

Determination of the HMG-CoA Reductase Inhibitors Simvastatin, Lovastatin, and Pravastatin in Plasma by Gas Chromatography/Chemical Ionization Mass Spectrometry

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A general method for the assay of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors lovastatin, pravastatin, and simvastatin in plasma has been developed and validated. The analytes are isolated from plasma by a solid-phase extraction procedure which separates the lactone and acid forms of the drugs. The lactone is converted to the acid form, which is subsequently derivatized by pentafluorobenzoylation of the carboxyl group, and trimethylsilylation of the hydroxyl functions. Derivatized samples of intrinsic and converted acid are assayed by gas chromatography/mass spectrometry using negative chemical ionization mass spectrometry. The method has sufficient sensitivity, precision, accuracy, and selectivity for the analysis of clinical samples containing the drugs administered at therapeutic doses. The method thus permits determination of both the lactone and hydroxy acid forms of lovastatin and simvastatin, and is also applicable to the assay of pravastatin.

INTRODUCTION

Recently, novel cholesterol-lowering drugs have been developed which are competitive inhibitors of the synthesis of mevalonic acid by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase.^{1,2} The potency of these substances necessitates the use of sensitive and reliable assays for their determination in biological fluids. The molecular structures of the drugs precludes the attainment of the requisite sensitivity through simple chromatographic and/or derivatization techniques. In addition, with two of these drugs, simvastatin and lovastatin, the compounds are administered as lactones (Fig. 1) rather than as the open acids which are the pharmacologically active entities. Such a strategy is desirable since the primary target is the liver—a major site of cholesterol synthesis.¹ The drug must first pass this organ after being absorbed from the gastro-

intestinal tract. Both the lactone and open acid are present in plasma following the administration of the lactones.³

A sensitive method using gas chromatography with negative chemical ionization mass spectrometry has previously been reported for the determination of pravastatin.⁴ In order to make the drug amenable to this technique, it was derivatized with pentafluorobenzyl bromide (PFB) to make an ester for negative ion chemical ionization mass spectrometry. In addition, to improve chromatographic behavior, the hydroxyl groups were silylated. In contrast to lovastatin and simvastatin, the parent drug is the open acid. There is evidence that pravastatin can be converted to the corresponding lactone to a small extent *in vivo*.⁵ However, no analytical methodology was presented to differentiate between the two forms.

A procedure for the analysis of lovastatin using the same derivatization technique as that employed for pravastatin has recently been described.⁶ Since a simple solid-phase extraction was employed in the isolation scheme, only the open acid form of the drug and that

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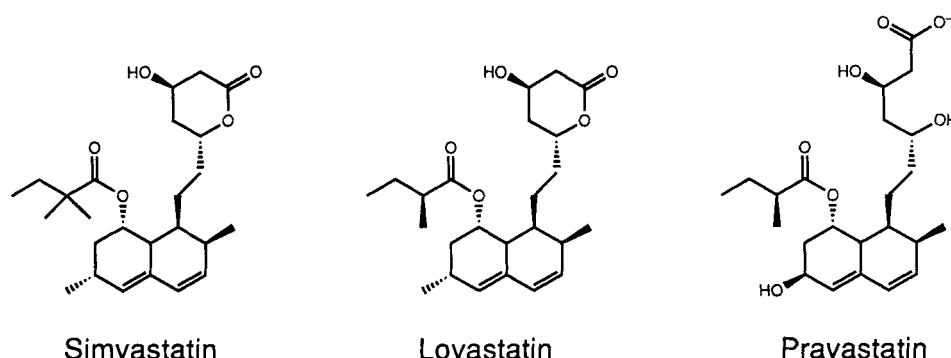


Figure 1. Structures of simvastatin, lovastatin, and pravastatin.

which may have been hydrolytically converted to the acid during storage and/or processing was analyzed. The unhydrolyzed lactone cannot be derivatized under such conditions. With lovastatin and simvastatin significant levels of the lactones have been shown to occur *in vivo* along with the open acid forms.³ Since the lactones are susceptible to hydrolysis, controls must be in place to ensure that one or both forms can be quantified in the presence of the other.

Recently, a sensitive method based on gas chromatography/mass spectrometry (GC/MS) with electron ionization was described in which both the lactone and open acid forms of simvastatin could be quantified.⁷ The lactone and open acid were separately isolated from plasma using a solid-phase extraction scheme. The lactone fraction was subsequently hydrolyzed to the acid prior to derivatization. A boronate derivative was made by cyclizing the 3- and 5-hydroxyl groups (1,3-diol) of the heptanoic acid side chain.⁸ In order to generate fragments with a high *m/z* ratio by mass spectrometry and potentially increase sensitivity, the high-molecular-weight ferrocene boronate derivative was employed. The carboxyl group was converted to the methyl ester. This method is suitable for the analysis of both acid and lactone forms of lovastatin and simvastatin. However, the method is not suitable as such for ring-hydroxylated inhibitors, such as pravastatin, which would require derivatization of the isolated hydroxyl group in addition to the 1,3-diol.

Accordingly, an alternative procedure based on GC/MS was developed which could separately quantify the lactone and open acid forms of simvastatin and lovastatin and the ring-hydroxylated inhibitor pravastatin. The method could, potentially, be extended to hydroxylate metabolites of lovastatin and simvastatin which exhibit inhibitory activity, some of which have been shown to be major circulating species.⁹ In the pursuit of this goal, several aspects of the prior methodologies were incorporated. Lovastatin and lovastatin acid served as internal standards for the assays of simvastatin and simvastatin acid, and vice versa.

EXPERIMENTAL

Materials

Simvastatin, lovastatin, 6' β -hydroxymethylsimvastatin, and the corresponding hydroxyacids of the lactones were synthesized by Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey. All were >99% pure as determined by high-performance liquid chromatography (HPLC). *N,N*-Diisopropylethylamine (DIEA) was purchased from Aldrich Chemical (St Louis, Missouri), tetradecane from Sigma Chemical (St Louis, Missouri), and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) from Pierce Chemical (Rockford, Illinois). Potassium hydroxide, potassium hydrogen phosphate, potassium dihydrogen phosphate, and phosphoric acid were obtained from Mallinckrodt (Paris, Kentucky). Methanol, acetonitrile, toluene, hexane, and heptane were all purchased from Baxter Healthcare (McGaw Park, Illinois) as HPLC-grade sol-

vents. The capillary column used was an Ultra 2 (methyl, 5% phenyl, 12 m \times 0.2 mm i.d. \times 0.11 μ m film thickness) from Hewlett-Packard (Paramus, New Jersey). The carrier gas was helium (UHP 99.999%) from MG Industries (Valley Forge, Pennsylvania) and ammonia (electronic grade, 99.999%) from Air Products (Trexlerstown, Pennsylvania). Disposable mini-reaction vials were purchased from Rainin Instruments (Woburn, Massachusetts). Solid-phase extraction (SPE) cartridges containing C₁₈ (3 ml \times 200 mg) and C₈ (3 ml \times 200 mg) sorbents were obtained from J. T. Baker (Phillipsburg, New Jersey) and Analytichem International (Harbor City, California), respectively.

Standard and sample preparation

Stock solutions of simvastatin and lovastatin were prepared by dissolving the lactones in acetonitrile at concentrations of 1 mg ml⁻¹. The corresponding hydroxy acids (as their ammonium salts) were prepared as solutions in acetonitrile-water (60 : 40) at concentrations of 1 mg ml⁻¹. Equal volumes of the appropriate acid and lactone were combined and subsequent dilutions were made in acetonitrile. Plasma standards were prepared by the addition of known amounts of standard solutions of acid and lactone in acetonitrile (0.1 ml) to 1 ml of control human plasma containing 20 μ l of phosphate buffer (1 M, pH 7.0). The concentrations of the analytes used in the assay for simvastatin and lovastatin were 0.2, 0.5, 1, 2, 5, 10 and 20 ng ml⁻¹. Standard plasma samples containing pravastatin were prepared similarly at concentrations of 0.5, 1, 2, 5, 10, 20, and 50 ng ml⁻¹.

Quality-control samples were prepared from independently prepared stock solutions. Appropriate aliquots of the diluted solution were added to 40.0 ml of control human plasma to yield the concentrations indicated in Table 3. The concentrations of drug in quality-control samples were selected according to the anticipated plasma concentrations in test samples. Aliquots (1.2 ml) of the well-mixed plasma were stored at -20°C until taken for assay with a set of test samples.

Extraction procedure

The sample preparation procedure used for the determination of lovastatin and simvastatin is shown schematically in Fig. 2. The plasma sample (1.0 ml) was placed in an 8 ml polypropylene tube containing 20 μ l of phosphate buffer (1 M, pH 7.0). After the addition of internal standards (20 ng of both acid and lactone in acetonitrile, 50 μ l), the sample was diluted with 2 ml of water and loaded onto a C₈ SPE cartridge at a rate of 1 ml min⁻¹ using gravity. The column was washed with 3 ml phosphate buffer (0.01 M, pH 7.0), followed by 3 ml water, and the washings discarded. The acids (acid fraction) were eluted with 2 \times 1 ml methanol-water (60 : 40) and the lactones (lactone fraction) with 2 \times 1 ml acetonitrile. The eluates were reduced in volume in a SpeedVac Model SVC200H (Savant, Farmingdale, New York) at 40°C, the acid fraction to less than 1 ml and the lactone fraction to dryness. The extracts were diluted to approximately 2 ml with water. Forty microliters of potassium hydroxide (0.5 M) were added to

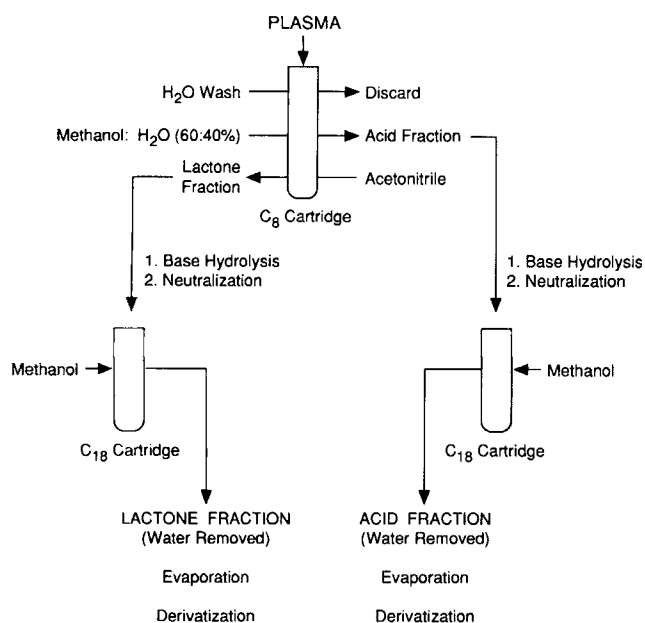


Figure 2. Sample preparation scheme for the separate isolation of the acid and lactone forms of simvastatin and lovastatin from plasma.

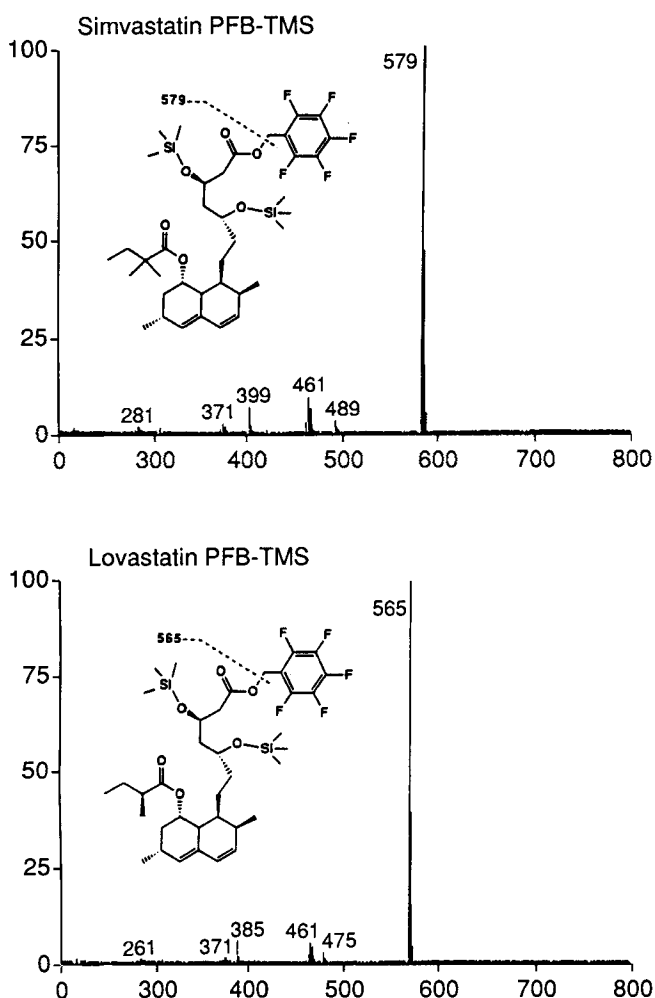


Figure 3. Ammonia negative chemical ionization spectra of the PFB bis-TMS derivatives of simvastatin and lovastatin acids.

hydrolyse the lactone. Base was also added to the acid fraction to mimic the treatment of the lactone fractions. The tubes were incubated at 20 °C for 15 min. Phosphoric acid (30 μ l/0.5 M) and water (1 ml) was added to each tube. C₁₈ solid phase cartridges were used to isolate the acids from the aqueous environment. The sample was loaded onto a cartridge under gravity at a rate of 1 ml min⁻¹. The cartridge was washed with water (3 ml) and hexane (3 ml) and the washings discarded. The analyte was eluted in 2 \times 400 μ l methanol, and the methanol eluate transferred to derivatization vials and evaporated to dryness under a stream of nitrogen at 40 °C. When assaying pravastatin, where no separation of acid and lactone was required, use of the C₈ solid-phase cartridge was omitted. The test plasma (1.0 ml) was mixed with 20 μ l of phosphate buffer (1 M, pH 7) and 50 μ l of internal standard solution (6' β -hydroxymethylsimvastatin, 5 ng in acetonitrile) was added. After dilution with water (2 ml) the sample was applied to a conditioned C₁₈ solid-phase cartridge and extracted as described above.

Aliquots (100 μ l) of a 2.5% solution of DIEA in acetonitrile and PFB (160 μ l) were added to the residues, which were incubated at 40 °C for 15 min. Excess reagent was removed at 40 °C under a stream of nitrogen. Silylating reagent (100 μ l MSFTA) was added to each tube and the mixture incubated for 15 min at room temperature. The reagent was removed at 40 °C under a stream of nitrogen and the residue reconstituted in 20 μ l tetradecane, transferred to an autosampler vial, and briefly vortexed to remove air bubbles prior to analysis.

GC/MS analysis

The mass spectrometer was a VG model Trio 2 interfaced by a direct transfer line to a Hewlett Packard 5890A gas chromatograph equipped with a Hewlett Packard 7673A autoinjector. Data acquisition was accomplished using a PDP 11/73 data system incorporating RSX operating software and VG System Task Files. The carrier gas (helium) pressure was 10 p.s.i. and injections were made in the splitless mode. The injector purge was opened after 1 min at a split ratio of 15:1. The injector and transfer line temperatures were set at 280 °C and 300 °C, respectively. The injection volume was 1 μ l. The oven temperature was held at 210 °C for 1 min, then increased at 10 °C min⁻¹ to 300 °C.

Under these conditions the derivatives of lovastatin, simvastatin, and pravastatin eluted with approximate retention times of 8.2, 8.4 and 9.1 min, respectively. The derivative of 6' β -hydroxymethylsimvastatin acid, which was used as an internal standard in the assay for pravastatin, was retained for about 9.4 min.

The mass spectrometer was operated in the negative ion chemical ionization mode using ammonia as reagent gas. The ion source temperature was maintained at 280 °C and the source housing pressure at 2 \times 10⁻⁴ mbar. The electron energy and photomultiplier settings were 70 eV and 600 V, respectively.

Selected ion monitoring was employed using the fragment ions [M - pentafluorobenzyl]⁻ at m/z 579 (simvastatin), m/z 565 (lovastatin), m/z 639 (pravastatin),

and m/z 667 (6'- β -hydroxymethylsimvastatin). The dwell and reset times were 80 ms and 20 ms, respectively. Peak area ratios of analyte/internal standard were computed using VG's software. The calibration curves were calculated using least-squares regression, weighted to the reciprocal of concentration, versus the measured ratios.

Extraction recovery

The recoveries of the analyte from plasma were determined by extracting spiked samples of control plasma containing known quantities of each analyte, adding the appropriate internal standard after extraction, and comparing the peak area ratios with ratios from unextracted analytes to which internal standard was added.

The recoveries of simvastatin were 82.4% (lactone) and 89.9% (acid). The corresponding recoveries of the lactone and acid of lovastatin were 83.7% and 78.9%, respectively.

RESULTS

Fragmentation patterns of the derivatives

The mass spectra of derivatized lovastatin, simvastatin, pravastatin, and 6'- β -hydroxymethylsimvastatin are shown in Figs 3 and 4. The principal fragment ions are listed in Table 1. As is common with pentafluorobenzyl derivatives, molecular ions are absent and the base peaks, resulting from electron transfer and cleavage with charge retention on the carboxyl group, and those corresponding to the loss of the pentafluorobenzyl function, $[M - 181]$.

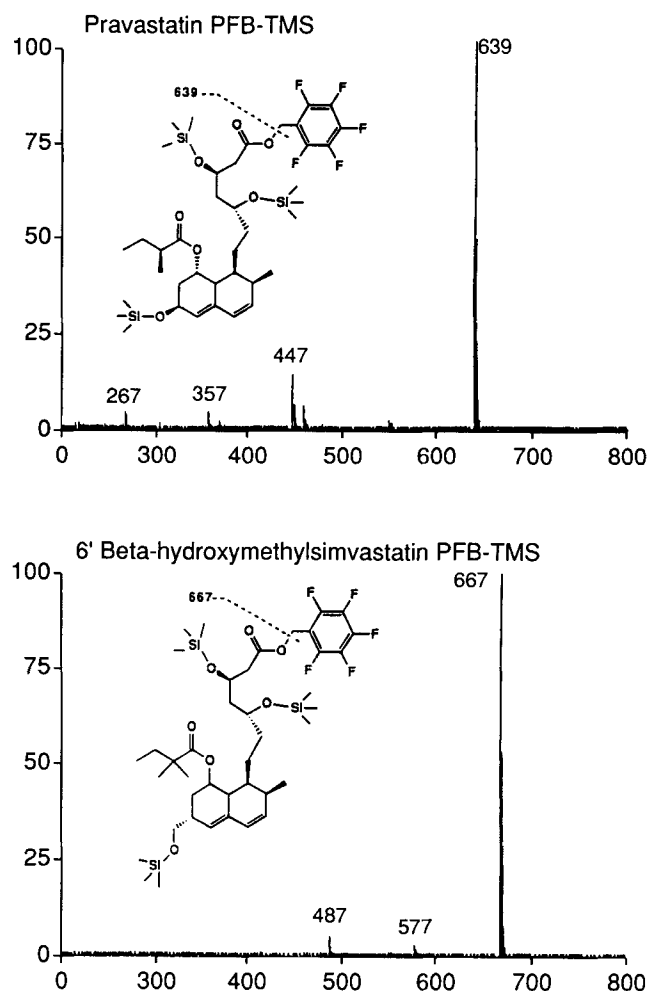


Figure 4. Ammonia negative chemical ionization spectra of the PFB tris-TMS derivatives of pravastatin and its internal standard.

The mass spectrum of derivatized simvastatin exhibits fragments characteristic of the further fragmentation of the m/z 579 ion. Elimination of dimethylbutyric acid ($-116 m/z$) with further loss of hydrogen from the hexa-

Table 1. Principal ions in the negative ion chemical ionization mass spectra of the pentafluorobenzyl trimethylsilyl (TMS) derivatives of lovastatin, simvastatin, pravastatin, and 6'- β -hydroxymethylsimvastatin. Instrument conditions are provided in the text

| Parent substance | Retention time (min) | $[M - 181]$ | Principal ions in mass spectrum ^a | | | |
|--|-------------------------|-------------|--|----------------------|----------------------|-----------------------|
| | | | Other significant ions | | | |
| Simvastatin-PFB-bis-TMS | 8.4 | 579 (100) | 489 ^b (4) | 399 ^c (7) | 463 ^d (6) | 461 ^e (10) |
| Lovastatin-PFB-bis-TMS | 8.2 | 565 (100) | 475 ^b (3) | 385 ^c (6) | 463 ^d (4) | 461 ^e (6) |
| Pravastatin-PFB-tris-TMS | 9.1 | 639 (100) | 447 ^f (15) | 357 ^g (5) | 267 ^h (5) | |
| 6'- β -Hydroxymethyl simvastatin-PFB-tris-TMS | 9.4 | 667 (100) | 577 ^b (3) | 487 ^c (5) | | |

^a Relative abundances are shown in parentheses.

^b $[M - 181] - \text{Si}(\text{CH}_3)_3\text{OH}$.

^c $[M - 181] - 2\text{Si}(\text{CH}_3)_3\text{OH}$.

^d $[M - 181] - \text{RCO}_2\text{H}$.

^e $[M - 181] - \text{RCO}_2\text{H} - 2\text{H}$.

^f $[M - 181] - \text{RCO}_2\text{H} - \text{Si}(\text{CH}_3)_3\text{OH}$.

^g $[M - 181] - \text{RCO}_2\text{H} - 2\text{Si}(\text{CH}_3)_3\text{OH}$.

^h $[M - 181] - \text{RCO}_2\text{H} - 3\text{Si}(\text{CH}_3)_3\text{OH}$.

Simvastatin: R = $\text{CH}_3\text{CH}_2\text{C}(\text{CH}_3)_2$;

Lovastatin: R = $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)$;

Pravastatin: R = $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)$;

6'- β -hydroxymethylsimvastatin: R = $\text{CH}_3\text{CH}_2\text{C}(\text{CH}_3)_2$.

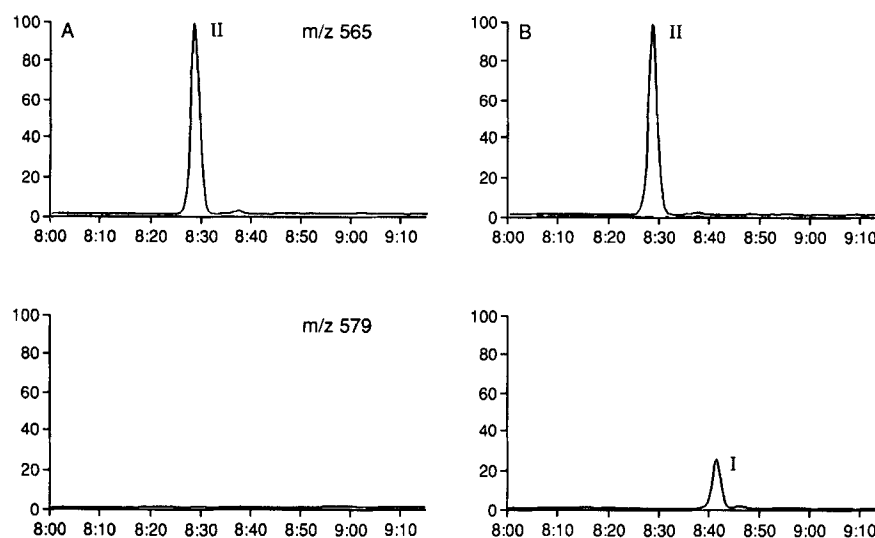


Figure 5. Chromatograms obtained by selected ion monitoring for the determination of simvastatin (lactone) in the plasma of a volunteer receiving 40 mg of the drug. (a) Pre-dose sample; (b) sample collected at 2 h post-dose containing 4 ng ml^{-1} . Peaks I and II correspond to derivatized simvastatin and its internal standard, observed at their diagnostic fragment, m/z 579 and m/z 565, respectively.

hydronaphthalene ring system results in a fragment at m/z 461. Successive losses of trimethylsilanol from the heptanoic acid side chain yields fragments at m/z 381 and 281. The loss of the dimethylbutyric acid side chain, hydrogen, and the trimethylsilyl groups can be rationalized on the basis of increasing conjugation. In addition, there are fragments at 399 m/z and 489 m/z which indicate that losses of trimethylsilanol from the heptanoic acid side chain can occur before loss of the dimethylbutyric acid side chain.

Similar patterns of fragmentation were observed for the derivatives of lovastatin, pravastatin, and 6'-hydroxymethylsimvastatin. In contrast to the acid of simvastatin, pravastatin has a chiral methylbutyric acid side chain in the 8' position and a β -hydroxy group rather than an α -methyl group in the 6' position. Thus,

the base peak for the pravastatin derivative is at m/z 639, and contains a silylated hydroxyl group in the 6' position. Mass chromatograms of derivatized extracts of plasma from subjects receiving simvastatin, lovastatin, and pravastatin are shown in Figs 5–7.

Calibration

Typical calibration curves for simvastatin, lovastatin, and pravastatin are shown in Figs 8 and 9. All calibration curves demonstrated good fit to the regression lines, weighted to the reciprocal of concentration. Calibration standards of plasma containing no drug showed peak area ratios that were essentially zero. No interferences from either endogenous plasma components or metabolites were apparent.

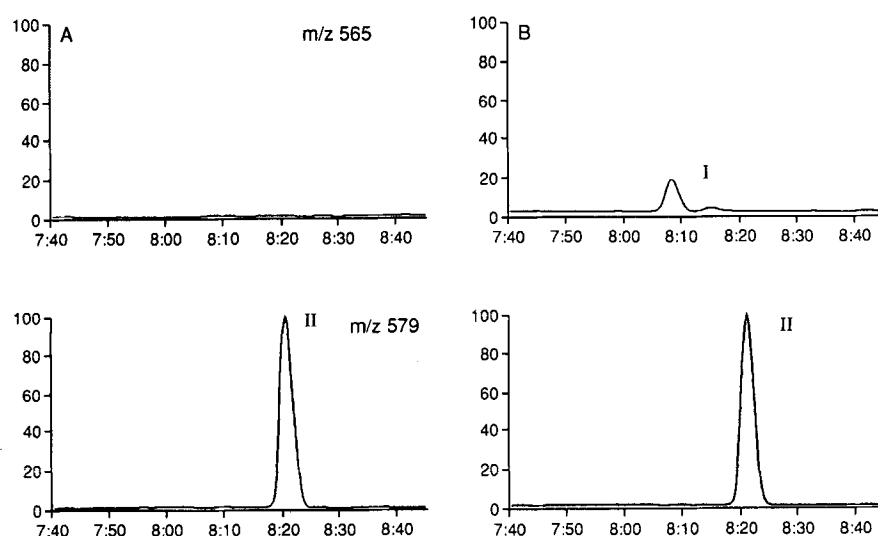


Figure 6. Chromatograms obtained by selected ion monitoring for the determination of lovastatin (lactone) in the plasma of a volunteer receiving 40 mg of the drug. (a) Pre-dose samples; (b) sample collected at 2 h post-dose containing 1.4 ng ml^{-1} . Peaks I and II correspond to derivatized lovastatin and its internal standard, observed at their diagnostic fragments m/z 565 and 579, respectively.

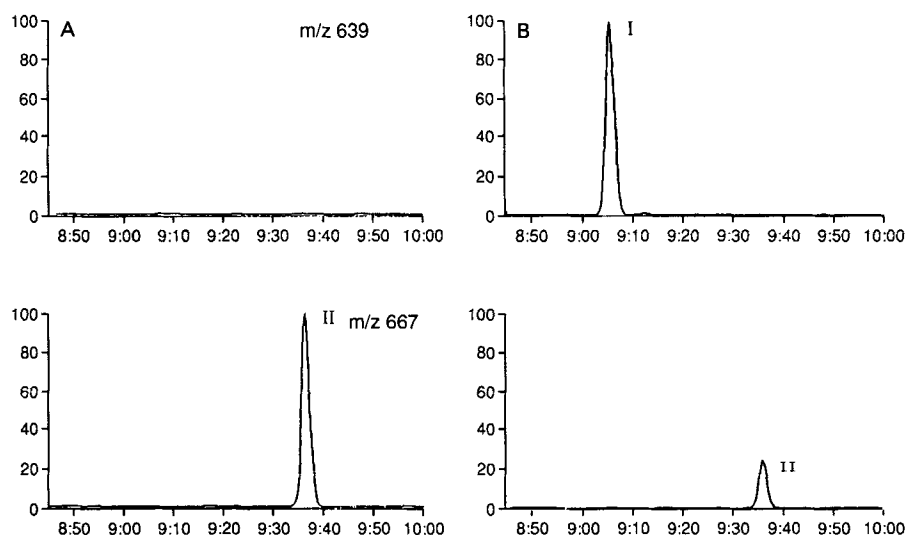


Figure 7. Chromatograms obtained by selected ion monitoring for the determination of pravastatin in the plasma of a volunteer receiving 40 mg of the drug. (a) Pre-dose sample; (b) Sample collected at 2 h post-dose containing 30 ng ml^{-1} . Peaks I and II correspond to derivatized pravastatin and its internal standard observed at their diagnostic fragments m/z 639 and m/z 667, respectively.

The assays for simvastatin lactone and its hydroxy acid were evaluated for their ability to quantify one of the molecular species (lactone or hydroxy acid) in the presence of the other. Standard curves were constructed

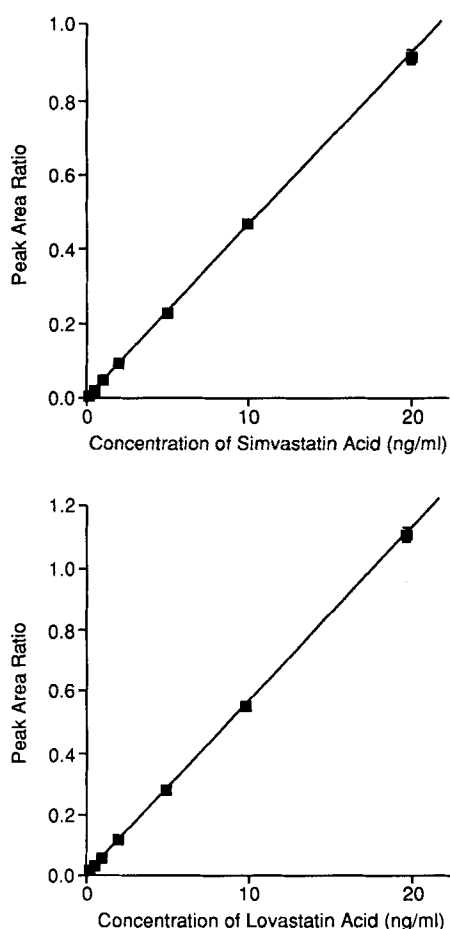


Figure 8. Calibration curves in plasma used for the determination of simvastatin (top: $y = 3.1629e-3 + 4.537e-2x$, $R^2 = 1.000$) and lovastatin (bottom: $y = 6.4129e-3 + 5.3977e-2x$, $R^2 = 1.000$).

in plasma over the range $0.2\text{--}2.0 \text{ ng ml}^{-1}$ for both simvastatin and simvastatin acid. Each sample contained, in addition to the species (lactone or hydroxy acid) used to construct the standard curve, 20 ng ml^{-1} of the other molecular entity. The standard curves obtained for simvastatin and simvastatin acid in the presence of a fixed amount of the other entity were linear, with the same slope. Significant interconversion would have been expected to result in significant changes in slope and deviation from linearity. In addition, quantification of the compound added as a constant amount to each sample gave consistent results of 20 ng ml^{-1} within experimental error.

As a further check on the specificity of a given assay standard curves were constructed with simvastatin or simvastatin acid in the absence of the other entity. Fractions expected to be devoid of compound (acid fraction

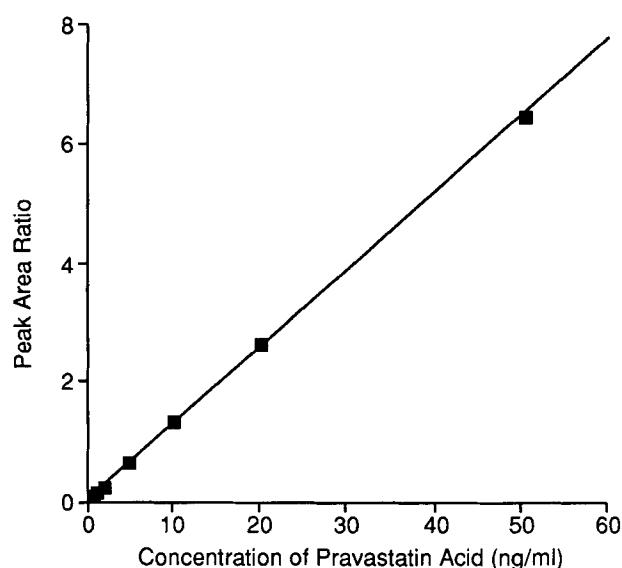


Figure 9. Calibration curve in plasma for the determination of pravastatin in plasma ($y = -1.6204e-3 + 0.13073x$, $R^2 = 1.000$).

Table 2. Intra-day accuracy (% found/added) and precision (coefficient of variation (CV) $n = 5$) for the determination of lovastatin, simvastatin, and pravastatin in plasma

| Concentration (ng ml ⁻¹) | Lovastatin acid | | Lovastatin lactone | | Simvastatin acid | | Simvastatin lactone | | Pravastatin | |
|---|--------------------|------------------|-----------------------|------------------|---------------------|-------------------|------------------------|------------------|-------------|-----|
| | % Accuracy | CV | % Accuracy | CV | % Accuracy | CV | % Accuracy | CV | % Accuracy | CV |
| 0.2 | 101.7 | 3.0 ^a | 105.8 | 6.8 ^a | 91.7 | 10.0 ^a | 101.8 | 5.0 ^a | — | — |
| 0.5 | 101.6 | 5.5 | 91.2 | 1.8 | 99.6 | 1.7 | 102.0 | 5.0 | 117.6 | 6.3 |
| 1.0 | 102.3 | 3.0 ^a | 100.2 | 5.2 | 95.6 | 3.4 | 99.1 | 2.3 | 110.0 | 3.1 |
| 2.0 | 99.4 | 2.0 | 99.3 | 1.3 | 100.1 | 1.0 | 101.8 | 1.8 | 101.4 | 2.6 |
| 5.0 | 102.4 | 2.7 ^a | 101.8 | 4.8 ^a | 98.8 | 4.6 ^a | 99.9 | 2.9 | 101.2 | 3.6 |
| 10.0 | 100.2 | 1.9 | 101.4 | 4.1 | 101.6 | 1.1 | 101.7 | 0.5 | 99.4 | 3.4 |
| 20.0 | 98.6 | 5.0 ^a | 98.6 | 5.7 ^a | 102.3 | 6.2 ^a | 101.2 | 2.5 ^a | 98.0 | 4.7 |
| 50.0 | — | — | — | — | — | — | — | — | 100.3 | 3.8 |

^a $n = 10$.

from lactone standard curve and vice versa) were evaluated for its presence. No significant quantities were observable in these fractions, indicating that conversions between molecular entities or incomplete separation were minimal under the conditions of the assay.

Precision and accuracy

Assay accuracy and intra-day precision were determined by analysis of replicates ($n = 5$) of control plasma

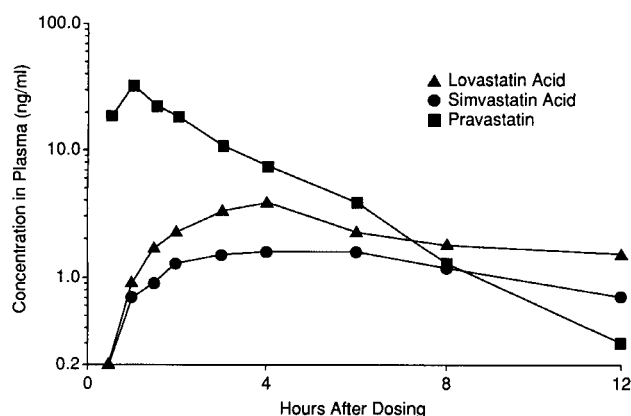


Figure 10. Mean concentration-time profiles ($n = 12$) of lovastatin, simvastatin, and pravastatin in the plasma of healthy volunteers receiving single oral doses (40 mg) of each drug.

containing known concentrations of the analytes. For lovastatin and simvastatin, additional QCs were included at 0.2, 1.0, 5.0, and 20.0 ng ml⁻¹. Results are shown in Table 2. Inter-day precision was calculated from quality-control data obtained during five successive analyses (Table 3).

The limits of reliable determination for lovastatin and simvastatin (acid and lactone) were 0.2 ng ml⁻¹. Pravastatin could be reproducibly quantitated over the range 1–50 ng ml⁻¹. Plasma samples containing in excess of 50 ng ml⁻¹ were diluted as appropriate with control plasma and re-assayed.

DISCUSSION

The derivatization procedure presently employed, including that for pravastatin, differed from that previously described for pravastatin,⁴ where it was reported that amines could not be used as catalysts to derivatize pravastatin with PFB because a bulky precipitate formed after the addition of the trimethylsilylating reagent, N,O-bis-trimethylsilyltri-fluoroacetamide (BSTFA). Therefore, pravastatin was esterified with PFB using acetone containing a few grains of potassium carbonate as a catalyst. In the present assays, DIEA was successfully employed as a catalyst for the derivatization. After pentafluorobenzyla-

Table 3. Inter-day precision and accuracy ($n = 5$) of the assays for the determination of simvastatin, lovastatin, and pravastatin in plasma

| Species | High range | | | Low range | | |
|---------------------|---|-------------------------|-----------|---|-------------------------|-----------|
| | Nominal concentration (ng ml ⁻¹) | % Accuracy ^a | CV (%) | Nominal concentration (ng ml ⁻¹) | % Accuracy ^a | CV (%) |
| | | | | | | |
| Simvastatin acid | 10.0 | 100.2 | 3.6 | 1.0 | 103.1 | 5.5 |
| Simvastatin lactone | 10.0 | 97.5 | 3.7 | 1.0 | 93.4 | 5.2 |
| Lovastatin acid | 20.0 | 105.9 | 5.5 | 0.2 | 118.8 | 7.2 |
| Lovastatin lactone | 20.0 | 90.4 | 7.2 | 0.2 | 87.5 | 11.3 |
| Pravastatin | 10.0 | 106.1 | 5.5 | 1.0 | 102.6 | 4.6 |

^a % (found/nominal).

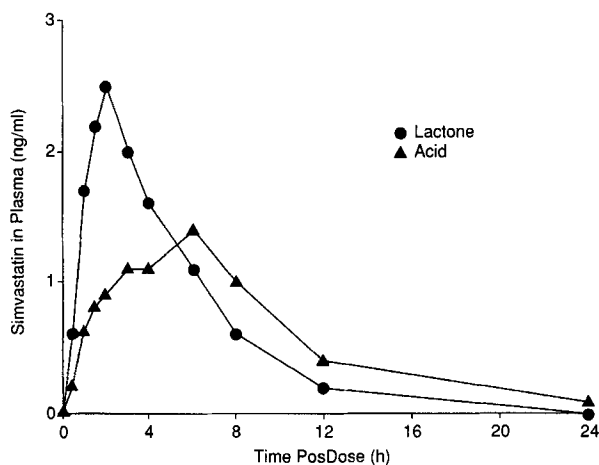


Figure 11. Plasma concentration-time curves for simvastatin acid and lactone following oral administration of 40 mg to a volunteer.

tion, excess reagent and amine were removed under a stream of nitrogen prior to derivatization with MSTFA and no bulky precipitate was observed. The use of an organic amine rather than potassium carbonate was specifically selected to eliminate the possibility of injecting particulate potassium carbonate into the chromatographic system and degrading the chromatography.⁶

A method previously reported for the determination of lovastatin acid had a lower quantifiable limit of about 25 ng ml⁻¹.⁶ Linearity was reported to a lower limit of 1 ng ml⁻¹ but the reliable sensitivity of the reported method was not sufficient to quantify either

lovastatin acid or simvastatin acid after the administration of therapeutic doses of the drugs. The plasma levels were generally less than 5 ng ml⁻¹, and to map the full pharmacokinetic profile methods were required to be valid at the low tenths of a nanogram per milliliter. The plasma concentration-time profiles (Figs 10 and 11) clearly demonstrate the need for such a level of validated sensitivity.

As mentioned previously, methods for simvastatin and its acid have been previously reported.⁷ These methods used the ferrocene boronate and methyl ester derivatives of simvastatin acid, which produced an abundant high-mass fragment under electron ionization suitable for quantitation at sub-nanogram per milliliter concentrations. However, residual retention of this derivative in the splitless injection system led to some variability at low concentrations, which was overcome by the addition of a silylating agent to the solvent used for injection. The assays were suitable for the separate determination of both the parent lactone and its hydroxy-acid in plasma at therapeutic doses. However, the derivatization procedure is not applicable to pravastatin, which contains a hydroxyl group in the hexahydronaphthalene ring besides the 1,3-diol in the heptanoic acid side chain. The present derivatization procedure shows no tendency for adsorption or carry-over, has been shown to be suitable for pravastatin, and has the potential to be extended to other active, hydroxylated metabolites such as 6' β -hydroxy-methylsimvastatin, which was employed as an internal standard in the assay for pravastatin.

REFERENCES

1. A. W. Alberts, in *Atherosclerosis Reviews*, Vol. 18; *Proceedings of a Symposium on Hypercholesterolemia: Clinical and Therapeutic Implications* held in Paris, France, 4-5 December 1987, ed. by J. Stokes III and M. Mancini, p. 123. Raven Press, New York (1988).
2. P. O'Connor, J. Feely and J. Shepherd, *Br. Med. J.* **300**, 667 (1990).
3. T. J. Thornton, M. S. Schwartz and R. J. Stubbs, *AAPS Abstract 5*. AAPS Joint Eastern Regional Meeting, Atlantic City, New Jersey (1988).
4. P. T. Funke, E. Ivashkiv, M. E. Arnold and A. I. Cohen, *Biomed. Environ. Mass Spectrom.* **18**, 904 (1989).
5. D. W. Everett, T. J. Chando, G. C. Didonato, S. M. Singhvi, H. Y. Pan and S. H. Weinstein, *Drug Metab. Dispos.* **19**, 740 (1991).
6. D. Wang-Iverson, E. Ivashkiv, J. Jemal and A. T. Cohen, *Rapid Commun. Mass Spectrom.* **3**, 132 (1989).
7. T. Takano, S. Abe and S. Hata, *Biomed. Environ. Mass Spectrom.* **19**, 577 (1990).
8. K. Blau and G. S. King, *Handbook of Derivatives for Chromatography*, p. 270. Heyden, London (1977).
9. R. J. Stubbs, M. S. Schwartz, R. J. Gerson, T. J. Thornton and W. F. Bayne, *Drug Invest.* **2** (Suppl. 2), 18 (1990).