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Validated RP-HPLC method for the quantification of aprepitant in bulk and pharmaceutical dosage forms

K. Krishna Chaitanya*, D. Gowri Sankar, D. Samson Israel and Ch. Narasimha kumar

Pharmaceutical Analysis & Quality Assurance, A. U. College of Pharmaceutical sciences Andhra University, Visakhapatnam-530003, India

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ABSTRACT

A reverse phase HPLC method has been developed and validated for the estimation of Aprepitant in Pharmaceutical dosage forms. An X-Terra RP -8, 150x4.6 mm, 5 µm particle size, with mobile phase consisting of Buffer (weigh and transfer 0.34g of Tetra-n butyl ammonium Sulphate in 1000ml of purified water, Add 1.1ml of concentrated Ortho phosphoric acid (88%) Mix properly) and Acetonitrile in the ratio of 50:50% v/v was used. The flow rate was 1.0 ml/min and the effluents were monitored at 210 nm. The retention time was 4.86 min. The detector response was linear in the concentration of 25-150 µg/ml. The respective linear regression equation being y = 22769x – 1489 (R^2 = 0.999). The limit of detection and limit of quantification was 0.28 and 0.84 µg/ml respectively. The percentage assay of Aprepitant capsules was found to be 100.02 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Aprepitant in bulk drug and in its pharmaceutical dosage form.

Key Words: Aprepitant, RP-HPLC, Estimation, and Capsules.

INTRODUCTION

Aprepitant is a substance P (SP) /neurokinin 1 (NK1) receptor antagonist and chemically described as 5-[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2dihydro-3H-1,2,4-triazol-3-one (fig.1). It is a white to off-white crystalline solid, with a molecular weight of 534.43 and empirical formula of $C_{23}H_{21}F_7N_4O_3$. Aprepitant is a selective high affinity antagonist of human substance P/neurokinin 1 (NK1) receptors and it has little or no affinity for serotonin (5-HT3), dopamine, and corticosteroid receptors. A large number of drugs are available for prevention of PONV[1], of which 5-HT3 receptor antagonists have occupied an important position because of their better efficacy and side effect profile with a disadvantage that it prevents only acute emesis. A newer class of drugs namely neurokinin receptor antagonists provides an additional advantage of preventing both acute and delayed emesis. Various NK1 receptor antagonists studied include Aprepitant, GR-205171, CP-122721 and CJ-11974, of which Aprepitant has been approved for PONV and treatment of nausea in cancer chemotherapy. Aprepitant has been shown in animal models to inhibit emesis induced by cytotoxic chemotherapeutic agents, such as cisplatin, via central actions. Animal and human Positron Emission Tomography (PET) studies with Aprepitant have shown that it crosses the blood brain barrier and occupies brain NK1 receptors[2] and also showed that Aprepitant augments the antiemetic activity of the 5-HT3 receptor antagonist ondansetron and the corticosteroid dexamethasone and inhibits both the acute and delayed phases of cisplatin-induced emesis. It has

been recently demonstrated that substance P (SP) and neurokinin -1 (NK1) receptor antagonists induce cell proliferation and cell inhibition in human melanoma cells. Literature review reveals that very few analytical methods has been established for the estimation of Aprepitant in human plasma[3] and estimation of its metabolites in human plasma[4], HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound[5], estimation of Aprepitant in rhesus macaque plasma[6], characterization and quantitation of Aprepitant drug substance polymorphs by attenuated total reflectance fourier transform infrared spectroscopy[7], stability of an extemporaneous oral liquid Aprepitant formulation[8] ,estimation of Aprepitant capsules by RP-HPLC[9] were reported. The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date[10]. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life[11, 12]. Only one method was reported for the determination of Aprepitant in presence of its degradation products in oral liquid formulation in the literature. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate reverse phase HPLC method for quantitative analysis of Aprepitant, and to validate the method in accordance with ICH guidelines[13] showed advantages of shorter retention time, runtime and economic mobile phase.

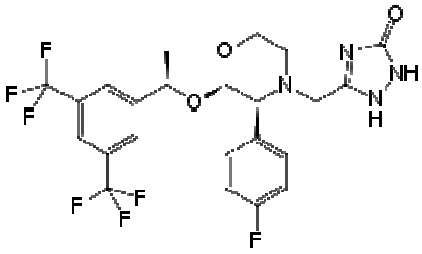

Fig: 1 Structure of Aprepitant

MATERIALS AND METHODS

Aprepitant was obtained as a gift sample from Hetero Drugs Ltd, Hyderabad. Tetra-n butyl ammonium hydrogen sulphate was of analytical grade, and supplied by Merck Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Aprepitant Capsules (Aprecap 80, 120 mg, Glenmark) were procured from local market.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

Quantitative HPLC was performed on Shimadzu LC -20AD VP series model chromatograph equipped with YMC pack Pro C18 (150mm X 4.6mm id, 5 µm Particle size) was employed for the study. Sample Injection was done with a Rheodyne 7725 injection value via a 20 µL loop and the output signal was monitored and intergrated by spinchrome software.

PREPARATION OF BUFFER:

Weigh and transfer 0.34g of Tetra-n butyl ammonium sulphate in 1000ml of purified water, add 1.1ml of concentrated Orthophosphoric acid (88%). Mix properly

MOBILE PHASE PREPARATION

Mix buffer and Acetonitrile in the ratio of 50:50 v/v and degas in a sonicator for 10min. Filter through 0.45µm Nylon 66 membrane filter or 10µm full flow Filter of sample collector of instrument

DILUENTS

Mix purified water and Acetonitrile in the ratio 50:50 volume by volume. Mix properly.

PREPARATION OF APREPITANT WORKING STANDARD SOLUTION

Weight and transfer about 25 ±2.5 mg of Aprepitant working standard into a 200mL volumetric flask, add about 5mL of Acetonitrile and sonicated to dissolve. Dilute to volume with dilute and mix.

The solutions were injected in triplicates using 20 µL fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area against respective concentration of Aprepitant was found to be linear in the range of 25-150 µg/ml. with correlation coefficient 0.999.

ASSAY METHOD FOR FORMULATIONS:

Weigh 5 capsules and take equivalent weight of 125mg of Aprepitant into 200ml volumetric flask, add 150ml of diluents and Sonicate for 30minutes with intermittent shaking. Dilute to volume with diluent. Centrifuge the above solution at 4000rpm at 5minutes. Further dilute 5ml of this solution into 25ml volumetric flask. Dilute to volume with diluents and mix well. Filter a portion of above solution through 0.45 µm nylon membrane filter and separately inject 20 µL of the Sample solution (five injections) into the HPLC, record the chromatographs and data is observed statistically(table:1)

Fig: 2 A TYPICAL CHROMATOGRAM OF APREPITANT BLANK

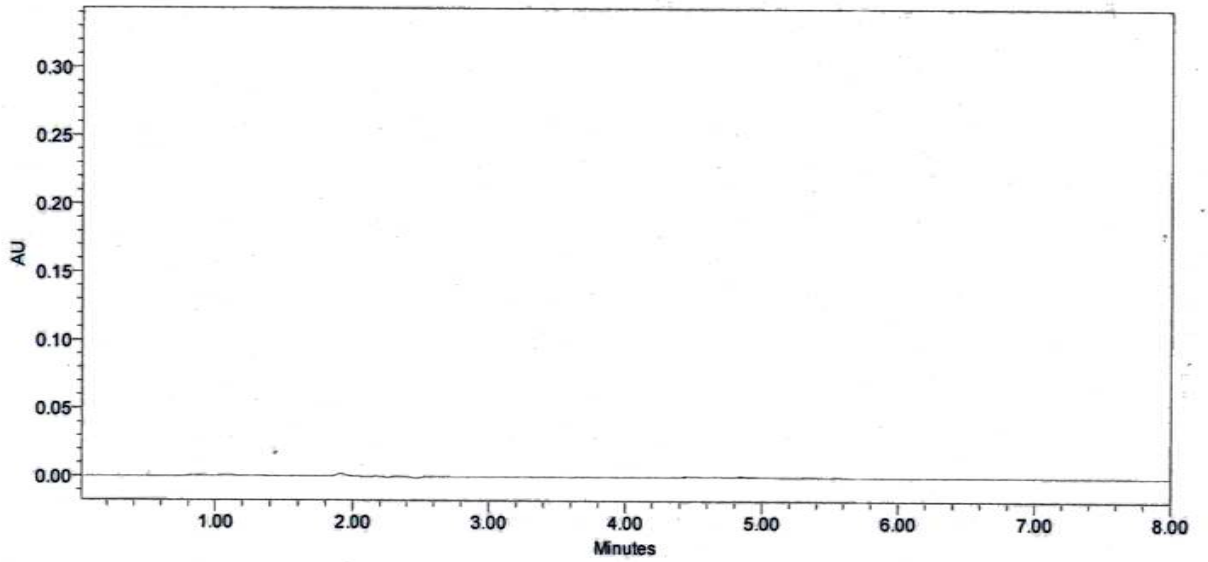

Fig: 3 A TYPICAL CHROMATOGRAM OF APREPITANT PLACEBO

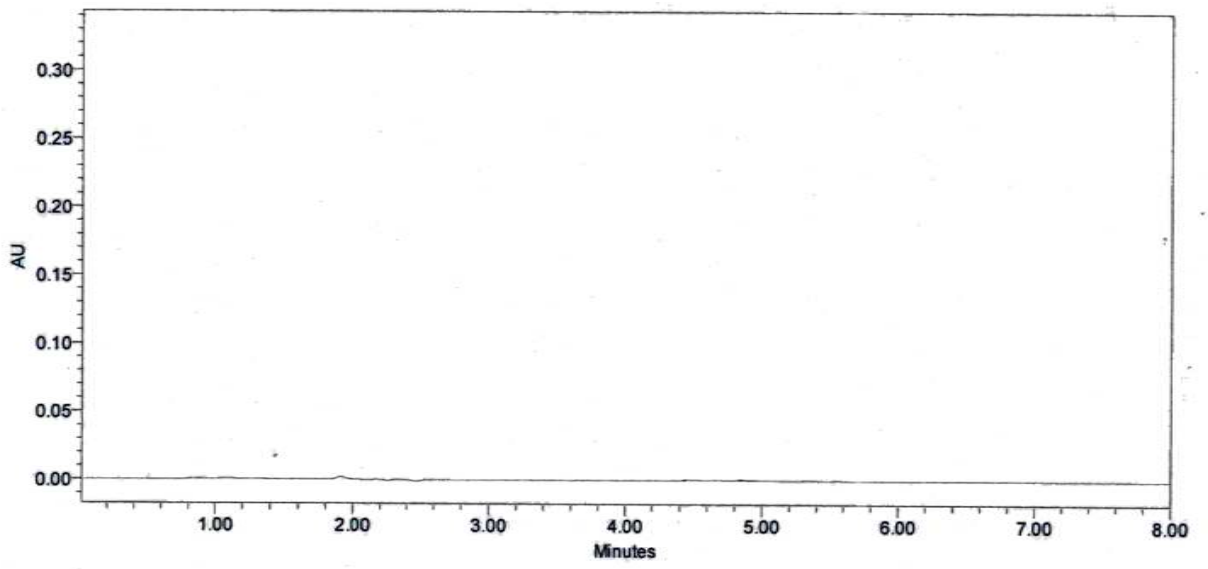

Fig: 4 A TYPICAL CHROMATOGRAM OF APREPITANT STANDARD

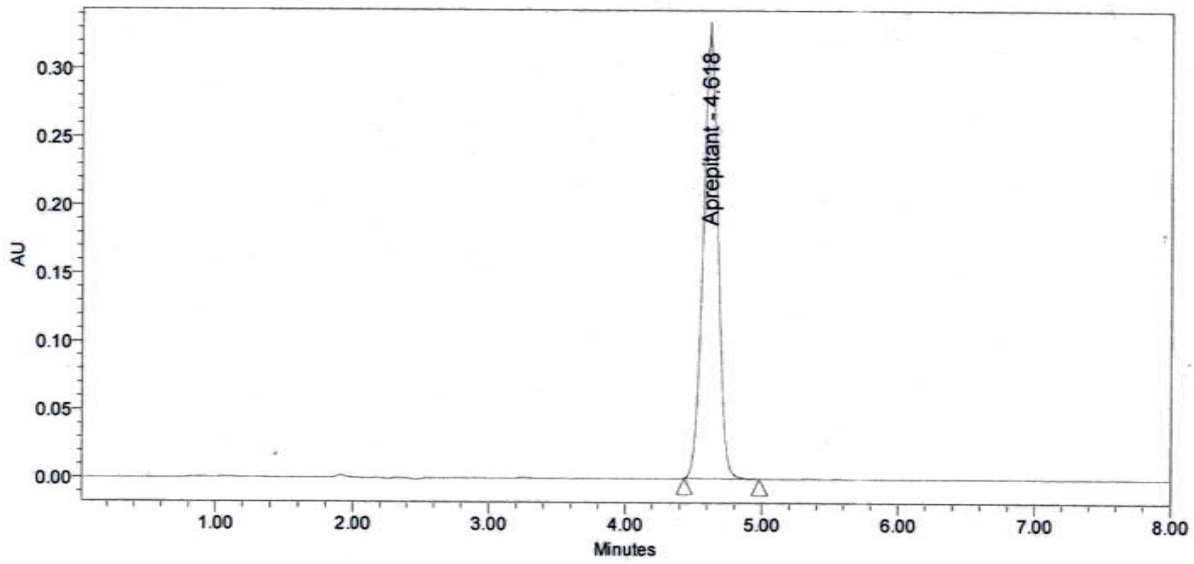

Fig: 5 A TYPICAL CHROMATOGRAM OF APREPITANT CAPSULE (APRECAP)

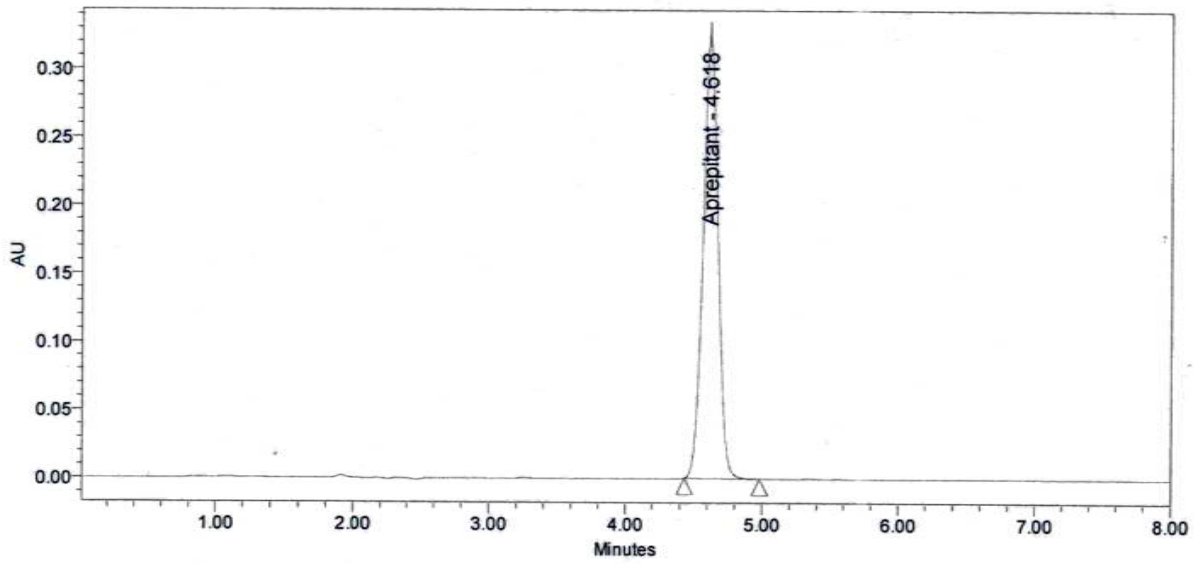

Table: 1 ASSAY OF APREPITANT CAPSULES (80mg)

Drug	Labeled Amount (mg/ml)	Amount taken(mg/ml) Mean(± S.D)	% Label Claim	%RSD
80 mg (Aprecap)	40	39.993±**0.01527**	99.982	0.03819
	80	79.986±**0.00577**	99.983	0.00721
	120	120.003±**0.01154**	100.002	0.00962

METHOD VALIDATION:

The accuracy, precision and robustness were determined by analyzing a set of laboratory sample (n=3) with each of the five concentrations ranging from 25-150 µg/ml for both drugs.

RESULTS AND DISCUSSION

Initially Buffer: Methanol was tried as mobile phase, in which Aprepitant shows greater peak asymmetry which was not satisfying the system suitability criterion and the resolution requirement for the estimations of Aprepitant. The tailing for both the peaks was reduced considerably by changing acetonitrile in mobile phase and brought close to 1, which is ideal requirement for chromatographic analysis. Acetonitrile can help in reducing the viscosity of the mobile phase and hence reduce the backpressure and increase the column life.

SYSTEM SUITABILITY:

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, precision and peak tailing. The result obtained is shown in Table 2. The number of theoretical plate for Aprepitant is 9290. All these parameters were evaluated with the background of regulatory requirements, which also suggests good chromatographic condition.

TABLE: 2 SYSTEM SUITABILITY PARAMETERS OF APREPITANT

Injection	RT	Peak Area	USP Plate Count	USP Tailing
1	4.86	2844351	9485	1.00
2	4.85	2841589	9254	1.02
3	4.88	2845025	9821	1.04
4	4.85	2842047	8991	1.07
5	4.87	2841628	9521	1.02
6	4.85	2845950	9021	1.00
7	4.86	2843328	9079	1.05
8	4.87	2842573	9248	1.01
9	4.86	2845590	9348	1.03
10	4.86	2843429	9127	1.08
Mean	4.861	2843551	9290	1.032
SD	0.0099	1617.81	-	-
%RSD	0.204	0.057	-	-

PRECISION:

The ICH documents recommended that repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e., three concentrations and three replicates of each concentration) precision was studied to find out intra and interday variations of the proposed method at three different levels (50, 100, 150 µg/ml for Aprepitant) on the same and on three different days respectively. The results were interpreted by statistical analysis by calculating % RSD values and all the results were within the acceptance criteria of not more than 2 % and the results are tabulated in the table 3

Table: 3 Summary of Inter-Day and Intra-day precision

Conc. (µg/ml)	Intraday Precision			Interday Precision		
	Mean Amt Found (µg/ml)	±SD	%RSD	Amt. Found (µg/ml)	±SD	%RSD
50	49.98	0.0100	0.020	49.99	0.0152	0.0305
100	100.01	0.0173	0.017	100.01	0.0057	0.0057
150	149.99	0.0152	0.0101	149.99	0.0208	0.0138

Table: 4 Recovery studies of Aprepitant

Amount added (µg/ml)	Amount found (µg/ml)	% Recovery	Statistical Analysis of % recovery	
50	49.99	99.980	**Mean**	100.01
50	50.02	100.04	**SD**	0.0305
50	50.01	100.02	**%RSD**	0.0305
100	100.01	100.01	**Mean**	100.01
100	100.02	100.02	**SD**	0.0057
100	100.02	100.02	**%RSD**	0.0057
150	150.01	100.01	**Mean**	99.99
150	149.99	99.99	**SD**	0.0101
150	149.98	99.98	**%RSD**	0.0101

ACCURACY:
The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard Aprepitant. The result is summarized in Table 4.The percent recovery for Aprepitant ranges from 99.33 to 99.93%

LINEARITY:
Aliquots of standard Aprepitant stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Aprepitant are in the range of 25-150 µg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed at 210nm and a Calibration graph was obtained by plotting peak area versus concentration of Aprepitant (Fig 6).The respective linear regression equation being y = 22769x – 1489 (R^2 = 0.999). The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table**: 5**

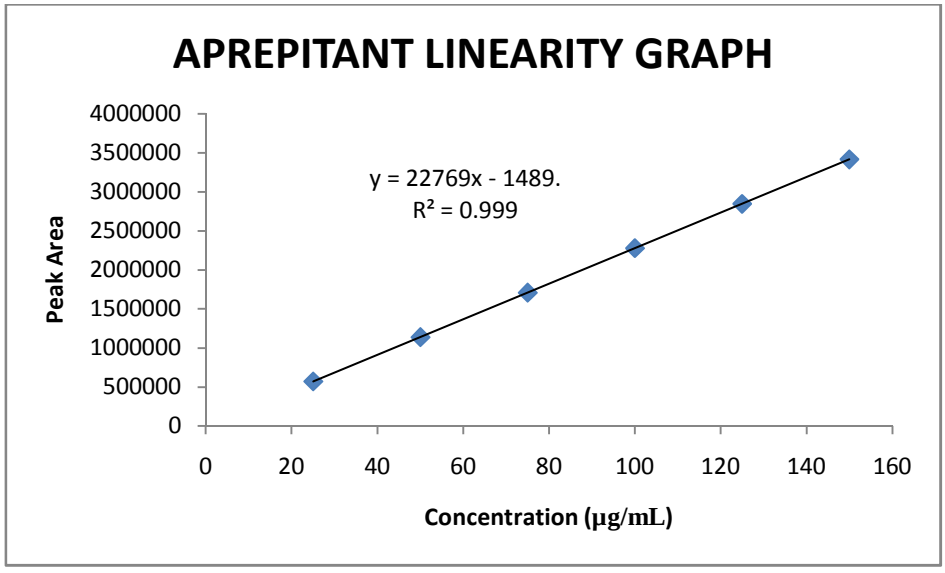

Fig no: 6 CALIBRATION CURVE FOR APREPITANT

Table: 5 LINEARITY DATA

Linearity Conc.	Peak Area	Average area	SD	%RSD
25	568409	568868	408.126	0.071
	569005			
	569190			
50	1135010	1135736	955.127	0.084
	1136818			
	1135380			
75	1705015	1705938	1417.648	0.083
	1707570			
	1705228			
100	2273637	2275472	1631.942	0.071
	2276760			
	2276020			
125	2845950	2844674	1482.987	0.052
	2845025			
	2843047			
150	3415140	3414209	856.098	0.025
	3413456			
	3414030			

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

The limit of detection (LOD) and limit of quantification (LOQ) for Aprepitant were found to be 0.28µg/ml and 0.84µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

RUGGEDNESS:

The sample solution was analyzed after a period of 8 hrs at room temperature. The assay of Aprepitant in standard solution was estimated against freshly prepared standard each time. The difference in % assay of standard and test solution from initial to 24 hrs was calculated and the results are given in the table: 6 from the above study, it is established that the sample solutions are stable for a period of 24 hrs at room temperature (25±2ºC).

Table No: 6 SOLUTION STABILITY

Time (hrs)	Assay	Difference
Initial (0)	100.02	0
12	99.98	0.04
18	99.89	0.09
24	99.52	0.37

ROBUSTNESS

The robustness of the method was determined as per ICH guidelines under a variety of conditions including changing the composition of organic phase ± 2%, detection wavelength by ±2nm, change in flow rate by ± 10% or ±0.1ml, pH of buffer by ± 0.05. No marked changes were observed in the system suitability parameters and peak area. The results obtained by deliberately variation in method parameters and data are summarized below table: 7

Table: 7 ROBUSTNESS

Parameter		%RSD of peak area	Theoretical Plates	Asymmetry
Flow rate ± 10% (1.0ml/min)	**0.8 ml/min**	0.032	2573884	1.2
	1.2 min/min	0.073	2573910	1.3
Organic phase Variation ± 2% Buffer: ACN 50:50 % v/v	**(Buffer: ACN) 40:60**	0.059	2573936	1.1
	(Buffer: ACN) 60:40	0.047	2573962	1.2
Temperature variations ±5 °c	**35**	0.061	2573988	1.2
	25	0.086	2573884	1.2
Column variation	**Symmetry C_{18} (150X 4.6mm, 5µ)**	0.038	2573910	1.3
	Inertsil ODS-3V (150X 4.6mm,5µ)	0.056	2573981	1.2

CONCLUSION

From the typical chromatogram of Aprepitant as shown in fig:4, it was found that the retention time was 13.73 min. Mixture consisting of Buffer (weighs and transfer 0.34g of Tetra-n butyl ammonium sulphate in 1000ml of purified water, add 1.1ml of concentrated Ortho phosphoric acid (88%). Mix properly) and Acetonitrile in the ratio of 50:50% v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (R^2=0.9999) was observed between the concentration range of 25-150 µg/ml. Low values of standard deviation are indicative of the high precision of the method. The assay of Aprepitant Capsules was found to be 99.85%. From the recovery studies it was found that about 102.4 % of Aprepitant was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the Capsules. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method

A validated stability-indicating HPLC analytical method has been developed for the determination of Aprepitant in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method was selective and stability-indicating. The proposed method was simple, accurate, precise, specific, and has the ability to separate the drug from degradation products. In the absence of a stability indicating assay in the literature, the proposed method was suitable to use for the routine analysis of Aprepitant in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and or GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method. In addition, the

HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals. The method had proved its importance in terms of sensitivity, rapidity, economy in the stability indicating estimation of Aprepitant.

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