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Validated HPLC–MS/MS method for simultaneous determination of simvastatin and simvastatin hydroxy acid in human plasma

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Abstract

Cholesterol lowering statin drugs are the most frequently prescribed agents for reducing morbidity and mortality related to coronary heart disease. This publication presents a validated, highly sensitive and selective isocratic HPLC method for the quantitative determination of the major statin drug simvastatin (SIM) and its metabolite simvastatin hydroxy acid (SIMA). Detection was performed on an electrospray ionization triple quadrupole mass spectrometer equipped with an ESI interface operated in positive and negative ionization mode. The multiple reaction-monitoring mode (MRM) was used to provide MS/MS detection. The linearity for the calibration curve in the concentration range of 0.10–16.00 ng/mL for SIM and 0.10–16.00 ng/mL for SIMA is presented. Inter- and intra-day precision and accuracy of the proposed method were characterized by relative standard deviation (R.S.D.) and percentage deviation, respectively; with both lower than 7% for all analytes. The limit of quantitation was 0.03 ng/mL for SIM and 0.02 ng/mL for SIMA. The devised method was employed in the pharmacokinetic study of SIM and the pharmacokinetic parameters of all analytes are also presented.

Keywords: Simvastatin; HPLC; MS/MS; Plasma; Pharmacokinetic study

1. Introduction

Simvastatin (SIM) is a reversible inhibitor of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase, widely used in the treatment of various types of hypercholesterolaemias. SIM—a methylated analogue of lovastatin—is synthesized from a fermentation product of *Aspergillus terreus*. The parent drug is a pharmacologically inactive lactone (prodrug form), which is absorbed from the stomach, extracted by the liver, and largely converted to several active metabolites. The most notable is simvastatin hydroxy acid (SIMA) [1–5]. The active metabolites tend to concentrate in the liver, a major site of cholesterol endogenous synthesis. The inhibition of HMG-CoA reductase results in a moderate reduction in cholesterol synthesis and, more importantly, in an increase of low-density lipoprotein (LDL) receptors located on the cell membranes of the liver [6] and extrahepatic tissues.

In humans, a linear increase in the inhibitory activity of simvastatin occurs in the dose range from 5 to 120 mg daily [4,7].

Gas chromatography–mass spectrometry (GC–MS) methods are highly sensitive and selective enough to determine the therapeutic plasma levels of both SIM and SIMA [8,9], but the operation and clean up procedure prior to analysis is complicated. Several HPLC methods for SIM and SIMA determination have been reported. Liquid chromatography–UV detection (LC–UV) methods are simpler than those in the GC–MS but they are not sensitive enough for determining the drug levels in plasma at therapeutic dose [10,11]. Although LC with fluorescence detection is a highly sensitive method, the samples need complex derivatization before their analysis, which is inconvenient [12].

Rapid and effective ways for the determination of drugs and metabolites in biological fluids are desirable. HPLC–MS/MS is a method that is suitable for the quantitative determination of drugs. MS/MS detection is sensitive and enables the effective elimination of interferences from endogenous components. Recently, several HPLC–MS/MS methods have been published for the determination of SIM.

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The first paper [13] describes an HPLC method with MS/MS detection for the determination of SIM in aqueous samples with LOQ 1 ng/mL, which is too high for the purpose of the pharmacokinetic study (PK study). The next paper [14] presents an HPLC–MS method for the determination of SIM in plasma with suitable sensitivity, however, none of these papers take into consideration the metabolites of SIM. SIM together with its metabolite were quantified using the method published by Jemal et al. [15]. The authors coupled on-line SPE with LC–MS/MS and presented a fairly sensitive method with a limit of quantitation of 0.5 ng/mL. However, on-line SPE is not suitable for routine use where hundreds of samples may need to be analyzed.

The goal of our work was to develop an HPLC–MS/MS method for simultaneous determination of SIM and SIMA in human plasma that could be used for routine analysis of hundreds of samples in a PK study and to use the results to evaluate the PK parameters of the investigated drug. According to the literature, SIM and SIMA concentrations between 0.2 and 15.0 ng/mL in human plasma could be expected after the administration of an 80 mg SIM dose, which was the dose tested. Confident monitoring of the concentration levels published in the literature would require a limit of quantification of 0.10 ng/mL.

In this paper we describe a rapid, selective and sensitive HPLC–MS/MS method for the determination of SIM and SIMA in human plasma. The developed method was successfully applied to a PK study that was approved by the appropriate Ethics Committee and performed in 72 healthy subjects after obtaining their written informed consent. More than 3000 samples were assayed using the proposed method.

2. Experimental

2.1. Chemicals and reagents

Simvastatin and simvastatin hydroxy acid, ammonium salt were obtained from Ranbaxy (India), lovastatin (LOV) and lovastatin hydroxy acid, amonnium salt (LOVA) from Biocon (India). Acetonitrile (MS grade), methanol (HPLC grade), ammonium acetate and acetic acid were purchased from Sigma (Germany). Water was deionized and further purified for HPLC with a Milli-Q system (Millipore, Germany).

2.2. Mass spectrometry

Mass spectrometry was performed using a Quattro micro™ triple quadrupole mass spectrometer (Micromass, UK) equipped with an ESI source. The multiple reaction monitoring (MRM) mode was used for the determination of SIM and SIMA due to its high selectivity. Collision-induced dissociation (CID) was carried out using 2.4×10^{-3} mbar argon. The cone voltage was operated at an optimal value (80 kV) in the positive ion mode for SIM and internal standard (LOV) and negative ion mode for SIMA and internal standard (LOVA). The capillary voltage was 1.5 kV for both analytes and the entrance and exit energies of the collision cell were set at −1 and 1, respectively. Nitrogen was used at flow rates of 350 and 35 L/h, respectively. The source and desolvation temperatures were optimized and were kept at 100 and 450 °C, respectively. The system was controlled by Masslynx V 4.0 software (Waters, UK).

2.3. Liquid chromatography

A Waters 2695 liquid chromatograph (Waters, USA) with a Discovery C18 column (50 mm × 4.6 mm, 5 μm) (Supelco, USA) were used for the separation of SIM and SIMA. The mobile phase was a mixture of acetonitrile:methanol:0.1 M ammonium acetate (62:10:28, v/v/v), pH was not adjusted. Before use, the mobile phase was degassed by vacuum filtration through a 0.45 μm filter (nylon 66). The flow rate was 0.8 mL/min and the injection volume was 70 μL.

2.4. Preparation of standard and quality control solutions

The stock standard solutions of SIM and SIMA were prepared by dissolving the accurately weighed SIM and SIMA standard in acetonitrile:H_2O (75:25, v/v). The stock standard solutions were then diluted with acetonitrile:H_2O (75:25, v/v) to achieve standard working solutions at concentrations of 10, 50, 100, 200, 400, 800 and 1600 ng/mL for both SIM and SIMA. The working quality control solutions were prepared in the same way at concentrations 15, 300 and 900 ng/mL.

The standard working solutions (100 μL) were used to spike blank plasma samples (9.8 mL) either for the calibration standards (S7–S1) of SIM and SIMA or for the quality control samples (QC3–QC1) in prestudy validation and during pharmacokinetic study. All plasma samples were stored at -75 ± 5 °C. The samples were confirmed to be stable at -75 ± 5 °C for 3 months.

Stock Internal Standard solution was prepared by accurately weighing LOV and LOVA which were then dissolved in acetonitrile:H_2O (75:25, v/v). Working Internal Standard (W.I.S.) containing both LOV and LOVA was prepared by accurate dilution of the Stock Internal Standard with acetonitrile:H_2O (75:25, v/v) to a final concentration of 1 μg/mL of each compound. Stocks I.S. was stored at 4 °C and confirmed to be stable for 5 days. A volume of 50 μL W.I.S. was added to 1.0 mL plasma samples.

2.5. Preparation of plasma samples

Solid phase extraction (SPE) was used for sample pretreatment.

W.I.S. (50 μL) was added to 1 mL of the plasma sample containing the analytes. The sample was diluted with 1 mL of H_2O and vortex-mixed. The mixture was loaded on Oasis HLB (hydrophilic–lipophilic balance). The cartridges (30 mg, 1 mL) were previously activated with 1 mL of MeOH and conditioned with 2 mL H_2O. The SPE cartridge with loaded sample was washed with 1 mL H_2O, and subsequently the analyte was eluted with 250 μL of acetonitrile:0.1 M ammonium acetate, 75:25 (pH was adjusted at 4.5 with acetic acid). The eluate was centrifuged at 10,000 rpm for 10 min. Seventy microliters of eluate was injected onto the HPLC system with MS/MS detection.

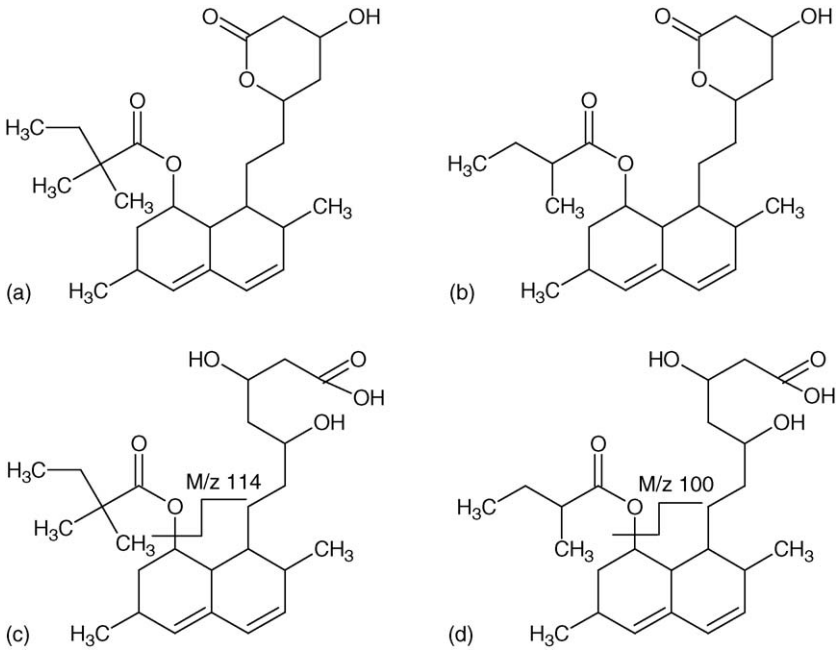

Fig. 1. Chemical structures of (a) simvastatin, (b) lovastatin, (c) simvastatin hydroxy acid and (d) lovastatin hydroxy acid.

2.6. Pharmacokinetic study

A single center, open, randomized, bioequivalence study on simvastatin 80 mg in healthy volunteers was performed.

Plasma samples were obtained from 72 volunteers at various time intervals within 32 h of drug administration. Each analytical batch consisted of a blank, a blank with internal standard (S0), seven calibration standards (S7, S6, S5, S4, S3, S2 and S1). The plasma samples obtained from the two volunteers in the study followed and were interspersed with six quality control (QC) samples (two series of QC3, QC2, and QC1).

3. Results and discussion

3.1. Performance of MS/MS

The electrospray interface was used to obtain good sensitivity, fragmentation and linearity. We have tested atmospheric pressure ionization (APCI) and no obvious improvement was observed. The first step in developing the detection method was to select the precursor ion to be fragmented. The chemical structures of SIM (molecular weight 418.57), SIMA (molecular weight 436.57), LOV (molecular weight 404.55) and LOVA (molecular weight 422.55) are presented in Fig. 1. Positive ion mode is appropriate for the lactone compounds whereas negative ion mode is normally used to monitor acids [12] therefore SIM and LOV were monitored in a positive-ion mode while SIMA and LOVA were monitored in a negative-ion mode due to their high signal intensity. To achieve this the positive–negative switch function available on most LC–MS/MS instruments was used to monitor statin drugs as acid and lactone forms simultaneously.

$[M-H]^-$ of SIMA (*m/z* 435.3) and LOVA (*m/z* 421.6) had the most intense signal in negative mode, therefore they were chosen as the precursor ion for the determination of the metabolite. Capillary, cone, extractor and RF voltages were optimized to obtain the maximum signal intensity. The product ion spectra of SIMA and its internal standard LOVA were obtained after optimization of these parameters. The base peaks of product ions were observed at *m/z* 114.0 and 100.2 for SIMA and LOVA in negative ion mode, respectively. Both substances show identical fragmentation behavior with the most important fragment ions arising from cleavage next to the ring (Fig. 1).

Many attempts including changing the mobile phase (solvent), ionization energies and gas flow were performed to detect the parent ions $[M+H]^+$ of the lactone compounds, however, contrary to the published results [15] our attempts did not result in the expected signal intensity. The only signal that could be employed to ensure the requested sensitivity of the detection of SIM and LOV was provided by the adduct ions $[M+CH_3CN+Na]^+$ (SIM *m/z* 481.2, LOV *m/z* 467.1).

Product ions of SIM and LOV with the most intense signal were generated by neutral loss of acetonitrile to gain $[M+Na]^+$ ions (SIM *m/z* 440.9, LOV *m/z* 426.7). Collision energy was optimized with respect to the intensity of the selected product ions. The signal corresponding to the product ion generated by the cleavage next to the ring as in the case of the hydroxy acids was observed with LOV in some of the optimization experiments, however, the intensity of such a response was low for the intended purpose.

The fragmentation of adducts of parent ions proved to be reproducible and useful for SIM and LOV determination.

The SIM and SIMA product ion spectra together with spectra of corresponding I.S. (LOV and LOVA) are shown in Fig. 2.

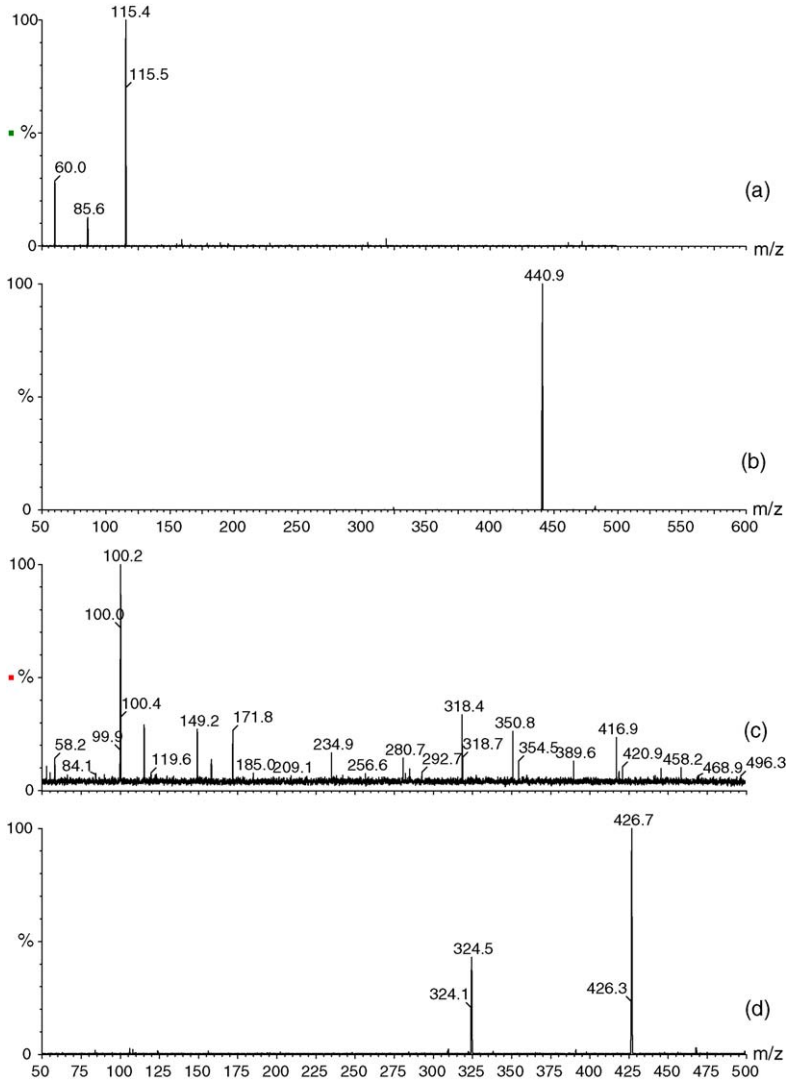

Fig. 2. Product ion scans (a) SIMA (ESI−), (b) SIM (ESI+), (c) LOVA (ESI−) and (d) LOV (ESI+) in acetonitrile.

3.2. Performance of LC

Several analytical columns (Discovery C8, C18 and Amide C16 embedded phase, Supelco) were tested to obtain maximal response of SIM and SIMA and reasonable time of analysis. Symmetrical peak shapes of SIM, SIMA and internal standards could not be obtained easily with the C8 stationary phase, while retention times on the Amide C16 phase were unacceptably long. The Discovery C18 column (50 mm × 4.6 mm, 5 μm) (Supelco) was eventually selected for all assays because it exhibited excellent peak shape and it had the highest response of SIM and SIMA with an acceptable run time.

The selection of mobile phase components was also critical factor. As phosphate buffer cannot be used for MS, we employed ammonium acetate to supply the ionic strength. Increasing the percentage of buffer in the mobile phase enhanced analyte peak symmetry and resolution but simultaneously prolonged the retention time of the SIMA and LOVA. Finally, a three component mobile phase containing methanol, acetonitrile and buffer was used with satisfying results.

3.3. Validation of the SIM and SIMA assay

3.3.1. Specificity and selectivity

Plasma samples from six different drug-free persons were tested for presence of endogenous components, which might interfere with SIM and SIMA or internal standards (LOV and LOVA). These samples were pre-treated according to the sample preparation procedure, apart from addition of internal standard solution. Chromatograms of blank plasma and plasma sample spiked with SIM and SIMA (0.10 ng/mL) and LOV and LOVA (1 μg/mL) were compared to show the specificity and selectivity of the proposed procedure. The chromatograms are presented in Figs. 3 and 4. The retention times of SIM and SIMA were 3.5 and 1.7 min, respectively, while LOV and LOVA were detected in 3.0 and 1.6 min, respectively. No endogenous components interfering with analytes and their internal standards were found in the chromatograms of blank plasma samples.

In addition, the "cross-talk" between MS/MS channels used for monitoring analytes and their internal standards was

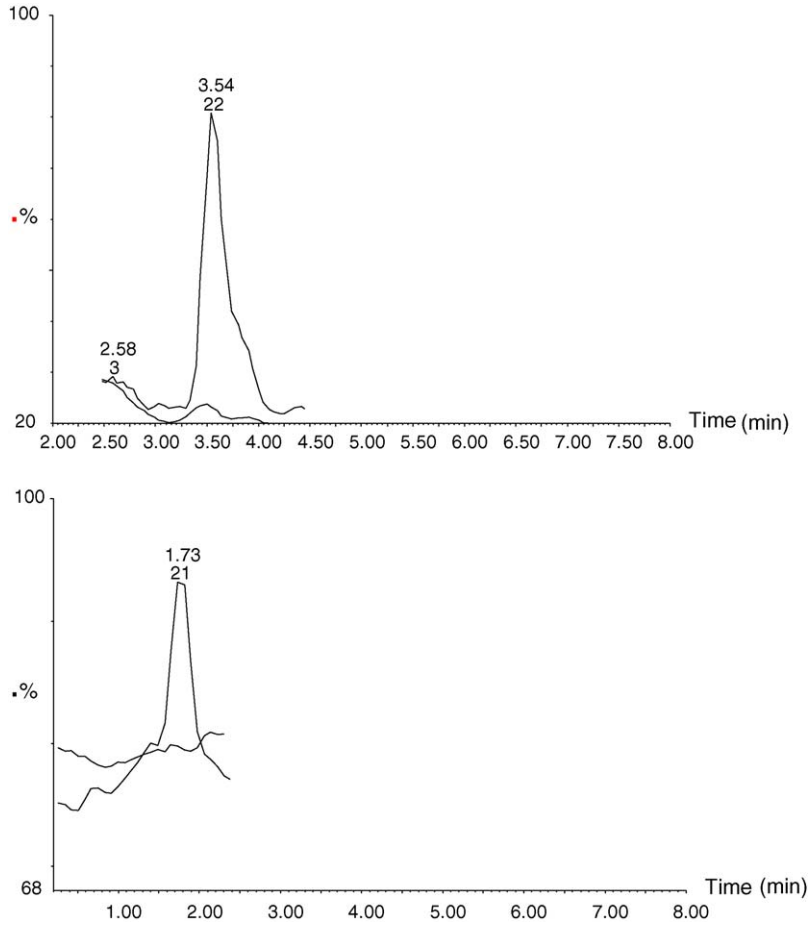

Fig. 3. Typical MRM chromatograms of blank plasma and plasma spiked with SIM at the LLOQ ($c = 0.1$ ng/mL) (top panel). MRM chromatogram of blank plasma or plasma spiked with SIMA at the LLOQ ($c = 0.1$ ng/mL) (bottom panel).

assessed by separately injecting SIM and SIMA (16 ng/mL) and monitoring the response in the LOV and LOVA channels and by injecting a plasma sample spiked only with LOV and LOVA and monitoring the response in the SIM and SIMA channels. No "cross-talk" between channels was observed.

3.3.2. Recovery and matrix effect

Three sets of seven calibration standards and a blank with internal standard (S0) were prepared for evaluation of recovery and ionization suppression or enhancement. Set 1 was prepared to evaluate the MS/MS response of working standard solutions injected in mobile phase. Working standard solutions (SIM and SIMA) were diluted 1:25 with acetonitrile:H_2O (75:25, v/v) to reach the concentration expected in plasma samples. Set 2 consisted of eight plasma samples spiked with 10 μL of each working standard solutions (SIM and SIMA) *after* extraction. Plasma samples spiked *before* extraction and S0 were processed and analyzed to obtain Set 3. Three replications of each set were used for determination of recovery and matrix effects. Internal standard was not added to standards.

The matrix effect (ME)—the possibility of ionization suppression or enhancement and recovery (RE) were evaluated by comparing the results of analysis of three sets of samples as follows:

$$\mathrm{ME}\,(\%) = \frac{B}{A} \times 100, \qquad \mathrm{RE}\,(\%) = \frac{C}{B} \times 100$$

where A is the mean peak area of Set 1, B the mean peak area of Set 2 and C is the mean peak area of Set 3.

A significant matrix effect was observed (8.1% for SIM and 3.9% for SIMA) but it was consistent over whole calibration range. Therefore, despite the matrix effect, the sensitivity of the assay was still sufficient to detect SIM and SIMA in the human PK study samples. Recovery of 88.8% for SIM and 85.6% for SIMA with low variability was evaluated to be consistent over whole calibration range and consequently the published method was proved to be reliable.

In addition the matrix effect was evaluated by analyte determination in six replicates (six different plasma sources) of two different concentrations (S7 and S1). Samples were processed using the described sample pretreatment method and the matrix effect was assessed as recovery to nominal value of S7 and S1.

No significant matrix effect was evaluated in six different lots of plasma. Therefore it was concluded that matrix does not affect the accuracy and precision of determination of SIM and SIMA.

The results of the recovery and matrix effect study are summarized in Tables 1 and 2.

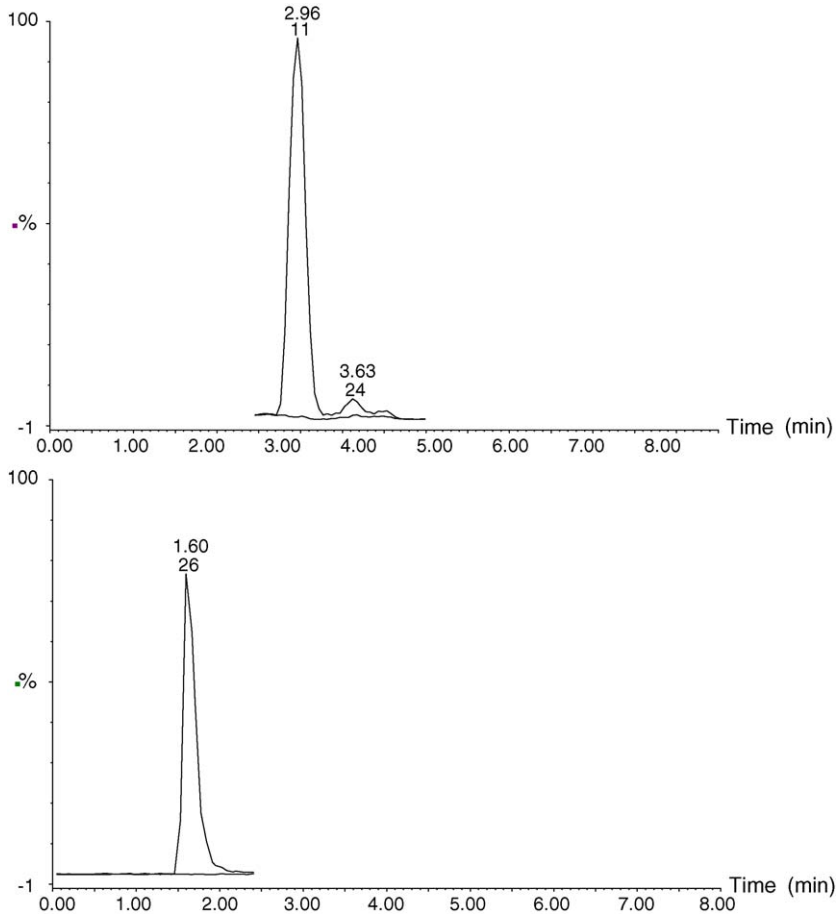

Fig. 4. MRM chromatograms of blank plasma and plasma spiked with I.S.: LOV (top panel) and LOVA (bottom panel).

Table 1
Recovery (RE) of SIM and SIMA from human plasma using SPE, and determination of the matrix effect (ME)

Nominal concentration (ng/mL)	Mean peak area			ME (%)	RE (%)
	Set 1	Set 2	Set 3		
SIM					
0.10	2068	1915	1722	92.6	89.9
0.50	5498	4948	4358	90.0	88.1
1.00	12547	11656	10035	92.9	86.1
2.00	30286	27842	24486	91.9	87.9
4.00	63841	58922	53416	92.3	90.7
8.00	133459	124301	111684	93.1	89.8
16.00	262945	238092	212587	90.5	89.3
I.S. (LOV)	65243	66251	61578	101.5	92.9
SIMA					
0.10	2231	2143	1764	96.0	82.3
0.50	4068	3930	3358	96.6	85.4
1.00	5598	5334	4598	95.3	86.2
2.00	16046	15572	13682	97.0	87.9
4.00	32584	30513	26492	93.6	86.8
8.00	53245	51063	43329	95.9	84.9
16.00	120894	118880	101781	98.3	85.6
I.S. (LOVA)	88159	78154	76218	88.7	97.5

Table 2
Recovery of SIM and SIMA from human plasma using six different plasma sources, and determination of the matrix effect

	SIM		SIMA	
	S7	S1	S7	S1
Amount added (ng/mL)	0.1	16.0	0.1	16.0
Amount found (ng/mL)	0.11	16.33	0.10	16.59
	0.11	16.15	0.09	16.35
	0.09	16.28	0.11	15.28
	0.10	15.86	0.09	15.62
	0.10	16.06	0.09	16.09
	0.11	15.65	0.10	16.59
Mean	0.10	16.06	0.10	16.09
% Deviation[a]	3.3	0.3	−3.3	0.5
% R.S.D.[b]	7.2	1.5	7.7	3.1

[a] Accuracy.
[b] Precision.

3.3.3. Limit of detection and quantitation

The limit of detection (LOD) was estimated as the amount of SIM or SIMA, which caused a signal three times to noise (S/N = 3/1). The limit of detection was calculated to be 0.03 ng/mL for SIM and 0.02 ng/mL for SIMA, respectively.

The lower limit of quantitation (LLOQ) defined as the lowest concentration analyzed with acceptable accuracy and precision (20%) was 0.10 ng/mL for both analytes, which was sufficient for the purpose of the PK study.

The proposed method is slightly more sensitive than the previously published method with on-line SPE [15]. The sensitivity of the latter method could probably be improved by increasing the injection volume. This possibility should be tested, however, with respect to the persistence of the SPE column.

3.3.4. Linearity, accuracy and precision

The seven point calibration curve obtained by weighted linear regression (1/*X*) showed good linearity over the whole concentration range (0.10–16.00 ng/mL), which covered the concentrations typically found in human plasma after administration of SIM and SIMA in the pharmacokinetic study. Correlation coefficient was better then 0.999.

Inter- and intra-day assays were performed to evaluate precision (R.S.D.) and accuracy (% deviation). Each assay batch consisted of blank, blank with internal standard (S0), seven calibration standards (S7, S6, S5, S4, S3, S2 and S1) and tested plasma samples of the same concentration as S7, S5, S3 and S1. Intra-day precision and accuracy were assessed by the analysis of four plasma samples in six series. Inter-day precision and accuracy were determined by analyzing six series of four plasma samples (S7, S5, S3 and S1) in four runs within 4 days. The intra- and inter-day assay results (summarized in Tables 3 and 4) show acceptable precision and accuracy for the proposed method.

3.3.5. Stability

Freeze-thaw stability (−75 ± 5 °C) was determined as percent recovery compared to the nominal value of QC3 and QC1 (0.15 and 9.00 ng/mL SIM and SIMA) in triplicate. The test was carried out over 4 days in four runs. Deviation was less than 15% of the nominal value. It was concluded that four cycles of freeze-thaw could be carried out with no loss of SIM and SIMA.

The long-term stability test at −75 ± 5 °C was performed in four runs over 3 months. Percent recovery of QC3 and QC1 (0.15 and 9.00 ng/mL SIM and SIMA) in triplicate was determined and compared to the nominal value. The obtained data showed no loss of the analytes.

Room temperature stability was assessed by analyte determination of five sets QC2 (3.00 ng/mL) in triplicate. Each set was left at room temperature for various lengths of time (0, 15, 30, 60 and 120 min) before sample processing. No significant difference (<15% of the nominal value) was found in SIM or SIMA concentration.

Autosampler Stability (stability of SIMA and SIMA in eluate) was estimated by analysis of QC samples (three series of QC3, QC2 and QC1). Samples were analyzed after 24 h while stored in autosampler at 10 °C in order to establish the stability of extracted SIM and SIMA. The results of both data sets differed by less then 15% from the nominal value, which proved the desired stability of the analyte during storage in autosampler.

All plasma samples for stability evaluation were prepared as described in the Section 2. To guarantee the reliability of the method, measured concentrations should not differ by more than 15% from the nominal value. All results of stability tests presented in Tables 5–7 show good stability of SIM and SIMA concentration over all steps of determination; therefore the method is proved to be applicable for routine analyses.

Table 3
Intra-day assay summary

Amount added (ng/mL)	SIM mean amount found (ng/mL)	% Deviation[a]	% R.S.D.[b]	SIMA mean amount found (ng/mL)	% Deviation[a]	% R.S.D.[b]
0.1	0.10	1.7	7.4	0.1	−1.7	7.7
1	1.04	3.5	6.3	1.01	0.7	4.9
4	4.09	2.2	4.6	4.07	1.8	2.6
16	15.96	−0.3	3.1	16.14	0.8	1.6
Overall		1.8	5.4		0.4	4.2

n = 6.
[a] Accuracy.
[b] Precision.

Table 4
Inter-day assay summary

	Amount added (ng/mL)			
	0.10	1.00	4.00	16.00
SIM mean amount found (ng/mL) / % R.S.D.				
Run #1	0.10 / 7.4	1.04 / 6.3	4.09 / 4.6	15.96 / 3.1
Run #2	0.10 / 6.3	1.00 / 5.0	4.13 / 3.0	16.11 / 2.2
Run #3	0.10 / 8.9	1.03 / 4.7	4.06 / 3.5	16.12 / 2.2
Run #4	0.10 / 7.4	1.00 / 5.5	4.13 / 3.5	15.78 / 2.2
Mean	0.100	1.020	4.090	16.050
Standard deviation	0.007	0.054	0.149	0.437
% Deviation[a]	0.8	1.6	2.3	0.3
% R.S.D.[b]	7.0	5.2	3.6	2.7
Overall % deviation	1.3			
% R.S.D.	7.0–2.7			
SIMA mean amount found (ng/mL) / % R.S.D.				
Run #1	0.10 / 7.7	1.01 / 4.9	4.07 / 2.6	16.14 / 1.6
Run #2	0.10 / 7.7	1.02 / 4.1	4.07 / 4.6	16.39 / 2.1
Run #3	0.10 / 4.2	1.02 / 4.7	4.03 / 3.4	15.78 / 2.2
Run #4	0.10 / 8.9	1.01 / 3.7	4.12 / 3.5	16.33 / 2.8
Mean	0.10	1.01	4.07	16.03
Standard deviation	0.007	0.043	0.144	0.364
% Deviation[a]	−1.3	0.9	1.7	0.2
% R.S.D.[b]	6.7	4.2	3.5	2.2
Overall % deviation	0.4			
% R.S.D.	6.7–2.2			

[a] Accuracy.
[b] Precision.

Table 5
Freeze-thaw stability of SIM and SIMA

Cycle	Concentration	Nominal value (ng/mL)	SIM mean amount found (ng/mL)	Difference (%)	SIMA mean amount found (ng/mL)	Difference (%)
0	Low	0.15	0.15	0.0	0.15	0.0
	High	9.00	9.11	1.2	9.11	1.2
1	Low	0.15	0.16	6.7	0.15	0.0
	High	9.00	9.24	2.7	8.83	−1.9
2	Low	0.15	0.16	6.7	0.15	0.0
	High	9.00	9.04	0.4	8.98	−0.2
3	Low	0.15	0.15	0.0	0.15	0.0
	High	9.00	9.17	1.9	9.15	1.7

Table 6
Long-term stability of SIM and SIMA

Day	Concentration	Nominal value (ng/mL)	SIM mean amount found (ng/mL)	Difference (%)	SIMA mean amount found (ng/mL)	Difference (%)
0	Low	0.15	0.16	6.7	0.15	0.0
	High	9.00	9.16	1.8	9.15	1.7
15	Low	0.15	0.15	0.0	0.15	0.0
	High	9.00	9.26	2.9	9.20	2.2
39	Low	0.15	0.16	6.7	0.15	0.0
	High	9.00	8.98	−0.2	8.98	−0.2
91	Low	0.15	0.15	0.0	0.15	0.0
	High	9.00	9.10	1.1	9.18	2.0

Table 7
Autosampler stability of SIM and SIMA

Cycle	Concentration	Nominal value (ng/mL)	SIM mean amount found (ng/mL)	Difference (%)	SIMA mean amount found (ng/mL)	Difference (%)
Day 1						
	QC 3	0.15	0.16	6.7	0.15	0.0
	QC 2	3.00	0.03	1.0	2.94	−2.0
	QC 1	9.00	9.01	0.1	8.85	−1.7
Day 2						
	QC 3	0.15	0.15	0.0	0.14	−6.7
	QC 2	3.00	0.13	4.3	2.84	−5.3
	QC 1	9.00	9.01	0.1	8.92	−0.9

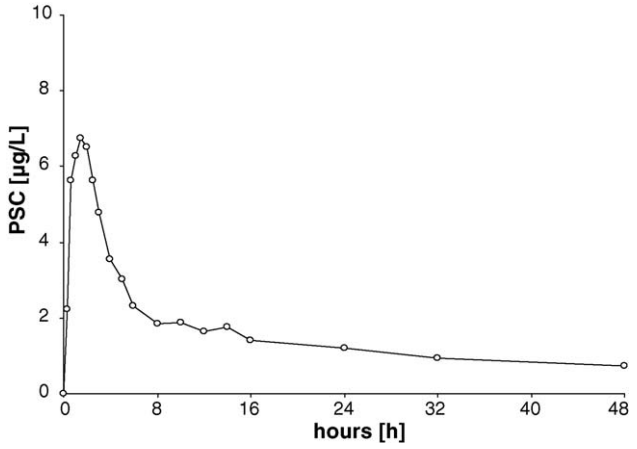

Fig. 5. Geometric means of plasma simvastatin concentration (PSC, μg/L) vs. time curves following single oral 80 mg dose of simvastatin (72 volunteers).

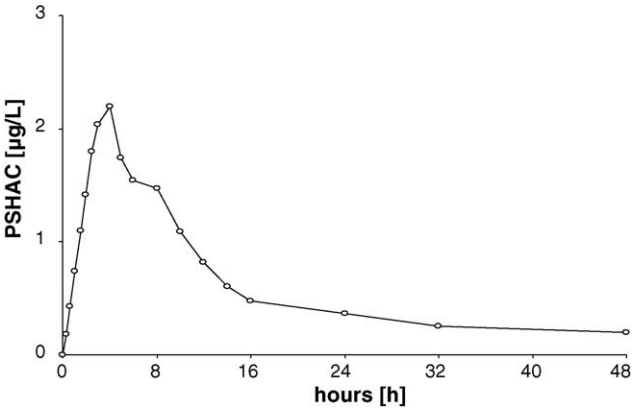

Fig. 6. Geometric means of plasma simvastatin hydroxy acid concentration (PSHAC, μg/L) vs. time curves following single oral 80 mg dose of simvastatin (72 volunteers).

3.4. Pharmacokinetic study

Figs. 5 and 6 show the pharmacokinetic profile obtained following single dose administration of 80 mg simvastatin; the data shown represent the concentration of SIM and SIMA determined in 72 volunteers. The limit of quantitation of SIM and SIMA (0.10 ng/mL) was low enough to assess SIM and SIMA over 48 h after drug administration.

Table 8
Pharmacokinetic Results of SIM and SIMA (as geometric means for AUC and C_{max}, as medians for t_{max} and $t_{1/2}$)

Parameter	$AUC_{0\text{-last}}$ (μg/L·h)	$AUC_{0\text{-inf}}$ (μg/L·h)	C_{max} (μg/L·h)	t_{max} (h)	$t_{1/2}$ (h)
SIM	57.40	71.29	10.34	1.50	10.79
SIMA	27.54	32.11	2.51	4.00	12.88

The basic PK characteristics of SIM and SIMA are summarized in Table 8. The geometric mean of peak concentration C_{max} of SIM is somewhat lower than the reported data [5,16] while geometric mean of peak concentration of SIMA is similar to the reported data for 60 mg dose, but lower than other highly variable published concentrations [16,17]. The median values found for SIM and SIMA t_{max} are in a good agreement with the references [5,16].

4. Conclusion

The method for the simultaneous determination of simvastatin and its metabolite simvastatin hydroxy acid in human Na_2EDTA plasma covering the concentration range 0.10–16.00 ng/mL, using 1.0 mL of plasma was proposed and validated. No interferences from endogenous plasma components or other sources were found and no "cross-talk" effect was observed in plasma samples. SIM, SIMA and their internal standards (LOV and LOVA) were well separated and their peaks were narrow and symmetric. The assay showed good precision and accuracy. Simple preparation procedure and short retention time allow determination of more than 250 samples per day.

The analytical method presented here has proved to be useful for the investigation of the pharmacokinetic characteristics of SIM and SIMA in human plasma.

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