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VALIDATED STABILITY INDICATING METHOD BY RP-HPLC FOR DETERMINATION OF ATAZANAVIR SULFATE IN BULK AND DOSAGE FORM

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ABSTRACT

In this study, a simple, precise and accurate RP-HPLC Method was developed for the estimation of atazanavir sulfate in bulk and pharmaceutical dosage forms. A reverse phase Hypersil BDS C_{18} column (150mm x 4.6 mm, 5µ) with mobile phase consisting of mixed buffer and acetonitrile in the ratio of 55:45% v/v was used. The flow rate was 1 mL/min and the system was monitored at 248 nm. The retention time was found to be 2.11 min. The linearity of the drug was obtained in the range of 25-150 µg/mL. The %Recovery value was found to be in between 98.0 % to 102.0 % and % RSD values were found to be less than 2.0 %.The results of analysis have been validated according to ICH guideline requirements.

INTRODUCTION:

Atazanavir sulfate (ATZ) is an inhibitor of the human immunodeficiency virus (HIV) protease. Atazanavir sulfate was the first protease inhibitor approved by the Food and Drug Administration (FDA). Protease inhibitors are mostly used in combination with atleast two other anti-HIV drugs. HIV-1 with advanced immunodeficiency, together with antiretroviral nucleoside analogues Atazanavir sulfate, an azapeptide HIV-I protease inhibitor selectively inhibits the virus- specific processing of viral Gag and gag-pol polyprotein in HIV-I infected cells, preventing formation of mature virions. Atazanavir sulfate exhibits anti HIV-I activity with mean EC50 in the absence of human serum of two to five nm against a variety of laboratory and clinical HIV-I isolates grown in peripheral blood mono nuclear cells, macrophages, CEM-SS cells, MT-2 cells.

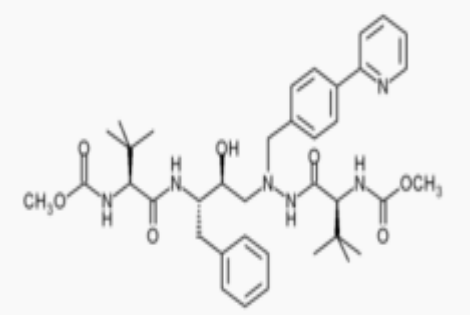

Fig.1: Structure of Atazanavir (ATZ)

MATERIALS AND METHODS:

Chemicals:

The reference standard Atazanavir sulfate was obtained from Matrix Ltd. Acetonitrile, sodium dihydrogen Orthophosphate, triethylamine were of HPLC grade, while potassium di hydrogen phosphate was of GR grade (Merck Ltd. Mumbai, India) milli-Q water was used throughout the analysis.

Instrument:

The liquid chromatographic system consisting of the following components was used for analysis. LC -10AT VP series model chromatograph equipped with a Hypersil BDS C_{18} (150mm x 4.6 mm, 5µ) was employed for the study. Sample Injection was done with a Rheodyne 7725 injection value via a 50 µL loop and the output signal was monitored and integrated by spinchrome software. Detection of the drug was done by using a SPD-20A UV-Visible detector.

Preparation of Buffer (0.01N NaH_2PO_4): Accurately weighed and transferred 1.36gm of sodium dihydrogen ortho phosphate into a 1000ml of volumetric flask, added about 900ml of milli-Q water. Sonicated for 5minutes to degas and finally made up the volume with water.

Preparation of Mobile phase: Mixed Buffer and Acetonitrile in the ratio of 55:45% v/v and sonicated for 5minutes to degas.

Preparation of Diluent: Mixed water and Acetonitrile in the ratio of (50:50% v/v) and sonicated to degas for 5minutes.

Preparation of Atazanavir sulfate Standard:

Accurately Weighed and transferred 10mg Atazanavir sulfate working Standard into a 10 ml clean dry volumetric flask, add 10ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pippetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Preparation of Atazanavir sulfate Sample:

Weighed 5 tablets of 200mg labelled claim and calculate the average weight of each tablet then sample equivalent to 500mg tablet was transferred into a 100ml volumetric flask, 20ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 2ml was pipette out into a 100 ml volumetric flask and made up to 100ml with diluent.

RESULTS AND DISCUSSION

Optimization of method: Equilibrate the column for at least 30 minutes with mobile phase flowing through the system with flow rate of 1.0ml/minute. Column temperature was monitored at 30°C and detection was set at a wavelength of 248nm with the optimized chromatographic conditions a steady base line was recorded. Separately inject appropriate aliquots (10µL) of diluent standard preparations and sample preparations into the chromatograph, record the chromatograms and measure the peak area responses for the major peak. Calculate the quantity in percentage of Atazanavir sulfate in the portion of Atazanavir sulfate tablets.

System suitability: According to USP, system suitability tests are an integral part of chromatographic method validation. The tests were used to verify whether the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution containing 100µg/ml of Atazanavir sulfate. 10 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the parameters like retention time (RT), plate number (N), peak area and peak asymmetry of sample were calculated these results are presented in the Table-1 for Atazanavir sulfate.

Linearity:

Preparation of Linearity Stock solution:

The linearity was established with a series of working standard solutions prepared by diluting the stock solution with mobile phase. A linear response of peak area was observed over the concentration range 25-150 µg/mL for Atazanavir sulfate respectively.

Table: 1: System Suitability of Atazanavir sulfate

Injection	Retention time	Peak Area	USP Plate Count	USP Tailing
1	2.11	589462	3180	1.10
2	2.11	592259	3185	1.11
3	2.11	589716	4059	1.12
4	2.14	585892	4215	1.10
5	2.12	588316	3254	0.96
6	2.15	587589	3458	1.11
Mean	2.12	588872	3559	1.08
SD	0.02	2161.84	-	-
%RSD	0.82	0.37	-	-

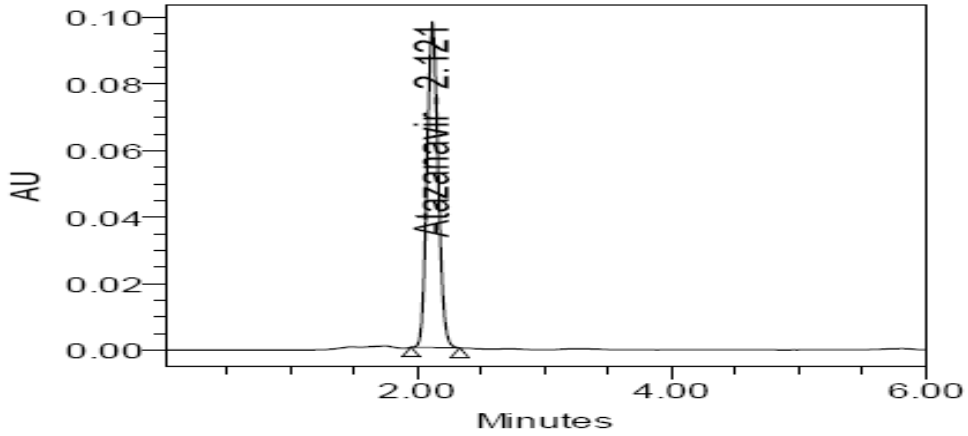

Fig 2: A typical chromatogram of Standard Atazanavir Sulfate

Table 2: Linearity data of Atazanavir sulfate

Linearity Conc. (µg/mL)	Peak Area	Average Area	SD	RSD
25	148146	145734	145734	1.55
	145403			
	143653			
50	279502	281792	281792	1.81
	287621			
	278253			
75	419721	420139	420139	1.14
	415577			
	425120			
100	578168	576419	576419	0.92
	570449			
	580639			
125	708285	713965	713965	0.81
	713762			
	719848			
150	852147	848693	848693	0.84
	853412			
	840520			

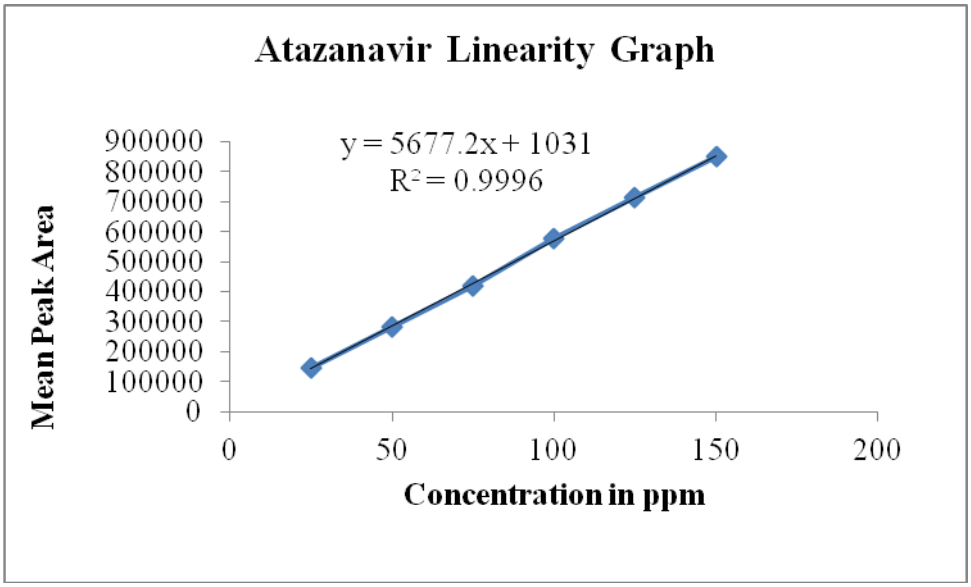

Table-3 Accuracy of Atazanavir sulfate

Amount added (µg/mL)	Amount found (µg/mL)	Peak area	% Recovery	Statistical Analysis of % recovery	
50	50.01	295926	100.02	MEAN	100.01
50	49.99	293395	99.98	SD	0.03
50	50.02	394718	100.04	%RSD	0.03
100	100.2	589493	100.02	MEAN	100.02
100	99.9	587728	100.01	SD	0.01
100	100.01	588375	100.02	%RSD	0.01
150	150.01	885982	100.007	MEAN	100.00
150	149.99	880710	99.993	SD	0.01
150	149.98	882651	99.987	%RSD	0.01

Intraday		
S. No.	**Peak Area**	**% Assay**
1	585224	99.0
2	596125	100.8
3	597973	101.1
4	595599	100.7
5	590384	99.9
6	588031	99.5
Average	592223	100.2
±SD	5091.68	0.83
RSD (%)	0.86	0.83

Interday		
S. No.	**Peak Area**	**% Assay**
1	592471	100.2
2	594817	100.6
3	592660	100.2
4	590030	99.8
5	592580	100.2
6	612593	103.6
Average	595859	100.8
±SD	8337.31	1.41
RSD (%)	1.40	1.40

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Drug	LOD µg/mL	LOQ µg/mL
Atazanavir sulfate	0.19	0.57

Chromatographic conditions		Atazanavir sulfate		
		% assay	Theoretical Plates	Asymmetry
Wave length (248nm±2nm)	246	99.59	3182	1.1
	250	99.80	3658	1.2
Flow rate (1.0ml/min ±0.1ml)	0.9	100.50	3574	1.1
	1.1	99.89	3248	1.1
Temperature (30°C ±2°C)	28°C	99.25	3687	1.2
	32°C	99.51	3524	1.1

Degradation data of Atazanavir sulfate:

Degradation conditions	Atazanavir sulfate			
	%Assay	% Degradation	Purity angle	Purity Threshold
Unstressed Sample	100.2	N/A	0.235	0.512
2N HCl at 60°C for 30min	96.92	3.28	0.276	0.405
2N NaoH at 60°C for 30min	97.68	2.52	0.208	0.262
Thermal 105°C for 48 h	99.15	1.05	0.190	0.280
UV Light at 254nm for 168 h	99.38	0.82	0277	0.405
3% H_2O_2at 60°C for 30 min	94.33	5.87	0.173	0.268

Calibrated curves were constructed by plotting average peak area Vs respective concentration of Atazanavir sulfate. Keeping the values to the straight line equation of calibration curve, quantification was carried out. The linearity data and calibration curves of Atazanavir sulfate are presented in table-2.

Accuracy:

To determine the accuracy of the proposed method, different amounts of bulk sample of Atazanavir sulfate within linearity limits were taken and analysed by the proposed method. The results are present in Table-3. Accuracy for Atazanavir sulfate was conducted by spiking the drug to the placebo powder at three different levels of the target concentration (i.e. 50%, 100%, and 150%) and each level three times. The mean % Recovery and % RSD values were calculated. The %Recovery value was found to be in between 98.0 % to 102.0 % and % RSD values were found to be less than 2.0 %.

Precision:

The ICH documents recommended that repeatability should be assessed by using six replicate measurements/injections of standard preparation were analysed for intra and inter day variations and the average % assay was found to be 100.2 % and 100.8. The results were interpreted by statistical analysis by calculating % RSD values which are within the acceptance criteria of not more than 2 % and the results are tabulated in the Table:4

Robustness:

Robustness of the method was checked by small deliberate changes made in the method parameters such as wavelength (±2nm) flow rate (±0.1 mL) and temperature (30°C ±2°C) but these changes did not affect the method results. The results are presented in Table: 5

Limit of Detection (LOD) and Limit of Quantification (LOQ):

A study to establish the limit of detection and limit of quantification was conducted. Limit of detection and Limit and quantification were established based on signal to noise ratio. A series of dilutions of the test solution were injected. Limit of detection was established by identifying the concentration which gives signal to noise ratio of about 3. Limit of quantification was established by identifying the concentration

which gives signal to noise ratio of about 10.The results of the LOQ and LOD are given in table.

CONCLUSION:

The method developed for Atazanavir sulfate was found to be simple, precise, sensitive, rapid, robust and economical. The analytical conditions developed with good resolution within a short analysis time. The %RSD for all parameters was found to be within the limit. This indicates the result and assay obtained by this method are in good agreement. Thus the method developed can be used for the routine analysis of Atazanavir sulfate in laboratories and quality control purpose.

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