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Case Report

Case reports of synthetic cannabinoid XLR-11 associated fatalities

Kevin G. Shanks [a,*], David Winston [b], John Heidingsfelder [c], George Behonick [a]

[a] AIT Laboratories, Indianapolis, IN 46241, USA
[b] Pima County Office of the Medical Examiner and University of Arizona, Tucson, AZ 85714, USA
[c] Fayette County Coroner's Office, Vandalia, IL 62471, USA

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ABSTRACT

Synthetic cannabinoids have been available in herbal incense and potpourri products over the Internet and in smoke shops for the last several years. We report the deaths of two individuals that were associated with XLR-11. Specimens were extracted via a liquid–liquid extraction at basic pH into hexane:ethyl acetate and analyzed by liquid chromatography tandem mass spectrometry. For these two case reports, we describe the instrumental analysis and extraction methods for XLR-11, toxicological results for postmortem blood specimens, relevant case information and autopsy findings. We also briefly review any previously published peer-reviewed reports in which XLR-11 was analytically confirmed and determined to be an intoxicating agent.

1. Introduction

XLR-11 ((1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)-methanone) is a synthetic cannabimimetic compound and is a 5-fluorinated derivative of the Abbott Laboratories' research chemical UR-144. UR-144 acts as a selectively binding full agonist of the CB_2 receptor, but it still retains some activity at CB_1 [1–3]. Wiley et al. studied the in vivo and in vitro pharmacology of XLR-11 and determined that the substance was a full agonist of both CB_1 and CB_2 receptors. It has K_i at CB_1 equal to 24.0 ± 4.6 nM and K_i at CB_2 equal to 2.1 ± 0.6 nM [4]. The compound has no formal history in academic research but does fall within Abbott's patent WO 2006/06916.

Chemical structures of XLR-11 and related synthetic cannabinoids are shown in Fig. 1. The first reports of the detection of XLR-11 in herbal incense products occurred in Japan in 2012 [5]. The United States Federal government passed legislation controlling 15 synthetic cannabinoids in July 2012 and it was made official on January 4, 2013. The scope of the legislation did not include XLR-11 [6]. XLR-11, along with UR-144, became prevalent in our nonbiological product casework during the 2012–2013 [7]. In May 2013, the US Federal government and Drug Enforcement Administration (DEA) used its emergency scheduling powers to place XLR-11 into Schedule I of the Controlled Substances Act. Reported symptoms of those who use XLR-11 include anxiety, agitation, hallucinations, hypertension, irritability, seizures, and tachycardia [8].

Herein we describe two postmortem case reports where cause of death was associated with XLR-11. We also briefly describe a liquid chromatography tandem mass spectrometry (LC/MS/MS) analytical method for the detection of XLR-11 and review clinical toxicology and human performance toxicology casework in which XLR-11 was detected.

2. Case reports

2.1. Case 1

A 29-year-old female was found dead on the floor of the bedroom of her apartment. She was last seen alive the day prior by her boyfriend who described signs of intoxication and agitation. The decedent was a known user of synthetic cannabinoids and associated herbal incense/potpourri products. Empty packages of a product named "Black Dragon" were found at the scene.

2.2. Case 2

A 32-year-old female, who had a history of drug abuse, including methamphetamine, heroin, and synthetic cannabinoids, presented to the emergency room with chest pain, nausea, and agitation. She was diagnosed with anxiety and left the hospital to

* Corresponding author. Tel.: +1 3177158893; fax: +1 3177158893.
E-mail address: kshanks@aitlabs.com (K.G. Shanks).

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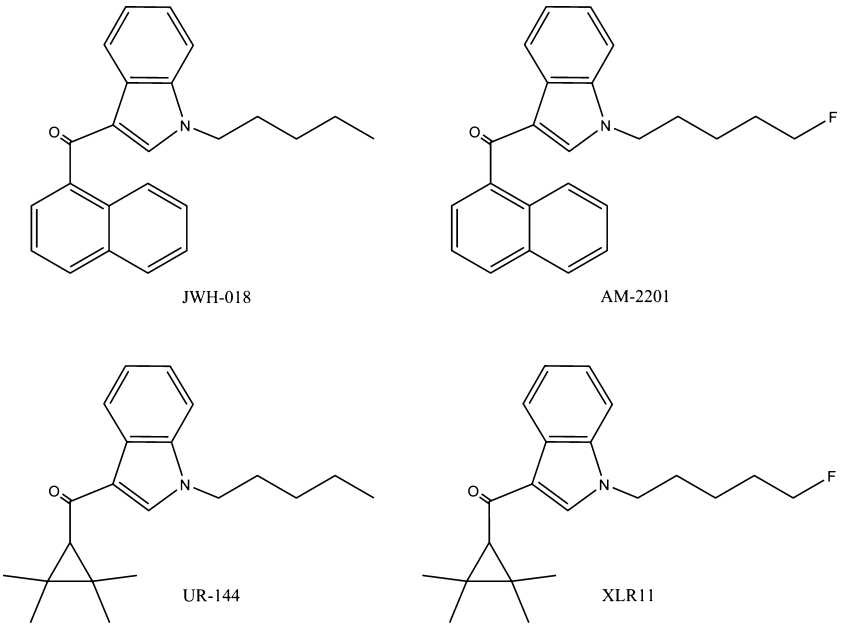

Fig. 1. Chemical structures of various synthetic cannabinoids.

travel to a friend's house to take a shower. Later in the day, she was found unresponsive in a bedroom at the friend's house. After transport to the hospital, resuscitation was attempted, but she was pronounced dead.

3. Specimen collection and testing protocol

At autopsy, the medical examiners collected blood specimens in polypropylene tubes which contained sodium fluoride and EDTA as additives. The specimens were sent to AIT Laboratories' facility in Indianapolis, IN at ambient temperature for systematic toxicological analyses. No other specimens were collected at autopsy.

A comprehensive toxicological testing scope was undertaken. Initial screening analyses included an enzyme linked immunosorbent assay (ELISA) for classical cannabinoids and opiates/oxycodone/oxymorphone, a liquid chromatography time of flight mass spectrometry (LC/ToF) assay for other drugs of abuse, prescription drugs, and/or therapeutic agents, and a headspace gas chromatography with flame ionization detection (GC-FID) assay for volatile compounds. Synthetic cannabinoids were analyzed via a directed LC/MS/MS assay.

4. Materials

The XLR-11 reference standard, along with the internal standard, JWH-073-d_7, were obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Formic acid (98%) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Other solvents such as acetonitrile (HPLC grade), ethyl acetate (GC/MS grade), hexane (GC/MS grade), methanol (HPLC grade), sodium bicarbonate (USP grade), and sodium carbonate (ACS grade) were acquired from Fisher Scientific (Pittsburgh, PA, USA).

5. Methods

The generalized extraction procedure was previously published in our case reports regarding the synthetic cannabinoid, 5F-PB-22 [9]. In summary, an aliquot of internal standard solution in acetonitrile and an aliquot of sodium bicarbonate buffer, pH 10.2 were added to a 500 μL specimen volume of blood. Hexane:ethyl acetate (98:2) was added to the tube and the specimens were mixed and centrifuged. The organic layer was evaporated to dryness under nitrogen gas and the residue was reconstituted in a mixture of deionized (DI) water:acetonitrile.

Instrumental analysis was performed via a Waters (Milford, MA) Acquity UltraPerformance® Liquid Chromatograph coupled to a Waters Quattro Premier XE tandem mass spectrometer. Chromatographic separation was performed by injecting 10 μL of vial extract onto a Waters Acquity UPLC® BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size), held at 60 °C, using a gradient elution. Electrospray ionization (ESI) mass spectrometry was performed in positive ionization multiple reaction monitoring mode (MRM). Summaries of the instrumental methods are shown in Tables 1 and 2.

The overall validation methodology used was previously published and is a standard procedure for quantitative mass spectrometry-based assays in our laboratory [10]. The analytical method for determination XLR-11 in blood was validated as a quantitative assay and the following attributes were assessed: linearity, imprecision and accuracy, ion suppression, exogenous drug interferences, and carryover. Results are summarized in Table 3.

6. Results

6.1. Case 1

There was no evidence of significant natural disease upon gross and microscopic examination. Peripheral blood was submitted to

Table 1
LC parameters.

Total time (min)	Flow rate (mL/min)	% A	% B
Initial	0.500	58.0	42.0
0.30	0.500	58.0	42.0
5.60	0.500	34.0	66.0
8.00	0.500	24.0	76.0
8.50	0.500	0.0	100.0
8.51	0.500	58.0	42.0
Mobile phases	A (0.1% formic acid in DI water); B (0.1% formic acid in acetonitrile)		
Retention time (min)	XLR-11 (5.5) JWH-073-d7 (5.2)		