Research Article

Stability Indicating RP-HPLC Method for Development and Validation of Atogepant and its Application in Dissolution Studies

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ABSTRACT

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Atogepant bulk and pharmaceutical formulations. Separation of Atogepant was successfully achieve Dona: WATERS 150X4.6mm, 5µm, C18 or equivalent in an isocratic mode utilizing K_2HPO_4: Methanol (55:45) at a flow rate of 1.0 mL/min and eluate was monitored at 272nm, with a retention time of 2.860 minutes for Atogepant respectively. The method was validated and their response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Atogepant. The values of the correlation coefficient were found to 1.000 for Atogepant respectively. The LOD and LOQ for Atogepant were found to be 0.050 and 0.165 respectively. This method was found to be good percentage recovery for Atogepant were found to be 100 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Accuracy, Precision, Specificity and Robustness.

Keywords: Atogepant, High performance liquid chromatography.

QUICK RESPONSE CODE →

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INTRODUCTION

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Most of the drugs can be analyzed by HPLC method because of several advantages like rapidity, specificity, accuracy, precision, reproducibility, ease of automation and eliminates tedious extraction and isolation procedures.[1-10]

Atogepant, sold under the brand name Qulipta, is a medication used to treat migraines. It is a gepant, an orally active calcitonin gene-related peptide receptor (CGRPR) antagonist. It was approved for medical use in the United States in September 2021.[11-12]

MATERIALS AND METHODS

Apparatus

HPLC device set with detector photodiode array detector (make-"Waters Alliance company"),Version two empower software (make - "Waters Alliance company"),Thermal oven, Membrane filter having 0.45 µm dimension pore, Ultra-Sonicator, Lab India-Dissolution apparatus.

Materials

Atogepant (ATG), K_2HPO, Acetonitrile, methanol, water, potassium dihydrogen phosphate. Columns used: Agilent C18, Thermo C18, Inertsil C18, Waters C18.

Stock Solution

Accurately weighed and transfer 60mg Atogepant working standards into a 100ml clean dry volumetric flask respectively, add 30ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Atogepant.

Working Solution

Working standard solutions were prepared by taking 1ml of stock solutions of Atogepant to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 60µg/ml of Atogepant.

Formulation Solution

One vial powder was weighed and powder equivalent to 60mgof Atogepant was taken into 100 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up with the diluent. The above sample solution was filtered, 1ml of filtrate was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method Validation

The proposed methodology was verified in keeping with "International Conference on Harmonization" strategies

Specificity

Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

Linearity

Aliquots of about 30mg, 45mg, 60mg, 75mg, and 90mg mL were taken from mixed ternary standard stock solution (solution-A) concurrently transferred into different volumetric flasks of 10 mL capacity. These solutions were diluted upto the mark with diluent. Such that the final concentrations were in the range of 30-90 µg/mL for Atogepant, respectively. Volume of 10µl of sample was injected in six times for each concentration level and calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak response vs. concentration was observed in the range of study.

LOQ and LOD

Both the LOQ and the LOD were calculated using a signal-to - noise concept. LOQ was described as the minimal level of quantity of analyte leading to a peak height of ten times the baseline noise (i.e signal-to-noise ratio is ten). LOD was described as the minimal level of quantity of analyte leading to a peak height of three times the baseline noise (i.e signal-to-noise ratio is three).

Precision

Precision was obtained by the assessment of combined working solution (60 µg/ml ATG) on the same day in six replicates. Determined the ATG mean peak.

Accuracy

The accuracy was assessed using standard technique of addition. In this technique, previously analysed placebo solution was spiked with extra 50% (30 µg/ml - ATG), 100% (60 µg/ml - ATG) and 150% (90 µg/ml - ATG) contents of analytes. Read values and relative standard deviation values of ATG peak areas.

Robustness

The working ATG solution (ATG - 60 µg/ml) was appraised via consciously fluctuating the chromatographic settings.

Stability of ATG

The formulation stock ATG solution with ATG quantity 100 µg/ml was stressed out consistent using ICH directions with situations like: Acid hydrolysis, Base hydrolysis, Dry heat, Oxidation, Sun light.

- **Acidic stress:** 1 ml of 0.1N hydrochloric acid followed by 30 min sonication at room temperature
- **Basic stress:** 1 ml of sodium hydroxide followed by 30 min sonication at room temperature
- **Oxidative stress:** 1 ml of 30% hydrogen peroxide followed by 30 min sonication at room temperature
- **Neutral stress:** 1 ml of distilled water followed by 30 min sonication at room temperature
- **Photo stress:** Exposing 1 ml of tablet stock solution to direct sunlight for 24 hr
- **Dry heat stress:** Exposing 1 ml of tablet stock solution to 105 °C for 30 min in hot air oven.

Dissolution Study

Dissolution Media

Weigh and transfer about 2grm of sodium Perchlorate in beaker containing mixture of 0.5ml of triethylamine and 800ml of water. Adjust pH 3.6 (±0.05) with phosphoric acid.

RESULTS

Optimized Method Conditions

Complete resolution of atogepant were obtained by employing Waters C18 (250 × 4.6 mm, particle dimension of 5 µm) column set with 25°C temperature and with mobile phase system of K_2HPO_4: Methanol (55:45)

Flow rate was 1.0 ml per min with 10 µl of sample was injected for one analysis. Quantification of atogepant simultaneously was done with photodiode array detector fine-tuned to 226 nm. Typical chromatogram of atogepant using optimized method conditions was displayed in Figure 1.

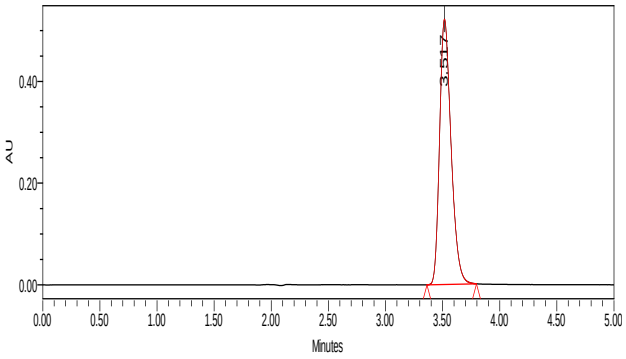

Figure 1: Optimized chromatogram

Validation

Specificity

Chromatograms explain that retention time for standard, sample and commercial product of Atogepant are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So, the method is highly selective.

Displayed in table 1.

Table 1: Specificity data for atogepant

S .no	Sample name	Atogepant area	Rt
1	Standard	3165241	2.860
2	Sample	3153623	2.851
3	Blank	-	-
4	Placebo	-	-

Linearity

Linearity scope was 50 – 150 µg/ml for ATG. The obtained regression equations along with regression coefficient where:

ATG linearity equation: y = 31569x-25761 R^2 = 0.999, displayed in fig 2.

Table 2: Linearity data for ATG

S.No	Conc (µg/ml)	RT	Area
1.	50	2.840	1562606
2.	75	2.845	2360967
3.	100	2.847	3158263
4.	125	2.851	3940901
5.	150	2.853	4747366
Correlation coefficient (r^2)			0.999

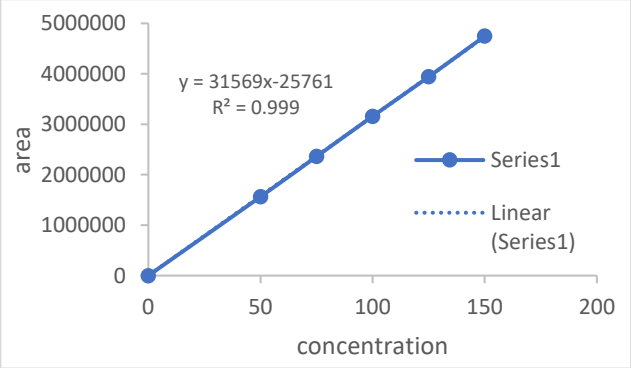

Figure 2: Linearity graph for Atogepant

Limit of Detection

Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD.

- Atg's Detection limit – 0.050 µg/ml having S/N proportion estimate – 3.8 µg/ml.

Limit of Quantification

Minimum concentration of standard component in which the peak of the standard gets detected and quantification

- ATG's Quantitation limit – 0.165 µg/ml having S/N proportion estimate – 10.1 µg/ml.

Precision

The mean peak area value is 1847672. The relative standard deviation values were 0.3 for ATG. in below table 3.

Table 3: Precision data for Atogepant

Response reached	
3153623	**Mean response peak area**
3152649	1847672
3161263	**Standard response Deviation**
3150696	6061.6
3156699	**RSD**
3167397	0.3

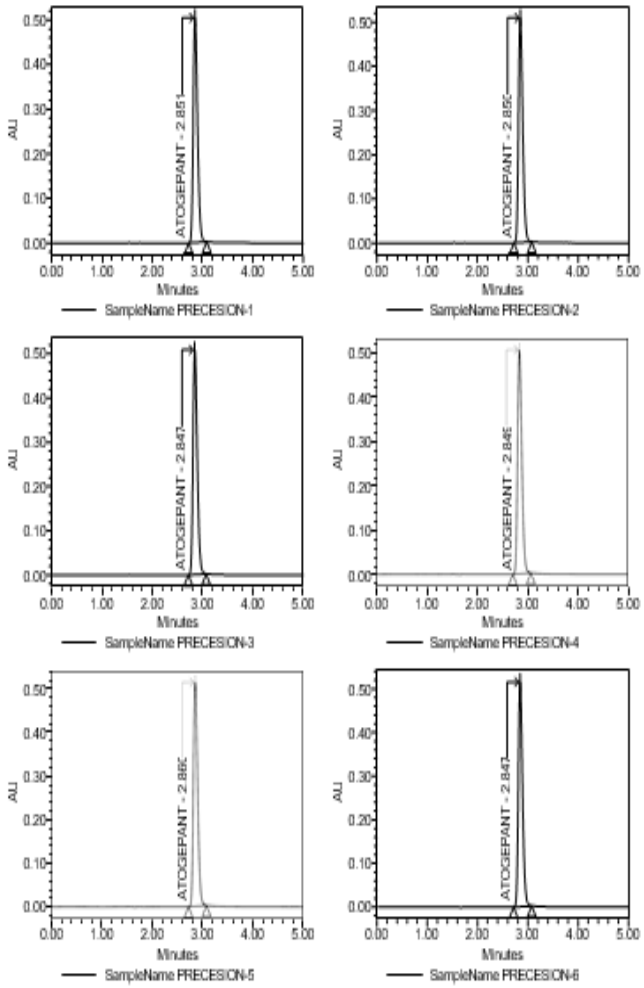

Figure 3: Graphs for precision

Accuracy

The average recovery of ATG detected in the spiked placebo solution was 100%, 100% and 101% at 50%, 100% and 150% spiked levels, respectively (Table 04). As shown below,

Table 4: Accuracy data for Atogepant

S.NO	Accuracy Level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	30.00	29.700	29.52	99	100
		2	30.00	29.700	29.50	99	
		3	30.00	29.700	29.71	100	
2	100%	1	60.00	59.400	59.56	100	100
		2	60.00	59.400	59.55	100	
		3	60.00	59.400	59.71	101	
3	150%	1	90.00	89.100	89.46	100	101
		2	90.00	89.100	89.76	101	
		3	90.00	89.100	89.54	100	

Robustness

Table 5: Robustness data for Atogepant

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	3.515	5837	1.30
Increased flow rate(1.2ml/min)	2.371	5158	1.28
Decreased temperature(20^0c)	3.133	5450	1.29
Increased temperature(30^0c)	2.593	5275	1.27
Decreased comp rate(5%)	2.371	5158	1.28
Increased comp rate(5%)	3.133	5450	1.29
Decreased pH(0.2)	2.851	5415	1.27
Increased pH(0.2)	2.850	5545	1.26
Decreased nm(2)	2.861	6099	1.24
Increased nm (2)	2.860	5996	1.26

Degradation Study

The stability investigations been performed out employing 60ml of ATG stock solution utilizing ICH eligibility requirement conditions like: Degradation accelerated by acid, Degradation accelerated by alkali, Oxidation accelerated by peroxide, Degradation accelerated by temperature, and Degradation accelerated by sun. The results for stability study are in below table

ATG stability direction:105° C >0.1N HCl > 0.1N NaOH > Sunlight >30% H_2O_2

Table 6: Degradation data for Atogepant

Condition	Percent recovered	Percent degradation
	Atogepant	Atogepant
0.1 N HCl	88.05	11.95
0.1N NaOH	91.70	8.30
30% H_2O_2	94.23	5.77
105°C	90.47	90.47
Sunlight	93.72	6.28
Water	98.83	1.17

Dissolution studies

Tablets are evaluated in vitro with a Dissolution Test. In order to identify how the medication should be taken, release information is provided by the Dissolution. The assay contents of ATG in dissolution formulation solution were set on below table:

Table 7: Dissolution data for Atogepant

Atogepant Drug	Area	%Drug Dissolved	Acceptance criteria
D1	2983623	2983623	Not less than 80%
D2	2942649	2942649	
D3	3001263	3001263	
D4	2890696	2890696	
D5	2916699	2916699	
D6	2967397	2967397	

CONCLUSION

A thorough literature survey has revealed that NO analytical methods using RP-HPLC were reported for the estimation of ATOGEPANT in tablet formulation. RP-HPLC method for the estimation of Atogepant in its dosage form was established and validated for system suitability, accuracy, linearity, precision, LOD, LOQ, robustness, stability, dissolution as per the ICH guidelines. The present analytical method in turn employed a technique to evaluate product stability and monitor validation parameters and degradation of formulation and to apply the developed method in dissolution studies.

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