STANDARD OPERATING PROCEDURE

Extraction of cannabis and its metabolites from wastewater

Deliverable D4.2 & D4.3

EUSEME

EUROPE-WIDE SEWAGE ANALYSIS TO MONITOR EMERGING DRUG PROBLEMS

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Executive Summary

This Standard Operating Procedure (SOP) for the extraction of cannabis and its metabolites in wastewater is delivery 4.2 (Analytical Methodology) and 4.3 (SOP; sample pretreatment) of WP 4 “Refine the use of wastewater-based epidemiology to monitor cannabis consumption” within the framework of the EuSeME project funded by the European Union’s Justice Programme - Drugs Policy Initiatives (project number 861602). This SOP describes the sample treatment applied for the determination of cannabis biomarkers in the dissolved phase and suspended solids of urban wastewater for the subsequent measurement of these biomarkers by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) based methods.

Introduction

The purpose of the SOP is to describe the sample treatment applied for the determination of THC, THC-OH and THC-COOH in (raw) influent wastewater (IWW), in the dissolved phase and in (suspended) solids of the IWW. The procedure is applied for the subsequent measurment of these compounds in wastewater by LC-MS/MS using isotope-labelled internal standards for matrix effects correction.

Reagents and chemicals

High purity analytical standards were purchased from Sigma-Aldrich (Cerilliant Corporation, Texas, USA). **Table 1** shows the analytes selected together with their corresponding isotope labeled internal standards (ILIS) and CAS numbers.

Table 1. Analytes and ILIS used

Compound	Abbreviation	CAS-Number
DELTA9-TETRAHIDROCANNABINOL	THC	1972-08-3
(+/-)-11-NOR-9-CARBOXYDELTA9-THC	THC-COOH	104874-50-2
(+/-)-11-HYDROXY-DELTA9-THC	THC-OH	34675-49-5
(-)-DELTA9-THC-d3	THC-D3	81586-39-2
(+/-)-11-NOR-9-CARBOXY-DELTA9-THC-d3	THC-COOH-D3	136844-96-7
(+/-)-11-HYDROXY-DELTA9-THC-d3	THC-OH-D3	130410-26-3

Individual standard stock solutions were prepared at 100 mg L^{-1} or 10 mg L^{-1} in methanol (MeOH) and stored in amber glass vials at −20 ºC. Multi-compound working solutions were prepared by appropriate dilution of the standard stock solutions in MeOH. The analytes mix work solution was prepared at 500 µg L^{-1} and the ILIS mix work solution was prepared at 200 µg L^{-1}. LC-MS grade MeOH, hexane (HX), ethyl acetate (EA), chloride acid, formic acid (purity, 98%) and sodium chloride (NaCl) were supplied by Scharlab. HPLC-grade water was obtained by purifying demineralised water using a Milli-Q system from Millipore (Bedford, MA, USA).

Instrumentation

A Waters Acquity UHPLC system (Milford, MA, USA) was interfaced to a triple quadrupole mass spectrometer (Xevo TQS, Waters Micromass, Manchester, UK) equipped with T-wave devices and an electrospray ionization interface (ESI) operated in positive-ion mode. Chromatographic separation was carried out using an Acquity UPLC BEH C18 column, 1.7 µm, 50 mm × 2.1 mm (i.d.) (Waters) at a flow rate of 0.3 mL min^{-1}. The column was kept at 40 °C and the sample manager was maintained at 5 °C. Mobile phase consisted of water with 5 mM ammonium acetate and 0.01 % formic acid (solvent A) and MeOH (solvent B). The percentage of MeOH changed linearly as follows: 0 min, 60 %; 3.5 min, 95 %; 5 min, 95 %; 5.1 min, 60 % and until 7 min at 60 % for re-equilibration of the column. Cone gas as well as desolvation gas was dry nitrogen. The cone gas and the desolvation gas flows were set to 250 and 1200 L h^{-1}, respectively. For operation in the MS/MS mode (Table 2), collision gas was argon 99.995 % (Praxair, Madrid, Spain) with a pressure of 4×10^{-3} mbar in the collision cell (0.15 mL min^{-1}). Other parameters optimized were: capillary voltage, 1.5 kV; source temperature, 150 °C and desolvation temperature, 650 °C. Dwell times of 0.01 s/transition were selected. All data were acquired and processed using MassLynx v 4.1 software (Waters, Manchester, UK).

Table 2. Analytes and ILIS used

Compound	Precursor ion	Cone (V)	Product ions	Collision energy (ev)
THC	315	25	259	15
			193	20
			123	35
THC-OH	331	25	313	15
			193	25
THC-COOH	345	25	193	15
			299	20
			327	25
THC-D3	318	25	196	25
THC-OH-D3	334	25	196	25
THC-COOH-D3	348	25	196	25

Procedure

Several extraction techniques were applied for the sample treatment of raw IWW, the liquid phase and the suspended solids previously to the LC-MS/MS determination of cannabis biomarkers. These techniques included liquid–liquid extraction (LLE), solid phase extraction (SPE) and solid-liquid extraction (SLE). Figure 1 shows the different extraction methods used.

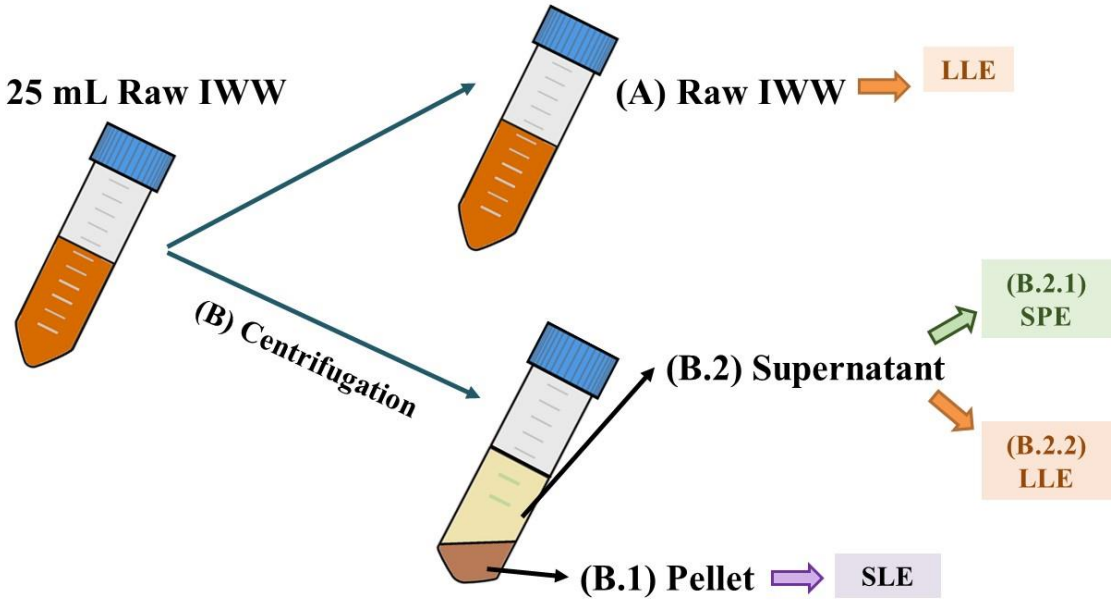

Figure 1. Extraction methods described in this SOP.

A. Extraction of cannabis and its metabolites from raw IWW

LLE for sample treatment of raw IWW has been based on two previous publications [1, 2]. In this SOP: Take 25 mL of unfiltered IWW sample and add 50 µL of the ILIS working mix solution (200 µg L-1). Then, transfer the sample to a 50 mL falcon tube that contains a spatula tip of NaCl and acidify to pH ~2 with HCl 1M (400 µL). Subsequently, add 10 mL of HX:EA (2:1, v/v), vortex for 30 s and sonicate 5 min. Centrifuge the content of the vessel at 5000 rpm for 5 min. Transfer 5 mL of the organic layer to a glass test tube and evaporate at 40°C under a gentle stream of nitrogen. Reconstitute in 300 µL of MeOH and 200 µL of Milli-Q, vortex and transfer the final extract to a vial for LC-MS/MS analysis. Table 3 (Annex I) shows the validation parameters of the LLE procedure of raw IWW.

B. Extraction of cannabis and its metabolites from the solid and liquid phase of the IWW

B.1. Suspended solids: SLE

For the extraction of the analytes in the suspended solid , take 25 mL of unfiltered IWW sample and centrifuge at 5000 rpm for 5 min, remove the liquid phase and extract the pellet (suspended solids) by SLE as follows: Add 50 µL of the ILIS working mix solution (200 µg L-1) to the pellet. Then, add 10 mL of HX:EA (2:1, v/v), vortex for 1 min and sonicate 5 min.

Centrifuge the content of the vessel at 5000 rpm for 5 min. Transfer 5 mL of the organic layer to a glass test tube and evaporate at 40°C under a gentle stream of nitrogen. Reconstitute in 300 µL of MeOH and 200 µL of Milli-Q, vortex and then transfer the final extract to a vial for LC-MS/MS analysis. Table 4 (Annex I) shows the validation parameters of the SPE procedure.

B.2. Liquid phase: SPE and LLE

For the extraction of the analytes in the liquid phase, two extraction procedures were validated B.2.1 a conventional SPE and B2.2 LLE. For both procedures it is required to centrifuge 35 mL of unfiltered IWW at 5000 rpm for 5 min, and take 25 mL of the centrifuged IWW for subsequent extraction by LLE or SPE.

B.2.1 Solid phase extraction

Dilute the sample x4 to give a final volume of 100 mL (i.e. 25 mL of centrifuged IWW and 75 mL of Milli-Q water) and add 50 µL of the ILIS working mix solution (200 µg L-1). Oasis HLB cartridges (3 cc, 60 mg) were employed for SPE. The cartridges were conditioned by washing and rinsing them with 6 mL of MeOH and 6 mL of Milli-Q water. After conditioning, the samples were percolated through the cartridges by gravity (flow rate of ~ 3 mL min−1), and then vacuum dried for approximately 15 min. The analytes were eluted with 5 mL of MeOH and the extract was evaporated to dryness at 40°C under a gentle stream of nitrogen. Finally, the residue was reconstituted in 0.5 mL MeOH:water (60:40, v/v). Table 5 (Annex I) shows the validation parameters of the SPE procedure.

B.2.2 Liquid-liquid extraction

Take 25 mL of centrifuged IWW sample and add 50 µL of the ILIS working mix solution (200 µg L-1). Then, transfer the sample to a 50 mL falcon tube that contains a spatula tip of NaCl and acidify to pH ~2 with HCl 1M (400 µL). Add 10 mL of HX:EA (2:1, v/v),vortex for 30 s and sonicate 5 min. Centrifuge the content of the vessel at 5000 rpm for 5 min. Transfer 5 mL of the organic layer to a glass test tube and dry at 40°C under a gentle stream of nitrogen. Reconstitute in 300 µL of MeOH and 200 µL of Milli-Q, vortex and transfer the final extract to a vial for LC-MS/MS analysis. Table 6 (Annex I) shows the validation parameters of the LLE procedure.

Observations

In section B.1 it is necessary to remove the supernatant for the extraction of the pellet. After centrifugation, let the content of the falcon tube decant and carefully remove the supernatant with a pasteur pipette until the maximum amount of liquid is eliminated. A few microliters of supernatant will remain that cannot be removed.

References

1. Iria González-Mariño, Kevin V. Thomas, Malcolm J. Reid. Determination of cannabinoid and synthetic cannabinoid metabolites in wastewater by liquid–liquid extraction and ultra-high performance supercritical fluid chromatography-tandem mass spectrometry. Drug Testing and Analysis (2018), 10, 222-228.

2. Benjamin J. Tscharke, Chang Chen 1, Jacobus P. Gerber, Jason M.White. Temporal trends in drug use in Adelaide, South Australia by wastewater analysis. Science of the Total Environment (2016), 565, 384-391.

Annex 1: Validation data of the presented extraction procedure

A. Extraction of cannabis and its metabolites in raw IWW

The validation was performed by spiking IWW, the procedure for fortification of the samples was as followed: take 25 mL of unfiltered IWW sample, and add 50 µL of the analytes mix solution at 50 µg L-1 (spiked level 0.1 µg L-1) or 40 µL of the analytes mix solution at 500 µg L-1 (spiked level 0.8 µg L-1). Then continue the procedure as indicated above.

Table 3. LLE method validation in raw IWW (n=5)

Compound	LOQ (ng/L)	LOD (ng/L)	Concentration in "blank“ (ng/L)	Recovery, % (RSD)		q1/Q ratio deviation (%)		q2/Q ratio deviation (%)	
				100 ng/L*	800 ng/L	100 ng/L	800 ng/L	100 ng/L	800 ng/L
THC	10	3	51	*	65 (4)	3	7	28	1
THC-OH	5	2	104	*	80 (9)	3	0.3	4	3
THC-COOH	3	1	246	*	73 (10)	10	7	1	2

*Not estimated due to the high concentration of the analyte in the spiked “blank” sample.

B. Extraction of cannabis and its metabolites from the solid and liquid phase of the IWW

B.1. Suspended solids: SLE

The procedure for fortification of the samples was as followed: to the pellet of 25 mL centrifuged IWW, 50 µL of the analytes mix solution at 50 µg L-1 was added (spiked level 0.1 µg L-1) or 40 µL of the analytes mix solution at 500 µg L-1 (spiked level 0.8 µg L-1). Then continue the procedure as indicated above.

Table 4. SLE method validation in the pellet of 25 mL of IWW (n=3)

Compound	LOQ (ng)	LOD (ng)	Concentration in "blank“ (ng/L) [a]	Recoveries (RSD) at 20 ng in pellet of 25 mL IWW
THC	0.22	0.07	79	97 (12)
THC-OH	0.21	0.07	175	101 (16)
THC-COOH	0.18	0.06	338	106 (15)

[a] Calculate from the ng obtain in pellet of 25 mL IWW

B.2.1 Liquid phase: solid phase extraction

The procedure for fortification of the samples was as follow: take 25 mL of centrifuged IWW sample, and add 50 µL of the analytes mix solution at 50 µg L^{-1} (spiked level 0.1 µg L^{-1}) or 40 µL of the analytes mix solution at 500 µg L^{-1} (spiked level 0.8 µg L^{-1}). Then continue the procedure as indicated above.

Table 5. SPE method validation in the liquid phase of IWW (n=3)

Compound	Estimated LOQ (ng/L)	LOD (ng/L)	Concentration in "blank" (ng/L) [(a)]	Recoveries, % (RSD)	
				100 ng/L	800 ng/L
THC	20	6	-	84 (9)	82 (4)
THC-OH	12	4	35	95 (3)	90 (1)
THC-COOH	26	8	183	*	98 (6)

*Not estimated due to the high concentration of the analyte in the "blank" sample.

[(a)]Average value of the "blank" concentration obtained by SPE and LLE extraction methods

B.2.2 Liquid phase: liquid-liquid extraction

The procedure for fortification of the samples was as follow: take 25 mL of centrifuged IWW sample, and add 50 µL of the analytes mix solution at 50 µg L-1 (spiked level 0.1 µg L-1) or 40 µL of the analytes mix solution at 500 µg L-1 (spiked level 0.8 µg L-1). Then continue the procedure as indicated above.

Table 6. LLE method validation in the liquid phase of IWW (n=3)

Compound	LOQ (ng/L)	LOD (ng/L)	Concentration in "blank" (ng/L) [(a)]	Recoveries (RSD) (n=3)	
				100 ng/L	800 ng/L
THC	10	3	-	70 (6)	72 (3)
THC-OH	5	2	35	71 (4)	78 (5)
THC-COOH	3	1	183	*	69 (6)

*Not estimated due to the high concentration of the analyte in the "blank" sample.

[(a)]Average value of the "blank" concentration obtained by SPE and LLE extraction methods