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Stability Indicating Analytical Method Development and Validation for the Estimation of Rimegepant in Bulk and Its Tablets Using Rp-HPLC

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Authors' contributions

This work was carried out in collaboration between both authors. Author HMSK done the experimental work, statistical analysis and wrote a first copy of manuscript. Author KBC has proof read and approved the manuscript.

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ABSTRACT

Aim: Ramegepant is a novel molecule belongs to the class of calcitonin gene – related peptide (CGRP) receptor antagonist, which was developed for the prevention and treatment of migraine. Ramegepant was reported to act at the CGRP receptor with good oral bioavailability. The objective of this study was to develop a simple and fast stability indicating method for the determination of Ramegepant in bulk and tablets.
Methodology: Ramegepant was eluted on a Waters C18 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size with a mobile phase of Potassium dihydrogen orthophosphate buffer pH 7.0 : Methanol 30:70 v/v in isocratic mode at a flow rate of 0.8 ml/min. The analyte was quantified using a 272 nm PDA detector.
Results: The chromatograms of Ramegepant obtained with this method showed a well resolved retention time at 3.29 min of its excipients and degradation products. The area of the peak with respect to the concentration calibration curves, which were linear from 70 to 210 µg / ml, had a

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regression coefficient (r2) greater than 0.999. Accuracy and precision have been determined and perfectly matched to the ICH standards.
Conclusion: The study showed that the proposed Rp-HPLC method was simple, fast, robust and reproducible, which can be used for the evaluation of the purity and stability of the drug without interference from excipients or decomposition products of active pharmaceutical ingredients.

Keywords: Rimegepant; migraine; zorbax; potassium dihydrogen orthophosphate; methanol.

1. INTRODUCTION

Migraine is one of the most common chronic neurologic diseases [1]. The condition is characterized by recurrent headache from moderate to severe accompanied by photophobia, phonophobia, cutaneuous allodynia and nausea [2,3]. The headache attack last from 4 to 72 h typically once or twice in a month. It is second most disabling and third most prevalent neurological condition which prevails for life time in 33% women and 12% men [4,5]. Migraine is associated with a series of various neurological and systemic symptoms which occurs most frequently between the ages of 25 to 50 years [6]. Migraine affects both quality of life and productivity which affects approximately 11% of the adult world population [2,7]. In generally acute migraine treatments are largely abortive in nature. Migraine is treated with triptans and/or non-steroidal anti-inflammatory drugs (NSAIDS), while central analgesic may be used in few cases [8-10]. The basic disadvantage with triptans causes vaso constriction of the cranial blood vessels by binding to effective receptors, so these drugs are contraindicated in patients suffering from vascular diseases [11,12]. Furthermore, NSAIDS exert non specific anti-inflammatory effects and also causes gastritis and renal failure, so they are not indicated for long-term usage [13-15].

Calcitonin gene related peptide (CGRP) is a novel member of calcitonin family of peptides that is produced in both peripheral and central neurons [16]. Research has shown that CGRP levels were found to increase during migraine attacks [17-18]. Several research groups have aimed to determine the clinical potential of CGRP receptor antagonists. Rimegepant (RPT) is a novel gene related peptide receptor antagonist got approved by FDA in Feb 2020, used for the treatment of acute management of migraine, rapid pain relief at 2 h post dose of 75 mg [19]. Chemically RPT is [(5S,6S,9R)-5-amino-6-(2,3-difluorophenyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridin-9-yl]4-(2-oxo-3H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxylate [20]. Fig. 1 shows the chemical structure of RPT.

Upon reviewing the literature thoroughly no analytical method was reported for the determination of RPT. To the best of authors' knowledge, stability indicating assay method for the determination of RPT in bulk and its tablets has not been reported in literature. For this purpose, the present study is aimed to develop and validate a simple and rapid Rp-HPLC-PDA method for quantification of RPT.

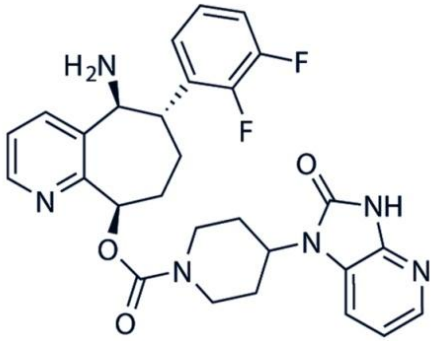

Fig. 1. Chemical structure of Rimegepant

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Rimegepant (purity:>99.99%) drug standard was gifted from Biohaven Pharmaceuticals, United States. Methanol, water and acetonitrile (LC grade) were purchased from Sigma-Aldrich, USA. Analytical grade sodium hydroxide (NaOH), hydrogen peroxide (H2O2), hydrochloric acid (HCl) and a 0.22 mm membrane filter were purchased from Sigma-Aldrich, USA. Nurtec ODT containing RPT with the label claim of 75 mg was purchased from the native pharmaceutical market at Kurnool. All chemicals were analytical or LC grade.

2.2 HPLC–PDA Instrumentation and Chromatographic Conditions

The HPLC system was an LC Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 Controller), in line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower-2 software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Waters C18 Column with 250 mm × 4.6 mm i.d and 5 µm Particle size with a mobile phase of Potassium dihydrogen orthophosphate buffer pH 7.0 : Methanol 30:70 v/v in isocratic mode at a flow rate of 0.8 ml/min. The analyte was quantified using a 272 nm PDA detector. Mobile phase was used as diluent for the preparation of working standards of RPT.

2.3 Preparation of Standard and Sample Solutions

A stock solution of RPT (10 µg/mL) was prepared by dissolving the appropriate amount of RPT solid in the diluent. Working solution of 140 µg/mL was prepared from the above stock solution for the determination of assay and stability studies.

20 tablets were weighed in a clean dry mortar and powdered using a pestle. The powder equivalent to 10 mg of drug was transferred into a 10 ml volumetric flask and 7 ml of diluent was added. The flask was attached to a rotary shaker and shaken for 10 mins to disperse the powder completely. The mixture was sonicated for 10 min and then diluted to the appropriate volume with diluent to make a solution containing 1.0 mg/ml. This solution was further diluted to obtain final concentration of 140 µg/mL. The resulted solution was filtered through a 0.45 µ nylon 66 membrane filter.

2.4 Method Validation

Method validation was performed as per ICH guidelines [21-23]. The following validation parameter was addressed system suitability, precision, accuracy, specificity, limit of detection and quantitation, robustness and stability of RPT in various stress conditions.

2.4.1 System suitability

The system suitability test is an integral part of the validation of the liquid chromatography method performed to verify and assure the continuous performance of a chromatographic system. The repeatability of the system was estimated by 6 repeated injections of standard working solution at 100% of the test concentration (140 µg/mL of RPT). The suitability parameters of the system have been calculated according to the recommendation of ICH.

2.4.2 Specificity

Specificity is the ability to unequivocally evaluate the analyte in the presence of components that can be assumed to be present (impurities, degrading, matrix, etc). Specificity has been demonstrated by determining RPT in the presence of excipients of the dosage form.

2.4.3 Linearity

For the evaluation of linearity, the calibration curve was obtained at 5 concentration levels of standard RPT solutions (70–210 µg/mL). The solutions (3 µL) were injected in triplicate in a chromatographic system with the chromatographic conditions previously provided. For linearity assessment, the peak area and concentrations were subjected to a least squares regression analysis to calculate the calibration equation and the coefficient of determination.

2.4.4 Precision

The precision of the analytical procedure (intra-assay precision) was investigated by analyzing six sample solutions obtained by multiple sampling of the same homogeneous sample under the prescribed conditions (at 100% of the test concentration of RPT (140 µg/mL)) on the same day, by the same analyst and using the same equipment. The Interday precision of the

analytical procedure was investigated by analyzing sample solutions on three consecutive days. The precision of the analytical procedure was expressed as the relative standard deviation of a series of measurements.

2.4.5 Accuracy

To study the accuracy of the proposed analytical method, recovery tests were conducted. To discover whether excipients interfered with the analyte, equivalent amounts at 50, 100 and 150% of RPT were evaluated from tablet formulation and the resulting mixtures were analyzed by the proposed methods. The percent of recovery was calculated as follows;

Amount Added:

$$\frac{\mu g}{ml} Added = \frac{Sample\ Weight}{Sample\ Dilution} X \frac{Average\ Assay}{100} X \frac{Labelled\ Claim}{Average\ Weight} X\ 1000$$

Amount Found:

$$\frac{\mu g}{ml} Found = \frac{Sample\ Area}{Standard\ Mean\ Area} X \frac{STD\ Weight}{STD\ Dilution} X \frac{STD\ Potency}{100} X\ 1000$$

% Recovery:

$$\%\ Recovery = \frac{\frac{\mu g}{ml}\ Found}{\frac{\mu g}{ml}\ Added} X100$$

2.4.6 Robustness

The ability of the proposed method to remain unaffected by small (deliberate) variations in parameters was evaluated in order to determine method robustness. Changes were made to the following method parameters: flow rate (±0.1 ml min–1), temperature (± 5°C) and wavelength of detection (± 2 nm).

2.4.7 Forced degradation studies

Forced degradation studies were performed on RPT. Intentional degradation was attempted to stress conditions of Acid (0.1 N HCl), Base (0.5 N NaOH), Peroxide (3% H_2O_2), thermal (55°C) and UV light (256 nm) to determine the ability of the proposed method to separate RPT from its degradation products generated during forced degradation studies. For thermal and UV light, study period was 8 and 30 H respectively, whereas for acid, base and oxidation it was 5 H, 5H and 8 H respectively. Peak purity test was carried out on the stressed samples by PDA. Assay studies were carried out for stress samples against qualified reference standard and mass balance (% assay + % degradation products) was calculated.

3. RESULTS AND DISCUSSION

3.1 Method Development

Various mobile phases have been studied in the development of an HPLC method for RPT analysis. These include: methanol - water, 50:50 (V/V), acetonitrile - water, 30:70 (V / V), methanol - orthophosphoric acid buffer (pH 4.5–6.5), 50:50 (V / V), Methanol buffer - phosphate (pH 3.0–6.5), 25:75 (V / V) and acetonitrile - phosphoric acid buffer (pH 3.2-4.5) 60:40 v / v. The suitability of the mobile phase was decided based on the sensitivity of the assay, the suitability for stability studies, the ease of preparation and the use of readily available solvents. Therefore, the mobile phase consisting of Potassium dihydrogen orthophosphate buffer pH 7.0: Methanol 30:70 v/v, has been found to be optimal for isocratic determination of RPT in pharmaceutical products. The wavelength was selected by scanning the standard RPT solution in between 200-400 nm and the wavelength of 272 nm was chosen for the detection.

RPT has been identified as a function of retention time compared to the RPT standard. Furthermore, RPT was identified by adding the standard to the sample prior to analysis, which resulted in an increase in the sample peak area that was proportional to the amount added. The mean RPT retention time was approximately 3.29 minutes at a flow rate of 0.8 ml min-1. RPT was rapidly determined as a single sharp peak. No interference was observed from other degradation products. Figs. 2 and 3 shows the standard and sample chromatograms respectively.

3.2 Method Validation

3.2.1 System suitability

The results of system suitability test were found within the acceptable range indicating that the system was suitable for the intended analysis (Table 1).

3.2.2 Specificity

In the specificity study, standard RPT solutions and the sample solution were injected and a

single peak was obtained, indicating that there was no interference from the excipients used or from the mobile phase. Representative chromatograms of standard and sample were shown in Figs. 2 and 3 respectively.

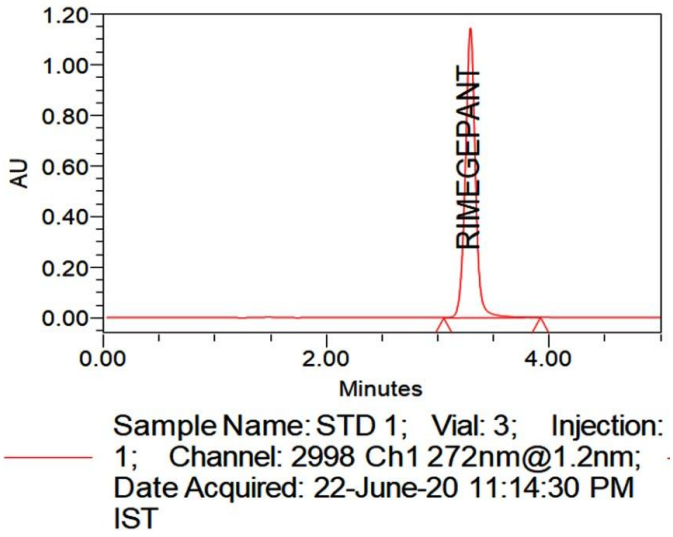

Fig. 2. Standard chromatogram of rimegepant

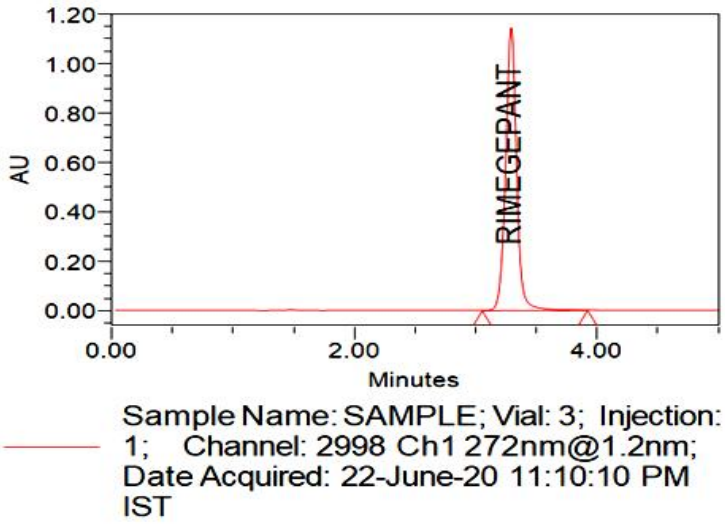

Fig. 3. Sample chromatogram of rimegepant

Table 1. Result of system suitability study

S. no	Parameter*	Result	% RSD
1	Theoretical Plate Count	6696.00	1.71
2	Average Peak Area	7145647.667	1.51
3	Peak Height	1135984	1.62
4	RT	3.281	0.10
5	Tailing	1.0	0.75
6	S/N	1045	1.01

** Average of 6 replicates*

3.2.3 Linearity

In the present study, linearity was studied in the concentration range 70-210 µg/mL RPT and the following regression equation was found by plotting the peak area (y) expressed in mAU versus the RPT concentration (x) expressed in µg/mL. The coefficient (r^2) demonstrates the excellent relationship between the peak area and concentration of DLT. The excipients had no influence and there was no matrix effect observed. Fig. 4 shows the linearity curve of RPT.

3.2.4 Precision and accuracy

Precision was demonstrated by Interday and intraday variation studies. In the intraday and Interday studies the solutions were injected 6 times and %RSD was calculated which was found to be less than 2%. Accuracy of the proposed methods was demonstrated by analyzing different concentrations covering the points in the calibration range. The mean percentage recovery was found to be 99.97, 99.94, and 99.52% at 50, 100 and 150% accuracy levels. The precision and accuracy was shown in Tables 2 and 3 respectively.

3.2.5 Robustness

Based on the obtained results the proposed HPLC analytical method was demonstrated to be robust (Table 4).

3.2.6 Method application

The validated method was applied for the determination of RPT in commercially available tablets. Figs. 2 and 3 shows two typical HPLC chromatograms obtained from the test of the standard RPT reference solution and of the tablet sampling solution, respectively. The results of the trial (n = 6) produced 100.00% (RSD = 0.46%) of the label claim for RPT in Nurtec ODT 75 mg tablets. The test results indicate that the method is specific for the analysis of RPT without interference from the excipients used to formulate and produce these tablets. Assay results were shown in Table 2.

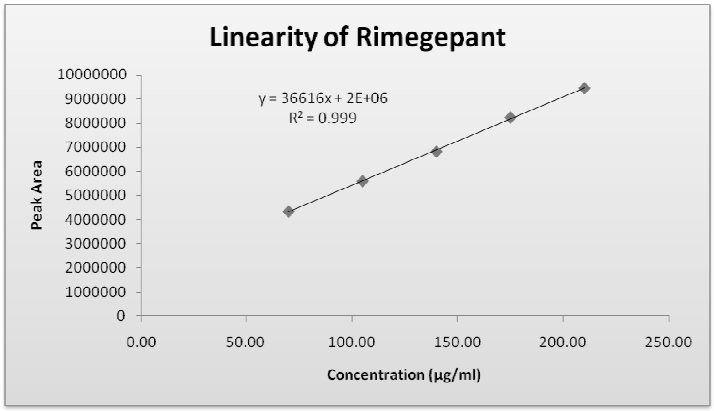

Fig. 4. Linearity curve of darolutamide

Table 2. Results of precision

S. no	Intraday precision		Inter day precision	
	Peak area*	% Assay*	Peak area*	% Assay*
1	7145637	99.87	7122699	99.55
2	7167389	100.18	7142606	99.83
3	7165372	100.15	7118974	99.50
4	7153907	99.99	7176367	100.30
5	7093764	99.15	7165881	100.15
6	7098352	99.21	7132006	99.68
Average	7137403.50	99.76	7143088.83	99.84
STDEV	33018.02	0.46	23440.36	0.33
% RSD	0.46	0.46	0.33	0.33

Table 3. Results of accuracy

Accuracy level	Wt. of sample(mg)	Peak area	Amount added	Amount found	% Recovery	Mean % recovery
50*	16.665	3570606.667	69.884	69.866	99.974	99.811
100†	33.33	7138896.667	139.768	139.685	99.941	
150*	49.995	10662863.167	209.652	208.639	99.517	

*Mean of 6 replicates; †Mean of 3 replicates

Table 4. Results of robustness

Parameter	Condition	RT (min)	Peak area	% Assay
Flow	0.6 ml/min	1.172	7157652	100.04
	0.8 ml/min	3.281	7145648	99.87
	1.0 ml/min	0.747	7208459	100.75
Temp	25 °C	0.826	7125339	99.59
	30 °C	3.281	7145648	99.87
	35 °C	0.83	7202750	100.67
Wave length	270 nm	0.831	7125339	99.59
	272 nm	3.281	7145648	99.87
	274 nm	0.83	7142750	99.83

Table 5. Forced degradation studies

S. no	Condition	Peak area	% Assay	% Degradation	Purity threshold	Purity angle
1	Acid	6389022	89.30	10.70	0.829	1.071
2	Base	6416189	89.68	10.32	0.757	0.989
3	H2O2	6554002	91.60	8.40	0.981	1.082
4	UV	6492252	90.74	9.26	0.763	0.921
5	Heat	6472842	90.47	9.53	0.638	0.932

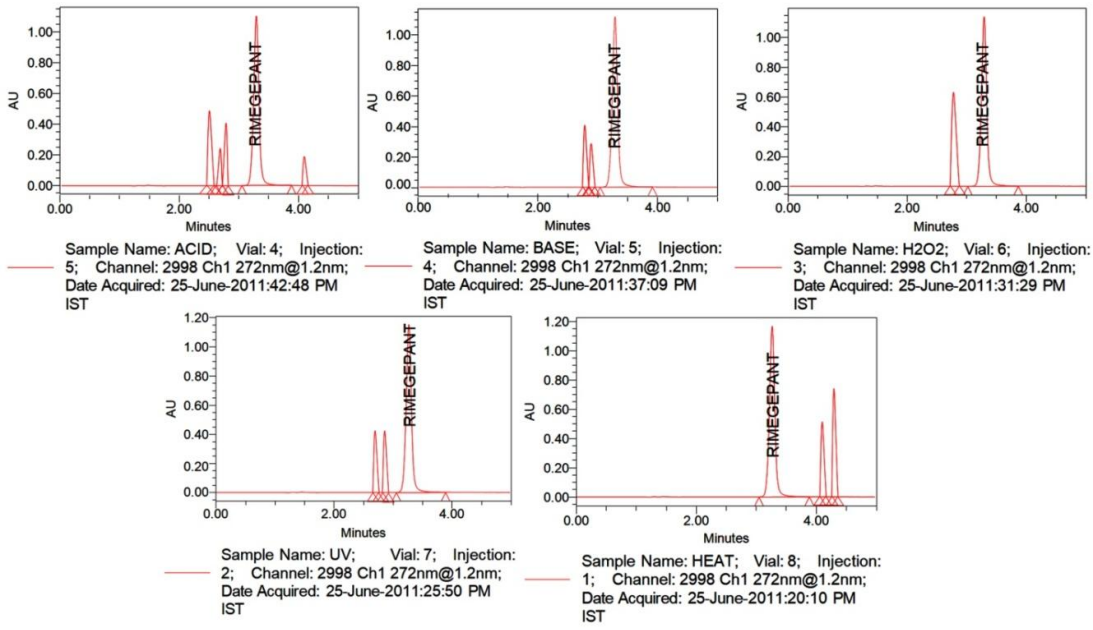

Fig. 5. Chromatograms of forced degradation studies

3.2.7 Forced degradation studies

All forced degradation samples were analyzed at an initial concentration 140 µg/mL of RPT with HPLC conditions mentioned earlier using PDA detector to ensure the homogeneity and purity of RPT peak. Significant degradation of RPT was observed in acid and base conditions leading to the formation of degradants. Assay studies were carried out for stress samples (at 140 µg/mL) against RPT qualified reference standard. The mass balance (% assay + % sum of all compounds + % sum of all degradants) results were calculated for all stressed samples and found to be more than 99%. The purity and assay of RPT was unaffected by the presence of its degradation products and thus confirms the stability-indicating power of the developed method. Table 5 shows the results of forced degradation studies. Fig. 5 shows the chromatograms of forced degradation studies.

4. CONCLUSION

In conclusion, a sensitive and selective stability indicating RP-HPLC method has been developed and validated for RPT in API and tablets. Based on the peak purity results obtained from the analysis of force degraded samples using the described method, it can be concluded that the absence of a co-eluent peak together with the main RPT peak indicated that the developed method it is specific for the estimation of RPT in the presence of degradation products. Furthermore, the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. Although no attempt has been made to identify degraded products, the proposed method can be used as a stability indicator method for Rimegepant dosage.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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