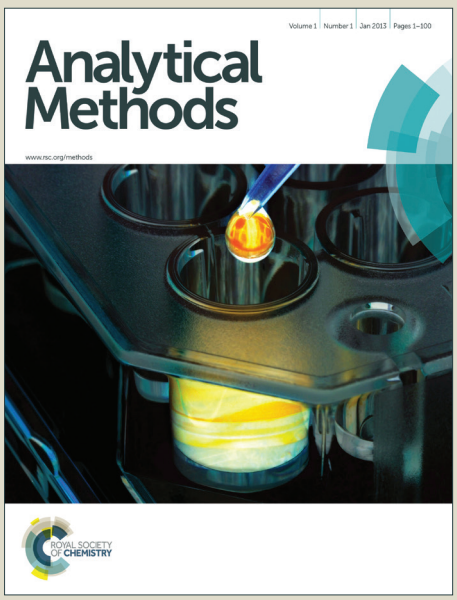
Analytical Methods

Accepted Manuscript



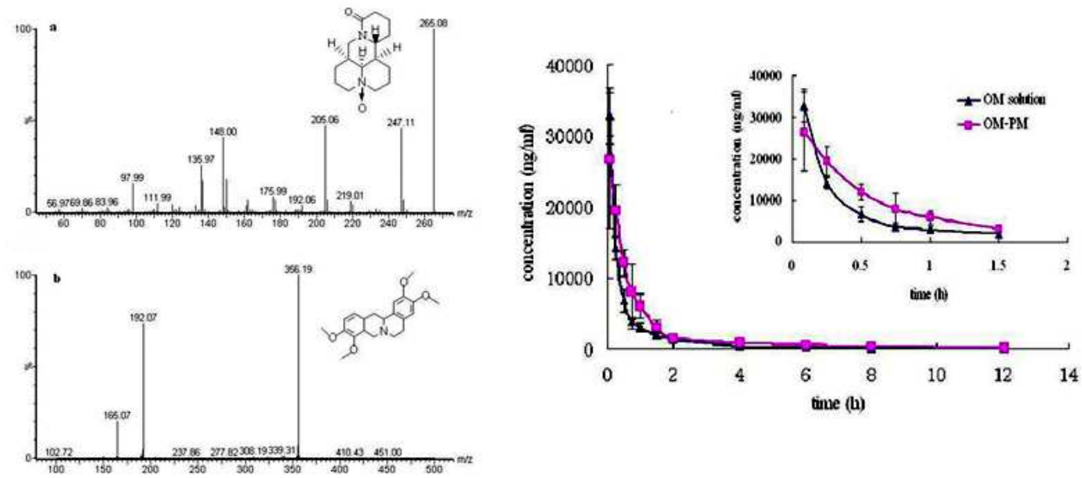
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

✎⑨♋⑦♑⓪♎♋③ ☛♎⑩⓿⑨♋♎⓿

Quantification of oxymatrine in rat plasma by UPLC-MS/MS to support pharmacokinetics of oxymatrine loaded polymersomes

Jianhong Yang [a, b], GuiDong Dai [b], Yanhui Hou [b], Zhihua Song [a], Fang Wang [c], Gangjian Ji [a], Jianhai Chen [a,*]



We developed a UPLC-MS/MS method that could efficiently extract oxymatrine (OM) from polymersomes and provided for the determination of OM in rat plasma to support pharmacokinetics study of OM-loaded polymersomes.

Full Length Article

Full Title

Quantification of oxymatrine in rat plasma by UPLC–MS/MS to support the pharmacokinetic analyses of oxymatrine-loaded polymersomes

Author:

Jianhong Yang [a, b], Guidong Dai [a], Yanhui Hou [a], Zhihua Song [b], Fang Wang [c], Gangjian Ji [b], Jianhai Chen [b,*]

Affiliation:

a: Department of Pharmaceutics, School of Pharmacy, Ningxia Medical University, No.1160 Shengli South Street, Yinchuan, 750004, P R China;

b: Department of Pharmaceutical Science, Nanfang Hospital, Southern Medical University, No.1838 Guangzhou Ave., Guangzhou, 510515, P R China;

c: Clinical Medicine School of Ningxia Medical University, No.804 Shengli South Street, Yinchuan, 750004, P R China;

*Corresponding Author:

Prof. Jianhai Chen Ph D

Department of Pharmaceutical Science, Nanfang Hospital, Southern Medical

University, No.1838 Guangzhou Ave., Guangzhou, 510515, P R China

Tel/Fax: +86-20-61642173; E-mail: jhchen06@126.com

Abstract

Oxymatrine (OM) is an alkaloid extracted from a Chinese herb that has been found to possess anti-hepatic fibrosis effects. To support a pharmacokinetic study for OM-loaded polymersome-based poly(ethylene glycol)-b-poly(ε-caprolactone) (mPEG-b-PCL) in rats, a rapid, highly selective ultra-performance liquid chromatography–tandem mass spectrometry method (UPLC–MS/MS) to quantify OM in rat plasma was developed and validated. Tetrahydropalmatine was employed as the internal standard (IS). Protein precipitation with acetonitrile was developed for preparation of plasma samples. OM and the IS were separated on an ACQUITY UPLC^{TM} BEH C18 column (2.1 mm × 50 mm i.d., 1.7 μm), with a gradient elution of acetonitrile and 0.1% formic acid (aq), within a run time of 2.5 min. Tandem mass detection was carried out electrospray ionization (ESI) in positive ion-selected multiple reaction monitoring (MRM) mode. The peak area of the *m/z* 265.20→148.07 transition of OM was evaluated *versus* that of the *m/z* 356.15→192.07 transition of the IS to generate the standard curve. In plasma, the linear range was 4–1000 ng mL^{-1}, with a lower limit of quantitation (LLOQ) of 4.0 ng mL^{-1} for OM using 100 μL of plasma. The intra- and inter-day precision (RSD) were <8.8%, and the relative accuracy (RE) was from –1.6% to 8.2%. The extraction recoveries were, on average, 79.92% for OM and 79.2% for the IS. Matrix effects and stability were investigated and found to be acceptable at all three concentration levels. This assay was applied to support a pharmacokinetic study of OM-loaded polymersomes in rats after intravenous administration.

Key words: UPLC–MS/MS; oxymatrine; polymersomes; tetrahydropalmatine; pharmacokinetic

1. Introduction

Oxymatrine (OM, Fig. 1a) is an alkaloid extracted from a traditional Chinese herb that is used for the treatment of infection by the hepatitis-B and -C viruses, and is known to be hepatoprotective [1, 2]. OM can relieve hepatic fibrosis or severe injury independently [3, 4]. However, if OM is administered parenterally, it has a short elimination half-life and poor distribution in the liver, resulting in low biological availability and some side-effects [5, 6]. To overcome some limitations of OM *in vivo*, several drug delivery systems (DDS), namely liposomes, polymeric micelles, dendrimers and nanoparticles, have been employed.

Polymersomes (PM) are self-assembled polymeric vesicles. They have received great interest in recent years for their potential applications as a novel class of nanocarriers in DDS [7]. OM has been successfully loaded into polymersomes based on diblock copolymers of methoxy poly(ethylene glycol) (mPEG) and ε-caprolactone (ε-CL).

Pharmacokinetic studies of delivery of OM using PEG-b-PCL polymersomes are critical because they provide forecasts, rational utilization, as well as mechanisms of enhancing antifibrosis efficacy.

Several methods have been described for the quantification of OM in biological samples: gas chromatography-mass spectrometry (GC–MS) [8], high-performance capillary electrophoresis (HPCE) [9] and high-performance liquid chromatography with ultraviolet detection HPLC/UV [10, 11]. However, these methods have some disadvantages: lack of quantitative precision and reproducibility, complicated sample

pretreatment, or low sensitivity of OM in pharmacokinetic studies [12, 13].

In recent years, there have been a few reports about the quantification of OM in biological samples utilising liquid chromatography-mass spectrometry (LC–MS) methods. Liquid–liquid extraction (LLE) of samples by chloroform is time-consuming in addition to the requirements of large plasma volume and extraction solvent [12-14]. The method reported by Xiaoluan et al. [15] involved protein precipitation with a large volume of methanol for sample preparation and long chromatographic run times. In addition, compared with conventional high-performance liquid chromatography–mass spectrometry (HPLC–MS), ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) produces obvious improvements in sensitivity, speed and resolution. Moreover, those LC-MS methods have been described only for the determination of free OM in biological samples, whereas the method for OM-loaded nanocarriers has not.

The aim of the present study was to develop a method that could efficiently extract OM from polymersomes and determine OM in rat plasma to support a pharmacokinetic study of OM-loaded polymersomes. Here, a rapid, specific, highly sensitive, and validated UPLC–MS/MS method was developed for the quantification of OM in rat plasma. This is the first study focusing on the *in vivo* pharmacokinetics of OM-loaded polymersomes.

2. Experimental

2.1 Materials and animals

OM was obtained from Zi Jinhua Pharmaceuticals (Ningxia, China). The internal standard (IS) tetrahydropalmatine (98% purity, Fig. 1b) was ordered from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile and formic acid were obtained from Dikma (Richmond Hill, NY, USA). Deionized water was from the Barnstead EASYpure® IIRF/UV Ultrapure Water system (Dubuque, IA, USA). All other reagents were of analytical or chromatographic grade. Deionized water, solvent and samples were passed through a 0.22-μm filter before use in UPLC–MS/MS analyses.

The animals used for experimentation were treated according to protocols that had been evaluated and approved by the ethical committee of Shenyang Pharmaceutical University (Shenyang, China).

Male Sprague–Dawley rats (220–240 g) were from Shenyang Laboratory Animal Center (Shenyang, China). Rats were housed in a controlled environment (temperature, 25±3°C; relative humidity, 45–60%) with a 12-h light–dark cycle and fasted for 12 h before experimentation but had free access to water.

2.2 Preparation of OM-loaded polymersomes

Polymersomes based on poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL) were prepared employing the film hydration method as reported by Discher et al. [16]. OM was loaded into polymersomes using a pH-gradient method

developed for liposomes [17].

2.3 Preparation of standard and quality-control (QC) samples

Stock solutions of OM and IS were prepared at 5 and 2 μg mL^{-1} in methanol, respectively. For OM, further dilutions were carried out with methanol-water (1/1, *v/v*) to make a set of working solution ranging from 20 to 5000 ng mL^{-1}. A working solution of the IS (98 ng mL^{-1}) was also prepared by diluting the stock solution with methanol-water (1/1, *v/v*). All solutions were stored at –20°C. Blank plasma samples should be defined as plasma samples lacking analyte and IS. Calibration standard and QC samples should be defined as fortified plasma standard and fortified plasma QC samples. The standard solutions (20 μL) were used to spike 100 μL of blank plasma samples either for calibration standard and QC samples, both in the preliminary study and during the pharmacokinetic study. The calibration samples for OM were 4, 10, 20, 40, 100, 200, 400 and 1000 ng mL^{-1}. QC samples were prepared in the same way at concentrations of 4, 10 and 100 and 800 ng mL^{-1} and represented the lower limit of quantification (LLOQ), low, medium and high concentrations, respectively.

2.4 Preparation of plasma samples

Plasma was collected with heparin sodium from Male Sprague-Dawley rats and stored at −20 °C until use. To 100 μL of plasma sample (or a calibration standard or QC sample) were added 20 μL of IS working solution (98 ng mL^{-1}) and 350 μL of acetonitrile. The mixture was vortex-mixed at 3000 rpm for 3 min and centrifuged at

15,000 rpm for 10 min at room temperature. An aliquot of the supernatant (250 µL) was transferred into a clean tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was reconstituted with 100 µL of acetonitrile: 0.1% formic acid (aq) (90: 10, v: v) and vortex-mixed at 1200 rpm for 3 min. After centrifugation at 15,000 rpm for 10 min at room temperature, the supernatant was transferred into an autosampler vial. Then, 5-µL each of the samples prepared above were injected into the UPLC-MS/MS system for analyses.

2.5 UPLC–MS/MS

Chromatography was conducted on an ACQUITY™ UPLC system (Waters, Milford, MA, USA) maintained at 4°C. Separation was achieved on an ACQUITY UPLC™ BEH C18 column (50 × 2.1 mm i.d., 1.7 µm; Waters) using gradient elution. The mobile phase was composed of solvent A (0.1% formic acid–water) and solvent B (acetonitrile) at a flow rate of 0.2 mL/min. The gradient elution was: 0 min, 90% B; 0–0.9 min, linear from 90% to 30% B; 0.9–1.6 min, holding at 30% B for 0.7 min and then an immediate reduction to 90% B at 1.7 min; 1.7–2.5 min for re-equilibration of the column. The column temperature was maintained at 40°C and the injection volume was 5 µL.

A Waters ACQUITYTM TQD triple quadrupole tandem mass spectrometer (Waters Corp, Manchester, UK) with an electrospray ionization (ESI) interface was used for mass analysis. The ESI source was operated in positive ionization mode for OM and IS. The optimal parameters for the ionization source were: capillary voltage,

3.0 kV; cone voltage, 40 V; source temperature, 100°C; desolvation temperature, 400°C. The optimized collision energy was 26 and 25 V for OM and IS, respectively. The cone and desolvation gas flow rates were 50 and 500 L h^{-1}, respectively. Argon was used as the collision gas at a flow rate of 0.24 mL min^{-1}. Multiple reaction monitoring (MRM) was used to monitor the fragmentation transitions of *m/z* 265→148 for OM and *m/z* 356→192 for IS, respectively, with a scan time of 0.2 s. All data were aquired using Masslynx^{TM} ver4.1 (Waters).

2.6 Method validation

Method validation was undertaken according to guidelines set by the Food and Drug administration [18]. The validation parameters were selectivity, linearity, LLOQ, accuracy, precision, recovery, matrix effect, and stability.

The selectivity of the method was observed by analyzing blank plasma from 6 rats, QC plasma samples, and plasma samples after intravenous administration. Chromatograms were assessed to quantify the presence of endogenous constituents that could potentially interfere with the analysis of OM and IS. To assess linearity, calibration curves were prepared using eight standard plasma samples in the range from 4 to 1000 ng mL^{-1}. The LLOQ was defined as the lowest concentration on the standard curve that can be detected with acceptable accuracy and precision (six replicates with a relative standard deviation (RE) below 20% and relative error (RSD) within ±20%). Intra- and inter-run precision and accuracy were determined by analyzing QC samples using six replicates at three concentrations (10, 100 and 800 ng

mL^{-1}) on 3 validation days. Precision was calculated as the RSD within a single run and between different runs. Accuracy was expressed by RE, i.e. (determined concentration – nominal concentration)/(nominal concentration) × 100. The extraction recoveries of OM were evaluated by comparing the peak areas of six replicates of blank plasma samples spiked with QC samples at three levels (10, 100 and 800 ng mL^{-1}) before extraction with those of blank plasma samples spiked with the corresponding QC samples after extraction. The recovery of the IS was also assessed in a similar fashion at 98 ng mL^{-1}. To evaluate the matrix effect of the ionization of analyte, OM at three concentrations (10, 100 and 800 ng/mL) was added to the extract of 100 µL blank plasma sample six different lots, dried and reconstituted with 100 µL of acetonitrile: 0.1% formic acid (aq) (90: 10, v: v). The corresponding peak areas (A) were compared with those of the OM standard solutions dried directly and reconstituted with the same volume of mobile phase (B). The ratio (A/B×100) % was used to evaluate the matrix effect. The matrix effect of the IS was evaluated using the same procedure.

The stability of sample storage (at room temperature for 4 h, at –20°C for 14 days), processing (three freeze–thaw cycles) and for 12 h in processed samples at 4°C in auto-sampler trays were determined by analyzing three replicate QC samples (at 10 and 100 and 800 ng mL^{-1}).

2.7 Pharmacokinetic experiments

Twelve male SD rats were divided randomly into two groups. One group was

given a single dose of OM solution (30 mg/kg) *via* the tail vein, whereas the other rats were given a single dose of OM-loaded polymersomes at the same dose (both preparations contained 3 mg mL^{-1} OM in solution or polymersomes). At 0.083, 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 8, and 12 h after dosing, blood samples (0.3 mL) were collected from the retro-orbital sinus, transferred to heparinized tubes (1.5 mL), and inverted slowly several times to ensure full blending with the anticoagulant. Blood samples were centrifuged at 4000 rpm for 10 min at 4±2°C to separate plasma and stored at −20 °C until analyses.

3. Results and Discussion

3.1 IS and extraction solvent

A stable isotope-labeled analyte is the optimal IS for an UPLC–MS/MS quantification assay to correct for unknown losses during the procedure. Achieving a reference standard that exhibits a high response and rapid elution under the same UPLC–MS/MS conditions as the analytes is difficult. Some candidate compounds (e.g., theocin, ephedrine and caffeine that are much closer in structure to the analytes) may be used as the IS. However, these compounds were found to be unsatisfactory for very low extraction recovery or poor ionization under the specific conditions [15]. Finally, tetrahydropalmatine was chosen as the IS because of its similar ESI ionization response, appropriate chromatographic retention time and extraction recovery to that of OM.

The reported extraction methods of OM from biological matrices include LLE and protein precipitation (PPT). These LLEs by chloroform needed samples and

chloroform with large volumes, and involved tedious preparation of samples [12-14]. A plasma sample of 0.25 mL was processed using PPT by the addition of 0.75 mL methanol and the chromatographic run time was 6.0 min [15]. With regard to determination of drugs entrapped in polymersomes, completely dissociating the drug from polymersomes with an extraction solvent was very important. With dissolution of mPEG-PCL, the structural integrity of the polymersomes was destroyed, and OM release ensued.

In the present study, several organic solvents were assessed for their suitability for the precipitation of protein and extraction of analytes from rat plasma. These solvents were methanol, methanol-ethyl acetate (1:2, *v/v*) and acetonitrile, and their volumes were 1–5-times that of the biological sample. The results showed that the highest extraction recovery was obtained when acetonitrile was used at a ratio of 1/3 (*v/v*) with plasma. Acetonitrile was employed concurrently for precipitation and as an emulsion-breaking agent compared with the conventional emulsion-breaking agent Triton X-100 [19]. We did not require addition of other agents that could have interfered with determination of the analytes. This method was similar to the methanol precipitation–ultrasonic emulsion breaking of polymer micelles in rat plasma described previously-[20]..

3.2 Chromatography and mass spectra

MRM was set for the detection of OM and IS. To obtain the most intense protonated molecular ions under positive ionization, the parameters for fragmentor

voltages and collision energies were optimized. The MRM transition of OM and IS was determined to be *m/z* 265.1→148.1 and 356.2→192.1, respectively (Fig. 1).

Insert Fig. 1 Here

Chromatographic separation was carried out by gradient elution, which can lead to an excellent peak shape, short analysis time, and strong response. To obtain an optimized response, satisfactory retention time and good peak shapes for the analytes, various mobile-phase additives at various concentrations and ratios were investigated: ammonium acetate, acetic acid and formic acid. Finally, acetonitrile and 0.1% formic acid (aq) were selected to obtain satisfactory ionization conditions and chromatographic run times.

3.3 Method validation

3.3.1 Selectivity

Typical MRM chromatograms obtained from blank plasma, blank plasma spiked with OM and the IS, and a rat plasma sample after intravenous administration of OM-loaded polymersomes are presented in Fig. 2. The retention times of OM and the IS were approximately 1.12 and 1.67 min, respectively, and the overall chromatographic run time was 2.5 min. There were no interfering peaks observed at the retention times of the analyte or IS. As presented in Fig. 3, chromatograms were a blank plasma sample after the upper limit of quantitation (ULOQ) and a blank plasma sample spiked with analyte at the LLOQ and IS.

Insert Fig. 2 Here

Insert Fig. 3 Here

3.3.2 Linearity and LLOQ

Linear responses were obtained for OM ranging from 4 to 1000 ng mL^{-1}. Best-fit calibration lines of the ratio of OM to IS peak area versus the concentration of calibration standards were quantified by least-squares regression analysis with weighting factors of $1/x^2$. The typical equation for the calibration curve and its correlation coefficient was:

$y = 2.01\times10^{-3}x + 4.07\times10^{-2}$ ($r^2 = 0.9959$)

where y refers to the peak area ratio of OM to the IS, and x refers to the plasma concentration of OM in serum (ng mL^{-1}). The LLOQ was set at 4.0 ng mL^{-1}. The precision and accuracy at this concentration were acceptable, with a RSD of <14.1% and with a RE of <13.5%.

3.3.3 Precision and accuracy

Intra-day and inter-day precision and accuracy for OM were calculated by measuring QC samples. They were within the acceptable range of ±15%, indicating

that the method was accurate and precise (Table 1). Intra-day and inter-day precision for IS were 11.46% and 12.92%, respectively.

Insert Table 1 Here

3.3.4 Extraction recovery and matrix effects

The extraction recoveries of OM determined using six replicates of QC samples at 10, 100 and 800 ng/mL were calculated to be 77.25±3.7, 80.58±5.28 and 81.94±4.48%, respectively. The mean recovery of the IS was 79.12±6.72%. The matrix effects were found to be 94.78±2.21, 93.56±3.44 and 101.1±2.46% for OM at three concentrations and 94.77±3.59% for the IS. There was no significant matrix effect on the analyte and IS in this method.

3.3.5 Stability

The stability of OM in rat plasma under different conditions is presented in Table 2. These results indicated that OM was stable in plasma at room temperature for 4 h, at −20 °C for 14 days, at 4°C in an autosampler for 12 h, and in processed samples after three freeze–thaw cycles.

Insert Table 2 Here

3.4 Application

The developed method was employed successfully to the pharmacokinetic study of OM solution and OM-loaded polymersomes in rat plasma after intravenous administration at an equal dose of 30 mg/kg OM. The curves of the plasma concentrations of OM for the two formulations are shown in Fig. 4. The pharmacokinetic parameters (mean ± SD, n=6) were summarized. For OM solutions, they were: area under the curve (AUC) 0–12 h, 16234.27±1385.34 ng.h.mL^{-1}; C_{max}, 32847.57±4041.06 ng.mL^{-1}; T1/2z, 1.41±0.57 h; CL, 3.10±0.27 L.h^{-1}. For OM-loaded polymersome formulations, they were: AUC 0–12 h, 30602.95±3982.03 ng.h.mL^{-1}; C_{max}, 26607.631±9599.203 ng.mL^{-1}; T1/2z, 3.12±0.25; CL, 1.62±0.2 L h^{-1}.

Insert Fig 4 Here

4. Conclusion

The UPLC–MS/MS method described here for OM determination in rat plasma is the first procedure for quantitative determination of OM in the presence of OM polymersomes. In plasma, the LLOQ was 4 ng mL^{-1}, and the assay was linear over a concentration range of 4–1000 ng mL^{-1}. In addition, the method required only 100 µL of plasma for OM determination and the total run time was only 2.5 min per sample. Overall, a validated rapid, selective, highly sensitive, and reliable UPLC–MS/MS method was developed and applied to study the pharmacokinetic profiles of OM-loaded polymersomes in rats.

Acknowledgements

We thank Professor Xing Tang and Zhonggui He (Shenyang Pharmaceutical University, China) for useful instructions, as well as the National Natural Science Foundation of China (81260645) and the Science and Technology Program of Ningxia Province (201087) for financially supporting this work.

References

1. L.G. Lu, M.D. Zeng, Y.M. Mao, J.Q. Li, M.B. Wan, C.Z. Li, C.W. Chen, Q.C. Fu, J.Y. Wang, W.M. She, X. Cai, J. Ye, X.Q. Zhou, H. Wang, S.M. Wu, M.F. Tang, J.S. Zhu, W.X. Chen, H.Q. Zhang. *World. J. Gastroenterol.*, 2003, **9**, 2480-2483.
2. J.Q. Li, C.Q. Li, M.D. Zeng, J.G. Fan, J. Hua, D.K. Qiu, S.D. Xiao. *Chin. J. Integr Med.*, 1998, **18,** 227-229.
3. Y.C. Jian, W. L., Y. He, M. Jiang, Y.B. Liu, W.J. Xiong. *Chin. J. Integr Med.*, ,2012, **18**, 445-450.
4. Y.M. Mao, M.D. Zeng, L.G. Lu, M.B. Wan, C.Z. Li, C.W. Chen, Q.C. Fu, J.Y. Wang, W.M.She, X. Cai, J. Ye, X.Q. Zhou, H. Wang, S.M. Wu, M.F. Tang, J.S. Zhu, W.X. Chen, H.Q. Zhang. *World. J. Gastroenterol.*, 2004, **10**, 3269-3273.
5. Y. Wang, G.D.L. Meng, l. W.Y. Zheng, Q.D. Liu. *Chin. J. Clin Pharmacol.*, 2003, **19**, 301-305.
6. C.S. Wu, X.X. Piao, D.M. Piao, Y.R. Jin, C.H. Li. *World. J. Gastroenterol.*, 2005, **11**, 122-126.
7. K. K. Upadhyay, H.G. Agrawal, C. Upadhyay, C. Schatz, J.F. Le Meins, A. Misra,

S. Lecommandoux. *Crit Rev. Ther Drug Carrier Syst.*, 2009, **26**, 157-205.

8. D.S.T. Sit, G.h. Gao, F.C.P. Lawa, P.C.H. Li. *J. Chromatography. B.*, 2004, **808**, 209-214.

9. Y.R. Ku, L.Y. Chang, J.H. Lin , L.K. Ho. *J. Pharm. Biomed. Anal.*, 2002, **28**, 1005–1010.

10. X.N. Wu, F. Yamashita, M. Hashida, X. Chen, Z. Hu. *Talanta.*, 2003, 59, 965-971.

11. G.D.L. Meng, Y.Wang, Q.F.Q. Jia. *Chin. J. Pharm Anal.*, 2003, **23**, 440-443.

12. S. Wang, G. Wang, X. Li, J. Sun, R. Ma, L. Sheng. *J. Chromatography. B.*, 2005, **817**, 319-325.

13. Y. Wang, Y. Ma, X. Li, F. Qin, X. Lu, F. Li. *Biomed. Chromatogr.*, 2007, **21**, 876-882.

14. W. Zhang, B.R. Xiang, P.C. Ma. *J. Chromatogr. SCI.*, 2008, **46**, 529-533.

15. X.L. Wu, T.J. Hang, J.P. Shen, Y.D. Zhang. *J. Pharm. Biomed. Anal.*, 2006, **41**, 918-924.

16. J.C.M. Lee, H. Bermudez, B.M. Discher, M.A. Sheehan, Y.Y. Won, F.S. Bates, D.E. Discher. *Biotechnol Bioeng.*, 2001, **73**, 135-145.

17. P.R. Harrigan, K.F. Wong, T.E. Redelmeier, J.J. Wheeler, P.R. Cullis. *Biochim. Biophys. Acta.*, 1993, **1149**, 329-338.

18. Guidance for Industry, Bioanalytical Method Validation, USFDA, http://www.fda.gov/cder/guidance/4252fnl.pdf, 2001

19. K. Pang, W. Gong, X.G. Wang, R.J. Zheng, C.Y. Zeng, R. Yao, X.G. Mei. *Asian J Pharm Sci.*, 2011, **6**, 36-42.

20. W. Zhang, J. Sun, Z. Feng, X. Su, M. Zhu, X. Sui, Y. Liu, M. Tao, Y. Sun, G. Chen, Z. He, *Chromatographia*, 2011, **74**, 333-340.

Table 1 Precision and accuracy data for the determination of oxymatrine in rat plasma samples, ((n = 3 days, six replicates per day).

Spiked concentration (ng/mL)	Average measured concentration (ng/mL)	RSD (%)	RE (%)
Intra-day of oxmatrine			
4	4.38	11.8	9.7
10	10.48	8.8	4.8
100	107.45	5.9	7.4
800	865.69	7.6	8.2
Inter-day of oxmatrine			
4	4.52	14.1	13.5
10	10.26	6.0	2.5
100	98.41	5.2	-1.6
800	842.52	4.8	5.3

Table 2 Stabilities of oxymatrine in rat plasma (n = 6).

stability	Spiked concentration (ng/mL)	Observed mean concentration (ng/mL)	RSD (%)	RE (%)
Short-term stability (at room temperature, 4 h)	10	10.56 ±1.14	10.79	5.65
	100	103.23±12.50	12.11	3.23
	800	756.24±54.12	7.16	-5.47
4 °C in autosampler for 12 h in processed samples	10	10.37±1.39	13.45	3.69
	100	97.05±7.88	8.12	-2.95
	800	819.47±81.32	9.92	2.43
Three freeze-thaw cycles (−20 °C to room temperature)	10	9.52±1.26	13.26	-4.75
	100	96.21±9.81	10.19	-3.79
	800	742.47±74.99	10.10	-7.19
Long-term stability (− 20 °C, 14 days)	10	9.31±1.06	11.43	-6.87
	100	91.14±8.15	8.94	-8.86
	800	762.14±44.22	5.80	-4.73

Figure legends

Fig. 1. Product ion mass spectra of $[M+H]^+$ ions of oxymatrine (a) and tetrahydropalmatine (b).

Fig. 2. Representative MRM chromatograms of oxymatrine and tetrahydropalmatine (IS) in rat plasmas: (a) blank plasma; (b) blank plasma spiked with oxymatrine (100 ng mL^{-1}) and IS (98 ng mL^{-1}); (c) the plasma sample collected 4 h after intravenous administration of 30 mg kg^{-1} oxymatrine. The retention time for oxymatrine and IS was 1.12 min and 1.67 min, respectively.

Fig. 3. Chromatograms for OM and IS in rat plasma samples: (A) a blank sample run right after the upper limit of quantitation (ULOQ, 1000 ng/mL); (B) a blank plasma sample spiked with analyte at the LLOQ and IS (98 ng/mL).

Fig. 4. Plasma oxymatrine concentration–time profiles following intravenous injection of oxymatrine solution (▲) and oxymatrine-loaded polymersomes (■) in rats. The dose for two formulations was 30 mg kg^{-1} of oxymatrine. Each time point represents mean ± SD (n = 6).

Fig.1

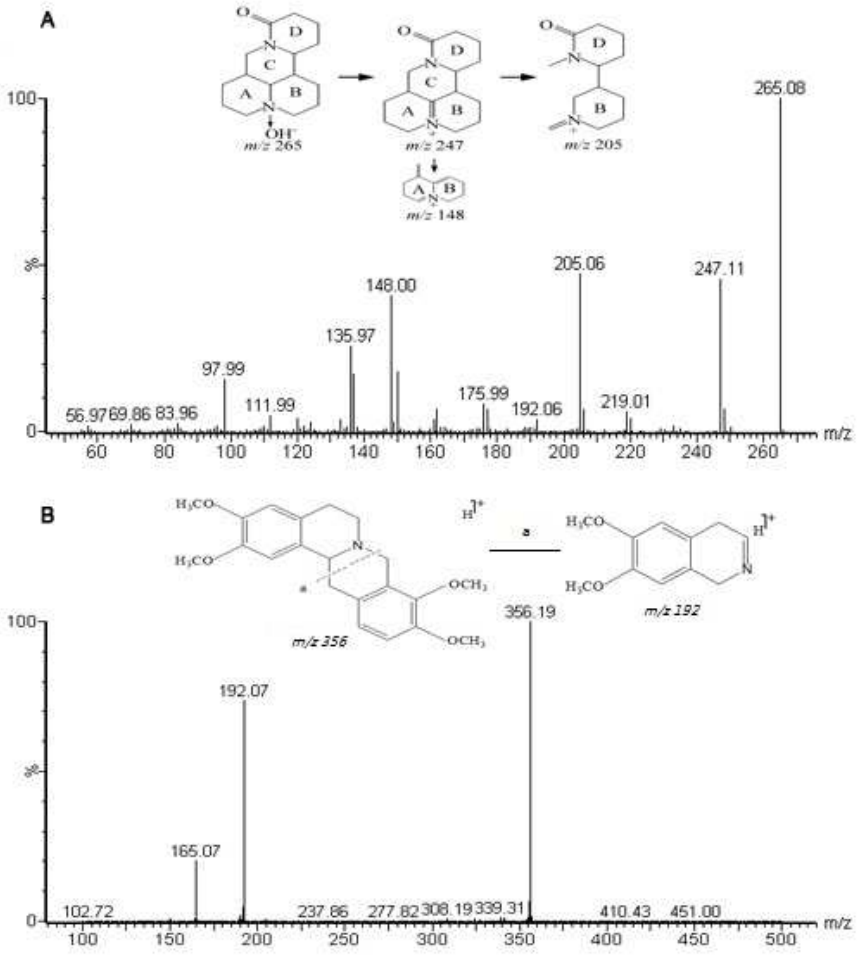


Fig.2

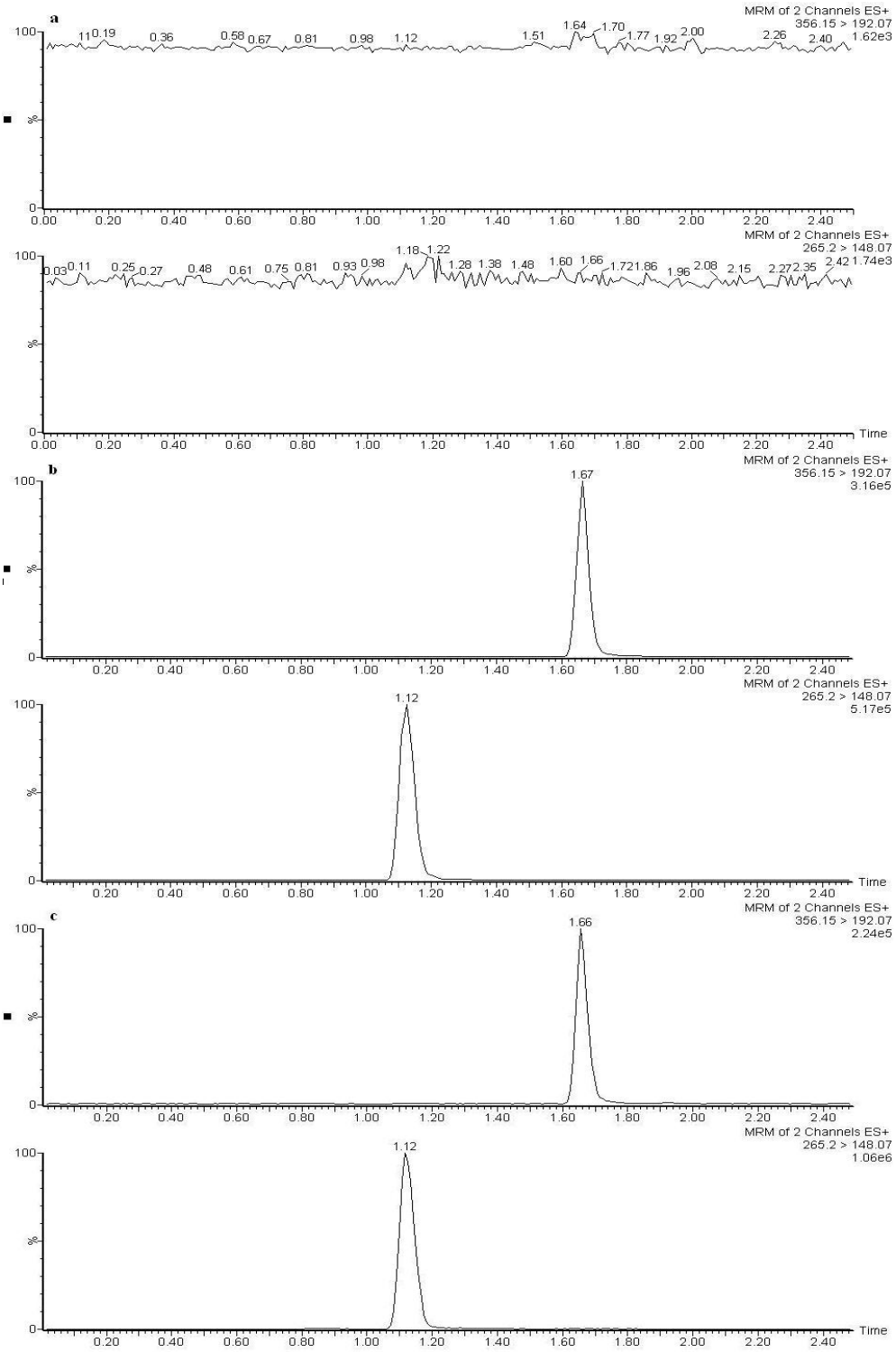


Fig.3

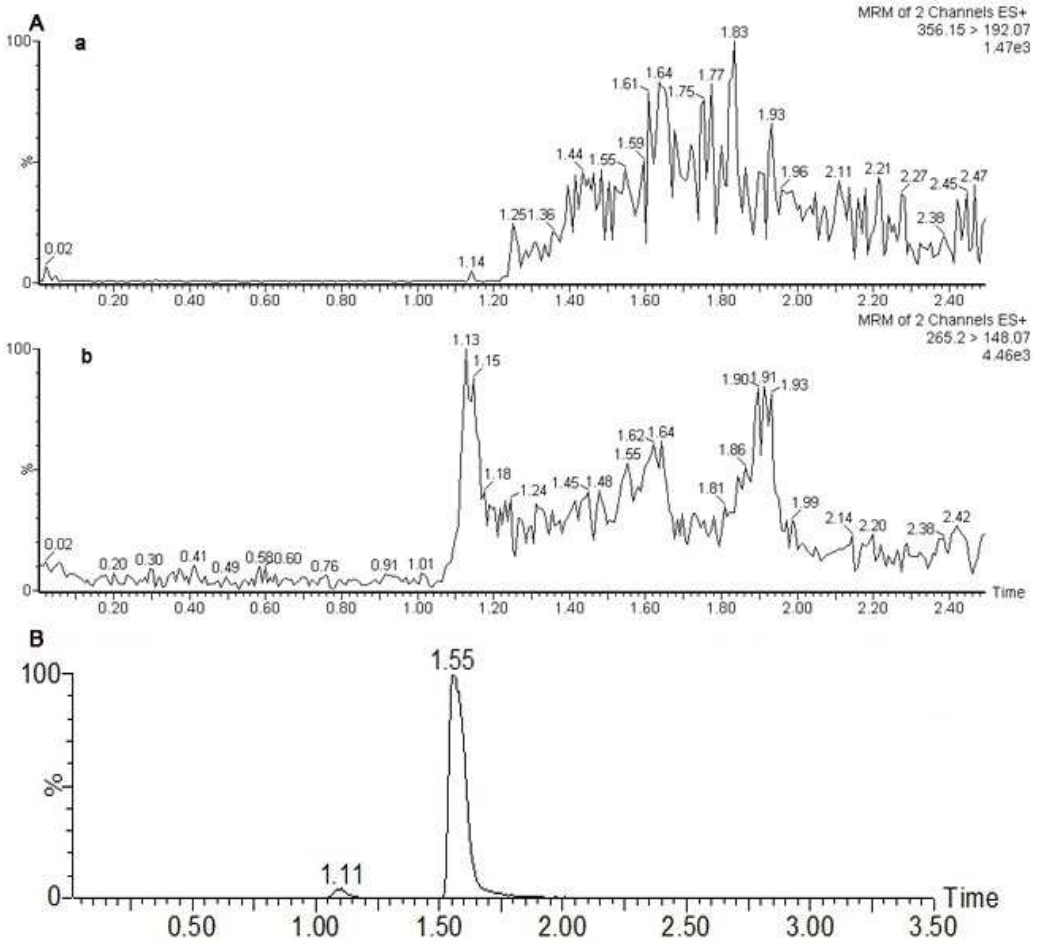


Fig.4