Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 785 (2003) 369–375
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

# Determination of Simvastatin in human plasma by liquid chromatography–mass spectrometry

Haitao Yang[a,*], Yan Feng[b], Yiwen Luan[a]

[a]*Laboratory of R&D, Nanjing Kangxing Pharmaceutical Ltd., Nanjing, China*
[b]*Drug Control Institute of Jiangsu Province, Jiangsu, China*

Received 8 May 2002; received in revised form 7 October 2002; accepted 8 October 2002

**Abstract**

A simple, sensitive and selective liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI/MS) method for the determination of simvastatin (I) has been developed. After extraction by ethyl acetate, using lovastatin (II) as internal standard, solutes are separated on a $C_{18}$ column with a mobile phase consisting of methanol–water (9:1). Detection is performed on an atmospheric pressure ionization single quadruple mass spectrometer equipped with an ESI interface and operates in positive ionization mode. Simvastatin quantification was realized by computing peak area ratio (I/II) of the extracts analyzed in SIM mode ($m/z$: 441 and $m/z$: 427 for I and II, respectively) and comparing them with calibration curve ($r=0.9997$). Accuracy and precision for the assay were determined by calculating the intra-batch and inter-batch variation at three concentrations 0.1, 5.0, 10.0 ng/ml; the intra batch relative standard deviation (RSD) was less than 10% and ranged from 1.8 to 8.5%, respectively; the inter-batch RSD was less than 20% and ranged from 4.1 to 16.5%. The limit of detection was 0.05 ng/ml.


*Keywords:* Simvastatin

## 1. Introduction

Simvastatin (I), an analogue of lovastatin, is the lactone form of 1′,2′,6′,7′,8′,8a′-hexahydro-3,5-dihydroxy-2′, 6′-dimethyl-8′(2″, 2″dimethyl-1″-oxobutoxy)-1′-naphthaleneheptanoic acid, which lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase. It is a highly effective cholesterol-lowering agent, which is widely used in the treatment of hypercholesterolemia. Recently it was reported that simvastatin is also effective in reducing lethality in coronary heart disease. Plasma levels of simvastatin following therapeutic oral doses are reported to be very low compared to levels observed after intravenous dosing [1,2], probably because only 5% of the dosed simvastatin reaches the systemic circulation [3]. Therefore, sensitive and selective methods for the determination of simvastatin have been required for therapeutic drug level monitoring.

Several methods for simvastatin determination

*Corresponding author.Present address: 904 Salk Hall, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15260, USA.
*E-mail address:* htyang2000@yahoo.com (H. Yang).

1570-0232/02/$ – see front matter 
PII: S1570-0232(02)00800-0

including gas chromatography–mass spectrometry (GC–MS) [4], liquid chromatography–UV detection (LC–UV) [5,6] and LC with fluorescence detection [7] have been reported. GC–MS methods are highly sensitive and selective enough to analysis the therapeutic plasma level of simvastatin, but the operation and clean-up procedure is complicated. Two LC–UV [5,6] methods require lengthy extraction procedures and they are not sensitive enough for determining the drug levels in plasma at therapeutic dose. Although LC with fluorescence detection is a highly sensitive method, the samples need complex derivativization before their analysis, which is inconvenient [7].

According to the literature, plasma concentrations could be expected to be between 0.1 to 15 ng/ml. This paper describes a simple, rapid and sensitive LC/ESI/MS method for direct quantification of simvastatin in the concentration range 0.1–20 ng/ml in human plasma. LC has numerous advantages, e.g. very selective separations, comparatively short analysis times, and simple preliminary treatment of the sample [8–10]. Electrospray ionization mass spectrometry (ESI-MS) has the advantage [11–13] that it can be easily coupled to LC as the ion source is at atmospheric pressure and the detection is very sensitive and specific. Due to the technique of pneumatic-assisted electrospray, the flow-rate was set at 1.0 ml/min. Under such conditions, the peaks are symmetrical and sharp, which is suitable for quantitative analysis. Another more sensitive method is LC/MS/MS, it can achieve lower concentration in plasma, but it is too expensive to use for the routine measurements in the clinical laboratory [14,15].

## 2. Experimental

### 2.1. Materials

Simvastatin (I, Fig. 1) ($M_r$ = 418.3) was obtained from Merck Research Laboratory and the internal standard (I.S.) lovastatin (II, Fig. 1) ($M_r$ = 404.3) were obtained from Sigma Chemical Company.

All solvents were of HPLC grade,

Methanol was purchased from Tedia Company, (USA).

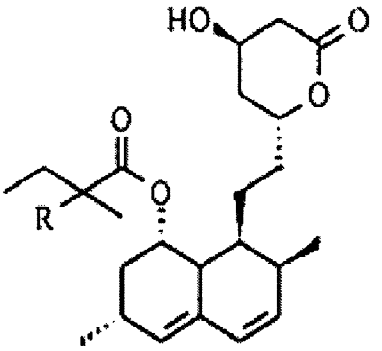


Fig. 1. Structures of simvastatin (I) and lovastatin (II).

### 2.2. Instrumentation and chromatographic condition

All analyses were performed using Agilent 1100 LC–MS system (Hewlett-Packard). The system components included a binary pump, mobile phase vacuum degassing unit, autosampler, temperature controlled column compartment, UV–visible diode array detector, and Agilent 1100 mass spectrometric detector (LC–MSD). A single Agilent Vectra 150/PC under the Windows NT operating system using Agilent LC–MSD Chemstation software performed the system control and data acquisition for both DAD and MSD. A Shim-pack SHIMADZU ODS (5 μm, 4.6×150 mm I.D.) column was used for all chromatographic separations. Mobile phase was methanol–water (9:1, v/v). The LC system was operated isocratically at 800 μl/min and at 35 °C. The column eluent was split and approximately 300 μl/min were introduced into the electrospray ionization source. The nebulizing gas flow-rate was set at 11 l/min. Air (purity grade 99.999%, USA) was the nebulizing gas at pressure of 45 p.s.i. (1 p.s.i. = 6894.76 Pa). And drying gas temperature was 350 °C and capillary voltage 4000 V. The injection volume was 5 μl and represented no more than 10% of the total sample available for injection. Signal intensity could be increased by injecting more of the final extract.

The responses of simvastatin and lovastatin were measured in the positive mode with a fragmentor voltage of 70 V. In the single ion monitoring (SIM) experiments, the ion for simvastatin was $[M+Na^+]$

($m/z$ 441.3) and that for lovastatin was $[M+Na^+]$ ($m/z$ 427.3), both ions had a dwell time of 500 ms $ion^{-1}$.

### 2.3. Preparation of stock solution and calibration standard and quality control samples

Stock solutions of simvastatin (I) and lovastatin (II) were prepared in methanol (50 μg/ml) and were diluted with methanol to obtain the desired concentrations. The stock solutions were kept refrigerated and were discarded 1 month after their preparation.

The nominal plasma concentrations of calibration standards were 0.1, 0.2, 0.5, 1.0, 3.0, 5.0, 10.0, 12.0, 15.0, 20.0 ng/ml. The II stock solution (1.0 ml) was diluted (to 100 ml) with 100% methanol. Three levels of quality controls (QCs) at 0.1,5.0,10.0 ng/ml (very low, medium and high) were prepared.

### 2.4. Extraction procedure

Adding 10 ng of internal standard solution in 1.0 ml of plasma, then vortex-mixed for 30 s, after that 5 ml ethyl acetate was added. Then vortex-mixed for 3 min. QC plasma samples and human plasma samples for the determination of I were prepared as describe above, but were not spiked with I. Following centrifugation at 3000 *g* for 10 min. The organic layer was transferred to a 10 ml tube, and evaporated to dryness in a water bath at 40 °C under a nitrogen stream. Before injection, 50 μl of methanol was added to the dried human plasma extract. This was vortex mixed for 60 s and a 5 μl portion was injected into the chromatograph.

### 2.5. Method validation

Plasma calibration curves and six replicates of QCs were analyzed. The ratios of the peak areas of I to the peak areas of II were calculated. The calibration curves were constructed by weighted ($1/y$) least-squares linear regression analysis of the peak area ratios of I/II vs. the concentrations of I. Calibration curve equations were used to calculate the concentrations of I in the samples and QCs from their peak area ratios. The intra-batch precision and accuracy were determined by analyzing a set of QC samples ($n=6$) at each of the three levels 0.1, 5.0 and 10.0 ng/ml. The inter-day batch precision and accuracy studies were also carried out by analyzing QC samples at the above three concentrations.

### 2.6. Extraction efficiency

The absolute recovery (extraction-efficiency) of I through the extraction procedures was determined at 0.5, 5.0, 10.0 ng/ml (low, medium and high concentrations) by external method. A known amount of I was added to human plasma prior to extraction as described in Section 2.4. The I.S. was added after extraction to eliminate bias introduced by sample processing. Concentration of I was calculated using calibration curve prepared on the same day.

## 3. Results and discussion

### 3.1. Assay development

Flow injection analysis (FIA) is a sample introduction technique where the sample is introduced into the LC/MSD without passing through a column for separation. FIA was used to optimize LC/MSD parameters and perform ion selection rapidly. The fragmentor voltage is especially important, as it not only affects the transmission of the ions; it also affects the dissociation of molecules into fragments.

In order to minimize undesirable fragmentation and achieve highest response, various fragmentor voltages were tested from 30 to 140 V. MS detector response of simvastatin is shown in Table 1 (concentration of Simvastatin was 1.0 ng/ml). Because 70 V could achieve both minimal undesirable frag-

Table 1
Response of Simvastatin at different fragmentor voltage

| Fragmentor voltage (V) | Area of simvastatin $[M+Na^+]$ |
|---|---|
| 30 | 95998 |
| 50 | 227123 |
| 70 | 680109 |
| 90 | 417797 |
| 110 | 224461 |
| 140 | 45759 |

mentation and highest response, the fragmentor voltage was set at 70 V. Protonated molecule of I $[M+H^+]$ ($m/z$ 419) and the sodium adduct of I $[M+Na^+]$ ($m/z$ 441) were tested at 70 V. Because the latter is more sensitive than the former, I $[M+Na^+]$ ($m/z$ 441) was selected for determination. Chromatogram of these two tested ions is shown in Fig. 2. (TIC: total ion chromatogram). Other mass spectrometric parameters (gas temperature, gas pressure, and gas flows) were adjusted to get a maximum signal for the simvastatin $[M+Na^+]$.

### 3.2. Separation and specificity

Two typical chromatograms from the study of I in human plasma are shown in Fig. 3. Short retention times of less than 5 min were achieved for both I and II. I eluted at 4.2 min and internal standard at 3.7 min. Ion chromatograms for I and II from blank (Fig. 3a), plasma spiked with I and II (Fig. 3b) and a sample from a volunteer 3 h after oral administration of 40 mg of simvastatin (Fig. 3c) are shown in Fig. 3 (concentration of Simvastatin was 3.5 ng/ml). For

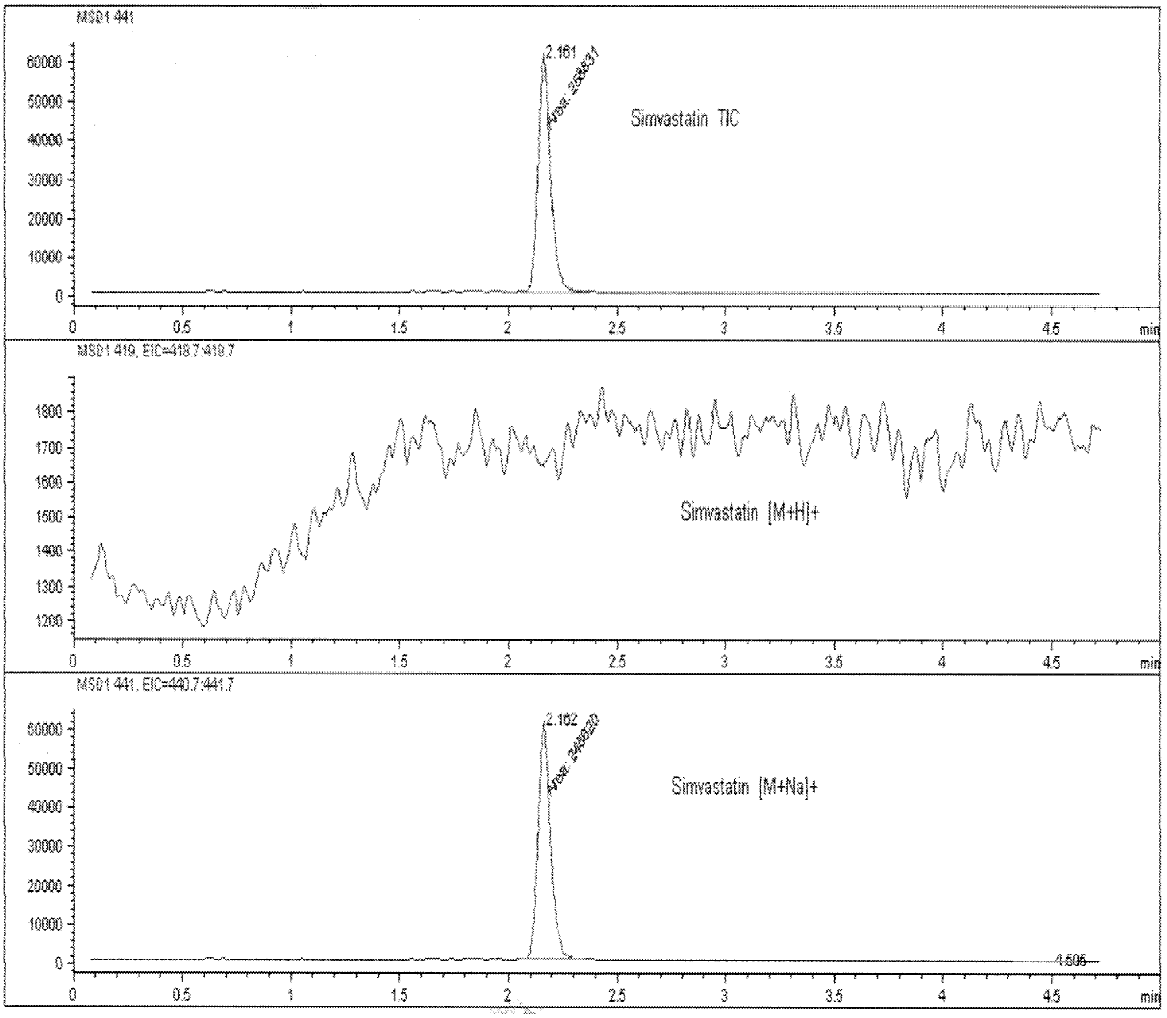


Fig. 2. Ion selection of Simvastatin.

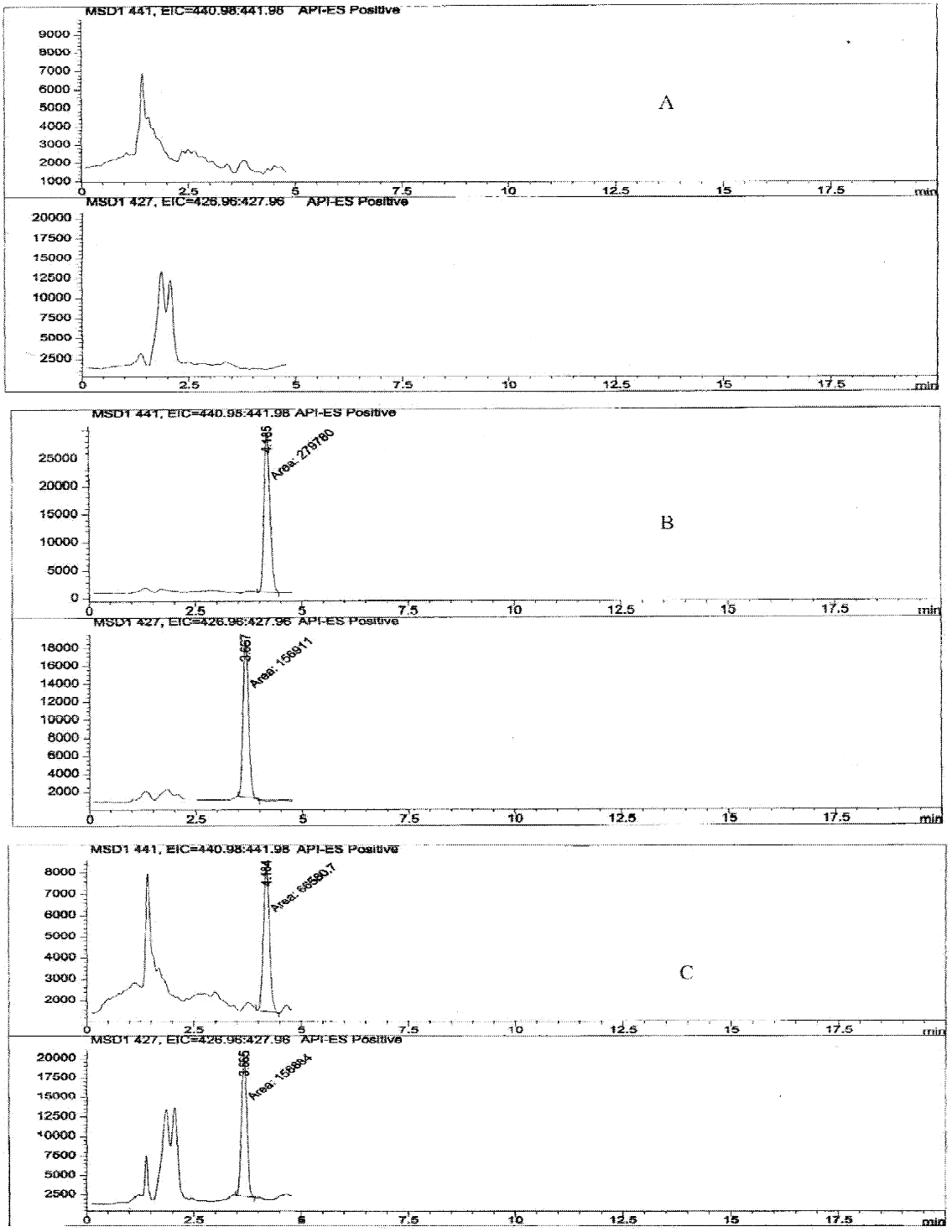


Fig. 3. Representative chromatograms of simvastatin in human plasma. (a) Blank human plasma; (b) plasma spiked with simvastatin and lovastatin; and (c) a sample from a volunteer 3 h after oral administration of two tablets of simvastatin.

Table 2
Intra-batch precision and accuracy for simvastatin ($n=6$)

| Normal conc. (ng/ml) | Calculated conc. (ng/ml) | RSD(%) |
|---|---|---|
| 0.1 | 0.1±0.1 | 8.5 |
| 5.0 | 4.4±0.1 | 3.2 |
| 10.0 | 9.3±0.2 | 1.8 |

both the drug and I.S., the chromatograms were free of interfering peaks at their respective retention times.

### 3.3. Linearity, precision and accuracy

Accuracy and precision for the assay were determined by extracting and assaying human plasma with simvastatin at 0.1, 5.0 and 10.0 ng/ml (each in six replicates).

Calibration curves were plotted as the peak area ratio (drug/I.S.) vs. drug concentration. Results for the calibration curve ($n=6$) showed good linearity ($r=0.9997$) over the concentration range of 0.1–20.0 ng/ml, with an equation of $y=0.17x-0.0324$ ($y=$I concentration in ng/ml; $x=$I area/II area)

Accuracy and precision for the assay were determined by calculating the intra-batch and inter-batch variation at three concentrations 0.1, 5.0 and 10.0 ng/ml in six replicates. As shown in Table 2, the intra-batch RSD was less than 10% and ranged from 1.8 to 8.5%, respectively. As shown in Table 3, the inter-batch RSD was less than 20% and ranged from 4.1 to 16.5%. These results indicate that the method was reliable within the analytical ranges and the use of the internal standard was very effective for

Table 3
Inter-batch precision and accuracy for simvastatin ($n=6$)

| Normal conc. (ng/ml) | Calculated conc. (ng/ml) | RSD(%) |
|---|---|---|
| 0.1 | 0.2±0.3 | 16.5 |
| 5.0 | 4.8±0.3 | 7.1 |
| 10.0 | 9.7±0.4 | 4.1 |

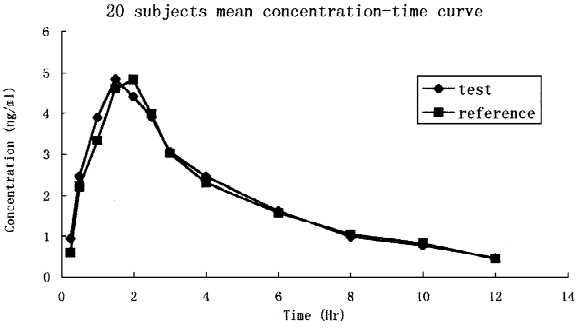


Fig. 4. Plasma concentration of simvastatin for two dosage forms.

reproducibility by LC–MS. The lower limit of detection, defined as a signal-to-noise ratio of 3, was reached at 0.05 ng/ml.

### 3.4. Recovery

The mean recovery of I from human plasma was 101.4±8.9% (range 96.3–107.7%). The recovery data reported here is the average for the three QC standards shown in Table 4.

### 3.5. Application to human subjects

Statistical analysis of I plasma samples was performed for a randomized, two treatment, cross-over study in which 20 healthy subjects between the ages of 20 and 40 years received 40 mg of Simvastatin. Fig. 4. shows a profile of mean plasma concentrations ($n=20$) of simvastatin, (test drug) and Zocor (reference drug, MSD, USA) vs. time. Quantifiable levels of I were detected for up to 12 h after each treatment. The maximum mean plasma concentration was 4.9–13.4 ng/ml for test and reference substances. The apparent elimination half-life was 3.6–3.5 h. No statistically significant difference was observed between the two drugs using a 90% confidence interval by two one-side test pro-

Table 4
Extraction efficiency of simvastatin ($n=6$)

| Added conc. (ng) | Measured conc. (ng) | Recovery (%) | RSD(%) |
|---|---|---|---|
| 0.503 | 0.54±8.5 | 107.7 | 15.2 |
| 5.03 | 4.84±3.7 | 96.3 | 5.7 |
| 10.06 | 10.09±3.4 | 100.3 | 6.7 |

cedures. The plasma concentrations were similar to those found earlier using a GC–MS method [2,4] and LC with fluorescence detection [7].

## 4. Conclusions

In conclusion, the use of LC–MS allows for accurate, precise and reliable measurement of simvastatin concentrations in human plasma for up to 12 h after oral administration of 40 mg to healthy volunteers. The assay has proven to be fast and simple, with each sample requiring less than 5 min of analysis time. The assay method is specific due to the inherent selectivity of mass spectrometry. The major advantage of this method is the sample preparation procedure, which is a simple extraction procedure method, and the rapidity of separation.

## References

[1] A.P. Mansur, C.V. Serrano Jr., J.C. Nicolau, L.A. Cesar, J.A. Ramires, Heart 82 (6) (1999) 689.
[2] M.J. Morris, J.D. Gilbert, J.Y. Hsieh, B.K. Matuszewski, H.G. Ramjit, W.F. Bayne, Biol. Mass Spectrom. 22 (1) (1993) 1.
[3] J. De Graaf, P.N. Demacker, A.F. Stalenhoef, Neth. J. Med. 43 (5-6) (1993) 254.
[4] C.H. Sun, S.M. Li, L. Liu, Chin. J. Pharmacol. 15 (1999) 431.
[5] L. Wang, M. Asgharnejad, J. Pharm. Biomed. Anal. 21 (6) (2000) 1243.
[6] G. Carlucci, P. Mazzeo, L. Biordi, M. Bologna, J. Pharm. Biomed. Anal. 10 (9) (1992) 693.
[7] H. Ochiai, N. Uchiyama, K. Imagaki, S. Hata, T. Kamei, J. Chromatogr. B Biomed. Sci. Appl. 694 (1) (1997) 211.
[8] S. Bala, G.C. Uniyal, Phytochem. Anal. 13 (4) (2002) 211.
[9] Y. Feng, L. Zhang, F. Wu, Z. Zhang, J. Chin. Pharm. Univ. 32 (1) (2001) 44.
[10] W. Li, J.F. Fitzloff, J. Pharm. Biomed. Anal. 2230 (1) (2002) 67.
[11] Y. Feng, L. Zhang, Z. Shen, J. Chromatogr. Sci. 40/1 (2002) 49.
[12] M. Sato, M. Hida, H. Nagase, Forensic Sci. Int. 128 (3) (2002) 146.
[13] B. Shao, J.Y. Hu, M. Yang, J. Chromatogr. A 950 (1–2) (2002) 167.
[14] R.A. Yokley, L.C. Mayer, S.B. Huang, J.D. Vargo, Anal. Chem. 74 (15) (2002) 3754.
[15] J. Henry, C. Zatylny, Peptides 23 (6) (2002) 1031.