

Note d'application

## UPLC-MS/MS Method for Drug Detection in Exhaled Breath

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*For forensic toxicology use only.*

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### Abstract

This application brief demonstrates that, using exhaled breath as specimen for drug testing the Xevo TQ-XS instrument provides the needed sensitivity and is a robust and suitable system for routine application.

### Benefits

- A safe and convenient specimen to collect
- Detection time related to time of impairment
- Easy procedure for sample preparation
- Method covers large number of analytes
- Screening and identification at the same time

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## Introduction

Drug testing with urine as the specimen has been in clinical and forensic use ever since the development of analytical technologies based on immunochemistry in the 1970's allowed for cost-effective screening of common drugs of abuse and gas chromatography-mass spectrometry methods for evidential confirmation of positive outcomes. Developments in mass spectrometry technology have now made it possible to avoid immunoassay screening and directly make an analytical investigation with evidential mass spectrometry methods.<sup>1</sup>

Interest in alternative matrices to urine can be traced back to the early days of drug testing, but it is not until more recent times that these alternative specimens became used in routine applications, *e.g.* oral fluid. This can to a large extent be credited to the development of more powerful technologies for bioanalysis based on hyphenated liquid chromatography and mass spectrometry instruments that have set a new standard of method performance.<sup>2</sup>

One such alternative specimen is exhaled breath, which is non-invasive and readily available.<sup>3</sup> The specimen can be collected by convenient procedures which are easy to supervise without any need for intrusion of privacy. Apart from volatiles, exhaled breath contains aerosol particles that carry non-volatile components from deeper parts of the lung.<sup>4</sup>

In 2010, the finding that amphetamine and methamphetamine are detectable using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in exhaled breath from illicit drug users following recovery from intoxication many hours after intake were published.<sup>5</sup> This initiated renewed interest and a new series of work aimed at exploring the possibility of using exhaled breath for drug testing with modern day analytical technology.

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## Experimental

### LC Conditions

LC system:

ACQUITY UPLC I-Class PLUS

Column:	ACQUITY UPLC BEH Phenyl (150 mm x 2.1 mm I.D, 1.7 µm)
Mobile phase A:	Water containing 20 mM ammonium formate + 0.1% formic acid (pH=3)
Mobile phase B:	Methanol containing 0.1% formic acid
Wash solvent:	Methanol:acetonitrile:2-propanol:water containing 0.2% formic acid (25:25:25:25 v/v)
Purge solvent:	Water:methanol (80:20 v/v)
Injection volume:	3 µL
Gradient elution :	Table 1

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.5	85.0	15.0	Initial
3.00	0.5	45.0	55.0	8
4.00	0.5	45.0	55.0	6
5.00	0.5	0.0	100.0	6
6.00	0.5	0.0	100.0	6
6.50	0.5	85.0	15.0	1

*Table 1. Gradient conditions. The cycle time injection to injection was 7.5 minutes, giving the column adequate time to re-equilibrate.*

## MS Conditions

MS system:	Xevo TQ-XS
Data acquisition and processing:	MassLynx with TargetLynx
Ionization mode:	UniSpray, positive mode
Impactor voltage:	2.2 kV
Acquisition mode:	Multiple reaction monitoring (MRM ) Table 2

All analytes were detected in positive ionization mode using UniSpray ionization and the MRM transitions stated in Table 2.

Compound	Precursor ion ( <i>m/z</i> )	Product ions ( <i>m/z</i> )	Cone voltage (V)	Collision energy (eV)	Retention time (min)	QC 20 pg CV (%)
Alpha-PVP	232.1	126.1/105.1	5	24/24	3.53	7.6
Amphetamine	137.1/136.1	92.1/119.1	8	14/8	2.20	14
Benzoyllecgonine	290.1	168.1/105.1	30	18/28	3.27	9.0
Cocaine	304.1	182.1/82.1	30	18/26	3.59	8.7
Delta-9-THC	315.1	193.1/123.1	5	24/32	5.76	20
Gabapentin	172.1	137.1/95.1	18	14/22	1.94	6.5
Ketamine	238.1	125.1/207.1	20	24/12	3.34	6.3
MDA	180.1	105.1/133.1	10	20/15	2.35	8.9
MDMA	194.1	163.1/133.1	18	11/19	2.58	4.8
Methadone	311.1	266.1/105.1	20	14/26	4.92	3.1
Methamphetamine	151.1/150.1	92.1/119.1	15	14/8	2.47	5.6
Methylphenidate	234.1	84.1/56.1	30	20/37	3.52	6.1
Oxazepam	287.1	241.1/269.1	10	21/14	4.98	6.5
Oxycodone	316.1	241.1/256.1	25	281/24	2.43	8.1
Pentedrone	192.1	132.1/161.1	5	16/11	3.30	5.8
Pregabalin	160.1	55.1/83.1	18	20/15	1.84	11
Temazepam	301.1	255.1/177.1	25	22/37	5.18	3.5
Tramadol	264.1/265.1	58.1/58.1	20	14/14	3.49	7.3
Zopiclone	389.1	245.1/217.1	10	18/32	3.65	4.4

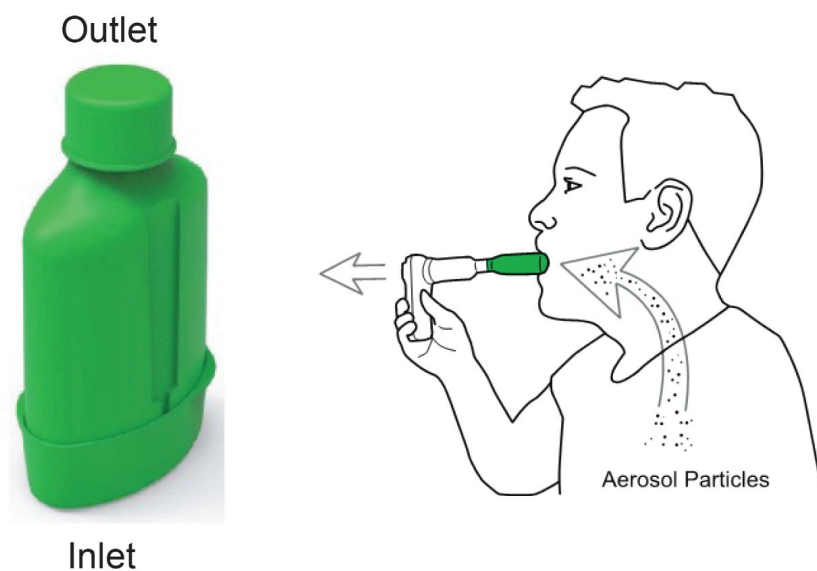
\*47 analytes were included in the complete method, Table 2 includes conditions for the analytes that were found positive in the study.

Table 2. Analytical parameters used for MRM monitoring and results from the lowest quality control specimen (*n*=21). Quantifier ions and parameters are indicated in bold font.

The internal standards; alpha-PVP-d8, amphetamine-d5, benzoylecgonine-d3, cocaine-d3, delta-9-THC-d3, gabapentin-(13C)3, ketamine-d4, MDA-d5, MDMA-d5, methadone-d9, methamphetamine-d5, methylphenidate-d9, oxazepam-d5, oxycodone-d3, pregabalin-(13C)3, temazepam-d5, tramadol-13C-d3, and zopiclone-d4 were analyzed with parameters corresponding to their individual analytes' according to Table 2. but with *m/z* adjusted to the number of stable isotopes respectively. For pentedrone, MBDB-d5 was used as internal standard, monitoring the transition 213.1>136.1.

## Collection of breath particles

Particles in exhaled breath are collected by impaction technology. A simple commercial device is available for this. The BreathExplor device was validated for collecting methadone and characteristic lung lipids.<sup>6</sup>



*Figure 1. The BreathExplor sampling device and sampling procedure. The sampling can be documented using a spirometer.*

Particles from twelve breaths are collected. The collection device contains three parallel collectors, providing three subsamples. After sampling the device is capped, labelled, and sent to the lab.

## Sample preparation

The device is taken apart and one collector is used for analysis, while two are being stored.

The collector is put in a test tube and 2 mL of methanol containing internal standards and 10  $\mu$ L ethylene glycol is added.

The test tube is vortexed for 10 seconds and the collector is taken out.

The methanol is evaporated using a vacuum centrifuge and the residue is dissolved in 60  $\mu$ L 50% methanol and transferred to autosampler vial and centrifuged.

Standards and controls were prepared by fortifying blank collectors.

## Application

The method was applied in studies where exhaled breath specimens were collected at music events, nightclubs, and festivals. In total, 1204 unknown samples were investigated for 47 analytes.

## Results and Discussion

During a period of 4 months 21 batches of unknowns were analyzed without any major problems. The fluctuation of response and retention time was small over time and is shown in Figure 2 for cocaine. The extracts proved to be clean and no indication of matrix effects on response was observed. The same column was used for about 2000 injections and no increase in back pressure was observed over time. The data for QC samples supported the stability and good performance of the method (Table 2). Example chromatograms from authentic specimens are shown in Figure 3.

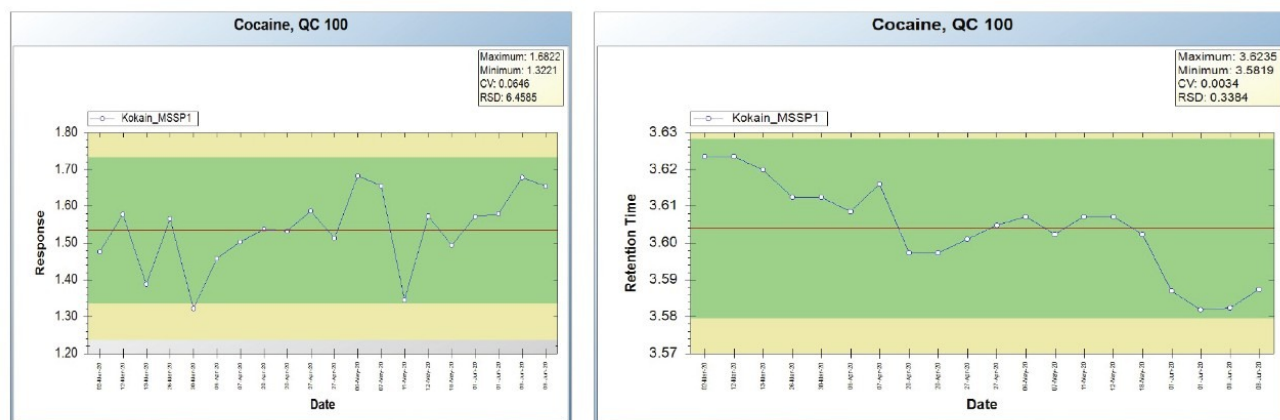


Figure 2. Fluctuation of response and retention time over time.

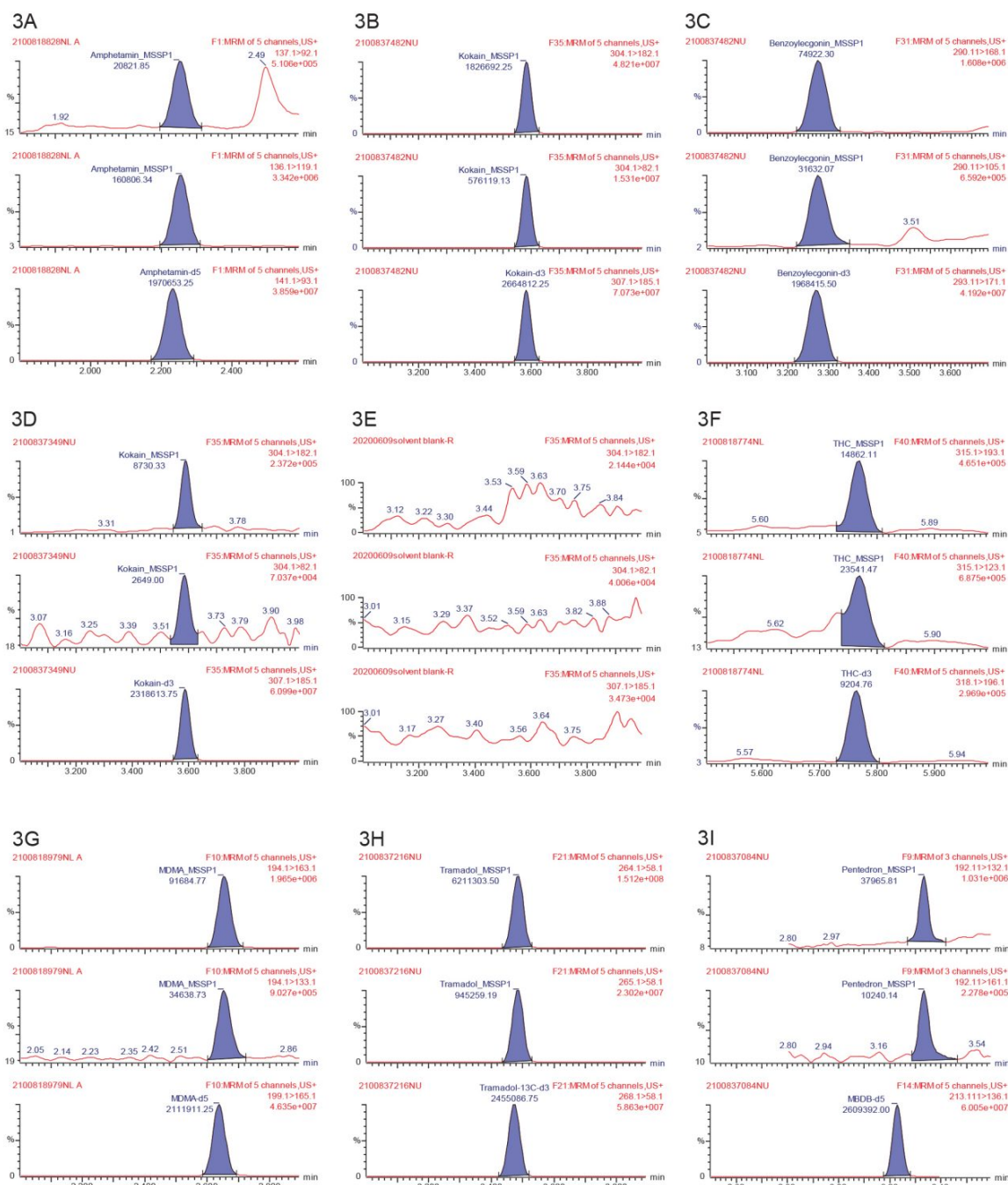


Figure 3. Chromatograms selected from the study samples.

a) Amphetamine at 20 pg/collector



- b) Cocaine at 38 pg/collector
- c) Benzoyl ecgonine from same sample as b)
- d) Cocaine response from a negative study sample
- e) Cocaine response in a system blank
- f) THC at 102 pg/collector
- g) MDMA at 2.1 pg/collector
- h) Tramadol at 163 pg/collector
- i) Pentadrone at 3.1 pg/collector

Out of the 47 analytes monitored in the method 20 was identified in the study samples (Table 3).

Substance	Number of positive samples	%	Lowest concentration pg/collector	Highest concentration pg/collector
Cocaine*	77	6.4	1.0	375
Amphetamine	46	3.8	1.1	2040
MDMA	22	1.8	1.2	117
THC	10	0.8	1.9	630
Methylphenidate	7	0.6	1.0	88
Tramadol**	5	0.4	1.2	163
Pentedrone	2	0.2	1.0	3.0
Alpha-PVP	2	0.2	1.0	2.0
Temazepam	2	0.2	1.0	1.1
Pregabalin	2	0.2	4.2	5.4
Ketamine	2	0.2	45	58
Methamphetamine	2	0.2	1.0	3.0
MDA	1	0.1	1.0	
Oxazepam	1	0.1	1.0	
Gabapentin	1	0.1	3.9	
Oxycodone	1	0.1	1.0	
Zopiclone	1	0.1	2.0	
Methadone	1	0.1	3.5	

\* Benzoyllecgonine was sometimes also detected

\*\* O-desmethyl-tramadol was sometimes also detected

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*Table 3. Analytical findings in 1204 exhaled breath samples.*

The use of exhaled breath as specimen made it possible to perform a study with biological sampling in the nightlife scene and provided new insight into drug use in this setting and a valuable complement to self-reports.

Cocaine was the most prevalent finding. It was noted that peaks for cocaine were present in all samples, but at a

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very low concentration. The background peaks fulfilled the criteria for identification but was lower than 10% of the applied LLOQ of 1 pg per collector. For comparison a system blank is also shown. This observation may agree with the presence of cocaine in bank notes, sewage, and drinking water, as well as in the free air in major cities.<sup>7</sup> The breath samples were collected in an environment where cocaine users were present, and this might explain the observed background of cocaine.

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## Conclusion

The collection procedure for aerosol particles in exhaled breath is rapid, simple, and safe. There is no inconvenience for the individual being tested. The sampling procedure can be performed without any need of special facility and is well suited for situations outside the health care system, *e.g.* workplaces, roadside.

For using exhaled breath as specimen for drug testing the Xevo TQ-XS instrument provides the needed sensitivity and is a robust and suitable system for routine application.

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