



## DETERMINATION OF SIMVASTATIN IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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### ABSTRACT

A simple, sensitive and selective liquid chromatography with mass spectrometry used for determination simvastatin in human plasma has been developed, after extraction simvastatin by ethyl acetate and hexane (90:10%, v/v) using lovastatin as internal standard. Solutes are separated on a C<sub>18</sub> column with mobile phase consisting of mixture of acetonitrile and water (75:25%, v/v) 500 µL/min. Adduct product ions was detected by selected reaction monitoring in positive ion mode (m/z: 436 and m/z: 422 for simvastatin and lovastatin respectively). The calibration curve was linear from 0.5-20 ng/mL. The entire run time for analysis was only 5 min. The lower limit of quantitation of 0.5ng/mL was achieved. Precision and accuracy for the assay were determined by the intra-day and inter-day variation at three concentrations of 3, 6 and 12 ng/mL. The intra-day coefficients of variation were found to be less than 10% and the accuracies were between 97.50% and 109.50%. The inter-day coefficients of variation were found to be less than 10% and accuracies were found to be between 98.30% and 106.60%. The liquid chromatography with mass spectrometry method for the determination of simvastatin in human plasma offers several unique aspects. The separation is simple and rapid. The volume of plasma sample used in the assay is only 200 µL.

**KEY WORDS:** Liquid chromatography; mass spectrometry; Simvastatin; human plasma



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## INTRODUCTION

Simvastatin is one of the major statin drugs inhibits 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase that catalyzes the conversion of HMG-CoA to mevolanate, which is an early rate-limiting step in cholesterol biosynthesis in body. This agent is highly effective in reducing total cholesterol and the low density lipoprotein level. It is a highly effective cholesterol decreasing agent, which is generally used in the treatment of hypercholesterolemia<sup>1,2,3,4,5,6,7</sup>. The quantitative determination of simvastatin has been reported using high performance liquid chromatography (HPLC) with ultra violet (UV) detection<sup>8</sup>. HPLC with fluorescence detection<sup>9</sup>, gas chromatography/mass spectrometry (GC/MS)<sup>10,11</sup>, liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS)<sup>12,13,14</sup> and liquid chromatography-mass spectrometry (LC-MS)<sup>15</sup>. The HPLC-UV method is not sensitive enough for determining the drug levels in plasma at therapeutic dose. As for the HPLC-fluorescence detection, the samples need complex derivatization before their analysis. Even though the GC-MS and LC-MS-MS methods are highly sensitive and selective enough to analyze simvastatin at therapeutic level, but the solid phase extraction and clean-up procedure were complicated. This method describes a simple, sensitive and rapid LC-MS method for direct quantification of simvastatin in human plasma at the concentration ranges between 0.5 to 20 ng/mL.

## MATERIALS AND METHODS

### Chemicals

Simvastatin with molecular weight 418.57 and its internal standard lovastatin with molecular weight 404.54 were supplied by Ranbaxy (Sungai Petani, Malaysia). HPLC grade acetonitrile was obtained from Merck Darmstadt Germany. Hexane (analytical grade) was obtained from Merck Darmstadt Germany. Ethyl acetate and formic acid (analytical grade) were obtained from Fisher scientific international

company UK. Water was purified and deionized using a Milli-Q ion exchange filtration system. Water was filtered through WCN 0.45 um filters Whatman Ltd, UK.

### Chromatographic conditions

Chromatographic and mass detection system was performed using Finnigan LCQ-DUO system, comprising a quaternary pump, mobile phase vacuum degassing unit, UV-visible diode array detector, mass spectrometry detector and an autosampler. The system was controlled using Excalibur software (Finnigan) that runs under Windows NT operation system. The separation was performed in an analytical column C<sub>18</sub>, 5µm (3.9,i.d x 50 mm, 5 µm) at room temperature which supplied by Symmetry (USA). The mobile phase consisted of a mixture of 3mM formic acid and acetonitrile (75:25%, v/v) and is prepared daily. The LC system was operated isocratically at 500 µL/min. The mobile phase was introduced into the electrospray ionization source with the nebulizing gas flow rate was set at 30 mL/min while the pressure was set at 40 ± 10 psi. The drying gas temperature and capillary voltage were set at 250<sup>o</sup>C and 4000 V, respectively. The injection volume was 20 µL and represented no more than 20% of total sample available for injection. During method development, determination of ions were carried out for simvastatin and its internal standard lovastatin in the positive mode with a mass range was selected between 200–500 m/z, by injecting different concentrations of both compounds with mobile phase into mass spectrometry.

### Preparation of stock solution and calibration standard

Stock solution of simvastatin was prepared in acetonitrile (1mg/mL) and was diluted with acetonitrile and water 80:20%. Stock solution of lovastatin was prepared in acetonitrile and water 80:20% (1mg/mL) and was diluted with acetonitrile and water 80:20% to obtain desired concentrations. The stock solutions were kept at -20<sup>o</sup>C and were discarded one month after their

preparation. A calibration standard of 0.5, 1, 5, 10, 15 and 20 ng/mL in plasma was arranged by adding known quantities of simvastatin into blank plasma (200  $\mu$ L). The calibration curve for simvastatin was generated by measuring peak area ratio of the analyte to the internal standard.

#### **Extraction procedure**

The human plasma sample (0.2mL) was transferred into glass test tube and followed by the addition of 50  $\mu$ L of internal standard (10 ng/mL). 3 mL mixture solvent of ethyl acetate and hexane (90:10%, v/v) was added to the tubes and was vortexed for 30 second. Following a centrifugation at 4000 rpm for 10 min, the upper organic phase is transferred into another tube and evaporated to dryness under nitrogen at 40°C. The dry residue was reconstituted with 100  $\mu$ L of acetonitrile and water (80:20%, v/v), vortexed for 15 second and 20  $\mu$ L of the mixture was injected into the chromatograph.

#### **Assay validation**

The intra-day coefficient of variation (CV) and accuracy of the quantification were assessed using six replicates at 3, 6 and 12 ng/mL. While the inter-day assay were evaluated using five replicates at the same concentrations, repeated for five different days.

#### **Extraction efficiency**

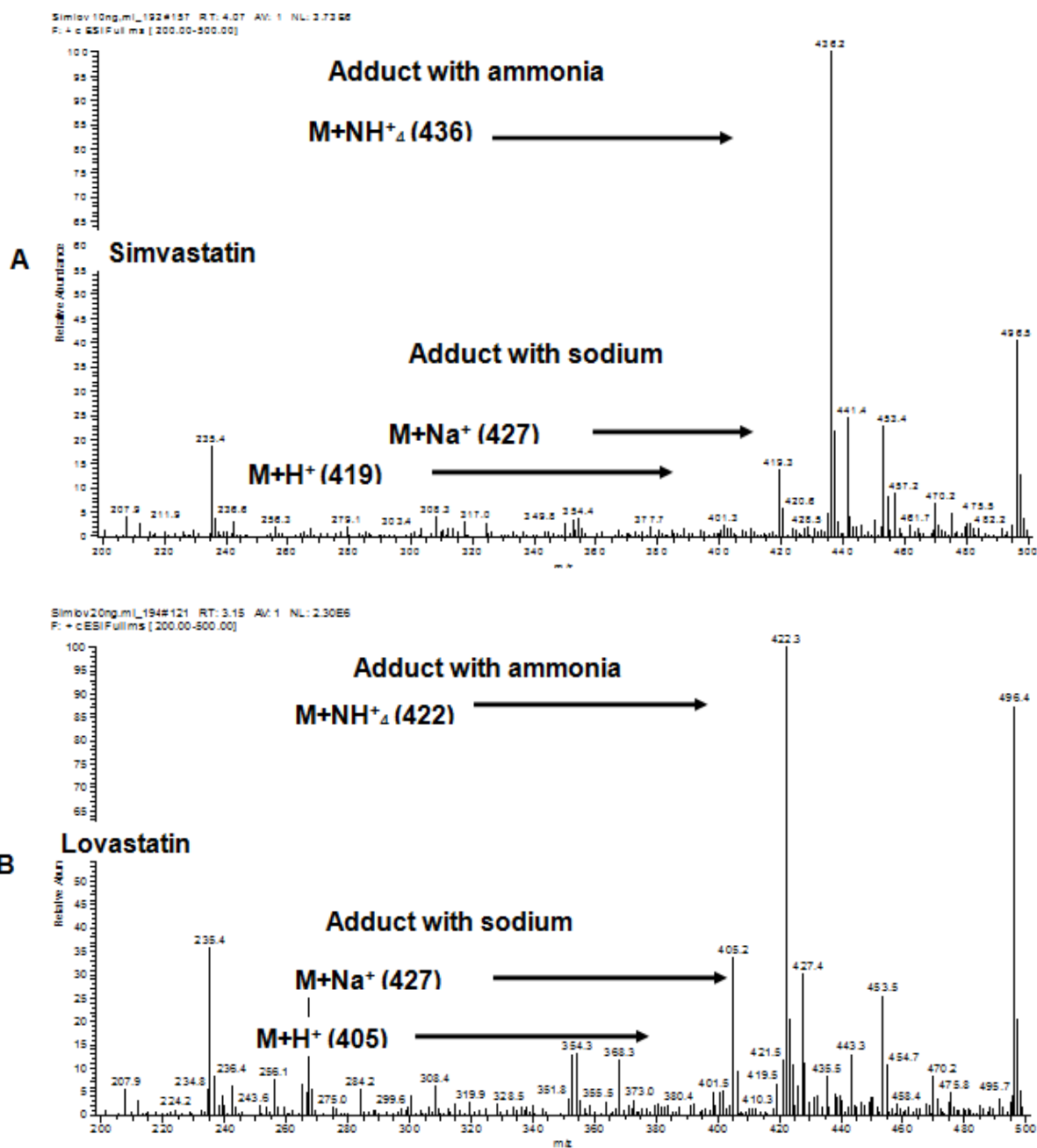
The extraction efficiencies of simvastatin was determined at low, medium and high concentrations of 3, 6 and 12 ng/mL,

respectively, by comparing peak area of solutions of extracts versus aqueous standards.

## **RESULTS AND DISCUSSION**

#### **Mass Spectrometric Conditions in LC-MS**

The protonated simvastatin  $M+H^+$  and lovastatin  $M+H^+$  were detected at m/z 419 and 405 respectively however, due to less reproducibility of these ions observed in the assay, other adducts ions were selected for quantitation of simvastatin and lovastatin. Ions of  $M+NH_4^+$  and  $M+Na^+$  were also detected, although the mobile phase contained no known sources of the ammonium or sodium ions, but due to reaction with heated capillary in the ion source. As ions of  $M+NH_4^+$  of simvastatin and lovastatin showed the highest abundance in the spectrum at m/z 436 and 422, respectively, as compared with  $M+H^+$  and  $M+Na^+$  ions, they have been selected for the quantitation of simvastatin and lovastatin. Both ions had a dwell time of 500 ms  $ion^{-1}$ . The adducts ionisation condition of mass spectrometry in this method is similar with LC-MS-MS method previously reported by Jemal *et al*<sup>[12]</sup>  $M+NH_4^+$  of simvastatin with m/z 436, while other LC-MS-MS methods previously reported by Wu *et al*<sup>[13]</sup> and Barrett *et al*<sup>[14]</sup>, the adducts ionisation for simvastatin was  $M+Na^+$  with m/z 441, although LC-MS method previously reported by Yang *et al*<sup>[15]</sup>, the adducts ionisation for simvastatin was  $M+Na^+$  with m/z 441. Fig. 1 shows electrospray positive ion mass spectra and adducts ionisation condition of mass spectrometry for both simvastatin and lovastatin.



**Figure 1**

*Electrospray positive ion mass spectra for simvastatin (A) and its internal standard (B) in mobile phase of 75% acetonitrile and 25% of aqueous 3 mM formic acid*

**Separation**

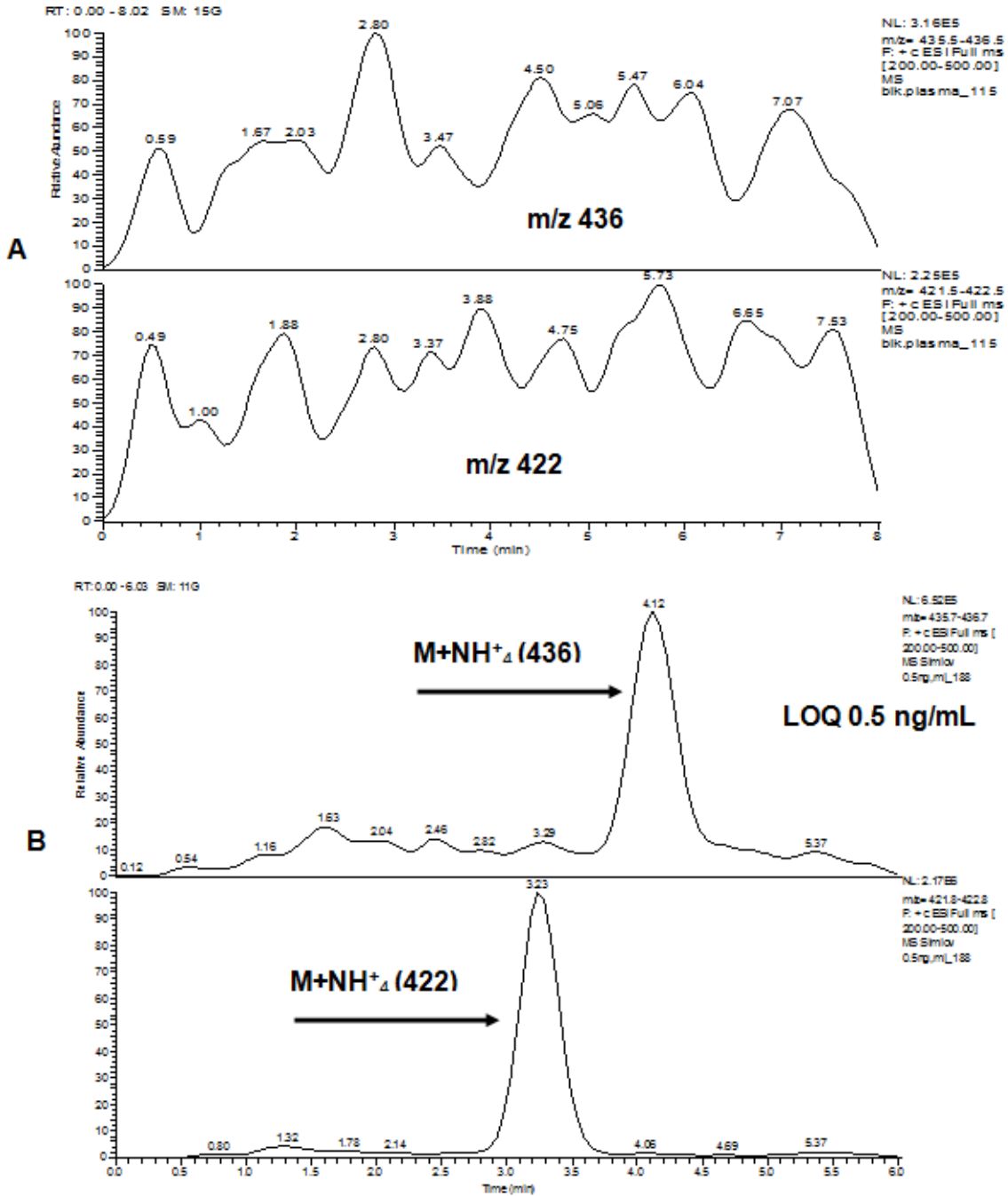
Chromatograms from the study of simvastatin in human plasma are shown in Fig. 2 and 3 with retention times of less than 5 min were achieved for both simvastatin at 0.5 and 12 ng/mL with internal standard lovastatin at 10 ng/mL. Simvastatin eluted at 4.1-4.2 min and internal standard lovastatin at 3.2-3.3 min.

**Calibration curves, coefficient of variations and accuracy**

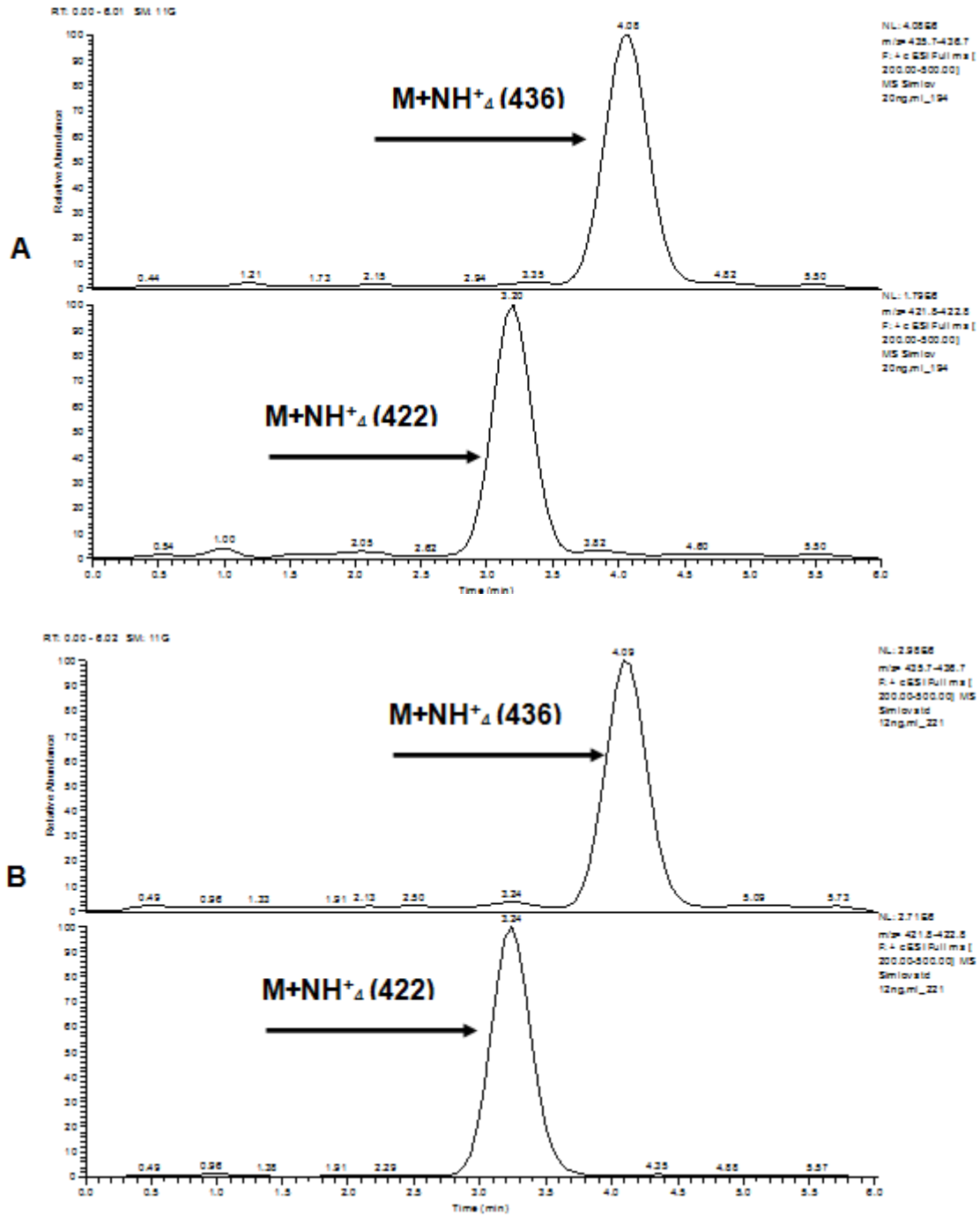
Results for the calibration curve (n = 5) showed good linearity (r = 0.995) over concentration of 0.5-20 ng/mL, with an equation of  $y = 0.0873x + 0.0425$  (y = simvastatin concentration in ng/mL; x = simvastatin area/lovastatin area). The results of intra-day and inter-day are tabulated in Table 1 and Table 2, respectively.

The intra-day coefficients of variation do not exceed 10.00% and the intra-day accuracies were between 97.50 and 109.90%. The inter-day coefficients of variation do not exceed 10.00% and the inter-day accuracies were between 98.30 and 106.70%. The analyzed method that has been developed shows good

sensitivity for simvastatin. The lowest detection limit (LOD) here is considered to be about 3 times the signal-to-noise (S/N) ratio is 0.4 ng/mL. The lowest quantification limit (LOQ) of 0.5 ng/mL analyte has been determined with acceptable precision and accuracy.



**Figure 2**  
 Chromatograms of drug free plasma (A) and plasma spiked with simvastatin (0.5 ng/mL) and lovastatin (10 ng/mL) (B). The m/z of simvastatin  $M+NH_4^+$  436 and m/z of lovastatin  $M+NH_4^+$  422



**Figure 3**

**Chromatograms of the quality control sample after spiking 12 ng/mL of simvastatin in 1mL human plasma with lovastatin 10 ng/mL (A) and simvastatin at 12 ng/mL with lovastatin 10 ng/mL in aqueous working solution (B)**

**TABLE 1**  
**INTRA-DAY COEFFICIENT OF VARIATION AND ACCURACY OF MEASUREMENT OF SIMVASTATIN IN HUMAN PLASMA OF INDIVIDUAL SAMPLES (N=6)**

Spike concentration (ng/mL)	Found concentration (ng/mL)	Coefficient of variation (CV) (%)	Accuracy (%)
3	3.30 ± 0.33	9.97	109.50
6	5.90 ± 0.53	9.00	97.50
12	12.00 ± 0.80	6.67	99.90

*The value are mean ±S.D.*

**TABLE 2**  
**INTER-DAY COEFFICIENT OF VARIATION AND ACCURACY OF MEASUREMENT OF SIMVASTATIN IN HUMAN PLASMA OF INDIVIDUAL SAMPLES (N=5)**

Spike concentration (ng/mL)	Found concentration (ng/mL)	Coefficient of variation (CV) (%)	Accuracy (%)
3	3.20 ± 0.30	9.40	106.70
6	5.90 ± 0.39	6.50	98.30
12	11.90 ± 0.26	2.20	99.20

*The value are mean ±S.D.*

### **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 during extraction, and comparing the peak area ratios with ratios from non-extracted analytes to which internal standard was added. The recovery of simvastatin from human plasma is between 87.50 to 97.00% and is depicted in Table 3.

**TABLE 3**  
**EXTRACTION EFFICIENCY OF SIMVASTATIN (N=3)**

Sample concentration (ng/mL)	Extracted Concentration Mean ( CV%)	Non-extracted Concentration Mean (CV%)	Recovery %
3	3.20 (3.1)	3.30 (6.2)	97.00
6	5.90 (3.7)	6.40 (8.4)	92.20
12	11.90(10)	13.60 (2.2)	87.50

### **Re-injection Process Validation**

The mean of each quality control concentration for the re-injection process was calculated in order to approve the precision of the re-injection process. The precision obtained from mean values of each re-injection set is not exceeding 15% (tolerance until 20% at LOQ concentration). The re-injection process validation data is presented in Table 4.

**TABLE 4**  
**RE-INJECTION PROCESS DATA USING LC-MS METHOD**

Concentration.	Mean Original value	CV(%)	Mean Re-injection value	CV (%)	Variation (%)
Low (3 ng/mL)	3.19	10.80	3.21	5.00	5.80
Medium (6 ng/mL)	6.00	7.70	5.99	6.04	1.66
High (12 ng/mL)	11.97	2.50	11.82	4.36	1.86

## CONCLUSION

The LC-MS method for the determination of simvastatin in human plasma offers several unique aspects. The separation is simple and rapid. The volume of plasma sample used in the assay is only 200  $\mu$ L, which is significantly less than in previously published methods. The LC-MS method developed here has proven to be sensitive, accurate, selective and reproducible.

## REFERENCES

1. Corsini A, Bellocchia S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic properties of statins. *J Pharmacol Ther*;64: 413-428 (1999)
2. Plosker LG, Mctavish D. Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolemia. *Drugs*; 50: 334-363 (1995)
3. Todd AP, Goa LK. Simvastatin A Review of its pharmacological Properties and Therapeutic Potential in Hypercholesterolaemia. *Drugs*; 40: 583-607 (1990)
4. Vickers S, Duncan CA, Chen I, Rosegay A, Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. *Drug Metab Dispos*;18: 138-145 (1990)
5. Vickers S, Duncan CA, Vyas KP, Kari PH, Arison B, Prakash SR, Ramjit HG, *et al.* In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG-CoA reductase. *Drug Metab Dispos*; 18: 476-483 (1990).
6. Mauro VF. Clinical Pharmacokinetics and Practical Applications of Simvastatin. *J Clin Pharmacokinet*; 24: 195-202 (1993).
7. Desager JP, Horsmans Y. Clinical Pharmacokinetics of 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase Inhibitors. *J Clin Pharmacokinet*; 31: 348-371 (1996).
8. Carlucci G, Mazzeo P, Biordi L, Bologna M. Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high performance liquid chromatography with UV detection. *J Pharm Biomed Anal*; 10: 693-697 (1992)
9. Ochiai H, Naotaka U, Iyama KI, Shunsuke H, Toshio K. Determination of simvastatin and its active metabolite in human plasma by column-switching high-performance liquid chromatography with fluorescence detection after derivatization with 1-bromoacetylpyrene. *J Chromatogr Bull*; 694: 211-217 (1997)
10. Morris MJ, Gilbert JD, Hsieh JYK, Matuszewski BK, Ramjit HG, Bayne WF. Determination of the HMG-CoA reductase inhibitors simvastatin, lovastatin and pravastatin in plasma by gas chromatography/chemical ionization mass spectrometry. *Biol Mass Spectrom*; 22: 1-8. (1993)
11. Takano T, Abe S, Hata S,. A Selected ion monitoring method for quantifying simvastatin and its acid form in human plasma, using the ferroceneboronate derivative. *Biomed Environ Mass Spectrom*; 19: 577-581. (1990)
12. Jemal M, Ouyang Z, Powell ML. Direct injection LC-MS-MS method for high throughput simultaneous quantitation of simvastatin and simvastatin acid in human plasma. *J Pharm Biomed Anal*; 23: 323-340 (2000)
13. Wu Y, Zhao J, Henion J, Korfmacher WA, Lapiguera AP, Lin CC. Microsample determination of lovastatin and its hydroxy acid metabolite in mouse and rat plasma by liquid chromatography tandem mass spectrometry. *J Mass Spectrom*; 32: 379-387 (1997)
14. Barrett B, Huclova J, Borek-Dohalsky V, Nemeč B, Jelinek I. Validated HPLC-MS-MS method for simultaneous determination of simvastatin and simvastatin hydroxyl acid in human plasma. *J Pharm Biomed Anal*; 41: 517-526 (2006)
15. Yang H, Yan F, Yiwen L. Determination of simvastatin in human plasma by liquid chromatography-mass spectrometry. *J Chromatogr Bull*; 785: 369-375 (2003)