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Case report

Death after use of the synthetic cannabinoid 5F-AMB

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ABSTRACT

The use of synthetic cannabinoids and related products has been associated with adverse effects including seizure, acute kidney injury, and sudden death. We report the death of an individual that was associated with the synthetic cannabinoid 5F-AMB. Specimens were extracted via a liquid–liquid extraction at pH 10.2 into hexane:ethyl acetate. Analysis was completed via liquid chromatography tandem mass spectrometry. For this case report, we briefly describe the extraction and instrumental methods for 5F-AMB as well as the blood toxicology results (5F-AMB, 0.3 ng/mL) and case circumstances and autopsy findings. Cause and manner of death was certified as accidental death due to synthetic cannabinoid toxicity. We also briefly review any previously published reports in which 5F-AMB was analytically confirmed and determined to be involved with cause of death.

1. Introduction

(S)-Methyl-2-(1-(5-fluoropentyl)-1H-indazole-3-carboxamido)-3-methylbutanoate, otherwise known as 5F-AMB, 5F-AMB-PINACA, or 5F-AMP, is a newer synthetic cannabinoid that was first reported as a constituent in herbal incense and potpourri products on the Japanese drug market in 2014 [1]. The substance has a chemical formula $C_{19}H_{26}FN_3O_3$ and a molecular weight equal to 363.4 g/mol. 5F-AMB is a derivative of an older synthetic cannabinoid receptor agonist AB-PINACA and differs with a replacement of a primary amine with a methoxy group and the addition of a fluorine atom on the terminus of the pentyl alkyl chain. The addition of the methoxy group leads to a moiety structurally based off the amino acid, valine, otherwise known as 2-amino-3-methylbutanoic acid. Although 5F-AMB is suspected to be a full cannabinoid receptor agonist, no pharmacological and toxicological data exists for the compound. There have been no reports regarding the detection of 5F-AMB in clinical toxicology casework, but the compound has been detected in two multi-substance deaths [2,3]. The chemical structures of 5F-AMB and AB-PINACA are shown in Fig. 1.

Herein we briefly report an analytical method for the detection of 5F-AMB in blood specimens and describe a fatality in which 5F-AMB was the only substance detected and ruled as associated with cause of death.

2. Case report

A 34-year-old male was last seen alive 17 h prior to discovery of his body. The decedent was found supine on the floor by a chair and fully clothed; he was cool to the touch. No medical history was noted by the decedent's family, but they did report that he had a history of ethanol abuse. An opened bag of "Apollo" brand herbal incense was found in his pocket.

3. Specimen collection and testing protocol

At autopsy, a blood specimen from the subclavian vein was collected in a polypropylene tube which contained sodium fluoride and potassium oxalate. The specimen was sent to our toxicology laboratory facility at ambient temperature for routine systematic toxicological analyses. Urine was not available for collection. No other specimens were obtained at autopsy by the pathologist. The herbal incense product was not available from law enforcement for testing as well.

The routine comprehensive toxicological testing scope included initial screening analyses for classical cannabinoids and opiates/oxycodone/oxymorphone by enzyme linked immunosorbent assay (ELISA), volatile substances by headspace gas chromatography with flame ionization detection (GC–FID), and other drugs of abuse, prescription medications, and therapeutic agents by liquid chromatography time of flight mass spectrometry (LC/ToF). Synthetic cannabinoid analysis was completed via a directed liquid chromatography tandem quadrupole mass spectrometry (LC/MS/MS) assay. The scope of the LC/MS/MS assay included 48 synthetic cannabinoid compounds.

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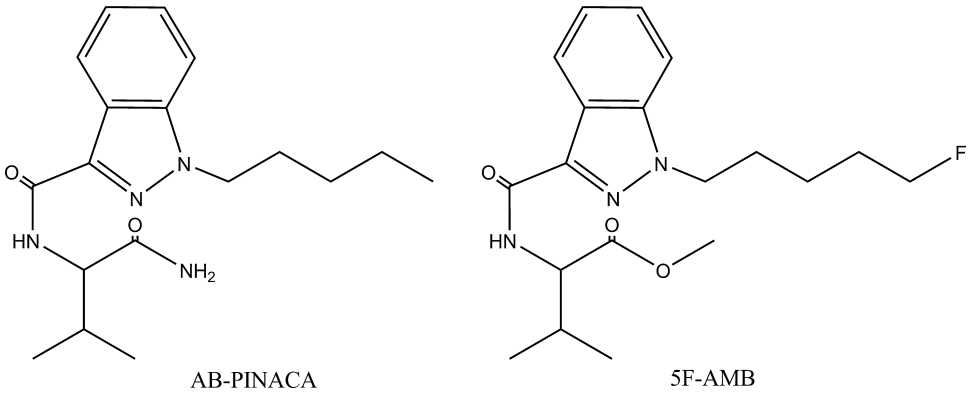

Fig. 1. Chemical structure comparison of 5F-AMB and AB-PINACA.

4. Materials

The 5F-AMB reference standard and JWH-122-d_9 internal standard were purchased from Cayman Chemical (Ann Arbor, MI, USA). All other solvents including acetonitrile, ethyl acetate, formic acid, hexane, methanol, and sodium bicarbonate were obtained from Fisher Scientific (Pittsburgh, PA, USA). Deionized (DI) water was obtained from the laboratory's water treatment system.

5. Methods

The general extraction and instrumental procedures for the synthetic cannabinoids analysis were previously published [4]. Briefly, the specimens were prepared by a liquid–liquid extraction at pH 10.2 into hexane:ethyl acetate. Instrumental analysis was completed via liquid chromatography and electrospray ionization tandem mass spectrometry. The instrument was a Waters Acquity UltraPerformance Liquid Chromatograph coupled to a Waters Quattro Premier XE tandem quadrupole mass spectrometer. Mobile phases were 0.1% formic acid in DI water and 0.1% formic acid in acetonitrile. The stationary phase was a Waters Acquity UPLC BEH C18, 2.1 mm × 100 mm, 1.7 μm analytical column. Initial conditions were 42% organic and held for 0.3 min. Between 0.3 min and 5.6 min, a gradient elution was used and the organic was linearly increased to 66%. Between 5.6 min and 8 min, the organic was increased linearly to 76%. Between 8 min and 8.5 min the organic was increased linearly to 100%, where it was returned to initial conditions. Total run time was 9.3 min.

The specific method parameters for 5F-AMB were 364.4 > 233.0 (Quantifying ion transition – cone voltage, 32 V and collision energy, 20 eV) and 364.4 > 304.2 (Qualifying ion transition – cone voltage, 32 V and collision energy, 14 eV). The ion transition for the JWH-122-d_9 was 365.3 > 169.1 (cone voltage, 44 V and collision energy, 29 eV). Dwell times for all ion transitions were 20 ms. Capillary voltage was 0.6 kV. Extractor voltage was 3 V. Source and desolvation temperatures were 140 °C and 450 °C respectively. Desolvation gas was nitrogen and collision gas was argon. Desolvation gas flow and collision gas flow were 850 L/h and 0.30 mL/min respectively.

The analytical method was validated as a quantitative analysis via a standard in-house laboratory method validation procedure. The generalized laboratory validation protocol has been previously published [5]. The following attributes were assessed: linearity, accuracy and precision, carryover, exogenous drug interferences, and ion suppression. The retention time for 5F-AMB was 3.3 min. The retention time for the JWH-122-d9 internal standard was 6.6 min. A representative chromatogram (postmortem blood specimen from the presented case) for each transition of 5F-AMB and the internal standard is shown in Fig. 2. The average ion transition ratio for 5F-AMB during method validation was 1.7 (n = 15). The assay was linear from 0.2 ng/mL (lower limit of quantitation or LLOQ) to 20 ng/mL (upper limit of quantitation or ULOQ), with an administratively set limit of detection (LOD) at 0.1 ng/mL. No concentrations lower than 0.1 ng/mL were tested for LOD analysis. During linearity testing, all coefficient of determination (R^2) values were between 0.9975 and 0.9999. For imprecision and accuracy testing, five quality control (QC) replicates were prepared at two different concentrations and analyzed over three days. Intrarun imprecision for the high control specimen (6 ng/mL) was 7.8, 0.8, and 6.6% CV with standard deviations 0.50, 0.05, and 0.40 ng/mL respectively. The high control interrun imprecision was 5.6% CV with standard deviation 0.35 ng/mL. The high control intrarun accuracy was 100.7–105.6% and the interrun accuracy was 103.9%. Intrarun imprecision for the low control specimen (1.5 ng/mL) was 2.9, 4.4, and 8.8% with standard deviations 0.04, 0.06, and 0.14 ng/mL respectively. The low control interrun imprecision was 7.9% CV with standard deviation 0.12 ng/mL. The low control intrarun accuracy was 95.1–108.8% and the interrun accuracy was 101.1%. Of the 180 routine drugs and metabolites and 47 synthetic cannabinoids analyzed as possible interferences for 5F-AMB, no quantifiable signals were observed for the two ion transitions monitored. No carryover for 5F-AMB was detected in three blank extracts injected immediately after a specimen spiked with 100 ng/mL of the analyte of interest. Ion suppression/matrix effects testing results are summarized in Table 1.

6. Results

Other than reported ethanol abuse, the medical history of the decedent was unremarkable. Observed findings at the scene included full rigor mortis, dependent fixed livor mortis and a nasal frothy purge substance. During autopsy, no remarkable findings

Table 1
Additional validation results.

Parameter	5F-AMB
Ion suppression	
1.5 ng/mL	
Matrix effect	1.10 (6.8% CV)
Response effect	1.09 (6.1% CV)
10 ng/mL	
Matrix effect	1.02 (7.2% CV)
Response effect	0.98 (3.2% CV)
16 ng/mL	
Matrix effect	0.94 (2.2% CV)
Response effect	0.97 (8.2% CV)