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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND CELECOXIB BY RP-HPLC

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ABSTRACT

A stable, rapid, accurate and selective method has been developed for the estimation of Atorvastatin calcium and Celecoxib by using buffer (pH3.7) and acetonitrile in ratio(40:60 v/v) in combination as mobile phase and at the flow rate of 1.5 ml/min at λ_{max} 254 nm. Chromatographic separation was performed on Shimadzu LC-2010 ATVP prominence liquid chromatograph and using Shimadzu SPD-10AVP UV-Visible detector, an Qualisil GOLD C_{18} 5µ (250× 4.60 mm) column used as stationary phase. Validation of the method was done according to the guidelines ICH Q2 (R1). Calibration curve was linear at concentration of 1-10 µg/ml of Atorvastatin calcium and Celecoxib respectively; detection was carried out at λ_{max} 254 nm; linear regression equation for Atorvastatin calcium was Y=33546x-192.6; R^2=0.998 and linear regression equation for Celecoxib was Y=51830x-5320; R^2=0.998 respectively. Retention time for Atorvastatin calcium and Celecoxib was 4.544 min and 6.064 min respectively. LOD for Atorvastatin calcium and Celecoxib was 0.01399 µg/ml and 0.01460 µg/ml respectively and LOQ for Atorvastatin calcium and Celecoxib was 0.0424µg/ml and 0.04426µg/ml respectively. The results of present study suggested that proposed method provides good peak resolution of Atorvastatin calcium and Celecoxib within short analysis time (<10 min) and high percentage of

recovery shown that method is free from interference of excipients. The % RSD of each parameter lies below the limit of 2% proves the suitability. The statistical analysis proved that the proposed method is precise, accurate, selective and rapid for the estimation of both drugs.

Keywords: Atorvastatin calcium, Celecoxib, RP-HPLC

INTRODUCTION

Atorvastatin calcium is [R-(R*,R*)]-2-(4-fluorophenyl)-ß,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate **[1]**. Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, which is a rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver. Atorvastatin also increases the number of hepatic LDL receptors **[2]**.

Celecoxib is p-[5-p-methylphenyl-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzene sulfonamide **[3]**. Celecoxib is a selective cycloxygenase-2 (PTGS2/ COX2) inhibitor used for treatment of osteoarthritis and rheumatoid arthritis. It acts by reducing prostaglandin synthesis through inhibition of COX-2 **[4]**. Based on recent studies it is also concluded that atorvastatin in combination with celecoxib have stronger effects on growth inhibition and apoptosis of PC-3 cells than either used individually **[5]**.

Many spectroscopic methods are used for determination of atorvastatin as single drug **[6-7]** and in combination with other drugs **[8-12]**. Celecoxib has been determined by HPLC and stability indicating methods **[13-17]**. A stability indicating method was also reported for validation of atorvastatin calcium and celecoxib in bulk and niosomal formulation by RP-HPLC **[18]**.

The aim of the present work was to develop simple, precise, accurate and reproducible RP-HPLC method for simultaneous determination of atorvastatin calcium and celecoxib. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines (ICH, 2005).

MATERIALS AND METHODS

Chemical and reagents

The drug Atorvastatin calcium was gifted fromLupin Laboratories, Aurangabad, Biharand Celecoxib was procured as a gift sample from Symbiosis Pharmaceuticals Pvt. Ltd, Sirmour, Himachal Pradesh. Acetonitrile

and triethylamine were purchased from ThermoFisher Scientific India Pvt. Ltd, Mumbai.

Apparatus

Absorbance measurements were made on Shimadzu UV-1800 UV/Visible spectrophotometer with 10 mm matched quartz cells. Chromatography was performed on Shimadzu HPLC equipped with LC-10 ATVP pumps, SPD-10AVP UV-Visible detectors and Rheodyne injector with a 50µL loop. Separation was performed on Qualisil GOLD C_{18} [250mm×4.6mm× 5 µm particle size]. Data acquisition and integration were performed using LC-solution version 6.42 software.

Chromatographic conditions

The method development for analysis of Atorvastatin calcium and Celecoxib was performed using various solvents. Final separation was achieved using a mobile phase consisting of buffer: acetonitrile [40:60 v/v], pH adjusted to 3.7 using orthophosphoric acid, pumped at a flow rate of 1.5 ml/min. The eluent was monitored using a Shimadzu SPD-10AVP UV-Visible detector at a wavelength of 254 nm. The mobile phase was vacuum filtered through 0.22 µm nylon membrane filter followed by degassing in an ultrasonic bath prior to use.

Preparation of solutions

Preparation of buffer solution

The buffer solution was prepared by dissolving 0.4 ml glacial acetic acid and 0.1 ml triethylamine in 250 ml of volumetric flask containing water and the pH was adjusted to 3.7 by adding orthophosphoric acid dropwise.

Preparation of blank solution

Acetonitrile and water in ration of 50: 50 was used as blank solution.

Preparation of standard solution

The quantity of powder equivalent to 10 mg of Atorvastatin calcium and Celecoxib were weighed and transferred into 10 ml volumetric flask , 5 ml of diluent was added and sonicated for 15 minutes and the volume was made upto the mark with diluent. From this further dilution was made to get the final concentrations of Atorvastatin calcium and Celecoxib.

Validation of the developed method

The optimized analytical method was validated for system suitability, linearity and range, precision, limit of detection [LOD], limit of quantitation [LOQ] and accuracy in accordance with ICH guidelines for analytical procedures Q2[R1].

System suitability

System suitability parameters were studied to verify the system performance. Six replicate samples of both the drugs of concentration 10

µg/ml were analysed using the developed method. Factors such as theoretical plate count, tailing factor, percent relative standard deviation [%RSD] of peak area and retention time were taken into consideration for testing system suitability.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of other drugs, excipients and their potential impurities.

Accuracy and precision

To determine the precision, triplicate injections of selected concentrations were analyzed, and the values of % RSD were calculated. In order to demonstrate applicability and accuracy of the proposed method, recovery tests are also carried out by analyzing samples at three different concentration level, i.e., 80, 100 and 120% mixtures of the Atorvastatin calcium and Celecoxib. After five repeated experiments, the recoveries from these sample mixtures were calculated for each compound.

Linearity

Linearity of the method was established by triplicate injections of the solutions containing the drugs in the range of 1-10 µg/ml for each drug. The calibration curves were constructed and the acceptable fit to the linear regression was demonstrated and reported by the necessary parameters.

LOD and LOQ

Limit of detection and quantification of the developed method were calculated from the standard deviation of the y-intercepts and slope of the calibration curve of Atorvastatin calcium and Celecoxib using the following formula:

Limit of detection = 3.3 α/S

Limit of quantitation = 10 α/S

Where α is the standard deviation of the y–intercepts and S is the slope of the calibration curve.

RESULT AND DISCUSSION

A) RESULTS OF METHOD

Method development

Optimization of mobile phase

Method development for Atorvastatin calcium and Celecoxib was started with a different combination of solvents with different ratios. Wehave tried various buffer-solvent and solvent-solvent ratios [80:20, 85:15, 65:35, 60:40, and 70:30]. However, finally a combination of 0.4 % glacial acetic acid and 0.1 % triethylamine buffer [pH adjusted to 3.7 using orthophosphoric acid]: acetonitrile 40:60 v/v has shown good resolution for Atorvastatin calcium and Celecoxib. Initially 1.0 ml/min flow rate was tried but to get adequate separation further

increase and decrease in the flow rate was applied and finally 1.5 ml/min shows good resolution between the peaks of Atorvastatin calcium and Celecoxib. The present work was developed by using a low-cost solvent buffer with acetonitrile in ratio 40:60.

Chromatographic conditions

The analytical conditions were selected, keeping in mind the chemical nature of Atorvastatin calcium and Celecoxib. The development trails were taken using different conditions. The column selection has been done on the basis of back pressure, peak shape, theoretical plates and day-to-day reproducibility of the retention time. After evaluating all these factors, the chromatographic separation of Atorvastatin calcium and Celecoxib were carried out on Qualisil GOLD C_{18} [250mm× 4.6mm× 5 µm particle size] using a mobile phase consisting of a combination of 0.4 % glacial acetic acid and 0.1 % triethylamine buffer [pH adjusted to 3.7 using orthophosphoric acid]: acetonitrile 40:60 v/v, the flow rate 1.5 ml/min and the injection volume was 10 µl, the detection was carried out at 254 nm. The peak retention time of Atorvastatin calcium and Celecoxib were found to be 4.544 min and 6.064 min respectively. Hence this method was finalised as an optimized method for the estimation of Atorvastatin calcium and Celecoxib. The optimised chromatographic condition was shown in **Table 1**.

Method validation

System suitability

The developed method has produced theoretical plate above 2000 for Atorvastatin calcium and Celecoxib with tailing factor less than 2.Similarly, the percent relative standard deviation [%RSD] of peakarea and retention time of Atorvastatin calcium and Celecoxib were less than 2, which ensure the suitability of the developed method. The results of the system suitability study were summarised in **Table 2**.

Specificity

The specificity of the method was performed by mixing the excipients with the drugs and injecting the samples and it was observed the retention time of Atorvastatin calcium does not interfere with the retention time of the Celecoxib.

Accuracy

The percentage recovery of the sample ensures the accuracy of the developed method. The results of recovery studies were summarised in **Table 3 and 4**.

Precision

The developed method has shown percent relative standard deviation [% RSD] less than 2 for intra-day and inter-day precision study,

which ensures precision of the developed method. The results of the precision study were summarised in **Table 5 and 6**.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 1-10 µg/ml for both the drugs. Peak areas of Atorvastatin calcium and Celecoxib were plotted versus their respective concentrations in the mobile phase, and linear regression analysis performed on the resultant curves and was confirmed by the high value of the correlation coefficients 0.998 for both drugs. Results of Atorvastatin calcium and Celecoxib were summarised in **Table 7**.

Table 1: Optimised chromatographic condition

Parameters	Condition
Mobile phase	0.4 % glacial acetic acid and 0.1 % triethylamine buffer: acetonitrile [40:60 v/v], [pH adjusted to 3.7 using orthophosphoric acid]
Diluent	acetonitrile: water [50:50 v/v]
Column	Qualisil GOLD C_{18} [250mm×4.6mm× 5 µm particle size]
Column temperature	40 ºC
Detection wavelength	254 nm
Injection volume	20 µl
Flow rate	1.5 ml/min
Run time	10 minutes

Table 2: System suitability of developed method

Parameters	Name of drug		Acceptance criteria
	Atorvastatin calcium	Celecoxib	
Retention time, min	4.591	6.044	-
Theoretical plates [N]	17927.481	19807.562	>2000
Tailing factor	1.153	1.168	<2
%RSD peak area	0.585624	1.041011	-
%RSD of retention time	0.755849	1.948099	<2

Table 3: Accuracy data for Atorvastatin calcium

Description	80%	100%	120%
Amount present	4	5	6
	4	5	6
	4	5	6
Amount recovery	4.066842843	4.961673821	6.155944375
	4.091108031	5.038076373	6.149445836
	4.053368807	5.025615871	6.146643713
% Recovery	101.671071	99.2334764	102.599073
	102.277701	100.761527	102.490764
	101.33422	100.512317	102.444062
% Mean recovery	101.761	100.169	102.511
SD	0.47812	0.81980	0.0795
% RSD	0.46985	0.818421	0.07757

Table 4: Accuracy data for Celecoxib

Description	80%	100%	120%
Amount present	4	5	6
	4	5	6
	4	5	6
Amount recovered	4.092271157	5.101793503	6.123525956
	4.101697512	5.109300031	5.956865689
	4.098049839	5.104865228	5.978041391
% Recovery	102.3067789	102.0358701	102.0587659
	102.5424378	102.1860006	99.28109481
	102.451246	102.0973046	99.63402319
Mean % recovery	102.4335	102.1064	100.3246
SD	0.118829	0.075477	1.512139
% RSD	0.116006	0.075477	1.507246

Table 5: Intraday precision for Atorvastatin calcium and Celecoxib

Concentration	Atorvastatin calcium		Celecoxib	
	Peak area	% amount found	Peak area	%amount found
5	166845	99.3575	264068	101.3455
5	166812	99.3378	267126	102.5197
5	168258	100.1999	265223	101.7889
5	167413	99.6961	265866	102.0358
5	165666	98.6546	263390	101.0852
5	165912	98.8012	260603	100.0151
Mean	166817.7	99.3412	264773.5	101.4650
SD	956.6953	0.57038	2738.448	0.87222
%RSD	0.573498	0.57416	1.034261	0.85962

Table 6: Interday precision of Atorvastatin calcium and Celecoxib

Concentration	Atorvastatin Calcium		Celecoxib	
	Peak area	%amount found	Peak area	%amount found
5	166514	99.1601	266991	102.4678
5	166543	99.1774	266089	102.1214
5	163160	97.1605	267244	102.5649
5	163902	97.6028	261330	100.2942
5	163191	97.1789	264410	101.4768
5	165491	98.5502	260623	100.0227
Mean	164800.2	98.1384	264449.8	101.4913
SD	1445.58	0.9441	2875.112	1.1039
% RSD	0.877171	0.962018	1.087213	1.0877

Table 7: Linearity data for Atorvastatin calcium and Celecoxib

Parameters	Atorvastatin calcium	Celecoxib
Dilutions	1-10	1-10
Regression equation	Y=33546x-192.69	Y=52088x-123.78
Slope	33546	52088
Intercept	192.69	123.78
Correlation coefficient	0.9983	0.9987
LOD	0.01399	0.014605
LOQ	0.042395	0.044257

Robustness and ruggedness

As per ICH, the prepared solution was analysed as per the proposed method with a small but deliberate change in chromatographic conditions such as change in column temperature, change in flow rate and change in wavelength. These variations did not cause any significant difference in

resolution of RP-HPLC method. Similarly ruggedness was determined by carrying out the analysis by two different analysts as per ICH guidelines.

LOD and LOQ

Limit of detection [LOD] and limit of quantification [LOQ] was estimated from the standard deviation of the y-intercepts and slope of the calibration curve of Atorvastatin calcium and Celecoxib. The LOD and LOQ for Atorvastatin calcium were found to be 0.01399 and 0.042395 respectively and LOD and LOQ for Celecoxib were found to be 0.014685 and 0.044257 respectively.

B) DISCUSSION

The mobile phase consisting of 0.4 % glacial acetic acid and 0.1 % triethylamine buffer: acetonitrile in (40:60, v/v) pH 3.7 adjusted with *o*-phosphoric acid, at 1.5 ml/min flow rate was optimized which gave two sharp, well resolved peaks with minimum tailing factor. The retention times for Atorvastatin calcium and Celecoxib were 4.544 min and 6.064 min, respectively. UV overlain of Atorvastatin calcium and Celecoxib showed that both drugs absorbed appreciably at 254 nm so this wavelength was selected as detection wavelength. Calibration curve was linear over the concentration range of 1-10 µg/ml for Atorvastatin calcium and Celecoxib, respectively. The HPLC method developed for the analysis of mixture of Atorvastatin calcium and Celecoxib in their pharmaceutical preparations is stability indicating, precise, accurate and with a short run time than other developed method. Recovery data states that the proposed method is accurate and reproducible. These results concluded that the proposed method is better in comparison to previously reported method. In present method retention times of the Atorvastatin calcium is least as compare to others method such as Surekha M *et al*., 2012 **[7]**; Ahmed M *et al*., 2012 **[8]**; Jadhav PS *et al*. 2015 **[17]** that state the retention time as 11.95 min, 7.09 min and 6.19 min, respectively. The LOD & LOQ values of atorvastatin calcium are0.01399 µg/ml and 0.0424µg/ml respectively which are least as compare to other methods reported such as Surekha M *et al*., 2015 [0.617 and 0.013 µg/ml, respectively] **[7]**; Ahmed M *et al.,* 2012 [0.025 and 0.076 µg/ml, respectively] **[8]**; Jadhav PS *et al*., 2015 [1.149 and 3.4823 µg/ml, respectively] **[17]**. The LOD and LOQ values of celecoxib are found to be 0.01460 µg/ml and 0.04426µg/ml respectively which are least as compare to other method such as Jadhav PS *et al*., 2015 [0.0993 and 3.00982 µg/ml respectively] **[17]**. This shown that the less sample is required for the quantitation and quantification of the drug. These data represent that this method is better than existing method and have less runtime

compared to the previously reported method.

CONCLUSION

The HPLC method developed for the analysis of mixture of Atorvastatin calcium and Celecoxibin their pharmaceutical preparations is stability indicating, precise, accurate and with a short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently applied for the routine estimation of Atorvastatin calcium and Celecoxib.

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