AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

SIMULTANEOUS DETERMINATION OF ROSUVASTATIN AND FENOFIBRIC ACID IN HUMAN PLASMA BY LC-MS/MS AND ITS APPLICATION TO A HUMAN PHARMACOKINETIC STUDY

Seshagiri Rao JVLN [1*], Nageswara Rao Pilli [2], Vijaya Kumari Karra [2], Jaswanth Kumar Inamadugu [3]

1. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India
2. University College of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500 085, India
3. Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, India

ABSTRACT

A rapid and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method has been developed and fully validated for simultaneous quantification of two lipid lowering agents rosuvastatin and fenofibric acid in human plasma. Lovastatin was used as an internal standard. Analytes and the internal standard were extracted from human plasma by liquid-liquid extraction technique using a 50:50, v/v mixture of ethyl acetate and diethyl ether. The reconstituted samples were chromatographed on a C_{18} column by using a 80:20, v/v mixture of acetonitrile and 0.1% formic acid as the mobile phase at a flow rate of 1.0 mL/min. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 0.10-80.00 ng/mL for rosuvastatin and 50-9003 ng/mL for fenofibric acid. The multiple reaction monitoring mode was used for quantification of ion transitions at *m/z* 482/258, 319/233 and 405/199 for the rosuvastatin, fenofibric acid and the internal standard, respectively. The results of the intra-day and inter-day precision and accuracy study results were well within the acceptable limits. A run time of 3.0 min for each sample made it possible to analyze more than 300 plasma samples per day. The proposed method was successfully applied for the determination of the fenofibric acid in real time plasma samples for pharmacokinetic studies.

Key words: Rosuvastatin, fenofibric acid, human plasma, LC-MS/MS quantification, pharmacokinetics.

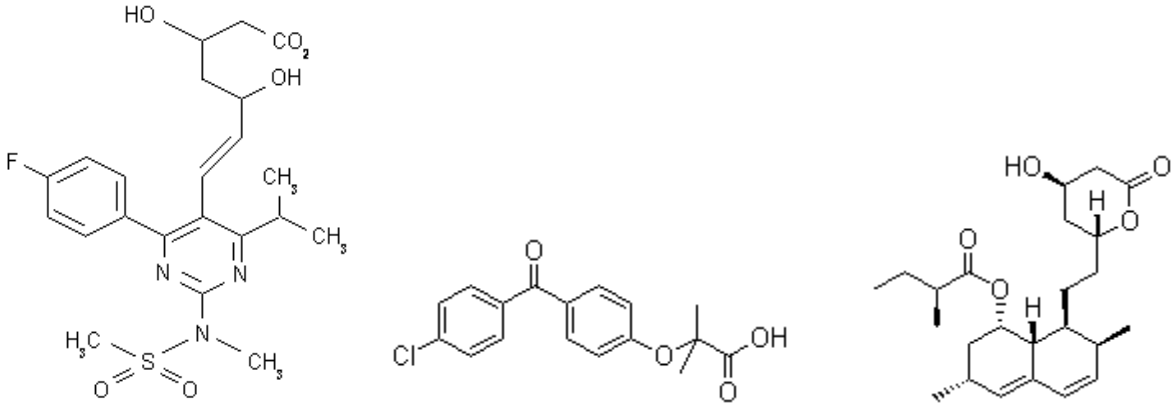
*Corresponding Author Email: jvlnsrao@rediffmail.com

Received 1 December 2011, Accepted 30 December 2011

Please cite this article in press as: Seshagiri RJVLN *et al.*, Simultaneous Determination of Rosuvastatin and Fenofibric Acid in Human Plasma by LC-MS/MS and Its Application to a Human Pharmacokinetic Study. American Journal of PharmTech Research 2012.

INTRODUCTION:

Achievement of balanced cholesterol levels is an important objective of lipid lowering therapy [1]. Many patients taking this therapy fail to achieve their cholesterol levels, especially those with coronary heart disease (CHD). Statins are the most commonly used lipid lowering drugs[2,3]. Statin drugs reduce cholesterol biosynthesis by competitively inhibiting the activity of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is essential for converting HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol synthesis[4,5]. Rosuvastatin (Figure 1) is a potent statin, has been developed for the treatment of dyslipidemia [5-9]. Rosuvastatin protects from ischemic stroke, reduces in the low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides (TG) and increases the high-density lipoprotein cholesterol (HDL-C) [10-12]. Fenofibrate (Figure 1) is a lipid-lowering agent used in the treatment of dyslipidemia [13]. After absorption, fenofibrate is metabolized by CYP 3A4 isozyme to its active metabolite, fenofibric acid, contributing to the reduction in the total cholesterol, apolipoprotein B, total triglycerides and triglycerides rich lipoprotein [14-15]. After oral dosing of fenofibrate, the maximum plasma concentrations of fenofibric acid were observed within 4-5 hours. No unchanged fenofibrate was detected in plasma after oral dosing. Rosuvastatin and fenofibrate regulate the lipid levels in the body by different modes of action and make a well-tolerated combination [16]. Patients with dyslipidemia who have not achieved results with monotherapy may benefit from the combination of these agents.



Rosuvastatin Fenofibric acid Lovastatin

Figure 1. Chemical structures of Rosuvastatin, Fenofibric acid and Lovastatin (IS).

Only one LC-MS/MS [17] method is reported in literature for the simultaneous quantification of rosuvastatin and fenofibric acid in human plasma. This method has several limitations like lack of sensitivity and longer chromatographic run time. In the present study, a simple, rapid and

reproducible method has been developed and fully validated to estimate rosuvastatin and fenofibric acid concentrations simultaneously in human plasma without compromising on the sensitivity reported earlier for both the drugs. Indeed in the present study we have achieved a higher sensitivity for fenofibric acid. The method meets the requirements and provides a high degree of accuracy, sensitivity and specificity by simple liquid-liquid extraction technique with commercially available internal standard using high performance liquid chromatography and detection by electrospray tandem mass spectrometry. The proposed assay method was successfully applied to a pharmacokinetic study in humans.

MATERIALS AND METHODS

Reagents and chemicals

The reference sample of rosuvastatin (>99.60%), fenofibric acid (>99.20%) and lovastatin (Figure 1) (>99.70%) were purchased form Neucon Pharma Pvt. Ltd, Goa, India. Water used for the LC-MS/MS analysis was prepared from Milli Q water purification system. Acetonitrile and methanol were of HPLC grade (J.T Baker, USA) and ethyl acetate and diethyl ether was purchased from Merck (Darmstadt, Germany). Analytical grade formic acid and acetic acid were purchased from Merck (Darmstadt, Germany). The control human plasma (K2. EDTA) sample was procured from Cauvery Diagnostics & Blood Bank (Secunderabad, India).

Instrumentation and chromatographic conditions

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary LC-20AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20A_3) were used for the study. Aliquots of the processed samples (20 µL) were injected into the BDS Hypersil, C_{18} (100 X 4.6 mm, 5µm column) which was kept at room temperature. The isocratic mobile phase, a 80:20, v/v mixture of acetonitrile and 0.1% formic acid was delivered at 1.0 mL/min into the electrospray ionization chamber of the mass spectrometer. Quantitation was achieved with MS-MS detection in positive ion mode for both the analytes and the internal standard using a MDS Sciex API-4000 mass spectrometer (Foster City, CA, USA) equipped with a Turboionspray ™ interface at 500 ºC. The ion spray voltage was set at 5000 V.

The source parameters viz. the nebulizer gas, curtain gas, auxillary gas and collision gas were set at 30, 10, 35 and 10 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 90, 48, 10, 5 V for rosuvastatin, 60, 22, 10, 5 V for fenofibric acid and 55, 17, 10, 12 V for IS. Detection of the ions was carried out in the multiple-reaction monitoring mode (MRM), by

monitoring the transition pairs of *m/z* 482.3 precursor ion to the *m/z* 258.3 for rosuvastatin, *m/z* 319.1 precursor ion to the *m/z* 233.1 for fenofibric acid and *m/z* 405.2 precursor ion to the *m/z* 199.2 product ion for the IS. Quadrupoles Q1 and Q3 were set on unit resolution. The analysis data obtained were processed by Analyst software™ (version 1.4.2).

Preparation of stock solutions of analytes and IS

Stock solutions of rosuvastatin, fenofibric acid and lovastatin (internal standard, IS) were prepared separately by dissolving in methanol at 1 mg/mL concentration. The stock solutions of rosuvastatin and fenofibric acid were serially diluted with 60:40, v/v mixture of acetonitrile and water (diluent) to produce working solutions. Another set of working solutions of rosuvastatin and fenofibric acid was made in diluent (from primary stock) at appropriate dilutions for preparation of QC samples. A working IS solution (2056 ng/mL) was prepared in diluent. Working solutions were stored approximately at 2-8 °C for fifteen days (data not shown).

Preparation of calibration curve standards and quality control samples

Calibration samples were prepared by spiking 950 µL of control human plasma with the appropriate working solution of the each analyte (25 µL dilution of rosuvastatin and 25 µL of fenofibric acid). Calibration curve standard consists of a set of nine non-zero concentrations ranging from 0.10 to 80.00 ng/mL for rosuvastatin and 50 to 9003 ng/mL for fenofibric acid were prepared. Samples for the determination of precision and accuracy were prepared by spiking control human plasma in bulk with rosuvastatin and fenofibric acid at appropriate concentrations and 700 µL plasma aliquots were distributed into different tubes. The QCs prepared for each analyte are: for rosuvastatin – 0.10 (LLOQ), 0.30 (LQC), 10.11 (MQC1), 40.44 (MQC2) and 70.33 ng/mL (HQC) and for fenofibric acid – 50 (LLOQ), 153 (LQC), 1017 (MQC1), 4520 (MQC2) and 7793 ng/mL (HQC). All the prepared plasma samples were stored at -70 ± 10 °C.

Sample preparation

A 500-µL volume of the plasma sample was transferred to a 15-mL glass test tube, and to it 50 µL of working concentration of the IS (2056 ng/mL) was spiked. To this 100 µL of 10% acetic acid was added. After vortexing for 30 s, a 4-mL aliquot of the extraction solvent (ethyl acetate – diethyl ether, 50:50, v/v) was added using Dispensette Organic (Brand GmbH, Wertheim, Germany). The sample was shaken for 10 min using a reciprocating shaker (Scigenics Biotech, Chennai, India) and then centrifuged for 2 min at 4000 rpm using a Heraeus Megafuse 3SR, Japan centrifuge. The organic layer (3.0 mL) was transferred to a 5-mL glass test tube and

evaporated at 40ºC under a stream of nitrogen. The dried extract was reconstituted with 250-µL of the mobile phase and a 20-µL aliquot was injected into the column.

Validation parameters

A thorough validation of the method was carried out as per the US FDA guidelines (US DHHS, FDA, CDER, 2001). The method was validated for selectivity, sensitivity, matrix effect, linearity, precision, accuracy, recovery, dilution integrity and stability. Selectivity of the method was assessed by analyzing six blank human plasma matrix samples. The responses of the interfering substances or background noises at the retention time of the rosuvastatin and fenofibric acid are acceptable if they are less than 20% of the response of the lowest standard curve point or LLOQ. The responses of the interfering substances or background noise at the retention time of the internal standard are acceptable if they are less than 5% of the mean response of internal standard.

Sensitivity was established from the background noise or response from six spiked LLOQ samples. The six replicates should have a precision of ≤20% and an accuracy of ±20%. Matrix effect is investigated to ensure that precision, selectivity and sensitivity are not compromised by the matrix. Matrix effect was checked with six different lots of EDTA plasma. Three replicate samples each of LQC and HQC were prepared from different lots of plasma. (36 QC samples in total).

Linearity was tested for rosuvastatin and fenofibric acid in the concentration range of 0.10-80.00 and 50-9003 ng/mL, respectively. For the determination of linearity, standard calibration curves containing at least 9 points (non-zero standards) were plotted and checked. In addition, blank plasma samples were also analyzed to confirm the absence of direct interferences, but these data were not used to construct the calibration curve. The acceptance limit of accuracy for each of the back-calculated concentrations is ±15% except LLOQ, where it is ±20%. For a calibration run to be accepted at least 75% of the standards, including the LLOQ and ULOQ are required to meet the acceptance criterion otherwise, the calibration curve is rejected. Five replicate analyses were performed on each calibration standard. The samples were run in the order from low to high concentration.

Intra-assay precision and accuracy were determined by analyzing six replicates at five different QC levels on two different days. Inter-assay precision and accuracy were determined by analyzing six replicates at five different QC levels on five different runs. The acceptance criteria included accuracy within ±15% deviation (SD) from the nominal values, except LLOQ QC,

where it should be ±20% and a precision of ≤15% relative standard deviation (RSD), except for LLOQ QC, where it should be ≤20%.

Recovery of the analytes from the extraction procedure was determined by comparing the peak areas of the analytes in spiked plasma samples (six each of low, medium2, and high QCs) with the those of the analytes in samples prepared by spiking the extracted drug-free plasma samples with the same amounts of the analytes at the step immediately prior to chromatography. Similarly, recovery of the IS was determined by comparing the mean peak areas of the extracted QC samples (n=6) with those of the IS in samples prepared by spiking the extracted drug-free plasma samples with the same amounts of IS at the step immediately prior to chromatography.

The dilution integrity exercise is performed with an aim to validate the dilution test to be carried out on higher analyte concentrations above the ULOQ during real time analysis of subject samples. Dilution integrity experiment was carried out at 1.7 times the ULOQ concentration for both the analytes. Six replicates each of 1/2 and 1/4th concentrations were prepared and their concentrations were calculated by applying the dilution factor 2 and 4.

Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The stock solution stability at room temperature (8 h) and refrigerated conditions for fifteen days (2-8 °C) was performed by comparing the area response of the analytes (stability samples) with the response of the sample prepared from fresh stock solution. Bench top stability (10 h), processed samples stability (Autosampler stability for 46 h, wet extract stability for 24 h and reinjection stability for 24 h), freeze-thaw stability (three cycles), long-term stability (60 days) were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% SD) and precision (≤15% RSD).

RESULT AND DISCUSSION

Mass spectrometry

Mass parameters were tuned in both positive and negative ionization modes for the analytes. Good response was found in positive ionization mode. Data of the MRM mode was considered to get better selectivity. Protonated form of each analyte and IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition for rosuvastatin was monitored from *m/z* 482.3 to 258.3, for fenofibric acid was monitored from *m/z* 319.1 to 233.1 and for IS was monitored from *m/z* 405.2 to 199.2.

Some earlier publications have discussed the details of fragmentation patterns of rosuvastatin [18], fenofibric acid [19] and lovastatin [20] we are not presenting the data pertaining to this.

Method development

Separation was attempted using various combinations of acetonitrile and buffer with varying contents of each component on different columns like C_8 and C_{18} of different makes like Grace, Chromolith, Hypersil, Hypurity advance, Zorbax, Kromasil, Ace and Intertsil etc. Use of a 0.1% formic acid helped in achieving good response for MS detection in the positive ionization mode. A mobile phase consisting of acetonitrile and 0.1% formic acid (80:20, v/v) was found suitable, as the analytes were protonated and well separated in this phase. BDS Hypersil, $C_{18,}$ 100 X 4.6 mm, 5 µm column (Make: Thermo Corporations) gave a good peak shape and response even at LLOQ level for both the analytes and IS. The mobile phase was operated at a flow rate of 1.0 mL/min. The retention time of rosuvastatin, fenofibric acid and IS are low enough (1.20, 1.60 and 2.40 min) allowing a small run time of 3.0 min.

Liquid-liquid extraction (LLE) technique was employed for the sample preparation in this work. LLE is helpful in producing a spectroscopically clean sample and avoiding the introduction of non-volatile materials onto the column and MS system and also minimizing the experimental cost. Clean samples are essential for minimizing ion suppression and matrix effect in LC-MS/MS. Among the different solvents checked alone and in combination for their suitability, ethyl acetate and diethyl ether in ratio of 50:50, v/v was found to be optimal, which can produce a clean chromatogram for a blank sample and yields the good recovery for the analytes from the plasma.

A good internal standard must mimic the analyte during extraction and compensate for any analyte on the column. For LC-MS/MS analysis, use of stable isotope-labeled drugs as internal standards proves to be helpful when a significant matrix effect is possible. Isotope-labeled analyte was not available to serve as IS, so, in the initial stages of this work, several compounds were investigated to find a suitable IS and finally lovastatin was found to be best for the present purpose.

Selectivity and chromatography

The degree of interference by endogenous plasma constituents with the analytes and IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in Figure 2 & 3, no significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free plasma at the retention time of the analytes.

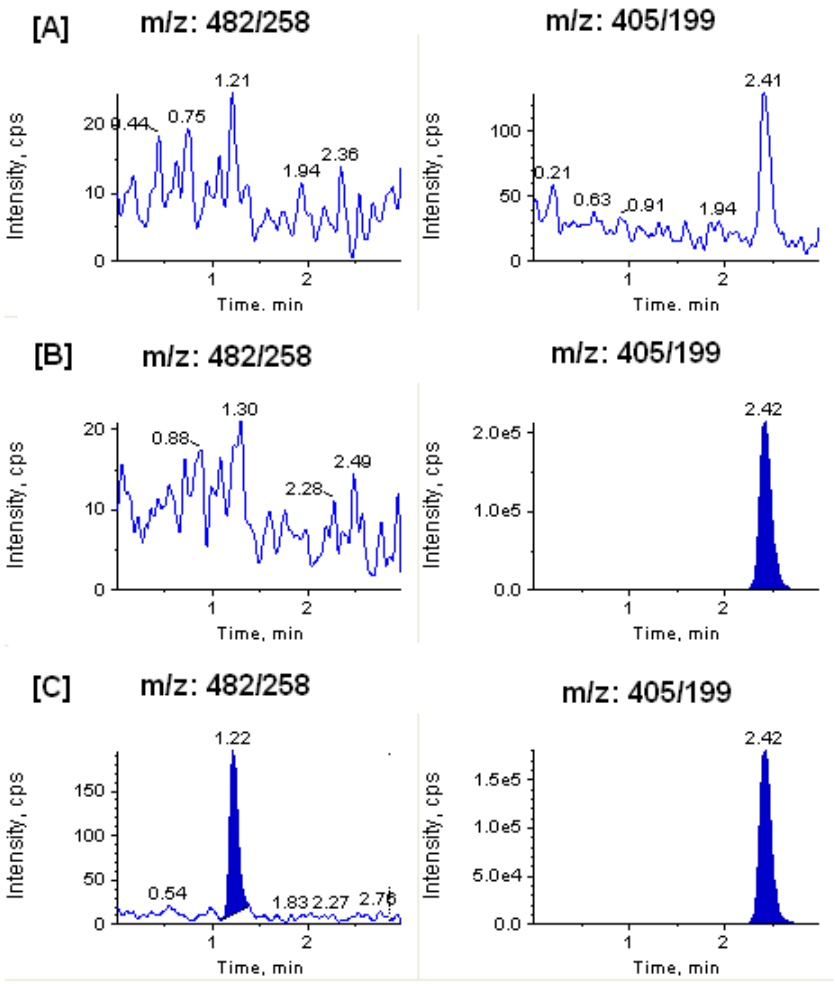


Figure 2. Typical MRM chromatograms of rosuvastatin (left panel) and IS (right panel) in (A) human blank plasma (B) human plasma with IS (C) a LLOQ sample along with IS.

Sensitivity

The lowest limit of reliable quantification for the analytes was set at the concentration of the LLOQ. The precision and accuracy at LLOQ concentration were found to be 6.5% and 100.5%, 3.8% and 103.6% for rosuvastatin and fenofibric acid, respectively.

Extraction efficiency

A simple liquid/liquid extraction with ethyl acetate and diethyl ether combination proved to be robust and provided cleanest samples. The recoveries of analytes and IS were good and reproducible. The mean overall recoveries (with the precision range) of rosuvastatin, fenofibric acid and IS were 65.2 ± 1.8% (0.5-7.4%), 67.5 ± 2.0% (1.7-6.6%) and 62.5 (3.0-6.0%), respectively.

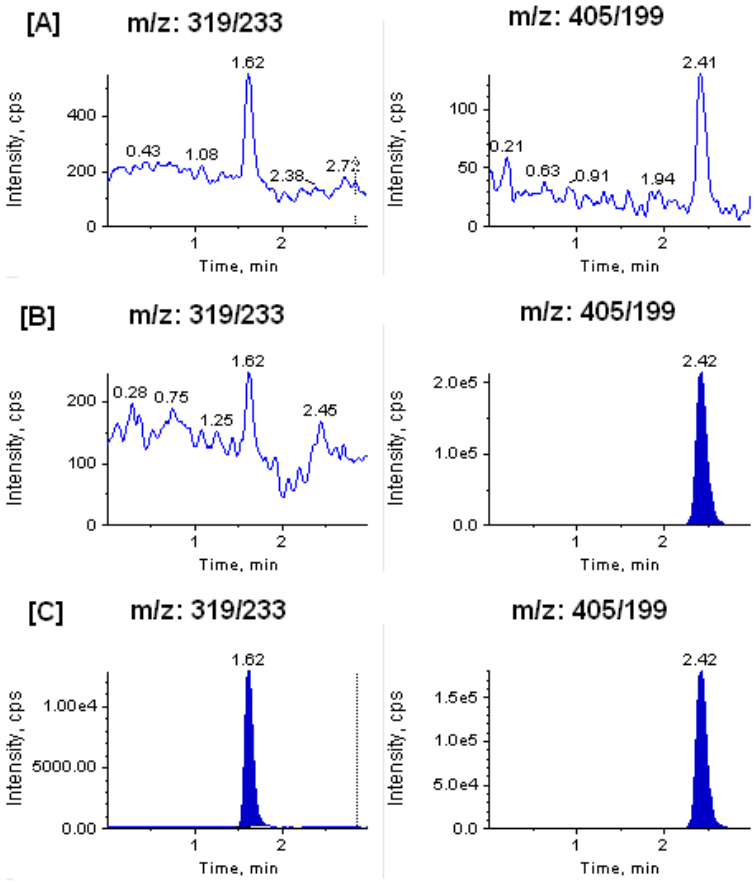


Figure 3. Typical MRM chromatograms of fenofibric acid (left panel) and IS (right panel) in (A) human blank plasma (B) human plasma with IS (C) a LLOQ sample along with IS.

Matrix effect

No significant matrix effect was observed in all the six batches of human plasma for the analytes at LQC and HQC concentrations. The precision and accuracy for rosuvastatin at LQC concentration was found to be 2.7% and 102.0%, and at HQC level was 2.1% and 101.2%, respectively. Similarly, the precision and accuracy for fenofibric acid at LQC concentration was found to be 1.4% and 100.6%, and at HQC level was 0.7% and 101.3%, respectively.

Linearity

Nine-point calibration curve was found to be linear over the concentration range of 0.1–80 ng/mL for rosuvastatin and 50–9003 ng/mL for fenofibric acid. After comparing the two weighting models (1/x and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration was found to produce the best fit for the concentration-detector response relationship for both the analytes in human plasma. The mean correlation coefficient of the weighted calibration curves generated during the validation was 0.99.

Precision and accuracy

As shown in Table 1, the precision and accuracy of each analyte in the intra-day and inter-day runs were within ± 15% at LQC, MQC1, MQC2 and HQC concentrations and within ± 20% at LLOQ QCs.

Table 1. Precision and accuracy of the method for determining rosuvastatin and fenofibric acid in plasma samples

		Intra-day precision and accuracy (*n*=12; 6 from each batch)			Inter-day precision and accuracy (*n*=30; 6 from each batch)		
Analytes	**Concentration added (ng/mL)**	**Concentration found (mean; ng/mL)**	**Precision (%)**	**Accuracy (%)**	**Concentration found (mean; ng/mL)**	**Precision (%)**	**Accuracy (%)**
Rosuvastatin	0.10	0.11	8.15	106.17	0.11	10.53	102.82
	0.30	0.30	8.48	98.97	0.31	8.81	103.27
	10.11	10.63	3.70	105.14	10.21	7.12	101.02
	40.44	42.31	5.71	104.61	41.89	4.52	103.57
	70.33	69.38	4.12	98.65	70.32	4.58	99.98
Fenofibric acid	50.34	52.88	3.93	105.04	52.15	7.36	103.59
	152.54	152.61	2.97	100.04	151.60	4.45	99.38
	1016.96	1030.41	1.73	101.32	1028.63	6.43	101.15
	4519.83	4764.43	3.75	105.41	4744.34	5.53	104.97
	7792.81	7533.60	4.88	96.67	7651.69	7.59	98.19

Dilution integrity

The upper concentration limits can be extended to 135.8 ng/mL, for rosuvastatin and 15259 ng/mL for fenofibric acid by 1/2 and 1/4 dilutions with screened human blank plasma. The mean back calculated concentrations for 1/2 and 1/4 dilution samples were within 85-115% of their nominal value. The coefficients of variation (%CV) for 1/2 and 1/4 dilution samples were less than 10%.

Stability studies

In the different stability experiments carried out viz. bench top stability (10 h), autosampler stability (46 h), repeated freeze-thaw cycles (3 cycles), reinjection stability (24 h), wet extract stability (24 h at 2-8 °C) and long-term stability at –70 °C for 60 days the mean % nominal values of the analytes were found to be within ±15% of the predicted concentrations for the analytes at their LQC and HQC levels (Table 2). Thus, the results were found to be within the acceptable limits during the entire validation.

Table 2. Stability samples result for rosuvastatin and Fenofibric acid (*n*=6)

Analytes	Stability test	QC (spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Accuracy/ Stability (%)	Precision (%)
Rosuvastatin	Process[a]	0.30	0.32 ± 0.03	108.19	9.23
		70.33	70.49 ± 2.64	100.23	3.75
	Process[b]	0.30	0.33 ± 0.03	111.13	7.97
		70.33	69.01 ± 4.82	98.13	6.98
	Bench top[c]	0.30	0.30 ± 0.01	102.17	4.62
		70.33	71.99 ± 4.90	102.36	6.80
	FT[d]	0.30	0.27 ± 002	92.07	5.60
		70.33	73.86 ± 2.28	105.02	3.08
	Reinjection[e]	0.28	0.29 ± 0.01	105.88	2.88
		67.67	68.11 ± 1.86	100.65	2.74
	Long-term[f]	0.31	0.33 ± 0.02	104.26	5.19
		71.09	70.45 ± 1.50	99.10	2.13
Fenofibric acid	Process[a]	152.54	148.99 ± 7.97	97.67	5.35
		7792.81	6924.95 ± 547.85	88.86	7.91
	Process[b]	152.54	155.07 ± 6.85	101.66	4.42
		7792.81	7689.13 ± 390.86	98.67	5.08
	Bench top[c]	152.54	141.61 ± 10.25	92.83	7.24
		7792.81	7861.38 ± 399.64	100.88	5.08
	FT[d]	152.54	143.38 ± 6.69	93.99	4.67
		7792.81	7629.06 ± 594.07	97.90	7.79
	Reinjection[e]	149.73	141.05 ± 3.48	94.20	2.47
		7600.98	7740.14 ± 344.71	101.83	4.45
	Long-term[f]	155.48	153.81 ± 7.74	98.93	5.03
		7466.22	7553.49 ± 175.67	101.17	2.33

[a] after 46 h in autosampler at 10°C; [b] after 24 h in refrigerator at 2-8°C; [c] after 10 h at room temperature; [d] after three freeze and thaw cycles; [e] after 24 h of Reinjection; [f] at -70°C for 60 days

Pharmacokinetic study

In order to verify the sensitivity and selectivity of this method in a real-world situation, the present method was used to test for Fenofibric acid concentrations in human plasma samples collected from healthy male volunteers (n = 6). The ethics committee approved the protocol and the volunteers provided with informed written consent. Blood samples were collected following oral administration of 145 mg tablet of Fenofibrate at pre-dose and 1, 1.5, 2, 2.5, 3, 3.33, 3.67, 4, 4.33, 4.67, 5, 5.5, 6, 8, 12, 24 and 72 h. The mean plasma concentrations vs time profiles of fenofibric acid is shown in Figure 4. The maximum concentration in plasma (C_{max}), time point of C_{max} (T_{max}), half-life ($t_{1/2}$), area under the plasma concentration time curve from zero hour to the last measurable concentration (AUC_{0-t}) and area under the plasma concentration-time curve from

zero hour to infinity ($AUC_{0-\infty}$) for fenofibric acid was 8102 ± 1033 ng/mL, 4.53 ± 0.60 h, 17.4 ± 3.35 h, 193690 ± 48234 ng*h/mL and 206602 ± 54216 ng*h/mL, respectively.

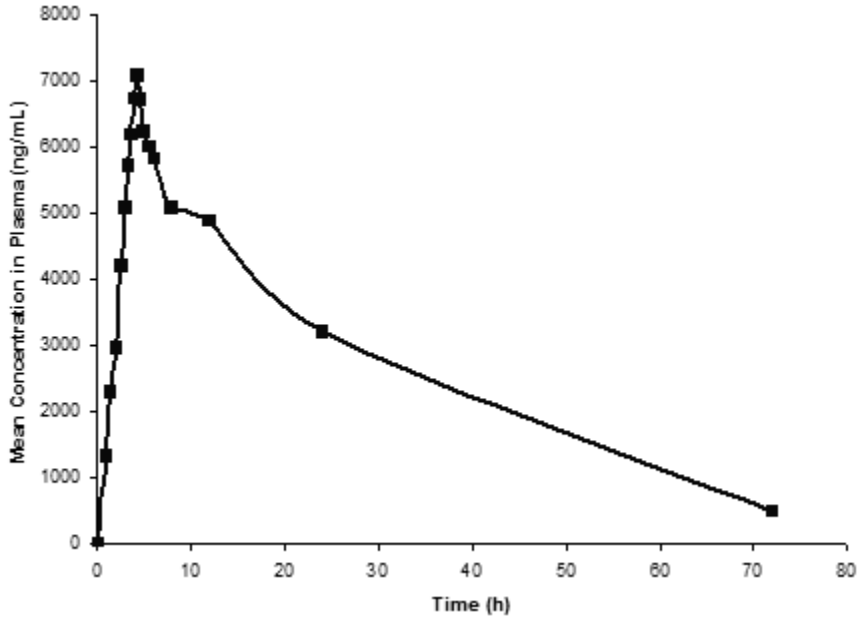


Figure 4. Mean plasma concentration-time profile of fenofibric acid in human plasma following oral dosing of 145 mg fenofibrate tablet to healthy volunteers.

CONCLUSION

In summary, we have developed and validated a highly sensitive, specific, reproducible and high-throughput LC-MS/MS method to quantify rosuvastatin and fenofibric acid simultaneously using single IS. The cost-effectiveness, simplicity of the assay and using liquid-liquid extraction, and sample turnover rate of less than 3.0 min per sample, make it an attractive procedure in high-throughput bioanalysis of rosuvastatin and fenofibric acid. From the results of all the validation parameters, we can conclude that the developed method can be useful for BA/BE studies and routine therapeutic drug monitoring (TDM) with desired precision and accuracy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Wellquest Laboratory, Hyderabad for providing necessary facilities to carry out this work.

REFERENCES

1. Wood D, De Backer G, Faergeman O, Graham I, Mancia G, Pyorala K. Prevention of coronary heart disease in clinical practice. Recommendations by the second joint task force of the European Society of Cardiology, European Atherosclerosis Society and European Society of Hypertension. Eur Heart J 1998; 19: 1434-1503.

2. Pearson TA, Laurora I, Chu H, Kafonek S. The Lipid Treatment Assessment Project (L-TAP): a multicentre survey to evaluate the percentages of dyslipidaemic patients receiving lipid-lowering therapy and achieving low-density lipoprotein cholesterol goals. Arch Intern med 2000; 160: 459-467.
3. Sueta CA, Chowdhury M, Boccuzzi SJ, Smith SC Jr, Alexander CM, Londhe A, Lulla A and Simpson RJ Jr. Analysis of the degree of undertreatment of hyperlipidemia and congestive heart failure secondary to coronary artery disease. Am J Cardiol 1999; 83: 1303-1307.
4. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990; 343: 425-430.
5. Olsson AG, McTaggart F, Raza A. Rosuvastatin: a highly effective new HMG-CoA reductase inhibitor. Cardiovasc drug Rev 2002, 20, 303-328.
6. Chapman MJ, McTaggart F. Optimizing the pharmacology of statins: characteristics of Rosuvastatin. Atheroscler Suppl 2002; 2: 33-36.
7. Blasetto JW, Stein EA, Brown WV, Chitra R, Raza A. Efficacy of rosuvastatin compared with other statins at selected starting doses in hypercholesterolemic patients and in special population groups. Am J Cardiol 2003; 91: 3C-10C.
8. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, Cain VA, Blasetto JW. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol 2003; 92: 152-160.
9. Martin PD, Warwick MJ, Dane AL, Hill SJ, Giles PB, Phillips PJ, Lenz E. Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. Clin Ther 2003; 25: 2822-2835.
10. Olsson AG, Pears J, McKellar J, Mizan J, Raza A. Effect of rosuvastatin on low-density lipoprotein cholesterol in patients with hypercholesterolemia. Am J Cardiol 2001; 88: 504-508.
11. Davidson M, Ma P, Stein EA, Gotto AM Jr, Raza A, Chitra R and Hutchinson H. Comparison of effects on low-density lipoprotein cholesterol and high-density lipoprotein cholesterol with rosuvastatin versus atorvastatin in patients with type IIa or IIb hypercholesterolemia. Am J Cardiol 2002; 89: 268-275.

12. Laufs U, Gertz K, Dirnagl U, Bohm M, Nickenig G and Endres M. Rosuvastatin, a new HMG-CoA reductase inhibitor, upregulates endothelial nitric oxide synthase and protects from ischemic stroke in mice. Brain Res 2002; 942: 23-30.
13. Goldberg AC, Schonfeld G and Feldman EB, Ginsberg HN, Hunninghake DB, Insull W Jr, Knopp RH, Kwiterovich PO, Mellies MJ, and Pickering J. Fenofibrate for the treatment of type IV and V hyperlipoproteinemias: a double-blind, placebo-controlled multicenter U.S study. Clin Ther 1989; 11: 69-83.
14. Kirchgassler KU, Schmitz H, Bach G. Effectiveness and tolerability of 12-week treatment with micronised fenofibrate 200mg in a drug-monitoring programme involving 9884 patients with dyslipidaemia. Clin Drug Invest 1998; 15: 197-204.
15. Miller DB, Spence JD. Clinical pharmacokinetics of fibric acid derivatives (Fibrates). Clin Pharmacokinet 1998; 34: 155-162.
16. Martin PD, Dane AL, Schneck DW, Warwick MJ. An open-label, randomized, three-way crossover trial of the effects of coadministration of rosuvastatin and fenofibrate on the pharmacokinetic properties of rosuvastatin and fenofibric acid in healthy male volunteers. Clin Ther 2003; 25: 459-471.
17. Trivedi RK, Kallem RR, Mullangi R, Srinivas NR. Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC–MS/MS with electrospray ionization: Assay development, validation and application to a clinical study. J Pharma Biomed Anal 2005; 39: 661-669.
18. Xu D-H, Ruan ZR, Zhou Q, Yuan H, Jiang B. Quantitative determination of rosuvastatin in human plasma by liquid chromatography with electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 2006; 20: 2369-2375.
19. Bhavesh D, Shah S and Shivprakash. Determination of fenofibric acid in human plasma by ultra performance liquid chromatography–electrospray ionization mass spectrometry: application to a bioequivalence study. Biomed Chromatogr 2009; 23: 922-928.
20. Li L, Sun J, Sun Y, He Z. LC–ESI–MS determination of lovastatin in human plasma. Chromatogr 2008; 67: 621-625.
21. US DHHS, FDA, CDER. Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CV), 2001. Available at: http://www/fda.gov/cder/guidance/index.htm.