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Data Article

Data in brief: Dataset of residues of drugs of abuse in wastewaters from Educational Institutions

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ABSTRACT

Metabolic residue concentration data for two licit drugs (nicotine and alcohol), three medications of abuse (morphine, methadone and codeine) and six illicit drugs (cannabis, cocaine, amphetamine, methamphetamine, ecstasy and heroin) were obtained from raw wastewater samples collected from 44 Slovenian educational institutions are presented. Also, concentrations obtained at one secondary school during a preliminary study is provided. The wastewater samples were collected at the end of the 2018/2019 academic year using time proportional sampling and analysed for 16 drug residues, extracted using solid-phase extraction and analysed using ultra-performance liquid chromatography hyphenated to tandem mass spectrometry (UPLC-MS/MS). Residues of nicotine and alcohol were determined by direct injection of filtered wastewater onto the UPLC. Concentrations data were studied based on educational level (primary, secondary and tertiary) and institution type (secondary schools: gymnasiums, vocational and technical schools, multi-programme schools; higher education institutions: natural sciences and social sciences), geographic location (municipalities) and degree of urbanisation (urban and non-urban areas). Due to the

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large number of different educational institutions included in the study, provided datasets are valuable for further studies on drug consumption patterns among young people. Drug presence and prevalence data for primary schools (6–15 years) offer an objective insight into drugs present in the early stage of a young person's development and help establish effective prevention programs. More details on the study can be found in [1].

Specifications Table

Subject	Health and medical sciences
Specific subject area	Determination of licit drugs, medications of abuse and illicit drugs in educational institutions
Type of data	Table
How data were acquired	Instruments: Shimadzu ultra-performance liquid chromatograph hyphenated to AB Sciex 4500 QTRAP detector mass spectrometer (UPLC-MS/MS)
Data format	Raw, anonymised
Parameters for data collection	The data was obtained using UPLC-MS/MS analysis of seven-hour composite raw wastewater samples (n=40) obtained from different educational institutions (primary, secondary, and tertiary) in urban and non-urban areas in seven Slovenian municipalities. Also, data on chemical analysis conducted during the preliminary study (one secondary school sampled over one week) is provided.
Description of data collection	In total, 16 metabolic residues of licit drugs (nicotine, alcohol), medications of abuse (morphine, codeine, methadone) and illicit drugs (cannabis, cocaine, amphetamine, methamphetamine, ecstasy and heroin) were determined in wastewater samples using UPLC-MS/MS. Residues of medications of abuse and illicit drugs were enriched using solid phase extraction, while residues of licit drugs were filtered and analysed directly. Ion-pair reagent (tetrabutylammonium bromide) was added to the samples when analysing alcohol residues.
Data source location	Institutions: 44 educational institutions; 19 primary schools (6–15 years.), ten secondary schools (15–19 years), nine higher education institutions (19+ years.) and six mixed secondary and higher education institutions (15+ years.). City/ Town/Region: seven municipalities (including the capital) from five statistical regions (Coastal-Karst, Central Slovenia, Southeast Slovenia, Savinja, Drava and Mura) Country: Slovenia
Data accessibility	With the article
Related research article	T. Verovšek, I. Krizman-Matasic, D. Heath, E. Heath., Investigation of drugs of abuse in educational institutions using wastewater analysis, Science of The Total Environment, 799 (2021) 150013. https://doi.org/10.1016/j.scitotenv.2021.150013

Value of the Data

- Compared to similar datasets, this dataset provides concentrations of residues of licit drugs, medications of abuse and illicit drugs obtained from educational institutions of different educational levels (n=44), located in urban and non-urban areas within different municipalities across Slovenia.
- The data can be valuable for researchers studying drug use patterns among young people. Also, it may help with establishing prevention programmes and intervention strategies for young people since the data covers ages from 6–19+ years.

- Collected data can be used in comparison studies or as a base for (additional) experiments to study the variables influencing drug consumption trends in educational institutions, i.e. among young people.
- For the first time, wastewater analysis data on drugs present in primary schools (6–15 years) was obtained.

1. Data Description

Here the data for the wastewater samples, applied analytical methods, validation parameters, and concentrations (raw data) of residues of licit drugs (nicotine and alcohol), medications of abuse (morphine, methadone and codeine) and illicit drugs (cannabis, cocaine, amphetamine, methamphetamine, ecstasy and heroin) in wastewater samples are presented. No further calculation from concentration, i.e., to mass loads and consumption estimations [2], was possible due to a lack of data on wastewater flows.

In total, 40 wastewater samples were obtained from educational institutions offering different levels and types of education (Table 1) from urban and non-urban areas in six statistical regions in Slovenia (Table 2).

Standards solutions of targeted analytes (16 drug residues) and their labelled analogues used for identification and quantification by UPLC-MS/MS are listed in Table 3. Retention times and ionisation mode utilised are presented in Table 4 along with optimised UPLC-MS/MS parameters, namely declustering potentials (DP), collision energies (CE 1 and CE 2) and collision cell exit potential (CXP 1 and CXP 2), for each precursor–product ion pair.

Linearity, the limit of detection (LOD), the limit of quantification (LOQ), extraction recovery, matrix effect (ME), accuracy and repeatability were addressed during method validation. Signal suppression was observed (Table 5) for licit drug residues (2-115%) except for nicotine, for which signal enhancement (77%) was observed at low concentration (5 ng/mL). Accuracy values were in the 84-136% range. Repeatability was below 10% (RSD) for all compounds, except for ethyl sulphate (14% RSD). A linear response for nicotine residues was obtained between the LOQ and 1000 ng/mL, while for ethyl sulphate, linearity was achieved in LOQ–500 ng/mL range. LODs were in the range 19 to 305 ng/L, and LOQs were between 64 and 1020 ng/L.

Table 6 shows validation parameters for residues of medications of abuse and illicit drugs. Extraction recoveries were in 71–110% range (exception: 23% for 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EDDP) and signal suppression was observed (between 15% and 70%). Accu-

Table 1
Wastewater samples obtained in educational institutions of different level.

Educational institution (age of attendants)	Number of obtained samples
Primary schools (age 6–15)	19
Secondary schools (age 15–19)	8; - Three from gymnasiums (general upper secondary education); - Two from vocational and technical schools (vocational and technical education); - Three from multi-programme schools (general upper secondary education, vocational and technical education).
HEIs (age 19+)	6; - Four from institutions offering natural sciences; - Two from institutions offering social sciences.
SHEIs (age 15+)	7

HEIs – higher education institutions, SHEIs – mixed secondary and higher education institutions.

Table 2
Wastewater samples from each municipality and urban and non-urban areas divided based on the level of education offered by the institutions.

Geographic location/area	Statistical region of Slovenia	Total number of obtained samples	Number of primary school samples	Number of secondary school samples	Number of HEI samples	Number of SHEI samples
Inter-municipality comparison						
Municipality 1 (M1)	Costal-Karst	2	1	1	n.a.	n.a.
Municipality 2 (M2) – Ljubljana (Slovenian capital)	Central Slovenia	16	5	4	5	2
Municipality 3 (M3)	Central Slovenia	6	3	2	1	n.a.
Municipality 4 (M4)	Southeast Slovenia	4	2	n.a.	n.a.	2
Municipality 5 (M5)	Savinja	6	3	1	n.a.	2
Municipality 6 (M6)	Drava	3	3	n.a.	n.a.	n.a.
Municipality 7 (M7)	Mura	3	2	n.a.	n.a.	1
Urban *vs* non-urban areas						
Urban areas	Central Slovenia, Drava, Mura, Costal-Karst, Savinja, Southeast Slovenia	33	13	8	6	6
Non-urban areas	Central Slovenia, Drava, Mura, Savinja, Southeast Slovenia	7	6	n.a.	n.a.	1

n.a. – not applicable (no obtained samples).
HEI – higher education institution, SHEI – mixed secondary and higher education institution.

Table 3
Standard solutions of analytes and their deuterated analogues.

Analyte	[mg/mL] *(solvent)*	Labelled analogues (internal standards)	[mg/mL] *(solvent)*
Licit drug standard solutions			
HCOT	1 *(methanol)*	(±)-Cotinine-d3	1 *(methanol)*
(-)-Cotinine	1 *(methanol)*		
(S)-(-)-Nicotine	1 *(methanol)*		
Ethyl sulphate sodium salt	1 *(methanol)*	Ethyl-d5-sulphate sodium salt	1 *(methanol)*
Basic drug standard solutions			
Morphine	1 *(methanol)*	Morphine-d3	1 *(methanol)*
Codeine	1 *(methanol)*	Codeine-d3	1 *(methanol)*
(±)-Methadone	1 *(methanol)*	(±)-Methadone-d3	1 *(methanol)*
EDDP perchlorate	1 *(methanol)*	EDDP-d3 perchlorate	1 *(methanol)*
Cocaine	1 *(acetonitrile)*	Cocaine-d3	1 *(acetonitrile)*
Benzoylecgonine	1 *(methanol)*	Benzoylecgonine-d3	1 *(methanol)*
Cocaethylene	1 *(acetonitrile)*	Cocaethylene-d8	0.1 *(acetonitrile)*
(±)-Amphetamine	1 *(methanol)*	(±)-Amphetamine-d6	1 *(methanol)*
(±)-Methamphetamine	1 *(methanol)*	(±)-Methamphetamine-d5	1 *(methanol)*
(±)-MDMA	1 *(methanol)*	(±)-MDMA-d5	1 *(methanol)*
6-Acetylmorphine	1 *(acetonitrile)*	6-Acetylmorphine-d3	1 *(acetonitrile)*
Cannabinoid standard solutions			
(±)-THC-COOH	1 *(methanol)*	(±)-THC-COOH-d3	1 *(methanol)*

EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, HCOT – trans-3'-hydroxycotinine, MDMA – 3,4-methylenedioxymethamphetamine, THC-COOH – 11-nor-9-carboxy-Δ9- tetrahydrocannabinol.

racy values were in the 90-112% range. Repeatability was below 10% (RSD). For the majority of residues of medications of abuse and illicit drugs, a linear response was observed between LOQ and 1000 ng/mL, except for methamphetamine, EDDP (LOQ-500 ng/mL) and methadone (LOQ-200 ng/mL). LODs were between 0.31 and 3 ng/L, and LOQs were between 1 and 9.60 ng/L.

During a preliminary study, four daily wastewater samples obtained in one secondary school were analysed for nicotine residues, residues of medications of abuse and illicit drug residues. Out of 15 biomarkers (Table 7), on average, ten were detected in individual samples. 3,4-methylenedioxymethamphetamine – MDMA (Monday sample: 6.24 ng/L), benzoylecgonine (Monday sample: 42.0 ng/L and Friday sample: <LOQ but above LOD) and 6-acetylmorphine (<LOD only in Monday sample) were detected in different daily samples. Nicotine residues (nicotine: 4400–7500 ng/L, cotinine: 3000–5600 ng/L and trans-3'-hydroxycotinine – HCOT: 6700–9900 ng/L), 11-nor-9-carboxy-Δ9-tetrahydrocannabinolcocaine – THC-COOH (158–3232 ng/L), amphetamine (1.96–7.60 ng/L), morphine (up to 9.72 ng/L) and codeine (up to 29.84 ng/L) were detected in all samples and methamphetamine, EDDP, and cocaethylene were detected in none of them. Methadone was under LOD in three samples, while on a Wednesday (midweek), it was slightly higher (0.680 ng/L).

Concentrations of 16 drug residues obtained in 40 wastewater samples are presented in Table 8. Samples are grouped based on geographic location (municipalities: M1–7). Additional properties, such as level (PS – primary school, SS – secondary school, SHEI – mix secondary and higher education institution, HEI – higher education institution), type (G- gymnasiums, VTS – vocational and technical schools, MPS – multi-programme schools, IN- institutions offering natural science, IS – institutions offering social science) of educational institution and urbanisation (U – urban, NU – non-urban area) are also stated. As can be seen from Table 8, methadone (methadone, EDDP) and heroin (6-acetylmorphine, 6-AM) residues were always under LOD, while the highest concentrations were obtained for licit drug and cannabis residues.

Table 4
UPLC-MS/MS parameters.

Analyte	Retention time (min)	Ionisation mode	Precursor ion (m/z)	DP	Product ion 1 (m/z)	CE 1	CXP 1	Product ion 2 (m/z)	CE 2	CXP 2
Analytes										
HCOT	0.7	ESI+	**193**	61	**80**	47	6	**134**	27	4
Cotinine	1.2	ESI+	**177**	16	**80**	39	6	**136**	11	10
Nicotine	0.8	ESI+	**163**	41	**117**	32	8	**130**	27	10
Ethyl sulphate	3.3	ESI-	**125**	-5	**97**	-22	-15	**80**	-41	-7
Morphine	1.6	ESI+	**286**	96	**152**	79	10	**165**	51	8
Codeine	3.1	ESI+	**300**	81	**152**	83	14	**165**	55	12
Methadone	8.4	ESI+	**310**	16	**265**	21	10	**105**	35	10
EDDP	7.7	ESI+	**278**	66	**234**	40	10	**219**	57	14
THC-COOH	11.6	ESI-	**343**	-100	**299**	-30	-9	**245**	-36	-9
Cocaine	5.5	ESI+	**304**	51	**182**	27	6	**82**	39	8
Benzoylecgonine	4.3	ESI+	**290**	81	**168**	26	8	**77**	77	10
Cocaethylene	6.3	ESI+	**318**	101	**196**	25	10	**82**	41	8
Amphetamine	1.8	ESI+	**136**	51	**91**	23	8	**119**	11	8
Methamphetamine	2.4	ESI+	**150**	11	**91**	24	8	**119**	15	6
MDMA	3.1	ESI+	**194**	46	**163**	17	8	**105**	33	8
6-acetylmorphine	3.6	ESI+	**328**	101	**165**	51	6	**211**	35	8
Labelled internal standards										
Cotinine-d3	1.2	ESI+	**180**	76	**80**	37	6	**101**	29	8
Ethyl-d5-sulphate	3.3	ESI-	**130**	-45	**98**	-22	-13	**80**	-42	-11
Morphine-d3	1.6	ESI+	**289**	106	**152**	79	10	**165**	54	6
Codeine-d3	3.1	ESI+	**303**	81	**152**	85	8	**165**	55	6
Methadone-d3	8.4	ESI+	**313**	26	**268**	21	10	**105**	36	8
EDDP-d3	7.7	ESI+	**281**	56	**234**	42	10	**249**	32	8
THC-COOH-d3	11.6	ESI-	**346**	-90	**302**	-28	-11	**248**	-38	-7
Cocaine-d3	5.5	ESI+	**307**	86	**185**	27	8	**85**	43	6
Benzoylecgonine-d3	4.3	ESI+	**293**	81	**171**	27	8	**77**	75	6
Cocaethylene-d8	6.3	ESI+	**326**	96	**204**	27	8	**85**	43	8
Amphetamine-d6	1.8	ESI+	**142**	41	**93**	19	6	**125**	12	4
Methamphetamine-d5	2.4	ESI+	**155**	36	**92**	27	8	**91**	27	8
MDMA-d5	3.1	ESI+	**199**	56	**165**	17	6	**107**	32	8
6-Acetilmorphine-d3	3.6	ESI+	**331**	106	**165**	48	12	**211**	37	8

CE – collision energy, CXP – collision cell exit potential, DP – declustering potential, EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, HCOT – trans-3'-hydroxycotinine, MDMA – 3,4-methylenedioxymethamphetamine, THC-COOH – 11-nor-9-carboxy-Δ9- tetrahydrocannabinol

2. Experimental Design, Materials and Methods

2.1. Preliminary study design, participants and wastewater sampling

A preliminary study was conducted at one of the participating secondary schools (15th–19th April 2019). Wastewater was obtained using an autosampler operating in time-proportional mode (100 mL in five minutes). Seven-hour composite raw wastewater samples were collected from Monday to Friday during lesson time (7:30–14:30). A technical error meant that Tuesday's sample was lost, and only four daily samples were obtained.

Forty-four educational institutions were included in the study; 19 primary schools (6–15 years.), ten secondary schools (15–19 years.), nine HEIs (19+ years.) and six SHEIs (15+ years.). Thirty-seven institutes were located in urban and seven in non-urban areas [3,4] of seven municipalities (M1–7) from six statistical regions of Slovenia. The intention was to collect one sample per participating institution (n=44) at the end of the 2018/2019 academic year. The sewer layout meant it was impossible to collect wastewater from just the institution at specific sam-

Table 5
Validation results for licit drug residues.

Analyte	Linearity - range in ng/mL (R^2)	LOD [ng/L]	LOQ [ng/L]	Concentration level used for validation [ng/mL]	Matrix effect [%]	Repeatability [%RSD]	Accuracy [%]
HCOT	LOQ–1000 (0.9991)	224.5	747.6	5	−28	2	86
				10	−20	3	87
				50	−26	3	84
Cotinine	LOQ–1000 (0.9967)	19.1	63.6	5	−22	3	90
				10	−20	5	91
				50	−19	3	94
Nicotine	LOQ–1000 (0.9993)	78.6	261.7	5	77	5	136
				10	−7.0	7	124
				50	−27	1	93
Ethyl sulphate	LOQ–500 (0.9996)	305.3	1016.8	5	−114.9	14	105
				20	−2.0	5	91
				50	−6.9	2	91
				100	−2.3	2	93

HCOT – trans-3'-hydroxycotinine.

pling sites. For this reason, forty composite raw wastewater samples covering all educational institutions were obtained (Tables 1 and 2). Wastewater samples were collected mid-week on either a Tuesday, Wednesday or Thursday using an autosampler (100 mL every five minutes). The samples were stored at −20°C until analysis.

2.2. Chemicals and materials

Standard solutions of targeted analytes (1 mg/mL) and labelled analogues (1 or 0.1 mg/mL) were purchased from Cerilliant (Round Rock, Texas, USA) and stored in the dark at −20°C (Table 3). Working standards were prepared by diluting the stock standards with methanol to give final concentrations of 10 mg/L for analytes, 2 mg/L for basic drug and cannabinoid standards, and 0.5 mg/L for alcohol and nicotine residues standards. All solutions were stored in the dark at −20°C. All HPLC solvents were purchased from JT Baker (Philipsburg, USA), while LC-MS grade formic (HCOOH) and phosphoric acid (H_3PO_4) were purchased from Fluka (Switzerland). Aqueous ammonia solution (NH_3, 25%) was purchased from Merck (Darmstadt, Germany) and ammonium formate and tetrabutylammonium bromide (ion-pair reagent) from Sigma Aldrich (Missouri, USA). Milli-Q water was obtained by Millipore Direct-Q purifying system.

2.3. Sample preparation

For nicotine and alcohol residues determination, the samples were filtered through three different-pore-size filters (2.7 µm – GF/D, 1.2 µm – GF/C, Whatman, USA, and 0.45 µm cellulose membrane filters, Sartorius, Gottingen, Germany) and spiked with labelled internal standards (final concentration of 10 ng/mL). To determine alcohol residue, tetrabutylammonium bromide (TBA) as an ion-pair reagent was added to the sample (final concentration of 50 mM) [5].

The method used for basic drugs and cannabinoids determination is based on Senta et al. [6]. Briefly, 125 mL of sample was spiked with labelled internal standards (60 ng/mL in final extracts) and filtered through two different-pore-size glass microfiber filters (GF/D and GF/C, Whatman, USA). The samples were then acidified to pH 2 using concentrated H_3PO_4. Drug residues were extracted and pre-concentrated on Oasis MCX (150 mg/6 mL, Waters, Milford, MA, USA) solid-phase extraction cartridges conditioned with 5 mL methanol, 5 mL Milli-Q water and 5 mL 25 mM H_3PO_4. A two-step elution followed sample loading. In the first fraction (6 mL of methanol), cannabinoids were eluted, while in the second fraction (6 mL of 0.5% ammonium solution in

Table 6
Validation results for residues of medications of abuse and illicit drugs.

Analyte	Extraction recovery [%]	Matrix effect [%]	Repeatability [% RSD]	Accuracy [%]	Linearity - range in ng/mL (R^2)	LOD [ng/L]	LOQ [ng/L]
Codeine	91	−41	3	98	LOQ–1000 (0.9997)	1.98	6.58
Methadone	88	−28	3	112	LOQ–200 (0.9958)	0.63	2.09
EDDP	23	−15	7	105	LOQ–500 (0.9944)	1.81	6.04
Morphine	110	−69	5	96	LOQ–1000 (0.9998)	1.39	4.61
THC-COOH	71	−70	5	104	LOQ–1000 (0.9985)	0.83	2.77
Cocaine	90	−30	2	97	LOQ–1000 (0.9952)	0.48	1.61
Benzoylecgonine	80	−28	5	90	LOQ–1000 (0.9936)	2.88	9.60
Cocaethylene	89	−23	4	100	LOQ–1000 (0.9941)	0.48	1.59
Amphetamine	101	−55	5	108	LOQ–1000 (0.9915)	0.31	1.03
Methamphetamine	81	−69	4	102	LOQ–500 (0.9955)	1.00	3.33
MDMA	72	−49	5	106	LOQ–1000 (0.9914)	0.83	2.78
6-acetylmorphine	76	−47	7	98	LOQ–1000 (0.9985)	1.44	4.80

EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, LOQ – limit of quantification, MDMA – 3,4-methylenedioxymethamphetamine, THC-COOH – 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

Table 7
Concentrations (ng/L) of drug residues obtained during the preliminary study.

Day	HCOT	COT	NIC	MOR	COD	MTHD	EDDP	THC-COOH	COC	BE	COE	AMP	MAMP	MDMA	6-AM
Monday	6700	3000	4400	<4.61	29.84	<0.63	<1.81	158	10.52	42.0	<0.48	7.60	<1.00	6.24	<1.44
Wednesday	7300	4400	5300	5.68	<6.58	<2.09	<1.81	3232	2.40	<2.88	<0.48	2.00	<1.00	<0.83	<4.80
Thursday	9900	4900	5400	9.88	<6.58	<0.63	<1.81	728	2.40	<2.88	<0.48	4.64	<1.00	<0.83	5.16
Friday	8900	5600	7500	9.72	<6.58	<0.63	<1.81	248	2.16	<9.60	<0.48	1.96	<1.00	<0.83	<4.80

6-AM – 6-acetylmorphine, AMP – amphetamine, BE – benzoylecgonine, COC – cocaine, COD – codeine, COE – cocaethylene, COT – cotinine, EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, HCOT – trans-3'-hydroxycotinine, MAMP – methamphetamine, MDMA – 3,4-methylenedioxymethamphetamine, MOR – morphine, MTHD – methadone, NIC – nicotine, THC-COOH – 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

Table 8
Concentrations (ng/L) of drug residues obtained in 40 wastewater samples.

No.	Properties of the sample	COT	HCOT	NIC	EtS	MOR	COD	MTHD	EDDP	THC-COOH	COC	BE	COE	AMP	MAMP	MDMA	6-AM
								M1									
1	PS, U	1040	2870	1630	2240	<1.39	<1.98	<0.63	<1.81	504	5.24	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
2	SS (MPS), U	3700	6770	3680	11200	12.4	<1.98	<0.63	<1.81	381	3.50	<9.60	<0.48	<0.31	<1.00	<0.83	<1.44
								M2 – Ljubljana									
3	PS, U	263	<747.6	1070	<305.3	<1.39	<1.98	<0.63	<1.81	<2.77	<1.61	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
4	PS, U	1860	3840	2290	<305.3	14.0	<1.98	<0.63	<1.81	1460	3.16	28.8	<0.48	<0.31	<1.00	<0.83	<1.44
5	PS, U	1100	1970	1710	8240	<1.39	<1.98	<0.63	<1.81	76	48.8	69.2	<0.48	<0.31	<1.00	<0.83	<1.44
6	PS, U	289	<747.6	926	<1016.8	<1.39	<1.98	<0.63	<1.81	5.84	<1.61	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
7	PS, NU	878	1730	1300	1110	<1.39	<1.98	<0.63	<1.81	9.24	<0.48	<2.88	<0.48	<0.31	<3.33	<0.83	<1.44
8	SS (G), U	964	1480	1670	228000	<1.39	16.2	<0.63	<1.81	163	<1.61	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
9	SS (G), U	674	1560	1650	1920	<1.39	24.6	<0.63	<1.81	128	3.57	<9.60	<0.48	<0.31	<1.00	<0.83	<1.44
10	SS (G), U	1000	2050	1350	10500	<1.39	<1.98	<0.63	<1.81	244	66.0	74.4	<0.48	<0.31	<1.00	<0.83	<1.44
11	SS (VTS), U	3640	6950	3260	4390	37.4	18.8	<0.63	<1.81	1330	63.6	1340	<0.48	<0.31	<1.00	10.8	<1.44
12	HEI (IN), U	1290	2840	1300	40000	<1.39	<1.98	<0.63	<1.81	333	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
13	HEI (IN), U	1820	3010	1970	6290	12.0	<1.98	<0.63	<1.81	1140	10.2	196	<0.48	<0.31	<1.00	<2.78	<1.44
14	HEI (IS), U	1640	2950	3010	2420	11.2	<1.98	<0.63	<1.81	1460	12.6	25.7	<0.48	40.4	<1.00	7.56	<1.44
15	HEI (IS), U	3610	6090	3450	4250	19.1	<1.98	<0.63	<1.81	512	15.6	21.6	<0.48	180	<1.00	4.84	<1.44
16	HEI (IN), U	1880	5170	2550	4900	14.9	9.80	<0.63	<1.81	856	5.04	<9.60	<0.48	<0.31	<1.00	<0.83	<1.44
17*	SHEI, U	4730	8945	3035	5415	<1.39	<1.98	<0.63	<1.81	1890	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
18*	SHEI, U	3945	7500	3755	12550	58.0	19.2	<0.63	<1.81	454	9.12	270	2.42	<0.31	<1.00	<0.83	<1.44
								M3									
19	PS, U	721	1590	1080	<305.3	36.4	111	<0.63	<1.81	31.4	2.26	62.8	<0.48	<0.31	<1.00	<0.83	<1.44
20	PS, U	1470	6260	2520	2910	<1.39	<1.98	<0.63	<1.81	21	<0.48	<2.88	<0.48	<0.31	<3.33	<0.83	<1.44
21	PS, NU	<63.6	<747.6	829	<305.3	8.20	<1.98	<0.63	<1.81	74	2.06	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
22	SS (MPS), U	6200	9120	5260	7790	<1.39	<1.98	<0.63	<1.81	672	10.8	<2.88	<0.48	<0.31	<3.33	<0.83	<1.44
23	SS (MPS), U	1400	3690	1590	3860	<1.39	<1.98	<0.63	<1.81	1130	4.52	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44

(*continued on next page*)

Table 8 (*continued*)

No.	Properties of the sample	COT	HCOT	NIC	EtS	MOR	COD	MTHD	EDDP	THC-COOH	COC	BE	COE	AMP	MAMP	MDMA	6-AM
24	HEI (IN), U	3850	6560	5520	22600	14.9	<1.98	<0.63	<1.81	67.6	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
								M4									
25	PS, U	1260	2000	2580	3680	<1.39	<1.98	<0.63	<1.81	8.12	2.83	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
26	PS, NU	364	<747.6	1450	7150	<1.39	<1.98	<0.63	<1.81	<0.83	2.44	<9.60	<0.48	<0.31	<1.00	<0.83	<1.44
27	SHEI, U	3850	7160	3510	54000	<1.39	<1.98	<0.63	<1.81	524	5.08	<9.60	<0.48	<0.31	4.20	5.60	<1.44
28	SHEI, NU	3760	5460	3680	27100	<1.39	<1.98	<0.63	<1.81	130	3.68	18.7	<0.48	<0.31	<1.00	39.6	<1.44
								M5									
29	PS, U	1380	2950	8320	2210	<1.39	<1.98	<0.63	<1.81	35.6	177	1640	2.40	<0.31	<1.00	<0.83	<1.44
30	PS, U	516	1360	1130	4060	<1.39	<1.98	<0.63	<1.81	153.2	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
31	PS, NU	529	772	1530	<305.3	<1.39	6.96	<0.63	<1.81	27.9	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
32	SS (VTS), U	2140	3460	1830	4350	<1.39	<1.98	<0.63	<1.81	106	36.5	476	<0.48	<0.31	<1.00	<0.83	<1.44
33	SHEI, U	5860	10400	4340	9110	<1.39	<1.98	<0.63	<1.81	235	2.60	<9.60	<0.48	<0.31	<1.00	<0.83	<1.44
34	SHEI, U	3270	4870	2150	4970	<1.39	<1.98	<0.63	<1.81	14600[†]	<0.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
								M6									
35	PS, U	101	<224.5	1750	<305.3	9.72	8.96	<0.63	<1.81	<0.83	5.48	<2.88	<0.48	<0.31	<1.00	<0.83	<1.44
36	PS, U	701	968	32800	<305.3	12.0	47.6	<0.63	<1.81	4.44	3110	1530	<1.59	<0.31	<1.00	<0.83	<1.44
37	PS, NU	1630	3160	2070	2790	<4.61	<1.98	<0.63	<1.81	16.0	33.6	19.8	<0.48	<0.31	<1.00	<0.83	<1.44
								M7									
38	PS, U	209	554	1040	4440	113.2	<1.98	<0.63	<1.81	4.48	<0.48	<2.88	<0.48	<0.31	<1	<0.83	<1.44
39	PS, NU	1710	2650	1680	<305.3	<1.39	<1.98	<0.63	<1.81	<0.83	<0.48	<2.88	<0.48	<0.31	<3.33	<0.83	<1.44
40	SHEI, U	9390	20000	3730	72600	10.44	<1.98	<0.63	<1.81	304.4	2.10	<2.88	<0.48	<0.31	<1	<0.83	<1.44

[*] – average of two sampling days/samples,

[†] – estimated from the extrapolation of the calibration curve, G – gymnasiums, HEI – higher education institution, IN- institutions offering natural science, IS – institutions offering social science, MPS – multi-programme schools, NU – non-urban, PS – primary school, SHEI – mix secondary and higher education institution, SS – secondary school, U – urban, VTS – vocational and technical schools;6-AM – 6-acetylmorphine, AMP – amphetamine, BE – benzoylecgonine, COC – cocaine, COD – codeine, COE – cocaethylene, COT – cotinine, EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EtS – ethyl sulphate, HCOT – trans-3'-hydroxycotinine, MAMP – methamphetamine, MDMA – 3,4-methylenedioxymethamphetamine, MOR – morphine, MTHD – methadone, NIC – nicotine, THC-COOH – 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.

methanol), basic drugs were eluted. The cannabinoid fraction was further purified by acidification with concentrated HCOOH (60 µL) and passed through a Strata NH_2 cartridges (200 mg/3 mL, Phenomenex, Torrance, California, USA). The analyte was eluted using 2 × 2 mL of 1% HCOOH in methanol. The fractions were combined and reduced to dryness (40°C, N_2) and reconstituted in either 500 µL of Milli-Q and methanol, 80:20, v/v with 0.1% formic acid (basic drug residues extract) or Milli-Q water and methanol, 30:70, v/v (cannabinoids-containing extract).

2.4. Sample analysis

Samples were analysed using a Shimadzu ultra-performance liquid chromatograph hyphenated to an AB Sciex 4500 QTRAP detector mass spectrometer (UPLC-MS/MS). Ionisation was achieved with an electron ionisation (ESI) interface. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Retention times, both transitions and the ratio between the transition peak areas were used for identification [7]. Quantification was performed based on the relative response factors of the analyte to its isotopically labelled standard. Optimised LC-MS/MS parameters for analytes and labelled internal standards are presented in Table 4.

Alcohol residues were separated by injecting 10 µL of sample on Ascentis® Express C18 (2 µm, 50 mm × 2.1 mm, Supelco, Pennsylvania, USA) column at 40°C. Milli-Q water (A) and methanol (B), containing 0.1% formic acid, were used as eluents at a flow rate of 0.3 mL/min. The gradient elution was performed as follows: 2% B at 0 min, increase to 15% B at 10 min, 95% B at 11 min and hold the conditions for 1 min, then decreased to 2% B at 13 min. The ionisation of the compounds was achieved using electrospray ionisation in negative ionisation mode (ESI–).

For nicotine and basic drug residues, the analysis was based on Senta et al. [6]. Briefly, 10 µL of the extracted sample was injected onto the UPLC-MS/MS system. Analytes were separated on Synergi Polar-RP column (2.5 μm, 30 mm × 2 mm, Phenomenex, Torrance, California, USA) temperate at 40°C. Milli-Q water (A) and Methanol (B) containing 5 mM ammonium formate and 0.1% formic acid were used as eluents at a flow rate of 0.3 mL/min. The gradient elution was performed as follows: 2% B at 0 min, increase to 50% B at 6.9 min, 55% B at 7.3 min, 85% B at 8.7 min, 88% B at 10.7 min, and 100% B at 11 min, hold the condition till 11.4 min, then decrease to 2% B at 11.7 min and hold that percentage till 15.3 min. The ionisation of the compounds was conducted in positive ionisation mode (ESI+). During acquisition Scheduled MRM™ algorithm (MRM detection window: 120 s) was applied.

For cannabinoids, 10 µL of the sample was injected onto UPLC-MS/MS system, where the separation was performed on Supelco Ascentis® Express C18 (2 µm, 50 mm × 2.1 mm, Supelco, Pennsylvania, USA) column temperate at 40°C. Gradient elution using Milli-Q water (A) and methanol (B) at a flow rate of 0.3 mL/min was used as follows: 10% B at 0 min, increase to 50% B at 1.5 min, 60% B at 3.0 min and hold the conditions for 4 min and a half, increase to 85% B at 12.5min, then decrease to 10% B at 13 min and hold the condition for two minutes. The ionisation of the compounds was conducted in negative ionisation mode (ESI–).

2.5. Method validation

The method validation included parameters such as linearity, limits of detection (LOD), limits of quantification (LOQ), extraction recovery (for basic drug resides and cannabinoid), matrix effect (ME), accuracy and repeatability (Tables 5 and 6). The method performance was assessed in raw wastewater collected from a wastewater treatment plant (WWTP), while linearity was tested in a solvent. Matrix effect, accuracy and repeatability for alcohol residues were tested on four (5, 20, 50 and 100 ng/mL) and nicotine residues on three concentration levels (5, 10 and 50 ng/mL). In comparison, the functionality of transferred methods for basic drug residues and cannabinoids was confirmed at one concentration level (250 ng/mL).

The response's linearity was determined from a seven-to-twelve-point calibration curve and described using the linearity range and coefficient of determination (R^2). A calibration curve was obtained from plotting the peak area ratio of the analyte and its deuterated analogue as a function of analyte concentration.

Limits of detection (LOD) and quantification (LOQ) were determined by calculating the signal-to-noise ratio (S/N=3 and S/N=10) in real wastewater, spiked with deuterated analyte analogues at low concentration (2 ng/mL for nicotine, basic drug residues and cannabinoid, and 5 ng/mL for alcohol resides). The LOD and LOQ were calculated as an average S/N ratio obtained from five replicates.

The extraction recovery was assessed using two sets of spiked RW samples (four replicates). One set was spiked prior (RW spike) and one after the extraction (eluate spike). An additional set of samples (four replicates) was prepared only with labelled internal standards (RW original) and used to correct drug metabolites concentration already present in the wastewater. The extraction recovery was calculated based on obtained analyte peak areas as shown in Eq. (1), where A represents peak areas of analytes:

$$Extraction\ recovery\ (\%) = \frac{A(RW\ spiked) - A(RW\ original)}{A(eluate\ spiked) - A(RW\ original)} \times 100 \quad (1)$$

The matrix effect (ME) was evaluated by preparing two sets of spiked samples (each in four replicates). One set was spiked with analytes after the sample preparation procedure (final extract spiked), while the second was spiked only with labelled internal standards (RW original). Additionally, four replicates of Milli-Q water spiked with analytes were prepared (STD spiked). Matrix effects were evaluated based on a comparison between analytical response for biomarkers in the reconstructed sample (final spiked) and response for standard solutions as is shown in Eq. (2), where A represents the average peak areas of the analytes:

$$ME\ (\%) = \frac{A(final\ extract\ spiked) - A(RW\ original) - A(STD\ spiked)}{A(STD\ spiked)} \times 100 \quad (2)$$

Method accuracy was assessed by spiking a set of raw wastewater samples with analytes labelled internal standards at the beginning of the sample preparation procedure (RW spiked). One set of samples was spiked only with deuterated internal standards (RW original). All sample sets were prepared in four replicates and undergone the whole sampling preparation procedure. Additionally, four replicates of Milli-Q water spiked with analytes and deuterated standards were prepared (STD spiked). Method accuracy was evaluated by comparing the measured concentration of biomarkers in spiked wastewater influent and measured concentration in the standard solution as shown in Eq. (3), where c represents the average measured concentration of analytes:

$$Accuracy\ (\%) = \frac{c(RW\ spiked) - c(RW\ original)}{c(STD\ spiked)} \times 100 \quad (3)$$

Repeatability was assessed as relative standard deviation (RSD) of four replicate analyses of the spiked raw wastewater samples.

Quality control was performed by preparing and analysing procedural blanks (Milli-Q), analysing instrument blanks (Milli-Q water spiked only with deuterated internal standards), and quality control samples (points of calibration curve: 20 ng/mL for nicotine and alcohol residues, 30 ng/mL for basic drug residues and cannabinoid) after every 14th sample per batch.

Ethics Statement

Wastewater analysis requires no ethical approval for its application since individuals cannot be identified, it poses little risk of harming the participants. Accordingly, no approval from the ethics committee was needed prior to the study. However, following "The Ethical research guidelines for wastewater-based epidemiology and related fields", an informed consent form and an anonymity agreement were both signed by the Heads of participating institutions [8].

CRediT authorship contribution statement

Taja Verovšek: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Writing – original draft. **Ivona Krizman-Matasic:** Conceptualization, Methodology, Writing – review & editing. **David Heath:** Conceptualization, Writing – review & editing. **Ester Heath:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

CRediT Author Statement

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.107614.

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