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Development and validation of a HPTLC method for simultaneous estimation of Atorvastatin calcium and Losartan Potassium in combined dosage form

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ABSTARCT
A simple, precise, rapid and accurate HPTLC method has been developed for the simultaneous estimation of Atorvastatin calcium (ATO) and Losartan Potassium (LOS) in bulk and pharmaceutical dosage form. Precoated silica gel 60 F254 plate was used as stationary phase. The separation was carried out using Toulene: Methanol (8:2 v/v) as mobile phase. The densitometric scanning was carried out at 260 nm. The Rf values was found to be 0.15 ± 0.01 for ATO and 0.09 ± 0.01 for LOS. The linearity was obtained in the range 200-1200 and 500-3000 ng/band ng/band with correlation coefficients (r2 = 0.9994) and (r2 = 0.9999) for ATO and LOS. The method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. The percentage recovery obtained for ATO and LOS were in the range of 99.25-101.5 % and 100.75-101.26 %, respectively. The proposed method was optimized and validated as per the ICH guidelines.

Keywords: Atorvastatin calcium, Losartan Potassium, HPTLC, Method Validation.

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Introduction:

Atorvastatin calcium chemically [R-(R, R*)] -2- (4-flurophenyl)-β, δ- dihydroxy -5 (1-Methyl ethyl) -3-phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is a synthetic HMG –CoA reductase inhibitor[1]. Atorvastatin calcium is an inhibitor of 3-hydroxy-3methyl glutaryl coenzyme A (HMG-Co A) reductase. This enzyme catalyses the conversion of HMG-Co A to mevalonate, an early and rate-limiting step in cholesterol biosynthesis[2].

Several analytical methods that have been reported for the individual determination of ATO in pharmaceutical formulations which include Methods such as HPLC[2-4], UPLC[5], LC-MS[6] and simultaneous UV spectrophotometric methods[1] as well as physicochemical properties and oral bioavailability[7] are reported for estimation of ATO alone or in combination with other agents. Losartan potassium (LOS), chemically, is 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt. It is an angiotensin II receptor blocker and chemically is used as an antihypertensive agent[8]. Several analytical methods have been reported for individual analysis of LOS which includes UV spectrophotometric methods[9-11], liquid chromatography coupled with spectrofluorimetric liquid chromatography[11], HPLC[12-15], HPTLC[16-17].

Since no HPTLC method is reported for simultaneous estimation of ATO and LOS in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously. The present work describes a new method for simultaneous estimation of ATO and LOS in tablets using HPTLC-densitometry. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines[18].

Material and Methods

Pure drug sample of ATO, % purity 98.80 and LOS, % purity 99.92 was kindly supplied as a gift sample by Litaka pharmaceuticals Ltd. Pune and Emcure Pharmaceuticals Ltd. Pune, respectively. These samples were used without further purification. Two tablet formulations (Lot 302 and 304) were supplied by JPLC Pharma ltd., Jalgaon were used for analysis containing ATO 10 mg and LOS 25 mg per tablet.

HPTLC precoated plates silica gel 60 F_{254} 20×10 cm, layer thickness 0.2 mm (Merck, Germany). Analytical grade methanol and toulene was procured by Merck Chemicals (Mumbai, India).

Instrumentation and chromatographic conditions

The standard solution ranging from 200-1200 ng/band ATO and 500-3000ng/band for LOS were applied on precoated silica gel 60 F_{254} plate in the form of bands with 100 µl sample syringe using CAMAG Autosampler. It was developed in a twin trough glass chamber without saturation. The mobile phase consisted of Toulene:Methanol(8:2 v/v). After development, plate was immediately dried with the help of dryer and was observed under CAMAG TLC Visualizer. The well resolved bands of drugs were scanned at 260 nm with CAMAG TLC scanner III densitometer controlled by WINCAT's software version 4.

Preparation of standard solutions and calibration curve

Mixture of standard stock solution of ATO and LOS (100µg mL^{-1}) were prepared separately in methanol. The solution was suitably diluted with methanol to get of 20 µg/ml of ATO & 50 µg/ml of LOS. The standard solutions were applied to reach a concentration range of 200-1200 ng/band ATO and 500-3000ng/band for LOS. The plate was developed on previously described mobile phase and well resolved band of drug were scanned at 260 nm with scanner. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve.

Analysis of tablet formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of LOS (20 mg of ATO) was weighed and dissolved in the 40 ml of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman filter paper No. 41 into a 50 ml volumetric flask. Filter paper was washed with the methanol, adding washings to the volumetric flask and volume was made up to mark. The solution was suitably diluted with methanol to get of 20 µg/ml of ATO & 50 µg/ml of LOS. The amount of each drug present per tablet was estimated from the respective calibration curves. A typical densitogram obtained from a sample solution shown in Fig. 1.

Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness and specificity.

Linearity:

Linearity of the method was studied by spotting eight concentrations of the drug prepared in the mobile phase in the range of 200-1200 ng/band ATO and 500-3000ng/band for LOS, respectively and noting the peak areas.

Accuracy:

For accuracy of method, recovery study was carried out

by applying the method to drug samples to which known amount of ATO and LOS was added at level of 80, 100 and 120% of label claim (standard addition method). At each level of the amount, three determinations were performed and the results obtained were compared with expected results.

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 repeated measurements of standard and sample solutions were made in a day and percentage RSD were calculated. In the inter day variation studies, 3 repeated measurements of standard and sample solutions were made on 3 consecutive days and percentage RSD were calculated.

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response and Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOD and LOQ was calculated using the following formula:

LOD = (3.3 x σ)/ b

LOQ = (10 x σ)/ b

Where σ = Standard deviation of the response

b = Slope of the calibration curve

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. Mobile phases having different composition like toluene: methanol (8.2: 1.9 v/v) and toluene: methanol (7.8: 2.1 v/v) were tried and chromatograms were run. Robustness of the method was checked at three different concentration levels 200, 400, 600 ng/spot.

Specificity:

The specificity of the method was ascertained by overlaying UV spectra of spots for standard drug and sample.

Result and Discussion

Optimization of Solvent System and Chromatographic Conditions:

Chromatographic separation studies were carried out on the stock solution of ATO and LOS. Initially the plates were spotted with 10 µL of stock solution and developed by linear ascending development method using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc. without chamber saturation. Based on the results of these initial chromatograms, binary and ternary mixtures of solvents were tried to achieve optimum peak parameter. The final mobile phase consisting of toluene: methanol in the ratio of (8: 2 v/v) was optimized since good Rf value of 0.15 for ATO and 0.09 for LOS was obtained as shown in Fig. 1. The samples were applied in form of bands on precoated aluminum sheets of silica gel 60 F_{254}. Linear ascending development was carried out in a twin trough glass chamber (20cm x 10 cm, 10 x 10 cm), without saturation. The length of chromatogram run was 85 mm. The developed plates were dried in the current of dry air with the help of a drier. Densitometric scanning was performed in the absorbance mode at 260 nm as shown in fig. 2.

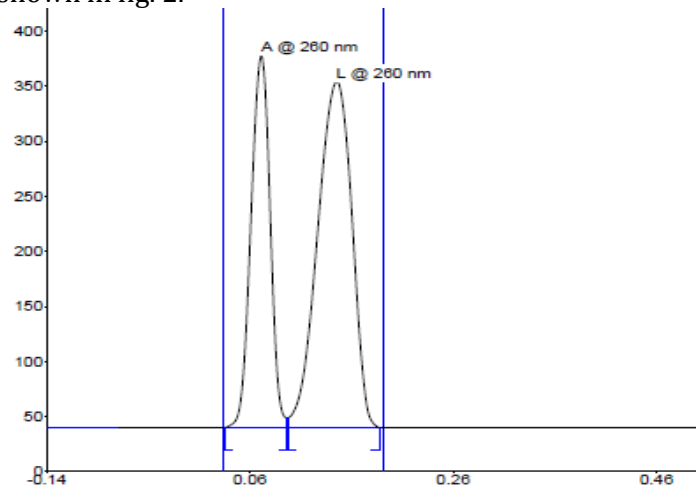

Fig.1: Densitogram of standard Atorvastatin calcium (200ng/spot) and standard Losartan potassium (500ng/spot); peak 1 (R_F: 0.15), peak 2 (R_F: 0.09).

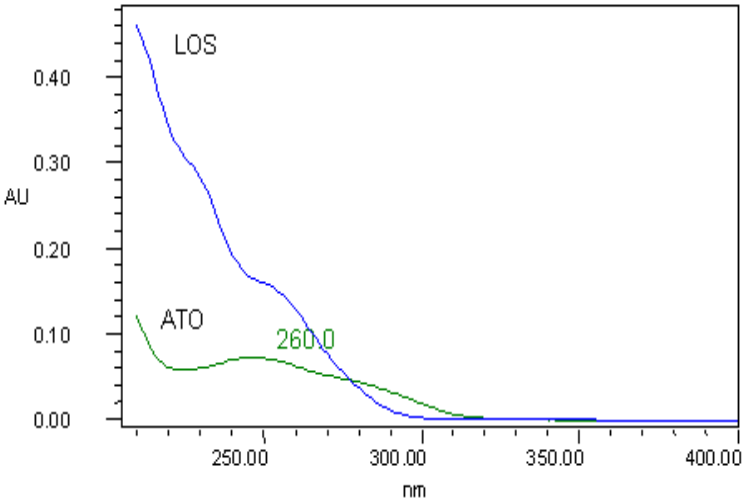

Fig.2: Densitometric scan of Atorvastatin calcium and Losartan potassium.

Linearity:

When peak area was plotted Vs Concentration (ng/spot) ATO and LOS showed good correlation coefficient in concentration range of 200-1200 ng/band ATO and 500-3000ng/band for LOS,. Linearity was evaluated by determining eight standard working solutions. Table.1 summarizes Beer's law limit, linear regression equation and correlation coefficient for the method.

Analysis of tablet formulation:

The proposed method was also evaluated in terms of assay of commercially available tablets containing ATO and LOS. Three replicate determinations were performed on the accurately weighed amounts of tablets. The results obtained are shown in Table. 2.

Parameters	ATO	LOS
Detection Wavelength (nm)	260	260
Beer's Law Limit (ng/band)	200-1200	500-3000
Regression equation	0.1578x+59.76	5.365x+56.68
Correlation Coefficient (r^2)	0.99924	0.9999
Slope (m)	0.1578	5.365
Intercept (c)	59.76	56.68
Limit of detection (ng/spot)	66.66	166.6
Limit of quantitation (ng/spot)	200	500

Table. 1: Regression analysis of calibration curves for HPTLC

Compound (Label Claim)	Formulation Study (n=6)		Recovery (accuracy) study	
	Batch no.	% Assay Found, % RSD	Recovery Level	% Recovery, % RSD (n=3)
Atorvastatin calcium (10mg)	Batch I	100.4, 1.05	80	101.41, 0.93
	Batch II	100.01, 1.26	100	99.24, 0.62
			120	101.75, 0.97
Losartan Potassium (25mg)	Batch I	99.80, 0.97	80	100.08, 0.72
	Batch II	100.2, 1.32	100	100.74, 0.47
			120	99.99, 0.88

Table. 2: Results of Tablet analysis and accuracy study

Parameter	SD of peak area		% RSD	
	ATO	LOS	ATO	LOS
Mobile phase composition	8.04	4.97	0.44	0.43
Amount of mobile phase	15.46	18.44	1.28	1.89
Time from spotting to chromatography	8.14	6.16	0.41	0.57
Time from chromatography to scanning	11.82	7.66	0.59	0.71
Plate from different lot no.	12.85	10.64	1.47	0.82

Table. 3: Result of robustness study

Precision:

The proposed method was found to be precise as indicated by percent RSD (Relative Standard Deviation) not more than 2.

Accuracy:

The proposed method when used for estimation of ATO and LOS from pharmaceutical dosage form after spiking with working standard afforded recovery of 98–102% and result of recovery for ATO and LOS from the marketed formulation are listed in Table. 2.

Limit of Detection and Limit of Quantification:

The limit of detection was found to be 66.66 ng/spot and 166.6 ng/spot, while the limit of quantitation was found to be 200 ng/spot and 500 ng/spot for ATO and LOS respectively.

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. The result obtained is shown in Table 3.

Specificity:

The method was found to be specific since no interfering spots were seen when R_f values of standard and sample were compared. There is no difference in the spectra of sample and standard solution which indicate the specificity of the method.

Conclusion

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and sensitive, thus can be used for routine analysis of Atorvastatin calcium and Losartan potassium in tablet dosage form.

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