Determination of Phenylisothiocyanate Derivatives of Amphetamine and its Analogues in Biological Fluids by HPLC–APCI-MS or DAD

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Abstract

Amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDE), and methylenedioxymethamphetamine (MDMA), as well as eight other sympathomimetic amines (benzyl-l-phenylethylamine, ephedrine, fenfluramine, norfenfluramine, phentermine, phenylethylamine, phenylpropanolamine, and propylhexedrine), were extracted from serum or urine with ether, derivatized with phenylisothiocyanate, and subjected to high-performance liquid chromatographic (HPLC) examination in isocratic mode. Two detection arts were applied: atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) and UV-spectrometry as diode array detection (DAD) or single wavelength at 250 nm. The derivatives were well-separated and showed good chromatographic behavior. Full-scan mass spectra of drugs examined by means of APCI with collision induced dissociation (APCID) contained protonated molecular ions (M+H)+ and fragments typical for particular drugs. APCID-liquid chromatography–mass spectrometry (LC-MS) appeared very selective for differentiation of all drugs involved. The quantitation with APCID was performed using selected ion monitoring (SIM) of (M+H)* ions and selected fragments of drugs involved and their deuterated analogues. The limits of detection ranged from 0.001 mg/L (MA, MDMA, and MDE) to 0.005 mg/L (A and MDA). In HPLC-DAD, the spectra of MDMA and MDE were practically identical with maxima of 236–240 nm. Other amphetamines showed slightly different spectra with maxima of 245–250 nm. The limits of detection in UV detection amounted to 0.01–0.03 mg/L (single wavelength detector at 250 nm) or 0.05–0.1 mg/L (DAD).

Introduction

Amphetamine (A) and its analogues, methylenedioxyamphetamine (MDA), methylenedioxyethylamphetamine (MDE), and methylenedioxymethamphetamine (MDMA), play an increasing role on the illicit drug market in the United States, Japan, and Europe (1–6). Acute intoxication and fatal cases involving not only amphetamine, but also MDMA or MDE were reported (7–11).

Not one of the analytical procedures applied for detection and quantitation of amphetamines, immunoassays, gas chromatography (GC) (usually with mass spectrometric detection), and high-performance liquid chromatography (HPLC), is devoid of some analytical flaws. The immunochemical procedures showed one definite drawback: N- or ring-substituted amphetamine analogues have very low crossreactivity with the available antibodies. In three commercial amphetamine radioimmunoassays, only MDA revealed crossreactivity comparable with amphetamine or methamphetamine (12–14). The FPIA amphetamine immunoassay showed pretty high crossreactivity for MDMA and MDE, which is in contrast to the EMIT immunoassay (15). Significant crossreactivity to phenylpropanolamine and ephedrine was observed for some amphetamine and methamphetamine immunoassays. These drugs appear in over-the-counter medications (16). Considering all these findings, the use of immunoassays as screening method for amphetamine analogues seems limited.

Gas chromatography–mass spectrometry (GC–MS) is regarded as a reference method for amphetamine derivatives and is routinely used for confirmation and quantitation (17–21). However, the electron impact (EI)–GC–MS showed questionable specificity. Base and second peaks of some derivatives of common over-the-counter sympathomimetic amines were identical with amphetamine and methamphetamine (22). The artifactual degradation of ephedrine to methamphetamine and methamphetamine to amphetamine was reported (23). Chemical ionization GC–MS appeared superior to EI–GC–MS and allowed discrimination of all relevant sympathomimetic amines from amphetamine and methamphetamine (24).

HPLC had less application in the analysis of amphetamines. The direct determination of these compounds by means of selected wavelength UV (HPLC–UV) or diode array detection (HPLC–DAD) is of limited use because of their low absorbptivity. Developed derivatization methods enabled more sensitive detection of drugs by means of UV-spectrophotometry (25–27) or fluorimetry (28). These HPLC methods, however, did not
Figure 1. Mass spectra of amphetamine (A), methamphetamine (B), MDA (C), MDMA (D), MDE (E), fenfluramine (F), norfenfluramine (G), ephedrine (H), phenylpropanolamine (I), phentermine (J), propylhexedrine (K), phenylethylamine (L), and benzyl-1-phenylethylamine (M), taken by means of APCID. Mass range, m/z 50 > 400; scan time, 3 s; offset 40 V.
ensure the selectivity appropriate for forensic analysis of biological fluids.

The purpose of this study was to develop a simple and rugged method for the determination of amphetamine derivatives in body fluids for forensic purposes. After consideration of existing procedures and pilot experiments, liquid-liquid extraction with derivatization with phenylisothiocyanate (PIT) (25) was selected for the following reasons: the sample pretreatment was simple, robust, and inexpensive, and the derivatives were stable and showed good chromatographic properties. HPLC–DAD or HPLC–UV and atmospheric pressure chemical ionization mass spectrometry (APCI-LC–MS) were applied as detection methods. HPLC–DAD/UV is currently a standard technique in toxicological laboratories, and APCI-LC–MS shows high selectivity and growing applicability in forensic analysis (29–32).

Experimental

Materials

Drug standards. Amphetamine, methamphetamine, MDMA, MDA, MDE, ephedrine, 1-phenylethylamine (PEA), phenylpropanolamine, propylhexedrine, and phentermine were obtained from Sigma Chemical (St. Louis, MO). Fenfluramine and norfenfluramine were supplied by Servier (Orleans,
France), and N-benzyl-1-phenylethylamine (BEA) were supplied by Fluka (Buchs, Switzerland).

Derivatizing reagent. Phenylisothiocyanate was supplied by Fluka (Cat.# 78781).

Deuterated internal drug standards. Amphetamine-d_{10}, methamphetamine-d_{10}, MDE-d_{7}, and MDMA-d_{5}, all from High Standard Products (Inglewood, CA), was used as internal standards for APCI-LC-MS in the concentration of 100 ng/10 μL.

Nondeuterated internal drug standards. BEA or one of amphetamines not present in the sample was used as the internal standard for HPLC-DAD/UV in the concentration of 500 ng/10 μL.

Serum and blood standards. Serum and blood were obtained from the local blood bank and spiked with amphetamine (A), methamphetamine (MA), MDMA, MDA, and MDE to the concentrations of 0.005, 0.010, 0.025, 0.050, 0.1, 0.2, 0.5, and 1.0 mg/L.

For accuracy control, the Medidrug BTM S-plus serum (Medichem, Stuttgart, Germany) was used with an assigned amphetamine concentration of 0.149 mg/L.

### Table I. Observed Fragmentation and Retention Behavior of Examined Amphetamines and Other Phenylethylamines

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula of derivative</th>
<th>m/z of fragments*</th>
<th>Rt t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>C_{16}H_{17}SN</td>
<td>271, 239, 136, 119, 91, 58</td>
<td>8.1</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>C_{16}H_{17}SN_{2}O_{2}</td>
<td>285, 253, 192, 167, 150, 119, 91</td>
<td>8.6</td>
</tr>
<tr>
<td>MDA</td>
<td>C_{16}H_{17}SN_{2}O_{2}</td>
<td>315, 283, 212, 180, 163, 133, 98, 94</td>
<td>7.0</td>
</tr>
<tr>
<td>MDMA</td>
<td>C_{16}H_{17}SN_{2}O_{2}</td>
<td>329, 297, 236, 194, 163, 135, 105</td>
<td>7.5</td>
</tr>
<tr>
<td>MDE</td>
<td>C_{16}H_{17}SN_{2}O_{2}</td>
<td>343, 311, 250, 208, 163, 133, 105, 72</td>
<td>10.4</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>C_{16}H_{17}SN</td>
<td>367, 335, 274, 232, 208, 187, 159, 136</td>
<td>20.6</td>
</tr>
<tr>
<td>Norfenfluramine</td>
<td>C_{16}H_{17}SN</td>
<td>339, 307, 283, 204, 178, 159, 94</td>
<td>13.6</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>C_{16}H_{17}SN_{2}O</td>
<td>301, 283, 267, 208, 166, 149, 133</td>
<td>3.6</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>C_{16}H_{17}SN_{2}O</td>
<td>387, 287, 269, 233, 175, 134, 105, 58</td>
<td>4.7</td>
</tr>
<tr>
<td>Phentermine</td>
<td>C_{16}H_{17}SN</td>
<td>285, 239, 219, 153, 133, 94</td>
<td>13.1</td>
</tr>
<tr>
<td>Propylhexedrine</td>
<td>C_{16}H_{17}SN</td>
<td>291, 259, 167, 156, 125, 71, 69</td>
<td>24.4</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>C_{16}H_{17}SN</td>
<td>257, 225, 136, 122, 105, 94, 58</td>
<td>5.9</td>
</tr>
<tr>
<td>Benzylphenylethylamine</td>
<td>C_{16}H_{17}SN</td>
<td>347, 288, 243, 212, 176, 149, 108, 105</td>
<td>22.1</td>
</tr>
</tbody>
</table>

* (M+H)^+ ions are indicated in bold italics; base peaks are underlined.
* Retention time.
* Abbreviations: MDA, methylenedioxyamphetamine; MDMA, methylenedioxyamphetamine; MDE, methylenedioxyethylamphetamine.

### Methods

#### Sample pretreatment

One milliliter of serum, blood, or urine was mixed with 3 mL of 0.6M NaOH in 20-mL screw-capped test tube. An appropriate internal standard (A-d_{10}, MA-d_{10}, MDE-d_{7}, MDMA-d_{5}, 100 ng each in 10 μL methanol) was added to the samples, and they were analyzed by APCI-LC-MS.

After 5 min, 10 mL diethyl ether was added, and the tube was closed, vortex mixed for 2 min, and centrifuged 5 min at 4000 rpm. The sample was then frozen at about -35°C for 30 min. The ethereal phase was decanted to another tube and 20 μL of 0.5% freshly prepared solution of PIT in dichloromethane was added. After 30 min of incubation at room temperature, the extract was evaporated at 37°C to dryness under a stream of nitrogen and reconstituted in 50 μL of mobile phase. A sample (5–10 μL) was injected into the HPLC.

If the sample was not determined immediately, it was stored at −20°C without reconstitution.

### HPLC

The mobile phase for HPLC-DAD/UV examinations was as follows: 50 mM ammonium formate buffer (pH 3.0) plus acetonitrile (60:40). The flow rate was 0.8 mL/min.

The mobile phase for APCI-LC-MS examinations was as follows: 50 mM ammonium formate buffer (pH 3.0) plus acetonitrile (55:45). The flow rate was 0.9 mL/min.

The column used was Superspher Select B a ECOcart (125 × 3 mm; E. Merck, Darmstadt, Germany).

### Detection and identification

Atmospheric pressure chemical ionization-LC-MS. A SSQ 7000 instrument from Finnigan MAT (San Jose, CA) was used in positive ionization mode. For the fragmentation study, full scan spectra were taken in the mass range m/z 50 > 400 at 3 s scan time using octapole offset voltage 15 V and 40 V. The latter offset voltage was associated with distinct fragmentation (collision induced dissociation = CID). On the basis of information obtained from CID, the Interactive Control Language (ICL) procedure was written for selective detection of A, MA, MDMA, MDA, and MDE in biological samples. The ICL procedures enable the tailoring of the data acquisition parameters (mass, scan time, offset voltage) for particular purposes. An alternative switching from octapole offset 15 V (no fragmentation) to 40 V (CID present) was applied. The scan time was 0.2 s. At offset 15 V, the protonated molecular ions (M+H) of PIT-derivatives of A (m/z 271), MA (m/z 285), MDMA (m/z 315), MDE (m/z 329), and MDE (m/z 343) were monitored, and at offset 40 V, the most specific fragments of each compound...
Figure 2. SIM chromatograms of serum extract spiked with A, MA, MDA, MDMA, and MDE to the concentration of 0.050 mg/L. (M+H)+ masses are monitored.

Figure 3. SIM chromatograms of serum extract spiked with A, MA, MDA, MDMA, and MDE to the concentration of 0.050 mg/L; mass-to-charge ratios of typical fragments are monitored.
were m/z 119 (A and MA), 150 (MA), 163 (MDA and MDMA), 194 (MDMA), and 208 (MDE). This procedure was used for routine screening for amphetamine derivatives in biological samples. The identification was done on the base of molar peak (M+H), fragments, and retention time of derivative. The quantitation was performed using deuterated analogues of compounds involved added to the sample before extraction as an internal standard (100 ng in 10 μL methanol). For MDA, a MDMA-d₅ was used as an internal standard. For quantitative analyses of particular compounds, the appropriate ICL procedures were written for monitoring (M+H)⁺ ions and fragments of drug and internal standard involved.

**HPLC-DAD/UV.** A series 900 diode array detector and a series 400 spectrophotometrical detector set at 250 nm (Waters Milipore, Eschborn, Germany) were used. Identification of particular compounds was done on the base of retention times (relative to the retention time of PTT, which is detectable in UV) and UV spectra. After identification, BEA or one of the drugs that was not present in the sample was used as an internal standard (500 ng/10 μL) for quantitation.

**Results and Discussion**

**APCID-LC-MS**

Figures 1A–1M and Table I show the results of APCID-LC-MS examinations of amphetamines and sympathomimetic stimulants using CID (octapole offset 40 V) and scan range m/z 50 > 400. A protonated molecular ion and several characteristic fragments were observed for each compound. The fragmentation was very reproducible in applied conditions. The chromatographic separation was satisfactory. Only in the cases of A

![Mass spectra of A-d₁₀ (A), MA-d₁₀ (B), MDMA-d₅ (C), and MDE-d₂ (D), taken in the same conditions given in Figure 1.](image)

**Table II. Observed Fragmentation and Retention Behavior of Deuterated Analogues of Amphetamines used as Internal Standards**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Formula of derivative</th>
<th>m/z of fragments*</th>
<th>Rt¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine-d₁₀</td>
<td>C₆H₁₁O₂N₂</td>
<td>295, 281, 249, 207, 163, 146, 129, 94</td>
<td>8.0</td>
</tr>
<tr>
<td>Methamphetamine-d₁₀</td>
<td>C₇H₁₁O₃N₂</td>
<td>295, 263, 239, 202, 160, 129, 98</td>
<td>8.3</td>
</tr>
<tr>
<td>MDMA-d₅</td>
<td>C₁₄H₂₁O₂N₂O</td>
<td>334, 302, 241, 199, 165, 135</td>
<td>7.5</td>
</tr>
<tr>
<td>MDE-d₂</td>
<td>C₁₁H₁₄O₂N₂</td>
<td>350, 300, 265, 215, 165, 151, 132, 122, 98</td>
<td>10.3</td>
</tr>
</tbody>
</table>

* m/z of deuterated fragments are indicated in bold italics; base peaks are underlined.

* Retention time.

*Abbreviations: MDMA, methylenedioxyamphetamine; MDE, methylenedioxyethylamphetamine.
Figure 5. SIM chromatograms of serum extract (case 4 on Table III), showing (M+H)^+ traces of A, A-d_{10}, MDE, and MDE-d_7.

Figure 6. SIM chromatograms of serum extract (case 6 in Table III), showing (M+H)^+ traces of A, A-d_{10}, MDMA, MDMA-d_5, MDE, and MDE-d_7.
and MDMA was a partial peak overlapping observed, which was fully compensated through completely different mass spectra.

The problem of unequivocal differentiation of amphetamine, methamphetamine, and other illicit amphetamines from commonly used sympathomimetic amines is of critical importance. The comparison of observed fragmentation of these compounds with GC–MS data indicates the higher specificity of APCI-LC–MS. Thurman et al. (22) have observed very similar fragmentation patterns for A, MA, and several sympathomimetic amines in EI-GC–MS, irrespective of derivatization applied. Paul et al. (23) demonstrated artifactual demethylation of MA to A during periodate degradation of sympathomimetic amines before EI-GC–MS. APCID-LC–MS also seems to be superior to CI-GC–MS, showing a more differentiated fragmentation pattern than the latter technique as used by Dasgupta et al. (24).

Figures 2 and 3 show the chromatograms of molecular ions and fragments of the extract of serum spiked with all five drugs to the concentration of 0.05 mg/L each. The chromatograms were taken using the ICL screening procedure described.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Material</th>
<th>HPLC-DAD/UV</th>
<th>APCID-LC-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>serum</td>
<td>MDE*, 0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>MDE, 50.0; MDA, 4.09</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>urine</td>
<td>A, 0.64; MDE, 0.65</td>
<td>A, 0.49; MDE, 0.70</td>
</tr>
<tr>
<td>3</td>
<td>urine</td>
<td>MDE, 4.84; MDA, 0.29</td>
<td>MDE, 5.20; MDA, 0.42</td>
</tr>
<tr>
<td>4</td>
<td>serum</td>
<td>A, 0.49</td>
<td>A, 0.36; MDE, 0.45</td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>A, 172.0</td>
<td>A, 151.6; MDE, 0.33</td>
</tr>
<tr>
<td>5</td>
<td>serum</td>
<td>MDE, 9.03; MDMA, 0.14</td>
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</tr>
<tr>
<td></td>
<td>urine</td>
<td>MDE, 70.5; MDMA, 28.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>serum</td>
<td>A, 1.06; MDE, 0.21; MDMA, 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>A, 13.8; MDE, 1.98; MDMA, 0.36</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>serum</td>
<td>MDE, 0.11; MDMA, 0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>A, 10.2; MDMA, 0.27; MDE, 3.64; MDA, 1.4</td>
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</tr>
<tr>
<td>8</td>
<td>serum</td>
<td>A, 0.43; MDMA, 0.12; MDE, 0.10</td>
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</tr>
<tr>
<td></td>
<td>urine</td>
<td>A, 10.7; MDMA, 10.6; MDE, 7.1; MDA, 0.5</td>
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</tr>
<tr>
<td>9</td>
<td>serum</td>
<td>A, 0.12; MDMA, 0.06</td>
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<tr>
<td></td>
<td>urine</td>
<td>A, 1.0; MDMA, 0.2; MDE, 0.05</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>serum</td>
<td>A, 0.04; MDMA, 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>A, 6.2; MDMA, 22.7</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>urine</td>
<td>MDMA, 23.22; MDA, 2.44; MDE, 0.04</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: MDE, methylenedioxyethylamphetamine; MDA, methylenedioxyamphetamine; A, amphetamine; MDMA, methylenedioxymethamphetamine.

Figure 7. Area ratios: standard–IS for A, MA, MDA, MDMA, and MDE over the concentration range 0.005–1 mg/L serum.
The quantitations were performed using appropriate deuterated analogues as internal standards. Figures 4A–4D present the full scan spectra (m/z 50–400; scan time, 3 s; octapole offset 40 V) of deuterated amphetamines. The fragmentation of deuterated internal standards is shown in Table II. Apart from (M+H)+ ions, four to five fragments typical of deuterated analogue, were observed. The use of A-d10, MA-d10, MDMA-d8, and MDE-d7 prevented the cross-contribution of fragments common for drugs and analogues with lesser extent of deuteration and assured high specificity of internal standards postulated by several authors (33,34).

For quantitation in SIM mode, the ICL procedures were written that allowed the detection of the (M+H)+ ions and characteristic fragments of drugs involved and appropriate internal standards. The following ions were chosen: m/z 271 and 119 for A; 281 and 129 for A-d10; 285 and 150 for MA; 295 and 160 for MA-d10; 315 and 163 for MDA; 329 and 194 for MDMA, 334 and 199 for MDMA-d8; 343 and 208 for MDE; and 350 and 215 for MDE-d7. Figures 5 and 6 illustrate the application of these procedures in forensic cases.

The sensitivity and linearity of the applied procedure was checked using serum standards spiked with A, MA, MDA, MDMA, and MDE to the concentrations of 0.005, 0.010, 0.025, 0.050, 0.1, 0.2, 0.5, and 1.0 mg/L. The response ratios for all drugs were linear in the examined range (Figure 7). Following values were found for respective calibrations curves: A, slope, 160.1, intercept, -11.6, fit (r2), 0.994; MA, slope, 67.1, intercept, 3.9, fit (r2), 0.999; MDA, slope, 123.4, intercept, 16.5, fit (r2), 0.998; MDMA, slope, 119.0, intercept, 3.1, fit (r2), 0.999; MDE, slope, 50.0, intercept, -1.3, fit (r2), 0.999.

The limits of detection (LOD), defined as peak area exceeding three times the noise level, for A and MDA in applied conditions were 0.005 mg/L and for MA, MDMA, and MDE were 0.001 mg/L. These values show that APCI-LC-MS is at least as sensitive as GC-MS (18,25).

Ten serum samples spiked with A, MA, MDMA, and MDE to the concentration of 0.050 mg/L each were tested for five days. The day-to-day precision, expressed as RSD, was 5–7%.

The concentration of A in reference serum Medidrug S (target value, 0.149 mg/L) was determined 10 times during several weeks and averaged 0.142 ± 0.009 mg/L.

The reconstituted extracts were stable for 4 days at -20°C; nonreconstituted extracts were stable for at least 10 days.

The APCID-LC–MS was applied in over thirty cases for serum and urine examinations. Selected cases are shown in Table III. Often two or more amphetamines were detected. MDA arises in urine as a desalkylation product of MDMA or MDA (35). Moreover, MDMA is frequently found as a "congener" in illicit MDE preparations.

HPLC–DAD and UV

Figure 8 presents the chromatogram of serum extract spiked with the mixture drugs to the concentration of 0.5 mg/L (MA, MDMA, and MDE) or 1 mg/L (A and MDA). The respective UV spectra are shown in Figure 9. The spectra of MDMA and MDE were practically identical with maxima at 236–240 nm. Other amphetamines showed slightly different spectra with maxima at 245–250 nm. The examination of several blank serum and urine extracts demonstrated an absence of matrix peaks in the elution range of amphetamine derivatives. Therefore, the method assured fairly high specificity as a screening procedure. For quantitative analysis, the extraction was repeated, adding BEA or this amphetamine derivative that was absent in the sample, as
an internal standard. In the earlier phase of the study, PEA was used as an internal standard for spectrophotometric examinations. However, in a number of blank urine extracts and aged serum or blood samples, the peaks with retention time and UV-spectrum identical with PEA were observed. Recently, PEA was identified in some street drug samples (36). For these reasons, the use of PEA as the internal standard was abandoned.

The HPLC-DAD method was applied in several cases. In three cases, an APCID-LC-MS procedure was also performed, showing concordant results (Table III).

The detection limits for DAD were as follows: A and MDA, 0.1 mg/L and MA, MDMA, and MDE, 0.05 mg/L. For UV detection at 250 nm, the limits of detection (LOD) were 0.03 and 0.01 mg/L for A and MDA and MA, MDMA, and MDE, respectively. Therefore, HPLC-DAD could detect relatively high concentrations of drugs, which are generally associated with massive abuse or intoxication. Using the spectrophotometrical detector at 250 nm, a lower LOD was reached, but it was at the expense of specificity.

The day-to-day precision was checked by the repetitive (at least three times) examination of nine forensic samples and averaged 27% RSD at concentrations of 0.2–5 mg/L.

The absolute extraction recovery was checked through the comparison of UV absorbance of phenylethylamine base and BEA base subjected to derivatization without extraction with the samples that were extracted. The recovery averaged 70–75%.

**Conclusion**

The applied method of sample pretreatment is simple and robust. The derivatives obtained are stable for several days and show good chromatographic behavior in HPLC.

APCID-LC-MS proved a very selective and sensitive method of determination of all amphetamine derivatives and related phenylethylamines. The selectivity was better than both EI- and CI-GC-MS. APCID-LC-MS seems to be the method of choice for screening for amphetamines and related sympathomimetic amines in biological material.

In direct comparison with HPLC-DAD/UV, it appeared that APCID-LC-MS was far more specific and sensitive. The HPLC-DAD/UV method may be used for serum examination in poisoning cases, for urine screening, or for examination of samples taken from subjects arrested for drugs.

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