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Sensitive determination of sertraline by capillary electrophoresis with dispersive liquid–liquid microextraction and field-amplified sample stacking

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ABSTRACT

A novel method for the determination of sertraline using dispersive liquid–liquid microextraction (DLLME) coupled with capillary electrophoresis (CE) was developed. Acetone and dichloromethane were used as the disperser solvent and extraction solvent, respectively. A mixture of the extraction and disperser solvents was rapidly injected into a 1.0 mL aqueous sample to form a cloudy solution. After the extraction, sertraline was analyzed using CE that was equipped with UV detection. A 74-fold improvement in the sensitivity was observed when DLLME was used to extract sertraline. Since the DLLME extract residue was redissolved with 5 μL of water that contained 20% methanol, the detection sensitivity was further enhanced through the use of field-amplified sample stacking (FASS). A 11-fold improvement in the sensitivity was obtained when FASS was used to on-line concentrate sertraline. Under optimal extraction and stacking conditions, the calibration curve, which ranged from 0.01 to 1 μM was observed to be linear. The limit of detection (LOD) at a signal-to-noise ratio of 3 was 2.5 nM for sertraline. An approximately 814-fold improvement in the sensitivity was observed for sertraline compare with injection of standard solution without the DLLME and FASS procedures. This developed method was successfully applied to the determination of sertraline in human urine samples.

1. Introduction

Sertraline is a recently introduced antidepressant drug that belongs to the class of selective serotonin reuptake inhibitors (SSRIs). The mechanism of action of sertraline involves the blockage of serotonin reuptake at the presynaptic nerve terminal. Sertraline has become one of the most widely prescribed medications for treating depression and other psychiatric disorders because of its minimal side effects [1]. Unfortunately, when the concentration of sertraline in plasma is higher than 2.9 μg/mL (9.5 μM) intoxication may occur [2]. Therefore, a simple and sensitive analytical procedure for monitoring the concentration of sertraline in biological fluids is required to ensure optimum efficacy while minimizing the risk of toxicity and adverse effects.

The analytical methods used to monitor sertraline have been reviewed by Bosch et al. [3] and Foley et al. [4]. Different methods have been reported for analyzing sertraline in biological fluids and environmental waters. In a gas chromatography–mass spectrometric (GC/MS) analysis [5,6], sample derivatization is necessary to enhance the volatility of the analyte. High-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) absorption [7,8], fluorescence [9,10], and MS detection [11,12] has also been reported. Among these methods, each has disadvantages that limit its application, such as complicated derivatization steps for fluorescence detection, poor sensitivity for UV detection, and expensive equipment for MS detection. CE has become a popular technique for drug analysis because of its high efficiency, rapidity, and small sample volume. The major drawback of CE is its low sensitivity, especially when it is coupled with a UV detector [13,14]. This low sensitivity is due to the short optical length of the capillary and the small injection volume. To enhance the sensitivity of CE, a LIF detector was used to detect FITC-derivatized sertraline in human plasma [15]. An on-line concentration technique was also used to improve the sensitivity of CE. Cation-selective exhaustive injection (CSEI)-sweeping-MEKC provided a 10-fold improvement in the sensitivity [16]. Moreover, CE/MS has been reported for the quantitation of sertraline in water samples [17,18].

The difficulties associated with establishing analytical methods for the analysis of sertraline in biological samples primarily arise from the complexities of the sample matrices and the low sertraline concentrations in the samples. According to the literature, a sequence of sample pretreatment steps is generally necessary prior to instrumental analysis. Liquid–liquid extraction

Abbreviations: CSEI, cation-selective exhaustive injection; DLLME, dispersive liquid–liquid microextraction; FASS, field-amplified sample stacking; SSRIs, selective serotonin reuptake inhibitors

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(LLE) [19,20] and solid-phase extraction (SPE) [5,12,15] have become widely utilized procedures for extracting sertraline from plasma samples and surface water. In addition, solid-phase microextraction (SPME) [6,11], stir bar sorptive extraction (SBSE) [7], exhaustive electromembrane extraction (EME) [21], and microextraction by packed sorbent (MEPS) [8] have also been reported as methods for extracting sertraline from plasma, urine and environmental samples. These sample pre-treatment procedures, however, are tedious and time consuming. Recently, dispersive liquid–liquid microextraction (DLLME) has become an important sample preparation technique because of its rapidity, ease of operation, and low cost [22,23]. DLLME is based on a ternary solvent system in which the extraction and disperser solvents are rapidly injected into the aqueous sample to form a cloudy solution. Extraction equilibrium is quickly achieved due to the large surface contact between the droplets of the extraction solvent and the aqueous sample. Therefore, the extraction time is very short. After centrifugation of the cloudy solution, the extraction solvent generally settles at the bottom of the tube and is aspirated with a microsyringe for instrumental analysis. The DLLME technique, coupled with GC and LC, has been widely applied to the analysis of PAH and pesticides [24,25]. Because of the incompatibility of the DLLME solvent with the CE buffer, there are few reports on the use of DLLME coupled with CE [26–28]. When DLLME coupled with CE, it is necessary to evaporate the extraction solvent and reconstitute it in a suitable media to avoid current breakdown at the beginning of CE analysis.

In this paper, we describe a simple, rapid and sensitive CE–UV method for the determination of sertraline. To suppress the matrix effect and to enhance sensitivity, DLLME was employed to extract sertraline from the aqueous solution. The detection sensitivity could be further enhanced using an on-line concentration technique during CE, first introduced by Mikkers et al. in 1979 [29]. Field-amplified sample stacking (FASS) is the simplest form of on-line concentration in CE [30]. The most critical factor in FASS is that the conductivity of the background electrolyte (BGE) must be at least 10 times higher than that of the sample. Because the sample zone is less conductive than the BGE, the electric field strength is much higher than in the BGE. The higher electric field strength causes analytes in this zone to move rapidly toward the sample/BGE interface, where their velocity is reduced and they stack on the boundary. Various parameters that affect the extraction efficiency and sample stacking were investigated. The applicability of this method for the analysis of sertraline in human urine was also demonstrated. To the best of our knowledge, this is the first report demonstrating the use of DLLME coupled with CE for the analysis of sertraline.

2. Experimental

2.1. Chemicals and solutions

Sertraline hydrochloride was purchased from the Tokyo Chemistry Industry (Tokyo, Japan). Citalopram hydrochloride and carbon tetrachloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citalopram hydrochloride was used as the internal standard (I.S.). Dichloromethane and chlorobenzene were obtained from J. T. Baker (Phillipsburg, NJ, USA). All chemicals were used as received without further purification. Water that was purified using a Millipore Synergy water purification system (Billerica, MA, USA) was used for all experiments.

A stock standard solution (1 mM) of sertraline was prepared in methanol and was diluted to the desired concentrations with 5 mM phosphate buffer (pH 10). The sertraline solution was stored at 4 °C for a month.

2.2. DLLME procedure

An aliquot (1 mL) of phosphate buffer (10 mM) solution that contained the sertraline was placed into a 1.5 mL sample vial. A total of 200 μL of acetone the disperser solvent containing 30 μL of dichloromethane the extraction solvent was rapidly injected into the sample solution using a 1.0 mL syringe (Hamilton, USA). The rapid injection created a cloudy solution. The mixture was gently shaken for 1 min. The mixture was then centrifuged at 4000 g for 5 min, and the dispersed fine droplets of the extraction solvent settled at the bottom of the sample vial. Twenty microliters of the sediment phase was transferred to a separate sample vial using a 25 μL HPLC syringe (Hamilton, USA). The extract was evaporated to dryness at room temperature and then redissolved with 5 μL of water that contained 20% methanol.

2.3. CE system

The CE system was assembled in house. The separation voltage was supplied using a 0–30 kV power supply (Glassman High Voltage, Whitehouse Station, NJ, USA). A 75 μm I.D. × 360 μm O.D. × 65 cm total length capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separation. The effective length of the capillary was 53 cm. The electrophoretic separation condition was modified from previously described method [14]. The capillary was equilibrated with 50 mM phosphate buffer (pH 3.5) under an electric field of 339 V/cm for 10 min before use. Samples were injected by raising the anodic end 21 cm above its normal position for 10–120 s. The UV absorption of sertraline was detected at 214 nm using a commercial UV detector (Model: SSI 500; Thermo Separation Products, San Jose, CA, USA) that was equipped with a CE flow cell module. A computer that was connected to the Peak-ABC chromatography data handling system (Great Tide Instrument Company, Taipei, Taiwan) was used to record the electropherograms and for the quantitative measurements of the peak area.

2.4. Preparation of urine samples

Human urine samples were collected from healthy volunteers who were not receiving any pharmaceutical treatment at the time of sampling. The urine samples were stored at −20 °C until analysis. A 990 μL aliquot of the urine sample was spiked with 10 μL of the sertraline standard. Urine samples of various sertraline concentrations were similarly prepared by spiking the urine with the desired amounts of sertraline. A blank urine sample was prepared by spiking 10 μL of DI water into 990 μL of urine. The pH of the urine samples was adjusted to 10 by the addition of 2 μL of concentrated NaOH solution (5 M). The sertraline-spiked urine (1 mL) was treated with DLLME following the procedure described above.

3. Results and discussion

3.1. Optimization of DLLME

The selection of the disperser solvent and the extraction solvent are critical factors in DLLME. Various extraction solvents (dichloromethane, carbon tetrachloride, chloroform and chlorobenzene) and disperser solvents (acetone, methanol, and acetonitrile) were evaluated for extraction efficiency. Thirty microliters of the extraction solvent were dissolved in 200 μL of the disperser solvent. The combination of dichloromethane and acetone provided the highest extraction efficiency. The effect of the disperser solvent volume on the signal intensity was next investigated.

The results indicated that the signal intensities increased as the disperser solvent volume was increased from 160 to 200 μL. When lower volumes of acetone were used, the cloudy solution did not form well, which resulted in a decrease in the signal intensities. When higher volumes of acetone were used, the solubility of the sertraline in water increased and the signal intensities decreased. To investigate the effect of the extraction solvent volume, different volumes of dichloromethane (i.e., 30, 40, 50, 60, and 70 μL) were subjected to identical DLLME procedures. From those samples, 20, 30, 40, 50, and 60 μL, respectively, of the sediment phases were collected for analysis. The peak areas for the different extraction solvent volumes were all similar, and a 30 μL volume of the extraction solvent volume was chosen for all subsequent experiments.

The pH of the aqueous solution significantly affects the analyte extractions using the DLLME method. Fig. 1 shows the effect of pH on the peak area of sertraline. The signal intensities of sertraline increased as the pH of the solution was increased from 7.0 to 10.0 and then remained constant at pH values between 10.0 and 11.0. Neutral analytes are more efficiently extracted by organic solvents than analytes in their ionic forms because of their high affinity for the organic solvent. According to the literature [16], the pK_a value of sertraline is 8.9. When the pH of the solution was greater than the pK_a value of sertraline, the sertraline was existed in its neutral form and was efficiently extracted using the DLLME method. Therefore, the pH of the solution was set to 10. The extraction time is also an important factor in DLLME. In DLLME, the extraction time is defined as the time interval between the injection of disperser and extraction solvent mixture before starting the centrifugation process. The optimal extraction time was investigated by plotting the signal intensities of sertraline versus the extraction time in the range of 1–5 min while keeping all other parameters constant (data not shown). The results indicated that the extraction equilibrium was achieved within 1 min. Due to the large surface contact between the solvent droplets and the aqueous sample, the mass transfer from the sample solution to the extraction solvent is very rapid. This rapid mass transfer is the most important advantage of the DLLME technique.

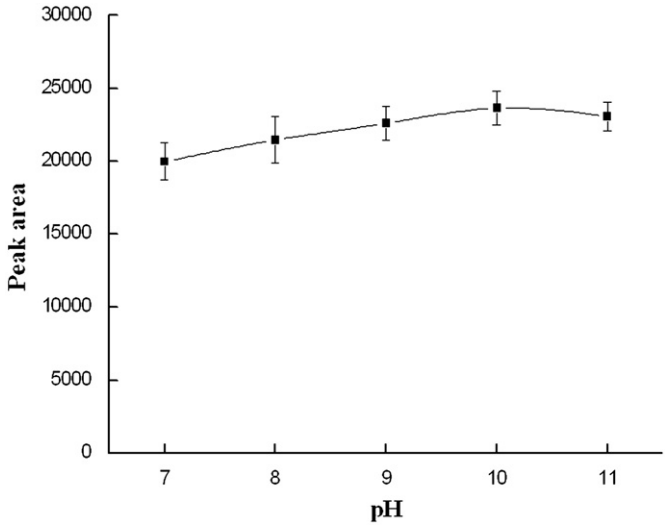

Fig. 1. The effect of pH on the DLLME efficiency. Conditions: aqueous sample volume, 1 mL; dispersive solvent, acetone (200 μL); organic extraction solvent; dichloromethane (30 μL); extraction time, 1 min; collected extraction solvent volume, 20 μL; sertraline concentration, 1.0 μM; CE buffer, 50 mM sodium phosphate (pH 3.5); CE voltage, 22 kV; hydrodynamic injection, 10 s at a height of 21 cm.

The repeatability of the DLLME procedure was examined by injecting three standard 1 μM sertraline solution one time on the same day. The RSD values of migration time and peak area were 0.6 and 4.8%, respectively. For injection of sertraline standard solution without DLLME, a calibration curve for sertraline was constructed over a concentration range of 5–300 μM. The plot of the ratio of the sertraline/I.S. peak area versus concentration presented good linearity ($y=0.16x+0.05$) with a correlation coefficient (r) of 0.9992 ($n=8$). The LOD ($S/N=3$) value for sertraline was calculated to be 2.1 μM. Using DLLME to extract the sertraline from a 1 mL buffer solution, a calibration curve for sertraline was constructed over the concentration range of 0.1–10 μM. The plot of the ratio of the sertraline/I.S. peak area versus concentration presented good linearity ($y=11.32x+0.22$) with a correlation coefficient (r) of 0.9978 ($n=8$). The LOD value for sertraline was calculated to be 28.3 nM. These results are summarized in Table 1. The sensitivity enhancement was estimated based on a previously reported method [31]. Compared with the injection of standard solution without DLLME, a 74-fold improvement in the sensitivity was observed when DLLME was used to extract sertraline. The theoretical sensitivity enhancement was calculated to be 133. In the future, the sensitivity enhancement may be improved by using organic solvent with different polarity.

3.2. FASS

The detection sensitivity could be further enhanced using FASS during CE. The most critical factor in FASS is that the conductivity of the electrolyte must be at least 10 times higher than that of the sample. This is often achieved by diluting the sample or reconstituting the extract residue with water [32,33]. In our experiment, the solubility of sertraline in water was low. Therefore, the DLLME extract residue was dissolved with 5 μL of water that contained 20% methanol.

The effect of the injection time on the enrichment of sertraline was investigated by changing the injection time from 10 to 150 s, which corresponded a change in the injection volume from 20 to 300 nL. Fig. 2 shows the electropherograms for different injection times. The peak heights and areas increased as the injection time was increased from 10 to 120 s. A further increase in the injection time did not increase the peak height of sertraline. In addition, the peaks of sertraline and the I.S. overlapped for injection time longer than 120 s. When compared with the 10 s injection, the theoretical plate number of the 120 s injection time decreased from 151,182 to 55,733. The signal, however, was enhanced by a

Table 1
Analytical characteristics of the determination of sertraline in aqueous solution.

Method	Linear range (μM)	Regression equation[a]	r[b]	LOD (nM)	Sensitivity enhancement
Injection of standard solution	5–300	$y=0.16x+0.05$	0.9992	2100.0	
DLLME	0.1–10	$y=11.32x+0.22$	0.9978	28.3	74
DLLME and FASS	0.01–1	$y=6.74x+0.13$	0.9991	2.5	814

[a] y, peak area ratio of analyte to I.S.; x, analyte concentration.
[b] Correlation coefficient.

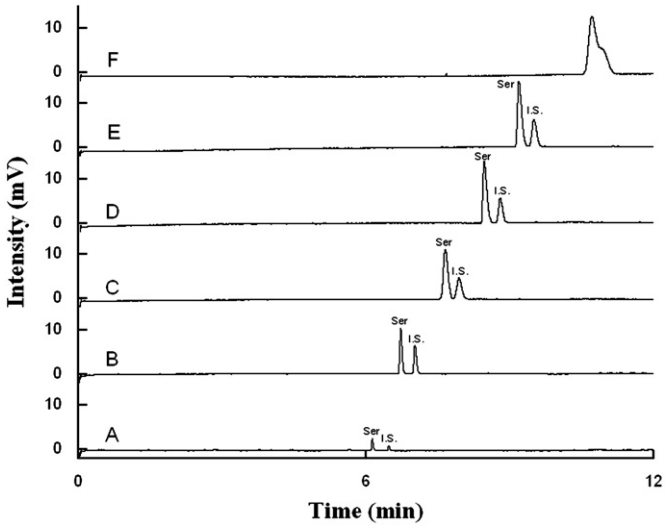

Fig. 2. Electropherograms of different injection times. (A) 10 s (B) 30 s (C) 60 s (D) 90 s (E) 120 s (F) 150 s. Conditions: sertraline concentration, 0.5 μM; I.S., 10 μM. All other conditions were the same as in Fig. 1.

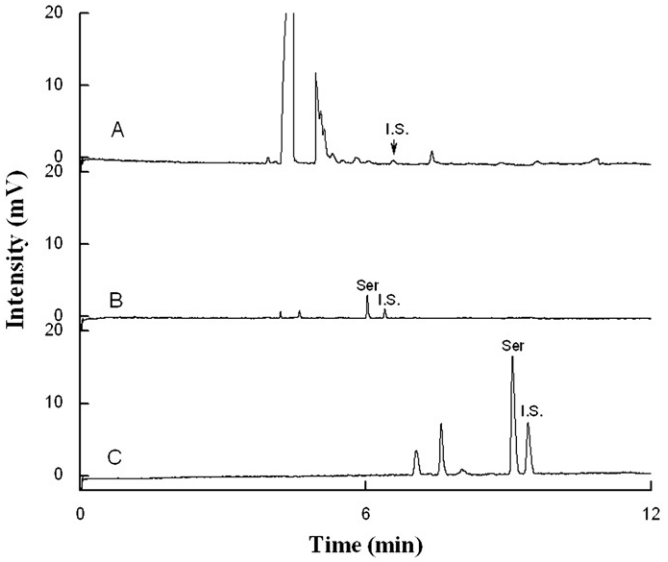

Fig. 3. Electropherograms of sertraline-spiked urine samples obtained by (A) direct analysis (B) with DLLME, and (C) with DLLME and FASS. Conditions: urine sample volume, 1 mL; dispersive solvent, acetone (200 μL); organic extractant; dichloromethane (50 μL); extraction time, 1 min; collected extraction solvent volume, 20 μL; sertaline concentration spiked, 0.5 μM; I.S., 10 μM. The other conditions were the same as in Fig. 1.

factor of 11. Therefore, the injection time was set to 120 s for subsequent experiments. The total capillary volume was calculated to be 2871 nL. As a result, the injection volume of a 120 s injection time is approximately 8.4% of the capillary volume. The repeatability of the FASS procedure was examined using seven injections of a standard sertraline solution (0.5 μM) on the same day. The RSD values of migration time and peak area were 1.1 and 6.4%, respectively.

3.3. Analytical characteristics

A calibration curve for sertraline was constructed over the concentration range of 0.01–1 μM using citalopram (10 μM) as an I.S. A plot of the ratio of the sertraline/I.S. peak area versus concentration presented good linearity ($y=10.49x+0.18$) with a correlation coefficient (r) of 0.9969 ($n=8$). The LOD and LOQ ($S/N=10$) values for sertraline in aqueous solutions were calculated to be 2.5 and 8.3 nM, respectively. By combining the described DLLME and the FASS technique, an 814-fold improvement in the sensitivity over the injection of standard solution without the DLLME and FASS procedures was observed for sertraline. The repeatability of this method was examined by injecting three standard 0.5 μM sertraline solution two times on the same day. The RSD values of migration time and peak area were 1.7 and 7.1%, respectively.

3.4. Applications

To evaluate the applicability of this method for biological and clinical analyses, human urine spiked with sertraline was used as a test sample. The sertraline-spiked urine (1 mL) was treated with the DLLME technique using the procedure described above. For the urine samples, the sediment phase volume was less than 20 μL. Therefore, 50 μL of the extraction solvent was used, and 20 μL of the extraction solvent was collected. Fig. 3A presents the result from the injection of the sertraline-spiked urine sample without DLLME procedure. There was no sertraline peak present without DLLME. After treatment using the DLLME method, the sertraline peak was obtained, as shown in Fig. 3B. When DLLME was coupled with FASS, the sertraline signal intensity was greatly enhanced (Fig. 3C). In addition, the use of DLLME effectively eliminated the interferences that were due to the complex matrix in the urine samples. Calibration curves were constructed using the sertraline-spiked urine in the concentration range of 0.05–1 μM. The plot of the ratio of the sertraline/I.S. peak area versus concentration presented good linearity ($y=4.00x+0.07$) with a correlation coefficient (r) of 0.9977 ($n=7$). The LOD and LOQ values for sertraline in urine were calculated to be 5.1 and 16.9 nM, respectively. The LOD obtained with this method was lower than that reported using LC/MS coupled with SPME [7] for urine analysis. Although GC/MS coupled with SPME [34] provided a lower LOD value for sertraline in urine, more complicated sample preparation and instrumentation are required.

Table 2
Within-day and between-day recovery and precision for CE–UV analysis of sertraline in human urine ($n=5$).

Nominal concentration (μM)	Within-day		Between-day	
	Recovery[a] (%)	Precision[b] (%)	Recovery (%)	Precision (%)
0.05	92.2	7.5	90.6	8.7
0.50	95.0	5.5	93.8	7.1
1.00	97.7	3.5	96.3	4.5

[a] Recovery expressed as [(mean observed concentration)/(nominal concentration)] × 100.
[b] Precision expressed as RSD.

The selectivity of the method was tested by analyzing blank urine samples obtained from six healthy adults. There were no endogenous compounds found at the migration times of sertraline or I.S. for the six tested samples that were tested. The within-day and between-day recovery and precision of the method were evaluated using urine samples that were spiked with low (0.05 μM), medium (0.50 μM) and high (1.00 μM) concentrations of sertraline. The results of these analyses are presented in Table 2. The within-day and between-day recoveries for the three spiked levels of sertraline in urine were 92–98 and 91–96%, respectively. The within-day and between-day precisions, which are represented as RSDs, for the three spiked levels of sertraline were 3.5–7.5 and 4.5–8.7%, respectively. These values meet the

accuracy and precision criteria set forth in the guidelines on bioanalytical method validation [35].

4. Concluding remarks

We have developed a DLLME method for the extraction of sertraline from aqueous solutions. Coupled with CE detection and the FASS technique, this method provides the advantages of simplicity, rapidity, and high sensitivity. The analysis of sertraline in an aqueous solution can be readily performed in less than 15 min. We also successfully applied this new developed method to the determination of sertraline in a human urine sample. The use of DLLME effectively eliminated the interferences caused by the complex biological matrix of the urine sample. This developed CE–UV method, with its speed and ease of operation, is suitable for monitoring of sertraline levels in human urine samples.

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References

[1] J. Guy Edwards, I. Anderson, Drugs 57 (1999) 507–533.
[2] Y. Sasajima, L.W. Lim, T. Takeuchi, K. Suenami, K. Sato, Y. Takekoshi, Anal. Sci. 26 (2010) 693–698.
[3] M.E. Bosch, A.J. Ruiz Sánchez, F. Sánchez Rojas, C. Bosch Ojeda, J. Pharm. Biomed. Anal. 48 (2008) 1290–1302.
[4] M.X. Zhou, J.P. Foley, J. Chromatogr. A 1052 (2004) 13–23.
[5] A. Khraiwesh, I. Papoutsis, P. Nikolaou, C. Pistos, C. Spiliopoulou, S. Athanaselis, J. Chromatogr. B 879 (2011) 2576–2582.
[6] J.P. Lamas, C. Salgado-Petinal, C. García-Jares, M. Llompart, R. Cela, M. Gómez, J. Chromatogr. A 1046 (2004) 241–247.
[7] L.P. Melo, A.M. Nogueira, F.M. Lancas, M.E.C. Queiroz, Anal. Chim. Acta 633 (2009) 57–64.
[8] A.R. Chaves, F.Z. Leandro, J.A. Carris, M.E.C. Queiroz, J. Chromatogr. B 878 (2010) 2123–2129.
[9] G. Bahrami, B. Mohammadi, A. Farshchi, G. Ghiasi, Chromatography 70 (2009) 323–327.
[10] N.Y. Khalil, A.M. Mahmoud, I.A. Darwish, A.A. Al-Majed, Chromatography 71 (2010) 825–831.
[11] M.-M. Zheng, S.-T. Wang, W.-K. Hu, Y.-Q. Feng, J. Chromatogr. A 1217 (2010) 7493–7501.
[12] J.M. Conley, S.J. Symes, S.A. Kindelberger, S.M. Richards, J. Chromatogr. A 1185 (2008) 206–215.
[13] D. Schaller, E.F. Hilder, P.R. Haddad, Anal. Chim. Acta 556 (2006) 104–111.
[14] V. Pucci, S. Fanali, C. Sabbioni, M.A. Raggi, J. Sep. Sci. 25 (2002) 1096–1100.
[15] A. Musenga, E. Kenndler, L. Mercolini, M. Amore, S. Fanali, M.A. Raggi, Electrophoresis 28 (2007) 1823–1831.
[16] H.-L. Su, Y.-Z. Hsieh, J. Chromatogr. A 1209 (2008) 253–259.
[17] M. Himmelsbach, C.W. Klampfl, W. Buchberger, J. Sep. Sci. 28 (2005) 1735–1741.
[18] Y. Sasajima, L.W. Lim, T. Takeuchi, K. Suenami, K. Sato, Y. Takekoshi, J. Chromatogr. A 1217 (2010) 7598–7604.
[19] K.M. Fried, P.E. Nolan, M. Anthony, R.L. Woosley, M.P. Freeman, Chromatography 73 (2011) 749–754.
[20] L. Labat, M. Deveaux, P. Dallet, J.P. Dubost, J. Chromatogr. B 773 (2002) 17–23.
[21] L.E.E. Eibak, G.A. jelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Pharm. Biomed. Anal. 57 (2012) 33–38.
[22] M. Rezaee, Y. Assadi, M.-R.M. Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[23] A. Zgola-Grześkowiak, T. Grześkowiak, Trends Anal. Chem. 30 (2011) 1382–1399.
[24] S.S. Caldas, F.P. Costa, E.G. Primel, Anal. Chim. Acta 665 (2010) 55–62.
[25] A.V. Herrera-Herrera, M. Asensio-Ramos, J. Hernández-Borges, M.A. Rodríguez-Delgado, Trends Anal. Chem. 29 (2010) 728–751.
[26] L. Meng, B. Wang, F. Luo, G. Shen, Z. Wang, M. Guo, Forensic Sci. Int. 209 (2011) 42–47.
[27] S. Zhang, C. Li, S. Song, T. Feng, C. Wang, Z. Wang, Anal. Methods 2 (2010) 54–62.
[28] C. Zhou, S. Tong, Y. Chang, Q. Jia, W. Zhou, Electrophoresis 33 (2012) 1331–1338.
[29] F.E.P. Mikkers, F.M. Everaerts, Th.P.E.M. Verheggen, J. Chromatogr. 169 (1979)1–10 169 (1979).
[30] S.L. Simpson, Jy., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504–541.
[31] M.-D. Li, T.-L. Cheng, W.-L. Tseng, Electrophoresis 30 (2009) 388–395.
[32] D.S. Burgi, R.-L. Chien, Anal. Chem. 63 (1991) 2042–2047.
[33] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A–496A.
[34] C. Salgado-Petinal, J.P. Lamas, C. Garcia-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 382 (2005) 1351–1359.
[35] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Rockville, MD, USA 2001.