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Microsample Determination of Lovastatin and its Hydroxy Acid Metabolite in Mouse and Rat Plasma by Liquid Chromatography/Ionspray Tandem Mass Spectrometry

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A sensitive and specific method was developed and validated to quantitate lovastatin and its hydroxy acid in mouse and rat plasma. This method employs a solid-phase extraction procedure to isolate lovastatin and its hydroxy acid metabolite from the biological matrices (0.1 ml of mouse or rat plasma). The reconstituted extracts were analyzed by liquid chromatography/ionspray tandem mass spectrometry (LC/MS/MS). Simvastatin and simvastatin hydroxy acid were used as internal standards for lovastatin and lovastatin hydroxy acid, respectively. The assay has a lower limit of quantitation (LLQ) of 0.50 ng ml^{-1} in mouse and rat plasma for both lovastatin and its hydroxy acid based on 0.1 ml aliquots of plasma. The intra-and inter-assay precision (RSD), calculated from quality control (QC) samples, was <7% for lovastatin and <6% for lovastatin hydroxy acid in both matrices. The inter-assay accuracy as determined from QC samples was less than 6% for lovastatin and less than 8% for lovastatin hydroxy acid in both matrices. The overall recovery of lovastatin was 54% in mouse plasma and 55% in rat plasma, and the overall recovery of lovastatin hydroxy acid was 100% in mouse plasma and 67% in rat plasma. © **1997 by John Wiley & Sons, Ltd.**

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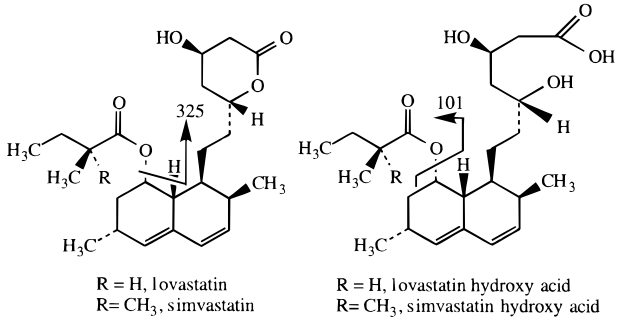
INTRODUCTION

Lovastatin (Lov) is a novel cholesterol-lowering drug which competitively inhibits the biosynthesis of mevalonic acid by HMG–CoA reductase.[1,2] The drug is administered as a lactone which is rapidly converted in the liver into its β-hydroxy acid (Lov-OH), the active metabolite of lovastatin (Fig. 1). The determination of lovastatin and its hydroxy acid metabolite in biological matrices has been reported using high-performance liquid chromatography with ultraviolet detection (HPLC/UV)[3] and gas chromatography/mass spectrometry (GC/MS).[4–6] A liquid chromatographic/atmospheric pressure chemical ionization tandem mass spectrometic (LC/APCI/MS/MS) method has been reported for the quantitation of simvastatin and its hydroxy acid in plasma using lovastatin and its hydroxy acid as the internal standards.[7] Both the GC/MS and LC/APCI/MS/MS methods showed good sensitivities for both compounds. However, time-consuming derivatization procedures were required for all analytes. In both methods, the lactone was separated from its β-hydroxy acid by solid-phase extraction, and then the two compounds were converted into the appropriate esters for the mass spectrometric analysis. Therefore, two separate analyses were required in order to determine the concentrations of drug and its hydroxy acid metabolite. Recently, an LC/APCI/MS/MS method was developed by Korfmacher *et al.*[8] for the determination of lovastatin and its hydroxy acid in dog plasma.

In this paper, we present an alternative, more sensitive and specific liquid chromatographic/ionspray tandem mass spectrometric (LC/MS/MS) method which can simultaneously quantitate lovastatin and its hydroxy acid in mouse and rat plasma to the required levels. Owing to the specific matrices used in this study, sample sizes as small as 0.1 ml were used to achieve the



R = H, lovastatin
R= CH_3, simvastatin

R = H, lovastatin hydroxy acid
R= CH_3, simvastatin hydroxy acid

Figure 1. Structures of Lov, Lov-OH, Sim and Sim-OH.

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desired LLQ. The assay has an LLQ of 0.50 ng ml^{-1} plasma for both lovastatin and its hydroxy acid based on 0.1 ml aliquots of mouse or rat plasma. Simvastatin (Sim) and simvastatin hydroxy acid (Sim-OH) were used as the internal standards for lovastatin and its hydroxy acid, respectively.

EXPERIMENTAL

Materials and chemicals

Lovastatin (USPC Reference Standard), lovastatin hydroxy acid ammonium salt, simvastatin and simvastatin hydroxy acid ammonium salt were obtained from the Department of Drug Metabolism, Schering-Plough Research Institute (Kenilworth, NJ, USA). HPLC-grade acetonitrile and methanol were obtained from Baxter/Scientific Products (McGaw Park, IL, USA). Acetic acid and ammonium acetate were purchased from J. T. Baker (Danvers, MA, USA). Formic acid was obtained from EM Science (Gibbstown, NJ, USA). HPLC-grade water was prepared using a Barnstead (Dubuque, IA, USA) Model D7331 ultrapure water system. Bond Elut C_8 SPE cartridges (1 ml/100 mg) were purchased from Varian/Sample Preparation Products (Harbor City, CA, USA).

LC/MS/MS instrumentation

A Hewlett-Packard (Avondale, PA, USA) Model 1090L HPLC pump was used. Chromatography was performed on a Kromasil C_{18} column (50 × 2 mm i.d., 5 μm) (Keystone Scientific, Bellefonte, PA, USA) using gradient elution with aqueous 1 mM ammonium acetate (pH 4.0, adjusted with glacial acetic acid) and acetonitrile (ACN) ($t = 0$ min, 60% ACN; $t = 3$ min, 80% ACN; $t = 3.1$ min, 90% ACN; $t = 4.1$ min, 90% ACN; $t = 5$ min, 60% ACN). The flow rate was maintained at 200 μl min^{-1} for all LC/MS/MS experiments. A PE-SCIEX (Thornhill, Ontario, Canada) API III^+ triple-quadrupole mass spectrometer equipped with an ionspray LC/MS interface was used for all analyses. Mass axis calibration was performed daily by the infusion of a 10^{-4} M PPG (polypropylene glycols, average M_r 425, 1000 and 2000) calibration solution in methanol–water (50:50) (PE-SCIEX API III^+ operator's manual) at a flow rate of 10 μl min^{-1} using a Harvard Apparatus (South Natick, MA,USA) Model 55-1111 syringe pump. Mass axis calibration was performed daily on m/z 59.0, 325.3, 520.4 and 906.7. Peak widths were maintained at ~0.7 u at half-height in the single MS mode.

All infusion spectra were summed over 10–20 scans. The full-scan single mass spectra and product ion mass spectra of the analytes and the internal standards were obtained by infusion of each test sample in a solution (in methanol for Lov and Sim; in acetonitrile-water (90:10) for Lov-OH and Sim-OH) into the mass spectrometer at a flow rate of 10 μl min^{-1}. Full-scan single mass spectra were acquired by scanning Q1 from m/z 150 to 500 at unit mass resolution with a step size of 0.1 u and a dwell time of 2 ms. The product ions were analyzed by scanning Q3 from m/z 100 to 500 at unit mass resolution with a step size of 0.1 u and a dwell time of 2 ms. Peak widths of the precursor and product ions were manitained at ~1 u at half-height in the selected reaction monitoring (SRM) mode. The SRM mode was used for all LC/MS/MS analyses. For the analysis of each sample, the mass spectrometer was operated in the negative-ion mode for the first 3 min, and then switched to the positive-ion mode for the rest of the run. The argon collision gas thickness was maintained at 2.5×10^{14} atoms cm^{-2}.

Sample preparation

Stock solutions of lovastatin and simvastatin were prepared in acetonitrile and of lovastatin hydroxy acid and simavastatin hydroxy acid in acetonitrile–water (90:10). The concentrations of lovastatin hydroxy acid and simvastatin hydroxy acid are based on the free acids.

Duplicate calibration standards were prepared fresh for each analysis. The concentration of the standards at the respective points on the calibration graphs were 0.50, 0.75, 1.00, 10.0, 50.0 and 100 ng ml^{-1} in mouse or rat plasma based on 0.1 ml of plasma for both lovastatin and lovastatin hydroxy acid. To 0.1 ml of plasma, 50 μl of diluted drug solution were added. The double blank sample was fortified with 50 μl of acetonitrile–water (90:10) and the blank sample was fortified with 50 μl of the internal standard working solution. Then each sample was diluted with 0.4 ml of water to provide the calibration samples.

The quality control (QC) samples were prepared by spiking the analytes (from separately prepared stock and diluted solutions) into mouse or rat plasma at concentrations of 0.80, 40.0 and 80.0 ng ml^{-1} of plasma for both analytes. Owing to the instability of lovastatin in mouse and rat plasma at ambient temperature, all sample preparation procedures were carried out in an ice–water bath.

Extraction procedure

Each solid-phase extraction cartridge (1 ml/100 mg) was conditioned with two 1 ml portions of methanol and two 1 ml portions of water. The diluted sample (see Sample preparation) was loaded on to each cartridge. Low vacuum was applied after 1 min, and then each cartridge was washed with 1 ml of water, 1 ml of 5% formic acid and 1 ml of water. Next, the cartridge was dried for 1 min. Each cartridge was then eluted with 1 ml of methanol–water (70:30) solution. Low vacuum was applied 1 min after the addition of the elution solvent, and the cartridge was dried for 15 s. Each cartridge was then eluted with 1 ml of acetonitrile. Low vacuum was applied 1 min after the addition of acetonitrile and the cartridge was dried for a final 1 min. The combined eluates were evaporated to dryness under nitrogen in a TurboVap at 45 °C. The dry residues were reconstituted in 50 μl of ammonium acetate (1 mM, pH 4.0)–acetonitrile (30:70) and then mixed in a vortex mixer for 1 min. The reconstituted extracts were transferred into autosampler vials and 20 μl of sample were injected on to the LC column.

Data acquisition and analysis

Data acquisition was performed using RAD software (version 2.4, PE-SCIEX, Foster City, CA, USA) residing on a Macintosh Quadra 650. Peak area integration wasperformed by MacQuan software (version 1.3, PE-SCIEX). Linear regression was performed by DMLIMS$^+$ (Drug Metabolism Laboratory Information Management System, PennComp, Wayne, PA, USA). Data for all samples were electronically transferred to an EXCEL spreadsheet for further statistical calculations. All calculations were based on the peak area ratios of lovastatin to simvastatin and lovastatin hydroxy acid to simvastatin hydroxy acid.

RESULTS AND DISCUSSION

The objective of this work was to develop a sensitive and specific method for the determination of the title compounds in small volume of mouse and rat plasma. From the full-scan product ion mass spectra of lovastatin determined in the positive-ion mode [Fig. 2(A)] and lovastatin hydroxy acid determined in the negative-ion mode [Fig. 2(B)], it is clear that both compounds produce abundant product ions under the described experimental conditions. It should be noted that the sodiated adduct ions predominated in the positive-ion mode for both Lov and Sim [Fig. 2(A) and (C)]. Since no sodium was deliberately added to the samples, its origin is believed to be from either the glass containers used in this work or residual sodium derived from the biological matrix. It was found that the best sensitivity was achieved with the detection of the two analytes under different ionization polarity conditions. With the ability to switch automatically the polarity of ionization during data acquisition by the PE-SCIEX API III$^+$ instrumentation, the conditions were optimized to separate Lov/Sim and Lov-OH/Sim-OH within a short gradient analysis period. Therefore, one injection of a solution containing all four compounds will provide analytical data for both analytes and internal standards. For quantitation in the SRM mode, the precursor → product ions monitored in the negative-ion mode were m/z 421.3 → 101 (Lov-OH) and m/z 435.3 → 115 (Sim-OH), and the precursor → product ions monitored in the positive-ion mode were m/z 427.4 → 325 (Lov) and m/z 441.4 → 325 (Sim). An example of SRM ion chromatograms of Lov, Lov-OH, Sim and Sim-OH analytical standards is shown in Fig. 3. These results were obtained from an injection of a synthetic mixture containing10 pg of each compound.

For plasma samples, a solid-phase extraction procedure was developed to isolate lovastatin, its hydroxy acid and the corresponding internal standards from the biological matrix. The combined fractions were evaporated to dryness and the reconstituted extracts were injected into the LC/MS/MS system. When SRM was

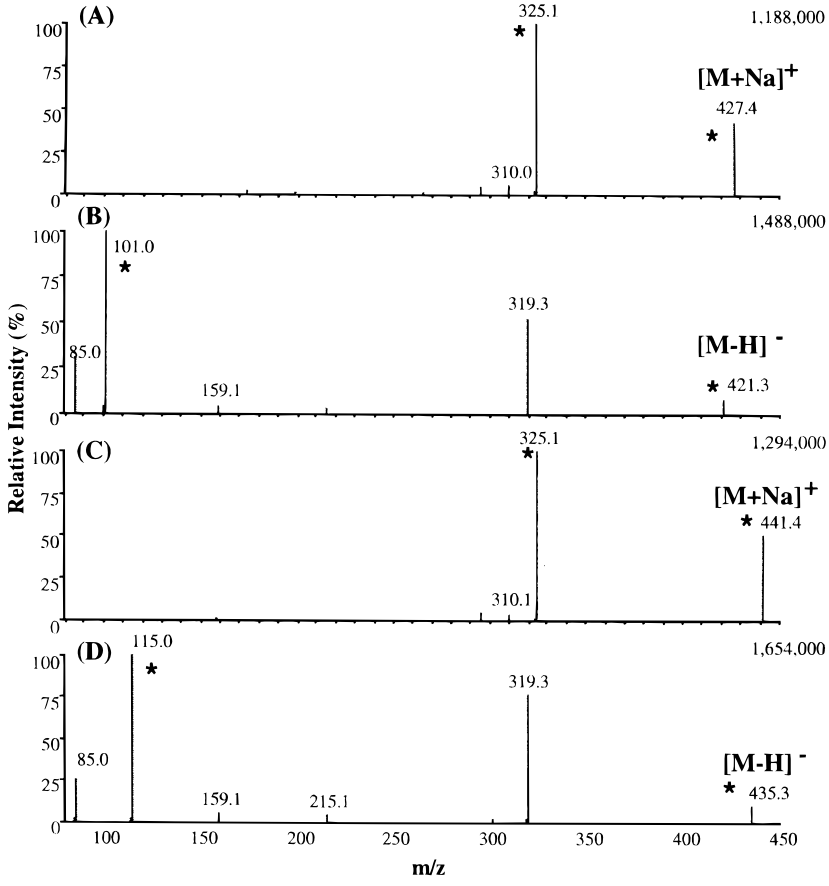

Figure 2. Full-scan product ion spectra of (A) Lov, (B) Lov-OH, (C) Sim and (D) Sim-OH. Peaks labeled with asterisks are the precursor and product ions used in quantitation in the selected reaction monitoring mode.

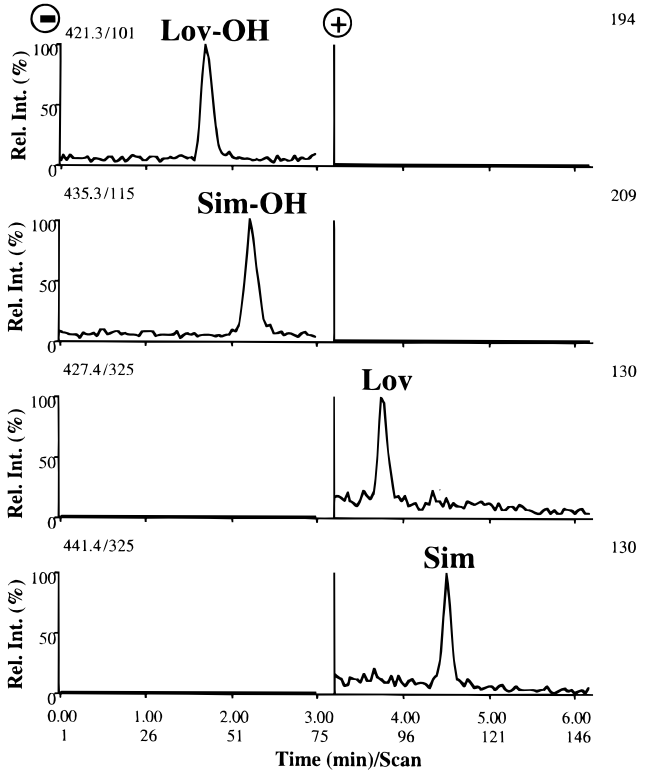

Figure 3. SRM LC/MS traces of Lov, Lov-OH, Sim and Sim-OH analytical standards. Injection of 10 pg of each standard was made.

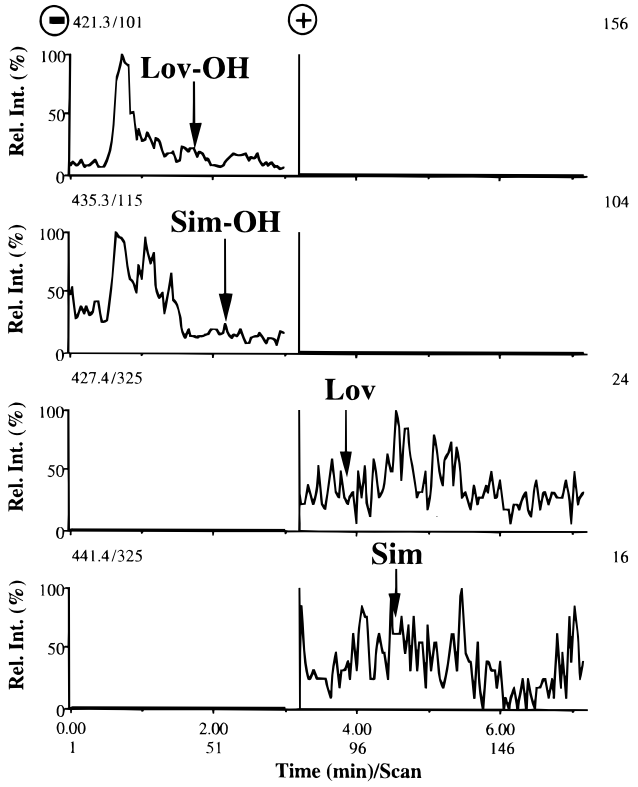

Figure 4. SRM LC/MS traces of double blank mouse plasma extract.

combined with polarity switching, an LLQ of 0.50 ng ml^{-1} for both lovastatin and its hydroxy acid was achieved with the use of 0.1 ml aliquots of mouse and rat plasma.

Assay specificity

The specificity of the method is documented by the absence of interferences from endogenous substances from drug-free plasma. Chemical interference from plasma was minimized by the combination of HPLC, MS/MS, the choice of ionization mode and the selected transitions. Representative ion chromatograms for the double blank mouse and rat plasma extracts (i.e. without either the analytes or the internal standards) are shown in Figs 4 and 5. Figures 6 and 7 show representative chromatograms of mouse and rat plasma spiked with Lov and Lov-OH at 0.50 ng ml^{-1} level. Assay specificity was confirmed by the absence of interferences observed in the retention time region of the analytes and internal standards (see Figs 4 and 5). SRM LC/MS traces for a representative mouse study sample are shown in Fig. 8. The Lov and Lov-OH concentrations determined with this sample were 88.2 ng ml^{-1} and 1.25 μg ml^{-1} (successive dilution of 20-fold with control mouse plasma), respectively.

Linearity

All mouse and rat validation analyses displayed linear calibration graphs from 0.50 to 100 ng ml^{-1} in plasma for both Lov and Lov-OH. Linear calibration graphs were constructed by weighted $(1/y)$ least-squares regression of concentration versus peak area ratios (analyte/internal standard) of the calibration standards, and the slope and the intercept were calculated. Tables 1 and 2 summarize the slope, intercept and r^2 values for three

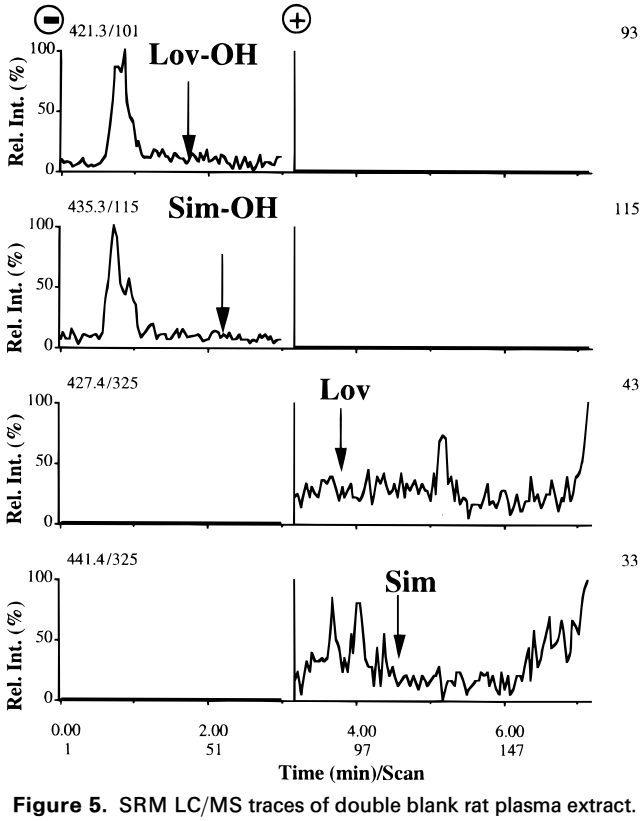

Figure 5. SRM LC/MS traces of double blank rat plasma extract.

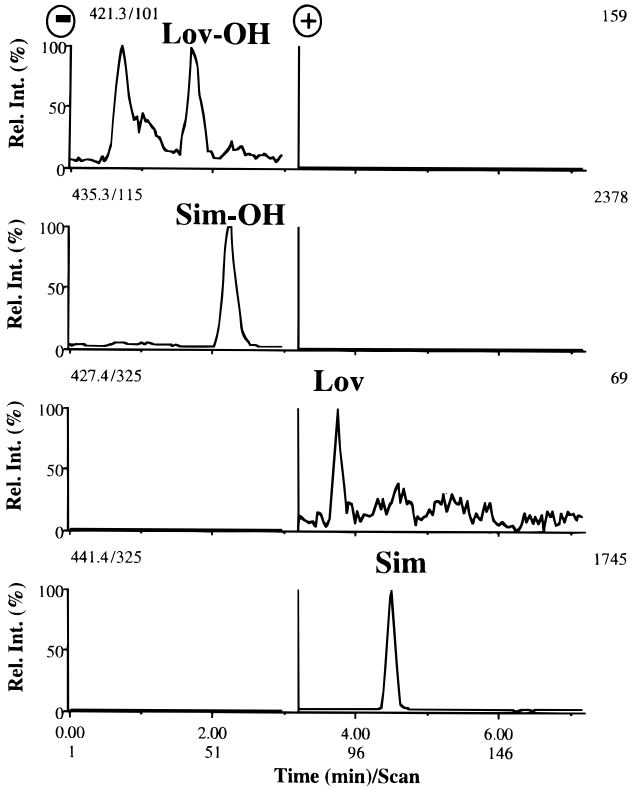

Figure 6. SRM LC/MS traces of extract from mouse plasma spiked with Lov at 0.50 ng ml^{-1}, Lov-OH at 0.50 ng ml^{-1}, Sim at 10.0 ng ml^{-1} and Sim-OH at 10.0 ng ml^{-1}.

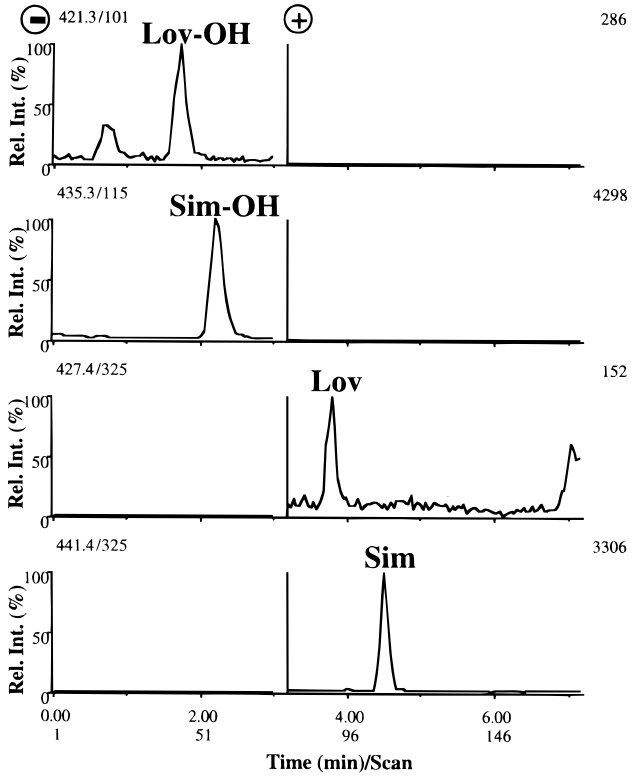

Figure 7. SRM LC/MS traces of extract from rat plasma spiked with Lov at 0.50 ng ml^{-1}, Lov-OH at 0.50 ng ml^{-1}, Sim at 10.0 ng ml^{-1} and Sim-OH at 10.0 ng ml^{-1}.

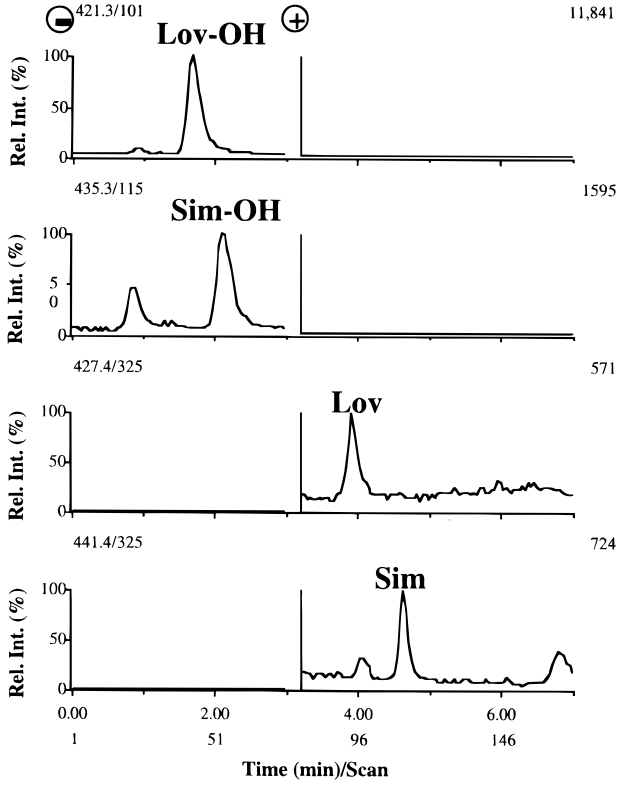

Figure 8. SRM LC/MS traces of extract from an unknown mouse plasma study sample spiked with Sim at 10.0 ng ml^{-1} and Sim-OH at 10.0 ng ml^{-1}.

validation analyses from mouse and rat plasma extracts, respectively. Representative calibration graphs for Lov and Lov-OH from mouse and rat plasma are shown in Figs 9 and 10, respectively.

Table 1. Summary of regression equation and r^2 for three calibration graphs from inter-assay precision and accuracy analyses in mouse plasma

Analyte	No. of analysis	Intercept	Slope	r^2
Lov	1	0.005198	0.065354	0.9814
	2	0.003842	0.049441	0.9941
	3	0.006788	0.065490	0.9969
Lov-OH	1	0.015982	0.094487	0.9987
	2	0.011406	0.086997	0.9996
	3	0.011418	0.090011	0.9996

Table 2. Summary of regression equation and r^2 for three calibration graphs from inter-assay precision and accuracy analyses in rat plasma

Analyte	No. of analysis	Intercept	Slope	r^2
Lov	1	0.104567	0.051988	0.9995
	2	0.104348	0.059534	0.9996
	3	0.103738	0.034795	0.9979
Lov-OH	1	0.121124	0.013679	0.9997
	2	0.122388	0.018527	0.9999
	3	0.108189	0.015479	0.9991

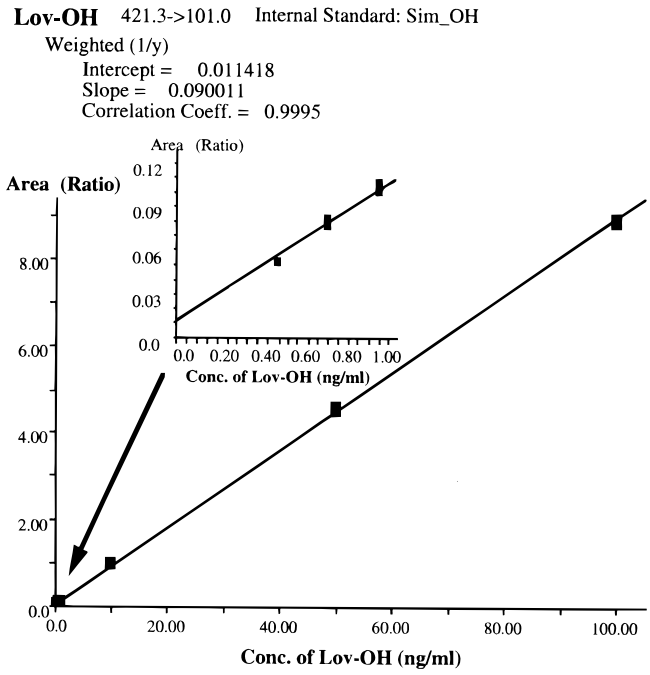

Figure 9. Representative calibration graph for Lov-OH extracted from mouse plasma from 0.50 to 100 ng ml^{-1}.

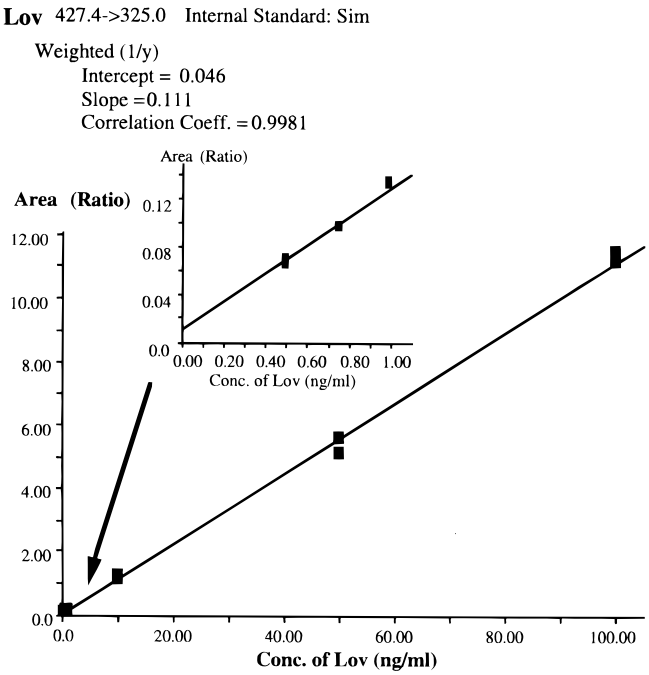

Figure 10. Representative calibration graph of Lov extracted from rat plasma from 0.50 to 100 ng ml^{-1}.

Assay precision and accuracy

The intra- and inter-assay precision represent the precision observed within one day's analysis and between different days of analyses, respectively. Intra- and inter-assay precision were assessed from the results with QC samples. The mean values and RSD for QC samples were calculated over three analyses. At least five replicates of each QC level were determined in each analysis. These data were then used to calculate the intra- and inter-assay precision (RSD) by using a one-way analysis of variance (ANOVA). Inter-assay precision was also assessed from the calibration standard results. The found concentrations of each standard level from three analyses were averaged. The inter-assay precision for the calibration standards was then calculated from the six found values at each level.

The intra- and inter-assay accuracy of the method were assessed by determining the percentage error observed in the analysis of QC samples. The theoretical value of each QC level was subtracted from the mean (for intra-assay) or grand mean (for inter-assay) concentrations determined from three validation analyses. The residual was divided by the theoretical value and converted to a percentage (DEV). Therefore, DEV represents the average percentage difference between the

Table 3. Summary of intra- and inter-assay precision and inter-assay accuracy from QC samples of mouse plasma extracts

Analyte	No. of analysis	QC 1 (0.80 ng ml^{-1})[a]	QC 2 (40.0 ng ml^{-1})[a]	QC 3 (80.0 ng ml^{-1})[a]
Lov	1	0.81	41.7	83.9
	2	0.77	39.0	73.3
	3	0.73	39.8	78.3
	Grand mean	0.77	40.2	78.5
	DEV (%)	−3.75	0.50	−1.88
	Inter-assay precision [RSD (%)]	4.47	2.67	6.19
	Intra-assay precision [RSD (%)]	4.83	5.47	4.32
Lov-OH	1	0.77	42.9	85.2
	2	0.80	42.8	81.7
	3	0.79	42.9	83.2
	Grand mean	0.79	42.9	83.4
	DEV (%)	−1.25	7.25	4.25
	Inter-assay precision [RSD (%)]	1.47	0.68	2.02
	Intra-assay precision [RSD (%)]	2.76	1.71	1.61

[a] Concentration (ng ml^{-1}) are the means of six replicates from each analysis.

determined value for a QC sample and its theoretical value.

Table 3 summarizes the intra- and inter-assay precision and inter-assay accuracy for Lov and Lov-OH from the QC samples of mouse plasma extracts. The results were calculated using a one-way ANOVA. The intra-assay precision, calculated from QC samples, was $<6\%$ for each QC level of lovastatin and $<3\%$ for each QC level of lovastatin hydroxy acid. The inter-assay precision, calculated from QC samples, was $<7\%$ for each QC level of lovastatin and $<3\%$ for each QC level of lovastatin hydroxy acid. The inter-assay accuracy as determined from QC samples did not exceed 3.9% for each QC level of lovastatin and 7.2% for each QC level of lovastatin hydroxy acid. Table 4 lists the inter-assay precision for Lov and Lov-OH from the calibration standards of mouse plasma extracts. The RSD was $<14\%$ for each concentration of the Lov standards and $<5\%$ for each concentration of the Lov-OH standards.

Similar calculations were performed for the data obtained from rat plasma extracts (Tables 2, 5 and 6). The intra-assay precision was $<7\%$ for each QC level of lovastatin and $<6\%$ for each QC level of lovastatin hydroxy acid. The inter-assay precision was $<1\%$ for each QC level of lovastatin and $<2\%$ for each QC level of lovastatin hydroxy acid. The inter-assay accuracy as determined from QC samples was $<6\%$ for each QC

Table 4. Summary of inter-assay precision from calibration standards of mouse plasma extracts

		Calibration standard concentration (ng ml^{-1})					
Analyte	No. of analysis	0.50	0.75	1.00	10.00	50.00	100
Lov	1	0.47	0.71	0.90	9.55	49.6	91.5
		0.47	0.75	1.12	11.0	68.0	96.3
	3	0.46	0.74	1.01	10.6	55.4	—[a]
		0.46	0.69	1.00	11.5	50.7	93.2
	3	0.55	0.78	0.91	9.70	48.4	94.2
		0.44	0.76	0.99	10.9	54.0	104
	Mean	0.48	0.74	0.99	10.5	54.4	95.8
	RSD (%)	8.07	4.49	8.08	7.29	13.2	5.09
	DEV (%)	−4.00	−1.33	−1.00	5.00	8.80	−4.20
Lov-OH	1	0.46	0.69	1.07	10.4	51.2	98.1
		0.49	0.71	1.03	11.1	47.4	102
	2	0.46	0.77	1.06	10.5	49.5	—[a]
		0.45	0.73	1.03	10.7	50.0	99.4
	3	0.45	0.73	1.05	10.5	49.8	99.7
		0.45	0.78	1.00	10.9	50.9	98.4
	Mean	0.46	0.74	1.04	10.7	49.8	99.5
	RSD (%)	3.37	4.69	2.43	2.54	2.70	1.55
	DEV (%)	−8.00	−1.33	4.00	7.00	−0.40	−0.50

[a] Standards which were eliminated from the calibration curves due to an injector problem.

Table 5. Summary of intra- and inter-assay precision and inter-assay accuracy from QC samples of rat plasma extracts

Analyte	No. of analysis	QC 1 (0.80 ng ml^{-1})[a]	QC 2 (40.0 ng ml^{-1})[a]	QC 3 (80.0 ng ml^{-1})[a]
Lov	1	0.76*	39.4	80.4
	2	0.74	40.5	81.4
	3	0.78	38.6	79.9
	Grand mean	0.76	39.5	80.6
	DEV (%)	−5.00	−1.25	0.75
	Inter-assay precision [RSD (%)]	0	0.67	0
	Intra-assay precision [RSD (%)]	6.83	5.94	4.07
Lov-OH	1	0.80	37.7	77.5
	2	0.77	38.3	79.1
	3	0.80	39.0	78.0
	Grand mean	0.79	38.3	78.2
	DEV (%)	−1.25	−4.25	−2.25
	Inter-assay precision [RSD (%)]	0	1.22	0
	Intra-assay precision [RSD (%)]	5.73	2.74	2.91

[a] Concentrations (ng ml^{-1}) are the means of six replicates from each analysis.

Table 6. Summary of inter-assay precision from calibration standards of rat plasma extracts

		Calibration standard concentration (ng ml^{-1})					
Analyte	No. of analysis	0.50	0.75	1.00	10.00	50.00	100
Lov	1	0.49	0.79	0.99	9.70	50.0	98.1
		0.50	0.76	0.93	10.3	52.3	99.9
	2	0.51	0.74	0.98	10.3	50.2	102
		0.51	0.72	1.02	9.91	49.6	97.8
	3	0.46	0.78	0.96	10.4	52.6	104
		0.58	0.74	0.97	9.50	48.6	95.1
	Mean	0.51	0.76	0.98	10.0	50.6	99.5
	RSD (%)	7.81	3.53	3.09	3.70	3.11	3.21
	DEV (%)	2.00	1.33	−2.00	0.00	1.20	−0.50
Lov-OH	1	0.48	0.76	1.04	9.86	51.6	99.7
		0.45	0.79	1.01	10.5	49.3	99.1
	2	0.48	0.71	1.00	10.2	50.6	98.9
		0.52	0.72	1.05	10.1	50.6	99.7
	3	0.43	0.72	1.01	10.8	51.1	97.5
		0.46	0.75	1.05	10.8	51.4	98.7
	Mean	0.47	0.74	1.03	10.4	50.8	98.9
	RSD (%)	6.59	4.13	2.19	3.73	1.63	0.82
	DEV (%)	−6.00	−1.33	3.00	4.00	1.60	−1.10

level of lovastatin and $<5\%$ for each QC level of lovastatin hydroxy acid. The RSD was $<8\%$ for each concentration of the Lov standards and $<7\%$ for each concentration of the Lov-OH standards.

Lower limit of quantitation (LLQ) and recovery

The lower limit of quantitation is defined as the lowest concentration on the calibration graph for which an acceptable accuracy of $100 \pm 20\%$ [(mean observed concentration/theoretical concentration) $\times$ 100] and a precision of 20% (RSD) were obtained. The current assay has an LLQ of 0.50 ng ml^{-1} in mouse and rat plasma for both Lov and Lov-OH based on 0.1 ml aliquots of plasma. The RSD and DEV at LLQ concentration were within 17% and 9% for Lov and Lov-OH in mouse plasma and within 11% and 3% for Lov and Lov-OH in rat plasma.

The extraction recoveries of Lov and Lov-OH at three QC levels were determined by comparing the peak area ratio of Lov or Lov-OH to the internal standard (Sim or Sim-OH) in samples that had been spiked with both analytes prior to extraction (pre-extraction) with samples to which both analytes had been added post-extraction. The internal standards were added to both sets of samples post-extraction.

The extraction recoveries of Sim-OH and Sim-OH were determined only at a level of 10.0 ng ml^{-1} of plasma using Lov and Lov-OH as internal standards at 40.0 ng ml^{-1} of plasma. The peak area ratios of Sim or Sim-OH to Lov or Lov-OH in samples that had been spiked with Sim and Sim-OH prior to extraction (pre-extraction) were compared with those from samples to which Sim and Sim-OH had been added post-extraction. Lov and Lov-OH were added to both sets of samples post-extraction.

In mouse plasma, the overall recoveries of Lov and Lov-OH were 54% (RSD = 10.78%) and 100% (RSD = 1.73%), respectively. The recoveries of Sim and Sim-OH were 66% and 96%, respectively. In rat plasma, the overall recoveries of Lov and Lov-OH were 55% (RSD = 12.65%) and 67% (RSD = 8.24%), respectively. The recoveries of Sim and Sim-OH were 69% and 74%, respectively. These results indicate that the recoveries of lovastatin and its hydroxy acid are not concentration dependent in the tested range in mouse and rat plasma.

Stability

The stability of Lov and Lov-OH in plasma and reconstitution buffer were investigated. Lov-OH was found to be stable in all experiments, whereas Lov was found to be unstable in both mouse and rat plasma at ambient temperature. Therefore, plasma samples need to be placed in an ice–water bath at 4 °C prior to solid-phase extraction. Lov was also found to be unstable in the reconstitution buffer (acetonitrile–ammonium acetate (1 mM, pH 4.0) (70:30) at ambient temperature for 24 h. However, Lov did appear to be stable in the reconstitution buffer at 4 °C for 24 h (the sample holder in the HP1090 was maintained at 4 °C). Both analytes were also shown to be stable after three freeze (−70 °C)–thaw (4 °C) cycles in both plasma and to be stable up to 4 days as dry extracts at 4 °C.

CONCLUSIONS

The proposed LC ionspray MS/MS method for the simultaneous determination of lovastatin and its

hydroxy acid offers several unique aspects. First, the sample preparation procedure is relatively simple. No time-consuming derivatization step is needed. Second, the volume of plasma sample is only 100 μl, which is significantly less than in previously published methods. Finally, excellent sensitivity and selectivity can be achieved using the described polarity switch during a single LC/MS/MS analysis. This LC/MS/MS assay procedure has proven to be sensitive, accurate, selective and reproducible. It is likely that this method could be amenable to the determination of lovastin and its hydroxy acid metabolite in plasma from other species. In addition, it is proposed that the use of a small biological sample volume as described here may be both applicable and desirable for miniaturized sample preparation strategies that will likely enjoy increased use in the future.[9]

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