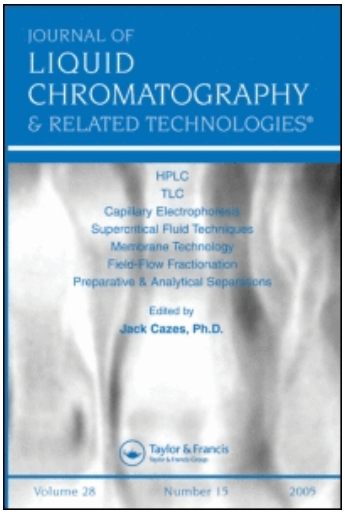

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A RAPID METHOD FOR DETERMINATION OF FOUR THIOAMPHETAMINE DESIGNER DRUGS (ALEPH-4, ALEPH-8, ALEPH-13, ALEPH-17) IN HUMAN URINE

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A RAPID METHOD FOR DETERMINATION OF FOUR THIOAMPHETAMINE DESIGNER DRUGS (ALEPH-4, ALEPH-8, ALEPH-13, ALEPH-17) IN HUMAN URINE

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□ *An analytical procedure for the simultaneous determination in human urine of four thioamphetamine designer drugs (ALEPH series) is reported. The quantitative analysis was performed by capillary electrophoresis with diode array detector (CE-DAD), using 2,5-dimethoxy-4-methylthioamphetamine-D_3 (ALEPH-D_3) as internal standard. In order to minimize interferences with matrix components and to preconcentrate target analytes, solid phase extraction was introduced in the method as a clean-up step. The method was validated according to international guidelines. Data for accuracy and precision were within required limits. Calibration curves were generated ranging from 1 to 500 $\mu g\,mL^{-1}$ and correlation coefficients always exceeded 0.998. The method was demonstrated to be specific, simple, and reliable for the analysis of these derivatives in urine samples.*

Keywords ALEPH-13, ALEPH-17, ALEPH-4, ALEPH-8, ALEPH-D_3, urine

INTRODUCTION

The continuous search for new psychoactive compounds has provided the drug-of-abuse market with more and more amphetamine designer drugs. New designer drugs are being introduced because these compounds are not covered by existing legislation. Therefore, these new drugs cannot be considered illicit drugs until their names are officially recognized.

The most recent development in Europe is the marketing of thio-substituted phenethylamines as 2C-T-2 (2,5-dimethoxy-4-ethylthiophenethylamine) and 2C-T-7 (2,5-dimethoxy-4-(n)-propylthiophenethylamine).[1] A number of analytical procedures for identification of these compounds by GC-MS has been reported.[2–4]

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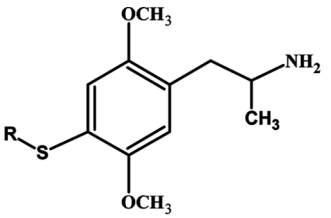
In our previous papers, a method for identification and quantification in human plasma and urine of several 2,5-methylenedioxy-derivatives of 4-thioamphetamine (ALEPH-series) and 4-thiophenethylamine (2C-T series) has recently been reported.[5–7] The determination of these substances is important for the protection and prevention of the risk to human health, mainly for young people who are the most exposed categories. Monitoring of amphetamines and designer drugs in biological fluids is successfully used for clinical and forensic application and in surveillance of drug substitution. The excretion of amphetamines and related stimulants mainly occurs in urine, where substantial amounts of unchanged drug are present.[8]

This paper describes a method for the identification and quantification of other four active compounds of ALEPH-series (ALEPH-4, ALEPH-8, ALEPH-13, and ALEPH-17) in human urine (Figure 1). The quantitative analysis was performed by capillary electrophoresis with diode array detector (190–350 nm), using 2,5-dimethoxy-4-methylthioamphetamine-D_3 (ALEPH-D_3) as internal standard (IS). The identification using migration time was confirmed by UV spectra. This procedure is simple, clean, and can easily be applied to epidemiological and clinical studies. In addition,



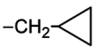
Code	Compounds	R
ALEPH-4	2,5-dimethoxy-4-(i)-propylthioamphetamine	-CH $(CH_3)_2$
ALEPH-8	2,5-dimethoxy-4-cyclopropylmethylthioamphetamine	$-CH_2-$ 
ALEPH-13	2,5-dimethoxy-4-(2-methoxyethyl) thioamphetamine	$-CH_2CH_2OCH_3$
ALEPH-17	2,5-dimethoxy-4-(i)-butylthioamphetamine	$-CH_2CH(CH_3)CH_3$
ALEPH-D_3	2,5-dimethoxy-4-methylthioamphetamine-D_3	$-CD_3$

FIGURE 1 Chemical structures of 2,5-dimethoxy-4-thioamphetamines analysed.

this method can be useful for their future identification in biological matrices as well as in confiscated tablets.

EXPERIMENTAL

Reagent and Chemicals

The 2,5-dimethoxy-derivatives of 4-thioamphetamine (Figure 1) were synthesized in our laboratory at their maximum level of purity using a slight modification of a method described in the literature.[9] Following the synthesis, the final products were identified by IR and NMR. IR spectra were recorded as Nujol mulls on NaCl plates with a Perkin-Elmer 1760-X IFT. The product characterization by ^{1}H-NMR spectrometry was carried out using a Bruker AMX 400.

ALEPH-D$_3$ (IS): ^{1}H NMR ($CDCl_3$) δ: 1.08–1.14 (d, 3H, CH_2-CH($C\mathbf{H_3}$)-NH_2); 1.59 (br s, 2H, exch. with D_2O, 2H, $N\mathbf{H_2}$); 2.40–2.77 (m, 2H, $C\mathbf{H_2}$-CH(CH_3)-NH_2); 3.10–3.23 (m, 1H, CH_2-C**H**(CH_3)-NH_2); 3.80 (s, 3H, O-$C\mathbf{H_3}$); 3.85 (s, 3H, O-$C\mathbf{H_3}$); 6.68 (s, 1H, arom); 6.76 (s, 1H, arom).

ALEPH-4: ^{1}H NMR ($CDCl_3$) δ: 1.08–1.18 (d, 3H, CH_2-CH($C\mathbf{H_3}$)-NH_2); 1.21–1.31 (d, 6H, CH-($C\mathbf{H_3}$)$_2$); 1.57 (br s, 2H, exch. with D_2O, 2H, $N\mathbf{H_2}$); 2.42–2.80 (m, 1H, $C\mathbf{H_2}$-CH(CH_3)-NH_2); 3.10–3.23 (m, 1H, CH_2-C**H**(CH_3)-NH_2); 3.39–3.56 (m, 1H, C**H**-(CH_3)$_2$); 3.78 (s, 3H, O-$C\mathbf{H_3}$); 3.84 (s, 3H, O-$C\mathbf{H_3}$); 6.70 (s, 1H, arom); 6.91 (s, 1H, arom).

ALEPH-8: ^{1}H NMR ($CDCl_3$) δ: 0.20–0.26 (q, 2H, $C\mathbf{H_2}$ cyclopropyl); 0.53–0.59 (q, 2H, $C\mathbf{H_2}$ cyclopropyl); 1.08–1.11 (d, 3H, CH_2-CH($C\mathbf{H_3}$)-NH_2); 1.12–1.30 (m, 1H, C**H** cyclopropyl); 1.68 (br s, 2H, exch. with D_2O, $N\mathbf{H_2}$); 2.42–2.77 (m, 2H, $C\mathbf{H_2}$-CH(CH_3)-NH_2); 2.79–2.88 (d, 2H, S-$C\mathbf{H_2}$-cyclopropyl); 3.05–3.23 (m, 1H, CH_2-C**H**(CH_3)-NH_2); 3.78 (s, 3H, O-$C\mathbf{H_3}$); 3.85 (s, 3H, O-$C\mathbf{H_3}$); 6.67 (s, 1H, arom); 6.88 (s, 1H, arom).

ALEPH-13: ^{1}H NMR ($CDCl_3$) δ: 1.00–1.15 (d, 3H, CH_2-CH($C\mathbf{H_3}$)-NH_2); 1.70 (br s, 2H, exch. with D_2O, $N\mathbf{H_2}$); 2.43–2.80 (m, 2H, $C\mathbf{H_2}$-CH(CH_3)-NH_2); 3.02–3.13 (t, 2H, S-$C\mathbf{H_2}$); 3.14–3.26 (m, 1H, CH_2-C**H**(CH_3)-NH_2); 3.36 (s, 3H, CH_2-O-$C\mathbf{H_3}$); 3.50–3.61 (t, 2H, $C\mathbf{H_2}$-O-CH_3); 3.78 (s, 3H, O-$C\mathbf{H_3}$); 3.84 (s, 3H, O-$C\mathbf{H_3}$); 6.69 (s, 1H, arom); 6.93 (s, 1H, arom).

ALEPH-17: ^{1}H NMR ($CDCl_3$) δ: 1.00–1.06 (d, 6H, CH-($C\mathbf{H_3}$)$_2$); 1.08–1.15 (d, 3H, CH_2-CH($C\mathbf{H_3}$)-NH_2); 1.70–1.90 (m, 1H, CH_2-C**H**-(CH_3)$_2$); 1.99 (br s, exch. with D_2O, $N\mathbf{H_2}$); 2.40–2.70 (m, 2H, $C\mathbf{H_2}$-CH(CH_3)-NH_2); 2.73–2.80 (m, 2H, S-$C\mathbf{H_2}$-CH-(CH_3)$_2$); 3.16–3.23 (m, 1H, CH_2-C**H**(CH_3)-NH_2); 3.78 (s, 3H, O-$C\mathbf{H_3}$); 3.85 (s, 3H, O-$C\mathbf{H_3}$); 6.67 (s, 1H, arom); 6.82 (s, 1H, arom).

Deionized and distilled water was purified through a Milli Q water system (Millipore). Other reagents and solvents used were of the highest commercial quality. Aqueous stock solutions (1.0 mg mL^{-1}) of

thioamphetamine derivatives were prepared, stored at −20°C, and diluted with Milli Q water to appropriate concentrations before use.

Quality control (QC) solutions containing all the analytes at three working concentrations (low-, medium-, and high-QC samples containing 50, 200, and 400 μg mL^{-1} of each analyte) and solutions of 50 μg mL^{-1} of the IS were prepared in blank urine.

Drug-free urine collected from 9 healthy adults male was used to make blank and spiked samples containing thioamphetamine derivatives.

Apparatus

Separations in capillary electrophoresis were performed using model HP (Hewlett-Packard) capillary electrophoresis system (Agilent Technologies).

Uncoated fused-silica capillary (50 cm × 50 μm ID) was used for the capillary electrophoresis separation. The running buffer consisted of 100 mM sodium phosphate adjusted pH 2.5 with phosphoric acid. A separation voltage of 10 kV was applied. Samples were injected hydrodynamically with a pressure of 50 mbar for 10 s. The detection was made at 210 nm.

Extraction Procedure from Urine

Amphetamines were extracted using our previously described procedure for other amphetamine analogous.[7,10–13] Briefly, urine samples (1 mL) were spiked with 50 μg of IS and mixed with hydrogencarbonate buffer (100 mM, pH 10, 1 mL). The mixture was applied to a Bond Elut C_{18} extraction column, previously activated and conditioned with 1 mL of methanol and 1 mL of 100 mM hydrogencarbonate buffer (pH 10). After the application of the sample, the column was washed with 2 mL of Milli Q water and dried by passing a stream of air for 5 min. The analytes were then eluted with 2 mL of methanol and the eluate was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 1 mL of the separation buffer.

Method Validation

The method validation was performed according to the accepted guidelines.[14–16]. The selectivity of the method was evaluated by analyzing urine from 9 healthy non-drug-consuming subjects.

Blank urine samples, extracted as described previously, were fortified with 50 μg mL^{-1} of IS and appropriate amounts of amphetamines, at concentrations ranging from 1 to 500 μg mL^{-1}. The linearity of the compound-to-IS peak ratio versus the theoretical concentration was verified in urine by using a $1/x$ weighted linear regression. The correlation coefficients (r^2)

and the curvature were tested on a set of five calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated on the calibration curve as $\mu + 3\sigma$ and $\mu + 10\sigma$, respectively, where μ is the average signal value of the noise. The precision and the accuracy of the method were evaluated at three concentrations over the linear dynamic range (50, 200, 400 $\mu g\,mL^{-1}$). Precision was expressed as the percent relative standard deviation (%RSD), where the sample standard deviation (s) was calculated for five replicates for each level for the within-day (intra-day) precision and over 5 days for the between-day (inter-day) precision. Accuracy was evaluated using the percentage of the measured concentration value versus the target concentration. Finally, reproducibility of migration time (t_M) was evaluated by calculating RSD (%) of the migration times of a standard solution (200 $\mu g\,mL^{-1}$) in ten sample injections (with washing every third injection).

Recoveries were determined at three concentrations (50, 200, 400 $\mu g\,mL^{-1}$) for each compound. Nine blank samples for each concentration were fortified with the appropriate amount of mixed standard solution. The recoveries were calculated by comparing the peak areas obtained from the extract of the spiked urine sample with those obtained by direct injection of standard solution at the same concentration.

RESULTS AND DISCUSSION

The calibration curves showed linearity in the range of 1–500 $\mu g\,mL^{-1}$ for all phenethylamines analyzed and the correlation coefficients (r^2) were higher than 0.998 (Table 1). The limits of detection (LOD) and quantification (LOQ) ranged from 7.0 to 14.5 $\mu g\,mL^{-1}$ and from 33.4 to 65.9 $\mu g\,mL^{-1}$, respectively (Table 1).

The extractive procedure from urine allowed one to obtain electropherograms free from interfering extraneous peaks. Figure 2 shows a full scan electropherogram of 50 $\mu g\,mL^{-1}$ spiked urine. Qualitative analysis was performed according to migration times (t_M) and UV spectra. Data for precision and accuracy (Table 2) were within required limits.[14–16] The intra-day and inter-day RSD (%) for three different concentrations were from 2.3 to 6.6% (Table 2).

TABLE 1 Validation Parameters

Analyte	Slope ± SD (n = 5)	Intercept ± SD (n = 5)	r^2	LOD ($\mu g\,mL^{-1}$)	LOQ ($\mu g\,mL^{-1}$)
ALEPH-4	1.163 ± 0.005	−0.056 ± 0.009	0.9997	7.9	36.0
ALEPH-8	1.286 ± 0.012	−0.007 ± 0.002	0.9982	14.5	65.9
ALEPH-13	0.883 ± 0.050	−0.079 ± 0.003	0.9998	7.0	33.4
ALEPH-17	1.080 ± 0.015	−0.211 ± 0.009	0.9985	8.7	43.3

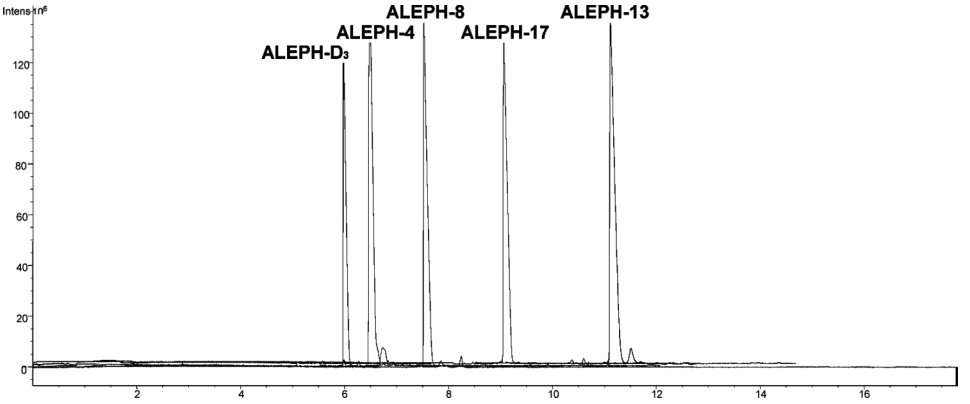


FIGURE 2 Overlay chromatograms of urine samples spiked with 50 μg mL^{-1} of 2,5-dimethoxy-4-thioamphetamines.

TABLE 2 Accuracy and Repeatability (Intraday and Interday)

Analyte	Conc. (μg mL^{-1})	Intraday RSD (%) (n = 5)	Interday RSD (%) (n = 5)	Accuracy (%) (n = 5)	Repeatability of instrument (RSD)
ALEPH-4	200	4.5	5.0	99	0.23
	400	2.3	4.9	101	
ALEPH-8	200	5.4	6.3	97	0.35
	400	3.2	5.4	97	
ALEPH-13	200	3.5	4.7	96	0.25
	400	3.9	5.0	98	
ALEPH-17	200	5.9	6.6	101	0.33
	400	6.4	7.4	99	

RSD = Relative Standard Deviation.

TABLE 3 Recovery

Analyte	Spiked Conc (μg mL^{-1})	Recovery (% ± RSD) (n = 9)	Mean Recovery (%)
ALEPH-4	50	72.2 ± 2.1	
	200	89.1 ± 3.9	82.6
	400	86.4 ± 3.4	
ALEPH-8	50	62.1 ± 1.3	
	200	66.5 ± 3.4	69.8
	400	80.7 ± 2.6	
ALEPH-13	50	76.7 ± 4.0	
	200	75.0 ± 2.7	77.9
	400	82.0 ± 1.3	
ALEPH-17	50	54.5 ± 4.2	
	200	60.2 ± 2.9	64.2
	400	78.0 ± 3.6	

Recovery percentages obtained from spiked urine were better than 64.2%. The values of recoveries at three fortification levels were reported in Table 3.

With regard to the analytical procedure, this is the first method that allows the simultaneous determination of these four compounds in human urine. The main advantage of our method is that it allows for simple, clean, and reliable SPE extraction of these amphetamines from human urine.

Abbreviations

2,5-dimethoxy-4-(i)-propylthioamphetamine (ALEPH-4); 2,5-dimethoxy-4-cyclopropylmethylthioamphetamine (ALEPH-8); 2,5-dimethoxy-4-(2-methoxyethyl) thioamphetamine (ALEPH-13); 2,5-dimethoxy-4-(i)-butylthiothioamphetamine (ALEPH-17); 2,5-dimethoxy-4-methylthioamphetamine-D_3 (ALEPH-D_3).

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