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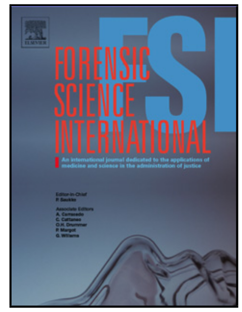

Title: Case Reports of Synthetic Cannabinoid XLR-11 Associated Fatalities

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Highlights

- We report the detection of XLR-11 in two postmortem whole blood cases.
- We describe a liquid chromatography tandem mass spectrometry method for XLR-11.
- We summarize clinical and human performance toxicology casework involving XLR-11.

Case Reports of Synthetic Cannabinoid XLR-11 Associated Fatalities

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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.

Keywords: Synthetic cannabinoid, XLR-11, Death, Fatality, LC/MS/MS

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 Figure 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.

Case Reports

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.

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 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.

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.

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).

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 x 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.

Results

Case 1

There was no evidence of significant natural disease upon gross and microscopic examination. Peripheral blood was submitted to the laboratory for toxicological analyses. The ELISA for cannabinoids/opiates/oxycodone/oxymorphone was negative. The LC/ToF screen was presumptively positive for diphenhydramine. The volatiles screen was negative. Diphenhydramine confirmed positive by LC/MS/MS at 81.0 ng/mL. The directed analysis for synthetic cannabinoids had positive findings for XLR-11 (1.4 ng/mL). The medical examiner certified the cause of death as synthetic cannabinoid toxicity and the manner of death as accident.

Case 2

Remarkable pathological findings at autopsy were significant pulmonary edema and congestion, along with acute visceral congestion and mild pulmonary anthracosis. The ELISA for cannabinoids/opiates/oxycodone/oxymorphone was negative. The LC/ToF screen was presumptively positive for naloxone and caffeine. The volatiles screen was negative. The directed analysis for synthetic cannabinoids had positive findings for XLR-11 (0.6 ng/mL). Naloxone was administered during resuscitative attempts by emergency personnel. Both naloxone and caffeine were reported as screen-only/presumptive positive results. The medical examiner ruled the cause and manner of death as undetermined, with significant findings of positive toxicology for XLR-11.

Discussion

Although other synthetic cannabinoids (JWH-018, JWH-073, JWH-210, and 5F-PB-22) have been reported in literature and attributed to be the cause of death, until this report XLR-11 has not been reported in literature as causing or contributing to death (9-11). Neither decedent in this current case report had sufficient natural disease to explain death. This absence of significant pre-existing natural disease was also documented in four cases reported with the synthetic cannabinoid 5F-PB-22 (9).

XLR-11 (or metabolites) were analytically confirmed in biological specimens and associated with acute kidney injury in cases in four states - Kansas, New York, Oregon, and Wyoming. Two of the cases had hospital serum specimens that were positive for XLR-11 (33 and 35 ng/mL). The symptoms upon presentation at the emergency department included nausea, vomiting, abdominal pain, and back pain, and significant lab values included elevated serum creatinine (12). XLR-11 was also detected in the admission serum specimen of a 26 year old male who presented to the emergency department and had symptoms of abdominal and lower back pain, nausea, and vomiting. He had used a product named “Mr. Happy”, which was found to contain both UR-144 and XLR-11. The XLR-11 concentration was 36 ng/mL, while UR-144 concentration was 5 ng/mL and XLR-11 metabolite was 102 ng/mL were also detected in the serum specimen. A second serum specimen drawn 3.5 hours after admission revealed XLR-11 at 34 ng/mL, as well as UR-144 at 3 ng/mL and XLR-11 metabolite at 62 ng/mL (13).

When compared to the concentrations detected in the postmortem blood cases, the XLR-11 concentrations in the clinical toxicology serum casework were considerably greater (23-60 times higher). Even though the levels were lower in the two cases described in this report, the lack of natural disease and essentially negative toxicology except for XLR-11, the certifying medical examiners felt that XLR-11 caused the death in Case 1 and probably contributed to death in Case

2. Both decedents were observed to be agitated and chest pain was described in Case 2, which are symptoms associated with XLR use (8).

XLR-11 also has been implicated in human performance toxicology casework. In 2014, Lemos reported a single case report of driving under the influence of XLR-11 where the driver's blood specimen was positive for the compound at a concentration of 1.34 ng/mL. No other substances were detected in the blood specimen. During his interview with a drug recognition expert, the suspect admitted to using "blueberry spice". Observations made during the evaluation included slow and mumbled speech, bloodshot and watery eyes, low body temperature, rigid muscle tone, lack of convergence in left eye, no presence of horizontal or vertical gaze nystagmus, eye tremors, inability to maintain balance and sway (14). Louis et al. reported a series of 18 driving under the influence cases involving synthetic cannabinoids – 12 of which included the detection of XLR-11. Of those 12 cases, eight were single drug intoxication cases. The other four included other synthetic cannabinoids (UR-144, AM-2201, JWH-018, and JWH-022). In the eight single drug intoxication cases, slow or poor coordination, bloodshot and watery eyes, and slurred or mumbled speech were commonly observed (15). XLR-11 was reported as qualitatively positive in these cases; no quantification was undertaken.

Conclusion

The two case reports presented describe fatalities in which XLR-11 was the only substance of toxicological significance detected. A generalized LC/MS/MS method for synthetic cannabinoids was described for the detection of XLR-11. Relatively non-specific pathological findings were observed at autopsy. Cause of death in the first case was explicitly listed as intoxication with XLR-11, while the cause of death in the second case was undetermined, but XLR-11 was noted as a significant finding.

References

1. Pace, J.M., Tietje, K., Dart, M.J., Meyer, M.D. (2006) 3-cycloalkylcarbonyl indoles as cannabinoid receptor ligands. Abbott Laboratories. WO Application 2006069196
2. Frost, J.M., Dart, M.J., Tietje, K.R., Garrison, T.R., Grayson, G.K., Daza, A.V., El-Kouhen, O.F., Miller, L.N., Li, L., Yao, B.B., Hsieh, G.C., Pai, M., Zhu, C.Z., Chandran, P., Meyer, M.D. (2008) Indol-3-yltetramethylcyclopropyl ketones: effects of indole ring substitution on CB2 cannabinoid receptor activity. *Journal of Medicinal Chemistry*, **51**, 1904-1912.
3. Frost, J.M., Dart, M.J., Tietje, K.R., Garrison, T.R., Grayson, G.K., Daza, A.V., El-Kouhen, O.F., Yao, B.B., Hsieh, G.C., Pai, M., Zhu, C.Z., Chandran, P., Meyer, M.D. (2010) Indol-3-ylcycloalkyl ketones: effects of N1 substituted indole side chain variations on CB(2) cannabinoid receptor activity. *Journal of Medicinal Chemistry*, **53**, 295-315.
4. Wiley, J.L., Marusich, J.A., Lefever, T.W., Grabenauer, M., Moore, K.N., Thomas, B.F. (2013) Cannabinoids in disguise: Δ9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. *Neuropharmacology*, **75**, 145-154.
5. Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., Goda, Y. (2013) URB-754: a new class of designer drug and 12 synthetic cannabinoids detected in illegal products. *Forensic Science International*, **227**, 21-32.
6. Drug Enforcement Administration. (2013) Establishment of drug codes for 26 substances. Federal Register. Vol. 78, No. 3.
7. Shanks, K.G., Behonick, G.S., Dahn, T., Terrell, A. (2013) Identification of novel third-generation synthetic cannabinoids in products by ultra-performance liquid chromatography and time-of-flight mass spectrometry. *Journal of Analytical Toxicology*, **37**, 517-525.
8. Drug Enforcement Administration. (2013) Schedules of controlled substances: temporary placement of three synthetic cannabinoids into schedule I. Federal Register. Vol. 78, No.71.
9. Behonick, G., Shanks, K.G., Firchau, D.J., Mathur, G., Lynch, C.E., Nashelsky, M., Jaskierny, D.J., Meroueh, C. (2014) Four postmortem case reports with quantitative detection of the synthetic cannabinoid 5F-PB-22. *Journal of Analytical Toxicology*, **38**, 559-562.
10. Shanks, K.G., Dahn, T., Terrell, A. (2012) Detection of JWH-018 and JWH-073 by UPLC-MS-MS in Postmortem Whole Blood Casework. *Journal of Analytical Toxicology*, **36**, 145-152.
11. Kronstrand, R., Roman, M., Andersson, M. Eklund, A. (2013) Toxicological Findings of Synthetic Cannabinoids in Recreational Users. *Journal of Analytical Toxicology*, **37**, 534-541.
12. Centers for Disease Control and Prevention (CDC). (2013) Acute kidney injury associated with synthetic cannabinoid use – multiple states, 2012. *MMWR Morbidity and Mortality Weekly Report*, **62**, 93-98.
13. Thornton, S.L., Wood, C., Friesen, M.W., Gerona, R.R. (2013) Synthetic cannabinoid use associated with acute kidney injury. *Clinical Toxicology (Philadelphia),* **51**, 189-190.

14. Lemos, N.P. (2014) Driving under the influence of synthetic cannabinoid receptor agonist XLR-11. *Journal of Forensic Sciences*, **59**, 1679-1683.
15. Louis, A., Peterson, B.L., Couper, F.J. (2014) XLR-11 and UR-144 in Washington State and State of Alaska Driving Cases. *Journal of Analytical Toxicology*, **38**, 563-568.

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 (Minutes)	XLR-11 (5.5) JWH-073-d7 (5.2)		

Analyte	Ion Transition	Type	Dwell Time (ms)	Cone Voltage (V)	Collision Energy (eV)
XLR-11	330.3 > 124.8	Quantifying	0.02	58	26
XLR-11	330.3 > 54.8	Qualifying	0.02	58	42
JWH-073-d7	335.2 > 127.0	Internal Standard	0.02	40	48
Capillary Voltage	Extractor Voltage	Source Temperature	Desolvation Temperature	Desolvation Gas Flow	Collision Gas Flow
(0.60 kV)	(3 V)	(140°C)	(450°C)	(850 L/Hr)	(0.30 mL/min)

Table 2. MS Parameters

Table 3. Method Validation Results for XLR-11

Parameter	Acquired Result
Limit of Detection (LOD)	0.1 ng/mL
Limit of Quantitation (LOQ)	0.2 ng/mL
Linearity	0.2-10 ng/mL
Coefficient of Determination (R^2)	0.9980-0.9999
Accuracy	
1.5 ng/mL	
Intrarun	96.6-106.0%
Interrun	102.0%
6 ng/mL	
Intrarun	105.0-117.0%
Interrun	111.0%
Imprecision	
1.5 ng/mL	
Intrarun	3.6-7.8% CV
Interrun	6.6% CV
6 ng/mL	
Intrarun	0.6-4.4% CV
Interrun	5.4% CV
Ion Suppression	
0.6 ng/mL	
Matrix Effect	1.07 (5.2% CV)
Response Effect	1.06 (6.7% CV)
5 ng/mL	
Matrix Effect	1.09 (4.5% CV)
Response Effect	1.07 (6.9% CV)
8 ng/mL	
Matrix Effect	1.02 (3.4% CV)
Response Effect	1.04 (5.5% CV)
Carryover	None detected at 200 ng/mL
Exogenous Interferences	None detected
Ion Transition Ratio (Mean)	1.0

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Figure 1. Chemical structures of various synthetic cannabinoids