Application of Gas Chromatography–Mass Spectrometry for the Identification and Quantitation of Three Common Synthetic Cannabinoids in Seized Materials from the Jordanian Market

Laith N. AL-Eitan,* Abdelqader S. Asa’ad, AbdelKader H. Battah, and Hanan A. Aljamal

ABSTRACT: Synthetic cannabinoids (SCs) were developed to mimic the effects of Δ⁹-tetrahydrocannabinol on humans. SCs were distributed in the form of herbal blends, with smoking being the main method of consumption. These synthetic compounds have a wide range of physical, behavioral, and harmful effects on the body. However, this study aimed to identify and quantify three common SCs including AB-FUBINACA, AB-CHMINACA, and XLR-11 in the seized materials from the Jordanian market by gas chromatography coupled with mass spectrometry (GC–MS). A liquid–liquid extraction sample preparation technique was applied to 100 different seized samples obtained from the Anti-Narcotics Department of Public Security in a period between 2017 and 2018. Profiling of the seized samples revealed different distributions of the targeted SCs in the obtained samples. Upon quantitation, concentrations of these SCs varied greatly within and among the samples. The use of GC–MS analysis provided a powerful technique in the detection and identification of SCs. This study revealed the current and trends of SC use in the Jordanian illicit substance market, which was previously unclear. Future studies are required to explore new SCs and their influence in different biological samples.

INTRODUCTION

Synthetic cannabinoids (SCs) are a class of designed drugs that simulate the effects of Δ⁹-tetrahydrocannabinol; the active substance in cannabis. SCs differ structurally from natural cannabis, but they affect and bind to the same cannabinoid receptors (CB₁ and CB₂) as the latter.¹,² Hundreds of SCs were initially developed for research purposes on the endocannabinoid systems and to investigate their possible therapeutic effects.³,⁴ Both of these G-protein coupled receptors have orthosteric and allosteric sites where ligand can bind to enhance or inhibit their activation.⁵ Activation of CB₁ receptors generally reduces or inhibits the neuronal release of γ-aminobutyric acid (GABA) and glutamate.⁶,⁷ GABA is the most prevalent inhibitory neurotransmitter in the central nervous system, whereas glutamate is a major neurotransmitter utilized in most of the brain’s fast excitatory synapses.⁸ Sixty different SCs were reported by UN member states, whereas most of them belong to the John W. Huffman (JWH) class (JWH-018, JWH-250, JWH-073).⁹ Among them, AB-FUBINACA ([N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide) is a synthetic indazole cannabinoid drug that contains a substituted indazole core.⁹ AB-FUBINACA was originally developed by Pfizer in 2009 as an analgesic medication.¹⁰ In addition, AB-CHMINACA ([N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide) is a cannabinoid receptor modulator synthesized by Pfizer for its potential therapeutic use.¹¹ XLR-11 ([(1-(5-fluoropentyl)-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone) is a nonclassical SC introduced to the drug market as a CB₂ receptor ligand that plays an important role in pain relief.¹⁰,¹¹ Recent studies reported the association of acute kidney injury after XLR exposure.¹²,¹³ Unlike cannabis, the chronic abuse of SCs has been associated with multiple deaths, more dangerous side effects, and toxicity in general.

To focus on the content of herbal blends that were mixed or sprayed with SCs because the vast majority of these blends are toxic materials or poor manufacturing.¹⁴ This work was conducted to identify the presence of three common SCs (AB-FUBINACA, AB-CHMINACA, and XLR-11) and measure their concentrations in the obtained seized samples by gas chromatography–mass spectrometry (GC–MS) method that provides a high level of selectively and sensitivity. In addition, this study may provide a preliminary database that could be utilized for comparative purposes of seized materials from the Jordanian Anti-Narcotics Department of Public Security with other available databases.

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RESULTS

Sensitivity of the GC–MS instrument was daily optimized on perfluorotributylamine according to the instrument manual recommendations. The validation method was modified from the United Nations Office on Drugs and Crime (UNODC) method, by changing the preparation procedures and program temperatures and avoiding derivatization. Linear calibration curves (Figure 1) and linear regression equations with excellent correlation coefficients near 0.999 were obtained for all drug standards (Table 1). The results indicated a good linear proportionality between concentration and response of drug standards (Figure 1 and Table 1).

Our samples were recently seized by the Anti-Narcotics Department on suspicion for containing cannabis-like substance. These products primarily appear in three forms: dried leaves, cigarettes, and powder (Figure 2). The targeted SCs; AB-CHMINACA, AB-FUBINACA, and XLR-11; are characterized by major ions (m/z), molecular weight, retention time (RT), and molecular formula in Table 2. Quantitation was performed for the three most common SCs in Jordanian markets. Detected concentrations of the same SC were varied greatly among the samples. The active AB-FUBINACA was quantified with a RT of 16.00 min (Figure 3). AB-FUBINACA was revealed in the 100 seized samples but could be quantified only in 27 samples with different concentrations, where the highest concentration was 0.11808 mg/g and the lowest concentration was 0.0015 mg/g (Table 3, Figures 4 and 5, respectively). Ninety-nine samples were found to contain AB-CHMINACA at a RT near to 17.00 min (Figure 6) with the highest concentration of 0.03721 mg/g, whereas the quantitation was applicable in five samples only (Figure 7). The lowest concentration of AB-CHMINACA was revealed in sample number 41 (0.00065 mg/g) (Figure 8 and Table 3). Although a suitable column and carrier gas parameters were selected, there is a considerable increase in background/baseline during analysis around 12–18 min RT in the calibration curve of AB-CHMINACA (Figure 6). This limitation resulted from baseline disturbance, which could affect the quantitation of AB-CHMINACA. XLR-11 was the least detected SC where it was found in 42 samples with a RT of 14.00 min, but it was quantified only in 4 samples (Figure 8 and Table 3).

Table 1. Linearity Equations, Relation Coefficients ($R^2$), Precision Values, and LOD of the Three Drugs Standards

| substances | linearity equations | $R^2$ | precision values | LOD (μg/mL) |
|------------|---------------------|-------|------------------|-------------|
| AB-FUBINACA | $\hat{y} = 44,287.60X - 98,577.77$ | 0.999 | 16.10 ± 0.01 | 0.15 |
| AB-CHMINACA | $\hat{y} = 17,748.59X - 7681.52$ | 0.999 | 17.58 ± 0.04 | 0.65 |
| XLR-11 | $\hat{y} = 20,896.07X + 112,053.33$ | 0.999 | 14.83 ± 0.09 | 17.89 |
The highest concentration was 0.19238 mg/g in sample number 62 while the lowest one was found in sample number 89 at 0.01789 mg/g (Figures 10 and 11, respectively, and Table 3). In most samples, the targeted SCs were either below the calibration curve (BC) where the concentration could not be quantified or not detected at all (reported as ND in the Table S1).

![Figure 3. Chromatogram of the reference (stock) standard, AB-FUBINACA. The abundance is observed as the Y-axis and the RT is observed as the X-axis.](https://dx.doi.org/10.1021/acsomega.9b03881)

Table 3. Quantified AB-FUBINACA, AB-CHMINACA and XLR-11 in the Seized Samples

| sample no. | RT (min) | targeted SCs | con. (mg/g) | sample no. | RT (min) | targeted SCs | con. (mg/g) |
|------------|----------|--------------|-------------|------------|----------|--------------|-------------|
| 1          | 16.117   | AB-FUBINACA  | 0.01461     | 41         | 17.560   | AB-CHMINACA  | 0.00065     |
| 2          | 16.105   | AB-FUBINACA  | 0.00433     | 42         | 17.560   | AB-CHMINACA  | 0.00139     |
| 3          | 16.105   | AB-FUBINACA  | 0.00038     | 44         | 16.111   | AB-FUBINACA  | 0.04757     |
| 5          | 16.123   | AB-FUBINACA  | 0.09516     | 47         | 17.577   | AB-FUBINACA  | 0.03721     |
| 6          | 16.105   | AB-FUBINACA  | 0.00920     | 49         | 16.117   | AB-FUBINACA  | 0.05724     |
| 9          | 16.105   | AB-FUBINACA  | 0.00678     | 50         | 16.111   | AB-FUBINACA  | 0.03842     |
| 14         | 16.105   | AB-FUBINACA  | 0.01868     | 52         | 16.099   | AB-FUBINACA  | 0.00548     |
| 16         | 16.105   | AB-FUBINACA  | 0.01775     | 55         | 16.105   | AB-FUBINACA  | 0.03064     |
| 17         | 16.099   | AB-FUBINACA  | 0.00015     | 58         | 16.123   | AB-FUBINACA  | 0.08923     |
| 18         | 16.105   | AB-FUBINACA  | 0.01597     | 59         | 16.123   | AB-FUBINACA  | 0.11808     |
| 19         | 16.099   | AB-FUBINACA  | 0.00215     | 60         | 16.099   | AB-FUBINACA  | 0.00334     |
| 23         | 16.105   | AB-FUBINACA  | 0.01023     | 62         | 14.824   | XLR-11        | 0.19238     |
| 25         | 16.111   | AB-FUBINACA  | 0.04874     | 76         | 16.099   | AB-FUBINACA  | 0.00815     |
| 28         | 17.566   | AB-CHMINACA  | 0.00413     | 81         | 16.105   | AB-FUBINACA  | 0.01643     |
| 29         | 16.111   | AB-CHMINACA  | 0.04110     | 89         | 14.801   | XLR-11        | 0.01789     |
| 30         | 17.566   | AB-CHMINACA  | 0.01000     | 90         | 16.105   | AB-FUBINACA  | 0.04182     |
| 36         | 16.099   | AB-FUBINACA  | 0.00266     | 91         | 14.818   | XLR-11        | 0.13703     |
| 38         | 14.812   | XLR-11       | 0.14537     | 94         | 14.807   | XLR-11        | 0.06648     |
| 39         | 16.111   | AB-FUBINACA  | 0.05457     | 96         | 16.099   | AB-FUBINACA  | 0.02203     |

9). The highest concentration was 0.19238 mg/g in sample number 62 while the lowest one was found in sample number 89 at 0.01789 mg/g (Figures 10 and 11, respectively, and Table 3). In most samples, the targeted SCs were either below the calibration curve (BC) where the concentration could not be quantified or not detected at all (reported as ND in the Table S1).

**DISCUSSION**

The analytical procedure was evaluated and proved to be applicable for SC identification and quantitation. This method helped in avoiding contaminations from derivatizing reagents because derivatization was excluded. The obtained coefficients indicated the linearity of the results, and an excellent correlation between concentration and response for each drug standard was produced. Sensitivity and accuracy are vital factors in drug profiling procedures because any variation in chromatograms can affect the interpretation of the comparison process. Therefore, the accuracy of the entire method was investigated by the precision of RT where sensitivity was measured by the limit of detection (LOD). The LOD was determined to be the lowest concentration yielding an integrated height corresponding to three times the height measured after injection of each drug standard.

The variability among SCs in addition to their rapid structural and appearance changes make it difficult to obtain a single extraction and analytical technique for both identification and quantitation of the newly introduced substances. The extraction procedures adopted in this study were compatible and suitable for the quantitation of the three targeted SCs in the seized samples. The use of this quantitation technique allowed an accurate and rapid determination of the SCs. Based on the GC–MS profiling, some identified chemicals represent the natural composition of the tobacco plant, which is the only type of dried leaf used as a medium for SC consumption.16 Most of the seized samples have more than one SC and the reasons behind such observation can be attributed to the poor manufacturing processes or it can also be...
Figure 4. GC−MS chromatogram of the sample with the highest concentration of AB-FUBINACA (number 59). The abundance is observed as the Y-axis and the RT is observed as the X-axis.

Figure 5. GC−MS chromatogram of the sample with the lowest concentration of AB-FUBINACA (number 17). The abundance is observed as the Y-axis and the RT is observed as the X-axis.

Figure 6. Chromatogram of the reference (stock) standard, AB-CHMINACA. The abundance is observed as the Y-axis and the RT is observed as the X-axis.
Figure 7. GC–MS chromatogram of the sample with the highest concentration of AB-CHMINACA (number 47). The abundance is observed as the Y-axis and the RT is observed as the X-axis.

Figure 8. GC–MS chromatogram of the sample with the lowest concentration of AB-CHMINACA (number 41). The abundance is observed as the Y-axis and the RT is observed as the X-axis.

Figure 9. Chromatogram of the reference (stock) standard, XLR-11. The abundance is observed as the Y-axis and the RT is observed as the X-axis.
attributed to dealers wanting to enhance the impact of their mixtures by adding additional SCs.

Nowadays, SCs are widely used and have become an alternative to marijuana because they are mimicking the effect of its active compound: tetrahydrocannabinol in herbal smoking blends.17,18 AB-FUBINACA is one of our targeted SCs that is commonly used by the drug designers in herbal blends. Its concentration varied greatly in 27 samples ranging from 0.00015 up to 0.11808 mg/g. Variation in the concentrations of active SCs is common and it was reported in the first, second, and third-generation cannabinoids where the variation was reported to be up to sixfold in the studied brands.19−21 There are no or limited published studies investigating the directly associated adverse effects of AB-FUBINACA, whereas several hospitalization reports on its closely related structure, ADB-PINACA.22 ADB-PINACA, a structurally similar indazole carboxamide of AB-FUBINACA, has been found to introduce vomiting, somnolence, hyperkalemia, myocardial infarction, rhabdomyolysis, nausea, seizures, hyperglycemia, tachycardia, and pneumonia following its intake in several hospitalized cases.22 AB-CHMINACA is another SC detected in the seized samples with a concentration range of 0.00065−0.03721 mg/g. This synthetic compound has first appeared in Germany in 2014.23 Different concentrations of AB-CHMINACA and/or its metabolites were reported and quantified in different blood, urine, and tissue samples in several cases including fatal intoxication, diabetic ketoacidosis, suspected impaired driving, acute delirium, and seizures.23−28 Symptoms of acute AB-CHMINACA intoxication were characterized by seizures, delirium, hallucinations, tachycardia, altered mental status, confusion, lack of coordination, and unintelligible speech.27,28 For XLR-11, the assessed quantities were varied greatly among the samples with a minimal detected concentration of 0.01789−0.19238 mg/g. A recent study conducted on several compounds including XLR-11 revealed variation in both the ingredients and concentrations within and among samples of 21 studied brands.29 XLR-11 was identified in herbal mixtures and considered among the top 10 most frequently abused drug in the United States.29 XLR-11 and/or its metabolites were confirmed to be associated with acute kidney injury in different cases in addition to being a cause of death in postmortem
cases.21 XLR-11 intake is represented by nausea, vomiting, elevated serum creatinine, abdominal and back pain, anxiety, seizures, and tachycardia, agitation, irritability, hypertension, and hallucinations.30,31 The amount of SCs in herbal products were recently increased with more than one SCs identified in the product. Between 2011 and 2015, narcotic cases assessed by the Council of Forensic Medicine in Turkey reported that XLR-11, AB-FUBINACA, and AB-CHMINACA were present in 2.20, 4.94, and 6.95%, respectively, in herbal products.32 Data on SC containing herbal product in eight samples analyzed by GC–MS revealed five different compounds with total concentrations from 72 to 303 mg/g. Among those SCs, XLR-11 concentrations were 15 ± 22 and 15 ± 1 mg/g in two of the samples.33 Dunne et al. quantified SC in 63 herbal blends using NMR showing variations depending on the active cannabinoid type with concentrations from 13 to 84 mg/g including AB-FUBINACA and AB-CHMINACA.17 In addition, Fowler et al. measured concentrations from 1.5 to 119 mg/g in 12 products with 50.6 and 118.6 mg/g for the XLR-11.34 Inhomogeneity in herbal mixtures investigated suggested a serious risk for the consumer. XLR-11 was among the detected cannabinoids with concentrations of 40, 39, and 42 mg/g in different herbal mixtures.20 Changes in both the quantity and the quality of SCs in smoking blends may harmfully endanger the consumers where it becomes difficult to estimate the appropriate dose of consumption and the predictable associated symptoms that can dramatically differ. According to an international monitoring study of new psychoactive substances (NPS), SCs continue to account for the majority of NPS followed by synthetic cathinones.36 The Russian Federation Laboratory revealed over the past seven years that there is an increase of nearly 130 times the volume of seizures of synthetic substances in the country from 165 kg up to 22 tons.37 The Turkish National Police reported over 240 NPS including a large number of SCs that have been placed under national control such as AB-CHMINACA, FUB-144, 5F-AMBICA, AM-6257, and various JWH compounds.38 The new SC N-(1-amino-3,3-dimethylxobutan-2-yl)-(1-cyclohexylmethyl)-1H-indazole-3-carboxamide, also known as MAB-CHMINACA has been scheduled as a controlled substance in the State of Louisiana to avoid an imminent peril to public health, safety or welfare.39

Scientific examination evidence, forensic data, and information are necessary to generate an effective response to rapidly growing synthetic drug markets and to complete the currently available international synthetic drug monitoring mechanism.35 Early warning advisory is recommended for enabling timely and comprehensive sharing of exchanging information on the new psychological effects of these illegal SCs as well as analytical methodologies and reference documents with international and regional organizations that are concerned with controlling drug trafficking.

# CONCLUSIONS

This study will help in the development of SC library related to the consumed materials in the Jordanian market for a better understanding and trafficking. In addition, a validated method was developed for the identification and quantitation of SCs in seized samples that proved to be sensitive, precise, accurate, and linear. For prevention and protection, it could be very useful to conducting researches on the toxicological effects of these illegal synthetic compounds in obtained urine and blood samples in the upcoming studies.

# MATERIALS AND METHODS

## Chemicals and Reagents

All reagents and solvents were high performance liquid chromatography- or analytical-grade to get high purity compared to MS solvent. Diphenylamine (DPA), dichloromethane (CH2Cl2), water (H2O), and methanol (CH3OH) were obtained from Merck (AnalaR, Merck BDH, Poole, UK). Hydrochloric acid (HCl) and ammonium hydroxide (NH4OH 25%) were purchased from Fluka (Fluka, Buchs, Switzerland). The SC standards of AB-FUBINACA, AB-CHMINACA, and XLR-11 were adopted from Cayman Chemical Company (Cayman Chemical, Ann Arbor, Michigan).

## Instrumentation

The experimental method was conducted at the Jordanian Forensic Laboratory using an Agilent 7890B gas chromatograph system equipped with an Agilent 5977B mass selective detector where data was processed on Agilent ChemStation software. Sample solutions were quantitatively analyzed using the GC–MS in electron impact mode with an Agilent HP capillary column (30 m × 0.25 mm i.d. and a 0.25 μm film thickness).36 The initial column temperature was 240 °C (hold time 3 min), then it increased gradually at a rate of 5 °C/min until it reaches 330 °C (hold time 23 min). The detector conditions included an ion source temperature of 225 °C and a transfer line temperature of 280 °C. The MS parameters instruct solvent delay for 3 min, scan mode total ion scan, and scanning mass range 40–600 amu at 2.17 scan/s.15,39

## Sample Selection and Extraction

One hundred randomly chosen samples of different herbal blends among samples seized in the period between 2017 and 2018 were used in this study (Figure S1). Official approval was obtained from Forensic Laboratory Directorate in Jordan (FLD) for the use of seized materials supplied by the Anti-Narcotics Department of Public Security.

The extraction procedure was applied to the samples by adding methanol that has the preferred requirements and recovery with the GC–MS method to 100 mg of the seized materials (dried leaf or cigarette) or 1–2 mg of the powder form. Mixtures were vortexed for 5 min and afterward centrifuged to isolate the organic layer which was stored or directly injected into the GC–MS.15

## Sample and Standard Preparation

The experimental procedures applied in this thesis were adopted from a published manual of the National Drug Analysis Labortoratories.15

## Internal Standard Preparation

DPA was used as an internal standard (IS) to verify suitable method performance. Homogenization was carried out using a 1000 mL volumetric flask where 100 mg of the IS diluted with ethanol to give a concentration of 100 μg/mL (100 ppm).15,90 This solution is prepared to meet a good linear calibration curve and to dilute the standard stock solution into different concentrations (1, 5, 10, 20, 100, and 200 ppm).

## Reference Standard Preparation

Different concentrations of the three reference standards including AB-FUBINACA, AB-CHMINACA, and XLR-11 were prepared and used started with the lowest possible detection limit of each standard. Standards were commercially available at a concentration of 1000 ppm. For each standard, 1 mg was diluted to a volume of 1 mL where it can be used directly or stored in the refrigerator for at least one year.

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Sample Solution Preparation. Using the electrical grinder, 200 mg of each sample was crushed into powder. The homogenized solution was prepared by diluted each sample with the IS into a 100 mL volumetric flask wherein 5 μL of each homogenized solution was injected into the GC using an Agilent 7693 autosampler.

Method Validation. The GC–MS method of detection in this study was slightly modified from the methods described by the UNODC.15 The obtained seized samples were processed with the liquid–liquid extraction procedure and then chromatographed to determine which SCs match the selected standards with a determined concentration. Validation of the quantitative and/or qualitative analysis requires certain parameters including, selectivity, the LOD, linearity, accuracy, and precision. The method was revalidated to adopt the changes in preparation of the reference standards, preparation of the IS and preparation of the samples, program temperatures, and exclusion of the derivatization, whereas LOD were determined by S/N (signal-to-noise) ratio > 3.

■ ASSOCIATED CONTENT

+ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03881.

Pictures of the seized samples and program of the targeted SCs in the 100 seized samples (PDF)

■ AUTHORS INFORMATION

Corresponding Author

Laith N. AL-Etian — Department of Applied Biological Sciences and Department of Biotechnology and Genetic Engineering, Jordan University of Science and Technology, Irbid 22110, Jordan; orcid.org/0000-0003-0064-0190; Phone: +962-2-7201000 ext. 23464; Email: lneitan@just.edu.jo

Abdelqader S. Asa‘ad — Department of Legal Medicine, Toxicology and Forensic Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan; Drug and Alcohol Analysis Department, Forensic Science Laboratories, Public Security Directorate, Amman 11942, Jordan

Abdel Kader H. Battah — Department of Pathology, Microbiology and Forensic Medicine, School of Medicine, The University of Jordan, Amman 11942, Jordan

Hanan A. Aljamal — Department of Applied Biological Sciences, Jordan University of Science and Technology, Irbid 22110, Jordan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03881

Notes

The authors declare no competing financial interest.

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