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On-line clean-up and screening of oxacillin and cloxacillin in human urine and plasma with a weak ion exchange monolithic column

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Abstract

A weak ion exchange monolithic column prepared by modifying the GMA–MAA–EDMA (glycidyl methacrylate–methacrylic acid–ethylene glycol dimethacrylate) monoliths with ethylenediamine was applied to remove matrix compounds in biological fluid. Using this monolithic column, on-line clean-up and screening of oxacillin and cloxacillin in human urine and plasma samples had been investigated. Chromatography was performed by reversed-phase HPLC on a C_{18} column with ultraviolet detection at 225 nm. Results showed that the ion exchange monolithic column could be used for deproteinization and retaining oxacillin and cloxacillin in human urine and plasma, which provided a simple and fast method for assaying drugs in human urine and plasma.

Keywords: Oxacillin; Cloxacillin; On-line clean-up; Weak ion exchange monolithic column

1. Introduction

HPLC analysis of drugs in biological fluids usually involves treatments of the samples before their injection into the chromatograph. Typical treatments include precipitation by acids or organic solvents [1], liquid–liquid extraction (LLE) [2–6] and off-line solid phase extraction (SPE) [7–9]. These conventional procedures are complicate, time-consuming, not friendly to environment, and drugs may be partly lost in sample preparation steps.

On-line SPE can solve these problems. On-line SPE is a clean, fast, efficient and sensitive trace-level determination pre-treatment procedure. It is particularly attractive since it allows the simultaneous removal of matrix compounds and preconcentration of the analytes [10]. SPE usually employs conventional ion exchange cartridges, small C_{18} pre-packed cartridges, restricted access material (RAM) [11–17], molecularly imprinted polymers [18–25] and immunoaffinity solid-phase extraction sorbents [26–28]. Our recent work [29] reported a method using a weak cation (carboxyl groups) exchange monolithic column for on-line simultaneous removal of human serum albumin and enrichment of doxazosin.

Oxacillin and cloxacillin are β-lactam antibiotics that are effective against a broad spectrum of gram positive and negative bacteria. The two drugs are widely used for prevention and treatment of bacterial infections in human and animals. By using traditional sample treatment methods, several chromatographic methods have been reported for the determination of oxacillin and cloxacillin in food as residuals such as in milk [30,31,37], bovine muscle [32–36], kidney and liver [32,36,37]. In this paper, a weak ion exchange monolithic column (ethylenediamine and carboxyl groups) was developed and validated for simultaneous determination of oxacillin and cloxacillin in human urine and plasma for on-line sample clean-up coupled to a HPLC-UV system.

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2. Experimental

2.1. Chemicals and reagents

Oxacillin sodium and cloxacillin sodium were provided by Zhongnuo Pharmaceutical Industry (Shijiazhuang, China). Ethylene dimethacrylate (EDMA) was purchased from Acros (New Jersey, USA). Glycidyl methacrylate (GMA) and methacrylic acid (MAA) were purchased from Suzhou Anli Chemical Limited Company (Suzhou, China). 2, 2-Azobisisobutyronitrile (AIBN) was purchased from the fourth Shanghai Chemical and Reagent Factory (Shanghai, China). Potassium dihydrogen phosphate was purchased from Tianjin Medical Company (Tianjin, China). Acetonitrile, tetrahydrofuran, ethylenediamine, dodecyl and cyclohexanol of analytical grade were purchased from Tianjin Beilian Co. (Tianjin, China). All solutions were filtered through a 0.45 μm membrane before use.

2.2. Equipments

Chromatography was performed on an Agilent 1100 system (Agilent Technology, Palo Alto, CA, USA), which included a quaternary pump, an autosampler, a micro vacuum degasser and a UV detector. The chromatographic data were analyzed using the Chemstation Software (Agilent).

2.3. Chromatography

Chromatographic separations were performed at ambient temperature on a C_{18} diomonsil column (250 mm × 4.6 mm I.D.; 5 μm, Dikma, NY, USA). The mobile phase consisted of a mixture of acetonitrile and 25 mM KH_2PO_4 (26:74, v/v) at pH 4.54 and the flow rate was 1.0 mL/min. The chromatogram was monitored at a wavelength of 225 nm.

2.4. Standard solutions

The mixed stock solution of oxacillin and cloxacillin was prepared at a concentration of 1.0 mg/mL in water and stored at −18 °C until use. The stock solution was diluted in order to obtain three solutions at 100, 10 and 1.0 μg/mL. These intermediate solutions were used to spike free drug samples at five concentration levels covering a range of 1.0–60 μg/mL for urine samples and 1.0–30 μg/mL for plasma samples, respectively. Quality control samples were prepared at concentrations of 5.0, 25 and 50 μg/mL for the evaluation of recovery, precision and accuracy in urine samples and prepared at concentrations of 2.5, 15 and 25 μg/mL in plasma samples.

2.5. Human urine and plasma samples

Human urine and plasma were obtained from the Hospital of Hebei University and were centrifuged at 4500 × *g* for 10 min, then stored under −18 °C before use. Urine and plasma samples were prepared daily by spiking with the intermediate solutions.

2.6. Preparation of weak ion exchange monolithic material

The monolithic polymer was prepared by an "in situ" polymerization method [38]. A mixture consisted of 1.0 mL dodecanol, 1.0 mL cyclohexanol, 0.2 mL EDMA, 0.4 mL GMA, 0.1 mL MAA and 60 mg AIBN. After purging with nitrogen for 10 min, a stainless steel column (10 mm × 4.6 mm I.D.) was filled with the mixture and sealed at the two ends. The polymerization was allowed to proceed at 55 °C for 24 h. The seals were removed from the tube and the monolithic column was provided with fittings and attached to the HPLC system. Methanol was pumped through the column at a flow rate of 0.1 mL/min to remove the porogens and other soluble compounds presented in the polymer rod. The epoxide groups of the polymerized glycidyl methacrylate were allowed to react with ethylenediamine using on-line modification method: the mixture of ethylenediamine and tetrahydrofran (1:1, v/v) was pumped through the column at a flow rate of 0.1 mL/min at 60 °C for 24 h (Fig. 1). Then the column was washed with tetrahydrofran until ethylenediamine could not be detected. In this way a new weak ion exchange monolithic column was obtained.

2.7. Sample pretreatment and chromatographic separation

The weak ion exchange monolith was used as a solid-phase extraction (SPE) column, which was placed in the sample-loop position of the injection valve and used for sample clean-up. In the "load" position, 0.1 mL urine or 0.2 mL plasma samples were directly injected into the SPE, and were washed with 10 mL washing liquid (water). Then valve was switched to the "injection" position. Due to the high ionic strength of the analytical mobile phase, the analytes were transferred to the analytical column and separated.

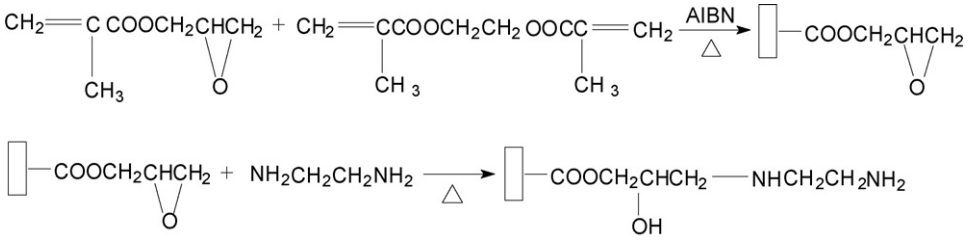

Fig. 1. Preparation of the weak ion exchange monolithic column.

3. Results and discussion

3.1. Effect of monomer ratio on the ion exchange monolithic column

Carboxyl groups, oxacillin (pK_a 2.8), cloxacillin (pK_a 2.7) and biomolecules such as human serum albumin (HSA, pI 4.9) were negatively charged and the ethylenediamine groups were positively charged when water was used as the washing liquid. So the electrostatic attraction and repulsion between drugs (or HSA) and the two kinds of groups (carboxyl and ethylenediamine groups) played a key role in the retention of drugs (or HSA) in the column. The competition between electrostatic attraction and electrostatic repulsion decided the retaining of drugs and HSA. The monomer ratio (MAA/(GMA + MAA)) was a critical factor for the properties of the weak ion exchange monolithic column. Seven monoliths were synthesized with different ratio of MAA/(GMA + MAA) in the polymerization mixture while keeping other experimental conditions constant. The deproteinization (or enrichment) efficiency of the seven ion exchange monoliths was tested by directly injecting 0.2 mg HAS (or 10 μL volume of the mixture of oxacillin and cloxacillin at the concentration of 1.0 mg/mL) into the ion exchange monoliths which was connected to the UV detector monitored at 280 nm (or 225 nm) when water was used as the mobile phase. The recoveries of HAS (or drugs) in these ion exchange monoliths were determined by comparing the peak areas measured in these ion exchange monoliths with the peak area found in a MAA–EDMA monolith (0.5 mL MAA in the mixture for the polymerization procedure). As there were only carboxyl groups in the MAA–EDMA monolith, so both drugs and HSA were absolutely flushed out of the monolith because of electrostatic repulsion. Experiments showed that when the volume ratio increased (0, 5%, 10%, 15%, 20%, 30%, 40%, respectively), the mean recoveries of HSA increased too (0, 28.1%, 59.3%, 78.9%, 97.0%, 97.9%, 98.4%, respectively), but the enrichment efficiency decreased (99.7%, 99.3%, 98.7%, 98.0%, 89.2%, 72.8%, respectively). As a result, the optimal MAA/(GMA + MAA) volume ratio was found to be 20%. Under the selected conditions, most of the matrixes in human urine and plasma could be flushed out of the SPE column whereas oxacillin and cloxacillin remained there.

3.2. Impact of pH on the retention of analytes

The pH of regular urine is about 6.0; because of different diet, pH value changes from 4.5 to 8.0. The mixture of oxacillin and cloxacillin (non-biological samples) at the concentration level of 1.0 mg/mL placed in vials were added with appropriate volume of 10% HCl or 10% NaOH solution to obtain pH values of 4.5–8.0 with steps of 0.5 as a unit. Then 10 μL volume of the mixture with different pH was directly injected into the ion exchange monolith connected to the UV detector monitored at 225 nm when water was the mobile phase. The drug samples with water as solvent could not be flushed out of the ion exchange monoliths in 10 min. Experiments showed that when pH was in the range of 4.5–8.0, the electrostatic repulsion between drugs and carboxyl groups was weaker than the electrostatic attraction between drugs and the ethylenediamine groups, and drugs were retained on the monolith. So the pH of human urine samples could not affect the retention of analytes.

3.3. Efficiency of on-line sample pretreatment

The efficiency of the selected washing liquid (water) to wash out the sample matrix was tested by directly injecting 0.1 mL urine or 0.2 mL plasma into the SPE which was connected to the UV detector monitored at 280 nm (Fig. 2A and B). The elimination of the biological matrix could be considered as complete when the detector signal reached the baseline, so a washing volume of 10 mL was sufficient for sample clean-up. During the washing period, oxacillin and cloxacillin were retained on the SPE column (Fig. 2C).

3.4. Chromatography

Typical chromatograms resulting from the HPLC-UV analysis of 0.1 mL urine extracts obtained after the ion-exchange monolithic material extraction was depicted in Fig. 3, including a blank urine sample (A), a standard urine sample spiked at a concentration of 1.0 μg/mL for both oxacillin and cloxacillin

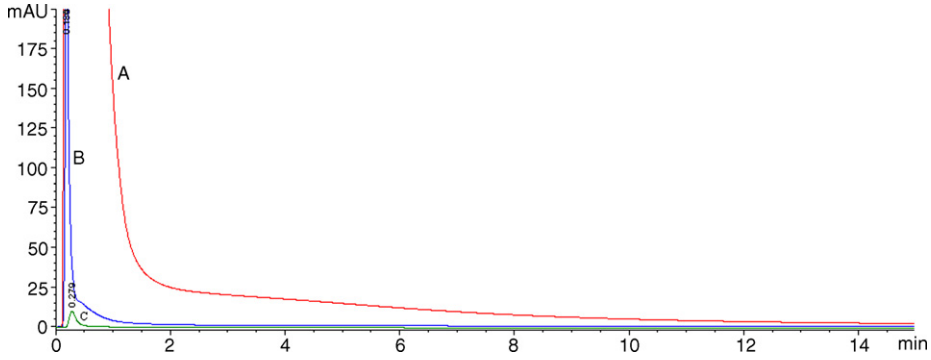

Fig. 2. Chromatograms of 0.2 mL plasma (A), 0.1 mL urine (B) and 10 μL the mixture of oxacillin and cloxacillin at the concentration of 1.0 mg/mL (C) directly injected into the weak ion exchange monolithic column when water was the mobile phase. (Detection: A, 280 nm; B, 280 nm; C, 225 nm).

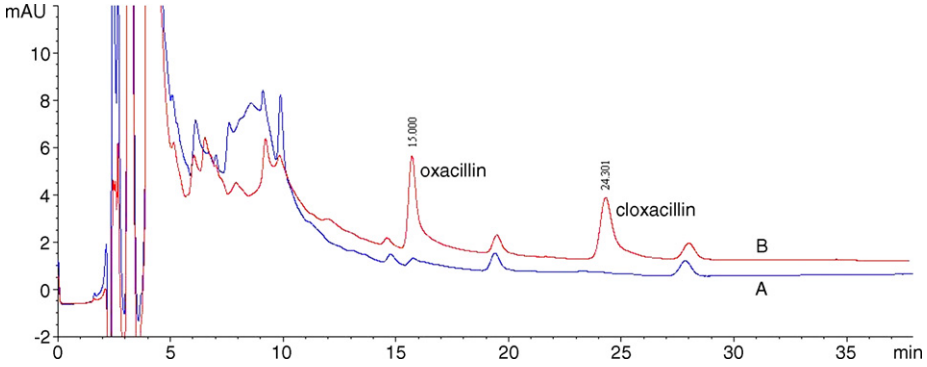

Fig. 3. Chromatograms of (A) blank urine sample; (B) a sample spiked with oxacillin and cloxacillin at a concentration of 1.0 μg/mL.

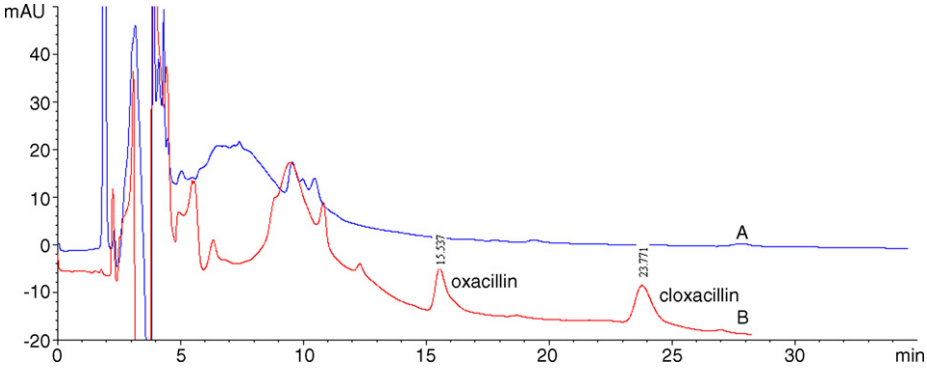

Fig. 4. Chromatograms of (A) blank plasma sample; (B) a sample spiked with oxacillin and cloxacillin at a concentration of 1.0 μg/mL.

(B). The retention times were about 15.7 min for oxacillin and 24.3 min for cloxacillin with an overall run time of 38 min. Blank urine and plasma sample were used to determine whether there were any interfering peaks around the retention time of oxacillin and cloxacillin. The peaks of oxacillin and cloxacillin showed a good separation and no interferences, reflecting the high specificity and sensibility of the described method. 0.2 mL plasma extracts obtained after ion-exchange material extraction was depicted in Fig. 4.

3.5. Calibration

Calibration graphs were calculated by linear regression analysis of the peak area of oxacillin and cloxacillin versus the drug concentrations, respectively. Urine and plasma samples spiked with five different concentrations of oxacillin and cloxacillin were analyzed. All analyses were performed three times. The peak area showed a linear relationship with the concentration over the range of 1.0–60 μg/mL for urine samples and 1.0–30 μg/mL for plasma samples, respectively. Statistical data reported in Table 1 represented the correlation coefficient, slope and intercept.

3.6. Precision and accuracy

Precision of the method could be expressed as intra-day and inter-day variability in the concentration ranges of oxacillin and cloxacillin in urine and plasma samples. The accuracy of the method was reported in% of the nominal concentrations. Precision and accuracy were determined by analyzing quality control samples with oxacillin and cloxacillin in the low, mid and high concentration ranges of the calibration curve. These results were shown in Table 2.

3.7. Recovery

The recovery of oxacillin and cloxacillin at three different concentration levels were determined by comparing the concentrations measured after analysis of spiked urine and plasma samples according to the procedure in Section 2.7 with those targeted concentrations. All analyses were performed five times. The results were shown in Table 3.

Table 1
Calibration curves for oxacillin and cloxacillin in spiked urine and plasma samples

Concentration range (μg/mL)	Correlation coefficient	Slope	Intercept	LOQ (μg/mL)
Oxacillin				
1–60	0.9938	132.9	−387.3	0.09
1–30	0.9995	81.09	−73.45	0.10
Cloxacillin				
1–60	0.9949	143.0	−474.5	0.09
1–30	0.9963	95.14	−105.7	0.10

Table 2
Intra- and inter-day precisions and accuracies of oxacillin and cloxacillin in human urine and plasma samples spiked with three different concentrations

The drugs	Spiked concentration (μg/mL)	Intra-day			Inter-day		
		Measured concentration (μg/mL)	Accuracy (%)	Precision RSD (%)	Measured concentration (μg/mL)	Accuracy (%)	Precision RSD (%)
Oxacillin							
Urine	5.0	5.02 ± 0.38	100.40	7.57	4.83 ± 0.18	96.60	3.72
	25	24.60 ± 1.36	98.40	5.53	23.26 ± 1.59	93.04	6.81
	50	48.81 ± 1.42	97.62	2.90	48.21 ± 2.01	96.42	4.17
Plasma	2.5	2.39 ± 0.31	95.60	12.97	2.42 ± 0.26	96.80	10.74
	15	14.36 ± 0.90	95.73	6.27	14.12 ± 1.04	94.13	7.37
	25	25.61 ± 3.10	102.44	12.10	24.39 ± 2.84	97.56	11.64
Cloxacillin							
Urine	5.0	5.19 ± 0.59	103.80	11.37	4.91 ± 0.42	98.20	8.55
	25	24.26 ± 2.27	97.04	9.36	24.49 ± 2.45	97.96	10.00
	50	50.69 ± 1.39	101.38	2.74	50.49 ± 1.49	100.98	2.95
Plasma	2.5	2.56 ± 0.13	102.40	5.08	2.37 ± 0.11	94.80	4.64
	15	14.71 ± 0.61	98.07	4.15	14.39 ± 0.74	95.93	5.14
	25	24.79 ± 0.88	99.16	3.54	24.52 ± 1.23	98.08	5.02

Data were expressed as mean ± SD ($n = 5$).

Table 3
Recovery of oxacillin and cloxacillin in spiked urine and plasma samples

Spiked concentration (μg/mL)	Recovery (%)		Average recovery (%)	
	Oxacillin	Cloxacillin	Oxacillin	Cloxacillin
Urine				
5.0	100.5 ± 7.6	103.9 ± 11.8	98.8 ± 1.5	100.8 ± 3.5
25	98.4 ± 6.0	97.0 ± 9.1		
50	97.6 ± 2.8	101.4 ± 2.7		
Plasma				
2.5	95.6 ± 3.2	102.4 ± 1.7	97.9 ± 3.9	99.7 ± 1.9
15	95.8 ± 6.0	97.6 ± 4.2		
25	102.4 ± 11.9	99.2 ± 3.5		

Data were expressed as mean ± SD ($n = 5$).

4. Conclusion

A weak ion exchange monolithic column (ethylenediamine and carboxyl groups) was successfully used as on-line clean-up material for simultaneous determination of oxacillin and cloxacillin in human urine and plasma. The method described required small sample volume and samples could be directly injected into chromatoraphy system. The application of the weak ion exchange monolithic column allows not only selective extraction of the target analytes but also deproteinization of the sample, which is important, particularly when the sample is complex and impurities can interfere with quantification.

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References

[1] J.M. Lobenhoffer, S.M. Bode, J. Chromatogr. B 819 (2005) 197.
[2] H.J. Jia, W. Li, K. Zhao, J. Chromatogr. B 837 (2006) 112.
[3] E. Ban, J.E. Maeng, J.S. Woo, C.K. Kim, J. Chromatogr. B 831 (2006) 230.
[4] Y.Y. Kou, Y.X. Xu, M. Xue, J.X. Ruan, Z.Q. Zhang, K.L. Liu, J. Chromatogr. B 828 (2005) 75.
[5] Y.W. Jia, G.J. Wang, H.T. Xie, J. Chromatogr. B 847 (2007) 72.
[6] X.R. Xia, N.A. Monteiro-Riviere, J.E. Riviere, J. Chromatogr. A 1129 (2006) 216.
[7] S. Ramanathan, S. Karupiah, N.K. Nair, P.L. Olliaro, V. Navaratnan, W.H. Wersdorfer, S.M. Mansor, J. Chromatogr. B 824 (2005) 45.
[8] N.J. Lakhani, A. Sparreboom, W.L. Dahut, J. Venitz, W.D. Figg, J. Chromatogr. B 806 (2004) 289.
[9] M.A. Saracino, L. Mercolini, G. Flotta, L.J. Albers, R. Merli, A. Raggi, J. Chromatogr. B 843 (2006) 227.
[10] W.M. Mullett, J. Biochem. Biophys. Methods 70 (2007) 263.
[11] K.S. Boos, A. Rudolphi, S. Vielhauer, A. Walfort, D. Lubda, F. Eisenbei, Fresenius J. Anal. Chem. 352 (1995) 684.
[12] S. Vielhauer, A. Rudolphi, K.S. Boos, D. Seidel, J. Chromatogr. B 666 (1995) 315.

[13] P. Chiap, O. Rbeida, B. Christiaens, Ph. Hubert, D. Lubda, K.S. Boos, J. Crommen, J. Chromatogr. A 975 (2002) 145.
[14] O. Rbeida, B. Christiaens, Ph. Hubert, D. Lubda, K.S. Boos, J. Crommen, P. Chiap, J. Chromatogr. A 1030 (2004) 95.
[15] E. Yamamoto, S. Takakuwa, T. Kato, N. Asakawa, J. Chromatogr. B 846 (2007) 132.
[16] E. Yamamoto, H. Igarashi, Y. Sato, I. Kushida, T. Kato, T. Kajima, N. Asakawa, J. Pharm. Biomed. Anal. 42 (2006) 587.
[17] A.J. Santos Neto, J.C. Rodrigues, C. Fernandes, G.M. Titato, C. Alves, F.M. Lanças, J. Chromatogr. A 1105 (2006) 71.
[18] E. Caro, R.M. Marce, F. Borrull, P.A.G. Cormack, D.C. Sherrington, Trends Anal. Chem. 25 (2006) 143.
[19] J. Hantash, A. Bartlett, P. Oldfield, G. Dénès, R. O'Rielly, D. Roudiere, S. Menduni, J. Chromatogr. A 1125 (2006) 104.
[20] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 562 (2006) 145.
[21] L.Q. Lin, J. Zhang, Q. Fu, L.C. He, Y.C. Li, Anal. Chim. Acta 561 (2006) 178.
[22] C. Chassaing, J. Stokes, R.F. Venn, F. Lanza, B. Sellergren, A. Holmberg, C. Berggren, J. Chromatogr. B 804 (2004) 71.
[23] P. Martin, G.R. Jones, F. Stringer, I.D. Wilson, J. Pharm. Biomed. Anal. 35 (2004) 1231.
[24] K. Möller, U. Nilsson, C. Crescenzi, J. Chromatogr. B 811 (2004) 171.
[25] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, J. Chromatogr. B 813 (2004) 137.
[26] P. Songsermsakul, G. Sontag, M. Cichna-Markl, J. Zentek, E. Razzazi-Fazeli, J. Chromatogr. B 843 (2006) 252.
[27] H.L. Lord, M. Rajabi, S. Safari, J. Pawliszyn, J. Pharm. Biomed. Anal. 40 (2006) 769.
[28] N. Delaunay-Bertoncini, M.C. Hennion, J. Pharm. Biomed. Anal. 34 (2004) 717.
[29] 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.
[30] B. Perez, C. Prats, E. Castells, M. Arboix, J. Chromatogr. B 698 (1997) 155.
[31] K. Takeba, K. Fujinuma, T. Miyazaki, H. Nakazawa, J. Chromatogr. A 812 (1998) 205.
[32] L.K. Sorensen, L.K. Snor, T. Elkar, H. Hansen, J. Chromatogr. B 734 (1999) 307.
[33] Y. Ito, Y. Ikai, H. Oka, T. Kagami, K. Takeba, J. Chromatogr. A 855 (1999) 247.
[34] S. Lihl, A. Rehorek, M. Yetz, J. Chromatogr. A 729 (1996) 229.
[35] M. Becker, E. Zittlan, M. Petz, Anal. Chim. Acta 520 (2004) 19.
[36] Y. Ito, T. Goto, H. Oka, H. Matsumoto, T. Kazue, J. Chromatogr. A 1042 (2004) 107.
[37] T.A.M. Msagati, M.M. Nindi, Food Chem. 100 (2007) 836.
[38] Q.C. Wang, F. Svec, J.M.J. Frechet, Anal. Chem. 65 (1993) 2243.