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Analytical method development and validation for the estimation of Revaprazan in bulk form and marketed pharmaceutical dosage form by RP-HPLC

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Abstract
Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Revaprazan.
Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Revaprazan. The chromatographic strategy utilized Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consists of 48:52% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 248 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.
Results: LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.
Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

Keywords: Revaprazan, RP-HPLC, method development, validation, accuracy, robustness

Introduction

Revaprazan is a member of isoquinolines. Revaprazan is under investigation in clinical trial NCT01750437 (Phase 2 Clinical Trial to Investigate the Safety, Tolerability and Efficacy of YH1885L in Patients with Non-erosive Reflux Disease (nerd)).Revaprazan [1] (trade name Revanex) is a drug that reduces gastric acid secretion which is used for the treatment of gastritis. It acts as an acid pump antagonist (Potassium-competitive acid blocker). Revaprazan is approved for use in South Korea, but is not approved in Europe or the United States. Revaprazan is under investigation in clinical trial NCT01750437 (Phase 2 Clinical Trial to Investigate the Safety, Tolerability and Efficacy of YH1885L in Patients with Non-erosive Reflux Disease (nerd)).Revaprazan [2] is a member of isoquinolines. Revaprazan is a proton pump inhibitor that is currently being investigated for the management of gastric and duodenal ulceration, functional dyspepsia and GERD. Revaprazan is prescribed for the treatment of duodenal ulcer, gastric ulcer and gastritis. Revaprazan [3] is prescribed for the treatment of duodenal ulcer, gastric ulcer and gastritis. Revaprazan is classified a reversible acid pump antagonist (APA or a potassium-competitive acid blocker) since it acts in a mechanism different from irreversible proton pump inhibitors, such as omeprazole. Revaprazan is not dependent upon secretion status of a proton pump or acid activation of a drug in a stomach. Revaprazan[4] has long-lasting acid-suppressive effects. Revaprazan is approved for use only in Korea and India.

Revaprazan, a novel acid pump antagonist, exerts anti-inflammatory action against Helicobacter pylori-induced COX-2 expression by inactivating Akt signaling. Revaprazan [5] is used for the short term treatment of gastric ulcer, duodenal ulcer and for improvement of mucosal lesion in acute and chronic gastritis. It decreases the acid produced in the stomach and helps in promoting the healing of ulcers. The IUPAC Name of Revaprazan [6] is N-(4-fluorophenyl)-4,5-dimethyl-6-(1-methyl-3,4-dihydro-1H-isoquinolin-2-yl)pyrimidin-2-amine. The Chemical Structure of Revaprazan is shown in figure-1.

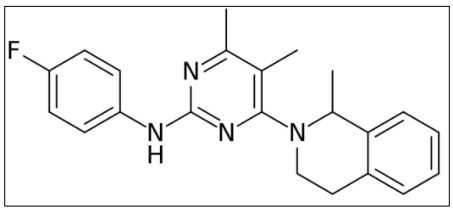

Fig 1: Chemical Structure of Revaprazan

Materials and Methods

Materials

Revaprazan API and Marketed Formulation were provided by Synpharma Research Lab, Dilsuknagar, Hyderabad. HPLC grade acetonitrile and phosphoric acid, Dipotassiumhydrogen orthophosphate, Potassium dihydrogen orthophosphate, Sodium hydroxide, Hydrochloric acid, 3% Hydrogen Peroxide were purchased from SD fine-Chem ltd; Mumbai. HPLC grade water was obtained from SD fine-Chem ltd; Mumbai.

Apparatus and Chromatographic Conditions

Analyses were performed using a Waters HPLC with Empower2 Software with Isocratic with UV-Visible Detector was provided from Synpharma Research Lab, Dilsuknagar, Hyderabad. This Instrument is connected to a Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm particle size) supplied with an isocratic pump and an auto-sampling device. The experiments were operated at ambient temperature. The mobile phase was composed of a mixture of Phosphate Buffer (0.02M) and Acetonitrile in the ratio of 48:52% v/v (pH-2.80). The mobile phase flow-rate was 1.0 mL/min. The detection was performed using a UV detector at wavelength 248 nm.

Preparation of Standard Solution

The standard solution was prepared by weighing 10 mg of Revaprazan in volumetric flask and dissolving in mobile phase and obtaining the concentration of 1000 µg/ml and further diluting to appropriate concentration. After filtration [7] through Ultipore 0.45 µm Nylon 6 filter, aliquots were combined to get a final concentration of Revaprazan is 50µg/ml. This concentration was further diluted with mobile phase before injection into HPLC system.

Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution

About 2.72172 grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

Preparation of Mobile Phase

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic [8] water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

Results and Discussion

Development of Analytical Method

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima[9]of Revaprazan, so that the same wave number can be utilized in HPLC UV detector for estimating the Revaprazan. While scanning the Revaprazan solution we observed the maxima at 248 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

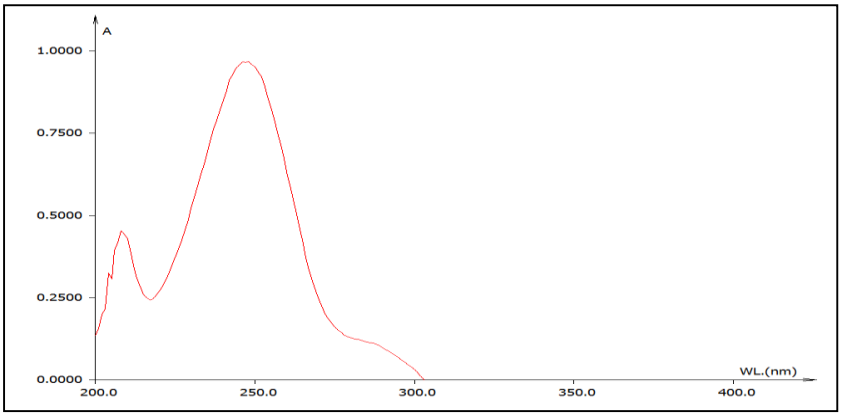

Fig 2: UV Spectrum for Revaprazan (248 nm)

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.5 ml of the above Revaprazan stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions [10, 11] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [33].

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 Revaprazan equivalent 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 [12]. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.5 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas [13].

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column [14], different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Summary of Optimized Chromatographic Conditions:

The Optimum conditions [15] obtained from experiments can be summarized as below:

Table-1: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80)
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	248 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.649 minutes

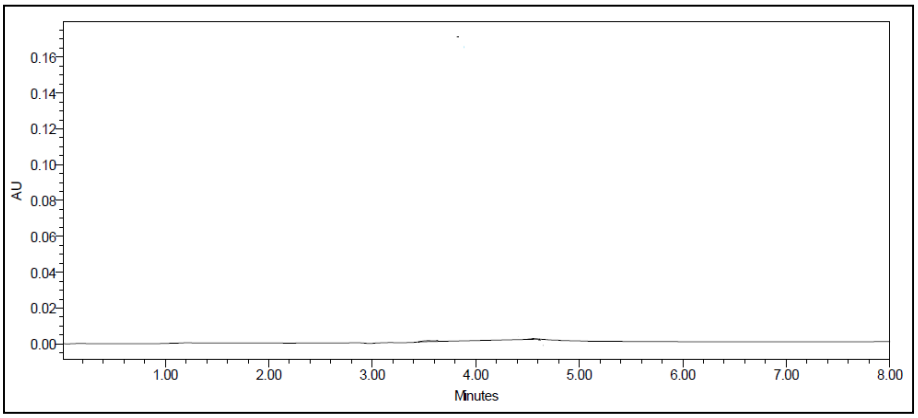

Fig 3: HPLC Spectrum of Revaprazan (Blank Solution)

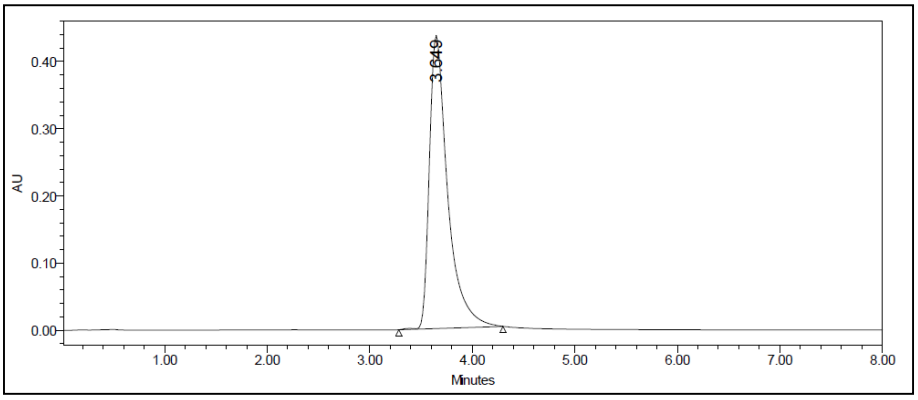

Fig 4: Chromatogram of Revaprazan in Optimized Chromatographic Condition

Method Validation

The method was validated [16] according to ICH guidelines [17] with respect to accuracy, precision, specificity, linearity, solution stability, robustness, sensitivity, and system suitability.

1. Accuracy

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.5 ml of the above Revaprazan stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

For Preparation of 80% Standard Stock Solution
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10 ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.4 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 100% Standard Stock Solution
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.5 ml of stock solution in to 10 ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 120% Standard Stock Solution
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.6 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Recovery Study
To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Revaprazan were taken and extra to the pre-analyzed formulation of concentration 50 µg/ml. From that proportion recovery values [18-19] were calculated. The results were shown in table-2.

Table 2: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80%	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319% R.S.D.=0.071038
S_2 : 80%	40	40.191	503214	100.477	
S_3 : 80%	40	40.142	502656	100.355	
S_4 : 100%	50	50.044	614215	100.088	Mean= 99.98533% S.D. = 0.183045% R.S.D.=0.183071
S_5 : 100%	50	49.887	612451	99.774	
S_6 : 100%	50	50.047	614254	100.094	
S_7 : 120%	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574% R.S.D.=0.407308
S_8 : 120%	60	59.939	725698	99.898	
S_9 : 120%	60	60.429	731211	100.715	

2. Precision
2.1. Repeatability
Preparation of Revaprazan Product Solution for Precision
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

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.
The exactitude [20] of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Revaprazan (API). The % relative variance was calculated for Revaprazan square measure bestowed within the table-3.

Table 3: Repeatability Readings

HPLC Injection Replicates of Revaprazan	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		5657177
Standard Deviation		11369.72
% RSD		0.200979

2.2. Intermediate Precision/Ruggedness
2.2.1. Intra-Day & Inter-Day
The intra & inter day variation [21-22] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Revaprazan revealed that the proposed method is precise.

Procedure

Analyst 1: 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.

Analyst 2

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.

Analyst-1/Intra Day/Day-1

Table 4: Results of Ruggedness for Revaprazan Analyst 1

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Revaprazan	3.687	584968	65982	4985	1.42
2	Revaprazan	3.688	582479	66354	4876	1.46
3	Revaprazan	3.688	586236	67425	4896	1.48
4	Revaprazan	3.687	586985	65982	4986	1.47
5	Revaprazan	3.684	582679	65932	5016	1.45
6	Revaprazan	3.649	583989	65874	4987	1.43
Mean			584556			
Std. Dev.			1846.658			
%RSD			0.315908			

Analyst 2/Inter Day/Day-2

Table 5: Results of Intermediate Precision Analyst 2 for Revaprazan

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Revaprazan	3.649	598698	66985	5265	1.49
2	Revaprazan	3.684	596847	67458	5168	1.47
3	Revaprazan	3.687	596354	66985	5436	1.46
4	Revaprazan	3.688	598676	67854	5369	1.45
5	Revaprazan	3.688	596874	68521	5247	1.48
6	Revaprazan	3.687	598989	67898	5375	1.42
Mean			597739.7			
Std. Dev.			1168.098			
%RSD			0.195419			

3. Linearity & Range

Preparation of Drug Solutions for Linearity

Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5 ml of the above Revaprazan stock solutions into a 10 ml volumetric flask and dilute up to the mark with Mobile Phase [23].

Preparation of Level – I (30 ppm of Revaprazan)

Take 0.3 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (40 ppm of Revaprazan)

Take 0.4 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (50 ppm of Revaprazan)

Take 0.5 ml of stock solution in to 10 ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (60 ppm of Revaprazan)

Take 0.6 ml of stock solution in to 10 ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (70 ppm of Revaprazan)

Take 0.7 ml of stock solution in to 10 ml of volumetric flask and make up the volume up to 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 [24].

The calibration curve showed good linearity in the range of 0-70 µg/ml, for Revaprazan (API) with correlation coefficient (r^2) of 0.999 (Fig-5). A typical calibration curve[25] has the regression equation of y = 11266.x + 50416 for Revaprazan.

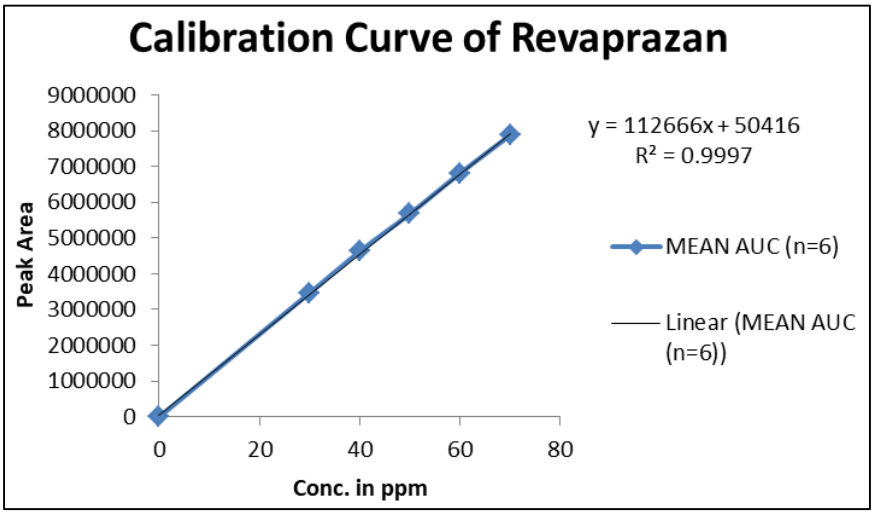

Fig 5: Calibration Curve of Revaprazan (API)

Table 6: Linearity Results

CONC. (µg/ml)	MEAN AUC (n=6)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

Linearity Plot
The plot of Concentration (x) versus the Average Peak Area (y) data of Revaprazan is a straight line.

Y = mx + c

Slope (m) = 112666
Intercept (c) = 50416
Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.
Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 50416. These values meet the validation criteria.

4. Method Robustness
The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Revaprazan. The method is robust[26] only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Revaprazan were injected by changing the conditions of chromatography [27]. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.5 ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1 ml/min, remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition
The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 10 µl of the above sample was injected and chromatograms were recorded.

Table 7: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	584624	3.649	1.42	4765
Less Flow rate of 0.9 mL/min	598676	3.687	1.49	4856
More Flow rate of 1.1 mL/min	612543	3.649	1.46	4965
Less organic phase	578642	3.688	1.49	4758
More organic phase	569896	3.684	1.47	4962

5. LOD & LOQ
LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected [28] but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve
LOQ: The quantitation limit of an individual analytical

procedure is the lowest amount of analyte in a sample which can be quantitatively [29] determined.

$LOQ = 10 \times \sigma/S$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Table 8: Results of LOD & LOQ

SE of Intercept	48846.22527
SD of Intercept	109223.4801
LOD	3.199168
LOQ	9.694449

Observation
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 3.19 & 9.69 µg/ml respectively.

6. System Suitability Parameter
System quality testing is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. Following system quality check parameters [30] were established. The information square measured shown in Table-9 & 10.

Preparation of Standard Solution
Accurately weigh and transfer 10 mg of Revaprazan working standard into a 10ml of clean dry volumetric flasks add about 7 ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.5 ml of the above Revaprazan stock solutions into a 10 ml volumetric flask and dilute up to the mark with Methanol.

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: Knowledge of System quality Parameter

S. No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Revaprazan =0.98
2	Theoretical plate	N > 2000	Revaprazan =4782
3	Tailing Factor	T<2	Revaprazan =1.49

Table 10: Results of System Suitability for Revaprazan

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Revaprazan	3.644	584635	65847	4857	1.48
2	Revaprazan	3.645	582695	65421	4955	1.42
3	Revaprazan	3.644	587432	65369	4875	1.47
4	Revaprazan	3.662	589687	65748	4796	1.46
5	Revaprazan	3.660	582547	65398	4952	1.49
6	Revaprazan	3.660	589656	652418	4896	1.47
Mean			586108.7			
Std. Dev.			3275.654			
%RSD			0.558882			

7. Specificity
Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific [31].
The chromatograms representing the peaks of blank, Revaprazan and the sample containing the one drug was shown in following figures respectively.
Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

8. Estimation of Revaprazan in Pharmaceutical Dosage Form
Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to work out the typical weight. On top of weighed tablets were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45 µm) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded.
A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The information square measure shown in Table-11.

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

Where:
AT = Peak space of drug obtained with check preparation
AS = Peak space of drug obtained with normal preparation
WS = Weight of operating normal taken in mg

WT = Weight of sample taken in mg
DS = Dilution of normal resolution
DT = Dilution of sample resolution
P = proportion purity of operating normal

Table 11: Recovery Data for Estimation Revaprazan

Brand Name of Revaprazan	Labelled amount of Drug (mg)	Mean (± SD) Amount (mg) found by the Proposed Method (n=6)	Assay % (± SD)
Revanex 200 Mg Tablet (Zydus Cadila)	200mg	199.462 (± 0.485)	99.639 (±0.287)

Result & Discussion

The amount of drug in Revaprazan Tablet was found to be 199.462 (±0.485) mg/tab for Revaprazan & % assay [32] was 99.639 (±0.287).

Summary and Conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 248nm and the peak purity was excellent. Injection volume was selected to be 20 µl which gave a good peak area. The column used for study was Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm particle size because it was giving good peak. Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 ml/min because of good peak area and satisfactory retention time. Mobile phase is Phosphate Buffer (0.02 M) and Acetonitrile were taken in the ratio of 48:52 % v/v (pH-2.80) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8.0 min because analyze gave peak around 3.649 min and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 30-70 ppm of the Revaprazan target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Revaprazan in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Revaprazan in bulk drug and in Pharmaceutical dosage forms.

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