Development and Validation of HPTLC Method for the Estimation of Atazanavir Sulphate in Bulk Drugs and Combined Dosage Form

Sujatha Kuppusamy*, Kavitha Karunakaran, Apurva Thambichetty, Dokka Manasa, Karuturi Bhargavi, Vanipriyanka Kagitala, Sridhanakiran
Department of Pharmaceutical chemistry, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, India.
*Corresponding author’s E-mail: ksujamano@yahoo.co.in

Accepted on: 05-02-2014; Finalized on: 31-03-2014.

ABSTRACT
A new, simple, rapid and accurate high performance thin layer chromatography method for the estimation of Atazanavir sulphate in bulk and combined dosage form was developed and validated. The chromatogram were developed using a mobile phase of n-hexane: ethyl acetate (30:70) on precoated plate of silica gel 60 F254 aluminum TLC plate (20X10 cm, layer thickness 0.2 mm) and quantified by densitometric absorbance mode at 281nm. The Rf value was found to be 0.48±0.02. The calibration curve was linear from 200-1400ng/band for Atazanavir with the regression coefficient (r²) of 0.9998. The limit of detection and quantification were 100 ng/band and 300 ng/ band. The method was validated for the precision, specificity and recovery as per the ICH guidelines. The proposed method can also be used for the routine analysis of Atazanavir in bulk and combined dosage form.

Keywords: Atazanavir, HPTLC, ICH guidelines, Validation.

INTRODUCTION
Atazanavir Sulphate is one of the oral antiretroviral protease inhibitors used for the treatment of HIV/AIDS. It is chemically (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[(4-(2-pyridinyl)phenyl)methyl]-2,5,6,10,13-pentaazatetrade anedioic acid dimethyl ester,1-[(4-pyridine-2yl)phenyl]-55,2,5-bis[[N-(methoxycarbonyl)-L-turtleucinyl]amino]-4S hydroxyl-6-phenyl-2-azahexane (figure 1). The molecular weight is 802.9 and the empirical formula is C₃₈H₃₂N₄O₇.H₂SO₄. Atazanavir sulphate is a white to pale yellow crystalline powder. It is slightly soluble in water. It is official in Indian Pharmacopoeia. Atazanavir is an azapeptide HIV-1 protease inhibitor. The compound selectively inhibits the virus specific processing of viral Gag and Gag-pol polyproteins in HIV-1 infected cells, thus preventing formation of mature virions.

Literature survey reveals Spectrophotometric and HPLC methods for the estimation of Atazanavir in bulk and combined dosage form. No HPTLC method so far has been reported for its analysis in combined dosage form. We herein report for the first time a new validated HPTLC method for the determination of Atazanavir in bulk and combined dosage form.

Figure 1: Structure of Atazanavir sulphate

MATERIALS AND METHODS

Chemicals and reagents
All the chemicals including ethyl acetate, methanol and n-hexane used were of analytical grade (E. Merck, Mumbai, India). Pure Atazanavir powder was procured as gift sample from Hetero labs, Hyderabad, India. Tablet containing Atazanavir sulphate was procured from local pharmacy.

Instrumentation
HPTLC glass plates are pre-coated with silica gel 60F254 (20X10cm) were from E. Merck. Densitometry was carried out with CAMAG TLC scanner 3, fitted with win CATS planar chromatography manager software. Samples were applied on the HPTLC plates using the spray on technique under nitrogen gas flow and developed in a CAMAG 20X10cm twin trough chambers.

Standard preparation
A stock solution of standard Atazanavir sulphate (mg/ml) was prepared in methanol and further dilution was made in methanol to get the final concentration of 100 µg/ml.

Preparation of sample solutions
For the analysis of Atazanavir in combined dosage form, twenty tablets were accurately weighed and their average weight was calculated. The tablets were finely powered and powder equivalent to 50mg of Atazanavir was accurately weighed and dissolved in 50ml of methanol. The solution was sonicated for 30min. Filtered through the whatmann no.41 filter paper. From this solution an aliquot of 5ml was pipetted out and transferred to 50ml volumetric flask. The volume was made up to the mark by using methanol to yield the final concentration of 100 µg/ml.
Optimum Chromatographic condition

Stationary phase: Aluminum plates precoated with silica gel 60F254 (Merck)

Mobile phase: n-Hexane: ethyl acetate (30:70)

Plate size: 20 X 10 cm

Development chamber: Twin through glass chamber 20 X 10 cm

Scanning speed: 20mm/s

Slit dimensions: 4.00 X 0.30mm

Separation technique: Ascending

Saturation Time: 5 min

Migration distance: ≈ 85 mm

Application type: Band

Band length: 8.0mm

Temperature: 20 ± 5°C

Scanning wavelength: 281 nm

Data resolution: 100µm / step

Scanning mode: Absorption

Method validation

The analytical method was validated as per ICH guidelines. The parameters studied for validation were Accuracy, Precision, Linearity, Specificity, LOD and LOQ. HPTLC chromatogram of Atazanavir and UV spectrum of Atazanavir standard and sample are shown in figure 2 and Figure 3 respectively.

Linearity

The Atazanavir working standard solutions (200 – 1400 ng / band) were prepared for the linearity studies. The plates were then developed and scanned under chromatographic conditions. The response was measured as per peak areas. The experiment was repeated for five times. The calibration curve was plotted over the concentration range of 200-1400ng/band (figure 4).

Accuracy

Accuracy of the method was calculated by performing recovery studies. Accuracy was determined by adding known amounts of each analyte corresponding to three concentration levels, 80%, 100%, and 120% of the labeled claim. The diluents were prepared and applied to the HPTLC plates and analyzed under the chromatographic conditions. The amount of drug present in the sample solution was obtained by comparing the sample bands with that of standard bands. Accuracy was expressed as percent of analyte recovered by the proposed method. Accuracy study was performed for five times and percentage of recovery of Atazanavir was calculated.

Limit of detection and Limit of Quantification

LOD and LOQ was calculated as per ICH guidelines by using the equation 1 and 2;

LOD = 3.3(SD) / S

LOQ = 10(SD) / S

Where,

SD - Standard deviation

S - Average of the slope of the curve

Figure 2: HPTLC chromatogram of Atazanavir

Figure 3: UV spectrum of Atazanavir Standard and Sample

Precision

Precision of the method was performed as inter day and intraday precision. The intraday precision was assessed by analyzing drug solutions with in the calibration range, six times on the same day. Inter day precision was assessed by analyzing drug solutions with in the calibration range on six different days over a period week.

Specificity

The specificity of the proposed method was determined by comparing the test results from the analysis solution containing active substances.

Analysis of the tablets samples

To determine the content of Atazanavir, 10 tablets are accurately weighed (Each tablet containing Atazanavir 300mg), their average weight was calculated and finely powdered. The powder equivalent to 50mg Atazanavir
sulphate was accurately weighed and transferred into 50 ml volumetric flask containing 20 ml of methanol and ultrasonicated for 30 minutes. The solution was diluted to 50ml with methanol. The above solution was filtered through the whatmann no.41 filter paper. From this solution 5ml of solution was transferred to 50 ml volumetric flask and made up to the mark with methanol to give the final concentration of 100µg/ml of Atazanavir. Appropriate volume of aliquots was applied on the TLC plates followed by the development and measured at 281nm. The analysis was repeated for three times.

Table 1: Results of Validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (percentage of recovery)</td>
<td>98.85%</td>
</tr>
<tr>
<td>at 80%</td>
<td>99.00%</td>
</tr>
<tr>
<td>100%</td>
<td>99.53%</td>
</tr>
<tr>
<td>120%</td>
<td></td>
</tr>
<tr>
<td>% of RSD</td>
<td>Intra Day 0.78 - 1.06%</td>
</tr>
<tr>
<td>Linearity concentration</td>
<td>200-1400ng / band</td>
</tr>
<tr>
<td>Equation for regression line</td>
<td>Y=33.287+3.021x</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9997</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.51%</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>100 ng / band</td>
</tr>
<tr>
<td>Limit of Quantification</td>
<td>300 ng / band</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
<tr>
<td>Analysis of Tablet formulation</td>
<td>99.21%</td>
</tr>
<tr>
<td>Label claim 300mg</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

To optimize the HPTLC parameters, the mobile phase composition of Chloroform: methanol (90:10 v/v) and n-hexane: ethyl acetate (70:30 v/v) was tried. The results were not good and the peak was not sharp. The combination of Ethyl acetate: n-hexane in the ratio 30:70 v/v resulted in sharp peaks of Atazanavir with Rf value 0.48±0.02. Well defined spots were obtained when the chamber was saturated with mobile phase vapour for 20 min at room temperature. Excellent linearity was obtained for compounds between peak areas and concentrations in the range from 200-1400ng/band. Linearity was evaluated by plotting peak area against concentration for Atazanavir. The accuracy of the developed method was expressed in % of recovery of the active pharmaceutical ingredient. The deviation among the results and RSD values was calculated. The low RSD values indicate the reproducibility of the method. The limit of detection and quantification were determined separately based on the standard deviation of the curve Y intercept and the slope of the calibration curves. The chromatogram of the solution containing inactive ingredients did not show any interference or spots at RF values of the examined drugs. Therefore this confirms the specificity of the proposed method. The results of Validation parameters were presented in table 1.

CONCLUSION

A new HPTLC method for the estimation of Atazanavir in combined dosage form was developed and validated as per the ICH guidelines for the first time. This method has some advantages over the reported HPLC methods. It consumes less amount of mobile phase and also requires less time and cost effective to evaluate. Specificity showed there is no interference with the main peak of Atazanavir due to the excipients. The low % RSD values confirm the method is précised. The proposed method is simple, fast, accurate, précise and specific. It can be used for the routine determination of Atazanavir in bulk and pharmaceutical formulations.

Acknowledgement: The authors are thankful to the Management and Central Research Facility, SRU for providing necessary facilities to carry out the research.

REFERENCES

1. Indian Pharmacopoeia, published by the controller of publication, New Delhi, I-II, 2010, 149, 844-845.


Source of Support: Nil, Conflict of Interest: None.