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Quantitation of cerivastatin and its seven acid and lactone biotransformation products in human serum by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A method for the simultaneous quantitation of cerivastatin (acid) and its biotransformation products, cerivastatin lactone, M-1 (acid), M-1 lactone, M-23 (acid), M-23 lactone, M-24 (acid) and M-24 lactone, in human serum by high-performance liquid chromatography (LC) with positive ion electrospray tandem mass spectrometry (MS–MS) was developed and validated. The method involves extraction of cerivastatin and its biotransformation products from acidified human serum (0.5 ml) using methyl *tert*.-butyl ether. The standard curve ranges in human serum were from 0.0100 to 10.0 ng/ml for cerivastatin and cerivastatin lactone, 0.0500 to 10.0 ng/ml for M-1 (acid) and M-1 lactone, 0.100 to 10.0 ng/ml for M-23 (acid) and M-23 lactone, and 0.500 to 10.0 ng/ml for M-24 (acid) and M-24 lactone. The lactone compounds in human serum at room temperature underwent considerable conversion to the corresponding acid compounds after only 4 h. Lowering the serum pH with a pH 5.0 buffer stabilized the lactone compounds for up to 24 h at room temperature. The degree of lactonization of the acid compounds was ≤3.5% and the degree of hydrolysis of the lactone compounds was ≤6.0% during the entire assay procedure. All the eight analytes eluted within 2.0 min and the total run time was only 3.5 min.

Keywords: Cerivastatin

1. Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase have been shown to reduce plasma cholesterol levels [1]. Cerivastatin (Fig. 1) is a synthetic HMG-CoA reductase inhibitor [2,3]. A liquid chromatography (LC) method, based on post-column induced fluorometric detection, has been reported for the quantitation of cerivastatin in human plasma, with the lower limit of quantitation (LLQ) of 0.025 ng/ml [4]. Methods based on radioimmunoassay, HMG-CoA reductase inhibition assay and capillary gas chromatography for the quantitation of cerivastatin in human plasma have also been reported [5]. Since it has been reported that cerivastatin has a number of biotransformation prod-

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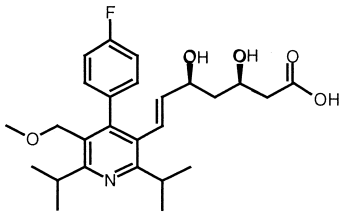
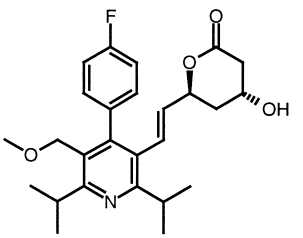


cerivastatin



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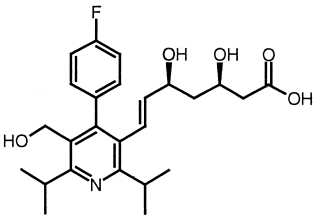

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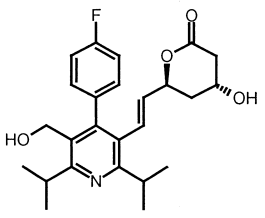
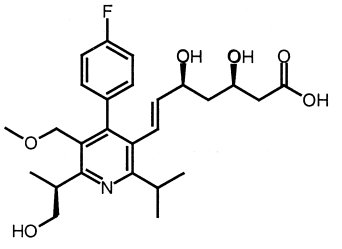


M-1 lactone



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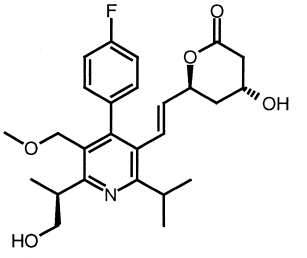

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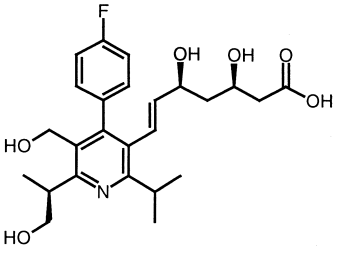

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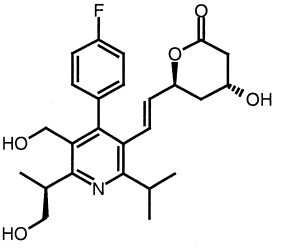

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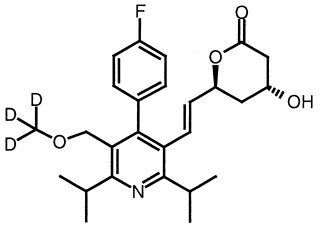
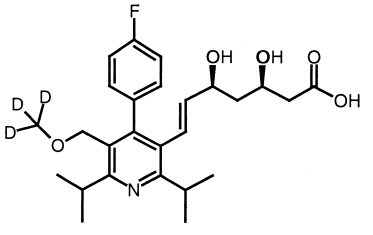


D_3-cerivastatin lactone (Int. Std 2)



D_3-cerivastatin (Int. Std 1)

Fig. 1. Chemical structures of cerivastatin, its biotransformation products and the internal standards.

ucts [3], a method was required for the quantitation of cerivastatin and the following biotransformation products in human serum: cerivastatin lactone, M-1 (acid), M-1 lactone, M-23 (acid), M-23 lactone, M-24 (acid) and M-24 lactone (Fig. 1). Because of the low oral dose used for cerivastatin due to its relatively high HMG-CoA reductase inhibitory activity [4], it was essential that the method possess a low-picogram LLQ for cerivastatin. We now report an LC–MS–MS method developed and validated for the simultaneous quantitative determination of cerivastatin and its seven biotransformation products in human serum.

2. Experimental

2.1. Chemicals and reagents

Cerivastatin (acid) and its biotransformation products, cerivastatin lactone, M-1 (acid), M-1 lactone, M-23 (acid), M-23 lactone, M-24 (acid) and M-24 lactone, were characterized products obtained from the Bristol-Myers Squibb Pharmaceutical Research Institute. D_3-cerivastatin, used as the internal standard for the four acid analytes, and D_3-cerivastatin lactone, used as the internal standard for the four lactone analytes, were also synthesized at the Bristol-Myers Squibb Pharmaceutical Research Institute. The reference standards for all the acid compounds were in their sodium salt form. However, the concentrations in solutions prepared from these reference standards were expressed in terms of the free acid forms. Acetonitrile (HPLC grade), formic acid (88%), ammonium acetate (HPLC grade), methyl *tert*.-butyl ether (HPLC grade) were purchased from Fisher Scientific (Tustin, CA, USA). Sodium acetate was from Aldrich Chemical (St. Louis, MO, USA). Water was purified using Barnstead Nanopure II. Drug-free human serum was purchased from Golden West Biologicals (Temecula, CA, USA).

A sodium acetate buffer (0.1 *M*, pH 5.0) was prepared by dissolving 2.05 g of sodium acetate in 250 ml water and the pH was adjusted to 5.0 with glacial acetic acid. Ammonium formate buffer (10 m*M*, pH 4.0) was prepared by dissolving 0.63 g of ammonium formate in 1 l of water with the pH adjusted to 4.0 with formic acid. Reconstitution solution was prepared by mixing 50 ml of acetonitrile and 50 ml of 10 m*M* ammonium formate, pH 4.0. Mobile phase A was ammonium formate (10 m*M*, pH 4.0) and mobile phase B was acetonitrile.

2.2. Equipment

Shimadzu LC-10AD pumps and a Perkin-Elmer Series 200 autosampler were used for HPLC. The analytical column used was a YMC Basic, 5 μm particle size, 2.0×50 mm (Wilmington, NC, USA) and the guard column used was YMC Basic 2.0×10 mm. A Sciex API 365 (Toronto, Canada) TurbolonSpray mass spectrometer, equipped with an atmospheric pressure ionization (API) electrospray interface was used for detection. Beckman TJ-6 centrifuge from Beckman Instruments was used. The shaker used was a reciprocating shaker, purchased from Eberbach (Ann Arbor, MI, USA). For evaporation, Zymark TurboVap was used.

2.3. Preparation of standard and quality control samples

Separate standard stock solutions of cerivastatin (acid), M-1 (acid), M-23 (acid) and M-24 (acid) were prepared in 50% acetonitrile–water. Similarly, separate standard stock solutions of cerivastatin lactone, M-1 lactone, M-23 lactone and M-24 lactone were prepared in 100% acetonitrile. Working standard solutions of varying concentrations, with each solution containing all the analytes, were prepared in 50% acetnitrile–water. The solutions were kept refrigerated when not in use. Each day, before extraction, the calibration curve in human serum was prepared by using a 50-μl aliquot of each working standard solution as described in Section 2.4. The standard curve ranges in human serum were from 0.0100 to 10.0 ng/ml for cerivastatin (acid) and cerivastatin lactone, 0.0500 to 10.0 ng/ml for M-1 (acid) and M-1 lactone, 0.100 to 10.0 ng/ml for M-23 (acid) and M-23 lactone, and 0.500 to 10.0 ng/ml for M-24 (acid) and M-24 lactone.

For the preparation of the human serum quality control (QC) samples, the individual stock solutions and the mixed working solutions were prepared as described for the calibration standard curve, except

using a different weighing for each reference standard. Three groups of QC samples were prepared: combination, acid-only and lactone-only QC samples. In the combination QC group, each sample contained all the eight analytes, with the acid and lactone forms of each compound (e.g., M-1 and M-1 lactone) at equal concentrations. There were four levels of these combination QC samples, covering the entire region of the calibration standard curve: at four times the lowest point of the standard curve (the low-level QC ranging from 0.0400 to 2.00 ng/ml, depending on the analyte and hence the calibration curve range), at 40% of the highest point of the standard curve (mid-level QC at 4.00 ng/ml), at 80% of the highest point of the standard curve (high-level QC at 8.00 ng/ml), and at ten times the high-level QC (dilution QC). In the acid-only QC group, each sample contained only the four acid compounds as analytes. There was only a mid-level of QC concentration (4.00 ng/ml) of the acid-only QC samples. These samples were used to gauge the in-process lactonization of the acid compounds. In the lactone-only QC group, each sample contained only the four lactone compounds as analytes. As in the case of acid-only QC samples, there was only a mid-level of QC concentration (4.00 ng/ml) for lactone-only QC samples. These samples were used to gauge the in-process hydrolysis of the lactone compounds. All QC samples were stored at −70°C until analysis.

Separate stock solutions of the internal standards D_3-cerivastatin (acid) and D_3-cerivastatin lactone were prepared in 50% acetnitrile–water and 100% acetonitrile, respectively. From the two stock solutions, a working internal standard solution, containing both internal standards, was prepared in 50% acetnitrile–water. The solutions were kept refrigerated when not in use.

2.4. Sample extraction

Before extraction, 50 μl of each working standard solution (in duplicate), prepared as described in Section 2.3, was transferred to separate 13×100 mm screw cap culture tubes containing 0.50 ml of chilled (4°C) 0.1 *M* sodium acetate (pH 5.0) and 0.45 ml of blank human serum and mixed gently. The calibration standards prepared in this manner consisted of a number of concentrations, each in duplicate, with each concentration point containing all the analytes. Because of the difference in the LLQ of the different analytes, the standard curve range changed with the analytes: 0.0100–10.0 ng/ml for cerivastatin and cerivastatin lactone (nine points, each in duplicate); 0.0500–10.0 ng/ml for M-1 and M-1 lactone (nine points, each in duplicate); 0.100–10.0 ng/ml for M-23 and M-23 lactone (nine points, each in duplicate); 0.500–10.0 ng/ml for M-24 and M-24 lactone (seven points, each in duplicate). At each concentration point of the calibration standard curve, the concentrations of the acid and lactone forms of each compound (e.g. M-1 and M-1 lactone) were at equal concentrations. The QC samples (0.500 ml) were pipetted, each in six replicates, into 13×100 mm test tubes containing 0.500 ml chilled (4°C) 0.1 *M* sodium acetate (pH 5.0). The working internal standard solution, 50.0 μ1, was added to all tubes and the samples were vortexed. The concentration of each internal standard was 0.500 ng/ml of serum. Methyl *tert*.-butyl ether (5.0 ml) was then added to each tube. The tubes were capped, put on the shaker for 15 min and then centrifuged for 5 min. The tubes were then placed in a dry ice–methanol bath in order to freeze the aqueous layer. The organic layer from each tube was decanted into a clean 16×100 mm test tube. The tubes were then placed in the TurboVap at 35°C and evaporated to dryness with nitrogen (approximately 10 min). The dried samples were then redissolved in 50.0 μl of the reconstitution solution containing 1:1 acetonitrile–ammonium formate (10 m*M*, pH 4.0). The reconstituted samples were transferred to polypropylene conical microvials and centrifuged to remove any particulate matter. The supernatants were transferred to autoinjector vials for injection.

2.5. Chromatographic and mass spectrometric conditions

A gradient HPLC mobile phase was used by combining mobile phases A (10 m*M* ammonium formate, pH 4.0) and B (acetonitrile) in the following manner: start with 40% A and 60% B and change to 100% B between 0 and 0.3 min; hold for 0.7 min; change to the starting mobile phase composition (40% A and 60% B) between 1.0 min and 1.1 min;

hold for 2.4 min; then start the next run. The total run time was 3.5 min. The YMC Basic, 2×50 mm column, with 5-μm particle size, was maintained at room temperature. The flow-rate was 0.3 ml/min and the injection volume was 25 μl. The entire effluent was delivered to the mass spectrometer.

The mass spectrometer was operated in the positive TurboIonSpray mode at 400°C. The samples were analyzed via selected reaction monitoring (SRM) employing the transition of the $[M+H]^+$ precursor ion to a product ion for each analyte and internal standard, as shown in Table 1. The ion spray voltage was set to +5.5 kV and the orifice voltage was 45 V. The heated nebulizer gas (nitrogen) pressure was 5.5 bar and the TurboIonSpray gas flow-rate was 7.0 l/min. The dwell time was 200 ms for all the analytes, except for the two internal standards (50 ms). The collision cell energy was set at 45 eV.

3. Results and discussion

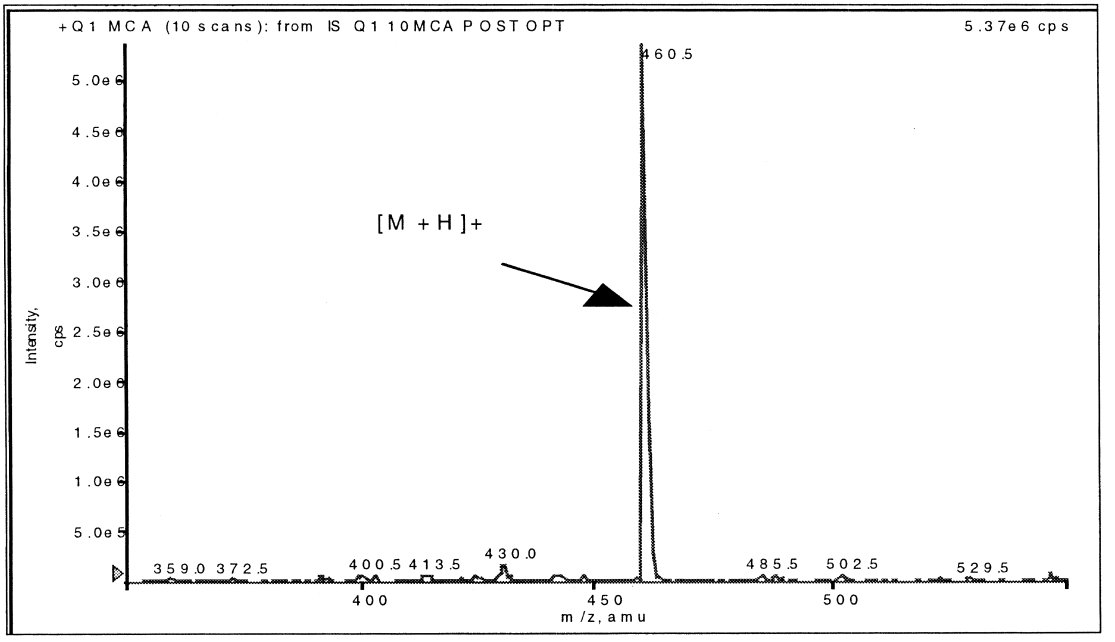
Full-scan single MS (Q1) and MS–MS (Q3) mass spectra of the eight analytes and the two deuterium labeled internal standards are shown in Figs. 2–11. For each compound, $[M+H]^+$ was the predominant ion in the Q1 spectrum, which was used as the precursor ion for obtaining product ion spectra in MS–MS scans. Fragmentation pathways proposed for the generation of the product ions used in the SRM transition of the acid and lactone compounds are shown in Fig. 12. For the acid compounds cerivastatin (acid), M-1 (acid) and D_3-cerivstatin (acid), the product ion formed is obtained by the neutral loss of the β-hydroxy butyric acid from the acid side chain. For the acid compound M-23 (acid), the product ion formed is obtained by the neutral loss of both the β-hydroxy butyric acid from the acid side chain and methanol from the methoxy group. For the acid compound M-24 (acid), the product ion formed is obtained by the neutral loss of both the 13-hydroxy butyric acid from the acid side chain and water from the OH of the *O*-demethylated group. For the lactone compounds cerivastatin lactone, M-1 lactone and D_3-cerivstatin lactone, the product ion formed is obtained by the neutral loss of $C_3H_4O_3$ (plausible structures for $C_3H_4O_3$ are shown in Fig. 12) from the lactone side chain. For the lactone compound M-23 lactone, the product ion formed is obtained by the neutral loss of both $C_3H_4O_3$ and methanol. For the lactone compound M-24 lactone, the product ion formed is obtained by the neutral loss of both $C_3H_4O_3$ and water.

The extraction recoveries of the analytes from serum were determined at three concentrations (0.500, 2.00 and 10.0 ng/ml) by comparing the area ratios of pre-extraction/post-extraction samples with the area ratios of post-extraction/post-extraction samples. The pre-extraction/post-extraction samples were prepared by spiking the analytes into the serum before extraction and spiking the internal standards after the extraction into the methyl *tert*.-butyl ether extracts of these samples. The post-extraction/post-

Table 1
Selected reaction monitoring (SRM) channels and the retention times for the analytes and internal standards

Compound	Precursor ion (m/z)	Product ion (m/z)	Retention time (min)
Cerivastatin (acid)	460.4	356.0	1.52
Cerivastatin lactone	442.2	354.0	1.75
M-1 (acid)	446.4	342.0	1.01
M-1 lactone	428.2	340.0	1.38
M-23 (acid)	476.4	340.0	0.87
M-23 lactone	458.2	338.0	1.08
M-24 (acid)	462.4	340.0	0.77
M-24 lactone	444.2	338.0	0.89
D_3-cerivastatin (acid)	463.4	359.0	1.49
D_3-cerivastatin lactone	445.2	357.0	1.72

Full Scan Single MS of Cerivastatin



Full Scan MS/MS Product Ion Spectrum of Cerivastatin

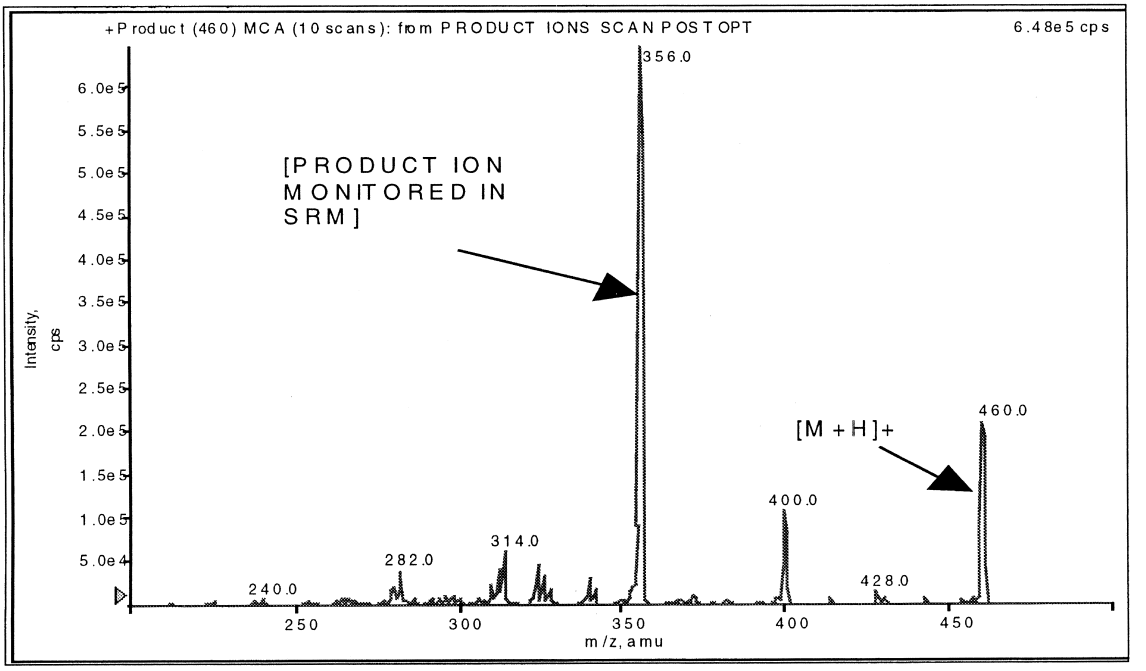
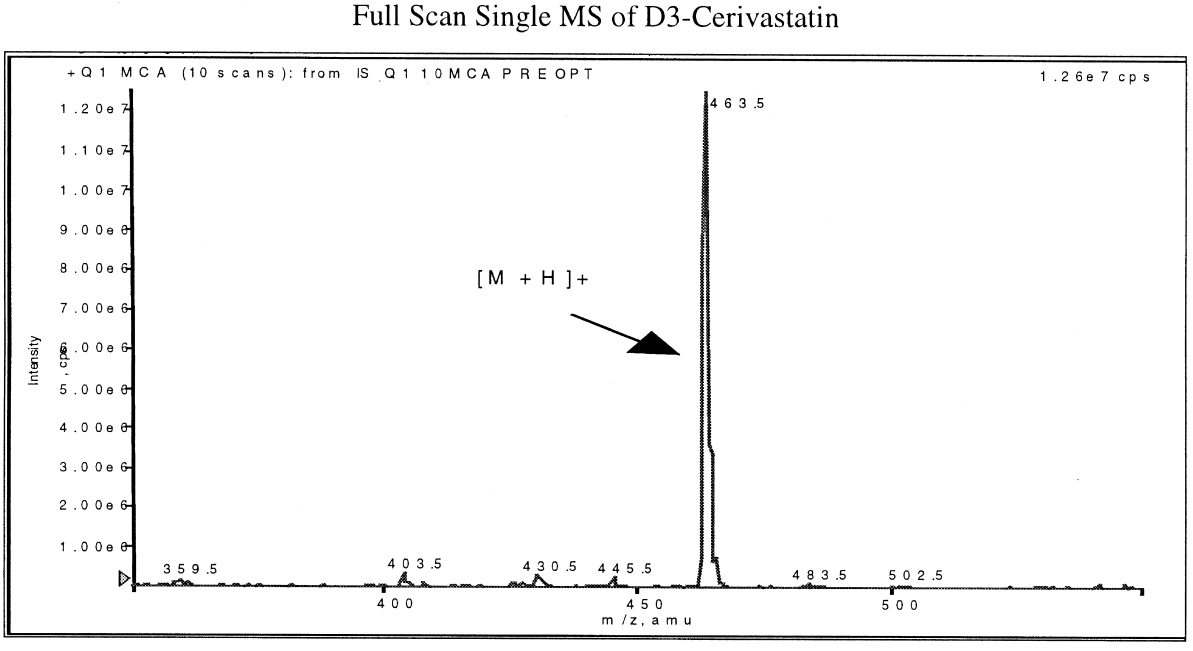


Fig. 2. Full scan single MS and full scan MS–MS product ion spectra of cerivastatin.



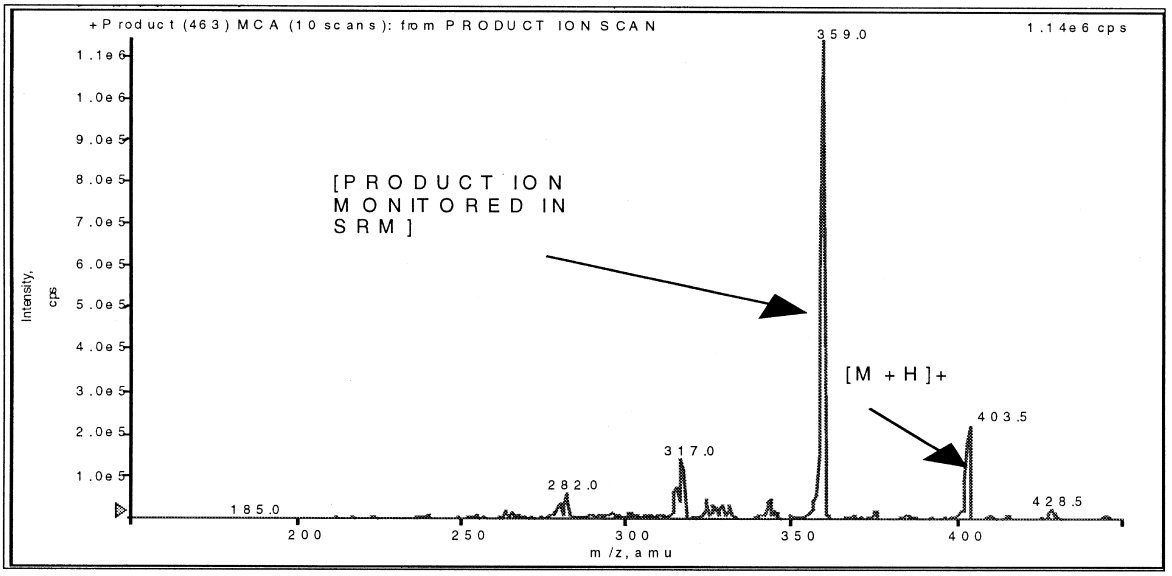
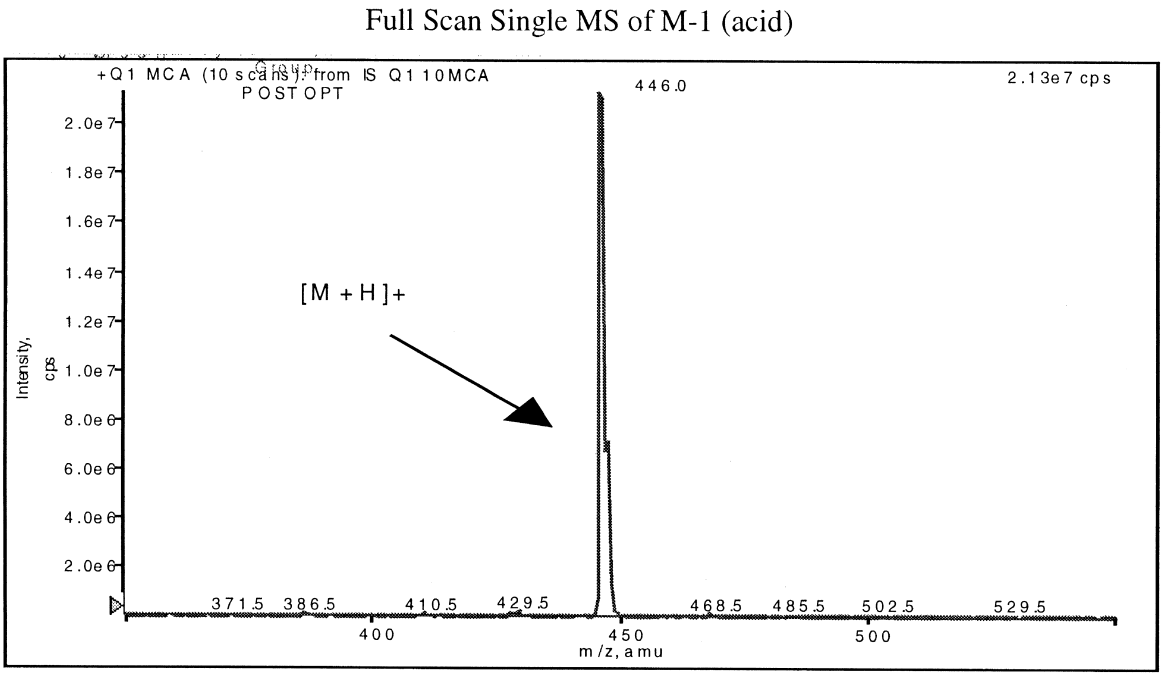


Fig. 3. Full scan single MS and full scan MS–MS product ion spectra of D_3-cerivastatin.



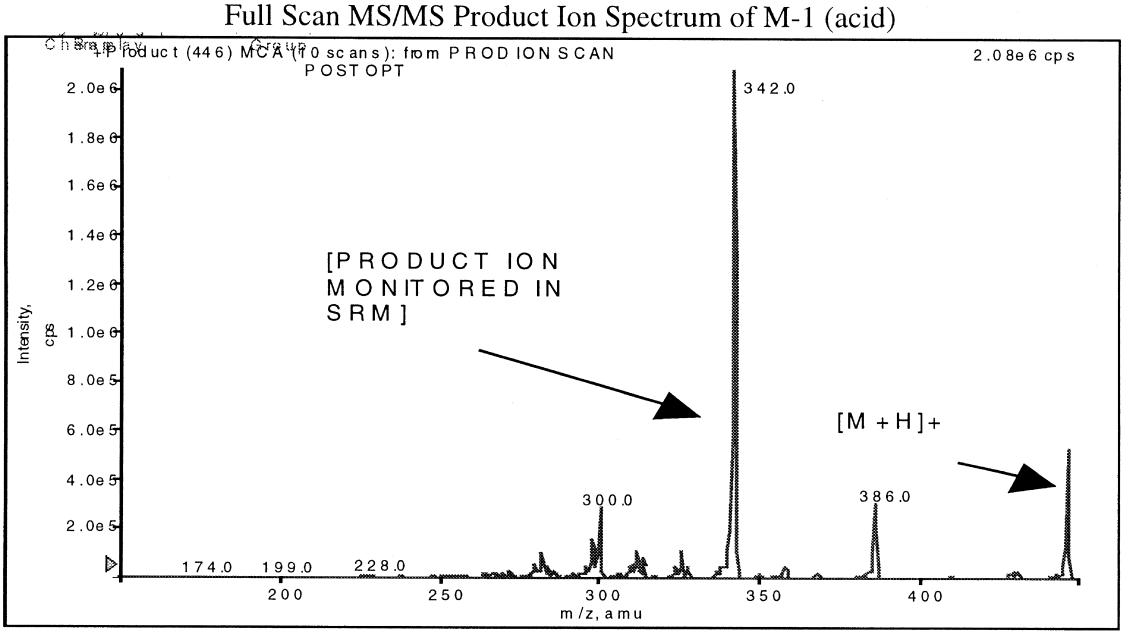
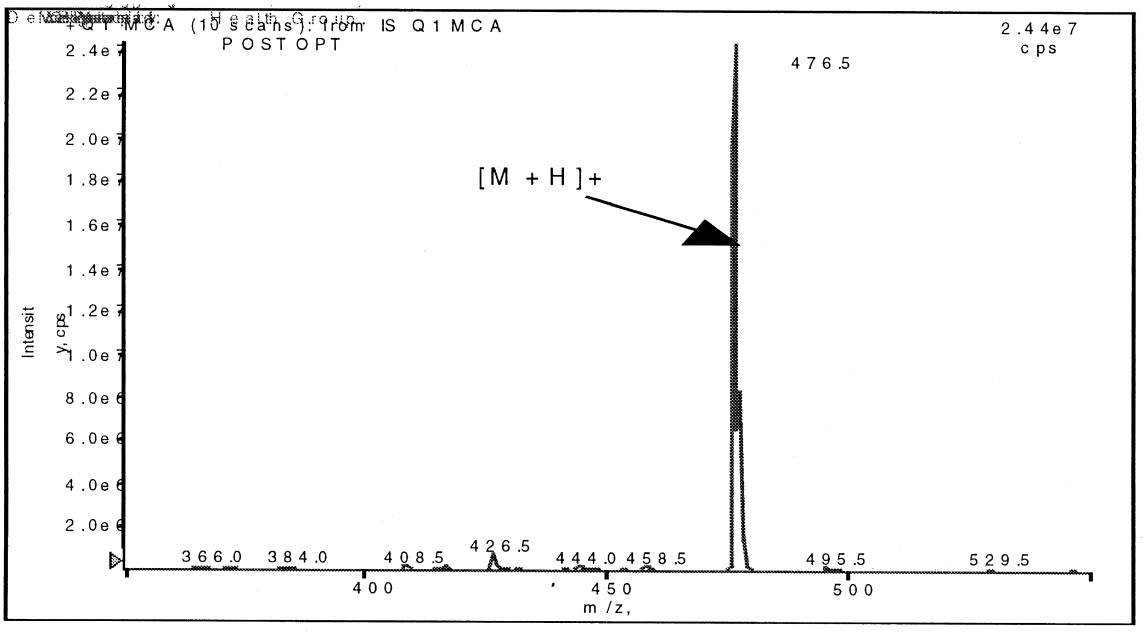


Fig. 4. Full scan single MS and full scan MS–MS product ion spectra of M-1 (acid).

Full Scan Single MS of M-23 (acid)



Full Scan MS/MS Product Ion Spectrum of M-23 (acid)

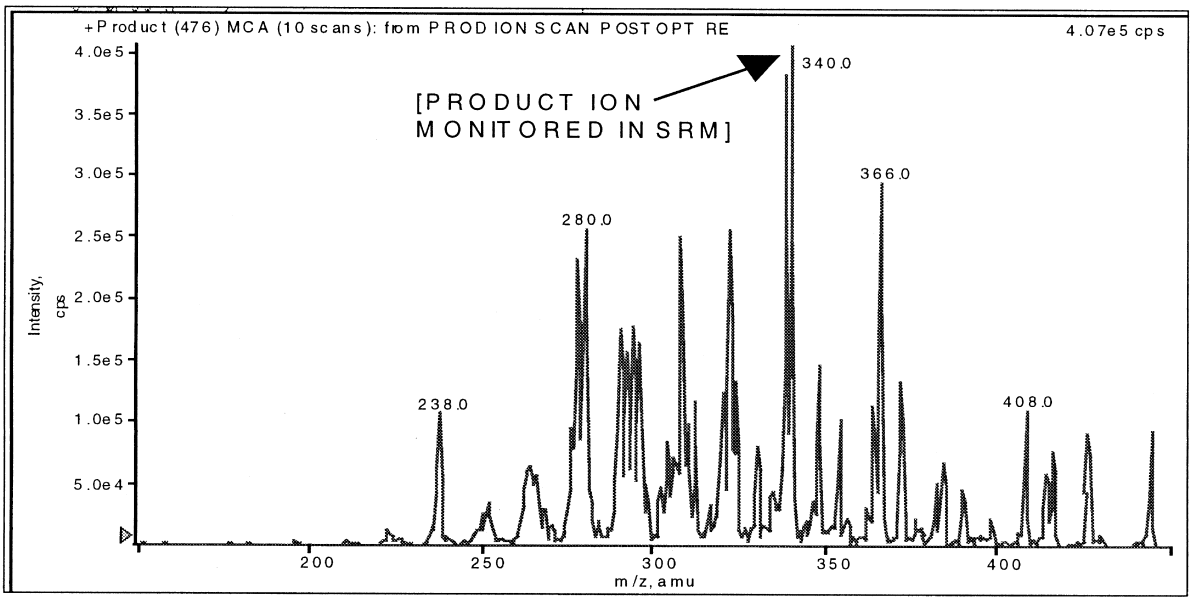


Fig. 5. Full scan single MS and full scan MS–MS product ion spectra of M-23 (acid).

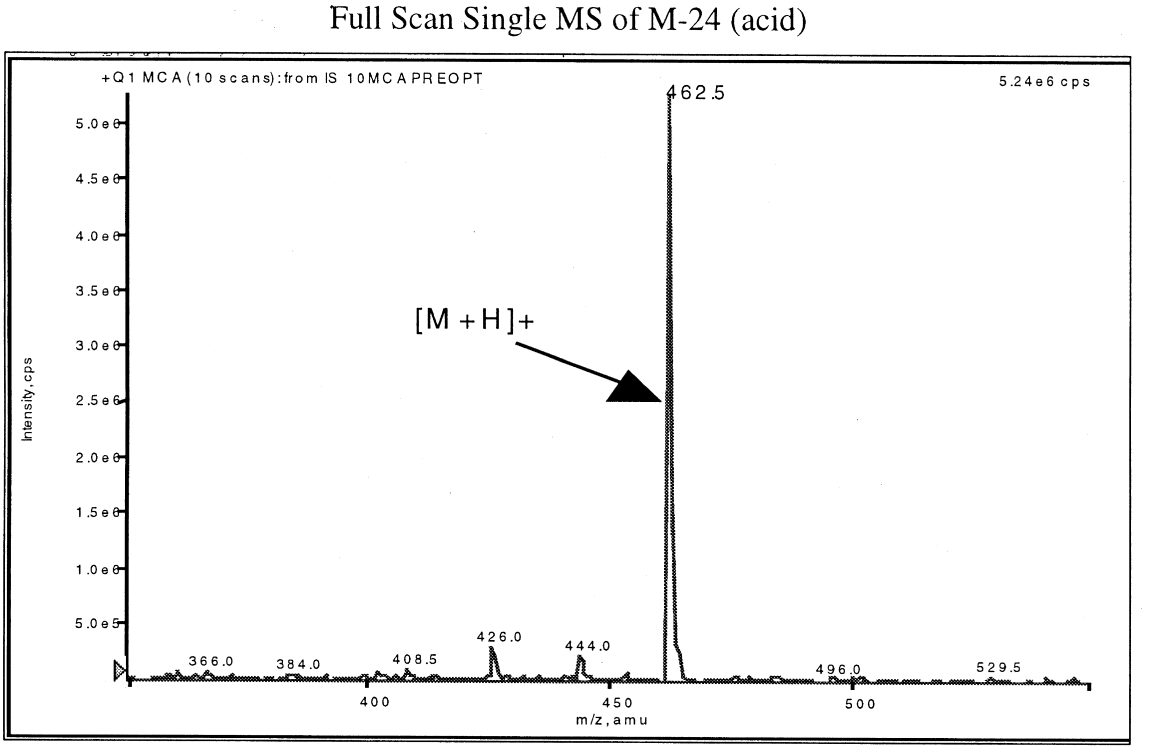
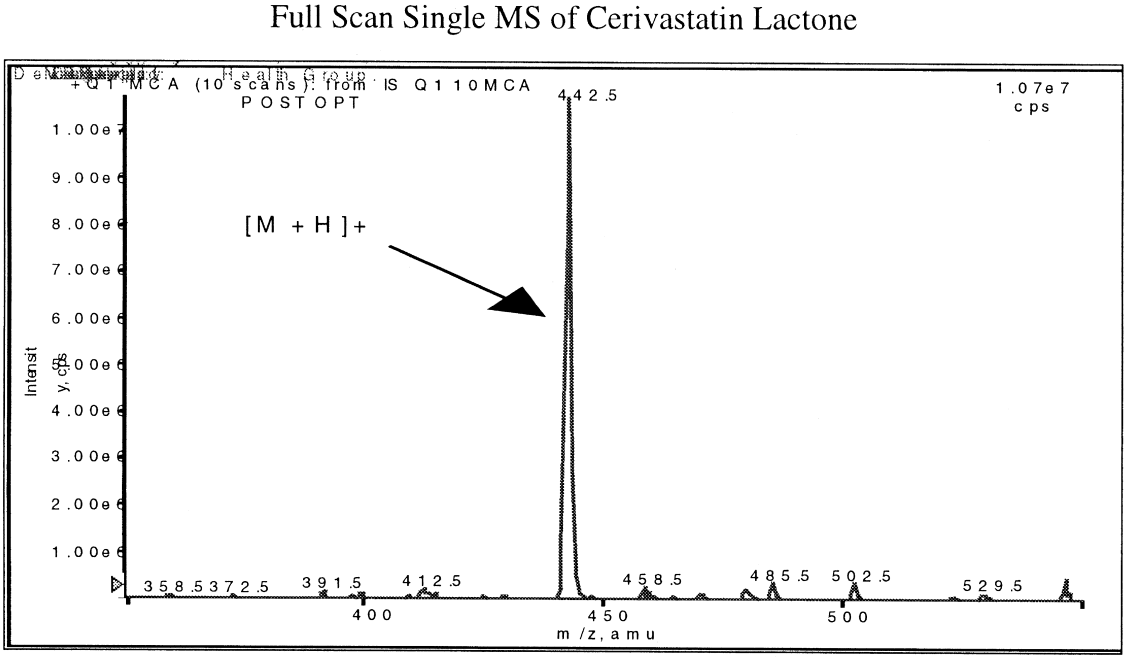


Fig. 6. Full scan single MS and full scan MS–MS product ion spectra of M-24 (acid).



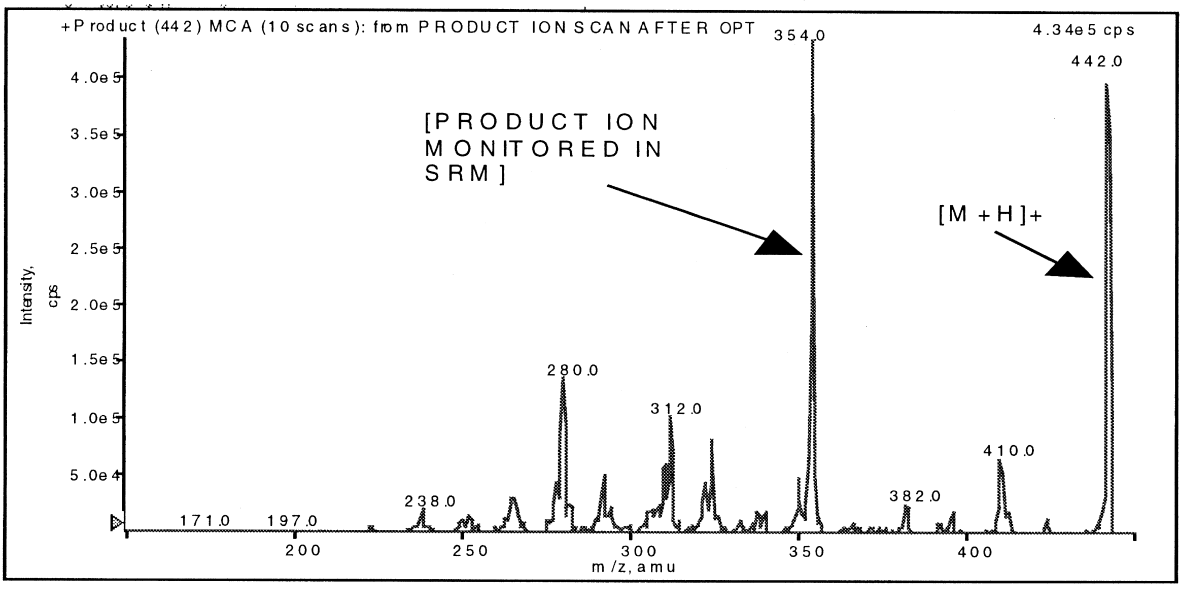
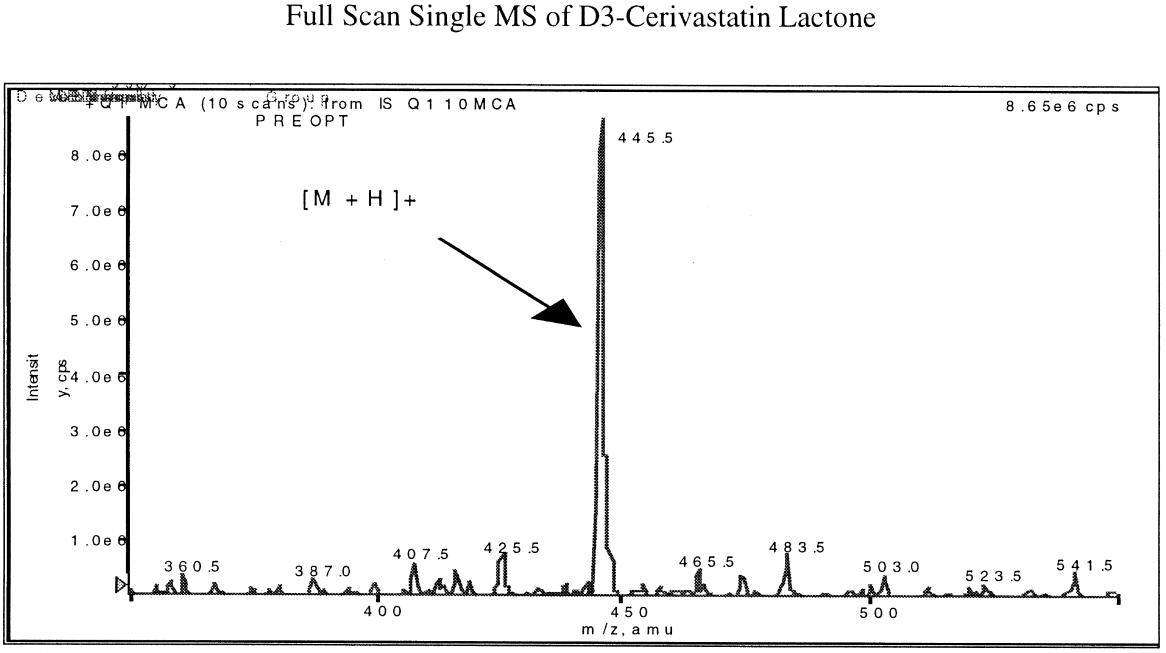


Fig. 7. Full scan single MS and full scan MS–MS product ion spectra of cerivastatin lactone.



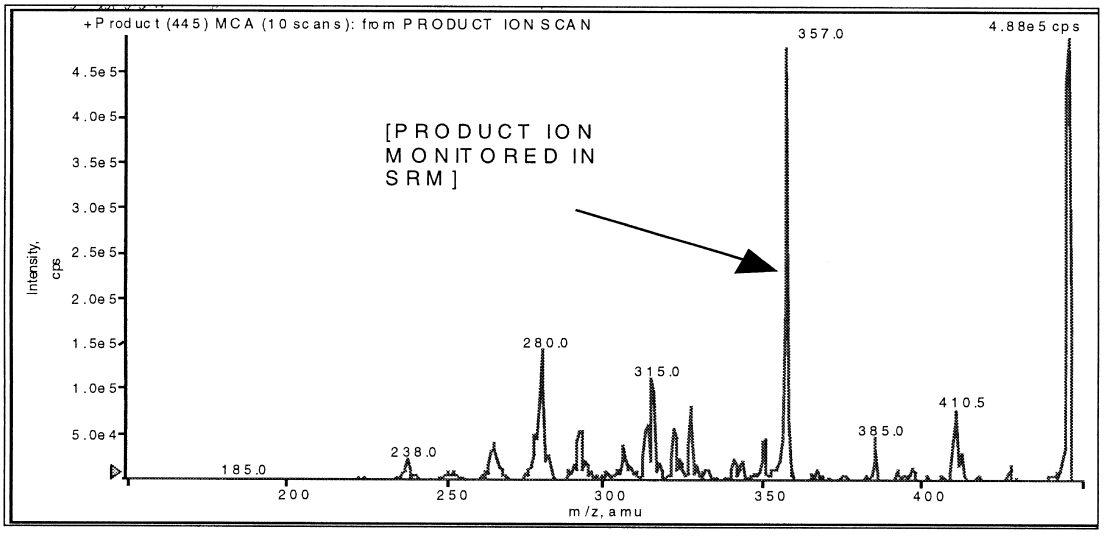
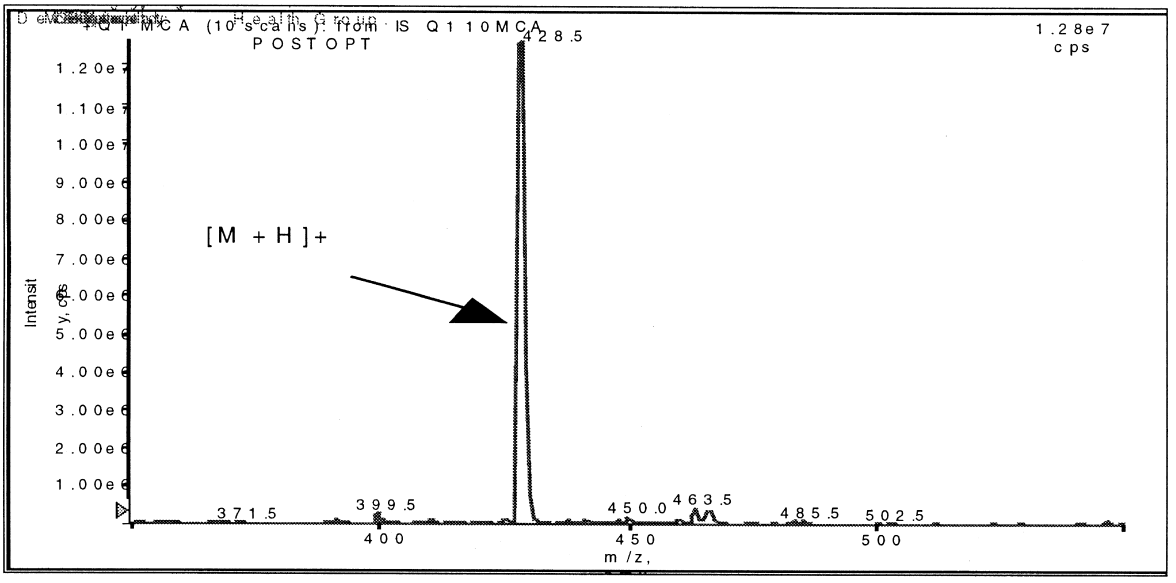


Fig. 8. Full scan single MS and full scan MS–MS product ion spectra of D_3-cerivastatin lactone.

Full Scan Single MS of M-1 lactone



Full Scan MS/MS Product Ion Spectrum of M-1 lactone

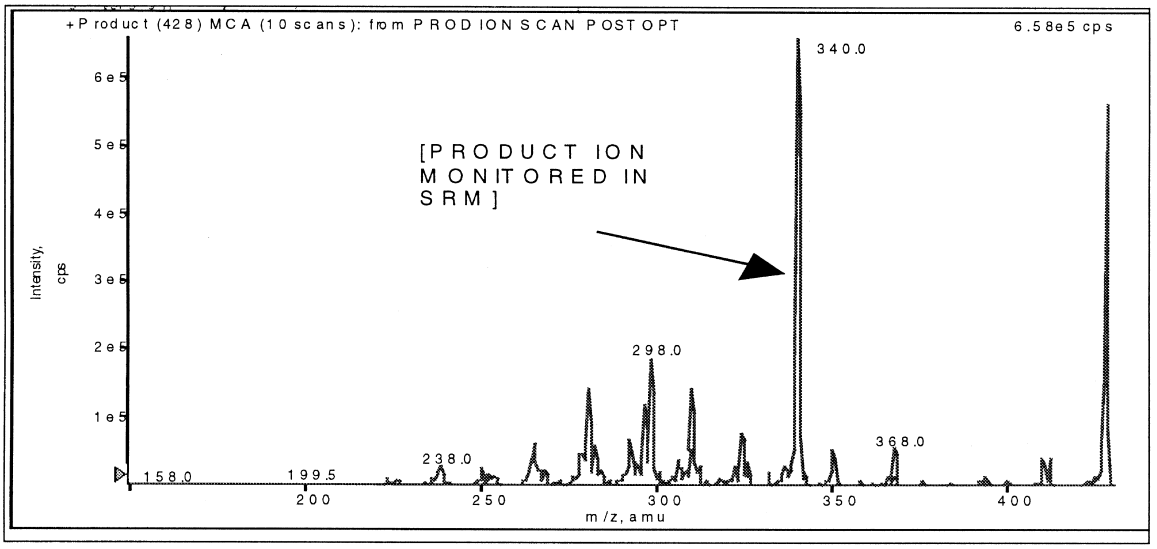
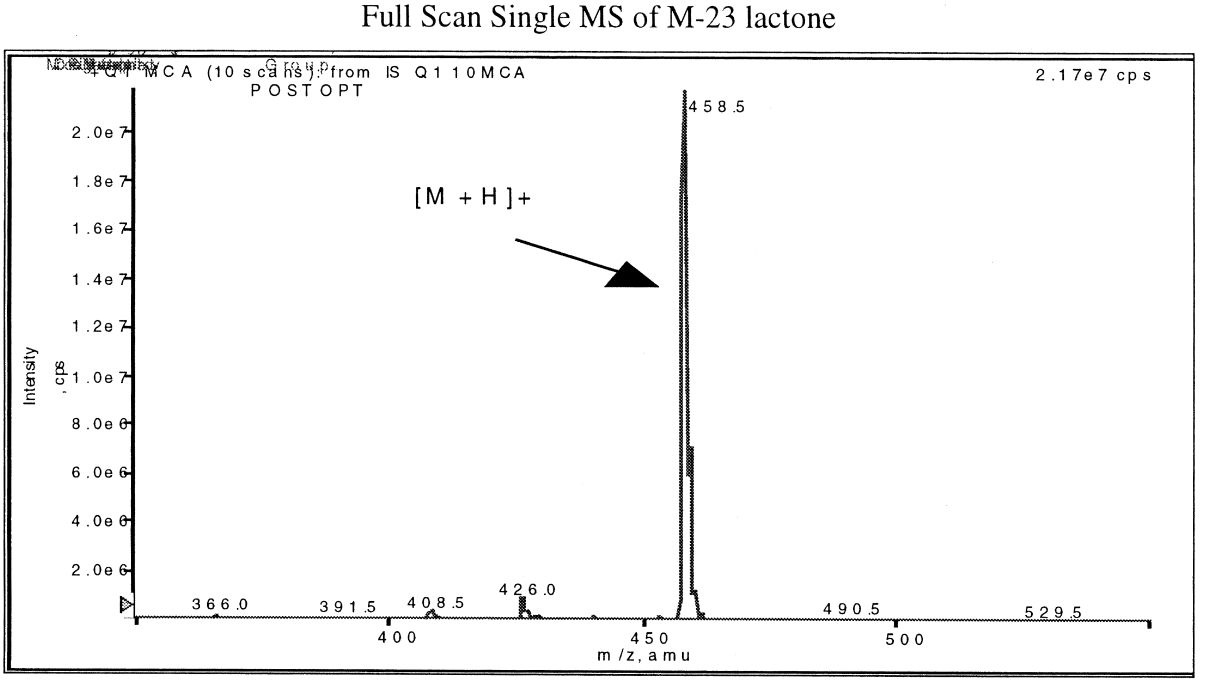


Fig. 9. Full scan single MS and full scan MS–MS product ion spectra of M-1 lactone.



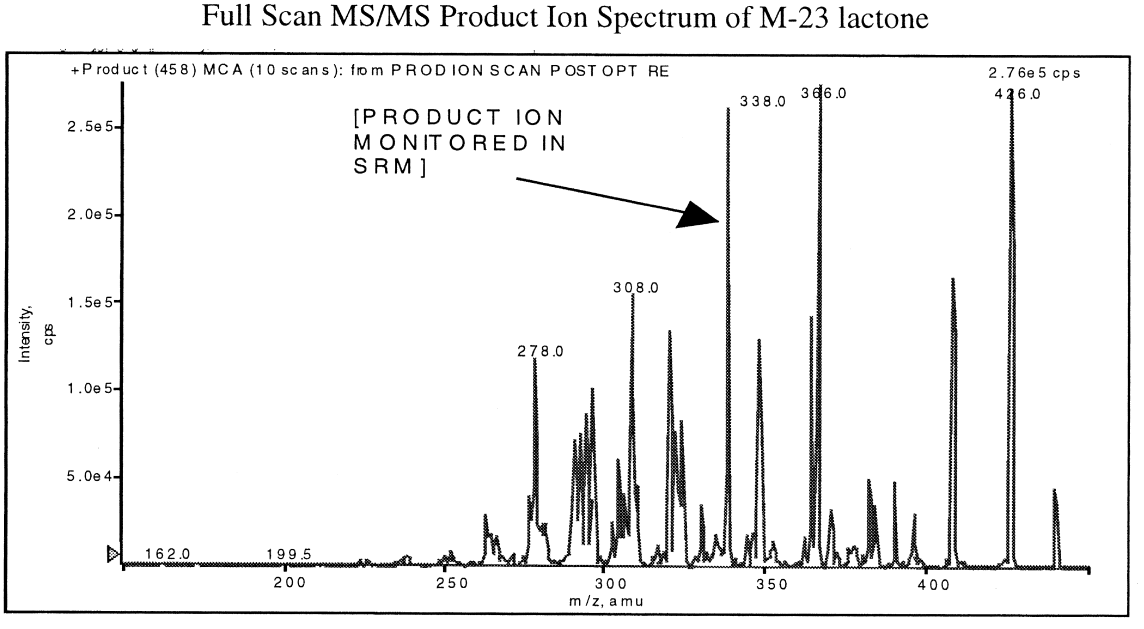
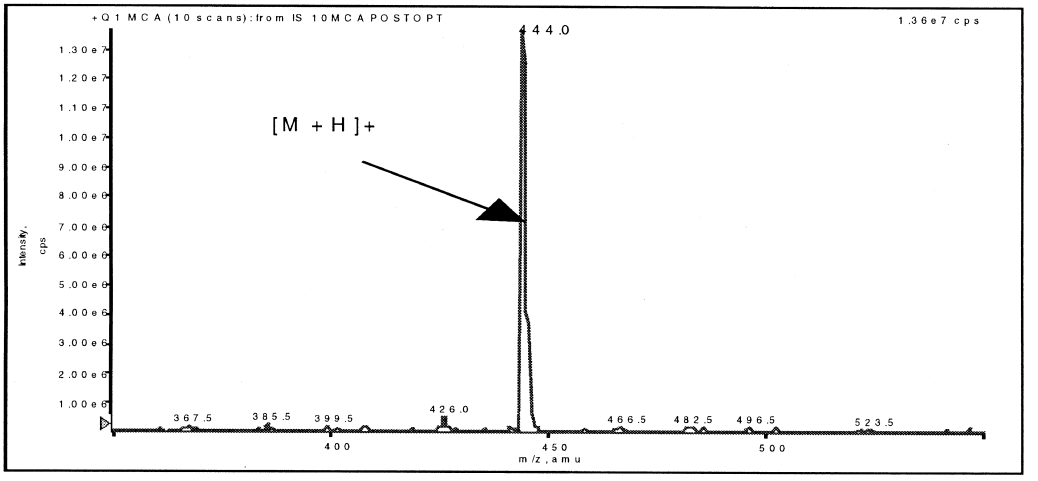


Fig. 10. Full scan single MS and full scan MS–MS product ion spectra of M-23 lactone.

Full Scan Single MS of M-24 lactone



Full Scan MS/MS Product Ion Spectrum of M-24 lactone

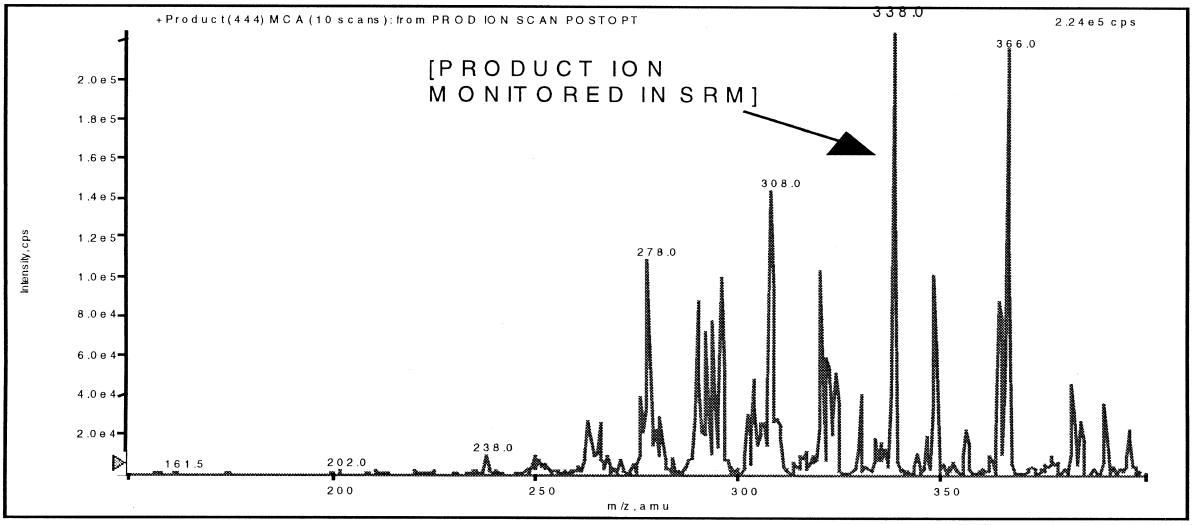


Fig. 11. Full scan single MS and full scan MS–MS product ion spectra of M-24 lactone.

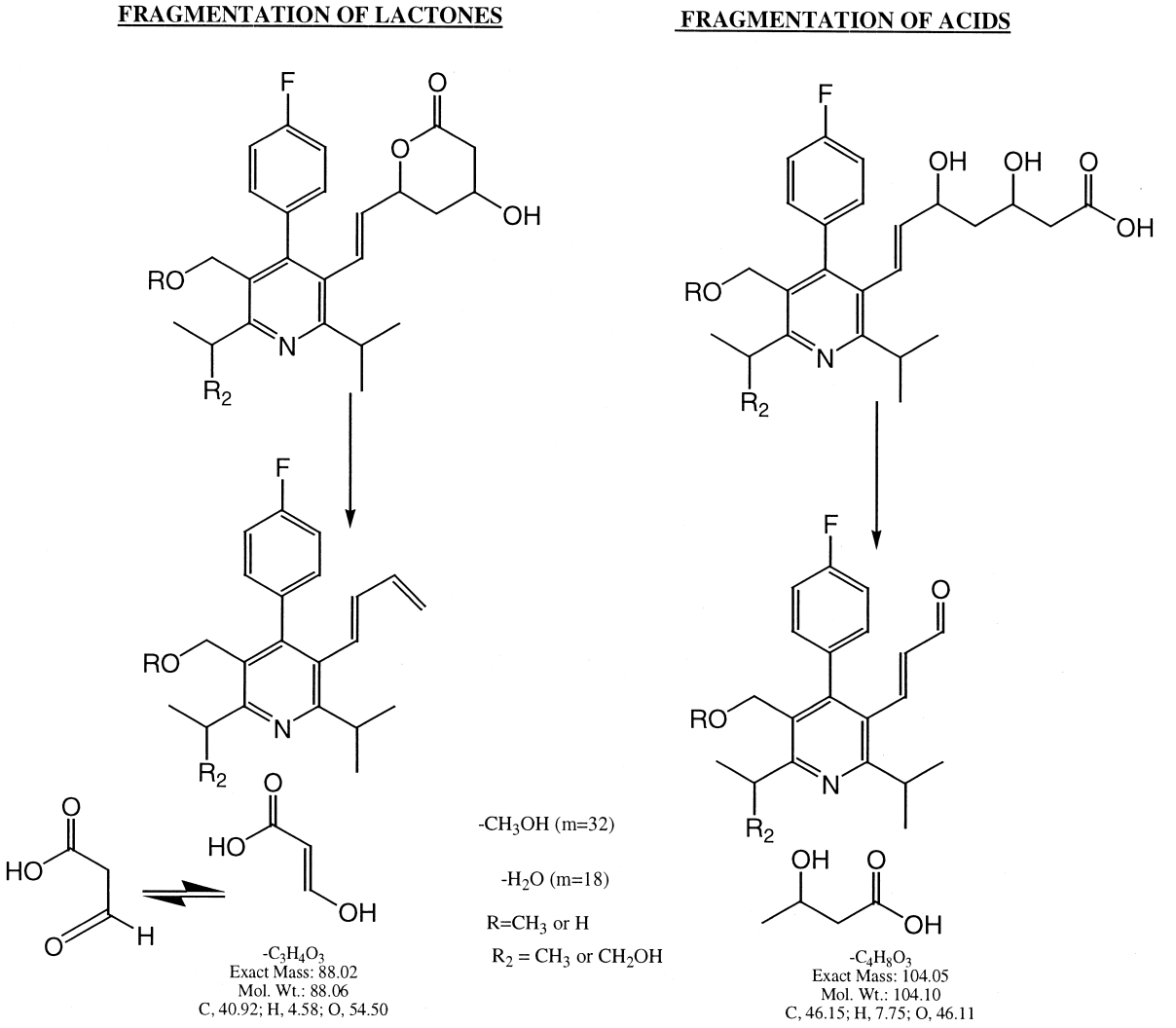


Fig. 12. Proposed pathways for the formation of product ions from the precursor ions of cerivastatin and its biotransformation products.

extraction samples were prepared by spiking both the analytes and internal standards after the extraction. The extraction recovery of the internal standards was also evaluated at the concentration used in the assay (0.500 ng/ml). The procedure described for the analytes was used, except for reversing the spiking scheme. The extraction recovery was ≥63% for all acid compounds and ≥92% for all the lactones.

For the assessment of the accuracy and precision of the method, a set consisting of calibration standards, four concentrations of combination QC samples, one concentration of acid-only QC sample, and one concentration of lactone-only QC sample was run on three different days. The lactone-only QC and acid-only QC samples were included to determine the extent of hydrolysis and lactonization, respectively, during the entire analytical process. In each set, the standards were run in duplicate and the QC samples were run in six replicate. For each analyte, the standard curve was fitted to a $1/x$ weighted linear regression, where x was the concentration of the analyte. A one-way analysis of variance was performed on the measured concentrations of the QC samples. The inter-day C.V., intra-day C.V. and

Table 2
Accuracy and precision of the method as measured by the performance of the combination QC samples analyzed on three different days at four concentrations[a]

Compound	Inter-day C.V. (%)[b]	Intra-day C.V. (%)	Deviation from nominal conc. (%)
Cerivastatin (acid)	3.2, –, 4.4, –	14, 6.0, 6.4, 5.6	−2.0, 2.1, 1.8, 0.73
Cerivastatin lactone	–, 3.1, 4.0, 4.2	11, 5.5, 7.2, 7.7	−0.91, −1.7, −4.9, −3.3
M-1 (acid)	4.3, 8.6, 8.8, –	11, 11, 10, 8.2	−4.l, −5.4, 0.13, 6.5
M-1 lactone	–, –, 2.0, 7.6	20, 9.5, 3.7, 7.7	6.3, 9.4, 16, 2.2
M-23 (acid)	–, 14, 15, –	17, 13, 12, 12	−12, −10, −8.0, −6.8
M-23 lactone	6.0, 4.1, 2.4, 7.1	8.2, 8.0, 5.5, 8.6	3.3, −0.86, 10, 1.1
M-24 (acid)	7.5, 15, 6.7, –	20, 11, 13, 12	−7.0, −10, −5.5, −0.56
M-24 lactone	10, 11, 7.0, 5.9	13, 11, 8.5, 6.0	2.8, −3.3, 4.1, −2.2

[a] The four values reported under the headings 'inter-day C.V.', 'intra-day C.V.' and 'Deviation from nominal conc.' are for the four concentrations of the low, mid, high and dilution QC samples, listed in that order.

[b] The inter-day C.V. values reported as '–' indicate that no significant additional variation was observed as a result of performing the assay on different days.

accuracy of the method, as measured by the performance of the QC samples for each analyte at all four levels of concentrations, are shown in Table 2. The precision for cerivastatin and cerivastatin lactone were within 15% and the accuracy was within 5%. For the other analytes, the precision and accuracy were all within 20% at all levels evaluated.

The specificity of the method was demonstrated by the absence of endogenous substances in the human serum that interfere with the quantitation of the analytes at the LLQ levels. For the verification of the LLQ, six different lots of blank human serum were spiked with the six analytes at the lowest level of the standard curve. The samples were spiked with the internal standard and then analyzed against these standard curves. Typical SRM chromatograms at the LLQ concentrations are shown in Figs. 13 and 14. Six different lots of blank human serum were also analyzed after spiking with the internal standard only (QCO) in order to determine if any endogenous plasma constituents coeluted with the analytes. Typical SRM chromatograms of QCO samples are shown in Figs. 15 and 16. The precision and accuracy for five out of the six LLQ samples were within 15 and 12%, respectively, for all the analytes. In all six QCO samples, the responses detected at the SRM channels and the retention times of the analytes were significantly lower than the LLQ responses.

The stability of the eight analytes in human serum was investigated using combination QC samples. The stability profile of each analyte in low-level and high-level QC samples at 4°C (ice) and at room temperature over a period of 24 h is shown in Table 3. The lactone compounds were found to be unstable at room temperature after only 4 h. This was indicated by the percentage decrease in the concentrations of the lactones and the concomitant percentage increase in the concentrations of the corresponding acids. Lowering the temperature to 4°C significantly increased the stability of the lactone compounds. Addition of a pH 5.0 buffer (0.1 *M* sodium acetate) to the serum stabilized all the lactones for up to 24 h even at room temperature (Table 4). The stabilization of the lactone compounds by lowering the pH was in line with the findings reported from the investigation of the effect of pH on the rate and equilibrium for the interconversion between the acid and lactone forms of atorvastatin, another HMG-CoA reductase inhibitor [6]. The in-process hydrolysis, as measured from the acids generated from the lactone-only QC samples, was within 6.0%. The in-process lactonization, as measured from the lactones generated from the acid-only QC samples, was within 3.5%. Cerivastatin and its biotransformation products were stable after three freeze-thaw cycles. The reconstituted extracted samples were stable for at least 24 h at room temperature.

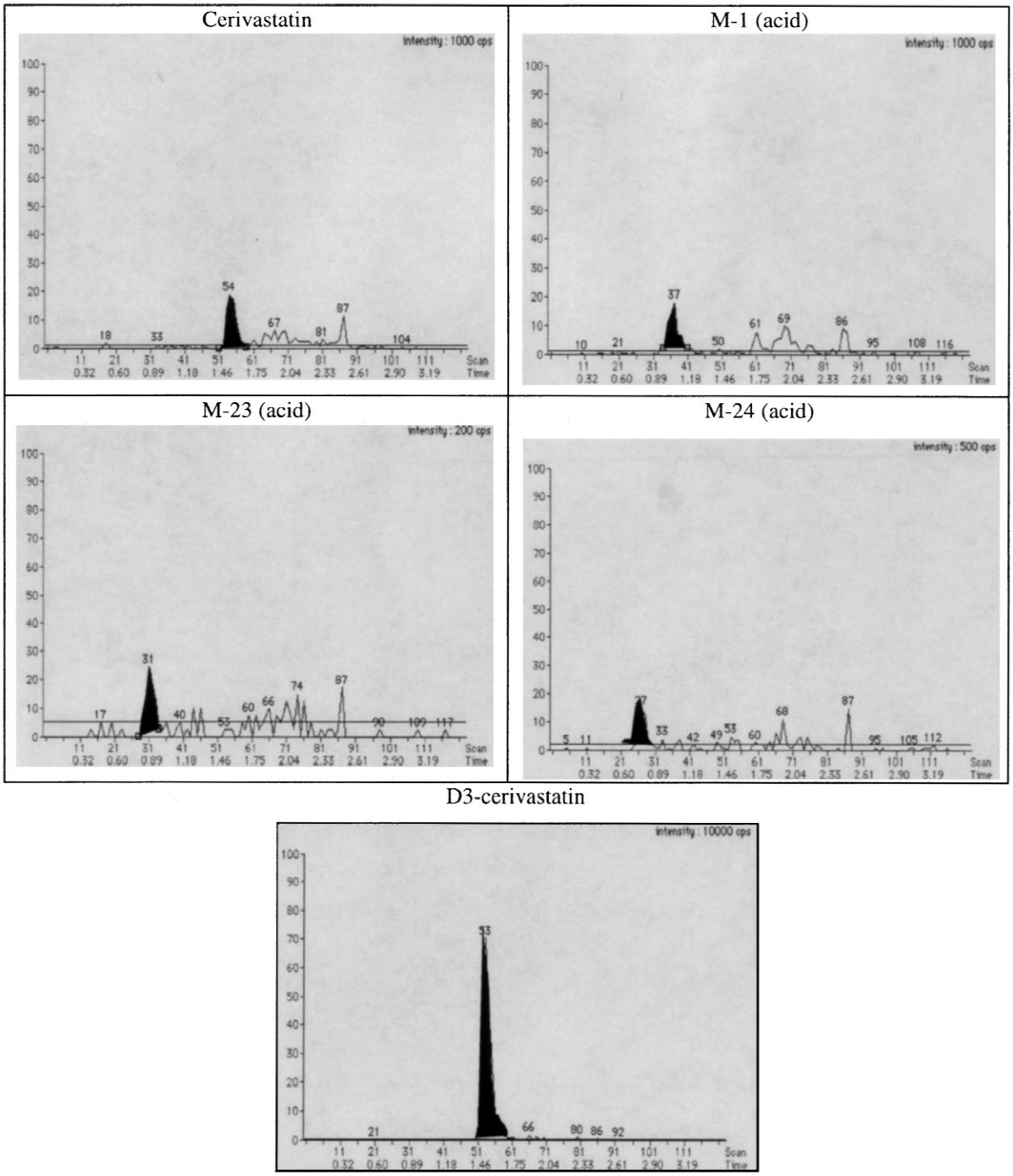
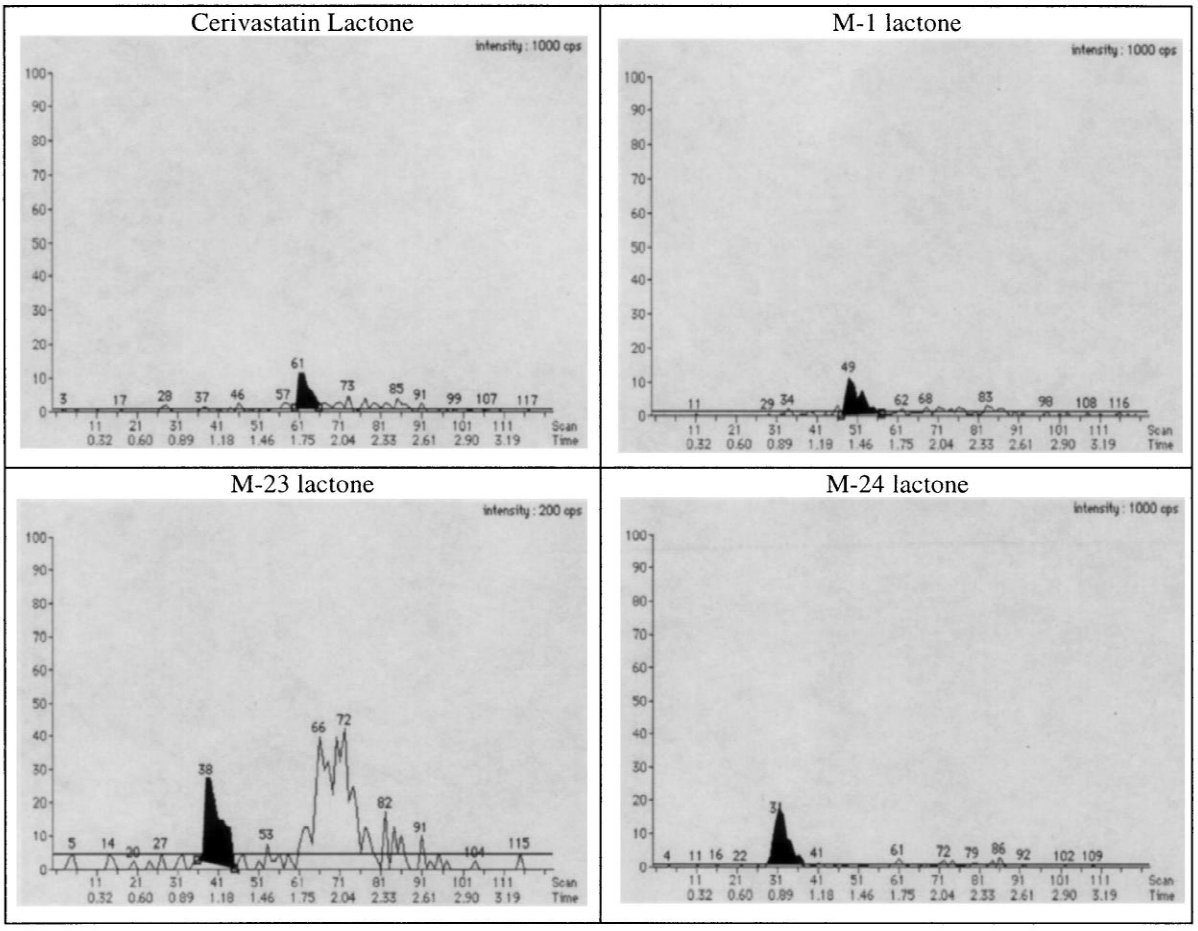


Fig. 13. SRM Chromatograms of cerivastatin (0.0100 ng/ml), M-l (0.0500 ng/ml), M-23 (0.100 ng/ml) and M-24 (0.500 ng/ml) at LLQ along with the internal standard, D_3-cerivastatin (0.500 ng/ml).



D3-cerivastatin lactone

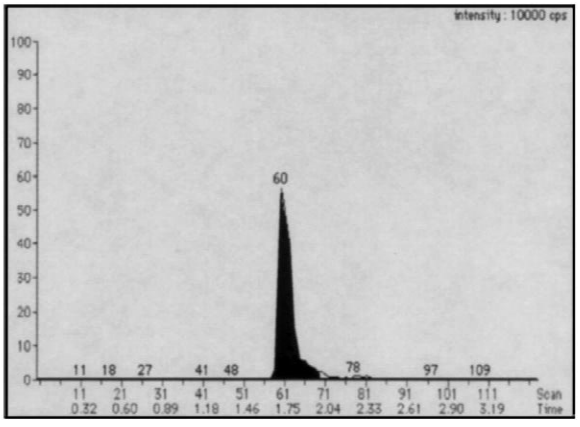


Fig. 14. SRM Chromatograms of cerivastatin lactone (0.0 100 ng/ml), M-1 lactone (0.0500 ng/ml), M-23 lactone (0.100 ng/ml) and M-24 lactone (0.500 ng/ml) at LLQ along with the internal standard, D_3-cerivastatin lactone (0.500 ng/ml).

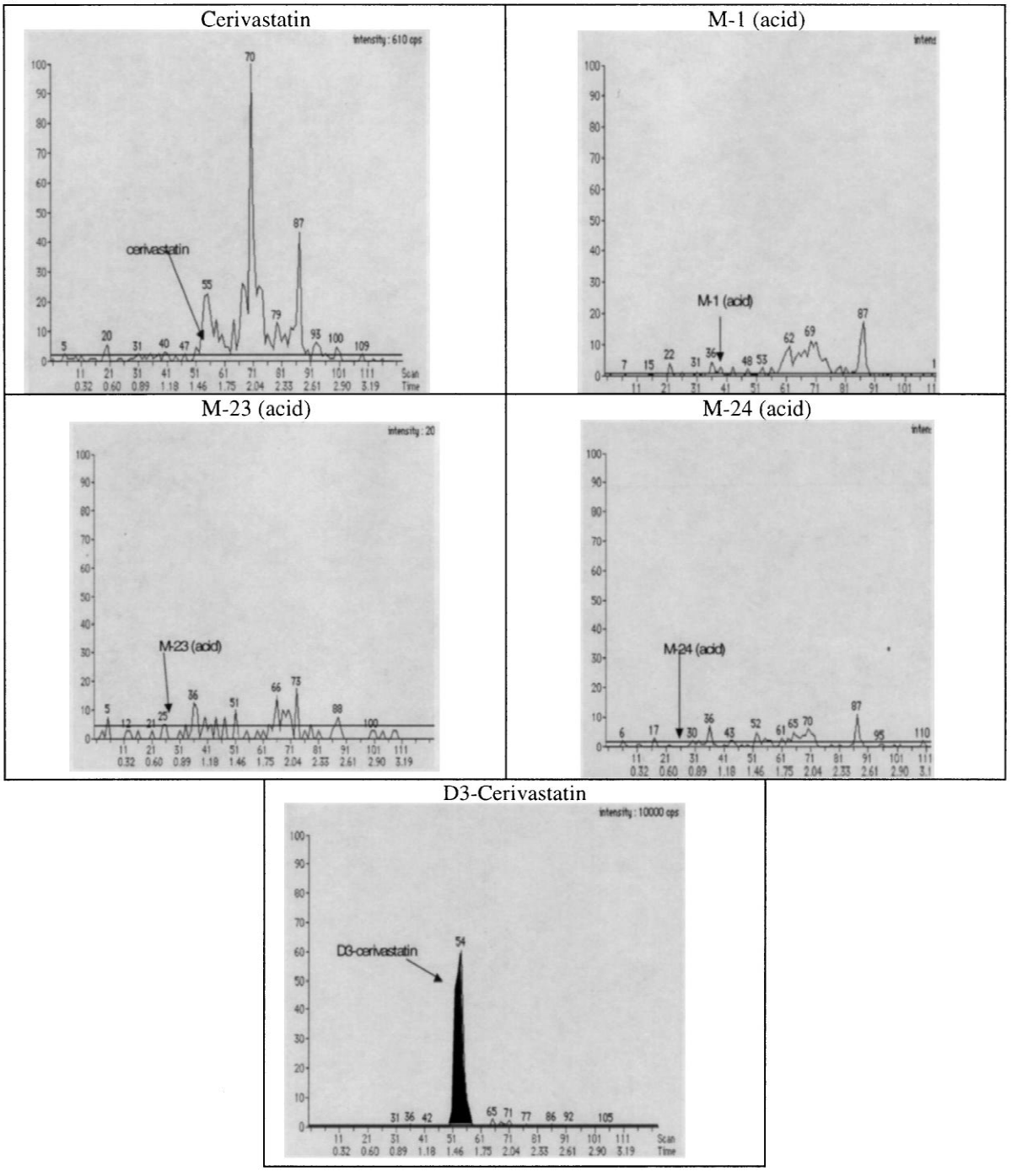


Fig. 15. SRM Chromatograms of a QCO sample for cerivastatin, M-1 (acid), M-23 (acid) and M-24 (acid) along with the internal standard, D_3-cerivastatin.

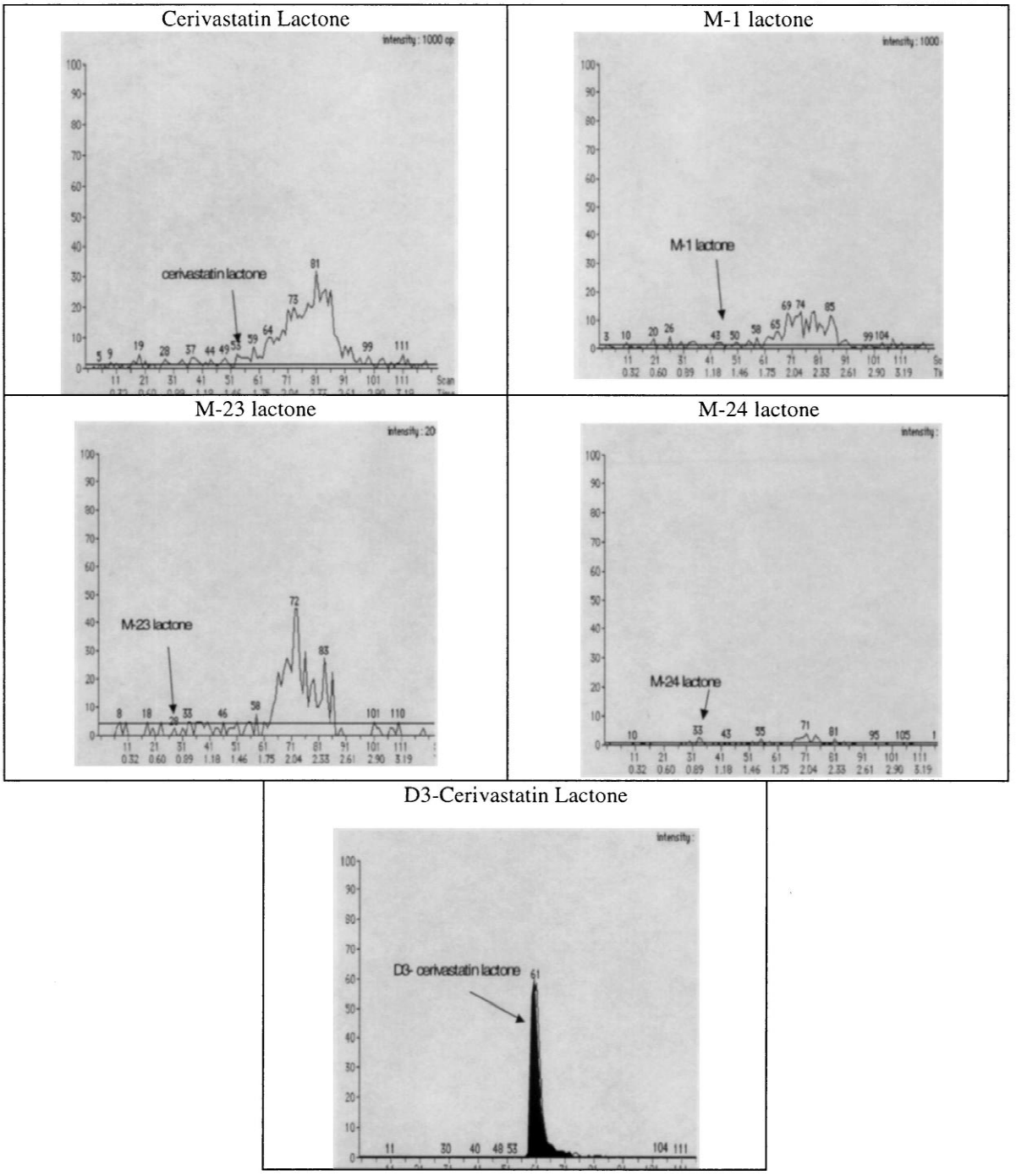


Fig. 16. SRM Chromatograms of a QCO sample for cerivastatin lactone, M-l lactone, M-23 lactone and M-24 lactone along with the internal standard, D_3-cerivastatin lactone.

Table 3
Percentage change[a] in concentrations of combination QC samples prepared in regular human serum and kept in ice and at room temperature (RT) for up to 24 h

	4 h ice		24 h ice		4 h RT		24 h RT	
	ng/ml		ng/ml		ng/ml		ng/ml	
	low[b]	8.00	low[b]	8.00	low[b]	8.00	low[b]	8.00
Cerivastatin (acid)	9	8	46	25	80	43	135	72
Cerivastatin lactone	−1	−9	−26	−24	−43	−50	−98	−98
M−1(acid)	10	8	9	5	37	29	81	58
M-1 lactone	5	17	3	14	−31	−32	−80	−88
M-23 (acid)	13	17	18	10	54	58	106	91
M-23 lactone	−6	14	−13	5	−52	−48	−95	−96
M-24 (acid)	−2	5	9	10	49	35	85	56
M−24 lactone	6	15	−15	−1	−46	−48	−91	−97

[a] Percent change is calculated against the zero-time concentration.
[b] Depending on the analyte, the low concentration varied between 0.0400 and 2.00 ng/ml: 0.0400 ng/ml for cerivastatin (acid) and cerivastatin lactone; 0.200 ng/ml for M-1 (acid) and M-1 lactone; 0.400 ng/ml for M-23 (acid) and M-23 lactone; 2.00 ng/ml for M-24 (acid) and M-24 lactone.

Table 4
Percentage change[a] in concentrations of combination QC samples prepared in human serum treated with pH 5 buffer and kept in ice and at room temperature (RT) for up to 24 h

	4 h ice		24 h ice		4 h RT		24 h RT	
	ng/ml		ng/ml		ng/ml		ng/ml	
	low[b]	8.00	low[b]	8.00	low[b]	8.00	low[b]	8.00
Cerivastatin (acid)	10	1	17	3	19	4	20	11
Cerivastatin lactone	–1	1	−7	0	0	2	−6	4
M-1 (acid)	1	−1	−5	4	10	3	9	9
M-l lactone	−4	9	−4	2	−7	14	7	−4
M-23 (acid)	−6	11	6	10	−4	19	13	15
M-23 lactone	−6	16	−8	1	−16	7	−17	−9
M-24 (acid)	−3	−3	−10	−l	7	−3	17	0
M-24 lactone	−9	11	−8	2	−14	14	−11	−5

[a] Percent change is calculated against the zero-time concentration.
[b] Depending on the analyte, the low concentration varied between 0.0400 and 2.00 ng/ml: 0.0400 ng/ml for cerivastatin (acid) and cerivastatin lactone; 0.200 ng/ml for M-1 (acid) and M-1 lactone; 0.400 ng/ml for M-23 (acid) and M-23 lactone; 2.00 ng/ml for M-24 (acid) and M-24 lactone.

4. Conclusions

A sensitive, specific, accurate and reproducible LC–MS–MS method for the simultaneous quantitation of the acid and lactone forms of cerivastatin and its biotransformation products (a total of eight analytes) in human serum was developed and validated. The desired sensitivity for cerivastatin and cerivastatin lactone was achieved with the LLQ of 0.0100 ng/ml. The LLQ for the other analytes was higher: 0.0500 ng/ml for M-1 (acid) and M-1 lactone; 0.100 ng/ml for M-23 (acid) and M-23 lactone; and 0.500 ng/ml for M-24 (acid) and M-24 lactone. The retention times for all eight analytes were within 2.0 mm and the total run time was only 3.5 min. The conditions chosen for the sample handling and extraction minimized the interconversion between the acid and lactone forms of the analytes. The

method has been successfully used to analyze human serum samples from a study of cerivastatin in humans.

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