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Development of A New RP-HPLC Method For Estimation of Aprepitant From Solid Dosage Form.

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ABSTRACT

The aim of the present work was to develop and validate a simple and efficient method for the analysis of Aprepitant in pharmaceutical dosage forms by reverse phase high-pressure liquid chromatography. A stainless steel column 75 mm long, 4.6 mm internal diameter filled with octasilyl silica chemically bonded with synthetic hybrid silica gel particles of 3.5 μm diameter was used for elution. The retention time of Aprepitant was 4.05 min. The method showed a good linearity in the concentration range of 0.02478 – 0.07434 mg/mL with a correlation coefficient of 0.9999. The validation characteristics included specificity, linearity, limit of detection, limit of quantification, precision, robustness and stability. Validation acceptance criteria were met in all cases. The method could be successfully used for the analysis of Aprepitant in pharmaceutical dosage forms.

Keywords: Aprepitant, Accuracy, Precision, Linearity, Mobile Phase and Validation

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INTRODUCTION

Aprepitant is a novel antiemetic agent used in cancer chemotherapy; with a chemical name 5-([(2R,3S)-2-((R)- 1-[3,5-bis (trifluoromethyl) phenyl] ethoxy)-3-(4-fluorophenyl) morpholino] methyl)-1H-1,2,4-triazol-3(2H)- one. Its molecular weight is 534.427 g/mol with molecular formula $C_{23}H_{21}F_7N_4O_3$. It mediates its effect by blocking the neurokinin receptor. RP-HPLC and LC-MS methods are used for the quantitative analysis. Literature review reveals that Aprepitant in the human plasma can be estimated by high performance liquid chromatography with tandem mass spectrometric methods [1-2], stability indicating RP-HPLC method in bulk and pharmaceutical dosage forms [3], rapid liquid chromatography method in solid dosage forms [4, 5]quantification of process related impurities by RP-LC method [6] and derivative spectroscopic methods [7]. Direct multivariate UV spectrophotometric method was used for the simultaneous determination of Aprepitant along with other two anti-emetic drugs [8].

Present study aims to develop simple, rapid, accurate, precise and validated stability indicating HPLC method for the determination of Aprepitant in solid dosage forms. The main objective of method development is to determine the drug content of formulations as well as purity. Using stability indicating analytical method, one can able to detect changes in the drug substance and the drug product that are specific active ingredient. In addition, the degradation of the drug substance can be estimated without interference.

MATERIALS AND METHOD

Chemicals

Aprepitant sample was obtained as generous gift from Dr Reddys Laboratories Private Limited, Hyderabad, India with the purity of 99.8%. Potassium Dihydrogen Phosphate (AR grade), Acetonitrile (HPLC grade), Orthophosphoric acid (AR grade) and Triethyl amine (GR grade) were purchased from Merck. Milli-Q-grade of water was used for the development.

Instrumentation

A High Performance Liquid Chromatographic system with gradient/isocratic elution capability, a Spectrophotometer UV detector and an auto sampler using a stainless steel column 75 mm long, 4.6 mm internal diameter filled with octadecylsilyl silica chemically bonded with synthetic hybrid silica gel particles with end capping and particle size of 3.5 µm diameter.

Preparation of mobile phase

Buffer preparation: Prepare 10mM Potassium dihydrogen phosphate solution containing 0.1% trimethylamine with final pH 3.0 using Orhophosphoric acid.

Mobile phase preparation: Mix Buffer and Acetonitrile in the ratio of 50:50 v/v and degas.

Diluent: Mix Water and Acetonitrile in the ratio of 50:50 v/v.

Chromatographic conditions

Column	C18, 75 X 4.6 mm, 3.5µ particle size or equivalent.
Flow rate	: 1.2 mL/min
Detection	: UV, 210 nm
Injection Volume	: 10 µL
Data acquisition time	: 8 minutes
Pump mode	: Isocratic

Preparation of solutions

Blank Preparation: Use diluent as blank solution.

Standard solution: Prepare a solution containing 0.05 mg/mL of Aprepitant in diluent.

Weigh accurately 25.0 mg of Aprepitant working standard into a 50 mL clean, dry volumetric flask. Add 30 mL of diluent and sonicate to dissolve. Make up to volume with diluent and mix. Dilute 5 mL to 50 mL with diluent and mix. Filter through 0.45µ PVDF syringe filter by discarding first 10 mL of filtrate. Prepare it in duplicate and label as standard solution-A and standard solution-B.

Sample solution: Prepare a solution containing 0.05mg/mL as Aprepitant in diluent.

Weigh and transfer powder equivalent to 250 mg of Aprepitant in to a 250 mL of clean and dry volumetric flask. Add 150 mL of diluent and sonicate for 15 minutes with intermittent shaking. Make up to volume with diluent and mix. Filter through 0.45µ PVDF syringe filter by discarding first 5mL of filtrate. Dilute 5 mL to 100 mL with diluent and mix. Prepare in duplicate (Concentration 0.05mg/mL as Aprepitant).

Evaluation of System Suitability

Equilibrate the column with initial composition for about 30 minutes. Inject the blank (diluent) solution into the liquid chromatograph and record the chromatogram. Separately inject 10µL of the standard solution-A, five times into the liquid chromatograph, record the chromatograms.

The symmetry factor should be not more than 2.0 from the standard chromatogram at least from the first injection. RSD for peak areas of five injections from standard solution-A should not be more than 2.0%. Inject 10µL of Standard solution-B in duplicate into the liquid chromatograph and record the chromatograms.

Calculate the similarity factor between two standard preparations. The similarity factor between two standard preparations should be within the range of 0.98 to 1.02.

Calculation of similarity factor:

$$\text{Similarity factor} = \frac{\text{Average area of STD-A}}{\text{Average area of STD-B}} \text{ X } \frac{\text{Weight of STD-B}}{\text{Weight of STD-A}}$$

Procedure:

Inject the sample solutions into the liquid chromatographic system and record the chromatogram. Retention time of Aprepitant is about 3.6 minutes. Calculate the assay for each preparation and report the average result.

Calculation:

$$\%\text{Label claim} = \frac{At}{As} \text{ X } \frac{Ws}{50} \text{ X } \frac{5}{50} \text{ X } \frac{250}{W_t} \text{ X } \frac{100}{5} \text{ X } \frac{A_W}{LC} \text{ X P}$$

Where,

At = Area of peak corresponding to Aprepitant in sample solution chromatogram.

As = Average area of peak corresponding to Aprepitant in STD-A chromatograms.

W_s = Weight of Aprepitant working standard in mg for standard solution-A.

P = % Assay of Aprepitant working standard on as is basis.

W_t = Weight of sample in mg.

AW = Average weight of the dosage form in mg.

LC = Label claim of Aprepitant film-coated tablets in mg.

VALIDATION OF THE HPLC METHOD

The proposed method was validated as per ICH guidelines

Linearity:

A series of standard dilutions of Aprepitant were prepared from stock solution. Linearity was evaluated by plot of peak areas as a function of analyte concentration and the test results were evaluated by appropriate statistical methods i. e. slope, intercept, regression (R^2) correlation coefficients (R).

Precision:

Precision is the measure of closeness of the data values to each other for a number of measurements under the same analytical conditions. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration. The standard deviation and the relative standard deviation were reported for precision.

Specificity:

The specificity of the method was determined by comparing the chromatograms obtained from the drug substance with that obtained from the tablet solution. The chromatograms of diluents,

standard and sample were shown.

Accuracy:

Accuracy was established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed method recovery studies were performed by the standard addition method by spiking 50%, 100%, and 150% of the known quantities of standard.

Robustness:

To determine the robustness of the method developed, the experimental conditions were deliberately altered and the chromatographic parameters viz., tailing factor, number of theoretical plates and percentage assay were recorded.

System suitability was carried out by injecting a standard concentration at different injection volumes. The system suitability test parameters were noted and percentage RSD was calculated.

RESULTS AND DISCUSSION

SPECIFICITY

Inference

The blank solution, placebo solution, standard solution, impurity solutions, sample solutions and Impurity spiked sample solutions are analyzed by HPLC system and checked for interference.

There is no interference peak was observed due to blank, placebo and known Impurity at the retention time of Aprepitant.

Acceptance criteria

No significant Interference of blank, placebo and known impurities should be observed at the retention time of analyte peak. Peak purity should pass for analyte. Purity angle should be less than the purity threshold.

Table 1: Interference table for blank, placebo, standard and assay sample

S. No.	Sample	Retention time (min)	Purity angle	Purity threshold	Peak Purity
1	Diluent (Blank)	-	-	-	-
2	Placebo	-	-	-	-
3	Standard (Aprepitant)	4.056	0.176	0.351	Pass
4	Aprepitant dosage form 80 mg strength	4.042	0.169	0.362	Pass
5	Aprepitant dosage form 125 mg strength	4.045	0.150	0.342	Pass
6	Impurity spiked sample (Peak purity for Aprepitant)	4.046	0.175	0.368	Pass

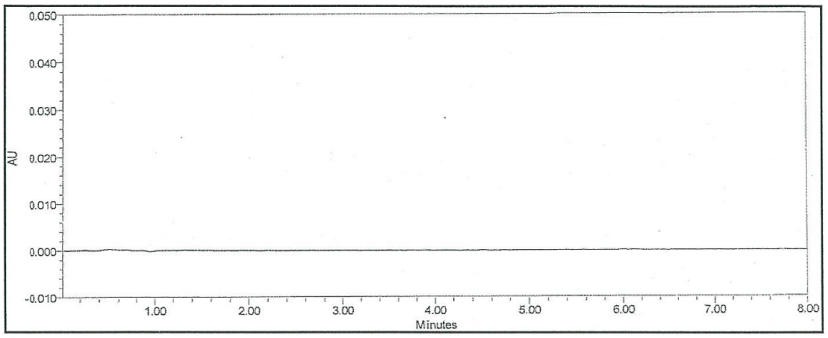

Figure 1: Blank solution – Chromatogram

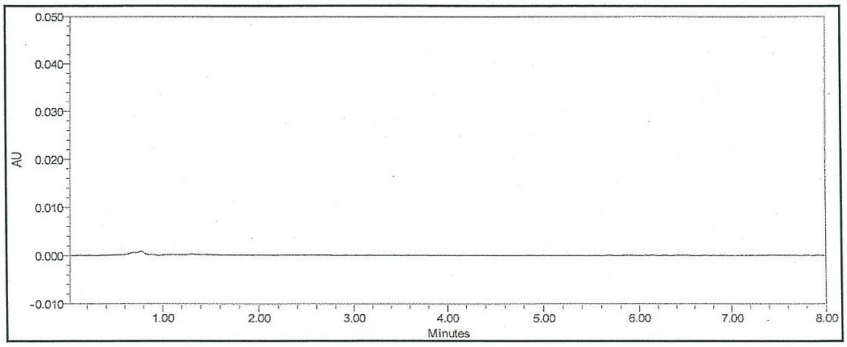

Figure 2: Placebo Solution – Chromatogram

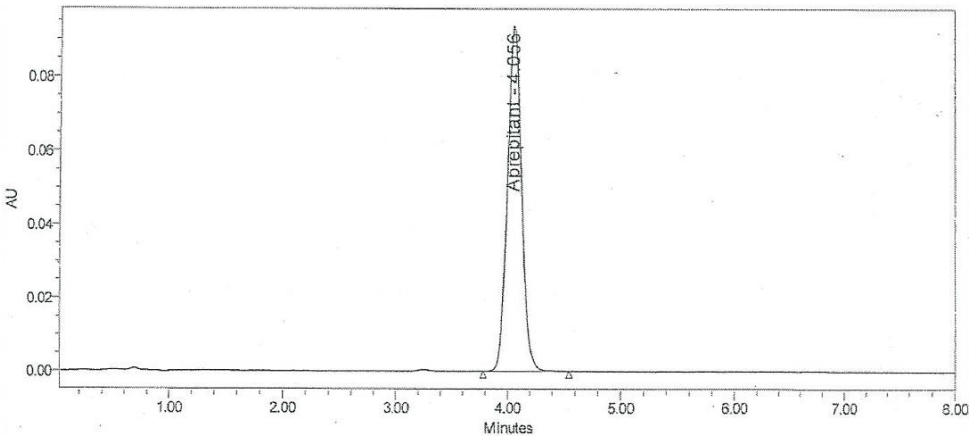

Figure 3: Standard solution

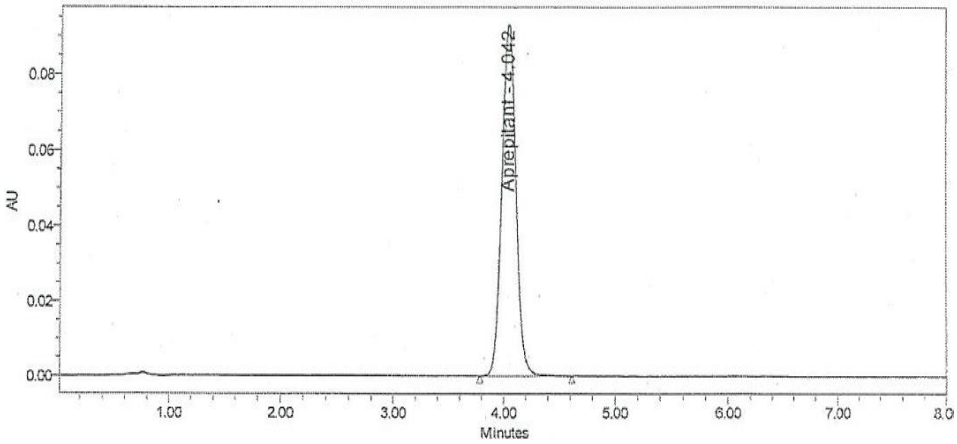

Figure 4: Chromatogram of sample solution-80 mg strength

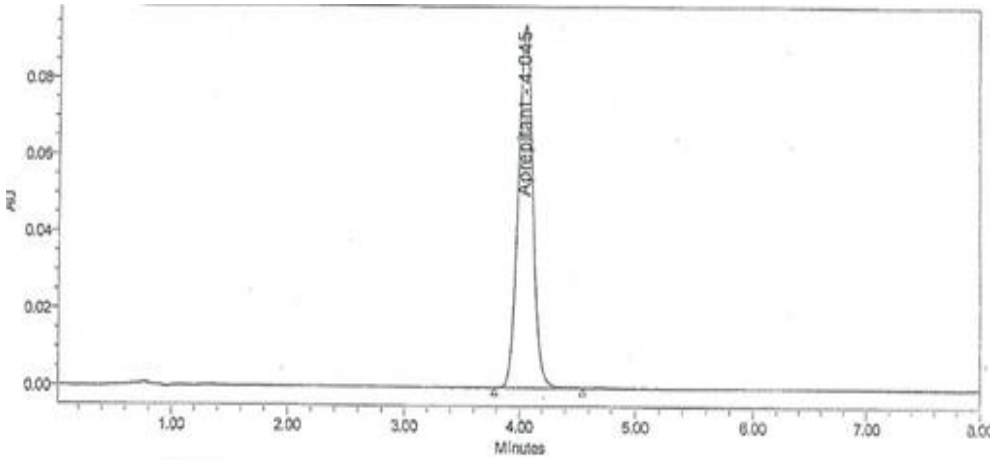

Figure 5: Chromatogram of sample solution-125 mg strength

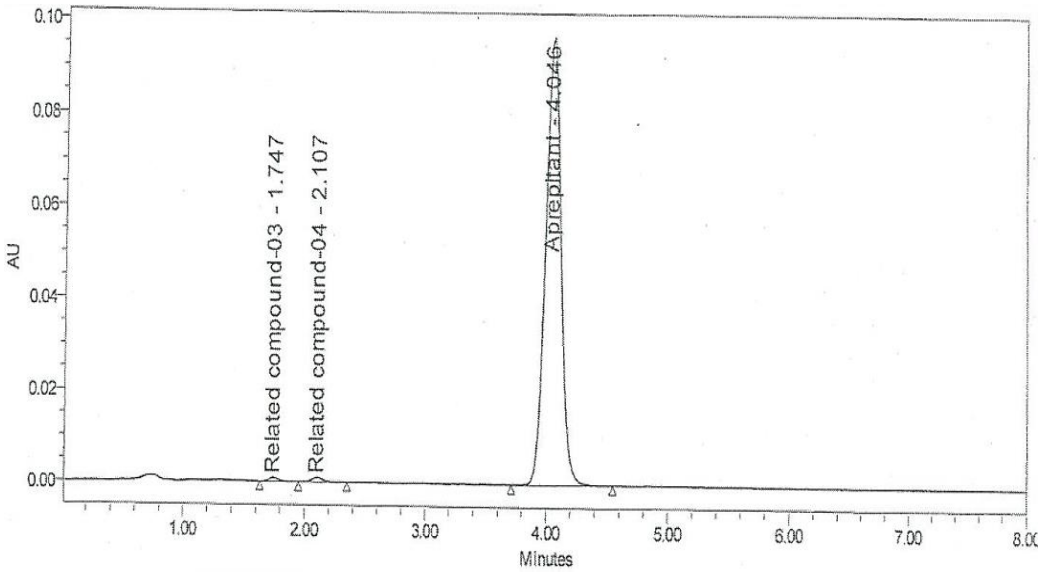

Figure 6: Impurity spiked sample solution-125 mg strength

LINEARITY

The linearity of response for Aprepitant was determined at different concentration levels as shown in the following table and enclosed graphically. The results were calculated from linearity graph using the linearity equation: Y = BX + A (Where as B is the slope and A is the intercept)

Table 2: Linearity Table

S. No.	Target concentration (%)	Concentration (mg/mL)	Area
1	50	0.02478	413051
2	80	0.03965	660876
3	100	0.04956	814819
4	120	0.05947	990889
5	150	0.07434	1236814
Correlation Coefficient [R]			0.9999
Regression Coefficient [R^2]			0.9998

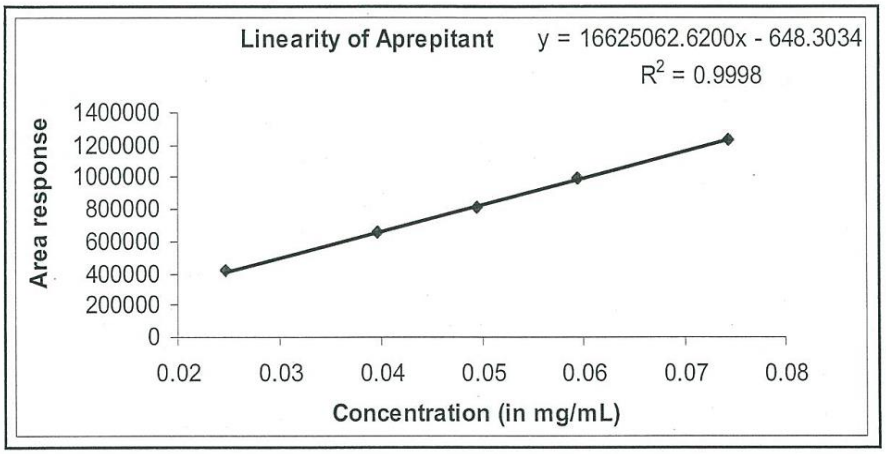

Figure 7: Linearity graph

Acceptance criteria

Correlation coefficient (R) should not be less than 0.99 within the specified range. Regression coefficient should (R^2) be not less than 0.98.

Conclusion: The correlation coefficient is 0.9999 and the regression coefficient is 0.9998. The regression analysis shows linear relationship between concentration and response of Aprepitant.

PRECISION

REPEATABILITY

Six preparations of sample solutions were prepared and injected into HPLC system. The % RSD value for assay of Aprepitant 80 mg and 125 mg strengths are shown below.

Table 3: Precision Table

Preparation No.	%Label claim	
	80 mg strength	125 mg strength
1	99.0	101.7
2	100.1	101.1
3	98.7	101.2
4	99.0	100.4
5	97.5	101.2
6	97.9	101.0
Mean	98.7	101.1
Standard deviation	0.9187	0.4195
% RSD	0.93	0.41

Acceptance criteria: % RSD for replicate analysis should not be more than 2.0.

Conclusion: The obtained percentage RSD value indicates a good degree of precision. The result indicates of precision of analytical method for the assay of Aprepitant in Aprepitant dosage form.

ACCURACY FOR ASSAY

A known amount of analyte, both above and below the normal levels expected in the sample

spiked with placebo and analyzed by the proposed HPLC method and the results are shown below.

Table 4: Accuracy for assay

Level	Theoretical Concentration (mg/mL, as Aprepitant)	Experimental Concentration (mg/ml, as Aprepitant)	% Recovery
50%-T1	0.0250682	0.0250345	99.9
50%-T2	0.0250068	0.0257472	103.0
50%-T3	0.0250229	0.0252000	100.7
100%-	0.0499767	0.0508234	101.7
100%-	0.0500016	0.0505891	101.2
100%-	0.0500204	0.0506592	101.3
150%-	0.0750396	0.0749546	99.9
150%-	0.0750339	0.0753405	100.4
150%-	0.0750480	0.0756308	100.8
Mean			101.0
Standard deviation			0.97
% RSD			0.96
Minimum			99.9
Maximum			103.0

Table 5: Precision and Accuracy at 50% Level

Level	Theoretical Concentration (mg/mL)	Experimental Concentration (mg/mL)	% Recovery
50%-T1	0.0250682	0.0250345	99.9
50%-T2	0.0250068	0.0257472	103.0
50%-T3	0.0250229	0.0252000	100.7
50%-T4	0.0250042	0.0250658	100.2
50%-T5	0.0250000	0.0251867	100.7
50%-T6	0.0250208	0.0251557	100.5
Mean			100.8
Standard deviation			1.11
% RSD			1.10
Minimum			99.9
Maximum			103.0

Table 6: Precision and Accuracy at 150% level

Level	Theoretical Concentration (mg/mL)	Experimental Concentration (mg/mL)	% Recovery
150%-T1	0.0750396	0.0749546	99.9
150%-T2	0.0750339	0.0753405	100.4
150%-T3	0.0750480	0.0756308	100.8
150%-T4	0.0750485	0.0756717	100.8
150%-T5	0.0750324	0.0759338	101.2
150%-T6	0.0750412	0.0761475	101.5
Mean			100.8
Standard deviation			0.57
% RSD			0.56
Minimum			99.9
Maximum			101.5

Acceptance criteria:

% Recovery of analyte should be 97 to 103 within specified Range.

Conclusion: The % Recovery was found in between 97 to 103 for all accuracy level. The results indicate the precision and accuracy of analytical method is good with in the specified range.

ROBUSTNESS

To establish the robustness of the HPLC method employed for analysis of assay of Aprepitant in Aprepitant dosage form, the method was challenged for various parameters like stability of analytical solutions, effect of mobile phase flow rate, effect of wavelength, effect of mobile phase ratio, effect of mobile phase pH and effect of filter interference. The observations in different conditions are tabulated below.

SOLUTION STABILITY

To establish the stability of analytical solutions of the proposed analytical method employed for analysis of Aprepitant in Aprepitant dosage form, stability of analytical solutions was evaluated during method validation. The observations in different conditions are tabulated below.

Table 7: Stability of analytical solutions

Parameter	Acceptance criteria	Initial	On Bench Top		In Refrigerator	
			After 24 hours	After 48 hours	After 24 hours	After 48 hours
% Labeled amount	NMT 2% of initial value	101.7	101.8	101.0	101.0	101.9
Symmetry factor	NMT 2.0	1.06	1.05	1.24	1.05	1.24
% RSD	NMT 2.0	0.16	0.55	0.42	0.55	0.42
Similarity factor with fresh standard	0.98 to 1.02	1.00	1.00	1.00	1.00	1.02

Conclusion: Analytical solutions were stable up to 48 Hours on bench top and in Refrigerator.

EFFECT OF WAVELENGTH (± 2nm)

Table 8: Effect of different wavelengths on % label claim

Parameter	Specification	Initial (At 210 nm)	Change-1 (At 208 nm)	Change-2 (At 212 nm)
% Labeled amount	95.0 to 105.0	97.3	98.0	98.0
Symmetry factor	NMT 2.0	1.22	1.08	1.09
% RSD	NMT 2.0	0.36	0.08	0.08
Similarity factor	0.98 to 1.02	1.00	1.00	1.00

Conclusion: No significant variation was observed in the results obtained by doing small variation in the wavelength.

EFFECT OF MOBILE PHASE FLOW RATE (± 0.2 mL/min)

Table 9: Effect of different flow rates on % label claim (+ 0.1mL of actual flow)

Parameter	Specification	Initial (1.2 mL/min)	Change-1 (1.1 mL/min)	Change-2 (1.3 mL/min)
% Labeled amount	95.0 to 105.0	97.3	98.6	98.7
Symmetry factor	NMT 2.0	1.22	1.10	1.09
% RSD	NMT 2.0	0.36	0.56	0.23
Similarity Factor	0.98 to 1.02	1.00	1.00	1.00

Conclusion: No significant variation was observed in the results obtained by small variation in the flow rate.

EFFECT OF MOBILE PHASE pH

Table 10: Effect of different mobile phase pH (± 0.2 pH of actual pH value)

Parameter	Specification	Initial (pH 3.0)	Change-1 (pH 2.8)	Change-2 (pH 3.2)
% Labeled amount	95.0 to 105.0	97.3	98.3	97.8
Symmetry factor	NMT 2.0	1.22	1.11	1.09
% RSD	NMT 2.0	0.36	0.18	0.26
Similarity factor	0.98 to 1.02	1.00	1.00	1.00

Conclusion: No significant variation was observed in the results obtained by small variation in the mobile phase ratio.

EFFECT OF MOBILE PHASE COMPOSITION

Table 11: Effect of different mobile phase composition

Parameter	Specification	Initial Buffer: Acetonitrile (50:50)	Change-1 Buffer: Acetonitrile (52:48)	Change-2 Buffer: Acetonitrile (48:52)
% Labeled amount	95.0 to 105.0	97.3	99.2	98.0
Symmetry factor	NMT 2.0	1.22	1.14	1.09
% RSD	NMT 2.0	0.36	0.73	0.26
Similarity factor	0.98 to 1.02	1.00	1.00	1.00

Conclusion: No significant variation was observed in the results obtained by small variation in the mobile phase ratio.

EFFECT OF FILTER

Table 12: Effect of different mobile phase composition

Parameter	Un filtered or centrifuged	Change -1 Filtered: 0.45µm (Axiva-PVDF)	Change -2 Filtered: 0.45µm (Millipore-PVDF)	Change -2 Filtered: 0.45µm (GHP Acrodisc)
% Labeled amount for sample	100.2	101.5	101.4	102.5
Response ration with unfiltered standard	-	1.00	1.00	0.99

Conclusion: No significant variation was observed in the results obtained by using different filters of membrane syringe filters.

SYSTEM SUITABILITY

The system suitability chromatograms were obtained during validation. System suitability parameters showed no significant difference in the values over a period.

Table 13: System suitability

Parameter	System Suitability Chromatogram 1	System Suitability Chromatogram 2	Acceptance value	Results
Precision of Area / No. injection	0.16/5 Injections	0.44/ 5 Injections	RSD <2%	Pass
Symmetry factor	1.06	1.11	NMT2.0	Pass
Similarity factor	1.00	0.99	0.98 to 1.02	Pass

Conclusion: The system suitability meets the required acceptance criteria.

CONCLUSION

The experimentally obtained results meet the limits of specificity, linearity, range, precision, and accuracy, stability of analytical solutions, robustness and system suitability. Therefore, determination of Assay of Aprepitant method of analysis for Aprepitant dosage forms can be used in routine analysis.

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