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Semiquantitative Activity-Based Detection of JWH-018, a Synthetic Cannabinoid Receptor Agonist, in Oral Fluid after Vaping

Annelies Cannaert, Maria del Mar Ramírez Fernández, Eef L. Theunissen, Johannes G. Ramaekers, Sarah M. R. Wille, and Christophe P. Stove*

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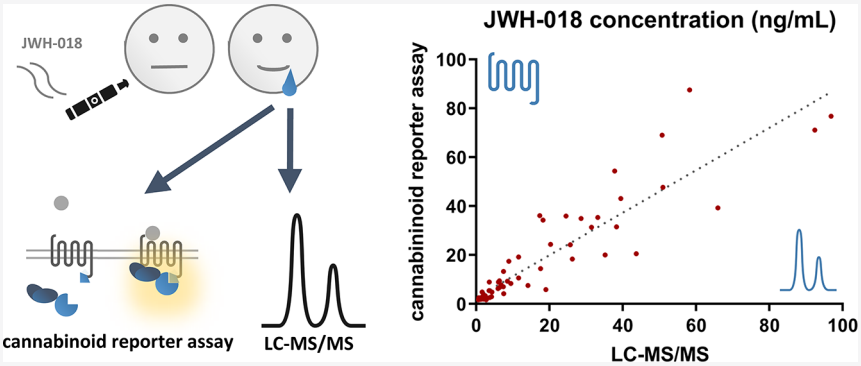

ABSTRACT: The rapid proliferation of new synthetic cannabinoid receptor agonists (SCRAs) has initiated considerable interest in the development of so-called "untargeted" screening strategies. One of these new screening technologies involves the activity-based detection of SCRAs. In this study, we evaluated whether (synthetic) cannabinoid activity can be detected in oral fluid (OF) and, if so, whether it correlates with SCRA concentrations. OF was collected at several time points in a placebo-controlled JWH-018 administration study. The outcome of the cell-based cannabinoid reporter system, which monitored the cannabinoid receptor activation, was compared to the quantitative data for JWH-018, obtained via a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. A total of 175 OF samples were collected and analyzed via both methods. The cannabinoid reporter assay correctly classified the vast majority of the samples as either negative (<0.25 ng/mL; 74/75 = 99%) or having low (0.25−1.5 ng/mL; 16/16 = 100% and 1.5−10 ng/mL; 37/41 = 90%), mid (10−100 ng/mL; 23/25 = 92%) or high (>100 ng/mL; 16/18 = 89%) JWH-018 concentrations. Passing−Bablok regression analysis yielded a good linear correlation, with no proportional difference between both methods (slope 0.97; 95% confidence interval 0.86−1.14) and only a small systematic difference. This is the first study to demonstrate the applicability of an untargeted, activity-based approach for SCRA detection in OF. Additionally, the outcome of the cannabinoid reporter assay was compared to the gold standard (LC-MS/MS), showing a good correlation between both methods, indicating that the cannabinoid reporter assay can be used for an estimation of drug concentrations.

By the end of 2019, 950 novel substances were reported to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory on new psychoactive substances (NPS) by governments, laboratories, and partner organizations. These substances include a broad range of drugs, such as synthetic cannabinoid receptor agonists (SCRAs), stimulants, opioids, and benzodiazepines.[1] The rise of NPS has put a huge strain on drug legislations and clinical and forensic laboratories worldwide in terms of legality and detectability. Several (new) technologies have been developed to cope with the problem of detection of NPS. One of these new screening technologies uses an untargeted approach and involves the activity-based detection of SCRAs and synthetic opioids in several biological matrices, including urine, serum, plasma, and vitreous.[2−6] In many cases though, for example, in the context of driving under the influence of drugs, workplace testing, or in drug abstinence monitoring, oral fluid (OF) is used as a matrix for the analysis of drugs of abuse.[7−10] OF offers the advantage that the sampling is noninvasive and that the parent compounds can be detected in the matrix. This study is the first to assess whether (synthetic) cannabinoid activity can be detected in OF. To evaluate the proof-of-

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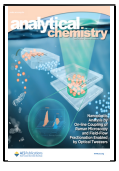

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principle, OF was collected at several time points in a controlled administration study with a number of subjects (n = 23) using a low dose of a well-known SCRA, JWH-018. The semiquantitative outcome of the activity-based assay was compared to the quantitative data for JWH-018 obtained via a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

MATERIALS AND METHODS

Chemicals and Reference Standards. The reference substance JWH-018 and its deuterated internal standard (IS) JWH-018-d_{11} were purchased from Cayman Chemical Company (Ann Arbor, MI, USA) and Lipomed AG (Arlesheim, Switserland), respectively. Methanol (ULC-grade) and hexane (HPLC-grade) were purchased from Biosolve (Valskenswaard, The Netherlands), and ammonium formate (powder) was obtained from VWR (Leuven, Belgium). Deionized water was prepared using Arium Comfort (Sartorius, Belgium). Dulbecco's modified Eagle's medium (GlutaMAX), Opti-MEMI reduced serum medium, penicillin−streptomycin (5000 IU/mL), and amphotericin B (250 μg/mL) were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Fetal bovine serum (FBS) and poly-D-lysine were supplied by Sigma-Aldrich (Overijse, Belgium).

Biological Samples. Twenty-three healthy cannabis-experienced participants took part in this placebo-controlled, crossover study at Maastricht University. Participants inhaled the smoke of 75 μg JWH-018/kg bodyweight (average 5.5 mg) or 50 mg Knaster Hemp (placebo, tobacco-replacement herbal blend, Future Necessities B.V., Sittard, The Netherlands) on two separate days. JWH-018 powder was heated via a vaporizer pen (Puffco Plus vaporizer, CA, USA), which reaches temperatures of approximately 380 °C. Participants inhaled the vapor in five intakes, according to a strict inhalation regimen. In case participants did not show a subjective response within 15 min after administration (i.e., a subjective high score <3), a booster dose of 50 μg/kg bodyweight was administered.[11] OF samples were obtained using the Intercept *i2* Oral Fluid Collection Device (OraSure Technologies, Inc., PA, USA, obtained from Meridian BioScience Europe, Braine-l'Alleud, Belgium) at several time points (5, 15, 45, 120 min) after inhalation. After collection, the devices were centrifuged and stored at −18 °C until analysis of JWH-018, as described below. For 20 participants, OF samples were collected during the 2 days. There were also 3 participants for whom only samples were collected on the day JWH-018 was administered. The study was approved by the standing Medical Ethics Committee of Maastricht University and was carried out in compliance with the current revision of the Declaration of Helsinki (Fortaleza, 2013) and the International Conference on Harmonization Guidelines for Good Clinical Practice. A written informed consent was obtained from all participants.

Standard Solutions, Calibrators, and Quality Control Samples. The primary stock solution (1 mg/mL) was prepared by dissolving 5 mg of the JWH-018 standard in 5 mL of methanol and was conserved for 6 months at −20 °C. Independently prepared solutions were used for the working solutions to prepare calibrators and quality control samples (QCs), which were prepared from drug-free OF, diluted 3-fold with the stabilizing solution in the Intercept *i2* Oral Fluid Collection Device. The primary methanolic working solution of JWH-018 was prepared just before use at a concentration of 0.333 μg/mL. Two additional methanolic working solutions were prepared via 1/10 and 1/100 dilution of the primary working solution. For the calibrators, 12.5−50 μL of the working solutions were added to 500 μL of the diluted oral fluid in buffer to obtain a concentration range from 0.25 to 100 ng of JWH-018 per mL in a neat oral fluid. For the QCs, separate working solutions (with the same concentrations) were made, and 12.5 or 37.5 μL was added to the diluted oral fluid to obtain the concentrations of 0.25 (lower limit of quantification; LLOQ), 0.75 (low), 7.5 (mid), and 75 ng/mL (high) of JWH-018 in neat oral fluid. The methanolic deuterated IS stock solution (JWH-018-d_{11}) was prepared at a concentration of 0.025 μg/mL and conserved for 6 months at −20 °C.

Sample Preparation. When during collection the volume adequacy indicator on the Intercept device turns blue, an OF volume of about 1 mL (n = 77; 95% confidence interval: 0.9635−1.097 mL) has been collected. The pad was inserted into 2 mL of the stabilizing solution, resulting in a 3-fold dilution. After centrifugation for 10 min at 3500 rpm, the diluted OF was collected and stored at −18 °C until analysis. For LC-MS/MS analysis, 16.7 μL of IS solution was added to a 500 μL aliquot of the diluted OF (to a theoretical concentration of 2.5 ng/mL in neat OF). Next, 2 mL of hexane was added, followed by shaking and centrifugation prior to transfer of the organic phase to a glass tube and evaporation to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 1 mL of mobile phase A/B (90/10, v/v) prior to LC-MS/MS analysis.

For the bioassay analysis, another 500 μL aliquot of diluted OF was processed as described above except that it was not spiked with IS. The evaporated extract was reconstituted in 100 μL of Opti-MEM I/methanol (50/50, v/v), of which 10 μL was used per well (see Cannabinoid Reporter Assay section). Calibration standards were prepared from drug-free OF, diluted 3-fold with the stabilizing solution in the Intercept device and mixed with methanolic standard solution containing JWH-018. The final concentrations of the calibrators were 0.25, 0.5, 1, 2.5, 5, 10, 50, and 100 ng/mL neat OF for the LC-MS/MS calibration curve and 0.5, 1, 2.5, 5, 10, 50, 100, 500, and 1000 ng/mL neat OF for the bioassay analysis.

LC-MS/MS Instrumentation and Analytical Conditions. The instrument used was an ACQUITY UPLC system coupled to a tandem mass spectrometer XEVO TQ MS (Waters, Milford, MA, USA). After injection of 1 μL of extract, the analyte was separated at 40 °C on an Acquity UPLC C18 1.7 μm (2.1 mm × 100 mm) column (Waters, Milford, MA, USA). The mobile phase consisted of water containing 1 mM ammonium formate (A), and methanol (B). The elution program with a flow rate of 0.35 mL/min started at 90% B, increasing for 2 min to 99%, after which it returned to 90% at minute 3, followed by re-equilibration for 2 min, resulting in a total run time of 5 min. Electrospray parameters were as follows: cone gas flow, 150 L/h; nebulizer gas flow, 7 bar; desolvation temperature, 650 °C; capillary voltage, 1kv; source temperature, 150 °C. The MS/MS was operated in a multiple reaction monitoring mode (MRM) with two transitions for the analyte and the internal standard. The transitions were (m/z, cone and collision energy in parentheses, quantifier underlined): JWH-018 342.1 → <u>127</u> (40,40), 342.1 → 154.9 (40,30); JWH-018-d_{11} 353.2 → <u>127.2</u> (20,40), 353.2 → 155.2 (20,20). Data evaluation was performed using the Targetlynx Software (Waters, Milford, MA, USA).

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Method Validation. In a previous JWH-018 administration study, it was already shown that the metabolites of JWH-018 were not present in OF (<limit of detection of 0.05 ng/mL);[9] therefore, these were not included in the LC-MS/MS method. The method was validated according to the protocol described by Wille et al.[12,13] In each analytical run, a control blank (i.e., sample prepared from a blank matrix that does not contain the IS), a zero sample (i.e., sample prepared from a blank matrix that includes the IS), and QC samples were included. Selectivity was assessed by analyzing 10 buffer/oral fluid mixtures using blank oral samples from 10 different individuals and two zero samples. The absence of interfering components was accepted if the response at the retention time of JWH-018 was less than 20% of the LLOQ for JWH-018. Carry-over was assessed by injection of a blank solvent after 3.5 times the highest calibrator (n = 10) using the same acceptance criteria as for selectivity. For evaluation of linearity, calibration curves with eight levels (0.25−100 ng/mL) using internal standardization were evaluated (in duplicate on five different days). Precision and bias of the method were evaluated over the linear dynamic range at four different concentration levels, i.e., the lower limit of quantification (LLOQ; 0.25 ng/mL), low level (0.75 ng/mL), medium level (7.5 ng/mL), and high level (75 ng/mL). Each concentration was analyzed in duplicate on five separate days. Accuracy and precision were considered acceptable if %RSD and %bias were within 15% and 20% for the LLOQ.[14,15] The evaluation of extraction efficiency (EE) and matrix effects (ME) was conducted according to the method published by Matuszewski et al.[16] at low (0.75 ng/mL JWH-018) and high (75 ng/mL JWH-018) concentrations (n = 10). For the evaluation of ME, analyte-free diluted oral fluid from 10 individual donors was used. Extracts (i.e., the 2 mL supernatants mentioned in the Sample Preparation section) prepared from blank diluted oral fluid were spiked at concentrations corresponding to low QC and high QC level (B). Neat solvent (methanol−ammonium formate (1 mM) in water, 90:10, v/v) was spiked at the same concentration levels (A). Absolute ME was calculated by dividing the peak area of (B) by the peak area obtained from (A), multiplied with 100% (n = 10). IS-compensated ME was calculated similarly by taking into account the IS peak area for (A) and (B). For the evaluation of EE, diluted oral fluid was spiked at low QC and high QC levels before (C) and after (B) extraction. The EE was determined by dividing the peak area ratio of (C) by the peak area ratio of (B), multiplied with 100% (n = 10). The recovery of JWH-018 from the oral fluid device was also evaluated at low, medium, and high levels (n = 6). Analyte stability determinations comprised an assessment of the freeze/thaw cycle stability (three cycles) and long-term stability (5 months at −20 °C) of the diluted oral fluid and the processed sample stability of the obtained extracts (autosampler stability 72 h at 4 °C). These were performed at low and high levels (same concentrations as for the EE and ME) in sextuplicate.

Cannabinoid Reporter Assay. A live cell-based reporter assay that monitors protein−protein interactions via the NanoLuc Binary Technology was used to assess the cannabinoid activity in the samples. Here, the receptor activation is evaluated via the interaction between a cytosolic truncated β-arrestin 2 (βarr2) protein and the cannabinoid receptor 1 (CB1).[3] Both βarr2 and CB1 are fused to an inactive part of nanoluciferase. Upon CB1 activation, βarr2 is recruited to the receptor, allowing interaction of the complementary nanoluciferase subunits, yielding a functional enzyme that generates a bioluminescent signal in the presence of the substrate furimazine. The original human embryonic kidney (HEK) T293 cell line was provided by Prof. O. De Wever (Laboratory of Experimental Cancer Research, Ghent University Hospital, Belgium) and was modified to stably express the cannabinoid reporter system.[2] The stability of the cell line (i.e., the expression levels of fusion proteins) was monitored by flow cytometric analysis.

The cells were routinely maintained at 37 °C and 5% CO_2, under a humidified atmosphere in Dulbecco's modified Eagle's medium (GlutaMAX) supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, 100 mg/L streptomycin, and 0.25 mg/L amphotericin B. For experiments, cells were seeded on poly-D-lysine coated 96-well plates at 5×10^4 cells/well and incubated overnight. The cells were washed twice with Opti-MEM I reduced serum medium to remove any remaining FBS, and 100 μL Opti-MEM I was added. The Nano-Glo live cell reagent (Promega, Madison, WI, USA), a nonlytic detection reagent containing the cell permeable furimazine substrate, was prepared by 20-fold dilution of the Nano-Glo live cell substrate using Nano-Glo LCS dilution buffer, and 25 μL was added to each well. Subsequently, the plate was placed into a TriStar^2 LB942 multimode microplate reader (Berthold Technologies GmbH & Co., Germany). Luminescence was monitored during the equilibration period until the signal stabilized (15 min). We added 10 μL of extract (see Sample Preparation section) per well. The luminescence was continuously detected for 120 min. For each 96-well plate, a calibration curve (0.5−1000 ng/mL) was used. Also, solvent controls were analyzed with all experiments.

Curve fitting of the calibration curves and statistics were performed using GraphPad Prism software (San Diego, CA, USA) and Medcalc software (Ostend, Belgium). The data are represented as mean areas under the curve (AUC) ± standard deviation (SD) with two replicates for each data point. Curve fitting of calibration curves was done via nonlinear regression (four parameter logistic fit; 4PL). This allowed us to calculate the concentration of JWH-018 in the participant samples using the following formulas:

$$x = c\left(\frac{a-d}{y-d} - 1\right)^{1/b}$$

where a is the minimal value, b the hill's slope, c the point of inflection, d the maximal value, x the concentration, and y the AUC.

For those samples with AUC values below the minimal (a) or above the maximal (d) values, no numerical value could be assigned via the bioassay. These were classified as either too low or too high to be calculated by the bioassay. All samples above a certain threshold were considered positive and were assigned a semiquantitative value for JWH-018. This threshold can be determined via a receiver operating characteristic (ROC) curve or can be calculated based on the standard deviation of the response and the slope[17] by using a specific calibration curve in the lower range (0.5−10 ng/mL) using the following formula

$$\text{Threshold} = \frac{3.3\sigma}{S}$$

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where σ is the standard deviation of the response (residual standard deviation of a regression line), and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

LC-MS/MS Method Validation. For the selectivity, no interfering components (<20% of LLOQ) were found at the retention time of JWH-018. There was no carry-over observed in the blanks following the injection of 3.5 times the highest calibrator (350 ng/mL). The calibration data were heteroscedastic. For the quantification of JWH-018 in diluted oral fluid, a linear model with a weighing factor of $1/x^2$ was the best fitting model based upon the procedure for the selection and validation of a calibration model by Desharnais et al.[18] The selected regression model met the preset acceptance criteria (%RE < 15% for the calibrators and <20% for the LLOQ). The accuracy, intraday precision, and total precision for QCs, analyzed in duplicate on five different days, fulfilled the predefined criteria (%bias and %RSD < 15%, 20% for LLOQ), as shown in Table 1.

Table 1. Accuracy (%bias), Intraday, and Total Precision (% RSD) Data for QC Samples Prepared at Four Concentration Levels for Diluted Oral Fluid Samples (n = 5 × 2)

QC	Accuracy (% bias)	Intraday precision (% RSD)	Total precision (% RSD)
LLOQ	7.35	6.63	9.11
Low	1.72	4.96	8.74
Medium	−1.84	5.80	6.99
High	0.82	4.70	6.55

The absolute ME and IS-corrected ME approximated 100% for both the low QC and high QC levels, indicating no significant enhancement or suppression of ionization. The relative ME fulfilled the predetermined acceptance criteria (<15%), with a maximum value of 6.66% (Table 2). The EE

Table 2. Absolute and Relative Matrix Effect at Low QC and High QC Levels Made from Spiked Diluted Oral Fluid from 10 Different Donors (n = 10)

	Absolute matrix effect (mean %)		Relative matrix effect (% RSD)	
QC	without IS	with IS	without IS	with IS
Low	102	104	2.49	6.66
High	99	103	6.16	4.30

values (mean ± SD) obtained were 80.9 ± 10.9% and 82.9 ± 9.9% for low and high QC samples, respectively. The actual recovery of JWH-018 from the oral fluid device (Intercept *i2* collector) was low, with rates of 29.5 ± 8.1%, 26.5 ± 8.1%, and 27.4 ± 11.6% at low, medium, and high levels, respectively, indicating adsorption by the collection pad. This problem is well-known, especially for lipophilic compounds[19−23] and has been observed with several devices (Quantisal, Dräger DCD 5000).[24−26] The IS, only added after the pad has been removed from the diluted oral fluid, does not compensate for this loss, implying there is a substantial underestimation of actual OF concentrations. Although this shortens the detection time, this was not an issue in our study, as we could still compare the semiquantitative outcome of the cannabinoid reporter assay with the quantitative data for JWH-018. For the purpose of this study (comparison of the cannabinoid reporter assay with LC-MS/MS), it was not possible to add IS already at an earlier stage, as also the IS will have cannabinoid activity.

For the diluted oral fluid samples, the freeze/thaw (three cycles) and long-term stability (5 months at −20 °C) data remained within 10% of the control (7.25%/7.73% and 9.26%/9.82% for low/high levels, respectively). The processed sample stability (72 h at 4 °C) samples showed less than 15% from the initial amount confirming stability of extracts in the autosampler (−14.8%/3.08% for low/high levels).

OF Sampling. In this study, JWH-018 was administered by inhalation via vaping, and OF was collected at several time points using the Intercept *i2* collector. For detection of JWH-018 in OF, the contamination of the oral cavity is very important as it was reported that SCRAs are only transferred at a very low rate from the bloodstream into the OF (and vice versa).[9] Therefore, OF is well suited for the detection of recent SCRA use.[25] The level of oral cavity contamination is highly variable between individuals,[25] as also observed in this study, where the maximal concentrations varied from 6.10 to >100 ng/mL JWH-018. The maximal concentrations that were found in this study (median >100 ng/mL) were higher than those in a similar JWH-018 administration study (median 25.7 ng/mL).[9] This may be due to the higher dose inhaled (average 5.5 mg JWH-018 versus 3 mg JWH-018) and/or due to the fact that in this study a vaping system was used to inhale the drug instead of a "crack pipe". The former was much more convenient to use for the participants, which might contribute to the higher concentrations found.

Cannabinoid Reporter Assay Model. The calibration curves (0.5−1000 ng/mL, prepared in matrix) obtained via the cannabinoid reporter assay are sigmoidal. Curve fitting was done via nonlinear regression (four parameter logistic fit; 4PL). The study participant samples were analyzed in five different runs alongside a calibration curve. Statistical analysis did not reveal any significant difference between the hill's slopes (b) and the points of inflection (c) of the calibration curves from the different runs (Figure 1A).

(Semi)quantification of OF Samples. A total of 175 OF samples were collected and analyzed via both the LC-MS/MS method and the cannabinoid reporter assay (Table 3). Via the LC-MS/MS analysis, 75 were below the LLOQ of 0.25 ng/mL, whereas 18 were above the ULOQ (upper limit of quantification) of 100 ng/mL JWH-018. The large fraction of samples below the LLOQ originated from the samples taken on placebo days. From the 76 (= 19 × 4) samples taken on the placebo days, 71 were, as expected, below the LLOQ, although in five cases low levels of JWH-018 (0.32−2.63 ng/mL) were found. In four of these cases, it was the first sample (5 min after inhalation) which showed this slight positivity, which could be explained if the mouth piece or the chamber of the vaping device was contaminated from previous use. For the actual comparison of the bioassay results with those from the LC-MS/MS assay, this apparent contamination issue did not matter.

On the basis of the 4PL model of the calibration curve, the JWH-018 concentrations from the participant samples could be calculated from their AUC values. All samples above a certain threshold (1.50 ng/mL) were considered positive and were assigned a semiquantitative value for JWH-018. This threshold was established using a receiver operating characteristic (ROC) curve to choose the most appropriate "cutoff", i.e., showing a combination of the best sensitivity and specificity

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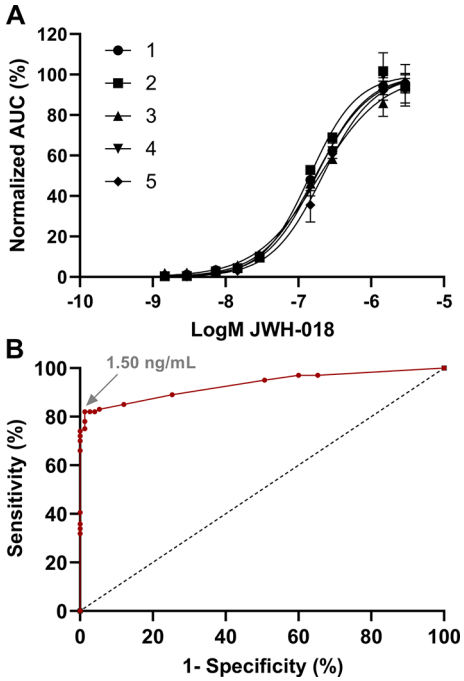

Figure 1. (A) Representation of the five different calibration curves (normalized to 100%), showing no significant difference between the different runs. (B) Receiver operating characteristic (ROC) curve to choose the most appropriate threshold for the cannabinoid reporter assay (= 1.50 ng/mL).

(Figure 1B). This threshold closely matches the theoretical threshold of 1.69 ng/mL, which can be calculated based on the standard deviation of the response and the slope by using a specific calibration curve,[17] in the lower range, run along with participants' samples (0.5−10 ng/mL, n = 5). When scoring samples being JWH-018 positive or negative on the basis of the quantitative LC-MS/MS results with 1.50 ng/mL as threshold, the data yielded an overall sensitivity and specificity of 82% (82/100) and 99% (74/75), respectively. This does not take into account the fact that the LLOQ of the LC-MS/MS method (0.25 ng/mL) is lower than the bioassay's threshold of 1.50 ng/mL. Overall, the bioassay scored 92 samples below the threshold of 1.50 ng/mL, 67 samples between 1.50−100 ng/mL, and 16 samples above 100 ng/mL (Table 3).

The cannabinoid reporter assay scored all 75 samples below the LLOQ of the LC-MS/MS method either below the bioassay's threshold (1.50 ng/mL; n = 74) or in the lower concentration range of the bioassay (1.71 ng/mL; n = 1). The 16 samples with a concentration between the LLOQ of the LC-MS/MS (0.25 ng/mL) and the threshold of the bioassay (1.50 ng/mL) were all scored as being below 1.50 ng/mL. From the 41 samples with concentrations between 1.50−10 ng/mL in the LC-MS/MS method, 90% (37/41) were also assigned as such in the cannabinoid reporter assay (Table 3). Within this set, two samples were scored higher in the cannabinoid reporter assay (17.42 and 13.3 ng/mL versus 8.89 and 7.48 ng/mL). Two other samples were scored below the bioassay's threshold of 1.50 ng/mL JWH-018, although the corresponding LC-MS/MS concentrations were 2.29 and 3.08 ng/mL. Twenty-three of the 25 samples (92%) that were measured between 10−100 ng/mL via the LC-MS/MS method were also calculated between that range in the bioassay. The two samples that were divergent had a concentration of 14.15 and 19.01 ng/mL in the LC-MS/MS method, while values of, respectively, 7.54 and 5.88 ng/mL were derived from the cannabinoid reporter assay. Sixteen of the 18 samples (89%) above the ULOQ of the LC-MS/MS method were also scored greater than 100 ng/mL in the cannabinoid reporter assay. The two remaining samples were assigned concentrations of 81.1 and 86.5 ng/mL in the bioassay, still matching the concentrations found via LC-MS/MS (semiquantitative: 109 and 120 ng/mL) quite well. Overall, these data show that the cannabinoid reporter assay allows a reasonably good estimate of the JWH-018 concentration, both at low and high concentrations. Next, we looked at the 64 samples that were quantifiable via both the LC-MS/MS and the cannabinoid reporter assay (bold in Table 3). The differences between measured concentrations in both methods can readily be deduced from Figure 2, depicting the Passing−Bablok regression analysis, yielding a good linear correlation (y = 0.9745x + 0.8244). The 95% confidence interval of the slope contains 1 (0.8573−1.1415), indicating that there was no proportional difference between the two methods, although there is a slight systematic difference as the 95% confidence interval of the intercept does not contain 0 (0.3747−1.1866).

Table 3. Overview of 175 Oral Fluid Samples Analysed via Both the LC-MS/MS Method and Cannabinoid Reporter Assay[a]

Cannabinoid reporter assay \ LC-MS/MS	<0.25 ng/mL JWH-018 (n = 75)	0.25 – 1.50 ng/mL JWH-018 (n = 16)	1.50 – 10 ng/mL JWH-018 (n = 41)	10 – 100 ng/mL JWH-018 (n = 25)	> 100 ng/mL JWH-018 (n = 18)
< 1.50 ng/mL JWH-018 (n = 92)	74	16	2		
1.50–10 ng/mL JWH-018 (n = 40)	1		**37**	**2**	
10–100 ng/mL JWH-018 (n = 27)			**2**	**23**	2
> 100 ng/ml JWH-018 (n = 16)					16
	99% (74/75)	100% (16/16)	90% (37/41)	92% (23/25)	89% (16/18)

[a]Gray boxes indicate correctly scored samples by the cannabinoid reporter assay. Bold numbers refer to the data used for the Passing−Bablok analysis (Figure 2).

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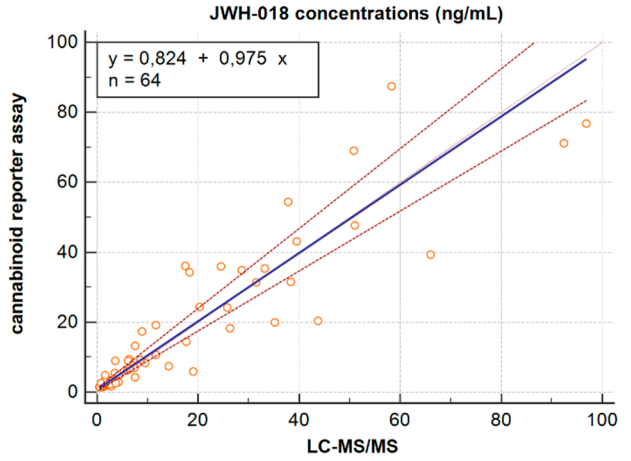

Figure 2. Passing–Bablok regression analysis of concentrations of JWH-018, measured via LC-MS/MS and calculated via the cannabinoid reporter assay.

Figure 3 shows the absolute differences between the JWH-018 concentrations determined via the cannabinoid reporter assay and LC-MS/MS. There is an overall mean difference of 0.4 ng/mL JWH-018 between both methods. As can be seen, the absolute difference between the two methods is limited at low concentrations (<10 ng/mL). At higher concentrations (10–100 ng/mL), these differences are more pronounced. This can be explained by the semiquantitative nature of the cannabinoid reporter assay. The concentration of JWH-018 is calculated via the 4PL fit, using a sigmoidal curve with a logarithmic scale in the *x*-axis (Figure 1A). A small deviation in the AUC (*y*-axis) can result in a large difference in the calculated concentration (*x*-axis), especially at higher concentrations. Additionally, one has to take into account that the cannabinoid reporter assay does not allow the use of an IS to cope with any variation during sample preparation. So, although the absolute deviation might seem pronounced (especially at higher concentrations (10–100 ng/mL)), the bioassay does allow for providing a semiquantitative estimate of the JWH-018 concentration in a sample.

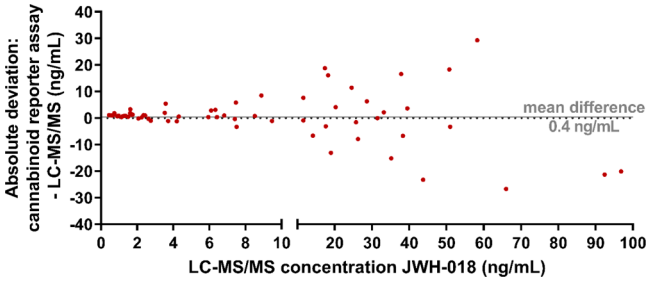

Figure 3. Absolute differences between the JWH-018 concentration determined via the cannabinoid reporter assay and the LC-MS/MS.

CONCLUSION

Although the number of new SCRAs has gone down in the last couple of years, they still dominate (together with cathinones) the number of seizures, indicating they remain drugs to be reckoned with.[1] The present study is the first to evaluate the application of an untargeted, activity-based approach for the detection of SCRAs in OF. It is also the first time that, using authentic samples, the potential of the bioassay to deliver semiquantitative results is demonstrated. The bioassay correctly classified the vast majority of the samples as either negative (<0.25 ng/mL; 74/75 = 99%), or having low (0.25–1.5 ng/mL; 16/16 = 100% and 1.5–10 ng/mL; 37/41 = 90%), mid (10–100 ng/mL; 23/25 = 92%), or high (>100 ng/mL; 16/18 = 89%) JWH-018 concentrations. This indicates that, besides urine, vitreous, plasma, serum, or blood, the activity-based cannabinoid reporter assay can also be used to screen OF for cannabinoid activity. Moreover, our results indicate that activity-based testing can not only be used to point out whether a sample is positive or not but also to get an estimate of drug concentrations. In the case presented here, JWH-018 was the sole analyte present at relevant concentrations in the evaluated matrix. In the future, it will be relevant to evaluate whether also the activity of a combination of analytes (main compounds and metabolites) can accurately be predicted. To achieve this, a proper "activity threshold" (corresponding to a "concentration threshold" of a reference compound) should be defined. This could be done by running a set of calibration lines (of e.g. JWH-018) at low concentrations and using the slope and standard deviation of the response to calculate this threshold. This would lend further support to the concept of "activity equivalents",[27] via which the combined activity of analytes present in a biological matrix can be used to get a better idea about the degree of intoxication. Although not within the scope of this study, we anticipate that also OF in which THC is present at relatively high concentrations may yield a positive response in the activity-based assay (as THC is only a relatively weak CB1 agonist we anticipate only a weak response, even at relatively high concentrations).[28] However, we do not envisage the use of activity-based screening to routinely detect the presence of THC in OF. Conventional approaches, either antibody-based screening or routine GC- or LC-MS/MS procedures, typically offer sufficient specificity and superior sensitivity for this purpose. However, as the higher penetration of these conventional tests in, for example, roadside testing may paradoxically cause an incentive to use SCRAs, which are typically not tested for in this context, screening OF for cannabinoid activity, as a complement, may allow closing this loophole.

AUTHOR INFORMATION

Corresponding Author

Christophe P. Stove – *Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium;* orcid.org/0000-0001-7126-348X; Phone: +32 9 264 81 35; Email: christophe.stove@ugent.be; Fax: +32 9 264 81 83

Authors

Annelies Cannaert – *Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium;* orcid.org/0000-0002-8654-1371

Maria del Mar Ramírez Fernández – *Laboratory of Toxicology, National Institute of Criminalistics and Criminology, 1120 Brussels, Belgium*

Eef L. Theunissen – *Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, 6229 Maastricht, The Netherlands*

Johannes G. Ramaekers – *Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, 6229 Maastricht, The Netherlands*

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Sarah M. R. Wille − *Laboratory of Toxicology, National Institute of Criminalistics and Criminology, 1120 Brussels, Belgium*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c00484

Author Contributions

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Notes

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