Journal of Chromatography B, 889–890 (2012) 55–60

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

On-line simultaneous deproteinization of biological samples and trace enrichment of three dipine series using a poly(N-isopropylacrylamide-co-ethyleneglycol dimethacrylate) monolith

Haiyan Liu*, Yanhui Duan, Yanhua Jia, Yanzhao Gu, Jia Li, Cuihong Yan, Gengliang Yang*

College of Pharmacy, Hebei University, Hebei Province Key Laboratory of Pharmaceutical Quality Control, Baoding 071002, China

ARTICLE INFO

Article history:
Received 29 July 2011
Accepted 25 January 2012
Available online 2 February 2012

Keywords:
P(NIPAAm-EDMA) monolithic column
On-line solid-phase extraction
Three dipine series
Human plasma
Human urine

ABSTRACT

A porous poly(N-isopropylacrylamide-co-ethyleneglycol dimethacrylate) [poly(NIPAAm-co-EDMA)] monolithic column was prepared by in situ free-radical polymerization. The morphology of monolithic column and pressure drop across the columns were characterized. The results showed excellent permeability and high selectivity. Nifedipine, nitrendipine and nisoldipine were simultaneously selected to validate the extraction efficiency of the prepared monolith both in plasma and urine. The extracted nifedipine, nitrendipine and nisoldipine from plasma and urine samples have been on-line tested quantitatively by using the prepared monolith connected with RP-C18 column. The total analytical run time was 38 min. For all analytes, linear calibration curves were obtained over a range of 2–500 ng/mL with coefficient of correlation > 0.997. Precision for inter- and intra-day assay showed acceptable results for quantitative assay with relative standard deviation (RSD) less than 12%. The accuracy and recovery was found to be in the range of 89–109% and 88–106%. The results indicated that the prepared monolith was feasible to be used as an on-line SPE sorbent material and the method was especially appropriate for multi-analytes monitoring in plasma and urine samples. Finally, the proposed method was successfully applied to simultaneously screen nifedipine, nitrendipine and nisoldipine in plasma.

1. Introduction

RPLC is one of the most used analytical techniques for quantitative analysis of biological samples such as in the plasma and urine [1]. The complex matrix components such as non-volatile endogenous substances in the biological samples can interfere with the analysis of the requisite drug in the process of HPLC analysis [2,3]. Therefore, sample pretreatment is a prerequisition prior to the HPLC analysis. Currently, sample pretreatment is typically achieved by protein precipitation [4–6], liquid–liquid extraction [7], supercritical fluid extraction [8,9], micellar liquid chromatography [10,11] and solid phase extraction (SPE) [12,13]. SPE is a widely accepted approach during the above-mentioned approaches for the trace enrichment of small-molecule drugs in biological samples and removing the matrix components at the same time [14,15]. Many different options exist for the off-line and on-line coupling of SPE to HPLC. However, off-line SPE and other commonly used approaches have disadvantages of time-consuming, tedious and error-prone and cost-intensive. Compared with these approaches, on-line SPE technique is clean, sensitive, selective and cost-effective; especially, it can incorporate the sample pretreatment and analyte enrichment. Meanwhile, there are minimal sample preparation steps required, only sample mixing [12,15]. On-line SPE employs column switching methods, which involves two columns: the first being used for sample cleanup with SPE column and the second being used for chromatographic separation with RPLC column [15–18]. So, sorbents in SPE column become an important factor. Since the late 1980s, monoliths have been subjected to intensive study in liquid chromatography because it has highly interconnected network structures with larger surface area and capacity [19,20]. Recently, monoliths are becoming increasingly popular as SPE sorbent [21,22]. Especially, the unique properties of easy preparation and high permeability for liquid biological samples at high flow rates, as well as selectivity, allow them to meet the requirements of SPE column [15].

Nifedipine, nitrendipine and nisoldipine (Fig. 1), dihydropyridine calcium channel blocker, are widely used in the treatment of hypertension and other cardiovascular disorders. Generally, nifedipine and nisoldipine are both used in the treatment of angina pectoris and hypertension, while nitrendipine is usually used to treat hypertension. They are all unstable compounds due to nitro functional group, which easily generate photodegradation when exposed to daylight. In addition, they are potent group of drugs which play desired effects at very low concentrations. So, sensitive

* Corresponding authors. Tel.: +86 312 5971107; fax: +86 312 5971107.
E-mail address: lhy1610@126.com (H. Liu).

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2012.01.030

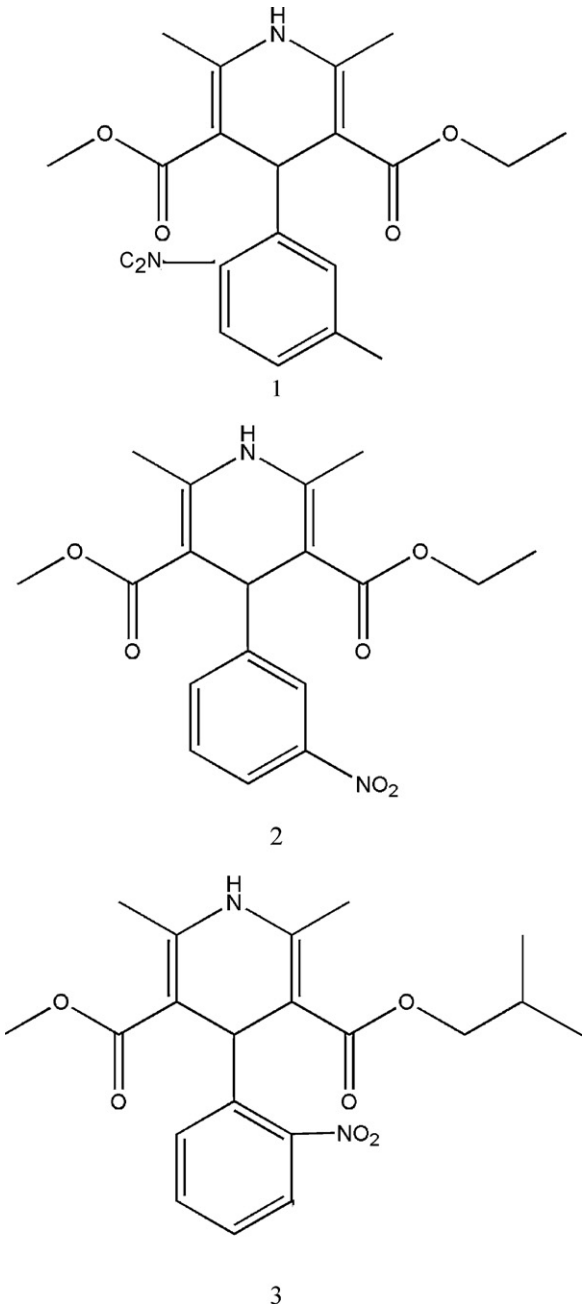

Fig. 1. The structure formula of the nifedipine (1), nitrendipine (2) and nisoldipine (3).

techniques are extremely required to detect and quantitate the levels of these drugs and their photolytic products in biological fluids. A number of analytical methods by using common sample pretreatment methods [5,23], combined with HPLC [5,24], GC [25] and other methods of chromatographic analysis have been described for the quantitative determination of three dipine series in plasma and urine.

In this paper, a porous poly(N-isopropylacrylamide-co-ethyleneglycol dimethacrylate)[poly(NIPAAm-co-EDMA)] monolithic column was prepared by in situ free-radical polymerization. The morphology of monolithic column and pressure drop across the columns were characterized. Then, the new monolithic column was operated as SPE column with column switching technology coupled to a HPLC-UV system for simultaneous determination of three dipine series in human and urine.

2. Materials and methods

2.1. Chemicals

N-isopropylacrylamide (NIPAAm) purchased from Kohjin (Tokyo, Japan). Ethylene dimethacrylate (EDMA) were purchased from Acros (NJ, USA). 2,2′-azobisisobutyronitrile (AIBN) was produced by Shanghai Chemical Plant (Shanghai, China) and refined before use. Dodecanol and methanol were purchased from Tianjin Kemiou Com (Tianjin, China). Nifedipine, nitrendipine and nisoldipine were purchased from Hebei Medical University. All reagents were of analytical reagent (AR) grade. Triple distilled water was used throughout all experiments. All media were filtered through a 0.45 μm membrane before injection for LC analysis.

2.2. Standard solutions

Nifedipine, nitrendipine and nisoldipine were dissolved with methanol to obtain a concentration of 1 mg/mL, respectively. Then, the mixed stock solution of nifedipine, nitrendipine and nisoldipine were also prepared at a concentration of 1 mg/mL in methanol. The mixed stock solution was diluted with methanol to yield intermediate solutions of 5 μg/mL, 2 μg/mL, 1 μg/mL, 0.5 μg/mL, 0.25 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.02 μg/mL. These intermediate solutions were used to prepare standards at concentration of 500 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 10 ng/mL, 5 ng/mL, 2 ng/mL by diluting with blank human plasma and urine for plasma samples and urine samples, respectively. Human plasma and urine obtained from the Hospital of Hebei University were centrifuged at 5000 r/min for 10 min. All the solutions mentioned above were stored at −20 °C and kept at 4 °C until use. Quality control samples at three different concentration levels of 5 ng/mL, 50 ng/mL, 200 ng/mL were prepared for the evaluation of precision, accuracy and recovery in analysis of plasma samples and urine samples.

2.3. Preparation and characterization of monolith

Monolith was prepared briefly as follows: 0.1164 g NIPAAm, 0.005 g AIBN and 0.4 mL EDMA were dissolved in the mixture of 0.5 mL dodecanol and 1.2 mL methanol. The mixture was shook for 1 min, sonicated and degassed briefly for 30 min. Then, the mixture was poured into the 50 mm × 4.6 mm I.D. stainless steel column sealed at one end and then sealed at the other end. After the mixture was left to polymerize at 60 °C in a water bath for 24 h, the column was flushed with methanol and water to remove unreacted monomers and porogens in the polymer rod for 1 h at a flow rate of 1 mL/min, respectively. The prepared monolith was cut into small pieces and dried under vacuum at 50 °C overnight. Then, the prepared monolith was characterized with regards to its macroporous and mesoporous structures as well as permeability. The morphological properties of this monolith were photographed using scanning electron microscope (SEM) by Hitachi (Hitachi High Technologies, Tokyo, Japan) S-4300 SEM instrument and the pressure drop across the column at different flow rates was also analyzed.

2.4. HPLC analysis

The Jasco HPLC system (Jasco Co., Japan) consisted of a PU-1580 pump and a variable wave-length UV-1570 detector. The data acquisition, handling and analysis were carried out using an HW-2000 chromatography Software (Nanjing Qianpu Software, China). The synthetic monolithic column was used as a pre-column and a C18 Dikma column (150 mm × 4.6 mm I.D.; 5 μm, Dikma, NY, USA)was used as the analytical column. The mobile phase for enrichment was deionized water; the mobile phase for separation

and analysis was methanol–water, which the proportion was 55:45 (v/v) for nifedipine and 75:25 (v/v) for nitrendipine and nisoldipine. The detection wavelength was set at 237 nm. The flow rate was set at 1 mL/min. The system was operated at ambient temperature.

2.5. Investigation of the pretreatment ability on the polymer monolith

The deproteinization ability of the monolithic column was also tested by directly injecting blank plasma into empty column and the monolithic column and eluted with deionized water at 280 nm. Meanwhile, ability of drug enrichment on the monolith was also investigated by injecting 1 μL of 1 mg/mL nifedipine, nitrendipine and nisoldipine solution into the monolithic column at 237 nm.

2.6. SPE

The resultant monolith, which was used as SPE column for sample enrichment, was placed in the sample-loop position of the injection valve. Prior to the extraction, the SPE column was equilibrated with deionized water at a flow rate of 1 mL/min for 5 min. Then, 50 μL of spiked plasma and urine standards were directly injected into the SPE column in the "load" position of six-port injector valve and were washed with 7 mL deionized water at a flow rate of 1 mL/min to remove protein and retain analytes. After 7 min of washing, the six-port valve was switched to "inject" position to connect the SPE column and the analytical column in series. As a result, the retained analytes on the SPE column were eluted from the SPE column onto the analytical C18 column with gradient elution of mixture of methanol–water at a flow rate of 1 mL/min for 38 min. For consecutive use, the monolith was washed with methanol and finally with deionized water before re-using the monolith for the subsequent SPE.

3. Results and discussions

3.1. Characteristic features of the monolith

To evaluate the characteristic features of the synthetic monolithic column, the column was washed with methanol until a stable baseline was observed. Subsequently, the monolith inside the column was pushed out of the column and cut into small pieces followed by drying under vacuum at 50 °C overnight. Then, using a small fragment of monolith to carry out SEM. Fig. S-1 exhibits the pore structure of the resultant monolith. The macropores offered a large number of channels, which allowed the mobile phase to flow through with lower column backpressure. Fig. S-2 shows the effect of flow-rate on the back pressure when water and methanol was used as the mobile phase and an excellent linear relationship was obtained.

3.2. Investigation of the pretreatment ability on the monolith

Fig. S-3A shows nearly identical peak area of blank plasma samples on the empty column (1) and the monolithic column (2) with deionized water as elution solution. In view of this result, the biological matrix compounds could be considered to largely remove when using deionized water as elution solution. Fig. S-3B shows that nifedipine, nitrendipine and nisoldipine could not be eluted when pure water (a) was used as the mobile phase. However, when methanol was used as mobile phase, nifedipine (b), nitrendipine (c) and nisoldipine (d) were eluted quickly from the monolithic column. Therefore, we could draw a conclusion that the monolithic column could be used as SPE column to completely eliminate matrix interferences and retain the analytes.

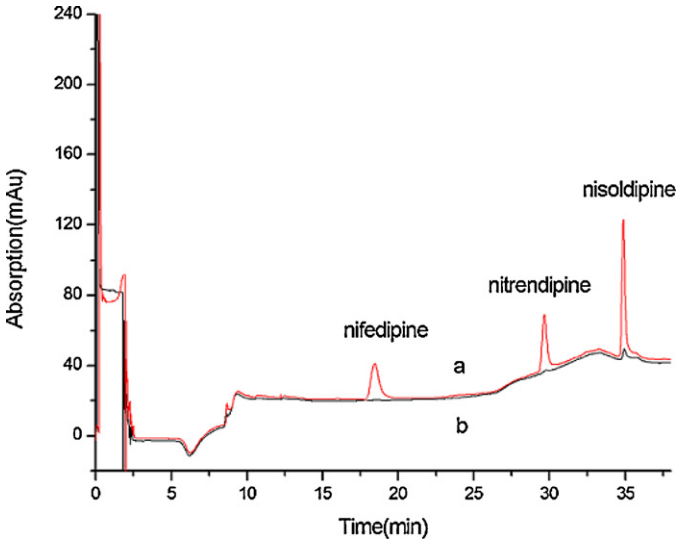

Fig. 2. Chromatograms for the gradient separation of mixture of nifedipine, nitrendipine and nisoldipine in the plasma sample at a concentration of 1.0 μg/mL (a) and blank plasma sample (b). Gradient elution: 0–20 min, 55% methanol; 20–29 min, 55–75% methanol; 29–40 min, 75% methanol. Flow rate: 1.0 mL/min; Column: RP-C18 Dikma, 150 mm × 4.6 mm I.D.; UV detection: at 237 nm.

3.3. SPE-HPLC

The plasma samples were passed through the SPE column with pure water as mobile phase and then analyzed on the RP-C18 column. The separation conditions on the RP-C18 column were optimized, the experiments demonstrated that the retention times of nifedipine, nitrendipine and nisoldipine on the RP-C18 column were suitable when using a gradient elution from 55% to 75% methanol at a flow rate of 1 mL/min. The total analytical run time was 38 min. Fig. 2 was the chromatogram which is obtained by the analysis of RP-C18 column after injecting plasma samples (a) containing nifedipine, nitrendipine and nisoldipine as well as blank plasma samples (b) into the monolithic column. Urine samples (Fig. 3a) containing nifedipine, nitrendipine and nisoldipine

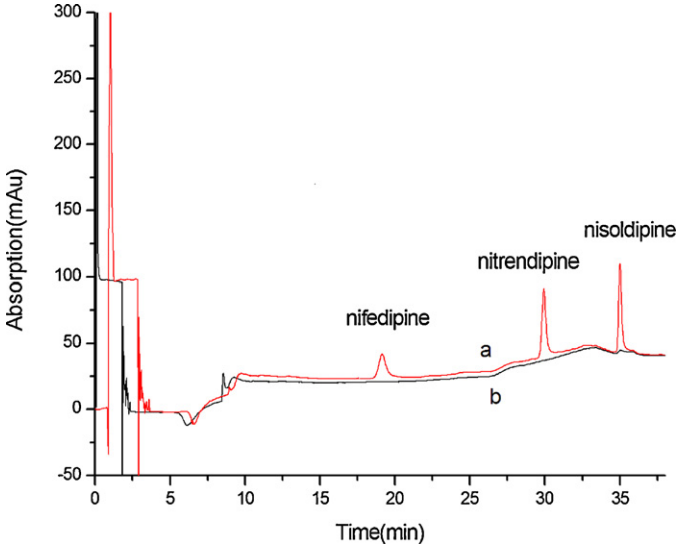

Fig. 3. Chromatograms for the gradient separation of mixture of nifedipine, nitrendipine and nisoldipine in the urine sample at a concentration of 1.0 μg/mL (a) and blank urine sample (b). Gradient elution: 0–20 min, 55% methanol; 20–29 min, 55–75% methanol; 29–40 min, 75% methanol. Flow rate: 1.0 mL/min; Column: RP-C18 Dikma, 150 mm × 4.6 mm I.D.; UV detection: at 237 nm.

Table 1
Calibration curve, LOD and LOQ of nifedipine, nitrendipine, nisoldipine from plasma and urine samples.

Analytes (samples)	Calibration equations	Correlation coefficient	LOQ (ng/mL)	LOD (ng/mL)
Nifedipine				
Plasma	$Y = 3.50 \times 10^4 x + 1.91 \times 10^2$	$r^2 = 0.9988$	10	3
Urine	$Y = 1.08 \times 10^4 x + 2.03 \times 10^2$	$r^2 = 0.9976$	5	2
Nitrendipine				
Plasma	$Y = 8.13 \times 10^4 x - 5.59 \times 10^2$	$r^2 = 0.9992$	6	2
Urine	$Y = 7.02 \times 10^4 x - 1.45 \times 10^2$	$r^2 = 0.9997$	5	1.5
Nisoldipine				
Plasma	$Y = 5.83 \times 10^4 x + 4.94 \times 10^2$	$r^2 = 0.9999$	6	2
Urine	$Y = 5.05 \times 10^4 x + 2.42 \times 10^2$	$r^2 = 0.9998$	5	1.5

as well as blank urine samples (Fig. 3b) obtained uniform results according to the operation procedure of the blood samples. It could be seen that nifedipine, nitrendipine and nisoldipine were separated very well in Figs. 2a and 3a; meanwhile, no interfering peaks from endogenous matrix components were observed near the retention time of nifedipine, nitrendipine and nisoldipine, which demonstrated the deproteinization and sample enrichment could be achieved at the same time by this approach and reflected the high specificity and sensibility of the described method.

3.4. Method validation

Selectivity, linearity, accuracy, precision (intra- and inter-day), recovery and reproducibility were assessed for the on-line SPE-HPLC method.

3.4.1. Selectivity

The selectivity of the method was evaluated by comparing the chromatograms obtained from the spiked samples containing nifedipine, nitrendipine and nisoldipine with those obtained from blank plasma and urine samples. As shown in Figs. 2 and 3, they were free from significant interfering endogenous substances at the retention times for the selected drugs. These results showed that the developed method is selective and specific.

3.4.2. Linearity

The calibration curve was constructed from standard solutions at different concentrations (500 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, 10 ng/mL, 5 ng/mL, 2 ng/mL) of nifedipine, nitrendipine and nisoldipine in plasma and urine. Each calibration sample of different concentrations was injected at least three times. As can be seen from Table 1, the results showed a linear relationship in the selected range. The correlation coefficients of all the equations were above 0.997. The limit of detection (LOD) and limit of quantification (LOQ) were defined as the concentrations, which yielded measure peaks with signal-to-noise ratio equal to 3 and 10, respectively.

3.4.3. Precision and accuracy

The precision and accuracy of the method was determined by using quality control (QC) samples at low, medium and high levels. The precision included an intra-day precision and inter-day precision was expressed as relative standard deviation (RSD) of analytes concentration. The intra-day precision was accessed with 5 replicates of standard-spiked samples (5, 50 and 200 ng/mL) within the same day; while inter-day precision was determined by repetitive analysis of standard-spiked samples over five consecutive days. The accuracy of this method was obtained by the measured concentrations of nifedipine, nitrendipine and nisoldipine in plasma and urine samples according to the on-line SPE to those targeted concentrations. Table 2 shows good intra- and inter-day precision with RSD and accuracy values less than 12%. The results showed that the reproducibility of method was excellent.

3.4.4. Recovery

It is clear that recovery of the analyte need not be 100%, but it is important to have reproducible recovery. To investigate the recovery, spiked samples of analytes in plasma and urine were prepared at low, medium, and high concentrations (5, 50, 200 ng/mL). The chromatographic peak areas of analytes in the plasma and urine samples were obtained according to the method of Section 2.6. All analyses were carried out five times. The absolute recovery was measured by comparing the peak area measured after SPE-LC analysis of spiked urine and plasma samples to the peak area obtained by direct injection of nifedipine, nitrendipine and nisoldipine dissolved in methanol without SPE pretreatment. To check the reliability of this method, the method recovery was measured by comparing the concentration of analytes obtained from the calibration curve to the initial concentration of analytes in the spiked plasma and urine standard. The results of recovery were shown in Table 3. The results showed that the recoveries were satisfactory and the method was acceptable for the analysis of plasma and urine samples.

3.4.5. Reproducibility

In order to evaluate the reproducibility of the prepared monolithic column as SPE column, four monolithic columns were

Table 2
Intra- and inter-day precisions and accuracies of nifedipine, nitrendipine and nisoldipine in human plasma and urine samples at three different concentrations.

Analyte (samples)	Concentration (ng/mL)	Precision RSD (%)		Accuracy (%)
		Intra-day	Inter-day	
Nifedipine				
Plasma	5	7.62	6.18	99.6
	50	8.25	6.41	99.4
	200	8.39	10.1	106.8
Urine	5	6.67	8.39	90.8
	50	5.7	4.73	99.7
	200	7.3	8.65	92.5
Nitrendipine				
Plasma	5	3.9	5.46	99.5
	50	6.52	8.59	100.3
	200	3.8	5.45	99.8
Urine	5	7.64	6.74	100.6
	50	3.55	4.26	97.8
	200	7.35	5.84	100.4
Nisoldipine				
Plasma	5	5.67	7.24	99.7
	50	7.98	9.28	108.5
	200	9.1	7.8	100.5
Urine	5	5.6	7.3	89.7
	50	10.1	11.5	100.1
	200	4.8	6.3	100.3

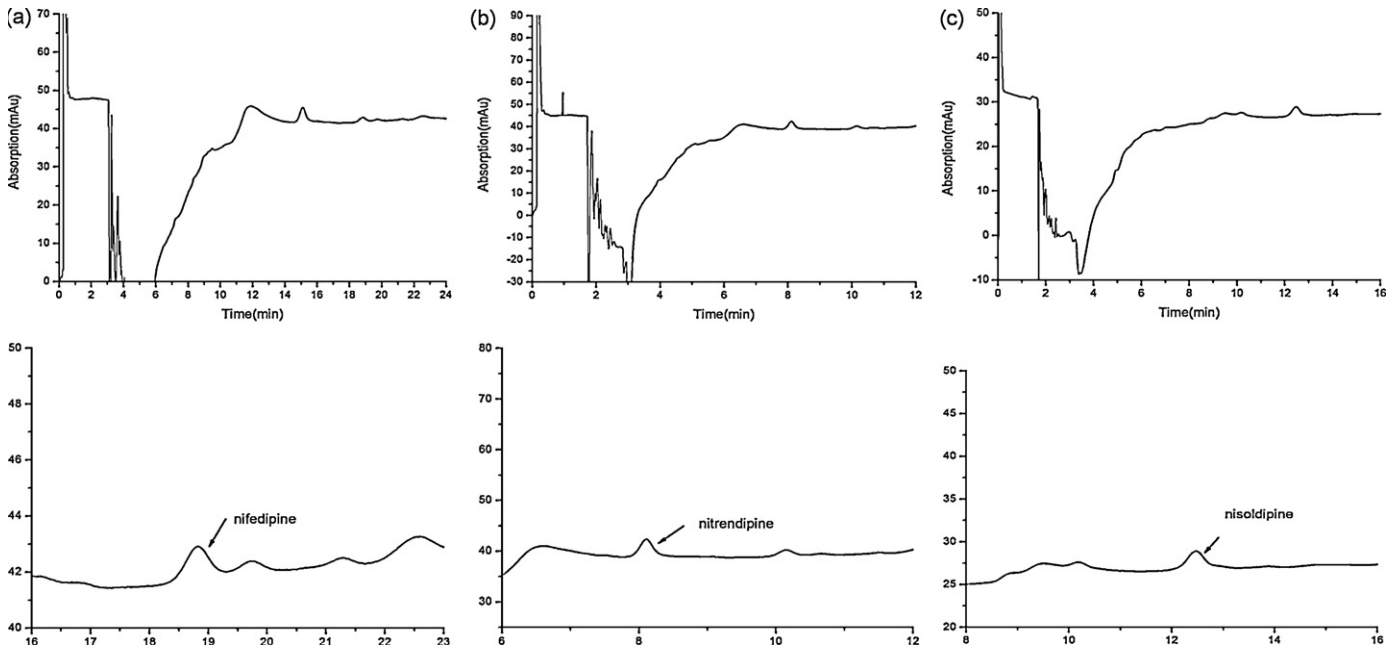

Fig. 4. Chromatograms of nifedipine (a), nitrendipine (b) and nisoldipine (c) in clinical plasma samples. Mobile phase: (a) 55% methanol; (b) 75% methanol; (c) 75% methanol. Flow rate: 1.0 mL/min; Column: RP-C18 Dikma, 150 mm × 4.6 mm I.D.; UV detection: at 237 nm.

prepared with the same polymerization process as described in Section 2.3 and used for extraction of nifedipine, nitrendipine and nisoldipine from plasma and urine samples. The reproducibility of the SPE monolithic column could be expressed through the RSD of the retention time and the peak area of three dipine series in plasma samples. The RSD of the retention time and the peak area were 2.71%, 3.25%, 2.56%, respectively, and the RSD for peak area were all less than 5%. These data revealed that the prepared monolithic column could provide excellent reproducibility and implied that the prepared monolith was feasible to be use as an on-line SPE sorbent material.

Table 3
Recovery of nifedipine, nitrendipine and nisoldipine in human plasma and urine samples.

Analyte (samples)	Concentration (ng/mL)	Absolute recovery (%)	Method recovery (%)
Nifedipine			
Plasma	5	99.8 ± 6.0	89.6 ± 5.5
	50	99.5 ± 4.4	93.6 ± 4.6
	200	101.6 ± 5.8	99.8 ± 6.2
Urine	5	100.2 ± 2.4	98.5 ± 1.8
	50	96.8 ± 4.5	89.0 ± 4.9
	200	99.5 ± 5.3	100.2 ± 4.5
Nitrendipine			
Plasma	5	99.5 ± 6.2	90.4 ± 5.5
	50	90.5 ± 5.8	95.1 ± 6.4
	200	100.2 ± 3.4	101.4 ± 1.2
Urine	5	101.3 ± 3.2	95.6 ± 5.8
	50	100.5 ± 5.4	96.9 ± 3.9
	200	96.4 ± 7.5	89.5 ± 7.9
Nisoldipine			
Plasma	5	88.5 ± 8.4	94.6 ± 5.2
	50	105.1 ± 4.6	100.7 ± 6.3
	200	90.9 ± 6.1	99.6 ± 7.6
Urine	5	92.6 ± 6.8	89.8 ± 5.6
	50	95.1 ± 3.8	98.4 ± 7.4
	200	101.3 ± 8.7	99.1 ± 2.8

Table 4
Concentrations of the target analytes in the clinical plasma samples.

Sample	Analytes	Dosage (mg)	Sampling time (h)	Concentration (ng/mL)
Sample 1	Nifedipine	5	1	15.56
Sample 2	Nitrendipine	10	1.5	31.01
Sample 3	Nisoldipine	5	1.5	26.42

3.5. Clinical application

The proposed on-line SPE-HPLC method was used to analyze real plasma samples. Samples were obtained from anonymous hypertensive patients who had taken orally 5 or 10 mg doses of nifedipine or nitrendipine, nisoldipine tablets, respectively. The contents of each analyte in clinical plasma samples were determined by interpolating the peak area on the calibration curve. The results were listed in Table 4, and the chromatograms were shown in Fig. 4.

4. Conclusions

A poly(NIPAAm-co-EDMA) monolithic column was used as SPE sorbent to simultaneously monitor three analytes in plasma and urine. The resultant monolith possessed unique advantages of ease preparation, high permeability and selectivity for specific analytes. The good linearity, precision, accuracy and recovery were also achieved. The study provided a re-confirmation in the application of polymer monolith in bioanalysis, particularly for the sample pretreatment of complex biological matrices like plasma and urine. In such approach, the sample pretreatment step was embedded into the LC chromatographic system and manual intervention was minimized. Meanwhile, the on-line simultaneous clean up of protein and enrichment of analytes also could be accomplished. Finally, the proposed method was successfully applied to simultaneously screen nifedipine, nitrendipine and nisoldipine in plasma. All these results showed that the established method is suitable for the analysis of plasma and urine samples collected during clinical studies.

Acknowledgements

This work was supported by the Funds of Education Department of Hebei Province (No. 2008416), the Natural Science Foundation of Hebei University (No. 2009-165), the Key Project Foundation of Hebei Province Higher Education (No. ZD2010234), the Key Basic Research Foundation of Hebei Province (No. 11966411D), the National Natural Science Foundation of China (No. 21175031), the Pharmaceutical Joint Research Foundation of the Natural Science Foundation of Hebei Province and China Shiyao Pharmaceutical Group Co., Ltd. (No. B2011201174) and the issue of second batch of "twelfth Five-year Plan" of Key Special Project for "significant Drug Discovery" of Ministry of Science and Technology of China (No. 2012ZX09103-101-057).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2012.01.030.

References

[1] N. Abello, P.P. Geurink, M. van der Toorn, A.J. van Oosterhout, J. Lugtenburg, G.A. van der Marel, H.A. Kerstjens, D.S. Postma, H.S. Overkleeft, R. Bischoff, Anal. Chem. 80 (2008) 9171.
[2] M. Wood, M. Laloup, N. Samyn, M. del Mar Ramirez Fernandez, E.A. de Bruijn, R.A. Maes, G. De Boeck, J. Chromatogr. A 1130 (2006) 3.
[3] J.H. Beijnen, W.J.M. Underberg, J. Pharm. Biomed. Anal. 6 (1988) 677.
[4] M.C. Breadmore, R. Theurillat, W. Thormann, Electrophoresis 25 (2004) 1615.
[5] N.C. van de Merbel, H. Poelman, J. Pharm. Biomed. Anal. 33 (2003) 495.
[6] J. Martens-Lobenhoffer, S.M. Bode-Böeger, J. Chromatogr. B 819 (2005) 197.
[7] X.R. Xia, N.A. Monteiro-Riviere, J.E. Riviere, J. Chromatogr. A 1129 (2006) 216.
[8] T. Sakamoto, A. Yamamoto, M. Owari, Y. Nihei, Surf. Interface Anal. 38 (2006) 317.
[9] R.J. Houben, J.G.M. Janssen, P.A. Leclercq, J.A. Rijks, J. High. Resolut. Chromatogr. 13 (1990) 669.
[10] U. Lövgren, M. Johansson, K. Kronkvist, L.E. Edholm, J. Chromatogr. B: Biomed. Sci. Appl. 672 (1995) 33.
[11] A. Szymański, Chem. Anal. (Warsaw) 48 (2003) 429.
[12] C. Baggiani, C. Giovannoli, L. Anfossi, C. Tozzi, J. Chromatogr. A 938 (2001) 35.
[13] D.O. Rumiantsev, T.V. Ivanova, J. Chromatogr. B: Biomed. Sci. Appl. 674 (1995) 301.
[14] L. Ortega, R. López, J. Cacho, V. Ferreira, J. Chromatogr. A 931 (2001) 31.
[15] K.C. Saunders, A. Ghanem, W. Boon Hon, E.F. Hilder, P.R. Haddad, Anal. Chim. Acta 652 (2009) 22.
[16] G.L. Yang, S. Feng, H.Y. Liu, J.F. Yin, L. Zhang, L.P. Cai, J. Chromatogr. B 854 (2007) 85.
[17] X.Y. Wei, J.F. Yin, G.L. Yang, C.X. He, Y. Chen, J. Sep. Sci. 30 (2007) 2851.
[18] G.L. Yang, H.Y. Liu, Y.H. Zhang, S.M. Wang, J.F. Yin, B.Y. Yin, Y. Chen, J. Chromatogr. A 1129 (2006) 231.
[19] J. Urban, P. Jandera, Z. Kučerová, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 1167 (2007) 63.
[20] M. Merhar, A. Podgornik, M. Barut, M. Žigon, A. Štrancar, J. Sep. Sci. 26 (2003) 322.
[21] O.G. Potter, E.F. Hilder, J. Sep. Sci. 31 (2008) 1881.
[22] Y. Fan, M. Zhang, Y.Q. Feng, J. Chromatogr. A 1099 (2005) 84.
[23] D.W. Shang, X.P. Wang, X.T. Zhao, F.M. Huang, G.Z. Tian, W. Lu, T.Y. Zhou, J. Chromatogr. B. 879 (2011) 3459.
[24] I. Niopas, A.C. Daftsios, J. Pharm. Biomed. Anal. 32 (2003) 1213.
[25] M.P. Marques, N.A. Santos, E.B. Coelho, P.S. Bonato, V.L. Lanchote, J. Chromatogr. B: Biomed. Sci. Appl. 762 (2001) 87.