL and 0.05–0.30 µg/mL for Ubrogepant and both the impurities, respectively. The regression equation was derived as $y = 2896.9x + 570.22$ ($R^2 = 0.9999$), $y = 315628x + 4223.9$ ($R^2 = 0.9993$) and $y = 288800x + 3864.8$ ($R^2 = 0.9993$) respectively for Ubrogepant, impurity 1 and 2. The peak area results identified in the linearity study are presented in Table 1.

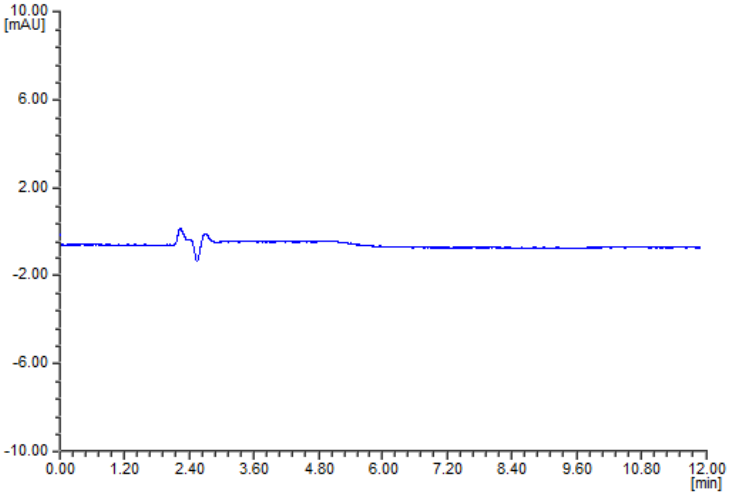


Fig. 4A (Blank chromatogram)

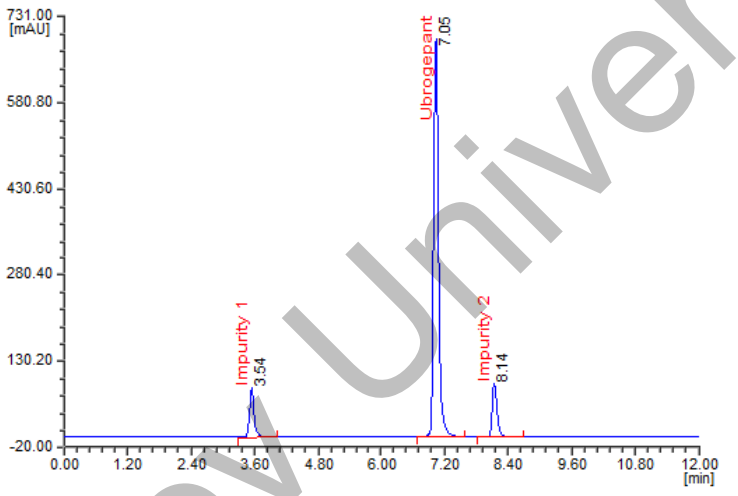


Fig. 4B (Standard chromatogram)

Figure 4. System suitability chromatograms of Ubrogepant and its impurities in the developed method

Table 1

Linearity results

S. No	Ubrogepant		Impurity 1		Impurity 2	
	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area
1	50	145159.1	0.05	19683.7	0.05	18010.6
2	100	290576.2	0.10	35683.1	0.10	32650.0
3	150	436957.4	0.15	51352.9	0.15	46987.9
4	200	575874.5	0.20	68961.5	0.20	63099.8
5	250	727493.1	0.25	82574.6	0.25	75555.8
6	300	869157.8	0.30	98496.9	0.30	90124.7

A 100 µg/mL Ubrogepant standard solution spiked with 0.1 % impurities was assessed in an optimized system suitability method. The system suitability parameters of the chromatographic results were summarized and the method system suitability was assessed. As shown in Table 2, the developed method passes the system suitability, which confirms the suitability of the developed method.

Table 2

System suitability results

S No	Parameter	Results achieved for		
		Ubrogepant	Impurity 1	Impurity 2
1	Concentration prepared	100µg/mL	0.10 µg/mL	0.10 µg/mL
2	Retention Time	7.05 min	3.54 min	8.14 min
3	Theo plate	7961	5127	9068
4	Tail Factor	1.07	0.95	0.98
5	Resolution	16.9	–	6.4

The 100 % solution of Ubrogepant containing 0.1 % of both impurities was evaluated in the precision and ruggedness study. The peak area response of each analyte was summarized in each study. The % RSD was calculated as 0.44, 0.96 and 1.07 for intraday precision, 0.59, 0.88 and 1.44 for interday precision and 0.62, 1.08 and 1.52 for ruggedness for Ubrogepant, impurity 1 and 2 respectively. The %RSD was achieved at acceptable levels for all the analytes in each study, proving the precision and ruggedness of the method.

The effect of the variations in the developed method conditions on the chromatographic response was assessed in robustness study. In robustness study, the composition of mobile phase was altered as 60:40 (MP 1) and 70:30 (MP 2) of buffer and methanol. The pH of buffer was altered as 4.4 (pH 1) and 4.6 (pH 2) as well as the detector wavelength was changed as 241 nm (WL 1) and 251 nm (WL 2) (Table 3).

Table 3

Robustness results

S No	Compound	Change	Peak Area	% Change	Plate Count	Tail factor	Resolution
1	Ubrogepant	MP 1	288484.1	99.28	7968	1.07	16.8
2		MP 2	287554.2	98.96	7847	1.09	16.5
3		pH 1	286944.0	98.75	7625	1.08	16.7
4		pH 2	288571.2	99.31	7691	1.07	16.8
5		WL 1	287815.7	99.05	7719	1.07	16.9
6		WL 2	288338.8	99.23	7835	1.08	16.8
7	Impurity 1	MP 1	35069.4	98.28	5230	0.96	–
8		MP 2	35194.2	98.63	5154	0.95	–
9		pH 1	35251.3	98.79	5196	0.95	–
10		pH 2	35533.2	99.58	5085	0.96	–
11		WL 1	35436.9	99.31	5176	0.96	–
12		WL 2	35358.4	99.09	5144	0.95	–
13	Impurity 2	MP 1	32539.0	99.66	9162	0.98	6.5
14		MP 2	32153.8	98.48	9418	0.99	6.4
15		pH 1	32235.4	98.73	9685	0.98	6.4
16		pH 2	32222.3	98.69	9472	0.98	6.5
17		WL 1	32016.6	98.06	9825	0.98	6.6
18		WL 2	32274.6	98.85	9326	0.99	6.5

The method accuracy was evaluated in a spiked recovery study and the experiment was performed at 50 %, 100 % and 150 % spiked levels. The solutions were evaluated by the optimized method and the peak area response was compared with the standard calibration results at the same level. The % recovery for each injection and the % RSD in each spiked level was calculated. The % recovery was observed to be at acceptable levels of 98–102 % and the % RSD was less than 2 for each spiked level (Table 4), confirming the method accuracy.

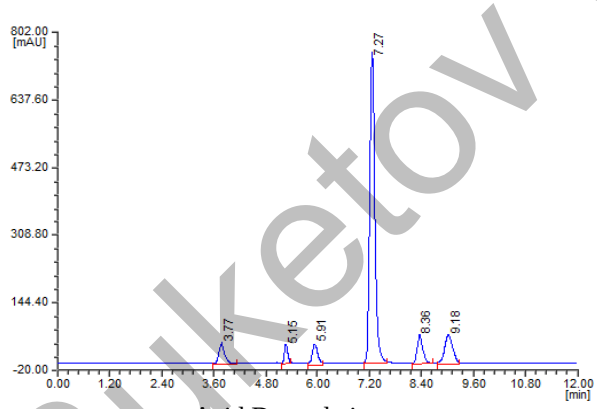
Table 4

Recovery results

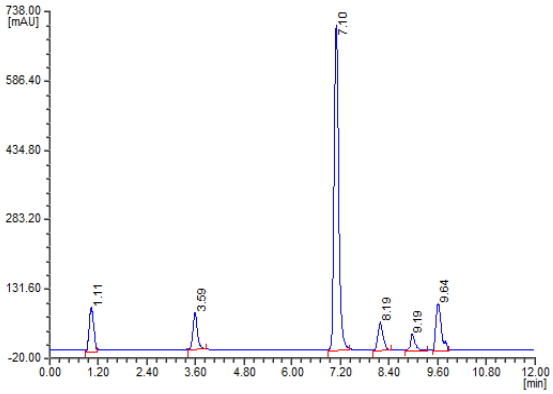
S. No.	Compound	Recovery Level	Concentration in µg/mL			Amount found* Mean ± SD	% recovered* Mean ± SD	% RSD of Recovery
			Target	Spiked	Final			
1	Ubrogepant	50 %	100	50	150	148.77±0.716	99.18±0.478	0.48
2		100 %	100	100	200	198.12±1.140	99.06±0.570	0.58
3		150 %	100	150	250	246.05±0.737	98.42±0.295	0.30
4	Impurity 1	50 %	0.10	0.05	0.15	0.148±0.0002	98.44±0.11	0.12
5		100 %	0.10	0.10	0.20	0.199±0.0004	99.56±0.18	0.18
6		150 %	0.10	0.15	0.25	0.247±0.0009	98.70±0.35	0.35
7	Impurity 2	50 %	0.10	0.05	0.15	0.147±0.0003	98.24±0.20	0.20
8		100 %	0.10	0.10	0.20	0.199±0.0007	99.37±0.33	0.33
9		150 %	0.10	0.15	0.25	0.249±0.0006	99.50±0.22	0.22

Note: *n = 3

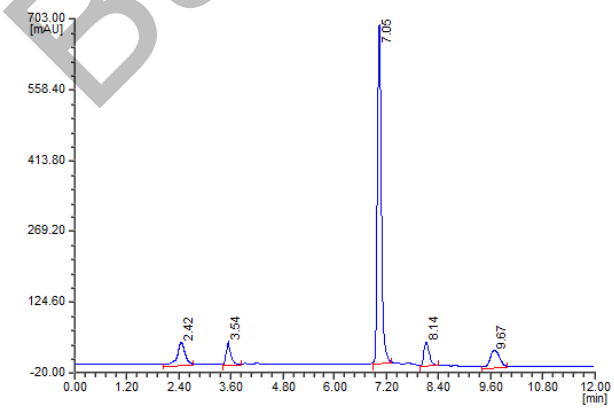
The method was evaluated for its applicability for the separation and analysis of various compounds generated due to stress degradation of Ubrogepant. The Ubrogepant standard drug was exposed to various stress conditions and then the stressed sample was evaluated by the developed method. The resultant chromatograms (Fig. 5) and their results were analyzed to evaluate their applicability for the separation of stress degradants. In acid degradation study, three additional compounds were identified at 5.1 min, 5.9 min and 9.1 min with % assay of 91.85 and % degradation of 8.15. In base degradation study, the % degradation was observed to be 7.32 % with three addition degradation products retained at 1.1 min, 9.1 min and 9.6 min. In peroxide degradation study, less % degradation of 3.8 min with two degradation compounds retained at 2.4 min and 9.6 min. In thermal and UV light degradation study one and four degradation compounds were identified with a % degradation of 4.15 % and 6.63 %. In all stress conditions, the standard Ubrogepant was detected along with the two impurities in the study. It was noticed that the % degradation is less than 10 under all stress conditions, and the method can effectively resolve the known studied impurities along with the stress degradants, effectively proving that the method is stable.



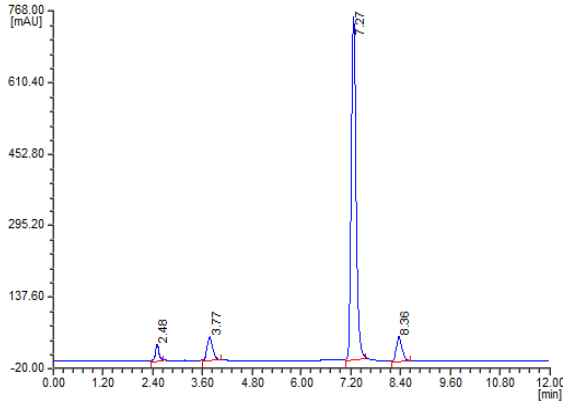
Acid Degradation



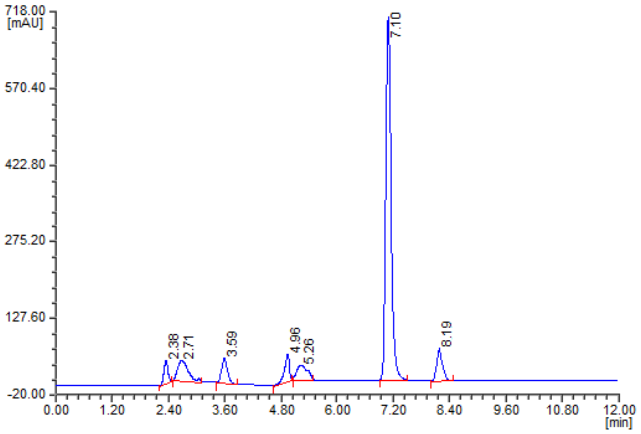
Base Degradation



Peroxide Degradation



Thermal Degradation



UV Light Degradation

Figure 5. Forced degradation chromatograms

The analytical method optimized in the study was applied for its applicability to the estimation of Ubrogepant and its impurities. The formulation solution prepared with 100 mg of Ubrelvy® was used for the formulation assay study. The resultant chromatogram (Fig. 6) shows clear identification and resolution of both impurities in the study along with standard Ubrogepant. The % assay was observed to be 98.75 % for Ubrogepant, 0.09 % for impurity 1 and 0.07 % for impurity 2. The impurities were observed to be under the permissible levels and there is no detection of additional compounds, additional impurities as well as the formulation excipients on the chromatogram. This confirms that the method was significantly used for the evaluation of studied impurities and Ubrogepant in dosage forms.

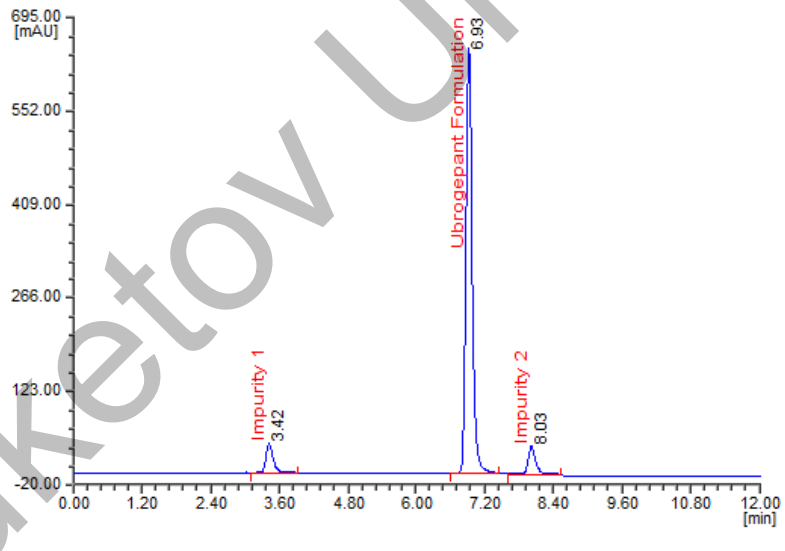


Figure 6. Formulation chromatogram

Conclusions

A simple and novel stability indicating analytical RP-HPLC method was optimized for separation and quantification of two potential impurities, i.e. impurity 1 and 2 of Ubrogepant. The method reports a very sensitive calibration range of 0.05 µg/mL to 0.30 µg/mL, which was achieved for impurity A and B. This confirms that the method can detect impurities at very low levels. Other validation parameters such as specificity, system suitability, accuracy/recovery, repeatability and reproducibility results were under the acceptable level. The method can efficiently resolve, detect and quantify unknown stress degradation products and known impurities of Ubrogepant. Based on the obtained validation results and method application studies, it can be concluded that the method can be effectively utilized for the analysis of Ubrogepant and its impurities in stress samples, bulk drug as well as in formulations.

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