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Quantitative determination of pravastatin and R-416, its main metabolite in human plasma, by liquid chromatography–tandem mass spectrometry

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Abstract

A quantitative method was developed and validated for rapid and sensitive analysis of pravastatin and R-416, the main metabolite of pravastatin, in human plasma. The analytes were extracted from plasma samples by a solid phase extraction method using a Bond Elut® C_8. The method involved the use of liquid chromatography coupled with atmospheric pressure chemical ionization (APCI) and selected reaction monitoring (SRM) mass spectrometry. A pravastatin analog, R-122798, was used as the internal standard (I.S.). Separation of pravastatin, R-416 and the I.S. was accomplished using a reverse-phase column (C_{18}). The components eluted were ionized by the APCI source (negative ion) and subsequently detected by a highly selective triple quadrupole mass spectrometer in the SRM mode. Linear standard curves were obtained from 0.1 ng/mL (lower limit of quantification, LLOQ) to 100 ng/mL. The intra-assay precisions (coefficient of variation) for the samples at the LLOQ were 1.8% for pravastatin and 1.6% for R-416. The intra-assay accuracy values were 95.8–107.6% for pravastatin, and 92.6–109.0% for R-416, respectively. Precision and accuracy of quality control (QC) samples were determined at concentrations of 0.5, 10 and 80 ng/mL for all analytes. The intra- and inter-assay precision calculated from QC samples were within 10% for pravastatin and within 11% for R-416. The overall recoveries for pravastatin and R-416 were 75.7–82.1% and 68.6–74.3%, respectively. Pravastatin and R-416 were stable in human plasma for 3 weeks at −20 °C in a freezer, up to 6 h at room temperature, and up to 48 h at 6 °C. This assay method was successfully used to evaluate the pravastatin and R-416 levels in healthy volunteers following oral administration of Mevalotin®.
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Keywords: Pravastatin; R-416

1. Introduction

Pravastatin sodium, a mono-sodium salt of 1S-(1α(βS,δS,2α6α,8β(R),8))-l,2,6,7,8,8-hexahydro β δ, 6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-1-naphthaleneheptanoic acid, is an anti-hypercholesterolemic agent having an inhibitory activity against 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-determining enzyme in the cholesterol synthesis. Its pharmacological action is due to the increase of low density lipoprotein receptors on hepatocytes as a consequence of decreased synthesis of mevalonic acid by inhibition of HMG CoA reductase, leading to an enhanced hepatic uptake of the plasma low density lipoprotein and to a decreased plasma cholesterol level. Chemical structures of pravastatin sodium, R-416 (the main metabolite) and R-122798, the internal standard substance (I.S.), are shown in Fig. 1. A sensitive and accurate quantitation of plasma levels of pravastatin (free form of pravastatin sodium) and R-416 (free form of R-416 sodium) in human plasma was required in order to investigate the clinical pharmacokinetics of pravastatin, especially from the viewpoint of elucidating the relationship between the pharmacodynamics and pharmacokinetics of the drug in patients. Several sensitive methods including ours [1] and those by others [2–4] using gas chromatography

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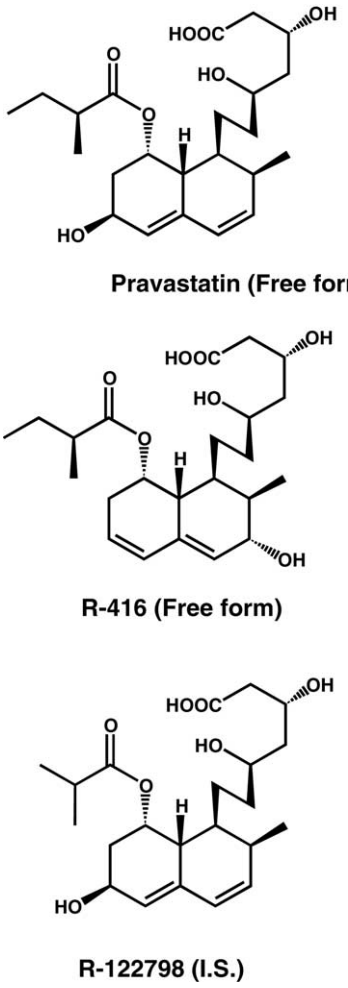


Fig. 1. Chemical structures of pravastatin, R-416 and the internal standard (I.S.).

with electron impact or negative chemical ionization-mass spectrometry (GC/EI-MS or GC/NICI-MS) have previously been reported for the determination of pravastatin and R-416 in human plasma. More recently, methods using liquid chromatography with electrospray ionization (ESI)-tandem mass spectrometry (LC/ESI-MS/MS) for the analysis of pravastatin, R-416 and pravastatin lactone in human plasma using solid-phase extraction or on-line purification have been reported [5–8]. We also reported previously an assay for plasma concentrations of pravastatin and R-416 by liquid chromatography with atmospheric pressure chemical ionization (APCI)-mass spectrometry (LC/APCI-MS), and compared the assay performance between LC/APCI-MS and GC/NICI-MS in analyzing the plasma samples collected from healthy volunteers after single, oral administration of pravastatin [9]. The lower limit of quantification (LLOQ) of these assay methods was in a range of 0.5–0.625 ng/mL, and the plasma concentrations of many patients were below the LLOQ at 12 and 24 h following oral administration of pravastatin. Since pravastatin is to be given to the patients once daily, monitoring of the plasma concentrations over a period of 24 h was considered necessary. This aim has not been accomplished by the previous methods, and there was a need for more sensitive assay method for pravastatin and R-416. In this paper, we report a highly sensitive assay method based on an LC-APCI-triple quadrupole mass spectrometer (LC/APCI-MS/MS) for the determination of pravastatin and R-416 in human plasma with an LLOQ of 0.1 ng/mL. Selective reaction monitoring (SRM) technique employed by this method was able to effectively eliminate background chemical interference arising from the complex plasma matrix, and achieved this low LLOQ value. The assay method has been adequately validated for application to pharmacokinetic studies of pravastatin sodium in humans.

2. Materials and methods

2.1. Standard substances and reagents

Pravastatin sodium (molecular weight: 446.5, purity: 94.8%), R-416 sodium (molecular weight: 446.5, purity: 93.1%) and R-122798 (molecular weight: 410.1, purity: 99.2%), the internal standard substance (I.S.), were synthesized by the Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan. Formic acid, ammonium acetate and triethylamine were obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Acetonitrile of analytical grade (Kanto Kagaku Co., Ltd., Tokyo) was used without further purification. Bond Elut® C_8 extraction cartridges (200 mg/3 mL) were obtained from Varian Incorporated (Harbor City, USA). Distilled water was passed through a Milli-Q® purification system (Millipore Corporation) before use. Blank plasma was prepared by centrifugation (3000 rpm, 10 min, 4 °C) of the blood obtained from five healthy volunteers, and stored frozen at −20 °C until used.

2.2. Operating conditions for liquid chromatography

Separation by high-performance liquid chromatography was conducted using a Waters 2690 Separations Module (Waters Co.) with an Inertsil® ODS-3 C_{18} (5 μm) column (4.6 mm i.d. × 150 mm, GL Sciences Inc.). A mixture of acetonitrile, water, ammonium acetate, formic acid and triethylamine (400/600/0.77/0.2/0.6, v/v/g/v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. The temperature of the auto-sampler chamber was held at 6 °C.

2.3. Operating conditions for mass spectrometry

Mass spectra were determined by a Finnigan TSQ-7000, API-2 MS/MS (Finnigan Corp., San Jose, CA) system in the negative-ion detection mode using the APCI-interface. The vaporizer was operated at a temperature of 520 °C with the heated capillary temperature set at 240 °C. Samples were ionized by reacting with reagent ions produced from the components in the mobile phase by the corona discharge (4 μA)

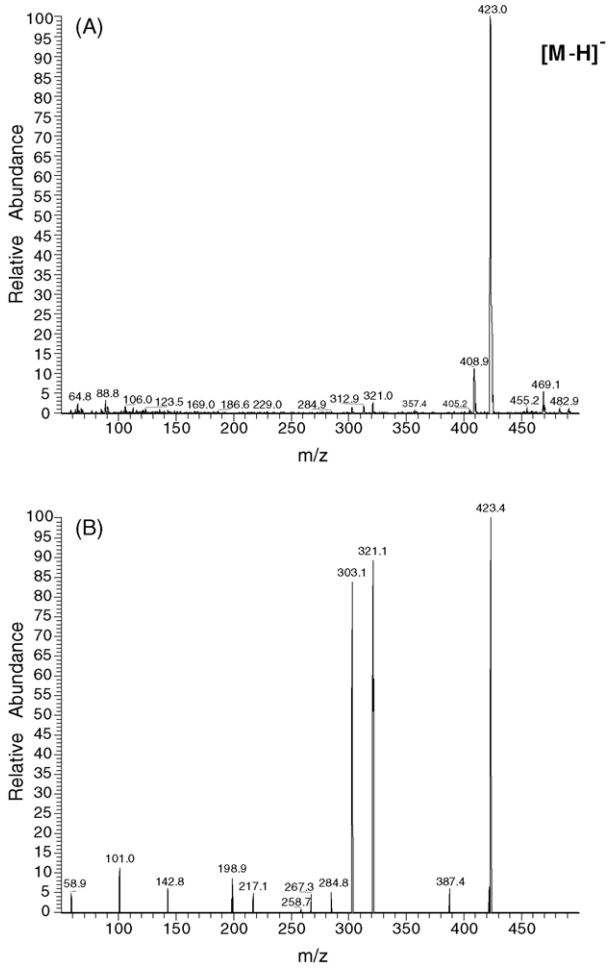


Fig. 2. APCI negative ion mass spectra of pravastatin. (A) Mass spectrum and (B) product ion spectrum.

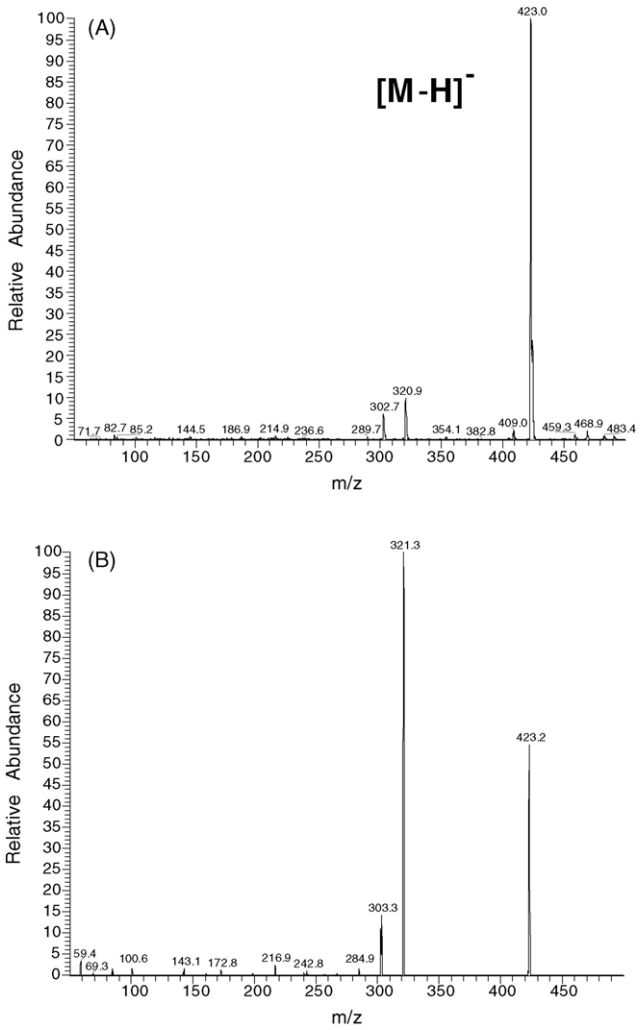


Fig. 3. APCI negative ion mass spectra of R-416. (A) Mass spectrum and (B) product ion spectrum.

in the chemical ionization (CI) mode. The pressure for the nitrogen sheath gas was 80 p.s.i., and the auxiliary gas was not used. The precursor ions of pravastatin $[M-H]^-$ and R-416 $[M-H]^-$ at *m*/*z* 423.0, and I.S. $[M-H]^-$ at *m*/*z* 408.9 (Figs. 2–4A) were admitted to the first quadrupole (Q1). After the collision-induced fragmentation in the second quadrupole (Q2), the product ions of pravastatin at *m*/*z* 321.1, R-416 at *m*/*z* 321.3 and I.S. at *m*/*z* 321.2 (Figs. 2–4B) were monitored in the third quadrupole (Q3). The collision offset energy was optimized at +20 eV for pravastatin and R-416 and at +25 eV for I.S. Mass peak width was 1.0 mass unit at half height for both Ql and Q3 and the mass span was 0.6 mass unit for all the compounds.

2.4. Preparation of stock solutions

Pravastatin sodium and R-416 sodium were dissolved in water to prepare a solution containing both pravastatin and R-416 at a concentration of 100 μg/mL. This solution was diluted to a concentration of 1 μg/mL to prepare the stock solution of pravastatin and R-416. The stock solution was further diluted serially to prepare the standard solutions for the calibration curve and quality control (QC) samples. The I.S. solution was made by dissolving the I.S. in water and further diluting with water to make a concentration of 800 ng/mL. These stock solutions were stored at 6 °C.

2.5. Calibration curve and quality control samples

The calibration curve was obtained by analyzing six plasma samples at each concentration spiked with the standard solutions of pravastatin and R-416 ranging from 0.1, 0.5, 5, 10, 50 to 100 ng/mL. Three levels of plasma quality control (QC) samples, 0.5, 10 and 80 ng/mL, were prepared in plasma spiked with the standard solutions of pravastatin and R-416. Peak areas of pravastatin, R-416 and I.S. were calculated using Finnigan Xcalibur LCquan^{TM} version 1.2 software. The calibration curve was generated by plotting nominal concentrations of pravastatin or R-416 (X) against the peak area ratios of pravastatin or R-416 versus I.S. according to a least-squares linear regression with a weighting index of $1/X^2$.

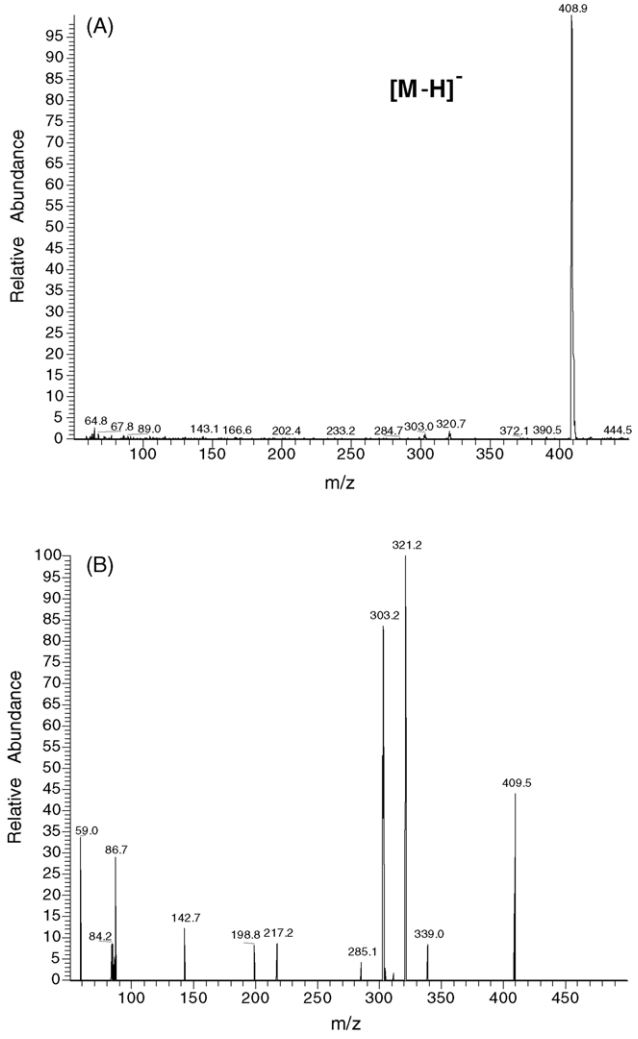
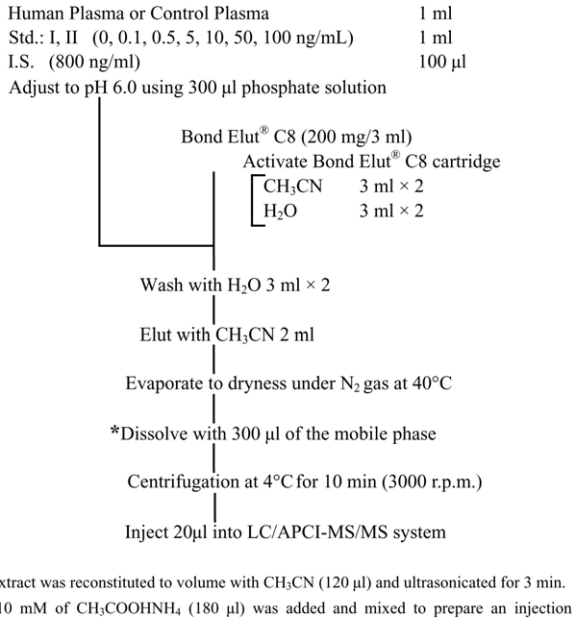


Fig. 4. APCI negative ion mass spectra of R-122798 (I.S.). (A) Mass spectrum and (B) product ion spectrum.

2.6. Procedure for extraction of plasma samples

To 1.0 mL of human plasma was added 1.0 mL of distilled water and 100 μl of the I.S. solution. The pH of this mixture was adjusted to 6.0 with 0.1 M phosphate solution (pH 4.0). It is important to keep the pH of the sample in a range of 3–6 in order to minimize the interconversion of the acid and lactone forms of pravastatin and R-416 [6,10]. The mixture was mixed briefly using a vortex mixer and applied to a Bond Elut® C_8 (200 mg/3 mL) cartridge, which had been preconditioned by washing successively twice with each 3 mL of acetonitrile and with each 3 mL of distilled water. The sample was applied to the column by vacuum. After washing the column two times each with 3 mL of water and removing the water from the column by vacuum, the analytes were eluted with acetonitrile (2 mL) into a round-bottomed tube (FALCON®). The eluate was evaporated to dryness under a nitrogen gas stream at 40 °C in a Turbo Vap LV® evaporator (Zymark, Corporation). The residue was reconstituted with acetonitrile (120 μl) by ultrasonication for 3 min. To this solution, 10 mM ammonium acetate (180 μl) was added to make an injection sample and this was transferred to a vial for autosampler. The typical injection volume was 20 μl. The sample preparation procedure for the determination of pravastatin and R-416 is shown schematically in Fig. 5. Plasma samples were thawed at 25 °C for 10 min or at 5 °C overnight.



*The extract was reconstituted to volume with CH_3CN (120 μl) and ultrasonicated for 3 min. Then, 10 mM of $CH_3COOHNH_4$ (180 μl) was added and mixed to prepare an injection sample.

Fig. 5. Assay procedure of pravastatin and R-416 in human plasma.

2.7. Method validation

The method was validated for precision (coefficient of variation, CV), accuracy, selectivity, specificity, linearity of the calibration curve, recovery, stability and reproducibility according to the recent paper by Shah et al. [11] on bioanalytical method validation, using plasma samples prepared at concentrations of 0.1, 0.5, 5, 10, 50 and 100 ng/mL. The intra-assay precision and accuracy were determined using these plasma samples in five replicates. The selectivity is the ability of an analytical method to quantify the analyte separately in the presence of other components in the sample. This test was performed by analyzing individually the blank plasma samples from five different donors to test for interference at the retention time of pravastatin, R-416 and I.S. The intra- and inter-assay precision and accuracy of QC samples at the lower (0.5 ng/mL), medium (10 ng/mL) and upper (80 ng/mL) concentrations ($n = 3$ for each concentration) were determined using these plasma samples in three replicates. The extraction recovery of pravastatin and R-416 was evaluated using three QC samples by comparing the peak area response of extracted in plasma analytes with that of the analytes in the standard solutions without extraction. The short-term stability of pravastatin and R-416 in human plasma was evaluated by analyzing three samples each at the concentrations of 0.5 and 80 ng/mL, after a storage period of 0, 2, 4 and 6 h in

an incubator (set at 25 °C). The long-term stability of pravastatin and R-416 in human plasma was evaluated by analyzing three samples each at the concentrations of 0.5 and 80 ng/mL, after a storage period of 0, 1 and 3 weeks in a freezer (set at −20 °C). The auto-sampler stability of pravastatin and R-416 in human plasma was evaluated by measuring three samples each at the concentrations of 0.5 and 80 ng/mL, after a storage period of 0, 6, 24 and 48 h in an auto-sampler (set at 6 °C).

3. Results and discussion

3.1. Lower limit of quantification, linearity and precision

The intra-assay precision in the analysis of the samples at the concentrations of 0.1, 0.5, 5, 10, 50 and 100 ng/mL was between 1.8 and 8.2% for pravastatin and 1.3 and 7.4% for R-416. The intra-assay accuracy was between 95.8 and 107.6% for pravastatin and 92.6 and 109.0% for R-416. The results of the intra-assay precision and accuracy are listed in Table 1. The LLOQ for both pravastatin and R-416 in human plasma was 0.1 ng/mL. The calibration curve was linear in the concentration range from 0.1 to 100 ng/mL for both pravastatin and R-416. The average coefficient of determination was 0.996 for pravastatin and 0.994 for R-416 ($n = 5$).

3.2. Specificity

Representative chromatograms of the extracted blank plasma containing only the I.S. are presented in Fig. 6. Representative chromatograms of the extracted plasma samples containing 0.1 ng/mL (LLOQ) of pravastatin and R-416 are presented in Fig. 7. Drug-free human plasma samples obtained from five different donors were screened and no endogenous peak interference was observed at the retention time of I.S., pravastatin and R-416.

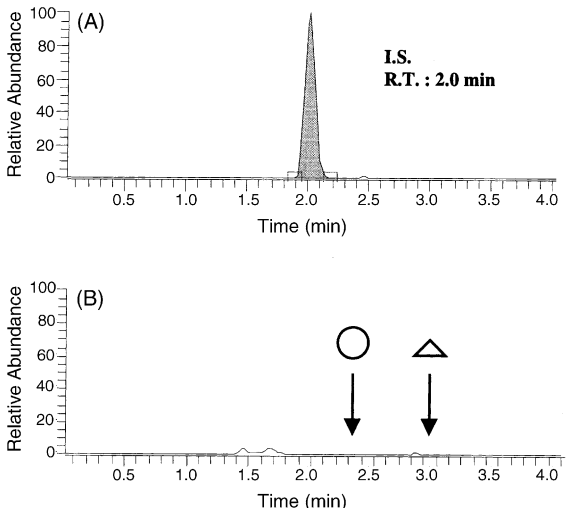


Fig. 6. SRM ion chromatograms of a human plasma extract spiked with the internal standard only. (A) I.S.: *m/z* 409 → *m/z* 321, (B) pravastatin and R-416: *m/z* 423 → *m/z* 321. Retention time of (◯) pravastatin and (△) R-416.

3.3. QC Samples

The intra-assay precision in the analysis of three QC samples at the concentrations of 0.5, 10 and 80 ng/mL was between 3.7, 1.0 and 6.0%, respectively, for pravastatin and 4.7, 2.6 and 6.7%, respectively, for R-416. The intra-assay accuracy was between 92.0, 105.4 and 106.0%, respectively, for pravastatin and 98.8, 101.4 and 95.0%, respectively, for R-416. The inter-assay precision was between 9.1, 5.3 and 3.8%, respectively, for pravastatin and 6.2, 3.2 and 11.0%, respectively, for R-416. The inter-assay accuracy was between 92.0, 103.1 and 104.4%, respectively, for pravastatin and 97.2, 104.2 and 97.3%, respectively, for R-416.

Table 1
Intra-assay precision and accuracy results of pravastatin and R-416 in human plasma

Nominal concentration (ng/mL)	Observed concentration (ng/mL)					Mean	S.D.	CV (%)	Accuracy (%)
	1	2	3	4	5				
Pravastatin									
0.1	0.101	0.098	0.103	0.101	0.101	0.101	0.002	1.8	100.8
0.5	0.484	0.550	0.435	0.488	0.482	0.488	0.041	8.2	97.6
5	4.81	4.90	4.72	4.56	4.95	4.79	0.15	3.2	95.8
10	9.47	9.61	9.86	10.0	10.0	9.79	0.24	2.4	97.9
50	51.4	47.6	52.5	51.3	49.1	50.4	2.0	3.9	100.8
100	109	103	112	108	106	108	3	3.1	107.6
R-416									
0.1	0.100	0.097	0.101	0.099	0.098	0.099	0.002	1.6	99.0
0.5	0.509	0.574	0.472	0.522	0.550	0.525	0.039	7.4	105.1
5	4.65	4.66	4.54	4.61	4.69	4.63	0.06	1.3	92.6
10	9.50	9.24	9.62	9.37	9.34	9.41	0.15	1.6	94.1
50	50.0	48.2	51.7	51.2	49.2	50.1	1.4	2.9	100.1
100	110	106	114	108	107	109	3	2.9	109.0

$\text{CV}\,(\%) = \frac{\text{Standard deviation (S.D.)}}{\text{Mean}} \times 100$; $\text{Accuracy}\,(\%) = \frac{\text{Observed concentration}}{\text{Nominal concentration}} \times 100$.

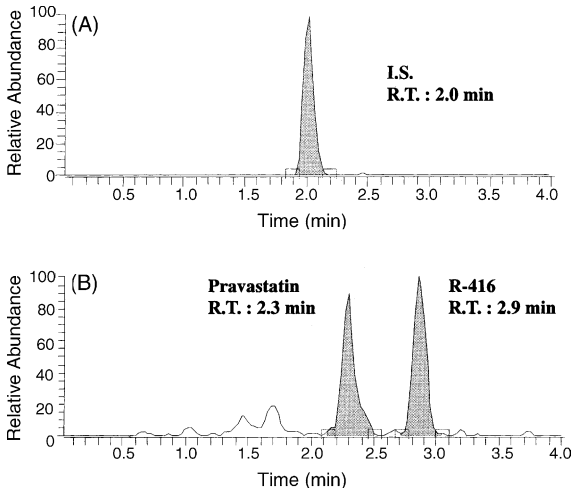


Fig. 7. SRM ion chromatograms of a human plasma extract spiked with the pravastatin and R-416 at 0.1 ng/mL. (A) I.S.: *m/z* 409 → *m/z* 321, (B) pravastatin and R-416: *m/z* 423 → *m/z* 321.

3.4. Extraction recovery

The mean recoveries of pravastatin and R-416 from the low, medium and high QC samples (0.5, 10 and 80 ng/mL) were 75.7–82.1% for pravastatin and 68.6–74.3% for R-416. The mean recoveries of I.S. samples (40 and 80 ng/mL) were 64.3 and 71.3% (Table 2). Effects of ion suppression on the peaks of pravastatin and R-416 were examined at a concentration of 80 ng/mL ($n = 3$), and were 5.0 ± 4.3% for pravastatin and 8.6 ± 4.6% for R-416.

3.5. Stability

Three low and three upper QC samples (0.5 and 80 ng/mL) were used to determine the sample stability. The back calculated values from time 0 to 6 h, expressed as percentages of the corresponding nominal values were 87.3–110.6% for pravastatin and 95.1–101.2% for R-416 which allowed us to conclude that the processed samples are stable for at least 6 h at room temperature (25 °C). The stability results for both pravastatin and R-416 showed that the residual percent of samples stored in a freezer (−20 °C) for 3 weeks is between 90.2 and 105.3% for pravastatin and 100.0 and 107.9% for R-416. Therefore, it was concluded that the plasma samples containing pravastatin and R-416 are stable for at least 3 weeks when stored in a freezer (−20 °C). The results of the test on auto-sampler stability ranged from 95.2 to 107.1% for pravastatin and 91.7 to 100.5% for R-416, and demonstrated that both pravastatin and R-416 are stable for at least 48 h at 6 °C.

3.6. Application of the assay method to pharmacokinetic study

After a single oral dose of 10 mg of Mevalotin® to 23 healthy male volunteers [12], the plasma concentrations of pravastatin and R-416 were simultaneously determined by the LC/APCI-MS/MS method described above. Representative SRM chromatograms of an unknown plasma sample collected from healthy male volunteer, at 1 h, the time of C_{max} (16 ng/mL), after a single oral dose of 10 mg Mevalotin® are shown in Fig. 8. The mean C_{max} of pravastatin was 23.9 ± 12.6 ng/mL (mean ± S.D.) ranging from 4.4 to 53.0 ng/mL. The mean C_{max} of R-416 was 16.5 ± 11.6 ng/mL ranging from 2.6 to 48.1 ng/mL. Fig. 9 shows the mean concentrations of pravastatin and R-416 in human plasma as a function of time. At 12 h after administration, the plasma concentrations of pravastatin and R-416 were below 0.625 ng/mL in 19 volunteers and 23 volunteers, respectively, and therefore, not possible to determine by the previous assay methods. The plasma concentrations at this time point were possible to determine by the LC/APCI-MS/MS method in all volunteers for pravastatin and 14 volunteers for R-416. At 24 h after administration, the plasma concentrations of these analyte were not possible to measure by the previous method, while the plasma concentrations of pravastatin in 9 volunteers and

Table 2
Summary of extraction recoveries for pravastatin, R-416 and I.S. in human plasma

Nominal concentration (ng/mL)	Extraction recovery (%)			Mean	S.D.
	1	2	3		
Pravastatin					
0.5	85.6	81.8	79.0	82.1	3.3
10	77.8	77.5	78.6	78.0	0.6
80	76.0	76.7	74.5	75.7	1.1
R-416					
0.5	69.8	76.6	76.4	74.3	3.9
10	72.4	73.4	72.4	72.7	0.6
80	69.5	69.2	67.1	68.6	1.3
I.S.					
40	71.7	73.3	69.0	71.3	2.2
80	64.9	65.2	62.7	64.3	1.4

Recovery calculated as $\frac{\text{peak area response of sample spiked pre-extractiony}}{\text{peak area response of sample spiked non-extraction}} \times 100$.

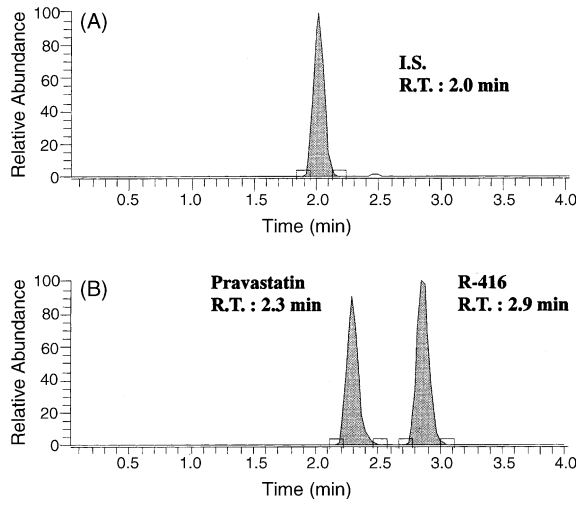


Fig. 8. SRM ion chromatograms of unknown plasma sample from a volunteer, at 1 h after an oral dose of 10 mg Mevalotin®. (A) I.S.: *m/z* 409 → *m/z* 321, (B) pravastatin and R-416: *m/z* 423 → *m/z* 321. The plasma concentrations of pravastatin and R-416 found were 43.4 and 45.2 ng/mL, respectively.

those of R-416 in one volunteer were still measurable by the present method. According to the paper by Zhu and Neirinck, the plasma concentration of pravastatin showed the C_{max} of 90 ng/mL at 0.75 h after a single oral administration of pravastatin to 18 healthy volunteers at a dose of 40 mg/subject [7]. Otter and Mignat reported that the C_{max} values of pravastatin were 14.0 and 67.4 ng/mL in two volunteers after a single oral administration of pravastatin at a dose of 40 mg/subject [13]. Our data on the plasma concentration of pravastatin extrapolated to a dose of 40 mg/subject were 95.6 ng/mL in C_{max} at 0.90 h. The C_{max} value in the Japanese volunteer seems to be comparable to or slightly higher than those in Caucasians most probably due to a lower body weight in the Japanese than in the Caucasians.

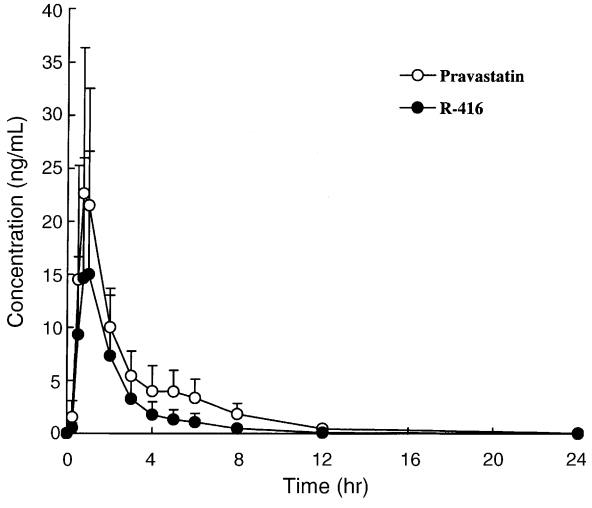


Fig. 9. Mean plasma concentrations of pravastatin and R-416 after oral administration of Mevalotin® at a dose of 10 mg to healthy male volunteers. (mean + S.D., $n = 23$).

4. Conclusions

A rapid and sensitive analytical method for the determination of pravastatin and R-416 in human plasma has been developed. The proposed method is the first LC/APCI-MS/MS method where precision and accuracy were included in the validation data. The method provided excellent specificity and linearity with a lower limit of quantitation of 0.1 ng/mL for both pravastatin and R-416, which were not achieved by the previous methods. The method was successfully used to provide bioanalytical data support for the human pharmacokinetic study of pravastatin sodium.

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References

[1] K. Kawabata, K. Sasahara, Proc. Jpn. Soc. Biomed. Mass Spectrom. 16 (1991) 149.
[2] P.T. Funke, E. Ivashkiv, M.E. Arnold, A.I. Cohen, Biomed. Environ. Mass Spectrom. 18 (1989) 904.
[3] D.B. Whigan, E. Ivashkiv, A.I. Cohen, J. Pharm. Biomed. Anal. 7 (1989) 907.
[4] M.J. Morris, J.D. Gilbert, J.Y. Hsieh, B.K. Matuszewski, H.G. Ramjit, W.F. Bayne, Biol. Mass Spectrom. 22 (1993) 1.
[5] M. Jemal, Y.Q. Xia, D.B. Whigan, Rapid Commun. Mass Spectrom. 12 (1998) 1389.
[6] M. Jemal, Y.Q. Xia, J. Pharm. Biomed. Anal. 22 (2000) 813.
[7] Z. Zhu, L. Neirinck, J. Chromatogr. B 783 (2003) 133.
[8] D. Mulvana, M. Jemal, S.C. Pulver, J. Pharm. Biomed. Anal. 23 (2000) 851.
[9] K. Kawabata, N. Matsushima, K. Sasahara, Biomed. Chromatogr. 12 (1998) 271.
[10] A.S. Kearney, L.F. Crawford, S.C. Mehta, G.W. Radebaugh, Pharm. Res. 10 (1993) 1461.
[11] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. Mckay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551.
[12] Y. Nishizato, I. Ieiri, H. Suzuki, et al., Clin. Pharmacol. Ther. 73 (2003) 554.
[13] K. Otter, C. Mignat, J. Chromatogr. B 708 (1998) 235.