



High Performance Thin Layer Chromatographic Estimation of Atorvastatin Calcium and Aspirin in Capsule Dosage Form

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ABSTRACT

A simple, fast, precise and accurate high performance thin layer chromatographic method has been developed for estimation of atorvastatin calcium (AST) and aspirin (ASP) simultaneously from a combined dosage forms. The chromatographic separation was developed using a pre coated silica gel 60 GF₂₅₄ TLC plate as stationary phase and the chromatogram was developed using Chloroform : toluene : methanol : glacial acetic acid (4:5.6:0.4:0.5v/v/v/v) as mobile phase. Densitometric evaluation was performed at 247 nm. Atorvastatin calcium and aspirin resolved satisfactorily at R_f values 0.61 ± 0.0084 and 0.84 ± 0.01647 respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines. The proposed method can be used for the estimation of these drugs in combined dosage form.

Keywords: Aspirin, Atorvastatin calcium, chromatographic method, HPTLC.

INTRODUCTION

Atorvastatin calcium is chemically a calcium salt of (β R, 8 R)-2-(4 – fluoro-phenyl) – α, δ di hydroxyl 5(1 methyl ethyl) 1, 3, phenyl, 4 (phenyl amino) carbonyl) -1 H pyrrole- heptanoic acid tri hydrate used as antihyperlipidaemic. It is official in Indian pharmacopoeia¹ Aspirin is chemically 2-(Acetyloxy) benzoic acid, best known as anti-platelet drug. It is official both in I.P and B.P.²

Detailed survey of literature for atorvastatin calcium (AST) revealed several methods based on different techniques like , HPLC, extractive Spectrophotometry, HPLC for its determination in human serum, capillary electrophoresis³⁻¹², HPTLC for its determination in pharmaceutical.¹³

Similarly literature survey for aspirin (ASP) reveals several methods based on Spectrophotometry¹⁴, Raman spectroscopy¹⁵. The combination of atorvastatin calcium and aspirin is used for the management of hypercholesterolemia. Many Spectrophotometric and RP HPLC methods¹⁶⁻²¹ are available for the estimation of these drugs. Since no HPTLC method was developed for this combination. This paper describes a simple, precise and accurate HPTLC method for the estimation of AST and ASP combination in a capsule dosage form.

MATERIALS AND METHODS

Chemical and reagents

AST was the generous gifts from Biocon Limited Bangalore, and aspirin was procured from Qualigens Fine Chemicals (Glaxo Ltd). Combination of these drugs was purchased from the local market (Ecosprin AV 75

containing Atorvastatin calcium 10 mg and aspirin 75 mg as per the label claim, marketed by USV limited, India). AR grade chloroform, toluene, methanol and glacial acetic acid were procured from Merck.

HPTLC instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on Merck TLC plates pre coated with silica gel 60F₂₅₄ (10cmx10 cm) with 250 μm layer thickness from E Merck, Germany. The samples were applied on to plates as a band with 6mm width with slit dimension of 5x0.45 mm micro using Camag 100 μl sample syringe (Hamilton, Switzerland) with the Linomat 5 applicator (Camag Switzerland) Linear ascending development was carried out in a twin through glass chamber (10 cm x 10cm) previously saturated with the mobile phase, chloroform: toluene: methanol: glacial acetic acid (4:5.6:0.4:0.5v/v/v/v) at room temperature, using 30 minutes of chamber saturation. The development distance was approximately 70mm. Densitometry scanning was performed using Camag TLC Scanner 3 in the range of 200-400nm and operated by Win cats software (V1.43, Camag) using deuterium lamp as source of radiation. Evaluation was by peak area with linear regression.

Preparation of standard stock solution

A standard stock solution of 500 μg/ml of AST and 750 μg/ ml of ASP were prepared separately using methanol as solvent.

Validation of the method²²⁻²³

The method was validated as per ICH guide line. The parameters checked were linearity, accuracy, precision,



limit of detection, limit of quantification, robustness and specificity.

Calibration curve

From the working standard solution of AST (500 µg/ml) and ASP (750 µg/ml), 1-3 µl solutions and 1.5-5.5 µl solutions were spotted for AST and ASP respectively on a HPTLC plate to obtain a final concentration of 500 ng/spot to 1500 ng/spot for AST and 1125 ng/spot to 4125 ng/spot for ASP. The plates were then developed as per procedure described above and the peak areas were plotted against corresponding concentration to obtain the calibration curves.

Specificity

The specificity of the method was determined by analysis of standard drug and samples. The band for AST and ASP in the sample was identified by comparing the R_f value and the spectrum of the band with those of the band obtained from a standard drug solution.

Accuracy: (% Recovery)

For accuracy of method, recovery studies were carried out by applying a known amount of standard AST and ASP at a level of 80,100,120 % to the sample solution (standard addition method). Three determinations were performed at each level, using same chromatographic condition as describe above.

Precision: (Reproducibility)

The precision of the method was verified by performing the intraday and interday precision. The intraday and interday precision of the proposed method was determined by estimating the corresponding response three times on the same day and on three different days over a period of one week for five concentration of AST (500,750,1000,1250,1500ng/spot) and ASP (1125,1875,2625,3375,4125ng/spot). The results are expressed in terms of relative standard deviation.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were calculated using following equations as per International conference on Harmonization guide line:

$$\text{LOD} = 3.3 \times \sigma/S \quad \text{LOQ} = 10 \times \sigma/S$$

Where σ is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure like slight change in the mobile phase, saturation time and the values were compared with the original chromatographic conditions.

Analysis of the marketed products

To find the content of the marketed formulation, (Ecosprin, Label Claim, 10 mg of AST and 75 mg of ASP),

twenty tablets were weighed and average weight was determined, powered, from this equivalent weight of 25 mg for AST and 37.5 mg of ASP was transferred into a 50 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 minutes, filtered through Whatmann filter paper No.41 and then volume was made up to 50 ml with methanol. From this stock solution 1000 ng/spot was spotted for AST and 2625 ng/spot was spotted for ASP on a HPTLC plate and chromatogram was developed as described earlier. The analysis was repeated for three times and interference for excipients was analyzed.

RESULTS AND DISCUSSION

To optimize the HPTLC parameters, several mobile phase were tried and satisfactory results were obtained by using the mobile phase chloroform: toluene: methanol: glacial acetic acid (4:5.6:0.4:0.5v/v/v/v). Quantification was achieved under UV detection at 247nm. A sharp and symmetrical peak was resolved with an R_f of 0.61 ± 0.0084 for AST and 0.84 ± 0.01647 for ASP as shown in the figure 1 and table 1. The proposed method was found to be simple and sensitive with linearity in the concentration range of 500 ng/spot to 1500 ng/spot for AST and 1125 ng/spot to 4125 ng/spot for ASP, the linearity curve are shown in the figure 2 and 3 respectively.

The method was found to be accurate and precise indicated by results of recovery studies and % RSD not more than 2%, LOD and LOQ for AST were found to be 1.299 ng/spot and 3.9374 ng/spot and for ASP were 2.8 ng/spot and 8.4857 ng/spot respectively as shown in the table 2. The proposed method was found to be specific as there is no interference from common capsule excipients. Peak purity values for peaks of both AST and ASP confirmed the specificity as shown in the figure 4 and 5.

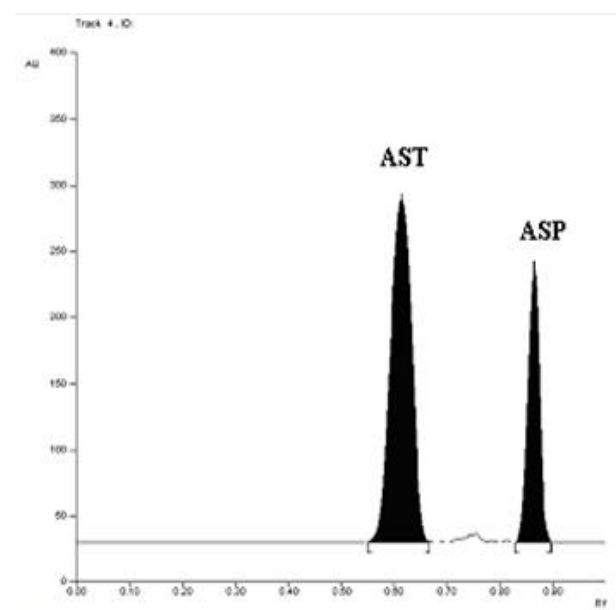


Figure 1: Chromatogram of AST and ASP

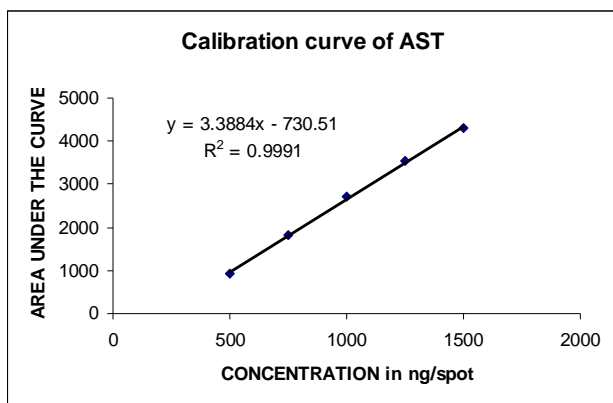


Figure 2: Representative of linearity of atorvastatin calcium. (Average of six trials)

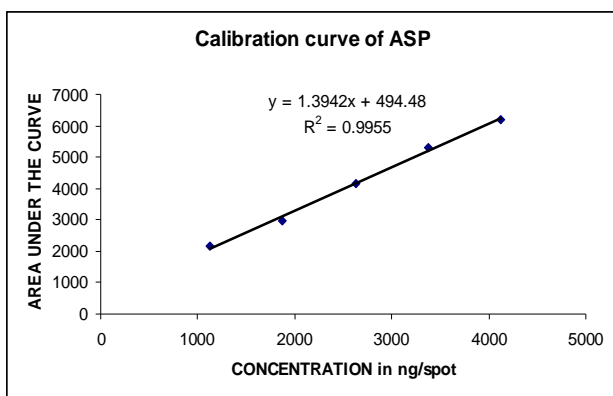


Figure 3: Representative of linearity of aspirin. (Average of six trials)

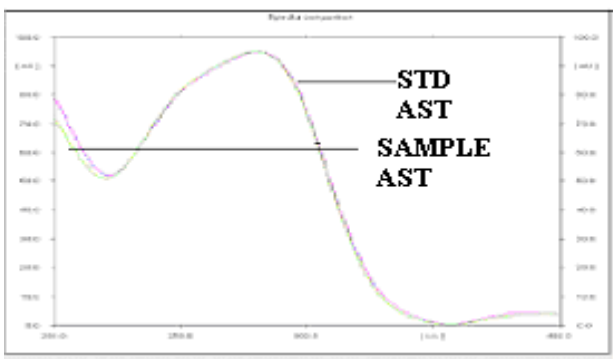


Figure 4: UV absorption spectra of Standard AST and Sample AST

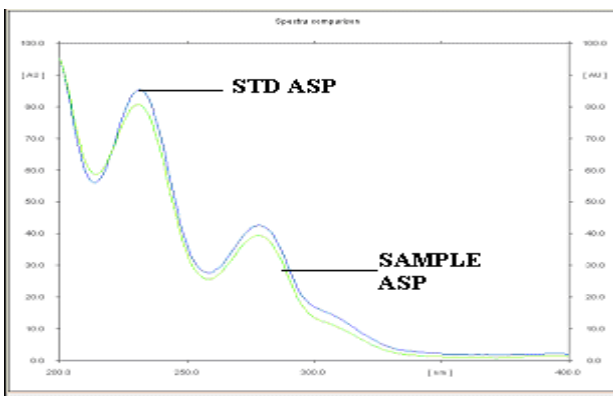


Figure 5: UV absorption spectra of Standard ASP and Sample ASP

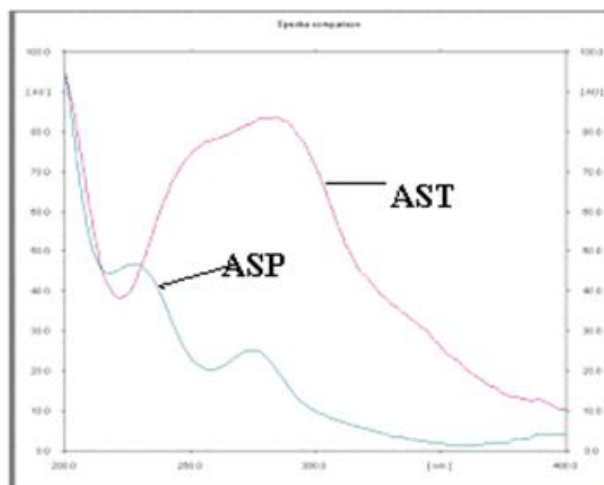


Figure 6: Overlay spectra of Standard AST and Standard ASP

Table 1: Regression analysis of the calibration curve of atorvastatin calcium and aspirin by the proposed HPTLC method

Parameters	AST	ASP
Linearity range	500-1500ng/spot	1125-4125ng/spot
Slope	3.3884	1.3942
Intercept	730.51	494.48
Correlation coefficient *	0.9991	0.9955

* Indicates mean of six observation

Table 2: Summary of validation parameters for atorvastatin calcium and aspirin by the proposed HPTLC method

Parameters	AST	ASP
Accuracy (% ± SD) #	101.31 ± 0.7066	99.42 ± 0.1986
Recovery studies(% ± SD)		
80%	99.61 ± 0.4350	98.24 ± 0.2427
100%	99.61 ± 0.4632	99.68 ± 0.1026
120%	99.39 ± 0.8989	99.46 ± 0.0296
LOD	1.299 ng/spot	2.8 ng/spot
LOQ	3.9374 ng/spot	8.4857 ng/spot
Precision (± RSD) #		
Inter day	0.2159-1.8226	0.0088-0.043
Intra day	0.3436-0.9199	0.019-0.2336

± indicates standard deviation # Average of three trials

CONCLUSION

The developed HPTLC method for the simultaneous determination of AST and ASP can be used for routine analysis of both these components in combined dosage form.

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