An Unusual Case of Drug-Facilitated Sexual Assault Using Aromatic Solvents

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Abstract

This report documents a case of drug-facilitated sexual assault (DFSA) under the influence of solvents. The victim was a 13-year-old female. Upon contact with law enforcement, she was still confused and could hardly explain the facts. She told authorities that she had been kidnapped 4 h previously when two individuals with covered faces put a cloth soaked in a solvent over her mouth. She spent a few hours in a room, during which she lost consciousness. The girl awakened semi-nude in the street with memory loss. No alcohol was present in the subject's body; no odor of alcohol was detected on the subject's breath. No lesions were observed during a gynecological exam. A blood sample was taken with the intent to investigate the use of chloroform or similar anesthetics. Toxicological analysis of the victim's blood revealed the presence of 7.6 mg/L of benzene, 24.8 mg/L of toluene, and 0.6 mg/L of xylene (mixture of isomers). As for other analytical findings, diazepam (0.02 mg/L) was also found. The aromatic solvents involved in this case were detected using gas chromatography with flame-ionization detection (GC-FID) and confirmed using GC-mass spectrometry (MS) in full scan mode after liquid-liquid extraction of the whole blood sample. Quantitation of the aromatic solvents was carried out using GC-FID. Diazepam was detected using GC with nitrogen-phosphorus detection (NPD) and confirmed using GC-MS with full scan mode after solid-phase extraction of the whole blood sample using Bond-Elut Certify columns. Quantitation of diazepam was carried out using GC-NPD. No other drugs, including ethanol, were detected. Recoveries for benzene, toluene, and xylene (mixture of isomers) in whole blood at 5 mg/L were 89.2%, 90.8%, and 93.4%, respectively. Intraday precisions were 5.3%, 5.0%, and 4.9%, respectively, and interday precisions were 12.1%, 11.6%, and 11.5%, respectively. The limits of detection (LOD) and quantitation (LOQ) were 30 and 100 pg/L, respectively. The linearity of the blood calibration curves was excellent with $r^2$ values of > 0.999 (range 0.1–2 mg/L). We want to alert other toxicologists about new or unexpected products that should be taken into account when the surreptitious use of substances in DFSA is suspected.

Introduction

The term sexual assault is generally used to describe a broad range of sexual offenses. Drug-facilitated sexual assault (DFSA) is a term used to define offenses in which victims are subjected to nonconsensual sexual acts while they are incapacitated or unconscious because of the effects of alcohol and/or drugs and are therefore prevented from resisting and/or are unable to consent. The defining element in DFSA is the victim's state of incapacitation resulting from the intake of substances. The other variable is if the disabling drugs are given surreptitiously or under fraudulent circumstances (1).

Drugs used for DFSA have one or more of the following properties: cause sedation, cause amnesia, are odorless and tasteless, dissolve readily in alcoholic or other beverages, and are rapidly absorbed after oral administration. Although the short-acting benzodiazepines probably come closest to the so-called "ideal" properties, the reality is that a wide range of other drugs readily available to the perpetrator of the assault have been used to "drug" potential sexual assault victims (1). Alcohol is the most common substance, and the two best-known "date-rape" drugs, gamma-hydroxybutyrate and flunitrazepam, were found in less than 4% of the samples in studies of prevalence in alleged sexual assault (2,3).

Benzene, toluene, and xylene are aromatic petroleum hydrocarbons with many commercial and industrial applications as solvent mixtures. They are present in paints, paint thinners, paint removers, inks, adhesives, lacquers, cleaning agents, pesticides, and other products likely to be found in the household (4). Their depressant effect on the central nervous system (CNS), which produces an inebriation similar to that of ethanol, along with their easy and legal accessibility and their relatively low cost, make them appealing substances for DFSA.
Case History

The victim was a 13-year-old female. She went to the police department and looked dazed when she reported the incidents. She said that she was kidnapped 4 h previously and spent a few hours in a room, during which she lost consciousness. Two individuals with their faces covered put a cloth soaked in a solvent on her mouth. The girl awakened semi-nude in the street and suffered memory loss. Her clothes were destroyed, and the kidnappers had left her a cellular telephone. The medical examiner did not see any lesions after the gynecological exam. He took a blood sample with the purpose to investigate chloroform or similar anesthetics.

Experimental

Materials

Benzene, toluene, xylene (mixture of isomers), and n-octylbenzene [internal standard, (IS)] standards were purchased from Fluka-Sigma Aldrich (Buch, Switzerland). Stock solutions (1 mg/mL) were prepared by dissolving the appropriate amount of each substance in methanol. These solutions were used to prepare whole blood calibration standards in a range of 0.1–10 mg/L by adding the appropriate amounts of benzene, toluene, and xylene to a pool of citrated human whole blood samples provided by Comunidad de Madrid Blood Bank (Madrid, Spain) and previously verified the possibility for use as blanks. The samples were kept frozen at −25°C until used. All other reagents and solvents were of analytical grade and obtained from Scharlau (Barcelona, Spain).

Sample preparations

All blood samples, including 1:3 dilution, blank, controls, and blood calibrators, were processed following the one-step liquid–liquid extraction procedure previously described (5–7). A 3-mL aliquot of cold whole blood sample (4°C) was transferred to a 10-mL screw-capped glass tube, and 100 μL IS solution (n-octylbenzene methanolic solution of 100 mg/L), 1 mL of diethyl ether (cold at 4°C), and 15 mg of anhydrous sodium sulfate were added. It was vortex mixed for 3 min, then cold centrifuged (4°C) at 4000 rpm for 10 min. The upper organic phase was collected and transferred to a gas vial; 3 μL was injected first for gas chromatography with flame-ionization detection (GC–FID) screening analysis and quantitation, followed by GC–mass spectrometry (MS) for confirmation of the obtained results.

Instrumentation

GC–FID analysis was performed with an HP 5890 series II apparatus, provided with an HP 7673A autosampler, linked to an HP 3396A integrator with a 25-m (0.20-mm i.d., 0.11-μm film thickness) Ultra-1 HP cross-linked methylsilicone column (Hewlett-Packard, Avondale, PA). An initial column temperature of 40°C was maintained for 3 min and then increased at a rate of 10°C/min to 280°C. The temperatures of the injection port and the detector were 280 and 300°C, respectively. Helium (Air Liquid, Madrid, Spain) was used as the carrier gas at a column head pressure of 22 psi. The split ratio was 1:24. The total chromatographic time, including 2 min of equilibration time, was 29 min. The detector gases were hydrogen and air (Air Liquid) delivered at a flow rates of 40 and 400 mL/min, respectively. Insert liners silanized with dimethylchlorosilane/toluene (5:100) and packed with Supelco silanized glass wool (Supelco Park, Bellefonte, PA) were used.

GC–MS analysis was performed with an HP 5971 mass-selective detector controlled by an HP G-1034 C ChemStation (Hewlett-Packard). The autosampler, GC, and column were as described. The MS conditions were as follows: total ion chromatogram mode (m/z 35–650), electron impact ionization with a 70 eV energy, and transfer line and ion-source temperatures were both maintained at 280°C. The presence of the aromatic solvents was confirmed using identical chromatographic conditions.

Drug screens and other analyses

Blood from the DFSA case was examined for ethanol and other common volatiles including sevoflurane, chloroform, halothane, and isoflurane, using headspace with GC–FID. The blood sample was also screened by immunoassay for propoxyphene, cocaine, benzylegonine, methadone, opiates, cannabinoids, benzodiazepines, amphetamine (and related compounds), barbiturates, and tricyclic antidepressants on a Hitachi 902 Automatic Analyzer (Tokyo, Japan) using Cedia® reagents (Microgenics, Fremont, CA). In addition, blood from the case was submitted to solid-phase extraction (SPE) using Bond-Elut Certify columns (Varian, Harbor City, CA) collecting together the acidic-neutral and basic eluates. The sample extract was analyzed by GC with nitrogen-phosphorus detection (NPD) for screening analysis and by GC–MS for confirmation of the results following an analytical method described in our previous work (8).

Results and Discussion

The DFSA victim’s blood sample was submitted to a comprehensive toxicological screening analysis including alcohol and other volatiles, illicit and pharmaceutical drugs, and solvents and related compounds. Results ruled out the presence of alcohol and other volatiles, although a significant carryover in the next blank sample analysis using headspace with GC–FID alert us about the probably presence of aromatic solvents. Modified immunoassay screening of the blood sample was negative for all illicit and therapeutic drugs tested. Acidic-neutral and basic drug screening of the blood with GC–NPD/GC–MS after SPE extraction revealed the presence of minimum quantities of diazepam and nordiazepam, which were undetectable with the previously employed immunoassay technique according to the urine cut-offs proposed by the manufacturers (110 and 150 μg/L for diazepam and nordiazepam, respectively). Solvent/hydrocarbon GC–FID/GC–MS blood screening revealed the presence of aromatic hydrocarbons. Figure 1 shows the GC–FID chromatograms of the case...
blood and blank blood samples. Figure 2 shows the GC–MS mass spectra of the analyte peaks eluting at 2.02, 3.07, and 4.98 min in the case blood sample. Toxicological findings are shown in Table I. Good recoveries, reproducibilities, sensitivities, and linealities were obtained, respectively, for all the compounds involved in the case using their respective analytical techniques. Validation data are shown in Tables II and III.

A number of the substances have been used to facilitate sexual assaults because they impair the victim's memory and his/her decision-making ability (3,9). The three solvents found in our study are similar in their spectrum of toxicity and well-absorbed both after inhalation and dermal contact (4). The systemic effects of acute exposure to toluene, benzene, and xyylene resembles alcohol intoxication, except for perceptual distortions, hallucinations, more rapid onset, and shorter duration (15-30 min) (10). Vapors produce transient effects of headache, nausea, lassitude, impairment of coordination that prevented the victim from escaping, and loss of memory. At higher concentrations, mental confusion, drunkenness and unconsciousness as well as myocardial sensitization to epinephrine are described (4). The threshold blood toluene concentration associated with neuropsychiatric events is lower than that associated with physical signs (11). Hallucinations were described with blood concentrations of 2.5–10 mg/L and unconsciousness with concentrations greater than 10 mg/L. In intoxicated toluene abusers, concentrations were of 0.3 to 30 mg/L. Concentrations higher than 2.5 mg/L indicated sufficient intoxication to require hospitalization in half of the patients, and levels above 10 mg/L were occasionally fatal (4). In fatal cases of acute toluene vapor exposure, blood concentrations ranged from 10 to 79 mg/L (4,12). Lethal blood concentrations of benzene were 0.9–120 mg/L (4,13,14). Blood xylene concentrations that exceed 3 mg/L caused significant impairment of equilibrium. In fatalities due to the ingestion of xyylene-containing products, blood xylene concentrations ranged from 3 to 40 mg/L (4).

When examining DFSA cases, many factors should be taken into account, including time lapse between the crime and blood sample and possible clinical impairment. Firstly, blood samples intended for toxin determinations are usually taken hours after the crime, sometimes even later. The interpretation of concentrations can be difficult because of the rapid elimination of these substances from the body (1) and the length of time between the reported incident and a sample being taken. Then, it is difficult to estimate the concentrations by back-calculations, as the pharmacokinetics of solvents are complex. The interpretation can be further hindered by the handling and storage of the biological samples prior to analysis because of the chemical properties and volatile nature of the substances.

Figure 1. GC-FID chromatograms of the DFSA case blood (A) and blank blood (B). Peak identification: 1, benzene; 2, toluene; 3, xyylene isomers; 4, n-octylbenzene (IS); 5, ionol (diethyl ether stabilizer); and 6, fatty acids.

Figure 2. GC-MS mass spectra of the aromatic solvents detected in the blood of the DFSA case with chemical structures.
involved (15).

Secondly, toxicologists are often asked by police to advice on the possible influence of the substance on actions or conditions during the crime. But it has to be recognized that the pharmacodynamics of most volatile substances following acute ex-

posure are poorly understood. Nevertheless, our case can be compare with those of exposure to toluene for 3 h, that is, showing slurred speak, stupor, and inability to walk or sit. The victims were amnesic for the duration of exposure until shortly after rescue (16). Furthermore, the concentrations were still high enough to predict an important CNS depression in the preceding hours.

Cases in which solvents have forensic relevance can be di-

vided into several groups: traffic violations or accidents where the driver's impairment was caused by solvent inhalation (17); crimes where volatiles may be related to the behavior of the in-

dividual involved, including volatile substance abusers when a crime is committed after a “sniffing” episode; and cases in which the presence of these solvents in blood or tissue has some relationship to the cause of death or the general health of the person, for instance sudden deaths or severe poisoning (7). A fourth etiology is shown here.

The committee of the Society of Forensic Toxicologists that was created to address DFSA prepared a list of drugs that could be used and included illicit, prescription, and over-the-counter drugs (9). Diazepam and other benzodiazepines have been used in DFSA because they are readily available (2, 3). Following a single oral 10-mg dose, peak blood concen-

trations of diazepam averaged 0.148 mg/L at 1 h, declining to 0.037 mg/L by 24 h; an average peak concentration of 0.029 mg/L was achieved for the major active metabolite, nor-

diazepam, at 24 h (4). The low concentration in our case could be due to a small dose administered or to a long period of time between ingestion and blood sampling. However, it was not possible to establish an explanation about the concentration or the circumstances of the chemical aggression because we do not know the quantity administered and time elapsed.

As far as we know, this is the first reported DFSA case in which a mixture of aromatic sol-

vents was involved. The investigation of the crime drives the toxicological analysis re-

quired for each individual case as reported here. We want to alert about new or unex-

pected products that should be taken into ac-

count when the surreptitious use of substances is suspected.

Table I. Toxicological Findings of the DFSA Case

<table>
<thead>
<tr>
<th>Compound</th>
<th>Blood Concentration (mg/L)</th>
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<tbody>
<tr>
<td>Benzene</td>
<td>7.6</td>
</tr>
<tr>
<td>Toluene</td>
<td>24.8</td>
</tr>
<tr>
<td>Xylene (mixture of isomers)</td>
<td>0.6</td>
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<tr>
<td>Diazepam</td>
<td>0.02</td>
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<td>Nordiazepam</td>
<td>&lt; LOQ*</td>
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* < LOQ: below the limit of quantitation.

Table II. Validation Data of the Aromatic Solvents from Fortified Whole Blood Samples Using Liquid-Liquid Extraction and GC-FID for Quantitation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level of Fortification (mg/L)</th>
<th>Percent Recovery (n = 6)</th>
<th>Intraday Precision RSD (%) (n = 6)</th>
<th>Interday Precision RSD (%) (n = 9)</th>
<th>Linearity r² (0.1–10 mg/L) (n = 6)</th>
<th>Limit of Detection (µg/L)</th>
<th>Limit of Quantitation (µg/L)</th>
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<td>Xylene†</td>
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† Mixture of isomers: m,p-xylene peak was used for calculations.

Table III. Validation Data of Diazepam from Fortified Whole Blood Using SPE with Bond-Elut Certify Columns and GC-NPD for Quantitation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Level of Fortification (mg/L)</th>
<th>Percent Recovery (n = 6)</th>
<th>Intraday Precision RSD (%) (n = 6)</th>
<th>Interday Precision RSD (%) (n = 9)</th>
<th>Linearity r² (0.1–2 mg/L) (n = 6)</th>
<th>Limit of Detection (µg/L)</th>
<th>Limit of Quantitation (µg/L)</th>
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<td>Diazepam</td>
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<td>2.0</td>
<td>10.8</td>
<td>0.999</td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

* RSD: relative standard deviation.

References


2. R.C. Baselt. Disposition of Toxic Drugs and Chemicals in Man, 7th ed. Chemical Toxicology Institute, Foster City, CA, 2004, pp 104–106 (benzene), 1120–1124 (toluene), 1198–1200 (xylene), 250–251 (chlorazepate), and 312–315 (diazepam).


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