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Determination of tryptamine derivatives in illicit synthetic drugs by capillary electrophoresis and ultraviolet laser-induced fluorescence detection

A method based on separation by capillary electrophoresis combined with UV-laser-induced fluorescence detection (λ_{ex} = 266 nm) was developed for the determination of nine tryptamine derivatives of forensic interest and potential matrix constituents. The composition of the separation electrolyte was optimized with respect to the resolution of solutes of interest and to the sensitivity of fluorescence detection. Native α-cyclodextrin was employed as a complex forming modifier of the electrophoretic separation and fluorescence-enhancing agent. With the help of a stacking procedure, limits of detection of 0.1–6 μg/L for all analytes were obtained. The repeatability for the peak area (at a concentration of the analyte about 100 times the LOD) was less than 2.3% RSD. A second HPLC method was developed, and its analytical parameters were evaluated for an estimation of the accuracy of the CE-LIF method and for method comparison. The results of the determination of tryptamine derivatives in the samples of forensic interest obtained with the two independent methods are in good agreement.

Keywords: Capillary electrophoresis / α-Cyclodextrin / Frequency-multiplied diode-pumped solid-state laser / Illicit drugs / Stacking / Tryptamine derivatives / (UV)laser-induced fluorescence detection
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1 Introduction

Recent years show an increase in the consumption of psychotropic biogenic drugs. The use of the so-called "magic mushrooms" (mainly species of *Psilocybe*, *Stropharia*, *Conocybe*, and *Panaeolus*), which contain the tryptamine derivatives psilocin and psilocybin as the active ingredients, is well known. Bufotenine (5-hydroxy-DMT), a positional isomer of psilocin (4-hydroxy-DMT), is the active psychotropic substance in ancient snuff powders of South America (typically prepared from seeds of the plant of genus *Anadenanthera*) and in secretions of toads (*Bufo marinus*, *Bufo vulgaris*, and others). Unlike psilocin and psilocybin, bufotenine is no controlled substance under German narcotics act, but a controlled Schedule I hallucinogenic substance in the United States. Their synthetic counterparts are different tryptamine derivatives, mainly *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), *N*,*N*-diethyltryptamine (DET), 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT), α-methyltryptamine, and α-ethyltryptamine (structures are depicted in Fig. 1), all of them influencing the serotonergic system and being relevant to forensic toxicologists as they are controlled substances under German narcotics act. Among these controlled tryptamines, DMT and 5-MeO-DMT not only occur in illicit synthetic drugs, but also in a large number of natural sources. To date, there is a lack of a state-of-the-art method for the determination of tryptamine derivatives in samples with complex matrices.

In the analysis of tryptamine derivatives, a wide range of analytical techniques has been employed in the past. Tryptamine derivatives being present in intoxicating snuffs used by native people in South America were determined by GC with argon ionization detection after derivatization with hexamethyldisilazane [1], by GC-FID [2], or by GC-flame-ionization detector (FID) in combination with TLC and GC-MS [3]. DMT in ayahuasca (a South American psychotropic beverage) and in human plasma after dosing with ayahuasca was quantified by TLC and HPLC-UV detector (UVD) [4] and by GC-nitrogen-phosphorus detector (NPD) [5, 6]. Tryptamine derivatives in plant and mushroom extracts were identified and quantified by RP-HPLC-UVD [7], RP-HPLC-DAD [8, 9], RP-HPLC-UVD combined with amperometric detection [10, 11], or ion-exchange chro-

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Abbreviations: DAD, diode array detection; **DET**, *N*,*N*-diethyltryptamine; **DMT**, *N*,*N*-dimethyltryptamine; **i. st.**, internal standard; **LSD**, lysergic acid diethylamide; **MDMA**, 3,4-methylenedioxymethamphetamine; **5-MeO-DIPT**, 5-methoxy-*N*,*N*-diisopropyltryptamine; **5-MeO-DMT**, 5-methoxy-*N*,*N*-dimethyltryptamine; **NMT**, *N*-methyltryptamine

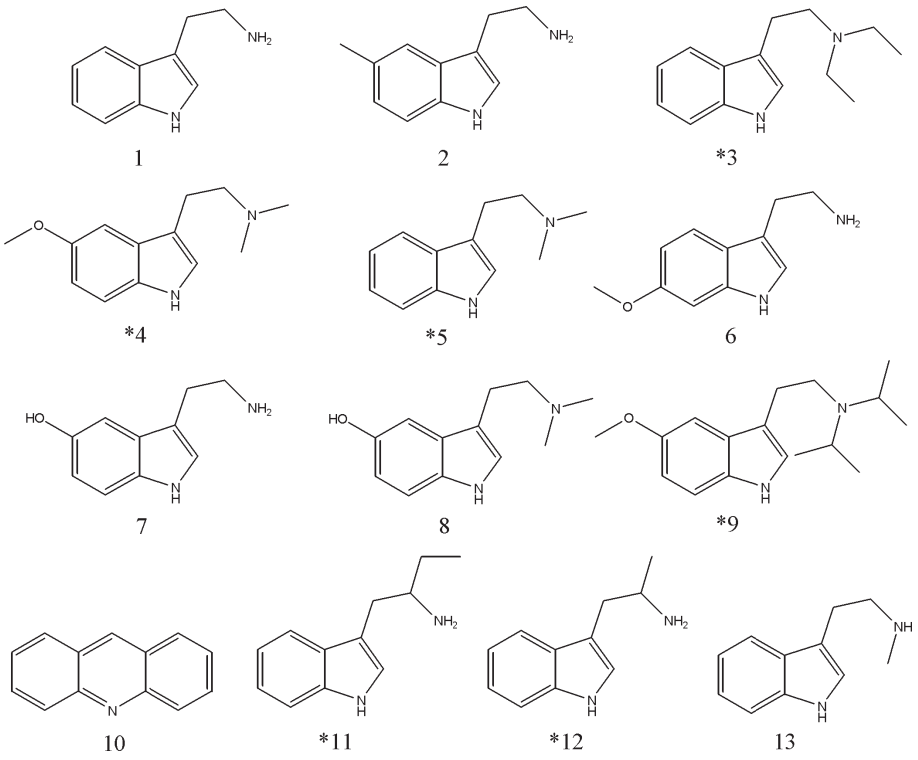

Figure 1. Structures of tryptamine derivatives quantified in this study: (1) tryptamine, (2) 5-methyltryptamine, (3) DET, (4) 5-MeO-DMT, (5) DMT, (6) 6-methoxytryptamine, (7) serotonin, (8) bufotenine, (9) 5-MeO-DIPT, (10) acridine (i.st.), (11) α-ethyltryptamine (α-ET), (12) α-methyltryptamine (α-MT), (13) NMT. Substances marked with an asterisk are controlled substances under German narcotics act.

matography combined with fluorescence detection (λ_{ex} = 267 nm, λ_{em} = 335 nm) [12]. In mouse or rat brain, tryptamine and related substances were quantified by GC-MS [13] or by RP-HPLC combined with fluorescence detection (λ_{ex} = 267 nm, λ_{em} = 335 nm) [14]. GC-MS was also used for the determination of bufotenine and related tryptamine derivatives in different samples of forensic interest [15, 16]. Most of the developed procedures are restricted to a limited number of tryptamine derivatives, however, in forensic applications analytical methods are desired, which make it possible to identify and quantify a large number of possible tryptamine derivatives within one single procedure.

In the present work, we develop a method for the determination of nine tryptamine derivatives (tryptamine, 5-methyltryptamine, DET, 5-MeO-DMT, DMT, 6-methoxytryptamine, serotonin, bufotenine, 5-MeO-DIPT) in items of evidence based on the separation of tryptamine derivatives and possible matrix constituents by CE combined with selective and sensitive laser-induced fluorescence detection. A low-cost frequency-multiplied diode-pumped solid-state laser emitting at 266 nm matching well the absorbance maxima of the analytes of interest is employed as the excitation source. The composition of the separation electrolyte is optimized concerning the resolution of solutes of interest and concerning the sensitivity of fluorescence detection. The first paper describing the use of CE-(UV-)LIF within a forensic background was published by Frost *et al.* [17] describing the determination of the structurally related lysergic acid diethylamide (LSD).

It is well-known that complexation with cyclodextrins (CDs) can be employed for an improvement of the sensitivity of fluorescence detection after an HPLC separation by adding CD to the eluent in a postcolumn mode. This effect is based on an enhancement of the fluorescence quantum yield due to a decrease in the rotation motion of the entrapped molecule and/or decrease in solvent relaxation [18]. In our work, we studied the influence of pH, the presence or absence of SDS micelles, and the influence of the α-CD concentration in the separation electrolyte on the selectivity of the separation and on the sensitivity of the detection.

In order to elucidate the accuracy of the method, a reference method employing HPLC-diode array detection (DAD) was developed for the baseline separation of eight tryptamine derivatives. Both methods were applied to the analysis of seized samples of illicit synthesis, showing a good correlation of results. The HPLC-DAD method was based on an HPLC method originally developed for the determination of indole alkaloids [19] (octadecyl silica gel, identical composition of the mobile phase).

2 Materials and methods

2.1 Chemicals

Serotonin, tryptamine, triethylamine, acridine, monosodium dihydrogenphosphate, sodium tetraborate, and hydrochloric acid were from Fluka (Buchs, Switzerland), SDS from Roth (Karlsruhe, Germany), α-CD, ammonium acetate, acetonitrile (LiChrosolv hypergrade), sodium hydroxide, and glacial acetic acid from Merck (Darmstadt, Germany), β-CD from Aldrich, (Milwaukee, WI, USA), and methanol (HPLC grade) from Riedel-de Haën (Seelze, Germany). Other tryptamine derivatives and seized samples of synthetic drugs containing tryptamine derivatives were provided by the Federal Criminal Police Office (Wiesbaden, Germany).

2.2 Buffer and sample preparation

For method development, the sample solutions were prepared by dissolving the analytes in methanol at a concentration of 2 mmol/L. The solutions with lower concentration of the analyte were prepared by dilution of the methanolic solution with separation buffer or water. The real samples were homogenized. Aliquots of 5–30 mg were dissolved in methanol, spiked with the internal standard, and directly injected after dilution. The buffer solutions were prepared in deionized water. The pH was adjusted by adding hydrochloric acid or sodium hydroxide, where necessary. pH measurements were carried out with a standard glass electrode with a Piccolo Plus (ATC pH/°C meter) from Hanna instruments (Kehl am Rhein, Germany) after a two-point calibration.

2.3 Instruments

Fluorescence spectra were measured with an FL3095 instrument (J&M Analytische Mess- und Regeltechnik, Aalen, Germany) with a Xe-lamp for excitation. Absorbance spectra were measured with a TIDAS II spectrometer (J&M Analytische Mess- und Regeltechnik). Quartz glass cuvettes of 10 mm optical path length were obtained from Hellma (Mülheim, Germany). A PrinCE CE instrument (Prince Technologies, Emmen, The Netherlands) with a modified buffer outlet and capillary holder was used. The sample injection was done hydrodynamically. Fused-silica capillaries from Polymicro Technologies LLC (Phoenix, AZ, USA) were used with an inner diameter of 75 μm and an outer diameter of 363 μm. The length was set to 66/46.5 cm. New capillaries were conditioned by flushing them first with an NaOH solution (0.1 mol/L) for 10 min and subsequently with a run buffer for 10 min. Rinsing with the running buffer for 1 min was used for cleaning the capillary between runs. A voltage of 15 kV was used for separation. The LIF detection system was a TIDAS LIF 266 system from J&M Analytische Mess- und Regeltechnik, which was described by Pütz [20]. The excitation radiation source was an FQSS266-S laser from Crystal (Berlin, Germany), which is a passively Q-switched frequency-converted diode-pumped solid-state microchip neodymium:yttrium-aluminum garnet laser with an optical quasi-cw power of about 0.5 mW at 266 nm. A photomultiplier detector was used for measuring the fluorescence intensity. Optical fibers connected the excitation source and the photomultiplier detector to the in-capillary detection cell, placed in a commercially available capillary holder (J&M, Analytische Mess- und Regeltechnik). The optical arrangement of this capillary holder was optimized with respect to maximizing the intensity of the excitation radiation in the inner volume of the detection cell, minimizing the instrumental band-broadening, and maximizing the fraction of emitted fluorescence light collected by one of the optical fibers [21]. The S/N was improved by optical filters (long pass filter LWP337 from Delta light & Optics, Lyngby, Denmark in combination with the dichroic band pass filter 505FD64 from LOT-Oriel, Darmstadt, Germany), which were placed between the detection window and the photomultiplier detector. The long pass filter (LWP337) absorbs radiation at the excitation wavelength of 266 nm and the dichroic band pass filter (505FD64) reflects the wavelength of the second harmonic (532 nm) of the exciting solid-state laser and the emission wavelength of the pump laser diode (808 nm). Original 6.0 Professional software was used for data analysis. A modular Shimadzu HPLC-DAD system consisting of an LC 10AT pump, a DGU-14A degasser, an SIL 10AD auto injector with a 20 μL sample loop, a CTO 10AC column oven, an SCL 10A system controller, and an SPD M10A diode array detector (Shimadzu, Japan) with a LiChroCART® 250–4 LiChrospher R 60 RP-select B (5 μm) HPLC cartridge (Merck) were used. A detection wavelength of 222 nm was selected.

3 Results and discussion

3.1 Selection of analytes

The analytes, which were selected as compounds of interest, are depicted in Fig. 1. All of them are structurally related tryptamine derivatives, some of them being controlled as psychotropic drugs in Germany (marked with an asterisk in Fig. 1), others relevant as precursors for controlled tryptamine derivatives (*e.g.*, tryptamine as the

most important precursor for synthetic DMT). Serotonin, an important neurotransmitter and tissue hormone, is very widespread in flora and fauna and has a close structural relationship with the hallucinogenic tryptamines bufotenine, 5-MeO-DMT, and 5-MeO-DIPT.

3.2 Optimization of separation buffer composition with regard to detection sensitivity

Possible buffer constituents can have a marked influence on the sensitivity of the method, due to the dependence of the fluorescence intensity on both the absorption coefficient at the wavelength of excitation and the fluorescence quantum yield.

3.2.1 Absorption

All analytes investigated have similar absorbance spectra (data not shown) due to their similarities in chemical structure. In neutral solution, they all exhibit a strong maximum in the range of 170–230 nm and a weaker maximum in the range of 250–300 nm, matching well the excitation wavelength of 266 nm. No significant changes in the absorption spectra relative to aqueous solutions were found upon addition of disodium tetraborate, SDS, β-CD, or methanol. For all the analytes, it holds true that the absorbance spectra are independent of the pH in acidic solution in a range of 2–7. In alkaline solution (pH 12), however, there is a shift to higher wavelengths for those analytes having a phenolic OH-group (see Fig. 1): serotonin and bufotenine, due to the dissociation of the OH-group (data not shown). However, the isosbestic point is around 266 nm. It can be concluded that the sensitivity in fluorescence detection for the analytes selected will not be influenced by the composition of the separation buffer *via* change in the absorption coefficient at 266 nm.

3.2.2 Fluorescence

We also investigated the influence of the composition of the solution in which the analytes are dissolved on the fluorescence quantum yield by determining the fluorescence spectra for solutions of the analytes at a constant analyte concentration and at a constant intensity of the excitation radiation at a wavelength of 300 nm. In Fig. 2 the fluorescence spectra for eight tryptamine derivatives of interest dissolved in an aqueous buffer (pH 7) are given. The maxima of the fluorescence spectra are between 350 and 375 nm. We investigated the influence of pH, the solvent composition, the presence of SDS micelles, and the presence of β-CD on the relative fluorescence intensity for selected analytes.

In Table 1, the data for the analytes investigated are given. There is only a moderate influence of the pH on the relative fluorescence intensity for basic tryptamine derivatives (*e.g.*, DMT), which is corroborated by results of Galian *et al.* [22] for tryptamine. In the case of the phenolic tryptamine derivatives (bufotenine and serotonin), there is a strong decrease in the relative fluorescence intensity at higher pH of the buffer solution, proportional to the degree of dissociation of the phenolic OH-group as shown in Fig. 3. This decrease has to be attributed to the decrease in fluorescence quantum yield rather than to the changes in the absorption coefficient.

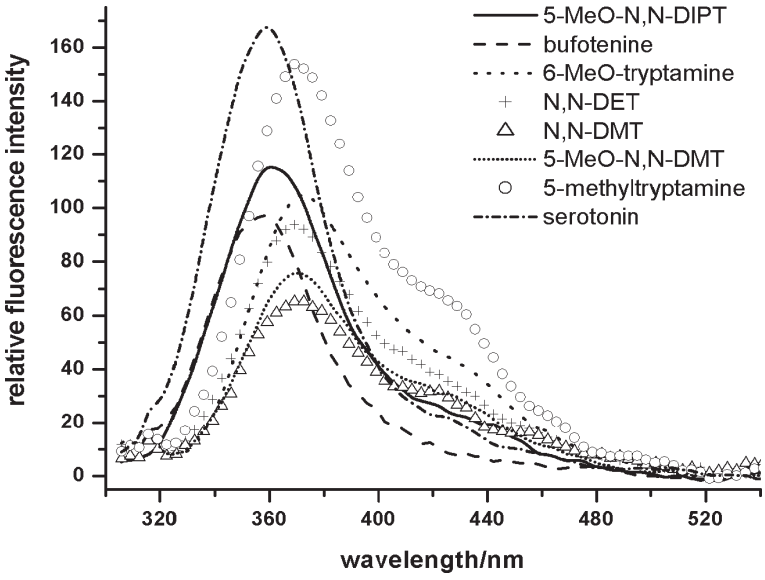

Figure 2. Fluorescence spectra, c = 10 μmol/L in an aqueous solution, pH 7, excitation at 300 nm.

Table 1. Relative fluorescence intensity at the peak maximum dependent on the composition of the sample solution (c(analyte) = 10 μmol/L), excitation at 300 nm, Xe-lamp

	pH 7[a]	pH 2[a]	pH 12[a]	Methanol	SDS buffer[b]	β-CD[c]
Serotonin	173	181	31	–[d]	–[d]	153
DMT	66	98	116	61	70	119
Bufotenine	103	204	21	169	181	198
5-Methyltryptamine	157	–[d]	–[d]	157	82	202
DET	99	–[d]	–[d]	97	161	170
6-Me0-tryptamine	111	–[d]	–[d]	169	106	213
5-Me0-DMT	78	–[d]	–[d]	83	78	112
5-Me0-DIPT	122	–[d]	–[d]	151	222	204

a) Aqueous solution, pH adjustment with HCl, NaOH
b) SDS buffer: c(SDS) = 60 mmol/L, c(disodium tetraborate) 10 mmol/L, pH 8.8
c) β-CD: aqueous solution of 5 mmol/L β-cyclodextrin
d) Data not available

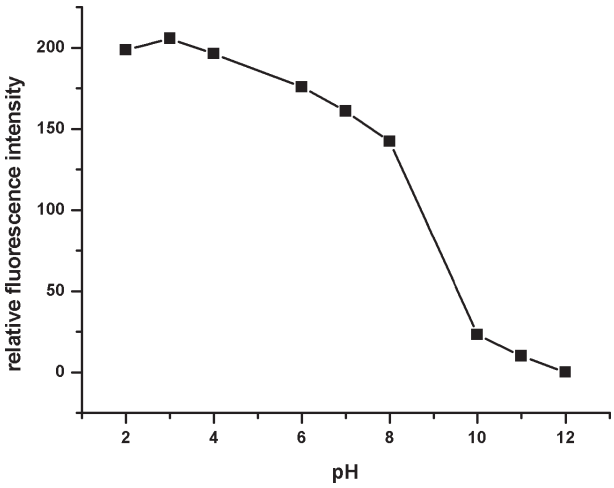

Figure 3. Dependence of the relative fluorescence intensity for an aqueous solution of bufotenine (10 μmol/L) on the pH, pH adjusted with HCl or NaOH solution, excitation at 300 nm, relative fluorescence intensity at a band maximum.

There is a moderate increase in the relative fluorescence intensity for most analytes investigated when replacing water as a solvent with methanol (see Table 1). The presence of SDS micelles has an unpredictable influence on the relative fluorescence intensity for the analytes investigated. Influences of the fluorescence quantum yield by the presence of micelles were also found for indole and indole derivatives [23]. In all cases there is a strong enhancement in the relative fluorescence intensity in the presence of 5 mmol/L β-CD in the sample solution. In some cases, an increase of about twofold in the relative fluorescence intensity was found for the aqueous solution containing β-CD compared to the aqueous solution of the analyte. A similar influence was found for α-CD (data not shown). The increase of the fluorescence quantum yield of tryptamine in the presence of β-CD was attributed to the formation of 1:1 inclusion complexes [22] and is explained by the restriction of intermolecular rotation in the rigid environment of the cavity and/or the exclusion of solvent relaxation [18].

From these data we decided to prefer separation by CE with an acidic buffer (protonated analytes) in the presence of α-CD or β-CD to other separation modes, because under these conditions we expected to obtain minimum limits of detection for all analytes of interest. The impact of the concentration of CD on the fluorescence quantum yield is a strong indicator that inclusion complexes between the tryptamine derivatives and CD are formed. Consequently, fine-tuning of the effective electrophoretic mobility of these analytes can be achieved by variation of the concentration of α-CD and/or β-CD.

3.3 Optimization of the separation conditions

In accordance with the results from the spectroscopic studies, separation by CD-modified CE was decided to be the method of choice. Following parameters were taken into consideration: buffer pH, concentration of an organic modifier, and concentration of α-CD and β-CD. The fact that the effective electrophoretic mobility for all analytes (data not shown) is independent of the pH (pH 3.3–6.0) shows that the degree of protonation remains constant (fully deprotonated) up to a pH of 6.0. In this range, no separation of all the analytes of interest is possible without further modifiers. With methanol as the organic modifier (50% v/v) there is an improved separation with one comigration of 5-MeO-DMT and 6-methoxytryptamine. In further studies, the pH was kept constant at 3.0, no organic modifier was added, and the concentration of α-CD or β-CD was varied in a concentration range up to 40 or 15 mmol/L, respectively.

As expected, the effective electrophoretic mobilities of the positively charged analytes were decreased by a complexation with the neutral host molecules. Optimum conditions were determined empirically. With β-CD comigration of analytes occurred, and the differences in the degree of complexation for the analytes of interest is too small with β-CD. The time difference between the migration of the fastest and the slowest analyte was less than 2 min compared to nearly 4 min time difference for α-CD. In Fig. 4, the separation of the nine tryptamine derivatives and acridine (internal standard) under optimum conditions (20 mmol/L monosodium dihydrogenphos-

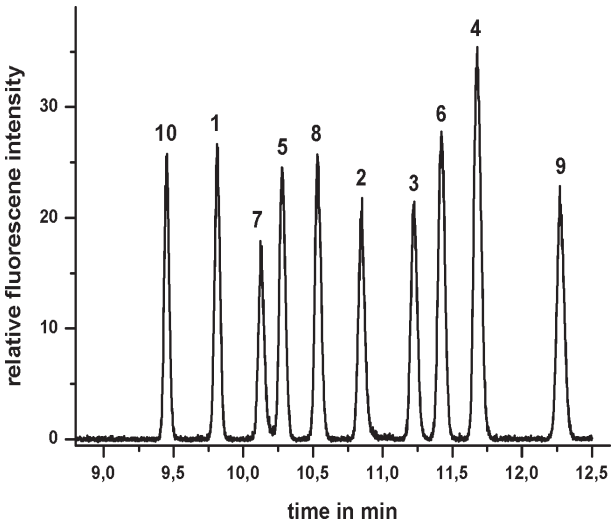

Figure 4. CE separation of standards, no stacking applied. Buffer: aqueous solution of 20 mmol/L NaH_2PO_4, pH 3.0, 15 mmol/L α-CD; voltage, 15 kV, 21.1 μA; sample solution: tryptamine (6.25 μmol/L), serotonin (25 μmol/L), 5-MeO-DIPT (20 μmol/L), bufotenine (25 μmol/L), 5-MeO-DMT (20 μmol/L), DMT (25 μmol/L), 6-methoxytryptamine (12.5 μmol/L), DET (12.5 μmol/L), 5-methyltryptamine (12.5 μmol/L); acridine (internal standard (i. st.) 0.75 μmol/L), injection, 3 s, 30 mbar, LIF detection, excitation 266 nm, peak assignment according to Fig. 1.

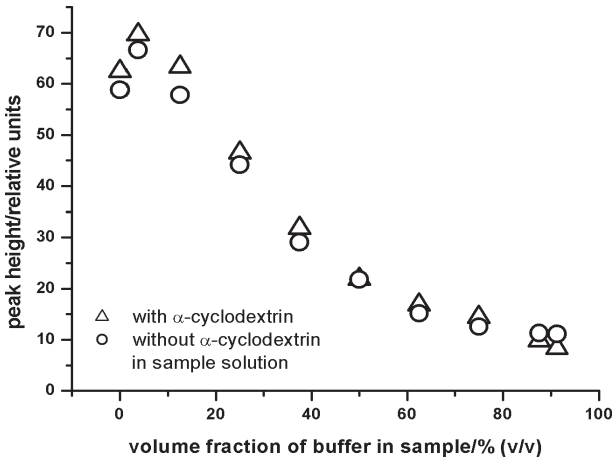

Figure 5. Dependence of peak height for 5-MeO-DIPT (20 μmol/L) on the volume fraction of separation buffer in the sample solution. Injection, 100 mbar for 6 s; buffer: aqueous solution of 20 mmol/L NaH_2PO_4, pH 3.0, 15 mmol/L α-CD; voltage, 15 kV, 20.8–22.1 μA; (Δ) sample dissolved in diluted buffer containing 15 mmol/L α-CD; (○) sample dissolved in diluted buffer without α-CD.

phate, pH 3.0, *c*(α-CD) = 15 mmol/L) is shown. A baseline separation of all the analytes of interest (and the internal standard acridine) is possible within 13 min. The migration order of the analytes is expected to correlate with the complex formation constants with α-CD (1:1 complex). Following structure units have been identified to influence the effective electrophoretic mobility *via* influence on the degree of complexation/complex forming constant: alkylation of the extracyclic amino group, hydroxy or methoxy substituent of the aromatic indole structure unit. We expect the indole unit to be included into the cavity of CD. Analytes with an alkylation of the extracyclic amino group have decreased electrophoretic mobilities (decrease dependent on the degree of alkylation). This decrease can be explained by an increase in the complex formation constants due to a stronger hydrogen bonding between the rim of the CD cavity and the more basic amino group. Analytes with a phenolic hydroxy group (serotonin and bufotenine) have high effective electrophoretic mobilities. They are expected to have very low complex formation constants due to a more polar aromatic structure unit. In contrast, molecules with an indolic methoxy-substituent have a decreased effective electrophoretic mobility (compared to their identical counterparts without methoxy group) explained by an increase in the complex formation constant due to steric reasons.

With sample solutions of low electric conductivity, stacking is possible. In Fig. 5 the peak height for 5-MeO-DIPT dependent on the volume fraction of separation buffer (diluted with water) in the sample solution is given. The sample injection parameters, the composition of the separation buffer, and all other separation parameters were kept constant. As expected for stacking, there is a decrease in the peak width at half height and an increase in the peak height with decreasing electric conductivity of the sample solution. The absence or presence of α-CD (component of the separation buffer) in the sample solution does not affect the peak shape. It can be concluded from this observation that the difference in the electric field strength (stacking) is the dominating on-line zone sharpening effect. Modification of the effective electrophoretic mobility by a buffer constituent which is not present in the sample solution (sweeping) is of negligible importance.

Figure 5 also shows that the injection parameters are close to optimum. With an injected zone of larger volume, instrumental band broadening will dominate the overall band broadening, while under the conditions selected there is an independence of the peak parameters of the electric conductivity of the sample solution for samples containing a volume fraction of separation buffer less than 10%. This independence indicates that in this region band broadening is dominated by the separation process (molecular diffusion).

In Fig. 6 the separation of standards employing the optimized stacking procedure is shown. Baseline separation was obtained except for serotonin and DMT due to the

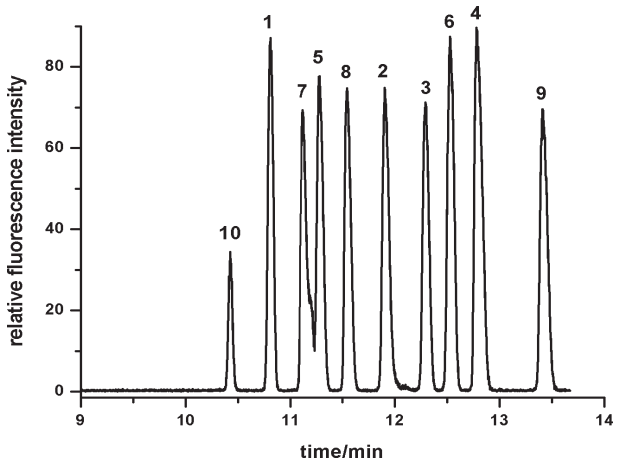

Figure 6. CE separation of standards under stacking conditions. Buffer: aqueous solution of NaH_2PO_4 20 mmol/L, pH 3.0, 15 mmol/L α-CD; voltage, 15 kV, 21.1 μA; sample solution: tryptamine (3.13 μmol/L), serotonin (12.5 μmol/L), 5-MeO-DIPT (10 μmol/L), bufotenine (12.5 μmol/L), 5-MeO-DMT (10 μmol/L), DMT (12.5 μmol/L), 6-methoxytryptamine (6.25 μmol/L), DET (6.25 μmol/L), 5-methyltryptamine (6.25 μmol/L); acridine (i.st. 0.075 μmol/L), injection 6 s, 100 mbar, LIF detection, excitation 266 nm, peak assignment according to Fig. 1.

presence of an impurity of unknown origin, which is also stacked by this procedure, migrating between serotonin and DMT. In Table 2, the mean plate numbers for seven consecutive runs dependent on the injection conditions are given. Although there is a significant decrease in the plate numbers when employing the stacking procedure, due to the EOF mismatch between the sample solution and the run buffer, the separation is sufficient and fit-for-purpose. The decrease in limits of detection (described in Section 3.4) justifies the limited decrease in efficiency obtained by the stacking procedure.

Table 2. Plate numbers obtained for different injection conditions

	Plate numbers	
	Without stacking	With stacking
Acridine	265 000	306 000
Tryptamine	287 000	182 000
Serotonin	307 000	169 000
DMT	280 000	147 000
Bufotenine	278 000	154 000
5-Methyltryptamine	265 000	151 000
DET	300 000	174 000
6-MeO-tryptamine	310 000	161 000
5-MeO-DMT	252 000	123 000
5-MeO-DIPT	265 000	149 000

Mean of seven consecutive runs, for additional parameters refer to Figs. 4 and 6.

3.4 Figures of merit for CE-(UV)LIF

Detection limits were calculated from smoothed electropherograms (data rate 12 Hz, averaging over five values) according to Eq. (1):

$$\mathrm{LOD} = c\frac{3s_R}{H} \quad (1)$$

with c being the concentration of the analyte, s_R the SD of the noise, and H the peak height.

The SD of the noise was calculated from an undisturbed interval of the baseline (2 min, about 1500 data points). The peak height was determined for seven consecutive injections of a sample containing the analyte at the concentration given in Figs. 4 and 6, respectively. The calculated limits of detection are listed in Table 3. Without the stacking procedure, detection limits between 1 and 64 μg/L were obtained. The stacking procedure lowered the detection limits by around one order of magnitude.

The repeatability of the peak height, the peak area, and the migration time was evaluated for seven consecutive measurements with concentrations of the analytes of about 100 times the LOD. In Table 4 the RSDs for the peak parameters and the migration times are given for the experimental parameters of Fig. 4 (without stacking conditions) and of Fig. 6 (employing stacking conditions). Acridine was employed as a fluorescing internal standard not comigrating with the analytes of interest. For the migration times there is a marked improvement in the repeatability, when the migration times of the analytes are normalized to the migration time of the internal standard. However, the same does not hold true for the peak parameters. It can be concluded that the migration times of the analyte and the internal standard are correlated to each other *via* the EOF velocity. However, there is no cor-

Table 3. Calculated detection limits depending on the injection procedure chosen (for experimental parameters refer to Figs. 4 and 6)

	Detection limits in μg L^{-1}		Detection limits in nmol L^{-1}	
	Without stacking	With stacking	Without stacking	With stacking
Acridine	0.7	0.1	4.1	0.3
Tryptamine	9.7	0.9	60	5.3
Serotonin	62	4.7	350	26
DMT	48	4.4	250	24
Bufotenine	50	5.0	250	25
5-Methyltryptamine	28	2.2	160	12
DET	32	2.8	150	13
6-MeO-tryptamine	22	2.0	110	11
5-MeO-DMT	31	3.6	140	16
5-MeO-DIPT	64	5.8	230	21

relation for the peak parameters (peak height, peak area), so that variations in the EOF velocity and variations in the injected sample volume can be excluded as the main sources of variation in the peak height and in the peak area. It can be assumed that fluorescence detector signal noise contributes mainly to the observed RSDs. From Table 4 it can also be deduced that the peak area is a better parameter for quantification than the peak height.

The linear working range (quantification by peak area) was determined by the graphical method (see Table 5). While the working range is limited for low concentrations by the limit of quantification, it is limited for higher concentrations by the limited linear measuring range of the photomultiplier detector used. At a photomultiplier voltage of 1000 V, the lowest possible limits of detection can be obtained (see Table 3). The linear range of the method can be shifted to higher concentrations (see Table 5) when decreasing the photomultiplier voltage. However, this shift of the upper limit to higher concentrations is accompanied by an increase in the limit of quantification.

Buffer solutions were stable for 2 weeks; storing them for a longer period resulted in turbid solutions, presumably due to decomposition of the CD. The pH of 3.0 chosen for the separation buffer was in the range of the high buffering capacity of phosphate (p*K*a = 2.14). No loss in separation efficiency due to changes at the capillary surface was observed during this project, thus capillaries were used for more than 500 runs. The capillaries were stored with the run buffer during night. The stability of runs was monitored *via* the electric current, which was found to be in the range of 20.9–21.3 μA for all measurements (for runs using a stacking procedure, these values were reached during the course of separation). The day-to-day precision was not evaluated; an internal standard was used to account for small variations in the laser beam intensity, as described by Schneede and Ueland [24].

3.5 Cross-validation *via* analysis of seized real samples by CE-(UV)LIF and HPLC-DAD

The accuracy of the method was estimated *via* cross-validation according to Wätzig *et al.* [25]: An isocratic HPLC-DAD procedure with an alkaline mobile phase was used as a second independent method for the determination of the tryptamine derivatives. The mobile phase consisted of methanol:acetonitrile 0.005 mol/L ammonium acetate (aqueous solution):triethylamine (1:2:2.5:0.02 v/v/v/v), adjusted to pH 8.5 with glacial acetic acid. The column oven temperature was kept at 25°C and the flow rate of the mobile phase at 0.8 mL/min. In Fig. 7 the separation of eight tryptamine derivatives is shown. Six tryptamine derivatives (α-MT, α-ET, 5-MeO-DIPT, DET, 5-MeO-DMT, and DMT, controlled substances under the German narcotics act) as well as bufotenine and NMT are separated within 13 min. The limits of detection were calculated from smoothed chromatograms (data rate 1.6 Hz, averaging over five values) according to Eq. (1); the LOD for the eight tryptamine derivatives of the HPLC procedure were in the range of 15.9–47.0 μg/L. The repeatability of the peak area and the retention time was evaluated for six consecutive measurements with concentrations of the analytes of about 500 times the LOD. The corresponding RSDs of the peak areas were 0.59% for 5-MeO-DIPT, 0.51% for DMT, and 1.13% for NMT. The RSDs of the retention times were 0.19% for 5-MeO-DIPT, 0.72% for DMT, and 0.16% for NMT.

Table 4. RSDs for migration times and peak parameters determined for seven consecutive injections of the standard samples (concentrations of analytes and further experimental parameters see Figs. 4 and 6)

	Without stacking (see Fig. 4)						With stacking (see Fig. 6)					
	Without normalization to IS			Normalized to IS			Without normalization to IS			Normalized to IS		
	Time	Height	Area	Time	Height	Area	Time	Height	Area	Time	Height	Area
Acridine (i.st.)	0.73	3.03	1.70				0.79	3.46	2.64			
Tryptamine	0.74	3.01	1.78	0.14	2.58	1.99	0.78	3.76	2.45	0.05	5.80	2.41
Serotonin	0.76	5.19	2.13	0.17	4.58	2.26	0.74	3.81	2.31	0.06	5.31	1.89
DMT	0.77	2.18	1.03	0.16	3.99	1.57	0.77	2.26	2.45	0.05	4.29	2.20
Bufotenine	0.78	4.85	2.08	0.19	4.67	2.19	0.77	2.12	2.22	0.07	4.06	2.91
5-Methyltryptamine	0.82	3.73	1.45	0.19	2.55	1.22	0.83	3.01	2.97	0.06	4.32	1.73
DET	0.87	2.00	1.47	0.23	3.26	1.65	0.81	3.12	2.61	0.09	4.96	1.73
6-MeO-tryptamine	0.85	2.47	1.84	0.22	3.63	1.12	0.90	2.38	2.19	0.09	3.87	1.83
5-MeO-DMT	1.30	4.17	0.24	0.24	4.17	1.30	0.88	2.44	2.40	0.07	4.62	1.87
5-MeO-DIPT	1.79	3.35	0.27	0.27	3.35	1.79	0.82	2.49	2.46	0.08	3.65	2.06

Table 5. Linear working ranges for the analytes of interest at different photomultiplier voltages (for experimental parameters refer to Fig. 4)

	Linear range in $\mu mol \cdot L^{-1}$	
	PM 1000 V	PM 900 V
Tryptamine	0.1–20	0.5–35
Serotonin	0.7–75	4–125
DMT	0.5–75	3–125
Bufotenine	0.5–80	3–120
5-Methyltryptamine	0.3–40	2–70
DET	0.3–40	2–70
6-MeO-tryptamine	0.2–40	1–70
5-MeO-DMT	0.3–60	2–100
5-MeO-DIPT	0.5–60	3–100

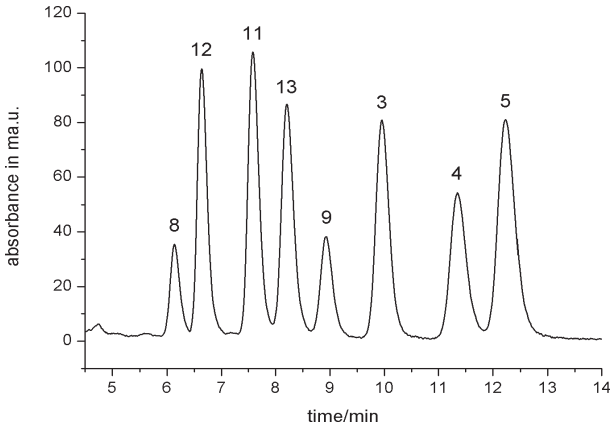

Figure 7. HPLC separation of standards. Mobile phase: methanol:acetonitrile 0.005 mol/L ammonium acetate (aqueous solution): triethylamine (1:2:2.5:0.02 v/v), adjusted to pH 8.5, flow rate of the mobile phase: 0.8 mL/min; sample solution: bufotenine (5.11 mg/L), α-methyltryptamine (4.97 mg/L), α-ethyltryptamine (18.92 mg; not chemically pure – seized sample also containing caffeine), NMT (5.14 mg/L), 5-MeO-DIPT (5.10 mg/L), DET (6.69 mg/L), 5-MeO-DMT (7.12 mg/L), DMT (7.67 mg/L); detection wavelength 222 nm; peak assignment according to Fig. 1.

The active compounds of three different seized samples of clandestinely produced synthetic drugs were determined by CE-(UV)LIF and HPLC-DAD.

3.5.1 Samples

Two of the samples were brownish crystalline powders containing DMT (sample 1) and 5-MeO-DIPT (sample 2) as active substances. The third sample was an ivory-colored Ecstasy tablet with the logo "N" (diameter: 7.2 mm, weight: 139.2 mg) that contained the active substances 5-MeO-DIPT and traces of 3,4-methylenedioxymethamphetamine (MDMA). The trace amount of MDMA (identified *via* NMR and ion mobility spectrometry) presumably was a contamination from the tableting process in the clandestine laboratory.

3.5.2 CE-(UV)LIF

The samples were finely ground, weighed (20–30 mg), and mixed with 10 mL methanol. Only the powder samples (samples 1 and 2) could be completely dissolved in methanol (homogeneous solution). The methanolic extract of the powdered tablet (sample 3) was separated from the insoluble adjuvants (mainly starch and fatty acids) by decanting. The sample solutions were diluted (1:10–25) and the internal standard was added. The tryptamine derivatives were quantified *via* an external calibration curve with double injections of the calibration solutions (concentrations: 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 μmol/L). The electropherograms for samples 1 and 2 are given in Fig. 8. Peak identification was done by spik-

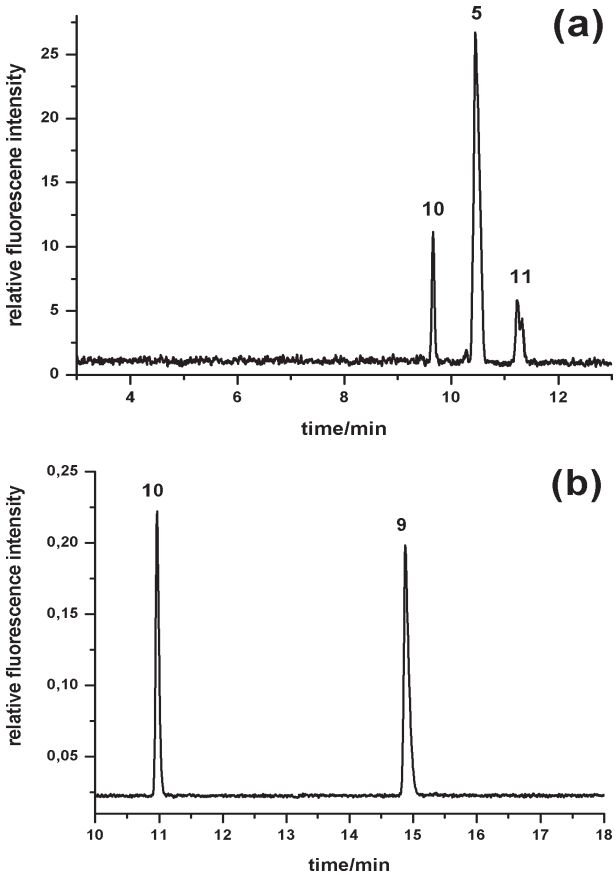

Figure 8. CE-(UV)LIF analysis of the powder samples of seized synthetic drugs, (a) sample 1, (b) sample 2, acridine as internal standard. Buffer: aqueous solution of 20 mmol/L NaH_2PO_4, pH 3.0, 15 mmol/L α-CD, voltage 15 kV, 27.1 μA, injection 3 s, 30 mbar, LIF detection, excitation 266 nm, peak assignment according to Fig. 1.

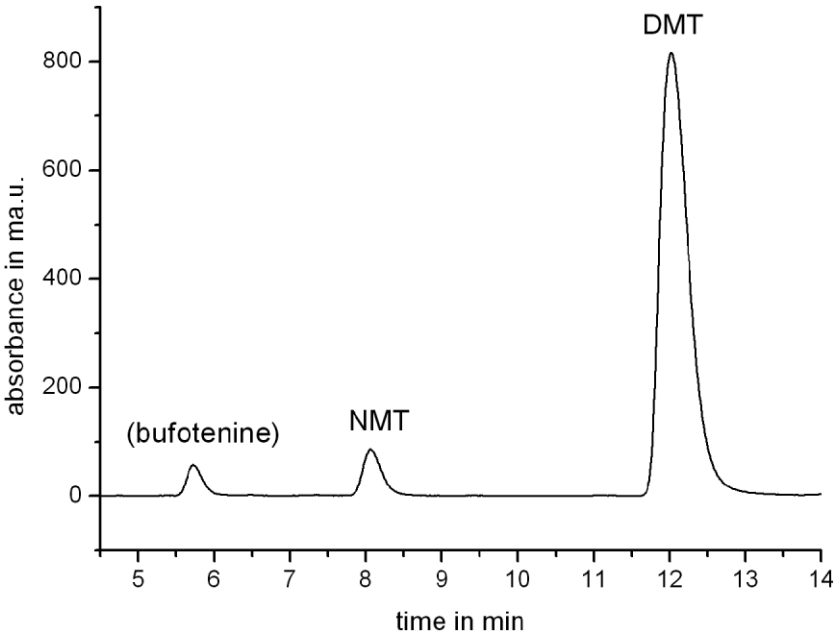

Figure 9. HPLC-DAD analysis of the seized synthetic drug Sample 1, peak detection wavelength: 222 nm; for separation conditions refer to Fig. 7.

ing. The peak migrating at 11.3 min (Fig. 8a) could be identified to be α-ethyltryptamine. For sample 2, it was confirmed that the repeatability for real samples (normalization on value for the internal standard acridine, seven consecutive injections) is not significantly lower than that obtained for standard samples (RSD (migration time) = 0.27%, RSD (peak area) = 1.8%, RSD (peak height) = 3.4%).

3.5.3 HPLC-DAD

About 1 mg of the homogenized real sample was mixed with 2 mL methanol, ultrasonificated, made up to a volume of 10 mL with mobile phase, and filtered *via* 0.45 μm syringe filter. The sample solutions were diluted with mobile phase (1:2–5) and directly analyzed. The tryptamine derivatives were quantified *via* an external calibration procedure with double injections of five calibration solutions (1.5–150 mg/L). Peak identification was done by comparison of the absorbance spectra and, if required, by spiking. In samples 2 and 3 only 5-MeO-DIPT was present. A chromatogram for Sample 1 (Fig. 9) reveals three main components: DMT (t_R = 12.02 min), NMT (t_R = 8.06 min), and a third component at t_R = 5.72 min. After spiking with bufotenine (5-OH-DMT), an increase in peak height but no increase in the full width at half maximum of the peak t_R = 5.72 min was observed, although the absorbance spectra for bufotenine and the third component are significantly different (data not shown). Psilocin (4-OH-DMT), being a positional isomer of bufotenine, shows a peak at t_R = 6.39 min and also differs from the absorbance spectrum of the analyte detected. The position isomers with the hydroxyl group in position 6 or 7 were not available as reference substances and thus could no be confirmed or excluded, but especially 6-OH-DMT should be closely related to bufotenine with respect to the chromatographic retention and the absorbance spectrum.

The presence of 5.3% (m/m) NMT in sample 1 points to a synthesis procedure for DMT, *via* demethylation of an *N*,*N*,*N*-trimethyltryptammonium salt (the di-demethylation also takes place and often the resulting NMT is not properly removed). Additionally, a trace of α-ethyltryptamine was detected in sample 1.

3.5.4 Results of the cross-validation

The figures of merit of both methods reveal lower limits of detection for CE-(UV)LIF with the stacking procedure. However, the repeatability of the peak area is better for the HPLC-DAD method. The repeatability of the migration time for CE-(UV)LIF without stacking (normalized to the internal standard) is comparable to the repeatability of the retention time for HPLC-DAD (without internal standard), however, the repeatability is even better for the normalized migration time for the CE-(UV)LIF stacking procedure with RSDs of 0.05–0.09% (Table 4). CE-(UV)LIF and HPLC-DAD were shown to be suitable for the determination of tryptamine derivatives in the samples of forensic interest. Calibration curves were linear with $R > 0.996$ for the two techniques. The CE-(UV)LIF

Table 6. Quantitative results (mg/g) for the main active substances in the samples of illicit synthetic drugs (for experimental parameters refer to Figs. 8 and 9)

Sample	Analyte	CZE	HPLC
1 (powder)	DMT	832.4	828.0
2 (powder)	5-MeO-DIPT	939.1	967.6
3 (tablet)	5-MeO-DIPT	108.0	91.3

and HPLC-DAD data for the quantification of the main active substances in samples 1 and 2 are in excellent accordance (see Table 6). Both samples exhibit a high content of the active tryptamine derivative, the 5-MeO-DIPT containing sample being of high purity, and the DMT containing sample showing considerable amounts of side products.

Regarding the data for the Ecstasy tablet "N" (sample 3), the relative deviation between the CE-(UV)LIF and HPLC-DAD amounts to about 10%. This might be attributed to slightly different procedures for the extraction of the powdered tablet. Based on the 5-MeO-DIPT content of 9.1% (HPLC quantification) in the tablet sample, an absolute amount of 12.7 mg 5-MeO-DIPT *per* tablet can be calculated. This value is consistent with doses of 8–12 mg (orally), which are reported in the relevant literature and in Internet sources to produce an LSD-like effect.

4 Concluding remarks

The CE-(UV)LIF method presented in this study enables a fast baseline separation of nine tryptamine derivatives, and their determination in samples of forensic interest, such as illicit synthetic drugs, but also pharmaceutical formulations. The (UV)LIF detection gives rise to a selective and sensitive determination of the analytes in real samples, which can be achieved with good repeatability. The accuracy of the method was verified by cross-validation with an independent HPLC-DAD method. The limits of detection obtained by the CE-(UV)LIF method are lower than those obtained by the alternative HPLC-DAD method.

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