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Research Article

HPLC Quantification of Aprepitant in Bulk and Capsules

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ABSTRACT

A simple, accurate, and precise Reverse Phase High-Performance Liquid Chromatography (RP HPLC) method has been developed and validated for the quantitative determination of Aprepitant in bulk and pharmaceutical dosage form(capsules). The chromatographic separation was carried out using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of Acetonitrile and Phosphate Buffer (pH 3.0 adjusted with 1% orthophosphoric acid) in the ratio of 85:15 v/v. The flow rate was maintained at 1.0 mL/min, and detection was carried out at a wavelength of 264 nm. The retention time of Aprepitant was found to be 3.75 minutes. The method showed good resolution with symmetrical peaks, a tailing factor less than 2, and theoretical plates greater than 2000, indicating high efficiency of the column. Linearity was observed in the concentration range of 200-1000µg/mL, with a correlation coefficient (R^2) greater than 0.997, confirming the method's linearity. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 39.63µg/mL and 120.09µg/mL, respectively. The accuracy of the method was confirmed by recovery studies at 80%, 100%, and 120% levels, with results ranging from 100.4% to 103.22% w/w, indicating excellent recovery. The % assay of Aprepitant in Capsule formulation was found to be 99-101.36% w/w, demonstrating that the method is suitable for routine analysis. The developed method was validated in accordance with ICH guidelines (Q2(R1))10 and found to be specific, accurate, precise, robust, and suitable for the routine quality control of Aprepitant in both bulk and pharmaceutical dosage form(capsules).

INTRODUCTION

Aprepitant (APT) is (SP) / neurokinin -1 (NK1) receptor antagonist chemically as 5-[(2R,3S)-2-[(1R)-1-(3,5-bis(trifluoromethyl)phenyl) ethoxy]-3- (4-fluorophenyl)-4 morpholinylmethyl-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 $C_{23}H_{21}F_7N_4O_3$. APT is a selective high affinity

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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 Postoperative nausea and vomiting[1] of which 5-HTs 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 include APT, GR-205171, CP-122721 and CJ-11974, of which APT has been approved for Postoperative nausea and vomiting and treatment of nausea in cancer chemotherapy, APT 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 APT have shown that it crosses the blood brain barrier and occupies brain NK1 receptors[2] and also showed that APT 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 APT and its metabolite in human plasma[3,4],and other methods[5-9]

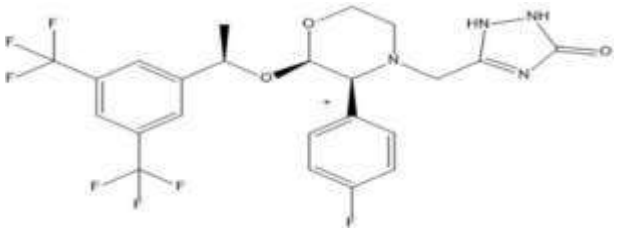

Figure 1: Chemical structure of Aprepitant

Only one method was reported for the determination of APT 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 HPLC method for quantitative analysis of Aprepitant and to validate the method in accor dance with ICH guidelines Q2(R1)[10]and apply the method to capsule formulation.

MATERIALS AND METHODS

Aprepitant pure drug (API) was procured from Glenmark Pharmaceuticals Ltd.

I. Instrument Used

- **Electronic Weighing Balance** : Sartorius TE 214 S
- **Ultrasonicator** : RC Systems MU 1700
- **UV–Visible Spectrophotometer** : Shimadzu 1900i (LabSolutions software)
- **Digital pH Meter** : Labman
- **Vacuum Pump** : Servewell Instruments Pvt. Ltd.
- **Membrane Filter** : Supor 200, 0.45 µm (Pall India Pvt. Ltd)
- **Hot Air Oven**

II. HPLC System

- **Liquid Chromatograph** : Shimadzu LC-10AT
- **Detector** : UV–Visible Detector (Shimadzu SPD-10A)

- **Analytical Column** : BRISA LC2 C18 (250 mm × 4.6 mm, 5 µm)
- **Data Processor** : LC Solution Software, Version 2.1.4.93
- **Injector** : Rheodyne 7725i (Fixed loop of 50 µL)
- **Syringe** : Hamilton, 20 µL

III. Chemicals and Reagents

- Acetonitrile (HPLC grade)
- HPLC Grade Water
- Potassium Dihydrogen Orthophosphate Buffer (20 mM, HPLC grade)
- Aprepitant (Standard API)
- Orthophosphoric Acid (1% v/v, AR grade)
- Aprepitant Capsules

EXPERIMENTAL METHODOLOGY

PREPARATION OF PHOSPHATE BUFFER

20mM of Phosphate buffer was prepared by dissolving 680.4 mg of potassium dihydrogen orthophosphate in 250 mL of water adjust the pH to 3.0 with 1% of Phosphoric acid. The working mobile phase was prepared in the ratio of 85:15 (Acetonitrile: Phosphate buffer) filtered, degassed and sonicated for 10 min.

PREPARATION OF STANDARD APREPITANT SOLUTION

Accurately weighed 10mg of Aprepitant standard was transferred into 10mL volumetric flask, 3-5mL of Mobile phase was added and sonicated for 5 min to dissolve it completely and the volume was made up to 10ml the volume with mobile phase to get 500µg/mL of standard Aprepitant solution and labelled as STD Stock.

PREPARATION OF SAMPLE APREPITANT SOLUTION

Aprepitant 1 capsule (Aprecap 80) were accurately weighed and their average weight was calculated. Accurately weighed a quantity of powder containing 114mg of Aprepitant and transferred to 10ml volumetric flask, solubilized in 10ml of mobile phase and sonicated for 15mins. After sonication the volume was made up to the 10ml mark with the mobile phase to obtain final concentration of 500µg/mL of Aprepitant and was labelled as ‘SMP STOCK’ and filtered through Whatman Filter paper (#41).

From ‘SMP STOCK’ 5mL was transferred to a 10mL volumetric flask and volume was made up to the mark with mobile phase to obtain a concentration of 500µg/mL of Aprepitant and labelled as ‘FINAL SMP’.

BLANK, FINAL STD STOCK and FINAL SMP solutions were filtered through 0.45µm nylon membrane filter and 20µL was injected into the HPLC system under standardized chromatographic conditions to get a stable baseline and to observe for peak of Aprepitant and any extra peak for 15 mins. The chromatograms obtained is represented.

SELECTION OF ANALYTICAL WAVELENGTH:

Using a Shimadzu 1900i UV-Visible Spectrophotometer, a standard solution of Aprepitant (500 µg/mL) made in a mobile phase of Acetonitrile and 20 mM Phosphate Buffer (pH 3.0) in a 85:15 (v/v) ratio was scanned in the UV range of 200–400 nm. At 264 nm, the highest absorbance (λmax) was recorded.

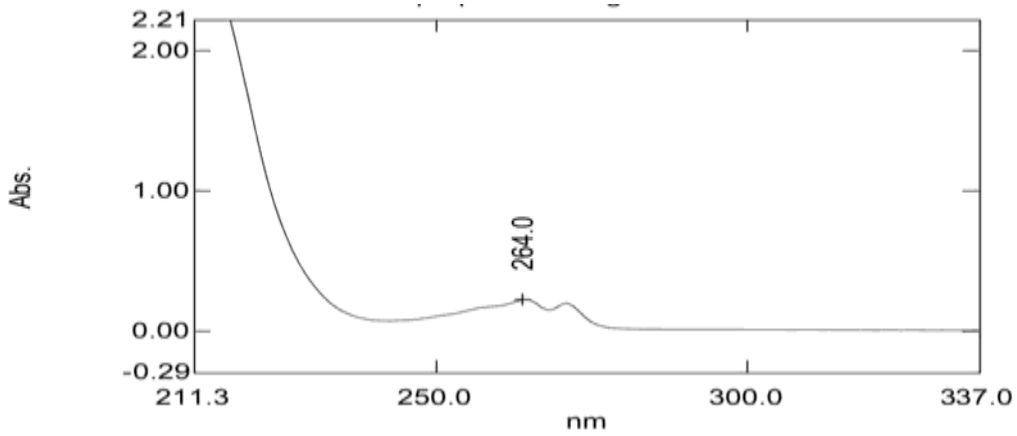

Fig 2: UV Spectra of Aprepitant(500µg/ml)

HPLC CHROMATOGRAPHIC CONDITIONS:

The stationary phase in reversed-phase chromatography was a Phenomenex BDS C18 column (250 mm× 4.6 mm, 5 µm). The mobile phase was made up of 85:15 (v/v) Acetonitrile and phosphate buffer (pH 3.0), filtered through a 0.2 µm nylon membrane, the mobile phase was filtered and sonicated to remove any dissolved gases. 1.0 mL/min was the fixed flow rate. Twenty minutes was set as run duration, and 20 µL of the sample was injected once the column had been equilibrated with the mobile phase, after a stable baseline. A wavelength of 264nm was used for the analyte's detection. The chromatogram is presented in Fig 3.

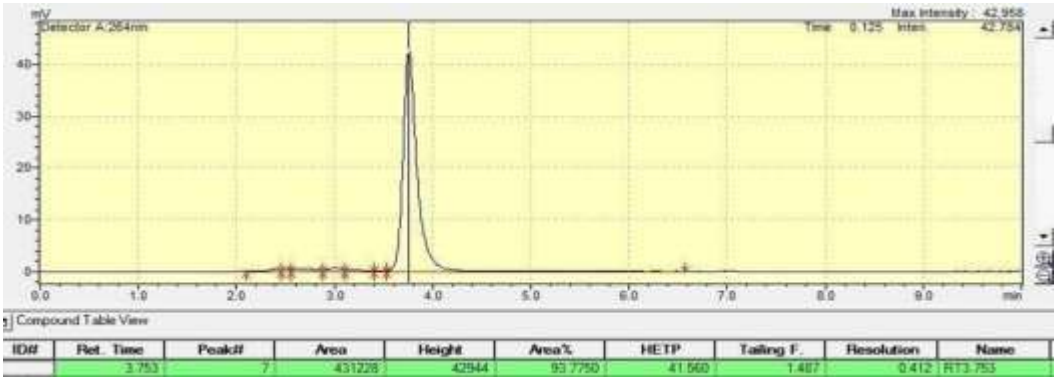

Fig3: Chromatogram for Aprepitant (500µg/ml) in MP containing Acetonitrile: Phosphate buffer 20mM pH 3.0, (85:15) at 264nm

VALIDATION OF RP-HPLC METHOD:

RP-HPLC method developed for determination of Aprepitant was Validated as per ICH guidelines Q2 (R1) for various parameters. Results obtained are presented below.

SPECIFICITY

Specificity was performed to determine that there is no interference of excipients with peak of Aprepitant in standard and sample solutions.

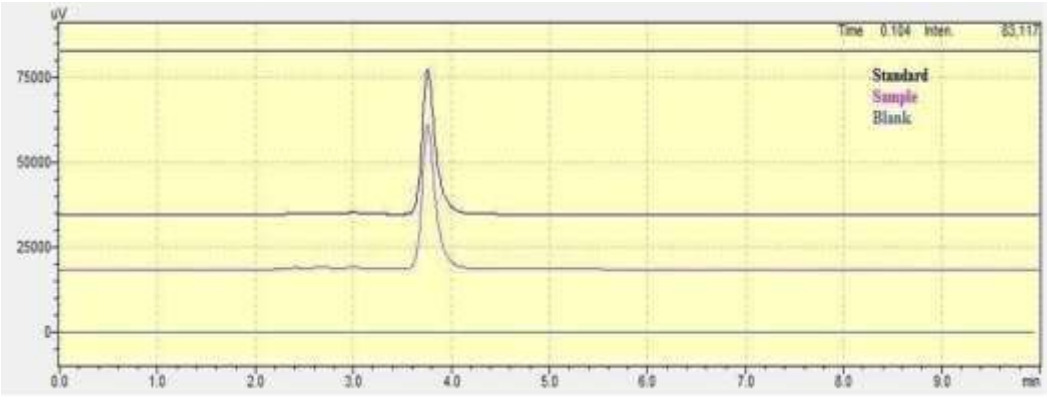

Fig 4: Overlain Chromatogram for specificity studies of Aprepitant (Standard and Sample)

LINEARITY AND RANGE

The linearity of an analytical procedure specifies the results which are directly proportional to the concentration of analyte in the sample. The linearity and range were determined from coefficient of correlation (R2) obtained by plotting AUC vs. CONCENTRATION at 264nm. The results obtained and calibration graph prepared is presented below.

Table 1: Data for concentration and peak area for the linearity studies of Apripetant

Sr. No.	Concentration (µg/mL)	Area under curve (n=3)
1	1000	1286552
2	800	949354
3	700	801567
4	600	632813
5	500	462228
6	400	307345
7	200	45175

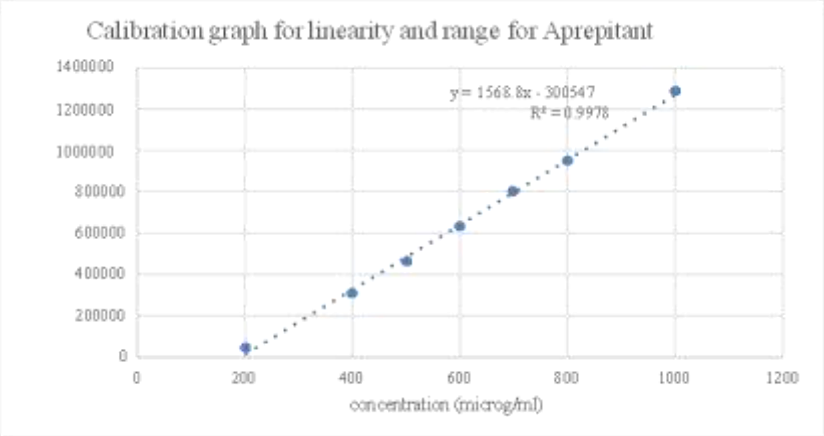

Fig5: Calibration graph for linear ity and range for Aprepitant

Table 2: Linear Regression Analysis Data for Calibration Curve of Apripetant

Parameters	Aprepitant
Linearity & Range (µg/mL)	200-1000
Correlation coefficient (R^2)	0.9978
Slope	1568.8
Intercept	300547

LOD and LOQ

The lowest amount that can be detected and quantified was calculated from the respective calibration curve.

The LOD and LOQ were calculated using

$$LOD = 3.3 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

$$LOQ = 10 \times \frac{\text{Standard Deviation of Y-intercept}}{\text{Average slope of six calibration curves}}$$

Table 3: Data for LOD and LOQ Studies for Aprepitant

Parameter	Aprepitant
SE	34601.19
SD of slope	415523.8
LOD(µg/ml)	39.62952
LOQ(µg/ml)	120.0895

ACCURACY

Accuracy studies were performed at three different levels (80%, 100% and 120%) and the % Recovery of Aprepitant were calculated and presented below.

Table 4: Percentage Recovery data for accuracy studies at three different levels

Drug	Conc. of STD (µg/ml) (A)	Conc of Sample (µg/ml) (B)	Total conc. (A+B) (µg/ml)	Peak Area* for A+B (µg/ml) (n=3)	Total amount (A+B) from graph (µg/ml)	Recovery of Std (µg/ml)	% Recovery of Std (%w/w)
Aprepitant	400	500	900	1114011	910	410	**102.5**
	500	500	1000	1307265.4	1016.1	516.1	**103.22**
	600	500	1100	1418294.9	1102.4	602.4	**100.4**

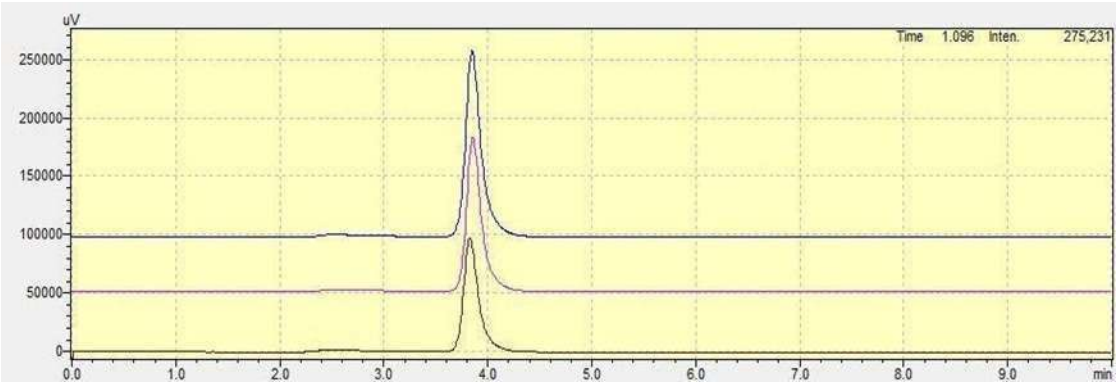

Fig 6: Overlain Chromatogram for Accuracy studies of Aprepitant at three different level

PRECISION

The precision of an analytical method was studied by performing intra-day, inter-day precision, repeatability and reproducibility studies.

Intra- Day and Inter- Day Precision

Intra-Day and Inter- Day Precision was performed to determine whether the developed method gives consistent results at different time intervals on the same day and for three consecutive days. The results obtained have been tabulated below.

Table5: Data for Intra-day and Inter-day precision studies for Aprepitant

Aprepitant (500 µg/ml) (n=3)			
Intraday		**Interday**	
Time hrs.	**AUC**	**Day**	**AUC**
0	426187	1	421418
1	426780	2	431228
2	426294	3	431510
Mean	426420.3	**Mean**	428052
Standard Deviation	316.041	**Standard Deviation**	5746.942
%RSD	0.0741	**%RSD**	1.342

REPEATABILITY

The test for repeatability was performed to check whether the developed method gives consistent

results with same solution on the same day and same time. The chromatogram and data obtained are presented below.

Table 6: Data for Repeatability studies of Aprepitant

Sr. No.	Aprepitant (500 µg/ml)	
	RT (min)	AUC
1	3.668	425418
2	3.653	431228
3	3.685	431510
4	3.670	426187
5	3.663	426780
6	3.650	421294
MEAN	3.664833	427069.5
SD	0.012703	3845.97534
%RSD	0.346	0.901

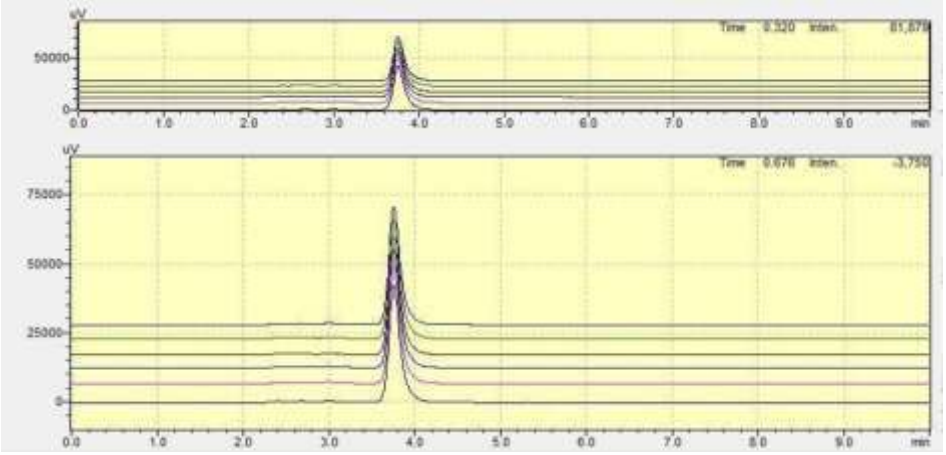

Fig7: Overlain Chromatogram for Repeatability studies of Aprepitant

REPRODUCIBILITY

Reproducibility was performed to ensure that the method is precise when different analysts performed the same analysis with the same method. The results obtained are presented below

Table 7: Data for reproducibility studies of Aprepitant

Sr. No	Analyst 1 (AUC) (n=3)	Analyst 2 (AUC) (n=3)
1	425420	425421
2	431229	431230
3	431513	431514
MEAN	429387.333	429388.333
SD	3438.74459	3438.74459
%RSD	0.8008	0.8008

ROBUSTNESS

Robustness was evaluated on the basis of system suitability parameters to assess whether the system suitability parameters change with small but deliberate variations. The system suitability parameters for standard solution of Aprepitant were studied with variation in flow rate and organic phase ratio.

A. CHANGE IN FLOW RATE

Small but deliberate changes in flow rate (±3) were tested and the chromatogram and the data obtained is presented below.

Table 8: Data for variation in Flow rate for Aprepitant

Drug	Acceptance Criteria	Flow Rate		
		STD (1ml/min)	+3% (1.03ml/min)	-3% (0.97ml/min)
Aprepitant (n=3)	TF (≤2.0)	1.477	1.554	1.487
		1.487	1.513	1.483
		1.464	1.533	1.496
	%RSD	**0.781**	**1.337**	**0.447**
	TP (≥2000)	3597.417	4244.732	3955
		3609.258	4287.401	3934.534
		3586.44	4222.497	3965.638
	%RSD	**0.3172**	**0.7758**	**0.400**

B. CHANGE IN WAVELENGTH

Small but deliberate changes in wavelength (±3nm) was tested and the chromatogram and the dat obtained are presented below:

Table 9: Data for variation in wavelength for Aprepitant

Drug	Acceptance Criteria	Wavelength (nm)		
		STD (264nm)	+3 (267nm)	-3 (261nm)
Aprepitant (n=3)	TF	1.489	1.493	1.490
		1.487	1.524	1.466
		1.464	1.5100	1.460
	%RSD	**0.9386**	**1.0287**	**1.0784**
	TP	3597.417	3598.416	3907.312
		3609.258	3659.254	3968.845
		3656.440	3609.258	3983.764
	%RSD	**0.862**	**0.895**	**1.025**

SYSTEM SUITABILITY PARAMETERS

Various system suitability parameters such as Retention time (RT), theoretical plates (TP), tailing factor (TF) and area under curve (AUC) were determined from the data obtained. The results are tabulated and presented below:

Table 10: Results of System Suitability parameters for Aprepitant

Sr. No.	Aprepitant (500 µg/ml)			
	RT (min)	AUC	TF	TP
1	3.744	425418	1.528	3655
2	3.756	431228	1.547	3636
3	3.740	431510	1.512	3643
4	3.761	426187	1.509	3647
5	3.753	426774	1.487	3629
6	3.746	421294	1.504	3645
Mean	**3.753333**	**424751.7**	**1.5**	**3649.333**
Standard Deviation	**0.007506**	**3008.777**	**0.011533**	**22.81082**
%RSD	**0.199**	**0.708**	**0.768**	**0.625**

APPLICATION OF DEVELOPED HPLC METHOD TO CAPSULE DETERMINATION ASSAY OF APREPITANT:

Capsule name: Aprecap 80

Label claim: 80 mg of Aprepitant (Glenmark pharmaceuticals Ltd.)

Determination of Capsule Formulation: Accurately weighed 0.0178 g of powdered drug was taken containing 10mg of Aprepitant and transferred into 10ml volumetric flask and diluted with Mobile phase and sonicated for 5 min and made up the mark with Mobile phase and filtered through 0.25µm Nylon membrane filter. From this 5 mL of the aliquot was pipetted out and transferred into 10ml volumetric flask volume made up to the mark with Mobile Phase which gives the concentration of Aprepitant (500µg/ml) of sample solution was injected into HPLC system at the flow rate of 1ml/min & detected at 264nm. The results obtained are tabulated below.

Table 11: Assay results of Aprepitant in capsule formulation

Sr. No.	AUC* (n=3)	% Assay (%w/w) (n=3)
1	421294	99.0
2	421287	100.18
3	431228	101.36
MEAN	424603	100.18
Standard Deviation	5737.419	1.18
% RSD	1.351	1.177

CONCLUSION

A simple and reliable RP-HPLC method was developed and validated for the estimation of Aprepitant in bulk and capsule dosage forms in accordance with ICH Q2(R1) guidelines. The method employed a BRISA LC2 C18 (250 × 4.6 mm, 5 µm) column with a mobile phase of acetonitrile: phosphate buffer (20 mM, pH 3.0) in the ratio of 85:15 v/v, a flow rate of 1 mL/min, an injection volume of 20 µL, and UV detection at 264 nm. The retention time was approximately 3.7 min. The method showed good specificity, linearity in the range of 200–1000 µg/mL (R^2 = 0.9978), accuracy (100.4–103.22%), and precision (%RSD < 2), with LOD and LOQ of 39.63 µg/mL and 120.09 µg/mL, respectively. Robustness and system suitability results were within acceptable limits. Hence, the developed method is suitable for routine quality control analysis of Aprepitant in bulk and capsule formulations.

Table 12: Results for RP-HPLC method

Parameters	Aprepitant (500□g/ml)
Retention time	3.753min
Linearity (ug/ml)	200-1000
Regression equation (y=mx+c)	y=1568.8x-300547
Correlation coefficient (R^2)	0.9978
LOD (µg/ml)	39.62952
LOQ (µg/ml)	120.0895
Accuracy (%w/w) Recovery at 80% level) Recovery at 100% level) Recovery at 120% level)	 102.5%w/w 103.22%w/w 100.4%w/w
Precision (%RSD) Intra-day Inter-day	 0.074% 1.342 %
Repeatability(%RSD)	0.901%
Robustness	TF, TP were found to be within the acceptance criteria
Analysis of Capsules (%Assay) %w/w	99-101.36%

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