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On-line coupling of automated solid-phase extraction with high-performance liquid chromatography and electrochemical detection Quantitation of oxidizable drugs of abuse and their metabolites in plasma and urine

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Abstract

The concentration effect of automated on-line solid-phase extraction (SPE) in combination with HPLC and very sensitive electrochemical detection was employed for the determination of *N*-ethyl-4-hydroxy-3-methoxy-amphetamine (HMEA, the main metabolite of the ecstasy analogue MDE), Δ^9 -tetrahydrocannabinol (THC) and 11-nor- Δ^9 -tetrahydrocannabinol-carboxylic acid (THC-COOH) in plasma and urine in comparison to a previously published psilocin assay. For the SPE either CBA (functional group: carboxypropyl)- or CH (functional group: cyclohexyl)-sorbent was used. The following separation was carried out on a reversed-phase column (LiChroCart, Superspher 60 RP select B from Merck). Depending on the hydrodynamic voltammogram of the analyzed substance the oxidation potential varied from 920 mV up to 1.2 V. In spite of using high potentials, precision and accuracy were always within the accepted statistical requirements. The limits of quantitation were between 5 ng/ml (THC, THC-COOH in plasma) and 20 ng/ml (HMEA in plasma). Advantages of on-line SPE in comparison with off-line methods were less manual effort, evidently smaller volumes ($\leq 400 \mu\text{l}$) of plasma or urine and almost always higher recovery rates ($>93\%$). The assays have been successfully proven with real biological samples and found suitable for use in routine analysis. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ecstasy metabolite; Tetrahydrocannabinol; Tetrahydrocannabinolic acid

1. Introduction

The sensitive determination of drugs of abuse or their metabolites in biological matrices is a matter of continuous research in the forensic chemistry. Several methods have been developed for the quantitation

of amphetamines in plasma and urine using high-performance liquid chromatography (HPLC) with ultraviolet [1] or fluorometric [2,3] detection or gas chromatography–mass spectrometry (GC–MS) [4]. The sensitive and reproducible simultaneous determination of the lipophilic Δ^9 -tetrahydrocannabinol (THC)-derivatives is quite difficult. A high recovery rate was achieved by some methods using GC–MS [5] or HPLC [6] with electrochemical detection (ED).

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The automated on-line solid-phase extraction (SPE) is appropriate for isolation and concentration for many substances even in complex matrices yielding good results for recovery and reproducibility. Successful couplings of liquid chromatography with UV, fluorometric and mass spectrometry detection have been published [7–12].

ED is suitable for the specific trace analysis of easily oxidizable or reducible substances [13–16]. On-line coupling between the automated sample preparator and the ED system have been published for psilocin, some drugs, catecholamines and lipophilic antioxidants [17–21]. None of these methods run at an oxidation potential above +900 mV. For the electrochemical determination of *N*-ethyl-4-hydroxy-3-methoxy-amphetamine (HMEA) and especially for THC and its main metabolite in urine, 11-nor- Δ^9 -tetrahydrocannabinol-carboxylic acid (THC-COOH) (cf. Fig. 1) high oxidative potentials must be applied at the working electrode [13,22]. Thus, in combination with on-line SPE difficulties concerning the robustness of the assays could be expected particularly in biological matrices.

The aim of this study was the coupling of the automated SPE on-line with liquid chromatography and ED at high oxidation potentials (up to +1.2 V). Further, the suitability of the method for use in routine analysis was investigated. Therefore, relevant drugs of abuse (ecstasy and cannabis) were chosen as examples.

2. Experimental

2.1. Materials

HMEA-HCl was synthesized according to Ref. [23]. 11-nor- Δ^9 -Tetrahydrocannabinol and 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid were obtained from Sigma (St. Louis, MO, USA). 4-Octylphenol (99%) was purchased from Aldrich (Milwaukee, WI, USA) and 4-dodecylresorcinol (97%) was obtained from Aldrich (Steinheim, Germany). Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, lithium acetate, sodium acetate and potassium dihydrogen phosphate were of analytical grade (Fluka, Buchs, Switzerland). Acetic acid (analytical-reagent grade), acetone (analytical-reagent grade), potassium hydroxide, sodium carbonate (analytical-reagent grade), 85% orthophosphoric acid (analytical-reagent grade), tetrabutylammonium hydrogen sulfate for ion pair chromatography, tetrahydrofuran for chromatography and urea (analytical-reagent grade) were supplied, together with the β -glucuronidase (30 U/ml)–arylsulphatase (60 U/ml) solution by Merck (Darmstadt, Germany). Methanol and acetonitrile were purchased from Rathburn (Zinsser Analytic, Frankfurt, Germany) and polyethylene glycol (PEG) 6000 in DAB quality from Merck-Schuchardt (Hohenbrunn, Germany).

Water was deionized and twice distilled. Human plasma as reference from three different donors was

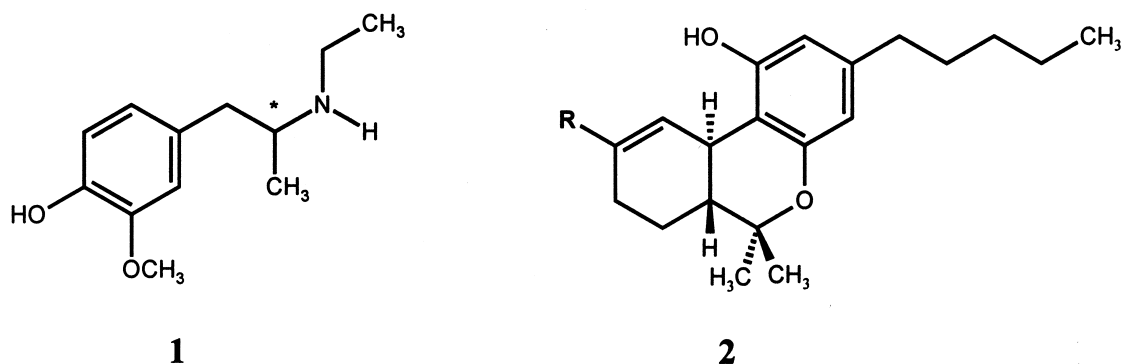


Fig. 1. Structure of (1) HMEA (*N*-ethyl-4-hydroxy-3-methoxy-amphetamine). (2) Structures of (a) THC (R=CH₃), (b) THC-COOH (R=COOH), the main metabolite of THC in urine.

obtained from a blood bank (University Clinic, Tübingen, Germany). The real plasma samples were provided by two different clinical studies (Department of Psychiatry and Psychotherapy, Technical University of Aachen, Germany). The real urine samples were obtained from the Rheinische Landesklinik, a state hospital, in Viersen. Plasma and urine were kept under -20°C before analysis.

OSP-2a cartridges 10 mm \times 4 mm (Merck) were loaded, dosed by volume, with CBA (functional group carboxymethyl) cation-exchange and CH (functional group: cyclohexyl) sorbent of particle size 60 μm (ICT, Frankfurt, Germany).

In all modifications, liquid chromatography was carried out on a LiChroCart Superspher 60 RP-select B column, 5 μm , 250 \times 4 mm I.D. using a guard column LiChrospher 60 RP-select B, 5 μm , 4 \times 4 mm I.D. (Merck).

2.2. Equipment

The HPLC station comprised an isocratic HP 1050 pumping system, a HP 1050 autosampler with a 1000 μl or 100 μl injection loop, a HP 1049 programmable electrochemical detector, a HP 3396 Series II integrator and a HP 9114 B disk drive all from Hewlett-Packard (Waldbronn, Germany). In addition a gradient pump L-6200 A, an autosampler L-7200 both from Merck–Hitachi (Darmstadt, Germany) and an OSP-2 sample preparator from Merck were used for the automated on-line SPE.

2.3. Sample preparation

2.3.1. HMEA in plasma

Aqueous standard solutions were prepared by dissolution of HMEA-HCl and further dilution in water yielding concentrations of 20, 70, 120, 170, 220, 270, 320, 370, 420, 470 and 520 ng HMEA/ml. Because most of the HMEA is submitted as ether-linked glucuronide in plasma, an enzyme-mediated cleavage of the conjugate must be performed to obtain the total amount of the free base. Therefore authentic plasma samples were analyzed by adding 20 μl of 1 M acetic acid– β -glucuronidase–arylsulphatase solution (1:1) to 40 μl freshly thawed plasma and incubating at 37°C for 24 h. Precipitation

of the plasma proteins was carried out with 120 μl lithium acetate, pH 7.2–20% PEG 6000 (2:1) under cooling in an ice-bath (10 min). Afterwards the samples were centrifuged at 10 000 $g/5$ min/ 20°C . Aliquots of 90 μl (corresponding to 20 μl plasma) were applied to previously conditioned CBA cartridges by means of an autosampler (details cf. Ref. [17]).

2.3.2. THC and THC-COOH in plasma

Ethanollic stock solutions of THC and THC-COOH were freshly diluted with the cheaper solvent methanol to get standard solutions ($c=5, 16, 27, 38, 49$ and 60 ng THC/ml; $c=5, 19.75, 34.5, 49.25$ and 64 ng THC-COOH/ml for working range I and $c=64, 123, 182, 241$ and 300 ng THC-COOH for working range II). Authentic plasma samples were analyzed by adding 40 μl internal standard solution (378 ng dodecylresorcinol/ml) to 400 μl freshly thawed plasma, adding 400 μl 16 M urea solution and precipitating the plasma proteins with 800 μl acetone under cooling in an ice-bath (5 min). Then the samples were centrifuged at 2875 $g/3$ min/ 20°C . Aliquots of 820 μl (corresponding to 200 μl plasma) were stepwise applied (8 \times 100 μl , 1 \times 20 μl ; injection every 0.7 min) to previously conditioned CH cartridges by means of an autosampler. For cleaning the cartridges were first purged with acetic acid, pH 2 (1 ml/min during 25 min) and then with a mixture of acetic acid, pH 2–acetonitrile (85:15) (0.8 ml/min during 1 min). Finally, the cartridge was inserted on-line with the chromatographic system by means of a switching valve in order to elute it onto the analytical column using the mobile phase. During the elution of one cartridge another cartridge was activated (methanol, 2 ml/min during 3 min) and conditioned with acetic acid, pH 2 (2 ml/min during 2 min).

2.3.3. THC-COOH in urine

Methanolic standard solutions were prepared by dilution of an ethanollic stock solution of THC-COOH ($c=15, 36.25, 57.5, 78.75$ and 100 ng THC-COOH/ml). Because most of the THC-COOH is excreted as esterlinked glucuronide, a basic hydrolysis step must be performed to obtain the total amount of the free acid. Therefore, authentic urine

samples were analyzed by adding 40 μl internal standard solution (200 ng 4-octylphenol/ml), 300 μl methanol and 30 μl potassium hydroxide solution (10 M) to 400 μl freshly thawed urine and heating the samples afterwards in a water-bath for 15 min. The pH of the solution was adjusted to 3–4 by adding 430 μl acetic acid, pH 2.2. Aliquots of 600 μl (corresponding to 200 μl urine) were applied stepwise (6 \times 100 μl , injection every 0.7 min) to previously conditioned CH cartridges by means of an autosampler. The time program for THC-COOH in urine was identical with that of THC and THC-COOH in plasma (see Section 2.3.2) apart from a shorter washing step with acetic acid, pH 2 (15 min only, instead of 25 min).

2.4. HPLC conditions and detection

2.4.1. HMEA in plasma

The separation was carried out isocratically on a LiChroCart, Superspher 60 RP select B column, 5 μm , 250 \times 4 mm. The mobile phase consisted of 150 mmol potassium dihydrogen phosphate buffer, pH 2.3–acetonitrile (94.5:5.5, v/v) with 160 μmol Na₂EDTA in the buffer–acetonitrile mixture. The flow-rate was 600 $\mu\text{l}/\text{min}$. The potential of the electrochemical detector was set to +920 mV.

2.4.2. THC and THC-COOH in plasma and THC-COOH in urine

The separation was carried out isocratically on a LiChroCart, Superspher 60 RP select B column, 5 μm , 250 \times 4 mm. The mobile phase consisted of 5.6 mmol tetrabutylammonium hydrogen sulfate, pH 2.3–acetonitrile–tetrahydrofuran (44:46:10) with 160 μmol Na₂EDTA in the mixture. The flow-rate was 850 $\mu\text{l}/\text{min}$. The potential of the electrochemical detector was set to +1.2 V.

2.5. Peak integration

The peaks of HMEA in plasma and THC-COOH in plasma and urine were integrated with standard construction from one valley point to the next valley point. In case of THC and its internal standard, dodecylresorcinol, the baseline was set time-programmed at the next valley point after the peaks and a horizontal baseline was drawn backwards.

2.6. Validation

For each determination method at least five equidistant calibration standard concentrations were measured in two different ways: (a) directly in aqueous or methanolic solution and (b) after sample pretreatment from spiked plasma or urine solutions. With this method the recovery rates in percent and the corresponding relative standard deviations (RSDs) could be calculated over the whole working range. The lowest calibration point of the working range inside the 95% confidence interval was defined as experimental limit of quantitation (LOQ) [24]. In addition ten spiked plasma concentrations of the highest calibration point as well as of the lowest calibration point were determined. The statistical parameters: homogeneity of variance, precision and accuracy were calculated from the received data. Homogeneity of variance is one of the requirements for a linear regression. An assumption for the simple, but reliable Hartley's test is the presence of equal sample sizes N . For its calculation the quotient of the variances of the high and low working range is compared with a tabulated F -value (degrees of freedom: $f_1, f_2 = N - 1$). If this quotient is smaller than the tabulated F for a defined confidence interval, a difference between the two variances is only probable but not statistically significant and the variances are accepted to be homogeneous [25]. The precision of a determination method is defined as the standard deviation of repeated, independent investigations of one concentration of a substance. In comparison to the precision the accuracy is calculated as percent difference between the real and the average of the determined values [26].

3. Results and discussion

3.1. Off-line sample preparation

The determination of THC-COOH in urine and HMEA in plasma starts with a cleavage of the conjugates. THC-COOH is mainly excreted as ester-linked glucuronide in urine. Therefore an acidic, an alkaline or an enzymatic hydrolysis is possible. The cleavage rate of the alkaline hydrolysis is the highest and the most confidential one as already shown by

Williams and Moffat [27]. The etherlinked HMEA-conjugate was split by enzymatic hydrolysis with a mixture of α -glucuronidase–arylsulfatase, because it is not known whether the conjugate is a glucuronide and/or a sulfate. Furthermore the enzyme-mediated cleavage is the more gentle procedure.

For the analysis of plasma samples a preliminary protein denaturation step was necessary. The precipitating agent in case of HMEA was a solution of an organic polymer (PEG 6000), which does not interfere with the subsequent SPE using ion-exchange. In contrast to this the extraction of THC and THC-COOH of plasma was carried out with a reversed-phase sorbent. Several protein denaturing substances have been tried yielding the best results with acetone. Without loss in recovery of the analytes most of the proteins could be deleted by adding a small volume of acetone.

3.2. Automated on-line solid-phase extraction

Two different sorbent materials were chosen for the extraction. The basic substance HMEA could be extracted specifically with the CBA-sorbent [17], a weak cation-exchange material. THC and THC-COOH, both rather lipophilic, ought to be extracted simultaneously in one assay. With regard to the following elution step CH-sorbent, a relatively polar reversed-phase material (comparable with C_2 -sorbent) was chosen. The stepwise application of biological samples to previously conditioned cartridges showed better results concerning the recoveries of the investigated cannabinoids. The washing step was carried out with a hydrophilic solution. In combination with the CH-sorbent a more lipophilic washing solution causes loss in recovery. Without success was the replacement of the extraction sorbent against a more lipophilic one – leading this time to difficulties concerning the elution especially of THC from the cartridge. Thus, using the on-line SPE the analyte is directly eluted by the mobile phase from the extraction sorbent. Yet the composition of the mobile phase is mainly determined by demands of chromatographic separation and detection.

The variations in on-line SPE as a result of differences in the self-filled extraction cartridges were negligible as well as peak broadening effects caused by on-line coupling. HMEA could be eluted

in the forward flush modus from the CBA cartridge taking advantage of the filter effect that prevents precipitates, proteins and other particulate matter from clogging the connective outlet capillary [7]. Yet for the determination of THC and THC-COOH a backflush-elution was chosen to avoid peak broadening effects especially of the THC peak, because of its high affinity to the CH material. The enzyme-mediated cleavage of the conjugate in case of HMEA and the less specific extraction method for the determination of THC and THC-COOH made it necessary to use a new cartridge for each analysis. Otherwise a reduction of the recovery rate was observed. Besides the conjugate cleavage and the protein denaturation step the on-line SPE methods were fully automated. Possible variations in analytical results caused by different operators were therefore minimized.

3.3. HPLC separation and detection

For all methods a RP select B column was used as stationary phase and a small quantity of Na_2EDTA was added to the mobile phase in order to complex interfering ions. This way the working electrode surface was protected against irreversible adsorption of heavy metal cations. The high buffer concentration as well as the use of the ion pair reagent (tetrabutylammonium hydrogen sulfate) was appropriate to obtain a stable baseline. No ion pair chromatography was processed in the acidic solution. Ion pair chromatography would have been a good possibility to bring the retention time of THC-COOH into line with the retention time of THC. But according to own investigations only the free acid of THC-COOH is electrochemical detectable at +1.2 V.

After integrating the extraction cartridge into the mobile phase stream (elution step) baseline disturbances were observed, which became higher and lasted longer with increasing potential at the electrochemical detector. Regarding the chromatographic separation of THC and THC-COOH the retention time of THC was rather long (55 min), since in consideration of the electrochemical detection only an isocratic separation was possible. Apart from HMEA an appropriate internal standard was found for each method. Ten substances had been examined as possible internal standards for the HMEA assay.

All of them had a basic partial-structure and were electrochemically detectable. However either the retention time overlapped with a plasma component or the oxidation potential of 920 mV laid under the limiting current of this substance. Yet a further increase in the potential would have caused a high baseline noise. Thus, HMEA was determined with the external standard method (cf. Fig. 2). After determination of hydrodynamic voltammograms for HMEA, THC and THC-COOH the oxidation potentials were set at +920 mV and +1.2 V, respectively, each time in the region of the limiting current, so that small variations in the potential did not influence the signal strength. The biological matrices led to a slow decrease in signal because of stains on the working electrode. Every three days the working electrode had to be polished with diamant suspension and acetone.

Considering the chromatogram of THC and THC-COOH in plasma a baseline drift could be observed starting at about 49 min after the elution of an unknown endogenous component of high concentration. Therefore for the following dodecylresor-

cinol peak (internal standard) and the THC peak a special integration was performed (see Section 2.5).

3.4. Statistical parameters

The described automated determination methods showed rather small linear ranges. Homogeneity of variance as a requirement of linear regression was proven with the Hartley's test (for details see Section 2.6). In order to pass this test with a defined statistical probability P of at least 99.9% the working range had to be split in two cases. Seeing that the oxidative potential was high and analytes were determined in a biological matrix leading to a decreasing working electrode performance followed by a higher variance in the results a restriction of the working range could not be avoided. The LOQs were 20 ng/ml (HMEA) and 5 ng/ml (THC, THC-COOH in plasma) and 15 ng/ml (THC-COOH in urine) depicting the lowest calibration points of the defined working ranges (inside the 95% confidence intervals of the calibration curves) [24]. The precision and

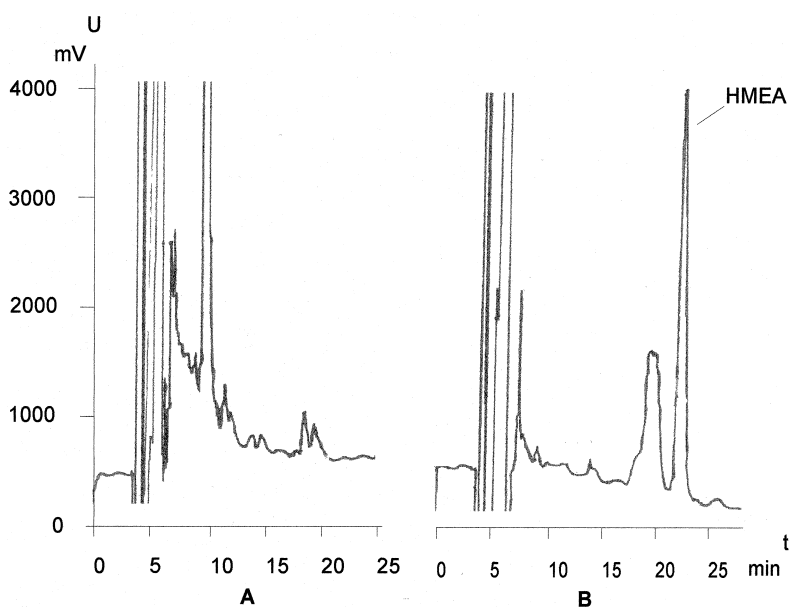


Fig. 2. HPLC chromatograms obtained from human plasma samples: (A) blank plasma, (B) real plasma sample from a study, $t_{R \text{ HMEA}} = 22.25$ min, $c_{\text{HMEA}} = 172$ ng/ml, 240 min after p.o. administration of 2 mg/kg MDE (*N*-ethyl-3,4-methylenedioxyamphetamine).

Table 1
Summary of statistical parameters

	HMEA in plasma		THC in plasma		THC-COOH in plasma		THC-COOH in urine	
	Working range I	Working range II	Working range I	Working range II	Working range I	Working range II	Working range I	Working range II
Working range (ng/ml)	20–270	270–520	5–60	5–64	64–300	15–100		
No. of calibration standard points	6	6	6	5	5	5		
Coefficient of correlation	0.9989	0.9987	0.9993	0.9994	0.9966	0.9980		
Mean recovery (whole working range)	104%	99.4%	96.7%	93.7%	93.3%	98.6%		
Relative standard deviation	6.8%	1.5%	3.6%	5.11%	4.3%	0.9%		
Homogeneity of variance	6.2	2.87	9.95	7.61	9.97	10.03		
	$P=99.9\%$ performed	$P=95\%$ performed	$P=99.9\%$ performed	$P=99.9\%$ performed	$P=99.9\%$ performed	$P=99.9\%$ performed		
Precision high working range (%) ($N=10$)	8.2	1.6	2.7	6.6	4.1	5.1		
Precision low working range (%) ($N=10$)	1.6	1.3	10.0	19.7	5.9	8.7		
Accuracy high working range (%) ($N=10$)	-2.5	-2.7	-0.3	+0.8	+2.8	+0.3		
Accuracy low working range (%) ($N=10$)	+0.5	-0.6	-16.4	+6.5	+13.1	-2.6		

Table 2

Parameters of calibration ($\hat{y}_i = a_0 + a_1 x_i$)

	a_0	a_1	s_y^a	$s_{x_0}^b$	$V_{x_0}^c$
HMEA working range I in plasma	136 160	18 663	93 070	4.99	3.44
HMEA working range II in plasma	-72 601	19 361	159 374	8.23	2.08
THC in plasma	-19 723	222 110	38 096	0.17	2.62
THC-COOH working range I in plasma	60 123	86 412	16 715	0.19	2.75
THC-COOH working range II in plasma	190 600	87 424	156 420	1.79	4.92
THC-COOH in urine	55 207	75 856	37 690	0.50	4.35

$$^a s_y = \text{Residual standard deviation, } s_y = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{N - 2}}$$

$$^b s_{x_0} = \text{Method standard deviation, } s_{x_0} = \frac{s_y}{a_1}$$

$$^c V_{x_0} = \text{Relative method standard deviation, } V_{x_0} = \frac{s_{x_0} \cdot 100\%}{\bar{x}}$$

accuracy were always inside the justifiable limits [28]. For each method a mean recovery rate of at least 93% was obtained (see Tables 1 and 2).

3.5. Determination of authentic biological samples

Each method has been proven successfully with real biological samples. Thirteen samples of one

volunteer were measured for HMEA, a time-concentration curve was designed and compared with data of another method using HPLC with off-line SPE and fluorometric detection [3] (Fig. 3). After passing a test of normal distribution (David) a Student *t*-test was carried out to compare the analytical results of the on-line and the off-line method. Calculation revealed $t=0.192$ compared to the tabu-

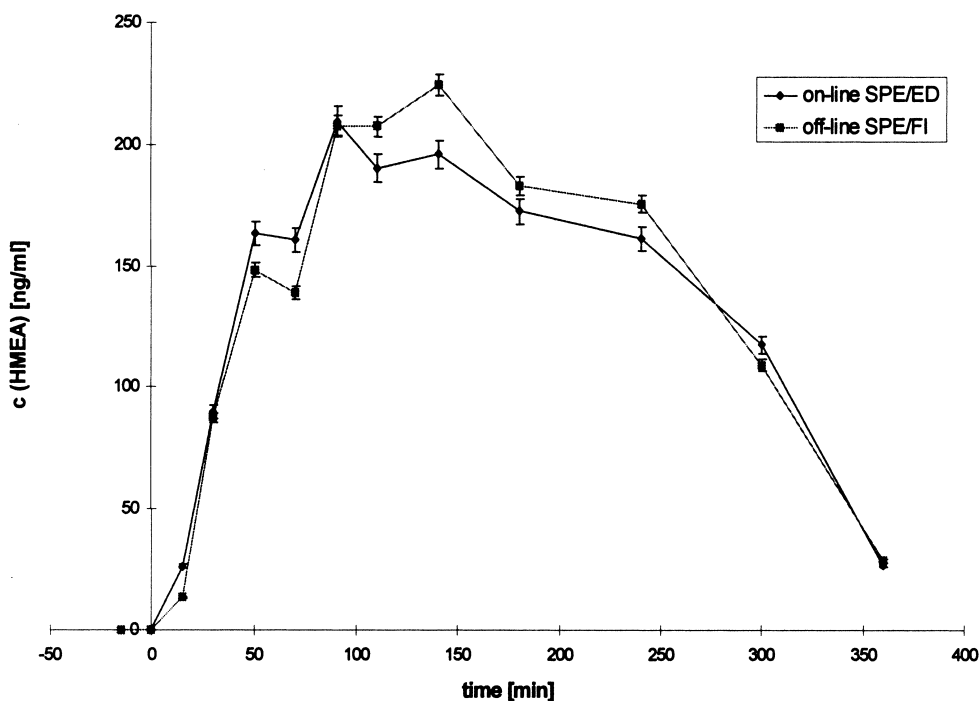


Fig. 3. Time-concentration curves of HMEA in plasma after p.o. administration of 2 mg/kg MDE, comparison of automated on-line solid-phase extraction with electrochemical detection (on-line SPE-ED) with off-line solid-phase extraction with fluorescence detection (off-line SPE-FI). Error bars: precision.

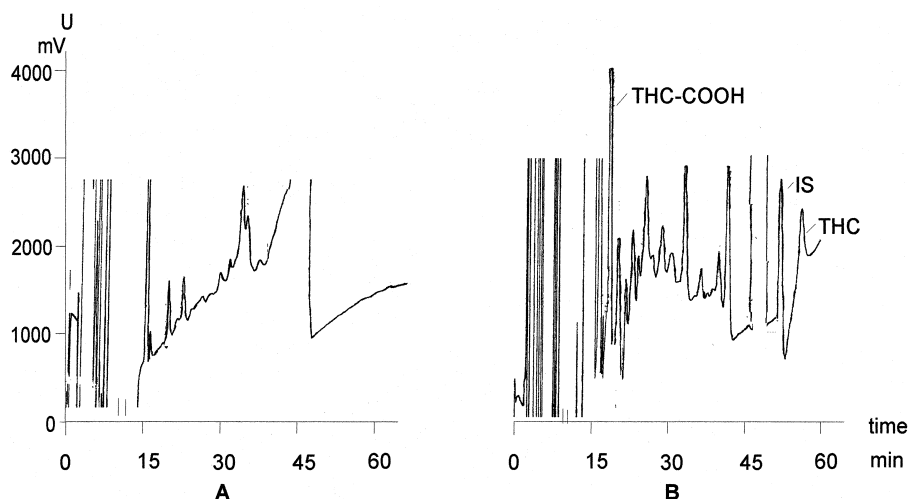


Fig. 4. HPLC chromatograms obtained from human plasma samples: (A) blank plasma, (B) real plasma sample from a study 33.5 h after last administration, $t_{R \text{ THC-COOH}} = 18.16$ min, $t_{R \text{ I.S.}} = 51.63$ min, $t_{R \text{ THC}} = 55.76$ min, $c_{\text{THC-COOH}} = 130.8$ ng/ml, I.S. = dodecylresorcinol, $c_{\text{THC}} < \text{limit of quantitation}$ (for integration parameters see Section 2.5).

lated $t = 2.18$ for $P = 95\%$, leading to acceptance of the zero hypothesis. This means that the automated solid-phase extraction on-line coupled with HPLC and ED and the off-line SPE with HPLC and fluorometric detection [3] yielded the same analytical results.

For THC and THC-COOH determination in plasma only one authentic biological sample was available (cf. Fig. 4). For THC-COOH a plasma concentration of 130.8 ng/ml was found. The signal of the THC peak was below the defined LOQ of 5 ng/ml and so the concentration for THC could not

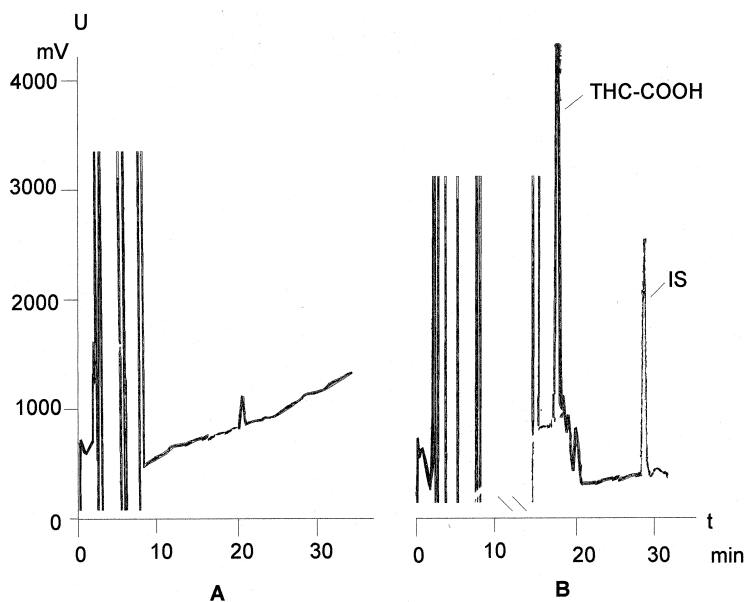


Fig. 5. HPLC chromatograms obtained from human urine samples: (A) blank urine, (B) real urine sample No. 108 (after dilution 1:20 with water), $t_{R \text{ THC-COOH}} = 18.0$ min, $t_{R \text{ I.S.}} = 31.28$ min, $c_{\text{THC-COOH}} = 1032$ ng/ml, I.S. = 4-octylphenol.

be calculated. The multitude of electrochemical detectable substances at +1.2 V is one reason for the big number of side peaks in these chromatograms. Another reason is the lack of specificity as consequence of the intended simultaneous determination of THC and THC-COOH in one assay.

The THC-COOH concentration was determined in two urine samples (cf. Fig. 5). The data achieved by immunoassays were for both samples above 270 ng/ml total amount of cannabinoids [29]. A prediction of the THC-COOH proportion was not possible concerning to inter-individual differences in the metabolic pathway [30]. The analytical results of the online SPE method were calculated as 1032 ± 56.7 ng THC-COOH/ml for sample No. 108 and 749.8 ± 41.2 ng THC-COOH/ml for sample No. 4. The reliability of these results could be demonstrated by comparison with the results of a method using off-line SPE with high-performance thin layer chromatography and in-situ UV detection [31]. The urine concentrations received by that method were for sample No. 108: 1331 ± 372 ng THC-COOH/ml and for sample No. 4: 622 ± 109 ng THC-COOH/ml [29] – showing for both samples an overlapping of the error bars (representing the precision).

4. Conclusions

The suitability of on-line coupling of automated SPE with HPLC and ED was proven even at high potentials. The high automatization minimizes possible variations in analytical results caused by different operators and also leads to less demands on staff time. Probably the greatest advantage of on-line SPE is the low plasma requirement. At least five-times the volume is required to reach the same determination limit by liquid–liquid extraction or off-line SPE. The described on-line SPE method for psilocin [17] and HMEA should be easily transferable to other basic substances. The assays were found suitable for use in routine analysis of psilocybin (“mushrooms”), ecstasy and its analogues and cannabis.

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