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Der Pharmacia Lettre, 2015, 7 (12):271-278
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ISSN 0975-5071
USA CODEN: DPLEB4

Stability indicating method development and validation for the estimation of atazanavir sulfate in pharmaceutical dosage forms by RP-HPLC

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ABSTRACT

A simple, precise, accurate, stability indicating method was developed for the estimation of Atazanavir sulfate in bulk drug and pharmaceutical dosage form by RP-HPLC. The separation was done using inertsil C-18 (250mm × 4.6mm, 5µ) column at a flow rate of 1mL/min with a column temperature of 30ºC on an isocratic mode. The detection wavelength used is 248nm. The mobile phase used is potassium dihydrogen phosphate (0.01N) and acetonitrile in the ratio of 55:45 (v/v). The performance of the method was validated according to the ICH guidelines for specificity, linearity, accuracy, precision, ruggedness and robustness. The retention time for Atazanavir sulfate was found to be 2.56mins. A good linear response was observed in the concentration range of 25µg/mL to 150µg/mL, with a correlation coefficient of 0.999. Atazanavir sulfate was subjected to stress conditions including acidic, alkaline, oxidation, neutral degradation, thermal degradation and photolysis, and the net degradation was found to be within the limits.

Keywords: Atazanavir sulfate, Stability indicating method, RP-HPLC, Method validation, Inertsil.

INTRODUCTION

Atazanavir sulfate (Figure 1) is chemically designated as (3S, 8S, 9S, 12S)-3, 12-Bis (1, 1-dimethyl ethyl)-8-hydroxy-4, 11-dioxo-9-(phenyl methyl)-6-[[4-(2-pyridinyl) phenyl] methyl]-2, 5, 6, 10, 13 penta azatetradecanedioic acid dimethyl ester, sulfate (1:1). It is an antiretroviral drug of the protease inhibitor class. It is used to treat the infection of human immunodeficiency virus (HIV) [1, 2]. According to the literature survey, it was found that few analytical methods like RP-HPLC method [3-8] and Spectrophotometric method [9-11] were developed for the determination of Atazanavir sulfate in bulk drug and pharmaceutical dosage forms. Hence, there is a need to develop an analytical method to estimate the drug. The objective of the proposed method was to develop and validate stability indicating method for the estimation of Atazanavir sulfate in Pharmaceutical dosage forms by RP-HPLC.

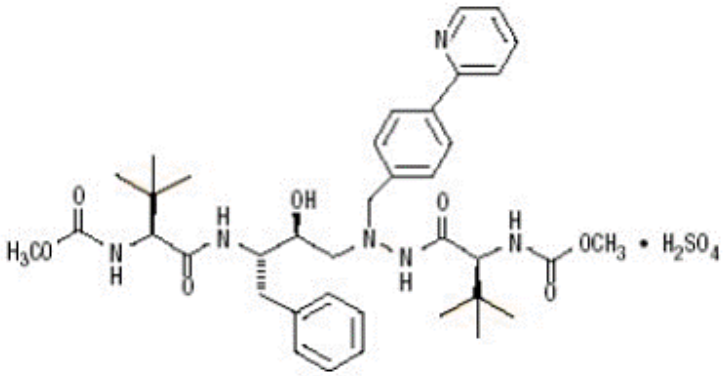

Figure 1: Chemical structure of Atazanavir sulfate

MATERIALS AND METHODS

Reagents and Chemicals: Atazanavir sulfate working standard was procured as gift sample. Atazanavir sulfate capsules 100mg were purchased from local pharmacies. Purified water was obtained from Millipore system. Acetonitrile (HPLC grade) was obtained from E-Merck. All other chemicals used in the analysis were of AR grade.

Instrumental and Analytical conditions: The HPLC analysis was performed using a Waters 2998 model equipped with an autosampler, PDA detector and running on empower software. Column used was Inertsil C-18 (250mm × 4.6mm, 5µ). UV detection was performed at 248nm. The injection volume of sample was 10µL. An isocratic mobile phase containing potassium dihydrogen phosphate buffer (0.01N) and acetonitrile in the ratio 55:45(v/v) was carried out with the flow rate of 1mL/min. Column was maintained at 30ºC.

Preparation of Buffer: (0.01N KH_2PO_4)
1.36gms of KH_2PO_4 was taken in a 1000mL of Volumetric flask add about 100mL of milli-Q water and final volume make up to 1000mL with milli-Q water.

Preparation of Mobile phase:
Mixture of Buffer and Acetonitrile in the ratio 55:45 (v/v) respectively

Preparation of Diluent:
Mixture of Water and Acetonitrile in the ratio 50:50 (v/v) respectively

Preparation of standard solution: 25mg of Atazanavir sulphate working standard was accurately weighed and transferred into a 25mL volumetric flask. 15mL of diluent was added, sonicated to dissolve and make up to final volume with diluent. From the above standard stock solution, 1mL was pipetted into a 10mL volumetric flask and the volume was made upto mark with diluent.

Preparation of sample solution: 20 Capsules (Reyataz) were weighed accurately and the average weight was calculated. Capsules were opened and fine powder was collected. An amount equivalent to 25mg of Atazanavir sulphate was weighed and transferred into 25mL volumetric flask. 15mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was filtered using HPLC filters. 1mL of the above solution was pipette into 10mL volumetric flask and made up with diluent.

METHOD DEVELOPMENT
Inertsil C-18 column (250mm × 4.6mm, 5µ) as stationary phase with a mobile phase of Potassium dihydrogen phosphate (0.01N) and acetonitrile (55: 45v/v) at a flow rate 1mL/min and a detection wavelength of 248nm afforded the best separation of drug. The standards solution and sample solution prepared as above were injected into the 10µL loop and the chromatograms were recorded as shown in the

figure 2, 3 and 4 respectively. The retention time of drug was found to be 2.56min. The % Assay of Atazanavir sulphate was calculated.

Calculation

The % Assay was calculated by comparing the peak area of the sample and standard.

% Assay of Atazanavir sulphate =

$$\frac{\text{Sample peak area} \times \text{Weight of standard} \times \text{Dilution of sample} \times \text{Average weight} \times \text{Purity} \times 100}{\text{Standard peak area} \times \text{Weight of sample} \times \text{Dilution of standard} \times \text{Label claim} \times 100}$$

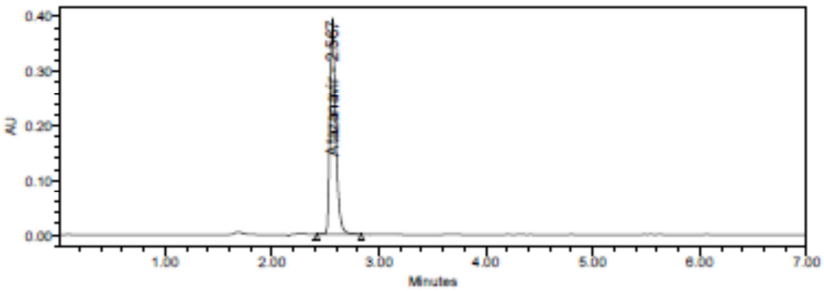

Figure 2: Standard chromatogram

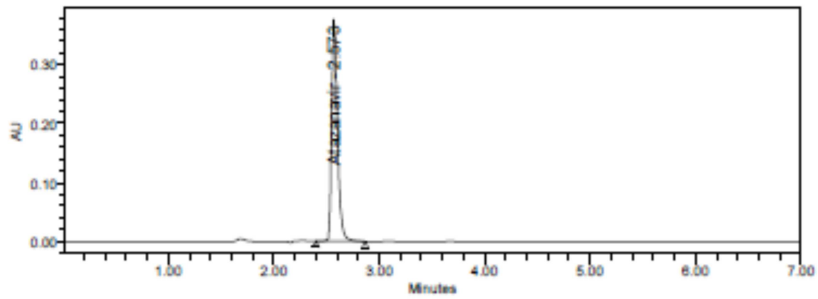

Figure 3: Sample chromatogram

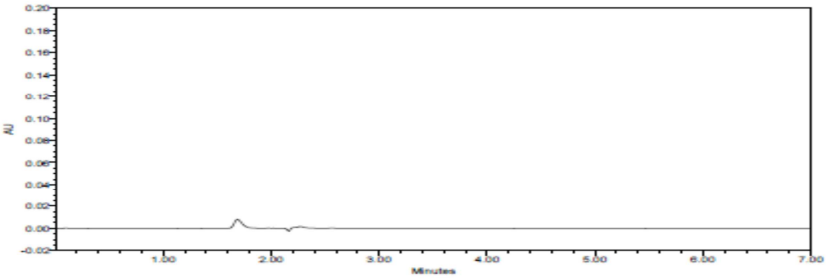

Figure 4: Blank chromatogram

METHOD VALIDATION [12]

SPECIFICITY: (Placebo interference)

The study of placebo interference from excipients was conducted. Placebo interference was checked for two strengths in duplicate, equivalent to about the weight of placebo as per the test method. The results were summarized in table 2 and chromatogram was recorded as shown in figure 6.

ACCURACY: (Recovery)

To determine the accuracy of the test method samples were prepared by spiking Atazanavir sulphate raw material with the equivalent amount of placebo at 50%, 100% and 150% of the target concentration.

Samples were prepared at each concentration levels in triplicate. The average % recovery of Atazanavir sulphate was determined. The results were summarized in table 2.

PRECISION:
Precision of the test method was determined by injecting six samples of standard Atazanavir sulphate solution. The %RSD was determined. The results were summarized in table 2.

LINEARITY:
A series of Standard solutions were prepared and injected into the HPLC system. A graph was plotted to "concentration" versus "peak area" in linearity section. The results and graph were summarized in table 2 and figure 7.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):
For determining LOD and LOQ, initially standard deviation and slope of calibration curve was calculated. Then by using these values as per formula maintained in ICH guideline these parameters were evaluated. The results were summarized in table 2.

RUGGEDNESS: (Analyst to analyst/ System to system variability)
Ruggedness of assay method was conducted on Atazanavir sulphate capsules using two different systems by different analysts and analyzed under similar conditions as per the test method. The results were summarized in table 2.

ROBUSTNESS:
Standard solution was prepared as per the test method, injected into HPLC system and analyzed using varied mobile phase composition (±10% of actual organic phase composition), flow rate (±0.2mL/min of actual flow rate) and column oven temperature (±5^{0}C of actual column oven temperature). The system suitability parameters were evaluated as per the test method. The results were summarized in table 2.

FORCED DEGRADATION STUDIES:
Acid stress study:
To 1mL of Atazanavir sulphate stock solution, 1mL of 2N hydrochloric acid was added and refluxed for 30mins at 60^{0}C. The resultant solution was diluted to obtain 100µg/mL solution and 10µL solution was injected into the chromatographic system and the chromatogram was recorded to assess the stability of sample as shown in figure 8a. The result was summarized in table 3.

Base stress study:
To 1mL of Atazanavir sulphate stock solution, 1mL of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}C. The resultant solution was diluted to obtain 100µg/mL solution and 10µL was injected into the chromatographic system and the chromatogram was recorded to assess the stability of sample as shown in figure 8b. The result was summarized in table 3.

Peroxide stress study:
To 1mL of Atazanavir sulphate stock solution, 1mL of 20% hydrogen peroxide (H_2O_2) was added. The solutions were kept for 30 min at 60^{0}C. For HPLC study, the resultant solution was diluted to obtain 100µg/mL solution and 10µL was injected into the system and the chromatogram was recorded to assess the stability of sample as shown in figure 8c. The result was summarized in table 3.

Dry heat exposure study:
The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/mL solution and 10µL was injected into the system and the chromatogram was recorded to assess the stability of the sample as shown in figure 8d. The result was summarized in table 3.

UV light exposure study:
The photochemical stability of the drug was also studied by exposing the drug solution to UV Light by keeping the beaker in UV Chamber for 7days or 200Watt hrs/m^2 in photo stability chamber. For HPLC study,

the resultant solution was diluted to obtain 100µg/mL solution and 10µL was injected into the system and the chromatogram was recorded to assess the stability of sample as shown in figure 8e. The result was summarized in table 3.

Water stress study:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 100µg/mL solution and 10µL were injected into the system and the chromatogram was recorded to assess the stability of the sample as shown in figure 8f. The result was summarized in table 3.

RESULTS

Selection of detection wavelength
From the UV spectrum, suitable wavelength considered for monitoring the drug was 248nm.

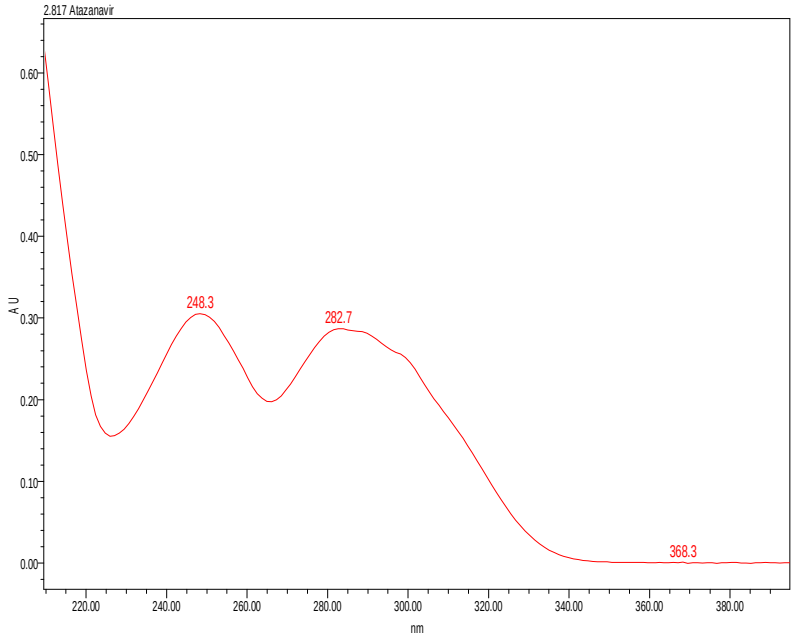

Figure 5: UV spectrum of Atazanavir sulphate

SYSTEM SUITABILITY:

Table 1: System suitability parameters

S.No.	System Suitability Parameter	Observed value	Acceptance criteria
1	% RSD	0.76	NMT 2.0
2	USP Tailing factor	1.31	NMT 2.0
3	USP Plate count	10112	NLT 2000

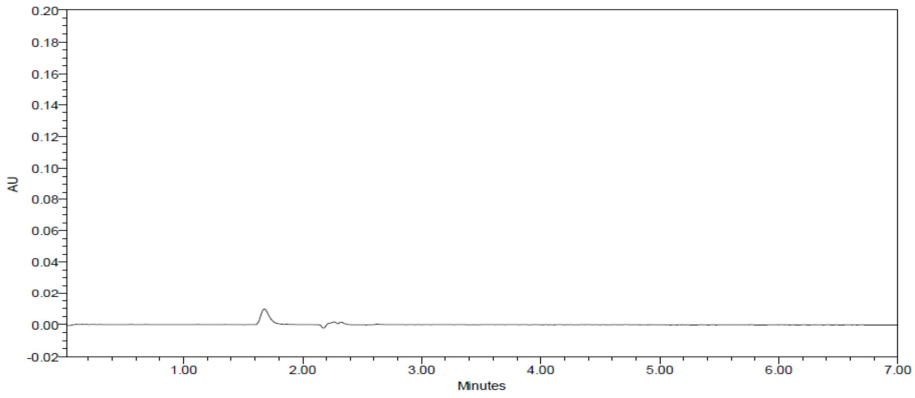

Figure 6: Placebo Chromatogram

Table 2: Results of Method Validation parameters

Parameter		Result
Specificity		Specific
Linearity	Concentration range, µg/mL	25- 150
	Regression equation, y=mx+c	y=15060x
	Slope, m	15060
	Correlation coefficient, r	0.999
Accuracy (%recovery) n=3	Level I, 50%	99.45
	Level II, 100%	99.70
	Level III, 150%	100.05
Precision (%RSD), n=6		0.86
Ruggedness (%RSD), n=6		< 2
Robustness (%RSD)	Variation in flow rate (±0.2mL/min)	< 2
	Variation in organic phase composition (±10%)	< 2
	Variation in column temperature (±5%)	< 2
Limit of Detection (LOD), µg/mL		4.39
Limit of Quantification (LOQ), µg/mL		13.32
Forced degradation studies (net degradation)		< 50%

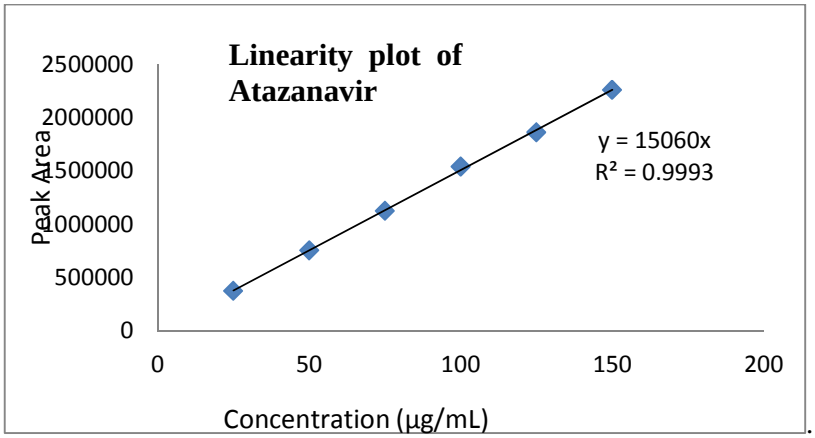

Figure 7: Linearity plot of Atazanavir sulphate

Table 3: Forced degradation studies

S.No.	Stress condition	% Assay	% area of degradation peak	Peak purity angle	Peak purity threshold
1	2N HCl for 30mins at 60°C	96.82	1.86	0.060	0.277
2	2N NaOH for 30mins at 60°C	97.82	1.95	0.060	0.277
3	20% H_2O_2 for 30mins at 60^0C	95.80	0.22	0.054	0.279
4	105^0C for 6hrs	99.43	-	0.054	0.280
5	UV light 200wts/hr or 7days	99.48	-	0.089	0.275
6	Water for 6hrs at 60^0C	99.56	-	0.093	0.287

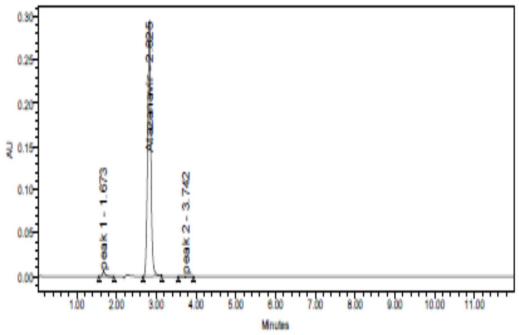

Figure 8a: Acid degradation chromatogram

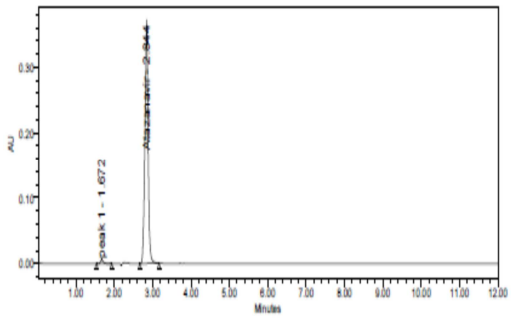

Figure 8b: Base degradation chromatogram

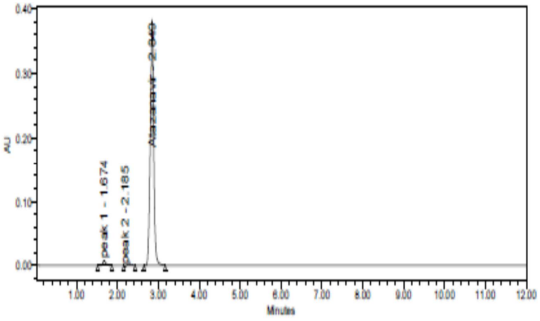

Figure 8c: Peroxide degradation chromatogram

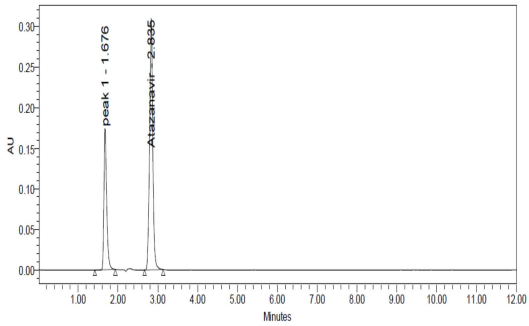

Figure 8d: Dry heat stress chromatogram

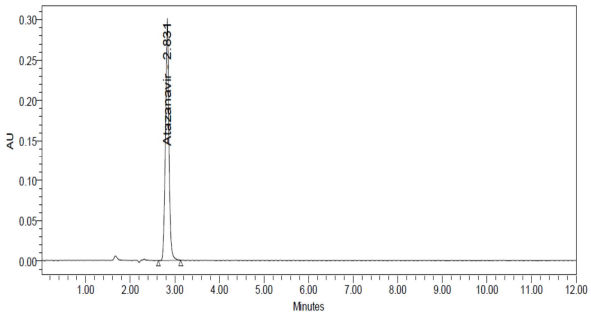

Figure 8e: UV degradation chromatogram

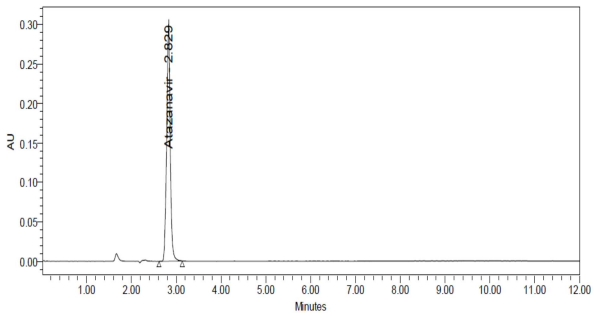

Figure 8f: Water Stress chromatogram

DISCUSSION

Initially, various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters (height, capacity, theoretical plates, tailing factor), run time and resolution.

The solution of 10ppm of Atazanavir sulphate in diluent (Acetonitrile: Water, 50:50) was prepared and the solution was scanned in the range of 200-400nm. At 248nm, the drug showed maximum absorbance and better detector response. After considering the entire system suitability parameters mobile phase potassium dihydrogen phosphate buffer (0.01N) and acetonitrile (55:45%v/v) run in isocratic mode and flow rate 1.0mL/min was selected. The retention time of Atazanavir sulphate was found to be 2.567min. The system suitability parameters are calculated.

The calibration was linear in concentration range of 25-150µg/mL, with correlation coefficient 0.999, indicates that the concentration of Atazanavir sulphate obeys Beer's – Lambert's law.

The method was found to be specific as there is no interference of placebo peak at the retention time of Atazanavir sulphate peak. Accuracy was confirmed by recovery studies by proposed method. The

percentage recovery of Atazanavir sulphate was found to be 99.45% - 100.05%. Hence the developed method was found to be accurate. To evaluate precision study, %RSD was calculated. The %RSD value was found to be 0.86. These results showed reproducibility of the assay.

The limit of detection and the limit of quantification were found to be 4.39µg/mL and 13.32µg/mL. To evaluate ruggedness study, %RSD was calculated and found to be less than 2. This indicates that the method is rugged. The values in the robustness evaluation study, indicated that the method was quite robust.

The stability of an analytical method was determined by forced degradation studies. The net degradation was found to be within the limits. Purity angle is found to be less than purity Threshold.

CONCLUSION

A simple, stability indicating method for the estimation of Atazanavir sulphate has been developed by RP-HPLC. The developed method was validated as per ICH guidelines. The proposed method shows good agreement with all validation parameters. The optimized method is precise, accurate, specific, rugged, robust and stable. A linear relation is observed between the concentration and the result. The developed method can be used for the analysis of routine quality control sample.

Acknowledgements
The authors are thankful to Mr. S. Pavan Raju, Research Associate, Sun Pharma Ltd., for providing the Atazanavir sulphate as gift sample.

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