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ORIGINAL RESEARCH PAPER

A simple HPLC method containing greener modifier and slighter temperature elevated for simultaneous determination of three statin drugs in tablets

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ABSTRACT

Statins drugs are thought to be among the most prescribed drugs worldwide for the treatment of hypercholesterolaemia. A simple and reliable RP-HPLC method has been successfully employed for simultaneously separating and qualifying three statin drugs including atorvastatin, rosuvastatin and simvastatin in pharmaceutical tablets. The optimal conditions were mobile phase 50:50 (v/v) (formic acid pH 2.50: ETOH), column temperature 40.00 °C, detection wavelength 238.00 nm, and flow rate 1.00 mL/min. The proposed method has been validated based on the ICH guidelines in terms of linearity, precision, accuracy, and limit of detection and limit of quantification. The linear range investigated 2.0–80.0, 4.0–100.00, and 12.00–120.00 µg/mL for rosuvastatin, atorvastatin and simvastatin respectively with coefficients of determination (R^2) within the range of 0.9993–0.9995. The LOD and LOQ for rosuvastatin, atorvastatin and simvastatin were (1.57, 4.76 µg/mL), (1.87, 5.66 µg/mL), (3.46, 10.49 µg/mL) respectively. In addition, in order to evaluate the feasibility of the method developed, it was employed towards the quantification of the pharmaceutical tablets for the analytes investigated and excellent recovery was obtained.

KEYWORDS

statins drugs, RP-HPLC, tablets

INTRODUCTION

Coronary heart disease (CHD) caused by the coronary atherosclerosis is considered to be a major trigger of death worldwide. The accumulation as well as the retention of cholesterol in arterial walls causing significant lipid modification to CHD prevention are the central to the pathogenesis of atherosclerosis. One of the main drugs prescribed to the treatment of hypercholesterolaemia are statins [1]. This is because statins drugs tend to enhance the stability of atherosclerotic plaques, hinder the thrombogenic responses, improve endothelial function, and decrease oxidative stress. Thus, statins have become among the widely prescribed drugs worldwide for reducing morbidity and mortality in CHD patients [2]. The statins drugs are thought to behave as hindrance to the enzyme HMG-CoA reductase which eventually obstruct the alteration of HMG-CoA to mevalonate. There are seven statins drugs available in the market utilised for the treatment including pravastatin, atorvastatin, lovastatin, rosuvastatin, simvastatin, and fluvastatin [3].

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Statins drugs have been investigated largely utilising a reversed-phase liquid chromatography (RP-LC) with either MS, UV detection [4] and various approaches were developed for their determinations in the pharmaceutical tablets form or biological matrixes [5, 6]. There is a huge tendency towards greening analytical approaches owing to its friendly impact towards the environment, which is a great of interest to the industry. Consequently, various protocols have been established so as to develop green HPLC approaches and the main principle to achieve a such goal was minimizing the solvent consumption [7]. This miniaturisation was achieved through the development of HPLC components illustrated in Nano-pump and column related parameters such as reducing the ID of the column, or reduction of the particle size of the column or its length. This approach of miniaturisation HPLC would, consequently, require the adaptation of its components including dwell volume, UV cell volume, connection tubes and so and so forth [7, 8] which is complicated and costly. Coutinho and co-authors [9] used a capillary liquid chromatography (CLC) for the separation of five statins which included fluvastatin, rosuvastatin, simvastatin, pravastatin, and atorvastatin. However, this technology cannot handle high back pressures limiting its applications with columns possessing low particles size ≥ 3 , which is considered as substantial limitation from this particular point of view. Another approaches for the green analytical methods are working at elevated temperatures leading to lower the viscosity of the solvents utilised, which ultimately results in a faster separation attained via using higher flow rates [8] or utilisation of green solvents/additives in the mobile phase [10]. A study towards a greener analytical approach by Karen Gauden et al. [10] was utilising ethanol instead of acetonitrile in order to resolve three statin drugs including pravastatin, atorvastatin, and fluvastatin. In this study, they used 50:50 (v/v) formic acid pH 2.50: ETOH. The organic modifier chosen, ethanol, is considered to be a “greener solvent” in comparison with the most common organic modifier, acetonitrile, due to its sourcing and safety. The method developed was merely focused on studying the analytes examined during pre- formulation phase of the pharmaceutical development. Then, the method was utilised to investigate the solubility's of the analytes in a organic solvent. In addition, the study also used UPLC which is expensive in comparison with the classic HPLC. However, there was no focus on the validation of the analytical methods including linearity, accuracy, stability, precision, LOD & LOQ, which is a crucial element in analytical industrial applications.

Therefore, the aim of this work was to simultaneously resolve and validate three statins including atorvastatin, rosuvastatin, and simvastatin using a simple RP-HPLC containing greener organic solvent and a slight elevated temperature compared with other published methods. In addition, the effect of pH, type and compositions of mobile phase, and column temperature were also examined. Additionally, the proposed was successfully applied to the separation of the analytes investigated in the commercial pharmaceutical tablets.

MATERIALS AND METHODS

Chemicals

Atorvastatin Calcium (95.30% purity) was purchased from Dr. Reddy's Laboratories Ltd, India. Simvastatin (99.80% purity) was obtained from Biocon, India. Rosuvastatin Calcium (95.10% purity) was bought from MSN Laboratories Private limited, India. Formic Acid (98.00% purity) was supplied from Acros, USA. Ethanol (98.00% purity) was purchased from Fisher-Sc., UK. Atorvastatin (10.00, 20.00 & 40.00 mg) tablets, simvastatin (10.00, 20.00 & 40.00 mg) tablets and rosuvastatin (40.00 mg) tablets were provided by Jamjoom Phram products, Saudi Arabia. Whereas, rosuvastatin (Resova 10.00 mg) and rosuvastatin (Crestor 20.00 mg) tablets were obtained from Jazeera Pharmaceutical Industries and a Local market in Saudi Arabia respectively. Hypersil BDS C18 (150.00 cm $\times$ 4.60 mm $\times$ 5.00 μ m) was obtained from Thermo – SCIENTIFIC, USA.

Instrumentations

Chromatographic separations have been conducted utilising SHIMADZU HPLC-18– LC-2010C-HT/diode array detector. A Mettler Toledo analytical balance (USA), a Mettler Toledo pH meter (USA), a Hettich centrifuge (Model D78532, Tuttlingen, Germany), A agitator orbital Shaker (Ivy men, Spain) and a sonicator (Model LUC-405/410/420, Korea) have been used for the preparation of samples and solutions as well as for the mobile phase's filtration. Karal Kolb pump (Scientific Technical Spplies (Model D-6072 Dreieich, West Germany). A Milipore Milford 18.00 M Ω system was utilised for the water purification. The Mini Tab and Excel 2007 software package (USA statistical software) has been employed in order to perform the statistical analysis of data generated.

Solutions and samples preparation

The stock solutions (10 mg/mL) were prepared by dissolving each standard of the drug investigated here in ethanol first then diluted with 50:50 (v/v) formic acid pH 2.50: ETOH. The reason of firstly dissolving with organic solvent was owing to the poor solubility of the drugs in pH used for the analysis. The working solutions were then prepared as per the required concentrations on a daily basis.

Ten tablets of each drug investigated were weighted and crushed using a mortar and pestle so that a true presentation of the tablet weight is obtained. Then, 0.50 mg/mL of each drug investigated were transferred into a 10 mL volumetric flask followed by the addition of 10.00 mL methanol. Subsequently, the solutions were shaken and sonicated for 15 min in order to ensure the solubility of the drugs and attain clear solutions. After that, the solutions were dissolved into the mobile phase 50:50 (v/v) formic acid pH 2.50: ETOH to obtain a sharp peak and avoid peak distortion.

Chromatographic conditions

The optimum chromatographic conditions were achieved with a mobile phase composed of 50:50 (v/v) formic acid pH 2.50: ETOH. The injection volume, column temperature, and flow rate were 5.00 μ L, 40.00 $^{\circ}$ C, and 1.00 mL/min respectively. The detection wavelength was fixed at 238.00 nm [10] and the column utilised was Hypersil BDS C18 (150.00 cm $\times$ 4.60 mm $\times$ 5.00 μ m) Thermo – SCIENTIFIC.

RESULTS AND DISCUSSIONS

Method optimisation

In order to select the best analytical methods, several parameters were investigated including mobile phase type, mobile phase composition and column temperature.

Mobile phase type and composition. The first attempt investigated in the mobile selection was pure water HPLC grade with ethanol HPLC grade at different ratios ranging

from 50.00 to 85.00% water. The point of choosing ethanol over the most used organic solvent, acetonitrile, in RPLC mode was to reduce the effects of solvents on the environment [11]. The best ratio found was 50.00% water and 50.00% ethanol, as observed in Fig. 1.

Effect of pH. Since the analytes studied are charged, it is well established to investigate the effects of pH. As the analytes investigated are acidic, it is suggested to select a pH lower than their pK_a by one-two units so as to accomplish satisfactory retention times for the analytes examined [12, 13]. The pK_a values for rosuvastatin, simvastatin, and atorvastatin are, 3.80, 4.21 and 5.20 respectively. Therefore, pH 2.50 was selected for the analysis, which is within the lower limit acceptable for use with such columns, as recommended by the manufacturer. This is because, with silica columns based, a pH that is lower than 2 could lead the silica to become hydrolysed, while a pH that is higher than 8 could lead to the solubility of the silica (silica dissolution) [14]. As seen in Fig. 2, on one hand, the retention times of rosuvastatin and atorvastatin had a minor shift compared with the conditions mentioned in Fig. 1 which could be overlooked. On the

Fig. 1. Demonstration of the chromatogram for the analytes investigated. Experimental conditions: column, Hypersil BDS C18 column (150 $\times$ 4.6 mm, 5 μ m) mobile phase 50:50 (H_2O : ETOH), detection wavelength 238 nm, flow rate 1 mL/min, injection volume 5 μ L, analytes, column temperature 40 $^{\circ}$ C, Rosuvastatin (1), Atorvastatin (2), Simvastatin (3)

Fig. 2. Demonstration of the chromatogram for the analytes investigated. Experimental conditions: mobile phase 50:50 (water buffered formic acid pH 2.5: ETOH), other conditions as mentioned in Fig. 1

other hand, the retention time of simvastatin has markedly decreased from 29.3 min at neutral media (Fig. 1) to 21.9 min at acidic media (Fig. 2). This behaviour could be due to the protonation of the hydroxyl group ($-OH$) in the cyclohexanol moiety in simvastatin at the pH investigated (pH 2.50). In addition, the value of pK_a (4.21) illustrates that the predominant form under the experiment conditions is $-OH_2^+$. In other words, at pH 2.50 the protonated hydroxyl group would be present in the form of $-OH_2^+$ leading to lesser retention compared with the neutral form of simvastatin.

Effects of column temperature. Column temperature is an effective tool utilised to investigate the resolution in HPLC. This is because this tool leads to a high extent the enhancement of the mass transfer phenomenon which is known as “C term” in van Deemter equation. This enhancement leads, in turn, to an increase in the separation efficiency illustrated in sharp peaks observed [15]. In addition, operating at elevated temperatures leads to decrease the backpressure generated due to a decrease in a solvent viscosity allowing the application of high flow rates [14, 15]. Therefore, several column temperatures were investigated ranging between 25.00 and 60.00 °C. Among the column temperatures studied, it was found that 40.00 °C column temperature was selected as the best values with good chromatographic separation were obtained, as shown in Table 1.

Method validation

One of the main parameters of an analytical method which requires investigation is a method validation [16]. This is because, a such parameter give rise to increase the level of confidence of the method established. Therefore, the method obtained was validated based on the International Conference on Harmonization guidelines (ICH) in terms of linearity, precision, accuracy, stability, limit of detection (LOD) and limit of quantitation (LOQ) [16]. Initially, the selectivity of the proposed RP-HPLC method for the simultaneous determination of atorvastatin, rosuvastatin and simvastatin in pharmaceutical tablets was investigated, utilising diode array detector (DAD) used in this study [17]. This was by comparing the similarity of the chromatogram of each drug in the sample solution with the spectrum received by the standard solution at the wavelength applied. In addition, the

peak identity of each drug was confirmed by spiking different concentrations of standard solutions of each drug in the studied matrix giving linearity equations similar to the calibration equations in aqueous media at the same retention time. Therefore, the obtained results could prove that the selectivity of the proposed method along with the adequate peak purity.

Linearity. It is highly recommended to dissolve the analytes investigated in the mobile phase used for the analysis. This is because, a such practice will lead to obtain a sharp peak and avoid peak distortion (high efficiency) [18]. Several stock solutions were prepared ranging from 2.0 to 80.00, 4.0–100.00, and 12.00–120.00 $\mu\text{g/mL}$ for rosuvastatin, atorvastatin and simvastatin, respectively. They were firstly dissolved in ethanol because of the poor statin stability in an acidic. Then, the solutions prepared were diluted in the acidic solution (mobile phase). The calibration curve was carried out using six concentrations in the appropriate concentrations, as stated in “Solutions and samples preparation” section previously. Each analyte peak area was plotted against the corresponding analyte concentrations in $\mu\text{g/mL}$. In addition, the regression parameters of slope and coefficients of determination were calculated by a least squares linear regression as illustrated in Table 2. Additionally, the calibration curves for rosuvastatin, atorvastatin and simvastatin are shown in Fig. 3a–c respectively. As seen from the figures illustrated, the method optimised is linear demonstrated through obtaining a high value of coefficient of determination, R^2 higher than 0.9993.

Precision and recovery. The precision criterion was examined through replicating the analysis for the analytes investigated within the intra-day (repeatability) and inter-day (reproducibility) under the optimum conditions. Table 3 illustrates the relative standard deviation (RSDs) calculated indicating the precision of the method developed. The recovery criterion was then investigated through the preparation of samples with known quantities of three levels of the target concentrations covering the linear range of analytes. Subsequently, the recovery obtained was calculated via comparing the spiked concentrations of mixed analytes (measured value) against the true value, using the Eq. (1). The recovery values attained indicates that the method developed is accurate, as shown in Table 3.

Table 1. Illustration of the chromatographic characteristics of the analytes investigated, experimental conditions as observed in Fig. 2

Analytes	R_t (min)	N/m (plates) ^a	R_s ^b	K ^c	α ^d
Rosuvastatin	2.50	2049.91	–	0.47	–
Atorvastatin	6.52	4,469.34	13.17	2.84	6.04
Simvastatin	21.85	8,346.03	22.76	11.85	4.17

^a Number of theoretical plates per meter (N/m) = $5.545 (t/W_{50\%})^2$.

^b Resolution of two given peaks (R_s): $R_s = 2 (t_{a2} - t_{a1}) / (W_{a1} + W_{a2})$.

^c Retention factor (k) = $t_R - t_0 / t_0$.

^d Selectivity (α) = $k_2/k_1 = t_{R2} - t_0 / t_{R1} - t_0$.

Table 2. Illustration of some parameters of the analytes investigated, experimental conditions as shown in Fig. 2

Parameters	Rosuvastatin	Atorvastatin	Simvastatin
Concentration range, $\mu\text{g/mL}$	2.0–80.0	4.0–100.0	12.0–120.0
Slope	11,537	11,465	15,671
R^2	0.9993	0.9994	0.9995
Limit of detection ($\mu\text{g/mL}$)	1.57	1.87	3.46
Limit of quantitation ($\mu\text{g/mL}$)	4.76	5.66	10.49

Fig. 3. Illustration of the linearity's calibration curves for Rosuvastatin (A), Atorvastatin (B) Simvastatin (C), experimental conditions as stated in Fig. 2

Limit of detection (LOD) and limit of quantification (LOQ). This criterion was determined via the Eqs. (1) and (2) illustrated below [16]. Table 2, shown previously above, demonstrate the values for the analytes investigated indicating that method developed are sensitive to a high degree compared with such methods for the same analytes studied.

$$\text{L.O.D} = 3.3 \sigma / S \quad (1)$$

$$\text{L.O.Q} = 10 \sigma / S \quad (2)$$

Where:

σ is the standard deviation of response of the calibration curve

S is the slope of the calibration plot.

Applications

When a method is developed, it is commonly practised to examine the feasibility of the method optimised through the application to real samples. Thus, this was conducted via the determination of atorvastatin, rosuvastatin and simvastatin in commercial tablets. The solutions were prepared on a daily basis and were analysed under the optimum conditions, as shown in Fig. 2. The recoveries of the target analytes were calculated are shown in Table 4A and B. As shown in the tables below, the values obtained are to a high extent in line with what reported on the commercial tablets qualifying the method developed to be utilised for a such use.

CONCLUSION

To the best of our knowledge, for the first time, a simple RP-HPLC method has been successfully validated and applied for simultaneous resolving and determining three statins drugs including atorvastatin, rosuvastatin and simvastatin in commercial tablets employing separation method containing greener solvent at a slight elevated temperature. The optimum method was mobile phase 50:50 (v/v) (formic acid pH 2.50: ETOH), column temperature 40.00 °C, detection wavelength 238.00 nm, and flow rate 1.00 mL/min. The linear range examined for rosuvastatin, atorvastatin and simvastatin were 2.0–80.0, 4.0–100.00, and 12.00–120.00 µg/mL respectively. The LOD and LOQ for rosuvastatin, atorvastatin and simvastatin were (1.57, 4.76 µg/mL), (1.87, 5.66 µg/mL), (3.46, 10.49 µg/mL) respectively. Moreover, to assess the practicability of the method established, it was

Table 3. Demonstration of accuracy and precision values for the analytes investigated, experimental conditions as shown Table 2

Parameter	Rosuvastatin			Atorvastatin			Simvastatin		
Concentration, µg/mL	10.00	20.00	40.0	10.00	20.00	40.00	10.00	20.00	40.00
Recovery %	101.36	97.55	112.91	105.08	101.07	104.22	99.25	101.08	98.16
Intra-day, RSD (n = 5)	0.90	1.00	0.80	1.70	1.00	0.90	2.50	1.70	1.00
Inter-day, RSD (n = 15)	4.50	3.20	2.70	5.00	3.50	2.80	4.50	2.90	2.00

Table 4A. Illustration of the quantification and recoveries values for the analytes investigated in tablets with different potencies individually, experimental conditions as stated in Fig. 2

Drug name	Tablet label claim (mg)			Found (mg)			Recovery (%)		
Atorvastatin	10	20	40	9.8	17.12	39.84	98.00	85.6	99.6
Rosuvastatin	10	20	40	10.36	18.72	37.37	103.60	93.6	93.4
Simvastatin	10	20	40	9.7	21.67	41.16	97.90	108.4	102.9

Table 4B. Illustration of the quantification and recoveries values for the analytes investigated in tablets with different potencies in synthetics samples Atorvastatin (1) rosuvastatin (2) simvastatin (3), experimental conditions as stated in Fig. 2

Mixture	Tablet label claim (mg)			Found (mg)			Recovery (%)		
	Drug 1	Drug 2	Drug 3	Drug 1	Drug 2	Drug 3	Drug 1	Drug 2	Drug 3
1	10	20	40	10.18	18.06	39.92	101.80	90.30	99.80
2	10	20	40	10.3	20.6	19.56	103.60	95.50	97.80
3	10	20	40	10.10	20.10	41.40	103.10	113.70	103.50

used for the quantification of the commercial pharmaceutical tablets for the analytes investigated and excellent recovery was obtained. Since the results obtained are promising, the method optimised and validated could be utilised as a potential simple method for the quality control of statins analysis for such pharmaceutical developments.

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