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Research Article

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Simultaneous estimation of statins like pravastain, atorvastatin and simvastatin in bulk and pharmaceutical dosage form by means of High-Performance Liquid Chromatography

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ABSTRACT

The aim of this study was to develop and validate a simple, rapid, precise, more accurate, reliable, least time consuming HPLC method for individual as well as simultaneous estimation of pravastatin, atorvastatin and simvastatin in bulk and pharmaceutical dosage form. The chromatographic separation was achieved by using a mixture of methanol and 0.1 % orthophosphoric acid in water as the mobile phase, with a C18 (150×4.6 mm i.d., 2.7 µm) reversed-phase column at flow rate of 1.0 mL/min and the eluents were monitered at 238 nm. A good linearity was found in the 0.12-0.24 mg/mL for both pravastatin and atorvastatin and 0.02-0.14 mg/mL simvastatin concentration range. The accuracy was good and recovery values for pravastatin, atorvastatin and simvastatin ranged from 99.21-100.40%, 99.87-100.39 and 98.84-100.66%, respectively. The proposed novel method was found to be efficient, accurate, precise, specific and economic and is suitable for individually as well as simultaneous estimation in quality control laboratories and research institutes.

Key words: Atorvastatin; HPLC; Pravastatin; Simvastatinb; Validation.

INTRODUCTION

The statins (or HMG-CoA reductase inhibitors) formed a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease. Statins are effective and often-prescribed drugs for the treatment of lowering cholesterol and cardiovascular diseases. Pravastatin (PRV), Atrovastatin (ATV), Simvastatin (SIM) are the member of the statin [1-16] class of pharmaceutical drugs, for chemical structures see figure1.

The primary uses of all these statins PRV, ATV, SIM is for the treatment of lowering cholesterol and the prevention of cardiovascular diseases. PRV acts as a lipoprotein-lowering drug. ATV is a competitive inhibitor of HMG-CoA reductase. SIM act by inhibiting 3-hydroxy-3-methylglutaryl coenzyme However, statins reduce cardiovascular disease and it works in lowering of cholesterol level. The aim of this study was to develop and validate a simple, rapid, precise, more accurate, reliable, least time consuming HPLC method for individually as well as simultaneous estimation of PRV, ATV and SIM in bulk and pharmaceutical dosage form. The analytical method was validated [17-19] as per current International Conference on Harmonization (ICH) guidelines [20].

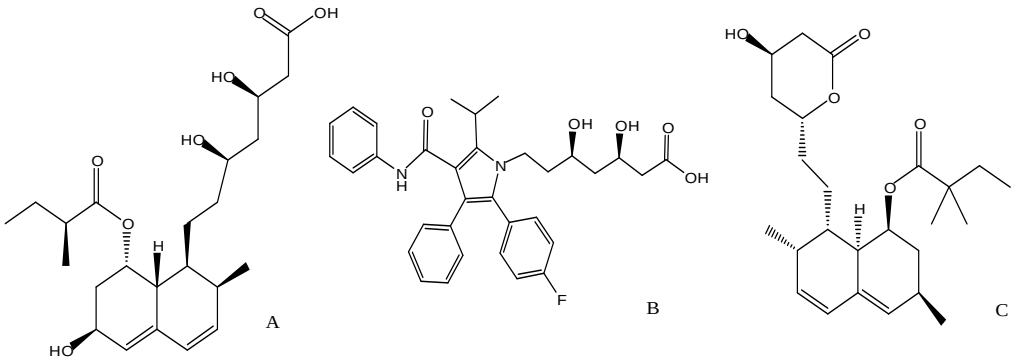

Figure 1:.Structure of statins like pravastatin (A), atorvastatin (B) and simvastatin (C).

EXPERIMENTAL SECTION

Apparatus and Chromatographic Conditions: Agilent 1200 series HPLC, G1331A Quaternary pump connected with G1314B Variable Wavelength detector, G1316A Thermostatted Column Compartment, G1329A ALS autosampler. The data acquisition was performed by Agilent Chemstation software. The chromatographic separation was performed using a Poroshell 120 SB C18 (150 mm×4.6 mm i.d., 2.7 µm particle size) column. Separation was achieved using a mobile phase consists of 0.1 % orthophosporic acid in water and methanol gradient elution mode, at a flow rate of 1 mL/min. The eluent was monitored using UV detection at a wavelength of 238 nm. The column temperature was maintained at 25 °C temperature and injection volume 5 µL was used. The total runtime was 15 min. The mobile phase was filtered through 0.45 µm nylon membrane (Millipore) prior to use.

Reagents: ortho phosphoric acid (AR grade, Spectrochem), methanol (HPLC grade, Thomas Baker), water (HPLC grade, Thomas Baker), PRA, ATV and SIM.

Preparation of Mobile Phase:
The mobile phase is composed of a mixture of methanol and 0.1% ortho phosphoric acid in water with gradient elution mode. The mobile phase were filtered separately through a 0.45 µm nylon membrane (Millipore) and degassed in an ultrasonic bath (Branson-5510) prior to use to avoid disturbances and column clogging due to small particles. Methanol used as diluent for standard and sample preparations.

Gradient elution mode of mobile phase

Time	Mobile phase	
in min	A %	B %
0	30	70
4	20	80
6	20	80
8	10	90
10	10	90
12	30	70
15	30	70

Preparation of Standard Solution:
The standard stock solution 1.0 mg/mL of both PRA, ATV and 0.5 mg/mL SIM was prepared in diluent. The working standard solution PRA, ATV (200 µg/mL) and SIM (100 µg/mL) was prepared by diluting the stock solution with diluent. The resulting solution was transferred to standard analytical HPLC glass vials and injected into the HPLC.

Preparation of Sample Solution:
To prepare a stock solution of sample, an accurately weighed amount, equivalent to 100 mg of both PRV, AVT and 50 mg of SIM drugs from composite of 20 powdered tablets, was transferred into a 100 mL volumetric flask and dissolved in 50 mL of diluent and the mixture was sonicated for 30 min. The contents of the flask were then left to return to room temperature and volume was adjusted with the same solvent mixture and mixed well. This solution 50 mL was filtered through a 0.45 µm nylon syringe filter. Pipette out 5 mL of above test stock solution and transfer

into 25 mL volumetric flask and dilute up to the mark with diluent. The concentration obtained is 200 µg/mL of PRV, ATV and 100 µg/mL of SIM of the test solution and injected to HPLC system for the analysis. All measurements were made at room temperature (25 ± 1°C) and 5 µL of clear solution was injected into HPLC system for the analysis.

RESULTS AND DISCUSSION

Method Development:

This study was directed towards developing an RP-HPLC method for separation of statins like PRV, AVT and SIM and to determine assay in bulk and its pharmaceutical formulations. The aim of this study was to develop a rapid, more accurate, precise, reliable, least time consuming HPLC method for the three drugs individually as well as simultaneously. This analytical method was developed and validated in accordance with ICH guidelines.

To optimize the operating conditions for RP-HPLC detection of all analytes, a number of parameters such as the mobile phase composition with different buffer solutions and the flow rate were varied. Various ratios of mobile phases like 60:40, 70:30, and 80:20 v/v of methanol: water was tested. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry, resolution and retention time. Orthophosporic acid was added to the mobile phase to optimize the peak shape with better resolution for all drugs analyzed with a detector wavelength 238 nm. The best peak shape and resolution were obtained when the mobile phase comprised of the methanol: water with orthophosphoric acid was used in the ratio of (70:30 v/v) in gradient elution mode at a flow rate of 1 mL/min. For simultaneous determination of PRV, ATV and SIM statin's drugs. A typical chromatogram obtained by using the aforementioned mobile phase from 5 µL of the assay preparation is illustrated in figure 2. The retention times of PRV, ATV and SIM were 3.646, 6.156 and 11.659 min, respectively.

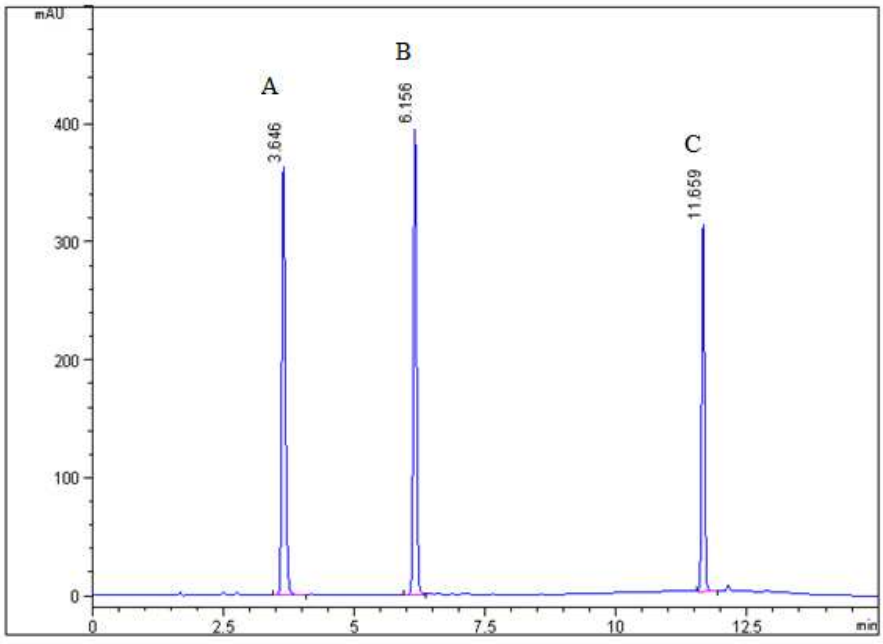

Figure 2: A typical chromatogram showing separation of statins like pravastatin (A), atorvastatin (B) and simvastatin (C).

Accuracy and Precision:

The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at three levels, viz. 50 %, 100 % and 150 % of the selected concentrations. Three samples were prepared for each recovery level. The recovery values for PRV, ATV and SIM ranged from 99-101 %, 99-101 % and 98-101 %, respectively (table 1). The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form. Intra and Inter day studies were performed by taking six replicates of sample concentrations. The results are shown in table 2.

Table 1: Accuracy study of pravastatin, atorvastatin and simvastatin

Parameter: Accuracy and Recovery					
Name	Accuracy Level	Amount added (mg/mL)	Amount Recovered (mg/mL)	Mean Recovery %	RSD %
PRV	50	50.7	50.3	99.21	0.56
	100	100.4	100.8	100.40	0.28
	150	150.9	151.1	100.13	0.09
ATV	50	50.7	50.9	100.39	0.35
	100	100.4	100.6	100.20	0.14
	150	150.5	150.3	99.87	0.12
SIM	50	25.9	25.6	98.84	0.83
	100	50.2	49.8	99.20	0.57
	150	75.7	76.2	100.66	0.46

Table 2: Precision study of pravastatin, atorvastatin and simvastatin

Parameter : Precision						
Set No.	PRV		ATV		SIM	
	Method	Inter mediate	Method	Inter mediate	Method	Inter mediate
1	99.53	99.68	99.81	99.73	99.83	99.45
2	99.26	99.51	99.75	99.56	99.78	99.59
3	99.47	99.65	99.67	99.48	99.64	99.88
4	99.56	99.58	99.83	99.37	99.76	99.55
5	99.38	99.75	99.77	99.72	99.69	99.63
6	99.59	99.57	99.83	99.69	99.83	99.83
Mean	99.47	99.62	99.78	99.59	99.76	99.66
SD	0.13	0.09	0.06	0.15	0.08	0.17
RSD %	0.13	0.09	0.06	0.15	0.08	0.17

System Suitability:

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested met the acceptance criteria on all days. The following system suitability criteria were fulfilled in the chromatograms- viz., % RSD of PRV, ATV and SIM were 0.40, 0.38 and 1.05, resolution between the two peaks were greater than 2, tailing factor is between 0.9 - 2.0 and theoretical plates more than 2000. As shown in the chromatogram, all the analytes are eluted by forming symmetrical single peaks well separated from the solvent front. Results are tabulated in table 3.

Table 3: System suitability test results of pravastatin, atorvastatin and simvastatin

Parameter: System suitability						
S. No.	PRV		ATV		SIM	
	RT	Area	RT	Area	RT	Area
1	3.628	1732.0	6.154	1656.9	11.640	1136.0
2	3.601	1731.0	6.112	1660.5	11.614	1154.1
3	3.583	1720.1	6.079	1654.3	11.598	1151.7
4	3.576	1720.4	6.072	1645.7	11.594	1124.3
5	3.585	1716.1	6.062	1645.0	11.562	1153.2
6	3.570	1717.5	6.058	1649.4	11.588	1148.9
Average	3.591	1722.9	6.090	1652.0	11.599	1144.7
SD	0.021	6.898	0.037	6.277	0.026	11.992
RSD %	0.59	0.40	0.61	0.38	0.23	1.05
Tailing factor		1.209	1.044		1.008	
Theoretical Plates		14532	51162		229615	
Resolution		-	21.813		53.289	

Linearity:

The linearity of the method was tested from 0.12- 0.24 mg/mL for PRV, 0.12- 0.24 mg/mL for ATV and 0.02- 0.14 mg/mL for SIM. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in mg/mL. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9997, 0.9996 and 0.9998 for PRV, ATV and MEL respectively see figure 3.

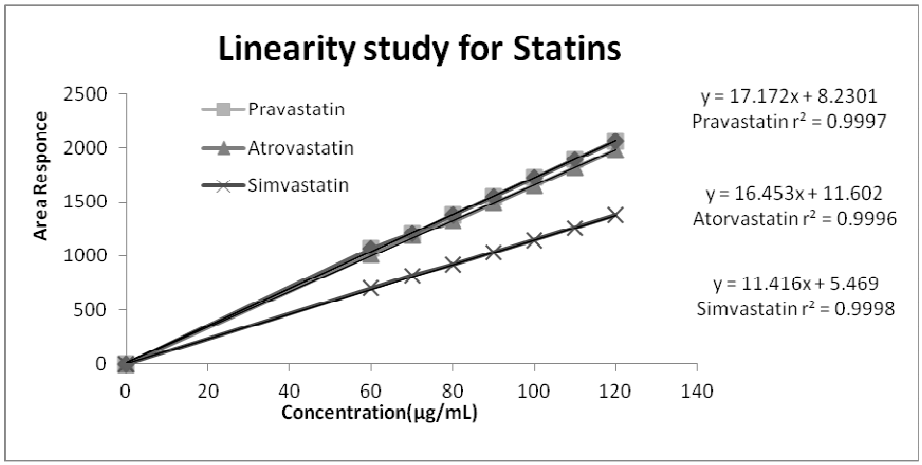

Figure 3: Graph showing linearity and range of statins like pravastatin, atorvastatin and simvastatin

Solution Stability:
Stability studies were carried out by keeping the prepared sample solution at room temperature for 24 hours. The prepared sample solution was analyzed at different time intervals and found that the solution is stable.

Robustness:
The assay results were found to be unaffected by small changes in the chromatographic conditions like concentration of the mobile phase, flow rate and wavelength.

Ruggedness:
Ruggedness of the method was determined between two different analysts and instruments. The value of % RSD was < 2, tailing factor and the theoretical plates found to be well within the system suitability limits. This indicates the ruggedness of the developed analytical method.

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of statins like PRV, ATV and SIM in bulk and pharmaceutical formulations using simple mobile phase with low buffer concentration compared to the reported method. The method gives good resolution between all the three statins with a short analysis time (<15 min). The method was validated and found to be simple, rapid, precise, more accurate, reliable, least time consuming HPLC method for three drugs individually as well as simultaneously. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation and bulk material. Therefore, the proposed method can be used for routine analysis of PRV, ATV and SIM in quality control laboratories and research institutes.

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