Case Report

Multiple Drug-Toxicity Involving Novel Psychoactive Substances, 3-Fluorophenmetrazine and U-47700

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Abstract

3-Fluorophenmetrazine (3-FPM) is a stimulant-like novel psychoactive substance (NPS) and fluorinated analog of phenmetrazine that has recently appeared on the recreational drug market, with limited published information. Likewise, the synthetic opioid U-47700 has gained popularity among recreational drug users and is frequently detected in postmortem casework. We present the case history, autopsy and toxicological findings of a fatality involving the designer drugs 3-FPM and U-47700 for the first time in the literature. A sensitive and specific liquid chromatography-tandem mass spectrometry method was developed and validated for the quantification of 3-FPM in whole blood, with a 0.001–0.100 mg/L analytical range. The method met the requirements for acceptable linearity, bias and precision. 3-FPM was detected along with U-47700 and other drugs including amitriptyline, nortriptyline, methamphetamine, amphetamine, diazepam, nordiazepam, temazepam, and the designer benzodiazepines flubromazolam and delorazepam. 3-FPM was quantified in the decedent’s peripheral (femoral) and central (aortic) blood at 2.4 and 2.6 mg/L, respectively. These concentrations are similar to reported concentrations in non-fatal intoxications. U-47700 was present in peripheral blood at a semi-quantitative concentration of 0.36 mg/L, consistent with reported U-47700 postmortem concentrations. The cause of death was considered multiple drug-toxicity (3-FPM, U-47700, amitriptyline, methamphetamine, diazepam, temazepam, flubromazolam and delorazepam) and the manner of death ruled an accident. This case illustrates the dangers of polysubstance use and discusses the potential overlap between recreational and fatal concentrations for some NPS.

Introduction

Despite active government regulation of novel psychoactive substances (NPS), new compounds with unknown toxicities are continually emerging, resulting in adverse events. These purported “legal highs” pose problems for law enforcement, military, and public health and safety officials, along with toxicologists who must identify an unending variety of new drugs of abuse. Since 2010, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has identified over 450 NPS in Europe (1). By the end of December 2014, the United Nations Office on Drug Crime (UNODC) Early Warning Advisory reported a total of 541 NPS (2).

In the United States, NPS, fentanyl and fentanyl-related compounds accounted for 8% of exhibits seized by the US Drug Enforcement Administration (DEA) in 2016, with 21 new compounds identified for the first time (3).

3-Fluorophenmetrazine (3-FPM) is a stimulant-like NPS that first emerged on the European drug market in 2014 (4). In the US, only three instances of 3-FPM seizures were reported by the DEA in 2016 (3). It is a fluorinated analog of phenmetrazine (Figure 1), a stimulant initially used as an appetite suppressant, now withdrawn from the market due to abuse liability (5). Limited information is available on the pharmacology of 3-FPM; however, based on its
structural similarity to phenmetrazine, one can hypothesize that 3-FPM would also increase the release of dopamine and norepinephrine (6). The duration of 3-FPM effects is short-lived, with peak effects observed up to 2h, thus increasing the likelihood of repeated dosing (7, 8). Reported physiological effects and clinical symptoms following 3-FPM intake include tachycardia, depressed consciousness, agitation/anxiety, delirium, mydriasis and in some instances seizures (8). However, 3-FPM clinical reports involve co-administration with other psychoactive substances; thus it is difficult to establish the unique clinical manifestations of 3-FPM. When compared to traditional stimulants like cocaine, amphetamine and 3,4-methylenedioxymethamphetamine (MDMA), 3-FPM users report effects that are weaker and less pronounced (9). To our knowledge, only two studies were published reporting 3-FPM blood concentrations from non-fatal intoxications (7, 8), none with postmortem concentrations.

U-47700 is a fentanyl-related synthetic opioid analgesic initially developed by the Upjohn pharmaceutical company in the 1970s (10), and is temporarily classified as a Schedule I controlled substance (11). It is a selective μ-opioid receptor agonist that exhibited 7.5 times higher potency than morphine in animal models (12, 13). U-47700 was the third most identified synthetic opioid by the US DEA in 2016, following only furanyl fentanyl and acetyl fentanyl, with 50 seizures reported (3). Recently, Mohr et al. described a series of postmortem cases containing U-47700 and/or other synthetic opioids, with U-47700 concentrations typically 10-fold higher than illicit fentanyl deaths (14, 15).

With this ever-changing landscape of designer drugs, few data are available on the pharmacology of these substances, and modifications to existing analytical methods are needed to detect emerging NPS and establish typical concentrations in human performance and postmortem cases. We describe the development and validation of an analytical method for the identification and quantitation of 3-FPM in whole blood. This method was subsequently applied to test blood collected postmortem from an individual suspected of using 3-FPM. For the first time, a death associated with the designer drugs 3-FPM and U-47700, and other drugs, is reported with postmortem blood concentrations.

**Methods**

**Specimen collection**

All specimens analyzed were collected at autopsy at the Travis County Medical Examiner’s Office. Peripheral blood (~30 mL) was drawn from the femoral/iliac veins and stored in gray-top tubes containing potassium oxalate and sodium fluoride. Central blood was collected from the aorta and placed in red-top tubes with no additives and a single gray-top tube. Vitreous humor samples were withdrawn from the eyes with a syringe and also stored in a red-top tube. Urine was collected and stored in 50 mL plastic conical centrifuge tubes. All specimens were stored at 4°C before analysis.

**Chemicals and reagents**

3-FPM hydrochloride (1000 mg/L), amphetamine-D5 (100 mg/L) and methadone-D9 (100 mg/L) were purchased from Cerilliant (Round Rock, TX, USA). U-47700 was purchased as a 1 mg powder standard from Cayman Chemical (Ann Arbor, Michigan, USA) and dissolved in 1 mL of acetonitrile just prior to use. All solvents employed in the extraction were of the high-performance liquid chromatography (HPLC) grade and LC–MS grade in the chromatographic system. Methanol, acetonitrile, N-butyl chloride, formic acid and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Chloroform was purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Initial toxicological analysis**

Postmortem femoral blood, vitreous and/or urine specimens were screened for volatiles utilizing headspace dual column gas chromatography (GC) with flame ionization detection (FID) for analysis of ethanol, acetone, isopropanol and methanol; and drugs of abuse by immunoassay (ELISA) for amphetamines, barbiturates, benzodiazepines, cocaine metabolite, fentanyl, opiates, oxycodeone/oxymorphine and THC in femoral blood specimens (Neogen, Lexington, KY). Acid-neutral and alkaline qualitative drug screens were also performed on femoral blood and/or urine specimens following liquid–liquid extraction procedures. All extracts were analyzed utilizing GC full-scan mass spectrometry (MS). Blood specimens suspected to contain NPS were initially screened with a targeted scheduled multiple reaction monitoring (sMRM) liquid chromatography-tandem mass spectrometry.
(LC–MS–MS) acquisition method for 35 NPS, including 3-FPM. Positive results were confirmed and/or quantified by targeted GC–MS or LC–MS–MS methods. For U-47700 quantification, a five-point GC–MS calibration (0.05–1.0 mg/L) was prepared in blank blood with methadone-D9 as the internal standard. Samples were extracted with N-butyl chloride at alkaline pH and back extracted into 1 N HCl. After alkalization of the HCl, chloroform was added to extract the compounds of interest. Data were collected in full-scan mode with ions 125 (U-47700) and 78 (methadone-D9) used for calibration.

The method is considered semi-quantitative due to lack of full method validation and unavailability of a quality control material at the time of analysis.

3-Fluorophenmetrazine confirmation and quantification

Sample preparation
A liquid–liquid extraction at alkaline pH with subsequent back-extraction into formic acid was utilized to isolate 3-FPM from whole blood and urine. Briefly, 10 μL of internal standard (1 mg/L amphetamine-D5) was added to 1 mL of specimen followed by the addition of 0.1 M sodium hydroxide (200 μL), while vortexing, and N-butyl chloride (5 mL). Samples were capped, rotated for 3 min, and then centrifuged at 2500 rpm for 2 min. The organic layer was transferred to conical glass centrifuge tubes and 100 μL of 0.1 M formic acid was added before samples were rotated (3 min) and centrifuged (2 min at 2500 rpm). The N-butyl chloride layer (top) was then removed and 65 μL of the formic acid layer was transferred to autosampler vials prior to injection (5 μL) on the LC–MS–MS for analysis.

Instrumental analysis
LC–MS–MS was performed on a Shimadzu LC-20AD XR LC system coupled with a Sciex 4500 QTRap® mass spectrometer with a TurboIonSpray source (Foster City, CA, USA). The instrument was operated in positive electrospray ionization, multiple reaction monitoring (MRM) mode (sMRM was utilized for initial screening of NPS). Chromatographic separation was achieved at 40°C with a Restek Allure PFP Propyl column (50 × 2.1 mm, particle size 5 μm) and identically packed guard cartridges (10 × 2.1 mm, particle size 5 μm). Gradient elution was performed with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) at 0.5 mL/min flow rate. Initial composition (10% B) was increased to 45% over 7.5 min, from 45% to 80% over 0.1 min, held at 80% for 0.9 min, and returned to initial conditions over 0.1 min and held for 1.4 min, with a total runtime of 10 min. For the NPS LC–MS–MS screening method, the gradient was extended for a total runtime of 17 min.

MS source parameters included IonSpray voltage 5 kV, capillary temperature 500°C, curtain gas 40, ion source gas 1 and 2 40 and 50, respectively, nitrogen collision gas medium for all experiments, and collision cell exit potential (CXP) 3. MRM transitions and MS compound parameters for 3-FPM and amphetamine-D5 are outlined in Table I.

| Table I. Liquid chromatography-tandem mass spectrometry parameters and retention times for 3-fluorophenmetrazine (3-FPM) and internal standard in blood |
|----------------|----------------|----------------|----------------|-----------------|-----------------|----------------|
| Analytes       | Precursor ion (m/z) | Product ions (m/z) | Declustering potential (V) | Entrance potential (V) | Collision energy (V) | Retention time (min) |
| 3-FPM          | 196.1            | 115.1, 135.1     | 41               | 8/10            | 39/29           | 3.31            |
| Amphetamine-D5 | 141.1            | 124.1            | 30               | 10              | 20              | 2.21            |

Quantitation ions are in bold.

Table II. Method validation summary for quantitation of 3-fluorophenmetrazine (3-FPM) in blood by liquid chromatography-tandem mass spectrometry at the limit of quantitation (LOQ, 1 μg/L), low quality control (LQC, 3 μg/L) and high quality control (HQC, 80 μg/L)

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>3-FPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Within-run imprecision (%CV)</td>
<td>6.3</td>
</tr>
<tr>
<td>Between-run imprecision (%CV)</td>
<td>7.3</td>
</tr>
<tr>
<td>% Bias</td>
<td></td>
</tr>
<tr>
<td>Ion suppression/enhancement % (%RSD) n = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (n = 14)</td>
<td></td>
</tr>
<tr>
<td>Within-run imprecision (%CV)</td>
<td>12.6</td>
</tr>
<tr>
<td>Between-run imprecision (%CV)</td>
<td>12.6</td>
</tr>
<tr>
<td>% Bias</td>
<td>-14.1</td>
</tr>
<tr>
<td>Ion suppression/enhancement % (%RSD) n = 3</td>
<td>16.2 (17.1)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>HQC (n = 10)</td>
<td></td>
</tr>
<tr>
<td>Within-run imprecision (%CV)</td>
<td>10.5</td>
</tr>
<tr>
<td>Between-run imprecision (%CV)</td>
<td>9.0</td>
</tr>
<tr>
<td>% Bias</td>
<td>-6.1</td>
</tr>
<tr>
<td>Ion suppression/enhancement % (%RSD) n = 3</td>
<td>39.4 (3.3)</td>
</tr>
</tbody>
</table>

Method validation
A limited fit-for-purpose validation was performed for 3-FPM quantification in accordance with laboratory standard operating procedures, as this report only involved one case where drug material was found at the death scene. Validation parameters included linearity, bias and precision (within- and between-run), specificity, selectivity, ion suppression/enhancement, carryover and autosampler stability. A summary of validation results is outlined in Table II. Linearity was assessed from matrix-matched five-point calibration curves on three separate days at the following concentrations: 0.001, 0.005, 0.020, 0.050 and 0.100 mg/L. Calibration curves yielded correlation coefficients >0.9985 and residuals <7.5%, with 1/x weighted linear regression. Bias and precision assessed at the limit of quantitation (LOQ), and low (0.003 mg/L) and high (0.080 mg/L) quality controls (QC) were within ±15% (Table II). No interferences from endogenous matrix components (n = 10) or exogenous interferences, including common acid/neutral and alkaline drugs, benzodiazepines, opiates and amphetamines, were observed. Ion enhancement was observed at the high QC (39.4%), however %CV was <15%. 3-FPM was stable in the autosampler (20°C) for 24 and 48 h and did not exhibit carryover at the highest calibrator.

Results
Toxicological results
Testing of the vitreous fluid demonstrated no abnormalities of glucose or electrolytes. Femoral blood screened positive by immunoassay for amphetamines and benzodiazepines at cutoffs of 0.050 mg/L (based upon d-amphetamine and oxazepam, respectively). Ethanol was not detected in the femoral blood by headspace GC–FID, and no drugs were detected by the acid/neutral GC–MS screen. Alkaline GC–MS results detected amitriptyline, nortriptyline, diazepam,
nordiazepam, temazepam, flubromazolam, delorazepam, methamphetamine, amphetamine, U-47700 and 3-FPM in the femoral blood. 3-FPM was also detected by our NPS screening method in both femoral blood and urine (Figure 2). Additionally, delorazepam and flubromazolam were further confirmed by our LC–MS-MS benzodiazepine screen in the femoral blood.

Amitriptyline and nortriptyline quantified at concentrations of 0.44 and 0.29 mg/L, respectively, in the peripheral blood; diazepam, nordiazepam and temazepam were quantified in the femoral blood at concentrations of 0.20, 0.18 and 0.011 mg/L, respectively; methamphetamine was <LOQ (0.040 mg/L), with amphetamine quantified at 0.070 mg/L; a semi-quantitative U-47700 concentration (0.36 mg/L) was obtained from the femoral blood; and 3-FPM concentrations of 2.4 and 2.6 mg/L were obtained from the peripheral and central blood, respectively, yielding a central to peripheral blood (C/P) ratio of 1.08. Results are summarized in Table III.

**Cause and manner of death**

Based on case history, autopsy and toxicology findings, the medical examiner determined the cause of death was a result of multiple drug-toxicity (diazepam, U-47700, temazepam, flubromazolam, delorazepam, methamphetamine, 3-FPM and amitriptyline) and the manner of death ruled accident. The decedent also exhibited an enlarged heart, which is more susceptible to cardiac arrhythmias especially under the influence of stimulant-type drugs listed above, and may be indicative of hypertensive heart disease.

**Discussion**

NPS identification in biological matrices is essential to aid in interpretation of results for both human performance and postmortem cases. As such, laboratories must be capable of responding quickly, adapting to changing NPS trends, and improving analytical methodologies for the detection of these highly abused compounds. Here we describe a sensitive and specific LC–MS-MS method for the detection of 3-FPM in blood and its application to a postmortem case suspected of 3-FPM use.

3-FPM was quantified in blood with an exceptional limit of quantitation (LOQ) of 0.001 mg/L and acceptable bias and precision. The linear range was established up to 0.100 mg/L based on typical concentrations observed for other stimulant-like NPS; however, upon analysis of the decedent’s blood and urine samples 50 and 200-fold dilutions were required. At the time of analysis there were no previously published methods for 3-FPM quantitation. Recently, Adamowicz et al. published an LC–MS-MS method for the quantitation of 3-FPM (7), however, the LOQ was higher (0.005 mg/L) and greater ion enhancement (51.6%) was observed compared to the method described in this current study (39.4%). Despite the observed ion enhancement in our method, %CV was <3.5%, and the LOQ and QCs were reproducible. 3-FPM matrix effects may decrease with a matched deuterated internal standard; however, this was not commercially available at the time of investigation.

Limited information is available on concentrations typically observed following 3-FPM use; however reported therapeutic, acute intoxication and fatal phenmetrazine concentrations range from 0.02–0.238 mg/L in plasma (16, 17), 0.5–4.0 mg/L in blood (18) and 0.1–4.9 mg/L in blood (19–21), respectively, demonstrating a propensity for acute and fatal concentrations to overlap one another. Following a fatal multiple drug intoxication, we detected 3-FPM in the decedent at 2.4 and 2.6 mg/L in the peripheral and central blood, respectively, with a C/P ratio of 1.08, suggesting minimal potential for 3-FPM postmortem redistribution (22). 3-FPM also was previously reported in cases of non-fatal intoxications (7, 8), and was detected in wastewater, mainly as unchanged parent drug

![Figure 2](image.png)

**Figure 2.** Multiple reaction monitoring chromatograms of 3-fluorophenmetrazine in the analyzed (a) urine and (b) peripheral blood specimens diluted 200 and 50-fold, respectively. The figure shows the two transitions monitored (m/z 196.1 > 115.1 and 196.1 > 135.1).
in addition to its N-oxide metabolite (23). In Sweden, analysis of STRIDA project samples from patients with suspected acute NPS exposure confirmed 3-FPM in 19 patients (8). Concentrations ranged from 0.003–1.4 mg/L (median 0.077 mg/L) in serum (n = 15) and 0.008–30 mg/L (median 1.8 mg/L) in urine (n = 14), respectively, much lower than the current study. Additionally, all samples confirming positive for 3-FPM also tested positive for one or more psychoactive substances (8). Diazepam and/or designer benzodiazepines, such as flubromazolam or clonazolam, were the most frequently encountered substances in combination with 3-FPM (n = 11). This polysubstance use was similar to what was observed in our current study, as 3-FPM was also found in combination with ten other substances including the designer drugs U-47700, flubromazolam and delorazepam (a potential metabolite of the designer benzodiazepine dclazepam). Additionally, Adamowicz et al. quantitated 3-FPM in the blood of a driver involved in a single vehicle crash at 2.77 mg/L, with no other psychoactive substance reported in the driver (7). This acute 3-FPM intoxication exhibited a concentration similar to our postmortem concentrations detected in the decedent, suggesting an overlap in recreational and fatal concentrations similar to what was previously observed for phenmetrazine. However, the use of additional psychoactive substances confounds the interpretation of these results, as lower 3-FPM concentrations were observed in non-fatal intoxications involving polysubstance use (8). Additional case studies are needed to establish typical 3-FPM concentration ranges. Moreover, the interpretation of these NPS concentrations in biological matrices is further hindered by lack of controlled administration studies as dose, time of ingestion, administration route and potency are typically unknown.

Recently, there has been an increase in designer opioid detection in both acute intoxications (24) and postmortem cases (14, 25–27). These compounds are also typically abused in combination with other psychoactive substances and/or NPS and may cause serious adverse events, such as respiratory depression and death. Along with 3-FPM, we also detected the designer opioid U-47700 in the decedent’s femoral blood with a semi-quantitative concentration of 0.36 mg/L. This is similar to other fatalities involving U-47700 (14, 26); however, this is the first reported instance where these two NPS were detected in combination. Mohr et al. examined U-47700 concentrations from deaths suspected of involving designer opioids and reported median U-47700 blood concentrations of 0.253 mg/L (0.017–0.490 mg/L, n = 16) (14). In five of these cases, the designer opioid furanyl fentanyl also was detected. U-47700 peripheral and central blood concentrations of 0.190 and 0.340 mg/L, respectively, were also recently observed in an accidental death involving multiple drugs (26).

This case report described a fatal accidental multiple drug-toxicity involving the designer drugs 3-FPM and U-47700, among other psychoactive substances, for the first time. This sudden death was attributed to the cardiac arrhythmias likely associated with 3-FPM use (8), as well as methamphetamine/amphetamine and amitriptyline use, in combination with the respiratory depression and sedation effects caused from U-47700, flubromazolam, delorazepam, temazepam, diazepam and amitriptyline. The results of our toxicological findings illustrate the dangers of polysubstance use and the potential overlap between recreational and fatal concentrations for some NPS, like 3-FPM. Furthermore, the continued emergence of new NPS warrants the need for laboratories to adapt and improve their analytical methods to detect these compounds and keep pace with the changing landscape to improve toxicological interpretation of these emerging compounds.

### Acknowledgments

The authors would like to thank the staff of the Travis County Medical Examiner’s Office for their technical assistance.

### References


<p>| Table III. Demographic information, case history, analytical findings, and cause and manner of death involving 3-fluorophenmetrazine (3-FPM) |</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Case history</th>
<th>Additional toxicology findings (mg/L)</th>
<th>3-FPM concentration (mg/L)</th>
<th>U-47700 concentration (mg/L)*</th>
<th>Cause; manner of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>M</td>
<td>The decedent, who had a history of depression, bipolar disorder and suicidal ideations, was found unresponsive at home. Emergency personnel were called to the scene and he was pronounced dead. Drug paraphernalia and an empty bag labeled “5582 mg 3-FPM” were found on scene</td>
<td>Femoral blood: Amitriptyline 0.44, nortriptyline 0.29, methamphetamine &lt;0.040, amphetamine 0.070, diazepam 0.20, nordiazepam 0.18, temazepam 0.011; detected flubromazolam and delorazepam</td>
<td>Femoral blood: 2.4</td>
<td>Femoral blood: 0.36</td>
<td>Multiple drug-toxicity; suicide</td>
</tr>
<tr>
<td>34</td>
<td>M</td>
<td>The decedent, who had a history of depression, bipolar disorder and suicidal ideations, was found unresponsive at home. Emergency personnel were called to the scene and he was pronounced dead. Drug paraphernalia and an empty bag labeled “5582 mg 3-FPM” were found on scene</td>
<td>Urine: 3-FPM detected</td>
<td>Central blood: 2.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Semi-quantitative concentration.