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Automated on-line dialysis and liquid chromatography of methylenedioxylated amphetamines in plasma and serum samples

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Abstract

An automated on-line dialysis coupled to a trace enrichment method has been developed for the separation and quantification of four methylenedioxylated amphetamines in serum and plasma, using liquid chromatography coupled to a fluorimetric detector. The on-line dialysis method was optimized and validated on fresh human serum and plasma samples. This sample preparation method allowed the quantification of methylenedioxylated amphetamines in serum or plasma, at concentrations as low as ca. 10 ng ml^{-1}, with good repeatability, reproducibility and accuracy. The automated on-line dialysis method took less than 30 min. This method was applied to seven toxicological cases and results showed that the concentration of methylenedioxylated amphetamines in blood was in the range of 20–484 ng ml^{-1}.

Keywords: On-line dialysis; Reversed-phase liquid chromatography; Methylenedioxylated amphetamines

1. Introduction

Drug monitoring of low concentrated substances in biological fluids, such as serum, plasma and whole blood remains a difficult problem. First, a direct injection of these matrices in an HPLC column is not possible, because proteins are strongly adsorbed on a conventional packing material [1]. Therefore, chromatographic performances decrease dramatically and the column lifetime is greatly shortened. In the past few years, a number of supports such as the restricted access media have been commercialized, in order to overcome the clogging problem due to the presence of high molecular weight proteins [2,3]. However, these packing materials are not yet currently used and only few articles present the quantitative determination of drugs at a low concentration [4,5].

Secondly, whatever the analytical method chosen, a concentration step is often necessary due to the tested concentrations and the sensitivity of the conventional detectors coupled to HPLC. There-

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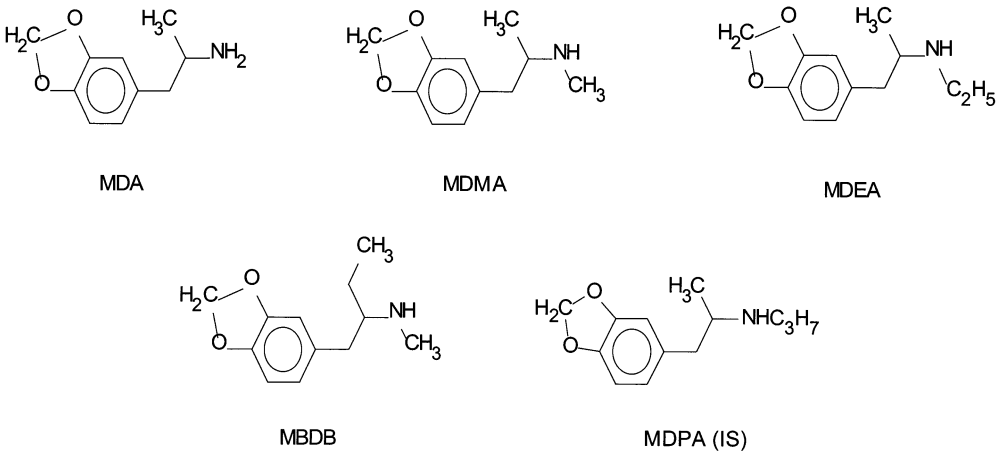

Fig. 1. Structure of analyzed methylenedioxylated amphetamines.

fore, a sample preparation is required to remove interfering substances from the matrix, especially proteins, and to concentrate the sample.

Blood samples are generally prepared by using liquid–liquid extraction (LLE) followed by a solvent evaporation step. This procedure is tedious, time consuming and can induce low precision. Moreover, LLE cannot be easily automated [6].

In order to avoid loss of time and limit manual intervention during sample preparation, a number of semi-automated and automated methods have been developed over the last decade. Commercially available automated solid phase extraction (SPE) workstations and on-line dialysis coupled to trace enrichment are examples of these developments. Indeed, in the last 10 years SPE has known a great expansion in the replacement of LLE procedures. Among the advantages of SPE vs. LLE, we can mention the wide choice of extracting supports and the possibility to couple on-line SPE with an HPLC separation [7–9]. As a drawback, SPE cannot directly extract biological proteineous samples. We have to note that with LLE and SPE methods, the total amount of analytes is analyzed, because the binding sites are destroyed by the denaturation of the proteins.

On-line dialysis coupled to SPE is one of the most attractive approaches for the analysis of drugs in biological samples with an automated method [10–18]. A semi-permeable membrane permits to remove macromolecular sample components. The coupling with a trace enrichment cartridge (TEC) overcomes the dilution of the sample caused by the dialysis step. It is noteworthy that dialysis can be performed by different modes. The static–static mode (both donor and receptor phases remain stagnant) performs equilibrium dialysis and allows the determination of free concentrations of the analytes (the fraction which is not bound to proteins) [17,19]. The dynamic mode (static–pulse: donor phase remains static while receptor phase is aspired continuously at a constant flow rate) maintains a high concentration gradient across the membrane. Hence, the recovery is maximized and a better sensitivity is obtained with this latter mode [17].

Methylenedioxy-*N*-methylamphetamine (MDMA, Ecstasy, Adam) and other related methylenedioxylated amphetamines are widely used and misused in many countries [20–22]. Analyses of illicit tablets sold as being 'Ecstasy', showed that they contained not only MDMA, but also other related phenylethylamines such as methylenedioxyamphetamine (MDA, 'love drug'), methylenedioxy-*N*-ethylamphetamine (MDEA, Eve) and more recently *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB, Eden) [22] (Fig. 1). Moreover, MDMA-related deaths [23–25] and acute clinical toxicological problems following its ingestion have been reported [20,26–28]. These compounds are detectable, after a single dose ingestion, for 24 h in blood and for 2–4 days in

urine. Toxicological analyses of fatal cases showed concentrations of MDMA and MDA in blood between 10 and 2900 ng ml^{-1} [22,29,30].

Several sample preparation methods prior to liquid chromatography of amphetamines in biological fluids have been developed [31]. Most of these methods are LLE procedures [32–38], but SPE is now widely used [39–44]. To our knowledge on-line dialysis has never been applied to the analysis of amphetamines. Therefore, in order to detect and quantify methylenedioxylated amphetamines in biological fluids, especially in serum and plasma, we developed an on-line dialysis method coupled to an enrichment cartridge and a liquid chromatography. This latter, coupled to a fluorimetric detector, was recently reported on for the determination of methylenedioxylated amphetamines (MDA, MDMA, MDEA and MBDB) at very low concentrations (limit of detection of 2–3 ng ml^{-1}) [45]. It is noteworthy that MDMA, MDEA and MBDB are natively fluorescent and that among the several metabolites of these latter, only MDA was detectable by fluorimetry.

This paper presents the development and validation of a fully automated on-line dialysis and trace enrichment method for the determination of methylenedioxylated amphetamines in serum and plasma samples by liquid chromatography with fluorimetric detection. The method was applied to the analysis of seven specific toxicological cases.

2. Experimentals

2.1. Chemicals

Standard solutions of 1 mg ml^{-1} of methylenedioxy-*N*-propylamine (MDPA, used as internal standard), MDA, MDMA and MDEA in methanol were purchased from Alltech (Deerfield, IL, USA). MBDB was obtained from RBI (Natick, MA, USA).Toxicological cases (citrated human blood) were kindly supplied by the Forensic Institute of Lausanne University (Switzerland). Blank human serum and plasma samples from healthy volunteers were obtained from the Clinical Chemistry Laboratory of the Geneva University Hospital (Switzerland). Acetonitrile and methanol were purchased from Maechler (Basel, Switzerland). Ultrapure water was supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA). All other reagents, solvents and substances were analytical-grade reagents from Fluka (Buchs, Switzerland).

2.2. Chromatography

Experiments were carried out on a Gilson 305/306 HPLC system (Gilson Medical Electronics, Villiers-le-Bel, France) equipped with a Varian Crococil oven (Palo Alto, CA, USA). The Gilson 712 HPLC software was used for instrument control, data acquisition and data analysis. Detection was carried out using a Merck-Hitachi F-1050 fluorescence detector (Darmstadt, Germany) operated at an excitation wavelength of 285 nm and an emission wavelength of 320 nm. The column was a C18-AB Nucleosil 100, 5 μm (Macherey-Nagel, Oensingen, Switzerland) 125×4 mm I.D thermostated at 40°C. Mobile phase was composed of acetonitrile/phosphate solution (pH 3.8; 20 mM Na_2HPO_4) (85:15 v/v). The separation was conducted at a flow rate of 1 ml min^{-1}.

2.3. Dialysis and trace enrichment

The dialysis and trace enrichment procedures were performed on an ASTED-XL unit (Gilson Medical Electronics, Villiers-le-Bel, France). This latter consisted of an autosampling injector equipped with two 401 dilutors fitted with 1 ml syringes. The dialysis cell had a donor channel volume of 100 μl and a receptor channel volume of 175 μl. Donor and receptor channels were separated by a cellulose acetate membrane (Cuprophane, Gilson) with a 15-kDa molecular mass cut-off. An automated six-port switching valve (Rheodyne, Berkeley, CA) connected the trace enrichment column either with the receptor channel or with the analytical column. The trace enrichment column (5×1.6 mm I.D.) was packed with 10 μm particles of ODS 2 Hypersil (Gilson). The temperature of the dialyzing block was controlled by using a thermostated water bath.

The donor washing solution was pure water. The various receptor solutions were composed of buffer solutions (acetate, citrate and phosphate buffers) at different pH (3.5–7.0) and percentages of acetonitrile (up to 10%). Dialysis procedure optimizations were first accomplished on standard aqueous solutions and then on spiked serum and plasma samples.

Before each run, donor and receptor channels were flushed with 1.0 and 1.75 ml of water and buffer solution respectively. The enrichment precolumn was preconditioned and regenerated successively with 0.5 ml of water, 0.9 ml of methanol, 0.5 ml water and 1.0 ml of receptor phase.

All dialysis experiments were performed in the static–pulse mode. 100 μl of sample were held stagnant in the donor compartment, while the receptor phase was continuously flushed. This continuous flow was performed through the pulse mode (at different flow rates 0.18–2.0 ml min^{-1} as specified) with no waiting time between pulses. After each pulse (1 ml), dialysates were dispensed through the precolumn (at 0.5, 1 or 2 ml min^{-1}) to be enriched. Regarding dialysis speed and dialysis time, one to six pulses were used. The precolumn was then washed with 2.7 ml and 3.6 ml of water for serum and plasma samples respectively. By switching the six-port valve, enriched analytes retained on the precolumn were back-flushed to the analytical column.

For the trace enrichment procedure, breakthrough volumes were determined by frontal analysis of a MDA aqueous solution at a specified pH through the enrichment precolumn.

2.4. Sample preparation and validation

The analyte-internal standard area ratios were plotted against the concentrations. Calibration curves were carried out for each amphetamine at a concentration range between 10 and 200 ng ml^{-1} (10, 20, 40, 60, 100 and 200 ng ml^{-1}) in fresh human serum and plasma samples containing 100 ng ml^{-1} of MDPA (internal standard).

Standard calibration lines were obtained from unweighted least-squares linear regression analysis of the data. The linearity of each method was statistically tested. Six duplicate determinations of each compound at two concentrations (10 and 200 ng ml^{-1}) were performed to estimate the precision of both methods within the same day (repeatability or run-to-run reproducibility). This procedure was repeated on three different days to test the intermediate precision (day-to-day reproducibility).

Toxicological cases were citrated whole blood samples. First, 10 μl of 10 μg ml^{-1} MDPA solution were added to 990 μl of blood samples and centrifuged at 4000 rpm for 10 min. Then, 100 μl of the supernatant plasma was loaded into the donor compartment.

3. Results and discussions

3.1. Trace enrichment and breakthrough volumes

Enrichment of dialysates is a main step of an on-line dialysis procedure. Before optimizing different dialysis parameters, the breakthrough volume (BTV) of the analytes has to be determined. Since MDA was the less retained substance, only the breakthrough volume of the latter was studied.

The use of an organic solvent, whatever the percentage, in the receptor phase was not possible, because its presence drastically decreased the breakthrough volumes with the selected precolumn. Therefore, the receptor phase was composed of a buffer solution only. The enrichment flow rate (0.5, 1 and 2.0 ml min^{-1}) and the ionic strength had no significant effect on breakthrough volume.

As amphetamines are basic drugs with pK_a values of about 10, on a C18 enrichment cartridge, the BTV increases with pH. Thus, a receptor phase with pH higher than 6.0 permitted to obtain BTVs larger than 10 ml. However, it is noteworthy that, even at pH 3.5, the BTV was ca. 6 ml (data not shown).

3.2. Dialysis

The effect of different parameters such as buffer type (acetate, citrate or phosphate), pH (3.5–7.0) and buffer concentration of the receptor

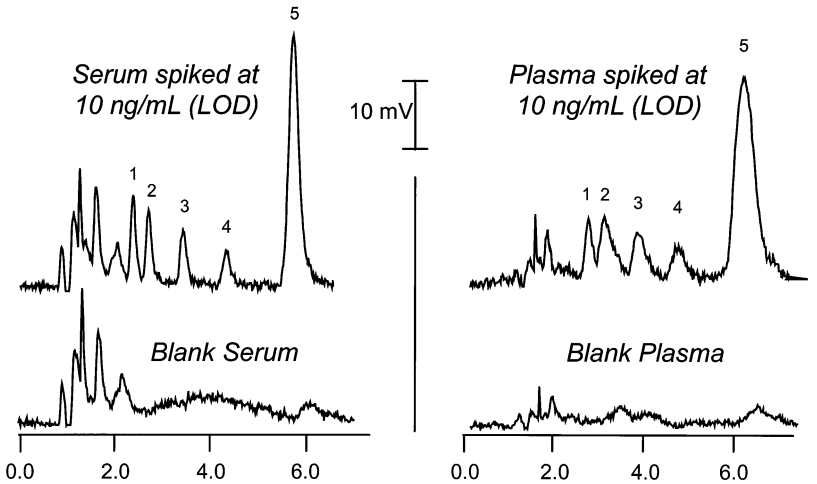

Fig. 2. Chromatograms of blank and spiked human serum and plasma (10 ng ml^{-1}) after an automated on-line dialysis and precolumn enrichment procedure. 1, MDA; 2, MDMA; 3, MDEA; 4, MBDB; 5, MDPA (internal standard).

phase as well as dialysis time, dialysis speed (i.e. the flow rate), dialysis volume (depending on dialysis time and dialysis speed) and temperature were investigated in order to optimize the dialysis in the dynamic mode.

Acetate buffer was not appropriate as a receptor phase. In fact, serious decrease of the fluorescence occurred (as already reported for the separation of amphetamines by HPLC with a UV detection at 200 nm [46]). Citrate buffer provided good results, but its instability and a rapid bacterial development after a few hours limited its use. Phosphate buffer seemed the most appropriate receptor phase. The pH of the acceptor phase did not have any significant influence on the dialysis recovery, but in order to obtain acceptable chromatograms, the pH must be lower than 4.0 (i.e. the same pH than the mobile phase: 3.8). Buffer concentration set at 20 mM gave the best recovery and chromatographic results.

The dialysis time, volume and speed are influential factors which are closely related. We observed that the minimum dialysis time to obtain the best recovery was five minutes. To study the dialysis speed, we fixed the total dialysis time at six minutes. The receptor was thus washed by successive pulses of a phosphate buffer solution phase at different speeds (0.2–2.0 ml min^{-1}). After each pulse, the dialysate (1 ml per pulse) was dispensed on the trace enrichment precolumn at 2.0 ml min^{-1}. The percentage of drug recovery increased as a function of the dialysis speed to reach a plateau (ca. 52%) for speeds above 0.7 ml min^{-1}. In order to reach a compromise between the dialysis efficiency and the breakthrough volume of the enrichment cartridge, the optimal dialysis speed was set at 1.0 ml min^{-1} and the dialysis volume at 4.0 ml (four 1 ml pulses).

The temperature of the dialyzing block is also an important factor. Dialysis recovery increased from ca. 30% at 30°C to ca. 50% at temperatures higher than 50°C. In fact, diffusion coefficient of the analyte is inversely related to the viscosity of donor and receptor phases. Since the viscosity decreases with the rise of the temperature, dialysis recovery directly depends on temperature [47]. Thus, the dialyzing block was thermostated in a water bath at 50°C during experiments.

We applied the optimized procedure to aqueous and serum samples containing equivalent amphetamines concentrations. In both cases, recoveries were similar and were ca. 52–54%. Recovery results were obtained by comparison with a direct injection (100 μl) of a standard aqueous solution of methylenedioxylated amphetamines.

3.3. Validation

Validation was performed on spiked human serum and plasma samples. The results are pre-

Table 1
Data analysis of the dialysis and trace enrichment method for four tested methylenedioxylated amphetamines in spiked fresh human serum

Compounds	Linear regression		LOD [ng ml^{-1}]	LOQ [ng ml^{-1}]	Repeatability R.S.D.[a] [%]		Intermediate precision R.S.D.[a] [%]		Accuracy [%]
	r	Intercept ± 2 S.D.[b]			200 ng ml^{-1}	10 ng ml^{-1}	200 ng ml^{-1}	10 ng ml^{-1}	
MDA	0.996	-0.056 ± 0.069	6.0	20.0	6.8	9.3	9.6	12.4	104.5
MDMA	0.994	-0.072 ± 0.074	7.5	24.0	9.0	12.3	11.7	13.9	114.6
MDEA	0.993	-0.002 ± 0.064	8.5	30.0	9.0	14.7	11.8	13.3	104.3
MBDB	0.993	0.028 ± 0.047	10.0	34.0	8.9	15.9	12.3	16.7	92.0

[a] R.S.D., relative standard deviation.
[b] S.D., standard deviation.

Table 2
Data analysis of the dialysis and trace enrichment method for four tested methylenedioxylated amphetamines in spiked fresh human plasma

Compounds	Linear regression		LOD [ng ml^{-1}]	LOQ [ng ml^{-1}]	Repeatability R.S.D. [%]		Intermediate precision R.S.D. [%]		Accuracy [%]
	r	Intercept ± 2 S.D.			200 ng ml^{-1}	10 ng ml^{-1}	200 ng ml^{-1}	10 ng ml^{-1}	
MDA	0.990	0.045 ± 0.071	5.5	17	7.5	11	10	14	111.0
MDMA	0.987	0.101 ± 0.124	6.1	20	5.2	11	8.1	15	122.4
MDEA	0.996	0.027 ± 0.039	6.6	22	5.9	10	9.5	14	104.6
MBDB	0.998	0.004 ± 0.032	9.2	31	4.4	9.0	13	11	105.6

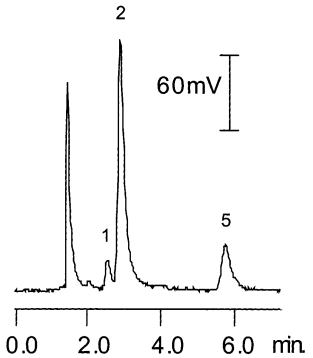

Fig. 3. Chromatograms of a toxicological case after the on-line dialysis procedure. 1, MDA 24.4 ng ml^{-1}; 2, MDMA 292.0 ng ml^{-1}; 5, MDPA 100 ng ml^{-1}.

sented in Tables 1 and 2. The lower limit of the calibration curves corresponded to the limit of detection. Nevertheless, the correlation coefficients (r) were always greater than 0.993 for serum and greater than 0.987 for plasma samples. A Student's t-test showed that the intercepts were not significantly different from 0.00. Therefore, simultaneous determination of these four tested methylenedioxylated amphetamines in serum or plasma can be performed with a single spiked standard serum or plasma sample. Validation results showed good repeatability, intermediate precision and accuracy as well as a high sensitivity for both matrices. We can also note that even if the limits of detection (LOD) and quantification (LOQ) obtained with serum and plasma samples were very close, linear equations were significantly different and chromatograms were not superposable. Thus, serum and plasma cannot be considered as being equivalent.

Fig. 2 shows chromatograms of serum and plasma samples spiked with 10 ng ml^{-1} of each amphetamine.

3.4. Application to toxicological cases

The on-line dialysis procedure was applied to seven toxicological cases. An example of chromatogram is shown in Fig. 3. Analytical results (Fig. 4) showed that MDMA was present in all cases and its concentration varied between 62 and 529 ng ml^{-1}. In four cases, we detected the presence of MDA (main metabolite of MDMA and MDEA) at a low concentration (20 and 60 ng ml^{-1}) and in one case MDA, MDMA and MDEA were simultaneously present. According to the analytical results obtained on illicit tablets [22,46,48,49], the concomitant presence, in a tablet, of MDMA and MDEA was not observed.

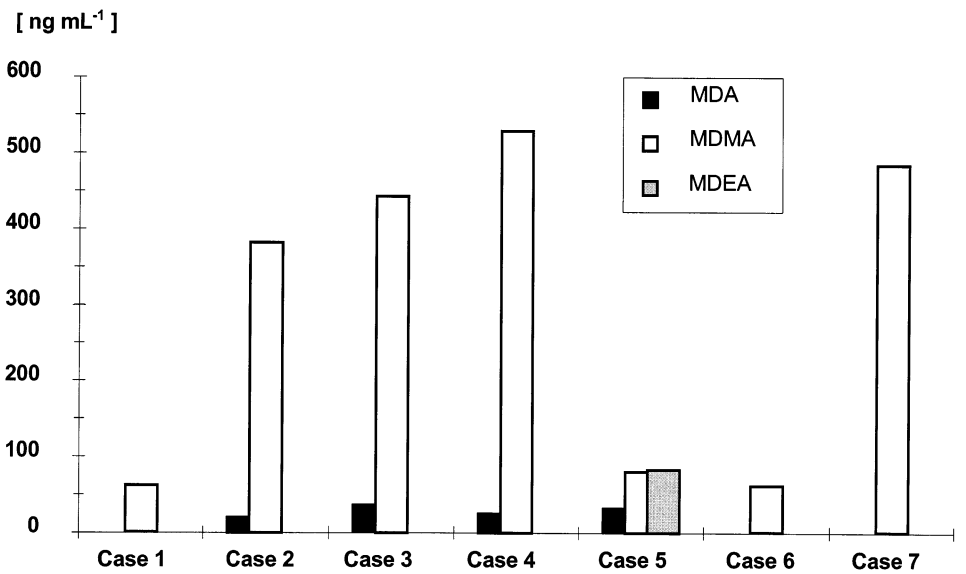

Fig. 4. Analytical results of seven toxicological cases after the on-line dialysis-trace enrichment procedure.

Thus, we can presume that Case 5 took different tablets each of which contained one of these amphetamines.

4. Conclusion

An automated on-line dialysis and trace enrichment procedure for the determination of methylenedioxylated amphetamines in human serum and plasma was developed and successfully validated.

For both matrices, we observed good linearity, repeatability, reproducibility and accuracy. Therefore, real cases can be analyzed and quantified with a single standard of serum or plasma. The sensitivity and the selectivity of the procedure for serum and plasma have been demonstrated. Considering the low detection limits (10 ng ml^{-1}), this method can be used for determining methylenedioxylated amphetamines in ante-mortem (monitoring) or post-mortem samples. Finally, the developed dialysis method was applied to seven toxicological cases and results showed the presence of MDMA and its main metabolite MDA at different concentrations (62–528 ng ml^{-1} and 20–60 ng ml^{-1}, respectively).

References

[1] J. Chamberlain, in: The Analysis of Drugs in Biological Fluids, CRC Press, London, 1995, p. 36
[2] T.C. Pinkerton, J. Chromatogr. 544 (1991) 13–23.
[3] L.J. Glunz, J.A. Perry, B. Invergo, H. Wagner, T.J. Szczerba, J.D. Rateike, P.W. Glunz, J. Liq. Chromatogr. 15 (1992) 1361–1379.
[4] P. Martin, E.D. Morgan, I.D. Wilson, J. Pharm. Biomed. Anal. 14 (1996) 419–427.
[5] R.A.M. van der Hoeven, A.J.P. Hofte, M. Frenay, H. Irth, U.R. Tjaden, J. van der Greef, A. Rudolphi, K.-S. Boos, J. Marko Varga, L.E. Edholm, J. Chromatogr. A 762 (1997) 193–200.
[6] D.C. Turnell, J.D.H. Cooper, J. Chromatogr. 492 (1989) 59–83.
[7] R. Herráez-Hernández, P. Campins-Falco, A. Sevillano-Cabeza, Anal. Chem. 68 (1996) 734–739.
[8] P. Campins-Falco, R. Herráez-Hernández, A. Sevillano-Cabeza, J. Chromatogr. 619 (1993) 177–190.
[9] R.B. Miller, J. Chromatogr. 616 (1993) 283–290.
[10] D.C. Turnell, J.D.H. Cooper, J. Aut. Chem. 7 (1985) 177–180.
[11] J.D.H. Cooper, D.C. Turnell, J. Aut. Chem. 7 (1985) 181–184.
[12] J.D.H. Cooper, D.C. Turnell, J. Chromatogr. 380 (1986) 109–116.
[13] T. Agasoster, K.E. Rasmussen, J. Chromatogr. 570 (1991) 99–107.
[14] A.T. Andresen, P.B. Jacobsen, K.E. Rasmussen, J. Chromatogr. 575 (1992) 93–99.
[15] T. Agasoster, K.E. Rasmussen, J. Pharm. Biomed. Anal. 5 (1992) 349–354.
[16] J.D.H. Cooper, C.T.C. Fook Sheung, A.R. Buick, J. Chromatogr. 652 (1994) 15–21.
[17] K. Johansen, M. Krogh, A.T. Andresen, A.S. Christophersen, G. Lehne, K.E. Rasmussen, J. Chromatogr. B 669 (1995) 281–288.
[18] R. Herráez-Hernández, N.C. van de Merbel, U.A.T. Brinkman, J. Chromatogr. 666 (1995) 127–137.
[19] A.T. Andresen, K.E. Rasmussen, H.E. Rugstad, J. Chromatogr. 621 (1993) 189–198.
[20] S.J. Peroutka, N. Engl. J. Med. 317 (1987) 1542–1543.
[21] J.D. Wright, L. Pearl, Br. Med. J. 300 (1990) 99–103.
[22] C. Giroud, M. Augsburger, F. Sadeghipour, E. Varesio, J.L. Veuthey, L. Rivier, Praxis 86 (1997) 510–523.
[23] G.P. Dowling, E.T. McDonough III, R.O. Bost, J. Am. Med. Assoc. 257 (1987) 1615–1617.
[24] J.A. Henry, Br. Med. J. 305 (1992) 5–6.
[25] J.A. Henry, K.J. Jeffreys, S. Dawling, Lancet 340 (1992) 384–387.
[26] G.T. Tucker, M.S. Lennard, S.W. Ellis, H.F. Woods, A.K. Cho, Biomed. Pharmacol. 47 (1994) 1151–1156.
[27] A.R. Green, A.J. Cross, G.M. Goodwin, Psychopharmacology 119 (1995) 247–260.
[28] F. Benazzi, M. Mazzoli, Lancet 338 (1991) 1520.
[29] J. Osterloh, L.L. Belle, Amphetamines in Abused Drugs Monographs, 1990
[30] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Mosby Year Book, Chicago, 1989.
[31] P. Campins-Falco, A. Sevillano-Cabeza, C. Molins Legua, J. Liq. Chromatogr. 17 (1994) 731–747.
[32] C.L. Hornbeck, R.J. Dzarny, J. Anal. Toxicol. 13 (1989) 144–149.
[33] P. Campins-Falco, F.B. Reig, A.S. Cabeza, C.M. Legua, Anal. Chim. Acta 287 (1994) 41–48.
[34] C. Molins Legua, P. Campins-Falco, A. Sevillano-Cabeza, Fresenius J. Anal. Chem. 349 (1994) 311–316.
[35] T.K. Wang, M.S. Fuh, J. Chromatogr. B 686 (1996) 285–290.
[36] B.J.A. Cooke, J. Anal. Toxicol. 18 (1994) 49–51.
[37] R.E. Michel, A.B. Rege, W.J. George, J. Neurosci. Med. 50 (1993) 61–66.
[38] L. Tedeschi, G. Frison, R. Giorgetti, S.D. Ferrara, Int. J. Leg. Med. 105 (1993) 265–269.

[39] B.K. Gan, D. Baugh, R.H. Liu, A.S. Walia, J. Forensic Sci. 36 (1991) 1331–1341.
[40] D.H. Fisher, A.J. Bourque, J. Chromatogr. 614 (1993) 142–147.
[41] S.D. Ferrara, L. Tedeschi, G. Frison, F. Castagna, J. Anal. Toxicol. 16 (1992) 217–222.
[42] S.D. Ferrara, L. Tedeschi, G. Frison, G. Brusini, F. Castagna, B. Bernardelli, D. Soregaroli, J. Anal. Toxicol. 18 (1994) 278–291.
[43] P. Campins-Falco, A. Sevillano-Cabeza, C. Molins-Legua, M. Kohlmann, J. Chromatogr. B 687 (1997) 239–246.
[44] H.J. Helmlin, K. Bracher, D. Bourquin, D. Vonlanthen, R. Brenneisen, J. Styk, J. Anal. Toxicol. 20 (1996) 432–440.
[45] F. Sadeghipour, J.-L. Veuthey, J. Chromatogr. A 787 (1997) 137–143.
[46] F. Sadeghipour, C. Giroud, L. Rivier, J.-L. Veuthey, J. Chromatogr. A 761 (1997) 71–78.
[47] F. Verillon, F. Qian, Analusis 19 (1991) 271–277.
[48] F. Sadeghipour, E. Varesio, C. Giroud, L. Rivier, J.-L. Veuthey, Forensic Sci. Int. 86 (1997) 1–13.
[49] C. Giroud, M. Augsburger, F. Sadeghipour, E. Varesio, J.-L. Veuthey, L. Rivier, P. Mangin, Toxicorama 9 (1997) 113–122.