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ESTIMATION OF ROLAPITANT IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

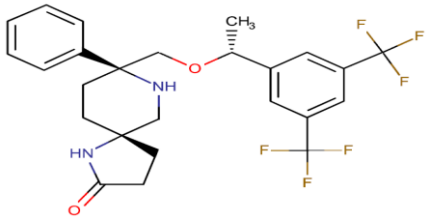
A simple, sensitive, isocratic and reproducible reversed phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the estimation of Rolapitant using UV detector. The composition of mobile phase was taken as Acetonitrile: Phosphate 3 (60:40 v/v) pumped at room temperature at a flow rate of 1ml/min which was eluted at 2.497min, and the detection was performed at a wavelength of 225 nm, using columns of X TERRA (4.6×150mm)5µ. The method was linear over the concentration range of rolapitant was found to be 20µg-100µg/ml and correlation coefficient (r^2) was found to be 0.999, % recovery was found to be 99.95%, %RSD for repeatability was 0.2, % RSD for intermediate precision was 0.1. The limit of detection (LOD) and the limit of quantification (LOQ) of rolapitant was found as 0.36µg/ml and1.10µg/ml. A new method was developed and validated according to ICH guidelines.

KEY WORDS

Rolapitant, Acetonitrile, Phosphate-3 buffer, HPLC

1. Introduction [1, 2]

Rolapitant is an Nk_1 receptor antagonist that has been approved for the treatment of chemotherapy-induced nausea and vomiting along the combination with the other antiemetic agents. Neurokinin-1 (NK-1) receptors are highly concentrated in the vomiting center of the brain, and bind a neurokinin called Substance P. Activation of NK-1 receptors by Substance P plays a central role in eliciting CINV. It is associated with initial and repeated courses of emetogenic cancer chemotherapy.



Chemical formula: $C_{25}H_{26}F_6N_2O_2$

IUPAC Name: (5S,8S)-8-{[(1R)-1-[3,5-bis(trifluoromethyl)phenyl] ethoxy] methyl}-8-phenyl-1,7 diazaspiro[4.5]decan-2-one

Molecular weight: 403.207g/mol

As there is no analytical method reported for the analysis of Rolapitant using RP-HPLC. Hence the work is done to develop a new analytical method by optimizing chromatographic conditions. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the analysis of Rolapitant. The developed method will be validated for various parameters specified in ICH guidelines, Q2 (R1).

2. Materials and Methods [3]

Pure sample of Rolapitant was gifted by Indu drugs pvt ltd (Hyderabad, T.G, India). All the solvents were of HPLC and analytical grade purchased from Merck.

Preparation of the Rolapitant standard and sample solution

Standard solution preparation

10 mg Rolapitant working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Sample solution preparation:

10 mg of Rolapitant tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Optimized HPLC Method:

The samples prepared were analyzed by an isocratic Hplc method. The HPLC analysis was performed on waters by injecting 20µl of sample Rheodyne syringe. The column used was X TERRA (4.6×150mm)5µ. The mobile phase consisted of a mixture of Acetonitrile: Phosphate 3 (60:40 v/v).The mobile phase was degassed using digital ultrasonic bath (Danwer Scales).The analysis was performed at ambient temperature with a flow rate of 1ml/min using UV detector. Detection was carried out at a wavelength of 225nm. The data analysis was performed by Empower-software version-2.

Column	:	X TERRA (4.6×150mm) 5µ
Mobile phase ratio (60:40 v/v)	:	Acetonitrile: Phosphate 3
Detection wavelength	:	225 nm
Flow rate	:	1 ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature:		Ambient
Run time	:	7.0min
Retention time	:	2.497min

3. Method Validation [4, 5]

The developed analytical method was subjected to validation with respect to various parameters such as specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision, Detection Limit, Quantitation Limit, Robustness were validated as per ICH guidelines using RP-HPLC.

3.1. Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

3.2. Linearity

Preparation of stock solution

10 mg of Rolapitant working standard was accurately weighed and was transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Five different levels of (20, 40, 60, 80,100) ppm of Rolapitant were prepared and diluted with mobile phase and each level was injected into the chromatographic system and peak area was measured. The linearity range of 20µg/ml-100µg/ml of Rolapitant was determined.

3.3. Accuracy

Preparation of standard stock solution

10mg of Rolapitant working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1 ml of the above stock solution into a 10-ml volumetric flask and was diluted up to the mark with diluent.

Preparation of sample solutions

For preparation of (50%, 100%, 150%) solutions 5mg,10mg,15mgof Rolapitant working standard was taken respectively into a 10-ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution). Further pipette out 10 ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

3.4. Precision and Intermediate precision (Repeatability / Ruggedness)

Preparation of stock solution

10 mg of Rolapitant working standard was accurately weighed and transferred into a 10ml clean dry

volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

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

3.5. Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:$\mathbf{LOD} = \mathbf{3.3\ X}\ \frac{\sigma}{S}$

Where σ - Standard deviation (SD), S - Slope

3.6. Limit of quantification (LOQ)

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:$\mathbf{LOQ} = \mathbf{10\ X}\ \frac{\sigma}{S}$

Where σ - Standard deviation, S - Slope

3.7. Robustness

As part of the robustness, deliberate change in the flow rate was varied at 0.4ml/min to 0.6 ml/min. Standard solution 60 ppm of rolapitant prepared and analysed using the varied flow rates along with method flow rate.

3.8. System suitability

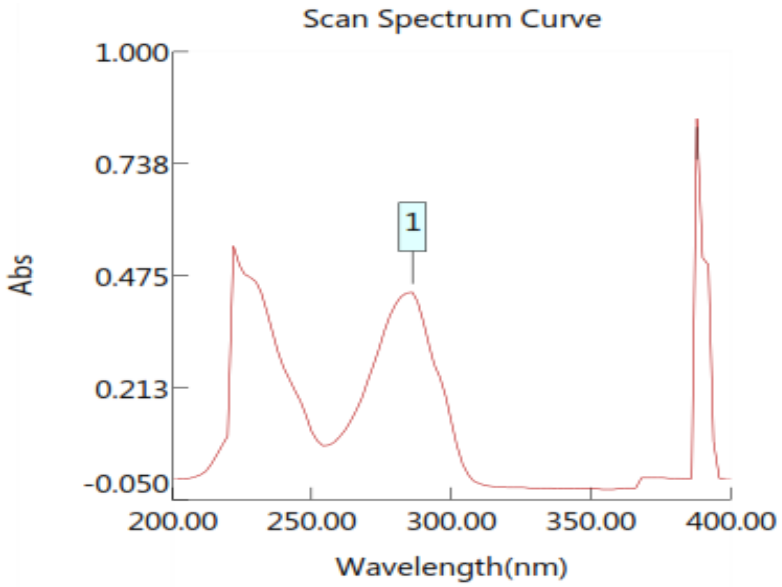
10 mg of Rolapitant working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of Rolapitant from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

4. Results and Discussions

4.1 Method Development

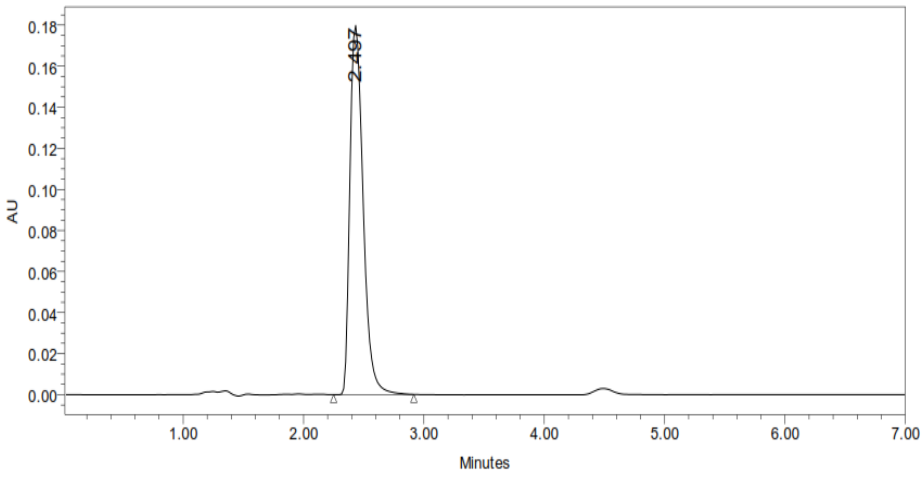
The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm.

Fig.No.1. Spectrum showing overlapping spectrum of Rolapitant



4.2. Optimized chromatographic conditions for simultaneous estimations of Rolapitant

Fig.No.2. Chromatogram showing trial-4 injection



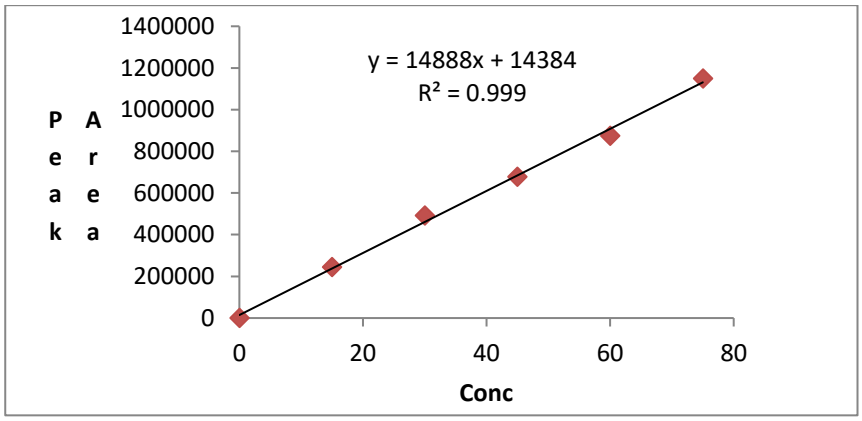
4.3. Linearity

The linearity study was performed for concentration range of 20µg-100µg Rolapitant and the correlation coefficient was found to be 0.999 (NLT 0.999)

Table.No.1. Linearity Results for Rolapitant

S. No	Concentration (µg/ml)	Area
1	20	264840
2	40	491415
3	60	677620
4	80	873311
5	100	1048958
Correlation coefficient		**0.999**

Fig.No.3. Showing calibration graph for Rolapitant



Rolapitant $r^2 = 0.999$

4.4. Accuracy

The accuracy study was performed for % recovery of Rolapitant. The % recovery was found to be 99.95% (NLT 98% and NMT 102%)

Table.No.2. Showing accuracy results for Rolapitant

% Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	%Recovery	Mean recovery
50%	728287	5	4.96	99.91%	
100%	1378202	10	9.98	99.18%	99.95%
150%	2115480	15	15.02	99.60%	

4.5. Precision

The Method precision study was performed for the %RSD of Rolapitant was found to be 0.24 (NMT 2).

Table.No.3. Showing% RSD results for Rolapitant

S. No	Name	Area
1	Rolapitant	693877
2	Rolapitant	696531
3	Rolapitant	693977
4	Rolapitant	695278
5	Rolapitant	697676
Mean		695468
Std. Dev.		1642.7
% RSD		0.24

4. 6. Intermediate precision

The intermediate precision was performed for %RSD of Rolapitant was found to be 0.15 (NMT2).

Table.No.4. Showing results for intermediate precision of Rolapitant

S. No	Name	Area
1	Rolapitant	693078
2	Rolapitant	693338
3	Rolapita	695080
4	Rolapitant	694843
5	Rolapitant	695336
Mean		694335
Std. Dev.		1047.5
% RSD		0.15

4.7. LOD

The LOD was performed for Rolapitant was found to be 3.04.

Fig.No.4. Chromatogram showing retention time

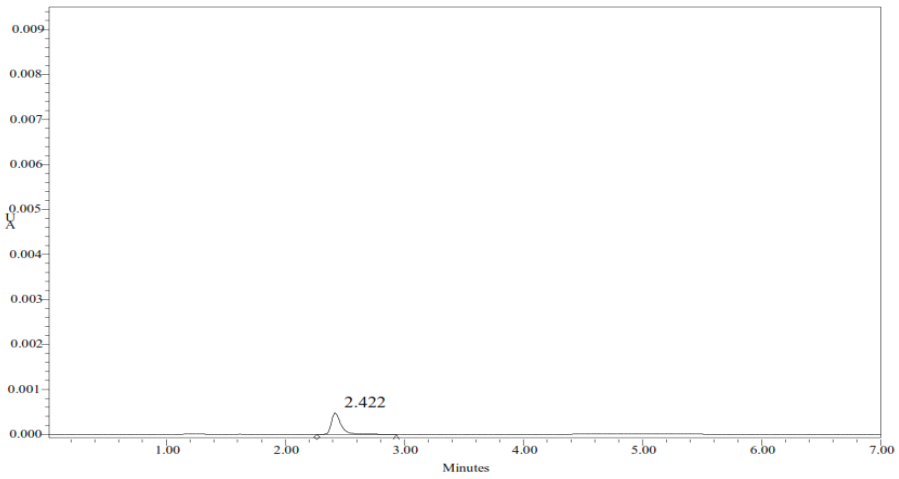


Table.No.6. Showing results for Limit of Detection

Drug name	Standard deviation(σ)	Slope(s)	LOD(µg/ml)
Rolapitant	1642	14888	0.36

4.8. LOQ

The LOQ was performed for Rolapitant was found to be 10.14.

Fig.No.5. Chromatogram showing retention time

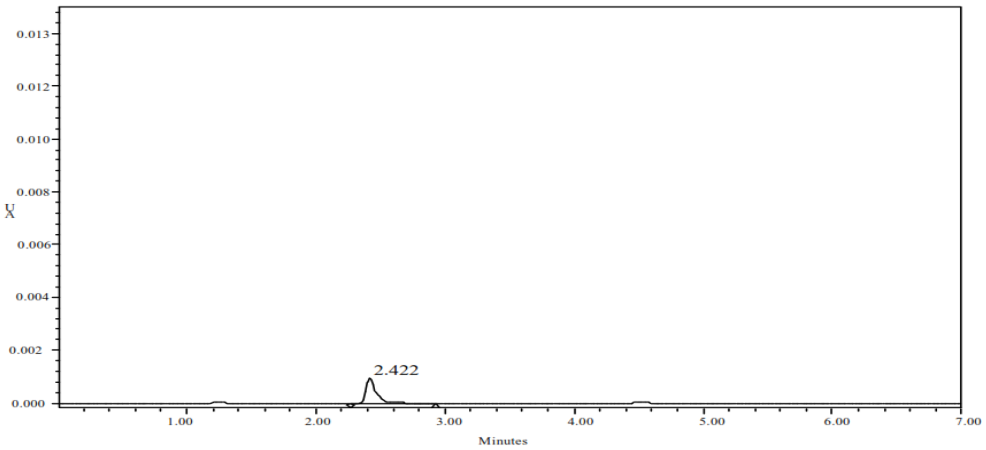


Table.No.7. Showing results for Limit of Quantification

Drug name	Standard deviation(σ)	Slope(s)	LOQ(µg/ml)
Rolapitant	1642	14888	1.10

4.9. Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rolapitant. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%.

4.10. System suitability

These tests are performed to verify resolution and reproducibility of the system and are adequate for the analysis.

Table.No.8. Showing system suitability results for Rolapitant

Change in organic composition in the mobile phase	System suitability results	
	USP Plate Count	USP Tailing
5 % less	4194	1.5
Actual	4524	1.5
5 % more	3097	1.4

Conclusion:

An accurate assay method was developed for the determination of Rolapitant by using RP-HPLC method. The results obtained from specificity, linearity range, LOD, LOQ, precision, accuracy, robustness, ruggedness and system suitability lie well within the acceptance criteria. Since all the results were within the limit, the developed and validated analytical method is suitable for anticipated use and all parameters are subjected as per the ICH guidelines. Hence the suggested RP-HPLC method can be used for routine analysis of Rolapitant in API and Pharmaceutical dosage form.

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