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A stability indicating RP-UPLC method for estimation of apripitant and its related impurities in bulk drugs and its pharmaceutical dosage forms

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ABSTRACT

A simple, precise, accurate and validated ultra performance liquid chromatography (UPLC) method has been developed for the estimation of Aprepitant and its impurities in capsule dosage form. The quantification was carried out using HSS C18 column, 100 x 2.1mm, 1.8µ and the mobile phase used was a mixture 0.01M Potassium di hydrogen phosphate and Acetonitrile (50:50 v/v) at a flow rate of 0.5 mL min-1. The detection wavelength was 210 nm at ambient temperature. The total run time was found to be 6 min. The method is linear with R^2 values more than 0.999. The results obtained showed a good agreement with the stated content. Recovery values for Aprepitant and its impurities were 90% to 110%. The proposed method is reliable, rapid, precise, accurate and selective. The method was shown equivalency with the USP Method (Pending monograph, correspondence Number C89258); it can be used for regular analysis of Aprepitant in capsule dosage form.

Keywords: Apripitant, UPLC, Forced Degradation, Validation, Stability Indicating.

INTRODUCTION

Aprepitant (APT) 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 $C_{23}H_{21}F_7N_4O_3$. APT 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 APT, GR-205171, CP-122721 and CJ-11974, of which APT has been approved for PONV 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 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 APT in rhesus macaque plasma [6],characterization and quantitation of APT drug substance

polymorphs by attenuated total reflectance Fourier transform infrared spectroscopy [7], stability of an extemporaneous oral liquid APT formulation [8] ,estimation of APT capsules by RP-HPLC [9-12],estimation of capsule by UPLC[13],other anti emetic agents estimations were reported[14-16]. 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 [17].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 [18]. Only one method was reported for the determination of APT in presence of its degradation products in oral liquid formulation in the literature. But there was no UPLC method reported stability-indicating analytical method for analysis of APT in the presence of its degradation products and impurities in bulk and pharmaceutical dosage forms. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating UPLC method for quantitative analysis of APT, and to validate the method in accordance with ICH guidelines [19].

MATERIALS AND METHODS

3.1 Chemicals:

Samples of Apripitant and its related impurities were obtained sample from MSN Laboratories (Hyderabad, India) (Figure 1). Commercially available 125 mg of Apripitant Capsules (Emend®) were purchased from Korean market. HPLC grades Acetonitrile, Tri ethyl amine, analytical reagent grade Potassium di hydrogen phosphate and ortho phosphoric acid were purchased from Merck.

Apripitant: 5-[[(2R,3S)-2-[(1 R)-1-[3,S-Bis(trifluoromethyl)phenyl] ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-l ,2dihydro-3 H-1 ,2,4-triazol-3-one,Molecular formulae:C_{23},H_{21},F_7,N_4,O_3. With molecular weight of 534.43, CAS No: 170729-80-3.

Morpholine impurity(Impurity-1): 2-(R)-(1(R)-(3,5,-Bis(Triflouro methyl)Phenyl)Ethoxy)3-(S)-(4-Flouro) phenyl morpholine,Mol.Formulae:$C_{22}H_{20}F_7NO_6$.Molecular weight:527.39.It is one of the starting material used for the preparation of apripitant.

Ester impurity(Impurity-2): 2-(R)-(1(R)-(3,5,-Bis (Triflouro methyl)Phenyl)Ethoxy)3-(S)- (4-Flouro) phenyl - 4-(2-(N-Methyl carboxy acetamidrzino) morpholine, Mol.Formulae:C24H25F7N4O4.Molecular weight: 566.47.It is intermediate for the preparation of apripitant.

Diasteriomer impurity(Impurity-3): 5-[[(2R,3R)-2-[(1(R)-1-[3,5,-Bis(Triflouro methyl)Phenyl]Ethoxy]3--(4-Flouro phenyl) - 4-morphonilyl]methyl]-1,2-Dihydro-3H-1,2,4,Triazole-3-one,Molecular formulae: Mol.Formulae:$C_{23}H_{21}F_7N_4O3$.Molecular weight:534.43.

It is a chiral impurity of apripitant.

Other isomer (Impurity-4): 5-[[(2S, 3R)-2-[(1S)-1-[3, 5,-Bis (Triflouro methyl) Phenyl] Ethoxy] 3-(4-Flouro phenyl) - 4-morphonilyl] methyl]-1, 2-Dihydro-3H-1, 2, 4, Triazole-3-one.Molecular Formulae: $C_{23}H_{21}F_7N_4O3$.Molecular weight: 534.43. It is a chiral impurity of apripitant.It is not detected in API. It is quantified with normal phase Liquid chromatography, which is different method from the proposed UPLC Method.

3.2 Equipments:

The Acquity UPLC system with Empower software used for method development, forced degradation studies (Waters Corporation, MA, and USA). The output signal was monitored and processed using Empower software on Pentium computer (Digital equipment Co). Water bath equipped with temperature controller was used to carry out degradation studies for all solution. Photo stability studies were carried out in a photo stability chamber (Newtronic, Mumbai, India). Thermal stability studies were performed in a dry air oven (Biotechnics Mumbai, India).

3.3 Chromatographic conditions:

The chromatographic column used was Acquity UPLC HSS C18 column (100 × 2.1) mm with 1.7 µm particles. Buffer consists of a mixture of 1.36 Grams of Potassium di hydrogen phosphate and 2 mL of Tri ethyl amine,pH adjusted to 2.0 using diluted phosphoric acid. The mobile phase consists of buffer and acetonitrile at 1:1 ratio. The flow rate of the mobile phase was 0.5 mL·min–1. The column temperature was maintained at 45°C and the detection was monitored at a wavelength of 210 nm. The injection volume was 2.0µL. Acetonitrile and buffer mixed in 90:10 ratio was used as diluent. The concentration is 1250 ppm for impurities and 125 ppm for assay.

3.4. Preparation of Solutions

3.4.1. Preparation of Standard Solutions

A stock solution of Apripitant (2.5 mg·mL–1) was prepared by dissolving appropriate amount in the diluent. Working solutions were prepared from above stock solution for related substances and stock solution of impurities (mixture of imp-1, imp-2 imp-3 and imp-4) at a concentration of 125 µg·mL–1 was also prepared in diluent. For assay 125 ppm conc. was prepared.

3.4.2. Preparation of Sample Solutions

Emend capsules contain 125 mg of Apripitant. The inactive ingredients present in Multaq® were Sucrose, microcrystalline cellulose, hydroxyl propyl cellulose, and sodium lauryl sulphate and gelatine. Twenty Emend capsule were emptied and the contents transferred into the paper and the average weight was calculated. The pellets were powdered in a mortar and a sample of the powder equivalent to 125 mg of the active pharmaceutical ingredient (Apripitant) was transferred to 50 mL volumetric flask. Approximately 40 mL diluent was added and the flask was placed on rotatory shaker for 10 min and sonicated for 30 min to dissolve the material completely. The solution was then diluted to 50 mL and centrifuged at 3000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm pore size Syringe filter. The filtrate was used as sample solution for impurities, the above solution on dilution of 5 mL to 50 mL used for assay.

3.5. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the Apripitant in the presence of its impurities namely imp-1, imp-2, imp-3, and degradation products was determined by developed UPLC method. Forced degradation studies were also performed on Apripitant to provide an indication of the stability indicating property and specificity of the proposed method [14-16]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60°C), acid hydrolysis (1 N HCl), base hydrolysis (1 N NaOH) and oxidation (10% H2O2). For heat study period was 1 day and for light studies, study period was to illuminate the sample for 1.2 million Lux hours, where as for acid, base and peroxide hydrolysis the test period was 24 h. Peak purity of stressed samples of Apripitant was checked by using Photo diode array detector of Waters Corporation, MA, and USA.

3.6. Analytical Method Validation

The developed chromatographic method was validated for linearity, precision, accuracy, sensitivity, robustness and Solution stability.

3.6.1. Precision

The precision of the related substance method was checked by injecting six individual preparations of 125 ppm for assay and (250 mg·mL–1) Apripitant spiked with 1% each imp-1, imp-2, and imp-3 for impurities. The %RSD area of Apripitant peak in assay and each imp-1, imp-2, and imp-3 was calculated. Precision study was also determined by performing the same procedures on a different day (intraday precision).

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

3.6.2. Sensitivity

Sensitivity was determined for impurities method by establishing the Limit of detection (LOD) and Limit of quantitation (LOQ) for imp-1, imp-2, and imp-3 estimated By using the linearity slope calculations of imp-1, imp-2, and imp-3.

3.6.3. Linearity and Range

A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels. From 10% to 150% for impurities and 50% to 150 % for Assay of the permitted maximum level of the impurity and Apripitant was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses.

Upper and lower levels of range were also established.

3.6.4. Accuracy
The accuracy of the related substance method was evaluated in triplicate sample preparations at 10% to 120% of the analyte concentration (5 ppm) for impurities and 25% to 200% for assay. The percentage of recoveries for imp-1, imp-2, imp-3and imp-4 and Apripitant were calculated.

3.6.5. Robustness
To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (*Rs*) between Apripitant imp-1, imp-2, imp-3, imp-3 were evaluated. The flow rate of the mobile phase was 0.5 mL·min–1. To study the effect of flow rate on the developed method, 0.05 units of flow was changed (*i.e.* 0.45 and 0.55 mL·min–1). The effect of column temperature on the developed method was studied at 35°C and 45°C instead of 40°C. The effect of % Acetonitrile on resolution of impurities was studied by varying ±5% (*i.e.* buffer % altered from 50% to 45% and 55%). In the all above varied conditions, the components of the mobile phase were held constant.

3.6.6. Solution Stability and Mobile Phase Stability
The solution stability of Apripitant and its related impurities were carried out by leaving both spiked sample solution in tightly capped volumetric flask at room temperature for 48 h.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions, at 24 hrs and 48 Hrs. Content of imp-1, imp-2, imp-3, and imp-4 and Apripitant was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

4.1. Method Development and Optimization
The UPLC method carried out in this study aimed at developing chromatographic system capable of eluting and resolving Apripitant from its process related impurities and degradation products that comply with the general requirements for system suitability. Initial trials were done with 0.01M KH_2PO_4 Buffer concentration at flow rate 0.5 mL·min–1. Longer retention times and poor peak shape of Apripitant was problem with the above method.

Different columns such as BEH C18 and different buffers such as potassium di hydrogen phosphate, trifluoro acetic acid were also tried with different isocratic and gradient methods to achieve the best chromatographic separation. But long retention times and poor peak shapes were still unavoidable. With 0.1% trifluoro acetic acid, impurity-3 and Main peak are co-eluting and long retention times are seen. Studied the separation and peak shape by varying pH from 2.5 to 7.0 with phosphate buffer, and observed that, as the pH is increasing towards 7.0, peaks were strongly retaining. Also at higher pH, Apripitant and impurity-3 are co eluting. Added tri ethyl amine to the mobile phase to study the separation on a HSS,C18 column at 2.0 pH. The peak shapes significantly improved and obtained better separations and peak shapes with 1:1 Buffer and Acetonitrile. The % of Acetonitrile played a key role in the retention times and resolution between impurities.

After many logical trials, chromatographic condition was established such that which could be suitable for separation of drug degradation products and three known impurities.

Using the optimized conditions, Apripitant and its known impurities were well separated with a resolution of greater than 2. The Chromatogram was given in figure1

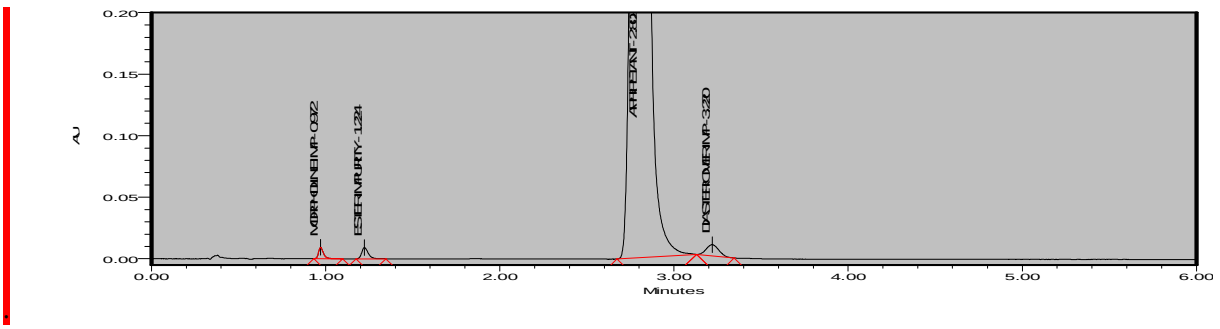


Figure 1: Impurity mixture chromatogram

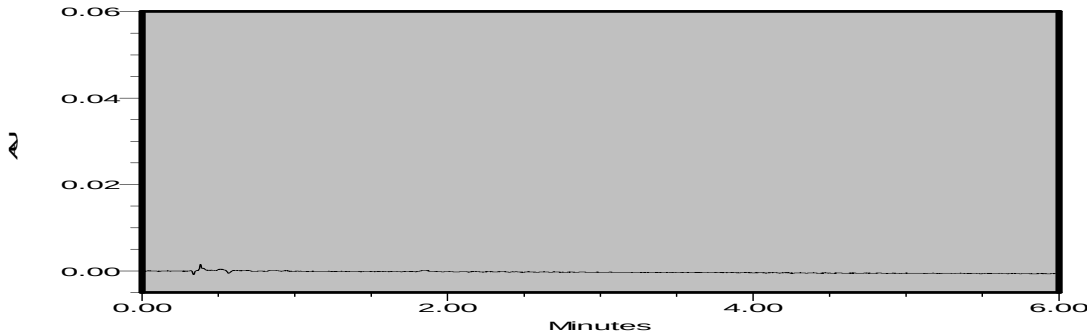


Figure 2: Blank chromatogram

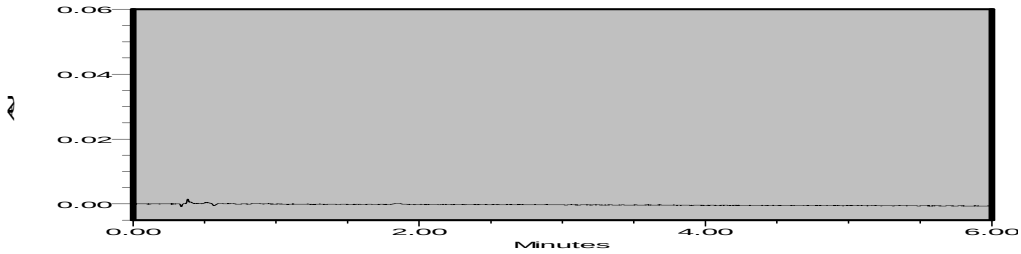


Figure 3: Placebo chromatogram

4.2. Results of Forced Degradation Studies

The forced degradations and the % degradations and Peak purities are given in Table

Table:1 forced degradation conditions and results

1.Conditions	%Degradation	Peak purity
Acid degradation 1N Hcl,60°C,24 Hrs	0.18%	Passed
Base Degradation 1N NaoH,60°C 24 Hrs	0.03%	Passed
Peroxide degradation 10%,50°C ,24Hrs	0.95%	Passed
Photo degradation 1.2 million Lux hours&200 W	0.0%	Passed
Thermal degradation 50°C,48 Hrs	0.0%	Passed
Hydrolysis Water, 50°C,24Hrs	0.0%	Passed

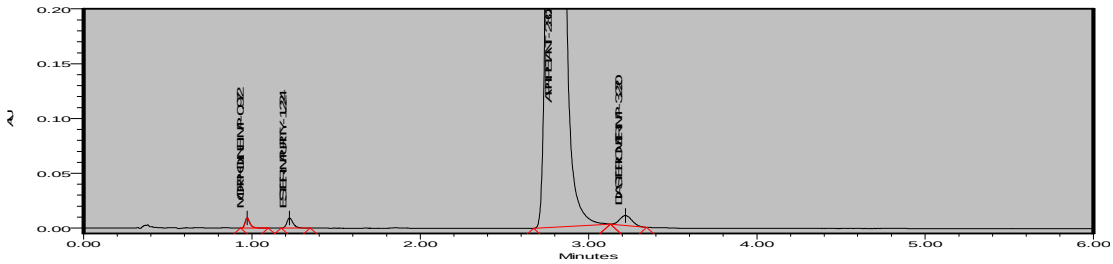


Figure 4: Acid degraded sample.

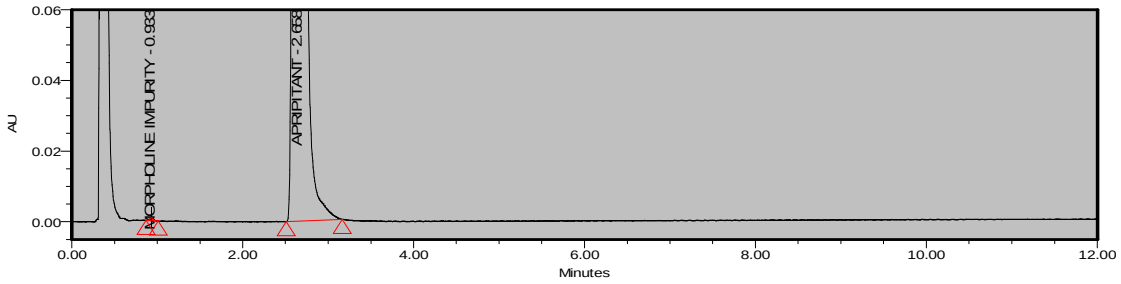


Figure 5: Base degraded sample.

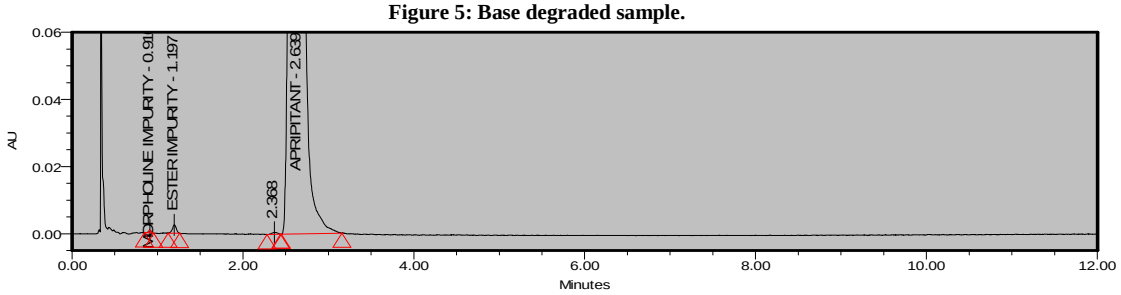


Figure 6: Peroxide degraded sample.

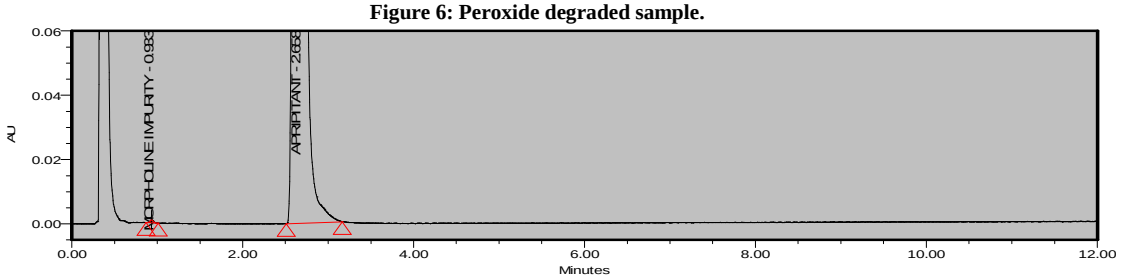


Figure 7: Water degraded sample.

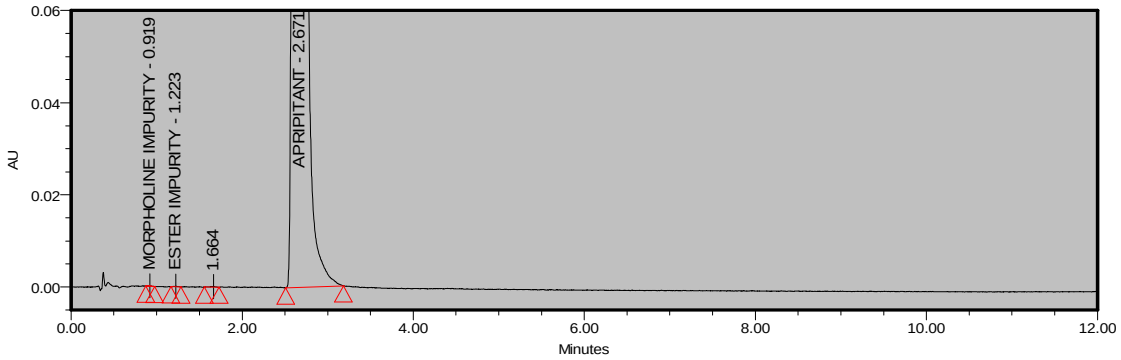
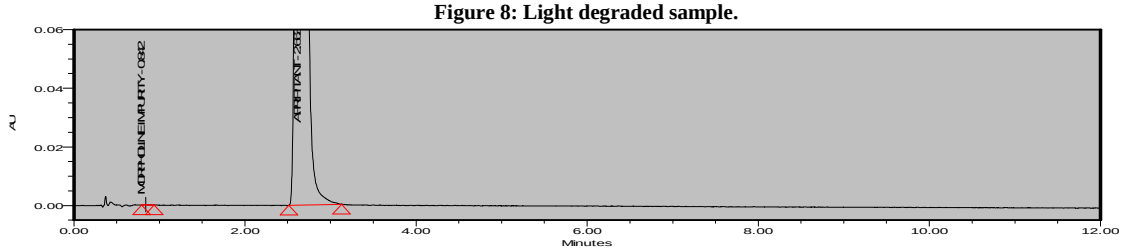


Figure 8: Light degraded sample.



4.3. Method Validation

4.3.1. Precision

The %RSD of Impurities in precision study was impurity-1(1.75), Impurity-2(2.29), Impurity-3(2.11) and Apripitant (0.59) respectively.

In intermediate precision study was Impurity-1(2.57), Impurity-2(2.83), Impurity-3(3.51) and Apripitant (1.21), in related substance method precision study were within 5.0 and in assay below 2.0and, confirming the good precision of the developed analytical method.

4.3.2. Sensitivity

The limit of detection and limit of quantification imp-1, imp-2, and imp-3 were listed in below table 3. The precision at LOQ concentration for imp-1, imp-2, and imp-3 were below 5%.

Table:2: LOD and LOQ Values of impurities

Impurity Name	LOD (ppm)	LOQ (ppm)
1.Impurity-1 (Morpholine)	0.02	0.05
2.Impurity-2(Ester)	0.17	0.51
3.Impurity-3 (Diasteriomer)	0.09	0.28

4.3.3. Linearity and Range

Linear calibration plot for related substance method was obtained over the calibration ranges tested, *i.e.* LOQ to 10 % to 400 %. The correlation coefficient obtained was greater than 0.999 for all impurities. The result given in table.

Table 3: Linearity concentrations and R^2 Values

Impurity Name	Conc. (ppm)	R^2 value
1.Morpholine impurity(Imp-01)	0.2 to 5.5	0.9998
2.Ester impurity(Imp-02)	0.2 to 5.5	0.9998
3.Diasteriomer(Impurity-03)	0.2 to 5.5	0.9992
4.Apripitant	50to 150	0.9999

The range of the method was found from 10% to 200% of the 2.7 ppm for impurities and 50 to 150 for assay method.

4.3.4. Accuracy

The percentage recovery of imp-1, imp-2, imp-3, and Apripitant in Formulation mentioned in the below table (4-5).

Table 4: Morpholine, Ester imp. Recovery.

Impurity	%Level	%Recovery
Morpholine impurity	10	96.8
	20	92.6
	50	102.1
	80	106.6
	100	107.5
	120	109.3
Ester impurity	10	97.2
	20	90.6
	50	108.5
	80	101.5
	100	108.9
	120	109.5

Table 5: Diasteriomer, Apripitant recovery.

Impurity	%Level	%Recovery
Diasteriomer Impurity	10	98.2
	20	101.3
	50	103.1
	80	99.5
	100	99.7
	200	105.2
Apripitant in assay	25	99.1
	50	98.6
	75	99.0
	100	100.2
	150	101.6
	200	99.4

4.3.5. Robustness

Close observation of analysis results for deliberately changed chromatographic conditions (flow rate, column temperature) revealed that the resolution between closely eluting impurity, namely imp-3 was always greater than 2.0, illustrating the robustness of the impurity method and the area %RSD was less than 2.0

3.6. Solution Stability and Mobile Phase Stability

No significant changes were observed in the content of imp-1, imp-2, imp-3, and imp-4 during solu- tion stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions were stable up to the study period of 48 h.

The values are given in table.

Table 6: Solution stability data.

Impurity	Morpholine	Ester	Diasteriomer	Apripitant
Initial	0.30	0.24	0.88	99.9
48 Hrs	0.31	0.24	0.88	99.8
%Difference	0.01	0.00	0.00	0.1

3.7 Equivalency with the USP Method:

The developed UPLC method (Pending monograph Draft 1issued for public comment, SM3:E.Goni berg, correspondence Number C89258) is tested for equivalency with the available USP Pending monograph.

The equivalency was shown in below table.

System suitability results:

Table 7:

S.No	Method	Parameter	Criteria	USP method Result	Proposed Method
1	Assay	Tailing factor	Not more than 2.0	1.23	1.18
2	Assay	Standard %RSD	Not more than 1.0	0.42	0.31
3	Impurities	Tailing factor for APT- Peak	Not more than 2.0	1.54	1.25
4	Impurities	Resolution between APT and Diasteriomer	Not Less than 2.0	2.59	2.8

API Batch analysis Results (B.NO:APm0031010)

Table 8:

S.No	Method	Criteria	USP method Result	Proposed Method
1	Assay	98.0-102.0%	100.1	100.3
2	Impurities			
3	Maximum individual impurity	Not more than 0.15%	0.03	0.03
4	Total impurity	Not more than 0.3%	0.08	0.08

Emend Batch analysis Results (B.No:R2822)

Table 9

S.No	Method	Criteria	USP method Result	Proposed Method
1	Assay	98.0-102.0%	100.1	100.3
2	Impurities			
3	Maximum individual impurity	Not more than 0.15%	0.03	0.03
4	Total impurity	Not more than 0.3%	0.08	0.08

CONCLUSION

The UPLC method developed for quantitative determination of apripitant and its impurities in both bulk drugs and pharmaceutical dosage forms are precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is equivalent to usp method and stability indicating It can be used for the routine analysis of production samples and also to check the stability of Apripitant samples.

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