Extraordinary long detection window of a synthetic cannabinoid metabolite in human urine – Potential impact on therapeutic decisions

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Abstract
Synthetic cannabinoids (SCs) have become established drugs of abuse. They play an increasing role in drug therapy, where abstinence control testing is required. Differentiation between recent drug uptake and uptake in the distant past is important for drug therapy. This study aimed to evaluate the detection window of a metabolite commonly used as a consumption marker for AB-FUBINACA and AMB-FUBINACA (synonym: FUB-AMB) in urine analysis. The acidic hydrolysis metabolite was quantified in urine samples of a drug user by applying a validated analytical method. The concentration profile of the metabolite was correlated with usage data of the subject. Pharmacokinetic properties of AB-FUBINACA were collected by analysis of serum and urine samples from a controlled administration study (single oral ingestion of AB-FUBINACA). Thirteen urine samples were taken without advance notice over 2 years. The metabolite was detected in the first urine sample at 0.77 ng/mg creatinine and subsequently in concentrations ranging from 0.06 to 0.29 ng/mg creatinine. Usage data showed credible abstinence from SCs during this period. The pharmacokinetic properties observed within the controlled self-administration study supported the hypothesis of distribution into deeper compartments and long-lasting elimination (serum concentration–time curve showing biphasic kinetics). An elimination phase of over 1 year after the last drug uptake seems plausible in cases of extensive consumption. To avoid misinterpretation of positive findings, we recommend testing patients with known SC use at the beginning of the abstinence program and to re-test continuously at short time intervals. These data enable the correct interpretation of analytical findings.

Keywords
AB-FUBINACA, AMB-FUBINACA, detection window, drug elimination, drug testing, FUB-AMB, synthetic cannabinoid receptor agonists, urine analysis
1 | INTRODUCTION

New psychoactive substances (NPS) have gained high popularity over the past decade and have become established drugs of abuse. With about 180 different compounds currently monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), synthetic cannabinoids (SCs) represent the largest group of NPS. Synthetic cannabinoid receptor agonists induce cannabis-like effects and are commonly consumed by smoking. Products containing SCs include ready-to-smoke herbal mixtures ("incense blends") and liquids for e-cigarettes ("cannabinoid liquids") but also highly pure substances in powder form ("research chemicals"). Such drug preparations are easily available via the Internet and are often believed to be legal, harmless, and non-detectable by drug tests. For these reasons SCs are increasingly being used as cannabis alternatives among populations undergoing drug abstinence control testing and among people with restricted access to classical drugs of abuse. In particular, the abuse of SCs has become a serious problem in penitentiaries and forensic-psychiatric clinics and probably also play a role in institutions incorporating drug withdrawal therapy. Besides the acute toxicity of these compounds, there are increasing concerns about the dependence potential and other psychiatric conditions (e.g. psychosis) associated with the consumption of SCs, making this class of drugs relevant for drug therapeutic casework in general.

Since undetected consumption of SCs may hamper the therapeutic process, abstinence control testing can be very important. Urine is typically the preferred sample material for abstinence control testing as sampling is non-invasive and urine usually provides a wider detection window for most drugs of abuse when compared with other body fluids (e.g. blood or oral fluid). In contrast to hair or oral fluid testing – where contamination can be a problem – a positive urine test result unambiguously proves drug uptake.

We received an increasing number of urine samples to be tested for SCs and their metabolites mainly from psychiatric institutions over the past few years that reflected the increasing relevance of these drugs within the abstinence control environment. Positive test results may indicate therapeutic non-compliance and may have serious consequences for the patient (e.g. cessation of therapy and dismissal from clinic, or prison for forensic patients). Hence, correct interpretation of analytical findings is of utmost importance.

A positive finding can typically raise questions about the time point of drug consumption to differentiate between recent and more distant substance use. Positive findings detected in consecutive samples with a time distance of several weeks and relatively low concentrations can be challenging in this context, in particular when the question arises as to whether the respective substance was consumed again between two sampling points. Another issue is the possibility of passive exposure to side stream smoke as a cause of positive test results. Unfortunately, patients’ statements regarding their substance use are often not reliable and the scientific data that could support a valid interpretation regarding the pharmacokinetic/toxicokinetic behavior of these drugs is scarce. During the past few years we encountered several exemplary cases with long elimination times over several months involving SCs and/or their metabolites after cessation of consumption. This observation indicates pronounced distribution into deeper compartments (e.g. fatty tissue) similar to other lipophilic drugs such as ∆9-tetrahydrocannabinol (THC) particularly following frequent and extensive use.

In the following we illustrate the difficulties in the differentiation between immediate drug intake and uptake in the distant past using the popular SCs AB-FUBINACA and AMB-FUBINACA (synonym: FUB-AMB) as examples. There is an extraordinarily long renal elimination time of a common main metabolite of both SCs (N-[1-(4-fluorobenzyl)-1H-indazol-3-yl][carbonyl]valine). This acidic hydrolysis metabolite is an important analytical target for urine screening as the parent compounds themselves are usually not excreted in urine to a relevant extent (Figure 1).

In order to verify the hypothesis of long terminal elimination, the pharmacokinetic properties of AB-FUBINACA were collected by a controlled low-dose administration study. This study was conducted in order to provide reliable data for correct interpretation of positive metabolite findings in abstinence control.

2 | METHODS

2.1 | Chemicals and reagents

Formic acid (Rotipuran® ≥ 98%, p.a.), sodium hydroxide (≥ 99%, p.a., pellets), potassium hydrogen phosphate (≥ 99%, p.a.) were obtained from Carl Roth (Karlsruhe, Germany), acetonitrile (LC-MS grade), ammonium formate 10 M (99.995%) were from Sigma Aldrich.

**FIGURE 1** Chemical structure of AB-FUBINACA, AMB-FUBINACA and their common hydrolysis metabolite N-[1-(4-fluorobenzyl)-1H-indazol-3-yl][carbonyl]valine.
AB-FUBINACA (N-[1-amo3-thyl-1-oxobutan-2-yl]-1-[4-fluorophenyl]methyl]-1H-indazole-3-carboxamide), AMB-FUBINACA (methyl 2-[[1-[4-fluorophenyl]methyl]-1H-indazole-3-carbonyl][amino]-3-methylbutanoate), the acidic hydrolysis metabolite (N-[1-(4-fluorobenzyl)-1H-indazol-3-yl][carbonyl]valine), and D9-ADBICA (N-[1-amino-3,3-dimethyl-1-oxobutan-2-yl]-1-[pentyl-2.2,3,3,4,4',5,5,5-d9]-1H-indole-3-carboxamide) were purchased from Cayman Chemical (Ann Arbor, MI, USA). Roche Diagnostics (Mannheim, Germany) produced the β-glucuronidase (E. coli K12) used for conjugate cleavage. Deionized water was prepared using a Medica® Pro deionizer from ELGA (Celle, Germany). Blank urine samples were donated by a volunteer and tested for the absence of SC metabolites prior to use. Mobile phase A (1% acetonitrile, 0.1% formic acid, 2 mM ammonium formate in water) and mobile phase B (0.1% formic acid, 2 mM ammonium formate in acetonitrile) were freshly prepared prior to analysis.

2.2 | Quantification of N-[1-(4-fluorobenzyl)-1H-indazol-3-yl]carbonylvaline in urine samples

An analytical method was developed and fully validated for the detection of N-[1-(4-fluorobenzyl)-1H-indazol-3-yl]carbonylvaline in urine. Method validation was performed according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh).

2.2.1 | Instrument

A Nexera X2 UHPLC (Shimadzu, Duisburg, Germany) coupled to a QTRAP™ 5500 triple quadrupole linear ion trap instrument (Sciex, Darmstadt, Germany) was utilized for the validation and analysis of the urine samples. Chromatographic separation was performed on a Kinetex® C18 column (2.6 μm, 100 Å, 100 × 2.1 mm; Phenomenex, Aschaffenburg, Germany) applying gradient elution as follows: Starting condition of mobile phase B was 30%, linearly increased to 40% in 2.5 min, further increased to 70% in 2.0 min, further increased to 90% in 0.5 min, held for 2.0 min, decreased to starting conditions of 30% in 0.5 min, and held for 1.5 min for re-equilibration. The flow rate was set to 0.5 mL/min. Autosampler and column oven temperature were set to 10°C and 40°C, respectively. The injection volume was 10 μL. The mass spectrometer was operated in positive electrospray ionization mode. Multiple reaction monitoring (MRM) scan mode was applied for analysis and the respective potentials for the monitored ion transitions were carefully optimized (Table 1). Analyst software 1.6.2 (Sciex, Darmstadt, Germany) and Valistat software 2.0 (Arvecon GmbH, Germany) were used for data evaluation.

2.2.2 | Urine samples from abstinence control

Thirteen urine samples were collected from a drug user without advance notice over a time period of 2 years (from June 2017 to June 2019) together with self-reported drug consumption behavior.

2.2.3 | Preparation of authentic urine samples

After the addition of 0.5 mL phosphate buffer (pH 6) and 30 μL β-glucuronidase to 0.5 mL of urine the samples were incubated at 45°C for 1 h. Afterwards, 1.5 mL ice-cold acetonitrile, 0.5 mL of a 10 M ammonium formate solution and 20 μL of a D9-ADBICA solution (10 ng/mL) were added. The mixture was shaken and centrifuged. 1 mL of the organic layer was transferred into a separate vial and evaporated to dryness under a stream of nitrogen at 40°C. Finally, the samples were reconstituted in 200 μL mobile phase A/B (50/50, v/v) prior to LC-ESI-MS/MS analysis. Negative control samples (blank urine) and calibration samples (blank urine spiked at 0.15, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, and 10 ng/mL) were prepared accordingly.

2.3 | Self-administration study

A controlled self-administration study was conducted to test the hypothesis of long terminal elimination of the drug metabolite, which was approved by the Ethics Committee of the University of Freiburg. Therefore, one of the authors (Caucasian male, 28 years old, 1.82 m body height, 75 kg body weight) orally ingested 5 mg of pure AB-FUBINACA powder in a capsule. Blood and urine samples were collected after a single drug ingestion over a time period of 19 and

| TABLE 1 | Optimized mass spectrometric parameters of the multiple reaction monitoring (MRM) ion transitions of N-[1-(4-fluorobenzyl)-1H-indazol-3-yl]carbonylvaline |
| Q1 [Da] | Q3 [Da] | DP [eV] | EP [eV] | CE [eV] | CXP [eV] |
|----------|----------|--------|--------|--------|--------|
| 370      | 253      | 66     | 10     | 27     | 10     |
| 370      | 109      | 66     | 10     | 49     | 16     |
| Q1       | m/z of the precursor ion |
| Q3       | m/z of the fragment ion |
| DP       | Declustering potential |
| EP       | Entrance potential |
| CE       | Collision energy |
| CXP      | Collision cell exit potential |
21 days, respectively. Control samples were collected directly before drug ingestion. Serum samples were analyzed using a partially validated, semi-quantitative LC–MS/MS method (LOD: 0.04 ng/mL, linearity: 0.1–1 ng/mL).11

The concentration of the acidic hydrolysis metabolite of AB-FUBINACA and AMB-FUBINACA was determined in all urine samples obtained from the authentic case and the administration study with the previously mentioned fully validated method.

3 | RESULTS

3.1 | Method validation

The method validated for the quantification of \(N\)-[[1-(4-fluorobenzyl)-1H-indazol-3-yl]carbonyl]valine in urine samples showed linearity in the concentration range between 0.15 and 10.0 ng/mL (coefficient of determination \(r^2\) of 0.9979). Selectivity of the method was proven by analysis of structurally similar SCs and their metabolites, where no interferences were observed. The limit of detection (LOD) and limit of quantification (LOQ) were 0.05 and 0.15 ng/mL, respectively. Precision (RSD 4.8–12%), accuracy (bias 0.6–2.1%), and matrix effects (recovery 110 ± 1.6%) were within the ranges recommended by the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh).

Metabolite concentration in urine was normalized to creatinine concentration for better comparability (ng/mg creatinine).

3.2 | User data

The drug user was a Caucasian male (November 2015: 19 years old, 1.95 m body height, 75 kg body weight) and stated extensive consumption of herbal mixtures between November 2015 and July 2016 of up to several joints per day. The stated motivation for consumption of SCs was the perceived non-detectability of the drug in standard drug tests as the person was subject to frequent abstinence control testing for alcohol and cannabis consumption as part of a psychological evaluation for driver license regranting. He successfully passed the evaluation in February 2017. The subject stated a period of abstinence from SCs from July 2016 to March 2017 followed by a new period of extensive consumption in April 2017 of about 2 weeks leading to another withdrawal of his driver license. He reported intermittent amnesia during the second period of consumption and pronounced withdrawal symptoms after stopping use, associated with a significant weight loss of about 20 kg within a couple of weeks. As driving under the influence of SCs was the cause of the second withdrawal of his driver license, an abstinence control program between June 2017 until June 2019 also covered urine screening for SCs and their metabolites.

3.3 | Metabolite quantification in urine samples (abstinence control)

The common acidic hydrolysis metabolite of the SCs AB-FUBINACA and AMB-FUBINACA was detected in all 13 urine samples. The first urine sample from June 2017 showed a concentration of 0.77 ng/mg creatinine. In the following 12 urine samples the metabolite was detected in lower concentrations ranging from approximately 0.06 to 0.29 ng/mg creatinine (Figure 2).

Since one of the urine samples (sampling date: 11/2019, Figure 2) showed a concentration below the LOQ (but above the LOD), the concentration was extrapolated. Prior to metabolite quantification, analysis of the urine samples with a screening method for SCs and their metabolites resulted in no additional findings (including specific metabolites of AB-FUBINACA and AMB-FUBINACA).12

3.4 | Self-administration study

The resulting serum concentration–time curve of AB-FUBINACA showed biphasic kinetics with a short primary distribution phase lasting about 5 hours (calculated half-life 9.8 h) and a longer terminal
elimination phase lasting about 60 hours (calculated half-life 42 h) (Figures 3 and 4). AB-FUBINACA was detected first 11 min after ingestion (test person was in a fasting state) and reached the maximum measured concentration after 1.9 h \( (t_{\text{max}}) \). The \( c_{\text{max}} \) was above the validated calibration range (upper limit of quantification: 1 ng/mL). The extrapolated concentration was approximately 2.3 ng/mL. In total, the drug was detected over a time period of 72.2 hours in serum. No AB-FUBINACA was detected in the control sample collected before drug ingestion. Apart from mild tachycardia, light vertigo, and a reduced ability to concentrate, no effects were noted by the volunteer. This was expected from the pronounced first-pass effect known from other SCs.\(^{13}\)

In urine, the respective metabolite showed a comparatively large detection window of about 13 days (311 h) after a single oral ingestion of the drug (Figure 3). The metabolite was detected in the first urine sample taken 100 min after drug ingestion. The maximum measured concentration was reached after 7.1 h \( (t_{\text{max}}) \). The \( c_{\text{max}} \) was above the validated calibration range (upper limit of quantification: 10 ng/mL). The extrapolated concentration was approximately 48 ng/mL (corresponding to 267 ng/mg creatinine). The parent compound AB-FUBINACA was not detected in urine to a relevant extent compared with the corresponding metabolite. The acidic hydrolysis metabolite was not detected in the control sample collected before drug ingestion. AB-FUBINACA-specific, hydroxylated metabolites were detected for maximum 97 h (data not shown).

4 | DISCUSSION

Evaluating the case samples, each analytical finding suggested intake of AB-FUBINACA or AMB-FUBINACA prior to the sampling date. However, maintaining this constantly low concentration level over such a large time period by controlled, low-dosed intake seems extremely unlikely. Users of herbal mixtures usually do not have information on the type and dose of SCs contained in the products. Consequently, a changing pattern of SCs as well as varying concentration levels have to be expected during persistent use of herbal mixtures.\(^{14}\) From this perspective, a prolonged renal elimination of this metabolite seems to be the most plausible explanation for the observed findings. Biphasic kinetics of AB-FUBINACA was demonstrated by the self-administration study. Furthermore, although a first-pass effect has to be expected after oral uptake, extraordinarily long detection windows for the drug and its acidic hydrolysis metabolite were noted. Both findings support the hypothesis of distribution into deeper compartments and prolonged renal elimination. Given a period of extensive consumption, an elimination phase of several months and even over 1 year after the last uptake seems plausible. Consequently, drug abstinence starting in April 2017 could be attested for the subject of the case presented here, although a metabolite of SCs was detected in all urine samples. The presented data enable correct interpretation of analytical findings and can support drug therapy (e.g. discussion of relapse causes) or justify disciplinary measures.

Considering the presented data for AB-FUBINACA and the few pharmacokinetic data available in scientific literature, it has to be assumed that detection windows can be extraordinarily long also for other structurally related SCs and their metabolites. Also self-administration studies of the well-known SCs JWH-018 and AM-2201 revealed long detection windows (several weeks for JWH-018,
From this experience, we recommend testing patients with known SC use at the very beginning of the abstinence program and to re-test continuously in short intervals in order to ensure reliable detection of a new event of consumption and to avoid misinterpretation of positive findings. Regarding the often stated "passive exposure defense": an exposure to the side stream smoke of cigarettes containing SCs is not necessarily perceivable by the exposed person due to the absence of typical odors. In a situation where, for example, a roommate shows urine concentrations of more than an order of magnitude lower than other patients, passive exposure should be taken into consideration as a cause of the finding. Again, repeated testing is recommended after stopping the exposure.

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CONFLICT OF INTEREST
The authors declare no competing financial interests.

CLINICAL TRIAL REGISTRATION DETAILS
The controlled self-administration study was approved by the Ethics Committee of the University of Freiburg under the registration number 411/14.

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