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Determination of drugs of abuse and their metabolites in human plasma by liquid chromatography–mass spectrometry An application to 156 road fatalities

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Abstract

A method, using 0.2 ml of plasma, was designed for the simultaneous determination of morphine, 6-monoacetylmorphine, amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, benzoylecgonine and cocaine. The drugs were analysed by LC–MS, after solid phase extraction in the presence of the deuterated analogues. Reversed phase separation on an Atlantis dC18 column was achieved in 10 min, under gradient conditions. The method was full validated, including linearity (2–250 ng/ml, $r^2 > 0.99$), recovery (>50%), within-day and between-day precision and accuracy (CV and bias <15%), limit of detection (0.5 and 1 ng/ml) and quantitation (2 ng/ml), relative ion intensities and no matrix effect was observed. The procedure showed to be sensitive and specific, and was applied to 156 real cases from road fatalities (7.1% cases positive to cocaine and 0.6% to designer drugs).

Keywords: Drugs of abuse; LC–MS; DUID; Plasma

1. Introduction

Concern over driving under the influence of drugs (DUID) as a risk factor and a cause of road accidents has recently risen. Illicit drugs can influence driving performance in different ways. Opiates induce sedation, indifference to external stimuli and increase reaction time. Stimulating drugs such as cocaine, amphetamines and designer drugs (MDMA, MDEA), induce a loss of concentration and attentiveness, produce dilated pupils, which increase sensitivity to blinding by light, and the euphoric phase may lead to increased risk-taking in traffic. Cannabis can influence perception, psychomotor performance, cognitive and affective functions, and finally, hallucinogens produce hallucinations, sleepiness and psychotic reactions incompatible with safe driving [1]. However, despite this knowledge of their effects, few epidemiological and experimental data are currently available on this issue. The influence of illicit drugs on driving performance and accident risk can be derived from epidemiological studies involving analysis of biological samples of fatally injured drivers for drugs. The most relevant matrices to be analyzed for this purpose, are plasma or blood, because their concentrations correlate best with the pharmacological or toxic effects. Moreover, blood samples are mandatory in cases of DUID in most European countries and some states of the USA [2].

Gas chromatography–mass spectrometry (GC–MS) is still the most widely method of reference used, but liquid chromatography coupled single-stage or tandem mass spectrometry (LC–MS, LC–MS–MS) is becoming increasingly important for the identification and quantification of analytes [3–6], especially for the more polar, thermolabile, or low-dosed drugs, as indicated by Maurer [7].

Many LC–MS methods for the determination of drugs of abuse in plasma have been published for the determination of opiates [8–15], cocaine and its metabolites [16–19], amphetamines and designer drugs [20–23], opiates and cocaine [24–26] and opiates, cocaine and LSD [27]. However, only

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two methods for the simultaneous determination of opiates, amphetamines, benzoylecgonine and cocaine based on LC–MS/MS in oral fluid analysis [28–30] and in urine analysis [31] have been published.

We have developed and full validated, simple and low-cost LC–MS method, which includes relative ion intensity data, for the determination of morphine, 6-monoacetylmorphine (6-MAM), amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, benzoylecgonine and cocaine in plasma after solid phase extraction (SPE). In an initial attempt to obtain epidemiological data, this method has been applied to 156 road fatalities.

2. Materials and methods

2.1. Reagents

Morphine, 6-acetyl-morphine, D,L-amphetamine, D,L-methamphetamine, D,L-MDA, D,L-MDMA, D,L-MDEA, D,L-MBDB, benzoylecgonine, cocaine and internal standards (IS) D,L-MDMA-d_5, D,L-MBDB-d_5, benzoylecgonine-d_3 and cocaine-d_3 were obtained from Lipomed (Arlesheim, Switzerland) in solid form. LC–MS Chromasolv® grade acetonitrile (99.98% pure) was from Riedel de Häen-Sigma-Aldrich Chemie (Schnelldorf, Germany). Purified water was obtained in the laboratory by using a Milli-Q water system (Le Mont-sur-Lausanne, Switzerland). Methanol, formic acid (99%), ammonia solution 25%, acetic acid (glacial) 100% anhydrous, H_3BO_3, KCl and NaOH were from Merck (Darmstadt, Germany). Ammonium formate was from Fluka-Sigma-Aldrich Chemie (Steinheim, Switzerland). Solid-phase extraction (SPE) cartridges OASIS® HLB (3 cc, 60 mg) were from Waters (Milford, MA, USA).

A pH 9.0 borate buffer was prepared by mixing 6.2 g of H_3BO_3 and 7.5 g of KCl with 420 ml of a solution of 0.1 M NaOH, and adding water until 1000 ml. Fresh and drug-free human plasma were obtained from a local blood bank.

2.2. Preparation of stock solutions and standards

Individual stock solutions of the compounds were prepared in methanol, except for cocaine and its deuterated analogue, which was prepared in acetonitrile, at a concentration of 1 g/l and stored at −20 °C in the dark for a maximum of 6 months. Working solutions were monthly prepared in methanol at 100 mg/l separately for each compound, and stored in the dark at 4 °C. Daily, mixed working solutions of non-deuterated compounds at 0.008, 0.04, 0.2 and 1 mg/l, and a mixed working solution of D,L-MDMA-d_5, D,L-MBDB-d_5 and benzoylecgonine-d_3 at 1 mg/l and cocaine-d_3 at 0.5 mg/l were prepared by appropriate dilution with methanol.

2.3. Specimens

Blood samples were obtained from femoral veins. The specimens were frozen at −20 °C until analysis, which was performed within 15 days.

2.4. Sample preparation

One milliliter volume of each sample was centrifuge for 10 min at 12,100 × *g* to remove cell debris. To 0.2 ml of supernatant were added 25 μl of a mixed working solution of IS (D,L-MDMA-d_5, D,L-MBDB-d_5 and benzoylecgonine-d_3 at 1 mg/l and cocaine-d_3 at 0.5 mg/l), and 1 ml of pH 9.0 borate buffer in a 10 ml borosilicate tube. The calibrating standards of plasma at 0, 2, 5, 10, 25, 50, 125 and 250 ng/ml were prepared by spiking blank plasma samples with the appropriate working solution volumes.

After conditioning with 2 ml methanol and 2 ml water, the samples previously prepared were applied onto the SPE cartridges. Clean-up was accomplished with successive 2 ml washes of water-5% methanol (95:5, v/v) and a mixture water–2%NH_4OH in methanol (80:20, v/v). The cartridges were dried by applying full vacuum for 10 min before elution with 2 ml of 2% acetic acid in methanol. The elution solution was evaporated to dryness at 35 °C under a stream of nitrogen. The dry extract was re-dissolved in 100 μl of a mixture of a pH 3.0 ammonium formate buffer (ammonium formate 0.002 M and formic acid 0.1%) and acetonitrile (95:5, v/v). The sample was transferred into autosampler vials, and 20 μl were injected into the LC–MS.

2.5. Liquid chromatography–mass spectrometry

The HPLC system was a Waters Alliance 2795 separation module with a Waters Alliance series column heater/cooler (Waters, Milford, MA, USA). Chromatographic separation was performed with an Atlantis dC18, 3 μm (100 mm × 2.1 mm i.d.) reversed-phase column. The mobile phase, delivered at a flow rate of 0.2 ml/min at 26 °C, was a gradient of acetonitrile and a pH 3.0 ammonium formate buffer, programmed as follows: 5% acetonitrile during 1 min, linearly increased to 50% in 10 min, kept that percentage for 1 min, decreased to 5% (original conditions) in 1 min and equilibrated for 4 min, which resulted in a total run time of 17 min.

The detection was performed by using a Micromass ZMD 2000 mass spectrometer (Micromass, Manchester, UK) fitted with a Z-spray ion interface. Ionisation was achieved by using electrospray in the positive ionisation mode (ESI+). Nitrogen was used as nebulisation and desolvatation gas. To optimise ionisation and ion transmission conditions for each compound and for the IS, separately 5 μl of a 10 μg/ml solution in the mobile phase were injected without HPLC separation into the ion source. In order to obtain the highest possible intensity for quantitation and confirmation ions, fragmentation energy (cone voltage) was optimised. During this experiment, a mass range from *m/z* 100 to 400 was monitored in SCAN mode, applying different cone voltages. Acquisition was made in the selected ion-monitoring mode (SIM). For the quantitation of each compound, the protonated molecule $[M + H]^+$ was selected as the quantifier ion and one main fragment was selected as the confirmation ion. In the case of deuterated IS only the protonated molecule was selected. Table 1 summarizes the conditions for the measurement of each compound and the

Table 1
SIR functions, time windows of SIR functions, selected ions, cone voltages, retention times and corresponding IS

Compound	SIR function	Time window SIR function (min)	Selected *m/z* ratios[a]	Cone voltage (V)	Retention time (min)	IS
Morphine	Funtion 1 SIR of 2 *m/z*	2.0–5.0	**286.0** 201.0	40 55	3.7	Cocaine-d3
Amphetamine	Funtion 2 SIR of 9 *m/z*	5.0–8.2	**136.1** 119.0	15 20	7	MDMA-d5
Methamphetamine			**150.1** 119.0	15 20	7.4	MDMA-d5
MDA			**180.2** 163.2	15 20	7.4	MDMA-d5
6-Acetylmorphine			**328.1** 211.0	30 60	7.5	MDMA-d5
MDMA			**194.2** 163.2	15 20	7.7	MDMA-d5
MDMA-d_5			**199.3**	15	7.7	MDMA-d5
MDEA	Funtion 3 SIR of 10 *m/z*	8.0–12.0	**208.3** 163.2	20 20	8.3	MBDB-d5
MBDB			**208.3** 177.1	20 25	8.7	MBDB-d5
MBDB-d_5			**213.3**	15	8.7	
Benzoylecgonine			**290.0** 168.2	20 25	8.6	Benzoylecgonine-d3
Benzoylecgonine-d_3			**293.2**	25	8.6	
Cocaine			**304.2** 182.1	20 30	9.5	Cocaine-d3
Cocaine-d_3			**307.1**	25	9.5	

[a] Quantifier ions are in bold characters.

deuterated IS. The other main parameters were: drying gas temperature 300 °C, source heater temperature 120 °C, nebulisation gas flow 500 l/h, cone gas flow 50 l/h and capillary voltage 3000 V.

Data acquisition peak integration and calculation were interfaced to a computer workstation running MassLynx NT 3.5 and QuanLynx 3.5 software.

2.6. Validation

The analytical validation was performed according the recommendations of Shah et al. [32] and Peters and Maurer [33].

The specificity of the method was evaluated by analysing plasma from 10 healthy non-drug-consuming subjects.

Linearity was obtained with an average determination coefficient (r^2) > 0.99 over a range from the lower limit of quantitation (LLOQ) up to the upper limit of quantitation (ULOQ). A weighting factor $1/x$ was used.

Within-day precision and accuracy were determinated at four concentration levels (the LLOQ, the ULOQ and two intermediate levels) by preparing and analysing same day six replicates for each level. Between-day precision and accuracy were assessed by analysing on 5 different days a set of plasma samples spiked at 2, 5, 10, 25, 50, 125 and 250 ng/ml. Precision, expressed as the coefficient of variation (CV) of the measured values, was expected to be less than 15% for all concentration levels, except for the LLOQ, for which 20% was acceptable. In the same way, accuracy was evaluated by using bias, measured as a percentage deviation from the accepted reference value, which had to be less than 15% for all concentration levels, except for the LLOQ, for which 20% was acceptable.

Lower limit of detection (LOD) was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 3:1. LLOQ was defined as the lowest concentration yielding within-day and between-day CV and bias less than 20%.

Recoveries were determined in quintuplicate at two concentration levels (low and high) for each compound in plasma. For each concentration, five blank samples were fortified with the appropriate amount of each compound. These fortified samples and five blank samples were extracted as previously described. The dry extracts of the fortified samples were re-dissolved in 100 μl of the reconstitution solvent containing the IS, while the extracts of the blank samples were re-dissolved with 100 μl of the reconstitution solvent containing the respective nominal amounts of the compounds and the IS. The latter were used as neat standards.

The ion suppression effect on the ESI response was evaluated by using a post-column infusion system [34–36] for all

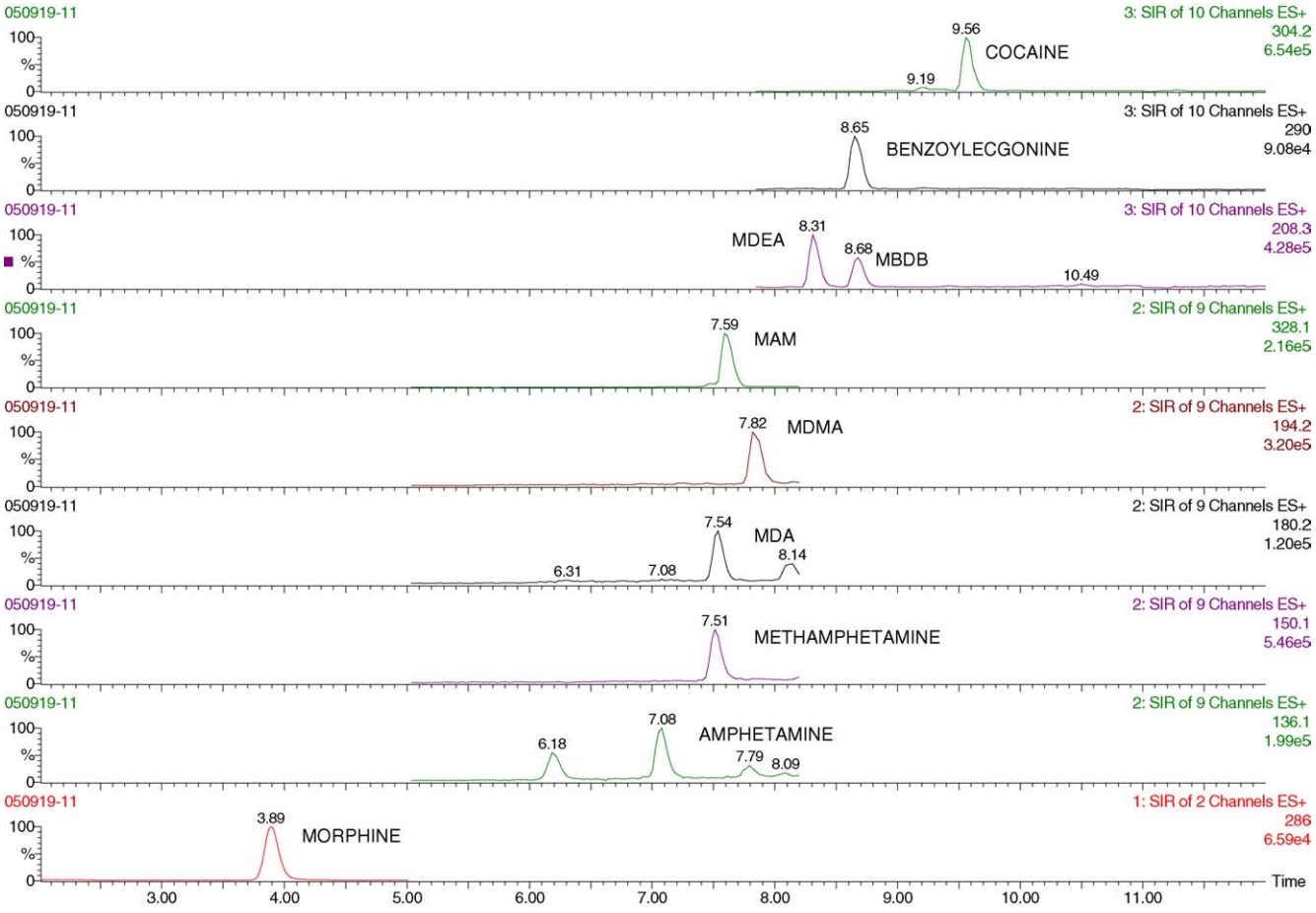

Fig. 1. LC–MS quantitative ion chromatograms of a plasma spiked at 5 ng/ml of all studied compounds.

the compounds and the deuterated internal standards. Mobile phase was delivered into the electrospray interface at a rate of 0.2 ml/min while analyte was being infused, post-column, through a Valco zero dead volume tee, by using a Harvard Apparatus Model 11 (South Natick, MA, USA) syringe pump. Five different blank plasmas were extracted as previously described and reconstituted with mobile phase, 20 μl of which were injected onto the column. Effluent from the HPLC column

Table 2
Calibration data, recovery, within-day precision and accuracy, between-day precision and accuracy and relative ion intensities for morphine and 6-monoacetylmorphine (6-MAM)

Compound	Concentration (ng/ml)	Recovery (%)	Within-day precision and accuracy ($n = 6$)			Between-day precision and accuracy ($n = 5$)			Relative ion intensities ($n = 5$)	
			Mean value	CV	Bias	Mean value	CV	Bias	Mean value	CV
Morphine	2		1.9	7.2	−4.2	1.7	10.3	−13.4	16	20.7
	5	96				4.8	8.4	−2.7	17.8	22.8
	10		10.9	7.4	8.5	10.9	7	9.2	18.7	27.5
	25					26.1	4.3	4.6	20.5	30.2
	50		53.5	7.1	7.1	51.9	4.3	3.8	21.1	36
	125	87.1				124.9	9.1	−0.1	20.9	33.1
	250		260.6	9.2	4.2	246.6	4.6	−1.4	21	34
Calibration curve ($n = 5$): slope = 0.2998 ± 0.0768, intercept = 0.3562 ± 0.1965, r^2 = 0.9954 ± 0.0034										
6-MAM	2		1.9	9.5	−3.3	1.8	7.5	−11	12.1	21.4
	5	88.8				5	7.3	−0.2	11.1	4.8
	10		10.8	4.9	7.5	10.7	5.2	7.3	11.5	9.6
	25					26.1	4.7	4.3	11.4	11.9
	50		53	5.6	6	49.6	9.6	−0.7	11.2	16
	125	73.5				127.2	7	1.8	10.9	13.2
	250		249.1	6.8	−0.4	246.6	3.3	−1.4	11.1	13
Calibration curve ($n = 5$): slope = 0.5517 ± 0.1570, intercept = 1.1254 ± 0.3899, r^2 = 0.9970 ± 0.0020										

combined with the infused analyte and entered the electrospray interface.

Relative intensities of the detected ions were expressed as a percentage of the intensity of the quantifier ion (relative ion intensities = intensity of confirmation ion × 100/intensity of quantifier ion).

All the concentrations above the corresponding linearity ranges should be considered as "semiquantitative", because the determination of such a high concentrations is not covered by the validated experiments. In our case, the plasma samples above the upper limit of quantitation (ULOQ) were diluted with drug-free plasma.

Carry-over effect was assayed by analyzing blank samples after a high positive sample (1000 ng/ml of each compound) and checking that in the blank samples the compounds were not detected.

3. Results and discussion

Deuterated analogues are commonly used as internal standards as they are essentially identical in chemical and chromatographic properties to the respective unlabelled compounds, whilst being readily distinguishable by mass spectrometry because of their mass differences. In our case MDMA-d_5, MBDB-d_5, benzoylecgonine-d_3 and cocaine-d_3 were chosen for this purpose. As expected, the deuterated substances eluted at the same time or a little earlier. In order to avoid cartridges clotting, samples were centrifuged before the extraction and the corresponding supernatant (0.2 ml) was diluted with a buffer (1 ml); in addition, the largest SPE cartridges were selected (3 cc instead of 1 cc cartridges).

The ion chromatograms of the 10 compounds are shown in Fig. 1. The retention times and the selected ions are reported in Table 1. Likewise, their respective optimised fragmentation voltages are shown in the same table.

Under chromatographic conditions used, there was no interference with the analytes by any extractable endogenous components of the plasma.

The linearity of the compound-to-IS peak area ratio versus the theoretical concentration was verified in plasma by using a $1/x$ weighted linear regression. The determination coefficients were above 0.99 and the curvature was tested on a set of five calibration curves. The within-day precision and accuracy, as well as the between-day precision and accuracy were satisfactory for all the tested concentrations. Recoveries obtained at 5 and 125 ng/ml for all compounds were >50%. These results and the relative ion intensities obtained are summarized in Tables 2–4.

The limit of detection (LOD) was 0.5 ng/ml for methamphetamine, MDMA, Benzoylecgonine and cocaine, and 1 ng/ml for morphine, 6-MAM, MDA, MDEA and MBDB. The LLOQ and ULOQ, which correspond to the lowest and highest concentration level of the calibration range, were 2 and 250 ng/ml for all the compounds.

The ion suppression effect, evaluated by the post column infusion system, was not detected in the region of interest for any compound or their deuterated analogues (Fig. 2).

Carry-over effect, tested in the previously described conditions, was not detected. Needle wash between injections was carried out with methanol.

The main advantage of using LC–MS versus GC–MS for the determination of these compounds is the non-use of derivatives, and so the whole procedure was simpler, faster and less expensive. Moreover, in the case of GC–MS methods, the simultaneous determinations of these drugs of abuse is very complex because different kinds of derivative agents have to be used for each group of compounds.

This method was applied to 156 road fatalities corresponding to 2004 and the first 6 months of 2005. Of this number,

Table 3
Calibration data, recovery, within-day precision and accuracy, between-day precision and accuracy and relative ion intensities for benzoylecgonine and cocaine

Compound	Concentration (ng/ml)	Recovery (%)	Within-day precision and accuracy ($n=6$)			Between-day precision and accuracy ($n=5$)			Relative ion intensities ($n=5$)	
			Mean value	CV	Bias	Mean value	CV	Bias	Mean value	CV
Benzoylecgonine	2		2	7.2	−1.6	1.9	17.1	−7.4	45.5	4.6
	5	99.7				4.98	10.9	−0.5	63.1	21.3
	10		9.8	3.4	−2.2	10.5	5.9	5	68.2	19.4
	25					25.7	6.1	2.8	73.8	11.6
	50		53.1	6.7	6.2	51.1	7.5	2.1	70.8	15.6
	125	103.4				121.8	4.2	−2.6	73.5	8.1
	250		263.8	8.6	5.5	251.2	2.4	0.5	75.1	4.2
Calibration curve ($n=5$): slope = 0.2256 ± 0.0928, intercept = 0.4062 ± 0.1525, r^2 = 0.9985 ± 0.0012										
Cocaine	2		1.9	10.3	−5.7	2	13.9	0.9	59.5	23.1
	5	92.7				4.8	4.2	−3.6	52.7	3.7
	10		11	3	9.9	10.4	6.2	4.4	49.6	4.2
	25					24.7	9.5	−1.4	52.2	4.4
	50		52.3	5.5	4.6	51.3	4.4	2.5	52.3	8
	125	83				119.3	3.5	−4.6	51	2.8
	250		250.8	5.2	0.3	254.5	2.8	1.8	53.3	6.1
Calibration curve ($n=5$): slope = 2.7447 ± 0.8394, intercept = −0.6488 ± 2.7220, r^2 = 0.9978 ± 0.0019										

Table 4
Calibration data, recovery, within-day precision and accuracy, between-day precision and accuracy and relative ion intensities for amphetamine, methamphetamine, MDA, MDMA, MDEA and MBDB

Compound	Concentration (ng/ml)	Recovery (%)	Within-day precision and accuracy ($n = 6$)			Between-day precision and accuracy ($n = 5$)			Relative ion intensities ($n = 5$)	
			Mean value	CV	Bias	Mean value	CV	Bias	Mean value	CV
Amphetamine	2		2.1	6.05	3.3	1.8	4.2	−9.7	118	14
	5	72.6				5	7.4	−1.1	125.8	9
	10		10.6	2.8	6.1	10.5	6.1	4.6	140.5	12.2
	25					26.5	7.9	5.9	142.9	0
	50		54.6	2.7	9.1	52.1	4.8	4.3	147.6	7.2
	125	78.8				118.7	5.1	−5	147.6	7.2
	250		269.4	2.5	7.8	252.5	2.9	1	142.9	0
Calibration curve ($n = 5$): slope = 0.5927 ± 0.1173, intercept = 0.4665 ± 0.2377, $r^2 = 0.9975 \pm 0.0033$										
Metamphetamine	2		1.9	8.2	−5.3	1.9	8.1	−5.7	51.2	5.9
	5	86				4.9	6.7	−1.6	54.5	5.1
	10		10.5	4.9	5.3	10.6	4.3	5.8	56.4	6.5
	25					25.9	6.4	3.5	59.1	7.7
	50		53.8	3.1	7.7	50.7	4.3	1.5	61.2	6.9
	125	92.3				118.5	6.2	5.2	61.8	2.7
	250		263.7	3.3	5.5	254.5	3.5	1.8	60.3	3.3
Calibration curve ($n = 5$): slope = 1.5822 ± 0.3101, intercept = 0.5984 ± 0.6689, $r^2 = 0.9966 \pm 0.0021$										
MDA	2		1.8	7.5	−10.3	1.7	4.5	−14.2	195	22.9
	5	74.2				4.8	7.3	−4.5	273.3	21.4
	10		10.9	5	9.1	10.5	4.5	5.1	273.3	21.4
	25					27.3	5.5	9.3	300	15.2
	50		53.9	4.1	7.8	53.3	4.7	6.6	283.3	16.1
	125	69.8				124.9	2.7	−0.1	283.3	16.1
	250		247.2	5.1	−1.1	244.5	1.2	−2.2	290	21.5
Calibration curve ($n = 5$): slope = 0.2433 ± 0.0381, intercept = 0.5748 ± 0.2830, $r^2 = 0.9985 \pm 0.0006$										
MDMA	2		1.9	8.1	−5.3	1.8	4.8	−12.3	80.2	23.6
	5	100				4.9	5.6	−2.9	87	21.7
	10		10.5	6	4.6	11	2.6	9.5	85.3	26
	25					25.7	4.4	2.6	93.4	19.9
	50		53.9	3	7.7	53.3	2.3	6.6	83.4	26.6
	125	78.3				120.2	4	−3.9	88.6	18.1
	250		266.9	2.4	6.8	250.3	2	0.1	92.2	13.3
Calibration curve ($n = 5$): slope = 0.9281 ± 0.0903, intercept = 0.7802 ± 0.5812, $r^2 = 0.9985 \pm 0.0007$										
MDEA	2		2	11.2	−2.3	1.8	11	−9.1	102.6	8.4
	5	92.5				4.9	6.44	−1.9	107.2	10.3
	10		10.5	4.5	5.3	10.7	5.7	6.6	113	15.6
	25					25.7	5.1	2.9	107.2	10.3
	50		53.4	4.3	6.8	52.4	3	4.9	107.2	10.3
	125	83				120.5	2	−3.6	109.4	9.4
	250		266.1	2.6	6.4	251	1.4	0.4	111.7	7.9
Calibration curve ($n = 5$): slope = 0.9979 ± 0.0921, intercept = 0.6042 ± 0.1015, $r^2 = 0.9991 \pm 0.0005$										
MBDB	2		1.8	5.5	−12.4	1.8	8.1	−8.7	77.2	33.4
	5	83.5				4.8	6.5	−3.2	66.7	14.1
	10		11	2.5	9.7	10.9	3.3	8.6	60.2	18.7
	25					25.7	3.5	2.7	61.6	3
	50		53.7	2.2	7.4	52.4	3.5	4.8	61.8	7.5
	125	69.7				118.3	2.3	−5.3	64.6	3.7
	250		256	2	2.4	253.1	0.8	1.2	64.7	6.9
Calibration curve ($n = 5$): slope = 0.6817 ± 0.0337, intercept = 0.2706 ± 0.1602, $r^2 = 0.9985 \pm 0.001$										

144 cases tested negative to opiates, amphetamines and cocaine, while 11 cases tested positive to cocaine (Fig. 3) and 1 case to amphetamine derivatives, which means 7.1 and 0.6%, respectively; 57 cases (36.5%) tested positive to alcohol. Table 5 shows the more important epidemiological parameters for the positive cases for drugs. More data should be collected for a more conclusive statistic study. Also, it would be interesting to establish whether the subjects were chronic or occasional drug consumers, since the difference in tolerance to the drugs could have a diverse influence on driving performance.

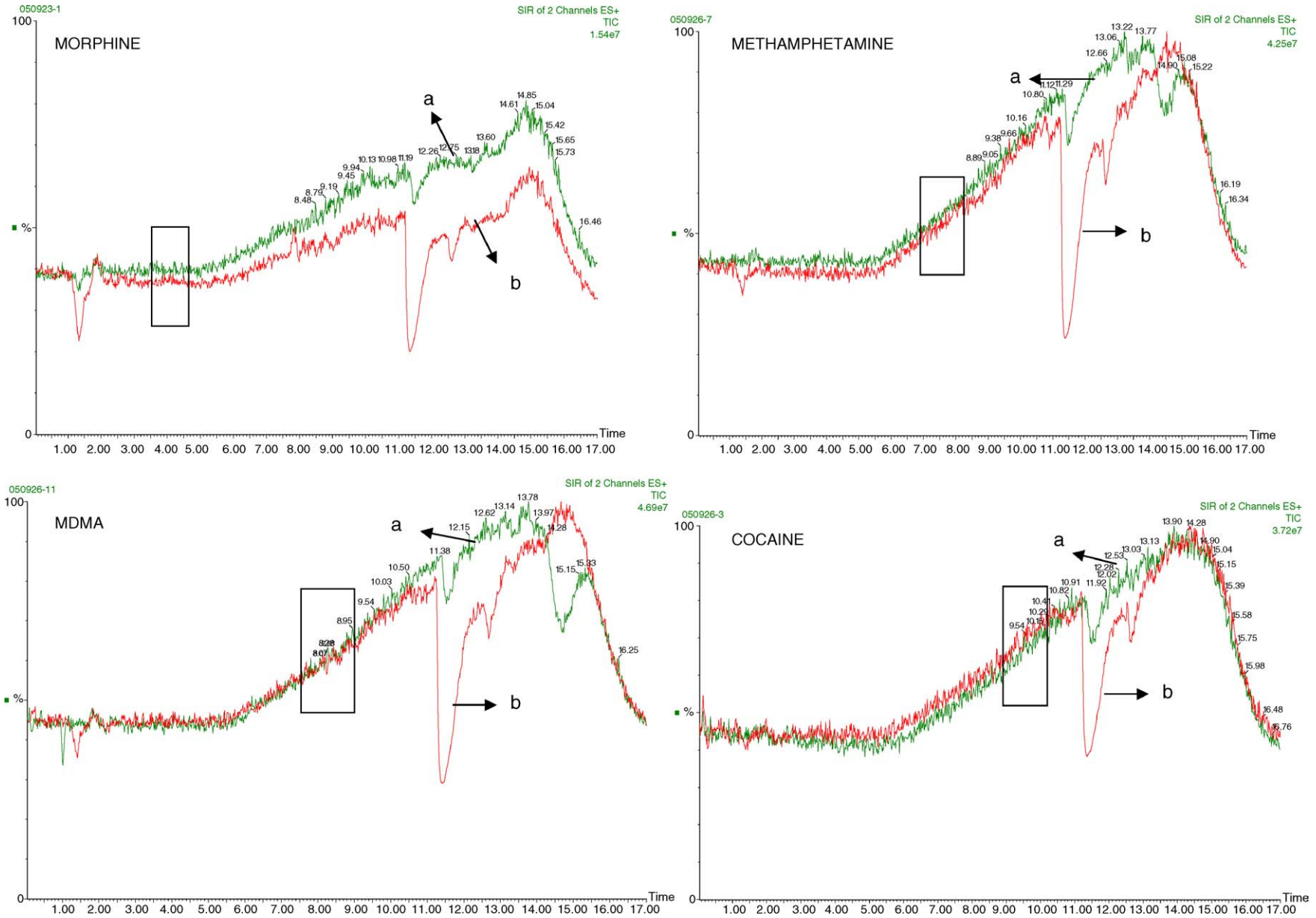

Fig. 2. Evaluation of the ion suppression effect on morphine, methamphetamine, MDMA and cocaine response by post-column infusion following a mobile phase injection (a) and an extracted blank sample (b). The dotted areas indicate the retention time for the corresponding compound.

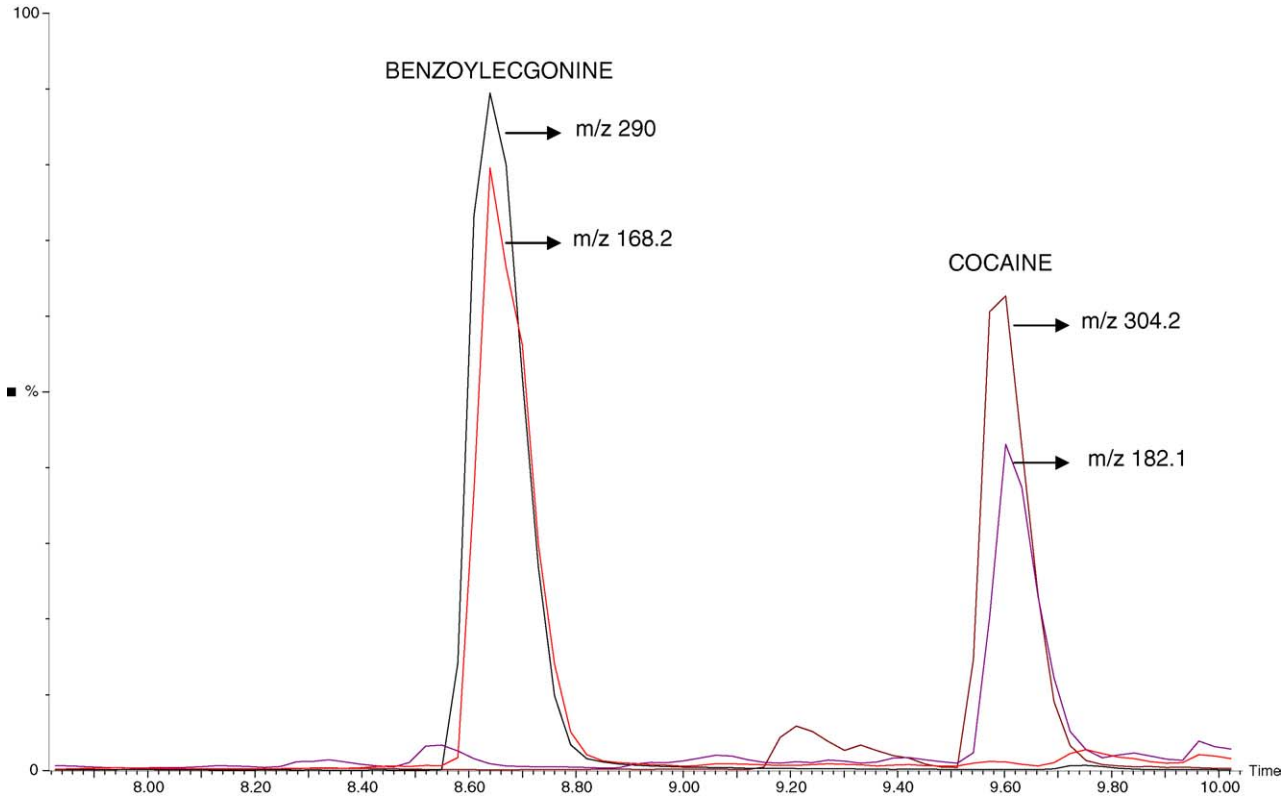

Fig. 3. LC–MS ion chromatograms of a real case from a decedent driver. The figure shows the quantifier and confirmation ion for benzoylecgonine (1041 ng/ml, semiquantitative result) and cocaine (109 ng/ml).

Table 5
Analytical results of the positive cases, indicating age, sex of the decedent, quantitative result of the corresponding drug and/or metabolite and alcohol in blood

Case (sex, age)	Drug and/or metabolite (ng/ml)	Alcohol (g/l)
1 (male, ? years)	Benzoylecgonine 47.3 ng/ml	0.2
2 (male, 19 years)	Benzoylecgonine 12.8 ng/ml	0
3 (male, 21 years)	Benzoylecgonine 38.7 ng/ml Cocaine 8.4 ng/ml	1.9
4 (male, 28 years)	Benzoylecgonine 36 ng/ml	1.7
5 (male, ? years)	Benzoylecgonine 680 ng/ml[a]	0
6 (female, 28 years)	Benzoylecgonine 391 ng/ml[a]	0
7 (male, 32 years)	Benzoylecgonine 15 ng/ml	3.2
8 (female, 26 years)	Benzoylecgonine 1600 ng/ml[a]	0
9 (male, 24 years)	Benzoylecgonine 1200 ng/ml[a] Cocaine 30 ng/ml	1.1
10 (male, 35 years)	Benzoylecgonine 1600 ng/ml[a] Cocaine 18 ng/ml	2.1
11 (male, 16 years)	Benzoylecgonine 1041 ng/ml[a] Cocaine 109 ng/ml	1
12 (male, 22 years)	Methamphetamine 6 ng/ml MDA 12.5 ng/ml	2.5

? years = unknown age.
[a] Semiquantitative results.

4. Conclusion

A method was developed and validated for the determination of morphine, 6-MAM, amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, Benzoylecgonine and cocaine by LC–MS after solid phase extraction. Only 0.2 ml of plasma/blood was needed to do the analysis. The method was successfully applied to 156 road fatalities, providing important data for epidemiological studies of DUID.

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