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Detection of drugs of abuse in exhaled breath using a device for rapid collection: comparison with plasma, urine and self-reporting in 47 drug users

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Abstract

Exhaled breath has recently been identified as a matrix for the detection of drugs of abuse. This work aims to further document this application using a new and simple collection device in patients following recovery from acute intoxication. Breath, plasma and urine samples were collected from 47 patients (38 males, age range 25–74) together with interview data. Analysis of breath and plasma samples was done by liquid chromatography–mass spectrometry methods. Urine was screened using immunochemical reagents and positive findings confirmed with liquid chromatography–mass spectrometry methods. The 12 analytes investigated were: methadone, amphetamine, methamphetamine, 6-acetylmorphine, morphine, benzoylecgonine, cocaine, diazepam, oxazepam, alprazolam, buprenorphine and tetrahydrocannabinol. In all 47 cases, recent intake of an abused substance prior to admission was reported, but in one case the substance (ketobemidone) was not investigated. In 40 of the remaining cases (87%) breath analysis gave a positive finding of any of the substances that were part of the analytical investigation. Identifications were based on correct chromatographic retention time and product ion ratios obtained in selected reaction monitoring mode. In general, data from breath, plasma, urine and self-reporting were in good agreement, but in 23% of the cases substances were detected that had not been self-reported. All substances covered were detected in a number of breath samples. Considering that breath sampling was often done about 24 h after intake, the detection rate was considered to be high for most substances. Analytes with low detection rates were benzodiazepines, and a further increase in analytical sensitivity is needed to overcome this. This study further supports use of exhaled breath as a new matrix in clinical toxicology.

(Some figures may appear in colour only in the online journal)

1. Introduction

The discovery that amphetamine, methadone and tetrahydrocannabinol are readily detectable in exhaled breath following intake has triggered further development of this matrix for drug testing [1–3]. For a long time, urine has been the principle specimen for drug testing, with important clinical and forensic applications [4, 5]. Today the gold standard is to perform screening with commercial immunochemical reagents followed by confirmation of positive findings using mass spectrometry methods.

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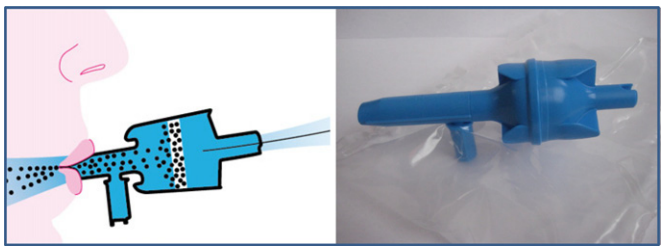

Figure 1. The SensAbues sampling device for exhaled breath. The sampling takes about 2 min. The mouth piece is discarded and the housing containing the micro-particle filter is sealed and subjected for analytical investigation.

In recent years, however, as the sensitivity of bioanalytical techniques has been much improved, there has been increased interest in the use of alternative specimens, including blood, hair, oral fluid, nails and sweat [4–7]. It would be advantageous to analyse a specimen that is non-invasive and possible to collect without risk of adulteration or invasion of privacy. Considering the popularity and acceptance of alcohol breath testing, exhaled breath might become a further alternative, offering new attractive features.

It is now well established that exhaled breath contains bioaerosol particles that carry non-volatile substances out of the body [8–10]. The microparticles contain components typical of the airway lining fluid and proteins typical of the surfactant phase [11–14]. The mechanism of formation is believed to occur when the airway opens during inhalation of breath [15]. It can be hypothesized that exogenous substances can contaminate the airway lining fluid and thereby represent a distribution compartment of drugs. Methadone used as a model substance was demonstrated to be exhaled in the particle fraction of exhaled breath [16].

Based on the initial results on drugs in exhaled breath and the studies on how the fraction carrying methadone can be collected, a sampling device was constructed to facilitate convenient and practical sampling. This paper aims to further evaluate the potential of exhaled breath analysis in patients recovering from acute intoxication at an emergency ward, using a new commercially available sampling device (figure 1).

2. Materials and methods

2.1. Chemicals and materials

Methadone, amphetamine, methamphetamine, 6-acetylmorphine (6-AM), morphine, diazepam, oxazepam, alprazolam, benzoylecgonine, cocaine, buprenorphine, tetrahydrocannabinol (THC), methadone-d3, amphetamine-d5, 6-AM-d3, morphine-d3, diazepam-d5, oxazepam-d5, cocaine-d3, buprenorphine-d4, and THC-d3 were obtained as ampouled methanol solutions from LGC Standards AB (Borås Sweden). Methanol and acetonitrile of LC-MS grade were from Fisher Scientific AB, Gothenburg, Sweden). 2-Propanol of 'normapur' grade was from VWR International (West Chester, PA). Ammonium formate, ammonia (25%), formic acid of analytical grade was from Merck KGaA (Darmstadt, Germany). The Milli-Q water was of ultra-pure quality (>18 MΩ cm^{-1}) and prepared in-house.

2.2. Preparation of standard solutions

The ampouled solutions of analytes and internal standards were diluted to concentrations of 360 ng mL^{-1} and 25 ng mL^{-1}, respectively, using methanol. These solutions were diluted further with methanol and used to fortify blank filters and plasma in order to prepare calibrators and quality controls.

2.3. Study subjects

Forty-seven patients undergoing recovery from acute intoxication (38 males, 9 females, age range 25–74 years) were recruited from the drug addiction emergency clinic at Beroendecentrum Stockholm. At the time of inclusion in the study, the subjects had recovered from intoxication and it was assessed to be about 24 h after the last intake of drugs. All subjects gave informed consent for participation. History of drug use was assessed by interviewing using two structured questionnaires: AUDIT (for alcohol) and DUDIT (for illicit or prescription drugs) [17–19]. The patients scored a median of 7 (range 0–37) in the AUDIT and 25 (range 0–39) in the DUDIT questionnaires. In the AUDIT questionnaire the limit for harmful drinking is 8. The low median AUDIT score and higher DUDIT score reflect a more limited use of alcohol and a heavy use of other substances in the studied patients.

Recent drug intake was further investigated by analysis of plasma and urine samples. In a number of cases, blood sampling or urine collection was not possible due to clinical circumstances. The urine and EDTA plasma samples were collected following the exhaled breath sampling and were stored at -20 °C (maximal storage time 3 months). Ethical approval was obtained from the Stockholm Regional Ethics Review Board (No 2008/1347–31).

2.4. Sampling of exhaled breath

A sampling procedure using a commercial sampling device was employed (SensAbues AB, Huddinge, Sweden) (figure 1). Micro-particles present in the exhaled breath were collected by letting the exhaled breath pass through a mouthpiece, separating saliva and larger particles from the micro-particles. The micro-particles passing through the mouth-piece were collected on a polymer filter inside the device. The sampling procedure was standardized by the filling of a plastic bag and collected about 20 L of exhaled breath (time required 2–3 min). Following the sampling the device was sealed with plugs and stored at -20 °C (maximal storage time 3 months).

2.5. Analysis of exhaled breath samples

Following storage the collection devices were prepared for analysis by placement onto a 10 mL glass test-tube. Following the addition of 75 μL internal standard working solution (0.25 ng of each) the extraction of analytes from the filter surface was performed by the addition of 2 mL of methanol, waiting 5 min, adding 2 $\times$ 2.5 mL methanol, sequentially, and finally pressing out the remaining methanol with slight over-pressure. The solvent was evaporated with nitrogen gas with gentle heating after the addition of 20 μL 10% formic

Table 1. Gradient system used in the LC-MS/MS method.

Time	Mobile phase composition	
(min)	Solvent A[a] (%)	Solvent B[a] (%)
0	70	30
0.6	70	30
1.5	45	55
3.1	1	99
3.6	1	99
4.0	70	30

[a] Solvent A consisted of 2 mmol L^{-1} ammonium formate with pH adjusted to 10 with 25% ammonia and solvent B was 100% methanol with ammonium formate and ammonia in same amounts as solvent A.

acid. The final dry residue was dissolved in 50 μL of methanol, mixed, and 50 μL of 2 mmol L^{-1} ammonium formate.

Calibration samples were prepared by fortifying blank filters with methanol solutions of analytes. These were prepared by adding 10–100 μL of methanol solutions containing 1–100 ng mL^{-1} of analytes. After drying, the discs were prepared for analysis as described above. The calibrators were in the ranges of 10–9000 pg/filter (10; 18; 36; 360; 900; 3600; 9000). Calibration curves were constructed using linear regression analysis, with weighting factor $1/x$.

2.6. Mass spectrometry analysis system

The LC-MS/MS system consisted of a Thermo Fisher Scientific TSQ Vantage triple quadropole mass spectrometer connected to a Dionex Ultima 3000 UHPLC. The liquid chromatography system comprised an Ultimate 2000 SRD degasser, Ultimate 3000 RS binary solvent pump system, column oven and Ultimate 3000 RS autosampler. The software used was Chromeleon Xpress 3, TraceFinder Clinical Research 2.1 and Thermo TSQ Tune Master 2.3.0. The heated electrospray interface (HESI) was used with the instrument operating in the positive ion mode. Nitrogen was used as sheath, auxillary and ion sweep gas, and argon as collision gas. Sampler needle wash between injections was performed with 100 μL of 0.5% formic acid in 90% methanol.

For all analytes the liquid chromatography system was operated in a gradient mode with a flow rate of 650 μL min^{-1} (table 1). Chromatography was performed using a 1.7 μm 100 $\times$ 2.1 mm (inner diameter) Ethylene Bridged Hybrid (BEH) phenyl column (Waters Co), preceded by a 0.2 μm column filter (Waters Co). Solvent A consisted of 2 mmol L^{-1} ammonium formate with pH adjusted to 10 with 25% ammonia, and solvent B was 100% methanol with ammonium formate and ammonia in same amounts as solvent A. The injection volume was 2 μL and the column oven temperature 65 °C. The total run time of the method was 4.0 min. The following conditions were used in the mass spectrometer: peak width 0.70 for Q1 and Q3, collision gas pressure 1.2 mTorr argon, capillary temperature 250 °C, vapourizer temperature 450 °C, sheath gas pressure 55 Arb units, ion sweep gas pressure 1.0 Arb units, aux gas pressure 18.0 Arb units, spray voltage 3000 V, DCV −4 V. The selected ions, S-lens voltage, collision energy and dwell time used for each compound are presented in table 2. Acquisition time was 0.5–3.6 min, with monitoring of each analyte in a time segment of $\pm$0.15 min of expected retention time.

2.7. Method validation

Six replicates of calibration curves were documented at different occasions.

Imprecision and accuracy in quantifications was estimated by repetitive analysis of samples prepared from fortified filters at two concentrations. The samples were analysed in triplicate for five consecutive days.

Limit of detection (LOD, signal/noise = 3) and limit of quantification (LOQ, s/n $\geqslant$ 10 and CV<20%) was estimated by using diluted calibrator extracts. The LOD was estimated at the qualifier transition and the LOQ at the quantifier transition. The lower limit of quantification (LLOQ) was determined experimentally by using calibrators at different concentration levels of 2, 6, 10 and 18 pg/filter.

Matrix effect was studied by using infusion of analytes post-column while injecting blank matrix extracts.

2.8. Urine analysis

Urine was screened for amphetamines, opiates, cannabis, cocaine, benzodiazepines, buprenorphine, and methadone using CEDIA immunoassay reagents applied on an Olympus 640 instrument according to the manufacturer's instructions. Confirmations were made with in-house LC-MS/MS methods. Creatinine concentrations were also measured. The LLOQs in ng mL^{-1}(screening/confirmation) were: benzoylecgonine 150/50; morphine 300/150 (6-AM 2); amphetamine and methamphetamine 500/300; benzodiazepines 200/60; cannabis 25/6; methadone 300/150; buprenorphine 5/5.

2.9. Plasma analysis

Analysis of plasma samples (0.2 mL) was carried out using protein precipitation with 0.6 mL of acetonitrile. Following centrifugation, the supernatant was evaporated to dryness under nitrogen and redissolved with 100 μL of 50% methanol in 0.1% ammonia. The same internal standards and LC-MS/MS conditions were used as for the breath extracts. Calibrators were prepared from blank plasma. The LLOQ was 1 ng mL^{-1} for all compounds.

3. Results

3.1. Method development and validation

A chromatographic method was developed that combined the measurement of all analytes in the same run. The gradient elution covered analytes ranging from benzoylecgonine to buprenorphine. The first eluting analyte, benzoylecgonine, eluted at ~1 min while the column void eluted at ~0.25 min. Matrix effect as studied by an infusion experiment suggested no effect to be expected for the analytes.

The phenyl column material was selected mainly due to favourable chromatography of all analytes when using

Table 2. Mass spectrometric parameters used in the LC-MS/MS method for breath and plasma.

Analyte	Precursor ion	Product ion*		Collision energy (eV)		S-lens (V)	Dwell time (s)
		1	2	1	2		
6-Acetylmorphine	328.2	165.1	211.1	40	26	105	0.05
Alprazolam	309.1	205.1	281.1	40	26	102	0.045
Amphetamine	136.1	91.0	119.1	16	5	45	0.12
Benzoylecgonine	290.1	105.0	168.1	29	18	87	0.13
Buprenorphine	468.3	396.2	414.3	38	33	153	0.05
Cocaine	304.2	105.1	182.2	32	18	103	0.05
Diazepam	285.1	154.0	193.1	26	32	98	0.05
Methadone	310.2	105.1	265.2	28	14	96	0.05
Methamphetamine	150.1	91.1	119.1	19	10	51	0.045
Morphine	286.1	165.1	201.1	38	25	113	0.12
Oxazepam	287.0	104.0	241.1	34	21	90	0.05
Δ-9-THC	315.3	123.1	193.1	32	21	112	0.095
Internal standards							
6-Acetylmorphine-d3	331.2	165.1		37		107	0.03
Amphetamine-d5	141.1	96.1		15		45	0.02
Buprenorphine-d4	472.4	400.2		37		128	0.02
Cocaine-d3	307.2	185.1		19		91	0.03
Diazepam-d5	290.1	198.1		31		86	0.02
Methadone-d3	313.2	268.2		13		71	0.02
Morphine-d3	289.1	152.1		62		106	0.04
Oxazepam-d5	292.0	246.2		21		93	0.02
Δ-9-THC-d3	318.3	196.2		21		107	0.01

* Product ion 1 = quantifier ion; product ion 2 = qualifier ion.

injection of a high concentration of organic modifier in the final extract, which was needed for dissolving THC. A suitable chromatographic performance of all analytes, as well as a reduced mass spectrometric background, was achieved by the use of a mobile phase at high pH. Mass spectrometric conditions were selected by infusing the analytes and using the automatic tune features of the system. Transitions were selected mainly for providing strongest response.

Six replications of the calibration curve demonstrated a linear relationship for all analytes between the analyte/internal standard peak area ratio and the amount of analyte applied on the filter. The mean r^2 values were >0.993 with RSD of <0.5% for analytes. The data for imprecision and accuracy studied over 5 days demonstrated total CVs at 100 pg/filter between 4.3 to 11.8% with accuracies between 85–100% (table 3). At 1000 pg/filter the total CVs were between 2.3 to 12.9% with accuracies between 86–99%. The lower limit of quantification, LLOQ, was set at 2–6 pg/filter with CVs <15% ($n = 6$). Chromatograms of all analytes extracted from filter at the LLOQ level are shown in figure 2. Carry-over in the injector system was found to be 0.34% for benzoylecgonine, 0.13% for buprenorphine and <0.1% for the other analytes.

Because of the nature of this method to measure very low amounts of analytes in exhaled breath extracts, all labware used was disposable, chemicals had to be tested for purity and all laboratory procedures were carefully performed with attention to contamination risks.

3.2. Study sample results

Among the 47 studied patients analytical findings were made in breath in 40 cases (85%). Of the other seven cases one involved intake of ketobemidone (according to self-report), which was not part of our analytical investigation. No plasma or urine was obtained in this case for further investigation. Omitting the one ketobemidone case gives a positive rate of 87% (40/46). In the remaining six cases (nos 9,18, 33, 35, 44, 45) analytical findings were made in plasma and/or urine to support intake of substances covered by our method. Out of all self-reported recent intakes, 79% were supported by analytical findings in plasma or urine. In 11 out of 47 (23%) cases, additional substances other than those self-reported were detected. In 87% of the cases, more than one substance was reported as recent intake.

Twelve cases involved methadone, either by self-report or by analytical findings (table 4). In nine of these cases methadone intake was self-reported and in an additional two cases this was suggested by the plasma or urine results. In all cases (100%) methadone was detected in breath. In one case (no 3) no additional support of methadone intake was present since plasma and urine samples were not available. The chromatograms obtained in case 8 (table 4) are shown in figure 3.

Ten cases involved buprenorphine, either by self-report or by analytical findings (table 5). In six of these cases buprenorphine intake was self-reported and in an additional three cases this was suggested by the plasma or urine results. In two cases the self-reported intake was not analytically supported. In all cases (100%) that involved a recent intake documented by self-report or analytically, buprenorphine was detected in breath but at an analytically low level (<LLOQ) in three of them. In one case (no 3) no additional support of buprenorphine intake was present since plasma and urine samples were not available.

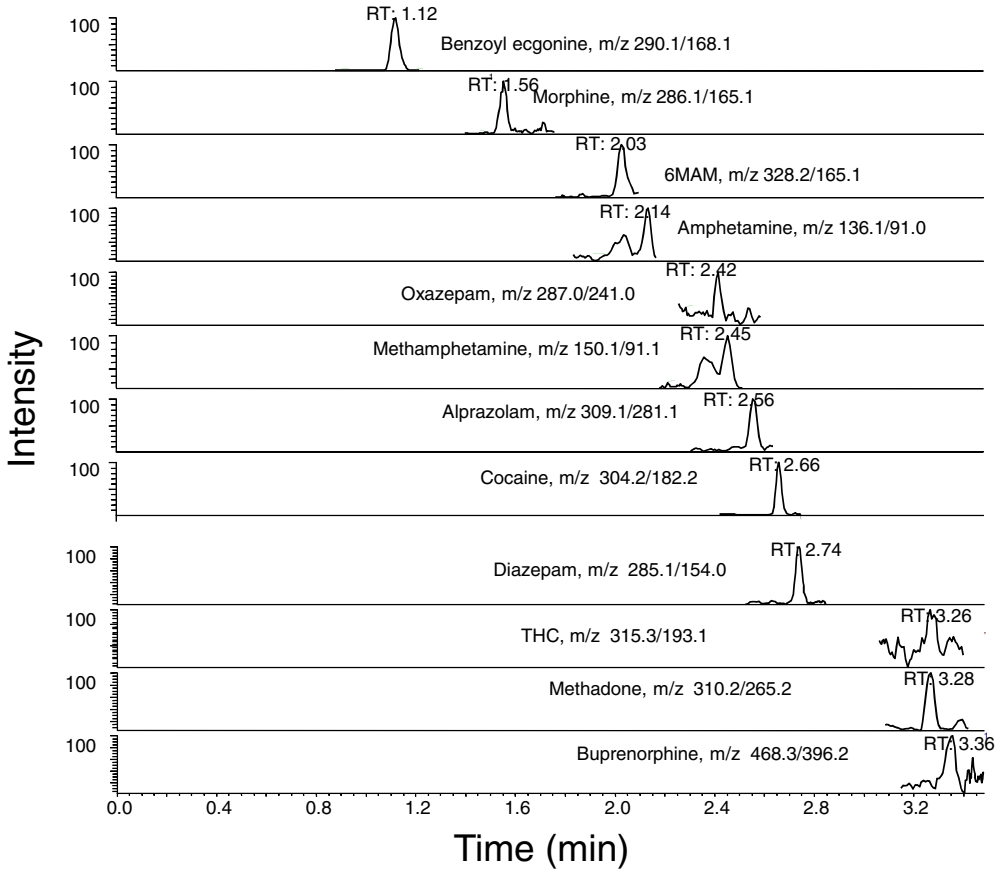

Figure 2. Chromatograms from an extracted filter fortified with all analytes at the LLOQ levels (see table 3). The chromatograms represent results from the quantifier transitions (product ion 1 of table 2).

Table 3. Summary of method validation results for breath analysis.

Analyte	LOD (pg/filter)	LLOQ CV<15% (pg/filter)	QC_{low} (pg/filter mean)	QC_{low} accuracy (%)	QC_{low} total CV (%)	QC_{high} (pg/filter mean)	QC_{high} accuracy (%)	QC_{high} total CV (%)
Amphetamine	3	6	90.7	90.7	6.0	925	92.5	2.3
Methamphetamine	1	6	90.6	90.6	6.2	917	91.7	2.5
Morphine	1	6	90.7	90.7	4,7	929	92.9	4.0
6-AM	1	6	93.1	93.1	4.9	927	92.7	3.1
Cocaine	2	6	91.5	91.5	7.3	924	92.4	2.6
Benzoylecgonine	0.5	6	89.2	89.2	4.3	931	93.1	7.9
Methadone	0.5	6	89.9	89.9	7.1	982	98.2	4.4
Buprenorphine	2	6	84.7	84.7	11.7	972	97.2	7.3
Diazepam	1	2	85.2	85.2	11.8	993	99.3	5.7
Oxazepam	1	2	89.0	89.0	7.4	913	91.3	5.0
Alprazolam	1	2	90.5	90.5	6.6	924	92.4	3.7
THC	3	6	95.9	95.9	8.8	982	98.2	12.9

Seventeen cases involved amphetamine, either by self-report or by analytical findings (table 6). In 13 of these cases amphetamine intake was self-reported and in an additional two cases this was suggested by the plasma or urine results. In all cases (100%) with documented recent intake, amphetamine was detected in breath. In two cases (no 14 and 24) no additional support of amphetamine intake was present. The analytical data fulfilled requirements for positive identification. In one case (no 7) the intake was most likely methamphetamine. In this case both amphetamine and methamphetamine were detected in breath. In a few cases amphetamine was detected in breath despite low levels in plasma and urine.

Eleven cases involved cocaine, either by self-report or by analytical findings (table 7). In five of these cases cocaine intake was self-reported. In four of these a recent intake was confirmed by plasma and urine data and in all four cases (100%) cocaine was detected in breath. In one case (no 33) no cocaine or its metabolite benzoylecgonine were detected in breath and the self-reported recent intake was not supported by plasma and urine data. Benzoylecgonine was less detectable and was only detected together with cocaine.

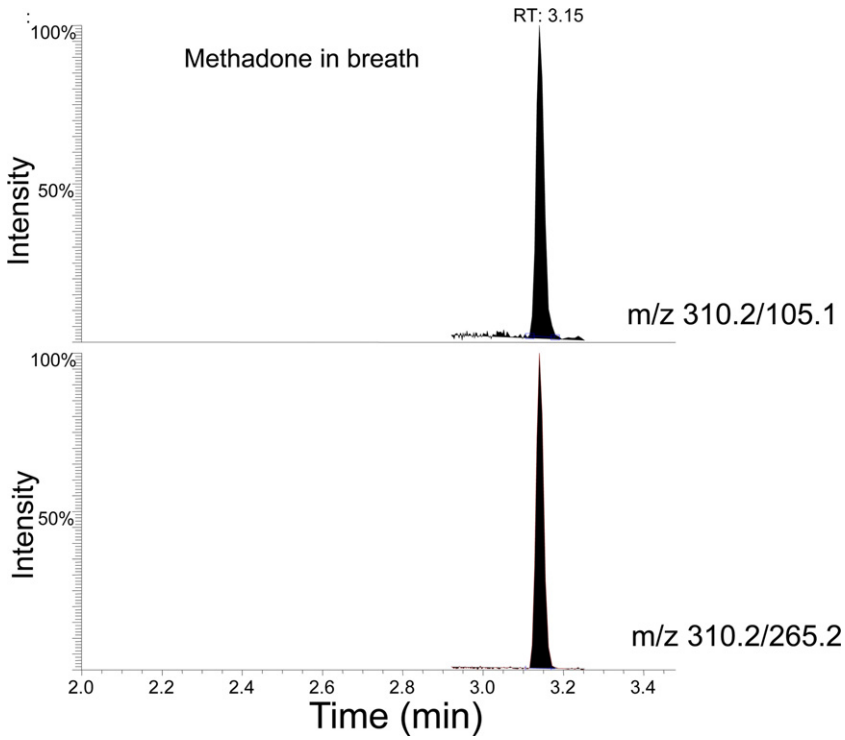

Figure 3. Chromatograms obtained from the analysis of methadone (case 8 of table 4) in exhaled breath using electrospray LC-MS/MS and monitoring of two product ions from the protonated molecular specie.

Table 4. Cases associated with methadone intake[a].

Case no	Breath (pg/sample)	Plasma (ng mL^{-1})	Urine (ng mL^{-1})	Self-report
3	58	No sample	No sample	No (heroin, alprazolam)
7	451	No sample	>42,000	Yes
8	69	21	>40,000	No (morphine, heroin)
16	58	24	4800	Yes
17	920	36	13,400	Yes
19	288	42	>36,000	Yes
20	2421	No sample	2900	Yes
22	360	34	17,000	Yes
26	431	43	5600	Yes
31	141	17	1180	No (heroin, benzodiazepine)
32	158	No sample	6900	Yes
41	61	27	500	Yes

[a] In the other 35 cases no methadone was detected in breath, plasma or urine.

Table 5. Cases associated with buprenorphine intake[a].

	Breath (pg/sample)	Plasma (pg mL^{-1})	Urine (ng mL^{-1})	
Case no	Bup	Bup	Bup; NorBup	Self-report
1	120	232	232; 837	No (heroin, alprazolam)
3	246	No sample	No sample	No (heroin, alprazolam)
4	n.d.	n.d.	n.d.	Yes
6	Detected	182	411; 429	Yes
14	114	189	62; 114	No (oxazepam)
21	221	162	94; 122	Yes
27	Detected	198	118; 192	Yes
29	Detected	No sample	100; 456	Yes
31	567	333	823; 733	No (heroin, benzodiazepine)
45	n.d.	n.d.	n.d.	Yes

[a] Thirty-seven cases with no detected buprenorphine in breath, plasma or urine.
Bup = buprenorphine; NorBup = norbuprenorphine (metabolite detected in urine).

Table 6. Cases associated with amphetamine intake[a].

Case no	Breath (pg/sample) Amph; MAmph	Plasma (ng mL^{-1}) Amph; MAmph	Urine (ng mL^{-1}) Amph; MAmph	Self-report
1	1752; n.d.	8.5; detected	35 000; 300	No (heroin, alprazolam)
4	1382; n.d.	Detected; n.d.	1800; n.d.	Yes
7	241; 213	No sample	1500; 16,000	Yes
13	338; 30	7.8; detected	7500; 725	Yes
14	20; n.d.	n.d.; n.d.	n.d.; n.d.	No (oxazepam)
15	1203; n.d.	14.5; n.d.	15 600; n.d.	Yes
24	109; n.d.	n.d.; n.d.	n.d.; n.d.	No (cocaine)
25	85; n.d.	2.2; n.d.	Detected; n.d.	Yes
27	170; 396	No sample	No sample	Yes
29	408; n.d.	No sample	20 600; detected	Yes
31	985; n.d.	1.8; n.d.	5600; 120	No (heroin, benzodiazepine)
38	2890; 420	6.9; 1.4	10 100; 2100	Yes
39	2000; n.d.	64.1; n.d.	50 000; 400	Yes
40	1174; n.d.	17.8; n.d.	60 600; detected	Yes
42	4700; 790	62; 9.3	56 000; 9800	Yes
46	135; n.d.	3.2; n.d.	7000; detected	Yes
47	72; n.d.	Detected; n.d.	n.d.; n.d.	Yes

[a] Thirty cases with no detected amphetamine in breath, plasma or urine.
Amph = amphetamine; MAmph = methamphetamine.

Table 7. Cases associated with cocaine intake[a].

Case no	Breath (pg/sample) Cocaine; BzE	Plasma (ng mL^{-1}) Cocaine; BzE	Urine (ng mL^{-1}) BzE	Self-report
2	73.8; detected	Detected; 13,8	3100	Yes
13	64; 18	n.d.; Detected	Detected	No (amphetamine)
14	56; 20	Detected.; detected	n.d.	No (oxazepam)
15	33; detected	Detected; detected	n.d.	No (amphetamine, cannabis)
23	560; 66	Detected; detected	140	Yes
24	74; n.d.	Detected; detected	n.d.	Yes
32	30; n.d.	No sample	n.d.	No (methadone, benzodiazepine)
33	n.d.; n.d.	n.d.; detected	n.d.	Yes
36	33; detected	n.d.; detected	n.d.	No (morphine, nitrazepam)
37	13 000; 560	Detected; 14.4	49 000	Yes
38	29	n.d.; n.d.	n.d.	No (amphetamine, cannabis, benzodiazepine)

[a] Thirty-six cases with no detected cocaine or benzoylecgonine in breath, plasma or urine.
BzE = benzoylecgonine (metabolite of cocaine).

Out of six cases where no self-report of recent intake was obtained, the detection of cocaine contradicted with the plasma and urine data in one case (no 38). The detection of cocaine and benzoylecgonine in the breath of case 14 was supported by both being detected in plasma, but at concentrations below LLOQ. Chromatograms from cases 2 and 14 in breath are shown in figures 4(*a*) and (*b*) and from case 14 in plasma in figure 4(*c*).

Nine cases involved opiate intake, according to self-report and analytical findings (table 8). In eight of these cases heroin intake was self-reported and in one case an opiate intake was self-reported. In this case (no 11) the analytical data supported a morphine intake rather than heroin since no 6-AM was detected in any specimen. In seven cases morphine and/or 6-AM was detected in breath. In one case (no 3) with very high levels of morphine and 6-AM, heroin intake was self-reported but no plasma or urine sample was available to support this.

Twenty cases involved cannabis, either by self-report or by analytical findings (table 9). In eight of those cases, recent cannabis intake was self-reported and this was confirmed by detected THC in the plasma in seven cases; in an additional two cases, recent intake was indicated by presence of THC in plasma. Out of these nine cases, eight (89%) had detectable THC in breath. In 10 cases the presence of THC carboxylic acid metabolite (THCA) urine might simply reflect a historical intake of cannabis.

Thirty-two cases involved benzodiazepine intake, either by self-report or by analytical findings (table 10). In all but two cases benzodiazepine intake was self-reported and in an additional two cases this was suggested by the plasma and urine results. Out of 24 cases where self-report of recent intake was supported by analytical findings in plasma, 15 (62%) had detectable benzodiazepines in breath. In the 11 cases with detected alprazolam in plasma, alprazolam was detected in breath in six (55%). Plasma levels of alprazolam were significantly higher ($p < 0.05$) in these six cases as compared to those with undetectable levels in breath. That was not the case for diazepam, although there was a tendency towards higher levels. Oxazepam was detected in only three

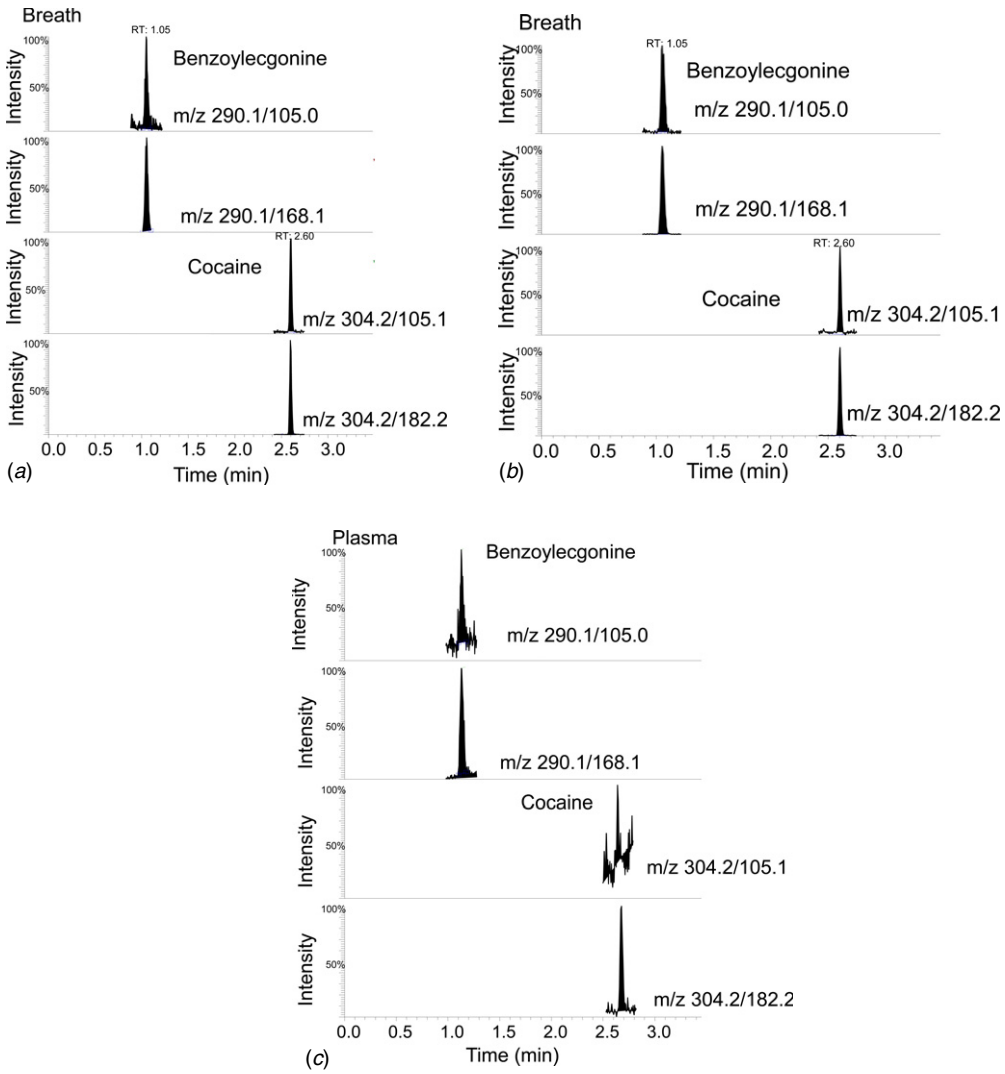

Figure 4. Chromatograms obtained from the analysis of benzoylecgonine and cocaine in exhaled breath ((*a*) case 2; (*b*) case 14 of table 7) and plasma ((*c*) case 14) using electrospray LC-MS/MS and monitoring of two product ions from each of the protonated molecular species. Note that the chromatograms shown for benzoylecgonine in 4(*a*) and both analytes in 4(*c*) represent concentrations below LLOQ.

Table 8. Cases associated with opiate intake[a].

Case no	Breath (pg/sample) Morphine; 6-AM	Plasma (ng mL^{-1}) Morphine; 6-AM	Urine (ng mL^{-1}) Total Morphine; 6-AM	Self-report
1	36; 134	Detected; n.d.	40 000; 475	Yes (heroin)
3	4650; 6080	No sample	No sample	Yes (heroin)
6	n.d.; n.d.	Detected; detected	6000; n.d.	Yes (heroin)
7	n.d.; detected	No sample	2000; n.d.	Yes (heroin)
8	28; 123	4.3; detected	30 000; n.d.	Yes (heroin)
11	25; n.d.	Detected; n.d.	30 000; n.d.	Yes (opiate)
17	69; 42	Detected; n.d.	160; n.d.	Yes (heroin)
31	59; 202	Detected; n.d.	4000; n.d.	Yes (heroin)
44	n.d.; n.d.	2.6; detected	8300; 290	Yes (heroin)

[a] Thirty-eight cases with no detected morphine or 6-AM in breath, plasma or urine.

cases. Chromatograms from the detection of alprazolam in plasma and breath in case 31 are shown in figure 5.

Only cases that had some indication of recent intake based on self-report or analytical findings are detailed in tables 4–10. Thus a total number of 352 negative analytical breath results were compliant with the other data.

4. Discussion

The results of this investigation provide further support to the possibility of using exhaled breath as a readily available specimen for drugs of abuse testing. There is a possibility that exhaled breath will develop into a new matrix for routine

Table 9. Cases associated with cannabis intake[a].

Case no	Breath (pg/sample) THC	Plasma (ng mL^{-1}) THC	Urine (ng mL^{-1}) THCA	Self-report
2	8	Detected	17	Yes
4	64	Detected	13	Yes
6	n.d.	n.d.	17	No (heroin, buprenorphine)
7	n.d.	n.d.	20	No (heroin, amphetamine, benzodiazepine, methadone)
12	n.d.	n.d.	78	No (diazepam)
15	192	Detected	147	Yes
17	n.d.	n.d.	12	No (heroin, amphetamine, benzodiazepine, methadone)
18	n.d.	n.d.	15	Yes
19	n.d.	n.d.	65	No (methadone, benzodiazepine)
26	n.d.	n.d.	22	No (methadone, benzodiazepine)
27	n.d.	n.d.	16	No (benzodiazepine, buprenorphine, amphetamine)
31	301	Detected	1300	No (heroin, benzodiazepine)
34	36	Detected	300	Yes
38	332	Detected	124	Yes
39	101	Detected	990	Yes
40	n.d.	n.d.	13	No (amphetamine, benzodiazepine)
41	n.d.	n.d.	76	No (methadone, benzodiazepine)
43	43	Detected	145	Yes
45	n.d.	Detected	102	No (buprenorphine, oxazepam)
46	n.d.	n.d.	18	No (diazepam, amphetamine)

[a] Twenty-seven cases with no detected THC in breath, plasma or urine.
THCA = tetrahydrocannabinol carboxylic acid (metabolite of THC).

Table 10. Cases associated with benzodiazepine intake[a].

Case no	Breath (pg/sample) Diaz; Oxaz; Alpr	Plasma (ng mL^{-1}) Diaz; Oxaz; Alpr	Urine (ng mL^{-1}) dDiaz; Oxaz; Temaz; OHAlpr	Self-report
1	n.d.; n.d.; 8	2.6; 1.2; 11	120; 160; 110; 4300	Alprazolam
2	n.d.	n.d.; n.d.; detected	n.d.; n.d.; n.d.; 60	Benzodiazepine
3	n.d.	No sample	No sample	Alprazolam
5	n.d.; 84; n.d.	n.d.; >130; n.d.	n.d.; 77 000; n.d.; n.d.	Oxazepam
8	n.d.; n.d.; 6	n.d.; detected; 27	n.d.; n.d.; n.d.; 1400	No
9	n.d.	n.d.; 16; n.d.	n.d.; 7600; n.d.; n.d.	Oxazepam
10	n.d.; n.d.; detected	n.d.; 38; 4.1	n.d.; 4800; n.d.; 90	Oxazepam, alprazolam
11	14; n.d.; n.d.	86; 8.0; n.d.	4300; 10 600; 8700; n.d.	Diazepam
12	10; n.d.; n.d.	84; 1.4; n.d.	630; 830; 3100; n.d.	Diazepam
14	6; 60; n.d.	Detected; 9.2; n.d.	n.d.; 5200; detected; n.d.	Oxazepam
16	n.d.	n.d.; n.d.; 7.8	n.d.; n.d.; n.d.; 500	Alprazolam
17	2; n.d.; n.d.	17; detected; 12	Detected; 310; 270; 420	Benzodiazepine
19	n.d.	n.d.	n.d.	Benzodiazepine
20	n.d.	No sample	n.d.	Diazepam, oxazepam
21	n.d.	n.d.	n.d.	Oxazepam
22	6; n.d.; n.d.	106; 49; detected	1420; 10 600; 4900; n.d.	Diazepam
25	n.d.	26; 4.1; n.d.	240; 700; 550; n.d.	Benzodiazepine
26	n.d.; n.d.; 4	n.d.; detected; 30	n.d.; 20; n.d.; 820	Benzodiazepine
27	n.d.	28; 4.5; n.d.	1500; 1250; 1700; n.d.	Diazepam
30	n.d.	9.2; detected; n.d.	Detected; 120; detected; detected	No
31	n.d.; n.d.; 53	23; detected; 69	n.d.; 70; n.d.; 8800	Benzodiazepine
32	n.d.	No sample	n.d.; n.d.; n.d.; 360	Benzodiazepine
34	145; n.d.; n.d.	119; 6.3; n.d.	2600; 3000; 5000; n.d.	Diazepam
35	n.d.	60; 87; n.d.	320; 93 000; 600; detected	Diazepam, Oxazepam
38	8; 4; n.d.	8.8; detected; n.d.	n.d.; 580; 140; detected	Benzodiazepine
39	6; n.d.; n.d.	66; detected; n.d.	n.d.; n.d.; 850; n.d.	Benzodiazepine
40	n.d.	46; detected; n.d.	430; 100; 730; n.d.	Diazepam
41	n.d.; n.d.; 20	n.d.; detected; 70	n.d.; 70; n.d.; 3700	Benzodiazepine
42	n.d.; n.d.; 16	n.d.; n.d,; 20	n.d.; n.d.; n.d.; 1200	Benzodiazepine
45	n.d.	n.d.; 35; n.d.	n.d.; detected; n.d.; n.d.	Oxazepam
46	n.d.	n.d.; 54; n.d.	n.d.; 46 500; n.d.; detected	Diazepam
47	n.d.	n.d.	n.d.; n.d.; n.d.; 420	Diazepam

[a] Fifteen cases with no detected diazepam, oxazepam or alprazolam in breath, plasma or urine.
Diaz = diazepam; Oxaz = oxazepam; Alpr = alprazolam; dDiaz = desmethyldiazepam; Temaz = temazepam; OHAlpr = hydroxyalprazolam.

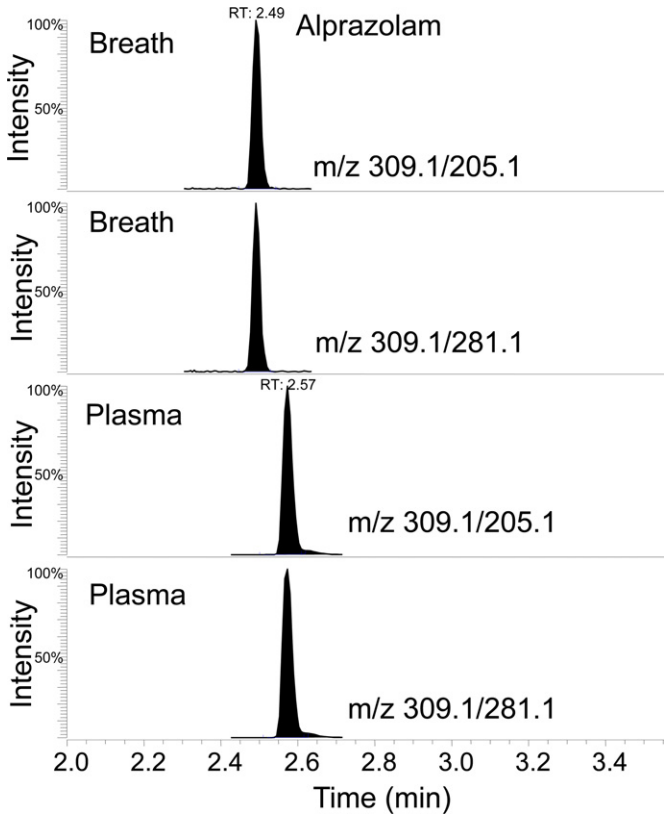

Figure 5. Chromatograms obtained from the analysis of alprazolam in exhaled breath and plasma (case 31 of table 10) using electrospray LC-MS/MS and monitoring of two product ions from the protonated molecular specie. The difference in retention time between breath and plasma was due to the fact that two different set of instruments were used.

drug testing and present an alternative to already used matrices like urine, blood, oral fluid, sweat and hair. Each matrix may have its specific advantages and disadvantages. Since exhaled breath may be as easy to collect as in alcohol breath testing, it may present a new, more accessible matrix than blood at the roadside and elsewhere when the sampling procedure is an obstacle. We previously observed that exhaled breath methadone increases after intake [2]. If a correlation to blood concentration can be shown for exhaled breath levels, it may become a substitute matrix for monitoring impairment. One advantage of exhaled breath may be the detection of 6-AM, which is problematic in blood.

Alprazolam and benzoylecgonine were detected in exhaled breath for the first time, whereas for methadone, amphetamine, methamphetamine, cocaine, morphine, 6-AM, THC, buprenorphine, diazepam and oxazepam, the results confirm previous observations [1–3, 20]. Breath was sampled using a new, simple and commercially available sampling device, that was evaluated for the first time in this study. The commercial device contains two critical parts, one being the mouthpiece that only lets micro-particles pass and traps larger particles. The other part is the filter that has the capability to trap the particles with a high efficiency (figure 1) [16].

The detection rate was generally higher in this study as compared to the previous [18]. This can be attributed to the improved sampling procedure as well as to the increased sensitivity of our modified LC-MS/MS method.

Exhaled breath contains aerosol micro-particles that carry non-volatile substances derived from the airway lining fluid, which is continuously produced and is a dynamic compartment [21]. Any compound present in blood or inhaled may contaminate this fluid. This is indicated by the detection of methadone and amphetamine that are rarely administered by inhalation while cannabis most often is.

The detection rate for most investigated substances appears to be high, and higher than previously reported, with the exception of benzodiazepines. Although only three benzodiazepines were investigated they may require increased analytical sensitivity for an acceptable detection rate. Oxazepam appears to be especially problematic. When evaluating the detection rate it should be considered that the time between intake and sampling was rather long (often 24 h). A higher detection rate is expected if sampling is made closer to the intake, but this was not possible in the present study due to the experimental design (i.e. a hospital setting with patients seeking treatment). However, a long detection time similar to that in urine is not to be expected for drug testing using exhaled breath.

Four analytical findings of amphetamine and cocaine in exhaled breath were not supported by self-report or findings in plasma and urine. The LC-MS/MS analytical results fulfilled requirements for positive identification and was reproduced by re-analysis. Even though great care was taken to eliminate contamination of extracts in the laboratory work, these cases may reflect false positive results, and attention should be given to this aspect when analysing these ultra-low concentrations.

In conclusion, this study confirmed the potential of exhaled breath as an alternative specimen for toxicological investigations. New analytes were detected in exhaled breath of intoxicated drug users and a higher detection rate than before [20] was observed, possibly due to improved sampling and analytical sensitivity. The availability of a simple and functional commercial sampling device may facilitate further development of this new method for drugs of abuse testing. In addition, it might be profitable to study whether this new sampling procedure can be applied to non-volatile analytes previously studied in exhaled breath condensates such as 8-isoprostane, leukotrienes and other small molecular weight compounds [22–24].

Acknowledgements

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