STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETECTION AND QUANTIFICATION OF ATOGEPANT IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Analytical method development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance departments. Analytical procedures play a critical role in equivalence and risk assessment, management. It helps in establishment of product-specific acceptance criteria and stability of results. In view of this, the present work is intended to develop an efficient and simple HPLC method for the determination of Atogepant in bulk and was applied on marketed Atogepant products. The pharmaceutical drug Atogepant is a gepant class drug and is an orally active calcitonin gene-related peptide receptor (CGRPR) antagonist. It was indicated for the preventive treatment of episodic migraine in adults. In the HPLC analysis, the mobile phase used for the chromatographic runs consisted of methanol, acetonitrile and triethyl amine in 55:40:5 (v/v). The separation was achieved on a Waters Spherisorb ods2 C18 (250 mm × 4.6 mm; 5µ)column using isocratic mode. Drug peak was well separated and were detected by a UV detector at 248 nm.The method was linear at the concentration range of 20–120 µg/mL for Atogepant. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.125 µg/mL and 0.413 µg/mL for Atogepant. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Atogepant in bulk drug, formulations as well as stability testing.

Key words: *Atogepant, Method validation, HPLC analysis, Liquid chromatography &Ultraviolet detector*

INTRODUCTION

Pharmaceutical analysis is basically concerned with the determination of the chemical composition of matter however, identification of substance, the elucidation of its structure and quantitative analysis of its composition are the aspects covered by modern analytical techniques.Analytical chemistry may be derived as the science and art of determining the composition of material in terms of the elements of compounds contained. [1]. Analytical chemistry involves the application of range of techniques and methodologies to obtain and access quantitative and structural information on the nature of matter [2, 3]. The quality of a drug is the degree of possession of all those characteristics designed and manufactured into it, which contributes in the performance of the intended function, when the drug is used as directed[4,5]. The most important aspect of analysis is quantitative chemical analysis. In the present age, the physical, chemical and biological analysis, involve computerized techniques to facilitate better result [6-8].Parameters usually examined in the validation process are limits of detection and quantization, accuracy, precision, selectivity/specificity, linearity, range and ruggedness[9].

Atogepant is a medication used to treat migraines. It is a gepant, an orally active calcitonin gene-related peptide receptor (CGRPR) antagonist. Atogepant is indicated for the preventive treatment of episodic migraine in adults. A study found that atogepant reduced the number of migraine days over twelve weeks. Figure 1 shows the molecular structure of Atogepant[10].

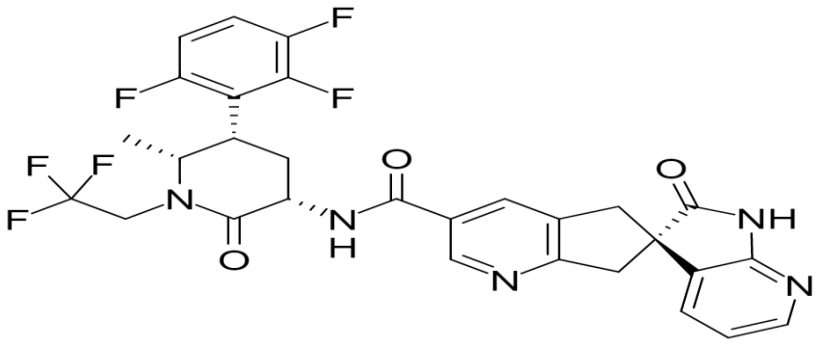

Figure 1:Molecular structure of Atogepant

The currently accepted theory of migraine pathophysiology considers dysfunction of the central nervous system, in particular the trigeminal ganglion, to be the root cause behind the condition.Activation of the trigeminal ganglion triggers the stimulation of trigeminal afferents that project to the spinal cord and synapse on various pain-sensing intra- and extracranial structures, such as the dura mater. Pain signals are then further transmitted via second-order ascending neurons to the brainstem, hypothalamus, and thalamic nuclei, and from there to several cortical regions (e.g. auditory, visual, motor cortices) [11]. The trigeminal ganglion appears to amplify and perpetuate the migraine headache pain through the activation of perivascular fibers and the release of molecules involved in pain generation, such as calcitonin gene-related peptide (CGRP).

Atogepant is an antagonist of the calcitonin gene-related peptide receptor - it competes with CGRP for occupancy at these receptors, preventing the actions of CGRP and

its ability to induce and perpetuate migraine headache pain [12].There are no data regarding overdosage with atogepant. Symptoms of atogepant overdose are likely to be consistent with its adverse effect profile and may therefore include significant gastrointestinal effects, such as nausea and constipation, as well as fatigue and somnolence. A single oral dose of 300mg (5x the maximum recommended dose) did not result in any serious adverse events and did not appear to impact cardiac function [13].

The literature survey was conducted for the available analytical methods for the estimation of atogepant in bulk drug as formulations using various analytical techniques. Based on the literature it was confirmed that there is no analytical method published for the estimation of atogepant in pharmaceutical formulations. As the literature review doesn't having adequate methods for the analysis ofatogepant, the present work was intended to develop a simple and precise HPLC method for the analysis of atogepant in bulk drug as well as in formulations. While developing the analytical method, along with ICH guidelines [14-17], the following literature was referred to obtain adequate knowledge for the development of HPLC method.

The aim of the research work to develop and validated analytical method for the determination of fexinidazole in single dosage form and estimation of degradants generated during storage of finished products using techniques such as High-performance liquid chromatography.

MATERIALS AND METHODS

The details of the instruments used in the study were given in table 1:

S No	Instrument	Model
1	HPLC system	LC – 7000, PEAK HPLC (India)
2	Pump	LC-P7000
3	Injection mode	Manual
4	Injector	Rheodyne type [model 7725]
5	Injection volume	20 µL fixed volume loop
6	Injection	Hamilton [USA]
7	Detector	UV Detector
8	Software	Autochro -3000 [Young Lin - Korea]
9	Weighing Balance	Denver - SI-234 [Bohemia]
10	UV-Visible spectrophotometer	Teccomp UV-2301 [India]
11	pH meter	Systronics - Sr No S 1326 [India]
12	Ultrasnic Bath Sonicator	GT Sonic [India]
13	Vacuum filtration	Borosilicate vacuum filtration kit
14	Membrane filter (0.2 µ)	Merck Millipore [USA]

Table 1: Instrumentation Details for the present study

Chemicals and solvents:

The working standard drug Atogepant (98.83 % purity) along with the formulation dosage form (Qulipta® - 60 mg) were obtained from Abbvie Biopharmaceuticals Private Limited, Mumbai. HPLC grade Methanol, Water and Acetonitrile were purchased from Merk chemicals private limited, Mumbai. The buffer solutions used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard drug solution:

Preparation of standard stock solutionwas the primary step prior to experimental work. A standard stock solution of 1000µg/mL was prepared by weighing accurately 10mg of the standard drug Atogepant and was taken in a 10mL volumetric flask having little amount of Methanol.Dissolve the drug in the solvent and make up to the mark.Then it was filtered through 0.45µ filter paper to remove un-dissolved particles or any solid substances.The solution was preserved safely and used when required. By diluting the standard solution with mobile phase, different concentrations (20, 40, 60, 80 &100µg/mL) of standard solutions was prepared and used in the present study.

Preparation of formulation solution:

Tablets of Qulipta®brand containing 450 mg of Atogepant was powdered using a sterile mortar and pestle. Then an amount of tablet powder equivalent to 50 mg of Atogepant was accurately weighed and dissolved in 50 mL solvent using sonicator and filtered through 0.45 µ membrane filter. Then it was diluted while doing the formulation analysis.

HPLC Method Development:

***Selection of wavelength*:**

To select an appropriate monitoring wavelength, the standard solutions of 10µg /mL was prepared and scanned by the UV-Vis spectrophotometer. The obtained wavelength maxima were selected as suitable wavelength for the detection.

***Selection of stationary phase*:**

Since the Atogepant is a Polar drug, a non-polar C18 column was selected for the separation of the drug. Different columns of different companies, manufactures and configurations were tested.

***Method Development trails*:**

Trail.no	Parameter	Condition	Trail.no	Parameter	Condition
I	MP	Methanol and water in 65:35 (v/v)	**IV**	MP	Methanol and Acetonitrile in 50:50 (v/v)
	Wavelength	248 nm		Wavelength	248 nm
	Stationary Phase	Zodiac c18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Spherisorb C18 column (250mm × 4.5 mm; 5µm)
	Flow Rate	1.0 mL/min		Flow Rate	1.0 mL/min
	Pump Mode	Isocratic		Pump Mode	Isocratic
	pH of MP	5.7		pH of MP	5.6
II	MP	Methanol and water in 65:35 (v/v)	**V**	MP	Methanol and Acetonitrile in 80:20 (v/v)
	Wavelength	248 nm		Wavelength	248 nm

	Stationary Phase	Kromasil C18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Spherisorb C18 column (250mm × 4.5 mm; 5µm)
	Flow Rate	1.0 mL/min		pH of MP	5.3
	Pump Mode	Isocratic		Flow Rate	1.0mL/min
	pH of MP	6.0		Pump Mode	Isocratic
III	MP	Methanol and Acetonitrile in 20:70 (v/v)	**VI**	MP	Methanol, acetonitrile and triethyl amine in 55:40:5 (v/v)
	Wavelength	248 nm		Wavelength	248 nm
	Stationary Phase	Kromasil C18 column (250mm × 4.5 mm; 5µm)		Stationary Phase	Waters Spherisorb ods2 C18 column (250 mm × 4.6 mm; 5µ)
	Flow Rate	1.0 mL/min		pH of MP	5.6
	Pump Mode	Isocratic		Flow Rate	1.0 mL/min
	pH of MP	5.5		Pump Mode	Isocratic

Table 02: Method Development trails HPLC method development

Analytical Method Validation:

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity, and specificity, according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the chromatograph.

***Specificity*:**

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Atogepant (standard), blank and sample solutions to those obtained for tablet solutions. Retention time of the Atogepant in standard solution, and in the sample solution was compared to determine the specificity of the method.

***System suitability*:**

The system suitability was determined by making six replicate injections of the standard solution and analyzing Atogepant for its peak area, peak USP tailing factor, and number of theoretical plates. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

***Sensitivity of the method*:**

The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Atogepant for LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

***Linearity and Range*:**

The calibration curve in the developed method was constructed from LOQ concentration. Atogepant standard stock solution of 100 mg/mL was used for preparation of subsequent aliquots. Various aliquots were prepared by serial dilution as given in figure 9. Sample solution was loaded and 20µL was injected into column. All measurements were repeated for each concentration. The calibration curve of the area under curve versus concentration were recorded. Form the calibration curve, correlation and regression values were calculated for Atogepant.

***Precision*:**

The precision studies were carried out by estimating response of Atogepant six times at a standard concentration of 80µg/mL and results are reported in terms of %RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and interday for three different days and it was expressed as the percentage relative standard deviation (%RSD) which was calculated as per the following expression

%RSD = (standard deviation / mean) x 100.

***Accuracy/ Recovery*:**

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Atogepant. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

%RSD = (standard deviation / mean) x 100.

***Ruggedness*:**

Two laboratory analysts carried out the precision of Atogepant at a standard concentration of 80 µg/ml was prepared by different analysts in the laboratory conditions, the prepared solution was analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of %RSD which must be less than 2.

***Robustness*:**

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The % change in each of the changed condition was calculated.

Methodology for Forced degradation study:

The forced degradation study is considered a vital analytical aspect of the drug development program for small molecules. Forced degradation, commonly known as stress testing, is carried out to demonstrate as specificity to developed a stability-indicating

analytical method, using high-performance liquid chromatography (HPLC), i.e., a single analytic method that is capable of separating the degradant peaks from the drug substance/drug product peak. As per International Conference on Harmonization (ICH) guidelines (Q1A), stability studies need to be performed to propose the shelf life of new drug substances and/or drug products.

Hence stress degradation studies such as acidic, base, peroxide, thermal and UV light was performed for standard Atogepant in the developed method.

Acid Hydrolysis:

50 mg of drugs were mixed with 50ml of 0.1N HCl solution. The solution was neutralized and diluted up to standard concentration (80 µg/ml) and was analyzed in the developed method condition

Base Hydrolysis: 50 mg of drugs were mixed with 50ml of 0.1N NaOH solution. The solution was neutralized and diluted up to standard concentration i.e 80 µg/ml and was analyzed in the developed method condition

Oxidative Degradation:

50 mg of drugs were with 50ml of 3% Peroxide solution. The solution was neutralized and diluted up to standard concentration (80 µg/mL) and was analyzed in the developed method condition

Photolytic Degradation:

50 mg of drug sample was kept in UV light [254 nm]. After the selected time of light expose, the drug solution was prepared and was analyzed

Thermal Degradation:

50 mg of drug sample was kept in oven at 60 ^{0}C. After the selected time of light expose, the drug solution was prepared and was analyzed

Formulation analysis:

This proposed method was applied to the determination of Atogepantin commercially combined tablets. The sample solution at a concentration of 80 µg/mL of Atogepant was analyzed in the optimized conditions. Peak area of the resultant chromatogram was used for the estimation of assay using label clime recovery method. The % assay was calculated for Atogepant using the standard calibration values.

RESULTS AND DISCUSSION

HPLC Method Development:

The spectrum of diluted solutions of the Atogepant in methanol was recorded separately on UV spectrophotometer.The ultraviolet absorption spectra of the Atogepant demonstrated that the maximum absorption at a wavelength near 248 nm, and it was therefore chosen during the entire study.

For developing the method,a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength by spectrophotometer and choice of stationary and mobile phases. The following studies were conducted for this purpose.

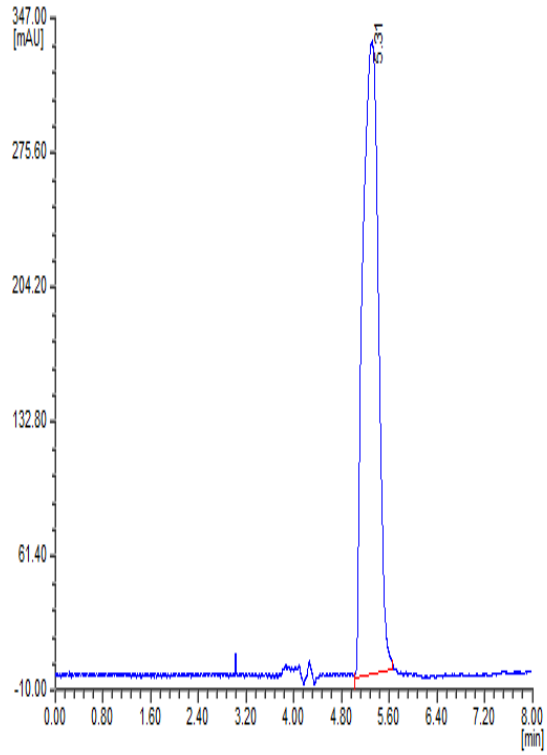

Figure 2: Chromatogram observed for Atogepantin trail 1

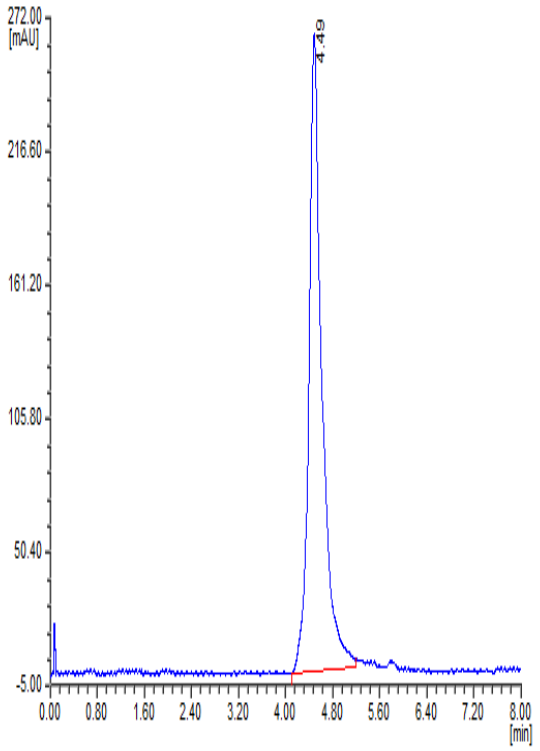

Figure 3: Chromatogram observed for Atogepant in trail 2

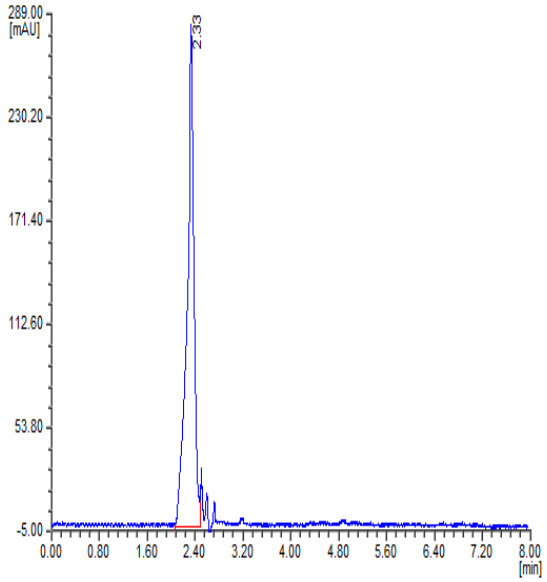

Figure 4: Chromatogram observed for Atogepant in trail 3

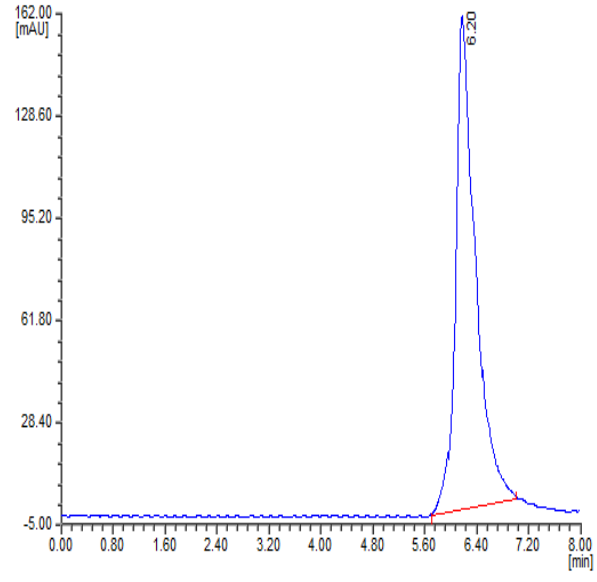

Figure 5: Chromatogram observed for Atogepant in trail 4

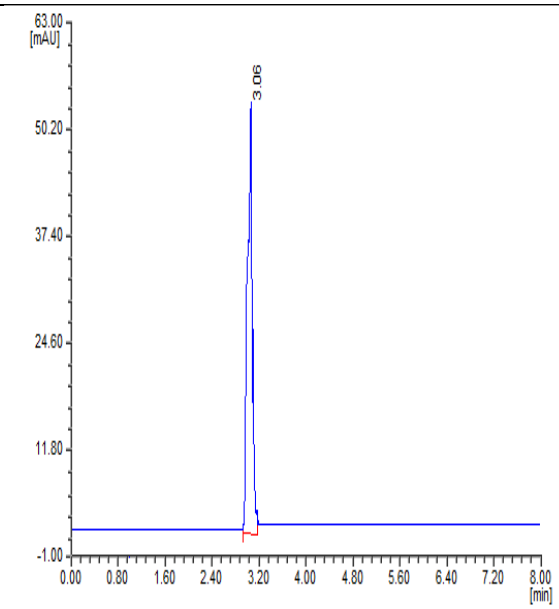

Figure 6: Chromatogram observed for Atogepant in trail 5

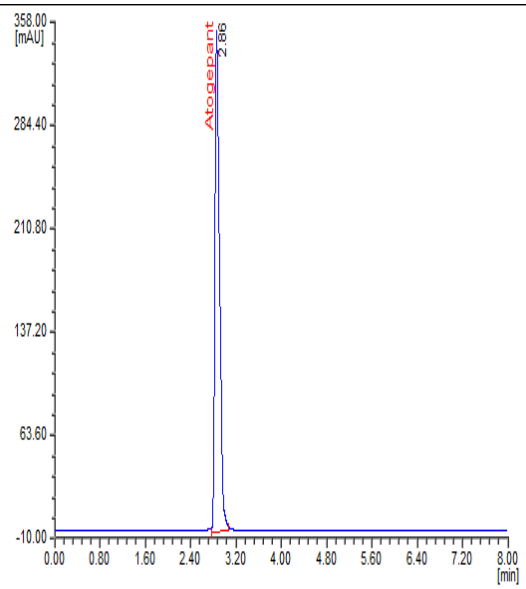

Figure 7: Optimized Chromatogram observed for Atogepant in trail 6

From trail1 to trail 5 the peak corresponding to Fexinidazole was not identified but in the trail 6, peak was sharp compared with previous trails with broad peak base.

In the optimized conditions, well resolved, retained and accepted system suitability was observed. The optimized conditions were given in table 3.

S.No	Parameter	Results
1	Mobile phase	Methanol, acetonitrile and triethyl amine in 55:40:5 (v/v)
2	Wavelength	248 nm
3	Stationary Phase	Waters Spherisorb ods2 C18 column (250 mm × 4.6 mm; 5µ)
4	pH of MP	5.6
5	Flow Rate	1.0 mL/min
6	Pump Mode	Isocratic
7	Pump Pressure	8.3 ±5 MPa
8	Run time	8 min

Table 3: Optimized chromatographic conditions

Method Validation:

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and stress degradation studies.

Specificity:

It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. Sharp peak was obtained for Atogepant at retention times of 5.35min. This peak was not detected in

the blank solution. Retention times of the drugs in standard solutions, in the mixed standard solutions and in the sample solutions were the same. This result indicated specificity of the method. Furthermore, there was no interference from the excipients present in the tablets; thus, the method was considered specific and selectivity.

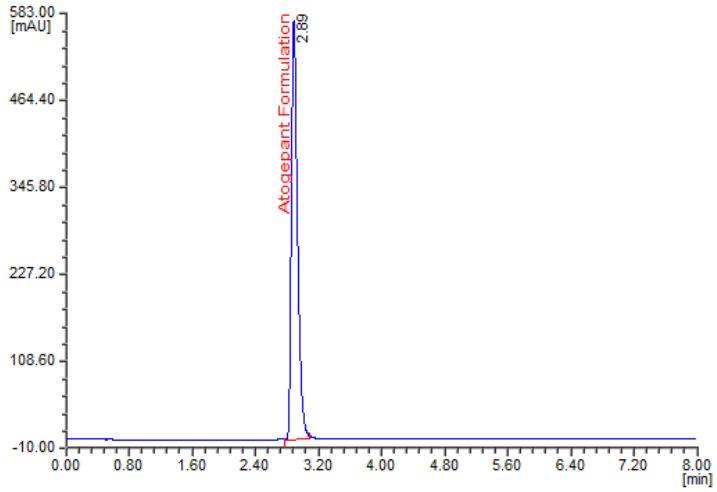

Figure 8: Chromatogram of Sample in the optimized conditions

System suitability:

In the optimized conditions, peak tailing factors of was found to be 0.95 for Atogepant whereas the number of theoretical plates was found to be 5784.

Linearity and Range:

Linearity was performed by preparing mixed standard solutions of Atogepant at different concentration levels including working concentration mentioned in experimental condition i.e.80 µg/ml. twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 248 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually.Linearity was observed in the concentration range of 20-120 µg/ml for Atogepant with Linear regression equation ofy = 9637.3x - 7673.2 (R^2 = 0.9998).

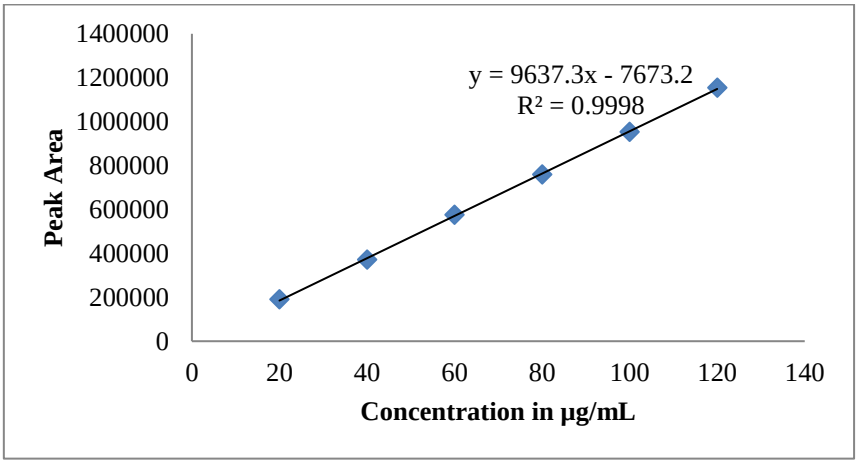

Figure 9: Linearity graph

Precision:

The %RSD was found to be 0.20 and 0.24 for Atogepant in intraday precision and interday precisionrespectively. The %RSD was found to be within the acceptance limit of less than 2. Hence the developed method was found to be precise. Results were given in table 4 for intraday and interday precision respectively.

S. No	Intraday Precision	Interday Precision
1	757485.3	759379.0
2	754582.9	757223.9
3	756339.1	757927.4
4	754857.2	755763.0
5	755485.2	757373.9
6	758462.1	760965.0
% RSD	**0.20**	**0.24**

Table 4: Precision results

Accuracy/ Recovery:

Accuracy was confirmed by carrying out recovery study as per ICH guidelines, where for a pre analyzed sample solution, known concentration of standard solution was added equivalent to 50%, 100%, 150% of labile claimed.Table 5 shows the recovery results of Atogepant in the developed method. The %recovery was fond to be within the range of 98.63 to 99.82 %. The %RSD in each spiked level was found to be 0.41, 0.21 and 0.54 % for Atogepant in 50 %, 100 % and 150 % spiked level respectively.

% Recovery	Concentration in µg/ml			Amount Found	% recovery	% RSD
	Target	Spiked	Total			
50%	40	20	60	59.153	98.59	0.46
	40	20	60	59.685	99.48	
	40	20	60	59.518	99.20	
100%	40	40	80	79.547	99.43	0.07
	40	40	80	79.635	99.54	
	40	40	80	79.528	99.41	
150%	40	60	100	99.854	99.85	0.80
	40	60	100	99.457	99.46	
	40	60	100	98.325	98.33	

Table 5: Accuracy results

Ruggedness:

Ruggedness was expressed in terms of %RSD which must be less than 2. The %RSD was found to be 0.36 in the developed method. Results found to be within the acceptance limit confirms that the method is rugged.

Robustness:

% change was found to be in the range of 0.59 to 1.21 % for Atogepant and the results were found to be within the acceptance limit of less than 2. This confirms that the small change in the analytical conditions doesn’t influence the results and hence the proposed

method was found to be suitable for the analysis of Atogepant when small change in the analytical conditions.

Method Sensitivity:

The sensitivity of the method was confirmed by determining the detection limit (LOD) and quantification limit (LOQ). LOD and LOQ were found to be 0.125 µg/mL and 0.413 µg/mL for Atogepant. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations.

Forced Degradation studies:

In all the stress degradation conditions i.e acidic, base, peroxide, thermal and UV light conditions, the standard drug Atogepant was effectively separated, identified and quantified. The % assay of Atogepant was found to be very high and the % degradation was found to be very less in the developed method. The degradation products were found to be 3, 3, 2, 3 and 3 in acidic, base, peroxide, thermal and UV light conditions respectively. Among the degradation conditions studied, very high % degradation was observed in base degradation condition in which the % degradation was observed to be 4.19 % with 3 additional degradation products. Very less % degradation was observed in peroxide condition in which the % degradation of 2.75 confirms that the drug was more stable in these conditions.

S No	Condition studied	No of degradation compounds separated	% assay	% degradation
1	Acid	3	96.25	3.75
2	Base	3	95.81	4.19
3	Peroxide	2	97.25	2.75
4	Thermal	3	96.32	3.68
5	UV light	3	95.91	4.09

Table 6: Forced degradation study results

Formulation analysis:

The developed method was applied for the estimation of Atogepant in its formulation solution prepared from Qulipta®brand tablets of Atogepant. The % assay in formulation analysis was found to be 99.16 for Atogepant in the developed method. More than 98% assay was observed in the developed method. Hence the method was found to be suitable for the routine analysis of Atogepant in bulk drug as well as formulations.

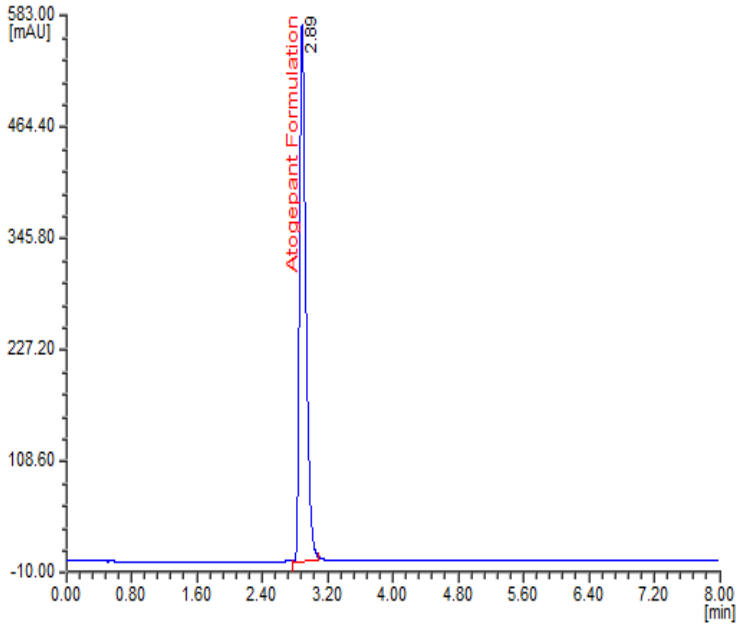

Figure 10:Formulation chromatogram

S.No	Drug	Brand	Label claim	Concentration prepared	Concentration found	% assay
1	Atogepant	Qulipta®	60 mg	80 µg/mL	79.329µg/mL	99.16

Table 7: Formulation results

CONCLUSION

An efficient and simple HPLC method has been developed and validated for the determination of Atogepant in bulk and was applied on marketed Atogepant products. The mobile phase used for the chromatographic runs consisted ofMethanol, acetonitrile and triethyl amine in 55:40:5 (v/v). The separation was achieved on Waters Spherisorb ods2 C18 (250 mm × 4.6 mm; 5µ)column using isocratic mode. Drug peak was well separated and were detected by a UV detector at 248 nm.The method was linear at the concentration range of 20–120 µg/ml for Atogepant. The method has been validated according to ICH guidelines with respect to system suitability, specificity, precision, accuracy and robustness. LOD and LOQ were found to be 0.125 µg/mL and 0.413 µg/mL for Atogepant. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations. The method can effectively separate the forced degradation products formed during the stress study confirms the stability indicating nature of the method. Hence it the method reported in the present study was used for the separation and quantification of Atogepant in bulk drug, formulations as well as stability testing.

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