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Simultaneous Quantification of 20 Synthetic Cannabinoids and 21 Metabolites, and Semi-quantification of 12 Alkyl Hydroxy Metabolites in Human Urine by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Clandestine laboratories constantly produce new synthetic cannabinoids to circumvent legislative efforts, complicating toxicological analysis. No extensive synthetic cannabinoid quantitative urinary methods are reported in the literature. We developed and validated a liquid chromatography tandem mass spectrometric (LC-MS/MS) method for simultaneously quantifying JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, RCS-4, AM-2201, MAM-2201, UR-144, CP 47,497-C7, CP 47,497-C8 and their metabolites, and JWH-203, AM-694, RCS-8, XLR-11 and HU-210 parent compounds in urine. Non-chromatographically resolved alkyl hydroxy metabolite isomers were considered semi-quantitative. β -glucuronidase hydrolyzed urine was extracted with 1 ml Biotage SLE+ columns. Specimens were reconstituted in 150 μ L mobile phase consisting of 50% A (0.01% formic acid in water) and 50% B (0.01% formic acid in 50:50 methanol:acetonitrile). 4 and 25 μ L injections were performed to acquire data in positive and negative ionization modes, respectively. The LC-MS/MS instrument consisted of a Shimadzu UFLCxr system and an ABSciex 5500 Qtrap mass spectrometer with an electrospray source. Gradient chromatographic separation was achieved utilizing a Restek Ultra Biphenyl column with a 0.5 ml/min flow rate and an overall run time of 19.5 and 11.4 min for positive and negative mode methods, respectively. Quantification was by multiple reaction monitoring with CP 47,497 compounds and HU-210 ionized via negative polarity; all other analytes were acquired in positive mode. Lower and upper limits of linearity were 0.1–1.0 and 50–100 μ g/l ($r^2 > 0.994$). Validation parameters were evaluated at three concentrations spanning linear dynamic ranges. Inter-day analytical recovery (bias) and imprecision (N=20) were 88.3–112.2% and 4.3–13.5% coefficient of variation, respectively. Extraction efficiencies and matrix effect (N=10) were 44–110 and –73 to 52%, respectively. We present a novel LC-MS/MS method for simultaneously quantifying 20 synthetic cannabinoids and 21 metabolites, and semi-quantifying 12 alkyl hydroxy metabolites in urine.

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Keywords

synthetic cannabinoids; urine; metabolites; analytical method; LCMSMS

1. INTRODUCTION

Synthetic cannabinoids bind CB1 and/or CB2 receptors and were originally developed for studying endocannabinoid pharmacology; however, now are abused drugs smoked or inhaled for psychoactive effects, but deceptively marketed as herbal incenses and air fresheners. Synthetic cannabinoid abuse resulted in increases in emergency room visits and occasional deaths [1–3]. Synthetic cannabinoid subclasses include naphthoylindoles (JWH-015, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210 and JWH-398), phenylacetylindoles (JWH-203, JWH-250, JWH-251, and RCS-8), benzoylindoles (RCS-4 and AM694), cyclohexylphenols (CP 47,497 C7 and C8 analogs) and dibenzopyrans (HU-210).

In July 2012 the United States Drug Enforcement Agency classified JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-250, JWH-398, AM694, AM2201, RCS-4, RCS-8, HU-210, CP 47,497-C7, CP 47,497-C8 and their analogs as schedule I controlled substances [4,5]. Recently, UR-144, XLR11 and AKB48 were temporarily added to the Schedule I controlled substance list [6]. Most countries enacted similar legislation. Clandestine laboratories constantly synthesize new compounds in response to legislative efforts, complicating drug testing.

New synthetic cannabinoid structures may not cross-react in antibody-based techniques, leading laboratorians to consider mass spectrometric screening [7–10]. Mass spectrometry is flexible, allowing incorporation of new analytes as rapidly as reference standards become available. We recently published a liquid chromatography tandem mass spectrometric (LC-MS/MS) qualitative screening method employing spectral library searching simultaneously targeting 9 synthetic cannabinoids and 20 metabolites in urine [8]. Urinary quantitative methods were only published for single parent analytes and metabolites [11,12] or for metabolites of JWH-018 and JWH-073 [13–15]. The most comprehensive urine quantification method reported to-date targets 8 parent analyte families [16]. A comprehensive, up-to-date quantitative confirmatory synthetic cannabinoid method is required for confirming presumptive positive and negative screening results, comparing screening techniques and evaluating optimal cutoff concentrations. We present a fully-validated LC-MS/MS method targeting 53 analytes: JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, RCS-4, AM2201, MAM2201, UR-144, CP 47,497-C7, CP 47,497-C8 and their metabolites, and JWH-203, AM694, RCS8, XLR11 and HU210 parent compounds in urine. Non-chromatographically resolved alkyl hydroxyl metabolite isomers were semi-quantitative.

2. METHODS

2.1. Reagents and supplies

All standards and deuterated internal standards were purchased from Cayman Chemical (Ann Arbor, MI), except 11-nor-9-carboxy-tetrahydrocannabinol-d₉ was from Cerilliant (Round Rock, TX). Ammonium acetate, formic acid, acetonitrile and ethyl acetate were obtained from Sigma-Aldrich (St. Louis, MO), and methanol from Fisher Scientific (Fair Lawn, NJ). Water was purified by an ELGA Purelab Ultra Analytic purifier (Siemens Water Technologies, Lowell, MA). All solvents were HPLC grade or better. Abalone beta-glucuronidase powder containing 1,500,000 units/gram beta-glucuronidase and 150,000

units/g sulfatase was diluted with distilled water to contain 100,000 units/ml beta-glucuronidase and 10,000 units/ml sulfatase activity for enzymatic hydrolysis (Campbell Science, Rockton, Illinois). 1-ml Isolute SLE+ cartridges were utilized for preparing samples (Biotage, Inc, Charlotte, NC). A Cerex System 48 positive pressure manifold (SPEware Corp, Baldwin Park, CA) was employed for specimen extraction. Resprep C18 (3 ml/200 mg, Restek Inc, Bellefonte, PA) and Strata C8 solid phase extraction columns (6 ml/500 mg, Phenomenex, Torrance, CA) were evaluated during method development. Analytical chromatography was performed on an Ultra Biphenyl HPLC column (100 × 2.1 mm; 3 μm particle size) combined with a 10 × 2.1 mm guard column of identical phase purchased from Restek.

2.2. Instrumentation

An ABSciex API 5500 QTRAP® triple quadrupole/linear ion trap mass spectrometer with a TurboIonSpray source operated in electrospray (ESI) mode (ABSciex, Foster City, CA) was coupled with an LC-20ADxr high performance liquid chromatography (HPLC) system (Shimadzu Corp, Columbia, MD). Analyst version 1.6.1 and Multiquant 2.1 were employed for data acquisition and analysis, respectively.

2.3. Calibrators, quality control and internal standards

Blank urine was evaluated to ensure absence of detectable synthetic cannabinoids or metabolites prior to fortification with working stock solutions to prepare calibrators and quality control samples. Primary stock solution containing 53 synthetic cannabinoids and metabolites at 1000 μg/l was prepared in methanol (see analyte list in Table 1). Dilutions of the stock solution created calibrators at 0.1, 0.2, 0.5, 1.0, 5.0, 10, 25, 50 and 100 μg/l when fortifying 20 μL standard solution into 200 μL blank human urine.

Quality control (QC) samples were prepared with different vials of reference standard solutions than calibrators. Three mixed QC working solutions containing the same analytes as present in calibrators (see Table 1), ranging from 0.3–30 μg/l, were prepared in methanol (see Table 2 for analyte QC concentrations).

Deuterated internal standard (N=24, see Table 1) stock solutions were diluted in methanol producing a mixed internal standard solution of 10 μg/l. 20μL of 10 μg/l mixed internal standard solution was added to blank urine, yielding 1 μg/l fortified urine internal standard concentrations.

All primary and working solutions were stored at –20°C in amber glass vials.

2.4. Specimen preparation approaches evaluated during method development

Preliminary synthetic cannabinoids recovery studies were conducted in triplicate during method development to evaluate potential sample preparation approaches with Resprep C18 columns, Strata C8 columns and SLE. Three sets of specimens were prepared: urine fortified prior to extraction, urine fortified after extraction and neat.

For Resprep C18 and Strata C8 sample preparations, 0.5 ml blank urine containing 10 μg/l JWH-018, RCS8, JWH-250 N-5-hydroxypentyl and JWH-250 N-5-carboxypentyl was diluted with 2.5 ml 400mM ammonium acetate buffer, pH 4.0 prior to addition of 50 μL glucuronidase solution (100,000 units glucuronidase activity/ml). Screwtop glass tubes were capped and incubated at 55°C for 2 h, then centrifuged at 1600g, 4°C for 5 min prior to application on conditioned columns. Resprep C18 and Strata C8 columns were conditioned with acetonitrile and 400 mM ammonium acetate buffer, pH 4.0 and washed with 3 ml water and 400 mM ammonium acetate buffer, pH 4.0:acetonitrile (80:20, v/v). Columns were

dried via 40 psi positive pressure for 5 min prior to elution with 3 ml 2% glacial acetic acid in acetonitrile followed by 3 ml hexane:ethyl acetate (90:10, v/v). Combined eluents were dried completely under nitrogen at 40°C prior to reconstitution with 150 µL mobile phase A:B 50:50 (v/v).

For SLE preparation, 200 µL blank urine containing 10 µg/l JWH-018, RCS8, JWH-250 N-5-hydroxypentyl and JWH-250 N-5-carboxypentyl was diluted with 0.3 ml 400 mM ammonium acetate buffer, pH 4.0 prior to addition of 40 µL glucuronidase solution (100,000 units glucuronidase activity/ml). Polypropylene microcentrifuge tubes were capped and incubated at 55°C for 2 h. Samples were centrifuged at 15,000g, 4°C for 5 min after addition of 0.5 ml acetonitrile. Samples were transferred onto SLE columns and gently driven onto column phase with fine pressure control by slowly increasing pressure up to 1 l/min (achieving 21 ml/min through each column). After equilibration at ambient pressure for 5 min, analytes were eluted with 6 ml ethyl acetate into 16×100 mm conical polypropylene tubes. Positive pressure was gradually applied up to 5 l/min (100 ml/min through each column) with fine pressure control until elution was complete. All sample extracts were completely dried at 45°C under nitrogen in a Zymark TurboVap. Samples were reconstituted in 150 µL mobile phase A:B 50:50 (v/v), vortexed 15 s prior to centrifugation at 4°C, 4000g for 5 min and transferred to autosampler vials containing 200 µL glass inserts.

2.5. LC-MS/MS

Chromatographic separation was performed on an Ultra Biphenyl column equipped with a guard column containing identical packing material. Two LC-MS/MS methods were required, a 19.5 min positive ionization mode method with 4 µL injection volume and a 11.4 min negative ionization mode method with 25 µL injection volume. For positive and negative mode methods, the column oven and auto-sampler were maintained at 40 and 4°C, respectively. Gradient elution was performed for both methods with (A) 0.01% formic acid in water and (B) 0.01% formic acid in acetonitrile:methanol (50:50, v/v) at a flow rate of 0.5 ml/min. The initial gradient conditions for the positive mode method were 40% B, held for 30 s, then increased to 90% B over 14.5 min, increased to 98% B at 15.1 min held until 17.6 min, returned to 40% B at 17.7 min and held until 19.5 min. Flow rate was ramped from 0.5 ml/min to 1.0 ml/min at 15.2 min and returned to 0.5 ml/min at 18.0 min. HPLC eluent was diverted to waste for the first 1.5 min and after 16.5 min of analysis. Initial gradient conditions for the negative mode method were 40% B, held for 30 s, then increased to 90% B over 6.5 min, increased to 98% B at 7.1 min held until 9.5 min, returned to 40% B at 9.6 min and held until 11.4 min. Flow rate was ramped from 0.5 ml/min to 1.0 ml/min at 7.3 min and returned to 0.5 ml/min at 9.9 min. The divert valve was directed to waste for the first 3.0 min and after 7.1 min.

Mass spectrometric data were collected in scheduled multiple reaction monitoring (MRM) mode with a target scan time of 0.5 s. Positive and negative mode method MRM detection windows were 50 and 40 s, respectively. CP 47,497-C7, CP 47,497-C7 C7-hydroxydimethylheptyl metabolite, CP 47,497-C8, CP 47,497-C8 C8-hydroxydimethyloctyl metabolite, HU210, CP 47,497-C7-d₁₁, CP 47,497-C8-d₇ and 11-nor-9-carboxy-tetrahydrocannabinol-d₉ were acquired in negative ionization mode; all other analytes and internal standards were acquired in positive ionization mode. MS/MS parameters (Table 1) were optimized via direct infusion of individual analytes at 10 or 50 µg/l in initial mobile phase for positive and negative mode, respectively. Optimized source parameters for positive and negative modes were: gas-1 60, gas-2 50, curtain gas 45, source temperature 500°C; ion spray voltage was 5500 and -4500 for positive and negative modes, respectively. Nitrogen collision gas was set at medium for all experiments. Quadrupoles one and three were set to unit resolution. Quantifier and qualifier ion transitions were monitored for each analyte and internal standard.

2.6. Hydrolysis optimization

Hydrolysis conditions were optimized with blank urine fortified to contain 2500 µg/l JWH-018 N-5-hydroxypentyl-glucuronide. Amount of enzyme, pH, temperature and duration of incubation were evaluated for optimally hydrolyzing glucuronides.

2.7. Data analysis

Peak area ratios of analytes to corresponding internal standards were calculated for each concentration to construct daily calibration curves via linear least-squares regression with a $1/x^2$ weighting factor. See Table 3 for calibration linear ranges.

2.8. Method validation

Specificity, sensitivity, linearity, imprecision, analytical recovery, extraction efficiency, matrix effect, stability, dilution integrity and carry-over were evaluated during method validation.

2.9. Specificity

Analyte peak identification criteria were relative retention time within ± 0.1 min of the lowest calibrator and qualifier/quantifier transition peak area ratios $\pm 20\%$ of mean calibrator transition ratios. We employed ± 0.1 min as a peak identification retention time requirement based upon our observations of calibrator retention time drift during method development. Retention times did not drift by more than ± 0.05 min, but we employed a wider ± 0.1 min retention time window requirement because larger retention time variation is expected with authentic specimen analysis over time. Potential endogenous interferences were assessed by analyzing ten blank urine specimens from different individuals. In addition, 83 potential interferences from commonly used drugs were evaluated by fortifying drugs into low QC samples. Final interferent concentrations were 500 µg/l (see Supplementary Table 1 for interferent list). Synthetic cannabinoids also were individually fortified into low QC samples at 200 µg/l (20 µg/l for parent analytes and hydroxyindole minor metabolites). No interference was noted if all analytes in the low QC sample quantified within $\pm 20\%$ of target concentrations with acceptable qualifier/quantifier transition ratios. At least 25 scans were acquired across each peak for accurate peak area determination.

2.10. Sensitivity and linearity

Limit of detection (LOD) was evaluated over three runs with duplicates from 3 different urine sources and defined as the lowest concentration producing a peak eluting within ± 0.1 min of analyte retention time for the lowest calibrator with signal-to-noise 3:1, Gaussian peak shape and qualifier/quantifier transition peak area ratios $\pm 20\%$ of mean calibrator transition ratios for all replicates. Limit of quantification (LOQ) also was evaluated in the same manner, and defined as the lowest concentration that met LOD criteria with signal-to-noise 10:1 and measured concentration within $\pm 20\%$ of target. Performance at the LOQ was confirmed in each batch of specimens and was each analytes' lowest limit of linearity.

Preliminary experiments with six sets of calibrators determined the most appropriate calibration model comparing goodness-of-fit via normalized residuals inspection for unweighted linear least squares, linear least squares employing $1/x$ and $1/x^2$ weighting. Calibration curves were fit by linear least squares regression with at least 6 concentrations across the linear dynamic range for each analyte. Calibrators were required to quantify within $\pm 20\%$ and during method validation correlation coefficients (R^2) were required to exceed 0.99.

2.11. Analytical recovery and imprecision

Intra- and inter-day analytical recovery (bias) and imprecision were determined from four replicates at three different QC concentrations across the linear dynamic range of the assay. Analytical recovery was determined by comparing the mean result for all analyses to the nominal concentration value (i.e. mean % of expected concentration). Inter-day imprecision and analytical recovery were evaluated on five different runs with four replicates in each run, analyzed on five separate days ($n=20$). Imprecision was expressed as % coefficient of variation (% CV) of calculated concentrations. One-way analysis of variance (ANOVA) was conducted on low, medium and high QCs to evaluate inter- and intra-day differences in analyte concentrations.

2.12. Extraction efficiency and matrix effect

Extraction efficiency and matrix effect were evaluated via three sets of samples as described by Matuszewski et al. ($n=10$ for each set) [17]. In the first set, urine samples were fortified with analytes and internal standards prior to SLE. In set 2, urine samples were fortified with analytes and internal standards after SLE, and the third set contained analytes and internal standards in mobile phase. Extraction efficiency, expressed as a percentage, was calculated by dividing analyte mean peak areas of set 1 by set 2. Absolute matrix effect was calculated by dividing the mean peak area of the analyte in set 2 by the mean analyte area in set 3. The value was converted to a percentage and subtracted from 100 to represent the amount of signal suppressed by the presence of matrix.

2.13. Analyte stability

Analyte stability also was evaluated with blank human urine fortified with analytes of interest at low and high QC concentrations ($n=3$). Analyte short-term temperature stability was evaluated for fortified human urine stored in the dark in polypropylene microcentrifuge tubes for 16 h at room temperature, 72 h at 4°C, 72 h on the autosampler (4°C), and after three freeze-thaw cycles at -20°C. On the day of analysis, internal standard was added to each specimen and analyzed as described. Analyte autosampler stability was assessed by re-injecting QC specimens after 72 h, and comparing calculated concentrations to values obtained against the original calibration curve.

2.14. Dilution integrity

Dilution integrity was evaluated by diluting a fortified urine sample ($n=3$) containing all analytes at 400 µg/l 1:20 (v/v). Internal standards were added and samples extracted as described. Dilution integrity was maintained if specimens quantified within $\pm 20\%$ of 20 µg/l.

2.15. Carry-over

Carry-over was investigated in triplicate by injecting extracted blank urine samples containing internal standards immediately after samples containing target analytes at 400 µg/l. Blank urine specimens could not meet LOD criteria to document absence of carryover.

2.16. Authentic specimens

Anonymous, randomly collected authentic urine specimens were analyzed to assess method utility.

3. RESULTS

3.1. Chromatography

Baseline chromatographic resolution between all analytes was not possible; all isobaric compounds were baseline resolved except for isomeric hydroxypentyl compounds and JWH-019, JWH-122, JWH-019 hydroxyhexyl and JWH-122 N-hydroxypentyl. Although, JWH-019, JWH-122 and JWH-019 hydroxyhexyl, JWH-122 hydroxypentyl isobaric pairs were not chromatographically baseline resolved, specific product ions differentiated the isobars. We added JWH-018 N-5-hydroxypentyl, JWH-019 N-6-hydroxyhexyl, JWH-073 N-4-hydroxybutyl, JWH-081 N-5-hydroxypentyl, JWH-122 N-5-hydroxypentyl, JWH-210 N-5-hydroxypentyl, JWH-250 N-5-hydroxypentyl, JWH-398 N-5-hydroxypentyl, AM2201 N-4-hydroxypentyl, MAM2201 N-4-hydroxypentyl, RCS-4 N-5-hydroxypentyl and UR-144 N-5-hydroxypentyl alkyl hydroxy metabolite standards, but since isomeric baseline separation was not possible, we can only identify these peaks as alkyl hydroxy metabolites without assigning hydroxy position on the pentyl chain. We employed ± 0.1 min as a peak identification retention time requirement based upon our observations of calibrator retention time drift during method development. Retention times did not drift by more than ± 0.05 min, but we employed a wider ± 0.1 min retention time window requirement because larger retention time variation is expected with authentic specimen analysis over time.

3.2. Evaluation of potential sample preparation approaches

For simplicity sake, we randomly selected two parent compounds and two metabolites for screening sample preparation approaches before method validation for all analytes. Resprep C18 and Strata C8 columns provided efficient recoveries for synthetic cannabinoid metabolites (83.1–98.7% recovery), but less than 27.3% synthetic cannabinoid parent analyte recovery from urine (Supplementary Table 2). SLE achieved 61.3–103.3% extraction efficiencies for all our urinary synthetic cannabinoid analytes of interest (Supplementary Table 2). Matrix effect was -14.0 – 6.0 for all sample preparations.

3.3. Hydrolysis optimization

We previously evaluated synthetic cannabinoid hydrolysis containing JWH-018, JWH-073, JWH-122, JWH-210, JWH-250, AM2201 and RCS-4 metabolites [8], but we wanted to verify optimal hydrolysis conditions for the larger urine specimen volume required in this method. We did not have fresh authentic specimens during hydrolysis optimization, therefore, we confirmed equivalent JWH-018 N-5-hydroxypentyl-glucuronide hydrolysis efficiency as observed during hydrolysis optimization for our previous qualitative method. We found that addition of 300 μ L 400 mM ammonium acetate, pH 4.0, 40 μ L 100,000 units beta-glucuronidase/ml and hydrolysis at 55°C for 2 h achieved optimal JWH-018 N-5-hydroxypentyl-glucuronide hydrolysis ($>95.8\%$ conversion to un-conjugated JWH-018 N-hydroxypentyl metabolite).

3.4. Specificity

Urine samples from ten synthetic cannabinoid-abstinent individuals contained no peaks fulfilling LOD criteria. None of the 83 potential exogenous interferences fortified at 500 μ g/l into low QC samples produced transition ratio or quantification criteria failure. MRM ion chromatograms from a blank urine specimen, a blank urine specimen fortified at low QC concentrations and anonymous, random urine specimens are depicted in Figures 1 and 2. No synthetic cannabinoid parent analytes or hydroxyindole metabolites fortified into low QCs at 20 μ g/l interfered with any other analytes. JWH-250 N-5-carboxypentyl interfered with JWH-210 5-hydroxyindole when fortified into low QCs at >100 μ g/l; no other hydroxypentyl

or pentanoic metabolites interfered with other analyte low QC quantification when fortified at 200 µg/l.

3.5. Sensitivity and linearity

Initial experiments were conducted with six sets of calibration curves fit via unweighted linear least squares and linear least squares with $1/x$ and $1/x^2$ weighting factor to identify the most appropriate calibration model. Inspection of residuals indicated linear least squares with $1/x^2$ weighting factor produced the best fit for the calibration data. All correlation coefficients exceeded 0.994 (Table 3).

Table 3 details LOD, LOQ, linearity and mean calibration results. LOD were between 0.05 and 1.0 µg/l; LOQ were between 0.1 and 1.0 µg/l. Assays were linear to 50 µg/l for all analytes except 100 µg/l for JWH-200 5-hydroxyindole, JWH-398 N-pentanoic acid and AM2201 6-hydroxyindole.

3.6. Analytical recovery and imprecision

Analytical recovery and imprecision were evaluated at three concentrations across the linear dynamic range. Analytical recovery in urine ranged from 83.3–118.3% of expected concentrations for intra-day and inter-day analytical recoveries (Table 2). Intra-day and inter-day imprecision were 0.8–9.1 and 4.3–13.5% CV, respectively (Table 2). There were few significant effects of day on most analyte QC concentrations ($F_{4,15} = 0.03$ – 3.02 , $p > 0.05$); however, in 25 cases day did have an influence ($F_{4,15} = 3.12$ – 9.98 , $p < 0.05$) for JWH-250 carboxypentyl, AM694 and RCS-4 carboxypentyl low QCs; JWH-019 5-hydroxyindole, JWH-203, JWH-210 5-hydroxyindole, JWH-398, RCS8, UR-144 N-hydroxypentyl, UR-144 N-pentanoic acid, XLR11 and CP 47,497-C8 mid QCs and JWH-018 6-hydroxyindole, JWH-019 N-hydroxyhexyl, JWH-203, JWH-210 5-hydroxyindole, JWH-250 N-hydroxypentyl, JWH-250 N-5-carboxypentyl, JWH-398, AM2201, RCS-4, RCS-4 N-5-carboxypentyl, RCS8, CP 47,497-C8 and HU210 high QCs.

3.7. Extraction efficiency and matrix effect

Extraction efficiencies and matrix effects for synthetic cannabinoids in urine are presented in Table 4. Mean extraction efficiencies were 43.7–109.3% ($n=10$). Mean matrix effects (% suppressed signal) were –73.1–51.7% ($n=10$, Table 4).

3.8. Analyte stability, dilution integrity and carryover

Analytes at low, mid and high QC concentrations in urine extracts were stable for 72 h at 4°C in the autosampler, $n=4$ (data not shown). All synthetic cannabinoid metabolites at low and high QC concentrations ($n=3$) were stable for 16 h at room temperature; all parent analytes were unstable except JWH-200, CP 47,497-C7, CP 47,497-C8 and HU210 (Table 5). All analytes were stable for 72 h at 4°C (Table 5). All synthetic cannabinoid metabolites at low and high QC concentrations ($n=3$) were stable after three freeze/thaw cycles; all parent analytes were unstable except JWH-200, JWH-398, AM694, CP 47,497-C7, CP 47,497-C8 and HU210 (Table 5).

Dilution integrity was acceptable (within $\pm 20\%$ of expected diluted concentration) for all analytes after diluting a sample containing analytes at 400 µg/l 1:20 with blank urine.

There was no evidence of carryover for synthetic cannabinoids. Negative specimens injected after samples containing analytes at 400 µg/l did not have analyte peaks satisfying assay LOD criteria ($n=3$).

3.9. Demonstration of method applicability

The method was applied to measurement of synthetic cannabinoids and metabolites in anonymous, randomly collected urine specimens (Figure 2).

4. DISCUSSION

Despite initial DEA scheduling of synthetic cannabinoids in March 2011, abuse is an ongoing problem, with the 2012 Monitoring the Future survey reporting that 11.3% of 12th graders ingested synthetic cannabinoids in the past year [18]. Another recent survey of undergraduate students at a southeastern university found 14% students reported lifetime synthetic cannabinoid use [19]. A validated and sensitive LC-MS/MS method for simultaneously quantifying synthetic cannabinoids and metabolites in urine is necessary for confirming intake by clinical and forensic laboratories. Few quantitative urinary synthetic cannabinoids methods exist; most published LC-MS/MS quantitative methods target JWH-018 and JWH-073 metabolites after β -glucuronidase hydrolysis [11–16]. We present a fully validated and sensitive quantitative LC-MS/MS method for simultaneously measuring the most comprehensive synthetic cannabinoid panel to-date in urine. This quantitative synthetic cannabinoid method is useful for evaluating cutoff concentrations to optimally document synthetic cannabinoid intake, for determining windows of synthetic cannabinoid detection and identifying optimal synthetic cannabinoid analytes for documenting recent intake.

Our goal during validation of the current method was to assess whether more β -glucuronidase enzyme was required to achieve similarly optimal hydrolysis as this method requires a larger 200 μ L urine volume compared to 100 μ L urine for the previous validated synthetic cannabinoid screening method [8]. We evaluated JWH-018 N-5-hydroxypentyl-glucuronide, the only synthetic cannabinoid glucuronide metabolite reference standard commercially available at the time of method development, for evaluating the effect of increasing urine volume on hydrolysis efficiency, as we did not have fresh authentic specimens. Using the current method's hydrolysis conditions achieved similar JWH-018 N-5-hydroxypentyl-glucuronide hydrolysis as observed during development of the previous qualitative synthetic cannabinoid assay inferring that the current conditions would also achieve optimal hydrolysis of JWH-073, JWH-122, JWH-210, JWH-250, AM2201 and RCS-4 metabolites. It is unknown whether these hydrolysis conditions are optimal for other synthetic cannabinoid glucuronide conjugates.

Previously, de Jager et al. presented a synthetic cannabinoid urinary method targeting JWH-018 N-5-hydroxypentyl, JWH-018 N-pentanoic acid, JWH-019 5-hydroxyindole, JWH-073 N-4-hydroxybutyl, JWH-073 N-butanoic acid, JWH-122 N-5-hydroxypentyl, JWH-200 5-hydroxyindole, JWH-250 5-hydroxyindole, JWH-250 N-5-carboxypentyl, JWH-398 N-hydroxypentyl and RCS-4 N-5-hydroxypentyl metabolites [16]. 500 μ L urine was hydrolyzed with β -glucuronidase prior to liquid-liquid extraction and analysis on an ABSciex 5500 QTRAP LCMSMS achieving linear ranges of 0.1–10 μ g/l. Our current LCMSMS method achieves similar LLOQ with increased linearity to 50 or 100 μ g/l with only a 200 μ L urine sample. Other methods targeting only JWH-018 and JWH-073 metabolites achieved LLOQ of 0.1–1.8 μ g/l, similar or lower LLOQ than our current method but only 2–14 analytes [11–15]. Two of the previous methods employed 1 and 2 ml sample volumes, 5–10 fold larger than our method [11,12], with Chimalakonda et al. and Yanes et al. only requiring 40 and 100 μ L urine, respectively [13,15].

Our method was unable to achieve baseline chromatographic resolution for alkyl hydroxy metabolite isomers (i.e. JWH-018 N-2, N-3, N-4, and N-5-hydroxypentyl compounds have similar retention times) providing semi-quantitative total alkyl hydroxy metabolite

concentrations for any synthetic cannabinoid. This is a potential limitation for distinguishing the synthetic cannabinoid ingested, as AM2201 metabolically forms JWH-018 N-5-hydroxypentyl with minimal JWH-018 N-4-hydroxypentyl, and JWH-018 forms less JWH-018 N-5-hydroxypentyl than JWH-018 N-4-hydroxypentyl. Our quantitative method includes the AM2201 specific metabolites AM2201 N-4-hydroxypentyl and AM2201 6-hydroxyindole, but these compounds are less abundant than JWH-018 N-5-hydroxypentyl after AM2201 intake. We added JWH-018 N-5-hydroxypentyl, JWH-019 N-6-hydroxyhexyl, JWH-073 N-4-hydroxybutyl, JWH-081 N-5-hydroxypentyl, JWH-122 N-5-hydroxypentyl, JWH-210 N-5-hydroxypentyl, JWH-250 N-5-hydroxypentyl, JWH-398 N-5-hydroxypentyl, AM2201 N-4-hydroxypentyl, MAM2201 N-4-hydroxypentyl, RCS-4 N-5-hydroxypentyl and UR-144 N-5-hydroxypentyl alkyl hydroxy metabolite standards, but since isomeric separation was not possible, we can only identify these peaks as alkyl hydroxy metabolites without assigning hydroxy position on the side chain.

We opted to include parent analytes, even though metabolites predominate in urine [20], to evaluate if parent analytes assist determining recent intake. This complicated sample preparation during method development, observing low recoveries of nonpolar parent analytes (<27%) with traditional C8 and C18 reverse phase solid phase extraction columns; SLE+ achieved >44% parent analyte recoveries.

Poor availability of analytical reference standards impedes method development for synthetic cannabinoids and other emerging drugs of abuse. Deuterated analogs compensate for variable analyte recovery or matrix effect enabling accurate quantification. Commercially available deuterated internal standards were utilized when available; deuterated analytes with similar functional groups and nearest retention time were utilized when matched deuterated internal standards were unavailable. There was one exception for this internal standard selection scheme; we selected deuterated JWH-018 parent analyte as an internal standard for JWH-210 5-hydroxyindole after observing poor QC performance with JWH-018 5-hydroxyindole- d_9 as internal standard. JWH-018- d_9 co-elutes with JWH-210 5-hydroxyindole better accounting for matrix effect.

ANOVA revealed 25 of 159 cases where statistically significant between-batch differences in measured QC concentration occurred. The explanation for these differences is unknown, possibly due to variable matrix effect, extraction efficiencies, or day-to-day variability in fortifying calibrators and QCs. Matched deuterated internal standards were not available for 16 of 25 cases that may explain between-day variability for these analytes. Although statistically significant, these effects are not likely clinically relevant since all QC concentrations were within 80–120% of target and maximum differences between days was 22.8%.

We are uncertain why >100 $\mu\text{g/l}$ JWH-250 N-5-carboxypentyl concentrations interfered with JWH-210 5-hydroxyindole quantification at 0.6 $\mu\text{g/l}$. JWH-210 5-hydroxyindole ion ratio criteria were within 80–120% of target, but the low QC quantified at 193% of target. Interference appears unlikely because retention times differ by 5.2 min and parent masses by 20 amu for these two analytes. It also seems unlikely that JWH-250 N-5-carboxypentyl would be converted to JWH-210 5-hydroxyindole during sample preparation or analysis. JWH-250 N-5-carboxypentyl standard may have a trace JWH-210 5-hydroxyindole impurity. False positive results could occur when both JWH-250 N-5-carboxypentyl and JWH-210 5-hydroxyindole co-occur in specimens, presence of JWH-210 N-hydroxypentyl and/or JWH-210 N-5-carboxypentyl is required for documenting JWH-210 intake.

All analytes were stable under all evaluated conditions except most parent analytes showed >20% loss after 16 h at room temperature or after three freeze/thaw cycles in fortified urine.

Similarly, Dresen et al. showed >15% JWH-073, JWH-081 and methandamide decreases in fortified serum after 72 h [21] and we previously showed THC instability in urine after 16h at room temperature [22]. Instability appears to be related to analyte polarity with more polar JWH-200, CP 47,497-C7, CP 47,497-C8 and HU210 being stable while other parent analytes were unstable. Parent analyte instability may partially explain not detecting parent synthetic cannabinoids in urine [20].

We present a fully-validated quantitative LC-MS/MS method for the most comprehensive synthetic cannabinoid urine method to-date targeting 53 analytes: JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, JWH-398, RCS-4, AM2201, MAM2201, UR-144, CP 47,497-C7, CP 47,497-C8 and their metabolites, and JWH-203, AM694, RCS8, XLR11 and HU210 parent compounds. 200 μ L urine was hydrolyzed with β -glucuronidase before SLE extraction. Two injections were required to acquire data for 53 analytes; 4 μ L positive mode injection with a 19.5 min runtime for 48 analytes and 25 μ L negative mode injection with a 11.4 min runtime for CP 47,497-C7, CP 47,497-C7 hydroxy metabolite, CP 47,497-C8, CP 47,497-C8 hydroxy metabolite and HU210. This method should accommodate new synthetic cannabinoids as certified reference standards become available. Inclusion of new analytes requires method re-validation for the new analytes; specificity, sensitivity, linearity, imprecision, analytical recovery, extraction efficiency, matrix effect, stability, dilution integrity and carry-over would need to be evaluated for new analytes. However, use of broad spectrum SLE+ sample preparation and scheduled MRM methodologies should eliminate re-optimization of sample preparation and most instrument settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Most comprehensive urine assay for 20 synthetic cannabinoids and 33 metabolites.
2. Enables cutoff concentration evaluation to optimally detect synthetic cannabinoids
3. Useful for determining best analytes to document synthetic cannabinoid intake
4. The method enables rapid addition of new synthetic cannabinoids.

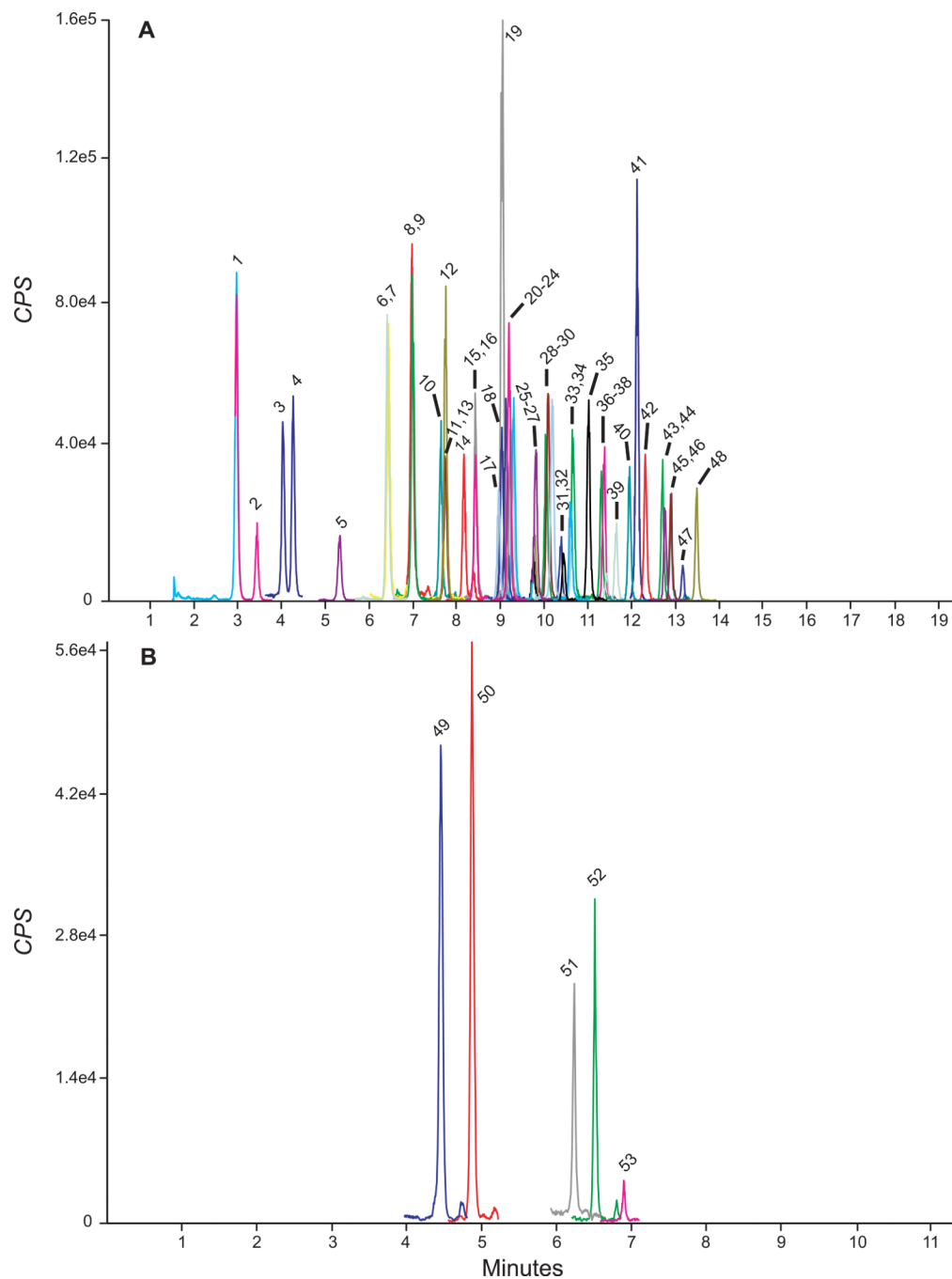


Figure 1. Extracted ion chromatograms showing quantification MRMs in blank urine fortified at low quality control concentrations (0.3 – 1.5 µg/l). Panels A and B are positive and negative mode injections, respectively. See Table 1 for peak numbering information and Table 3 for quality control concentrations.

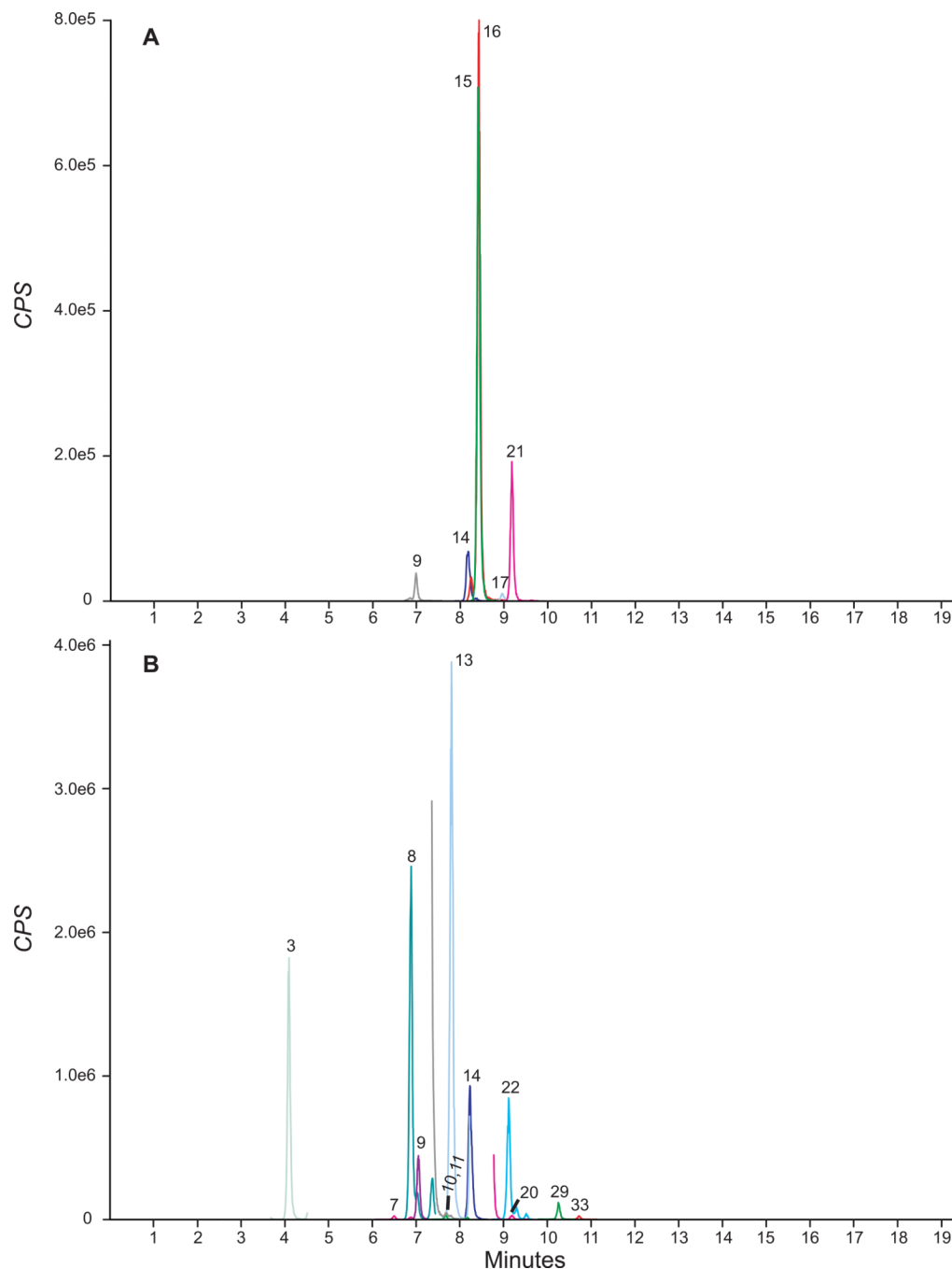


Figure 2. Extracted ion chromatograms showing quantification MRMs in authentic urine specimens containing A) 11.3, 18.3, 0.9, 1.8, 0.3 and 3.9 $\mu\text{g/l}$ JWH-018 N-hydroxypentyl, JWH-018 N-pentanoic acid, JWH-250 N-5-carboxypentyl, AM2201 N-hydroxypentyl, MAM2201 N-hydroxypentyl and MAM2201 N-pentanoic acid, respectively, B) 0.2, 0.7, 0.2, 45.5, 6.7, 0.2, 1.4, 5.0, 10.2, 0.3, 36.4 and 0.2 $\mu\text{g/l}$ JWH-018 5-hydroxyindole, JWH-018 6-hydroxyindole, JWH-073 N-hydroxybutyl, JWH-073 N-butanoic acid, JWH-122 N-hydroxypentyl, JWH-250 5-hydroxyindole, JWH-250 N-hydroxypentyl, JWH-250 N-5-carboxypentyl, AM2201 N-hydroxypentyl, RCS-4 N-5-carboxypentyl, RCS-4 M9 metabolite and UR-144 N-hydroxypentyl, respectively. Specimen B also contained 138.4

and 211.6 $\mu\text{g/l}$ JWH-018 N-hydroxypentyl and JWH-018 N-pentanoic acid (determined after diluted re-analysis, data not shown). See Table 1 for peak numbering.

Table 1

Liquid chromatography tandem mass spectrometry parameters for synthetic cannabinoids and metabolites in human urine.

Analyte	Peak #	Q1 mass (amu)	Q3 mass (amu)	DP (V)	EP (V)	CE (V)	CXP (V)	RT (min)
A. Positive Mode Method								
JWH-018	42	342.1	155.2 , 127.2	46	10	33, 53	12, 12	12.30
JWH-018 5-hydroxyindole	33	358.2	155.1 , 127.2	91	10	33, 65	16, 10	10.70
JWH-018 6-hydroxyindole	29	358.1	155.1 , 127.2	76	10	31, 69	16, 10	10.20
JWH-018 N-5-hydroxypentyl	16	358.2	155.1 , 127.2	56	10	29, 65	12, 14	8.48
JWH-018 N-pentanoic acid	15	372.2	155.0 , 126.9	106	10	31, 71	14, 14	8.47
JWH-019	45	356.0	154.9 , 127.0	26	10	33, 65	16, 14	12.90
JWH-019 5-hydroxyindole	36	372.0	155.0 , 127.0	11	10	33, 71	12, 12	11.40
JWH-019 N-6-hydroxyhexyl	23	372.0	154.9 , 127.0	86	10	29, 73	14, 12	9.34
JWH-073	39	328.0	155.2 , 127.1	51	10	31, 63	12, 14	11.70
JWH-073 5-hydroxyindole	27	344.2	155.0 , 127.0	51	10	33, 65	12, 18	9.88
JWH-073 6-hydroxyindole	24	344.2	155.1 , 127.0	46	10	31, 67	10, 14	9.37
JWH-073 N-4-hydroxybutyl	10	344.1	155.0 , 127.0	36	10	29, 51	12, 18	7.68
JWH-073 N-butanoic acid	13	358.1	155.0 , 127.0	61	10	31, 61	14, 12	7.80
JWH-081	43	372.1	185.1 , 157.2	181	10	33, 51	16, 10	12.70
JWH-081 N-5-hydroxypentyl	18	388.2	185.1 , 113.9	56	10	29, 99	16, 14	9.09
JWH-122	46	356.1	169.0 , 115.0	51	10	33, 91	16, 12	12.90
JWH-122 N-5-hydroxypentyl	22	372.1	169.0 , 115.0	51	10	29, 85	12, 14	9.29
JWH-200	5	385.0	155.1 , 126.8	126	10	29, 69	10, 14	5.41
JWH-200 5-hydroxyindole	1	401.1	155.0 , 76.9	31	10	29, 125	14, 8	2.99
JWH-200 6-hydroxyindole	2	401.1	155.0 , 127.0	120	10	29, 71	12, 16	3.48
JWH-203	38	340.9	124.9 , 89.1	6	10	35, 103	14, 8	11.50
JWH-210	48	370.1	183.1 , 214.1	51	10	33, 33	18, 18	13.50
JWH-210 5-hydroxyindole	41	386.2	183.0 , 155.1	51	10	35, 49	14, 14	12.20
JWH-210 N-5-hydroxypentyl	30	386.2	183.1 , 155.1	30	10	31, 47	16, 12	10.20
JWH-210 N-5-carboxypentyl	28	400.1	183.0 , 155.0	40	10	33, 49	14, 18	10.10
JWH-250	35	336.1	121.2 , 91.1	50	10	27, 61	12, 14	11.10

Analyte	Peak #	Q1 mass (amu)	Q3 masses (amu)	DP (V)	EP (V)	CE (V)	CXP (V)	RT (min)
JWH-250 5-hydroxyindole	20	352.1	121.0, 91.0	66	10	27, 65	14, 12	9.19
JWH-250 N-5-hydroxypentyl	8	352.2	121.0, 186.2	50	10	27, 21	12, 14	7.02
JWH-250 N-5-carboxypentyl	9	366.1	121.1, 200.1	56	10	27, 23	14, 16	7.04
JWH-398	47	377.0	188.8, 126.0	81	10	33, 97	22, 16	13.20
JWH-398 N-5-hydroxypentyl	26	393.1	188.8, 160.9	51	10	29, 59	18, 14	9.85
JWH-398 N-pentanoic acid	25	406.9	189.1, 161.1	31	10	31, 65	18, 14	9.82
AM2201	37	360.1	155.1, 127.2	106	10	33, 57	10, 10	11.40
AM2201 6-hydroxyindole	19	376.2	127.1, 77.0	66	10	67, 111	10, 12	9.09
AM2201 N-4-hydroxypentyl	14	376.1	155.0, 126.9	46	10	33, 69	14, 18	8.22
MAM2201	40	374.1	169.0, 115.0	30	10	35, 91	14, 14	12.00
MAM2201 N-4-hydroxypentyl	17	390.1	169.0, 141.1	166	10	35, 59	18, 12	9.02
MAM2201 N-pentanoic acid	21	386.1	169.1, 141.1	61	10	33, 49	20, 16	9.26
AM694	31	435.9	230.8, 202.9	196	10	35, 61	18, 20	10.40
RCS-4	34	322.1	135.1, 77.2	56	10	31, 73	12, 10	10.70
RCS-4 N-5-hydroxypentyl	6	338.2	135.0, 77.0	141	10	27, 73	12, 12	6.47
RCS-4 N-5-carboxypentyl	7	352.1	135.0, 107.0	51	10	31, 59	14, 12	6.49
RCS-4 M9 metabolite	3	324.1	120.9, 92.9	21	10	27, 63	14, 14	4.09
RCS-4 M10 metabolite	4	324.1	120.9, 93.0	16	10	31, 63	14, 14	4.31
RCS8	44	376.0	120.9, 90.9	146	10	31, 65	10, 12	12.80
UR-144 N-5-hydroxypentyl	11	328.0	124.9, 97.0	141	10	25, 37	18, 14	7.78
UR-144 N-pentanoic acid	12	342.0	125.0, 244.1	61	10	27, 31	10, 18	7.78
XLR11	32	330.1	125.1, 232.0	156	10	31, 33	6, 24	10.50
JWH-018-d9		351.1	155.0, 127.0	36	10	33, 53	14, 16	12.30
JWH-018 5-hydroxyindole-d9		367.1	155.0, 127.0	56	10	35, 73	18, 12	10.60
JWH-018 6-hydroxyindole-d9		367.1	155.0, 127.0	46	10	33, 73	14, 14	10.20
JWH-018 N-5-hydroxypentyl-d5		363.1	155.0, 127.0	56	10	29, 65	14, 14	8.43
JWH-073-d7		335.1	155.0, 127.0	76	10	33, 65	14, 16	11.60
JWH-073 5-hydroxyindole-d7		351.1	155.0, 127.0	51	10	33, 51	12, 16	9.81
JWH-073 6-hydroxyindole-d7		351.1	155.0, 127.0	36	10	33, 61	12, 10	9.31
JWH-073 N-4-hydroxybutyl-d5		349.1	155.0, 127.0	46	10	29, 57	14, 14	7.62

Analyte	Peak #	Q1 mass (amu)	Q3 masses (amu)	DP (V)	EP (V)	CE (V)	CXP (V)	RT (min)
JWH-073 N-butanolic acid-d5		363.1	155.0 , 127.0	61	10	31, 61	12, 16	7.74
JWH-081-d9		381.2	185.0 , 157.0	51	10	35, 53	18, 12	12.70
JWH-122-d9		365.2	169.0 , 114.9	86	10	35, 95	16, 14	12.90
JWH-122 N-5-hydroxypentyl-d5		377.1	169.0 , 114.9	56	10	29, 97	16, 14	9.23
JWH-200-d5		390.1	155.1 , 127.0	41	10	29, 65	14, 18	5.35
JWH-210-d9		379.2	183.0 , 223.1	36	10	33, 33	18, 18	13.40
JWH-250-d5		341.1	121.0 , 91.0	46	10	27, 63	14, 12	11.00
JWH-398-d9		385.9	189.0 , 125.9	36	10	37, 93	20, 18	13.10
AM2201-d5		365.1	155.0 , 127.0	31	10	35, 73	14, 12	11.30
AM2201 N-4-hydroxypentyl-d5		381.1	155.1 , 127.1	50	10	29, 77	16, 16	8.15
RCS-4-d9		331.1	135.0 , 77.0	61	10	33, 75	12, 10	10.60
UR-144-d5		317.0	125.0 , 218.6	61	10	31, 33	12, 18	11.40
XLR11-d5		335.1	125.1 , 237.1	161	10	31, 33	12, 20	10.40
B. Negative Mode Method								
CP 47,497-C7	51	317.1	245.1 , 159.1	-90	-10	-42, -64	-29, -15	6.26
CP 47,497-C7-hydroxy dimethylheptyl metabolite	49	333.2	261.2 , 158.9	-15	-10	-50, -76	-15, -15	4.49
CP 47,497-C8	52	331.1	259.1 , 159.0	-30	-10	-46, -68	-23, -15	6.54
CP 47,497-C8-hydroxy dimethyloctyl metabolite	50	347.1	159.1 , 185.0	-180	-10	-72, -66	-17, -19	4.91
HU210	53	385.6	301.3 , 281.1	-36	-12	-48, -58	-27, -26	6.93
CP 47,497-C7-d11		328.2	256.2 , 159.0	-160	-10	-44, -68	-23, -15	6.22
CP 47,497-C8-d7		338.2	266.1 , 159.0	-150	-10	-46, -64	-33, -17	6.52
11-nor-9-carboxy-tetrahydrocannabinol-d9		352.1	254.2 , 194.1	-50	-10	-40, -44	-21, -17	6.14

Q1= quadrupole 1, Q3= quadrupole 3, DP= declustering potential, EP= entrance potential, CE= collision energy, CXP= collision cell exit potential, RT= retention time.

Bold masses depict quantification transitions.

Table 2

Analytical recovery and imprecision data for synthetic cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry.

Analyte	Target Low µg/l	Intra-day, N=4			Inter-day, N=20		
		Low	Mid	High	Low	Mid	High
JWH-018	0.6	109.2 (8.1)	102.6 (7.9)	101.6 (9.8)	102.3 (7.6)	103.4 (7.6)	101.2 (8.1)
JWH-018 5-hydroxyindole	0.3	109.2 (6.3)	108.1 (8.7)	106.6 (5.0)	107.2 (7.9)	108.1 (6.9)	103.8 (7.5)
JWH-018 6-hydroxyindole	0.3	104.2 (4.8)	107.5 (6.8)	106.0 (7.4)	106.2 (7.3)	110.4 (6.5)	102.0 (10.0)
JWH-018 N-hydroxypentyl	0.3	97.5 (12.9)	98.9 (7.7)	96.1 (7.0)	96.0 (9.4)	102.4 (7.2)	92.8 (7.6)
JWH-018 N-pentanoic acid	0.3	110.8 (9.3)	112.4 (3.1)	109.8 (4.7)	111.0 (6.4)	112.0 (4.3)	109.6 (5.2)
JWH-019	0.3	104.2 (11.5)	100.2 (7.8)	100.2 (10.7)	101.3 (10.8)	103.3 (7.9)	99.6 (9.2)
JWH-019 5-hydroxyindole	0.3	100.8 (11.2)	99.8 (5.9)	96.7 (8.6)	100.0 (11.7)	103.6 (10.0)	96.3 (7.2)
JWH-019 N-hydroxyhexyl	0.3	111.7 (5.2)	111.8 (3.2)	107.3 (5.2)	107.5 (7.9)	112.0 (4.4)	105.6 (7.4)
JWH-073	0.3	108.3 (8.1)	100.7 (8.5)	100.2 (6.1)	98.2 (10.1)	101.4 (8.6)	97.3 (7.1)
JWH-073 5-hydroxyindole	0.3	105.0 (9.9)	106.0 (8.8)	105.3 (5.3)	103.5 (8.1)	107.6 (7.4)	100.7 (7.7)
JWH-073 6-hydroxyindole	0.3	102.5 (9.3)	99.3 (9.8)	99.7 (7.1)	100.7 (8.8)	103.0 (7.6)	96.0 (8.2)
JWH-073 N-hydroxybutyl	0.3	105.8 (4.0)	105.4 (6.7)	101.6 (5.7)	102.7 (8.6)	106.5 (7.8)	97.6 (5.9)
JWH-073 N-butanoic acid	0.3	102.5 (9.7)	103.8 (8.7)	101.4 (7.0)	105.5 (7.8)	105.4 (6.7)	99.0 (5.3)
JWH-081	0.3	105.8 (6.5)	102.0 (7.8)	99.8 (9.4)	98.8 (9.4)	104.1 (7.2)	96.4 (7.5)
JWH-081 N-hydroxypentyl	0.3	112.5 (7.8)	107.8 (6.4)	104.7 (2.2)	108.2 (8.1)	111.1 (5.6)	104.6 (7.0)
JWH-122	0.3	102.5 (6.7)	95.5 (7.2)	90.0 (8.1)	101.3 (9.8)	105.4 (8.7)	98.5 (8.7)
JWH-122 N-hydroxypentyl	0.3	101.7 (11.2)	100.9 (9.2)	99.3 (4.0)	99.7 (8.3)	103.7 (7.7)	97.4 (7.3)
JWH-200	0.3	103.3 (9.5)	102.0 (9.4)	98.4 (6.6)	99.3 (9.1)	103.1 (6.8)	97.5 (6.7)
JWH-200 5-hydroxyindole	1.5	109.2 (6.6)	107.2 (7.7)	108.7 (5.0)	104.5 (6.4)	104.1 (7.5)	103.2 (7.6)
JWH-200 6-hydroxyindole	0.3	110.0 (6.5)	107.3 (9.3)	108.7 (3.4)	104.3 (8.5)	106.0 (7.9)	104.8 (6.1)
JWH-203	1.5	94.0 (7.7)	87.9 (3.8)	88.8 (5.4)	98.0 (9.9)	96.8 (10.7)	96.2 (10.0)
JWH-210	0.3	110.0 (2.5)	102.9 (10.0)	101.2 (7.5)	105.0 (8.0)	106.3 (8.6)	101.5 (7.5)
JWH-210 5-hydroxyindole	0.6	100.0 (10.4)	94.3 (6.9)	94.5 (9.2)	98.7 (10.7)	106.8 (7.8)	104.6 (8.4)
JWH-210 N-hydroxypentyl	0.3	96.7 (13.5)	95.5 (7.1)	93.9 (10.0)	97.8 (9.3)	101.8 (9.2)	91.3 (7.6)
JWH-210 N-5-carboxypentyl	0.3	104.2 (13.7)	103.2 (3.3)	96.2 (8.0)	99.7 (10.3)	103.8 (6.8)	97.6 (7.2)
JWH-250	0.6	107.5 (5.1)	101.6 (7.9)	101.3 (5.6)	104.9 (7.3)	103.9 (8.3)	98.7 (6.9)

Analyte	Target Low µg/l	Intra-day, N=4			Inter-day, N=20		
		Low	Mid	High	Low	Mid	High
JWH-250 5-hydroxyindole	0.3	110.0 (6.5)	103.5 (7.1)	104.7 (8.0)	105.7 (7.3)	107.3 (7.0)	103.6 (8.3)
JWH-250 N-hydroxypentyl	0.6	108.8 (5.8)	109.3 (7.6)	104.5 (9.5)	106.6 (6.1)	107.9 (6.6)	99.1 (9.4)
JWH-250 N-5-carboxypentyl	0.6	115.4 (1.4)	111.5 (8.7)	111.8 (10.9)	111.3 (5.4)	111.4 (6.7)	105.2 (9.8)
JWH-398	1.5	98.3 (10.9)	102.6 (7.9)	101.4 (2.4)	100.1 (10.0)	105.9 (8.2)	101.7 (11.2)
JWH-398 N-hydroxypentyl	1.5	112.5 (4.5)	105.3 (6.2)	101.1 (5.1)	105.2 (8.5)	106.1 (5.4)	102.0 (6.1)
JWH-398 N-pentanoic acid	1.5	99.7 (9.1)	101.0 (9.7)	99.4 (5.9)	95.2 (10.2)	93.6 (7.6)	92.5 (7.2)
AM2201	0.3	105.0 (8.4)	105.0 (9.6)	104.1 (5.6)	105.3 (7.1)	107.9 (6.0)	102.6 (7.3)
AM2201 6-hydroxyindole	1.5	109.2 (7.8)	107.1 (8.4)	106.8 (6.6)	109.1 (6.7)	107.2 (6.9)	104.4 (8.2)
AM2201 N-hydroxypentyl	0.3	96.7 (12.95)	98.1 (10.0)	96.6 (6.5)	94.2 (10.0)	99.7 (9.2)	93.3 (6.3)
MAM2201	0.3	96.7 (9.8)	97.7 (5.1)	88.9 (3.3)	92.0 (7.7)	94.0 (6.3)	88.3 (5.3)
MAM2201 N-hydroxypentyl	0.3	110.0 (10.5)	110.0 (5.6)	107.3 (3.4)	104.7 (9.4)	110.4 (5.5)	105.3 (5.6)
MAM2201 N-pentanoic acid	0.6	104.6 (9.4)	105.9 (6.5)	101.9 (5.6)	103.1 (8.1)	105.6 (6.6)	101.2 (6.7)
AM694	0.3	97.5 (12.3)	103.5 (7.0)	102.2 (8.0)	100.8 (11.2)	107.3 (6.4)	103.9 (7.5)
RCS-4	0.3	106.7 (8.5)	105.1 (11.8)	101.2 (6.2)	104.7 (8.5)	108.4 (7.6)	101.6 (8.4)
RCS-4 N-hydroxypentyl	0.6	105.8 (6.9)	102.8 (7.7)	99.8 (8.7)	102.2 (8.3)	105.6 (8.1)	96.7 (7.4)
RCS-4 N-5-carboxypentyl	0.6	118.3 (1.1)	111.8 (6.8)	110.9 (10.2)	112.2 (5.7)	111.7 (6.1)	106.7 (8.2)
RCS-4 M9 metabolite	0.6	112.5 (4.4)	110.0 (8.4)	107.8 (7.6)	105.9 (7.5)	108.9 (6.6)	104.1 (6.1)
RCS-4 M10 metabolite	0.6	112.1 (6.5)	109.7 (8.7)	107.3 (7.2)	105.9 (7.9)	109.4 (7.0)	103.3 (6.6)
RCS8	0.3	110.0 (10.2)	103.6 (11.7)	98.9 (7.3)	97.5 (12.1)	99.0 (10.7)	92.7 (8.6)
UR-144 N-hydroxypentyl	0.3	95.0 (14.2)	91.6 (8.5)	88.0 (6.9)	90.0 (9.3)	97.7 (10.3)	88.5 (5.5)
UR-144 N-pentanoic acid	0.6	100.8 (10.3)	96.7 (8.6)	94.2 (4.2)	103.6 (8.0)	102.9 (8.4)	97.0 (5.2)
XLR11	0.6	101.7 (5.8)	100.6 (6.0)	100.6 (6.5)	101.7 (7.1)	101.7 (9.2)	97.2 (9.7)
CP 47,497-C7	1.5	113.7 (4.2)	103.0 (6.4)	95.6 (5.9)	106.2 (9.4)	106.4 (7.1)	97.4 (7.3)
CP 47,497-C7-hydroxy metabolite	1.5	105.8 (12.1)	112.6 (7.0)	111.0 (7.4)	103.7 (10.2)	108.8 (9.4)	105.2 (10.4)
CP 47,497-C8	1.5	112.7 (5.6)	105.3 (8.0)	105.1 (7.8)	106.2 (9.1)	105.4 (7.1)	103.1 (6.8)
CP 47,497-C8-hydroxy metabolite	1.5	101.0 (16.8)	93.6 (9.1)	95.7 (5.2)	99.0 (13.5)	99.9 (12.2)	98.1 (11.0)
HU210	1.5	95.5 (9.7)	95.9 (12.7)	86.5 (6.0)	97.3 (10.8)	101.0 (11.7)	99.2 (11.8)
% target (range)		94.0 – 118.3	87.9 – 112.6	86.5 – 111.8	90.0 – 112.2	93.6 – 112.1	88.3 – 109.6
%CV (range)		1.1 – 16.8	3.1 – 12.7	2.2 – 10.9	5.4 – 13.5	4.3 – 12.2	5.2 – 11.8

Mid and high quality control concentrations were 7.5 and 30 µg/l, respectively.

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Table 3

Synthetic cannabinoids and metabolites in human urine by liquid chromatography tandem mass spectrometry: limits of detection (LOD), linear ranges and calibration results ($N=6$).

Analyte	Internal Standard	LOD ($\mu\text{g/l}$)	Linear range ($\mu\text{g/l}$)	Y-intercept Mean (SD)	Slope Mean (SD)	R ² (range)
JWH-018	JWH-018-d9	0.2	0.2–50	0.78 (0.03)	0.02 (0.01)	0.995–0.999
JWH-018 5-hydroxyindole	JWH-018 5-hydroxyindole-d9	0.1	0.1–50	1.08 (0.06)	0.02 (0.01)	0.994–0.998
JWH-018 6-hydroxyindole	JWH-018 6-hydroxyindole-d9	0.1	0.1–50	0.96 (0.06)	0.02 (0.01)	0.994–0.998
JWH-018 N-hydroxypentyl	JWH-018 N-hydroxypentyl-d5	0.1	0.1–50	1.19 (0.04)	0.04 (0.01)	0.996–0.997
JWH-018 N-pentanoic acid	JWH-018 N-hydroxypentyl-d5	0.1	0.1–50	0.71 (0.04)	0.01 (0.01)	0.996–0.999
JWH-019	JWH-018-d9	0.1	0.1–50	0.63 (0.06)	0.01 (0.01)	0.995–0.999
JWH-019 5-hydroxyindole	JWH-018 5-hydroxyindole-d9	0.1	0.1–50	0.95 (0.11)	0.02 (0.01)	0.995–0.997
JWH-019 N-hydroxyhexyl	JWH-122 N-hydroxypentyl-d5	0.1	0.1–50	1.04 (0.05)	0.03 (0.01)	0.995–0.998
JWH-073	JWH-073-d7	0.1	0.1–50	0.48 (0.02)	0.01 (0.01)	0.995–0.998
JWH-073 5-hydroxyindole	JWH-073 5-hydroxyindole-d7	0.1	0.1–50	0.94 (0.06)	0.02 (0.01)	0.995–0.998
JWH-073 6-hydroxyindole	JWH-073 6-hydroxyindole-d7	0.1	0.1–50	0.94 (0.06)	0.02 (0.01)	0.995–0.998
JWH-073 N-hydroxybutyl	JWH-073 N-hydroxybutyl-d5	0.1	0.1–50	1.34 (0.11)	0.02 (0.01)	0.995–0.998
JWH-073 N-butanoic acid	JWH-073 N-butanoic acid-d5	0.1	0.1–50	1.43 (0.08)	0.02 (0.02)	0.996–0.998
JWH-081	JWH-081-d9	0.1	0.1–50	0.78 (0.04)	0.05 (0.01)	0.994–0.998
JWH-081 N-hydroxypentyl	JWH-122 N-hydroxypentyl-d5	0.1	0.1–50	1.06 (0.07)	0.02 (0.01)	0.995–0.997
JWH-122	JWH-122-d9	0.1	0.1–50	1.05 (0.08)	0.02 (0.01)	0.995–0.997
JWH-122 N-hydroxypentyl	JWH-122 N-hydroxypentyl-d5	0.1	0.1–50	0.99 (0.05)	0.02 (0.01)	0.995–0.997
JWH-200	JWH-200-d5	0.1	0.1–50	8.01 (0.38)	0.12 (0.05)	0.996–0.997
JWH-200 5-hydroxyindole	JWH-073 6-hydroxyindole-d7	1.0	1–100	0.77 (0.30)	-0.01 (0.03)	0.994–0.999
JWH-200 6-hydroxyindole	JWH-073 6-hydroxyindole-d7	0.1	0.1–50	0.81 (0.30)	0.01 (0.01)	0.996–0.998
JWH-203	UR-144-d5	0.5	0.5–50	0.37 (0.04)	0.08 (0.05)	0.994–0.998
JWH-210	JWH-210-d9	0.1	0.1–50	1.30 (0.05)	0.02 (0.02)	0.995–0.999
JWH-210 5-hydroxyindole	JWH-018-d9	0.2	0.2–50	1.24 (0.45)	0.09 (0.06)	0.995–0.999
JWH-210 N-hydroxypentyl	JWH-018 N-hydroxypentyl-d5	0.1	0.1–50	1.38 (0.06)	0.03 (0.01)	0.994–0.997
JWH-210 N-5-carboxypentyl	JWH-122 N-hydroxypentyl-d5	0.1	0.1–50	0.91 (0.09)	0.01 (0.01)	0.996–0.998
JWH-250	JWH-250-d5	0.2	0.2–50	0.61 (0.04)	0.02 (0.02)	0.995–0.998
JWH-250 5-hydroxyindole	JWH-073 6-hydroxyindole-d7	0.1	0.1–50	0.77 (0.02)	0.01 (0.004)	0.995–0.998

Analyte	Internal Standard	LOD (µg/l)	Linear range (µg/l)	Y-intercept Mean (SD)	Slope Mean (SD)	R ² (range)
JWH-250 N-hydroxypentyl	JWH-073 N-hydroxybutyl-d5	0.2	0.2-50	1.41 (0.09)	0.08 (0.02)	0.995-0.997
JWH-250 N-5-carboxypentyl	JWH-073 N-butanoic acid-d5	0.2	0.2-50	1.34 (0.09)	0.03 (0.03)	0.995-0.999
JWH-398	JWH-398-d9	0.5	0.5-50	0.93 (0.07)	0.06 (0.07)	0.996-0.999
JWH-398 N-hydroxypentyl	JWH-122 N-hydroxypentyl-d5	0.5	0.5-50	0.08 (0.0005)	0.01 (0.004)	0.995-0.999
JWH-398 N-pentanoic acid	JWH-122 N-hydroxypentyl-d5	1.0	1-100	0.05 (0.0006)	0.01 (0.007)	0.996-0.999
AM2201	AM2201-d5	0.1	0.1-50	7.60 (0.26)	0.10 (0.03)	0.994-0.999
AM2201 6-hydroxyindole	JWH-073 6-hydroxyindole-d7	1.0	1-100	0.56 (0.04)	-0.02 (0.04)	0.996-0.999
AM2201 N-hydroxypentyl	AM2201 N-hydroxypentyl-d5	0.1	0.1-50	1.33 (0.08)	0.03 (0.01)	0.995-0.998
MAM2201	AM2201-d5	0.1	0.1-50	10.14 (2.22)	0.22 (0.11)	0.995-0.996
MAM2201 N-hydroxypentyl	JWH-122 N-hydroxypentyl-d5	0.1	0.1-50	0.68 (0.03)	0.01 (0.004)	0.995-0.999
MAM2201 N-pentanoic acid	JWH-122 N-hydroxypentyl-d5	0.2	0.2-50	0.82 (0.07)	0.02 (0.01)	0.995-0.999
AM694	XLR11-d5	0.1	0.1-50	1.99 (0.52)	0.05 (0.03)	0.996-0.998
RCS-4	RCS-4-d9	0.1	0.1-50	0.73 (0.04)	0.01 (0.004)	0.995-0.997
RCS-4 N-hydroxypentyl	JWH-073 N-hydroxybutyl-d5	0.2	0.2-50	1.27 (0.09)	0.04 (0.01)	0.996-0.998
RCS-4 N-5-carboxypentyl	JWH-073 N-butanoic acid-d5	0.2	0.2-50	1.39 (0.06)	0.03 (0.02)	0.995-0.997
RCS-4 M9 metabolite	JWH-073 N-hydroxybutyl-d5	0.2	0.2-50	0.77 (0.13)	0.03 (0.02)	0.995-0.998
RCS-4 M10 metabolite	JWH-073 N-hydroxybutyl-d5	0.2	0.2-50	0.77 (0.13)	0.03 (0.02)	0.995-0.998
RCS8	RCS-4-d9	0.1	0.1-50	0.46 (0.05)	0.01 (0.01)	0.995-0.998
UR-144 N-hydroxypentyl	JWH-073 N-butanoic acid-d5	0.2	0.2-50	1.91 (0.17)	0.06 (0.03)	0.994-0.997
UR-144 N-pentanoic acid	JWH-073 N-butanoic acid-d5	0.2	0.2-50	1.30 (0.07)	0.05 (0.02)	0.995-0.998
XLR11	XLR11-d5	0.2	0.2-50	1.32 (0.03)	0.03 (0.01)	0.995-0.999
CP 47,497-C7	CP 47,497-C7-d11	0.5	0.5-50	1.10 (0.06)	0.11 (0.07)	0.995-0.998
CP 47,497-C7-hydroxy metabolite	11-nor-9-tetrahydrocannabinol-d9	0.5	0.5-50	1.50 (0.46)	0.21 (0.16)	0.995-0.999
CP 47,497-C8	CP 47,497-C8-d7	0.5	0.5-50	0.75 (0.07)	0.02 (0.02)	0.995-0.999
CP 47,497-C8-hydroxy metabolite	11-nor-9-tetrahydrocannabinol-d9	0.5	0.5-50	2.21 (0.61)	0.10 (0.16)	0.994-0.999
HU210	CP 47,497-C8-d7	0.5	0.5-50	0.09 (0.02)	0.03 (0.01)	0.996-0.998

Limit of quantification was the lower limit of linearity.

Table 4

Mean extraction efficiencies and matrix effects for synthetic cannabinoids and metabolites extracted from urine by supported-liquid extraction.

Analyte	Low QC (µg/l)	Extraction efficiency (%, N = 10)		Matrix effect (% of signal suppressed, N = 10)	
		Low	High	Low	High
JWH-018	0.6	65.1%	59.6%	-8.1%	-11.6%
JWH-018 5-hydroxyindole	0.3	89.8%	92.5%	22.6%	7.3%
JWH-018 6-hydroxyindole	0.3	89.7%	93.9%	20.6%	6.5%
JWH-018 N-hydroxypentyl	0.3	94.4%	96.1%	30.8%	12.3%
JWH-018 N-pentanoic acid	0.3	93.5%	97.5%	35.1%	16.4%
JWH-019	0.3	66.9%	58.9%	-15.4%	-19.1%
JWH-019 5-hydroxyindole	0.3	94.3%	90.5%	16.5%	6.7%
JWH-019 N-hydroxyhexyl	0.3	88.1%	91.0%	28.4%	12.5%
JWH-073	0.3	62.5%	60.0%	16.1%	6.7%
JWH-073 5-hydroxyindole	0.3	91.8%	93.9%	23.6%	7.8%
JWH-073 6-hydroxyindole	0.3	92.2%	93.1%	25.3%	10.3%
JWH-073 N-hydroxybutyl	0.3	92.5%	95.4%	31.4%	14.9%
JWH-073 N-butanoic acid	0.3	96.1%	99.4%	33.4%	16.6%
JWH-081	0.3	75.9%	68.7%	-10.4%	-15.1%
JWH-081 N-hydroxypentyl	0.3	90.9%	94.1%	31.2%	13.3%
JWH-122	0.3	70.7%	61.3%	-17.8%	-20.0%
JWH-122 N-hydroxypentyl	0.3	89.1%	93.6%	42.7%	11.0%
JWH-200	0.3	96.0%	94.6%	24.1%	10.3%
JWH-200 5-hydroxyindole	1.5	92.9%	97.7%	27.3%	10.0%
JWH-200 6-hydroxyindole	0.3	94.3%	95.1%	25.0%	9.8%
JWH-203	1.5	67.4%	52.9%	11.8%	11.0%
JWH-210	0.3	72.5%	58.1%	-24.8%	-23.1%
JWH-210 5-hydroxyindole	0.6	92.9%	91.7%	21.9%	3.1%
JWH-210 N-hydroxypentyl	0.3	92.4%	93.9%	36.4%	14.9%
JWH-210 N-5-carboxypentyl	0.3	99.2%	98.8%	44.4%	23.4%
JWH-250	0.6	63.4%	62.9%	14.9%	5.3%
JWH-250 5-hydroxyindole	0.3	92.3%	95.1%	29.0%	12.9%

Analyte	Low QC (µg/l)	Extraction efficiency (%, N = 10)		Matrix effect (% of signal suppressed, N = 10)	
		Low	High	Low	High
JWH-250 N-hydroxypropyl	0.6	91.5%	99.7%	51.7%	23.3%
JWH-250 N-5-carboxypropyl	0.6	85.3%	94.7%	41.8%	22.1%
JWH-398	1.5	70.8%	55.1%	-27.2%	-27.9%
JWH-398 N-hydroxypropyl	1.5	84.4%	89.5%	26.5%	7.7%
JWH-398 N-pentanoic acid	1.5	96.6%	96.5%	35.5%	13.8%
AM2201	0.3	76.1%	72.4%	8.9%	0.8%
AM2201 6-hydroxyindole	1.5	89.3%	94.3%	29.9%	14.5%
AM2201 N-hydroxypropyl	0.3	93.8%	97.6%	26.4%	12.5%
MAM2201	0.3	77.3%	73.8%	45.5%	26.6%
MAM2201 N-hydroxypropyl	0.3	90.2%	95.8%	34.1%	14.5%
MAM2201 N-pentanoic acid	0.6	94.3%	98.0%	31.8%	16.7%
AM694	0.3	77.2%	78.6%	25.9%	8.2%
RCS-4	0.3	62.9%	61.4%	9.7%	0.7%
RCS-4 N-hydroxypropyl	0.6	95.8%	97.4%	37.5%	21.4%
RCS-4 N-5-carboxypropyl	0.6	88.4%	94.3%	40.0%	18.4%
RCS-4 M9 metabolite	0.6	93.8%	99.1%	46.6%	24.8%
RCS-4 M10 metabolite	0.6	94.3%	98.9%	45.2%	23.6%
RCS8	0.3	74.6%	63.7%	-12.0%	-14.9%
UR-144 N-hydroxypropyl	0.3	95.0%	92.6%	30.2%	17.1%
UR-144 N-pentanoic acid	0.6	94.9%	96.0%	31.3%	14.3%
XLR11	0.6	57.3%	55.4%	5.0%	-0.8%
CP 47,497-C7 ^a	1.5	86.7%	82.3%	-34.4%	-41.1%
CP 47,497-C7-hydroxy metabolite ^a	1.5	91.0%	89.7%	-54.1%	-59.2%
CP 47,497-C8 ^a	1.5	92.9%	80.9%	-41.4%	-43.0%
CP 47,497-C8-hydroxy metabolite ^a	1.5	92.5%	85.0%	-44.8%	-50.6%
HU210 ^a	1.5	92.6%	88.7%	-68.6%	-73.1%
JWH-018-d9		69.8%	64.9%	-6.6%	-13.7%
JWH-018 5-hydroxyindole-d9		94.3%	97.9%	17.8%	5.9%
JWH-018 6-hydroxyindole-d9		95.0%	103.9%	26.9%	9.5%

Analyte	Low QC (µg/l)	Extraction efficiency (%, N = 10)		Matrix effect (% of signal suppressed, N = 10)	
		Low	High	Low	High
JWH-018 N-hydroxypropyl-d5		100.2%	107.0%	29.0%	16.1%
JWH-073-d7		64.1%	65.2%	23.1%	9.4%
JWH-073 5-hydroxyindole-d7		94.6%	102.8%	24.0%	8.5%
JWH-073 6-hydroxyindole-d7		96.9%	102.1%	21.9%	11.0%
JWH-073 N-hydroxybutyl-d5		97.1%	107.6%	29.7%	13.4%
JWH-073 N-butanolic acid-d5		99.0%	104.7%	34.8%	18.0%
JWH-081-d9		78.9%	74.2%	-9.3%	-13.8%
JWH-122-d9		72.3%	66.6%	-15.9%	-19.2%
JWH-122 N-hydroxypropyl-d5		94.8%	102.9%	32.8%	15.2%
JWH-200-d5		99.8%	105.3%	19.5%	7.4%
JWH-210-d9		78.1%	63.3%	-24.0%	-23.0%
JWH-250-d5		69.7%	71.9%	22.4%	11.2%
JWH-398-d9		72.6%	61.7%	-22.6%	-22.3%
AM2201-d5		76.2%	77.3%	10.6%	-4.1%
AM2201 N-hydroxypropyl-d5		96.6%	109.3%	30.8%	12.6%
RCS-4-d9		64.2%	66.1%	11.1%	-0.1%
UR-144-d5		53.9%	44.8%	-15.3%	-12.0%
XLR11-d5		60.4%	59.6%	5.4%	-1.2%
CP 47,497-C7-d11 ^a		92.7%	92.1%	-42.4%	-47.5%
CP 47,497-C8-d7 ^a		90.5%	89.7%	-41.2%	-44.5%
11-nor-9-carboxy-tetrahydrocannabinol-d9 ^a		82.3%	84.4%	-52.7%	-60.2%

^aData acquired in negative ionization mode

High quality control concentrations were 30 µg/l; deuterated internal standard concentrations were 1 µg/l.

Table 5

Synthetic cannabinoids and metabolites stabilities in human urine.

Analyte	Low QC µg/l	16 h room temperature (% target, n=3)		72 h 4°C (% target, n=3)		3 freeze and thaw cycles (% target, n=3)	
		Low	High	Low	High	Low	High
JWH-018	0.6	65.0%	73.4%	86.7%	93.9%	76.7%	79.0%
JWH-018 5-hydroxyindole	0.3	93.3%	84.9%	90.0%	89.4%	96.7%	85.0%
JWH-018 6-hydroxyindole	0.3	84.4%	83.5%	92.2%	85.5%	90.0%	82.9%
JWH-018 N-hydroxypentyl	0.3	91.1%	86.3%	86.7%	85.4%	92.2%	84.3%
JWH-018 N-pentanoic acid	0.3	115.6%	110.5%	108.9%	109.0%	114.4%	106.2%
JWH-019	0.3	56.7%	71.4%	81.1%	95.6%	73.3%	76.4%
JWH-019 5-hydroxyindole	0.3	88.9%	84.7%	92.2%	90.3%	84.4%	84.1%
JWH-019 N-hydroxyhexyl	0.3	93.3%	90.7%	95.6%	98.7%	100.0%	88.7%
JWH-073	0.3	72.2%	70.2%	82.2%	85.0%	70.0%	74.3%
JWH-073 5-hydroxyindole	0.3	90.0%	89.7%	95.6%	97.1%	98.9%	87.7%
JWH-073 6-hydroxyindole	0.3	87.8%	82.2%	92.2%	83.8%	87.8%	84.1%
JWH-073 N-hydroxybutyl	0.3	101.1%	91.0%	100.0%	91.1%	101.1%	86.2%
JWH-073 N-butanoic acid	0.3	105.6%	102.8%	104.4%	101.6%	111.1%	93.3%
JWH-081	0.3	67.8%	70.7%	86.7%	94.7%	74.4%	77.2%
JWH-081 N-hydroxypentyl	0.3	97.8%	93.2%	101.1%	101.2%	107.8%	91.7%
JWH-122	0.3	62.2%	70.6%	88.9%	99.8%	72.2%	75.2%
JWH-122 N-hydroxypentyl	0.3	92.2%	86.4%	87.8%	90.9%	91.1%	85.1%
JWH-200	0.3	90.0%	93.1%	90.0%	94.8%	90.0%	87.7%
JWH-200 5-hydroxyindole	1.5	107.3%	105.1%	106.7%	104.4%	109.1%	106.5%
JWH-200 6-hydroxyindole	0.3	103.3%	105.7%	102.2%	105.5%	105.6%	105.5%
JWH-203	1.5	76.4%	65.8%	84.2%	92.3%	82.0%	77.8%
JWH-210	0.3	57.8%	69.6%	87.8%	97.2%	74.4%	82.6%
JWH-210 5-hydroxyindole	0.6	85.6%	85.0%	91.1%	99.0%	101.7%	84.3%
JWH-210 N-hydroxypentyl	0.3	92.2%	83.3%	90.0%	85.9%	90.0%	81.8%
JWH-210 N-5-carboxypentyl	0.3	94.4%	93.8%	98.9%	98.2%	105.6%	92.9%
JWH-250	0.6	78.3%	77.3%	88.9%	90.3%	76.7%	80.8%

Analyte	Low QC µg/l	16 h room temperature (% target, n=3)		72 h 4°C (% target, n=3)		3 freeze and thaw cycles (% target, n=3)	
		Low	High	Low	High	Low	High
JWH-250 5-hydroxyindole	0.3	97.8%	90.5%	98.9%	90.9%	101.1%	91.2%
JWH-250 N-hydroxypentyl	0.6	108.3%	86.3%	100.6%	88.5%	106.7%	82.3%
JWH-250 N-5-carboxypentyl	0.6	107.2%	100.7%	107.2%	101.1%	111.7%	95.1%
JWH-398	1.5	57.3%	70.5%	82.2%	98.6%	80.9%	85.2%
JWH-398 N-hydroxypentyl	1.5	88.4%	85.6%	90.7%	94.1%	89.3%	85.7%
JWH-398 N-pentanoic acid	1.5	95.8%	85.4%	92.9%	89.3%	99.3%	85.2%
AM2201	0.3	85.6%	76.4%	92.2%	92.8%	82.2%	78.5%
AM2201 6-hydroxyindole	1.5	99.8%	93.4%	106.9%	95.4%	104.0%	92.9%
AM2201 N-hydroxypentyl	0.3	87.8%	92.8%	90.0%	93.6%	92.2%	86.7%
MAM2201	0.3	70.0%	72.1%	84.4%	84.5%	70.0%	77.0%
MAM2201 N-hydroxypentyl	0.3	95.6%	96.3%	94.4%	102.6%	96.7%	93.1%
MAM2201 N-pentanoic acid	0.6	102.2%	97.7%	101.1%	103.9%	104.4%	95.2%
AM694	0.3	78.9%	81.6%	87.8%	107.4%	82.2%	84.6%
RCS-4	0.3	80.0%	72.5%	96.7%	86.0%	70.0%	72.9%
RCS-4 N-hydroxypentyl	0.6	101.1%	92.4%	99.4%	96.5%	99.4%	88.4%
RCS-4 N-5-carboxypentyl	0.6	107.2%	103.9%	108.3%	105.7%	111.7%	97.0%
RCS-4 M9 metabolite	0.6	105.6%	101.9%	100.6%	105.1%	103.3%	97.5%
RCS-4 M10 metabolite	0.6	102.8%	101.6%	99.4%	103.0%	102.2%	97.0%
RCS8	0.3	56.7%	67.4%	85.6%	88.9%	65.6%	79.4%
UR-144 N-hydroxypentyl	0.3	86.7%	89.8%	86.7%	88.9%	83.3%	84.9%
UR-144 N-pentanoic acid	0.6	106.1%	101.9%	103.3%	102.0%	109.4%	93.6%
XLR11	0.6	76.1%	79.2%	85.0%	92.4%	77.8%	80.1%
CP 47,497-C7	1.5	84.4%	84.7%	82.9%	98.9%	80.9%	84.3%
CP 47,497-C7-hydroxy metabolite	1.5	101.3%	111.7%	108.2%	113.5%	95.6%	98.9%
CP 47,497-C8	1.5	85.6%	87.9%	89.3%	96.2%	83.3%	89.3%
CP 47,497-C8-hydroxy metabolite	1.5	103.1%	107.2%	103.6%	113.1%	97.1%	97.2%
HU210	1.5	88.4%	83.7%	93.6%	95.7%	91.6%	85.9%

Bold type indicates >20% analyte loss