

Identification of designer drug 2C-E (4-ethyl-2, 5-dimethoxyphenethylamine) in urine following a drug overdose

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In recent years, access to information regarding acquisition and synthesis of newer designer drugs has been at an all-time high due largely to the Internet. As these drugs have become more prevalent, laboratory techniques have been developed and refined to identify and screen for this burgeoning population of drugs. This provides a unique opportunity for learning about many of these methods. Laboratory testing techniques and instrumentation are obscure to many health care professionals, yet their results are crucial. Here, we present a case of an overdose of an uncommon designer drug (2C-E) and discuss the basics of liquid chromatography and mass spectrometry, two important techniques used in isolating and identifying the drug. Although often overlooked and taken for granted, these techniques can play a pivotal role in the diagnosis and subsequent management of select patients.

The drug scene is constantly changing and evolving. Traditionally, drugs of abuse are associated with popular street drugs such as marijuana, heroin, cocaine, and methamphetamine. In the 1990s, several other drugs were added to this list, including the so-called “club drugs”: ecstasy, ketamine, and gamma hydroxybutyric acid. In more recent years, “designer drugs” have emerged, which are either chemically altered natural substances or completely synthetic molecular structures that have psychotropic effects. Due to the widespread use of the Internet, information regarding synthesis of and access to novel compounds is more accessible than ever. This poses new challenges to the medical community in terms of treatment as well as identification of the abused substance, especially in patients unable to communicate. Here we present a case of a fatal overdose of the designer drug known as 2C-E (4-ethyl-2, 5-dimethoxy- β -phenethylamine), a phenethylamine derivative. Although previously published, the prior case report focused on radiographic findings (1). In the present article, we focus on how 2C-E is detected in the urine using basic chromatography and mass spectrometry principles.

CASE REPORT

A 26-year-old white man with known polysubstance abuse and psychiatric problems was found unresponsive at a friend's house. Initial emergency responders intubated the patient and treated him with naloxone with no change in clinical status.

According to the patient's friend, he had ingested a drug known as 2C-E, but was not aware of any other drug or alcohol use. The patient had prescriptions for various psychiatric medications including sertraline, clonazepam, gabapentin, and zolpidem.

In the emergency department, his clinical status remained unchanged. An initial urine toxicology screen was positive for marijuana metabolites and benzodiazepines. A head computed tomography scan was negative, and electroencephalography showed diffuse slowing. The patient's initial laboratory studies are shown in *Table 1*.

On admission, the patient had acute kidney failure and leukocytosis, and he subsequently developed a right lower lobe pneumonia, which resolved with antibiotics. Although the patient remained obtunded, his brain stem reflexes remained intact throughout his hospitalization. After discussing the patient's prognosis in detail, the family decided to withdraw life-supporting therapy. The patient died about 2 weeks after his initial admission. Subsequent specialized urine drug screens were positive for the compound known as 2C-E.

DISCUSSION

The 2C family of designer drugs is a large group of chemicals classified as *ring-substituted phenethylamines*, most of which were first synthesized in the 1970s by the chemist Alexander Shulgin (2). They are characterized by methoxy groups at positions 2 and 5 of the benzene ring. Within the 2C family, there are differences in the substitution of the fourth position on the benzene ring. For instance, at position 4, 2C-I substitutes iodine while 2C-B substitutes bromine. For 2C-E in particular, an ethyl group is substituted at position 4 (*Figure 1*). A full list of 2C compounds published by Shulgin is provided in *Table 2*.

Pharmacologically, 2C-E (and the 2C family) shows strong efficacy for the 5-HT_{2C} receptor, accounting for its hallucinogenic effects (3). Accounts from users typically report a dosage range from 10 to 30 mg (2). Although little is known of the pharmacokinetics of the drug, it seemingly takes effect within seconds of insufflation, with a slightly delayed action if taken

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Table 1. Initial laboratory values

Analyte	Value	Reference range	Flag
Glucose (mg/dL)	295	70–99	High
Sodium (mEq/L)	143	136–145	
Potassium (mEq/L)	4.5	3.5–5.1	
Chloride (mEq/L)	104	98–107	
Carbon dioxide (mEq/L)	29	21–32	
Anion gap (mEq/L)	10	6–16	
Blood urea nitrogen (mg/dL)	15	7–18	
Creatinine (mg/dL)	2.4	0.6–1.3	High
Calcium (mg/dL)	8.2	8.5–10.1	Low
Total protein (g/dL)	6.9	6.4–8.2	
Albumin (g/dL)	3.7	3.4–5.0	
Globulin (g/dL)	3.2	2.4–3.5	
Total bilirubin (mg/dL)	0.2	0.2–1.0	
Alkaline phosphatase (U/L)	66	50–136	
Aspartate transaminase (U/L)	124	15–37	High
Alanine transaminase (U/L)	122	12–78	High
Amylase (U/L)	76	25–115	
Lipase (U/L)	174	73–393	
Lactic acid (mmol/L)	3.7	0.9–1.7	High

Table 2. The 2C family of phenethylamine derivatives*

2C family	
2C-B	4-bromo-2, 5-dimethoxy-phenethylamine
2C-C	4-chloro-2, 5-dimethoxy-phenethylamine
2C-D	4-methyl-2, 5-dimethoxy-phenethylamine
2C-E	4-ethyl-2, 5-dimethoxy-phenethylamine
2C-F	4-fluoro-2, 5-dimethoxy-phenethylamine
2C-G	3, 4-dimethyl-2, 5-dimethoxy-phenethylamine
2C-G-3	3, 4-trimethylene-2, 5-dimethoxy-phenethylamine
2C-G-4	3, 4-tetramethylene-2, 5-dimethoxy-phenethylamine
2C-G-5	3, 4-norbornyl-2, 5-dimethoxy-phenethylamine
2C-G-N	1, 4-dimethoxynaphthyl-2-ethylamine
2C-H	2, 5-dimethoxy-phenethylamine
2C-I	4-iodo-2, 5-dimethoxy-phenethylamine
2C-N	4-nitro-2, 5-dimethoxy-phenethylamine
2C-O-4	4-isopropoxy-2, 5-dimethoxy-phenethylamine
2C-P	4-propyl-2, 5-dimethoxy-phenethylamine
2C-SE	4-methylseleno-2, 5-dimethoxy-phenethylamine
2C-T	4-methylthio-2, 5-dimethoxy-phenethylamine
2C-T-2	4-ethylthio-2, 5-dimethoxy-phenethylamine
2C-T-4	4-isopropylthio-2, 5-dimethoxy-phenethylamine
2C-T-7	4-propylthio-2, 5-dimethoxy-phenethylamine
2C-T-8	4-cyclopropylmethylthio-2, 5-dimethoxy-phenethylamine
2C-T-9	4-(t)-butylthio-2, 5-dimethoxy-phenethylamine
2C-T-13	4-(2-methoxyethylthio)-2, 5-dimethoxy-phenethylamine
2C-T-15	4-cyclopropylthio-2, 5-dimethoxy-phenethylamine
2C-T-17	4-(s)-butylthio-2, 5-dimethoxy-phenethylamine
2C-T-21	4-(2-fluoroethylthio)-2, 5-dimethoxy-phenethylamine

*Reprinted with permission from Shulgin (2).

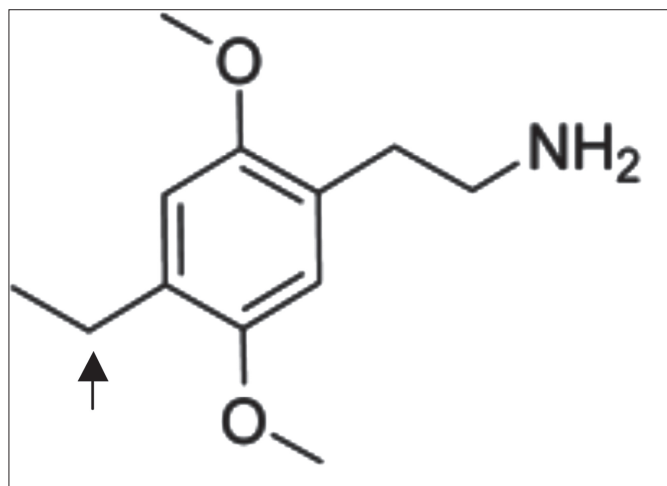


Figure 1. Structure of 2C-E. The arrow denotes the ethyl group attached to the fourth position of the benzene ring.

orally. The drug displays a marked dosage effect, and hallucinogenic activity typically lasts for 4 to 8 hours (2). Metabolism of 2C-E appears to have several pathways, although the main ones appear to be via hepatic oxidative deamination and/or O-demethylation (4–6).

The clinical presentation of users of the 2C group can be varied. At lower doses, the drugs act as a stimulant (2). However, at higher doses (>10 mg), there is a marked hallucinogenic and

psychoactive effect. Deaths have also been reported following the usage of 2C compounds including, but not limited to, 2C-T-7 (7) and 2C-I (8).

For diagnosis, an analytic method for detection and quantification is required for clinical chemistry and forensic toxicology. Many different techniques have been developed to screen for these newer designer drugs, including capillary electrophoresis–mass spectrometry (9–12), capillary electrophoresis coupled with a diode array detector (13), gas chromatography–mass spectrometry (14, 15), and liquid chromatography–mass spectrometry (LC-MS) (16). In more recent years, LC-MS in tandem (LC-MS/MS) has become the detection test of choice due to its ability to separate and identify small molecules with similar structures in one run (17).

In order to understand LC-MS/MS, it is easiest to break it up into its separate analytic components. The liquid chromatography phase, specifically high-performance liquid chromatography, separates chemicals by running a solvent

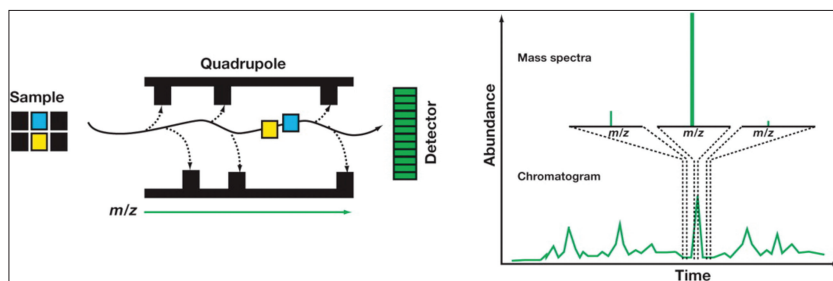


Figure 2. Schematic of a quadrupole mass spectrometer. The quadrupole is set to transmit only ions with a specific mass-to-charge ratio (m/z) that matches the analytes of interest. Because only ions matching the selected m/z are transmitted, the resultant chromatogram and mass spectra are clean and easy to interpret. However, because the two analytes of interest have identical m/z , the mass spectrometer is unable to differentiate between them. Reprinted with permission from Hill et al, 2011 (18).

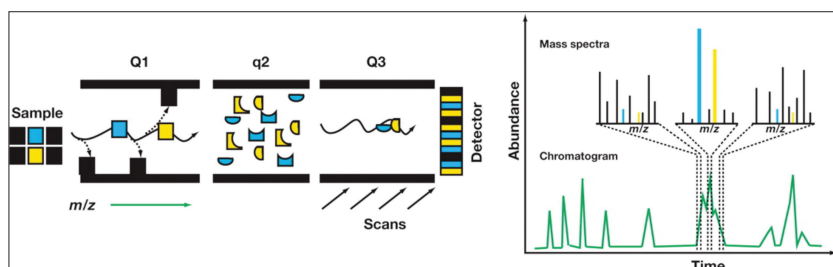


Figure 3. Schematic of a mass spectrometer in tandem. Q1 is set to only transmit ions with a specific mass-to-charge ratio (m/z) that matches the analytes of interest. The collision cell, q2, produces fragments from these transmitted ions, while Q3 constantly scans across the entire m/z range, allowing all fragments produced in q2 to reach the detector. The recording of all fragments results in a complex chromatogram and mass spectra but has allowed for the unique identification of each analyte at the appropriate sampled time point, indicated in blue and yellow. Reprinted with permission from Hill et al, 2011 (18).

(mobile phase) containing the chemicals of interest through a column (stationary phase). The chemical (2C-E) binds to the column through hydrophobic interactions. As can be expected, different molecules elute through the column at different times due to differences in molecular structure and hydrophobic interactions. This technique is very good at separating chemicals (even molecules with very similar masses); however, it does not necessarily identify the compound, especially if there are many unknown chemicals in the sample. This is where coupling of mass spectrometry is useful.

In typical non-tandem mass spectrometry, these unknown compounds enter the mass spectrometer, where they are initially ionized and given either a positive or a negative charge. Mass spectrometers identify the molecules present in a sample based on their mass-to-charge ratio (m/z). The molecules then move into the quadrupole chamber, which consists of four parallel magnetic rods arranged in a square formation (Figure 2) (18). The rods are calibrated in such a way that only the ions of interest are allowed to pass unimpeded through the chamber and hit the detector. For instance, 2C-E has a molecular weight of approximately 209 and a charge of 1+ following ionization; therefore, the m/z ratio will be 209. The mass spectrometer only allows compounds with this specific m/z ratio to pass through and be detected. However, other compounds can have very similar m/z ratios, detracting from the specificity of a mass spectrometer. For example, morphine and hydromorphone have an

identical m/z ratio of 286, thus making separation of the two compounds difficult by non-tandem mass spectrometry. Running mass spectrometry in tandem can help alleviate this issue.

Mass spectrometry in tandem is characterized by multiple quadrupoles (typically three) that are arranged in a series (Figure 3) (18). The first and third quadrupoles are similar in function to those described previously. The middle quadrupole differs in that it functions as the “collision cell.” It uses a combination of inert gases and changing frequencies. As the ions from the first quadrupole mass spectrometer enter this second chamber, they collide with the inert gases and fragment into even smaller ions. These molecules then enter the third quadrupole, where they are detected. By fragmenting parent ions with similar m/z ratios, new ions are created with differing m/z ratios, which are sent and detected in the third quadrupole. This functions to identify the parent molecule with great specificity.

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