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Simultaneous determination of rosuvastatin and atorvastatin in human serum using RP-HPLC/UV detection: Method development, validation and optimization of various experimental parameters

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ABSTRACT

A novel, precise, accurate and rapid isocratic reversed-phase high performance liquid chromatographic/ultraviolet (RP-HPLC/UV) method was developed, optimized and validated for simultaneous determination of rosuvastatin and atorvastatin in human serum using naproxen sodium as an internal standard. Effect of different experimental parameters and various particulate columns on the analysis of these analytes was evaluated. The method showed adequate separation for rosuvastatin and atorvastatin and best resolution was achieved with Brownlee analytical C18 column (150 × 4.6 mm, 5 μm) using methanol–water (68:32, v/v; pH adjusted to 3.0 with trifluoroacetic acid) as a mobile phase at a flow rate of 1.5 ml/min and wavelength of 241 nm. The calibration curves were linear over the concentration ranges of 2.0–256 ng/ml for rosuvastatin and 3.0–384 ng/ml for atorvastatin. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) for rosuvastatin were 0.6 and 2.0 ng/ml while for atorvastatin were 1.0 and 3.0 ng/ml, respectively. All the analytes were separated in less than 7.0 min. The proposed method could be applied for routine laboratory analysis of rosuvastatin and atorvastatin in human serum samples, pharmaceutical formulations, drug–drug interaction studies and pharmacokinetics studies.

1. Introduction

Rosuvastatin (Fig. 1a), bis{(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl-sulfonyl)amino]pyrimidin-5-yl}](3R,5S)-3,5-di-hydroxyhept-6-enoic acid calcium salt and atorvastatin (Fig. 1b), [R,(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt, belong to the statin class of drugs used to treat hypercholesterolemia both in patients with established cardiovascular disease as well as those who are at a high risk of developing atherosclerosis. These drugs inhibit the rate limiting key enzyme known as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase involved in cholesterol biosynthesis. Statins cause reduction in low density lipoproteins-C (LDL-C), total cholesterol (TC) and triglycerides (TG) and elevation in high-density lipoprotein-C (HDL-C) [1–5]. Besides lipid lowering effects, statins also have potential roles independent of cholesterol reduction as anti-oxidative [6–8], anti-tumor [9], anti-inflammatory [8,10,11], immunomodulator [7,12], anti-malarial [13] and bone forming agents [14]. Thus, due to their so many beneficial effects, there is growing interest in developing analytical methods for statins monitoring. Until the approval of rosuvastatin in 2003, atorvastatin was the most efficacious drug in the statins class [15] but recent studies reported rosuvastatin as a potent inhibitor of HMG-CoA reductase having a higher LDL-lowering effects as compared with other statins [16,17], which demonstrates that both rosuvastatin and atorvastatin are the leading drugs in the statins class.

To date several HPLC–UV and mass spectrophotometric methods have been developed for the quantification of both rosuvastatin and atorvastatin either alone or in combination with other drugs in different matrices. LC/MS/MS methods reported for quantification of rosuvastatin in biological matrices include its determination either alone [18–23], in combination with other drugs such as fenofibric acid [24], or its metabolite N-desmethyl rosuvastatin [25]. HPLC-UV methods have also been reported for the determination of rosuvastatin in pharmaceuticals [26], rat plasma [27] and in human plasma along with gemfibrozil [28].

Similarly atorvastatin has been determined along with its metabolites using LC/MS in biological matrices [29–34]. HPLC-UV methods have also been reported for the determination of atorvastatin alone in biological matrices [35–38], pharmaceutical preparations [36,39] along with impurities in pharmaceutical preparations [40,41] and in combination with amlodipine [42–44],

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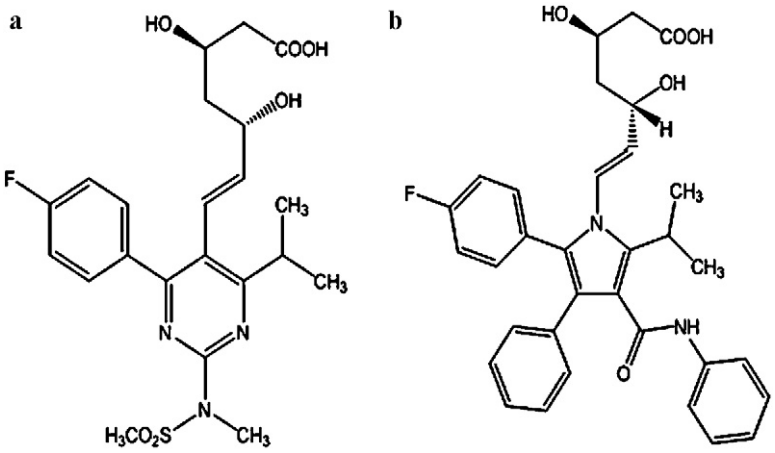

Rosuvastatin Atorvastatin

Fig. 1. Chemical structures of rosuvastatin and atorvastatin.

nicotinic acid [45] and ezetimibe [46,47] in dosage forms. An UPLC method [48] for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets has also been reported.

Several analytical methods have been developed for the determination of two or more than two statins simultaneously. LC/MS/MS and HPLC-UV methods have been reported for simultaneous determination of four statins, i.e. atorvastatin, pravastatin, lovastatin and simvastatin in aqueous samples [49] and five statins, i.e. atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin in pharmaceutical formulations [50], respectively. Similarly, GC/MS and UPLC–MS/MS methods for simultaneous determination of lovastatin, simvastatin, and pravastatin in plasma [51] and simvastatin and atorvastatin in human plasma [52], respectively, have also been reported. To our knowledge no HPLC/UV method has been reported in which both rosuvastatin and atorvastatin are determined simultaneously in human serum however a method in which five statins including rosuvastatin and atorvastatin in pharmaceutical formulations [50] has been reported.

Our suggested method is rapid, versatile, specific, precise and accurate for simultaneous determination of rosuvastatin and atorvastatin in human serum. The method was validated according to standard guidelines and various experimental parameters were optimized with the aim that the reported method could be applied for routine laboratory analysis of these statins, pharmacokinetic drug–drug interaction studies and in pharmaceutical dosage forms. Although neither of the statin is prescribed together with other statin, yet this method will provide ease in the quantification of rosuvastatin and atorvastatin without changes in the chromatographic procedures for individual statin.

2. Experimental

2.1. Chemicals and reagents

Atorvastatin calcium (purity 94.93%) was kindly provided by Pfizer Labs. Ltd. (Karachi, Pakistan), Rosuvastatin calcium (purity 98.4%) by Ferozsons Labs. Pvt. Ltd. (Nowshera, Pakistan) and Naproxen sodium (purity 99.3%) by Saydon Pharma Pvt. Ltd. (Peshawar, Pakistan). Methanol, diethyl ether, absolute ethanol, dichloromethane, chloroform, ethyl acetate, and n-hexane were purchased from Sigma–Aldrich (Oslov, Norway). HPLC grade ultra pure water was prepared by Milli-Q® system (Millipore, Milford, MA, USA).

2.2. Methods

Chromatography was performed with a Perkin Elmer Series 200 system (Norwalk, USA) comprising a Series 200 on-line vacuum degasser, Series 200 auto-sampler, Series 200 Peltier column oven and variable wavelength programmable Series 200 UV–VIS detector. The data acquisition was performed with Perkin Elmer Total-chrom workstation software (version 6.3.1) linked with the LC system via network chromatography interface (NCI) 900. Chromatographic separation was achieved using four different particulate columns: Perkin Elmer Brownlee analytical C18 column (150 mm × 4.6 mm, 5 μm; Shelton, USA), ThermoQuest Hypersil C8 column (150 mm × 4.6 mm, 5 μm; Runcorn, UK), Phenomenex Gemini C18 column (150 mm × 4.6 mm, 5 μm; California, USA) and Thermo Quest Hypersil C18 column (250 mm × 4.6 mm, 5 μm; Runcorn, UK). All columns were protected by a Perkin Elmer pre-column guard cartridge RP18 (30 × 4.6 mm, 10 μm; Norwalk, USA). Centrifugation was carried out with a temperature-controlling centrifuge (model: k-2080, Centurion, UK).

2.3. Preparation of standard solutions

The stock solutions of rosuvastatin, atorvastatin and naproxen sodium (I.S.) were prepared by dissolving appropriate amount corresponding to 1.0 mg/ml concentration of working standards in methanol. All stock solutions were stored at 2–8 °C. The stock solutions of rosuvastatin and atorvastatin were further diluted with the mobile phase methanol–water (68:32, v/v; pH adjusted to 3.0 with trifluoroacetic acid) to give a series of standard mixtures having a final concentration in the range of 2.0–256 ng/ml and 3.0–384 ng/ml, respectively. A working solution of the naproxen sodium (to give a final concentration of 400 ng/ml) was also prepared by diluting its stock solution and added to all standard mixtures and serum samples. A standard 1:1 mixture containing 200 ng/ml of rosuvastatin and atorvastatin was also prepared in methanol.

2.4. Sample preparation

A simple two step liquid–liquid extraction (LLE) procedure was carried out for the extraction of rosuvastatin and atorvastatin from serum samples. A volume (50 μl) of the working solution of the naproxen sodium (to give a final concentration of 400 ng/ml) was added to 200 μl of serum and mixed for approximately 10 s. Then

absolute ethanol (600 μl) was added and vortex-mixed for 2 min for deproteination. In step one, 1.0 ml of diethyl ether (extraction solvent 1) was added, vortex-mixed for 5 min and centrifuged at 3500 rpm at 0 °C for 5 min. The supernatant organic layer was separated in a test tube. In step two, 0.5 ml of dichloromethane (extraction solvent 2) was added, vortexed for 5 min followed by centrifugation at 3500 rpm at 0 °C for 5 min. The organic layer was separated, collected in the same tube and evaporated to complete dryness under the gentle stream of nitrogen on a heating block maintained at 40 °C. After drying, the residue was reconstituted in 500 μl of mobile phase, vortex-mixed for 2 min and 20 μl sample was injected onto HPLC system.

2.5. Chromatographic conditions

Chromatographic separation was performed with different proportions of acetonitrile–water and methanol–water as a mobile phase with different flow rates in the range of 1.0–1.5 ml/min in an isocratic mode. The injection volume was kept in the range of 10–50 μl. The column oven temperature was varied in the range of 25–35 °C and the eluate was monitored using UV detection at various wavelengths in the range of 210–260 nm. Various experimental parameters were optimized for simultaneous determination of rosuvastatin and atorvastatin.

2.6. Method validation

The suggested analytical method was validated according to international guidelines with respect to certain parameters such as specificity/selectivity, linearity, LLOQ, LLOD, precision, accuracy, sensitivity, recovery and robustness/ruggedness [53].

2.6.1. Linearity

The linearity of the method was established by spiking a series of standard mixtures of rosuvastatin (2.0–256 ng/ml), atorvastatin (3.0–384 ng/ml) and a working solution of the internal standard (400 ng/ml) into human serum samples, extracting and analyzing in triplicate. Calibration curves for standard solutions and spiked serum samples were then acquired by plotting their response ratios (ratios of the peak area of the analytes to internal standard) against their respective concentrations. Linear regression was applied and slope (a), intercept (b), correlation coefficient (r) and standard error (Es) were determined.

2.6.2. Precision

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility). In order to determine injection repeatability, serum samples spiked with 256 ng/ml of rosuvastatin and 384 ng/ml of atorvastatin were injected 10 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained. Similarly, analysis repeatability was verified by analyzing five serum samples spiked with 256 ng/ml of rosuvastatin and 384 ng/ml of atorvastatin prepared individually, determined as amount recovered and expressed as mean and %RSD calculated from the data obtained.

For the intermediate precision (intra-day and inter-days reproducibility), serum samples spiked at three different concentration levels were analyzed three times a day in triplicate injections over three consecutive days and expressed as mean ± SD and %RSD calculated from data obtained.

2.6.3. Specificity/selectivity

The specificity/selectivity of the analytical method was investigated by confirming the complete separation and resolution of all the desired peaks of the analytes in mobile phase, spiked human blank serum and standard 1:1 mixture of both statins.

2.6.4. Accuracy

Accuracy was determined in terms of percent recovery. Blank human serum was spiked with the analytes at three different concentration levels (2.0, 32, 256 ng/ml of rosuvastatin and 3.0, 48, 384 ng/ml of atorvastatin) keeping the naproxen sodium concentration constant (400 ng/ml). Another set of standard mixtures at the same concentration levels was also prepared in the mobile phase (methanol–water, 68:32, v/v; pH adjusted to 3.0 with trifluoroacetic acid). The serum was extracted with the procedure noted above and injected onto the HPLC system in triplicate. Percent recoveries for both statins were calculated using the following formula:

$$\%\,\text{Recovery} = \frac{A}{B} \times 100 \quad (1)$$

where A is the response ratio of the analyte with respect to the internal standard in serum sample, B is the response ratio of the analyte with respect to the internal standard in standard mixture.

2.6.5. LLOD and LLOQ

Detection and quantification limits were determined through dilution method using S/N approach by injecting a 20 μl sample. LLOD was considered as the minimum concentration with a signal to noise ratio of at least three ($S/N \approx 3$), while LLOQ was taken as a minimum concentration with a signal to noise ratio of at least ten ($S/N \approx 10$).

2.6.6. Stability

The stability studies of rosuvastatin and atorvastatin spiked serum samples were carried out over a period of 48 h at 25 °C (room temperature under laboratory light), 2–8 °C (refrigerator) and −80 °C (frozen) and standard solutions for one month at 2–8 °C.

2.6.7. Robustness

The robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like column oven temperature (±1 °C), flow rate (±5%) and pH of the mobile phase (±0.2 units).

3. Results and discussion

3.1. Sample preparation

Several organic solvents were tried for the preparation of stock solutions of all analytes. Methanol was selected due to greater solubility of analytes in it. The corresponding working solutions of rosuvastatin, atorvastatin and naproxen sodium were prepared by diluting their stock solutions with methanol–water (68:32, v/v; pH adjusted to 3.0 with trifluoroacetic acid).

Acetonitrile, methanol and methanol–ethanol in different ratios were tried for protein precipitation but complete protein precipitation was achieved with absolute ethanol at least three times the volume of serum. Dichloromethane, ethyl acetate, chloroform, n-hexane and diethyl ether were evaluated either alone or in different ratios for the extraction of all the analytes from the serum. Recovery of the rosuvastatin was better when extracted with diethylether, while atorvastatin was better extracted with dichloromethane. So best results in terms of recoveries were obtained with a simple two step LLE procedure involving extraction with 2 parts (1.0 ml) of diethyl ether (extraction solvent 1) followed by 1 part (0.5 ml) of dichloromethane (extraction solvent 2). Organic layers from both steps 1 and 2 were combined together and evaporated to dryness under gentle stream of nitrogen. The residue was reconstituted in

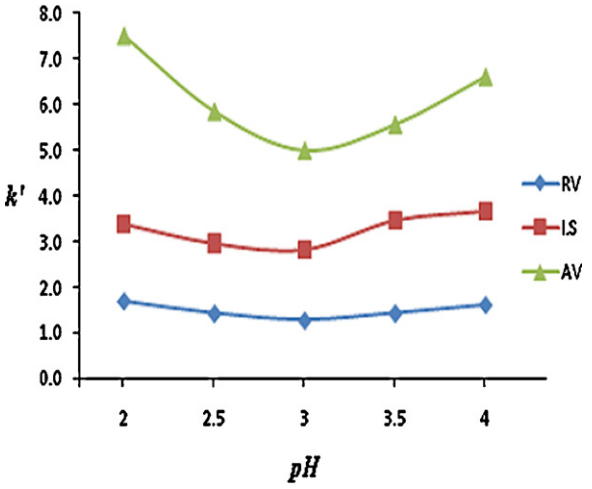

Fig. 2. Effect of variation in pH of the mobile phase on the retention factors (k') of the analytes.

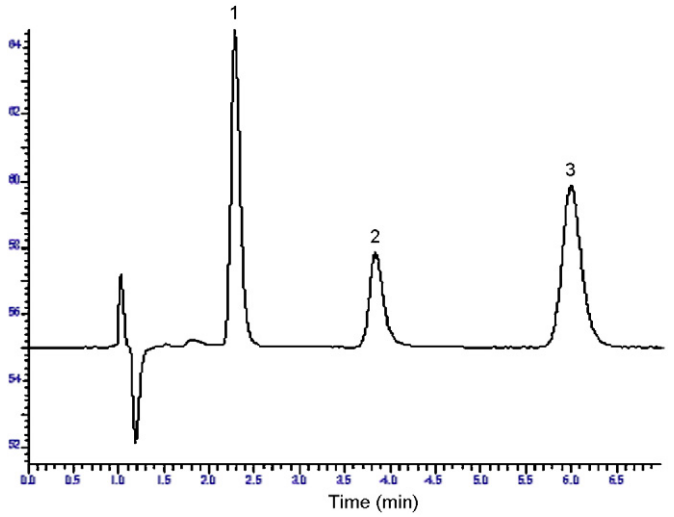

Fig. 3. RP-LC chromatogram on Perkin Elmer Brownlee analytical C18 column representing peaks of rosuvastatin (peak 1), internal standard (peak 2) and atorvastatin (peak 3).

500 μl of mobile phase and 20 μl sample was injected onto HPLC system.

3.2. Method optimization (experimental parameters optimization)

Feasibility of different solvent systems such as acetonitrile–water and methanol–water mixtures in different compositions, pumped at different flow rates (in the range of 1.0–1.5 ml/min) having variable pH range (2.0–4.0) and at different column oven temperatures (in the range of 25–35 °C) were evaluated. Best results were obtained using methanol–water in the ratio of 68:32, v/v (pH adjusted to 3.0 with trifluoroacetic acid) at a flow rate of 1.5 ml/min. While optimizing the composition of the mobile phase, the pH was fixed to 3.0 and while assessing the effect of pH of the mobile phase, the mobile phase composition was methanol–water (68:32, v/v). The retention of all analytes varied considerably by changing the pH of the mobile phase in the range of 2.0–4.0. Since both rosuvastatin ($pk_a = 4.6$) and atorvastatin ($pk_a = 4.46$) are acidic compounds so their retention on the column is likely to be pH dependant. When pH of the mobile phase was decreased from 4.0 to 3.0 the retention times of the analytes decreased unexpectedly and with further decrease in the pH to 2.0 the retention times increased once again. This behavior may be due to a change in the solubility of the analytes in the mobile phase or may be due to change in binding of the analytes to the stationary phase. Therefore, pH 3.0 was chosen as optimum pH because of the reasonable retention times, resolution and separation of all the compounds of interest. Retention factors or capacity factors (k') of both statins and naproxen sodium were plotted against various pHs of the mobile phase (Fig. 2).

The effect of column oven temperatures on the analysis of both statins was also evaluated in the range of 25–35 °C and best results were observed at 25 °C in terms of retention factor and resolution. Increasing the temperature above 25 °C resulted in the rapid elution of rosuvastatin closer to the solvent front.

Various detection wavelengths in the UV range of 210–260 nm were tried for monitoring of all analytes. Keeping in view the theoretical values of molar absorptivity co-efficients of rosuvastatin and atorvastatin, the wavelength 241 nm was selected as the optimum wavelength for simultaneous determination of rosuvastatin and atorvastatin.

Besides naproxen sodium other internal standards were also tested including paracetamol, diclofenac sodium and simvastatin. Paracetamol eluted with the solvent front, diclofenac sodium gave a peak which showed poor resolution from atorvastatin, while simvastatin had a very large retention time and poor recovery with the selected solvents. Therefore, naproxen was preferred on the basis of good resolution, compatibility and comparatively better recovery.

Four different types of analytical columns were also tested including both C8 and C18 having lengths of 15 cm and 25 cm, respectively from various manufacturers, Viz. Perkin Elmer Brownlee analytical C18 column (150 × 4.6 mm, 5 μm; Shelton, USA), ThermoQuest Hypersil C8 column (150 × 4.6 mm, 5 μm; Runcorn, UK), Phenomenex Gemini C18 column (150 × 4.6 mm, 5 μm; California, USA), Thermo Quest Hypersil C18 column (250 × 4.6 mm, 5 μm; Runcorn, UK). Other parameters were kept constant while evaluating all of the four columns. Perkin Elmer Brownlee analytical C18 column was selected as the best column on the basis of excellent peak parameters (separation, retention, height, asymmetry, tailing, and resolution) and run time. A typical chromatogram representing separation of rosuvastatin, atorvastatin and naproxen sodium on Perkin Elmer Brownlee analytical C18 column is shown in Fig. 3. In case of other analytical columns, Phenomenex Gemini C18 column had a comparatively larger analysis time, i.e. more than 8 min as compared to others. Thermo Quest Hypersil C18 column (25 cm) showed better efficiency due to its greater length but separation factor for rosuvastatin is relatively small and had a comparatively large run time. Although ThermoQuest Hypersil C8 column had shorter analysis time, i.e. less than 4 min than all others but retention factor of rosuvastatin is small and showed poor resolution and peaks separation in case of spiked serum samples. Retention factors (k') were plotted against various particulate columns (Fig. 4).

3.3. Method validation

3.3.1. Linearity

The response was found linear over a concentration range of 2.0–256 ng/ml for rosuvastatin and 3.0–384 ng/ml for atorvastatin. The correlation co-efficients for both statins were 0.999. The linearity equations and standard errors for the calibration curves of standard mixtures and spiked serum samples of both statins are presented in Table 1.

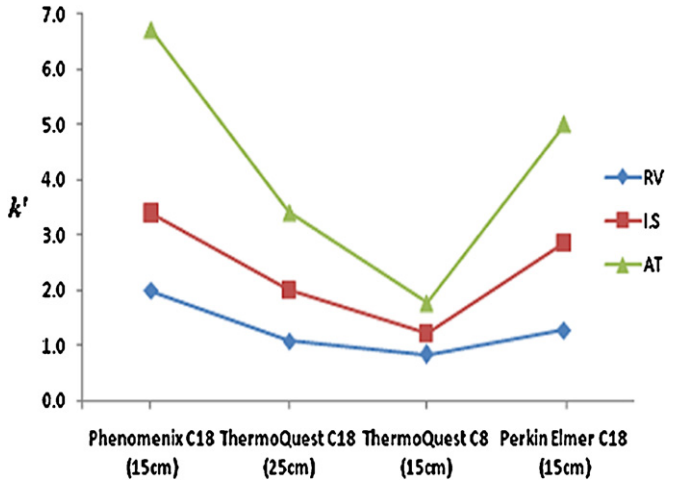

Fig. 4. Effect of different particulate columns on the retention factors (k') of the analytes.

3.3.2. Accuracy and recovery

Average percent recoveries for rosuvastatin and atorvastatin were above 97.0% and 98.0%, respectively, while %RSD values for both statins were less than 1% indicating accuracy of the reported method.

Table 1
Calibration range, linearity, sensitivity repeatability and accuracy of the method.

Parameters	Analytes	
	Rosuvastatin	Atorvastatin
Accuracy (mean % recovery ± SD)		
Spiked concentration level 1[a]	97.0 ± 0.2	98.4 ± 0.4
Spiked concentration level 2[a]	97.1 ± 0.3	97.9 ± 0.2
Spiked concentration level 3[a]	97.2 ± 0.3	98.3 ± 0.2
Accuracy (% RSD)		
Spiked concentration level 1[a]	0.24	0.43
Spiked concentration level 2[a]	0.28	0.22
Spiked concentration level 3[a]	0.35	0.15
Calibration range (ng/ml)	2.0–256.0	3.0–384.0
Linearity		
Standard mixtures		
Slope (b)	0.007	0.005
Intercept (a)	0.003	0.002
Correlation coefficient (r)	0.999	0.999
Standard error (Es)	0.006	0.003
Spiked serum samples		
Slope (b)	0.007	0.005
Intercept (a)	0.002	0.003
Correlation coefficient (r)	0.999	0.999
Standard error (Es)	0.008	0.019
Repeatability		
Injection repeatability (mean; %RSD)		
Spiked concentration level 3[b]	[c]2.30; 0.47	[c]6.00; 0.19
Spiked concentration level 3[b]	[d]53573; 0.45	[d]56330; 1.02
Analysis repeatability (mean; %RSD)		
Spiked concentration level 3[b]	248.50; 0.71	376.34; 0.57
Sensitivity		
Lower limit of detection, LLOD (ng/ml)	0.6	2.0
Lower limit of quantification, LLOQ (ng/ml)	2.0	3.0

Spiked concentration level 1 = rosuvastatin: 2.0 ng/ml and atorvastatin: 3.0 ng/ml; Spiked concentration level 2 = rosuvastatin: 32.0 ng/ml and atorvastatin: 48.0 ng/ml; Spiked concentration level 3 = rosuvastatin 256.0 ng/ml and atorvastatin: 384.0 ng/ml.

[a] $n = 5$.
[b] $n = 10$.
[c] Retention time (min).
[d] Peak area. [e] Amount recovered.

Table 2
Intra-day and inter-day precision data ($n = 3$).

Known concentration spiked	Concentration found (ng/ml)			
	Intra-day (mean ± SD)	%RSD	Inter-day (mean ± SD)	%RSD
Rosuvastatin				
2	1.94 ± 0.01	0.64	1.94 ± 0.02	1.15
32	30.18 ± 0.14	0.46	30.14 ± 0.26	0.87
256	249.13 ± 0.54	0.22	248.14 ± 1.43	0.58
Atorvastatin				
3	2.98 ± 0.01	0.44	2.96 ± 0.03	1.06
48	47.11 ± 0.24	0.50	47.06 ± 0.33	0.71
384	378.57 ± 0.72	0.19	377.90 ± 2.40	0.63

3.3.3. Precision

Precision data representing both repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility) are summarized in Tables 1 and 2, respectively. The %RSD values for both intra-day and inter-days were less than 2.0%, which indicates that the proposed method is precise.

3.3.4. Specificity/selectivity

Representative chromatograms of blank serum (b), serum spiked with internal standard (c), 1:1 mixture containing 200 ng/ml each of rosuvastatin and atorvastatin and 400 ng/ml of naproxen sodium as internal standard (d) and serum sample spiked with standard mixture containing 256 ng/ml of rosuvastatin and 384 ng/ml of atorvastatin and 400 ng/ml of naproxen sodium as internal standard (e) are shown in Fig. 5, confirming the absence of interference from any endogenous component of the serum. Rosuvastatin, naproxen sodium and atorvastatin were well resolved and completely separated at retention times of 2.3, 3.8 and 6.0 min, respectively.

3.3.5. LLOD and LLOQ

The LLOD for rosuvastatin and atorvastatin standard solutions were found to be 0.6 and 1.0 ng/ml, respectively, while LLOQ were

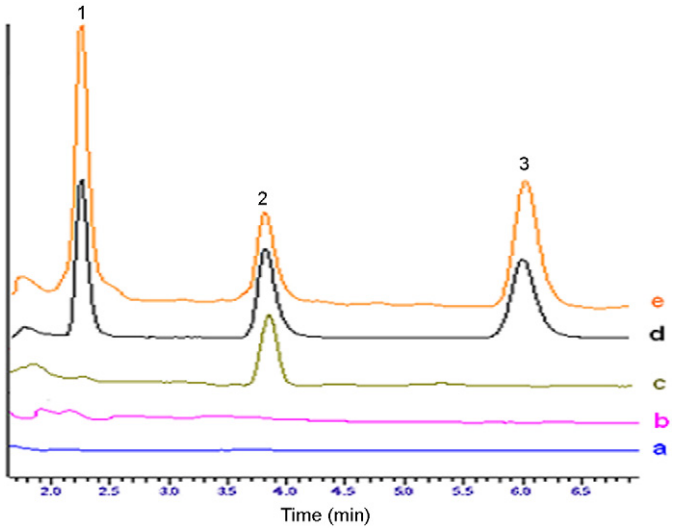

Fig. 5. RP-LC chromatograms of different samples showing complete resolution of all peaks. Peak—1: rosuvastatin, 2: internal standard and 3: atorvastatin. Chromatograms a: blank solvent; b: blank serum; c: blank serum spiked with 400 ng/ml of internal standard; d: standard 1:1 mixture containing 200 ng/ml of rosuvastatin (peak 1) and atorvastatin (peak 3) each and internal standard (peak 2) having concentration of 400 ng/ml; e: serum sample spiked with standard mixture containing 256 ng/ml of rosuvastatin and 384 ng/mlof atorvastatin and 400 ng/ml of internal standard.

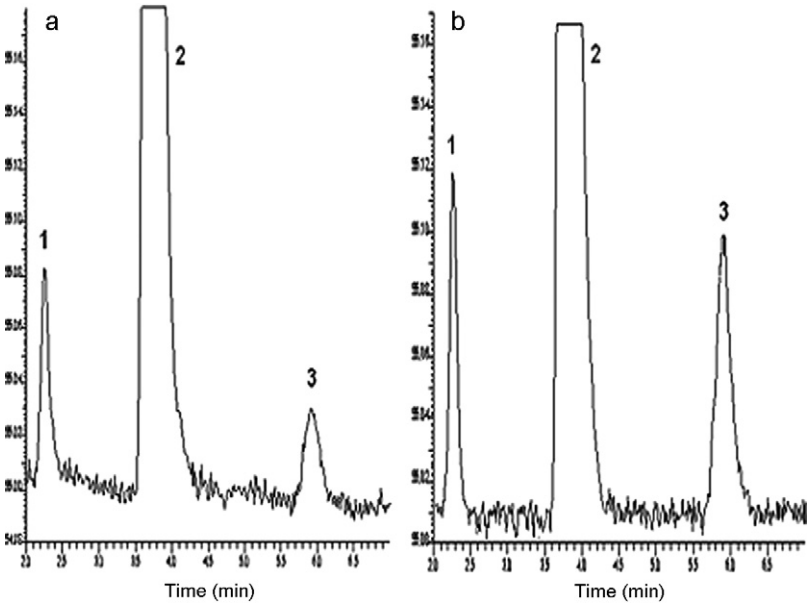

Fig. 6. RP-LC chromatograms showing peaks of rosuvastatin (peak 1) and atorvastatin (peak 3) at the level of LLOD (a) and LLOQ (b).

found to be 2.0 and 3.0 ng/ml, respectively, as presented in Table 1. Chromatograms representing peaks of rosuvastatin and atorvastatin at their LOD and LOQ levels are given in Fig. 6.

3.3.6. Stability

Results from the stability studies of both spiked serum samples and standard solutions indicated that spiked serum samples were stable for 48 h when stored at room temperature (25 °C), refrigerator (2–8 °C) and frozen (−80 °C), while the standard solutions demonstrated stability for one month at 2–8 °C.

3.3.7. Robustness

Minor deliberate changes in different experimental parameters such as column oven temperature (±1 °C), flow rate (±5%) and pH of the mobile phase (±0.2 units) did not significantly affect the recoveries, peak area and retention time of both statins indicating that the proposed method is robust.

4. Conclusion

A novel, simple, rapid and cost effective RP-HPLC/UV method was successfully developed for simultaneous determination of rosuvastatin and atorvastatin in human serum. The proposed method was optimized and validated for the various experimental parameters. Influence of pH of the mobile phase, column oven temperature and various particulate columns on the analysis of rosuvastatin, atorvastatin and naproxen sodium was evaluated. Retention factors (k') were plotted against the aforementioned parameters. In addition, the method employed a simple two step LLE procedure exhibiting excellent recoveries of both statins and internal standard. All the analytes were well resolved and separated in less than 7.0 min. This method offers advantage of simultaneous determination of two clinically important and widely prescribed statins in a single chromatographic run. There is no need of change in chromatographic procedures for the analysis of individual statin. Our developed method will be applied for assessing the pharmacokinetics and drug–drug interaction studies of these statins with other commonly prescribed drugs. This method could also be used for the analysis of these drugs in pharmaceutical preparations and routine laboratory analysis with slight modification in the extraction procedure. Overall, the proposed method provides high throughput for simultaneous determination of rosuvastatin and atorvastatin in human serum with excellent accuracy, precision, selectivity and reproducibility.

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