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Short communication

Rapid determination of piperazine-type stimulants in human urine by microextraction in packed sorbent after method optimization using a multivariate approach

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ABSTRACT

This paper describes the analysis of piperazine-type stimulants [1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP)] in low volume urine samples (0.1 mL) by microextraction in packed sorbent and liquid chromatography-diode array detection. Analyte extraction has been comprehensively optimized, and the influencing factors were screened by means of the fractional factorial design approach. Several parameters susceptible of influencing the process were studied, and these included extraction sorbent type (C_8 and C_{18}), sample dilution (1:2 and 1:4), number of aspirations through the device (2 and 8) and the amount of methanol on both the washing (0 and 10%) and eluting solvents (10 and 100%). The method was linear from 0.5 (lower limit of quantitation) to 5 $\mu g\,mL^{-1}$, with determination coefficients higher than 0.99 for all compounds. Intra- and interday precision ranged from 1 to 9%, trueness was within a ±11% interval for all analytes, and analyte recoveries were of about 70% for mCPP and TFMPP, and of about 10% for MeOPP and BZP. The method has shown to be selective, as no interferences from endogenous substances were detected by analysis of blank samples, and the analytes were stable in the samples for short periods at room temperature, after three freeze/thaw cycles and in processed samples. Due to its simplicity and speed, this method can be successfully applied in the screening and quantitation of these compounds in urine samples, and is suitable for application in forensic toxicology routine analysis.

1. Introduction

Piperazine-like stimulants are considered a new group of synthetic drugs, and have already been found in the illicit drug market as abused drugs, due to their stimulant effects and capability of producing euphoria. Benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) have been circulating among drug users since the beginning of the century, readily followed by 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP) [1]. Despite the fact that those compounds are usually controlled substances, their use is still legal in some European countries [2].

Due to their potential for use and abuse, identifying and quantifying piperazines in biological specimens assumes particular relevance in forensic toxicology scenarios, as important information regarding drug use can be obtained.

Sample preparation is usually the limiting step in bioanalysis, and as the number of sample increases, high throughput and automated analytical techniques are desired [3,4].

Microextraction by packed sorbent (MEPS) is a recent extraction technique which can be easily connected on-line to either gas or liquid chromatographic systems without the need of modifying the extracting device. This packed syringe can be reused several times, and more than 100 extractions have been reported using plasma or urine samples [5,6]. This approach for sample preparation is very promising because of its ease of use, full automation, speed, reduction of solvent volumes (being therefore more environmentally friendly) and the cost of analysis is minimal compared to conventional solid-phase extraction (SPE) procedures [7]. MEPS also presents advantages over other micro-sampling techniques, e.g. solid-phase micro extraction (SPME), namely the reduction of sample preparation time and sample volume, presenting also higher recoveries (usually above 50%) as well [8,9]. This technique

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has been used in bioanalysis, in the quantitation of local anesthetics in plasma and urine [3,6,9,10], cocaine and metabolites in urine [4], amphetamines in hair [12], olomoucine [13], antipsychotics [14–16] and antidepressants [17] in plasma, cotinine in oral fluid [18], and antiepileptics in plasma and oral fluid [19]. However, its use in the determination of piperazines is not documented yet. Indeed, analytical methods for the determination of those compounds are scarce and normally employ SPE [1,20–22] or liquid–liquid extraction (LLE) [22–24], which require larger amounts of organic solvents and are more time-consuming. A liquid chromatographic–tandem mass spectrometric method using direct injection of the diluted urine sample is also described [25].

This paper describes for the first time the analysis of several piperazines (BZP, TFMPP, mCPP and MeOPP) in urine samples by means of MEPS, after optimization using a multivariate approach. The method was fully validated, allowing its application in clinical and forensic scenarios where the compounds are involved.

2. Experimental

2.1. Reagents and standards

The analytical standards of BZP dihydrochloride, TFMPP hydrochloride and mCPP were purchased from Lipomed (Arlesheim, Switzerland) as 1 mg mL^{-1} solutions; MeOPP dihydrochloride and 1-(2-chlorophenyl)-piperazine (oCPP) hydrochloride (internal standard, IS) were purchased from Sigma–Aldrich (Steinheim, Germany). Ammonium formate and acetic acid (50% purity) were acquired from Sigma–Aldrich (Switzerland). Methanol (HPLC grade) was obtained from Merck Co. (Darmstadt, Germany). Ammonium hydroxide (analytical grade) was obtained from J.T. Baker (Holland). Ultrapure water was obtained from a Milli-Q System (Millipore, Billerica, MA, USA).

Stock solutions of MeOPP and oCPP were prepared at 1 mg mL^{-1} by weighing 10 mg of the compound to a 10 mL volumetric flask, and filling up to volume with methanol. Working solutions at 10 and 1 μg mL^{-1} were prepared for all analytes by proper dilution of the stock solutions with methanol. A working solution of the IS at 10 μg mL^{-1} was prepared also in methanol. All those solutions were stored light protected between 2 and 8 °C.

To prepare the 5 mM ammonium formate solution, 315.3 mg of ammonium formate was weighed into a volumetric flask and a final volume of 1 L was obtained with ultrapure water.

MEPS 250 μL syringe and MEPS BIN (Barrel insert and Needle) each of C_8 and C_{18} (SGE Analytical Science – Australia) were purchased from ILC (Porto, Portugal).

2.2. Biological samples

Blank urine samples were obtained from laboratory staff, and were stored between 2 and 8 °C.

2.3. Sample preparation

Before first use, the extraction sorbents were activated with 5 × 0.1 mL of methanol, and then conditioned with 4 × 0.1 mL of water. The extraction of the analytes was optimized previously (see Section 3.1), and the final conditions were as follows. 25 μL of the IS solution (10 μg mL^{-1}) was added to 0.1 mL urine previously diluted with 0.1 mL of deionised water, and the samples were slightly vortex-mixed for 30 s. The samples were afterwards aspirated and passed through the device 8 times (at an approximate flow rate of 10 μL s^{-1}). Endogenous interferences were removed with 1 × 0.25 mL of 10% methanol in water; the analytes were finally eluted with 1 × 50 μL of methanol and manually injected (off-line) into the HPLC system. After each extraction, the sorbent was cleaned with 5 × 0.25 mL of methanol followed by 4 × 0.25 mL of water, in order to avoid carryover, conditioning it for the next extraction.

Under these optimized conditions, each MEPS device could be used for about 100 extractions.

2.4. Liquid chromatographic conditions

Analyses were carried out using an UPLC system (Agilent 1290 Infinity LC) equipped with an Agilent 1290 Infinity Detector (G4212A DAD). The piperazines were separated in a Zorbax 300 SB-C_{18} (5 μm, 4.6 mm × 150 mm) column (Agilent, Santa Clara, CA, USA) at 25 °C with a mobile phase consisting of 5 mM ammonium formate (pH 6.4) and methanol (55:45, v/v), using the isocratic mode at a flow rate of 0.8 mL min^{-1}. The mobile phase was filtered under vacuum (0.2 μm hydrophilic polypropylene filter) and degassed in ultrasonic bath before use. The detector was set at 236 nm for MeOPP, 211 nm for BZP, 208 nm for mCPP and 246 nm for both TFMPP and IS, and their retention times were, in minutes, 3.2, 4.1, 6.2, 8.4 and 5.5, respectively.

3. Results and discussion

3.1. Optimization of the extraction conditions: fractional factorial design

At an initial stage, the factors which could influence the extraction of the analytes from urine, as well as their interactions, were screened by means of a two-level-five factor half fractional factorial design (2_v^{5-1}) [26]. The studied factors and respective levels (low, high) were sorbent type (C_8, C_{18}), sample dilution (2, 4), number of sample aspirations through the device (strokes) (2, 8), amount of methanol (%) to remove interferences (washing step) (0, 10) and amount of methanol (%) in the eluting solvent (10, 100). If a full factorial design (2^5) had been used in this particular study, 32 experiments would have to be conducted to cover all possible combinations of factors' levels. However, the use of the fractional factorial design allowed reducing this number of experiments to 16 by combining the main effects with the higher order interactions; this is made assuming that the observed effects are due to the main effects only. The amount of methanol in the eluting solvent was the most influencing factor for all analytes; indeed, for BZP, mCPP and TFMPP is the only factor whose influence was statistically significant at the studied levels, while for MeOPP no factor influenced significantly the response (data not shown).

This way, the used approach aided in eliminating 4 non-significant factors, and method optimization could be performed in a univariate fashion regarding the eluting solvent. These non-significant factors were set at those values which originated a better apparent response despite of the lack of significance of the observed effect (C_{18} for extracting sorbent, sample dilution of 1:2, 8 strokes and 10% methanolic wash), and the percentage of methanol in the eluting solvent was varied from 10 to 100. The best results were obtained when 100% methanol was used as eluting solvent, and therefore those were chosen as the final optimized conditions for analyte extraction.

3.2. Method validation

The methodology was fully validated according to the guiding principles of the Food and Drug Administration (FDA) [27] and International Conference on Harmonization (ICH) [28], and the parameters included selectivity, linearity and calibration model, limits, intra- and interday precision and trueness, recovery and stability.

Table 1
Linearity data ($n = 5$).

	Weight	Linear range ($\mu g\,mL^{-1}$)	Linearity		R^2	LLOQ ($\mu g\,mL^{-1}$)
			Slope	Intercept		
MeOPP	$1/y$	0.5–5	0.3457 ± 0.1231	0.0329 ± 0.0675	0.9961 ± 0.0025	0.5
BZP	$1/x$	0.5–5	0.3403 ± 0.1400	0.1029 ± 0.0801	0.9952 ± 0.0009	0.5
mCPP	$1/x^2$	0.5–5	1.3861 ± 0.1787	−0.0225 ± 0.0879	0.9957 ± 0.0017	0.5
TFMPP	$1/x^2$	0.5–5	0.9249 ± 0.0903	0.0616 ± 0.0544	0.9943 ± 0.0016	0.5

Mean values ± standard deviation; LLOQ, lower limit of quantitation.

3.2.1. Selectivity

The selectivity of the method was evaluated by analyzing blank urine samples of ten different origins, and it was checked for interferences at the retention times of the studied compounds. Furthermore, several other abused drugs and metabolites [including amphetamines (amphetamine, methamphetamine, MDMA, MDA, MBDB, MDEA), opiates (morphine, codeine, 6-acetylmorphine, tramadol) and cocaine and metabolites (cocaine, benzoylecgonine, ecgonine methyl ester)], therapeutic drugs (piracetam, trazodone, ketoprofen), alkaloids (papaverine, nicotine, narceine) and caffeine that might be also present in the samples was evaluated for interference at the retention times of the piperazines. No interferences from endogenous substances were observed, and the other compounds presented different retention times and/or could not be detected using our method's conditions.

3.2.2. Calibration curves and limits

Linearity of the method was established on spiked urine samples prepared and analysed using the described extraction procedure in the range of 0.5–5 $\mu g\,mL^{-1}$ (six calibrators evenly distributed, five replicates). Along with each calibration curve, a zero sample (blank sample with IS) and two quality control samples at medium (1.5 $\mu g\,mL^{-1}$) and high (3.5 $\mu g\,mL^{-1}$) concentrations ($n = 3$) were also analysed.

Calibration curves were obtained by plotting the peak-area ratio between each analyte and the internal standard against analyte concentration. To compensate for heteroscedasticity, weighted least squares regressions were adopted ($1/y$ for MeOPP, $1/x$ for BZP, and $1/x^2$ for both mCPP and TFMPP).

Linear relationships were obtained for all compounds, and the calibrators' residuals were considered adequate, being within ±15% of the nominal concentration for all levels, except at the lower limit of quantitation – LLOQ –, for which ±20% was considered acceptable. Calibration data is shown in Table 1.

The LLOQ was defined as the lowest piperazine concentration that could be measured reproducibly and accurately (coefficient of variation of less than 20% and bias within ±20% of the nominal concentration), and was determined by analysing six replicates of spiked urine samples independent from those of the calibration curve. The LLOQ has been found to be 0.5 $\mu g\,mL^{-1}$ for all analytes. Lower concentrations of the analytes could not be detected, and therefore these were also considered as the method's limits of detection.

These limits are in general in accordance to those published elsewhere for piperazines determination in urine samples [23,24], especially taking into account the lower sample amount used (0.1 versus 1 or 2 mL). Fig. 1 shows a chromatogram of a urine sample spiked with all analytes at their LLOQ.

Table 2
Intra- and inter day precision and trueness.

Compound	Spiked	Measured		CV%		RE%	
		Inter-day ($n = 5$)	Intra-day ($n = 6$)	Inter-day ($n = 5$)	Intra-day ($n = 6$)	Inter-day ($n = 5$)	Intra-day ($n = 6$)
MeOPP	0.5	0.50	0.49	7.38	4.92	−1.19	−1.31
	1	1.00	1.09	8.72	2.00	−0.29	9.47
	2	2.01		5.31		0.41	
	3	3.05	3.01	6.54	2.85	1.16	−0.25
	4	4.00		3.32		−0.02	
	5	4.97	5.30	1.46	3.13	−0.70	−5.92
BZP	0.5	0.52	0.44	2.48	1.98	3.30	−11.00
	1	0.98	0.97	7.52	5.31	−2.26	−3.15
	2	1.92		5.39		−4.06	
	3	3.11	3.13	4.91	2.37	3.69	−4.44
	4	3.96		4.00		−1.01	
	5	5.02	5.24	3.63	2.46	0.34	−4.74
mCPP	0.5	0.49	0.48	2.91	3.89	−2.11	3.95
	1	1.05	1.07	5.07	2.85	4.11	7.10
	2	2.02		3.18		1.03	
	3	2.96	2.80	2.89	2.78	−1.51	−6.53
	4	3.88		3.95		−3.12	
	5	5.04	4.86	5.31	1.23	0.48	2.71
TFMPP	0.5	0.49	0.49	1.26	5.12	−2.82	−1.42
	1	1.05	1.01	2.89	3.77	4.58	−7.01
	2	2.05		6.49		2.28	
	3	3.09	3.32	4.44	2.51	2.84	8.63
	4	3.84		3.13		−4.14	
	5	4.80	4.84	2.15	1.10	−4.22	3.13

All concentrations in $\mu g\,mL^{-1}$. CV, coefficient of variation; RE, relative error [(spiked concentration − nominal concentration/nominal concentration) × 100].

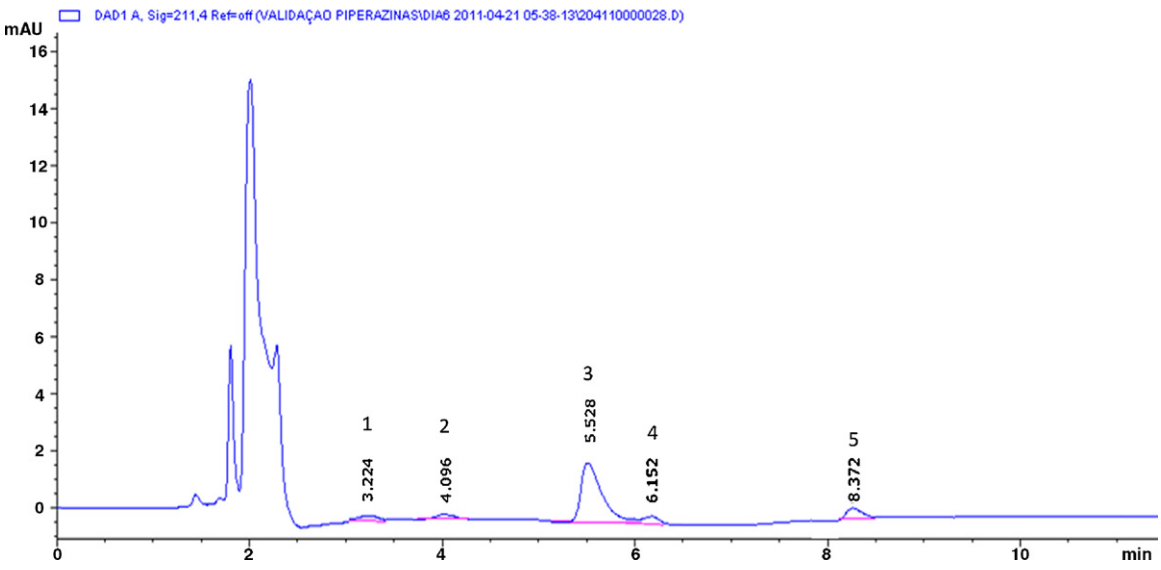

Fig. 1. Chromatogram of a sample spiked at the LLOQ (0.5 μg mL^{-1}). MeOPP (1), BZP (2), oCPP (IS) (3), mCPP (4) and TFMPP (5).

3.2.3. Intra- and interday precision and trueness

Intra-day precision and trueness were evaluated by analyzing in the same day 6 replicates of blank urine samples spiked with piperazines at 4 concentration levels (0.5, 1, 3 and 5 μg mL^{-1}). The obtained coefficients of variation (CVs) were in general lower than 6% for all compounds at all tested concentrations, presenting a mean relative error within a ±11% interval. Interday precision and trueness were evaluated at six concentrations within a 5-day period. The calculated CVs were lower than 9% for all compounds at all concentration levels, while trueness (in terms of mean relative error) was within a ±5% interval. Table 2 presents intra- and interday precision and trueness data.

In addition, combined intra- and interday intermediate precision was assessed by analysis of the quality control samples (1.5 and 3.5 μg mL^{-1}) which were analysed in triplicate over the same 5-day period (15 measurements) (Table 3).

3.2.4. Extraction efficiency

Extraction efficiency was evaluated in sixtuplicate at both 2 and 4 μg mL^{-1}. The samples were spiked at the desired concentrations and were extracted as previously described. The obtained peak areas for each analyte were compared to those peak areas obtained after methanolic injections (n = 6) of the analytes at the same concentrations. The obtained values for efficiency are presented in Table 4. While for mCPP and TFMPP high extraction efficiencies were obtained at both concentrations (70–80%), the extraction of MeOPP and BZP was much less efficient.

Table 3
Intermediate precision and trueness data (n = 15).

Compound	Spiked	Measured	CV%	RE%
MeOPP	1.5	1.56	4.66	4.00
	3.5	3.39	4.63	−3.14
BZP	1.5	1.51	6.14	0.51
	3.5	3.35	3.51	−4.36
mCPP	1.5	1.57	5.11	4.73
	3.5	3.34	4.28	−4.49
TFMPP	1.5	1.52	8.55	1.34
	3.5	3.35	5.22	−4.25

All concentrations in μg mL^{-1}. CV, coefficient of variation; RE, relative error [(spiked concentration − nominal concentration/nominal concentration) × 100].

Concerning piperazine determination in urine, Vorce et al. [23] reported recovery values from 97 to 102% for the extraction of BZP and TFMPP from urine by means of liquid–liquid extraction, while Tsutsumi et al. [22] reported about 40% for BZP and TFMPP using the same extracting approach. However, the latter authors reported higher values (96 and 99%) using SPE.

Our low recoveries for BZP and MeOPP were probably due to the fact that the retention of those compounds in the extracting sorbent is much weaker than that of mCPP and TFMPP, and therefore perhaps a different retention mechanism could be more adequate for these compounds, e.g. using cation exchange sorbents. For instance, Tsutsumi et al. [22] have used both OASIS® HLB and OASIS® MCX extraction cartridges for piperazine determination in urine, and concluded that the former presented better performance. When the mixed-mode cation exchange cartridges were used, analyte retention on the sorbent was improved using 0.1% hydrochloric acid, and the analytes were eluted with 5% ammonia in methanol. On the other hand, when using the regular reverse phase cartridges the solvents were only methanol and water, and the sample's pH was not modified. Perhaps in the herein described method the retention of those particular compounds (BZP and MeOPP) could have been further improved by modifying the sample's pH, but this would have increased the total number of experiments. However, one should take into account that in micro-sampling methods (e.g. MEPS, SPME) all the extracted amount of analyte is injected in the chromatographic system, counteracting low recoveries.

It is not possible to compare adequately our results to those obtained by other authors because the determination of piperazines using MEPS has not been published yet.

3.2.5. Stability

In order to study stability in processed samples at two concentration levels, urine was spiked with 1.5 and 3.5 μg mL^{-1} of each

Table 4
Extraction efficiency (%) for each studied analyte under the optimized conditions (n = 3).

Concentration (μg mL^{-1})	MeOPP	BZP	mCPP	TFMPP
2	4.3 ± 1.7	6.3 ± 1.4	78.8 ± 4.8	75.1 ± 6.3
4	6.1 ± 1.4	10.9 ± 1.4	73.1 ± 5.0	82.6 ± 8.7

Mean values ± standard deviation.

compound, and extracted using the above mentioned procedure ($n = 3$). However, after extraction the extract was left standing at room temperature in the autosampler for 24 h. Those samples were compared to freshly prepared samples, and the obtained coefficients of variation were less than 8% for all compounds, meaning that the analytes are stable in the extracts for at least 24 h at room temperature.

Short-term stability was evaluated at the same concentration levels ($n = 3$). Urine samples were spiked and were left at room temperature for 24 h. These were compared to freshly prepared samples, and the obtained coefficients of variation were less than 5% for all compounds, meaning that the analytes are stable in the samples for at least 24 h at room temperature.

Freeze and thaw stability was also evaluated in triplicate at the same concentration levels. Urine samples were spiked, and were stored at −20 °C for 24 h, after which they were thawed unassisted at room temperature. When completely thawed, the samples were re-frozen for 12–24 h under the same conditions. This freeze/thaw cycle was repeated twice more, and the samples were analysed after the third cycle. These samples were compared to samples prepared and analysed in the same day, and the analytes were stable for at least 3 freeze/thaw cycles (the obtained coefficients of variation were less than 14% for all compounds).

3.2.6. Method applicability (authentic urine samples)

After validation, the herein described procedure was applied to authentic samples obtained from autopsies performed at the National Institute of Legal Medicine – South Branch, Lisbon, Portugal. In addition, samples belonging to persons undergoing treatment with trazodone were also analysed, for the detection of its metabolite mCPP.

A total of 5 samples have been analysed so far (3 post-mortem samples and 2 belonging to living subjects); only the clinical samples tested positive for mCPP, and the concentrations obtained for this analyte in those samples were 0.55 ± 0.02 and 0.78 ± 0.05 μg mL^{-1} ($n = 3$).

4. Conclusions

A simple, rapid (<15 min) and fully validated procedure is described for the detection and quantitation of piperazine-like stimulants in human urine samples, using microextraction by packed sorbent and analysis by ultra high performance liquid chromatography-diode array detection. This method has shown to be linear within the adopted ranges for all analytes, and presented adequate precision and trueness. Furthermore, the procedure can be useful for those laboratories performing routine urine analysis in the field of both clinical and forensic toxicology. Moreover, it is sensitive and specific enough as to detect small amounts of the compounds using only 0.1 mL of sample and analytical instrumentation accessible in most laboratories nowadays.

References

[1] M. Barroso, S. Costa, M. Dias, D.N. Vieira, J.A. Queiroz, M. López-Rivadulla, J. Chromatogr. A 1217 (2010) 6274.
[2] European Monitoring Centre for Drugs and Drug Addiction, 2009 Annual Report on the State of Drugs Problem in Europe, 2009, http://www.emcdda.europa.eu/publications/annual-report/2009.
[3] M. Vita, P. Skansen, M. Hassana, M. Abdel-Rehim, J. Chromatogr. B 817 (2005) 303.
[4] E. Jagerdeo, M. Abdel-Rehim, J. Am. Soc. Mass Spectrom. 20 (2009) 891.
[5] M. Abdel-Rehim, Z. Altun, L. Blomberg, J. Mass Spectrom. 39 (2004) 1488.
[6] M. Abdel-Rehim, J. Chromatogr. B 801 (2004) 317.
[7] E. Gallardo, S. Costa, M. Barroso, Rev. Lusófona Ciênc. Tecnol. Saúde 61 (2009) 105.
[8] Z. Altun, M. Abdel-Rehim, Anal. Chim. Acta 630 (2008) 116.
[9] Z. Altun, M. Abdel-Rehim, L.G. Blomberg, J. Chromatogr. B 813 (2004) 129.
[10] R. Said, M. Kamel, A. El-Beqqali, M. Abdel-Rehim, Bioanalysis 2 (2010) 197.
[12] H. Miyaguchi, Y.T. Iwata, T. Kanamori, K. Tsujikawa, K. Kuwayama, H. Inoue, J. Chromatogr. A 16 (2009) 4063.
[13] M. Abdel-Rehim, P. Skansen, M. Vita, Z. Hassan, L. Blomberg, M. Hassan, Anal. Chim. Acta 539 (2005) 35.
[14] R. Mandrioli, L. Mercolini, D. Lateana, G. Boncompagni, M.A. Raggi, J. Chromatogr. B 879 (2011) 167.
[15] M.A. Saracino, A. de Palma, G. Boncompagni, M.A. Raggi, Talanta 81 (2010) 1547.
[16] M.A. Saracino, G. Lazzara, B. Prugnoli, M.A. Raggi, J. Chromatogr. A 1218 (2011) 2153.
[17] A.R. Chaves, F.Z. Leandro, J.A. Carris, M.E. Queiroz, J. Chromatogr. B 878 (2010) 2123.
[18] F. Lafay, E. Vulliet, M.M. Flament-Waton, Anal. Bioanal. Chem. 396 (2010) 937.
[19] M.A. Saracino, K. Tallarico, M.A. Raggi, Anal. Chim. Acta 661 (2010) 222.
[20] M. Wada, K. Yamahara, R. Ikeda, R. Kikura-Hanajiri, N. Kuroda, K. Nakashima, Biomed. Chromatogr., in press.
[21] A. Wohlfarth, W. Weinmann, S. Dresen, Bioanal. Chem. 396 (2010) 2403.
[22] H. Tsutsumi, M. Katagi, A. Miki, N. Shima, T. Kamata, M. Nishikawa, K. Nakajima, H. Tsuchihashi, J. Chromatogr. B 819 (2005) 315.
[23] S.P. Vorce, J.M. Holler, B. Levine, M.R. Past, J. Anal. Toxicol. 32 (2008) 444.
[24] A.J. Dickson, S.P. Vorce, J.M. Holler, T.P. Lyons, J. Anal. Toxicol. 34 (2010) 464.
[25] C. Bell, C. George, A.T. Kicman, A. Traynor, Drug Test. Anal. 3 (2011) 496.
[26] R.H. Myers, D.C. Montgomery, C.M. Anderson-Cook, Response Surface Methodology: Process and Product Optimization Using Designed Experiments, 3rd ed., John Wiley & Sons Inc, Hoboken, 2009.
[27] U.S. Department of Health and Human Services, Food and Drug Administration, Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf, May 2001 (accessed 29.03.11).
[28] International Conference on Harmonization (ICH), Validation of Analytical Procedures: Methodology ICH Q2 B. http://www.ich.org/LOB/media/MEDIA417.pdf, 2005 (accessed 29.03.11).