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Analytical Method Development and Validation for Estimation of Atazanavir Sulfate in Bulk and Dosage Form by Stability Indicating RP-HPLC

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ABSTRACT :

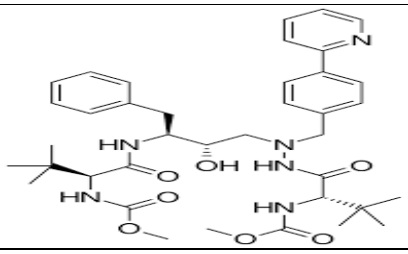
Atazanavir sulfate was effectively separated using the Agilent C18 Column (250 x 25 mm) 5µm, a flow rate of 1 ml/min, a mobile phase ratio of Methanol: Acetonitrile: Buffer (45:35:20 v/v), and a detection wavelength of 249 nm. Using a mobile phase and the Systronics 1170 apparatus with UV Win software, the spectroscopic technique was carried out in solvent. Agilent HPLC, the Uv-Vis detector, the separation module 2695, and Empower software version 2 were the instruments utilized. 3.73 min was determined to be the retention time. Atazanavir sulfate's system suitability characteristics, including theoretical plates and tailing factor, were determined to be 6754 and 1.62, respectively. In compliance with ICH criteria, the analytical procedure was verified (ICH, Q2 (R1))[1.] Using an internal standard, the linearity investigation of atazanavir sulfate revealed that its concentration ranged from 25 µg to 150 µg, with a correlation coefficient (r2) of 0.999 and a recovery percentage of 100.5%, respectively. The precision and repeatability RSDs were found to be less than 2. For atazanavir sulfate, the LOD value was 50 ng/mL and the LOQ value was 140 ng/mL.

Keywords: Atazanavir sulfate, HPLC.

INTRODUCTION:

Atazanavir sulfate, chemically known as 3,12-bis(1,1-dimethylethyl) 8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-((4-(2-pyridinyl)phenyl) methyl)-, dimethyl ester [1] Anazapeptide HIV-1 protease inhibitor (PI) is atazanavir2 (ATV). has anti-Human Immunodeficiency Virus Type 1 (HIV-1) activities. The proteolytic cleavage of the viral polyprotein precursors into the distinct functional proteins present in infectious HIV-1 is dependent on the enzyme HIV-1 protease. By attaching itself to the protease's active site, atazanavir stops the enzyme's function. This inhibition stops the viral polyproteins from cleaving, which leads to the creation of viral particles that are immature and not contagious. By attaching itself to the HIV active site, atazanavir specifically prevents the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1-infected cells.[3]Atazanavir does not work against HIV-2.

DRUG PROFILE :

Particulars	Description
Drug name	**ATAZANAVIR**
Molecular structure	
Molecular formula	**C38H54N6O11S**
Molecular weight	**802.9 g/mol**
IUPAC Name	**methyl N-[(2S)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2S)-2-**

	(methoxycarbonylamino)-3,3-dimethylbutanoyl]amino]-4-phenylbutyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate;sulfuric acid
Solubility	**limited solubility in water, typically around 4-5 mg/mL at a pH of 1.9**
Category	**antiretroviral (protease inhibitor (PI) class)**
Mechanism of Action	**HIV-1 Protease Inhibition** **• Binds to HIV-1 protease active site.** **• Prevents mature virions formation.**

II. MATERIALS AND METHOD :

Apparatus :	• Agilent HPLC • Separation module 2695 • Uv-Vis detector • Empower-software version-2
Reagents and Materials :	Solvents Used: Methanol, Acetonitrile, Sodium dihydrogenortho phosphate, Disodium hydrogen ortho phosphate, Tri Ethyl Amine, Ortho phosphoric acid, HPLC Water
Selection of chromatographic condition:	• Selection depends on sample nature, molecular weight, and solubility. • Polar drug selected for reversed phase or ion-pair or ion exchange chromatography. • Reversed phase HPLC chosen due to simplicity and suitability.
Selection of detection wavelength:	Uv-Vis Detection Method Selection • Sensitivity depends on wavelength selection. • Ideal wavelength maximizes absorbance and response. • Both drugs to be detected are detected. Atazanavir Sulfate Spectrometry Study • Standard solution scanned in UV range (200-400nm). • Spectrum recorded. • 249 nm selected as detection wavelength
Selection of mobile phase :	Mobile Phase Optimization • Initial phases: methanol and water, methanol and Acetonitrile, buffer and water. • Final phase: Methanol: Acetonitrile: Sodium dihydrogenortho phosphate buffer, 45:35:20 v/v.

III. Chromatographic condition (Optimized Method)

Coloumn	Agilent (25×250mm) 5µ
Ratio of Mobile phase	Methanol: ACN: Phosphate
Wavelength detection	249 nm
Flow rate	1.0 ml/min
Coloumn temperature	Ambient
Injection volume	10µl
Run time	10min
Retention time	3.73 min
Auto Sampler temperature	Ambient

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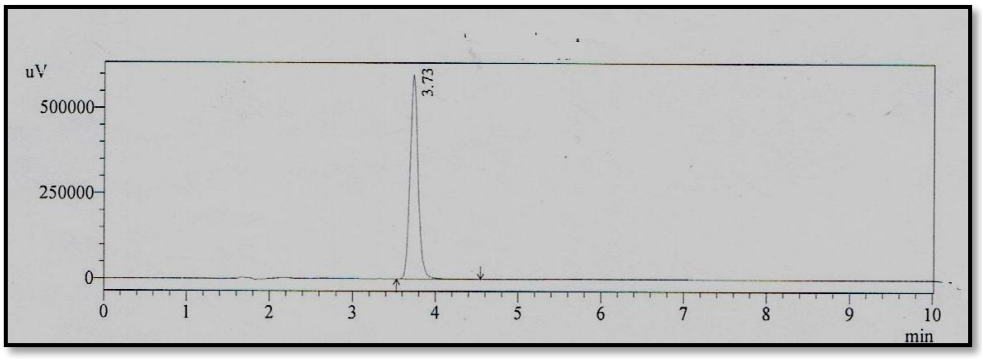

Fig.1: Chromatogram of Atazanavir sulfate

Procedure :

Buffer Preparation :

Sodium Dihydrogen Orthophosphate Preparation

* Dissolved in HPLC grade water.

* Adjusted pH 4.0 with Orthophosphoric acid.

* Filtered through 0.45µm nylon membrane.

* Degassed with sonicator.

* Used as diluent for sample and standard solution preparation.

Mobile phase preparation :

Mixing Process:

* 200ml Phosphate buffer (20%), 450ml Methanol (45%), 350ml Acetonitrile (35%).

* Degassed in ultrasonic water bath for 5 minutes.

* Filtered through 0.22 µ filter under vacuum filtration.

Preparation of Atazanavir sulfate standard preparation:

Atazanavir Sulfate Working Standard Distillation

* Accurately weigh 10mg of Atazanavir sulfate.

* Transfer to 10ml clean, dry volumetric flask.

* Add 2ml of diluent and sonicate.

* Make volume up to mark with same solvent.

* Pipette out 1.0ml from stock solution.

* Dilute with diluent up to mark.

Sample solution preparation :

Atazanavir Sulfate Tablet Preparation

* Accurately weighed 10 mg of Atazanavir sulfate tablet powder.

* Transferred to 10ml clean, dry flask.

* Add 2ml of diluent and sonicated.

* Volumetrically diluted to the mark with the same solvent.

* Pipetted into 100ml flask and diluted with diluent.

IV.RESULTS AND DISCUSSION :

- **Method Validation Parameters**

1. Specificity :

System Specificity Test

* Determined interference of impurities in analytical peak retention time.

* Specificity performed via Injecting blank.

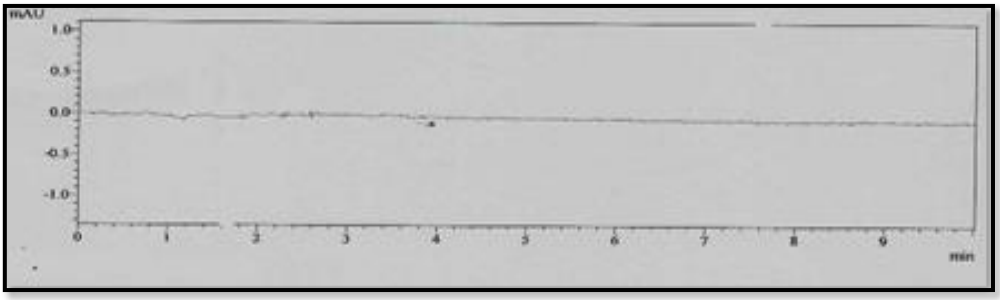

Fig.2: Chromatogram of blank

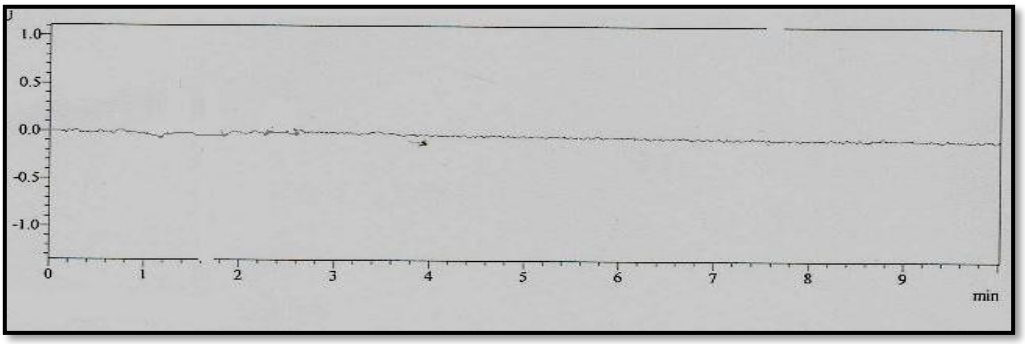

Fig.3: Chromatogram of Sample

1. Linearity :

Analytical Method Linearity and Atazanavir Sulfate

• Linearity elicits test results directly or mathematically.

• Test results proportional to analyte concentration in samples.

• Serial dilutions of Atazanavir sulfate detected at 249 nm wavelength.

• Calibration curve obtained by plotting concentration vs. peak area.

Acceptance criteria:

Correlation Coefficient must be less than 0.999.

2. Range :

- The assay method for Atazanavir sulfate is found to be precise, linear, and accurate within the range of 20-200 µg/ml, according to the data analysis.

3. Accuracy :

Assay Method for Atazanavir Sulfate Precise, linear, accurate. Range: 20-200 µg/ml.

4. Standard addition method :

Formulation Process:

• Addition of reference standard of known drug concentration.

• HPLC analysis.

• Comparison with standard drug concentration.

5. Percentage method :

Sample Preparation Method Preparation in 50%, 100%, 15% concentrations.

Acceptance criteria:

Atazanavir Sulfate Recovery

• Mean recovery: 95.0%-105.0% at each level.

6. Assay procedure :

Chromatographic System Analysis

• Injection of blank, standard, and sample.

• Atazanavir sulfate peak used for % assay calculation.

7. Precision:

Method Precision Demonstration

• Indicates consistent results for single batch.

• Demonstrated by preparing six test solutions at 100% concentration.

• Recorded chromatograms of six solutions.

Calculated % RSD of peak areas in six samples.

• Precision performed on Atazanavir sulfate formulation.

Acceptance criteria :

Sample Injection Results Ensure %RSD <2.

• Limit area of six injections.

Selection of solvent :

Drug Detection Process

• Dissolved drug in mobile phase for 10µg/ml concentration.

• Scanned solution in U.V range from 200-400 nm.

• Overlay spectrum of Atazanavir sulfate obtained.

• Isobestic point showed absorbance's maxima at 249 nm.

VALIDATION OF THE METHOD :

Linearity :

Atazanavir sulfate:

Atazanavir sulfate Dilution and Calibration

• Serial dilutions injected into column.

• Detection at 249 nm wavelength.

• Calibration curve plotted.

• Correlation coefficient found at 0.999.

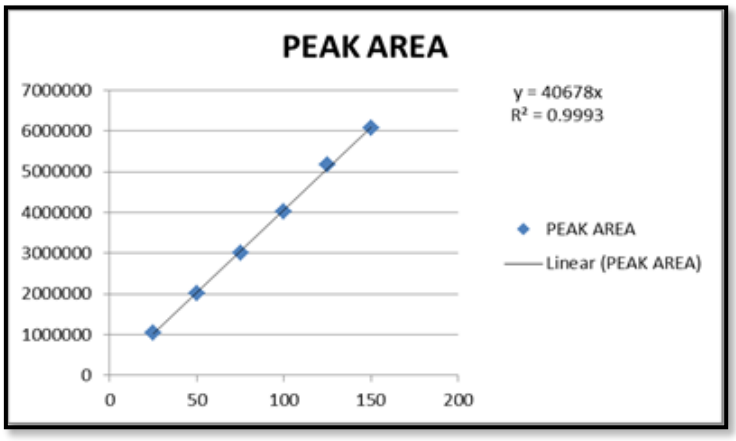

Fig.4: Calibration graph of Atazanavir sulphate.

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Table 1: Calibration data of Atazanavir sulphate :

Level	Conc(ug/ml)	Peak Area
Level-1	25	1053328
Level-2	50	2022943
Level-3	75	3019848
Level-4	100	4024196
Level-5	125	5167667
Level-6	150	6074920

- **Recovery studies :**

Proposed Method Requirements and Recovery Studies

• Carried out to ensure method suitability and reliability.

• Addition of standard Atazanavir sulfate at 50%, 100%, 150% levels.

• Contents re-analyzed using proposed method.

Table 2: Accuracy results for Atazanavir sulphate:

S.No	Concentration	Measured Concentration (mcg) ± S.D (n=3)
1.	0.1	0.09±0.05
2.	1	0.99±0.04
3.	10	9.99±0.11

Table 3 : Assay results for Atazanavir sulphate:

Formulation	Labeled amount(mg)	Mean S.D(amount recovered) (n=3)	%RSD	Mean S.D (% of recovery)	%RSD
Virataz	300	301.5±0.36	0.05	100.5±0.44	0.46

Table 4 : Recovery results for Atazanavir sulphate:

Concentration of specific level	Peak Response	Average Peak area	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	5613787	5616407	300	305.1	101.70	99.7%
	5610866					
	5624568					
100%	9928627	9924076	400	397.12	99.28	
	9915165					
	9928437					
150%	15384338	15340585	500	504.05	100.81	
	15288620					
	15348798					

- **Precision**

Table 5 : Results of Precision for Atazanavir sulphate :

S.No.	Concentration mcg/ml	Measured Concentration(mcg/ml)□S.D(n=3)	
		Intra day	Interday
1	0.1	0.099□0.0005	0.099□0.0005
2	1	0.99□0.005	0.99□0.005
3	10	9.99□0.005	9.99□0.005

- **LOD and LOQ**

Table 6 : LOD and LOQ for Atazanavir sulphate:

Drug name	LOD	LOQ
Atazanavir sulfate	50ng/mL	140ng/mL

1. Stress Degradation :

Atazanavir Sulfate Degradation Assay Method

• Utilized water methanol phases to separate Atazanavir sulfate and stressed degradation products.

• Achieved a rapid, simple assay method with a reasonable run time, suitable retention time, and sharp peak sharpness.

• Mobile phase system of Methanol: Acetonitrile: Sodium dihydrogen ortho phosphate buffer (45:35:20) achieved satisfactory resolution.

• Atazanavir sulfate showed maximum absorption at 249 nm, setting detector at 249 nm.

• Chromatogram of Atazanavir sulfate showed a single peak of around 3.7 minutes.

- "ICH Guideline on Stability Testing of New Drugs"

- Stress testing is needed to determine the stability of active substances and hydrolytic stabilities. HPLC studies and chromatograms of degradation products reveal drug degradation behavior.

- **Drug Chromatography Conditions**

1. Acidic Condition: Atazanavir sulfate solution heated with 1N HCl for 4 hours, causing parent peak disappearance and new chromatogram signals.

2.Alkali Degradation: Atazanavir sulfate solution exposed to basic hydrolysis for 4 hours, causing parent peak disappearance and new peaks.

3.Oxidative Conditions: Nelfinavir solution exposed to chemical oxidation with H2O2 for 4 hours, causing complete chromatographic peak disappearance and new signals.

Table 7 :Degradation details Atazanavir sulphate at Room Temperature:

Condition	**Time**	**% Degradation**	**R_t values of degradation products**
Acid, 1 N HCl	48hr	24	3.1,3.5,3.9
Base,1NNaOH	48hr	64	3.2,3.8
H_2O_2, 30%	48hr	100	3.5,3.9,4.2,4.5

Table 8 : Degradation details Atazanavir sulphate at 60^0C :

Condition	Time	% Degradation	R_t values of degradation products
Acid,1NHCl,(60 ^oC)	48hr	100	2.9,3.9,4.0
Base,1NNaOH,(60^oC)	48hr	100	3.3,3.5,4.1,4.7
H_2O_2, 30%, (60 ^oC)	48hr	100	3.1,3.4,4.2,4.9,5.1.

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Table 9: Stress induced stability studies of Atazanavir sulphate at Room temperature:

Time in hours	O.1NHCl		O.1NNaOH		O.3%H2O2	
	Amt.found	%ofdeg	Amt.found	%of deg	Amt.found	%of deg
0	9.97	0.2	9.57	4.1	9.53	4.2
2	9.66	3.7	9.02	9.4	8.76	12.8
4	9.10	8.9	8.62	13.8	8.08	19.3
8	8.53	14.2	7.49	25.3	6.70	32.5
24	7.82	21.6	5.09	48.4	5.55	64.1
48	6.59	24	3.56	64	0	100

Table 10 : Stress induced stability studies of Atazanavir at elevated Temperature(60^{0}C):

Time in hours	O.1NHCl		O.1NNaOH		O.3%H2O2	
	Amt.found	%of deg	Amt.found	%of deg	Amt.found	%of deg
0	9.97	0.2	9.77	3.2	9.52	4.2
2	9.75	3.7	8.74	12.2	9.36	12.8
4	8.62	12.7	7.62	23.4	6.89	29.3
8	6.97	29.3	5.89	41	4.61	52.5
24	3.86	61.3	2.18	78.6	1.58	84.1
48	0	100	0	100	0	100

Initial analyte concentration=10µg/ml

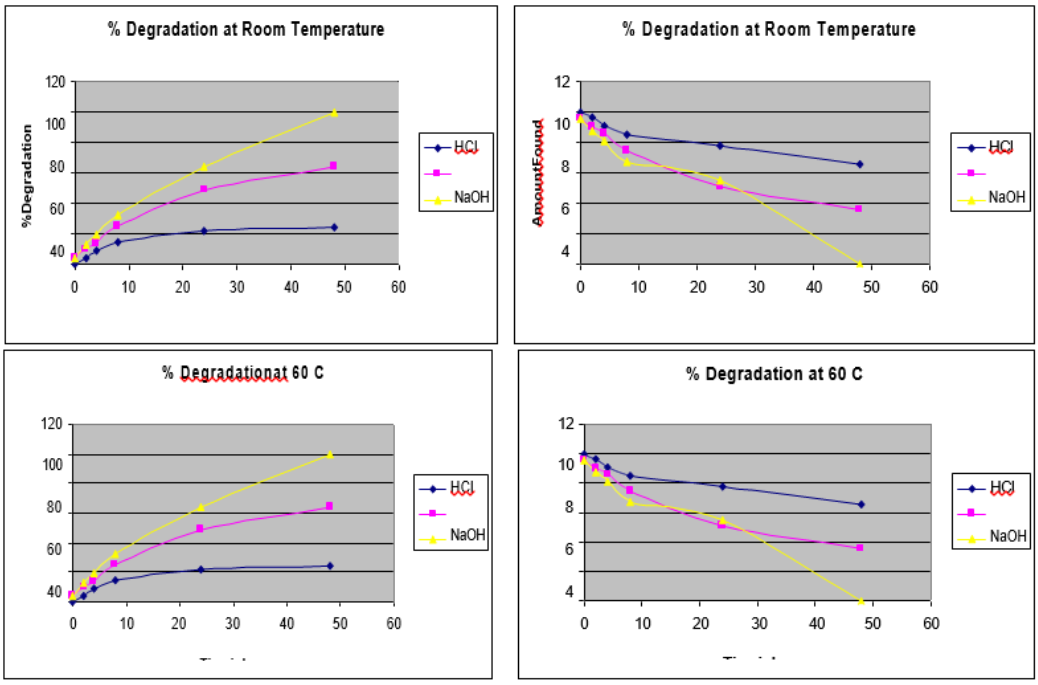

Fig 5: Degradation Pattern of Atazanavir sulphate

SUMMARY AND CONCLUSION:

A new RP-HPLC method was developed for estimating Atazanavir sulfate in pharmaceutical dosage form. The method was simple, specific, precise, accurate, rapid, and economical. It was validated for accuracy, precision, linearity, robustness, and ruggedness, and results will be statistically validated according to ICH guidelines. The RP-HPLC method is suggested for routine analysis of Atazanavir sulfate in API and Pharmaceutical dosage form.

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