Amphetamines Detected in Exhaled Breath from Drug Addicts: A New Possible Method for Drugs-of-Abuse Testing

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Abstract

Testing for drugs of abuse in sample matrices alternative to urine such as blood, sweat, and saliva have received increasing attention and is needed, for example, in traffic medicine. Human breath is known to contain a large number of substances including non-volatile molecules. We explore whether intake of amphetamines could be detected by analytical investigation of exhaled breath from drug addicts. Exhaled breath was collected from 12 drug addict patients after recovering from acute intoxication. Self-reported intake of “amphetamine” was confirmed by analysis of urine and plasma. The compounds were trapped by filtering the air through a modified silica surface and subsequently analyzed by a combined liquid chromatography–tandem mass spectrometry method. As a control, exhaled breath was collected in the same way from eight healthy volunteers. Here we report for the first time that amphetamine and methamphetamine are present in exhaled breath following ingestion of these compounds by drug addicts. Both amphetamine and methamphetamine were indisputably identified by means of the mass spectrometry technique in exhaled breath samples from all 12 patients. Identifications were based on monitoring two product ions in selected reaction monitoring mode and having correct relative ratios (± 20%). Excretion rates ranged from 0.2 to 139 pg/min. No amphetamine or methamphetamine was detected in the control subjects. This finding, using a yet non-validated sampling procedure, opens a new possibility for drugs-of-abuse testing.

Introduction

Combating drug abuse is of primary concern in societies all over the world. Testing for drugs of abuse is a common practice effective in detecting drug users and is an integral part of clinical treatment programs. Furthermore, drug testing is used in workplaces and has important forensic applications. Testing is usually performed by laboratory investigation on collected urine samples, but the use of on-site testing and sample matrices alternative to urine such as blood, sweat, and saliva have received increasing attention and is needed, for example, in traffic medicine (1–3). The ongoing interest and application of saliva testing in traffic medicine has demonstrated a true need for tests based on simpler and safer sampling procedures (4,5) and with immediately available results. Testing for alcohol is readily available using portable instruments for breath analysis. Apart from alcohol, exhaled breath has so far not been developed for testing of any other drugs of abuse. However, exhaled breath is known to contain a great number of substances including non-volatile compounds. It is known that non-volatile compounds can be detected in expired breath air, possibly carried as an aerosol (6,7). Altogether over 3000 analytes have been detected in human breath (8). Apart from alcohol testing, breath analysis is established for the diagnosis of H. pylori infection and for nitric oxide measurement in asthma monitoring, and its potential for use in other areas is evident (6,8).

The possibility of using exhaled breath for drugs-of-abuse testing is attractive as it would overcome the problem of sampling difficulties and produce a sample devoid of any complicated risk for adulteration or alternate explanations. Recent technological advances in mass spectrometry (MS) have enabled the combination of increased selectivity and sensitivity of bioanalysis. The interfacing of liquid chromatography (LC) with tandem MS has provided a new means for toxicology analysis and for analysis of trace analytes in biological samples in general (9). For these reasons, we investigate whether amphetamine and methamphetamine could be detected in exhaled breath from active drug users by employing a most sensitive and selective bioanalytical technology ultra-performance LC–MS–MS in selected reaction monitoring (SRM) mode.

Materials and Methods

Chemicals and materials

Amphetamine, methamphetamine, and amphetamine-d₅ was obtained as ampouled methanol solutions from Cerilliant...
Ammonia (25%) solution was obtained from VWR International (Darmstadt, Germany); methanol and acetonitrile of HPLC grade was from JT Baker (Mallinckrodt Baker BV, Deventer, Holland); ethyl acetate and formic acid of HPLC grade were from Merck (Darmstadt, Germany). The water was of ultra-pure quality (> 18 MΩ/cm) and prepared in-house. The AQUITY UPLC BEH C<sub>18</sub> column was from Waters (Milford, MA), and the 30 mg SPEC DAS solid-phase extraction (SPE) cartridges were from Varian (Palo Alto, CA).

Patients and control subjects

Twelve patients reporting recent use of amphetamine (7 male, 5 female, ages 22–51) were recruited from two addiction treatment clinics in Stockholm (Beroendecentrum Stockholm) after recovering from acute intoxication. History of drug use was assessed by interviewing and by using two structured questionnaires, AUDIT (for alcohol) and DUDIT (for illicit drugs) (10–12). The patients scored a median of 2.5 (range 0–34) in the AUDIT and 34.5 (range 12–43) in the DUDIT questionnaires. In the AUDIT questionnaire, the limit for harmful use is 8 (max 40), and for DUDIT, the limit was 6 (max 44). The low average AUDIT score and high DUDIT score reflect the limited use of alcohol and the heavy use of illicit drugs (e.g., amphetamine), respectively, in the studied patients.

Recent drug intake was further investigated by analysis of plasma and urine samples. In three of the cases, blood sampling was not possible because of clinical factors. The urine and EDTA plasma samples were collected following the exhaled breath sampling and stored at –80°C. As a control group, 8 drug-free healthy volunteers (3 male, 5 female, ages 29–67) were recruited. Ethical approval was obtained from the Stockholm Regional Ethics Committee (No 2008/1347-31).

Sampling of exhaled breath

Compounds in exhaled breath were collected for 10 min by suction through a SPEC DAS cartridge. The patients were asked to breathe normally in a face mask (no. 1516, Intersurgical, Berkshire, U.K.), and a three-way coupling was used to withdraw breath air (Figure 1). It was estimated that about half of the exhaled breath was collected through the SPEC DAS cartridge via a 3-m long plastic tubing. Following sampling, the cartridge was stored at –80°C.

MS analysis

Following storage, the SPE cartridge was subsequently eluted with 2% ammonia (25%) in a mixture of methanol and ethyl acetate (20:80, v/v) at the time of analysis. The eluate was evaporated to dryness under nitrogen gas after adding of formic acid (10 µL of 10% formic acid in MeOH), and the residue was redissolved in 30 µL of 0.1% formic acid containing internal standard (5.94 ng amphetamine-d<sub>5</sub>). An aliquot of 3 µL was subjected to analysis by SRM UPLC–MS–MS (Waters Quattro Premier XE). The chromatographic system was an ACQUITY UPLC BEH C<sub>18</sub> column (100 mm × 1.0 mm, particle size 1.7 µm) with a gradient system that consisted of A = 0.1% formic acid and B = acetonitrile. The linear gradient started at 100% A and ended at 70% A after 1.7 min. Thereafter, 100% was pumped for 0.49 min before returning to 100% A.

Two product ions from the protonated molecules were monitored for amphetamine (m/z 136→119; 136→91), two for methamphetamine (m/z 150→119; 150→91) and one for amphetamine-d<sub>5</sub> (m/z 141→124). This was done by SRM in the positive electrospray mode with 25 ms dwell time for each channel. Other instrumental settings are provided in Table I. The minimum detectable amount (signal-to-noise ratio = 3) injected on column was about 0.1 pg.

Methods used for plasma and urine analysis were in routine use in the laboratory and based on published LC–MS procedures (13,14).

Quantitation

Standards for quantitation were prepared by using the matrix from blank SPE cartridges.

Results

In all 12 studied patients, amphetamine and/or methamphetamine were detected in the exhaled breath sample, which was in accordance with self-reported drug intake (Table II). In
all cases, the self-reported intake was supported by analysis of plasma and urine. The presence and relative levels of amphetamine and methamphetamine indicated mixed drug use of both compounds, which is in accordance with a recent trend in Sweden observed in the clinical urine drug testing. None of the patients specifically reported the use of methamphetamine.

Identification of detected analytes was based on a correct relative (to amphetamine-d5) retention time (± 0.5%) and correct (< ± 20%) relative ion intensity ratio between the two product ions (Figure 2). Because levels were generally low, background signal resulted in failure to fulfill identification criteria in some of the samples, despite the fact that signal was actually present at the correct retention times (Figure 3). The amount of substance collected from exhaled breath ranged from 0.2 to 103 pg/min for amphetamine and < 0.3 to 139 pg/min for methamphetamine (Table II). In the eight healthy controls, no amphetamine or methamphetamine were detected (Figure 4).

When comparing response for internal standard between standards and samples, no indication of ion suppression from matrix was evident. No amphetamine or methamphetamine was detected in the trapping liquid (Figure 1).

Discussion

This study reports the original observation that self-administered amphetamine and methamphetamine can be detected in exhaled breath collected from drug addicts. The identification of amphetamine and methamphetamine followed commonly accepted criteria. These criteria for identification are in accordance with scientific standards and are being successfully applied in urine drug testing (15). No correlation between plasma and exhaled breath levels was evident from the results indicating that the exhaled breath sample might represent another compartment than blood. However, the sampling technique employed could not be validated for extraction efficiency, but a similar application has been used and validated for collection of airborne aromatics (16). The collection cartridge material used in the present study is normally used for extraction of analytes from aqueous solutions. It is, therefore,

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**Table II. Summary of Data Obtained for Plasma, Urine, and Exhaled Breath**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Self-Reported Recent Drug Use</th>
<th>AUDIT Score</th>
<th>DUDIT Score</th>
<th>Plasma† (ng/mL)</th>
<th>Urine† (µg/mL)</th>
<th>U-Creatinine (mmol/L)</th>
<th>Breath† (pg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amphetamine, diazepam</td>
<td>1</td>
<td>14</td>
<td>A = 166</td>
<td>A = 107</td>
<td>32.0</td>
<td>A = 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 1.9</td>
<td>M = 0.69</td>
<td></td>
<td>M &lt; 0.3†</td>
</tr>
<tr>
<td>2</td>
<td>Amphetamine, diazepam</td>
<td>3</td>
<td>26</td>
<td>A = 62.4</td>
<td>A = 14</td>
<td>15.2</td>
<td>A = 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 0.6</td>
<td>M = 0.08</td>
<td></td>
<td>M &lt; 0.3†</td>
</tr>
<tr>
<td>3</td>
<td>Amphetamine</td>
<td>4</td>
<td>19</td>
<td>A = 282</td>
<td>A = 30</td>
<td>12.6</td>
<td>A = 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 2.1</td>
<td>M = 0.12</td>
<td></td>
<td>M &lt; 0.3†</td>
</tr>
<tr>
<td>4</td>
<td>Amphetamine, methylphenidate</td>
<td>2</td>
<td>12</td>
<td>A = 110</td>
<td>A = 63</td>
<td>27.2</td>
<td>A = 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 27</td>
<td>M = 5.4</td>
<td></td>
<td>M = 1.2</td>
</tr>
<tr>
<td>5</td>
<td>Amphetamine, alprazolam</td>
<td>5</td>
<td>37</td>
<td>A = 53</td>
<td>A = 30</td>
<td>21.2</td>
<td>A = 0.4</td>
</tr>
<tr>
<td></td>
<td>zopiclone</td>
<td></td>
<td></td>
<td>M = 52</td>
<td>M = 19</td>
<td></td>
<td>M = 0.4</td>
</tr>
<tr>
<td>6</td>
<td>Amphetamine, alprazolam, buprenorphine,</td>
<td>0</td>
<td>32</td>
<td>No sample</td>
<td>A = 5.3</td>
<td>7.9</td>
<td>A = 102</td>
</tr>
<tr>
<td></td>
<td>flunitrazepam, morphine, zopiclone</td>
<td></td>
<td></td>
<td></td>
<td>M = 62</td>
<td></td>
<td>M = 139</td>
</tr>
<tr>
<td>7</td>
<td>Amphetamine</td>
<td>3</td>
<td>30</td>
<td>A = 4.3</td>
<td>A = 0.94</td>
<td>8.1</td>
<td>A &lt; 0.6†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 3.2</td>
<td>M = 0.40</td>
<td></td>
<td>M = 0.5</td>
</tr>
<tr>
<td>8</td>
<td>Amphetamine, diazepam, heroin, methadone</td>
<td>0</td>
<td>41</td>
<td>No sample</td>
<td>A = 20</td>
<td>31.6</td>
<td>A &lt; 0.3†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 119</td>
<td></td>
<td>M = 0.6</td>
</tr>
<tr>
<td>9</td>
<td>Amphetamine, alprazolam, diazepam,</td>
<td>0</td>
<td>40</td>
<td>No sample</td>
<td>A = 6.7</td>
<td>15.0</td>
<td>A = 0.4</td>
</tr>
<tr>
<td></td>
<td>methylphenidate</td>
<td></td>
<td></td>
<td></td>
<td>M = 0.02</td>
<td></td>
<td>M &lt; 0.3†</td>
</tr>
<tr>
<td>10</td>
<td>Amphetamine, flunitrazepam, methadone</td>
<td>10</td>
<td>43</td>
<td>A = 535</td>
<td>A = 229</td>
<td>33.3</td>
<td>A = 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M = 64</td>
<td>M = 15</td>
<td></td>
<td>M &lt; 0.3†</td>
</tr>
<tr>
<td>11</td>
<td>Amphetamine, cannabis, clonazepam,</td>
<td>0</td>
<td>40</td>
<td>A = 504</td>
<td>A = 163</td>
<td>22.1</td>
<td>A = 5.2</td>
</tr>
<tr>
<td></td>
<td>methadone</td>
<td></td>
<td></td>
<td>M = 274</td>
<td>M = 51</td>
<td></td>
<td>M = 1.3</td>
</tr>
<tr>
<td>12</td>
<td>Amphetamine, benzodiazepine, cannabis, heroin</td>
<td>34</td>
<td>40</td>
<td>A = 2.0</td>
<td>A = 1.3</td>
<td>19.0</td>
<td>A = 1.4</td>
</tr>
</tbody>
</table>

* Sampled from 12 drugs addicts after recovery from acute intoxication.
† A, amphetamine and M, methamphetamine.
‡ LOD was estimated for each chromatogram individually and was limited when observed peaks did not meet identification criteria.
unknown to what degree the amphetamines are trapped from exhaled breath and the reproducibility of the extraction efficiency, which may have contributed to the variability in detected amounts in the exhaled breath samples. It was not possible to validate the accuracy and precision in quantitation in the present study, but this will be the topic for further work. Firm conclusions regarding a possible correlation of exhaled breath excretion rate with blood levels can, therefore, not be made at this point.

The majority of the patients were sampled after hospitalization and recovery following acute drug intoxication. The urine and plasma data indicated that in most cases sampling was performed close to “amphetamine” intake (< 24 h), whereas in other cases low levels (< ~5 µg/mL) in urine indicated longer times since intake (Table I). Analytes were, however, still detected in the exhaled breath. The relative proportion of amphetamine and methamphetamine in the exhaled breath agreed well from a qualitative perspective with plasma results, which further support the validity of the findings. In cases 1, 2, and 3, there was a clear predominance of amphetamine in plasma, which was reflected in the breath sample; in cases 4, 5, and 11, a higher proportion of methamphetamine was reflected in both specimens.

Apart from alcohol, exhaled breath has so far not been considered for testing of any other drugs of abuse since earlier studies in the 1970s and 1980s on tetrahydrocannabinol in breath following cannabis smoking (17). However, exhaled breath is known to contain a great number of substances including non-volatile compounds (6–8). It is, therefore, reasonable to assume that also abused drugs can be excreted this way. Compounds exhaled in exhaled breath may originate from blood by a mechanism of producing a gas phase in the alveoli (18,19). Alternatively, compounds may originate from other parts of the airways (18,19). The importance and role of the aerosol fraction for the excretion of drugs of abuse in exhaled breath needs to be addressed in future work.

This original observation demonstrates that drug testing using exhaled breath is feasible and deserves further investigation. Exhaled breath sampling is more accessible and safer than other matrices presently used for drug testing. Our results demonstrate that current bioanalytical technology enables the use of exhaled breath for detecting and measuring drugs of abuse. A better and validated sampling technique is needed to extend these findings to other drugs of abuse in exhaled breath. The possible development of a miniaturized detector that would enable field testing for drugs of abuse in general is intriguing and would be expected to significantly contribute to the area of drug testing in, for example, traffic medicine.

Acknowledgments

We thank Inger Engman-Borg for assistance in the sam-

Figure 2. Chromatograms from the identification of amphetamine (A) and methamphetamine (B) in exhaled breath from one patient (case 11 of Table II) after intake of “amphetamine”. The urine and plasma data suggest possible intake of methamphetamine with amphetamine. Identification using LC-MS-MS was based on the presence of compounds with correct retention time and with correct relative abundance of two product ions.

Figure 3. Chromatograms from the identification of amphetamine (A) and failure to identify methamphetamine (B) in exhaled breath from one patient (case 10 of Table II) after intake of “amphetamine”. The urine and plasma data suggest intake of amphetamine with methamphetamine. Identification using LC-MS-MS was based on the presence of compounds with correct retention time and with correct relative abundance of two product ions. For methamphetamine, the product ion ratio (0.55) was outside the acceptance criterion 0.41 ± 20%.

Figure 4. Chromatograms from the analysis of amphetamine (A) and methamphetamine (B) in an exhaled breath extract from a control subject.
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References


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