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STABILITY INDICATING ASSAY DETERMINATION METHOD OF APREPITANT IN APREPITANT CAPSULES DOSAGE FORMULATIONS BY USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (UPLC)

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Abstract

A Precise, Economical, Accurate, Rapid, Linear, and Simple Stability Indicating reversed phase Ultra Performance Liquid Chromatography (UPLC) method was developed for the estimation of Aprepitant in Capsules dosage form. The separation was achieved on Acquity UPLC BEH C18 column 50 mm x 2.1 mm, 1.7 μ m particle size, with a mobile phase consisting of 0.1 % v/v ortho phosphoric acid in water and Acetonitrile in the ratio of 50:50 v/v. The flow rate was kept at 0.5 ml/min, detection was performed at UV 210 nm and Column temperature was maintained at 35°C. The retention time of Aprepitant was found to be about 1.20 min. and the total run time is 2.00 min. The detector response was linear in the concentrations of 25 – 200 μ g/ml. The correlation coefficient (R^2) was found to be 0.9998. The percentage assay of Aprepitant was 99.50 %. The method was validated by determining its precision, accuracy, linearity, stress degradation and system suitability. Stress testing showed degradation products were well separated from the parent compound, conforming by spectral purity plots. The results of the study showed that the proposed UPLC method is simple, rapid, precise, linear, accurate and stability indicating, which is useful for the routine determination of Aprepitant in bulk drug and in its pharmaceutical dosage form.

Key Words: Aprepitant, Capsules, Estimation, Stability Indicating, UPLC, Validation.

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, and molecular

weight is 534.427 g/mol with molecular formula $C_{23}H_{21}F_7N_4O_3$. The chemical structure of Aprepitant is shown in Figure -1. Many High Performance Liquid Chromatographic separation methods for the determination of Aprepitant pharmaceutical dosage formulations and bulk samples for assay procedures has been reported [1-5]. United States Pharmacopeia pending monograph corresponding number C89258 for determination of Aprepitant in drug substance described for HPLC and few methods using UPLC^[6-8]. Authors prefer to use Ultra Performance Liquid Chromatography (UPLC) for determination of Aprepitant in the formulated capsules using sub 2μ particle stationary phase technology. The availability of an UPLC method with high sensitivity will be very useful for the determination of Aprepitant in pharmaceutical formulations. The aim of the study was to develop Economical, Accurate, Rapid, Linear, and Simple Stability Indicating reversed-phase UPLC method for the estimation of Aprepitant in bulk drug samples and in pharmaceutical dosage forms.

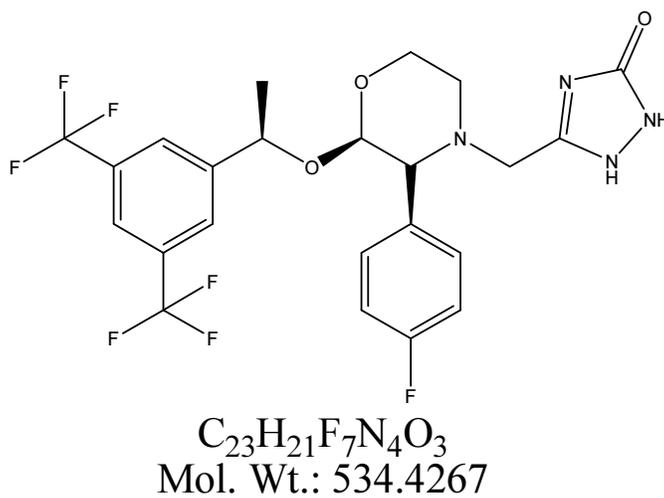


Figure-1, Chemical structure of Aprepitant.

According to current good manufacturing practices, all drugs must be tested with a stability-indicating method before release. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways. Intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance involved. Keeping into the view of susceptibility of Aprepitant under variety of conditions, it was felt that a LC method of analysis for the drug substance, its related compounds and degradation products should be separated from each other under ICH suggested guidelines^[9] (hydrolysis, oxidation and thermal stress), would be of general interest. These studies provide valuable information on drug's inherent stability and help in the validation of analytical methods to be used in

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stability studies to identify the degradation products during the study. Therefore, the aim of present study was to develop a stability-indicating assay method for Aprepitant. The developed method was validated as per ICH guidelines.

Materials and Methods

Samples of Aprepitant received as gift from MSN laboratories ltd, Hyderabad, India. HPLC grade acetonitrile and AR grade ortho phosphoric acid were purchased from Rankem, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Instrument: The method development, forced degradation studies and method validation was carried out using Waters Acquity UPLC system equipped Binary gradient solvent manager, Sample manager, column manager with Photo Diode Array (PDA) detector. The output signal was monitored and processed using Empower-2 software, Build 2154 (Waters).

Chromatographic conditions

The chromatographic separation was achieved on Acquity UPLC BEH C18, 50 mm x 2.1 mm, 1.7 μm column using 0.1 % v/v aqueous ortho phosphoric acid and acetonitrile in the ratio of 50:50 v/v as mobile phase. The mobile phase was filtered through 0.22 μm nylon 66 membrane filter and degassed. The flow rate of the mobile phase was kept at 0.5 mL min^{-1} . The column temperature was maintained at 35°C and the detection was monitored at a wavelength of 210nm. The injection volume was kept at 2.0 μL . The diluent was prepared by mixing Water and Acetonitrile in 50:50 v/v ratio. The standards and test samples were prepared in diluent and the test concentration was maintained at 100 $\mu\text{g mL}^{-1}$ concentration.

Preparation of standard solution

A stock solution of Aprepitant was prepared by dissolving 50 mg of Aprepitant in 50ml standard volumetric flask containing 30 ml of diluent, sonicated for about 5 minutes and then made up to the mark with diluent to get 1000 $\mu\text{g mL}^{-1}$ standard stock solution. Further 5 ml of standard stock solution was transferred in to 50 ml volumetric flask and made up to the mark with diluent to get a concentration of 100 $\mu\text{g mL}^{-1}$.

Preparation of sample solution

Twenty Capsules were weighed, and pellets are collected. A sample of pellets equivalent to 50 mg of active ingredient, was mixed with 30 ml of diluent. The mixture was sonicated for 30 minutes with intermittent shaking to ensure complete solubility of the drug. The solution was made up to the mark and then filtered through a 0.45 μm membrane

filter. Transferred 5 ml of this solution to a 50 ml volumetric flask and made up to the volume with diluent to give a concentration of $100 \mu\text{g mL}^{-1}$.

Results and Discussion

Optimization of chromatographic conditions

To design the experiment, we considered 0.1 % v/v aqueous ortho phosphoric Acid as mobile phase A and Acetonitrile as mobile phase B. Since Aprepitant is polar in nature, BEH C18, 50 mm x 2.1 mm, 1.7 μm column was selected since the BEH particles are fully end-capped, having operating pH range between 1 to 12 and small particle size.

Initial trials were performed with 0.1% v/v aqueous Ortho phosphoric acid as mobile phase A and acetonitrile selected as mobile phase B. Acetonitrile has strong elutropic strength when compared to methanol therefore Acetonitrile was use for the study. In the optimized analytical conditions Aprepitant and its degradants were separated from impurities of stressed samples which were well resolved form Aprepitant peak. The system suitability tests were carried out on freshly prepared standard stock solution of Aprepitant. Theoretical plates for Aprepitant were found to be 4892 and tailing factor was 1.70. Typical blank injection and Aprepitant standard injection chromatogram is shown in Figure -2 &3.

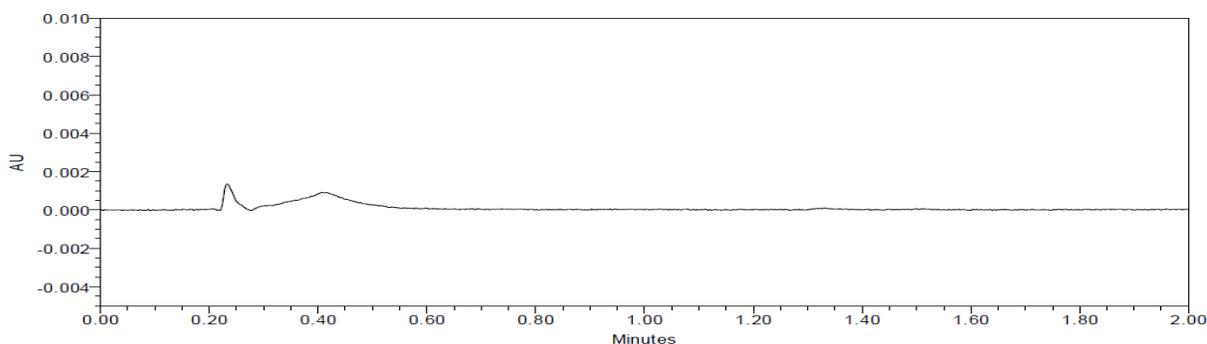


Figure-2: Blank injection chromatogram (Water: Acetonitrile(1:1))

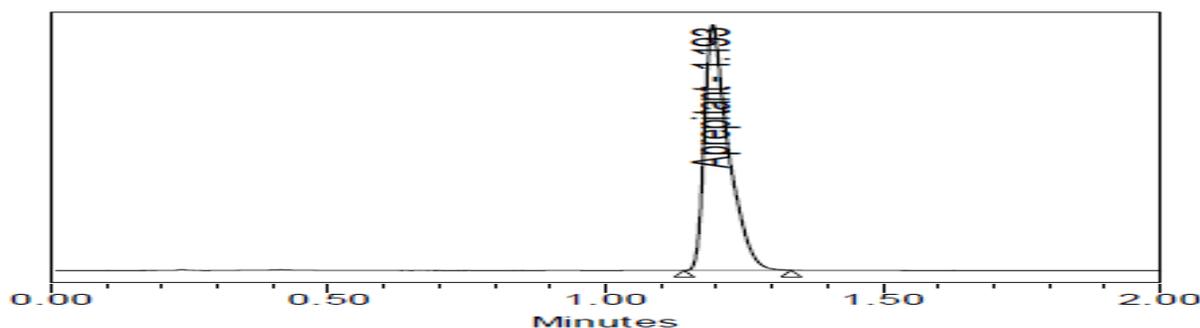


Figure-3: Aprepitant standard injection chromatogram.

Method validation

The Chromatographic method was validated according to ICH guidelines. Specificity, Precision, Accuracy and Linearity were established.

Specificity

Prepared $1000 \mu\text{g mL}^{-1}$ of API and Placebo spiked sample stock solutions separately, dissolved in 1.0 N hydrochloric acid (Acidic stressed sample and placebo injection chromatograms are shown in Figure-4 & 5), 1.0 N sodium hydroxide (Base stressed sample and placebo injection chromatograms are shown in Figure-6 & 7) and also $1000 \mu\text{g mL}^{-1}$ of API and Placebo spiked sample stock solution treated with 3 % H_2O_2 . The peroxide stressed sample and placebo injection chromatograms are shown in Figure-8 & 9. Samples were neutralized (for Acid stress and base stress) and diluted with diluent to a theoretical concentration of $100 \mu\text{g mL}^{-1}$ before injecting the samples in to UPLC system.

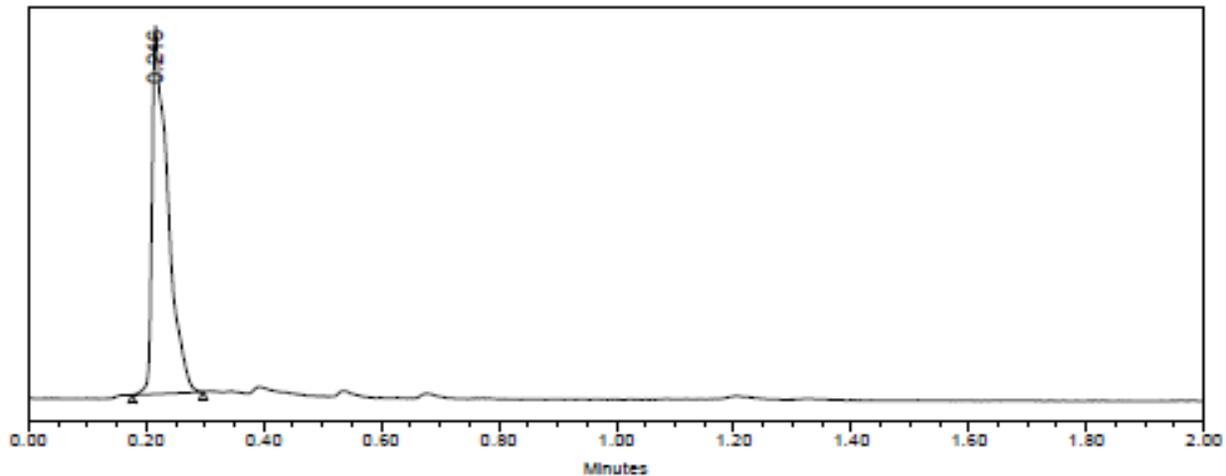


Figure-4: Acid degradation Placebo chromatogram.

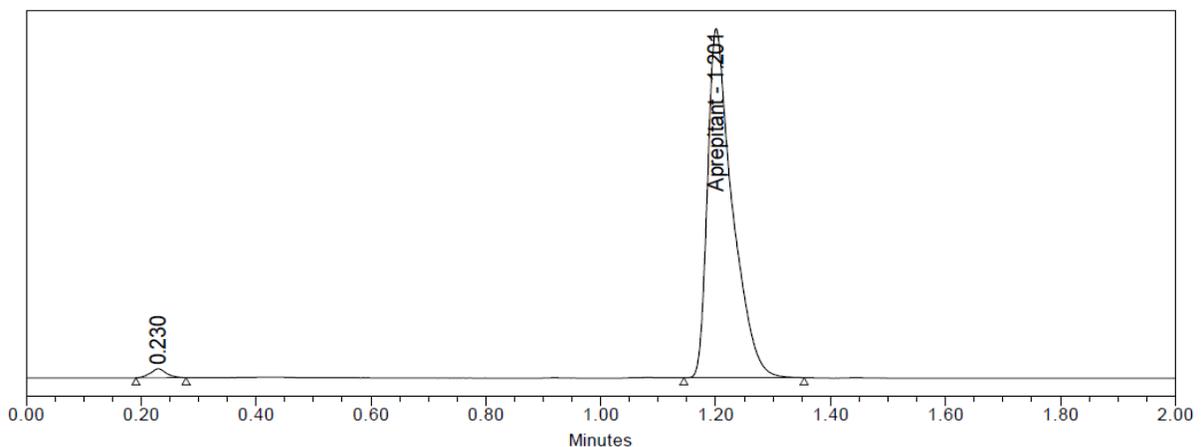


Figure-5: Acid degradation sample spiked chromatogram.

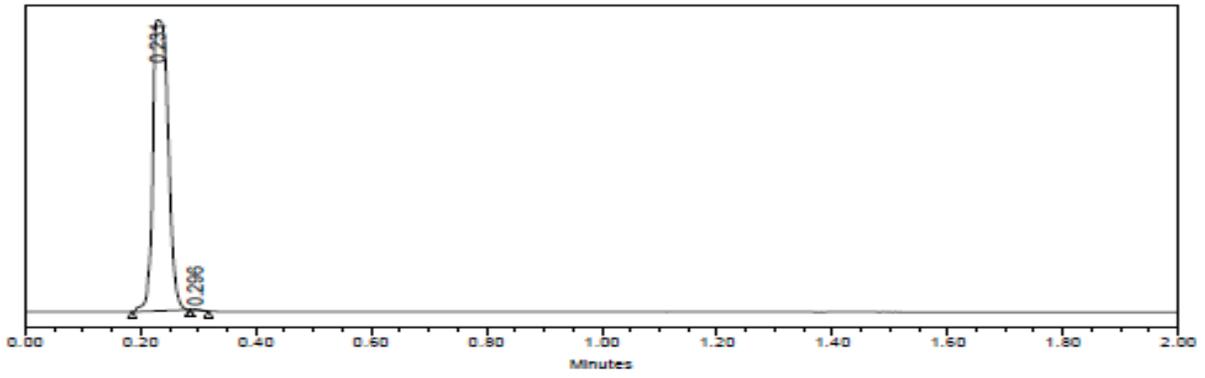


Figure-6: Base degradation Placebo.

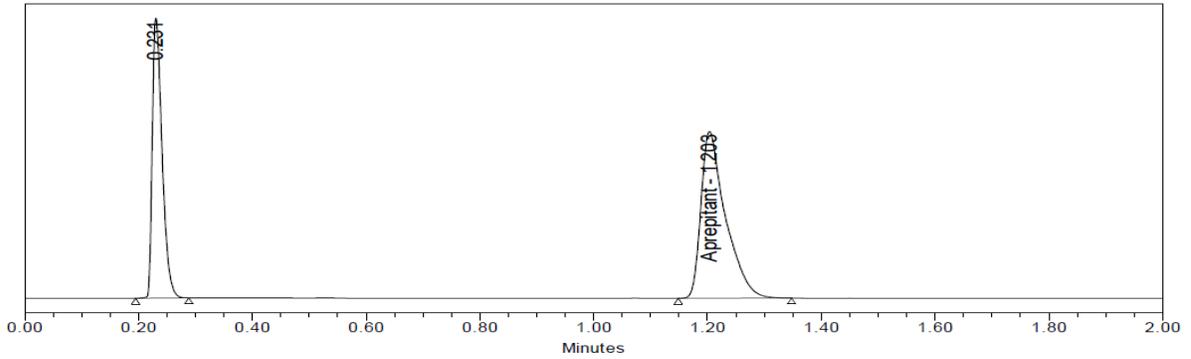


Figure-7: Base degradation Sample chromatogram.

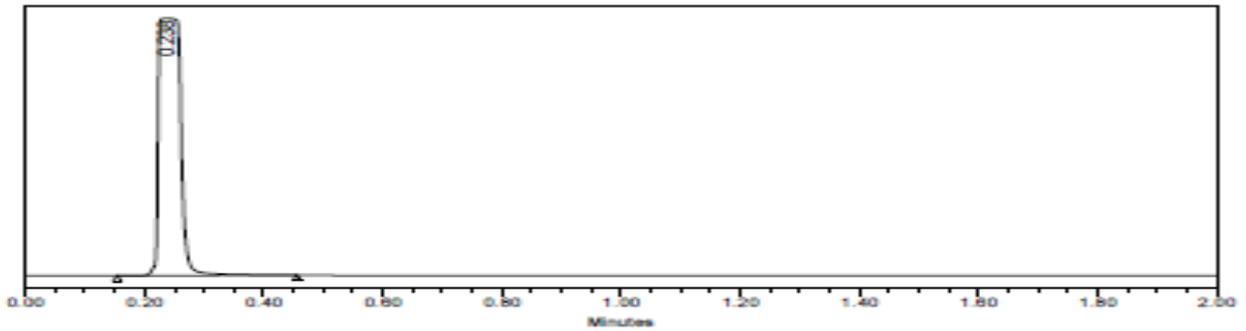


Figure-8: Peroxide degradation-Placebo.

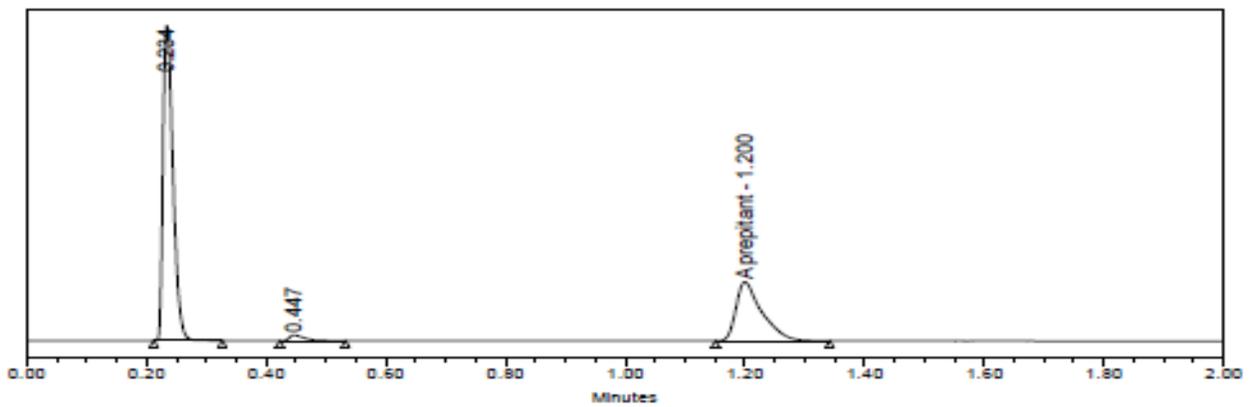


Figure-9: Peroxide degradation –sample chromatogram.

Degradation of less than 15.0 % was observed for Aprepitant when it was treated with Acid, Alkali and 3.0 % H₂O₂.

However, the decomposition was significant when Aprepitant was treated with acid, alkaline stress, and oxidation stress.

Aprepitant recoveries were 92.0, 89.7, and 88.2 % and the total degradation was 8.0, 10.3 and 11.8 % respectively.

Aprepitant was clearly separated from its degradants. Thus, the UPLC method presented in this study is specific for

Aprepitant.

Peak purity plot results shown in Figure - 10, 11 & 12 and confirmed that the Aprepitant peak is spectrally homogeneous

and pure in all the stressed samples, analyzed with Photo diode array detector which confirms the stability-indicating

power of the proposed method.

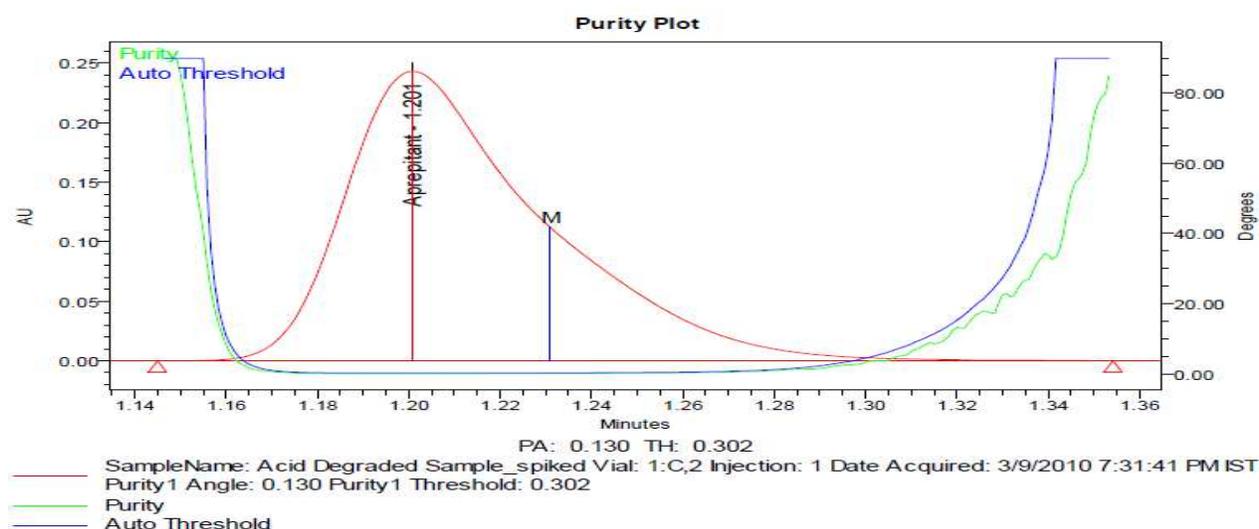


Figure 10: Purity plot for Acid degradation sample peak.

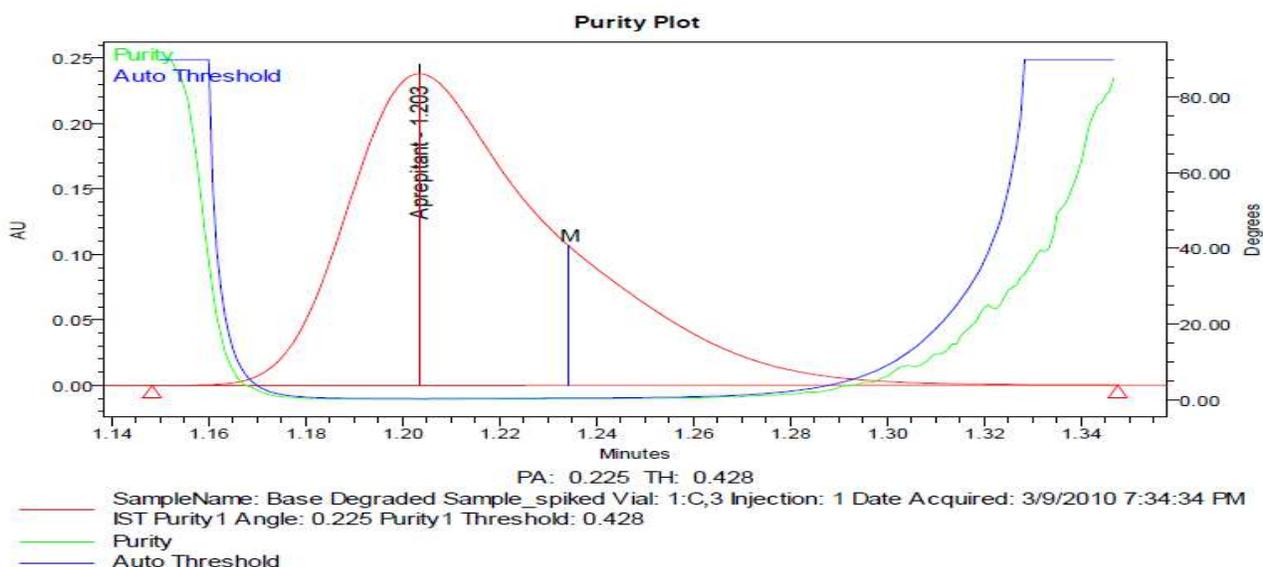


Figure 11: Purity plot for Base degradation sample peak.

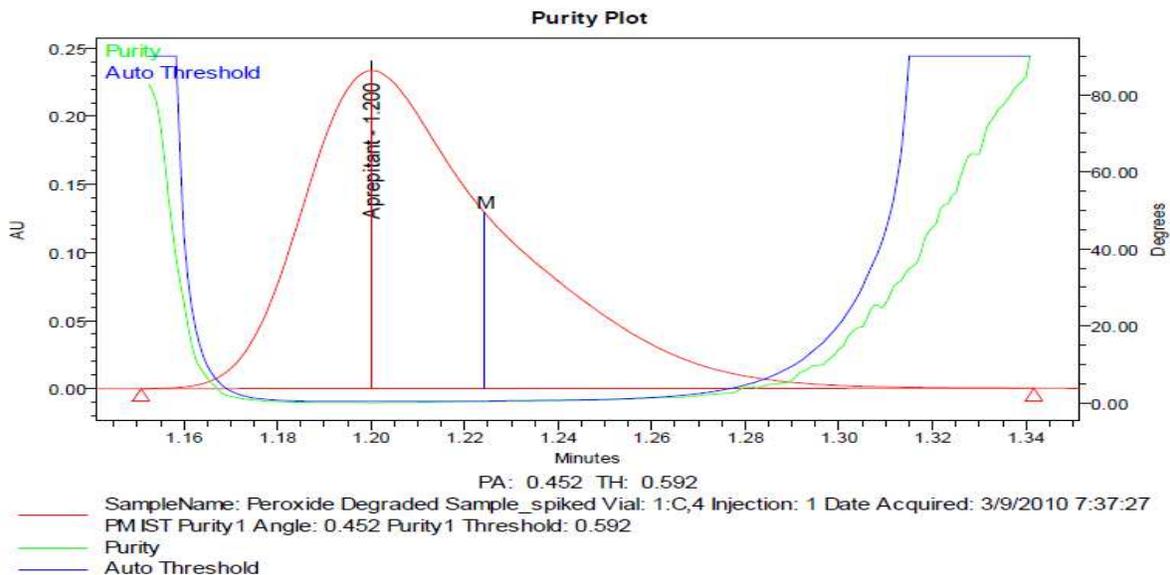


Figure 12: Purity plot for Peroxide degradation sample peak.

Precision

Method precision was evaluated by carrying out six replicate injections of test sample of Aprepitant against reference standard and calculated the relative standard deviation (RSD).

The RSD of assay of Aprepitant during method precision study was found to be 0.17 %, shown in Table - 1 confirming good precision of the method.

Table-1: Precision Data.

Injection	Area
1	793670
2	792173
3	790597
4	789979
5	789881
6	791602
Mean	791317
SD	1336.74
%RSD	0.17

Accuracy

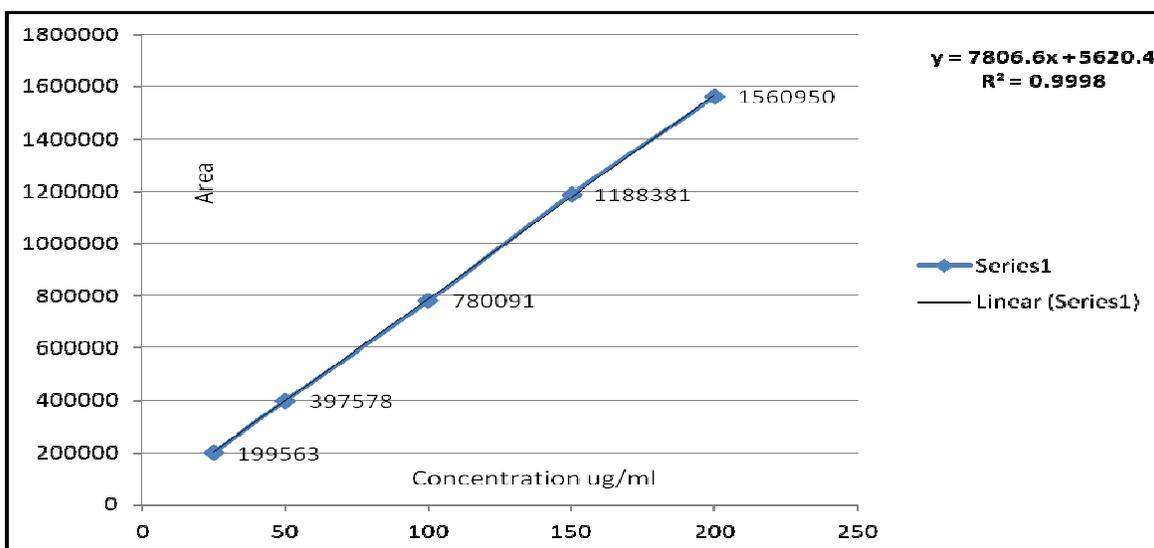
The accuracy of the method was evaluated in triplicate at five concentration levels i.e. 25 µg mL⁻¹, 50 µg mL⁻¹, 100 µg mL⁻¹, 150 µg mL⁻¹ and 200 µg mL⁻¹ (25, 50, 100,150 and 200% of the normal assay concentration) in sample. The percent recoveries were calculated from the slope and Y-intercept of the calibration plot and the results are shown in Table-2.

Table-2: Accuracy data.

Level	Concentration in (ug/mL)	Area of Replicate-1	Area of Replicate -2	Area of Replicate-3	Average	%RSD
Level-1	25	200577	199726	198385	199562.7	0.55
Level-2	50	397164	397612	397957	397577.7	0.10
Level-3	100	782539	781967	778168	780891.3	0.30
Level-4	150	1190095	1187870	1187179	1188381	0.13
Level-5	200	1561228	1560158	1561463	156-950	0.04
Correlation	0.9998					

The percentage recovery of Aprepitant in samples was ranged from 99.0 to 100.4 % w/w.

Calibration plot-1



Linearity

Linearity testing solutions were prepared from Aprepitant stock solution (1000 µg mL⁻¹) at five different concentration levels from 25, 50, 100, 150 and 200% of analyte concentration (25 µg mL⁻¹, 50 µg mL⁻¹, 100 µg mL⁻¹, 150 µg mL⁻¹

Mr. U. Satyanarayana* et al. *International Journal Of Pharmacy & Technology* and 200 $\mu\text{g mL}^{-1}$). The peak area versus concentration data was performed by least-squares linear regression analysis.

The calibration curve was drawn by plotting Aprepitant average area and the concentrations shown in calibration plot-1. Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 25 $\mu\text{g mL}^{-1}$ to 200 $\mu\text{g mL}^{-1}$ and the correlation coefficient obtained was greater than 0.9998. The results show that an excellent correlation existed between the peak area and concentration of the analyte.

Conclusion

A UPLC method for the assay determination of Aprepitant was validated in this study. Aprepitant and the other related compounds which may coexist with it as impurities or degradants gave very well resolved peak which indicate the specificity of the method and the possibility of using it as an indicator of stability. All the statistical values (percent of recoveries and RSD) calculated were within the acceptable limits. The validation data provided here indicated that the chromatographic assay for Aprepitant drug product is transferable method and is suitable for testing. The method satisfies the regulatory requirements of linearity, precision and selectivity to quantitate Aprepitant. The developed stability-indicating UPLC-UV method can be used for the pharmaceutical quality control of Aprepitant drug product.

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