

Data Hidden in Sewage: Advanced Methods for Identification and Quantification of Synthetic Cannabinoids in Urban Wastewater

Wiktoria Kurzeja ¹, Mariola Kuczer ¹, Jan Matysiak ² and Agnieszka Klupczyńska-Gabryszak ^{2,*}

¹ Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland; wiktoriakurzeja23@gmail.com (W.K.); mariola.kuczer@uwroclaw.edu.pl (M.K.)

² Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Poznań University of Medical Sciences, Rokietnicka 3, 60-780 Poznań, Poland; jmatysiak@ump.edu.pl

* Correspondence: aklupczynska@ump.edu.pl; Tel.: +0048-61-641-83-38

SUPPLEMENTARY MATERIALS

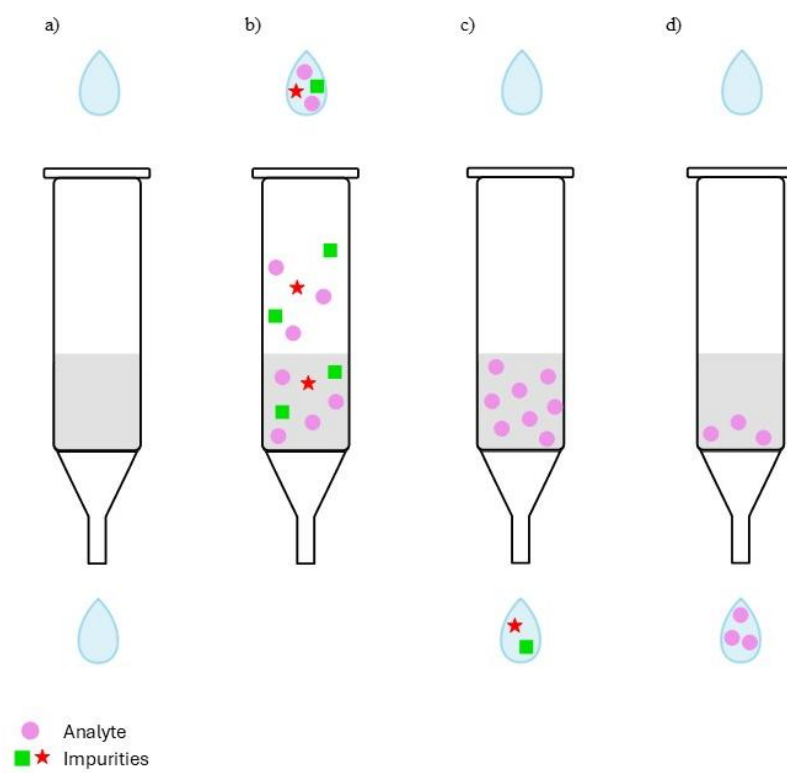


Figure S1. Solid phase extraction scheme: a) Conditioning, b) Sample loading, c) Washing, d) Analyte elution.

Table S1. Summary of the 16 methodologies of sampling, sample preparation and instrumental analysis aimed at the detection and quantification of synthetic cannabinoids and their metabolites in wastewater.

Sampling and storage	Sample preparation	Instrumentation	Validation	Reference
Raw wastewater sample	SPE	UHPLC-MS/MS		
	Oasis MCX	Analyzer: Triple quadrupole	Linearity	
24 h mixed sampling	C: 12 mL MeOH, 12 mL acidified MilliQ water (0.1% AA at pH 3)	Ionization: ESI+	LOD	
Volume-proportional sampling	SL: 500 mL sample	Separation: C ₈ column	LOQ	[15]
	W: 12 mL acidified water (0.1% AA at pH 3)	Injection volume: 7 µL	Accuracy	
Stored at -20°C	E: 12 mL of 2% NH ₄ OH in MeOH and 6 mL ACN R: 2 mL of mixture ACN, MeOH, and MilliQ water	Solvent A: 10mM ammonium acetate and 0.1% AA in water Solvent B: 10mM ammonium acetate and 0.1% AA in MeOH	Precision Recovery	
Influent and effluent wastewater sample	SPE	LC-MS/MS		
	Spherical pure mixed polymer sorbent	Analyzer: Triple quadrupole		
	C: 6 mL MeOH, 6 mL of acidified ultrapure water (HCl, pH 2.5)	Separation: PFP column		
Stored at 4°C	SL: 50 mL sample	Injection volume: 10 µL		
	W: 3 mL ultrapure water (pH 2.5)		Linearity	
	E: 2x2 mL MeOH and 4x2 mL MeOH:EtAc (50:50)	Ionization: ESI+	ILOD	[28]
	R: 500 µL MeOH:water (60:40)	Solvent A: 0.2% v/v FA in MilliQ water Solvent B: MeOH	ILOQ	
		Ionization: ESI- Solvent A: MeOH Solvent B: ACN		
Influent wastewater sample	SPE	LC-HRMS/MS		
	Oasis MCX (150 mg)	Analyzer: ion trap-Orbitrap		
24 h composite sampling	SL: 200 mL sample	Ionization: DESI+		
	W: 6 mL sodium acetate buffer, 2 mL of 0.1 M AA and 6 mL MeOH	Separation: C ₁₈ column	Linearity	[16]
Volume- or time-proportional sampling	E: 3 mL MeOH and 3 mL of 2% NH ₄ OH in MeOH R: ultrapure water:MeOH (80:20)	Injection volume: 2 µL Solvent A: 0.1% FA in ultrapure water Solvent B: ACN	ILOD	
Stored at -80°C				
Raw wastewater sample	LLE	UHPSFC-MS/MS	Linearity	
	Solvent: 10 mL HX: EtAc (1:1)	Analyzer: Triple quadrupole	MQL	[22]

24 h composite sampling		Ionization: ESI+	IQL	
Stored at -20°C		Separation: Torus 2-PIC Column	Precision	
		Injection volume: 2 µL	Repeatability	
		Mobile phase: CO ₂	Trueness	
		Cosolvent: 0.1% FA in MeOH:iPrOH (1:1 v/v)	Matrix effects	
Influent wastewater sample	SPE	LC-MS/MS		
	Mixed mode: Cs+benzenesulfonic acid	Analyzer: Quadrupole time-of-flight		
Stored at -20°C	C: 6 mL MeOH, 6 mL of 20 mM sodium acetate buffer (pH 5)	Ionization: ESI+		
	SL	Separation: PEP column		
	W: 6 mL sodium acetate buffer, 2 mL of 0.1 M AA and 6 mL MeOH	Injection volume: 10 µL	-	[33]
	E: 6 mL dichloromethane: isopropanol:ammonia (80:16:4)	Solvent A: water with 5% MeOH and 0.1% FA in water		
	R: 20 µL of 0.1% FA in MeOH and 180 µL of 0.1% FA in MilliQ water	Solvent B: MeOH with 5% water and 0.1% FA		
Raw wastewater sample	SPE	LC-MS/MS		
	Oasis MCX (6cc, 150 mg)	Analyzer: Triple quadrupole	LOD	
24 h composite sampling	C: 3 mL MeOH, 3 mL water (pH~2)	Ionization: ESI+	LOQ	
	SL: 100 mL sample	Separation: Biphenyl column	Precision	[17]
Time-proportional sampling	E: 5 mL 5% NH ₃ in MeOH	Injection volume: 1 µL	Matrix effects	
Stored at -20°C		Solvent A: 0.1% FA	Repeatability	
		Solvent B: MeOH		
Influent wastewater sample	SPE	LC-MS/MS		
	Oasis HLB (6cc, 150 mg)	Analyzer: Triple quadrupole		
24 h composite sampling	C: 6 mL MeOH, 6 mL ultrapure water	Ionization: ESI+	Linearity	
	SL: 20 mL sample	Separation: Biphenyl column	Accuracy	
Stored at -20°C	W: 6 mL of 5% MeOH in water (v/v)	Injection volume: 5 µL	Precision	
	E: 2 x 4 mL dichloromethane:isopropanol:ammonia (80:16:4)	Solvent A: 0.1% FA in ultrapure water and MeOH (95:5)	Matrix effects	[18]
	R: 100 µL MeOH and 100 µL of 0.1% FA in ultrapure water	Solvent B: 0.1% FA in MeOH with water (95:5)	Selectivity	
	LLE		Stability	
	Solvent: 10 mL EtAc		Recovery	
	R: 100 µL MeOH and 100 µL 0.1% FA in ultrapure water			
Effluent wastewater sample	Filtration (0.2 µm, regenerated cellulose filters)	LC-MS/MS	Linearity	
containing 5% activated sludge		Analyzer: Triple quadrupole ion trap	ILOD	[27]

Stored at -26°C	Dilution (ultrapure water:MeOH 80:20)	Ionization: ESI+ Separation: XSelect HSS T3 Injection volume: 20 µL Solvent A: ultrapure water with 5 mmol AF, pH 3 Solvent B: MeOH with 5% ultrapure water and 5 mM AF, pH 3 LC-HRMS Analyzer: Orbitrap Ionization: ESI+ Separation: XSelect HSS T3 Injection volume: 20 µL Solvent A: ultrapure water with 5 mmol AF, pH 3 Solvent B: MeOH with 5% ultrapure water and 5 mM AF, pH 3	ILOQ Matrix effects Recovery Precision	
Influent wastewater sample	SPE	LC-MS/MS	Linearity	
24 h composite sampling	Cleanert PEP C: 3 mL MeOH, 3 mL water SL: 50 mL sample	Analyzer: Triple quadrupole Ionization: ESI+ Separation: C ₁₈ column Injection volume: 2 µL Solvent A: 30 mM AF, 0.1% FA and ultrapure water Solvent B: MeOH	Recovery Sensitivity (LOD, LOQ) Matrix effects Accuracy Precision	[10]
Time-proportional sampling	E: 3 mL MeOH R: 250 µL MeOH			
Stored at -20°C				
Influent wastewater sample	SPE	LC-MS/MS	Linearity	
24 h composite sampling	Oasis MCX (6cc, 60 mg) C: MeOH, MilliQ water and MilliQ water acidified at pH 2 SL: 50 mL sample	Analyzer: Triple quadrupole Ionization: ESI+ Separation: C ₁₈ column Injection volume: 5 µL Solvent A: 10 mM/L AF and 0.1% FA in water Solvent B: 10 mM/L AF and 0.1% FA in MeOH	Precision Sensitivity Matrix effects Recovery	[19]
Stored at -20°C	W: 5 mL of 2% NH ₃ in water E: 8 mL of a 2% NH ₃ in MeOH R: 1 mL of mixture MeOH:MilliQ water (10:90, v/v).			
Influent wastewater sample	Filtration: 10 mL of the sample (0.2 µm RC filter)	LC-MS/MS	Linearity	
24 h period sampling	Transfer of 1 mL filtrate into an LC vial with 12 µL ISTD (12.5 µg/ L)	Analyzer: Triple quadrupole Ionization: ESI+ Separation: Biphenyl column Injection volume: 10 µL Solvent A: MilliQ water:MeOH with 0.1% FA (95:5 (v/v)) Solvent B: MeOH:MilliQ water with 0.1% FA (95:5 (v/v))	Range Precision LOD LOQ Filtration losses Matrix effects	[25]
Time- or flow-proportional sampling				

Stored at -20°C			
Influent wastewater sample	Homogenisation	LC-MS/MS Analyzer: Triple quadrupole	Selectivity Linearity
24 h composite sampling	Filtration: 10 mL of the sample (0.2 µm RC filter)	Ionization: ESI+ Separation: Biphenyl column	LOD LOQ [26]
Stored at -20°C	Transfer of 1 mL filtrate into an LC vial with 12 µL ISTD (12.5 µg/ L)	Injection volume: 10 µL Solvent A: 95% ultrapure water, 5% MeOH and 0.1% FA Solvent B: 95% MeOH, 5% ultrapure water and 0.1% FA	Accuracy Precision Matrix effects
Influent wastewater sample	MSPE	LC-MS/MS	
24 h composite sampling	20 mg magnetic graphene oxide + 1 mL ionic liquid solution (20 mg/mL) Mechanical stirring (30 min)	Analyzer: Triple quadrupole Ionization: ESI+ Separation: C ₁₈ column	LOQ Linearity Accuracy [23]
Stored at -20°C	Separation with a magnet E: 5 mL ACN R: 100 µL initial mobile phase	Injection volume: 5 µL Solvent A: 0.1% FA in water Solvent B: ACN	Precision Matrix effects
Influent wastewater sample	pH adjustment using aqueous-ammonia (pH 9)	UPLC-MS/MS Analyzer: Triple quadrupole	
24 h composite sampling	900 µL sample + 100 µL of IS mix Vortexing (30 s)	Ionization: ESI+ Separation: Biphenyl column	LOD Recovery Selectivity [29]
Flow-proportional sampling	Filtration: 400 µL of the supernatant (PTFE filter)	Injection volume: 20 µL Solvent A: 5 mM/L ammonium acetate and 0.01% FA in water Solvent B: ACN	Matrix effects
Stored at -20°C			
Influent wastewater sample	Filtration ("GE Whatman" nonadhesive, glass-fiber filter paper (Grade GF/CTM)).	LC-MS/MS Analyzer: Triple quadrupole	
Stored at -20°C	Transfer of 10 mL wastewater samples into 15 mL polypropylene centrifuge tubes with 20 µL IS solution. Vortexing.	Ionization: ESI+ Separation: C ₁₈ column Injection volume: 10 µL Solvent A: 0.1% FA Solvent B: ACN	Specificity Linearity LOQ Accuracy [24] Precision
	Introduction of 1 mL SUPRASS into the centrifuge tube		Recovery Matrix effects
	Agitation at 40°C for 5 min on a table thermostatic oscillator, followed by centrifugation.		

	Transfer of 0.4 mL of extracted SUPRASs into a 1.5 mL centrifuge tube.	
	Evaporation to dryness and subsequent reconstitution with 100 μ L of MeOH:water (2:1, <i>v/v</i>).	
Influent wastewater sample	Filtration	LC-MS/MS
24 h composite sampling	Acidification with HCl	Analyzer: Triple quadrupole
Stored at -20°C	SPE	Ionization: ESI+
	Oasis MCX	Separation: PFP column
	C: MeOH, MilliQ water, acidified MilliQ water (pH 2)	Solvent A: 0.1% FA in water
	SL: 50 mL sample	Solvent B: 0.1% FA in MeOH
	W: 5 mL 2% NH ₃ in water	Linearity
	E: 8 mL solution of 2% NH ₃ in MeOH	Precision
	R: 1 mL solution of MeOH:MilliQ water (10:90, <i>v/v</i>)	Sensitivity
		Matrix effect
		Recovery

[20]

AA: acetic acid; ACN: acetonitrile; AF: ammonium formate; C: conditioning; DESI+: desorption electrospray ionization in positive mode; E: elution; ESI+: electrospray in positive mode; ESI-: electrospray in negative mode; EtAc: ethyl acetate; FA: formic acid; HX: hexane; ILOD: instrumental limit of detection; ILOQ: instrumental limit of quantification; iPrOH: isopropanol; IQL: instrumental quantification limits; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; LLE: liquid-liquid extraction; LOD: limit of detection; LOQ: limit of quantification; MSPE: magnetic solid-phase extraction; MQL: method quantification limits; PFP: pentafluorophenyl; R: reconstitution; SL: sample loading; SPE: solid-phase extraction; SUPRAS: pentanol-type supramolecular solvents; UHPSFC-MS/MS: ultra-high performance supercritical fluid chromatography-tandem mass spectrometry; W: washing;