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Der Pharmacia Lettre, 2015, 7 (11):264-271
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Stability indicating method development and validation for the estimation of aprepitant by RP-HPLC in bulk and pharmaceutical dosage form

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ABSTRACT

The purpose of the investigation was to develop a new RP-HPLC Method for estimation of Aprepitant in pharmaceutical dosage forms. Chromatography was carried out on an Kromosil C18 column (4.6 x 250mm, 5µ particle size) with a isocratic mobile phase composed of 0.1% Perchloric acid, Acetonitrile (80:20v/v) at a flow rate of 1.1 mL/min. The column temperature was maintained at 30°C and the detection was carried out using a PDA detector at 210 nm. Validation parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), Stability of sample and standard stock solutions and robustness were studied as reported in the International Conference on Harmonization guidelines. The retention time for Aprepitant was 3.429 min. The percentage recovery of Aprepitant was 101.01%. The relative standard deviation for assay of capsule was found to be less than 2%. The Method was fast, accurate, precise and sensitive hence it can be employed for routine quality control of capsules containing drugs in quality control laboratories and pharmaceutical industries.

Keywords: Aprepitant, ICH guidelines.

INTRODUCTION

Aprepitant is a substance P/neurokinin 1 (NK1) receptor antagonist, an antiemetic agent, chemically described as 5-[[(2R,3S)-2-[(1R)-1-[3,5bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3one.

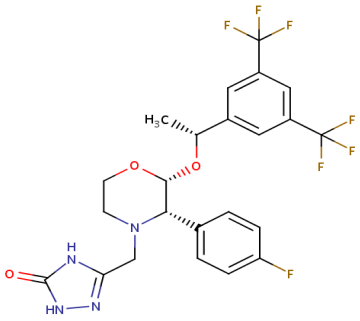
Its empirical formula is C23H21F7N4O3.[1]

Aprepitant is a white to off-white crystalline solid, with a molecular weight of 534.43. It is practically insoluble in water. Aprepitant is sparingly soluble in ethanol and isopropyl acetate and slightly soluble in acetonitrile [2]. Aprepitant is classified as an NK1 antagonist because it blocks signals given off by NK1 receptors. This, therefore, decreases the likelihood of vomiting in patients. Emend is usually taken as a preventative for chemotherapy-induced nausea and vomiting, which is a serious side-effect experienced by over 80% of patients who undergo chemotherapy[3-4].

Various HPLC assay Methods are also reported in the literature for the estimation of Aprepitant[5-9]. According to literature survey there is official Method for the estimation of Aprepitant by RP-HPLC in capsule dosage forms.

Hence, an attempt has been made to develop better Method for estimation and validation of Aprepitant in formulation in accordance with the ICH guidelines[10-14].

Fig.1.Chemical structures of drugs investigated in this study



Aprepitant

MATERIALS AND METHODS

Instrumentation: Chromatography was performed with Alliance waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and with class Empower-2 software.

Reagents and chemicals: The reference sample of Aprepitant was provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade Methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial formulation (**Emend**; Dosage: Aprepitant-40mg capsule) were purchased from the local pharmacy.

Chromatographic condition: The chromatographic separation was carried out under the isocratic conditions. Chromatographic separation was achieved by injecting a volume of 10µl of standard into Kromosil (250 x 4.6 mm, 5µ) column. The mobile phase composed of 0.1% Perchloric acid, Acetonitrile (80:20v/v) was allowed to flow through the column at a flow rate of 1.1 ml per minute for a period of 7 min at 30^0C column temperature. Detection of the component was carried out at a wavelength of 210 nm. The retention time of the component was found to be 3.429 min for Aprepitant.

Preparation of diluent solution: Diluent solution was prepared by mixing 500 ml of HPLC grade water with 500ml of Acetonitrile, in a 1000ml beaker and sonicated for 15min.

Preparation of standard stock solution: Accurately Weighed and transferred 4mg of Aprepitant working Standards into 10ml clean dry volumetric flasks, add 3/4th vol of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

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***Preparation of Working Standard Solutions*:** Aliquot of 0.25, 0.50, 0.75, 1, 1.25 & 1.5 mL were pipette out from stock solution into 10 mL voluMetric flask separately for both APREPITANT and volume was made up to 10 mL with diluent. This gives the solutions of 10, 20, 30, 40, 50and 60µg/mL for Aprepitant.

Sample preparation: 1 capsule was weighed and powdered and it was taken into a 100ml volumetric flask and made up with diluents and labeled as Sample stock solution. Sample stock solution was filtered by HPLC filters.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Method validation:

System suitability tests: To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions of Aprepitant was used for the evaluation of the system suitability parameters like tailing factor, the

number of theoretical plates, retention time.

Linearity: By appropriate aliquots of the standard Aprepitant solution with the mobile phase, six working solutions ranging between 10-60µg/mL was prepared. Each experiment linearity point was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of Aprepitant to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition Method were performed with a view to justify the accuracy of the proposed Method. Previously analyzed samples of Aprepitant to which known amounts of standard Aprepitant corresponding to 50%, 100% and 150% of target concentration were added. The accuracy was expressed as the percentage of analyte recovered by the proposed Method.

Precision: Precision was determined as repeatability and intermediate precision (ruggedness), in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of Aprepitant. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification: Limit of detection (LOD) and limit of quantification (LOQ) of Aprepitant was determined by calibration curve Method. Solutions of both Aprepitant was prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b

Where Syx is residual variance due to regression; b is slope.

Robustness: The robustness of Aprepitant was performed by deliberately changing the chromatographic conditions. The organic strength was varied by ±5%, column temperature was varied by ±5^{0}c and the flow rate was varied by ±0.1mL.

Stability: The sample and standard solutions injected at 0 hr (comparison sample) and after 24 hr (stability sample) by keeping at ambient room temperature. Stability was determined by determining %RSD for sample and standard solutions.

***Degradation studies*:**
***Oxidation*:**
To 1 ml of stock solution of Aprepitant, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^{0}c. For HPLC study, the resultant solution was diluted to obtain 40µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:
To 1 ml of stock ssolution Aprepitant, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^{0}c .The resultant solution was diluted to obtain 40µg/ml l solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:
To 1 ml of stock solution Aprepitant, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 40µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:
The standard drug solution was placed in oven at 105^{0}c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the 40µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber For HPLC study, the

resultant solution was diluted to obtain 40µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 40µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Statistical analysis:
Wherever applicable, results were expressed as the Mean±SD, %RSD and data were analyzed statistically by using t- test with aid of Microsoft excel-2007 software and data were considered not significantly different at 5% significance level of probability P ≤0.05**.**

RESULTS AND DISCUSSION

Aprepitant method development:
Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. Thereafter, 0.1% Perchloric acid: Acetonitrile were taken in isocratic ratio: 80:20 and with flow rate of 1.1 mL/min was employed. Kromosil column (4.6 x250mm, 5µ particle size) was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze drug detection were tried at wavelengths 210nm. Aprepitant showed maximum absorption at 210nm of wavelength and 210 nm was selected as the detection wavelength for PDA detector. The retention times were found to about 3.429 min for Aprepitant. The chromatogram obtained was shown in the Fig. 2.

Aprepitant and met method Validation:
System suitability and Specificity: System suitability parameters such as number of theoretical plates, peak tailing, and retention time was determined. The total run time required for the method is only 6 minutes for eluting Aprepitant. The results obtained were shown in Table No.1. The chromatogram obtained for blank and spiked was shown in the Fig. 3.

Linearity: Aprepitant showed a linearity of response between 10-60 µg/mL. These were represented by a linear regression equation as follows: y (Aprepitant) = 33686x + 955.0 (r^2=0.999) and regression line was established by least sqsuares method and correlation coefficient (r^2) for Aprepitant is found to be greater than 0.98. Hence the curves established were linear.

Accuracy: To pre analyzed sample solution, a definite concentration of standard drug (50%, 100% & 150 % level) was added and recovery was studied. The % Mean recovery for Aprepitant is 100.71% and these results are within acceptable limit of 98-102. The % RSD for Aprepitant was 0.64 and %RSD for Aprepitant is within limit of ≤2, hence the proposed method is accurate and the results were summarized in Table No.2.

Precision: Repeatability: Six replicates injections in same concentration (40µg/ml of Aprepitant) were analyzed in the same day for repeatability and the % RSD for Aprepitant found to be 0.56 and % RSD for Aprepitant found to be within acceptable limit of ≤2 and hence method is reproducible and the results are shown in Table No. 3**.**

Intermediate Precision: Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for Aprepitant is found to be 0.31 and it is within acceptable limit of ≤2. Hence the Method is reproducible on different days with different analyst and column. This indicates that the method is precise and the results are as shown in Table No. 3.

Robustness*:* The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean R_t and RSD is within limit of ≤2 .The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for Aprepitant. Hence the Method is reliable with variations in the analytical

conditions and the result of Aprepitant was shown in Table No.5.

Stability of sample solution:. The sample solution injected after 24 hr by keeping at ambient room temperature $30^{O}C$ did not show any appreciable change. The % Deviation in the assay is not more than 2 and the results are shown in table-6.

LOD and LOQ: LOD and LOQ for Aprepitant were 0.003 and 0.009 µg/mL respectively. The lowest values of LOD and LOQ as obtained by the proposed Method indicate that the Method is sensitive.

Capsule Analysis: The Content of Aprepitant in the capsules was found by the proposed method. RSD values for both Aprepitant was within limit of ≤2 and the results were shown in Table No. 7.

Degradation studies: The degradation studies for Aprepitant was performed by various conditions like Acid, Alkali, Oxidation, Thermal, Photolytic and Neutral Degradation Studies and their limits like purity angle and purity threshold values like purity angle< purity threshold and the results shown in table no.8.

Fig.2.A typical Chromatogram of Aprepitant

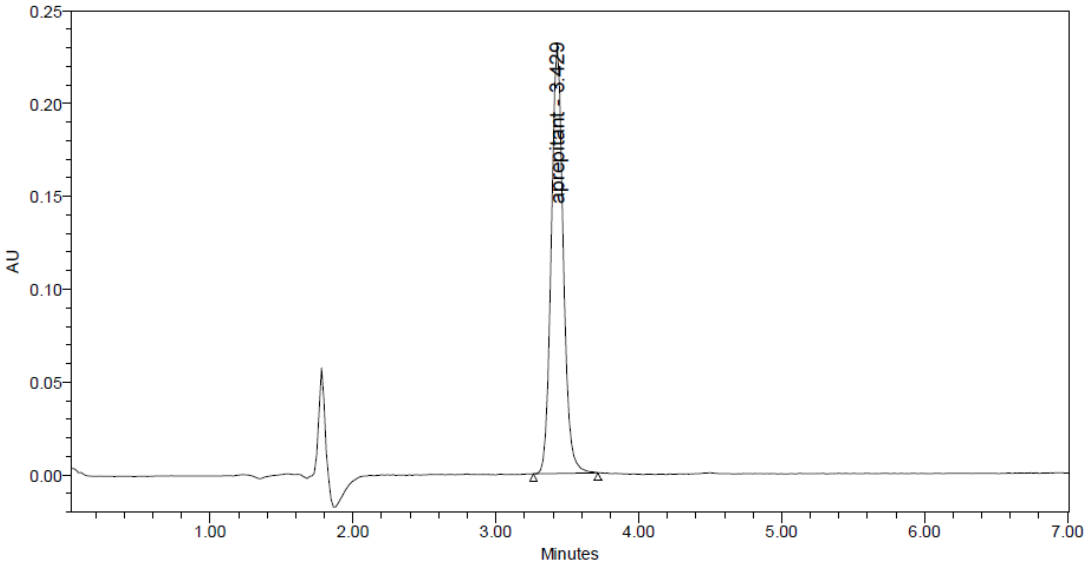
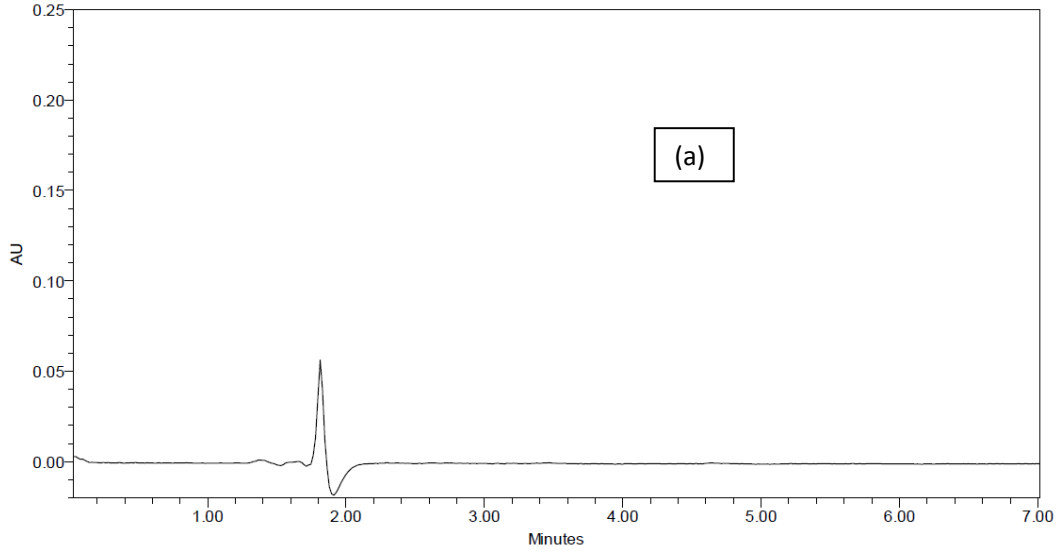


Fig.3. Chromatograms of Blank(a) and spiked (b)



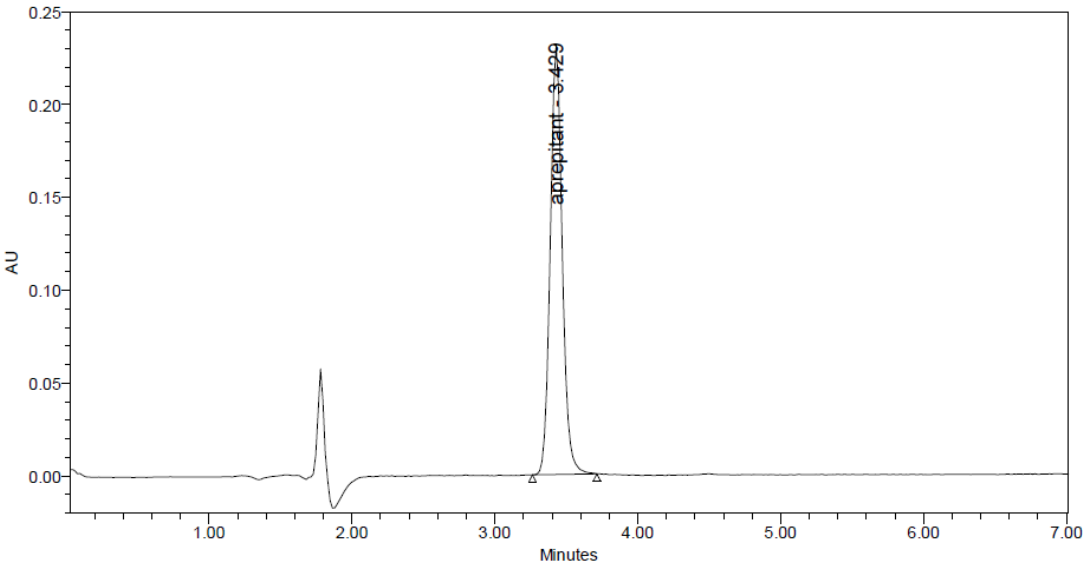


Fig.4. Degradation overlay chromatogram

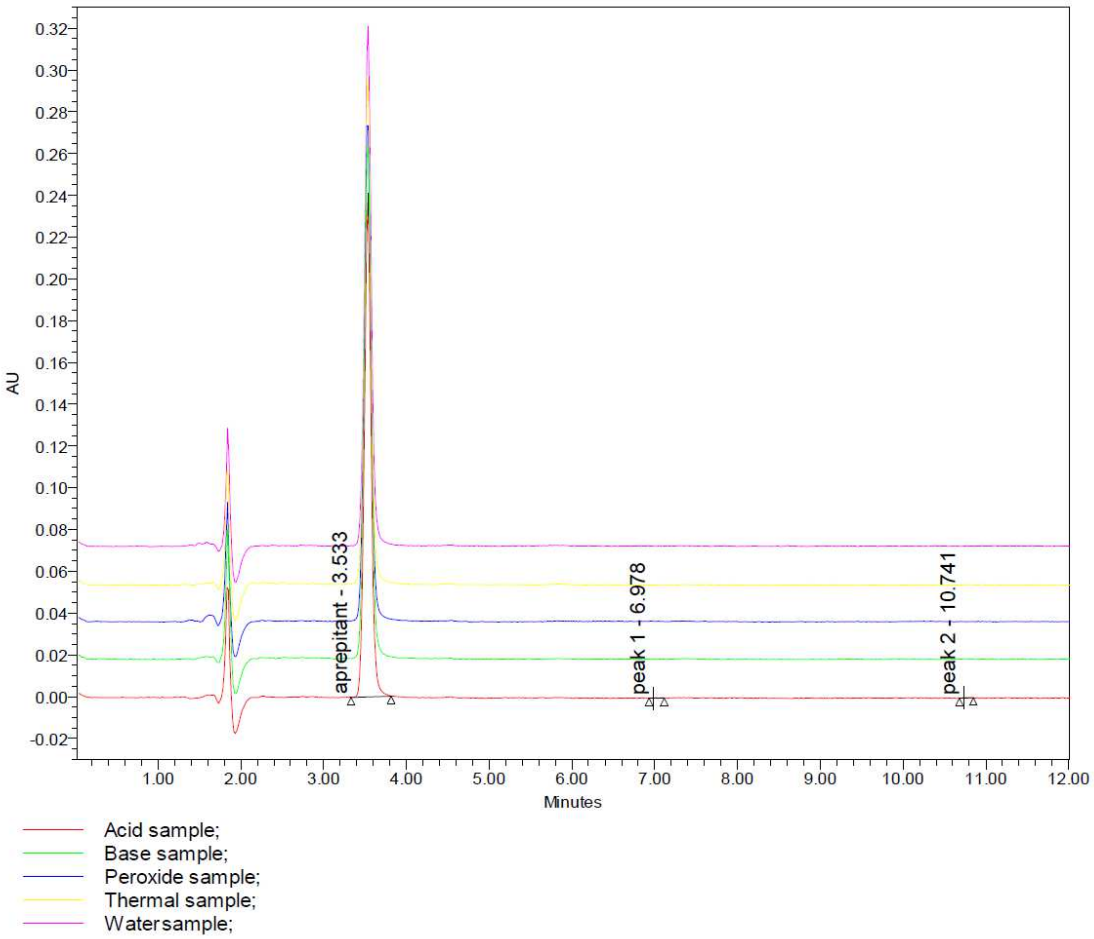


Table No.1: System suitability of Aprepitant

SYSTEM SUITABILITY PARAMETERS	APREPITANT
No of theoretical plates	8393
Tailing Factor	1.10
RT	3.429 min
Mean Area	1359704
%RSD	0.3

Table No.2: Results of accuracy of Aprepitant

Sample	Amount Taken (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Aprepitant	20	20.09	100.44	0.17
	40	40.30	100.75	0.80
	60	60.57	100.95	0.87

Table No.3: Results of Precision for Aprepitant

Repeatability data		Inter day precision	
S. No.	Aprepitant	Sr. No.	Aprepitant
1	1370337	1	1356168
2	1389543	2	1357898
3	1376469	3	1362264
4	1384602	4	1366546
5	1371100	5	1363373
6	1381619	6	1356443
Mean	1378945	Mean	1360449
Std. Dev.	7658.2	Std. Dev.	4240.3
%RSD	0.56	%RSD	0.31

Table No. 4: Results of Robustness for Aprepitant

Analytical conditions Evaluation paraMeters	Flow rate (ml/min)		Column temperature (^{o}c)		Mobile phase composition	
	1.2	1	35	25	+5%	-5%
Mean RT	3.123	3.891	3.661	3.891	3.661	3.472
Mean area	1216702	1564492	1406941	1568523	1405967	1409110
SD	8000.3	11130.9	5165.7	8811.9	5688.0	4952.5
RSD	0.66	0.71	0.37	0.56	0.4	0.35
Tailing factor	1.07	0.71	1.03	1.05	1.03	0.35
No. of theoretical plates	8617	8704	8663	8667	8668	9016

Table 6: Results of stock solution stability for Aprepitant

Drug	%Assay at 0 hr	%Assay at 24 hr	%Devaition
APREPITANT	100.01	99.34	1.67

Table 7: Results of HPLC Analysis of capsule for Aprepitant

Label amount (mg)	Amount found(mg) n=6	%Assay (Mean±SD)	RSD
40	4.013	101.01±1.098	0.56

Table 8: Results of HPLC Degradation of Aprepitant

Degradation parameter	Purity angle	Purity treshould	%Drug degradated
Acid degradation	0.265	0.498	2.57
alkali degradation	0.377	0.529	2.90
oxidative degradation	0.243	0.472	1.42
Thermal degradation	0.287	0.511	1.79
Photolytic degradation	0.308	0.517	0.37
Neutral degradation	0.261	0.476	.35

CONCLUSION

A new precise accurate and simple HPLC Method was developed and validated for estimation of Aprepitant

pharmaceutical dosage form. This Method is fast, accurate, precise and sensitive hence it can be employed for routine quality control of capsule containing drug in QC laboratories and industries.

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