Preparation of a Novel Glycidyl Methacrylate-Based Monolith and Its Application for the Determination of *m*-Nisoldipine in Human Plasma

Qiaoxia Zhang, Gengliang Yang*, Haiyan Liu, Jing Yang, Yongna Yan, and **Ligai Bai**
Key Laboratory for Drug Quality Control and Analysis of Hebei Province, College of Pharmacy, Hebei University, Baoding 071002, China

Abstract

A new method has been developed for preparing monolithic materials by polymerisation of the oil-in-water (O/W) emulsions with nonion-surfactant (Pluronic F68). Morphology of monolithic materials is studied by scanning electron microscopy. The properties of the column are investigated, and the column exhibits the ability of low backpressure and fast analysis. Using this monolithic column, on-line sample clean-up and screening of *m*-nisoldipine in human plasma samples have been investigated. Chromatography is performed by reversed-phase high-performance liquid chromatography (RP-HPLC) on a C_{18} column with UV detection at 237 nm. The linear range of *m*-nisoldipine in human plasma is 2–200 ng/mL (r = 0.9992, n = 7). And the limit of detection is 1.5 ng/mL. The 12-h pharmacokinetic profile of m-nisoldipine in mice after oral administration has been investigated. The results indicate that the method could be used for monitoring of *m*-nisoldipine and enabled simple and rapid assay of the drugs in human plasma.

Introduction

Nowadays polymer monolithic columns have held an impressively strong position and have been used in preparative purification (1–3) and on-line solid-phase extraction (SPE) (4,5). The size of the skeleton and the distribution of the pores of monolithic materials offer the possibility of developing efficient separation at higher flow rates due to the low pressure-drop across the column (6,7). Herein, methacrylate-based monoliths are applied in a variety of shapes and separation modes for the purification of large molecules like proteins, polynucleotides, or even viruses (8). The common method of preparing methacrylate monoliths is via bulk polymerization in the presence of porogenic solvents. Preparation of poly(glycidyl methacrylate) grafted polymerization of high internal phase emulsion (PolyHIPE) material for separation has been recently reported (9–11).

m-Nisoldipine (Figure 1) is a new dihydropyridine calcium ion antagonist (12). It is stable in solid form when exposed to light (13). *m*-Nisoldipine increases cardiac output and cardiac index significantly. The negative inotropic effect of *m*-nisoldipine on myocardium was dramatically less potent than that of nisoldipine. As a result, *m*-nisoldipine has relatively higher selectivity on the thoracic aorta than nisoldipine (14).

Analytic methods for the determination of m-nisoldipine in biologic samples were developed, mainly on the basis of liquid chromatography (LC) (15–17). A liquid chromatography–tandem mass spectrometric (LC–MS–MS) method (18) has also been developed to determine *m*-nisoldipine in rat plasma. The concentrations of these compounds in human plasma are relatively low as a consequence of their high pharmacological potency. These methods have mostly used liquid–liquid extraction with organic solvents after alkalization of the plasma to precipitate proteins, followed by evaporation of the organic supernatant. These conventional procedures are complicated and time-consuming; in addition, they are not environmentally friendly, and the addition of an internal standard is necessary. On-line SPE can solve these problems. On-line SPE is a clean, fast, efficient, and sensitive trace-level determination pretreatment procedure. It is particularly attractive because it allows the simultaneous removal of matrix compounds and preconcentration of the analytes (19).

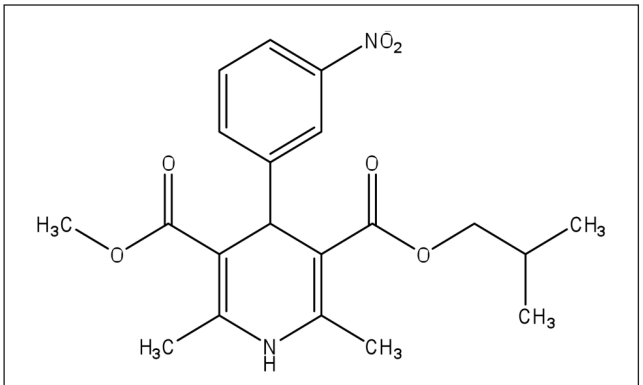

Figure 1. Chemical structure of *m*-nisoldipine.

*Author to whom correspondence should be addressed: email ygl@hbu.edu.cn.

In this work, an alternative method has been developed for preparing monolithic materials by polymerization of the oil-in-water (O/W) emulsions. The properties of the prepared column were investigated, and the column exhibited the ability of low backpressure and fast analysis. The monolithic column was developed and validated for the determination of *m*-nisoldipine in human plasma for on-line sample clean-up coupled to a HPLC–UV system. The potential of the method for performing pharmacokinetic studies was also demonstrated.

Experimental

Reagents and materials

Glycidyl methacrylate (GMA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ethylene glycol dimethacrylate (EDMA) was purchased from Acros (Morris Plains, New Jersey). Pluronic F68 (PF68) was obtained from Shenzhen Youpuhui Chemical Co., Ltd. (Shenzhen, China). Polyethylene glycol 400 was purchased from Beijing Huaboyuan Science and Technology Development Center (Beijing, China). Potassium persulfate was purchased from Huadong Chemical Reagents Factory (Tianjin, China). Anhydrous calcium chloride was purchased from Tianjin Beifangtianyi Chemical Reagents Factory (Tianjin, China). *m*-Nisoldipine was obtained from Hebei Medical University. Methanol of analytical grade was obtained from Kermel Chemical Co., Ltd. (Tianjin, China). Water was purified by a Millipore system. All solutions were filtered through 0.45-µm membrane filters (Millipore) before use.

Human plasma, donated by healthy volunteers, was centrifuged at 4500 rpm for 10 min, then stored below –18°C before use. The institutional review board (Hospital of Hebei University) approval was required and obtained, regulating the acquisition of plasma from human subjects. In all of the cases, an informed consent was obtained from each person.

Instrument and chromatography

Chromatography was performed with a PU-1580 pump and a variable-wave length UV-1570 detector (Jasco, Japan). Data processing was performed with an HW-2000 chromatography workstation (Nanjing Qianpu Software, China). Chromatographic separation was achieved on a C_{18} Diamonsil column (250 × 4.6 mm i.d., 5 µm, Dikma, NY) maintained at ambient temperature (25°C). The analytical mobile phase was methanol–water (90:10, v/v) at a flow rate of 1.0 mL/min. The chromatogram was monitored at a wavelength of 237 nm, the maximum absorption wavelength of *m*-nisoldipine.

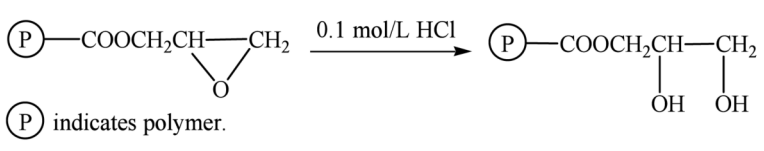

Figure 2. The preparation process of the monolith with diol groups.

Standard solutions

First, m-nisoldipine was dissolved with methanol to prepare working solutions of concentrations: 1, 2.5, 5, 10, 25, 50, 100 µg/mL. These working solutions were then diluted with blank human plasma to obtain standard solutions with concentration of 2, 5, 10, 20, 50, 100, and 200 ng/mL, respectively. Quality control samples at concentrations of 2, 50, and 200 ng/mL were prepared for the evaluation of precision, accuracy, and recovery in analysis of plasma samples.

Preparation and characterization of the monoliths

First, 0.5 mL of monomers (GMA), 0.6 mL of cross-linker (EDMA), and 0.9 mL of polyethylene glycol 400 were placed in a round-bottomed flask, and the mixture was stirred with an overhead stirrer at 400 rpm. Then the phase consisting of initiator (potassium persulfate, 0.7 mL, 3% w/v based on H_2O), electrolyte (anhydrous calcium chloride, 0.45 mL, 2% w/v based on H_2O), and 0.1 g of surfactant (PF68) was added dropwise. Stirring was continued for 1 h whereupon a white-milky emulsion was formed. The emulsion was transferred to a mold (stainless-steel column, 50 × 4.6 mm i.d.) and cured at 55°C for 48 h. After cooling to room temperature, the column was connected with the HPLC system to remove the soluble compounds by pumping deionized water (60 mL) and methanol (30 mL) through the column. The epoxy groups on the surface of the monolith were hydrolyzed by 0.1 mol/L hydrochloric acid at 40°C for 12 h to obtain diol groups. The preparation process is shown in Figure 2. After that, the column was washed completely by excessive methanol and deionized water. In this way, a novel monolithic column was obtained.

Images of the emulsion were captured by an optical microscope (Yongheng Optical Instrument Manufacturing Co., Ltd., Shanghai, China) with an automatic camera just after the emulsion formation. The monoliths were cut into small pieces followed by drying at 60°C overnight. Morphology of the dried monolith samples was observed by scanning electron microscope (SEM). FT-IR spectra were recorded on a FT-IR-8400 spectrometer (Shimadzu, Kyoto, Japan).

On-line SPE

The monolithic column was used as an SPE column, which was placed in the sample-loop position of the injection valve and used for deproteinization and sample enrichment.

With the switching valve in "load" position, a volume of 5 µL of plasma sample was directly injected into the SPE column and was washed with 5 mL of washing liquid (water). While the endogenous compounds were flushed to waste, *m*-nisoldipine was retained by the inner surface of the SPE column. During the sample clean-up step, the analytical column was equilibrated with the analytical mobile phase. Then the switching valve was switched to the "inject" position, and the SPE column and analytical column were series-connected. Thus, the retained analyte was swept on-line by the analytical mobile phase from the SPE column to the analytical column. *m*-Nisodipine was separated in the analytical column, which was connected to the UV detector monitored at 237 nm. The total analysis time was about 10 min.

Pharmacokinetic studies

Male Kunming mice (weighting 20 ± 5 g) were purchased from Hebei Medical University. The experiment was approved by the local bioethics committee. *m*-Nisoldipine (0.9 mg/mL) was prepared by 0.5% CMC-Na (sodium carboxymethylcellulose) shaking for 5 min for uniformity. Then m-nisoldipine was orally administrated at a single dose of 9 mg/kg, respectively. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 4, 8, and 12 h after the administration and collected in heparinized centrifuge tube, respectively. Then the samples were centrifuged at 4500 rpm for 10 min, and the separated plasma was frozen at –18°C prior to analysis.

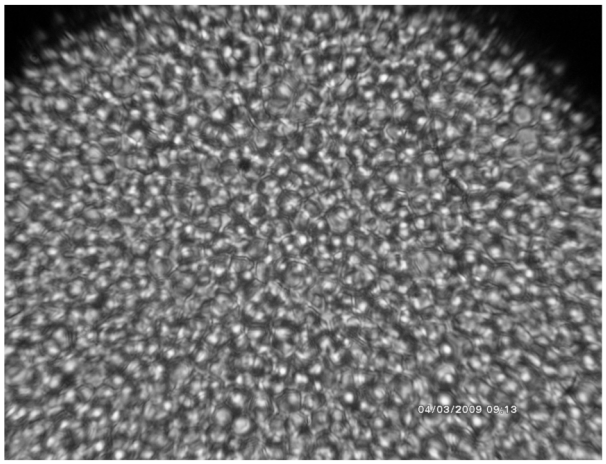

Figure 3. Optical photograph of the emulsion.

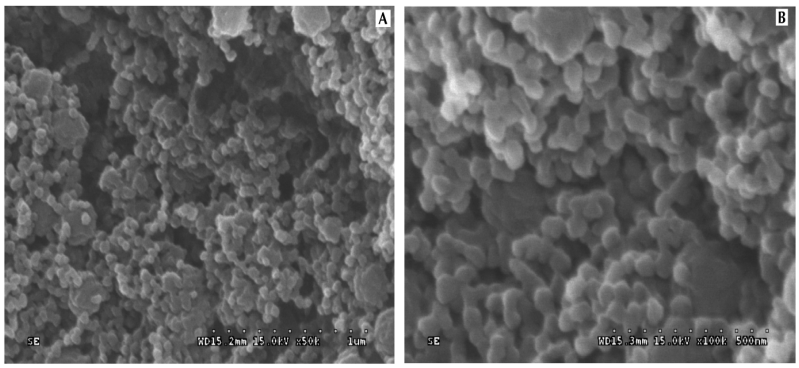

Figure 4. SEM images of of the monolithic products. SEM image × 50 (A) and SEM image × 100 (B).

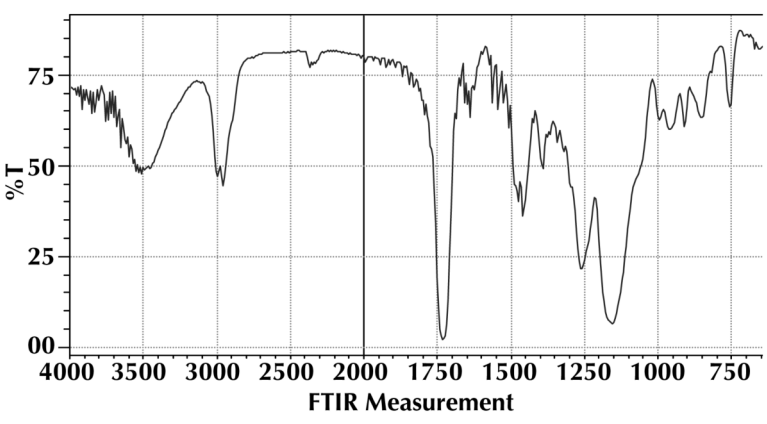

Figure 5. FT-IR spectrum of the monolith materials.

Results and Discussion

Characterization of the monoliths

The white-milky O/W emulsions were formed and significantly stable for 24 h after stirring was ceased at 25°C, and no settling occurred even after extended periods. The result indicated that this system was suitable for emulsion templating because the polymerization chemistry would be expected to occur before the emulsion became unstable. Images of emulsions captured by an optical microscope are displayed in Figure 3. This indicates that the emulsion has good internal structure. While the conditions changed, rapid separation appeared over a period of a few minutes, causing two distinct phases: a transparent upper phase and a white-milky lower phase. In order to provide further proof, PF68 was replaced separately by polyoxyethylene lauryl ether (Brij35), sorbitan monooleate (Span80), sodium dodecyl sulfate (SDS), while other factors remained unchanged. The experiments showed that steady emulsion could not be formed.

The morphology of the monolithic materials, which is studied by SEM, is shown in Figure 4. The monoliths are further characterized using FT-IR spectroscopy on all samples to confirm their chemical structure (Figure 5). Hydroxyl groups are clearly evident as the peak at 3400 cm^{-1}.

Performance of the monolithic column

The obvious advantage of the monolithic columns is their high permeability. Using deionized water as the mobile phase, the relationship between the flow rate and backpressure was studied, as illustrated in Figure 6. The results showed that a good linearity was obtained (r = 0.9994). The backpressure of the monolithic column was about 31 bar at the flow rate of 4.0 mL/min. It showed that the monolith could be used at high flow rate, which would provide a favorable possibility for fast analysis. To investigate the reproducibility of polymerization, 10 columns were prepared under the same conditions, using the same method. Similar results and excellent chromatographic separation and performance were realized. With regards to column stability, the columns prepared from this method have been used for hundreds of injections over a four month period, and no deterioration of performance both in terms of retention time and peak shape has been observed.

Efficiency of on-line sample pretreatment and chromatography

The efficiency of the selected washing liquid

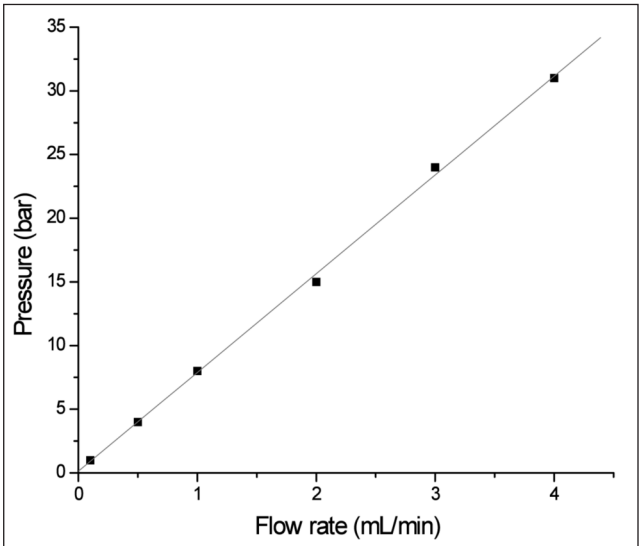

Figure 6. Relationship between mobile phase flow rate and backpressure for deionized water. Column: 50 × 4.6 mm i.d.

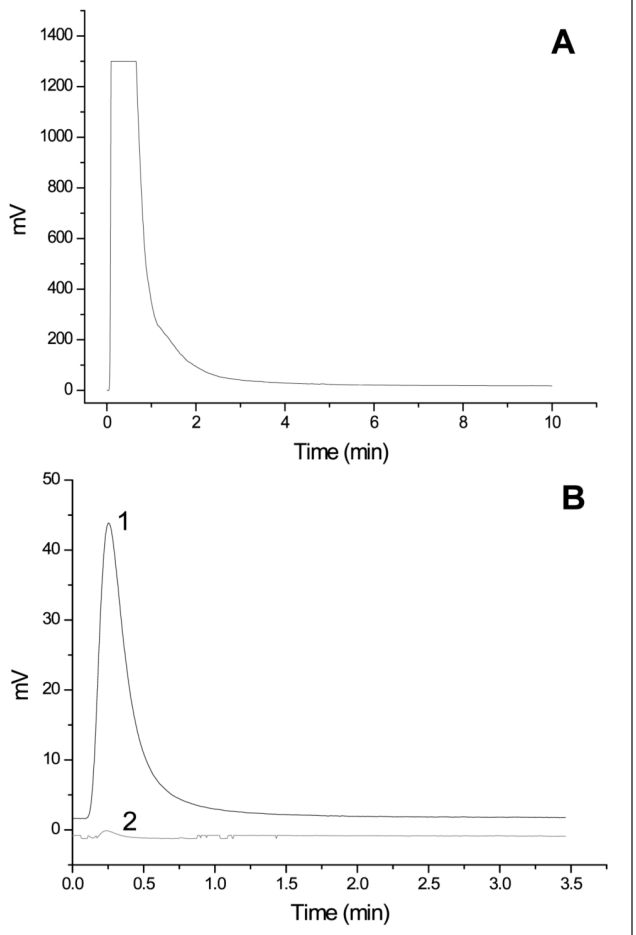

Figure 7. Chromatograms obtained after sample deproteinization and enrichment of m-nisoldipine with the monolithic column. Column: 50 × 4.6 mm i.d.; flow rate: 1.0 mL/min; temperature: room temperature: (A): 5 µL blank plasma samples inject into the monolithic column. Mobile phase: deionized water; detection wavelength: 280 nm; (B): 5 µL *m*-nisoldipine working solution with methanol (1) and deionized water (2) as the mobile phase. Detection wavelength: 237 nm.

(water) to wash out the sample matrix was tested by directly injecting 5 µL of blank plasma into the SPE column, which was connected to the UV detector and monitored at 280 nm (Figure 7A). The elimination of the biological matrix could be considered as complete when the detector signal reached the baseline, so a washing volume of 5 mL was sufficient for sample clean-up. The chromatograms in Figure 7B showed that when pure water was used as mobile phase, *m*-nisoldipine could not be eluted, whereas when methanol was used as mobile phase, *m*-nisoldipine was eluted quickly from the monolithic column. Therefore, during the washing period (washing liquid: water), biological matrix was simply removed and *m*-nisoldipine was well retained on the SPE column.

The selective adsorption of *m*-nisoldipine by the inner surface of the SPE column is probably because of hydrogen bond interactions and electrostatic interactions between the drugs and the column binding sites.

The chromatographic conditions were also investigated. Satisfactory separation could be achieved by the use of mixed solution of methanol and water as analytical mobile phase. The experiments also showed that when the proportion of methanol was increased, the drugs could be eluted more quickly. Considering of the retention time and separation effect, methanol–water (90:10, v/v) at a flow rate of 1.0 mL/min was selected for separation. Typical chromatograms resulting from the HPLC–UV analysis of 5 µL of plasma extracts obtained after the monolithic material extraction are depicted in Figure 8, including a plasma sample containing *m*-nisoldipine (Figure 8A) and a blank plasma sample (Figure 8B). The retention time of m-nisoldipine was approximately 9.530 min. Blank plasma sample was used to determine whether there were any interfering peaks around the retention time of *m*-nisoldipine. The peak of *m*-nisoldipine showed good separation and no interferences, reflecting the high specificity and sensibility of the described method.

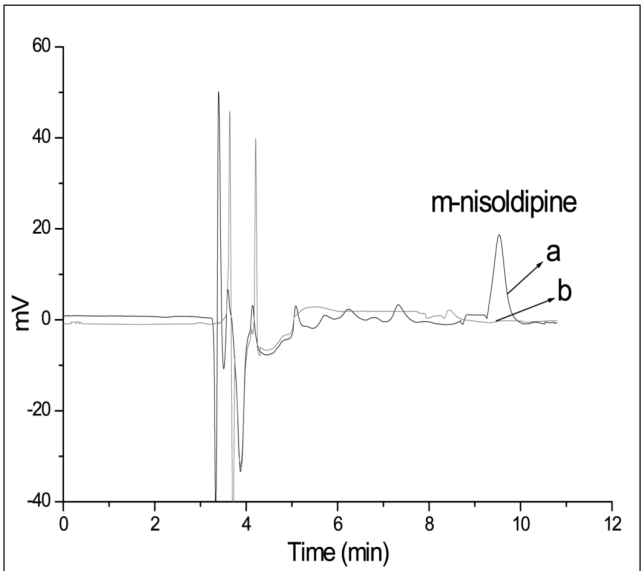

Figure 8. Chromatograms obtained from a plasma sample containing *m*-nisoldipine (A) and a blank plasma sample (B) after SPE. Column: 250 × 4.6 mm i.d., 5 µm; mobile phase: methanol–water (90:10, v/v); flow rate: 1.0 mL/min; detection wavelength: 237 nm; injection volume: 5 µL; temperature: room temperature.

Calibration, precision, and accuracy

Calibration graphs were calculated by linear regression analysis of the peak area of *m*-nisoldipine versus the drug concentrations. Plasma samples spiked with seven different concentrations (2, 5, 10, 20, 50, 100, and 200 ng/mL) of *m*-nisoldipine were analyzed. All analyses were performed three times. The peak area showed a linear relationship (r = 0.9992) with the concentration over the range of 2–200 ng/mL for plasma samples. The limit of detection (LOD) for *m*-nisoldipine in plasma at these concentrations, the amount for which the signal-to-noise ratio was 3, was calculated to be 1.5 ng/mL.

Precision of the method could be expressed as intra-day and inter-day variability in the concentration ranges of *m*-nisoldipine in plasma samples. Precisions were determined by five replicated injections of three spiked samples (2, 50, and 200 ng/mL). The accuracy of the method at these levels was determined by comparing the concentrations measured after analysis of the plasma samples with *m*-nisoldipine according to the procedure on-line SPE with those targeted concentrations. The results of precision and accuracy are shown in Table I.

The absolute recoveries of *m*-nisoldipine were determined by comparing *m*-nisoldipine peak area obtained by HPLC analysis of spiked plasma samples after SPE pretreatment with peak area obtained by HPLC analysis of *m*-nisoldipine dissolved in methanol without SPE pretreatment. All analyses were performed five times. The recoveries determined at three different concentrations (2, 50, and 200 ng/mL) were 81.7, 84.0, and 79.6%, respectively.

Table I. Precision and Accuracy of the Method to Determine m-Nisoldipine in Human Plasma

Conc. (ng/mL)	RSD (%)		Accuracy (%)
	Intra-day	Inter-day	
2	2.81	5.95	92.0
50	4.60	4.47	90.0
200	4.29	5.25	92.4

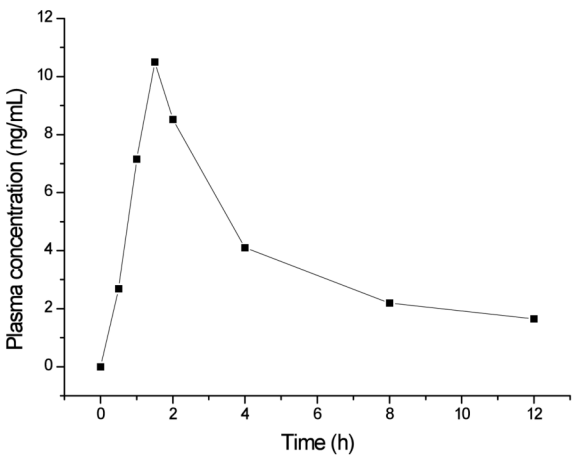

Figure 9. Mean plasma concentration-time curves of *m*-nisoldipine in mice after oral administration (n = 5).

Pharmacokinetic study of *m*-nisoldipine in mouse plasma

The described method was subsequently applied to study the pharmacokinetic of *m*-nisoldipine in mouse plasma. Figure 9 shows the mean plasma concentration-time curves of *m*-nisoldipine after oral administration of a single dose of *m*-nisoldipine (9 mg/kg) to mice. After oral administration, the highest concentration of *m*-nisoldipine was 10.5 ± 0.25 ng/mL after 1.5 h. The area under the curve ($AUC_{0-12\,h}$) was calculated to be 49.82 ± 4.37 h ng/mL.

Conclusions

In summary, an original route to achieve monolithic materials have been demonstrated, while epoxy groups in the polymer matrix offer possibilities of chemical modifications. The permeability of the prepared column was high, and the column could be used for rapid analysis at a high flow rate. Additionally, the monolithic column was successfully used as on-line sample clean-up material to deproteinize and enrich *m*-nisoldipine simultaneously without tedious pretreatment of samples. The method described required small sample volume, and plasma samples could be directly injected into chromatoraphy system. It was also used for therapeutic monitoring of *m*-nisoldipine in mice after oral administration of the drug. The results suggested that such a kind of monolithic column could be used as a simple, cheap, effective, and environmentally friendly method for assaying drugs in plasma sample.

Acknowledgments

The authors are grateful for financial support by the National Natural Science Foundation of China (Grant Nos. 20375010 and 20675084) and Hebei Province Programs for Science and Technology Development (Grant Nos. 06276479B and 07276407D).

References

1. M.Y. Wang, J. Xu, X. Zhou, and T.W. Tan. Preparation and characterization of polyethyleneimine modified ion-exchanger based on poly(methacrylate-co-ethylene dimethacrylate) monolith. *J. Chromatogr. A* **1147:** 24–29 (2007).
2. Q.Z. Luo, H.F. Zou, X.Z. Xiao, Z. Guo, L. Kong, and X.Q. Mao. Chromatographic separation of proteins on metal immobilized iminodiacetic acid-bound molded monolithic rods of macroporous poly(glycidyl methacrylate–co-ethylene dimethacrylate). *J. Chromatogr. A* **926:** 255–264 (2001).
3. R. Hernandez, S. Nelson, J.R. Salm, D.T. Brown, and A.J. Alpert. Rapid preparative purification of west nile and sindbis virus PCR products utilizing a microbore anion-exchange column. *J. Virol. Methods* **120:** 141–49 (2004).
4. G.L. Yang, S. Feng, H.Y. Liu, J.F. Yin, L. Zhang, and L.P. Cai. On-line clean-up and screening of oxacillin and cloxacillin in human urine and plasma with a weak ion exchange monolithic column. *J. Chromatogr. B* **854:** 85–90 (2007).

5. X.J. Feng, H.Y. Liu, G.L. Yang, B.N. Ren, X.R. Yang, and G.Q. Yang. Online purification and screening of nisoldipine with a novel poly(vinyl ester) resin monolithic column. *Chromatographia* **69:** 403–407 (2009).
6. M.R. Buchmeiser. Polymeric monolithic materials: syntheses, properties, functionalization and applications. *Polymer* **48:** 2187–2198 (2007).
7. G. Guiochon. Monolithic columns in high-performance liquid chromatography. *J. Chromatogr. A* **1168:** 101–168 (2007).
8. A. Strancar, A. Podgornik, M. Barut, and R. Necina. Advances in Biochemical Engineering/Biotechnology (Modern Advances in Chromatography), Vol. 76. R. Freitag, Ed. Springer, Berlin/Heidelberg, 2002, pp. 49–85.
9. P. Krajnc, N. Leber, D. Štefanec, S. Kontrec, and A. Podgornik. Preparation and characterisation of poly(high internal phase emulsion) methacrylate monoliths and their application as separation media. *J. Chromatogr. A* **1065:** 69–73 (2005).
10. C.H. Yao, L. Qi, H.Y. Jia, P.Y. Xin, G.L. Yang, and Y. Chen. A novel glycidyl methacrylate-based monolith with sub-micron skeletons and well-defined macropores. *J. Mater. Chem.* **19:** 767–772 (2009).
11. S.M. Zhang and J.D. Chen. Synthesis of open porous emulsion-templated monoliths using cetyltrimethylammonium bromide. *Polymer* **48:** 3021–3025 (2007).
12. F.Y. Yuan and X.Z. Huang. New type calcium channel blockers m-nisoldipine and nisodipine. *J. Shenyang Coll. Pharm.* **7:** 58–59 (1990).
13. Z.M. Chen, C.H. Bao, Z.B. Ma, and F.Y. Yuan. Separation and structure of the photodecomposition product of m-nisoldipine. *J. Hebei Med. Univ.* **23:** 227–228 (2002).
14. Y.J. Gao, Y.S. Li, and S.X. Fu. Effects of m-nisoldipine on rabbit cardiac and vascular preparations in vitro: a comparison with nisoldipine. *Chin. J. Pharmacol. Toxicol.* **4:** 21–25 (1990).
15. S.H. Wang, J.X. Chu, Q. Shang, Y.J. Zhang, and R.X. Wang. Determination of m-nisoldipine in Beagle dog's plasma by RP-HPLC and study of its pharmacokinetics. *J. Chin. Pharm. Univ.* **38:** 140–143 (2007).
16. Z.Q. Zhang, Y.L. Wang, X.N. Li, Z.K. Tian, and Y. Wang. Concentration determination of m-nisoldipine in the plasma of rhesus monkeys by RP-HPLC. *J. China Pharm.* **17:** 101–103 (2006).
17. Z.Q. Zhang, Y.L. Wang, and X.N. Li. Pharmacokinetics of m-nisoldipine after an intravenous administration in Beagle dogs. *Chin. Pharm. J.* **41:** 535–537 (2006).
18. H.R. Wang, L.T. Zhang, Q. Wang, Z.F. Yuan, and M. Li. Validated LC–MS–MS method for determination of m-nisoldipine polymorphs in rat plasma and its application to pharmacokinetic studies. *J. Chromatogr. B* **835:** 71–76 (2006).
19. Z. Kuklenyik, X.Y. Ye, L.L. Needham, and A.M. Calafat. Automated solid-phase extraction approaches for large scale biomonitoring studies. *J. Chromatogr. Sci.* **47:** 12–18 (2009).

Manuscript recieved September 28, 2009;
revision received January 15, 2010