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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF APREPITANT IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Sreekanth Nama[1*], Bahlul Z.Awen[2], Babu Rao Chandu[2], Mukkanti Khagga[3]

[1]*Department of Pharmacy, College of Public Health and Medical Sciences, Jimma University, Jimma, Ethiopia*
[2]*College of Pharmacy, Al-Jabal Al-Gharbi University, Al-Zawia, Libya*
[3]*Professor & Head, Centre for Chemical Sciences and Technology, IST, J.N.T.University, Kukatpally, Hyderabad, Andhrapradesh, India – 500 085*

Abstract

A stability indicating HPLC assay method has been developed and validated for the estimation of aprepitant in bulk and pharmaceutical dosage forms. A RP-HPLC isocratic separation was achieved on C_{18} column (250X4.6 mm i.d., 5µm) utilizing a mobile phase comprising of methanol and water in the ratio of 90: 10(v/v) and the eluents from the column were detected using a variable wavelength detector at 220 nm. The stress testing of aprepitant was carried out under acidic, alkaline hydrolysis, oxidation and thermal degradation (dry heat) conditions and apripitant was well resolved from its degradation products with good resolution. The proposed method has permitted the quantification of aprepitant in the linearity range of 1-100µg/ml and the flow rate was maintained at 1ml/min. The column was maintained at ambient temperature and the complete separation was achieved for aprepitant with all degradation products in an overall analytical run time of approximately 15 min and it was eluting at approximately 4.4 min. The retention times of aprepitant and rimonabant hydrochloride (IS) were found to be 4.383 min and 5.783 min, respectively. The limit of detection and limit quantification were found to be 0.130µg/ml and 0.395µg/ml, respectively. The percentage recovery was found to be in between 99.56 to 101.5 and the % RSD of system and method precision was found to be 1.20 and 0.561, respectively. The percentage amount of marketed commercial brand of aprepitant was found to be 99.97. The method was found to be suitable for the routine quality control analysis of aprepitant in bulk drug and formulation as well as for the stability indicating studies. The method was validated as per ICH guidelines.

Keywords: Aprepitant, RP-HPLC, Stability indicating assay, Forced degradation, Validation, ICH guide lines

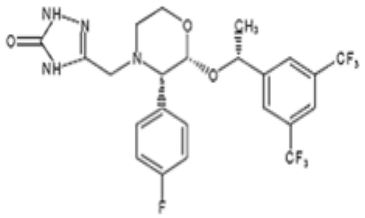
Introduction

Aprepitant (APT) is a substance P (SP) / neurokinin 1 (NK_1) 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 (NK_1) receptors and it has little or no affinity for serotonin (5-HT_3), dopamine, and corticosteroid receptors. A large number of drugs are available for prevention of PONV [1], of which 5-HT_3 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 NK_1 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 NK_1 receptors [2] and also showed that APT augments the antiemetic activity of the 5-HT_3 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 neuro kinin -1 (NK_1) 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

* Corresponding Author, *Email*: sreekanthpharma@yahoo.co.in, sreekanthnama@rediffmail.com, sreekanthpharma@gmail.com, Mob: +251917498574

fourier transform infrared spectroscopy [7], stability of an extemporaneous oral liquid APT formulation [8] , estimation of APT capsules by RP-HPLC [9] were reported.

Fig 1: Chemical structure of Aprepitant



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 APT in presence of its degradation products in oral liquid formulation in the literature. But there was no reported stability-indicating analytical method for analysis of APT in the presence of its degradation products in bulk and pharmaceutical dosage forms. The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC method for quantitative analysis of APT, and to validate the method in accordance with ICH guidelines [13] with rimonabant hydrochloride as an internal standard showed advantages of shorter retention time, runtime, and economic mobile phase.

Experimental

Materials

Pure standard of APT (Assigned purity 99.96%) was obtained as a gift sample from Dr.Reddys laboratories Pvt. Ltd, Hyderabad and pure sample of rimonabant hydrochloride (IS, assigned purity 99.87%) was obtained from Inventis drug delivery systems, Pvt. Ltd, Hyderabad along with certificate of analysis (COA). HPLC grade water and methanol (Qualigens), Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (S.D. fine chemicals, Mumbai, India), Hot air oven (Sky lab instruments & engineering Pvt. Ltd) Electronic analytical balance (DONA), Micro pipette (In labs, 10-100µl) and Aprecap capsules (80 mg, Glen mark pharmaceuticals Ltd) were employed in the study. All the glassware employed in the study was cleaned with hot water, followed by acetic anhydride and then with acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-22^{0}c. However, the chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC studies.

Instrumentation and chromatographic conditions

A HPLC system consisted of an Agilent LC 1200 HPLC pump, solvent degasser, a variable wavelength detector with deuterium lamp, a rheodyne injector and EZ chrom elite soft ware. Isocratic elution of mobile phase comprising of methanol and water in the ratio of 90: 10(v/v) with flow rate of 1ml/min was performed on C_{18} column (250x 4.6 mm i.d., 5µm). The column temperature was maintained at ambient and the volume of injection was 20 µl. Prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluents were monitored at 220 nm, data were acquired, stored and analysed with soft ware EZ chrom elite.

Preparation of mobile phase

The HPLC grade solvents of methanol and water were used for the preparation of mobile phase in the ratio of 90:10 (v/v). The contents of the mobile phase were filtered before use through a 0.45µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

Procedure recommended

Preparation of standard solution

A stock solution of drug and internal standard (IS) was prepared by dissolving 100 mg of APT and rimonabant hydrochloride in a 100 ml volumetric flasks containing 70 ml of methanol (HPLC grade), sonicated for about 15 min and then made up to volume with methanol. Daily working standard solutions of APT and rimonabant hydrochloride were prepared by suitable dilution of the stock solution with the mobile phase. Six sets of the drug solution were prepared in the mobile phase containing APT at a concentration of 1-100µg/ml along with a fixed concentration (0.4µg/ml) of rimonabant hydrochloride as the internal standard. Each of these drug solutions (20µl) was injected six times into the column, the peak area and retention times were recorded.

Procedure for pharmaceutical formulation

Twenty capsules were weighed and the contents were removed to obtain the average weight powder. A sample of the powder equivalent to 100 mg of the active ingredient was mixed with known amount of methanol and the mixture was allowed to stand for 30 min with intermittent sonication to ensure complete

solubility of the drug, then it was filtered through a 0.45 µm membrane filter, followed by adding methanol to obtain a stock solution of 0.1 mg/ml (100µg/ml). An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up to a sufficient volume with the mobile phase to give an expected concentration of 10 µg/ml. All determinations were conducted in triplicate.

Forced degradation of API and capsules of APT

In order to establish whether the analytical method and the assay were stability indicating, the capsules and pure active pharmaceutical ingredient of APT were stressed under various conditions to promote degradation. As this drug was freely soluble and stable in methanol and methanol was used as solvent in all forced degradation studies. All solutions were prepared to use in forced degradation studies were prepared by dissolving API or drug product in small volume methanol and later diluted with 3% hydrogen peroxide, 0.1 N hydrochloric acid and 0.1 N sodium hydroxide to achieve concentration of 100 µg/ml. After the degradation, these solutions were diluted with mobile phase to get starting concentration of 10µg/ml with the objective of evaluating stability of APT. The degradants were observed in the chromatogram and showing good resolution with the APT.

Hydrolysis (Acid and alkali)

Initially for hydrolytic degradation the APT was dissolved in known amount of methanol and diluted with 0.1N HCl or 0.1N NaOH to obtain a concentration of 100µg/ml. After completion of degradation process, both the solutions were neutralized with acid or base, as necessary and diluted with the mobile phase to achieve a concentration of 10µg/ml. The solutions for hydrolysis were prepared in methanol and 0.1 N HCl and 0.1N NaOH (60:40 v/v). The prepared solutions in acid were injected to the chromatographic system at 0 h (immediately after preparing the solution) and after reflux at 60^0c about 2h and the solutions prepared in alkali were injected at 0 h and after reflux at 60^0c about 24 h. The respective chromatograms were recorded for the study of extent of degradation.

Peroxide degradation

The solutions for peroxide degradation were prepared in methanol and 3% hydrogen peroxide (60:40 v/v). The prepared solution was refluxed at 60^0c about 2h and injected into chromatographic system after 2 h. The respective chromatogram was recorded for the study of extent of degradation.

Thermal degradation

The bulk and marketed formulation was subjected to heat treatment for the assessment of the thermal degradation and were exposed to dry heat in an oven at 70^0c about two days (48h).The capsules and bulk drug was removed from the oven and the contents of capsule were collected and mixed. The amount which was equivalent to 100 mg of active ingredient and bulk drug were prepared for analysis by the proposed method. The respective chromatograms were recorded for the study of extent of degradation.

Results and Discussions

Optimization of chromatographic conditions (method development)

A simple stability-indicating RP-HPLC method has been developed for determination of APT in presence of its degradation products. The method was optimized to provide a good separation of the components (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor < 2) in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules. The chromatographic separation was achieved using an RP C_{18} column because it was suitable to resolve the degradation products from APT with adequate resolution and gave symmetrical peak shapes. APT was easily soluble in methanol and it is a well known solvent for various pharmaceutical compounds. Data reported in the literature showed that 0.01 M ammonium Acetate containing 0.1% formic acid and methanol in the ratio of 35:65 v/v was used a mobile phase. But our experiments using methanol used along with water (HPLC grade) as mobile phase was eluted the APT in a significant shorter retention time of 4.383 min. Therefore, we selected methanol and water in the ratio of 90:10 (v/v) as a mobile phase. The method has many advantages, e.g., simplicity, isocratic conditions, and absence of buffers in the mobile phase that could damage the chromatographic column and equipment. Under these conditions, the retention time of APT was about 4.383 min, with a good peak shape (peak symmetry).

Stability indicating method development

Solutions used in forced degradation studies were prepared by dissolving API and drug formulation in small volumes of methanol and then diluting with 3% aqueous hydrogen peroxide, 0.1 N aqueous hydrochloric acid, and 0.1 N aqueous sodium hydroxide to promote degradation at concentration of 100µg/ml of APT. After degradation at suitable degradation conditions the samples were diluted with mobile phase and subjected to HPLC analysis. To study the effect of thermal stress, Aprecap capsules and API powder were exposed to dry heat at 70^0c in a convection oven for about 48 h. The samples removed

from the oven, the contents from the twenty capsules were removed, thoroughly mixed and the average weight calculated. Equivalent amount of powder was analysed after preparation of 10 µg/ml solutions in mobile phase. Chromatograms for all samples were inspected for the appearance of additional peaks and the APT peak was compared between samples for changes in concentration, retention time, peak shape, and UV spectral purity compared to an authentic undegraded standard. The degradant were well resolved from the APT, shows that the assay was stability indicating and specific.

Method validation

The method was validated as per the ICH guidelines for different validation parameters. The method was validated for its specificity, linearity, accuracy, precision, selectivity, LOD and LOQ.

Specificity

The results from the stress studies indicated that the method was highly specific for APT. The degradation products were completely distinguishable from the parent compound. Based on peak purity of APT, every degradation sample showed that the peaks were homogenous and there were no co eluting peaks indicating that the method was stability indicating and specific.

Linearity

The standard plot for the APT was constructed by plotting the ratio of the peak area of APT to the peak area of the internal standard (*Y*) against concentration (*X*). It was found to be linear with a correlation coefficient (r^2) of 0.999, the corresponding linear regression equation being y=0.288x + 0.32. In the linear range of 1-100µg/ml, the coefficients of variation (CV) based on the peak area ratios for six replicate injections, were found to be in between 0.16 to 0.72.

Statistical data of calibration curves

The regression characteristics, such as standard deviation of slope (S_b, 0.029), the % RSD of the slope (11.12), the standard deviation of intercept (S_a, 0.0576), regression equation (0.288x + 0.32) and correlation coefficient (r^2, 0.999) were calculated.

Precision

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Six replicate measurements/injections of standard preparation (System Precision), six replicate analysis of the samples through the complete analytical procedure from sample preparation (Method Precision) was performed. The % RSD of system precision and method precision were found to be 1.20 and 0.561, respectively and were shown in the table-1.

Table 1: Precision of the method

S.No	System precision	Method precision
1	8880239	100.3
2	8634069	99.84
3	8852531	100.5
4	8760932	99.97
5	8823094	98.93
6	8645867	99.56
Mean (n=6)	8766122	99.85
%RSD	1.20	0.561

Accuracy

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with preanalysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting five times at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of APT standard to a sample of known concentration and calculating the recovery of APT with RSD (%) and % recovery for each concentration. The mean % recoveries were in between 99.56 to 101.5 and shown in table -2. There was a high recovery 101.5% of APT indicating that the proposed method for the determination of APT in the capsule dosage forms was highly accurate.

Table 2: Accuracy of the method

APT	80% level	100% level	120% level
1	99.56	100.2	101.5
2	101.32	99.97	100.4
3	100.76	99.56	100.2
Mean (n=3)	100.54	99.91	100.7
% RSD	0.899	0.324	0.7

System suitability

To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, resolution, asymmetry factor, and asymmetry (10%), limit of detection and limit of quantification. The values were shown in table-3.

Table 3: System suitability

Parameter	Value
Retention time (min)	4.383
Theoretical plates	1230
Tailing factor	1.25
Linearity range (µg/ml)	1-100
Limit of detection (µg/ml)	0.130
Limit of quantification(µg/ml)	0.395
Resolution	3.5
Asymmetry factor	1.22
Asymmetry (10%)	1.18

Selectivity

The results of stress testing studies indicated a high degree of selectivity of this method for APT. The degradation of APT was found to be similar for both the capsules and API powder.

Limit of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were calculated by the method based on standard deviation (σ) of responses for triplicate blank injections and the slope (S) of the calibration plot, using the formulae $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$ as defined by ICH.

Stress degradation studies

To determine whether the method was stability indicating, APT capsules and APT active pharmaceutical ingredient (API) powder were stressed under different conditions to promote degradation. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 requires the development and validation of stability indicating potency assays. APT was exposed to different stress conditions and the degradation products were well separated with greater resolution. The drug was showed degradation in all degradation conditions .The drug was degraded in both acidic and alkali environments when it was exposed to 0.1N HCl (at 0h, 2 h) and 0.1N NaOH (at 0 h, 24h). In acidic degradation of APT at 0 h (immediately after preparing the solution) had shown 2 degradation products with the retention times (2.967 and 3.813 min) and the APT was eluted at retention time of 4.4 min with percentage of degradation 30.85. After reflux at 60^0c for about 2 h, APT had shown 8 degradation products with retention times (1.530, 2.960, 3.440, 3.650, 3.783, 5.257, 5.810, and 6.193 min) and the APT was eluted at retention time of 4.397min with percentage of degradation 32.52. In alkali degradation of drug at 0 h (immediately after preparing the solution) had shown 2 degradation products with the retention times (2.963 and 3.780 min) and the APT was eluted at retention time of 4.393 min with percent of degradation 23.7. After reflux at 60^0c for about 24 h the APT had shown six degradation products with retention times (2.400, 2.973, 3.223, 3.523, 3.873 and 5.613 min) and the drug was eluted with the retention time of 4.623 min with percentage of degradation 47.16. After reflux at 60^0c for about 2h in hydrogen peroxide the APT was completely degraded with 100% degradation. The degradation was observed in thermal treatment of APT for about 48 hr at 70^0 c in an oven (dry heat) and the API and capsule had shown degradation about 31.31% and 25.03%, respectively. The degradation of bulk had shown eight degradants with retention times (2.407, 3.047, 3.693, 3.917, 4.043, 5.293, 5.743 and 6.233 min)

and the APT was eluted at 4.603 min. The degradation of APT capsule had shown seven degradants with retention times (2.507, 3.060, 3.630, 4.383, 4.670, 5.530, and 6.810 min) and the APT was eluted 4.670 min. The percentage of degradation and the summary of forced degradation data were shown in the table-4, 5 and the chromatograms of degraded APT in different stress conditions were shown in fig.2 (a-g).

Table 4: % of degradation

Degradation mechanism	Degradation condition	Assay (mg/tab)	% Degradation
Un degraded	-	79.98	-
Acid hydrolysis	0.1 N HCl, at 0 hr, 2hr	55.94, 53.97	30.05%, 32.52%
Alkali hydrolysis	0.1 N NaOH, at 0 hr, 24 hr	61.02, 42.26	23.7%, 47.16%
H_2O_2 oxidation	3% H_2O_2 at 2 hr	---------	100%
Thermal solid	Dry heat, 48 hrs in oven	54.93	31.31%
Thermal liquid	Dry heat 48 hrs in oven	59.96	25.03%

Table 5: Forced degradation of APT

S.No	Exposure condition	Degradation Products (R_t, in min)	%Drug remained	% recovery	Fig no
1	0.1N HCl at 0 h& after reflux at 60^0c about 2h	2 (2.967 and 3.813)	69.95 67.48	69.95 67.48	2(a) 2(b)
2	0.1 N NaOH at 0 h& after reflux at 60^0c about 24h	8 (1.530, 2.960, 3.440, 3.650, 3.783, 5.257, 5.810 and 6.193)	76.30 52.84	76.30 52.84	2 (c) 2(d)
3	H_2O_2 (3% v/v), after reflux at 60^0c about 2h	----------	0	0	2(e)
4	Thermal treatment of API at 70^0 C about 48 h in an oven	8 (2.407, 3.047, 3.693, 3.917, 4.043, 5.293, 5.743 and 6.233)	68.69	68.69	2(f)
5	Thermal treatment of API capsule at 70^0 C about 48h in an oven	7 (2.507, 3.060, 3.630, 4.383, 4.670, 5.530 and 6.810)	74.97	74.97	2(g)

Fig 2 (a): A Chromatogram of APT degraded in 0.1 N HCl at 0 h

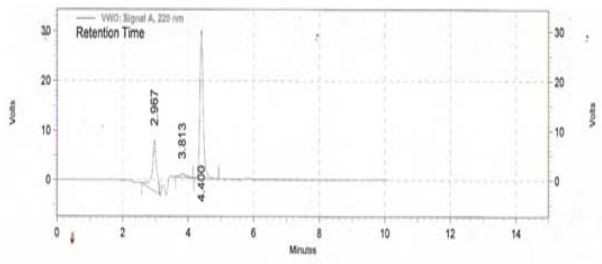


Fig 2(b): A Chromatogram of APT degraded in 0.1 N HCl after reflux at 60^0c about 2 hr

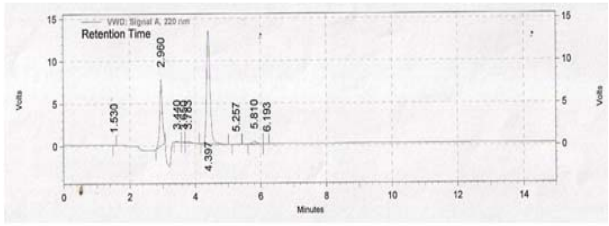


Fig 2(c): A Chromatogram of APT degraded in 0.1 N NaOH at 0 h

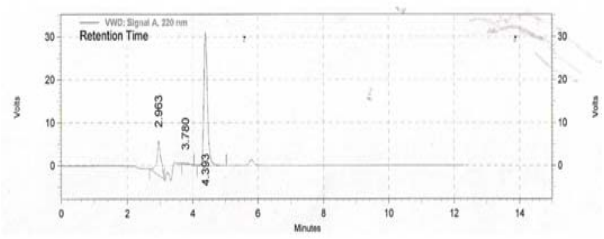


Fig 2(d): A Chromatogram of APT degraded in 0.1 N NaOH after reflux at 60^0c about 24 h

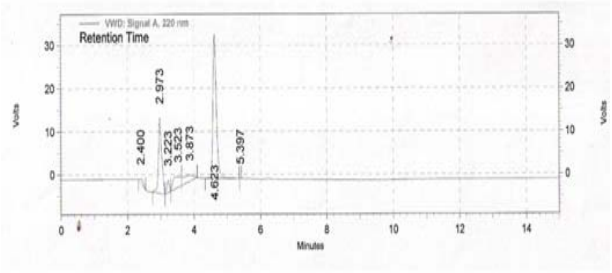


Fig 2(e): A Chromatogram of APT degraded in 3% H_2O_2 after reflux at 60^0c about 2 h

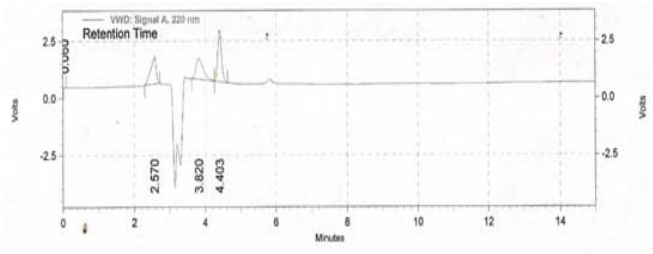


Fig 2(f): A Chromatogram of API degraded in an oven at 70^0c about 48 h

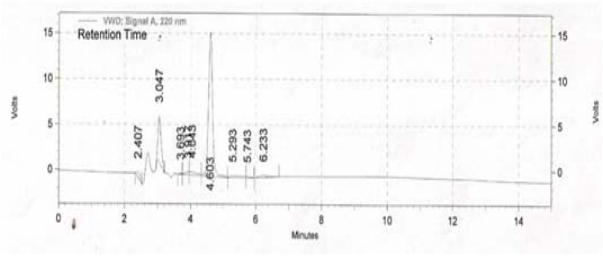
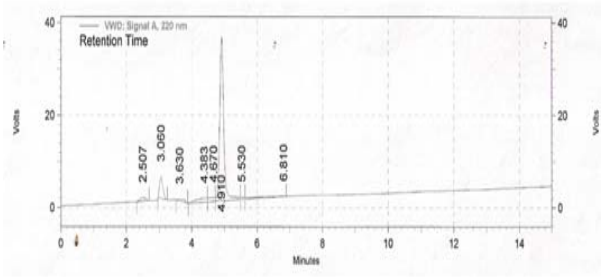


Fig 2(g): A Chromatogram of capsule of APT degraded in an oven at 70^0c about 48 h



Estimation of APT in marketed capsule formulation

The assay of commercial capsules was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 99.97 of the labeled claim. No interference peaks were found in chromatogram, indicating that estimation of APT free from interference of excipients. The results were shown in the table-6 and the chromatograms of APT with internal standard were shown in fig. 3, 4.

Table 5: Assay of the method

Brand name	Labeled claim	Mean amount found*± S.D	% purity
Aprecap	80 mg	79.976±0.12	99.97

*Mean of three values

Fig 3: A typical chromatogram of APT with rimonabant hydrochloride (IS)

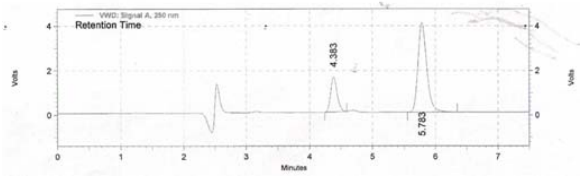
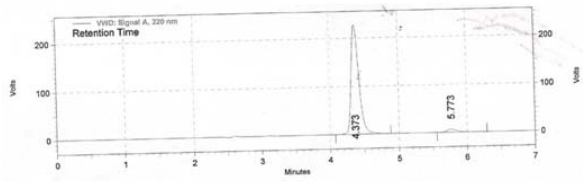


Fig 4: A typical chromatogram of APT capsule with rimonabant hydrochloride (IS)



Conclusion

A validated stability-indicating HPLC analytical method has been developed for the determination of APT 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 APT 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 APT.

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