STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE DETERMINATION OF ROLAPITANT IN BULK FORM AND PHARMACEUTICAL TABLET DOSAGE FORM

Chepuri Divya[1]*, Pasupuleti Sunitha[2], Dr. Vijaya Kuchana[3]

[1*]Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet (V), Balapur (M), Ranga Reddy (Dist), Hyderabad – 500097, Telangana

[2]Department of Pharmaceutical Analysis, Principal and Professor, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet (V), Balapur (M), Ranga Reddy (Dist), Hyderabad – 500097, Telangana

[3]Department of Pharmaceutical Chemistry, Associate Professor, Teegala Krishna Reddy College of Pharmacy, Medbowli, Meerpet (V), Balapur (M), Ranga Reddy (Dist), Hyderabad – 500097, Telangana

ABSTRACT: A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Rolapitant in bulk form and marketed formulation. Separation of Rolapitant was successfully achieved on a Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol : Phosphate buffer (0.02M, pH-3.6) in the ratio of 45:55% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Rolapitant. The correlation coefficient was found to be 0.9995 for Rolapitant. The LOD and LOQ for Rolapitant were found to be 5.004µg/mL and 15.164µg/mL respectively. The proposed method was found to be good percentage recovery for Rolapitant, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Rolapitant, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

I. INTRODUCTION

Rolapitant is an orally available antiemetic agent that is used to prevent cancer chemotherapy related nausea and vomiting. Rolapitant[1] therapy has not been associated with serum enzyme elevations or with instances of clinically apparent liver injury with jaundice. Rolapitant is an azaspiro compound that is 1, 7-diazaspiro [4.5] decan-2-one carrying additional phenyl and 1-{[3, 5-bis (trifluoromethyl) phenyl] ethoxy} methyl substituents at position 8. Used (in the form of the hydrochloride hydrate) for the prevention of delayed nausea and vomiting associated with initial and repeat courses of emetogenic cancer chemotherapy. It has a role as an antiemetic and a neurokinin-1 receptor antagonist. It is an ether, an azaspiro compound, a member of pyrrolidin-2-ones, a member of piperidines and an organofluorine compound. It is a conjugate base of a Rolapitant (1+). Rolapitant[2] is a potent, highly selective, long-acting Neurokinin-1 (NK-1) receptor antagonist approved for the prevention of delayed chemotherapy-induced nausea and vomiting (CINV) in adults. Delayed-phase CINV typically occurs >24 hours after chemotherapy treatment and is principally mediated by Neurokinin-1 and its ligand Substance P, which is released in the gut following chemotherapy administration. Neurokinin-1 is also known as Tachykinin Receptor 1 (TACR1), Neurokinin 1 Receptor (NK1R), and Substance P Receptor (SPR). By blocking Substance P from interacting with NK-1 receptors in the gut and the central nervous system, Rolapitant prevents late-phase CINV. Unlike other available NK-1 receptor antagonists, Rolapitant is not an inhibitor of Cytochrome P450 enzyme CYP3A4 and has a long elimination half-life, allowing a single dose to prevent both acute and late-phase CINV during the first 120 hours post-chemotherapy. The IUPAC Name of Rolapitant[3] is (5S, 8S)-8-[[(1R)-1-[3, 5-bis (trifluoromethyl) phenyl] ethoxy] methyl]-8-phenyl-1, 9-diazaspiro [4.5] decan-2-one. The Chemical Structure of Rolapitant is as following

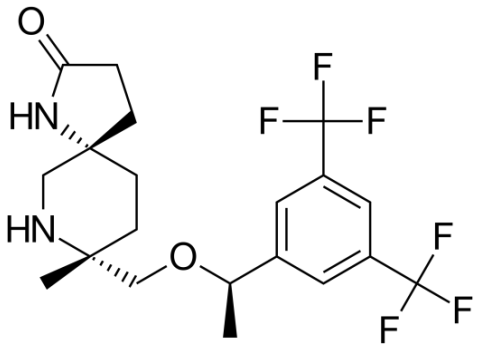

Fig.1. Chemical Structure of Rolapitant

II. EXPERIENTAL

Table-1: List of Instrument used

S. No.	Instruments/Equipment/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator(Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5 m, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai

HPLC METHOD DEVELOPMENT

Selection of Wavelength:

The detection wavelength[4] 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.

Table-3: Trials for the Method Development and Results

S.No.	Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
1	Symmetry C_{18}, 5µm, 25cmx4.6mm i.d.	ACN : Water = 70 : 30	0.8 ml/min	255nm	Early elution of peak	Method rejected
2	Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol: ACN = 40 :60	1.0 ml/min	255nm	Tailing Peaks	Method rejected
3	Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN: Phosphate buffer (0.02M) = 70:30	1.0 ml/min	255nm	Low resolution peak	Method rejected
4	Develosil ODS HG-5 RP C_{18}, 5µm,15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.01M) = 50:50 (pH-3.8)	1.0 ml/ min	255nm	Many Peaks	Method rejected
5	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.02M) = 65:35	1.0 ml/min	255nm	Many Peaks	Method rejected
6	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.	Methanol : Phosphate buffer (0.02M) = 45:55 (pH-3.6)	1.0 ml/min	255nm	Good Peaks	Method Accepted

Preparation of Standard Solution:

10 mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution[5] into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Rolapitant working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Preparation of Sample Solution:

Twenty tablets were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Rolapitant equivalent[6] to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Preparation of 0.02M Potassium dihydrogen orthophosphate Solution:

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 3.60 with diluted orthophosphoric acid.

Preparation of Mobile Phase:

550ml of Phosphate buffer (0.02M) pH 3.60 and 450ml of HPLC Grade Methanol were mixed well and degassed[7] in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

III. METHOD VALIDATION

System suitability

It is defined by ICH as "the checking of a system, before or during the analysis of unknowns, to ensure system performance." System suitability[8] criteria may include such factors as plate count, tailing, retention, and/or resolution. System suitability criteria should also include a determination of reproducibility (%RSD) when a system suitability "sample" (a mixture of main components and expected by-products/interferences) is run.

Specificity

One of the significant features of HPLC is its ability to generate signals free from interference. Specificity[9] refers to the strength of the analytical method to differentiate and quantify the analyte in complex mixtures. An investigation of specificity is to be conducted during the determination of impurities and validation of identification tests.

An ICH guideline[27] defines specificity as the ability to assess unequivocally the analyte in the presence of other compounds that may be likely to be present. Typically these might be impurities, degradants, matrix, etc.

Precision

The closeness of agreement between a series of measurements multiple samplings of the same homogeneous sample under prescribed condition. The precision[10,11] of test method is usually expressed as the standard deviation or relative standard deviation of a series of measurements.

Precision may be considered at three levels: Repeatability, Intermediate Precision[12] and reproducibility.

Method precision (Repeatability):

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate Precision:

It expresses with in laboratory variations; different days, different analysts, different equipment, etc.

Reproducibility:

Precision between laboratories (mostly performed during analytical method transfer).

Accuracy

It is the closeness of agreement between the actual value and measured value. Accuracy[13] is calculated as the percentage of recovery by the assay[14] of the known added amount of the analyte in the sample or the difference between the mean and accepted true value together with confidence intervals.

The ICH guidance recommended to take a minimum of 3 concentration levels covering the specified range and 3 replicates of each concentration are analyzed (totally 3 * 3 = 9 determination)

Linearity and Range

Linearity

The linearity[15] of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

The range[16] of analytical procedure is the interval between the upper and lower concentrations of analyte in the analytical procedure has a suitable level of precision, accuracy, and linearity.

Detection Limit

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated[17] under the stated experimental conditions.

Quantitation Limit

It is lowest amount of analyte in a sample, which can be quantitatively[18] determined with acceptable accuracy and precision.

Ruggedness

Ruggedness[19] is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions such as different laboratories, analysis, instruments, reagent lots, elapsed assay times, temperature, days, etc. It can be expressed as a lack influence of the operation and environmental variable on the test results of the analytical method[20].

Robustness

It is a measure of the method's ability to remain unaffected by small but deliberate variations[21] in method parameters and provides an indication of its reliability during normal usage. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

Forced Degradation Studies

This topic provides procedures for creating and managing stability studies[22,23] including when they are performed, and what essential guidelines exist for stability testing programs. The topic also provides an overview of the stability study lifecycle management, including the creation of test interval plans and the creation of a storage condition plan. You are given guidance on the creation and management of a stability study, including the assignment of its material sources, editing stability study variants[24] and time points, and defining its storage packages.

IV. RESULTS AND DISCUSSION

Selection of Wavelength:

The UV spectrum of Rolapitant was obtained and the Rolapitant showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

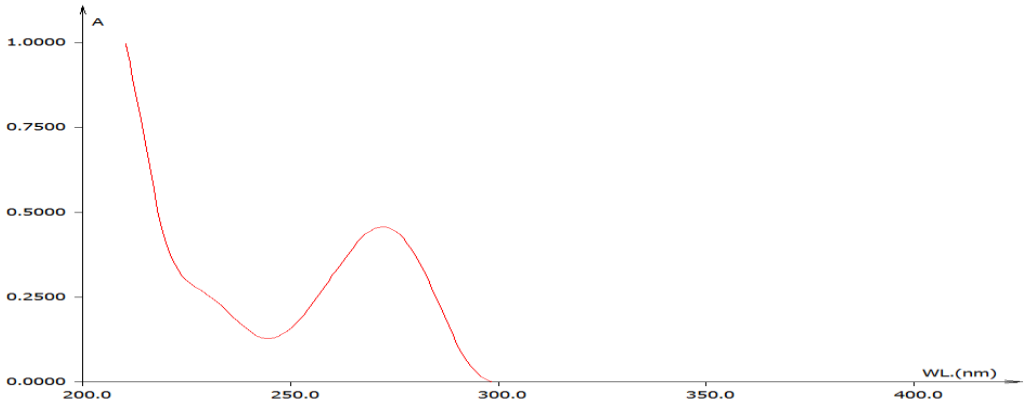

Fig.2. UV Spectrum of Rolapitant

Observation: While scanning the Rolapitant solution we observed the maxima at 255nm. The UV spectrum[25] has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Method Development

Optimized Chromatographic Method:

Table-4: Optimized Chromatographic Conditions

Mobile phase	Methanol : Phosphate buffer (0.02M, pH-3.6) = 45:55
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

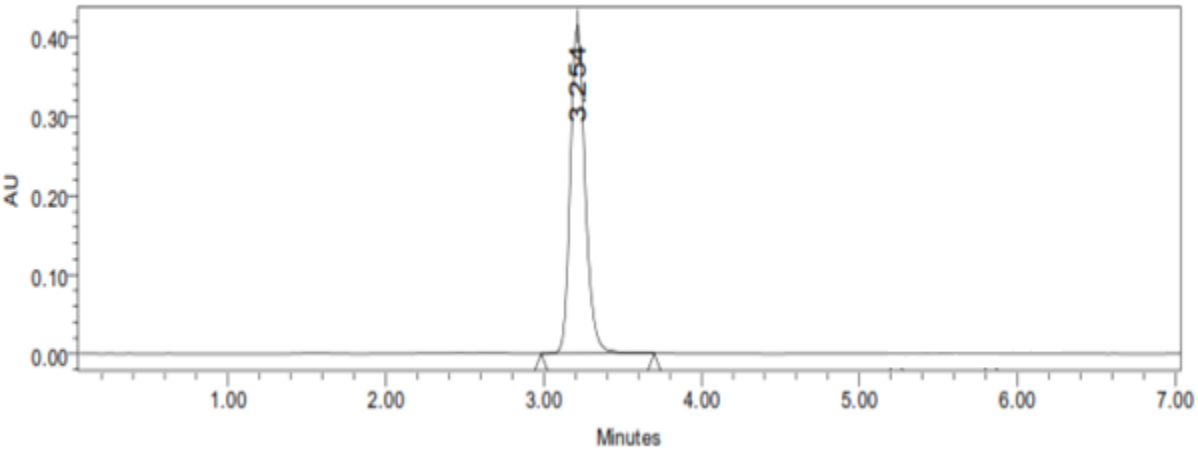

Fig.3. Chromatogram of Rolapitant in Optimized Chromatographic Condition

Validation of Method

Analytical method validation[26] establishes documented evidence that the procedure adopted for a test is fit for the

intended purpose in terms of quality, reliability and consistency of results.

System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-5 and 6.

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Rolapitant working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Procedure:

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-5: Data of System Suitability Test

S.No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	3.253	284568	7368	1.26
2	Injection 2	3.254	285684	7295	1.25
3	Injection 3	3.215	283659	7346	1.27
4	Injection 4	3.297	284754	7394	1.29
5	Injection 5	3.253	283695	7425	1.25
6	Injection 6	3.213	284578	7385	1.27
Mean			**284489.7**	**7368.833**	**1.265**
S.D			**752.5617**		
%RSD			**0.26453**		

Table-6: System suitability results for Rolapitant (Flow rate)

S.No.	Parameter	Limit	Result
2	Theoretical Plates	N > 2000	Rolapitant = 7368.833
3	Tailing Factor	(Tf) < 2	Rolapitant = 1.265

Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Rolapitant. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Preparation of Level – I (12ppm of Rolapitant):

Pipette out 1.2ml of Rolapitant above stock solution was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (16ppm of Rolapitant):

Pipette out 1.6ml of Rolapitant above stock solution was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Rolapitant):

Pipette out 2ml of Rolapitant above stock solution was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (24ppm of Rolapitant):

Pipette out 2.4ml of Rolapitant above stock solution was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (28ppm of Rolapitant):

Pipette out 2.8ml of Rolapitant above stock solution was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

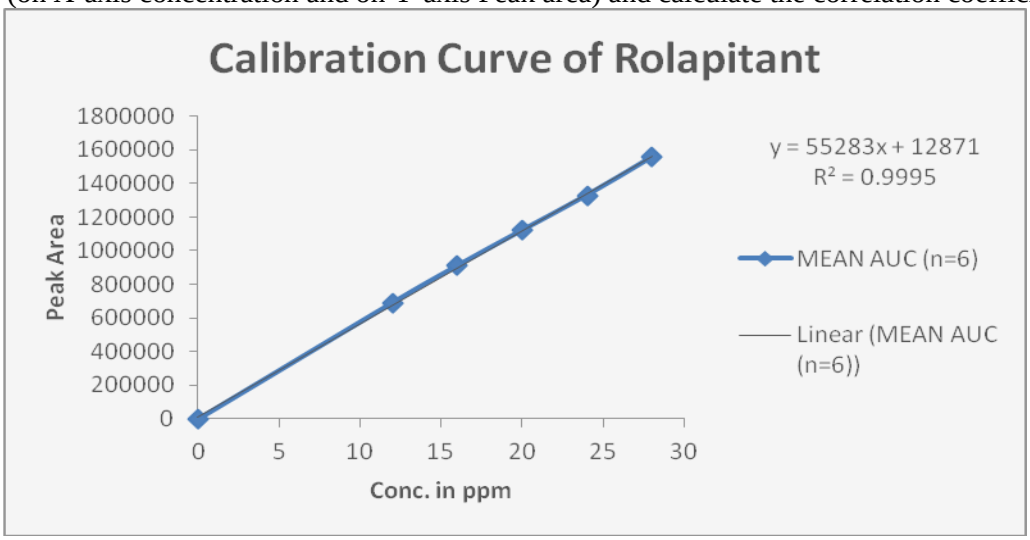

Fig.4. Standard curve for Rolapitant

Observation: Linearity range was found to be 0-28µg/ml for Rolapitant. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Rolapitant.

Table-7: Linearity Readings for Rolapitant

Conc. in µg/ml	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Accuracy:

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Rolapitant were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation y = 55283x + 12871. The results were shown in table-8.

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

For preparation of 80% Standard Stock solution:

Further pipette out 0.8ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard Stock solution:

Further pipette out 1.0ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 120% Standard Stock solution:

Further pipette out 1.2ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Rolapitant and calculate the individual recovery and mean recovery values.

Table-8: Accuracy results of Rolapitant

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113% S.D. = 0.473694346 % R.S.D.= 0.471753
S_2 : 80 %	8	7.843532	446485	100.637	
S_3 : 80 %	8	8.19449	465887	100.73	
S_4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S_5 : 100 %	10	9.978655	564521	100.868	
S_6 : 100 %	10	10.19623	576549	101.716	
S_7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S_8 : 120 %	12	12.16785	685546	100.69	
S_9 : 120 %	12	12.18644	686574	100.823	

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Rolapitant. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision: The precision of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Rolapitant. The percent relative standard deviations were calculated for Rolapitant are presented in the Table-9.

i) Repeatability

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Rolapitant working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Procedure:

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-9: Repeatability Results of Rolapitant

HPLC Injection Replicates	AUC for Rolapitant
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	**285081.3**
Standard Deviation	**1318.666**
% RSD	**0.462558**

Observation: The repeatability study which was conducted on the solution having the concentration of about 20µg/ml for Rolapitant (n =6) showed a RSD of 0.462558% for Rolapitant. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate Precision / Ruggedness:

The Intermediate Precision consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Method: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

For preparation of 80% Standard Stock solution:

Further pipette out 0.8ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard Stock solution:

Further pipette out 1.0ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 120% Standard Stock solution:

Further pipette out 1.2ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

DAY 1:

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

DAY 2:

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

Table-10: Ruggedness Results for Rolapitant

Conc. of Rolapitant (API) (µg/ml)	Observed Conc. of Rolapitant (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	8.21	0.76	8.23	0.46
10	10.37	0.33	10.36	0.57
12	12.56	0.23	12.56	0.75

Observation: Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

Robustness: Robustness is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents which gave 10 ppm Rolapitant working standard solution. The solution was mixed well and filtered through 0.45µm filter.

Procedure:

In every changing of the parameters standard solution was injected for two times individually and measured the area for all the injections in HPLC. The %RSD for the area of all the replicate injections was found to be within the specified limits.

Table-11: Result of Method Robustness Test for Rolapitant

Change in parameter	% RSD
Flow (0.8 ml/min)	0.554
Flow (1.2 ml/min)	0.867
More Organic	0.886
Less Organic	0.817
Wavelength of Detection (257 nm)	0.813
Wavelength of detection (253 nm)	0.794

Observation: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & organic phase (±5%) studied to determine the robustness of the method are also in favour of (Table-11, % RSD < 2%) the developed RP-HPLC method for the analysis of Rolapitant (API).

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

For preparation of 5.004ppm Standard solution:

Further pipette out 0.5004ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for one time and measured the LOD value for injection in HPLC.

Observation: The LOD was found to be 5.004µg/ml for Rolapitant.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ.

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Method: Accurately weigh about 10mg of Rolapitant working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and volume was made up to the mark with the same solvent which gave stock solution of 1000 ppm.

Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask was diluted up to the mark with diluents (100 ppm solution).

For preparation of 15.164ppm Standard solution:

Further pipette out 1.5164ml of Rolapitant from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for one time and measured the LOQ value for injection in HPLC.

Observation: The LOQ was found to be 15.164µg/ml for Rolapitant.

Assay of Marketed Pharmaceutical Dosage form:

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-12.

ASSAY:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

The assay was performed as explained in the previous chapter. The results which are obtained are following:

Table-12: Recovery Data for estimation Rolapitant in Varubi

Brand name of Rolapitant	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Varubi (Tesaro, Inc.)	90mg	89.823 (± 0.368)	99.698 (± 0.476)

Result & Discussion: The amount of drug in Varubi Tablet was found to be 99.823 (±0.368) mg/tab for Rolapitant & % Purity was 99.698 (± 0.476) %.

Forced Degradation Studies

Results of Forced Degradation Studies: The results of the forced degradation studies indicated the specificity of the developed method that has been developed. Rolapitant were stable only in acidic, thermal and basic stress conditions. The results of stability studies are given in the following Table-13.

Table-13: Results of Force Degradation Studies of Rolapitant API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	91.326	8.674	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	83.215	16.785	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	90.311	9.689	100.00
UV (254nm)	24Hrs.	81.322	18.678	100.00
3% Hydrogen Peroxide	24Hrs.	73.514	26.486	100.00

V. SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Rolapitant, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Rolapitant it is evident that most of the HPLC work can be accomplished in the wavelength range of 255 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Rolapitant in different formulations.

REFERENCES

[1] https://go.drugbank.com/drugs/DB09291
[2] https://pubchem.ncbi.nlm.nih.gov/compound/Rolapitant
[3] https://en.wikipedia.org/wiki/Rolapitant
[4] R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn., 2011.
[5] S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 452-474, 2005.
[6] H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi. 1-9, 157-167.
[7] H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods Of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi.: 430-440, 495-504,529-545.
[8] B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut: 286-300.
[9] Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK, 6-7, 2002.
[10] Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), P-2.566, 2005,.
[11] M. E. Swartz, Journal of liquid chromatography, 28(7/8), 1253-1263(2005).
[12] Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1; 863(2): 258-265. Published on Jan 18 2008.
[13] International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1). November 2005.
[14] International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A. 1994.
[15] J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, pp. 305a–309a, 1 May 1996.
[16] P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, pp. 361–367, 1997.
[17] R. Patil: J of Chromatographia, 67, 575, (2008).

[18] Baht and Leena: J of Liq. Chrom., 30, 309, (2007).
[19] H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.
[20] GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).
[21] Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), Vol:65 (169), 52776–52777, 2000.
[22] Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.
[23] Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.
[24] Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).
[25] Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.
[26] Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.
[27] ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
[28] Mounika Nerella1, Afzal Nazneen2, Ch. Sneha3, T. Manjula bai4, T.Supriya5 and N.Anusha6, Estimation of Rolapitant in its Bulk and Pharmaceutical Dosage Form By Rp-Hplc Method, International Journal of Pharmacy and Biological Sciences, IJPBS | Volume 7 | Issue 4 | OCT-DEC| 2017 | 60-66.
[29] Sreekanth Nama1*, Babu R Chandu2, Bahlul Z Awen2 and Mukkanti Khagga3, Development and Validation of a New RP-HPLC Method for the Determination of Aprepitant in Solid Dosage Forms, Tropical Journal of Pharmaceutical Research August 2011; 10 (4): 491-497.