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Stress Degradation Studies And Validation Method For Quantification Of Aprepitent In Formulations By Using RP-HPLC

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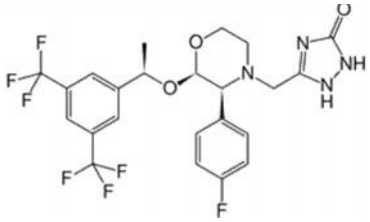
Abstract: A Stability indicating reverse phase high performance liquid chromatography method has been developed for the estimation of Aprepitant in tablet dosage form. A Thermosil symmetry C18 column having dimensions of 150×4.6mm and 5µm particle size, with mobile phase containing a mixture of water and Acetonitrile in the ratio of 30:70 v/v was used. The flow rate was 1ml/min and the column effluents were monitored at 220 nm. The retention time for Aprepitant was found to be 2.8 min. The proposed method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness. The LOD and LOQ were found to be 0.085µg/ml and 0.28µg/ml respectively. The method was found to be linear in the range of 50 – 90 µg/ml with regression coefficient (r=0.999). The % recovery for the Aprepitant was found to be 100.7 and the forced degradation studies were also carried out as per ICH guidelines. There was complete separation of degradation and Aprepitant peak, which demonstrate the specificity of assay method for estimation of Aprepitant in the presence of its degradation products and it can be employed as a stability indicating one. Due to simplicity, rapidity and accuracy of the proposed stability indicating HPLC method and it is useful for quality control analysis.
Keywords: Aprepitant, Stability indicating HPLC, forced degradation, validation, Formulation.

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. Its molecular weight is 534.427 g/mol with molecular formula $C_{23}H_{21}F_7N_4O_3$. It mediates its effect by blocking the neurokinin receptor. For the quantitative analysis of dosage forms mostly HPLC and LC-MS methods are used. Literature survey reveals that the drug can be estimated by RP-HPLC in Capsules [1], [2], stability indicating RP-HPLC [3], rapid liquid chromatography-tandem mass spectrometry method [4],[5],

quantification of process related impurities by RP-LC method [6] and UPLC methods[7]. Present study aims to develop simple, rapid, accurate, precise and validated stability indicating HPLC method for the determination of Aprepitant in Capsules. Literature survey reveals one stability indicating HPLC method with high retention time. The main objective of method development is to determine the drug content of formulations as well as purity. In analytical research, the time and cost of method development and validation are of great importance. The objective of this study was to develop and validate a simple, sensitive, rapid, economic and accurate RP-HPLC method for the estimation of Aprepitant in commercial tablet products. The stability indicating method is a validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanism, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product. Stress testing is a part of development strategy under the ICH requirements. This method most precise , accurate , reliable and take less time of analysis tan compared with the existing methods.

Experimental

Instrumentation

Quantitative HPLC was performed on liquid chromatography water separation 2695 DAD or UV detector module equipped with automatic injector with injection volume 20µl and 2487 pump. The Thermosil symmetry C18 column having dimensions of 150×4.6mm and 5µm particle size, was used. The HPLC system was equipped with empower software.

Chemicals And Reagents:

Aprepitant was obtained as a gift sample from Dr.Reddy`s Laboratories, Hyderabad. Acetonitrile and water used were of analytical grade and supplied by M/S S.D Fine chem. limited, Mumbai. Commercially available Aprepitant tablets were procured from local market.

Chromatographic Conditions:

Mobile phase containing a mixture of water and Acetonitrile in the ratio of 30:70 v/v was used and filtered through 0.45µ membrane filter. The mobile phase was pumped from the solvent reservoir in the ratio 30:70 to the column at a flow rate 1ml/min whereas runtime set was 6 min. the column was maintained at ambient and the volume of each injection was 20µl. Prior to injection of the solutions, column was equilibrated for at least 30 min with mobile phase flowing through the system. The eluent were monitored at 220nm.

Standard Preparation:

10 mg of Aprepitant was weighed and transferred to 10ml volumetric flask containing 7 ml of mobile phase. The solution was sonicated for 15 min to dissolve the drug completely and the volume made up with mobile phase to get the concentration of 1 mg/ml solution (stock solution). Further pipette 0.7ml of the above stock solution into 10ml volumetric flask and dilute up to the mark with diluents (working standard solution)

Sample Preparation

Weigh about five Aprepitent tablets and calculate the average weight. Accurately weigh and transfer the sample, equivalent 10mg of Aprepitent into 10 ml volumetric flask. Add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with mobile phase. Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Method Validation

System Suitability

A standard solution of Aprepitant working standard was prepared as per procedure and was injected 3times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the %RSD of retention times, tailing factor, theoretical plates and peak area from 3 replicate injections. The %RSD for the retention times of principal peak from 3 replicate injections of each standard

solution should be not more than 2%. The number of theoretical plates for Aprepitant peaks should be not less than 2000.

Linearity

Several aliquots of standard solutions of Aprepitant was taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of Aprepitant is 50 -90 µg/ml and evaluation of drug was performed with DAD detector at 220nm. Peak area is recorded for all the peaks. The slope and intercept value for calibration curve was Y= 12739+51034 (r^2=0.998). The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range 50-90µ g/ml. Regression data was shown in Table-2.

Precision

The precision of the method was demonstrated by Interday and Intraday variation studies. In intraday studies five repeated injections of working standard solutions were made and response factor of drug peaks and %RSD were calculated. In the Interday variation studies, five repeated injections of standard working solutions were made for different day and make column of same dimensions. The response factor of drug peaks and %RSD were calculated and present in table4. From the data obtained the developed RP-HPLC method was found to be precise.

Accuracy

The accuracy of the method was evaluated by determination of recovery of Aprepitant at three levels of concentrations. The sample solutions were spiked with Aprepitant standard solutions corresponding to 50% ,100% and 150% of nominal analytical concentrations. The results showed good recovery within limits (99.5 to 101.7%).

LOD And LOQ

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentrations of the standard solutions found to be 0.085µg/ml and 0.28µg/ml respectively. The LOD and LOQ values reveal that the developed method shows very good sensitivity.

Ruggedness And Robustness

Ruggedness test was determined between two columns or two analysts or two instruments. Robustness of the proposed method was determined by small deliberate changes in flow rate, change in composition of mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of RSD indicating that the method was rugged and robust. On evaluation of these results, it can be concluded that the variation of flow rate and variation of org. composition in mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in flow rate slightly.

Application Of Proposed Method To Solid Dosage Form

The assay of commercial Aprepitant tablets showed that the developed method, show in Table-5 was accurate and reliable with mean drug content of 100.3% of the labeled claim. No interference peaks were found in the chromatogram indicating that the determination of the drug content was free from interference by excipients.

Degradation Study

The degradation samples were prepared by transferring powdered tablets, equivalent to 1000µg/ml Aprepitant into a 10ml volumetric flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were allowed to room temperature and diluted with mobile phase up to the mark. Filter the solution with 0.45 microns syringe filters and place in vials. Specific conditions were described below.

Hydrolytic degradation under acidic condition: Acidic degradation study was performed by treating the drug content in 3ml of 0.1 N HCl at normal condition for 90 min and then the mixture was neutralized with 0.1 N NaOH

Hydrolytic degradation under alkaline condition: Alkaline degradation study was performed by treating the drug content in 3ml of 0.1N NaOH at normal condition for 90 min. and then the mixture was neutralized with 0.1N HCl

Thermal induced degradation: Thermal degradation study was performed by reflux the drug content for 1hr at 60 ^{0}C temp.

Oxidative degradation: Oxidative degradation study was performed by treating the drug content in 3 ml of 3%v/v H_2O_2 for 15 min. at room temperature.

The results of degradation studies were tabulated in Table-7 and the chromatograms are shown in Figures 2 to 5.

Table-1 : System suitability parameters.

Retention time	2.8min
USP plate count	3922
USP tailing	1.4
LOD	0.085
LOQ	0.28
Peak area	892717
%RSD	0.47

**mean of 3 determinations*

Table-2 : Calibration curve data of proposed method

Concentration (µg/ml)	Peak area
50	632417
60	766995
70	894737
80	1031164
90	1137297
Concentration range	50-90µg/ml
Slope	12739
Intercept	51034
Correlation coefficient	0.998

Table-3 : Accuracy results

Concentration %	**Peak area**	**Amount added**	**Amount found**	**% recovery**	**Mean recovery**
50%	928000	5.0 mg	5.08 mg	101.7%	100.7%
100%	1815326	10.0 mg	9.95 mg	99.5%	
150%	2764496	15.0 mg	15.1 mg	101.0%	

Table-4 : Precision and intermediate results

Concentration of Aprepitant (30µg/ml)	**Peak area**	
	Intraday	**Interday**
Injection 1	894562	895311
2	896754	896783
3	893627	895237
4	893750	894206
5	892682	895085
Average	894275	895324
S.D	1537.7	927.8
%RSD	0.17	0.10

Table - 5 : Assay results

Label claim in mg	**Amount found in mg**	**% recovery**
100	99.9	100.3

Table-6 : Robustness results of the method

Change in flow rate	**USP plate count**	**USP tailing**	**Retention time**
0.9	4921.2	1.4	3.1
1.0	3943.3	1.4	2.8
1.1	4493.3	1.4	2.5
Change in organic composition in the mobile phase	**USP plate count**	**USP tailing**	**Retention time**
10% less	5032.7	1.3	3.3
Actual	3943.3	1.4	2.8
10% more	3834.7	1.3	2.5

Table 7: Degradation characteristics of Aprepitant

Degradation parameter	**Degradation Time**	**Results of Degradation**							
		Peak area of degradated product	**Peak area of Standard**	**% of recovery**	**% of Degradation**	**Purity angle**	**Purity threshold**	**Tailing factor**	**Plate count**
Acid Degradation (0.1N HCl)	90 min	830037	892513	92.9	7.1	0.15	0.32	1.4	3946.8
Base Degradation (0.1NNaOH)	90 min	812187	892513	91.0	9.0	0.24	0.33	1.4	3846.5
Thermal Degradation	60 min.	794337	892513	89.0	11.0	0.19	0.64	1.4	3946.8
Peroxide Degradation (3% H_2O_2)	15 min.	758636	892513	84.9	15.1	0.24	0.39	1.4	3842.5

Results And Discussion:

From the typical chromatogram of Aprepitant shown in **Figure-1,** it was found that the retention time was 2.8 min. The mobile phase containing a mixture of water and Acetonitrile in the ratio of 30:70 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=0.999) was observed between the concentration range 50-90µ g/ml. Low values of S.D are indicative of high precision of the method. The assay of Aprepitant tablets was found to be 100.3%. From the recovery studies, it was found that about 100.7% of Aprepitant was recovered which indicates high accuracy of the method. The results of LOD and LOQ indicate that the method is reliable and the forced degradation studies were also carried out as per ICH guidelines. There was complete separation of degradation and Aprepitant peak, which demonstrate the specificity of assay method for estimation of Aprepitant in the presence of its degradation products, it can be employed as a stability indicating one.This demonstrates that the developed Stability indicating HPLC method is simple, linear, accurate, sensitive and reproducible.

Figure 1: Linearity curve of Aprepitant

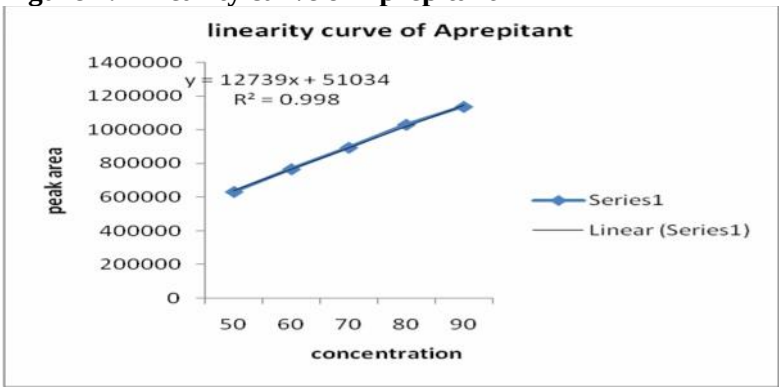


Figure 2: Chromatogram of Aprepitant

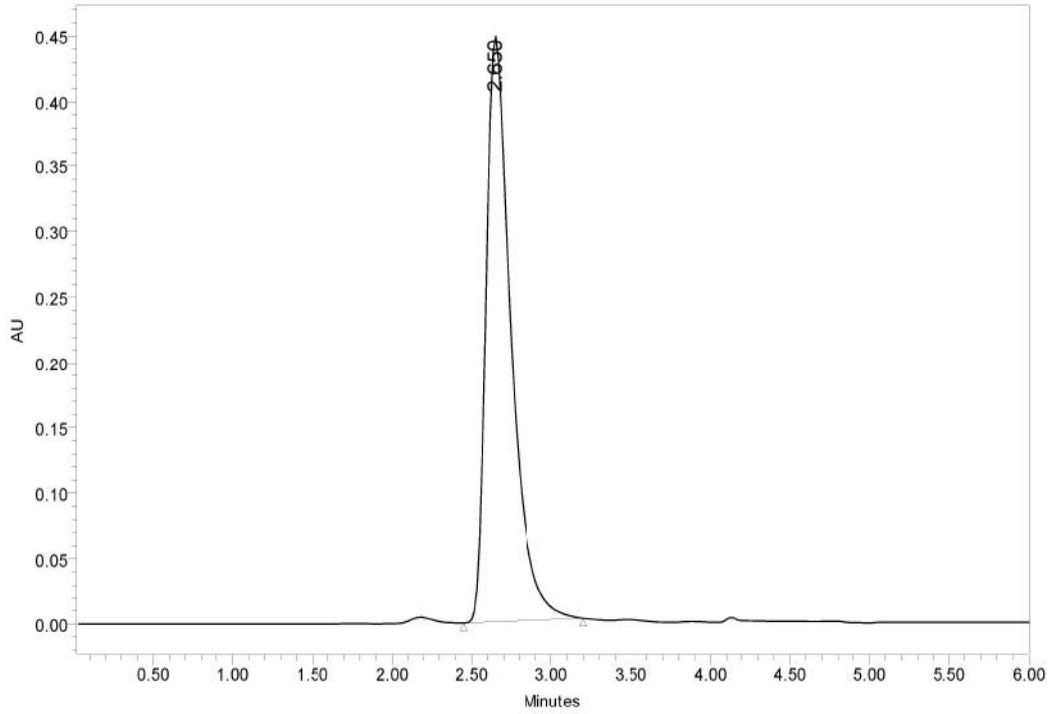
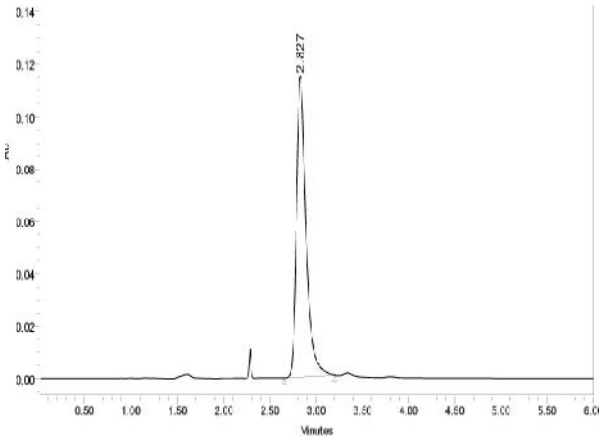
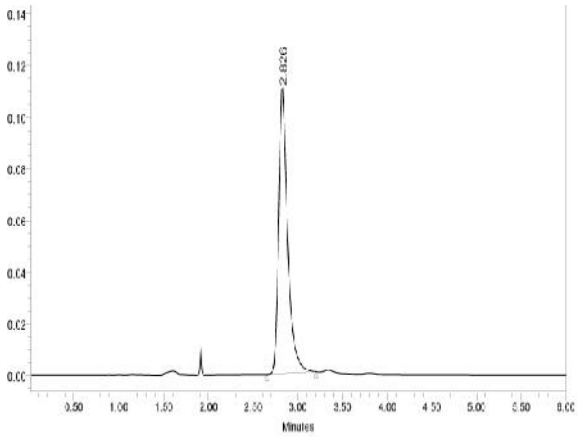


Figure 3: Acid degradation chromatogram



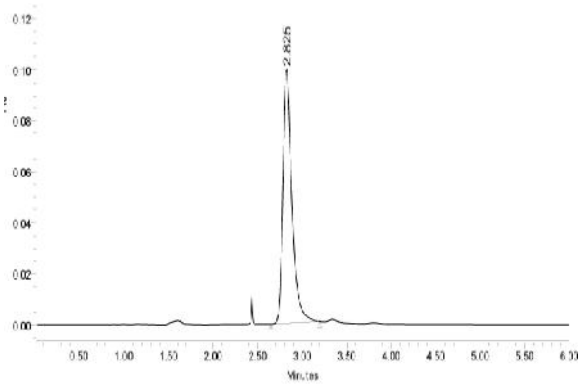
	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Purity Angle	Purity Threshold
1	aprepitant	2.827	830037	115447	3943.8	1.4	0.15	0.32

Figure 4: Base degradation chromatogram



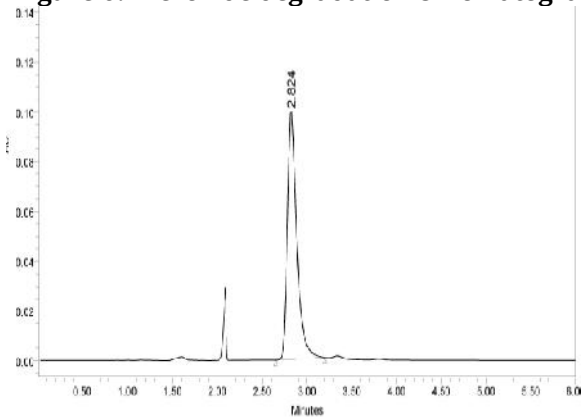
Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Purity Angle	Purity Threshold
aprepitant	2.826	812157	112964	3848.5	1.4	0.24	0.33

Figure 5: Thermal degradation chromatogram



	Name	Retention time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Purity Angle	Purity Threshold
1	aprepitant	2.825	794337	110481	3946.8	1.4	0.19	0.64

Figure 6: Peroxide degradation chromatogram



	Name	Retention time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Purity Angle	Purity Threshold
1	aprepitant	2.824	768836	105517	3842.5	1.4	0.24	0.39

Conclusion

This study presents a simple and validated stability-indicating HPLC method for estimation of Aprepitant in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability indicating. The method could be applied with success even to the analysis of marketed products Aprepitant tablet formulation, as no interference was observed due to excipients or other components present.

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