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Determination of free and total valproic acid in human plasma by capillary electrophoresis with contactless conductivity detection

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ABSTRACT

A new approach for the determination of free and total valproic acid in small samples of 140 μL human plasma based on capillary electrophoresis with contactless conductivity detection is proposed. A dispersive liquid–liquid microextraction technique was employed in order to remove biological matrices prior to instrumental analysis. The free valproic acid was determined by isolating free valproic acid from protein-bound valproic acid by ultrafiltration under centrifugation of 100 μL sample. The filtrate was acidified to turn valproic acid into its protonated neutral form and then extracted. The determination of total valproic acid was carried out by acidifying 40 μL untreated plasma to release the protein-bound valproic acid prior to extraction. A solution consisting of 10 mM histidine, 10 mM 3-(*N*-morpholino)propanesulfonic acid and 10 μM hexadecyltrimethylammonium bromide of pH 6.5 was used as background electrolyte for the electrophoretic separation. The method showed good linearity in the range of 0.4–300 μg/mL with a correlation coefficient of 0.9996. The limit of detection was 0.08 μg/mL, and the reproducibility of the peak area was excellent (RSD = 0.7–3.5%, $n = 3$, for the concentration range from 1 to 150 μg/mL). The results for the free and total valproic acid concentration in human plasma were found to be comparable to those obtained with a standard immunoassay. The corresponding correlation coefficients were 0.9847 for free and 0.9521 for total valproic acid.

1. Introduction

Valproic acid (2-propylvaleric acid, VPA) is an eight-carbon branched-chain fatty acid. Its structure is shown in Fig. 1 together with that of caproic acid which was used as internal standard. Valproic acid is used widely as an anticonvulsant [1] and as a mood-stabilizing drug in patients with bipolar disorder [2]. Although the mechanisms of action of valproic acid in epilepsy and bipolar disorder are currently not fully understood, the most widely accepted processes for its antiepileptic activity involve an increase in the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in certain brain regions and an inhibition of voltage-dependent sodium channels [3].

Taking into account the pK_a of VPA of 4.6, most valproate in serum is deprotonated under physiological conditions. Since VPA is highly bound to albumin (approximately 80–95%), only a small fraction of VPA exists in the free, pharmacologically active form [4,5]. The therapeutic range reported for total VPA in human plasma is 50–100 μg/mL [6]. Therapeutic drug monitoring (TDM) of VPA is commonly performed for guiding therapy as there is only a poor correlation between dose and steady state serum concentrations between patients [7] and the difficulty to monitor the clinical effect of valproic acid, since seizures are usually rare events. Detailed discussions are available regarding TDM of VPA in the treatment of epilepsy [7,8] and bipolar disorders [9].

Several methods have been published for the determination of free and total VPA in biological matrices. For the determination of the total concentration, VPA is usually released from proteins by acidification [10–12], which converts it into its protonated form. An alternative method of destroying the protein-binding is precipitation of the serum proteins, e.g. by addition of an organic solvent (see

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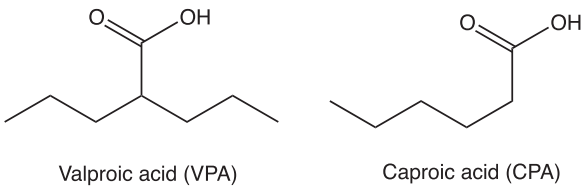

Fig. 1. Structures of valproic acid (VPA) and caproic acid (CPA) used as internal standard (IS).

for example [13]). For the determination of free VPA in the presence of serum proteins and protein-bound VPA, free VPA is removed by a separation step such as dialysis, ultrafiltration, ultracentrifugation or gel filtration [14–16].

In both approaches for the quantification step the most commonly used methods are enzyme immunoassays [17,18]. This technique is simple and reliable, but relatively expensive. A number of chromatographic techniques such as gas chromatography (GC) [19] and liquid chromatography (LC) [20–23] have also been reported, and have been used in conjunction with various sample pretreatment steps. Commonly used pretreatments are, for instance, liquid–liquid extraction (LLE) [24], solid phase extraction (SPE) [10], solid-phase microextraction (SPME) [25], liquid-phase microextraction (LPME) [12] and dispersive liquid–liquid microextraction (DLLME) [11]. A major drawback of the reported chromatographic approaches is the requirement of prior derivatization of VPA to either render it volatile or suitable for UV-detection.

More recently, capillary electrophoresis coupled with contactless conductivity detection (CE-C^4D) has become an attractive alternative analytical method due to its universal characteristics in detecting any charged species without requiring a chromophore. A further distinct advantage is the ability to carry out an analysis in very small sample volumes. Several recent general review articles on CE-C^4D are available [26–28]. A series of applications of the method for clinical analysis of diverse biological samples have been reported [29–40]. Recent reviews on the applications of CE-C^4D in pharmaceutical analysis [41,42] can also be found.

The potential usefulness of CE-C^4D for the determination of VPA in clinical samples has been shown by Belin et al. [13]. However, in these investigations, no distinction between free and protein-bound VPA was made and the amount of biological sample used was too high for monitoring pediatric patients. We therefore improved this method by reducing the plasma sample size needed and by making the method suitable for the determination of both free and total VPA.

2. Experimental

2.1. Reagents and materials

All chemicals were at least of analytical grade and purchased from Aldrich or Fluka (both Buchs, Switzerland). Ultrapure deionized water was produced using a Nano-Pure water purification system (Barnstead, IA, USA). Separation buffers were prepared daily. Stock solutions of VPA sodium salt and caproic acid sodium salt (CPA) as internal standard (IS) at the concentration of 1000 μg/mL were prepared in deionized water and kept at 4 °C. Working standard solutions of lower concentrations were prepared by dilution with deionized water.

2.2. Plasma samples

Blank and VPA containing plasma samples were obtained from the Clinical Pharmacology and Toxicology Laboratory of the University Hospital of Basel, Switzerland. All plasma samples were kept at −20 °C in a freezer until the experiments. The reference values for free and total VPA content in the collected plasma samples were measured using standard protocols adopted at the Clinical Chemistry Laboratory of the University Hospital of Basel. The total VPA concentration was determined using a homogenous enzyme immunoassay in a Cobas 6000 analyzer (Roche Diagnostics, GmbH, Mannheim, Germany) using reagents from Roche Diagnostics (Basel, Switzerland) instrument. The free VPA was determined by first carrying out ultracentrifugation for isolation of the free VPA followed by a fluorescence polarization immunoassay on a TDx analyzer (Abbott Laboratories, Abbott Park, IL, USA).

2.3. Sample pretreatment procedure

For the determination of free VPA, 100 μL of plasma sample was pretreated by ultracentrifugation using Amicon ultracentrifugal filters (cut off >10,000 Da) (Millipore Corporation, Billerica, MA, USA) for 15 min at 14,000 × *g*. After ultrafiltration, 40 μL of the filtrate, which contained free VPA, was placed into a 1.5 mL conical bottom polypropylene tube. Subsequently, 10 μL of a solution containing 25 μg/mL CPA (internal standard resulting in a final concentration of 5 μg/mL) was added and the sample acidified with 10 μL of 1 M HNO_3 to protonate VPA. The mixture was vortexed for 30 s and VPA extracted as described below. For the determination of total VPA, 10 μL internal standard and 10 μL 1 M HNO_3 were added directly to 40 μL of the raw plasma sample.

The optimization of the extraction step was carried out by using blank plasma samples into which VPA was spiked at the same level as the internal standard. For the extraction a mixture of extraction and dispersive solvent was rapidly injected into the sample tube, the solution vortexed for 30 s and finally centrifuged for 10 min at 6000 × *g* at room temperature. After centrifugation, the lower (organic) phase was withdrawn using a 100 μL microsyringe and transferred to a 200 μL polypropylene bullet tip tube. 20 μL of triethylamine (TEA) solution of different concentrations (see Section 3) was then added to the collected organic phase, vortexed for 30 s, and centrifuged for 10 min at 6000 × *g*. The target analyte was back-extracted into the diluted TEA solution and the supernatant was injected into the CE-C^4D system.

2.4. CE-C^4D analysis

The capillary electrophoresis instrument was purpose-built and utilized a commercial high voltage power supply module (CZE 2000R, Spellman, Pulborough, UK). The C^4D detector was built-in-house, details can be found elsewhere [43]. The detector signals were recorded with an e-corder data acquisition system (eDAQ, Denistone East, NSW, Australia). A bare fused silica capillary of 50 μm I.D. and 363 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 50 cm and effective length of 45 cm was employed. The new capillary was conditioned by first flushing with 0.1 M NaOH for 15 min and followed by water for 10 min. The pre-conditioned capillary was then rinsed with the separation buffer for 30 min. The running buffer employed was slightly modified from the previous work [13] and consisted of 10 mM 3-(*N*-morpholino)propanesulphonic acid (MOPS), 10 mM histidine (His), and 10 μM hexadecyltrimethylammonium bromide (CTAB) (pH 6.5). After each injection, the capillary was rinsed with separation buffer for 3 min to maintain the reproducibility of the analysis. Injections were performed by siphoning at 18 cm height difference for 10 s. The separation voltage was set at −16.5 kV.

3. Results and discussion

3.1. Optimization of the dispersive liquid–liquid microextraction

First tests were carried out using direct injection of plasma samples into the CE system as reported previously [13]. It was found however, that some samples showed overlaps with peaks of unknown origin. Therefore an extraction procedure was adopted in order to consistently obtain electropherograms free of undesired matrix elements. Dispersive liquid–liquid microextraction (DLLME) allows efficient extraction of small samples. In this procedure a mixture of two solvents, one soluble in water, the other not, is rapidly injected into an aqueous sample. This leads to the formation of finely dispersed droplets into which the extraction of the analytes occurs. Subsequently, phase separation is performed and the enriched analyte can then be determined in the sedimented phase [44,45]. Several factors affecting the extraction efficiency of DLLME were comprehensively examined to seek for optimum conditions. For these tests, valproate and caproate as internal standard were added to blank plasma samples (both at a final concentration of 5 μg/mL) and these were acidified in order to protonate, and thus neutralize, analyte and internal standard. Caproic acid (CPA) has a molecular structure which is very similar to that of valproic acid (VPA) (see Fig. 1).

3.1.1. Selection of extraction and dispersive solvents

An ideal extraction solvent in DLLME should demonstrate characteristics such as higher density than water, high extraction capability for analytes of interest, low solubility in water, and low volatility [44,46]. On the other hand, the dispersive solvent should be miscible with the extraction solvent as well as the sample solution to enlarge the contact area between the extraction solvent and the sample solution. Based on these requirements, 3 extraction solvents namely tetrachloroethylene (C_2Cl_4), chloroform ($CHCl_3$) and carbon disulfide (CS_2) were studied in combination with 4 dispersive solvents, i.e. acetonitrile (MeCN), methanol (MeOH), acetone (Ace), and 2-propanol (IPA). It was found that $CHCl_3$ hardly formed an emulsified solution when added to plasma regardless of the dispersive solvent being used. When CS_2 was employed, emulsified solutions were observed, but clear phase separation could not be achieved after centrifugation. Nevertheless, mixtures of C_2Cl_4 with various dispersive solvents studied were found to be able to form satisfactory emulsified solutions and phase separation was instantaneously achieved after the vortex and centrifugation processes. Hence, C_2Cl_4 was selected as extraction solvent and its performance with various dispersive solvents was evaluated. In order to maintain consistency, 13 μL of each dispersive solvent with 87 μL of C_2Cl_4 was always added to the 40 μL of the blank plasma to which acid as well as VPA and CPA had been added. As can be seen in Fig. 2, the highest VPA peak area response was obtained when IPA was used as dispersive solvent. The same result was obtained for CPA.

3.1.2. Effect of extraction/dispersive solvent ratio and volume of solvent mixture

Different ratios of C_2Cl_4:IPA solvent mixtures were studied to seek for optimum extraction conditions. The volume of the solvent mixture was fixed at 100 μL and this was again added to the 40 μL of the blank plasma which had then been acidified and spiked with VPA and CPA. As can be seen from Fig. 3, the peak area response for the VPA extract increased according to the increase of C_2Cl_4 percentage in the mixture. A significant increase of VPA responses was observed from 20% of C_2Cl_4 to 50% and ultimately reached its maximum at 87% of C_2Cl_4. When the percentage of C_2Cl_4 was further increased, no significant further enhancement of VPA and CPA response was observed. Hence, the C_2Cl_4:IPA ratio of 87:13 was adopted.

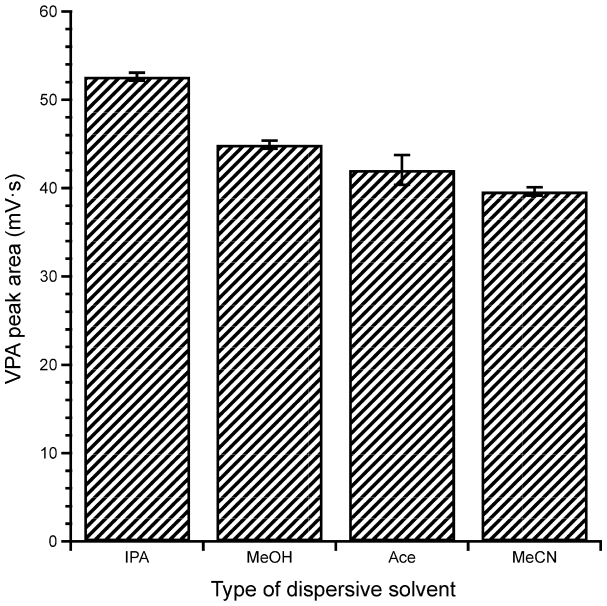

Fig. 2. Effect of dispersive solvents on the peak area response of VPA ($n = 3$). Extraction conditions: sample volume, 50 μL; extraction solvent, 87 μL C_2Cl_4; dispersive solvent, 13 μL; concentration of VPA, 5 μg/mL.

To consider the effect of the solvent volume on extraction efficiency, different volumes of C_2Cl_4:IPA mixtures with the optimum ratio of 87:13 were tested. The volumes ranged from 50 to 175 μL. It was found that when even smaller volumes were employed (<50 μL), the organic droplets were not properly formed and not well-dispersed in the relatively viscous plasma sample. As can be seen from Fig. 4, the amount of VPA detected increased significantly by increasing the solvent volume from 50 to 125 μL and then reached a maximum in the range from 125 to 175 μL. Although the total solvent volume used in this study is relatively high compared to the amounts used in most of the studies reported, the

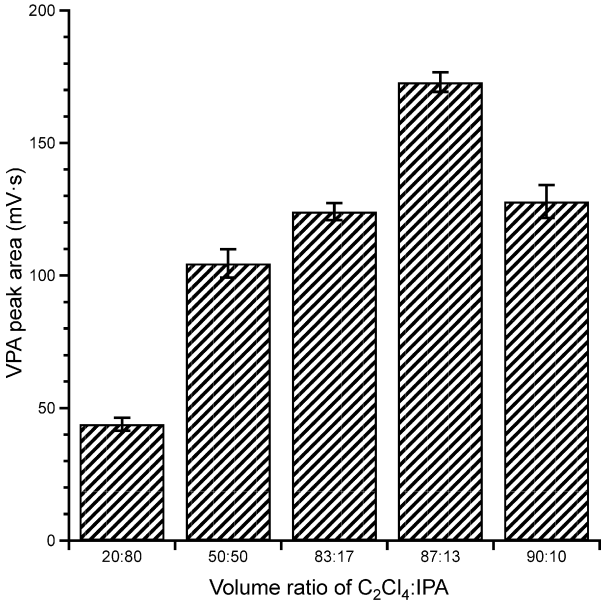

Fig. 3. Effect of the volume ratio of C_2Cl_4:IPA on the peak area response of VPA ($n = 3$). Extraction conditions: extraction solvent, C_2Cl_4; dispersive solvent, IPA; total solvent volume, 100 μL. Other conditions as for Fig. 2.

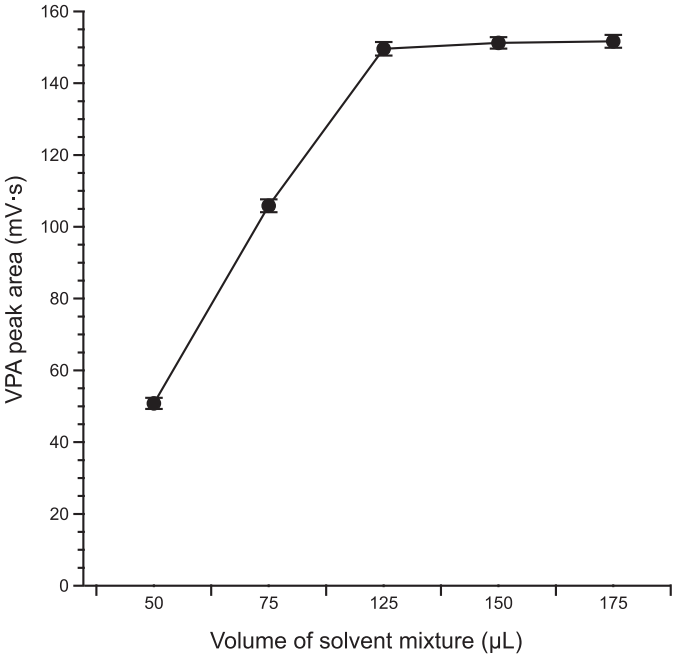

Fig. 4. Effect of the volume of solvent mixture on the peak area response of VPA ($n = 3$). Extraction conditions: ratio of extraction solvent (C_2Cl_4):dispersive solvent (IPA), 87:13. Other conditions as for Fig. 2.

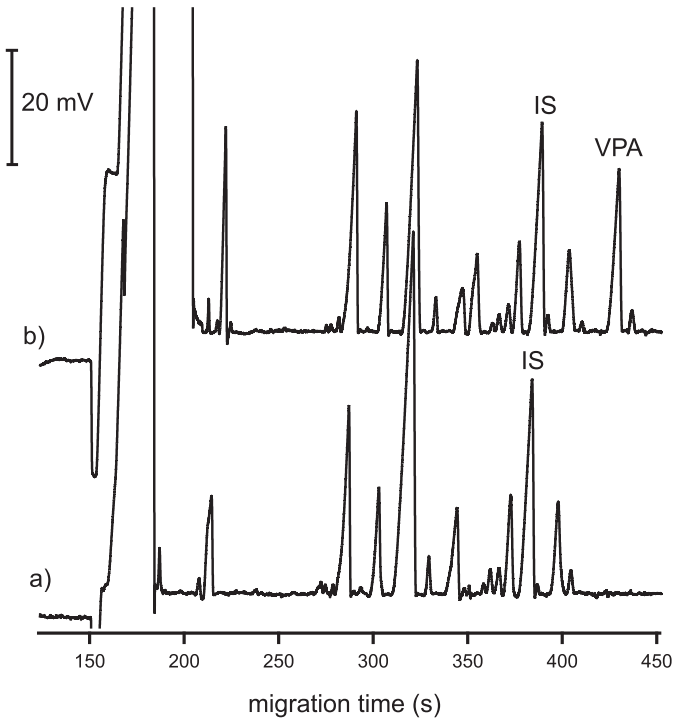

Fig. 5. Electropherogram for (a) blank plasma spiked with CPA (5 μg/mL, as internal standard, IS) and (b) blank plasma spiked with VPA (5.4 μg/mL) and CPA (5 μg/mL). CE conditions: buffer 10 mM MOPS/10 mM His, pH 6.5, CTAB 10 μM, siphoning injection at 18 cm height difference for 10 s, separation voltage −16.5 kV.

new approach involves an additional back-extraction procedure that effectively transfers the analyte into only 20 μL of TEA solution prior to CE-C^4D analysis. In order to ensure a high consistency of extraction performance, the solvent volume was fixed to 150 μL for the subsequent experiments.

3.1.3. Optimization of triethylamine percentage for back extraction

As mentioned previously, the VPA enriched in the organic phase was back-extracted into a diluted aqueous solution of TEA [24], which was compatible with the subsequent CE-C^4D analysis. 20 μL of this solution was used as this was the minimum volume which could be handled reliably with the CE-system employed. Concentrations of 0.05%, 0.1%, 0.25%, 0.5%, 1% and 2.5% were tested for their suitability. For TEA solutions of 0.05% and 0.1%, the extraction recoveries for VPA were generally unsatisfactory with values of 47–62%. An increase of the TEA percentage to 0.25% and 0.5% resulted in improved extraction recoveries of 86%. The result for CPA was identical. For higher concentrations, poor baseline stabilities resulted in the CE-C^4D analysis. Hence, a percentage of 0.5% of TEA was adopted for the back-extraction solution for the subsequent CE-C^4D analyses.

3.2. Method validation

The optimum DLLME parameters finally arrived at were as follows: 40 μL of plasma sample acidified with 10 μL 1 M HNO_3, 10 μL of 25 μg/mL CPA internal standard solution (5 μg/mL final concentration), 150 μL of 87% C_2Cl_4:13% IPA as solvent mixture, and 20 μL of 0.5% TEA solution as back-extraction medium. Normalization of the peak areas obtained for VPA with the peak areas for CPA resulted in a good linearity for VPA with a correlation coefficient of 0.9996 in the concentration range from 0.4 to 300 μg/mL (note that these tests were carried out for unfiltered plasma). This linear range covered the entire therapeutic range of VPA in human plasma which is 5–10 μg/mL for free and 50–100 μg/mL for total valproate. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.08 μg/mL and 0.24 μg/mL, respectively (calculated for signal-to-noise ratios of 3 and 10 from a comparison of peak heights with the maximum amplitude of the short term baseline deviations). The reproducibilities for peak area were found to be between 0.7% and 3.5% (RSD, $n = 3$) for the concentration range from 1 to 150 μg/mL. For illustration, electropherograms for an extract of blank plasma spiked with CPA and for an extract of blank plasma spiked with CPA and VPA are shown in Fig. 5.

Table 1
Quantitative results for free and total VPA in human plasma samples.

Sample ID	Free VPA (μg/mL)		Total VPA (μg/mL)	
	DLLME–CE-C^4D[a]	Immunoassay	DLLME–CE-C^4D[a]	Immunoassay
BVS11	16.2 ± 0.3	14.8	116.2 ± 1.0	99.5
EH4	5.4 ± 0.1	3.7	56.5 ± 1.6	56.5
AP10	4.8 ± 0.2	3.6	37.2 ± 0.7	33.8
JS09	5.4 ± 0.6	5.3	61.1 ± 4.2	63.0
GP14	7.0 ± 1.3	6.0	71.9 ± 10.7	75.7
EH5	4.6 ± 0.2	3.6	43.5 ± 5.3	44.2

[a] Errors are standard deviations ($n = 3$).

3.3. Analysis of human plasma samples

A total of 6 human plasma samples had been collected in a clinical study conducted at the University Hospital of Basel. The plasma samples were first tested using standard protocols based on enzyme immunoassay techniques employed in the Clinical Chemistry Laboratory of the University Hospital of Basel (see Section 2.3 for details), followed by measurement using the developed DLLME–CE-C^4D approach. Note that the appearance of the electropherograms of these samples containing VPA is very similar from those of blank plasma to which VPA had been spiked as shown in Fig. 5. The results for free (for the filtered sample) and total VPA (for the not filtered sample) are summarized in Table 1. It is observed that the overall results obtained using DLLME–CE-C^4D are comparable to the results obtained employing the standard enzyme immunoassay. The correlation coefficients, *r*, for the two pairs of data were determined as 0.9847 for free VPA and 0.9521 for total VPA, indicating an acceptable relationship.

4. Conclusion

In this work, the determination of free and total VPA in a total volume of only 140 μL of human plasma employing CE-C^4D for quantification was developed. The method requires a filtration step in order to be able to distinguish between free and bound analyte and an extraction procedure to avoid potential peak overlaps, but no chemical or enzymatic conversion steps, as needed for the established methods, are required to make the analyte amenable for quantification. The method is deemed suitable for the routine therapeutic drug monitoring (TDM), in particular for pediatric patients for whom the available sample volumes are limited.

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References

[1] C.U. Johannessen, S.I. Johannessen, CNS Drug Rev. 9 (2003) 199.
[2] D.J. Bond, R.W. Lam, L.N. Yatham, J. Affect. Disord. 124 (2010) 228.
[3] C.U. Johannessen, Neurochem. Int. 37 (2000) 103.
[4] C. DeVane, Psychopharmacol. Bull. 37 (2003) 25.
[5] E. Perucca, CNS Drugs 16 (2002) 695.
[6] T. Tatsuhara, H. Muro, Y. Matsuda, Y. Imai, J. Chromatogr. A 399 (1987) 183.
[7] E. Yukawa, Clin. Pharmacokinet. 31 (1996) 120.
[8] E. Kozer, D. Scolnik, W.M. Agamata, S.K. Weiss, Z.H. Verjee, G. Koren, Ther. Drug Monit. 25 (2003) 17.
[9] J. Fleming, M. Chetty, Clin. Neuropharmacol. 29 (2006) 350.
[10] S. Gao, H. Miao, X. Tao, B. Jiang, Y. Xiao, F. Cai, Y. Yun, J. Li, W. Chen, J. Chromatogr. B 879 (2011) 1939.
[11] H.R. Sobhi, A. Kashtiaray, H. Farahani, F. Abrahimpour, A. Esrafili, Drug Test. Anal. 2 (2010) 362.
[12] P. Shahdousti, A. Mohammadi, N. Alizadeh, J. Chromatogr. B 850 (2007) 128.
[13] G.K. Belin, S. Krähenbühl, P.C. Hauser, J. Chromatogr. B 847 (2007) 205.
[14] H. Kurz, H. Trunk, B. Weitz, Arzneimittelforschung 27 (1977) 1373.
[15] T.C. Kwong, Clin. Chim. Acta 151 (1985) 193.
[16] A.C. Metha, TrAC-Trends Anal. Chem. 8 (1989) 107.
[17] S. Cooreman, E. Cuypers, M. De Doncker, P. Van Hee, W. Uyttenbroeck, H. Neels, Immuno-Anal. Biol. Spec. 23 (2008) 240.
[18] A.A. Elyas, V.D. Goldberg, N. Ratnaraj, P.T. Lascelles, Ann. Clin. Biochem. 17 (1980) 307.
[19] J. Darius, J. Chromatogr. B 682 (1996) 67.
[20] C. Lucarelli, P. Villa, E. Lombaradi, P. Prandini, A. Brega, Chromatographia 33 (1992) 37.
[21] M. Nakamura, K. Kondo, R. Nishioka, S. Kawai, J. Chromatogr. B 310 (1984) 450.
[22] J.H. Wolf, L. Veenma-van der Duin, J. Korf, J. Chromatogr. B 487 (1989) 496.
[23] M.-C. Lin, H.-S. Kou, C.-C. Chen, S.-M. Wu, H.-L. Wu, J. Chromatogr. B 810 (2004) 169.
[24] H. Amini, M. Javan, A. Ahmadiani, J. Chromatogr. B 830 (2006) 368.
[25] M. Krogh, K. Johansen, F. Tønnesen, K.E. Rasmussen, J. Chromatogr. B 673 (1995) 299.
[26] W.K.T. Coltro, R.S. Lima, T.P. Segato, E. Carrilho, D.P. de Jesus, C.L. do Lago, J.A.F. da Silva, Anal. Methods 4 (2012) 25.
[27] P. Kubáň, P.C. Hauser, Electrophoresis 32 (2011) 30.
[28] P. Kubáň, P.C. Hauser, Electrophoresis 30 (2009) 3305.
[29] X.Y. Gong, P. Kubáň, A. Scholer, P.C. Hauser, J. Chromatogr. A 1213 (2008) 100.
[30] P. Tůma, K. Málková, E. Samcová, K. Štulík, J. Sep. Sci. 33 (2010) 2394.
[31] P. Tůma, K. Málková, Z. Wedellová, E. Samcová, K. Štulík, Electrophoresis 31 (2010) 2037.
[32] P. Tůma, E. Samcová, Chem. Listy 101 (2007) 200.
[33] P. Tůma, E. Samcová, F. Duška, J. Sep. Sci. 31 (2008) 2260.
[34] P. Tůma, E. Samcová, K. Štulík, Anal. Chim. Acta 685 (2011) 84.
[35] Q.J. Wan, P. Kubáň, J. Tanyanyiwa, A. Rainelli, P.C. Hauser, Anal. Chim. Acta 525 (2004) 11.
[36] P. Kubáň, P.C. Hauser, Lab Chip 8 (2008) 1829.
[37] W.S. Law, P. Kubáň, L.L. Yuan, J.H. Zhao, S.F.Y. Li, P.C. Hauser, Electrophoresis 27 (2006) 1932.
[38] W. Pormsila, S. Krähenbühl, P.C. Hauser, Anal. Chim. Acta 636 (2009) 224.
[39] W. Pormsila, R. Morand, S. Krähenbühl, P.C. Hauser, J. Chromatogr. B 879 (2011) 921.
[40] W. Pormsila, R. Morand, S. Krähenbühl, P.C. Hauser, Electrophoresis 32 (2011) 884.
[41] L. Suntornsuk, Anal. Bioanal. Chem. 398 (2010) 29.
[42] A.A. Elbashir, H.Y. Aboul-Enein, Biomed. Chromatogr. 24 (2010) 1038.
[43] L. Zhang, S.S. Khaloo, P. Kubáň, P.C. Hauser, Meas. Sci. Technol. 17 (2006) 3317.
[44] X.-H. Zang, Q.-H. Wu, M.-Y. Zhang, G.-H. Xi, Z. Wang, Chin. J. Anal. Chem. 37 (2009) 161.
[45] M. Rezaee, Y. Yamini, M. Faraji, J. Chromatogr. A 1217 (2010) 2342.
[46] A.R. Zarei, F. Gholamian, Anal. Biochem. 412 (2011) 224.