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Optimisation of a gas chromatography-mass spectrometry method for the simultaneous determination of tetrahydrocannabinol and its metabolites in rat urine

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In order to evaluate the effect of irinotecan (IRI) on urinary elimination of delta-9-tetrahydrocannabinol (THC) in a rat experimental model, we developed an analytical method for the determination of the mass concentration of THC and its metabolites [11-hydroxy-delta-9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH)] in the urine of rats treated only with THC and treated simultaneously with THC and irinotecan. For this purpose, hydrolysis and solid phase extraction conditions of the investigated analytes were optimised and a gas chromatography-mass spectrometry (GC-MS) method was developed to determine all three analytes in rat urine. The most effective hydrolysis method for THC, THC-OH, and THC-COOH conjugates was so-called tandem hydrolysis by the β-glucuronidase enzyme from *Escherichia coli* at 50 °C for 2 hours and followed by alkaline hydrolysis. The proposed method was then applied for determining concentrations of analytes in 24-hour rat urine. THC was not detected in either sample, THC-OH was detected in 50 % of samples, and THC-COOH in all of the samples. Enhanced urinary THC-COOH excretion was noted in rats administered combined treatment compared to single THC treatment. The method described herein was suitable for determining the mass concentration of THC metabolites in the rat urine due to its sensitivity (detection limits: 0.8-1.0 μg/L), accuracy (>96 %), and precision (RSD <6 %).

KEY WORDS: analytical validation; cannabis; GC-MS; hydrolysis; irinotecan

Delta-9-tetrahydrocannabinol (THC) is the main psychoactive substance in the plant *Cannabis sativa*. Lately, interest in the therapeutic effects of cannabinoids and the development of cannabinoid-derived medicine has risen, among others, for the purpose of alleviating symptoms such as diarrhoea, abdominal pain, nausea caused by chemotherapy, vomiting, etc. (1). Most of the aforementioned symptoms appear during therapy with the antitumor drug irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin, IRI, CPT-11) whose primary purpose was chemotherapy in metastatic colorectal cancer, but was also proven to be effective in treating lung cancer, ovarian cancer, leukaemia, and malignant glioma (2).

Although there are several conventional therapies that could relieve the undesired effects of antitumor drugs, we are faced with the growing use of cannabinoid-containing oral preparations administered for this purpose. Unlike approved and registered products that contain a standardised and defined dose of THC, illegal products with untested compositions may contain a high THC concentration. This exposes patients to increased risks of harmful effects, as well as to potential intoxication. Apart from insufficient data on the therapeutic efficacy of such products, there is also concern regarding the potentially negative effects that result from the pharmacokinetic and pharmacodynamic interactions of cannabis' main components with standard drugs (3). The quantification of cannabinoids in urine is necessary in pharmacokinetics studies in order to evaluate the potential impact of the chemotherapeutics (e.g. IRI) on THC metabolism.

Due to the fast metabolism of cannabinoids, concentrations of THC metabolites are much higher in urine than in blood, so urine is considered the biological sample of choice for the identification and quantification of cannabinoids (4). Furthermore, urine as a biological material is relatively easy to obtain (5).

Cannabinoids are rapidly and extensively metabolised by hepatic enzymes. The hydroxylation of THC yields the psychoactive component 11-hydroxy-delta-9-tetrahydrocannabinol (THC-OH), whereas further oxidation leads to the inactive 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) excreted by urine mostly as a conjugate of glucuronic acid (6). Glucuronidation

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is catalysed by uridine diphosphate-glucuronyltransferases (UGTs) (7).

The unambiguous identification of cannabinoids and metabolites in urine was carried out by a gas chromatograph (4, 8, 9) or a high performance liquid chromatograph (10-13) coupled with a mass spectrometer as a detector. High performance liquid chromatography-mass spectrometry (HPLC-MS) enables direct conjugate detection, while the widely used gas chromatography-mass spectrometry (GC-MS) requires hydrolysis of conjugates before extraction and analysis to quantify a total concentration (free and conjugated fraction) of the measured analytes. Considering that cannabinoids and their metabolites are present in urine mainly in the form of conjugates, it is necessary to hydrolyse these compounds. Enzymatic hydrolysis using β-glucuronidases is effective for the cleavage of the glucuronide bond in THC and THC-OH glucuronides, while alkaline hydrolysis is more effective for splitting THC-COOH glucuronides (14). In order to separate the desired analytes from the complex sample matrix before instrumental analysis, organic solvent extraction (*n*-hexane, ether, ethyl acetate, etc.) (10, 15) or solid phase extraction (SPE) (4, 8, 9, 12, 13) were employed.

Although rats have often been used as an experimental model for understanding the metabolism of drugs (16-20), to the best of the authors' knowledge, urinary THC and metabolite detection following oral application of THC has not yet been studied. In the existing literature we have found optimised and validated chromatographic methods only for the determination of concentrations of cannabinoids and metabolites in human urine. Considering that rat urine differs from human urine in composition, a reliable method for the detection and quantification of THC and its metabolites in rat urine is required. Therefore, this study optimised the hydrolysis and extraction conditions for THC and its metabolites (THC-OH and THC-COOH) and developed and validated a GC-MS method for the simultaneous determination of the mass concentration of all three analytes in rat urine. The proposed method was applied to determine the THC, THC-OH, and THC-COOH concentration in 24-hour urine of rats treated only with THC or THC and IRI simultaneously.

MATERIALS AND METHODS

Chemicals and reagents

THC (Dronabinol; CAS-No. 1972-08-3) was obtained from THC Pharm GmbH (Frankfurt, Germany). Before administration, THC was dissolved in sesame oil (Bio Primo, Ulm, Germany). IRI (CAS-No. 100286-90-6) was provided as the hydrochloride trihydrate salt by LC Laboratories (Woburn, MA, USA). It was diluted in sterile 0.9 % sodium chloride solution (Croatian Institute for Transfusion Medicine, Zagreb, Croatia).

Analytical standards of THC (1 g/L), THC-OH (0.1 g/L), THC-COOH (1 g/L), and THC-COOH-d_3 (1 g/L; internal standard) were purchased from Lipomed (Vienna, Austria). Intermediate stock solutions (1000 µg/L) were prepared by dilution with methanol and were stored at -20 °C. Methanol, dichloromethane, *n*-hexane, and ethyl acetate (HPLC grade) were purchased from Merck (Darmstadt, Germany), while glacial acetic acid (99.5 %), isopropyl alcohol, ammonium hydroxide, potassium phosphate monobasic, and potassium hydroxide were analytical-grade-purity products of Kemika (Zagreb, Croatia). N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) was obtained from Restek (Bellefonte, PA, USA).

β-glucuronidases [from *Escherichia coli* (type IX-A, lyophilized powder, 1,000,000-5,000,000 units/g protein) and from *Helix pomatia* (type H-2, aqueous solution, ≥85,000 units/mL)] were purchased from Sigma (St. Louis, MO, USA). A working solution of β-glucuronidase from *E. coli* was prepared by dissolving the enzyme in 0.1 mol/L phosphate buffer pH 6.8.

Ultra-pure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Animals

For the purpose of this study, adult male three-month old Wistar HsdBrlHan rats were supplied by the animal facility of the Institute for Medical Research and Occupational Health, Zagreb (Croatia). Animals were kept in standard clear polycarbonate cages (Ehret, Tulln, Austria) under pathogen-free and steady-state microenvironmental conditions, 12 h light/dark cycle, room temperature 20-22 °C and humidity 40-60 %, with *ad libitum* access to standard Good Laboratory Practice (GLP) certified food (Complete feed for mice and rats 4RF21, Mucedola, Italy) and tap water. Appropriate enrichment was provided in animal cages. The research procedures were carried out in compliance with international standards and the national law on the protection of animal welfare. The study was approved by the Ethics Committee of the Institute for Medical Research and Occupational Health, Zagreb, Croatia (approval number: 100-21/16-16, 30 June 2016).

Experimental design

Adult male rats with an initial body weight of 235 g to 249 g were randomly assigned to three groups, each comprising 5 animals. Rats were placed in metabolic cages for 24 hours for urine sample collection.

The THC group received a single dose of 7 mg of THC/kg body weight (bw) *per os* (*p.o.*). The IRI+THC group received IRI intraperitoneally (*i.p.*) via a single dose of 100 mg/kg bw and immediately afterwards 7 mg/kg of THC *p.o*. The control group was treated with sesame oil *p.o*. and kept in the same conditions. For the optimisation of the

hydrolysis and extraction procedures, the THC group (N=5) received a single dose of 20 mg THC/kg bw.

The urine samples were collected into 10-mL polypropylene tubes over 24 hours from the moment of applying the tested substances. The samples from each rat were pooled and stored at -20 °C until analysis.

Optimised hydrolysis and solid phase extraction

The urine samples of rats (3 mL) treated with THC or the THC and IRI combination were mixed with 6 mL of 0.1 mol/L phosphate buffer (pH 6.8) with the addition of 60 µL of internal standard at concentrations of 1000 µg/L and 300 µL of solution of β-glucuronidase from *E. coli* (25,000 U/mL). To create a calibration curve within the range of 3-100 µg/L, the urine of rats from the control group (negative for the tested analytes) was spiked with appropriate aliquots of analytical standards (THC, THC-OH, THC-COOH, and THC-COOH-d_3). The mass concentration of the internal standard was 20 µg/L in all of the samples. Samples were vortex mixed and the pH of the samples was confirmed to be 6-7. Enzymatic hydrolysis of samples was performed at 50 °C for 2 hours. After the samples were cooled, 300 µL of 10 mol/L KOH was added, the samples were vortex mixed and chemical hydrolysis was carried out at 60 °C for 15 min. After cooling, 165 µL of glacial acetic acid and 2 mL of 0.1 mol/L sodium acetate in 5 % methanol (pH 7.0) were added to the samples. The measured pH values had to be within 4.5-6.5. The samples were centrifuged at 1400 *g* for 15 min and a supernatant was used for further procedures.

The extraction procedure was adjusted according to a previously optimised method for determining the mass concentration of THC-COOH in human urine (21). Bond Elut Certify II (Agilent Technologies, USA) columns were used for the solid phase extraction (SPE) of cannabinoids and metabolites from the urine samples. SPE columns were conditioned with 2 mL of methanol and 2 mL of 0.1 mol/L sodium acetate in 5 % methanol (pH 7.0). The sample was passed through a column and the column was then washed with 2 mL of methanol and water (1:1, v/v) mixture. After drying the column for 2 min using vacuum, the analytes were eluted with 2 mL mixture of *n*-hexane:ethyl acetate:glacial acetic acid (75:25:1, v/v/v).

The eluents were collected in glass tubes and evaporated to dryness under a stream of nitrogen at room temperature. The dry residue was mixed with 0.5 mL of toluene and evaporated in the same manner. Then, 50 µL of BSTFA + 1 % TMCS were added to the residue and the sample was capped, shaken, and derivatised for 15 min at 90 °C.

To optimise the hydrolysis and extraction conditions, we used urine from rats that orally received a single dose of THC (20 mg/kg bw) and had a measurable level of all three analytes in their urine. The hydrolysis efficiency was tested by using β-glucuronidase (5000 U/mL urine) originating from *E. coli* and *H. pomatia* using the following hydrolysis conditions: 1) 37 °C for 16 hours and 2) 50 °C for 2 hours. Afterwards, the obtained results were compared with a) chemical hydrolysis (300 µL of 10 mol/L KOH, 60 °C for 15 min) and b) tandem hydrolysis [chemical hydrolysis following hydrolysis by β-glucuronidase from *E. coli* (5000 U/mL urine), at 50 °C for 2 hours]. Also, to evaluate hydrolysis and extraction efficiency we tested the volume (3 and 6 mL) of 0.1 mol/L phosphate buffer (pH 6.8) which was added before hydrolysis, activity of β-glucuronidase from *E. coli* (5000 and 7000 U/mL), which is sufficient to complete hydrolysis, and the effect of centrifugation (room temperature, 15 min, 1400 *g*) on extraction.

GC-MS analysis

The analyses were carried out using a Trace 1300 gas chromatograph (Thermo Scientific, Milan, Italy) coupled to a ITQ 700 ion trap mass spectrometer (Thermo Scientific, Austin, TX, USA). The analytes were separated on a TG-5MS capillary column (30 m×0.25 mm ID, 0.25 µm film thickness, Thermo Scientific, Runcorn, UK). Helium was used as the carrier gas at a flow rate of 1 mL/min. Samples (1 µL) were injected in the programmable temperature vaporizing (PTV) injector which temperature was held at 40 °C for 0.1 min and then increased to 280 °C at 3 °C/s. The initial oven temperature was set at 50 °C for 1 min, then increased to 250 °C at 50 °C/min, held for 1 min, increased to 280 °C at 5 °C/min, and held for 1 min. The transfer line temperature was set at 280 °C. The ion source temperature was set at 200 °C. The MS detector operated in electron impact ionisation mode. Three ions were monitored for each trimethylsilyl (TMS) derivative: THC-TMS: *m/z* <u>371</u>, 386, 303; THC-OH-2TMS: *m/z* <u>371</u>, 474, 459; THC-COOH-2TMS: *m/z* <u>371</u>, 488, 473, and THC-COOH-d_3-2TMS: *m/z* <u>374</u>, 491, 476. The underlined ions were used for quantitation.

Analytes were identified by a comparison of the retention time of each tested analyte with the retention time of the analytical standards followed by a comparison between the obtained mass spectrum of each tested analyte and the mass spectrum from our own database and NIST13 mass spectra database. An internal standard method was used to quantitatively determine the analytes.

Analytical validation of the GC-MS method

The calibration curve was prepared using the final experimental parameters in the concentration range of 3-100 µg/L. The limit of detection (LOD) was calculated using a signal-to noise ratio of 3. Precision and accuracy were evaluated by analysing the blank urine samples spiked with two different concentrations of THC, THC-OH, and THC-COOH (10 and 40 µg/L) in six replicates. Accuracy was estimated by comparing the analyte concentration calculated from the calibration curve using the internal

standard method with the theoretical value. Precision was expressed as relative standard deviation (RSD).

Statistical analysis

The statistical analysis of the results was carried out using the Dell™ Statistica™ 13.2 software (StatSoft, Tulsa, OK, USA). The normality of distribution for numerical data was tested by Shapiro-Wilk's test. Due to an asymmetrical distribution of the measured parameters, results within groups were expressed as median with ranges, while statistical significance between the groups was confirmed by Mann-Whitney U-test. In cases when the concentrations were lower than the limit of detection, values 0.5 x LOD were used for statistical analysis. The level of statistical significance was set at $p<0.05$.

RESULTS

The development of the analytical procedure for the determination of THC and metabolites in rat urine included the optimisation of hydrolysis and extraction conditions, choosing appropriate chromatographic conditions that enable the efficient separation of selected analytes, determining accuracy and precision as well as the linearity and sensitivity of the detector's response. The largest peak area for the target ion of a particular analyte corresponded to the most efficient hydrolysis and extraction. All of the analyses were conducted in triplicate.

In the first experiment, the efficiency of two enzymes of different origin (from *E. coli* and *H. pomatia*) and different conditions: 1) 37 °C for 16 hours and 2) 50 °C for 2 hours) for hydrolysis of conjugates of THC and metabolites in rat urine were tested. Hydrolysis using enzyme from *H. pomatia* did not enable the detection of THC, while all three analytes were detected when hydrolysis with enzyme from *E. coli* was performed using both hydrolysis conditions. There were no differences in the hydrolysis efficiency of THC and THC-OH conjugates in both of the applied conditions, while the efficiency of hydrolysis of THC-COOH conjugate was 45 % greater at the higher temperature over the shorter hydrolysis time. With this in mind, for further hydrolysis experiments, the enzyme from *E. coli* was chosen with hydrolysis conditions at 50 °C during 2 hours. For THC-COOH-glucuronide hydrolysis, alkaline hydrolysis with KOH at 60 °C for 15 minutes was the most efficient. Combined (tandem) hydrolysis (enzymatic followed by alkaline) resulted in the most effective hydrolysis for all three conjugates (Figure 1).

In the following experiment, the effect of the amount of β-glucuronidase from *E. coli* (5000 and 7000 U/mL) on the hydrolysis degree of the conjugates was tested. Considering that the hydrolysis was equally effective using both amounts of enzyme, the smaller quantity of enzyme (5000 U/mL) was used in further experiments.

A volume of 0.1 mol/L phosphate buffer (pH 6.8; 3; and 6 mL) added to the urine sample before enzyme hydrolysis was also tested. The twice lower chromatogram baseline noise and the 20 % larger surface of target peaks were achieved by using a more diluted sample (6 mL of buffer). An additional decrease of the chromatogram baseline noise (25 %) was achieved by centrifugation (15 min, 1400 *g*) of the sample before adding it to the SPE column.

The proposed GC-MS conditions enabled the efficient separation of all of the tested analytes without interferences. IRI treatment did not influence the quality of the obtained

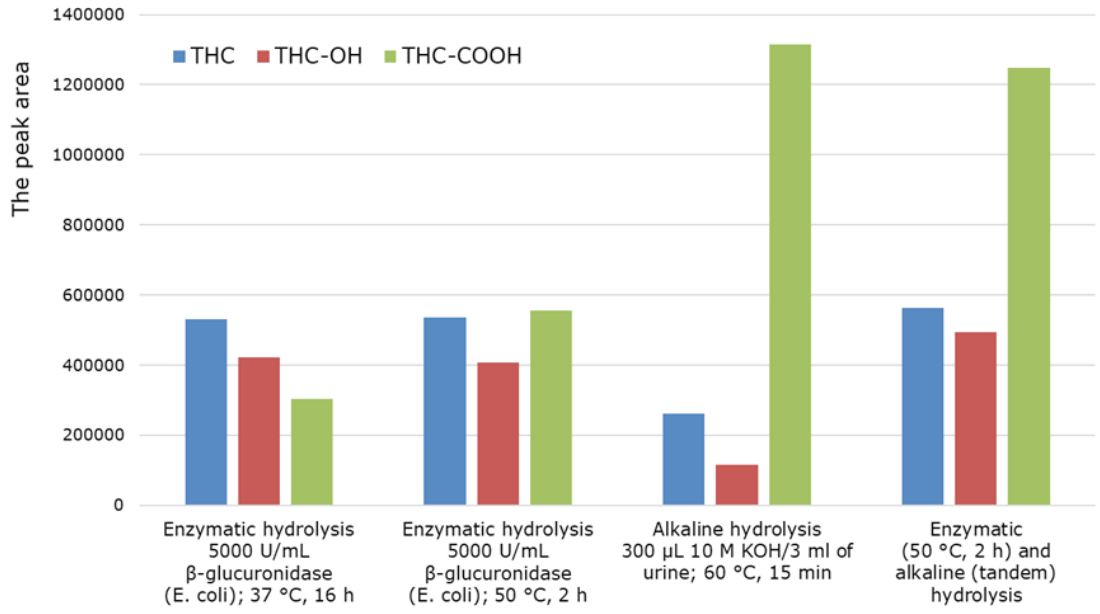

Figure 1 Comparison of different types of hydrolysis for cleavage of conjugates THC, THC-OH, and THC-COOH from 24-hour rat urine (pooled sample of five rats that received a single dose of 20 mg of THC/kg bw orally)

chromatograms. In order to avoid the appearance of additional peaks at higher column temperatures due to the impurities present in the complex sample matrix, after programmed heating to 280 °C, the column remained at that temperature for an additional 0.5 min.

Regressions were calculated for each of the calibration curves (THC, THC-OH, and THC-COOH, 3-100 μg/L) and they all showed good linearity with coefficients of determination (R^2)>0.9986, $p<0.01$. Table 1 shows the results of the analytical validation of the method for determining the mass concentration of cannabinoids and metabolites in rat urine. The limits of detection were within the range of 0.8-1.0 μg/L, whereas accuracy exceeded 96 % for both of the tested concentration levels. Precision, expressed as relative standard deviation (RSD), was < 6 %.

The suggested validated method was used to analyse 10 samples of 24-hour rat urine, five of which were treated with a single dose of THC (7 mg/kg), while another five were treated with a single dose of THC (7 mg/kg) and IRI (100 mg/kg). The cannabinoid and metabolite concentrations in the 24-hour rat urine from the aforementioned two groups are shown in Table 2. THC was not detected in any of the samples, THC-OH was detected in 50 % of samples, while THC-COOH was found in all of the samples. A statistically significantly higher mass concentration of the THC-COOH in urine was found in the THC+IRI group compared to the THC group.

DISCUSSION

Urine is the most frequently used biological sample for the analysis of metabolites, as it is easily available and contains larger quantities of metabolites than other body fluids (6). The metabolic profile of human and rat urine is quite different. Rat urine is richer in amino acids and is therefore thicker, more viscous, and less transparent (22). Considering the differences in the composition of human and rat urine, the complexity of the sample matrix, and differences in metabolic rate (rats metabolise THC more quickly due to the larger amount of enzymes from cytochrome system P450 per gram of body mass) (23), it was necessary to optimise and validate a GC-MS procedure for determining the THC and metabolite concentration in rat urine. To the best of our knowledge, the relevant literature does not contain a single mention of a validated method involving GC-MS used for the qualitative and quantitative determination of the aforementioned analytes in rat urine.

In the first experiment, we optimised the conditions of glucuronide hydrolysis, as during THC metabolism, THC and metabolite conjugation with glucuronic acid occurs and glucuronides are formed (9). Having in mind the polarity and size of a metabolite bound in the form of a glucuronide, it is not possible to analyse them using gas chromatography. For that reason, it is necessary to separate the analyte from the glucuronic acid by hydrolysis process. The enzyme β-glucuronidase isolated from *E. coli* bacteria showed better hydrolytic activity than the same enzyme isolated from the *H. pomatia* snale. The dependence of β-glucuronidase activity on the source of the enzyme is in accordance with literature sources that point to a weak hydrolytic activity of enzymes from *H. pomatia* towards THC and THC-COOH glucuronides (15). We monitored the efficiency of enzymatic hydrolysis by β-glucuronidase from *E. coli* over two time intervals at different temperatures (16 hours at 37 °C and 2 hours at 50 °C), non-enzymatic alkaline hydrolysis by potassium hydroxide, and combined (tandem) hydrolysis (enzyme followed by alkaline). While both conditions of enzymatic hydrolysis yielded similar results, with a slightly higher detected concentration of THC-COOH at 50 °C during 2 hours compared to enzymatic hydrolysis at 37 °C during 16 hours, potassium hydroxide hydrolysis exhibited the highest THC-COOH concentrations with extremely low THC-OH and THC values, suggesting that potassium hydroxide does not hydrolyse the ether bond in THC and THC-OH glucuronides, but only the ester bond in the THC-COOH glucuronide (8, 15). Enzymatic hydrolysis is more suitable for splitting the ether bond (15). Combined hydrolysis was shown to be the most effective, after which the highest concentrations for all of the analytes were detected, with special emphasis on the THC-COOH concentration, which was higher in comparison with individual hydrolyses. Therefore, tandem hydrolysis, using the enzyme from *E. coli* at 50 °C during 2 hours, followed by alkaline hydrolysis at 60 °C during 15 min, proved to

Table 1 The precision, accuracy, and limit of detection in determining the mass concentrations of cannabinoids and metabolites in rat urine (N = 6)

Analyte	γ (μg/L)	Precision (RSD %)	Accuracy (%)	Limit of detection (μg/L)
THC	10	4.4	96.7	0.9
	40	3.9	97.7	
THC-OH	10	5.9	97.6	1.0
	40	4.8	99.1	
THC-COOH	10	5.2	97.3	0.8
	40	4.4	98.2	

N=number of replicates at each concentration level; γ – mass concentration; RSD – relative standard deviation

Table 2 Mass concentrations of cannabinoids and metabolites in 24-hour rat urine from the THC and IRI+THC groups

Analyte	Mass concentration (μg/L) Median (range)	
	THC group (N = 5)	IRI+THC group (N = 5)
THC	ND	ND
THC-OH	0.5 (ND-8.5)	1.7 (ND-9.3)
THC-COOH	16.6 (15.1-18.4)	18.7* (18.5-22)

* statistically significant difference in comparison with the THC group ($p<0.05$; Mann-Whitney's U-test)

be the best with the highest detected concentrations of all the analytes.

During hydrolysis, for purposes of urine sample preparation, the effect of a volume of phosphate buffer (3 and 6 mL) added before hydrolysis to dilute the thick rat urine samples was examined. We concluded that greater dilution is more favourable for further analyses of samples on SPE columns, which resulted in lower chromatogram baseline noise and a larger surface of target peaks. The efficiency of extraction was also increased by sample centrifugation, which removes the tiny particles that originate from the enzymes and significantly endanger the quality of the chromatogram. Hydrolysis was also efficient with the smaller enzyme quantity (5000 U/mL) and our results were in accordance with the available literature (15).

GC-MS analysis of cannabinoids and metabolites in urine using optimised conditions of hydrolysis and extraction enabled an effective separation of all of the tested analytes. The sensitivity (LOD=0.8-1.0 μg/L), accuracy (>96 %), and precision (RSD<6 %) of the optimised and validated GC-MS method were similar to the values of these parameters obtained by other authors using GC-MS (8) and LC-MS (12, 13) for the determination of cannabinoids and metabolites in human urine.

The proposed GC-MS method was used to analyse 24-hour urine samples of rats administered only THC and THC in combination with IRI. Enhanced urinary THC-OH and THC-COOH concentrations were noted in rats administered combined treatment compared to single THC treatment. Possible explanations for such a result could be the potential redistribution of THC from fatty tissues into blood due to interaction with IRI (7), fatty tissue loss caused by IRI application, which also increases the free fraction of THC in blood available for metabolising (24), or the competition of THC and IRI for the same enzyme in phase II metabolism (conjugation with glucuronic acid mediated by UGT1A1) (25, 26).

CONCLUSION

To understand changes in THC metabolism as a consequence of its concomitant use with different chemotherapeutics, it was necessary to find a suitable method for quantifying THC and its metabolites in biological samples. The GC-MS method proposed within this study is sensitive, accurate, and precise and therefore acceptable for the quantification of THC metabolites in rat urine.

However, further studies are needed to elucidate the increase of the THC metabolites urinary concentrations in the concomitant use of IRI and THC.

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Conflicts of interest

The authors declare no conflict of interest.

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Optimizacija metode plinske kromatografije-spektrometrije masa za istovremeno određivanje tetrahidrokanabinola i njegovih metabolita u urinu štakora

S ciljem procjene učinka irinotekana (IRI) na eliminaciju delta-9-tetrahidrokanabinola (THC) mokraćom u eksperimentalnom modelu štakora, razvili smo analitičku metodu za određivanje masene koncentracije THC-a i njegovih metabolita [11-hidroksi-delta-9-tetrahidrokanabinola (THC-OH) i 11-nor-9-karboksi-delta-9-tetrahidrokanabinola (THC-COOH)] u mokraći štakora tretiranih samo THC-om i tretiranih istodobno s THC-om i IRI-jem. U tu svrhu optimizirani su uvjeti hidrolize i ekstrakcije na čvrstom nosaču za ispitivane analite i razvijena je metoda plinske kromatografije-spektrometrije masa (GC-MS) kako bi se kvantificirala sva tri analita u mokraći štakora. Najučinkovitija metoda hidrolize za konjugate THC, THC-OH i THC-COOH bila je tandemska hidroliza enzimom β-glukuronidazom iz *Escherichia coli* na 50 ° C u trajanju od 2 sata koju je slijedila alkalna hidroliza. Predložena metoda je primijenjena za određivanje masene koncentracije analita u 24-satnom urinu štakora. THC nije detektiran ni u jednom uzorku, THC-OH je kvantificiran u 50 % uzoraka, a THC-COOH u svim uzorcima. Uočeno je povećano izlučivanje THC-COOH u mokraći štakora koji su tretirani kombinacijom THC-a i IRI-ja u usporedbi sa štakorima tretiranim samo THC-om. Opisana metoda bila je pogodna za određivanje masene koncentracije metabolita THC-a u mokraći štakora zbog njene osjetljivosti (granice detekcije: 0,8-1,0 μg/L), točnosti (> 96%) i preciznosti (RSD <6%).

KLJUČNE RIJEČI: analitička validacija; GC-MS, irinotekan; hidroliza; kanabis