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METHOD DEVELOPMENT AND VALIDATION OF ATAZANAVIR IN PHARMACEUTICAL FORMULATION BY RP-HPLC METHOD

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ABSTRACT

Present work describes method for determination of Atazanavir in solid dosage form. The estimation was carried out on a Waters ODS (C_{18}) RP Column, 250 mm x 4.6 mm.column with a mixture of Buffer (triethylamine & pH adjusted to 3.0 with glacial acetic acid) and Acetronitrile in a ratio of 80 : 20(v/v) as mobile phase. UV detection was performed at 212 nm. The method was validated for linearity, accuracy, precision, specificity, limits of detection and quantitation and robustness as per ICH norms. The developed and validated method was successfully used for indicating the stability of the drug and quantitative analysis of commercially available dosage form. The retention time was 3.1min. The flow rate was 1.0 mL min^{-1}. The calibration curve was linear in the range of 10-100 µg/ml, for Atazanavir (API) with correlation coefficient (r^2) of 0.998. The LOD and LOQ values were found to be 0.05 µg/ml and 0.15 µg/ml. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the estimation of Atazanavir in pure and in capsule dosage form.

Key words: Atazanavir, High performance liquid chromatography, Acetonitrile.

INTRODUCTION

Atazanavir belongs to the class antiretroviral drug. Chemical name is methyl N- [(1S)-1-{N'- [(2S, 3S)-2-hydroxy-3-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethylbutanamido]-4-phenylbutyl]-N'-{[4-(pyridin-2-y) phenyl]methyl}hydrazinecarbonyl}-2,2-dimethyl propyl] carbamate. This is the protease inhibitor (PI) class. It is not official in any of the pharmacopoeia. Literature survey revealed estimation of Atazanavir by several techniques such as estimation by HPLC [1,2], Determination of related substance in Atazanavir by UV [3-9] method shave been reported.

In this present study an attempt was made to develop rapid and economical RP-HPLC method for estimation of Atazanavir in pharmaceutical formulation with better sensitivity, precision and accuracy using C_{18} column.

MATERIALS AND METHODS

Atazanavir capsules (300mg) were procured from Emcure Pharmaceuticals, Pune, India. All chemicals and reagents used were of HPLC grade, MERCK company Mumbai. A High Performance Liquid Chromatographic System (HITACHI L2130with D Elite 2000 Software) with Analytical Column-Waters ODS (C_{18}) RP Column, 250 mm x 4.6 mm. was used for the analysis. The mobile phase constituted of phosphate buffer (pH 3.4): acetonitrile (80:20) and the flow rate 1.0ml/min. Detection was performed at 212nm.

Preparation Standard Stock Solutions

Stock solution was prepared by transferring 25 mg of Atazanavir in 25ml volumetric flask and diluted with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Sample preparation

Powder equivalent to 25 mg of Atazanavir was weighed and transfer into 25 ml volumetric flask, mobile was added to it and sonicate and filtered through whatmann filter paper, resulting solution was diluted to 8 mcg/ml was injected into HPLC system.

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Chromatographic conditions

Chromatographic separation was performed a tambient temperature on a reverse phase Waters ODS (C_{18}) RP Column, 250 mm x 4.6 mm. The mobile phase used in this analysis consists of a mixture of Buffer (triethylamine& pH adjusted to 3.0 with glacial acetic acid) and Acetronitrile in a ratio of 80 : 20. The mobile phase was filtered, degassed before use. The flow rate was adjusted to 1ml/min. the detector wavelength was set at 212nm. The injector volume of the standard and sample was 20µl.

Method Development

Different concentrations of mobile phases were tried for the separation and resolution. Individual drug solution was injected into column and elution pattern of all the drugs and resolution parameters were studied. In addition to this, UV spectra of individual drugs were recorded at the wavelength from 200 to 400nm and the response for optimization was compared. The choice of wavelength 212nm was considered satisfactory, permitting the detection of the drugs with adequate sensitivity (Table 5).

RESULTS AND DISCUSSION

Method Validation

System suitability

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, retention time (RT) were determined. The results are shown in (Table 1), it indicates good performance of system.

Linearity

Under the experimental conditions described above, linear calibration curves for the two drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the two drugs (y) v/s concentration (x). The linearity range of Atazanavir is 10-100µg/ml as showed in (Table 2), (Fig.2).

Accuracy

Accuracy of the method was determined by applying the proposed method to synthetic mixture containing known amount of each drug to 80%, 100%, and 120% of the label claim. The accuracy was then calculated as the percentage of analyse recovered by the assay. The results of the recovery analysis are enclosed under (Table 3).

Precision

The assay was carried out of two drugs using proposed method in six replicates. The value of relative standard deviation lie well within the limits, it indicates the sample repeatability of the method enclosed in Table (4).

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in flow rate by ± 0.1ml/min.

Variation in wavelength by ± 2nm.

Detection limit and Quantitation limit (LOD AND LOQ) :

The Detection Limit and Quantitation Limit can be calculated based on the Standard deviation of the response and the Slope. The results obtained are presented in Table (5).

Stability of Solution

Acid hydrolysis

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Hydrochloric acid was added &make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions). The results obtained are presented in Table (6).

Basic hydrolysis

An accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask. To which 0.1 N Sodium hydroxide was added &make up to the mark & kept for 24 hrs. from that 0.2 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions). The results obtained are presented in Table (6).

Method Application

The validated high performance liquid chromatography method was applied for determination Atazanavir. Market available capsule dosage form contains Atazanavir 300mg. weigh powder equivalent to 25mg of Atazanavir and transfer into 25ml volumetric flask, add 25ml of mobile phase .it was sonicated at 35^{o}c for 6min to dissolve completely. This solution was further diluted to get a solution having concentration of 8µg/ml Atazanavir. 20µl of this solution was injected into the chromatograph under the specified chromatographic conditions. The analyte peaks were identified by comparisons with those of respective standard for their retention time. The peak areas were used to calculate the drugs. The assay results, expressed as % of the label claim in table 7.

Table 1. System Performance for Atazanavir

Drug substances	Retention time	Tailing factor	No of plates
Atazanavir	3.1	1.5	2994

Table 2. Linearity for Atazanavir

Conc. in µg/ml	AUC n=6
0	0
10	2124588
20	3124586
30	4258963
40	5258639
50	6541239
60	7586931
70	8521364
80	9874561
90	10923785
100	11548793
Co-relation coefficient ($r^{2)}$	0.993

Table 3. Accuracy-%Recovery of each Analyte Atazanavir

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	16	20	99.18	Mean= 98.97667%
S_2 : 80 %	16	20	98.58	S.D. = 0.200083
S_3 : 80 %	16	20	99.20	% R.S.D.= 0.202152
S_4 : 100 %	20	20	99.67	Mean= 99.54%
S_5 : 100 %	20	20	99.74	S.D. = 0.33
S_6 : 100 %	20	20	99.21	% R.S.D.= 0.331525
S_7 : 120 %	24	20	99.12	Mean= 99.567%
S_8 : 120 %	24	20	99.85	S.D. = 0.33
S_9 : 120 %	24	20	99.98	% R.S.D. = 0.331159

Table 4. Average of triplicateAnalysis-Precision

HPLC Injection Replicates of Atazanavir	Area	Retention Time
Replicate – 1	452867	3.19
Replicate – 2	452667	3.19
Replicate – 3	452567	3.19
Replicate – 4	452867	3.19
Replicate – 5	452633	3.19
Average	452720.2	3.19
Standard Deviation	138.74869	0.00
% RSD	0.0306478	0.00

Table 5. LOD and LOQ of Atazanavir

1.	Limit of Detection concentration in ppm (mgL)	0.05
2.	Limit of Quantitation concentration in ppm (mgL)	0.15

Table 6. Results of force degradation studies of Atazanavir API

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	90.36	8.54	98.36
Basic Hydrolysis (0.I MNaOH)	24Hrs.	96.32	4.34	98.32

Table 7. Assay of Atazanavir Tablets

Brand name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Atazor (Emcure Pharmaceuticals Ltd.)	100	100.34 (±0.06)	100.34 (±0.49)

Figure 1. Calibrated graph of Atazanavir shows the linearity of Atazanavir

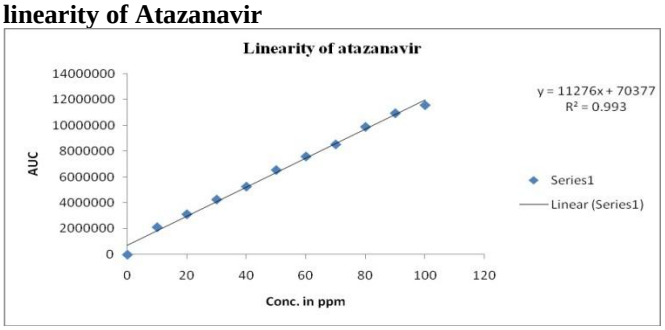
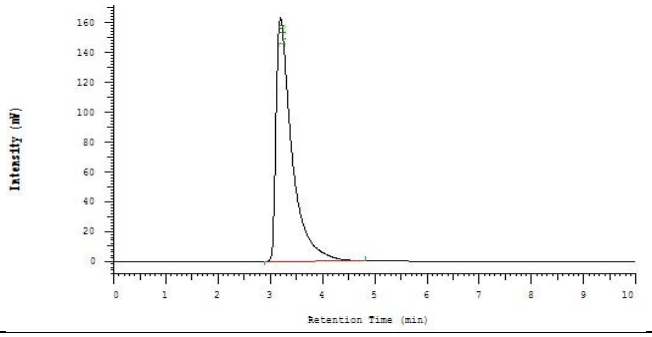


Figure 2. Chromatogram of Atazanavir



CONCLUSION

In this study a simple, fast and reliable HPLC method was developed and validated for the determination of Atazanavir in pharmaceutical formulations. As these proposed methods have the lowest LOD values and wider linear range is more sensitive method. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these methods were simple and inexpensive and these can be employed for the routine quality control of Atazanavir in pharmaceutical formulations.

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