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Development and Validation of HPTLC Method for Simultaneous Estimation of Atorvastatin Calcium and Aspirin In Bulk and Dosage Form

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ABSTRACT

A simple, accurate, and precise HPTLC method has been developed and validated for the simultaneous estimation of Atorvastatin Calcium (ATO) and Aspirin (ASP) from bulk drug and Dosage form. The method employed TLC aluminum plates precoated with silica gel 60 GF_{254} as the stationary phase. The solvent system comprised Toluene: Ethyl Acetate: Methanol: Acetic acid [7:2:1.5:0.1v/v/v/v]. This system was found to give good result for both the drugs (Rf value: of ASP 0.43cm and ATO 0.53cm). Spectrodensitometric scanning-integration was performed at a wavelength of 235nm.The calibration curve was found to be linear within the concentration range of 20ng/spot to 100ng/spot for ATO and 30 to 150ng/spot for ASP. The regression data for calibration curve shows good linear relationship with r^2 = 0.9989 and 0.9990 for ATO and ASP respectively. The method was validated in accordance with the requirements of ICH guidelines. The method was successfully applied for determination of drug in bulk and Dosage form. Thus, the proposed method can be used successfully for routine analysis of ATO and ASP from bulk and dosage form.

Keyword: Validation, HPTLC, Atorvastatin Calcium and Aspirin

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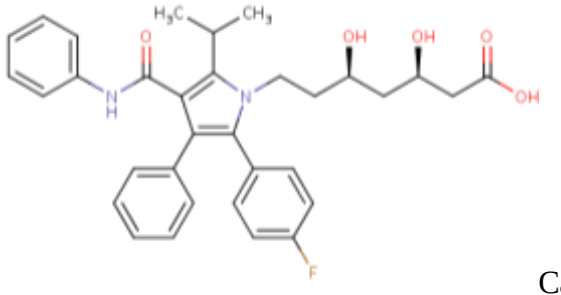
INTRODUCTION

Atorvastatin Calcium (ATO) is chemically (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid (Figure 2). It is Anticholesteremic Agent, HMG-CoA Reductase Inhibitor and Hydroxymethylglutaryl-CoA Reductase Inhibitor and used as primary prevention in individuals with multiple risk factors for coronary heart disease (CHD) and as secondary prevention in individuals with CHD to reduce the risk of myocardial infarction (MI), stroke, angina, and revascularization procedures[1-9].



Ca^{++}

Figure 1: Structure of Atorvastatin Calcium

Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Figure 1). It is non-selective cyclo-oxygenase inhibitor used as an antipyretic, analgesic, anti-inflammatory and antithrombotic agent. It reduces non-fatal myocardial infraction[1-9]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopoeia (USP) and European Pharmacopoeia (EP). It is estimated by acid-base titration method as per IP, BP, USP & EP [7-10]. Literature review reveals that UV Spectrophotometric[11], HPLC[12-14], HPTLC [16] methods has been reported for estimation of ASP in pharmaceutical dosage forms.

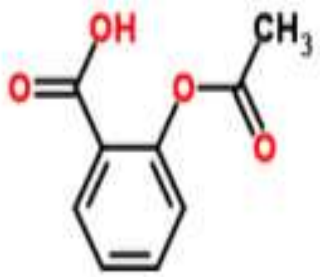


Figure 2: Structure of Aspirin

Literature review also reveals that UV Spectrophotometric [17-23], HPLC [24-31] methods has been reported for the estimation of Aspirin in pharmaceutical dosage forms. Literature survey does not reveal any HPTLC method for simultaneous determination of ASP and ATO in Pharmaceutical dosage form. The present developed method is simple, rapid, precise and accurate for simultaneous determination of both drugs in synthetic mixture as per International

Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Pure drug samples of Aspirin and Atorvastatin calcium was provided by TRC Ahmadabad as a gift sample. Methanol, Acetonitrile, Toluene, Ethyl acetate and Acetic acid of AR Grade were provided by SICART, Vallabh Vidyangar, Gujarat, India.

Chromatographic Conditions

Stationary phase was Precoated Silica gel G60 F_{254} aluminum Sheets 10×10 cm^2, layer thickness 0.2 mm. Activated the TLC plates by prewashing with Toluene and activated in Oven at 50^0C for 5minute. The Optimized Mobile phase was Toluene: Ethyl Acetate: Methanol: Acetic acid [7:2:1.5:0.1v/v/v/v]. Chamber saturation time: 30 minute at ambient temperature and migration distance was 75mm. The detection was done at 235nm.

Preparation of standard stock solutions

Accurately weighed 10mg of ATO and ASP was transferred into 10ml volumetric flask individually, dissolved and diluted up to the mark with methanol to get stock solution having 1000µg/ml concentration for both the drugs. From that individual flask pipette out 0.1 ml of ATO and 0.15ml of ASP from standard stock solutions of ASP and ATO was transferred to 10 ml volumetric flask and diluted to 10 ml with methanol to get ASP 15µg/ml and ATO 10µg/ml concentration as working standard solution. To obtain calibration curve, working standard solutions ranging from 2.0 – 10.0 µl was applied by Hamilton syringe with the help of Linomat V applicator on TLC plate that gave concentration in the range of 20-100 ng/spot for ATO and 30-150ng/spot for ASP.

Preparation of sample solution

Tablet Powder equivalent to 10mg of Atorvastatin Calcium and 75 mg of Aspirin was transferred in 100ml volumetric flask containing 50ml methanol, sonicated for 5 min and diluted to mark with same solvent to obtain 0.1mg/ml of ATO and 0.75mg/ml of ASP. The resulting solution was filtered using Whatman filter paper. 1ml filtrate was transfer in 10ml volumetric flask and from that, 2µl solution was injected which gave 20ng/spot of ATO and 140ng/spot of ASP and followed by development and scanning at 235 nm.

METHOD VALIDATION

Linearity

The calibration curve was linear over the concentration range of 20-00ng/spot for ATO and 30-

150ng/spot for ASP.

Precision

Precision of the method was determined in the terms of intra-day and inter-day variation (%RSD). Intra-day precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days over a period of 7 days.

Accuracy

To the pre-analyzed sample a known amount of standard solution of pure drug (ASP and ATO) was spiked at three different levels (50%, 100% and 150%). These solutions were subjected to re-analysis by the proposed method.

Sensitivity

The sensitivity of measurement of ASP and ATO by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formulae:

LOD= 3.3 σ/S Where, σ = the standard deviation of the response

S = the slope of the calibration curve

LOQ = 10 σ/S Where, σ = the standard deviation of the response

S = the slope of the calibration curve , LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Specificity

Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate, lactose,) were spiked in to a pre weighed quantity of drugs .The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPTLC method is illustrated in Figure 6. Where complete separation of ASP and ATO in presence of tablet excipients.

Repeatability

Repeatability of sample application was assessed by injecting 60ng/spot and 90ng/spot of Atorvastatin calcium and Aspirin respectively six times and statistical data was calculated.

RESULTS AND DISCUSSION:

Method development

The solvent system was developed and optimized using trial and error method. Various proportions of different solvents as mobile phase were tried to get resolution of both the compounds. The optimized mobile phase was Toluene: Ethyl Acetate: Methanol: Acetic acid [7:2:1.5:0.1v/v/v/v]. The optimized mobile phase could resolve both the compounds apart from each other and the bands obtained were compact too. The maximum absorption of ATO and ASP together as detected at 235 nm and this wavelength was chosen for the analysis.

The optimized solvent system yielded a symmetrical peak for the both drugs with R_f 0.43 and 0.53 for ASP and ATO respectively. The HPTLC chromatogram of ASP and ATO is shown in Figure 3.

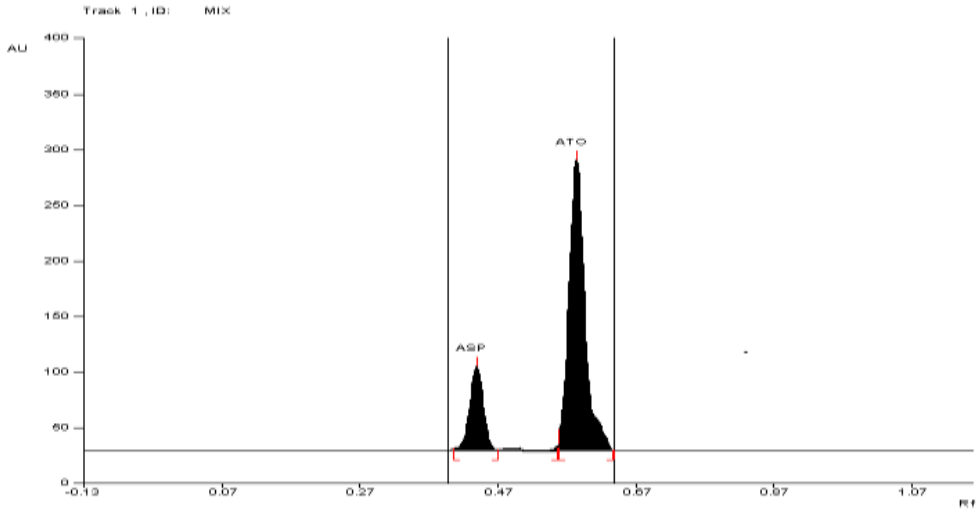


Figure 3: A Typical Chromatogram of ASP and ATO

The developed method was then validated and successfully applied for quantification of ASP and ATO from the formulation. Regression analysis data is shown in Table 1.

Table 1: Statistical Data of ASP and ATO drug

Parameters	**Results**	
	ASP	**ATO**
Linear Range(ng/spot)	30-150	20-100
Slope	20.79	40.37
Intercept	339.8	766.6
S.D. of Slope	0.27	0.97
S.D. of Intercept	14.03	47.08
Regression Equation	y = 20.793x + 339.8	y = 40.37x + 766.6
Correlation -Efficient (r^2)	0.9990	0.9989

Precision, expressed in terms of %RSD was determined in terms of intra-day and inter-day precisions, analyzing the drugs at three different concentrations, determining each concentration thrice summarized in Table 2 and 3.

Table 2: Intra- day Precision Data for ASP and ATO

Concentration (ng/spot) ASP	Mean Area (n=3) ± SD ASP	%RSD ASP	Concentration (ng/spot) ATO	Mean Area (n=3) ± SD ATO	%RSD ATO
60	1547 ± 26.22	1.69	40	2423 ± 39.80	1.64
90	2265 ± 25.71	1.13	60	3266 ± 36.22	1.10
120	2858 ± 38.88	1.36	80	3965 ± 61.99	1.56

Table 3: Inter- day Precision Data for ASP and ATO

Concentration (ng/spot) ASP	Mean Area (n=3) ± SD ASP	%RSD ASP	Concentration (ng/spot) ATO	Mean Area (n=3) ± SD ATO	%RSD ATO
60	1554 ± 27.79	1.78	40	2424 ± 43.91	1.81
90	2255 ± 29.51	1.30	60	3248 ± 38.19	1.17
120	2871 ± 53.25	1.85	80	3968 ± 65.13	1.64

To ensure accuracy of the method, recovery studies were performed by standard addition method at three different levels I, II and III (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed and the results obtained are shown in Table 4 and 5.

Table 4: Recovery data for determination of ASP

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)
45	30	75	74.91	99.88
45	45	90	90.53	100.59
45	60	105	104.96	99.96

Table 5: Recovery data for determination of ATO

Concentration of Sample Taken (ng/spot)	Concentration of Pure API spiked (ng/spot)	Total Concentration (ng/spot)	Mean Total Concentration Found (n=3) (ng/spot)	%Recovery Mean (n=3)
30	20	50	49.6	99.20
30	30	60	60.03	100.05
30	40	70	69.76	99.66

The sensitivity of measurement of ASO and ATO by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope values obtained are shown in Table 6.

Table 6: Results of sensitivity data for ASP and ATO

Parameter	Results	
	ASP	ATO
LOD (ng/spot)	3.85	2.96
LOQ (ng/spot)	11.57	8.88

The method was applied to the formulation and the Chromatogram of the formulation is shown in Figure 5.

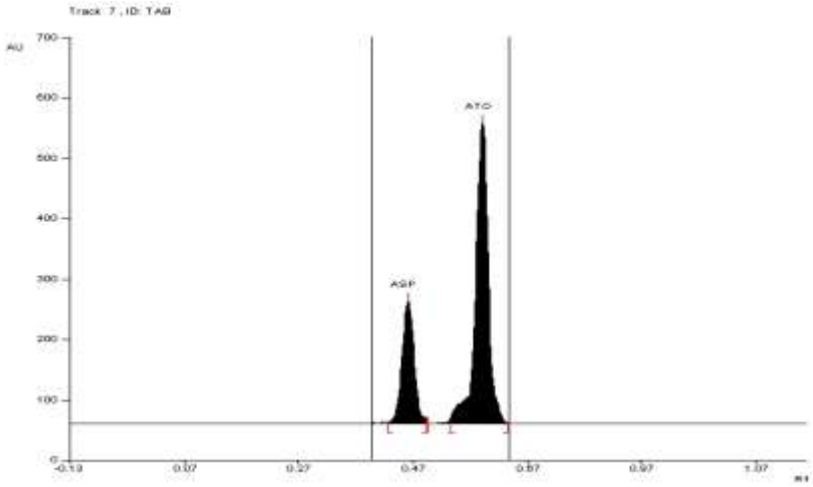


Figure 5: Typical HPTLC Chromatogram of ASP and ATO formulation

The peak purity of ASP and ATO were assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) and r (M, E) Figure 6.

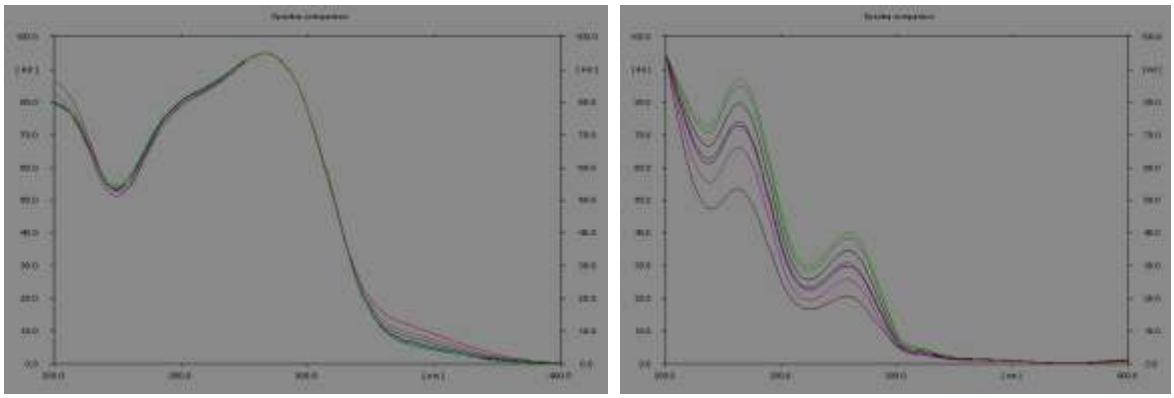


Figure 6: Purity Comparison Spectra of a. ASP and b. ATO in standard and formulation

Analysis of ASP and ATO in Formulation

Tablet powder equivalent to 75mg of Aspirin and 10mg of Atorvastatin Calcium was transferred in 100ml volumetric flask containing 50ml methanol, sonicated for 5 min and diluted to mark with methanol to obtain 0.75mg/ml of ASP and 0.1mg/ml of ATO. The resulting solution was filtered using Whatman filter paper no 42. From the above solution 1ml was transferred into 10ml volumetric flask and diluted to mark with same solvent. So, resultant solution was found to contain 10µg/ml of Atorvastatin Calcium and 75µg/ml Aspirin. 2 µl of this solution applied on TLC plate followed by development and scanning at 235 nm. The analysis was repeated for three times.

When the formulation was analyzed, ASP and ATO gave sharp and well defined peaks at R_f 0.43

± 0.02 and 0.52 ± 0.02, respectively, when scanned at 235 nm. The results in Table 7 indicate that there is no interference from the excipients. The % purity was 99.76% for ASP and 99.21% for ATO. Chromatogram is shown in Figure 7.

Table 7: Assay Result of Formulation

Parameters	ASP	ATO
Actual Concentration (ng/spot)	140	20
Concentration Obtained (ng/spot)	139.6	19.63
%Purity	99.76	98.21

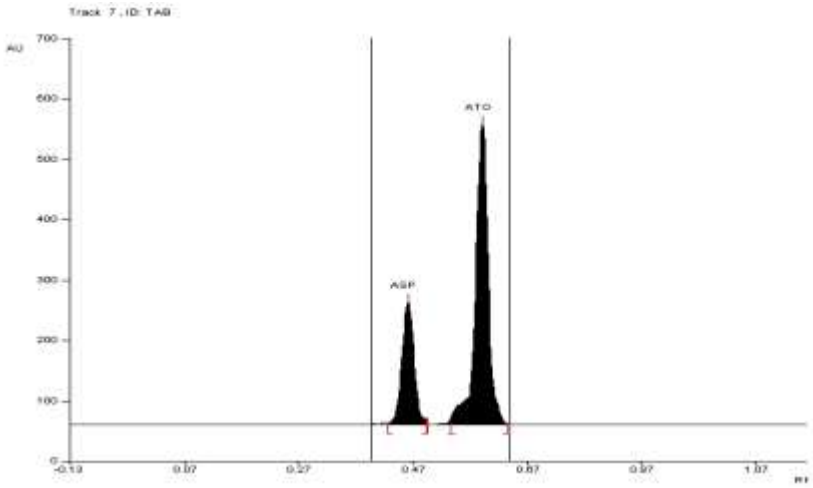


Figure 7: HPTLC Chromatogram showing peaks of ASP and ATO in Formulation

CONCLUSION

In this proposed method the linearity was observed in the concentration range of 30-150ng/spot for ASP and 20-100ng/spot for ATO with co-efficient of correlation, r^2 = 0.9990 and r^2 = 0.9989 for ASP and ATO, respectively at 235nm. The result of the analysis of combined mixture by the proposed method was found to be highly reproducible and reliable. The additives present in formulation did not interfere with determination of ASP and ATO. So, the developed HPTLC method is simple, precise and accurate and can be used for simultaneous determination of ASP and ATO in pharmaceutical dosage forms.

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