Simultaneous Detection of Ten Psychedelic Phenethylamines in Urine by Gas Chromatography–Mass Spectrometry

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Abstract

Psychedelic phenethylamines are an emerging class of designer drugs capable of producing a complex array of sought after adrenergic and hallucinogenic effects. Toxicological detection poses a number of challenges to laboratories. The purpose of this study was to develop a procedure for the detection of psychedelic amphetamines using techniques that are widely accepted in forensic toxicology laboratories. In all, 10 target analytes were selected: 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2), 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7), 4-methylthioamphetamine (4-MTA), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), and 2,5-dimethoxy-4-methylamphetamine (DOM). Target drugs in urine were analyzed by gas chromatography in selected ion monitoring mode after mixed-mode solid-phase extraction. Limits of detection for all analytes were 2–10 ng/mL, and limits of quantitation were 10 ng/mL or less. Precision evaluated at 50 and 500 ng/mL yielded CVs of 0.4–7.9% and accuracy in the range 91–116%. Calibration curves were linear to 1500 ng/mL using mescaline-d9 as the internal standard. No carryover was evident at 5000 ng/mL (the highest concentration tested) and no interferences were observed from the presence of other structurally related compounds or endogenous bases.

Introduction

The psychedelic phenethylamines described in this study are a series of psychoactive derivatives that produce sought after effects for recreational drug users. Many of these synthetic psychotropics are not scheduled and bypass controlled substance legislation in the United States. Hallucinogenic phenethylamines were first synthesized by Shulgin (1) and later emerged as illicit drugs in Europe and Asia before making an appearance in this country. Although the most widely abused amphetamine in the United States is d-methamphetamine, there is still significant interest in new designer amphetamines as the drug scene continues to evolve (2). These emerging designer drugs include the dimethoxyphenethylamine (2C, 2C-T) and dimethoxyphenylylpropylamine (DO) series of psychedelics, which includes 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxyamphetamine (2C-H), 2,5-dimethoxy-4-iodophenethylamine (2C-I), 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7), 2,5-dimethoxy-4-bromoamphetamine (DOB), 2,5-dimethoxy-4-ethylamphetamine (DOET), 2,5-dimethoxy-4-iodoamphetamine (DOI), and 2,5-dimethoxy-4-methylamphetamine (DOM). 4-Methylthioamphetamine (4-MTA) was also included in the study because of its structural similarity, toxicity, and reported use.

Methoxylated designer amphetamines are not new; para-methoxyamphetamine (PMA) and para-methoxymethylamphetamine (PMMA) were introduced in the late 1990s, and these were associated with fatal intoxications and acute toxicity. Dimethoxy derivatives of phenalkylamines are the subject of this study. These contain methoxy groups at positions 2 and 5 of the aromatic system and often a lipophilic substituent in the 4 position. Drugs in the 2C series contain two carbons separating the amine from the aromatic core. The 2C-T series of...
drug are sulfur containing, and many are derivatives of 4-thioamphetamine. Some drugs within this class differ by just one substituent or atom, and share very similar chemical and physical properties. Molecular data and structures for the psychedelic amphetamines described in this study are depicted in Table I and Figure 1, respectively.

A number of these drugs are Schedule I drugs in the Federal Controlled Substances Act (CSA) because of the high potential for abuse and absence of either medical use or accepted safety. Although most of these substances are considered “drugs or chemicals of concern” by the Drug Enforcement Administration (DEA), several remain unscheduled to date (2C-H, 2C-I, 2C-T-2, DOI, and 4-MTA). Instead, they may be regulated by the Federal Analogue Act, which states that any drug substantially similar to a scheduled drug may be treated as though it were scheduled, if intended for human consumption. Scheduling status, street names, dosages, and duration of action for the drugs included in this study are summarized in Table II (3,4).

There is very limited published scientific literature concerning the pharmacology or toxicology of these psychedelic amphetamines. A common route of administration is oral ingestion; however, insufflation, smoking, and rectal use are not uncommon, and intravenous and intramuscular administrations have been reported (2). Some of the psychedelic phenethylamines show affinity to 5HT₂ receptors, acting as potent and selective 5HT₂C receptor agonists and 5HT₂A receptor antagonists (5). Although these drugs are still the subject of ongoing study, it appears clear that their somewhat unique properties are mediated largely by serotonergic and adrenergic receptors. Many are capable of producing central nervous system effects, euphoria and enhanced visual, auditory, olfactory, or physical sensations similar to LSD; however, reported effects are highly dose-dependent (1,6). Overdose and death are of concern, and fatal intoxications have been associated with the use of 2C-T-7, 4-MTA, and DOB (7–12).

From 2004 through 2010, the DEA published numerous reports of drug seizures throughout the United States (13–45). Reports are not geographically

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**Table I. Chemical and Mass Spectral Data for Target Analytes**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Formula</th>
<th>Base Peak</th>
<th>Molecular Weight</th>
<th>Ions m/z* (Ion Ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-H</td>
<td>C₁₀H₁₅NO₂</td>
<td>152</td>
<td>181</td>
<td>152.1, 181.1 (19), 137.1 (53)</td>
</tr>
<tr>
<td>4-MTA</td>
<td>C₁₀H₁₅NS</td>
<td>44</td>
<td>181</td>
<td>148.0, 122.0 (31), 44.0 (497)</td>
</tr>
<tr>
<td>DOM</td>
<td>C₁₀H₁₅NO₂</td>
<td>166</td>
<td>209</td>
<td>166.1, 151.1 (35), 44.1 (158)</td>
</tr>
<tr>
<td>DOET</td>
<td>C₁₀H₁₅NO₂</td>
<td>180</td>
<td>223</td>
<td>180.1, 165.1 (32), 91.1 (11)</td>
</tr>
<tr>
<td>2C-B</td>
<td>C₁₀H₁₅BrNO₂</td>
<td>232</td>
<td>261</td>
<td>232.0, 216.9 (11), 216.9 (24)</td>
</tr>
<tr>
<td>DOB</td>
<td>C₁₀H₁₅BrNO₂</td>
<td>44</td>
<td>274</td>
<td>232.0, 216.9 (17), 77.1 (44)</td>
</tr>
<tr>
<td>2C-I</td>
<td>C₁₀H₁₅INO₂</td>
<td>278</td>
<td>307</td>
<td>278.0, 262.9 (19)</td>
</tr>
<tr>
<td>DOI</td>
<td>C₁₀H₁₅INO₂</td>
<td>44</td>
<td>321</td>
<td>278.0, 262.9 (12), 77.1 (19)</td>
</tr>
<tr>
<td>2C-T-2</td>
<td>C₁₂H₁₉NO₂S</td>
<td>212</td>
<td>241</td>
<td>212.1, 241.1 (29), 183.1 (39)</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>C₁₃H₂₁NO₂S</td>
<td>226</td>
<td>255</td>
<td>226.1, 255.1 (41), 183.0 (60)</td>
</tr>
<tr>
<td>Mescaline-d₉, IS</td>
<td>C₁₁H₈D₉NO₃</td>
<td>191</td>
<td>220</td>
<td>191.0, 220.0 (22), 173.0 (53)</td>
</tr>
</tbody>
</table>

* Quantitation ions are underlined, and ion ratios for qualifier ions are shown in parentheses.

**Table II. Scheduling Status, Street Names, and Common Dosages**

<table>
<thead>
<tr>
<th>Drug</th>
<th>CSA Schedule</th>
<th>Effective Year</th>
<th>Street Names</th>
<th>Common Dosage* (mg)</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-H</td>
<td>Not scheduled</td>
<td>N/A</td>
<td>N/A</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>4-MTA</td>
<td>Not scheduled</td>
<td>N/A</td>
<td>Flatliner, Golden Eagle</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DOM</td>
<td>I</td>
<td>1973</td>
<td>STP (Serenity, Tranquility, Peace)</td>
<td>3–10</td>
<td>14–20</td>
</tr>
<tr>
<td>DOET</td>
<td>I</td>
<td>1993</td>
<td>Hecate</td>
<td>2–6</td>
<td>14–20</td>
</tr>
<tr>
<td>2C-B</td>
<td>I</td>
<td>1995</td>
<td>2's, Bees, Bromo, Nexus, Spectrum, Toonies, Venus</td>
<td>12–24</td>
<td>4–8</td>
</tr>
<tr>
<td>DOB</td>
<td>I</td>
<td>1973</td>
<td>Bob, Dr. Bob</td>
<td>1–3</td>
<td>18–30</td>
</tr>
<tr>
<td>2C-I</td>
<td>Not scheduled</td>
<td>N/A</td>
<td>N/A</td>
<td>14–22</td>
<td>6–10</td>
</tr>
<tr>
<td>DOI</td>
<td>Not scheduled</td>
<td>N/A</td>
<td>N/A</td>
<td>1.5–3</td>
<td>16–30</td>
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<tr>
<td>2C-T-2</td>
<td>Not scheduled</td>
<td>N/A</td>
<td>N/A</td>
<td>T₂</td>
<td>6–8</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>I</td>
<td>2004</td>
<td>Beautiful, Blue Mystic, T7, Tripsastay, Tweety-Bird Mescaline</td>
<td>10–30</td>
<td>8–15</td>
</tr>
</tbody>
</table>

* Dosage and duration of action are reported from testimonial and nonscientific literature (1,3).

† May be regulated under the Federal Analogue Act (4).
### Table III. Summary of Published Procedures for Analysis

<table>
<thead>
<tr>
<th>Drugs of Interest</th>
<th>Matrix</th>
<th>Extraction</th>
<th>Internal Standard</th>
<th>Instrumentation</th>
<th>Derivatization</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-MTA</td>
<td>NB*</td>
<td>none</td>
<td>none</td>
<td>GC–MS, CE–DAD, ATR/FTIR, 1H-NMR</td>
<td>None</td>
<td>47</td>
</tr>
<tr>
<td>4-MTA</td>
<td>Blood</td>
<td>LLE</td>
<td>Fenfluramine</td>
<td>HPLC–DAD, GC–NPD</td>
<td>None</td>
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<tr>
<td></td>
<td>Urine</td>
<td></td>
<td>Diethylpropion</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4-MTA</td>
<td>Blood</td>
<td>LLE</td>
<td>Phentermine</td>
<td>LC–MS–MS</td>
<td>None</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitreous Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-MTA</td>
<td>Blood</td>
<td>LLE</td>
<td>Diphendylamine</td>
<td>GC–MS, HPLC–DAD</td>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td></td>
<td>2C-T-7</td>
<td>GC–MS</td>
<td>N-Butyric anhydride</td>
<td>49</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>Blood</td>
<td>LLE</td>
<td>None</td>
<td>GC–MS</td>
<td>None</td>
<td>10</td>
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<tr>
<td></td>
<td>Urine</td>
<td></td>
<td>TMA</td>
<td>GC–MS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C-B</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>HPLC–UV</td>
<td>None</td>
<td>50</td>
</tr>
<tr>
<td>2C-B, 2C-T-7</td>
<td>Blood</td>
<td>SPE</td>
<td>Mescaline-d9</td>
<td>GC–MS, LC–MS</td>
<td>PFPA</td>
<td>52</td>
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<tr>
<td></td>
<td>Urine</td>
<td></td>
<td>S-Fluorotryptamine</td>
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<td></td>
<td></td>
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<tr>
<td>DOB</td>
<td>Blood</td>
<td>SPE</td>
<td>Brompheniramine</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>12</td>
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<tr>
<td></td>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C-B, 2C-I, DOB, DOl, DOM</td>
<td></td>
<td>SPE</td>
<td>None</td>
<td>CE–MS</td>
<td>None</td>
<td>53</td>
</tr>
<tr>
<td>4-MTA</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>54</td>
</tr>
<tr>
<td>2C-B, 2C-I, 2C-T-2, 2C-T-7</td>
<td>Blood</td>
<td>SPE</td>
<td>AM-d5, MA-d5, MDA-d5, MDMA-d5, MDEA-d5</td>
<td>GC–MS</td>
<td>HFBA</td>
<td>5</td>
</tr>
<tr>
<td>2C-T-2, 2C-T-7</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>55, 56</td>
</tr>
<tr>
<td>DOB</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>57</td>
</tr>
<tr>
<td>2C-B, 2C-I, DOB, DOI, DOM</td>
<td></td>
<td>SPE</td>
<td>Medazepam</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>58</td>
</tr>
<tr>
<td>2C-I</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS, CE–MS</td>
<td>Acetic anhydride</td>
<td>59</td>
</tr>
<tr>
<td>2C-B, 2C-I, 2C-T-2, 2C-T-7</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>CE–NF, CE–LIF, GC–MS</td>
<td>Fluorescence derivatization</td>
<td>60</td>
</tr>
<tr>
<td>DOB</td>
<td>NB</td>
<td>None</td>
<td>None</td>
<td>CE–DAD, MS*, FTIR, GC–MS</td>
<td>None</td>
<td>61</td>
</tr>
<tr>
<td>DOI</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>62</td>
</tr>
<tr>
<td>DOM, DOET</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>CE–MS</td>
<td>None</td>
<td>63</td>
</tr>
<tr>
<td>2C-B</td>
<td>Urine</td>
<td>SPE</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>64</td>
</tr>
<tr>
<td>Tissue</td>
<td>SPE</td>
<td>MBDB</td>
<td>None</td>
<td>GC–MS</td>
<td>Acetic anhydride</td>
<td>65</td>
</tr>
<tr>
<td>2C-B, 2C-H, 2C-I, 2C-T-2, 2C-T-7, 4-MTA, DOB, DOET, DOM</td>
<td>SPE</td>
<td>AM-d5, MDMA-d5, MDEA-d5, cocaine-d5</td>
<td>LC–MS–MS</td>
<td>None</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: NB, nonbiological matrix; LLE, liquid extraction; SPE, solid-phase extraction; TMA, trimethoxyamphetamine; amphetamine-d5, AM-d5; methamphetamine-d5, MA-d5; 1,4-methylenedioxyamphetamine-d5, MDA-d5; 1,4-methylenedioxymethamphetamine-d5, MDMA-d5; 1,4-methylenedioxymethylamphetamine-d5, MDEA-d5; 2-methylamino-1-(3,4- methylenedioxyphenyl)-butane, MBDB; GC, gas chromatography; MS, mass spectrometry; CE, capillary electrophoresis; DAD, diode-array detection; ATR/FTIR, attenuated total reflection of Fourier transform infrared spectrometry; 1H-NMR, proton nuclear magnetic resonance; HPLC, high-performance liquid chromatography; NPD, nitrogen-phosphorus detection; LC, liquid chromatography; UV, ultraviolet spectrometry; NF, native fluorescence detection; LIF, light emitting diode-induced fluorescence detection; MS*, tandem mass spectrometry; PFPA, pentafluoropropionic anhydride; and HFBA, heptfluorobutyric anhydride.
isolated: they include Tennessee, Georgia, Arkansas, Kentucky, Florida, Pennsylvania, California, New Mexico, Wisconsin, Oklahoma, Oregon, Iowa, Michigan, New York, South Dakota, and Texas. Most of these designer amphetamine are recovered in powder, tablet, or blister form, although some have been encountered as liquids and capsules. LSD-like “blotters” are particularly common for 2C-I, DOB, and DOI. There are numerous reports of these psychedelic phenethylamines being sold as Ecstasy mimic tablets and “acid” blotter mimics (13–46).

Users report a variety of sought after effects including psychedelic ideation, a sense of well being, emotional awakening, profound insight, closed and open-eyed visuals, increased appreciation of music, introspection and empathogenesis. Other effects include increased blood pressure, blurred vision, dehydration, nausea, vomiting, headache, dilated pupils, muscle tension, and tachycardia.

These recreational drugs are not routinely assayed in forensic toxicology laboratories and there is limited data concerning their prevalence in toxicological casework. Existing published studies are summarized in Table III (47–67). Some describe the analysis of non-biological samples (i.e., seized drugs), others use techniques that are not in widespread use in toxicology laboratories, and most target some, but not all, of the drugs described in this study. Gas chromatography–mass spectrometry (GC–MS) is the most widely used technique for confirmatory toxicology analysis. Most of the published methods to date are limited in their ability to simultaneously identify more than a few of the psychedelic phenethylamines of interest. Ishida et al. (58) developed a method for the detection of 30 abused drugs in human urine using GC–MS, including 2C-B, 2C-I, 2C-T-2, 2C-T-7, and 4-MTA. However, there is no literature to date that describes a comprehensive screening procedure for some of the most common 2C, 2C-T, and DO series designer drugs using GC–MS.

Some published methods utilizing GC–MS derivatize these amphetamine-like drugs using acetic anhydride, n-butyric anhydride, isobutyric anhydride, heptafluorobutyric anhydride, and pentafluoropropionic anhydride (Table III). Derivatization has many advantages from the standpoint of improved detectability, volatility, specificity, and chromatographic separation. However, in this study drugs were not derivatized. Non-derivatized drugs can be advantageous if a laboratory is making an identification using a commercial or widely used mass spectral library, particularly if the laboratory conducts full scan screening by GC–MS. The purpose of this study was to establish a simple procedure for the separation and identification of the 10 target drugs in urine, using techniques and instrumentation already widely used in human performance and medical examiner’s toxicology laboratories.

### Experimental

#### Materials and methods

2C-B, 2C-H, 2C-I, 2C-T-2, 2C-T-7, (±)-4-MTA, (±)-DOB, (±)-DOET, and (±)-DOM were obtained from Lipomed (Cambridge, MA). DOI, phenethylamine, putrescine, tryptamine, and tyramine were obtained from Sigma-Aldrich (St. Louis, MO). Mescaline-<sub>d<sub>6</sub>, (±)-amphetamine, (±)-methamphetamine, (±)-methyleneedioxyamphetamine (MDA), (±)-methylene-dioxymethamphetamine (MDMA), (±)-methylene-dioxymethylamphetamine (MDEA), (±)-methylbenzodioxolylbutanamine (MBDB), (±)-epheprine, (±)-pseudoephedrine, phentermine, and (±)-phenylpropanolamine were obtained from Cerilliant (Round Rock, TX). PolyCrom Clin II (3 cc) solid-phase extraction (SPE) columns (catalog #691-0353) containing 35 mg polymeric sorbent were obtained from SPEware (Baldwin Park, CA). Deionized water was purified through a Millipore Milli Q water system (Billerica, MA). Acetic acid, hexane, ethyl acetate, methanol, methylene chloride, and isopropyl alcohol were obtained from Mallinckrodt-Baker (Hazelwood, MO). Ammonium hydroxide was obtained from Fisher Scientific (Pittsburgh, PA). Sodium phosphate monobasic monohydrate (ACS grade) and sodium phosphate dibasic heptahydrate (ACS grade) were purchased from Sigma-Aldrich (St. Louis, MO) and VWR (West Chester, PA), respectively, and used to prepare a 0.1 M phosphate buffer solution (pH 6). All inorganic reagents and solvents were ACS or HPLC grade or higher. A solution of methylene chloride and isopropanol alcohol was prepared at a ratio of 95.5 (v/v). The elution solvent was prepared with 95:5 v/v methylene chloride/isopropyl alcohol and ammonium hydroxide at a ratio of 98.2 (v/v).

Mescaline-d<sub>6</sub> internal standard solution was prepared in methanol at a concentration of 0.01 mg/mL. Working standards of 2C-B, 2C-H, 2C-I, 2C-T-2, 2C-T-7, 4-MTA, DOB, DOET, DOI, and DOM were prepared in methanol at concentrations appropriate for the fortification of calibrators and controls. An amphetamine interference solution consisted of amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine in methanol. An endogenous interference solution consisted of phenethylamine, putrescine, tryptamine, and tyramine in methanol. Pooled drug-free urine containing 1% sodium fluoride (w/v) was used to prepare all calibrators and controls.

#### Instrumentation

GC–MS analysis was performed using an Agilent HP 5975 MSD/6890 GC (Santa Clara, CA) with a DB-5MS (30 m × 0.25 mm × 0.25 μm) capillary column purchased from VWR (West Chester, PA). The injector and interface were both set at 280°C. Injections (2 μL) were made in split mode with a 5:1 split ratio. Ethyl acetate was used as the wash solvent, with a total of six pre-and post injection syringe washes between samples. The oven temperature was held at 130°C for 0.50 min, ramped to 170°C at a rate of 15°C/min with a hold time of 1 min, ramped to 180°C at a rate of 5°C/min with a hold time of 9 min, ramped to 200°C at a rate of 15°C/min and then ramped to 290°C at a rate of 30°C/min with a final hold time of 1 min. The total run time was 20.0 min. Helium was used as the carrier gas at a flow rate of 1.3 mL/min. The MS was operated in the electron impact (EI) ionization mode. The ion source and quadrupole were set at 230°C and 150°C, respectively. Data was acquired using selected ion monitoring (SIM) using quantitation and qualifier ions shown in Table I.
Extraction

A methanolic working standard was used to prepare all calibrators and controls. In the absence of deuterated analogues for each of the target drugs, a number of alternatives were evaluated. These included deuterated analogues of MDMA, MBDB, and mescaline. Mescaline-d₉ was structurally similar and yielded the most promising results during method development. After addition of internal standard (IS) solution to 2 mL urine (250 ng/mL, IS), 2 mL phosphate buffer (0.1M, pH 6.0) was added. Buffered urine samples were added to Poly-Crom Clin II columns (catalog #691-0353) and successively rinsed using 1 mL deionized water and 1 mL 1 M acetic acid. Columns were dried under full vacuum for 5 min and then rinsed with 1 mL hexane, 1 mL ethyl acetate, and 1 mL methanol. Drugs of interest were eluted using 1 mL of 2% ammonium hydroxide in 95:5 (v/v) methylene chloride/isopropyl alcohol in silanized conical borosilicate glass tubes. Extracts were evaporated to dryness under nitrogen at 50°C, reconstituted in 20 μL of ethyl acetate, and transferred to autosampler vials for analysis.

Assay performance

Although quantitative analyses are not routinely performed on urine samples, the new procedure was evaluated qualitatively and quantitatively in order to determine overall assay performance. The analytical recovery was estimated by comparison of the relative peak areas of target analytes. Urine containing internal standard was extracted with (250 ng/mL) and without target drugs. The extract containing internal standard alone was fortified with target drugs (250 ng/mL) immediately after the extraction, prior to the evaporation step. Samples were reconstituted and analyzed by GC–MS. The analytical recovery (extraction efficiency) was calculated from the relative peak area (drug/IS) of extracted and non-extracted samples.

The limit of detection (LOD) was defined as the lowest concentration of analyte that met the following criteria: signal-to-noise (S/N) ratio of at least 3:1 for the total ion chromatogram; ion ratios for both qualifiers within acceptable ranges (±20%); and a retention time within 2% of the expected value. The LOD and LOQ were assessed using urine fortified with psychedelic amphetamine working standard. For the purpose of the LOQ, the urine calibrators were prepared using independently prepared stock solutions at to give final concentrations of 2, 10, and 20 ng/mL.

Accuracy and precision were assessed by replicate analysis (n = 4) of drug-free urine fortified with target drugs at 50 and 500 ng/mL. Linear regression analysis was used to determine the limit of linearity of the assay, and carryover was evaluated using drug-free matrix injected immediately after extracts containing high concentrations of target drugs.

Interferences were evaluated using a number of structurally related substances, endogenous bases, and common drugs. From a quantitative standpoint, an interference was defined as a substance that caused the calculated concentrations of a target drug to deviate from the expected value by more than ±20%. The potential interference of other abused amphetamine-like drugs was investigated. Negative and positive (250 ng/mL) controls were assayed in the presence of 1 mg/L of amphetamine-like drugs (amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, ephedrine, pseudoephedrine, phentermine, phenylpropanolamine); endogenous bases (phenethylamine, putrescine, tryptamine, tyramine); and common basic drugs, including dextromethorphan, zolpidem, ketamine, diphenhydramine, cocaine, amitriptyline, diazepam, nordiazepam, oxycodone, hydrocodone, alprazolam, phencyclidine (PCP), methadone, tramadol, and codeine.

Results and Discussion

Analytical recovery, LOD, and LOQ

Analytical recoveries for each of the target drugs were 63–94% (Table IV). The lowest recoveries were generally observed

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recovery (%)</th>
<th>LOD (ng/mL)</th>
<th>LOQ (ng/mL)</th>
<th>Calculated Concentration at the LOQ (ng/mL)</th>
<th>Linear Range (ng/mL)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-H</td>
<td>70</td>
<td>10</td>
<td>10</td>
<td>11.1</td>
<td>0–1500</td>
<td>0.997</td>
</tr>
<tr>
<td>4-MTA</td>
<td>71</td>
<td>2</td>
<td>10</td>
<td>9.9</td>
<td>0–1500</td>
<td>0.994</td>
</tr>
<tr>
<td>DOM</td>
<td>63</td>
<td>2</td>
<td>5</td>
<td>5.8</td>
<td>0–1500</td>
<td>0.995</td>
</tr>
<tr>
<td>DOET</td>
<td>64</td>
<td>2</td>
<td>2</td>
<td>2.1</td>
<td>0–1500</td>
<td>0.993</td>
</tr>
<tr>
<td>2C-B</td>
<td>94</td>
<td>2</td>
<td>5</td>
<td>4.8</td>
<td>0–1500</td>
<td>0.997</td>
</tr>
<tr>
<td>DOB</td>
<td>74</td>
<td>2</td>
<td>2</td>
<td>1.9</td>
<td>0–1500</td>
<td>0.992</td>
</tr>
<tr>
<td>2C-I</td>
<td>92</td>
<td>2</td>
<td>5</td>
<td>5.1</td>
<td>0–1500</td>
<td>0.990</td>
</tr>
<tr>
<td>DOI</td>
<td>72</td>
<td>2</td>
<td>10</td>
<td>10.8</td>
<td>0–1500</td>
<td>0.988</td>
</tr>
<tr>
<td>2C-T-2</td>
<td>86</td>
<td>5</td>
<td>10</td>
<td>8.6</td>
<td>0–1500</td>
<td>0.990</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>78</td>
<td>5</td>
<td>10</td>
<td>8.1</td>
<td>0–1500</td>
<td>0.993</td>
</tr>
</tbody>
</table>
for the DO series of drugs. During the method development stage it was necessary to increase the polarity of the elution solvent (using isopropanol) in order to optimize recovery. An elution solvent consisting of 2% ammonium hydroxide in 95:5 v/v methylene chloride/isopropyl alcohol was optimal for methoxylated drugs. LODs ranged from 2 to 10 ng/mL, and LOQs were 10 ng/mL or less for all analytes (Table IV). Calculated concentrations for controls run at the quantitation limits are also shown, and Table V shows the corresponding signal-to-noise ratios for the total ion chromatogram (TIC) and acquired ions. Low LODs are preferable for this class of drug because of the limited pharmacological data in humans and the absence of metabolites from commercial sources.

The most challenging drugs to separate and identify were the following pairs of structurally related compounds: 2C-B and DOB; 2C-I and DOI. Despite the structural similarity, chromatographic and spectroscopic resolution was achieved for all 10 target drugs. Representative urine extracts containing 10 and 100 ng/mL of each drug are depicted in Figure 2 and extracted ion chromatograms are shown in Figure 3.

### Table V. Signal-to-Noise (S/N) Ratios and Calculated Concentrations at the LOQ

<table>
<thead>
<tr>
<th>Drug</th>
<th>m/z</th>
<th>S/N Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C-H</td>
<td>152</td>
<td>79:1</td>
</tr>
<tr>
<td></td>
<td>181</td>
<td>110:1</td>
</tr>
<tr>
<td></td>
<td>137</td>
<td>35:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>10:1</td>
</tr>
<tr>
<td>4-MTA</td>
<td>138</td>
<td>103:1</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>13:1</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>20:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>31:1</td>
</tr>
<tr>
<td>DOM</td>
<td>166</td>
<td>570:1</td>
</tr>
<tr>
<td></td>
<td>151</td>
<td>151:1</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>20:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>34:1</td>
</tr>
<tr>
<td>DOET</td>
<td>180</td>
<td>430:1</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>103:1</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>18:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>119:1</td>
</tr>
<tr>
<td>2C-B</td>
<td>232</td>
<td>212:1</td>
</tr>
<tr>
<td></td>
<td>261</td>
<td>122:1</td>
</tr>
<tr>
<td></td>
<td>217</td>
<td>24:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>32:1</td>
</tr>
<tr>
<td>DOB</td>
<td>232</td>
<td>398:1</td>
</tr>
<tr>
<td></td>
<td>217</td>
<td>19:1</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>18:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>63:1</td>
</tr>
<tr>
<td>2C-I</td>
<td>278</td>
<td>100:1</td>
</tr>
<tr>
<td></td>
<td>307</td>
<td>84:1</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>16:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>32:1</td>
</tr>
<tr>
<td>DOI</td>
<td>278</td>
<td>184:1</td>
</tr>
<tr>
<td></td>
<td>263</td>
<td>21:1</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>15:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>39:1</td>
</tr>
<tr>
<td>2C-T-2</td>
<td>212</td>
<td>67:1</td>
</tr>
<tr>
<td></td>
<td>241</td>
<td>76:1</td>
</tr>
<tr>
<td></td>
<td>183</td>
<td>10:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>10:1</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>226</td>
<td>49:1</td>
</tr>
<tr>
<td></td>
<td>255</td>
<td>46:1</td>
</tr>
<tr>
<td></td>
<td>183</td>
<td>10:1</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>17:1</td>
</tr>
</tbody>
</table>

* S/N ratios were evaluated for the total ion chromatogram (TIC) and for each acquired ion.

### Precision, accuracy, and linearity

Precision and accuracy data are summarized in Table VI. Accuracy was 91–116% and 98–109% at 50 and 500 ng/mL, respectively. Corresponding CVs were 0.9–6.5% and 0.4–5.6%, respectively. Calibrations were linear from 0 to 1500 ng/mL for all drugs, and correlation coefficients are given in Table IV. No carryover was evident following injection of an extract containing 5000 ng/mL of target drugs. During method development, a comparison of silanized and non-silanized glassware indicated the former to be preferable. This suggests that some of the methoxylated species may have a tendency to adsorb to the surface of glass.

### Interferences

Interferences were evaluated qualitatively and quantitatively using negative and positive controls fortified with potential interferants. None of the amphetamine-like drugs (am-
amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine) or endogenous bases (phenethylamine, putrescine, tryptamine, and tyramine) interfered with the assay. With the exception of MDMA, all amphetamine-like and endogenous bases eluted prior to data acquisition (solvent delay 5 min). Negative controls remained blank and quantitative controls containing target drugs at 250 ng/mL produced calculated concentrations within 82–104% of expected values for stimulants, and 81–116% for endogenous amines. In the presence of common alkaline drugs, both 2C-I and 2C-T-7 quantitated outside of the acceptable ±20% range (79% for both). Although this appeared marginal, negative controls were always drug-free, indicating the absence of interfering ions from these species. Although the quantitative discrepancy was very small, it was reproducible. There was no obvious source of the possible interference because none of the common drugs coeluted with the target analytes with the exception of 2C-I and phencyclidine at relative retention times of 1.80 and 1.79, respectively (Table VII).

Limitations

Pharmacological and toxicological data for many of these drugs are still somewhat limited. However, animal and, to a lesser extent human, studies for select drugs within the class suggest a number of common metabolic pathways. The DO series of drugs may undergo hydroxylation of the 4 methyl, followed by conjugation or oxidation to the corresponding acid, deamination (to a ketone), reduction to an alcohol, O-demethylation, or combinations of these pathways (57,62,65). In a somewhat similar fashion, proposed pathways for the 2C series include O-demethylation, deamination, alcohol formation, acid formation, reduction, and acetylation (48,49,59,64,66). Sulphur-containing drugs in the 2C-T series likely undergo similar transformations, in addition to S-depropylation followed by methylation of the resulting thiol (50,55,56). Conjugation (glucuronidation and sulfation) takes place and several metabolic studies employ a deconjugation step prior to the identification of proposed metabolites.

Figure 3. Extracted ion chromatograms for target analytes and internal standard in urine at 10 ng/mL (A) and 100 ng/mL (B). Internal standard (mescaline-d9) was present at 250 ng/mL.
A significant limitation, however, is the absence of commercial standards for these metabolites. From a practical standpoint, this limits most laboratories to the identification of the parent drug alone. Although concentrations of 2C-T-7 in heart blood and urine were 57 ng/mL and 1120 ng/mL following a fatality (10), concentrations in recreational drug users are not well established. DOB concentrations in serum following a fatal overdose were particularly low (19 ng/mL) (12), but this is perhaps not surprising considering the very low dose (1–3 mg) of this drug (Table II). Authors of this study tentatively identified urinary metabolites in addition to DOB, but were unable to identify them because of the absence of a commercial standard.

Conclusions

The 2C, 2C-T, and DO series of designer drugs pose a number of challenges to forensic toxicology laboratories. Although these drugs are seized by law enforcement agencies throughout the United States, they are not readily detected in forensic toxicology laboratories. It is not clear whether these drugs are rarely encountered due to overall low prevalence or limitations with respect to detectability. Commercial immunoassays have limited cross-reactivity towards these amphetamine-like drugs. As a consequence, laboratories that rely upon immunoassay rather than more broad spectrum chromatographic screening techniques may fail to detect these and other similar substances. In this study, we report a simple alkaline SPE to isolate drugs of interest, followed by GC–MS analysis of derivatized extracts. Although the metabolic transformation of these drugs has been preliminarily investigated and likely involves a number of common pathways, commercial standards are not readily available. Toxicology laboratories performing routine human performance or postmortem investigations must therefore rely upon detection of the parent drug. Using the approach described here, a total of 10 designer drugs were

![Figure 3 (continued). Extracted ion chromatograms for target analytes and internal standard in urine at 10 ng/mL (A) and 100 ng/mL (B). Internal standard (mescaline-d9) was present at 250 ng/mL.](https://academic.oup.com/jat/article-abstract/35/7/459/880209)

### Table VI. Precision and Accuracy at 50 and 500 ng/mL

<table>
<thead>
<tr>
<th>Drug</th>
<th>50 ng/mL</th>
<th>500 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated Concentration (ng/mL)</td>
<td>Calculated Concentration (ng/mL)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD (n = 4)</td>
<td>Mean ± 95% CI (n = 4)</td>
</tr>
<tr>
<td>2C-H</td>
<td>46.2 ± 0.5</td>
<td>46.2 ± 0.9</td>
</tr>
<tr>
<td>4-MTA</td>
<td>50.8 ± 4.0</td>
<td>50.8 ± 6.4</td>
</tr>
<tr>
<td>DOM</td>
<td>50.4 ± 1.3</td>
<td>50.4 ± 2.1</td>
</tr>
<tr>
<td>DOET</td>
<td>49.2 ± 1.3</td>
<td>49.2 ± 2.0</td>
</tr>
<tr>
<td>2C-B</td>
<td>47.4 ± 0.4</td>
<td>47.4 ± 0.7</td>
</tr>
<tr>
<td>DOB</td>
<td>46.3 ± 1.5</td>
<td>46.3 ± 2.3</td>
</tr>
<tr>
<td>2C-I</td>
<td>47.7 ± 0.5</td>
<td>47.7 ± 0.8</td>
</tr>
<tr>
<td>DOI</td>
<td>45.4 ± 1.3</td>
<td>45.4 ± 2.1</td>
</tr>
<tr>
<td>2C-T-2</td>
<td>48.0 ± 3.1</td>
<td>48.0 ± 4.9</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>57.8 ± 0.9</td>
<td>57.8 ± 1.5</td>
</tr>
</tbody>
</table>
targeted in one assay with very low detection limits, sufficient
to identify parent drug in urine samples. Using this approach,
2C, 2C-T, and DO series drugs could be incorporated somewhat
readily into a laboratory’s existing analytical procedures where
necessary.

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cation are those of the author(s) and do not necessarily reflect
those of the Department of Justice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Relative Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>0.71</td>
</tr>
<tr>
<td>2C-H</td>
<td>0.72</td>
</tr>
<tr>
<td>4-MTA</td>
<td>0.76</td>
</tr>
<tr>
<td>MDEA</td>
<td>0.78</td>
</tr>
<tr>
<td>DOM</td>
<td>0.83</td>
</tr>
<tr>
<td>MBDB</td>
<td>0.86</td>
</tr>
<tr>
<td>DOET</td>
<td>0.94</td>
</tr>
<tr>
<td>Mescaline-d9</td>
<td>1.00</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>1.13</td>
</tr>
<tr>
<td>2C-B</td>
<td>1.35</td>
</tr>
<tr>
<td>DOB</td>
<td>1.38</td>
</tr>
<tr>
<td>Ketamine</td>
<td>1.60</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>1.66</td>
</tr>
<tr>
<td>PCP</td>
<td>1.79</td>
</tr>
<tr>
<td>2C-I</td>
<td>1.80</td>
</tr>
<tr>
<td>DOI</td>
<td>1.84</td>
</tr>
<tr>
<td>2C-T-2</td>
<td>1.95</td>
</tr>
<tr>
<td>Tramadol</td>
<td>2.07</td>
</tr>
<tr>
<td>2C-T-7</td>
<td>2.23</td>
</tr>
<tr>
<td>Methadone</td>
<td>2.41</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>2.42</td>
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<tr>
<td>Amitriptyline</td>
<td>2.47</td>
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<td>Cocaine</td>
<td>2.47</td>
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<td>Codeine</td>
<td>2.62</td>
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<tr>
<td>Diazepam</td>
<td>2.66</td>
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<tr>
<td>Hydrocodone</td>
<td>2.67</td>
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<tr>
<td>NORDiazepam</td>
<td>2.72</td>
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<td>Oxycodeine</td>
<td>2.73</td>
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<tr>
<td>Zolpidem</td>
<td>2.98</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>2.82</td>
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</tbody>
</table>

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dimethoxyphenethylamine (2C-B, ”Nexus”) tablets seized in Ten-
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ually containing 4-bromo-2,5-dimethoxy-ethylamphetamine (DOB)
and 4-chloro-2,5-dimethoxy-ethylamphetamine (DOC)) in Pratt County,
15. U.S. Drug Enforcement Administration. Blotter acid mimic (act-
ually containing a mixture of 4-chloro-2,5-dimethoxyethylamphetamine
and 4-bromo-2,5-dimethoxyethylamphetamine in Warner Robins,
16. U.S. Drug Enforcement Administration. Ecstasy mimic tablets
(actually containing 4-iodo-2,5-dimethoxyphenethylamine hy-
drochloride (2C-I) in Sherwood, Arkansas. Microgram Bull. 41(8):
72 (2008).
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dimethoxyphenethylamine (2C-E) in dropper bottles in Kentucky.
18. U.S. Drug Enforcement Administration. LSD blotter acid mimics
(actually containing 4-iodo-2,5-dimethoxyethylamphetamine (DOI)
and 4-chloro-2,5-dimethoxyethylamphetamine (DOC) in Lantana,
19. U.S. Drug Enforcement Administration. Poly-drug seizure of hal-
ucinogens in Upper Darby Township, Pennsylvania. Microgram
20. U.S. Drug Enforcement Administration. LSD blotter acid mimic
(actually containing a mixture of 4-chloro-2,5-dimethoxy-
ethylamphetamine (DOC) and 4-iodo-2,5-dimethoxyethylamphetamine
DOI).
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