

DEVELOPMENT AND VALIDATION OF NOVEL HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND TELMISARTAN IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, precise normal phase high performance thin layer chromatography (HPTLC) method was developed and validated for simultaneous estimation of Atorvastatin Calcium (ATC) and Telmisartan (TEL) in tablet dosage form. Chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F₂₅₄ as the stationary phase and the solvent system consisted of Chloroform: Methanol: Toluene: Ammonia (5:2:1:0.2 v/v/v/v). Densitometric evaluation of the separated zones was performed at 289 nm. The two drugs were satisfactorily resolved with R_f values 0.17 ± 0.02 and 0.49 ± 0.02 for Atorvastatin Calcium and Telmisartan respectively. The linearity was studied in the concentration range 100-600 µg/ml for both Atorvastatin Calcium and Telmisartan with a correlation coefficient of 0.9998 and 0.9997 respectively. The method was validated for linearity, accuracy, precision, LOD, LOQ and robustness as per International Conference on Harmonisation (ICH) guidelines. The study shows that the developed method is repeatable and selective for the estimation of Atorvastatin calcium and Telmisartan in its pharmaceutical formulations.

Keywords: Atorvastatin Calcium, Telmisartan, HPTLC, Validation and Densitometry.

INTRODUCTION

Atorvastatin Calcium (ATC) chemically it is calcium salt of (βR, δR)-2-(4-fluorophenyl)-α, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl-amino) carbonyl]-1H-pyrrol-1-heptanoic acid trihydrate^[1]. It is an antihyperlipidemic, that is it reduces level of bad cholesterol (low-density lipoprotein or LDL) and triglycerides in the blood, while increasing of good cholesterol (high-density lipoprotein or HDL)^[4]. Telmisartan (TEL), chemically it is 4-[[4-methyl-6-(1-methyl-1H-benzimidazole-2-yl)-2-propyl-1H-benzimidazole-1-yl]methyl]-2-biphenyl carboxylic acid¹. It is an antihypertensive. It is a new angiotensin II receptor antagonist that is highly selective for type I angiotensin II receptor. Angiotensin II is the principle pressure agent of the rennin-angiotensin system with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone cardiac stimulation and renal reabsorption of sodium¹⁰. Telmisartan and Atorvastatin calcium are introduced into the

market in combined dosage form, which is widely used in the treatment of hypertension. Literature review reveals that the methods have been reported for Telmisartan and Atorvastatin alone or in combined dosage forms are such as RP-HPLC, Spectrophotometric, HPTLC, Fluorimetry and ion-pair Chromatographic method⁶⁻²⁰.

Chemical structure of Atorvastatin calcium and Telmisartan are shown in figure no. 1 and 2 respectively.

In the present study attempt were made to develop and validated rapid, precise and accurate new method for simultaneous estimation of ATC and TEL by HPTLC method. Now a day's HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, minimum sample preparation and high sample throughput. The major advantages of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase thus saving the analysis time as

well as cost per analysis. The developed method was validated according to ICH guidelines.

MATERIALS AND METHODS

Instrumentation

The system used for the analysis consisted of Camag HPTLC (Switzerland). The samples were spotted in the form of bands with a Camag 100 μ l sample (Hamilton Bonded, Switzerland) syringe on silica gel precoated aluminium plate 60 F₂₅₄ (20 × 10 cm, E. Merck, Darmstadt, Germany) using a Camag Linomat V sample applicator. The mobile phase containing Chloroform: Methanol: Toluene: Ammonia (5:2:1:0.2 v/v/v/v) and 15 ml mobile phase was used for each chromatographic run. Linear ascending development was carried out in a 20 × 10 cm plate twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase for 15 minutes. The developed TLC plates were dried in stream of air with the help of air dryer in wooden chamber. Densitometric scanning was performed using a Camag TLC scanner in the reflectance absorbance mode at 289 nm and operated by winCATS software (version 1.4.6, Camag). The source of radiation used was deuterium (D2) lamp emitting continuous UV spectrum between 190 nm and 400 nm. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Reagents and Chemicals

Working standards of ATC (potency = 95.70%) and TEL (potency = 99.94%) were obtained as a gift samples from Glenmark pharmaceutical Ltd. (Mumbai, India). HPLC grade of Chloroform, Methanol, Toluene and AR grades Ammonia were procured from Merck Ltd. Mumbai India. The commercial fixed dose combination product Telista plus 40 tablet (Marketed by Lupin Ltd. Mumbai) containing 10 mg ATC and 40 mg TEL was procured from the local market.

Standard solution preparation

To prepare a stock solution for assay, weight accurately equivalent to 10 mg of Atorvastatin working standard and 40 mg of Telmisartan working standard was transferred into 100 ml volumetric flask, to this 40 ml diluent was added to dissolve the substance by sonication for 5 minutes. Then volume was made upto the mark by diluent and mix well.

Sample solution preparation

20 tablets (Telista plus 40 Tablet) each contained 10 mg ATC and 40 mg TEL were accurately weighed. Their average weight determines and finally powdered. Quantity of the powder containing weight equivalent to 10

mg of ATC and 40 mg of TEL were transferred into 100 ml volumetric flask and 50 ml diluents was added followed by sonication for 10 minutes. Then volume was made upto the mark by diluent and mix well. The resulting solution stirred for 1 hour and after that centrifuged at 5000 RPM for 10 minutes. Upper supernatant solution was used for the further analysis.

Optimization of the HPTLC method

To optimize the HPTLC parameters, several mobile phase composition were tried. Good peak symmetry, satisfactory resolution for ATC and TEL was obtained with mobile phase Chloroform: Methanol: Toluene: Ammonia (5:2:1:0.2 v/v/v/v). To get better reproducibility and repeatedly optimization of method was done by changing mobile phase composition; the effect on R_f and peak shape were monitored for ATC and TEL. The results of validation studies of simultaneous estimation of ATC and TEL in the current study are given below. The chamber was saturated with the mobile phase for 15 min at room temperature and plates were activated at 80° C for 5 min to obtain well-define peaks.

METHOD VALIDATION

The developed method was validated according with ICH (Q2) B guidelines for validation of analytical procedures. The parameters assessed were System suitability, linearity, accuracy, precision, LOD, LOQ and robustness.

RESULTS AND DISCUSSION

System suitability

The system suitability test of the chromatographic system was performed before each validation run. Six replicate injections of standard preparation were injected and retardation factor and %RSD of peak area were determined for the same. Acceptance criteria for system suitability, R_f should not be more than ± 0.02 from its mean value and % RSD of peak area should not be more than 2.0%.

Linearity (Calibration Curve)

For constructing calibration curve, series of six dilutions in the concentration range 100-600 (100, 200, 3000, 400, 500, and 600) μ g/ml for ATC and TEL was taken. Calibration curve were constructed by plotting peak area vs. concentration of ATC and TEL and regression equation calculated from straight line equation. Linearity curves for ATC and TEL shown in figure no. 6 and 7 respectively.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of ATC and TEL by the

standard addition method. Known amounts of standards solutions of ATC and TEL added at 80,100 and 120% level to prequantified sample solution of ATC and TEL. Three samples were prepared for each recovery level solutions were then analysed and the results were recorded.

Precision

The precision of analytical procedure express the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of homogenous samples. Precision are considered at three levels that is system precision, method precision (repeatability) and intermediate precision (reproducibility).

System precision

The system precision checked by repeatedly injecting (n=6) standard solution of ATC and TEL under the same chromatographic conditions and calculating the percentage relative standard deviation (%RSD) for six determination of peak area which should be not more than 2%.

Method Precision (Repeatability)

The method precision of analytical method was determined by analysing six sets of sample preparation against the same standard. Assay of all six sample preparations was determined and mean %assay value, standard deviation and %RSD for the same was calculated.

Intermediate Precision (Reproducibility)

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different equipment (instrument) under same experimental condition. Assay of all replicate sample preparation was determined and mean % assay value, standard deviation and %RSD for the same was calculated. Precision study was established by evaluating system precision, method precision and intermediate precision.

Robustness

Robustness was assessed by introducing small changes in the mobile phase composition and measuring the effect on results. The amount of mobile phase was varied by ± 5 ml also saturation time of mobile phase change by 5 minutes. The results obtained from the robustness study are shown in Table no. 1.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products

and related substances. The specificity of the method was determined by analyzing standard drug and test samples. The spot for ATC and TEL in the sample was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of ATC and TEL was determined by comparing the spectrum at three different regions of the spot i.e. peak start, peak max and peak end.

Limit of Detection & Limit of Quantification

Limit of Detection (LOD) is the lowest concentration of analyte in the sample that could be detected under the stated experimental condition and Limit of Quantification (LOQ) is the lowest concentration of the active ingredients in a sample that could be determined with accepted precision and accuracy. According to ICH recommendation, the approach based on the standard deviation (SD) of the response and slope (M) was used for determining the detection and quantification limits. LOD can be calculated according to formula $LOD = 3.3 (SD/M)$ and $LOQ = 10(SD/M)$. The signal to noise ratio was determined. The LOD was regarded as the amount for which the signal to noise ratio was 3:1 & LOQ as the amount for which the signal to noise ratio was 10:1.

DISCUSSION

In order to achieve simultaneous estimation of the two components, initial trials were performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters such as ideal mobile phase and their proportions, detection wavelength and concentrations of the standard solutions were carefully studied. Several solvents were tested in varying proportions, finally a mixture of Chloroform: Methanol: Toluene: Ammonia (5:2:1:0.2 v/v/v/v) selected as the optimum mobile phase. The optimized chromatographic conditions were selected based on sensitivity, R_f values, peak shape and baseline drifts. The accuracy data shows that the method is accurate with in desired range. The LOD and LOQ values were low, which indicates that the method is sensitive. The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and R_f values.

CONCLUSION

A validated HPTLC method has been developed for the determination of Atorvastatin calcium and Telmisartan in tablet dosage form. The developed method is simple, rapid, linear, accurate, precise and specific. Results from the validation experiments showed that the method

is reliable and accurate therefore it can be successfully applied for the routine quality control analysis of Atorvastatin calcium and Telmisartan in tablet dosage form.

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Chemical structures of ATC and TEL

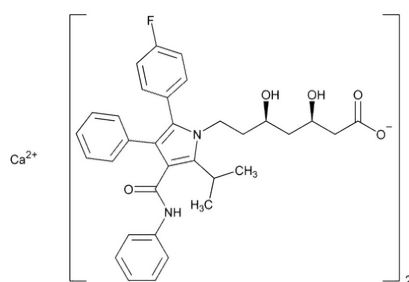


Fig. 1: Chemical structure of ATC

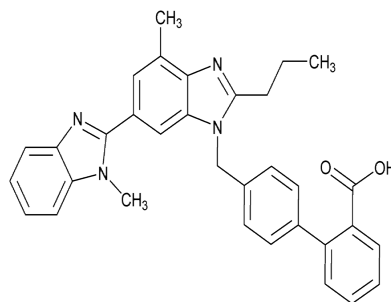


Fig. 2: Chemical structure of TEL

A typical chromatogram shown in figure no. 3 and 4.

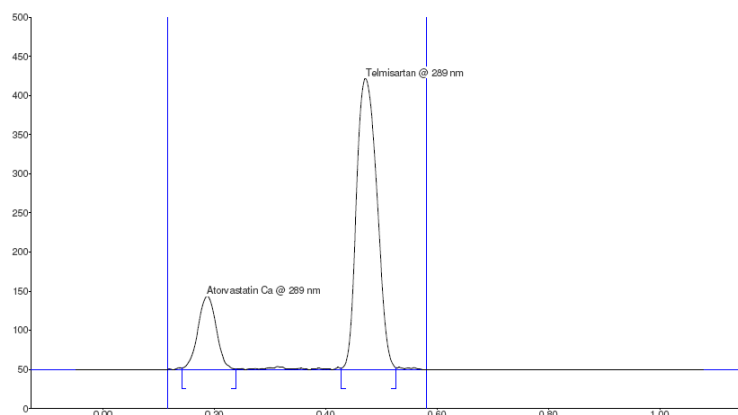


Fig. 3: Chromatogram of ATC and TEL in Standard solution

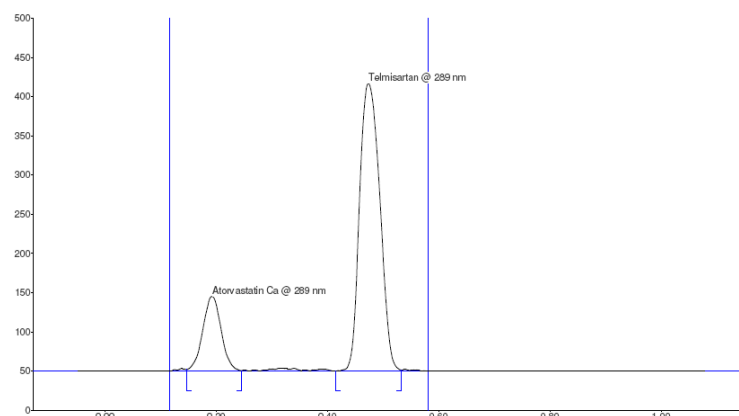


Fig. 4: Chromatogram of ATC and TEL in Sample solution

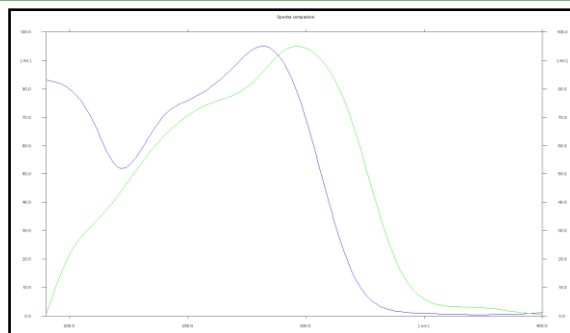


Fig. 5: Overlain spectra of ATC and TEL

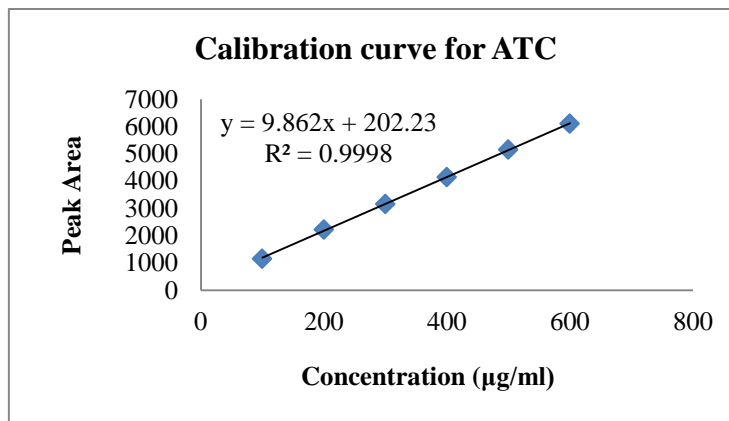


Fig. 6: Linearity curve of ATC

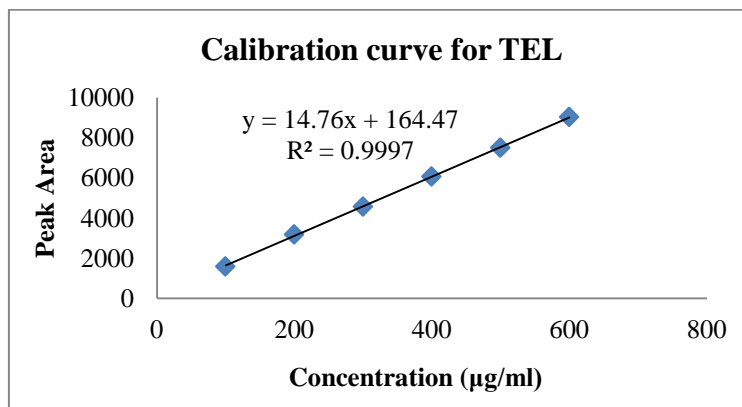


Fig. 7: Linearity curve of TEL

Table 1: System suitability parameters of ATC and TEL

Sr. No.	Peak Area		Retardation Factor	
	ATC	TEL	AT C	TEL
1	5196.9	6583.5	0.18	0.48
2	5245.4	6571.5	0.17	0.49
3	5252.1	6600.4	0.17	0.49
4	5109.3	6436.6	0.17	0.49
5	5191.7	6524.8	0.17	0.48
6	5162.1	6490.7	0.17	0.49
Mean	5192.9	6534.6	0.17	0.49
SD	53.322	62.785	0.004	0.005
%RSD	1.027	0.961	2.378	1.061

Table 2: Regression parameters of calibration curves

Parameters	ATC	TEL
Linear range ($\mu\text{g/ml}$)	100-600	100-600
Slope	9.862	14.76
Intercept	202.23	164.47
Correlation Coefficient (R^2)	0.9998	0.9997

Table 3: Data of system precision study

Sr. No.	Area of ATC	Area of TEL
1	5196.9	6583.5
2	5245.4	6571.5
3	5252.1	6600.4
4	5109.3	6436.5
5	5191.7	6524.8
6	5162.1	6490.7
Mean	5192.9	6534.6
SD	53.322	62.816
%RSD	1.027	0.961

Table 4: Data of method precision study

Sr. No.	Wt. of sample in mg	Avg. Area of ATC	Avg. Area of TEL	% Assay of ATC	% Assay of TEL
1	532.60	5014.1	6211.05	99.4	98.2
2	531.99	5096.8	6245.1	101.1	98.8
3	538.65	5063.3	6348.0	99.2	99.2
4	535.40	5008.9	6380.6	98.8	100.3
5	537.21	5074.5	6453.5	99.7	101.1
6	533.73	5089.0	6276.7	100.6	99.0
Mean				99.8	99.5
SD				0.905	1.084
%RSD				0.907	1.090

Table 5: Data of intermediate precision study

Sr. No.	Wt. of sample in mg	Avg. Area of ATC	Avg. Area of TEL	% Assay of ATC	% Assay of TEL
1	535.10	5134.9	6461.4	101.3	101.7
2	533.92	5087.4	6356.9	100.6	100.2
3	538.23	5095.0	6374.8	99.9	99.7
4	536.50	5087.3	6351.8	100.1	99.7
5	532.41	5024.1	6387.0	99.6	101.0
6	535.64	5034.5	6290.6	99.2	98.9
Mean				100.1	100.2
SD				0.736	1.004
%RSD				0.736	1.002

Table 6: Data of Accuracy study of ATC

Accuracy level	Set No.	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80%	1	40	40.61	101.5	100.9	0.630	0.624
	2	40	40.10	100.3			
	3	40	40.35	100.9			
100%	1	50	49.35	98.7	100.2	1.334	1.331
	2	50	50.63	101.3			
	3	50	50.31	100.6			
120%	1	60	59.57	99.3	100.3	0.901	0.898
	2	60	60.25	100.4			
	3	60	60.64	101.3			

Table 7: Data of Accuracy study of TEL

Accuracy level	Set No.	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80%	1	160	157.76	98.6	99.6	0.874	0.877
	2	160	160.48	100.3			
	3	160	159.68	99.8			
100%	1	200	202.60	101.3	100.1	1.387	1.385
	2	200	201.00	100.5			
	3	200	197.20	98.6			
120%	1	240	240.45	99.7	99.9	1.092	1.093
	2	240	237.36	98.9			
	3	240	242.54	101.1			

Table 8: Results of assay test

Drugs	Label claim (mg/tab)	Amount of drug estimated (mg/tab)	% Amount found
ATC	10.0	10.10	101.01
TEL	40.0	39.86	99.7

Table 9: Data of LOD and LOQ study

Drugs	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
ATC	10.695	32.409
TEL	11.374	34.467

Table 10: Data of robustness study

Parameters	Variation	ATC		TEL	
		Retardation factor	Assay (%)	Retardation factor	Assay (%)
Mobile phase composition	6:1:1:0.2	0.17	100.14	0.45	101.13
	5:2:1:0.2	0.19	99.38	0.48	98.19
	4:3:1:0.2	0.21	98.72	0.50	99.51
Saturation time (minutes)	10	0.19	99.53	0.48	100.32
	15	0.19	99.38	0.48	98.19
	20	0.18	99.96	0.47	99.21
Amount of mobile phase (ml)	10	0.18	100.74	0.48	100.41
	15	0.19	99.38	0.48	98.19
	20	0.19	98.11	0.49	101.03

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