OriginalArticle

Method Development And Validation Of Ubrogepant In Bulk And Pharmaceutical Dosage Form By Using Rp-Hplc

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Abstract

The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredients of Ubrogepant and their related substances. A simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Ubrogepant. The chromatographic strategy utilized Column of Dikma Diamsil (4.6 x 150mm, 3µm), using isocratic elution with a mobile phase of 0.1 percent orthophosphoric acid and Acetonitrile (70:30). A flow rate of 1 ml/min and a detector wavelength of 283 nm utilizing the 2487 Uv detector was given in the instrumental settings. Using the impurity-spiked solution, the chromatographic approach was streamlined. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. LOD and LOQ for the two active ingredients and their impurities were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R^2=0.999, which means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range. The proposed method to be fast, simple, feasible and affordable in RS condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Ubrogepant, RP-HPLC, Development, Validation.

INTRODUCTION

Ubrogepant, sold under the brand name Ubrelvy, is a medication used for the acute (immediate) treatment of migraine with or without aura (a sensory phenomenon or visual disturbance) in adults. It is not indicated for the preventive treatment of migraine. Ubrogepant is a small-molecule calcitonin gene-related peptide receptor antagonist. It is the first drug in this class approved for the acute treatment of migraine. The chemical name of Ubrogepant is (3'S)-N-((3S,5S,6R)-6-methyl-2-oxo-5-phenyl-1-(2,2,2,trifluoroethyl)piperidin-3-yl)-2'-oxo-1',2',5,7-tetrahydrospiro[cyclopenta[b]pyridine-6,3'-pyrrolo[2,3 b]pyridine]-3-carboxamide and has the following structural formula[1-2]

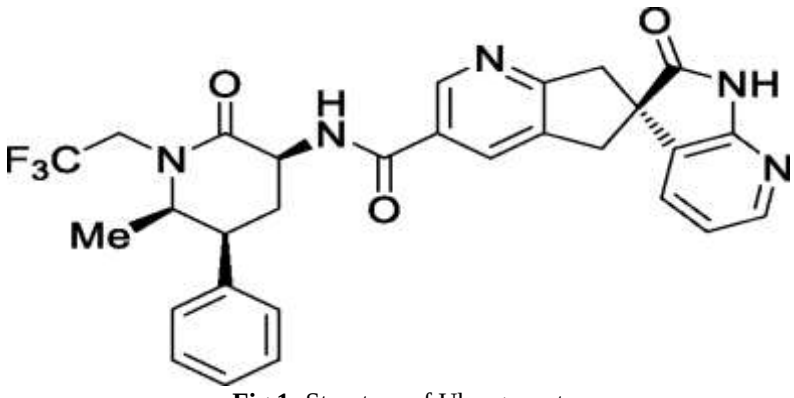

Fig 1: Structure of Ubrogepant

MATERIALS AND METHODS:

Chemicals

Acetonitrile, HPLC-grade Orthophosphoric acid, water, were purchased from Merck India Ltd, Mumbai, India. Ubrogepant standard was procured from Honour Labs, Hyderabad.

The instrumentation

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of UV (model 2487) was used for this study [3-6].

Method optimization

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to orthophosphoric acid with buffer (pH 3.0), Acetonitrile in proportion 70: 30 v/v respectively.UV spectrum of 10µg/ml Ubrogepant in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 283 nm. At this wavelength the drug showing good absorbance.The developed HPLC method was utilized for the estimation of the drug by *in vitro* method. [7].

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [8-16].

PREPARATION OF THEUBROGEPANTSTANDARD & SAMPLESOLUTION:

Standard Solution Preparation:

Accurately weigh and transfer 10mg of Ubrogepant working standard into a 50ml clean dry volumetric flask add about 30ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Ubrogepant)

Sample Solution Preparation:

Accurately weigh and taken the Lyophilized injection Powder equivalent to 10mg Ubrogepant(marketed formulation=30.12 mg Powder) sample into a 50ml clean dry volumetric flask add about 30 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 1.5 ml of Ubrogepant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. (30ppm of Ubrogepant)

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Ubrogepant peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients from their impurities. In order to provide a good performance, the chromatographic conditions were optimized.

Calculation: **(For Ubrogepant)**

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim mg/ml.

Assay Results: **(Ubrogepant)**

$$\frac{4857079}{4848095} * \frac{10}{50} * \frac{1.5}{10} * \frac{50}{240.4} * \frac{10}{1.5} * \frac{120.2}{5} * \frac{99.8}{100} * 100 = 99.98\%$$

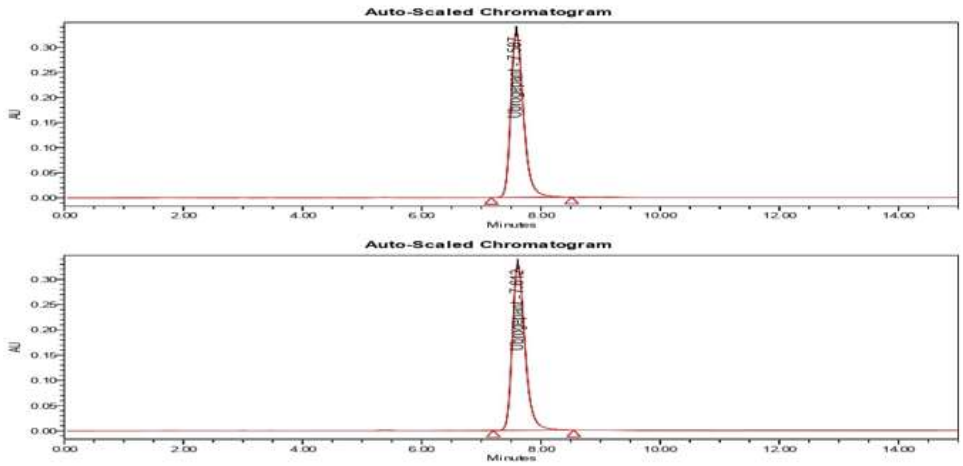

Fig:1 Chromatograms of Standard Ubrogepant

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Ubrogepant 10, 20, 30, 40, 50 µg/ml respectively. Linearity was performed in the range of 10-50 µg/ml of Ubrogepant. The correlation coefficients achieved 0.999.

Table: 1 Results of Linearity

S. No	Linearity Level	Concentration(µg/ml)	Area
1	I	10	2645369
2	II	20	4963544
3	III	30	7351889
4	IV	40	9716723
5	V	50	11948077
Correlation Coefficient			0.999

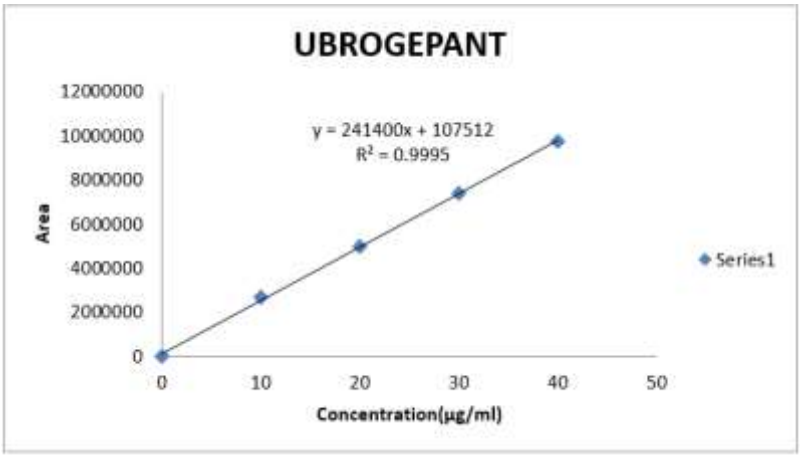

Fig: 2 Calibration Plot of Ubrogepant

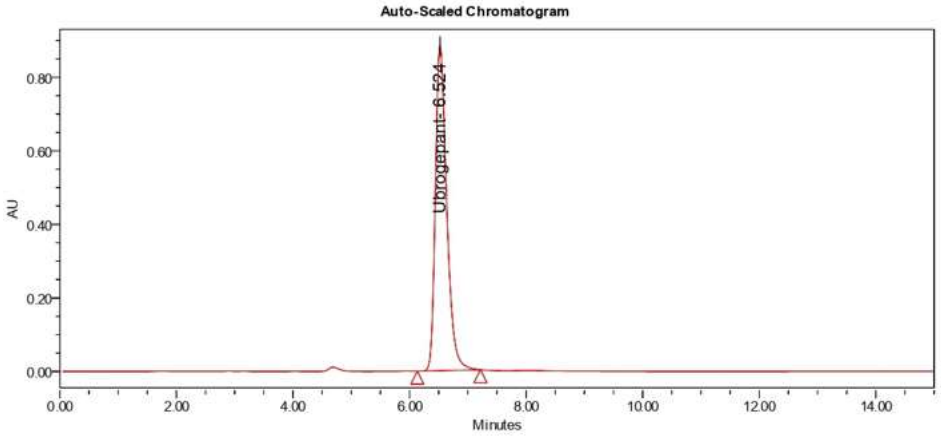

Fig. 3 Chromatogram of Linearity

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution spiked with known amounts of all the impurities at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. For all impurities, percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 97-100% for Ubrogepant. The results are given in table 2.

Table: 2 Results of Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2419060	5	4.98	99.59	98.30
100%	4766550	10	9.81	98.12	
150%	7081276	15	14.58	97.18	

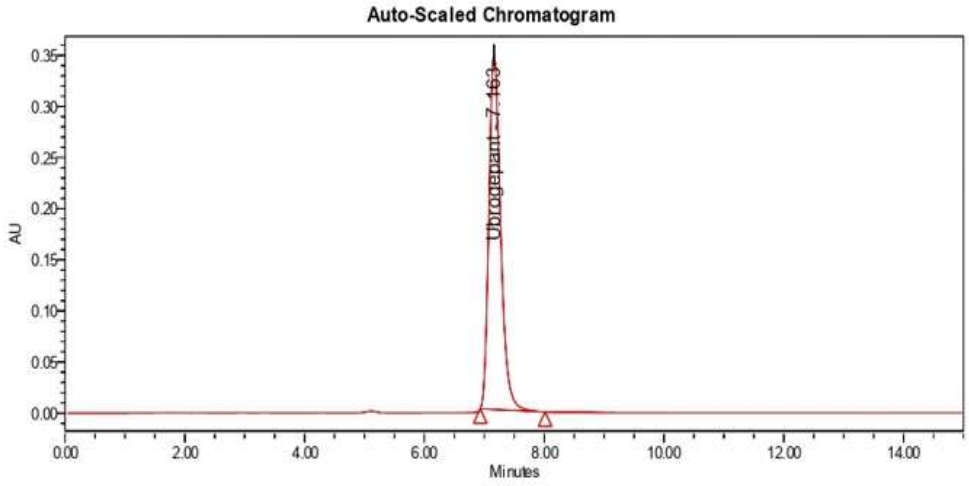

Fig:4 Chromatogram of Accuracy

Precision

The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table: 3 Results of Precision

Injection	Area for Ubrogepant
Injection-1	4843271
Injection-2	4858055
Injection-3	4853667
Injection-4	4855803
Injection-5	4860271
Injection-6	4871412
Average	**4857079.9**
Standard Deviation	**9170.9**
%RSD	**0.2**

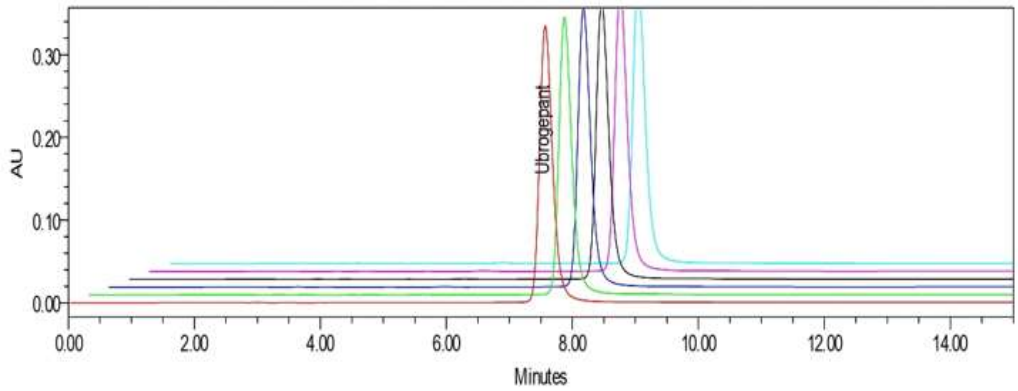

Fig:5 Chromatogram of Precision

Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day. The standard solutions prepared in the precision were injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table: 4 Results of ruggedness

Injection	Area for Ubrogepant
Injection-1	4843271
Injection-2	4858055
Injection-3	4853667
Injection-4	4855803
Injection-5	4860271
Injection-6	4871412
Average	**4857079.9**
Standard Deviation	**9170.9**
%RSD	**0.2**

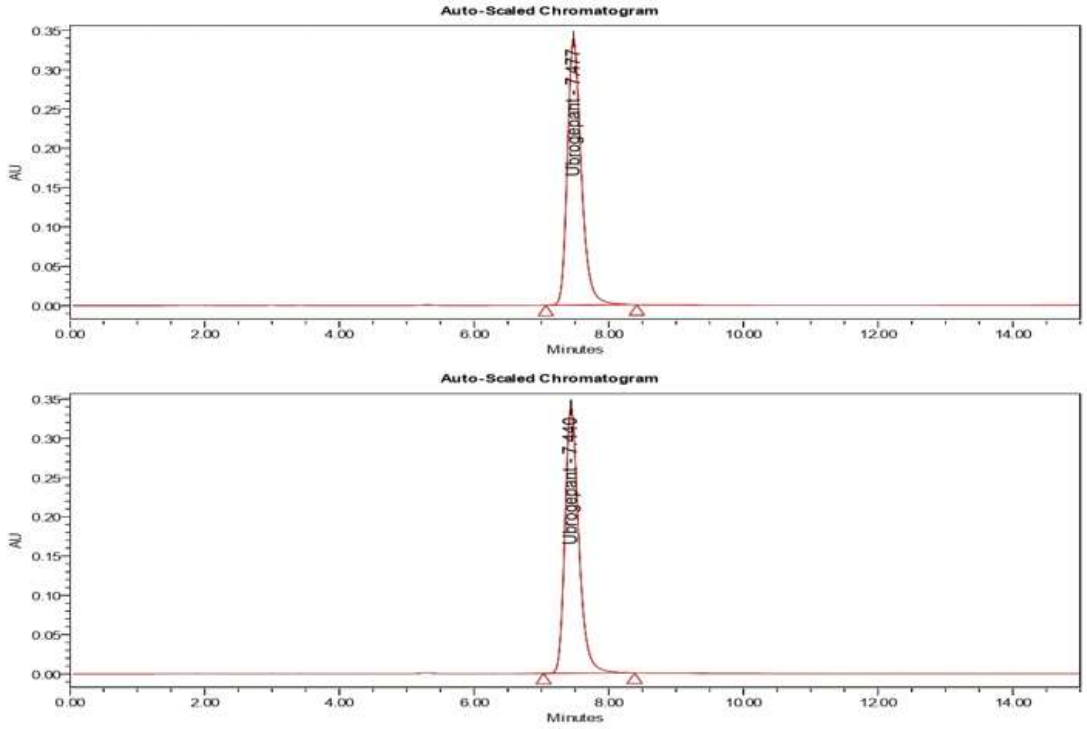

Fig: 6 Chromatograms of Ruggedness

Robustness

The conditions of the experiment were designed to test the robustness of the established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for Ubrogepant found to be within the limit and results are tabulated in table 5 and 6.

Table: 5 Results of Robustness (Flow rate)

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7026.47	1.30
2	1.0	6505.69	1.31
3	1.2	6391.66	1.27

Table: 6 Results of Robustness (Mobile Phase)

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	7026.41	1.30
2	*Actual	6505.69	1.31
3	10% more	6391.66	1.30

Degradation studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Ubrogepant using the proposed method [17-18].

Preparation of stock

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10mg Ubrogepant (marketed formulation=30.12 mg of tablet Powder) sample into a 50ml clean dry volumetric flask add about 30 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.3ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation

Ubrogepant sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation

Pipette 0.3ml above stock solution into a 10ml volumetric flask and 1ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 0.3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Table: 7 Results of degradation studies

Sample Name	Ubrogepant				
	Area	% Degraded	Purity Angle	Purity Threshold	Peak purity
Standard	4848095				
Acid	4481906	7.55	0.339	1.250	Passes
Base	4453667	8.14	0.208	1.252	Passes
Peroxide	4410116	9.03	0.123	0.262	Passes
Thermal	4526404	6.64	0.180	0.255	Passes
Photo	4545274	6.25	0.168	0.253	Passes

CONCLUSION

We present in this article simple, selective, validated and well- defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Ubrogepant. All the products of degradation formed during the stress conditions and the related active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in RS condition. Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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Conflict of Interest

No conflict of Interest.

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