GC–MS Identification of Sympathomimetic Amine Drugs in Urine: Rapid Methodology Applicable for Emergency Clinical Toxicology

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Abstract

A method was developed that permitted rapid identification in urine of the following sympathomimetic amines: amphetamine, benzphetamine, cathinone, desmethylselegiline, diethylpropion, ephedrine, fenfluramine, mazindol, methylenedioxymethamphetamine, methylenedioxyethylamphetamine, methylenedioxymethamphetamine, mescaline, methamphetamine, mephedrine, methcathinone, methylaminorex, methamphetamine, methcathinone, methyaminorex, methylphenidate, pemoline, phenidimetrazine, phenylephrine, phentermine, phenylpropanolamine, pseudoephedrine, and selegiline. In addition, two o-phenylethylamine-like monoamine oxidase inhibitors, phenelizine and tranylcypromine, were studied. Those sympathomimetic amines containing a primary or secondary amine, a hydrazine, and/or hydroxyl (except mazindol) functional groups were derivatized effectively using an on-column derivatization technique that used a reagent consisting of 10% fluoroanhydride in hexane, whereas the other sympathomimetic amines, including mazindol, were analyzed underivatized. Three different fluoroanhydrides, trifluoroacetic (TFAA), pentafluoropropionic (PFPA), and heptafluorobutyric (HFBA), and three different injection-port temperatures (160, 200, and 260°C) were investigated. Both TFAA and PFPA gave sympathomimetic amine derivatives with essentially identical retention times, whereas HFBA gave longer retention times and better separation of individual compounds. The base fragmentation ion was noted to increase 50 amu (CF₂) for each derivatized sympathomimetic amine as the length of the carbon-fluorine chain increased. Fragmentation ion abundance was maximized at an injection-port temperature of 260°C, and this enhanced sensitivity coupled with the better chromatographic resolution of the individual sympathomimetic amines prompted the selection of HFBA as the derivatizing agent of choice. Assignments were made for the fragmentation ions produced by each derivatized drug. The developed method was adapted to analyze urine specimens that might be encountered in emergency toxicology testing. For identification of sympathomimetic amines requiring derivatization, 0.1 mL of the patient specimen had amphetamine-d₃ and methamphetamine-d₃ added as internal standard followed by adjustment of pH to 9.3 with borate buffer, extraction with 9:1 chloroform/isopropanol, centrifugation and separation of the organic phase, addition of 10% methanolic HCl and evaporation under nitrogen, reconstitution with HFBA reagent, and on-column derivatization during gas chromatographic–mass spectrometric (GC–MS) analysis. For those sympathomimetic amines not requiring derivatization, 1.0 mL of urine specimen had diazepam-d₃ added as internal standard followed by the same extraction procedure and reconstitution accomplished with ethyl acetate. Because precolumn derivatization was eliminated and only 8 min was required for GC–MS analysis, complete analysis time was approximately 30 min, making the method suitable for clinical emergency toxicology purposes.

Introduction

Analyses for sympathomimetic amines in urine specimens produce some unique challenges for toxicologists. The phenylethylamine nucleus for this class of endogenous amines and exogenous drugs possesses physicochemical properties (1) that affect its removal from a biological matrix, as well as functional groups on the parent drugs and their metabolites that prevent effective chromatography without derivatization. Commonly available oral sympathomimetic amine drugs generally have the following pharmacological uses: central nervous system (CNS) stimulants, anorectics, anti-Parkinsonians, and nasal decongestants. Regardless of their intended use, each, in toxic overdose, has the potential to produce effects on the CNS and the cardiovascular system. Therefore, clinical toxicologists need a method for identifying sympathomimetic amine drugs that can be performed rapidly enough to assist the clinician in arriving at a proper differential diagnosis for the patient. Because amphetamine and methamphetamine are prominent members of the sympathomimetic amine drug class and are frequently abused, a great deal of effort has been expended on developing gas chromatography–mass spectrometry (GC–MS) assays that will detect and quantify the presence of each in urine (2–12), blood (13,14), saliva (15), sweat (15),...
Amphetamine was first related to sudden death in a young child (25). Screening detects the presence of a sympathomimetic amine—amphetamine at a cutoff value of 1000 ng/mL or greater. For example, Meatherall et al. (25) investigated either HFBA or 4-carboxyhexafluorobutyric acid (4-CB) derivatives to differentiate methamphetamine and ephedrine because both amphetamine and methamphetamine are regulated in the workplace drug-testing programs (26) and by the Department of Transportation (DOT) (24). GC-MS confirmation is required for each of these drugs whenever urine immunoassay screening detects the presence of a sympathomimetic amine-like substance. Concern in workplace urine drug-testing programs has centered around the ability of an assay method to differentiate methamphetamine and ephedrine because both have similar chromatographic and mass spectral properties and there was some evidence that large amounts of ephedrine would form methamphetamine under high injection-port temperatures when derivatized using 4-carboxyhexafluorobutyric acid (25,26) or heptafluorobutyric anhydride (26). One report (27) suggested removing the offending ephedrine by a metaperiodate oxidation prior to GC–MS analysis, and this has been recommended in the workplace drug-testing programs (26), but the pH must be controlled to prevent amphetamine artifact when methamphetamine is present (28). This application has little value in a clinical toxicology situation where the identity of an α-hydroxyl phenylethylamine is important in establishing a differential diagnosis, guiding subsequent therapeutic intervention, and providing effective patient or caregiver counseling.

Materials and Methods

**GC-MS instrumentation and chromatographic conditions**

Analyses were performed on a Hewlett-Packard model 5972B positive ion electron impact, quadrupole MS interfaced with a model 5890 GC, model 7653 autosampler, and model HP Vectra 90 MHz Pentium computer using the Drug-Quant software (revision A.00.00). The GC was equipped with a 12-m×0.2-mm i.d. capillary column containing a 0.33-μm film thickness cross-linked methyl silicone gum (HP-1) and operated in a program with an initial temperature of 80°C for 2 min, rate of 40°C/min, and with a final temperature of 280°C for 1 min. The
carrier gas was helium with a splitless time of 2 min followed by a 20:1 split, column head pressure of 10.8 psi, and a helium carrier gas flow rate of 1.15 mL/min. The injector port was operated at 260°C and was fitted with a glass mixing chamber assembly (HP-19251-06540) containing a 10-mm silane-treated glass-wool plug (HP-5080-8764) that was 30 mm from the end next to the gold-plated seal (bottom injector plate). The glass mixing chamber assembly was changed daily and cleaned by overnight soaking in concentrated H2SO4 saturated with K2Cr2O7, followed by washing with deionized water and methanol and drying at 110°C. Packing of each glass mixing chamber assembly with the silane-treated glass wool was accomplished using nylon gloves (HP-8650-0030) to prevent contamination, and the chambers were stored in the original shipping packets until use. The injector port was fitted with a Merlin microseal septum (HP-5182-3444). The gold-plated seal at the column end of the injector port was changed whenever the column was replaced. The MS was tuned daily with PFTBA and was operated either in the full-scan mode (SCAN) for acquiring mass spectra of derivatives or in the select ion mode (SIM) for analysis of extracted urine specimens. Additional parameters for both SCAN and SIM analyses are given in subsequent sections. The autosampler was set to perform five pumps of the syringe prior to drawing up 1 µL for injection. A viscosity delay of 1 was used along with five washes of each solvent A and B containing methanol and ethyl acetate, respectively.

Chemicals and reagents
Acetone, ethyl acetate, sulfuric acid, hydrochloric acid, and potassium dichromate were obtained from Mallinckrodt. D,L-Amphetamine-d5 (side-chain labeled), D,L-methamphetamine-d5 (side-chain labeled), and diazepam-d5 were obtained from Radian Corp. as methanolic solutions containing 100 µg/mL. Chloroform, hexane, isopropanol, methanol, and water were obtained from Burdick and Jackson. Sodium borate (Na2B4O7·10H2O) was obtained from Fisher, and 12.5 g was dissolved in 250 mL of reagent-grade water to prepare the pH 9.3 borate buffer. Trifluoroacetic anhydride (TFAA) was from Pierce, pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were from Aldrich. Fluoroanhydride reagents were prepared by mixing 1 mL of TFAA, PFPA, or HFBA and 9 mL of hexane in a 20-mL liquid scintillation vial fitted with a Polysel® cap (Scientific Products) and stored in a desiccator when not in use. The following were purchased from Sigma as 1 mg/mL of the free base (w/v in methanol): benzphetamine hydrochloride, (-)-ephedrine hydrochloride, fenfluramine hydrochloride, L-phenylephrine hydrochloride, phentermine hydrochloride, (±)-phenylpropanolamine hydrochloride, and d-pseudoephedrine hydrochloride. D-Amphetamine, 2-S-cathidine hydrochloride, D,L-MDA, D,L-methylenedioxyethylamphetamine (MDMA), D,L-MDMA, cl5(-)-4-methylaminorex, methylphenidate hydrochloride, D-methamphetamine, and 2-S-methcathinone hydrochloride were purchased from Radian as 1.0 mg/mL of the free base (w/v in methanol). Diethylpropion hydrochloride, mazidol, mescaline, phendimetrazine bitarate, pemoline, and selegiline were also purchased from Sigma in a powder form and were prepared as a 1 mg/mL of the free base (w/v in methanol).

Internal standard solutions. One ampule of each D,L-amphetamine-d5 and D,L-methamphetamine-d5 containing 100 µg/mL in methanol was transferred quantitatively to a 100-mL Class A volumetric flask and diluted to volume with methanol to give a final concentration of 1 µg/mL. The solution was transferred to a 100-mL Reipiper® (Labindustries) fitted with a 1-mL syringe, and the solution was stored at 5°C when not in use. The syringe was set to deliver 0.5 mL of the internal standard solution. Diazepam-d5 was prepared in the same manner.

Urine control
Urine collected from laboratory personnel was used for preparation of the control. Each lot of urine collected was shown to be devoid of sympathomimetic amine drugs by GC–MS analysis and had 30 mg of sodium azide added per 4 L of urine. The control urine was prepared by quantitatively transferring 0.5 mL of each sympathomimetic amine solution containing 1 mg/mL of the free base (w/v in methanol) to a 500-mL Class A volumetric flask and diluting to volume with urine free of sympathomimetic amine drugs to give a final concentration of 1000 ng/mL. Each lot of control was aliquoted as 1.5-mL portions into individual storage containers and frozen at −30°C until use.

Urine specimen or control urine analyses
For subsequent SIM analyses, either 0.1- or 1.0-mL aliquots were used. To each aliquot was added 0.9 mL of reagent-grade water (0.1-mL aliquots only), 0.5 mL of internal standard solution (either amphetamine-d5 and methamphetamine-d5, or diazepam-d5), 1 mL of pH 9.3 borate buffer, and 3 mL of 9:1 chloroform/isopropanol, followed by rotation on a hematologic mixer for 10 min then centrifugation at 5°C and 2600 rpm for 10 min. Following centrifugation, the lower organic layer from each aliquot was removed, 100 µL of 10% (v/v) methanolic HCl was added, followed by evaporation to dryness under nitrogen at 50°C. To the resultant residues was added 100 µL of either the HFPA reagent or ethyl acetate. Following vortex mixing, the solutions were each transferred using a disposable glass Pasteur pipette to autosampler vials for GC–MS analysis.

SCAN spectra
The program and instrument conditions for the analyses were given here previously, except the MS was operated in the SCAN mode using a threshold value of 100, 30–650 amu scan, sampling of 2, and 1.2 scans/s. The fragmentation patterns of some sympathomimetic amine drug standards were each evaluated in the following ways.

TFFA, PFPA, and HFBA. To 200 µL of 1 mg/mL methanolic solutions of each amphetamine, ephedrine, phentermine, phenylpropanolamine, pseudoephedrine, and 100 µL of methamphetamine was added 100 µL of 10% (v/v) methanolic HCl followed by evaporation to dryness under nitrogen at 50°C. To each of the resultant residues was added 100 µL of neat TFFA, PFPA, or HFBA followed by GC–MS analysis.

HFBA reagent. Volumes of each sympathomimetic amine methanolic solution listed, as well as 200 µL of most other sympathomimetic amines and the internal standards used in
the study were treated in an identical fashion except instead of using the neat derivatization chemical, 100 μL of the HFBA reagent (9:1, hexane/HFBA) was added, followed by GC–MS analysis to obtain SCAN spectra (Table I). Benzphetamine, diethylpropion, mazitol, phendimetrazine, and selegiline had 100 μL of ethyl acetate instead of HFBA reagent added to obtain SCAN spectra (Table II).

SIM analysis
Three characteristic ions were selected from each SCAN spectra of sympathomimetic amine standards obtained with the HFBA reagent or ethyl acetate for use in the SIM monitoring. Criteria for such ion selection were based where possible on the following: 1. base (most abundant) ion; 2. parent ion (dehydration product with side-chain hydroxyl-containing amines), if present; and 3. an ion with at least 10–50% of the abundance of the base ion that was characteristic of the amine or derivatizing agent coupled to an ion fragment of the drug. SIM acquisition was begun at 3 min, and the ions for internal standards and sympathomimetic amine drugs were monitored using a 50-ms dwell and eV volts 400 above the daily tune. The program and other instrument conditions for the analyses were given here previously.

Injector-port temperature evaluations
To each of the following volumes of drug standard solutions was added 100 μL of 10% methanolic HCl, followed by evaporation to dryness under nitrogen at 50°C and reconstitution in 100 μL of neat PFPA: 0.5 mL of the amphetamine-d5 and methamphetamine-d5 internal standard solution; 10 μL of d-amphetamine, (-)-ephedrine, phenetermine, and (±)-pseudoephedrine; and 5 μL of (+)-methamphetamine. SCAN analyses were conducted using the given GC–MS conditions and volumes of standards, except each evaluation was conducted at three different injection port temperatures: 160, 200, and 260°C. The abundance of the base ions for each sympathomimetic amine were compared at each injector port temperature (Table III).

Table I. Mass Spectral Characteristics of HFBA Derivitized Sympathomimetic Amines

<table>
<thead>
<tr>
<th>Standard</th>
<th>Retention time (min)</th>
<th>Ions m/z (abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine-d5*</td>
<td>3.77</td>
<td>244(100), 169(34), 123(41), 122(37), 92(33), 69(65)</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>3.77</td>
<td>240(95), 169(22), 118(100), 91(80), 69(15)</td>
</tr>
<tr>
<td>Methamphetamine-d5</td>
<td>4.13</td>
<td>254(100), 210(23), 169(20), 105(100), 77(41), 69(14)</td>
</tr>
<tr>
<td>MDEA (MDE)</td>
<td>5.36</td>
<td>344(8), 254(100), 210(23), 169(20), 105(100), 69(18)</td>
</tr>
<tr>
<td>MDMA</td>
<td>5.27</td>
<td>269(9), 268(100), 240(30), 187(71), 186(5), 169(19), 159(20), 69(13)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>4.13</td>
<td>357(30), 240(30), 169(20), 105(100), 77(30), 69(25)</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>4.36</td>
<td>403(13), 268(100), 240(93), 169(18), 162(97), 135(55), 77(19), 69(13)</td>
</tr>
<tr>
<td>Methyldopamine</td>
<td>5.37</td>
<td>389(18), 254(100), 210(79), 169(21), 162(99), 131(70), 77(20), 69(18)</td>
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<tr>
<td>Methylenedihydantil</td>
<td>5.56</td>
<td>407(31), 394(42), 181(100), 179(29), 169(12), 69(12)</td>
</tr>
<tr>
<td>Methamphetamine-d5*</td>
<td>4.15</td>
<td>258(100), 213(25), 169(19), 150(16), 120(13), 119(28), 100(18), 92(16), 69(38)</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>4.17</td>
<td>255(29), 254(100), 210(83), 169(20), 118(35), 91(38), 69(20)</td>
</tr>
<tr>
<td>Methylenedihydantil</td>
<td>5.37</td>
<td>399(100), 356(15), 328(12), 321(14), 169(20), 160(57), 142(21), 177(15), 70(47), 69(27)</td>
</tr>
<tr>
<td>MDEA (MDE)</td>
<td>5.27</td>
<td>281(36), 280(100), 226(13), 169(13), 91(12), 69(11)</td>
</tr>
<tr>
<td>Methamphetamine-d5</td>
<td>4.17</td>
<td>265(28), 252(9), 214(11), 169(29), 140(29), 139(13), 100(19), 96(16), 69(100), 68(42)</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>4.36</td>
<td>169(29), 105(29), 104(100), 91(40), 77(66), 69(19)</td>
</tr>
<tr>
<td>Methylenedihydantil</td>
<td>5.92, 6.04</td>
<td>542(44), 520(11), 317(13), 240(100), 169(80), 119(10), 69(50)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>4.91</td>
<td>254(100), 214(24), 169(15), 132(41), 91(82), 69(30)</td>
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<tr>
<td>Phenylpropanolamine</td>
<td>4.13</td>
<td>330(11), 240(100), 169(15), 117(7), 105(8), 69(16)</td>
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<tr>
<td>Pseudoephedrine</td>
<td>4.51</td>
<td>344(6), 254(100), 210(19), 169(13), 117(5), 69(9)</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>4.33, 4.37</td>
<td>238(12), 169(16), 117(25), 116(100), 113(39), 91(9), 69(20)</td>
</tr>
</tbody>
</table>

* Used as the internal standard.
Chromatographic peaks found in the SIM analyses were identified by their characteristic retention times and ions.

**Results and Discussion**

A number of different sympathomimetic amine drugs are available by prescription, over-the-counter, or illicitly. Many of these drugs have similar chemical structures, differing mainly in alkyl or hydroxyl substitution or in stereochemistry of the parent phenylethylamine skeleton (Table IV). For purposes of this study, those sympathomimetic amine drugs available in the United States as either prescription or over-the-counter drugs were evaluated. Fenfluramine and its d-isomer, dexfenfluramine, have recently been withdrawn from the United States market, but fenfluramine was evaluated because supplies may still be available for either accidental ingestion or suicide gesture. Propylhexedrine and (-)-methamphetamine are both marketed only as dilute solutions in over-the-counter nasal sprays and were omitted from this study because they would typically be of little importance in clinical toxicology evaluations. The other sympathomimetic amines evaluated are often associated with illicit use. These include amphetamine, cathinone, MDA, MDEA, MDMA, mescaline, methamphetamine, methcathinone, and methylaminorex. Two other phenylethylamine-like drugs, phenelzine and tranylcypromine, both monoamine oxidase inhibitors used to treat depression, were also evaluated. Because many of the sympathomimetic amine drugs can also be present in a patient specimen as a result of metabolism, knowing the metabolic relationships can be helpful in identifying the parent drug taken by the patient. To aid in this process, Figure 1 is a summarization of the known major metabolic routes for these drugs in humans based upon the following literature reports: amphetamine (41-45), benzphetamine (46,47), cathinone (48,49), diethylpropion (50-54), ephedrine (55-58), fenfluramine (59-65), mazindol (66), MDA (67), MDEA (67-70), MDMA (67,71-74), mescaline (75,76), methamphetamine (45,77-81), methcathinone, methylaminorex (82), methylphenidate (83-86), pemoline (87), phendimetrazine (88), phenylephrine (89,90), phenetermine (91-93), phenylpropanolamine (55,56), pseudoephedrine (94-96), and selegiline (97-102). In emergency clinical toxicology, the analyst does not normally hydrolyze a urine specimen because of time constraints; the Phase II metabolites that largely appear in urine (phenols and alcohols as glucuronides and acids as sulfates or glycinites) are not as important in making a rapid determination of the drug responsible for the clinical symptomatology as are the parent drugs and those metabolites that do not form conjugates.

Some of the parent drugs shown in Figure 1 have been shown to be of limited value in identifying the administered drug because little is excreted in the unmetabolized form. For example, benzphetamine is metabolized primarily to methamphetamine and amphetamine, and the parent drug can only be detected at low concentrations and only in specimens collected shortly after administration of the drug (46). For emergency toxicology purposes, benzphetamine should be detectable because clinical symptomatology causing the patient to seek medical assistance would generally occur in a time pe-
period within a few hours of dosing with the parent drug and be in the time window for detection. Another point to consider for clinical toxicology purposes is that metabolism studies have been performed in humans using therapeutic or subtherapeutic amounts of the parent drug, whereas a toxic overdose would be anticipated to be metabolized differently. Most cytochromes responsible for metabolism of drugs are saturable at rather low concentrations and therefore limited in how much parent drug can be processed. This usually gives rise to more of the parent drug being excreted than would be the case when a therapeutic dose is administered. We were not able to validate this point for benzphetamine in the present study because no patients presented with benzphetamine toxicity.

Prior to the initiation of the present work, our laboratory had employed an on-column method using neat PFPA for quantitating amphetamine and methamphetamine in urine specimens. This method had been successfully used in an extensive study of urine specimens from human subjects who had received d-methamphetamine (103). This method had also been successfully used to perform GC–MS confirmations of urines derived from workplace testing performed in our laboratory that had screened positive by immunoassay and external proficiency urine specimens. The method had proved to be reliable and permitted accurate and precise measurement of both amphetamine and methamphetamine. However, there was always concern that injection of a neat anhydride directly onto the GC–MS was not an appropriate maneuver. This suspicion was partially confirmed by the fact that attempts to process speci-
mens that had been derivatized with N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) immediately following analysis of the amphetamines were not successful unless the column was "washed" five or more times by repeated injections of ethyl acetate followed by execution of the temperature program to restore well-defined chromatographic peaks with appropriate areas for the internal standards. This finding limited the use of the same column for multiple analyte analyses with an efficient turnaround time. The effect on chromatographic resolution was not noted if analytes such as 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid or benzoylecgonine, both derivatized precolumn with PFPA in our standard laboratory protocols, were performed following the on-column derivatizations with neat PFPA. Because our laboratory was frequently requested to perform toxicology evaluations on urine specimens that needed to have a trimethylsilyl derivative prepared to effectively use available mass spectral libraries, the on-column method with neat PFPA was a potential liability. This prompted us to examine ways in which the advantages of rapid analysis for the sympathomimetic amines using on-column derivatization could be accomplished without the resultant aberrant effects on chromatography of subsequently analyzed silylated derivatives. The most obvious way was to reduce the amount of PFPA in-line with an amount slightly greater than a stoichio-

metric amount, yet have sufficient volume to permit removal of multiple aliquots for injection purposes. To accomplish both, PFPA was diluted in an inert solvent and introduced into the GC–MS. Hexane was chosen as the inert solvent because it was volatile but did not increase the injection-port pressure as did heptane or petroleum ether. Ethyl acetate, which was routinely used as the injection solvent for all the precolumn-formed derivatives in our laboratory, was not used because there might be a chance for transesterification reactions to occur at the elevated injection-port temperatures that were felt to be necessary to achieve efficient on-column derivatizations. A 10% (v/v) mixture of PFPA in hexane was found to produce the same mass spectra as had previously been observed with neat PFPA. Although a smaller percentage of PFPA in hexane would produce the same results, it was felt that 10% represented an amount that would not be totally consumed in the event that a specimen was derived from an overdose victim in whom large amounts of analyte could potentially be present.

Several reports had indicated that elevated injection-port temperatures were responsible for the artifactual conversion of ephedrine to methamphetamine with the use of precolumn derivatizations (25,26). Lower injection-port temperatures had been advocated by SAMHSA in directives to certified laboratories to counteract this problem (104). Thus, a study was con-

Figure 1. A schematic of the major human metabolism of sympathomimetic amines. Those drugs contained within a box are available in the United States and are the parent administered compound, whereas those drugs in bold and not in a box are illicit parent drugs. All compounds in non-bold type and not in boxes are metabolites of the parent compounds. Conjugates formed may be glucuronides, sulfates, or glycinates. Literature references for the various metabolic pathways are cited in the text. The metabolic route for methylaminorex is not shown because only rat studies have been reported.
ducted at three different injection-port temperatures, 160°C, 200°C, and 260°C, to determine the effect injection-port temperature had on the on-column derivatization for some of the sympathomimetic amine drugs with neat PFPA. As shown in Table III, base ion abundance varied at the different injection-port temperatures in a random fashion. With the exception of (+)-methamphetamine, the sympathomimetic amine drugs all gave greater abundances at 260°C when compared to 160°C, which was interpreted as being a more complete derivatization. Consistent increases in base ion abundance were also seen at 200°C when compared to 160°C with amphetamine and the deuterated internal standards but not with the other sympathomimetic amines. Reasons for this decrease in abundance at 200°C was not readily apparent. To specifically address whether ephedrine would be converted to methamphetamine at the 260°C injection-port temperature, another study was performed to determine if such a conversion would occur using the HFBA reagent. This study was conducted using repetitive 1-μL injections of 2 mg ephedrine dissolved in 100 μL of HFBA reagent followed by SIM acquisition and computer evaluation for m/z 91(tropolium ion) that was characteristic of (+)-methamphetamine-HFBA derivative and not found in ephedrine (Table I). No evidence of methamphetamine derivative was found (data not shown) thus demonstrating that with an injection port temperature of 260°C and limited amount of derivatizing agent, artifactual methamphetamine was not produced.

In developing a method that would be applicable to emergency clinical toxicology, adequate separation of the potential sympathomimetic amine drugs that might be found in an unhydrolyzed urine specimen was essential. To obtain adequate mass spectra and chromatographic separations, the sympathomimetic amine drugs, with the exception of benzphetamine, diethylpropion, mazindol, phendimetrazine, and selegiline required derivatization. The initial success with a mixture of PFPA in hexane prompted a study of two other fluoro-anhydrides, TFMA and HFBA, in an effort to optimize the chromatographic separation and identification of sympathomimetic amine drugs that might be present in an emergency toxicology specimen. Fragmentation patterns for each of the three different derivatives were remarkably similar for each sympathomimetic amine drug investigated. The major observable difference was a progressive increase of 50 amu (CF₂) for the base ion in the TFMA, PFPA, and HFBA derivatives. The TFMA and PFPA derivatives produced essentially identical retention times for each of the sympathomimetic drugs. Chromatographic peak resolution was good for each of the drugs except (+)-methamphetamine and (-)-ephedrine, which was < 0.1 min. Therefore, HFBA was chosen as the derivatization agent because these two drugs had separations of > 0.1 min (Table I).

Table I presents the chromatographic retention times and observed fragmentation patterns along with their relative intensity compared to the base ion which was set to 100 following on-column derivatization with the HFBA reagent. Table II presents the same data for the underivatized sympathomimetic amines. Those ions observed to be suitable for sub-
Though many of the sympathomimetic amine derivatives had similar retention times, this was not perceived as a problem because the mass spectra were dissimilar, and as was shown in Figure 1, identification of urinary metabolites would also help to confirm the parent drug.

Thurman et al. (25) had previously assigned the base m/z 254 fragment found in the mass spectra of HFBA derivatives of methamphetamine, propylhexedrine, ephedrine, pseudoephedrine, phentermine, and MDMA to [CH3CHN(CH3) COCF2CF2CF3]+, and the m/z 240 base fragment from amphetamine, phenylpropanolamine, phenylephrine, p-hydroxy-norephedrine, and MDA derivatives to [CH3CHN(H)COCF3 CF2CF3]+. In our studies, the same base ion fragments were found and in addition methcathinone and cathinone also produced 254 and 240 fragment ions, respectively (Table I). Although Thurman and his co-workers (25) had observed both m/z 169 and 69 ions, these were not assigned as [CF2CF2CF3]+ and [CF3]+ fragments, respectively, which would be consistent as the correct assignments. Either one or both of these ions appeared in all the HFBA-derivatized sympathomimetic amines studied. These ions were generally prominent and occasionally were useful in SIM analyses when other fragments were not deemed suitable for monitoring. Ion fragments of the additional sympathomimetic amine drugs studied in our work and not previously assigned by Thurman and his co-workers are given in Figure 2 (derivatized) and Figure 3 (underivatized). Mazindol was not included with the underivatized drugs since the assignments for the fragment ions shown in Table II would be consistent with the loss of the tertiary hydroxy group (Table IV) to produce m/z 266 and the isotopic effects of the chlorine atom giving rise to the adjacent ions, m/z 268, 267, and 265.

Two drugs, methylphenidate and tranylcypromine, produced two distinct chromatographic peaks, each of which had identical mass spectra. The piperidine ring of methylphenidate can exist in either the boat or chair conformation and the derivatization of these various confirmations would explain the differences in retention times but the same mass spectrum. In a similar manner, tranylcypromine HFBA derivatives can exist in the endo or exo forms, giving rise to two chromatographic peaks with identical mass spectra. This same effect was noted with larger amounts of phenelzine used to obtain SCAN spectra, but only one chromatographic peak was observed in the smaller amounts used for the SIM analyses, and thus Table I gives only the retention time for one chromatographic peak found in that analysis.

Emphasis in this work was placed on a method that would be useful for identifying but not quantitatively determining the various sympathomimetic amines in an overdose situation. Therefore, an exact determination of the limit of detection was not performed for each study drug. However, limited dilution studies with the 1000-ng/mL urine control clearly demonstrated that each analyte could be detected at 100 ng/mL if a 1-mL aliquot of specimen was analyzed (data not shown). As described, the method used only a 0.1-mL aliquot of specimen in anticipation that its use in emergency toxicology would require the lower amount of specimen to prevent column and detector overload. The developed methodology could be readily adapted to determinations of the sympathomimetic amines as a group or individually by comparison with calibrators over an appropriate concentration range.

Conclusions

A method was developed to permit the rapid GC-MS identification of a wide variety of sympathomimetic amines in urine specimens such that an impact can be made on the clinical treatment of a patient showing the effects of a toxic dose. The developed method was demonstrated to be robust in helping to identify potential overdoses or therapeutic amounts of a sympathomimetic amine drugs that had produced clinical symp-tomatology. For example, use of this methodology permitted the identification of adolescents that were experiencing chest pain following the use of ephedrine (36). Other drugs identified in children and adolescents experiencing various clinical symp-toms using the developed methodology have included in addition to ephedrine (data not shown), amphetamine, methamphetamine, methylphenidate, phentermine, phenylpropanolamine, and pseudoephedrine. Further experience with the developed method should permit other sympathomimetic amine drugs, with the potential for toxic manifestation, to be detected and correlations made with clinical presentations.

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References


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