Modified QuEChERS extraction and GC-MS analysis of selected cannabinoids from human urine

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Abstract: The aim of this work is to apply a modified QuEChERS method to extract cannabinoids from urine, using a mixture of salts for extraction in an appropriate ratio instead of commercially available cartridges. The analysis was performed on blank urine to which a known concentration of tetrahydrocannabinol (THC), cannabinol (CBN) and cannabidiol (CBD), 11-hydroxy-Δ⁹-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH) was added. Six solvents, as well as four solvent mixtures, were tested for extraction and the solvent mixture acetonitrile: dichloromethane (1:3) was selected for which the best recovery factor was obtained. Derivatization of all samples was done with MSTFA (N-trimethylsilyl-N-methyl trifluoroacetamide) + 1% TMCS (2,2,2-Trifluoro-N-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane) at room temperature. The prepared extracts were analyzed by a coupled system, gas chromatography-mass spectrometry (GC-MS) in full-scan mode. Peaks of selected cannabinoids are well separated indicating that there was no interference with the selected analytes. The results were calculated from a calibration curve ranging from LOQ to 1000 ng/mL for selected cannabinoids with a correlation factor over 0.998. The LOD and LOQ for THC are (3.0 ng/mL; 9.0 ng/mL), for CBN (5.0 ng/mL; 18.0 ng/mL) for CBD (5.0 ng/mL, 16.0 ng/mL), for THC-OH (2.6 ng/mL; 8.7 ng/mL) and for THC-COOH (5.0 ng/mL; 15.0 ng/mL). The recovery factor was recorded in the range of 79.40% for THC-COOH to 94.86% for CBN.

The modified QuEChERS extraction method can be used for routine analysis of selected cannabinoids. This method was successfully applied on real samples and thirty urine samples were analyzed.

INTRODUCTION

Cannabinoids are commonly present in Cannabis sativa L., and include compounds of which Δ⁹-tetrahydrocannabinol (THC) is the most psychologically active component. These psychoactive constituents are responsible for most of the pharmacological effects. Cannabis also known as marijuana has number of health
benefits for humans, including treating glaucoma, controlling epileptic seizures, and stopping cancer spreading. (Zhang, Wang, Mi, et al., 2016). Analysis of THC often includes the simultaneously analyses of other cannabinoids and its metabolites.

THC is a lipophilic compound and is widely distributed in the body. From the gastrointestinal tract, THC is absorbed, but absorption is slow and irregular. It is oxidised to the active metabolite THC-OH, which is further oxidized to the inactive metabolite THC-COOH. Up to almost a quarter of the dose is excreted in the urine in 3 days, mainly as THC-COOH in free and conjugated form (Moffat, Osselton, Widdop, et al., 2011).

Recently, the increasing use of marijuana for medicinal purpose has led to increased interest in the determination of cannabinoids in biological matrixes. Urine is a sample of choice for these analyses, because its simplicity and security of sampling and after metabolic process, the concentration of THC metabolites is higher in urine than in blood. Cannabinoids and their metabolites are in the form of conjugates, so they need to be hydrolyzed by enzymatic (Fuchs, Miljanić, Katić, et al., 2019) or alkaline hydrolysis (Battista, Sergi, Montesano, et al., 2014).

Many analytical techniques are available for determination of cannabinoids such as thin layer chromatography (Galand, Ernouf, Montigny, et al., 2004), radioimmunoassay (Clawthery, Oon, Smith, et al., 1990), HPLC method (Scheidweiler, Desrosiers, and Huestis, 2012), Aizpurua-Olaizola, Zarandona, Ortiz, et al., 2016), and GC-MS method (Nestić, Babić, Pavlović, et al., 2013, Heinl, Lerch and Erdmann, 2016).

The coupled system GC-MS ensures the necessary selectivity and sensitivity to confirm positive results induced by screening methods or by the quantification process needed in clinical studies (Kemp, Abukhalaf, Manno, et al., 1995). The analysis of cannabinoids in biological fluids is a challenging issue as it is very important to determine low concentrations for toxicology as well as for clinical use. Numerous analytical methods have been applied to analyze THC, CBD and CBN in urine samples, but the extraction procedure is expensive, (Raharjo and Verpoorte, 2004).

Many studies have investigated methods of extraction and determination of cannabinoids in body fluids, (Citti, Braghiroli, Vandelli, et al., 2018, Aizpurua-Olaizola, Zarandona, Ortiz, et al., 2017, Ramesh, Manjula, Bijargi, et al., 2015).

Legislation of marijuana has been increasingly talked about, increasing the need to find methods for determining the lowest concentrations of cannabinoids in biological samples, both for medical purposes and for determining the concentration of these compounds in case of marijuana abuse. Numerous extraction methods have been developed for the determination of cannabinoids in body fluids. The aim of this work was to determine the concentration of tetrahydrocannabinol (THC), and its metabolites, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) and 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH), as well as cannabinol (CBN), and cannabidiol (CBD) in human urine of patients on methadone therapy and in urine samples subjected to analysis as samples obtained from Laboratory for Toxicology Studies and Sanitary Work Environment, Institute of Occupational Health Sarajevo.

**EXPERIMENTAL**

**Chemicals and reagents**

All standards, THC, THC-OH, THC-COOH, CBN and CBD were purchased from Lipomed (Switzerland). Working solutions were prepared in methanol. The derivatization reagent was MSTFA+ 1% silicate derivative TMCS (N-Methyl-N-(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane), ≥99% from Sigma-Aldrich, Deisenhofen, Germany, while all solvents (hexane, methanol, hydrochloric acid, ethyl acetate, dichloromethane, methyl tert-butyl ether, chloroform, acetonitrile, glacial acetic acid) were of HPLC-purity and obtained from Sigma-Aldrich, Seelze, Germany. The solid salts used for QuEChERS extraction were magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen sesquihydrate, sodium tungstate and diatomaceous earth purchased from Sigma-Aldrich, Germany. Immunochromatographic test, Syva-RapidTest d.a.u. 10 were obtained from Dade Behring (Leusden, Netherlands) and Detox-tubes A from (Varian).

**Sample collection and pre-treatment**

Human urine samples (blank), which were used for the optimization and validation of the analytical method, were obtained from volunteers, healthy people not subjected to drugs or any pharmacological treatment.

Two milliliters of urine sample was pipetted into a glass test tube (15 mL). Volume of 200 μL 6 mol/L NaOH was added in urine, for hydrolysis for 30 min at room temperature (25°C). Samples of urine had a high pH, so 400 μL of concentrated acetic acid was added to neutralise and the pH value was adjusted using the acetate buffer (pH 4) to the total volume of 5 mL. The urine sample was briefly vortexed and then centrifuged at 2600 rpm for 10 min.

A fully optimized and validated method was applied to the urine samples of drug-positive patients who underwent methadone therapy at Institute for Addiction Disorders of Canton Sarajevo, and samples obtained from Laboratory for Toxicology Studies and Sanitary Work Environment.

First, all samples were tested on the presence of cannabinoids by immunochromatographic test, and only positive samples were stored at -20°C until further analysis.

**Extraction with QuEChERS salts – modified**

The extraction procedure was used according to the optimization results. Optimization of the procedure was performed with samples of blank urine (2 mL) spiked with 50 ng/mL of each standard, THC, CBD, CBN, THC-OH and THC-COOH.

The QuEChERS procedure was modified using mixture of salts in a 15 mL glass test tube. Content of 1.3 g of mixture was as follows, 0.4 g of MgSO₄; 0.1 g NaCl; 0.1
g of C₈H₆Na₃O₂·xH₂O and 0.05 g of C₈H₇Na₃O₄·xH₂O. Ratio of salts in mixture for extraction was MgSO₄ : NaCl : C₈H₆Na₃O₂·xH₂O : C₈H₇Na₃O₄·xH₂O (4:1:1:0.5). Solid salts and 3 mL of a solvent mixture of acetonitrile: dichloromethane (1:3) were placed in test tube for extraction. Aliquot of 5mL of prepared urine sample was added and shook gently for about 1 minute manually. Mixing was continued on a stir on the roller for 10 minutes, and finally centrifuged for 10 min at 3000 rpm. The top organic layer was separate into a vial and evaporated to dryness under a stream of nitrogen flow at room temperature. The derivatization was done with 30 μL MSTFA +1% TMCS, vortexed for 5 s and allowed to stand at 25°C for 30 min (Nadulski, Sporkert, Schnelle, et al., 2005) to accomplish silylation. The extract was transferred to 250 μL vial. An aliquot of 1 μL of the prepared extract was injected into the GC-MS system.

**GC-MS analysis**

The GC-MS analysis was performed on GC-MS Agilent Technologies. Inc. GC7890A; MS 5975C and Autosampler 7983. Chromatographic separation was achieved using a capillary column HP-5MS 30 m x 0.25 mm ID, 250 μm film thickness. The analyses were performed using simultaneous MS Scan (scan range 40–600 Da) and Single Ion Recording. Mass spectrometer mode: electron ionization (EI) conditions (70 eV). An example of volume of 1 μL was injected in splitless mode. Injector temperature was set to 250°C. Ultrapure helium gas was used as the carrier gas at a flow rate 1.5 mL/min.

Initial oven temperature was 100°C held for 1 min, raised to 175°C at 30°C/min, and then to 310°C at 12°C/min and held for 10.25 min (Alves, Agonia, Cravo, et al., 2017, Angeli, Casati, Ravelli, et al., 2018). The total run time was 25 min. Each analyte was identified according to their retention time and three characteristic ions (Table 1). The most abundant ion was used for quantification and the second and third ions were used for the confirmation. Samples were analyzed with GC-MS using the full-scan mode. The readings were compared with the Wiley Library of Mass Spectra of Designer Drugs (Rösner, Junge, Westphal, et al., 2015) and the free database of the Scientific Working Group for the Analysis of Seized Drugs SWGDRUG-3 (Committee 2001).

**Method validation**

The analytical parameters tested within the validation of the method were: selectivity, linearity, examination of extraction steps, accuracy, limit of detection (LOD) and limit of quantification (LOQ) and measuring range (Peters, Drummer and Musshoff, 2007, Shah, Midha, Findlay, et al., 2000). Due to the lack of deuterated cannabinoids as internal standards, validation was performed using the standard addition method (Sutlović, et al., 2011). For validation process, standard solutions of THC, CBD, CBN, THC-OH and THC-COOH in methanol and in blank urine, as well as urine samples (blank urine, positive and negative urine on THC content, CBD, CBN, THC-OH and THC-COOH) were used.

The selectivity was examined by extracting ten urine samples collected from ten different individuals who had not consumed cannabis preparations or other drugs. The resulting extracts were derivatized and analyzed by the GC-MS method. There were no interfering signals at retention times in the tested samples where peaks of interest were expected.

The linearity of the method and the measurement range were determined by the analysis of standard solutions of CBD, THC and CBN, THC-OH and THC-COOH prepared in the blank urine. For this purpose, blank urine samples were spiked with increasing concentrations of CBD, THC and CBN, THC-OH and THC-COOH, and subjected to GC-MS analysis after the extraction procedure was performed. Calibration curves were constructed based on the peak area of CBD, THC, CBN, THC-OH and THC-COOH as analytes, with respect to the corresponding concentration. All measurements were made in triplicate.

The effect of the extraction steps on the determination of CBD, THC and CBN, THC-OH and THC-COOH was investigated by comparative analysis of standard solutions of increasing concentrations (eight concentration points) of the non-extracted test analytes and standard solutions which were added to blank urine samples and extracted.

The accuracy and precision of the method was determined by dosing a standard solution at a concentration of 50 ng/mL for CBD, THC, CBN, THC-OH and THC-COOH in seven (7) different blank urine samples to include matrix influence. Accuracy was calculated as (mean concentration - nominal concentration)/(nominal concentration×100), while precision was calculated as - the relative standard deviation (RSD, %).

**Limit of detection (LOD) and Limit of quantification (LOQ)**

Determination of the limit of detection (LOD) and limit of quantification (LOQ) was performed on the basis of the standard deviation of the peak area of the urine and the slope of the direction obtained by analysing five blank urine analysis in triplicate, soldered with increasing concentrations of standard solutions CBD, THC, CBN, THC-OH and THC-COOH. The formulas used for the calculation were: LOD=3×SD/b LOQ=10×SD/b (SD – standard deviation, b-slope). The standard deviation of the CBD, THC, CBN, THC-OH and THC-COOH peak area was calculated based on linear regression.

**Measuring range**

The measuring range (determination range) covered by this method was the range from the quantification limit shown to the highest value on the calibration curve of 1000 ng/mL.
RESULTS AND DISCUSSION

In order to develop a sensitive and reliable method, it is important to obtain an efficient simple and inexpensive procedure of extraction. The aim of this work was to establish procedure for extraction of cannabinoids as a simple, sensitive, inexpensive and reliable method, using a prepared mixture of salts instead of commercial cartridges. Urine is the preferred sample for drug abuse testing because drugs and metabolites are present in higher concentration in urine than in plasma and because urine can easily be sampled.

In order to find the best conditions for extraction, optimisation was done with solvents, composition of salts mixtures for extraction, pH values and condition of derivatization.

Six solvents; hexane, ethyl acetate, methyl tert-butyl ether, acetonitrile, chloroform, dichloromethane, and four mixtures of solvents, hexane : ethylacetate (8:2), ethylacetate : hexane : acetic acid (49:49:2), ethylacetate : dichloromethane (1:3), acetonitrile : dichloromethane (1:3) were used. It was concluded that a mixture of acetonitrile : dichloromethane (1:3) gave best recovery for THC and its metabolites and this mixture is selected for further analyses. The mixture of hexane : ethylacetate (8:2) is often used for extraction (Abraham, Lowe, Pirnay, et al., 2007), but using this mixture recovery was 80%. Also, a mixture of chlorophorm : ethylacetate (6:4) showed good results (Nestić, Babić, Pavlović, et al., 2013).

The optimisation was done according to the total mass of QuEChERS salt used but in the same ratio, (4:1:1:0.5). The best results were with a mass of 1.3 g, while half of that mass was not sufficient for complete extraction, and twice of that mass was not suitable for extraction the process. Gas chromatogram showed that the use of half of the prepared mass of mixture of salt response for cannabinoids was lower and the extraction was insufficient. In addition to QuEChERS salt, sodium tungstate and diatomaceous earth were used for the extraction. The results of the analysis using sodium tungstate gave similar results to QuEChERS salts, while the extracts with diatomaceous earth were cloudy, colored, and there were many interferences on the chromatogram near the peaks of interest.

The extraction procedure was carried out with three pH values (3, 4, 5) using acetate buffer. Optimal pH value is important for preparation the sample for analysis, especially for the simultaneous analysis of analytes with different characteristics.

The best results were achieved with pH 4. Cannabinoids cannot be directly analyzed with GC without derivatization. After optimization, the best conditions for derivatization were found as follows, addition of a derivatization reagent, MSTFA + 1%TMCS, at room temperature for 30 min. Similar results were obtained using temperature of 75°C and time of derivatization of 10 min, but because of simplicity, conditions at room temperature were chosen.

Under specified chromatographic conditions, the retention times of standards prepared in blank urine corresponded to the retention times of standards prepared in methanol, shown in Table 1 with characteristic ions, ions in bold are target ions, while the others were confirmative ions.

| Analyte  | Retention time (min) | Ions (m/z) |
|----------|----------------------|------------|
| CBD      | 9.98                 | 390.3      |
| THC      | 10.75                | 386.3      |
| CBN      | 11.30                | 367.3      |
| THC-OH   | 12.30                | 474.3      |
| THC-COOH | 13.35                | 371.3      |

Figure 1 shows GC chromatogram of blank urine and GC chromatogram of blank urine spiked with all analytes. Peaks of selected cannabinoids are well separated and can be easily and reproducibly integrated because the other peaks do not influence the height and area, which indicates that there are no interferences of the extraction process. All analytes were sufficiently separated to allow simultaneous determination of THC, its metabolites as well as, CBD and CBN.

Figure 1. GC chromatograms of blank urine (black line) and mixture of canabinoids in human urine (blue line)
Calibration was performed over a wide range of concentration for all analytes and the obtained calibration parameters are presented in Table 2. These samples were then prepared in triplicate according to the procedure described above. The developed method was linear over the range from LOQ to 1000.0 ng/mL for CBD and THC, and the other three analytes CBD, THC-OH and THC-COOH was linear from LOQ to 500.0 ng/mL. For CBD, THC, CBN, THC-OH and THC-COOH, LOD was 5.0, 3.0, 5.0, 2.6, 4.5 ng/mL, respectively. LOQ was in range from 8.7 ng/mL for THC-OH to 18.0 ng/mL for CBN (Table 2). Linearity was determined using linear regression analysis. The correlation coefficient ($R^2$) exceeded 0.998 thus confirming the linearity of the method.

### Table 2. Calibration parameters for determination of cannabinoids

| Analyt | Linearity range (ng/mL) | Equation of calibration curve | $R^2$ | LOD (ng/mL) | LOQ (ng/mL) |
|--------|-------------------------|-------------------------------|-------|-------------|-------------|
| CBD    | 16.0-1000               | $y = 965942x-2760$            | 0.9999| 5.0         | 16.0        |
| THC    | 9.0-1000                | $y = 942573x-11427$           | 0.998 | 3.0         | 9.0         |
| CBN    | 18.0-500                | $y = 3E+07x-17697$           | 0.998 | 5.0         | 18.0        |
| THC-OH | 8.7-500                 | $y = 215980x-15096$          | 0.979 | 2.6         | 8.7         |
| THC-COOH | 15.0-500            | $y = 3E+07x-27881$           | 0.984 | 4.5         | 15.0        |

The accuracy and precision of the method was determined by dosing a standard solution of CBD, THC and CBN, THC-OH and THC-COOH in seven different blank urine samples to avoid matrix influence. The mean average recovery value was obtained for different matrices from 91.00% for CBD, to 79.40% for THC-COOH. The effect of the matrix is negligible, except slightly for THC-COOH.

### Table 3. Validation parameters – precision, accuracy, recovery of the method

| #    | $x$ (ng/mL) | CBD $x$ (ng/mL) | THC $x$ (ng/mL) | CBN $x$ (ng/mL) | THC-OH $x$ (ng/mL) | THC-COOH $x$ (ng/mL) |
|------|-------------|-----------------|-----------------|-----------------|--------------------|-----------------------|
| 1.    | 50.0        | 46.0            | 40.0            | 48.0            | 41.4               | 39.8                  |
| 2.    | 50.0        | 45.0            | 40.0            | 48.0            | 41.2               | 40.2                  |
| 3.    | 50.0        | 46.0            | 41.0            | 48.0            | 40.9               | 39.7                  |
| 4.    | 50.0        | 45.0            | 40.0            | 47.0            | 41.2               | 39.1                  |
| 5.    | 50.0        | 45.0            | 40.0            | 47.0            | 41.0               | 39.0                  |
| 6.    | 50.0        | 45.0            | 40.0            | 47.0            | 39.7               | 40.0                  |
| 7.    | 50.0        | 45.0            | 40.0            | 47.0            | 41.3               | 40.4                  |
| Average | 45.3        | 40.1            | 47.4            | 40.9            | 39.7               |
| SD    | 0.5         | 0.4             | 0.5             | 0.5             | 0.5                |
| Precision (%) | 1.077 | 0.941 | 1.127 | 1.239 | 1.197 |
| Recovery (%) | 91.00 | 80.29 | 94.86 | 81.88 | 79.40 |
| Accuracy (%) | -9.4 | -19.8 | -5.2 | -18.2 | -20.6 |

Following the validation of the method, 30 urine samples received at the Laboratory for Toxicology Studies and Sanitary Work Environment, which had previously been positive on THC by drug strips, were analyzed, and results are presented in Table 4.
Table 4. Content of cannabinoids in positive urine samples extracted by modified QuEChERS method

| Sample | CBN  | THC  | CBD  | THC-OH | THC-COOH |
|--------|------|------|------|--------|----------|
| 1.     | n.d. | 20.0 | <LOQ | <LOQ   | <LOQ     |
| 2.     | n.d. | 70.0 | <LOQ | 9.5    | n.d.     |
| 3.     | <LOQ | n.d. | n.d. | n.d.   | <LOQ     |
| 4.     | <LOQ | 81.0 | <LOQ | n.d.   | 36.0     |
| 5.     | n.d. | n.d. | n.d. | n.d.   | <LOQ     |
| 6.     | n.d. | 38.0 | <LOQ | n.d.   | 58.0     |
| 7.     | n.d. | <LOQ | n.d. | <LOQ   | 141.0    |
| 8.     | n.d. | 65.0 | n.d. | n.d.   | 18.5     |
| 9.     | <LOQ | n.d. | n.d. | n.d.   | 39.0     |
| 10.    | n.d. | <LOQ | n.d. | n.d.   | 90.0     |
| 11.    | n.d. | 9.7  | n.d. | n.d.   | <LOQ     |
| 12.    | n.d. | 12.5 | <LOQ | n.d.   | <LOQ     |
| 13.    | n.d. | 63.0 | n.d. | <LOQ   | 36.0     |
| 14.    | n.d. | 22.3 | <LOQ | n.d.   | 51.9     |
| 15.    | n.d. | <LOQ | n.d. | n.d.   | 26.4     |
| 16.    | n.d. | 13.8 | <LOQ | 16.4   | 32.0     |
| 17.    | n.d. | 35.0 | n.d. | 9.0    | 40.3     |
| 18.    | n.d. | <LOQ | n.d. | <LOQ   | 35.0     |
| 19.    | n.d. | <LOQ | n.d. | <LOQ   | <LOQ     |
| 20.    | <LOQ | n.d. | n.d. | n.d.   | 37.8     |
| 21.    | n.d. | <LOQ | n.d. | n.d.   | n.d.     |
| 22.    | n.d. | <LOQ | n.d. | <LOQ   | 34.0     |
| 23.    | n.d. | <LOQ | n.d. | 35.1   | 53.0     |

n.d.: not detected; <LOQ: lower than limit of quantification

For the analyzed urine samples, it is not known how often these people consumed cannabis, whether they consumed it occasionally or were chronic addicts, in what form and amount they consumed, or when they last consumed it.

Analysis of positive urine samples showed that in fourteen of the thirty samples, THC was quantified, in nine samples the concentration was lower than LOQ, while in seven samples THC was not detected.

The inactive metabolite THC-COOH was found in twenty two samples at a concentration higher than LOQ (9 ng/mL). THC-COOH was detected in seven samples at a concentration lower than LOQ. In one sample, THC-COOH was not detected, but the concentration of THC in this sample was high, so it could be assumed that this person had recently consumed cannabis, so that it was not metabolized.

The concentration of the active metabolite THC-OH was quantified in six samples with a concentration higher than LOQ (8.7 ng/mL), while in nineteen samples was not detected. Rarely, CBD and CBN are suitable analytes for proving cannabis consumption in human matrices, if CBD and CBN are positive, which undoubtedly indicates recent cannabis consumption, (Citti, Braghiroli, Vandelli, et al., 2018). Usually, the high concentration of CBD in Cannabis means that these samples were of good quality for medicinal purpose. In analysed samples CBD was not quantified, but in ten samples was detected at a concentration lower than LOQ. CBN was detected in two samples but at a concentration lower than LOQ.

Figure 2 shows the chromatogram of sample 13, where THC and THC-COOH were quantified, while THC-OH was detected.
CONCLUSIONS

A validated method of extraction and simultaneous determination of cannabinoids using a mixture of solid salts instead of the commercially available cartridges for QuEChERS extraction was described. The developed analytical method is a simple, fast, accurate and economical alternative to expensive cartridges, as well as the LLE method, in the analysis of cannabinoids and their metabolites. After extraction, the chromatograms of the obtained extracts indicate that there was no interference with the selected analytes. Acceptable parameters for characterization of analysis, such as LOD, LOQ, accuracy, were obtained and the method was successfully applied to the real samples.

The results of the analysis indicate the adequacy of the method for determining THC metabolites as well, which is of particular interest for determining the pharmacokinetic parameters of THC. Moreover, since marijuana, beside THC, contains other cannabinoids, this method is suitable for the determination of certain cannabinoid compounds in the same sample, which can be significant in determination of marijuana medical use.

The method is completely acceptable for the needs of toxicological analysis and can be applied for routine analysis of THC, CBN, CND, THC-OH, THC-COOH from human urine using the GC-MS method.

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Summary/Sažetak

Cilj ovog rada jeste primjena modificirane QuEChERS metode za ekstrakciju kanabinoida iz humanog urina, koristeći smjesu soli za ekstrakciju u odgovarajućem omjeru umjesto komercijalno dostupnih kertridža. Analiza je vršena na blank urinu u koji je dodata poznata koncentracija tetrahidokanabinola (THC), kanabinol (CBN) i kanabidiola (CBD), kao i metabolita THC-a, 11-hidroksi-Δ9-tetrahidrokanabinol (THC-OH) i 11-nor-9-karboksi-Δ9-tetrahidrokanabinol (THC-COOH). Testirano je šest rastvarača, kao i četiri smjese rastvarača, za ekstrakciju, a kao najefikasnija odabrana je smjesa rastvarača acetonitril : dihlormetan (1:3) za koju je dobiven najbolji recovery factor.

Derivatizacija svih uzoraka je urađena sa MSTFA + 1% TMCS na sobnoj temperaturi. Dobiveni ekstrakti su analizirani vezanim sistemom gasna hromatografija-masna spektrometrija (GC-MS) uz full-scan mod. Pikovi odabranih kanabinoida su dobro razdvojeni što pokazuje da nema interferenci sa izabranim analitima. Rezultati su izračunati iz kalibracione krive u rasponu od LOQ do 1000 ng/mL za izabrane kanabinide, sa korelacionim faktorom preko 0.998. Vrijednosti LOD i LOQ za THC su (3.0 ng/mL; 9.0 ng/mL), za CBN (5.0 ng/mL; 18.0 ng/mL) za CBD (5.0 ng/mL; 16.0 ng/mL), za THC-OH (2.6 ng/mL; 8.7 ng/mL) i za THC-COOH (5.0 ng/mL; 15.0 ng/mL). Zabilježen je recovery factor u rasponu od 79.40% za THC-COOH do 94.86% za CBN. Modificirana QuEChERS metoda ekstrakcije se može koristiti za rutinsku analizu izabranih kanabinoida. Metoda je uspješno primijenjena na realne uzorke, analizirano je trideset uzoraka urina.