water

MDPI

Article

Direct-Injection UHPLC-MS/MS Method for Simultaneous Determination of 78 Illegal Drugs and Psychoactive Substances in Domestic Wastewater

Kan Li, Yiling Hu, Yuke Jiang, Xing Han, Xin Liu and Mingluo Du *

Anti-Drug Technology Center of Guangdong Province, Guangzhou 510000, China; likan15@163.com (K.L.)
* Correspondence: philipdu@163.com

Abstract: The determination of illegal drugs and psychoactive substances in wastewater is increasingly being used to monitor the use of both by populations in specific areas. This article describes a method for the simultaneous determination of 78 illegal drugs and psychoactive substances in wastewater using direct-injection ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). This method includes the analysis of stimulants, opioids, antidepressants, antipsychotic drugs, anti-anxiety drugs, and hallucinogens. The method was validated in terms of the selectivity, calibration range, recovery, matrix effects, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The correlation coefficients were higher than 0.99 for all analytes, and the calibration range was from 0.2 to 500 ng/L. The LOD and LOQ of this method were 0.1–1 ng/L and 0.2–5 ng/L, respectively. The intra- and inter-day precisions were <9.3% and 11.4%, respectively, and the recovery ranged from 81.3% to 117.7%. The method was applied to real domestic wastewater collected from wastewater treatment plants, and the results showed that morphine, codeine, and ephedrine were detected in all samples. Some samples also contained other illegal drugs and psychoactive substances (such as etomidate, methamphetamine, ketamine, and tramadol). This indicates that the direct-injection UHPLC-MS/MS method can be used for the rapid determination of illegal drugs and psychoactive substances in wastewater.

Keywords: UHPLC-MS/MS; direct injection; illegal drugs; psychoactive substances; wastewater

Citation: Li, K.; Hu, Y.; Jiang, Y.; Han, X.; Liu, X.; Du, M. Direct-Injection UHPLC-MS/MS Method for Simultaneous Determination of 78 Illegal Drugs and Psychoactive Substances in Domestic Wastewater. *Water* **2024**, *16*, 1315. https://doi.org/10.3390/w16091315

Academic Editor: Ivan Senta

Received: 19 March 2024
Revised: 30 April 2024
Accepted: 1 May 2024
Published: 6 May 2024

1. Introduction

Drug abuse is a long-standing public health problem worldwide. According to the 2023 World Drug Report released by the United Nations Office on Drugs and Crime (UNODC), the number of people injecting drugs in the world in 2021 was estimated to be 13.2 million [1]. In 2021, more than 296 million people used drugs globally, with an increase of 23% over the previous decade. At the same time, the number of people suffering from drug abuse disorders surged to 39.5 million, with a 45% increase in 10 years, an outcome partly linked to the growing availability of new psychoactive substances on the global market.

Over the past 15 years, the cumulative number of new psychoactive substances reported to have been discovered reached 1165 in 2021 and, based on preliminary data, 1184 in 2022. Psychoactive substances refer to substances that have physiological and (or) psychological effects on the human body after administration, resulting in changes in emotions, feelings, and behaviors. They are mainly divided into stimulants, sedatives, hallucinogens [2–4], etc. Currently, there is no clear distinction and boundary when countries classify psychoactive substances as illicit drugs and psychotropic substances based on their medicinal use and addictive potential. Although the use of new psychoactive substances is still lower than that of traditional drugs, their use may be high in certain populations. Many countries have revised their laws and regulations to ban these addictive chemicals, including the United States, New Zealand, and China [5–7]. When these substances are

abused, part of them is metabolically transformed in the human body and excreted as metabolites, while the unreacted part is excreted as the parent drug. Both eventually pass through the sewer network to wastewater treatment plants. Given that the complex matrix in sewage samples can severely hinder the detection effectiveness, more sensitive and advanced detection methods are needed to monitor these trace substances.

Illicit drugs and psychotropic drugs are mostly polar organic compounds with different physicochemical properties. The analytical detection of these substances is usually performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) [8–11]. The advantages of LC-MS/MS analytical tandem technology lies in its ability to analyze compounds in the complex matrix while maintaining a good selectivity and sensitivity [12–14]. It has been widely reported in the literature that the concentration of these two classes of substances in environmental water and sewage samples ranges from ng/L to μg/L [15–17]. In order to meet this detection requirement, most methods adopt offline solid phase extraction (Offline-SPE) as the pre-treatment and concentration technology of sewage [18–20]. The process mainly includes pre-filtration, pH adjustment, solid phase extraction, elution, concentration, and re-dissolution. It is a time-consuming process and requires a large volume of samples. Moreover, most of the pre-treatment processes are open, which can easily introduce external pollution and interfere with the test results. In addition, special equipment and a large number of reagents and consumables are also required in Offline-SPE. Based on the cost and time factors of Offline-SPE, some researchers have developed online solid phase extraction with a large volume injection (Online-SPE), which saves time and some consumables, but also brings new problems: due to the large injection volume, the analysis system is prone to contamination and blockage, and the introduced matrix effect cannot be ignored [12,21–23].

To solve the related problems encountered by the above two SPE methods, researchers have developed a technical means that directly injects samples for analysis without SPE, which requires a small sample size, and sample pre-treatment only requires filtration. Compared with Offline-SPE and Online-SPE, it saves a lot of manpower, time, and consumables, while also avoiding the generation of a large amount of chemical waste. As far as we know, this method can detect only a dozen to fifty types of illegal drugs and psychoactive substances, which is far from enough compared to the total of over a thousand types [2,24,25]. Therefore, in this study, a new direct-injection ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) was developed and validated to analyze and detect 78 illegal drugs and psychotropic drugs in sewage. The illicit drugs and psychotropic substances included in this analysis cover a wide range of categories, including stimulants, opioids, hallucinogens, anti-anxiety drugs, narcotics, sedatives, antidepressants, and antipsychotics. This new method has been developed and validated in deionized water and wastewater. In addition, in order to prove the effectiveness and applicability of the method, sewage samples collected from 20 sewage treatment plants in a province were analyzed.

2. Experimental Section

2.1. Instruments and Equipments

SCIEX Triple Quad™ 7500 triple-quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA), EXION 2.0 ultra-high-performance liquid chromatography (AB SCIEX, USA), VORTEX 1 vortex oscillator (IKA, Staufen im Breisgau, Germany), electronic analytical balance (sensitivity 0.01 mg, METTLER TOLEDO, Greifensee, Switzerland), pipette (range 10–200 μL and 100–1000 μL. Eppendorf, Freiberg, Germany), and Milli-Q deionized water machine (Merck, Darmstadt, Germany) were used.

2.2. Materials and Reagents

Standard material: methamphetamine (MA), amphetamine (AM), 3,4-methylene dioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), codeine (COD), morphine (MOR), 6-monoacetylmorphine (6-MAM), tramadol (TRD), cocaine

(COC), benzoylaconine (BZE), ketamine (KET), norketamine (NK), 2-fluoro deschloroketamine (2-FDCK), norfluoramine (NFK), cathinone (CAT), methcathinone (MC), ethylcathinone (EC), 1-benzylpiperazine (1-BP), 2,5-dimethoxy-4-ethylphenylethylamine (2C-E), 2-(ethylamino-)-2-phenylcyclohexan-1-ketone (2-oxo-PCE), alprazolam (AZ), triazolam (TZ), 2-bromo deschloroketamine (2-BDCK), ethylfluramine (2-FXE), 2-methyl deschloroketamine (2-MDCK), cloprazolam (ClZ), dextromethorphan (DMO), dezocine (DZC), heroin (HRI), diazepam (DZ), flunitrazepam (FNZ), nimetazepam (NMZ), methadone (MTD), 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), ephedrine (EPD), etomidate (ET), metomidate (MET), etomidate acid (ETA), fentanyl (FT), oxycodone (OCD), norfentanyl (NFT), sufentanil (SFT), remifentanil (RFT), teletamine (TM), ergoethylenediamine (LSD), methoxymethamphetamine (MOP), 4-methoxymethamphetamine (PMMA), pethidine (PTD), quaalude (MQ), mephedrone (MD), 4-hydroxymethamphetamine (PHMA), Selegiline (SLGL), Amphilone (AFPM), methylphenidate (MPD), phentamine (PTM), cyclopropylmethylbuprenorphine (NBNP), tetrahydrocannabinic acid (THC-COOH), N-(adamantan-1-yl)-1-(4-fluorophenyl)-1H indole-3-formamide (4F-MDMB-BUTINACA, SC-096), 2-[1-(5-fluoropentyl)-1H indole-3-formamide]-3,3-dimethylbutyrate methyl ester (5F-MDMB-PICA, SC-078), N-(1-aminoformyl-2-methylpropyl)-1-(4-fluorobenzyl) indole-3-formamide (AB-FUBINACA), N-(1-amino-3,3-dimethyl-1-oxo-2-yl)-1-Butyl-1-H-indole-3-formamide (ADB-BUTINACA, SC-109), N-(1-aminoformyl-2,2-dimethylpropyl)-1-pentylindole-3-formamide (ADB-PINACA), 3,3-dimethyl-2-[1-(5-fluoropentyl) indole-3-formamide] ethyl butyrate (5F-EDMB-PICA, SC-106), 3,3-dimethyl-2-[1-(4-penten-1-yl)-1H-inazole-3-formamide] methyl butyrate (MDMB-4en-PINACA, SC-104), N-(1-amino-3,3-dimethyl-1-oxobutyl-2-yl)-1-(4-penten-1-yl)-1H indole-3-formamide (ADB-4en PINACA, SC-111), N-(-1-ethoxycarbonyl-2-methylpropyl)-1 (-5-fluoropentyl) indole-3-formamide (5F-EMB-PICA, SC-110), 1 (-4-cyanobutyl)-N (-1-methyl-1-phenylethyl)-1H indole-3-formamide (4CN-CUMYL-BUTINACA, SC-099), N-(1-adamantyl)-1-(4-fluorobutyl) Indole-3-formamide (4F-ABUTINACA, SC-107), 2-[1-(4-fluorobenzyl)-1H indole-3-formylamine]-3-methylbutyrate methyl ester (AMB-FUBICA, SC-095), 3,3-dimethyl-2-[1-(5-fluoropentyl) indole-3-formylamine] butyrate methyl ester (5F-ADB, SC-043), N′-(1-hexyl-2-oxoindole-3-subunit) benzoyl hydrazine (MDA-19), N′-(1-pentyl-2-oxoindole-3-subunit) benzoyl hydrazine (5C-MDA-19), N′-(1-(5-fluoropentyl) benzoyl hydrazine 2-oxoindole-3-subunit benzoyl hydrazide (5F-MDA-19), Ethyl 3,3-dimethyl-2-(1-pentyl-1H-indole-3-formamide) butyrate (EDMB-PINACA, SC-113), N-(1-adamantyl)-1-(5-chloropentyl)-1H-inindole-3-formamide (5Cl-APINACA, SC-100), 2-[1-(5-fluoropentyl)-1H-inindole-3-formamide]-3-phenylpropanoic acid methyl ester (5F-MPP-PICA, SC-098), and [1-(4-fluorobenzyl)-1H-inindole-3-yl] (2,2,3,3-tetramethylcyclopropyl) methyl Ketone (FUB-144, SC-094), 3,3-dimethyl-2-[1-(4-fluorobutyl) indole-3-formylamine] Methyl butyrate (4F-MDMB-BUTICA, SC-105)—all purchased from Cerilliant Corporation, Round Rock, TX, USA.

Isotope internal standard: morphine-D3 (MOR-D3), 6-monoacetylmorphine-D3 (6-MAM-D3), codeine-D3 (COD-D3), methadone-D3 (MTD-D3), 2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine-D3(EDDP-D3), fentanyl-D5 (FET-D5), amphetamine-d5 (AM-D5), methamphetamine-D5 (MA-D5), 3,4-methylenedioxymethamphetamine-D5 (MDA-D5), 3,4-methylenedioxymethamphetamine-D5 (MDMA-D5), ketamine-D4 (KET-D4), norketamine-D4 (NK-D4), cocaine-d3 (COC-D3), benzoylaconin-D3 (BEZ-D3), cathinone-D5 (CAT-D5), etomidate-D5 (ETA-D5), etomidate-D5 (ET-D5), and tetrahydrocannabinic acid-D5(THC-COOH-D5) all purchased from Cerilliant Corporation, Round Rock, TX, USA.

Ammonium formate (LC-MS grade), methanol (chromatographic pure), and acetonitrile (ACN, chromatographic pure) were purchased from Merck in Germany; formic acid (FA, chromatographic pure) was purchased from Shanghai Aladdin Company (Shanghai, China); deionized water was prepared by deionized water mechanism; and polypropylene (PP) needle filter (25 mm, 0.22 μm) was purchased from ANPEL Laboratory Technologies (Shanghai) Inc. (Shanghai, China).

2.3. Solution Preparation

The above 78 target substances were prepared in 1 μg/mL mixed stock solution with methanol and stored in the dark at −20 °C. Before use, working standard solutions were prepared by appropriately diluting mixed stock solutions in methanol to different concentrations. At the same time, the appropriate amount of the above 18 internal standard solutions was taken to prepare 5 ng/mL mixed internal standard working solution with methanol.

2.4. Sample Collection

An automatic sampling device was used to collect 20 sewage samples from sewage treatment plants in different areas, sampling every 2 h, and mixing 12 samples collected in one day as 24-h mixed samples (collection date was January 2024). Samples were stored in 500 mL polyethylene terephthalate (PET) containers and transported to the laboratory using a −20 °C cold chain. Samples were stored in a −20 °C refrigerator and thawed at room temperature before analysis.

2.5. Sample Pre-Treatment

Then, 4.95 mL of shaken wastewater sample and 50 μL internal standard working solution were mixed well to obtain sample solution, and then filtered through 0.22 μm PP syringe filter. The filtrate was transferred to the injection vial, and then analyzed. Each sample was run twice in parallel. The blank solution consisted of 4.95 mL ultrapure water and 50 μL internal standard working solution was processed in the same way as the sample.

2.6. Experimental Conditions

2.6.1. Chromatographic Conditions

EXION 2.0 Ultra-Performance Liquid Chromatograph (AB SCIEX, Malaysia, Singapore) equipped with Shimadzu Shim-pack Septer C18-120 (2.1 mm I.D. × 100 mm L, 3 μm) (Shimadzu Corporation, Kyoto, Japan); mobile phase: A was 2 mM ammonium formate buffer solution (containing 0.02% FA), and B was ACN; elution procedure: 0–0.5 min 5%B; 0.5–7.5 min 5–90%B; 7.5–9 min 90%B; 9–9.1 min 90–5%B; 9.1–12 min 5%B; the total analysis time was 12 min; the flow rate was 0.3 mL/min; and the injection volume was 20 μL.

2.6.2. Mass Spectrometric Conditions

SCIEX Triple Quad™ 7500 triple-quadrupole mass spectrometer equipped with electrospray ionization source (ESI+/−), scanning mode was multi-reaction monitoring (MRM), ion spray voltage were 1350 V(+)/1500 V(−), ion source temperature was 450 °C, collision gas (CAD) was set to 9, and the curtain gas (CUR), the nebulizing gas (GAS1), and the heater gas (GAS2) were set at 40, 30, and 60 psi, respectively. The mass spectrometric parameters and retention time (RT) of each target are listed in Table 1. The extracted ion chromatogram (EIC) of 78 target compounds is illustrated in Figure 1. From the graph, it can be seen that some substances elute at the same retention time, indicating that it is not possible to separate and detect all these substances simultaneously using ultra-high-performance liquid chromatography. It is necessary to use the MRM scanning mode in UHPLC-MS/MS to achieve this.

Table 1. MRM parameters and retention time of 78 target substances and isotopic internal standards.

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
1-BP	3.15	177.2	91.1	30	CAT-D5
			65.1	61	
2C-E	4.77	210.1	193.1	25	COC-D3
			178.1	25	

Table 1. *Cont.*

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
2-oxo-PCE	4.06	218.2	91.1	39	KET-D4
			173.2	17	
AB-FUBINACA	6.61	369.1	253.1	33	ET-D5
			109	55	
ADB-PINACA	7.46	345.3	215.2	34	ET-D5
			300.3	20	
AZ	5.99	309.5	281.2	39	ET-D5
			205.1	55	
AM	3.59	136.1	91.1	22	AM-D5
			119.1	11	
2-BDCK	4.16	282.1	172.2	25	KET-D4
			264.1	20	
BZE	3.89	290.1	168.1	27	BZE-D3
			105	41	
CAT	3.2	150.1	117.1	30	CAT-D5
			105.1	35	
2-MDCK	4.16	218.2	105	38	KET-D4
			159	28	
ClZ	6.05	316.2	270.1	36	ET-D5
			214.1	52	
COC	4.62	304.2	182.1	29	COC-D3
			150.1	35	
COD	3.38	300.1	165.1	52	COD-D3
			215.1	35	
DMO	5.15	272.3	147.2	42	COC-D3
			213.3	37	
DZC	4.32	246.2	147.1	27	AM-D5
			97.1	22	
DZ	6.97	285.1	193.2	40	ET-D5
			154.1	36	
EDDP	5.72	278.2	234.3	41	EDDP-D3
			249.3	33	
EPD	3.33	166.1	133.1	27	MA-D5
			117	30	
EC	3.61	178.1	160.1	18	CAT-D5
			130.1	25	
ET	6.58	245.2	141	15	ET-D5
			105.2	25	
ETA	3.48	217.1	113	13	ETA-D5
			95	34	

Table 1. *Cont.*

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
2-FDCK	3.83	222.2	191.1	20	KET-D4
			109	40	
FT	5.27	337.2	188.3	31	FT-D5
			105.2	55	
FNZ	6.36	314.1	268.2	35	ET-D5
			239.2	45	
HRI	4.39	370.1	268.1	38	MOR-D5
			165	63	
KET	4.06	238.1	125	39	KET-D4
			207.1	21	
LSD	4.74	324.2	223.1	32	COC-D3
			208.1	40	
MA	3.76	150.1	91.1	26	MA-D5
			119.1	13	
MDA	3.68	180	105.1	30	MDA-D5
			133.1	23	
MDMA	3.83	194	163.1	18	MDMA-D5
			105.1	34	
PTD	4.61	248.3	220.3	30	MA-D5
			174.1	28	
MD	3.96	178.2	145.2	26	AM-D5
			160.2	18	
MTD	5.97	310.2	265.2	20	MTD-D3
			105.1	35	
MQ	6.36	251.1	132.1	35	ET-D5
			91.1	58	
MC	3.37	164	146	18	CAT-D5
			131.1	27.5	
MET	6.09	231.1	127	23	ET-D5
			95	30	
MOR	2.67	286.1	201.1	34	MOR-D3
			165.1	50	
NMZ	6.42	296.1	250.2	36	ET-D5
			222.1	38	
NK	3.95	224.1	125	35	NK-D4
			179	22	
NFT	4.01	233.2	84	36	FT-D5
			150.1	25	

Table 1. *Cont.*

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
6-MAM	3.62	328.2	165.1	48	6-MAM-D3
			211.1	34	
MOP	4.1	180.2	149	20	MDMA-D5
			121	30	
OCD	3.59	316.2	298.2	26	MA-D5
			241.1	40	
PMMA	3.9	180.1	91	43	MDMA-D5
			121	28	
PHMA	2.85	166.1	107.1	30	MA-D5
			135.1	20	
PEPD	3.32	166.1	117	26	MA-D5
			133.1	27	
RFT	4.62	377.2	317.2	23	FT-D5
			228.1	29	
SC-104	8.56	358.2	213.1	34	ET-D5
			298.2	21	
SC-105	7.52	363.2	218.1	22	ET-D5
			144	55	
SC-109	7.06	331.2	201.1	35	ET-D5
			145	56	
SLGL	4.27	188.1	91.1	25	MA-D5
			119.2	16	
SFT	5.8	387.2	355.2	26	FT-D5
			238.1	27	
THC-COOH	8.06	343.2	299.1	−30	THC-COOH-D3
			245.1	−36	
TM	3.85	224.1	179.1	15	MA-D5
			151.1	25	
TRD	4.37	264.1	58.1	20	MA-D5
			246.1	13	
TZ	6.06	343.2	308.2	36	ET-D5
			315.2	36	
NBNP	4.7	414.2	223.1	59	COD-D3
			187	53	
AFPM	3.89	206.2	133.1	23	BZE-D3
			105	31	
MPD	4.35	234.1	84.1	26	COC-D3
			174.1	30	
PTM	3.72	150.1	133.1	13	MDA-D5
			91.1	30	

Table 1. *Cont.*

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
SC-78	7.73	377.2	232.1	20	ET-D5
			144	55	
SC-111	7.09	343.2	298.2	20	ET-D5
			171.1	53	
SC-096	7.9	364.2	219.1	33	ET-D5
			304.2	20	
SC-099	7.56	361.2	243.1	16	ET-D5
			145	56	
SC-106	8.04	391.2	232.2	23	ET-D5
			144	58	
SC-107	8.98	370.2	135.1	29	ET-D5
			93.1	71	
SC-110	7.73	377.2	144	54	ET-D5
			232.1	22	
SC-043	8.14	378.2	233.1	33	ET-D5
			318.2	22	
SC-095	7.57	383.2	252.1	21	ET-D5
			109	47	
SC-113	9.16	374.2	215.1	35	ET-D5
			300.3	22	
SC-100	9.73	400.2	135.1	29	ET-D5
			107.1	67	
SC-098	7.58	411.2	232.1	23	ET-D5
			144	57	
SC-094	8.84	350.2	109	60	ET-D5
			125.1	30	
2-FXE	4.01	236.1	163.1	22	K-D4
			109.1	51	
MDA-19	8.87	350.2	105	25	ET-D5
			77	80	
5C-MDA-19	8.53	336.2	105	25	ET-D5
			77	73	
5F-MDA-19	7.76	354.2	105	25	ET-D5
			77	76	
AM-D5	3.58	141.1	93.1	24	/ *
			124.1	12	
BZE-D3	3.88	293.1	171.1	28	/
			105	42	
CAT-D5	3.18	155.1	122.1	30	/
			110.1	24	

Table 1. *Cont.*

Target	RT (min)	Precursor Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (eV)	Internal Standard
COC-D3	4.62	307.2	185.1	29	/
			153.1	35	
COD-D3	3.38	303.1	165.1	63	/
			215.1	38	
EDDP-D3	5.72	281.2	234.3	41	/
			249.3	33	
ETA-D5	3.47	222.2	113	13	/
			95	34	
ET-D5	6.56	250.1	141	15	/
			95	35	
FT-D5	5.26	342.2	188.3	31	/
			105.2	31	
KET-D4	4.05	242.1	129	40	/
			211.1	22	
MA-D5	3.75	155.2	92.1	27	/
			121.1	14	
MDA-D5	3.67	185.1	110.1	31	/
			138.1	24	
MTD-D3	5.97	313.2	268.2	22	/
			105.1	38	
MDMA-D5	3.82	199	165.1	19	/
			107.1	35	
MOR-D3	2.66	289.1	201.1	36	/
			165.1	57	
NK-D4	3.94	228.1	129	36	/
			211.1	17	
6-MAM-D3	3.62	331.2	165.1	49	/
			211.1	35	
THC-COOH-D3	8.1	346.3	302.2	−30	/
			248.2	−36	

* It is an internal standard and does not need to be filled in.

2.7. Method Validation

In order to eliminate interference as much as possible, we selected blank wastewater matrix (the wastewater samples that do not contain analytical target substances or have extremely low content) for methodological validation and also subtracted the background value.

2.7.1. Selectivity

Selectivity was verified by analyzing 6 different blank wastewater samples and internal standard. The established method was used to prove that there was no interference of the target compound or internal standard in blank wastewater samples.

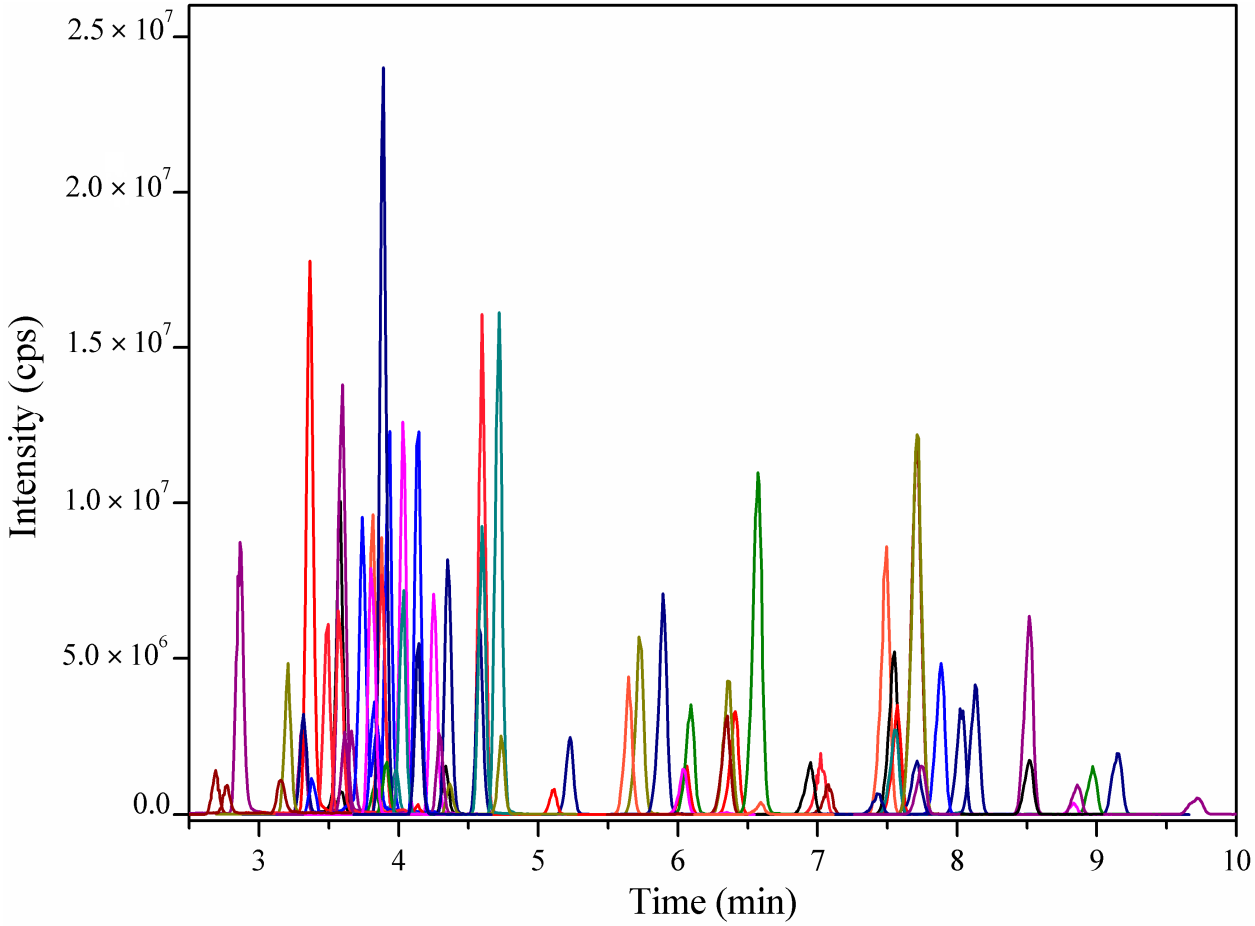

Figure 1. The extracted ion chromatogram (EIC) of 78 target substances (Different colors represent different compounds).

2.7.2. Calibration Curves and Range, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

The calibration curve standard solution was prepared by 4.9 mL blank wastewater with 50 µL of internal standard working solutions and 50 µL of standard solutions at different concentrations to obtain the concentration range from 0.2 to 500 ng/L, and then filtered in the same manner as the samples. Then, each of the calibration curve standards was measured according to the experimental method, and the linearity of the method was investigated. Linear regression equation and coefficient of determination were obtained using least-squares linear regression analysis. Linearity was acceptable when the coefficient of determination $R^2 \geq 0.99$. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using spiked wastewater samples containing a continuously decreasing concentration of analytes, and according to the International Union of Pure and Applied Chemistry (IUPAC), LOD and LOQ were calculated with 3 times signal-to-noise ratio (S/N) and 10 times S/N, respectively.

2.7.3. Accuracy and Precision

The accuracy was investigated using spiked wastewater at three different concentration levels of 5, 50, and 200 ng/L. The intra-day precision, calculated as relative standard deviation (RSD), was studied in six replicates at the same concentration level. The inter-day precision was evaluated at the same concentration level in 3 days.

2.7.4. Filtration Recovery and Matrix Effect

In order to eliminate interference as much as possible, we selected blank sewage matrix for methodological validation and also subtracted the background value. The filtration

recovery and matrix effects of the target compounds were determined using wastewater spiked with the analytes at three concentrations (low, medium, and high) with 6 replicates of each concentration in the following groups: $A_{sp\text{-}ww}$, wastewater spiked with the standard solution before filtration; $A_{sp\text{-}af}$, extracts spiked with the standard solution after filtration; and $A_{sp\text{-}water}$, the standard solution mixed with pure water. The extraction recovery and matrix effect were calculated using the following equations:

$$\text{Filtration recovery (\%)} = A_{sp\text{-}ww}/A_{sp\text{-}af} \times 100\%;$$

$$\text{Matrix effect (\%)} = (A_{sp\text{-}af}/A_{sp\text{-}water} - 1) \times 100\%$$

where $A_{sp\text{-}ww}$, $A_{sp\text{-}af}$, and $A_{sp\text{-}water}$ all represent the peak area of quantitative ion pairs of analytes.

2.7.5. Stability

Standard solutions with different concentration levels were added to the blank wastewater to obtain the quality control samples with low, medium, and high concentrations. The added concentrations of the target analytes were 5, 50. and 200 ng/L, respectively. The samples were analyzed according to the sample treatment process. After 24 and 48 h at room temperature and 4 °C, each sample was measured twice in parallel.

3. Results and Discussion

3.1. Method Optimization

The separation of the target compounds was investigated using three different chromatographic column: ACQUITY BEH C18 (1.7 μm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA, USA), ACQUITY Premier HSS T3 (1.8 μm, 2.1 mm × 100 mm, Waters Corporation), and Shim-pack GIST-HP C18-AQ (3.0 μm, 2.1 mm × 100 mm, Shimadzu Corporation, Kyoto, Japan). The results showed that there was no significant difference between the three chromatographic columns. From a cost perspective, we have chosen Shimadzu's.

Moreover, four different compositions of the mobile phase were evaluated: (1) HPLC-grade water containing 0.1% FA/ACN, (2) HPLC-grade water containing 0.02% FA/ACN, (3) HPLC-grade water containing 2 mM ammonium formate/ACN, and (4) HPLC-grade water containing 2 mM ammonium formate and 0.02% formic acid/ACN. HPLC-grade water containing 2 mM ammonium formate and 0.02% formic acid (solvent A) and ACN (solvent B) was selected as the most appropriate mobile phase because each analyte was adequately eluted and a low concentration of formic acid enhanced the response of the targets.

The MS/MS conditions of the optimum performance were in the ESI mode (+/−). Data for each illegal drugs and psychoactive substances were acquired in the MRM mode, where the transitions between the precursor ion and the two most abundant product ions were quantified and confirmed. To ensure high sensitivity, the collision energy of each selected product ion was optimized.

3.2. Selectivity

The selectivity of this method was validated using internal standard solution and blank wastewater samples. The results showed that no response of the target compound was found in the blank wastewater sample and internal standard solution, indicating that the selectivity of the method meets the requirements.

3.3. Calibration Curve, LOD, and LOQ

The results of the linearity parameters LOD and LOQ of 78 target substances are shown in Table 2. The detection range of 78 targets in this method was mostly 0.2~500 ng/L, the LOD was 0.1~1 ng/L, the LOQ was 0.2~5 ng/L, and all the coefficients of determination R^2 were greater than 0.995. Compared with the existing direct-injection method [2,24,25], our study exhibited a lower LOD and LOQs for almost all listed chemicals.

Table 2. Method validation data for 78 targets.

Target Compound	LOD/(ng/L)	LOQ/(ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
1-BP	0.5	1	1–500	y = 0.00488x − 0.000509	0.9996	5	98.1	3.1	5.2	99.8	−4.6
						50	98.9	3.0	6.6	111.8	−15.3
						200	102.5	2.3	3.4	106.4	−9.1
2-CE	0.2	0.5	0.5–500	y = 0.01451x + 0.00323	0.9992	5	108.8	6.2	7.5	115.7	1.0
						50	107.1	1.8	4.9	112.0	−19.4
						200	115.0	7.3	8.3	115.1	−6.8
2-oxo-PCE	0.5	1	1–500	y = 0.000177x + 0.0033	0.9999	5	102.9	2.0	8.9	100.1	4.0
						50	100.9	6.3	7.3	90.0	−1.1
						200	101.4	1.3	4.7	82.4	15.5
5-MeO-DiPT	0.1	0.2	0.2–500	y = 0.03212x + 0.000327096	0.9999	5	103.7	3.8	10.9	101.9	−3.0
						50	96.6	3.6	7.8	109.3	−1.8
						200	92.9	4.6	5.7	107.2	12.7
AB-FUBINACA	0.2	0.5	0.5–500	y = 0.00189x + 0.000261893	0.9994	5	89.9	3.8	10.8	98.8	−8.3
						50	104.5	1.0	5.3	99.4	9.9
						200	107.4	1.3	3.2	97.1	−1.5
ADB-PINACA	0.1	0.2	0.2–500	y = 0.00413x + 0.000605504	0.9991	5	89.0	1.6	10.3	83.6	3.8
						50	107.1	3.3	4.5	100.1	2.0
						200	100.5	1.7	3.8	95.6	−15.5
AZ	0.5	1	1–500	y = 0.00000628798x + 0.0000155935	0.9994	5	106.7	4.8	8.6	98.8	−7.4
						50	97.8	3.7	5.6	86.3	3.5
						200	105.4	4.1	6.4	102.9	−15.9
AM	0.2	0.5	0.5–500	y = 0.00311x + 0.000191977	0.9995	5	90.8	7.4	9.4	91.9	−3.3
						50	111.9	5.2	6.4	108.4	3.1
						200	108.9	5.0	7.4	106.1	4.4

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
BDCK	0.2	0.5	0.5–500	y = 0.01118x + 0.00544	0.9994	5	107.9	7.4	9.4	112.1	12.3
						50	106.6	5.2	6.4	105.5	3.7
						200	101.9	5.0	7.4	105.0	−3.7
BZE	0.2	0.5	0.5–500	y = 0.01753x + 0.00501	0.9996	5	105.6	5.9	7.1	107.4	−4.3
						50	114.4	6.6	6.9	115.0	5.5
						200	113.6	3.0	4.3	111.7	−2.2
CAT	0.2	0.5	0.5–500	y = 0.0214x + 0.00211	0.9997	5	111.5	2.8	4.2	106.7	2.9
						50	109.5	3.4	5.3	105.0	−0.2
						200	110.9	6.0	6.6	109.1	3.3
2-FXE	0.1	0.2	0.2–500	y = 0.03446x + 0.000533496	0.9985	5	111.1	6.1	8.7	108.6	−0.8
						50	109.2	5.3	7.4	112.8	−2.7
						200	105.7	7.6	8.2	106.9	2.4
2-MDCK	0.1	0.2	0.2–500	y = 0.03113x + 0.00479	0.9979	5	107.8	5.2	8.2	104.5	0.6
						50	110.7	8.1	10.2	108.2	−11.4
						200	104.8	4.5	10.9	100.1	−3.5
ClZ	0.2	0.5	0.5–500	y = 0.000623596x − 0.0000502649	0.9999	5	106.2	4.7	9.5	109.8	8.7
						50	98.5	6.1	8.8	99.4	−9.9
						200	103.1	5.9	9.1	102.7	−8.4
COC	0.2	0.5	0.5–500	y = 0.01869x + 0.06946	0.9998	5	97.2	8.6	10.9	101.5	3.5
						50	100.4	3.4	4.2	109.7	−14
						200	107.1	2.4	3.4	106.3	6.1
COD	0.2	0.5	0.5–500	y = 0.03039x + 0.00195	0.9996	5	98.7	5.6	14.0	102.4	−5.0
						50	116.3	5.5	6.3	111.7	−9.6
						200	113.1	7.2	8.0	112.1	−4.4

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
DMO	0.2	0.5	0.5–500	y = 0.00265x + 0.0000982837	0.9999	5	84.3	6.7	10.2	93.1	−59.8
						50	87.8	1.8	10.8	99.0	−29.6
						200	96.3	4.7	10.7	94.9	1.8
DZC	0.2	0.5	0.5–500	y = 0.00116x + 0.000497194	0.9981	5	103.8	4.7	10.5	100.7	−12.6
						50	95.4	4.8	9.4	98.3	−8.7
						200	95.2	5.9	9.9	102.5	−36.6
DZ	0.2	0.5	0.5–500	y = 0.00089018x − 0.0000298905	0.9998	5	100.9	3.9	10.1	97.8	−7.3
						50	99.0	3.4	5.7	93.9	−14.8
						200	104.4	2.6	4.1	100.5	−15.2
EDDP	0.1	0.2	0.2–500	y = 0.01918x + 0.00237	0.9995	5	97.4	1.9	7.1	103.0	8.5
						50	97.7	2.7	4.1	107.3	9.2
						200	91.5	0.9	2.3	102.8	6.7
EPD	0.1	0.2	0.2–500	y = 0.02086x − 0.000624722	0.9994	5	99.6	4.8	10.6	105.4	5.6
						50	110.7	4.3	10.7	111.5	15.4
						200	112.2	9.2	9.9	106.6	7.4
EC	0.2	0.5	0.5–500	y = 0.08437x + 0.02064	0.9995	5	102.7	5.8	8.9	100.2	−3.8
						50	108.5	4.9	6.4	99.1	−16.8
						200	108.8	7.0	7.4	102.9	−4.6
ETA	0.2	0.5	0.5–500	y = 0.0169x + 0.00358	0.9999	5	105.7	2.9	5.5	109.6	9.8
						50	102.6	3.4	6.5	109.1	9.3
						200	99.6	2.8	4.7	111.4	4.9
ET	0.1	0.2	0.2–500	y = 0.02322x + 0.01151	0.9986	5	97.4	1.9	2.8	108.4	6.9
						50	98.4	1.1	3.7	108.4	8.8
						200	97.7	1.4	2.7	107.1	6.5

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
FDCK	0.1	0.2	0.2–500	y = 0.01963x + 0.00234	0.9996	5	109.9	6.1	9.8	98.0	7.9
						50	107.7	4.8	10.2	109.5	11.8
						200	108.9	4.9	7.3	104.4	10.1
FT	0.1	0.2	0.2–500	y = 0.02289x + 0.01493	0.9996	5	110.4	4.5	8.5	95.7	−12.7
						50	93.8	1.8	3.0	115.9	12.2
						200	101.2	0.9	2.0	108.8	9.4
FNZ	0.1	0.2	0.2–500	y = 0.00172x + 0.0000995641	0.9998	5	107.9	2.7	8.6	114.5	6.9
						50	94.7	1.5	3.4	106.2	5.1
						200	95.9	1.8	1.9	111.0	9.8
HRI	0.2	0.5	0.5–500	y = 0.002285x + 0.01622	0.9993	5	97.8	4.8	11.4	89.9	−61.6
						50	91.0	3.7	11.1	83.8	−87.1
						200	100.6	4.1	12.3	88.7	−84.1
KET	0.1	0.2	0.2–500	y = 0.02473x + 0.00532	0.9998	5	105.7	6.2	8.8	104.9	12
						50	101.1	2.6	4.5	107.9	7.8
						200	102.3	4.0	5.5	108.3	6.7
LSD	0.1	0.2	0.2–500	y = 0.00606x + 0.00035567	0.9995	5	103.8	4.6	8.1	100.2	−51.6
						50	109.1	1.8	6.2	103.4	−65.4
						200	115.4	6.2	7.5	109.7	−62.5
MA	0.1	0.2	0.2–500	y = 0.03283x + 0.02160	0.9993	5	96.3	7.7	8.7	97.4	0.5
						50	100.3	3.3	5.9	92.9	−5.7
						200	102.8	3.3	5.2	102.8	2.2
MDA	0.1	0.2	0.2–500	y = 0.0209x + 0.00652	0.9998	5	92.4	9.3	9.6	104.4	3.5
						50	108.3	6.6	8.4	105.9	−1.7
						200	108.1	4.9	7.6	113.6	−2.4

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
MDMA	0.1	0.2	0.2–500	y = 0.0202x + 0.00033487	0.9998	5	105.5	7.3	7.5	111.5	17.7
						50	100.2	4.6	7.5	103.9	8.0
						200	95.2	3.8	6.7	106.0	0.5
SC-104	0.1	0.2	0.2–500	y = 0.00496x + 0.00166	0.9983	5	88.9	6.0	11.3	95.3	−7.9
						50	105.1	7.2	12.3	103.3	−16.7
						200	111.3	5.3	9.1	107.2	−27.3
PTD	0.1	0.2	0.2–500	Y = 0.01721x + 0.00406	0.9992	5	95.1	4.9	10.8	91.3	−83.4
						50	88.1	3.9	8.1	84.4	−85.7
						200	98.5	4.6	8.2	85.8	−74.7
MD	0.2	0.5	0.5–500	y = 0.000311955x + 0.000214614	0.9987	5	97.8	6.9	10.2	89.5	−6.4
						50	86.1	5.6	10.4	82.8	−23.9
						200	91.5	5.9	11.2	83.5	−18.3
MTD	0.1	0.2	0.2–500	y = 0.02181x + 0.00279	0.9986	5	84.9	4.1	4.9	90.7	−69.1
						50	94.2	2.0	3.8	88.5	−32.3
						200	95.2	2.1	2.4	85.7	−26.6
MQ	0.2	0.5	0.5–500	y = 0.00717x + 0.000741914	0.9997	5	102.4	1.5	8.9	102.3	5.4
						50	92.0	1.2	3.9	97.2	6.5
						200	91.2	1.7	3.8	103.4	10.5
MC	0.2	0.5	0.5–500	y = 0.09423x + 0.03338	0.9991	5	94.2	7.0	9.3	95.9	−2.9
						50	105.7	4.8	9.8	98.3	−16.9
						200	107.3	4.7	8.4	100.8	−2.6
MET	0.1	0.2	0.2–500	y = 0.00778x + 0.000411085	0.9999	5	108.8	2.3	2.7	109.8	−5.3
						50	103.9	1.1	1.6	105.7	−16.3
						200	107.2	1.3	1.7	109.9	−12.3

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
MOR	0.1	0.2	0.2–500	y = 0.02472x + 0.00319	0.9999	5	98.4	5.8	8.0	106.5	8.3
						50	96.9	4.1	5.1	105.8	6.3
						200	95.5	4.0	4.3	108.2	6.0
NMZ	0.2	0.5	0.5–500	y = 0.00624x + 0.000780312	0.9999	5	108.9	2.8	13.0	99.0	11.9
						50	107.0	2.3	4.3	101.0	7.8
						200	106.4	2.8	3.4	100.9	8.4
NK	0.1	0.2	0.2–500	y = 0.02854x + 0.00275	0.9999	5	100.5	3.2	6.9	105.7	6.6
						50	98.4	5.7	6.6	105.9	5.5
						200	95.3	3.9	7.0	105.1	2.9
NFT	0.2	0.5	0.5–500	y = 0.00892x − 0.00239	0.9981	5	96.1	6.2	13.3	108.8	−7.5
						50	90.0	5.4	14.2	117.7	15.2
						200	86.4	6.0	13.3	108.8	14.8
6-MAM	0.2	0.5	0.5–500	y = 0.02506x − 0.00115	0.9999	5	100.8	7.1	11.8	103.8	−3.6
						50	104.3	4.5	6.7	109.6	13.6
						200	99.6	3.8	10.9	110.4	10.6
MOP	0.1	0.2	0.2–500	y = 0.01739x + 0.00142	0.9999	5	104.5	8.0	9.2	102.3	−11.3
						50	114.1	7.2	8.4	112.2	−18.5
						200	111.2	5.0	7.4	110.9	−13.4
OCD	0.2	0.5	0.5–500	y = 0.02409x + 0.00176	0.9999	5	100.1	3.7	8.0	87.0	−84.9
						50	94.3	7.4	10.9	92.7	−88.0
						200	103.9	2.9	6.1	104.4	−82.8
PMMA	0.1	0.2	0.2–500	y = 0.00437x + 0.00422	0.9992	5	102.6	7.6	10.6	99.6	2.4
						50	104.9	4.8	7.0	105.2	−10.3
						200	97.3	5.1	9.4	103.6	−20.6

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
PEPD	0.1	0.2	0.2–500	y = 0.00129x + 0.0000528161	0.9991	5	99.8	4.4	9.8	100.4	4.0
						50	96.3	5.5	7.0	96.4	0.5
						200	98.0	3.1	5.0	101.3	4.9
PHMA	0.1	0.2	0.2–500	y = 0.02333x + 0.0000697922	0.9997	5	95.7	4.0	6.0	102.6	1.1
						50	105.6	4.2	4.5	105.0	11.3
						200	109.7	7.9	8.6	108.3	8.0
RFT	0.1	0.2	0.2–500	y = 0.04546x + 0.01092	0.9967	5	108.5	1.9	8.9	94.1	−15.7
						50	109.6	2.5	7.7	98.7	−9.6
						200	112.1	2.1	12.2	96.6	−10.8
SC-105	0.1	0.2	0.2–500	y = 0.02111x + 0.00362	0.9955	5	99.4	2.4	11.2	93.0	10.1
						50	112.3	2.4	2.7	104.2	11.1
						200	112.9	1.5	1.6	104.4	−2.5
SC-109	0.2	0.2	0.2–500	y = 0.00589x + 0.000772020	0.9993	5	99.6	4.0	9.7	99.4	3.5
						50	98.7	2.6	5.2	96.2	4.2
						200	100.6	1.2	3.6	94.4	−4.9
SLGL	0.1	0.2	0.2–500	y = 0.02149x + 0.0019	0.9996	5	98.4	4.1	9.4	98.7	28.3
						50	83.7	1.6	6.0	106.1	16.0
						200	83.3	6.8	6.9	113.1	39.4
SFT	0.1	0.2	0.2–500	y = 0.02381x + 0.0029	0.9996	5	84.0	5.7	8.2	83.8	16.1
						50	94.3	6.4	11.4	82.0	20.6
						200	100.0	1.7	8.1	84.9	−4.8
TM	0.2	0.5	0.5–500	y = 0.01902x + 0.01675	0.9999	5	105.4	3.7	5.3	93.7	−8.8
						50	96.3	3.3	4.2	104.2	0.9
						200	94.4	4.6	6.7	112.0	10.7

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
TRA	0.2	0.5	0.5–500	y = 0.00886x + 0.0000594916	0.9996	5	101.6	5.5	7.3	103.0	7.2
						50	107.8	6.2	7.4	109.6	0.7
						200	115.0	3.9	7.0	114.5	13.2
TRZ	0.2	0.5	0.5–500	y = 0.00425x + 0.000483134	0.9993	5	99.3	5.3	7.2	107.0	8.9
						50	109.5	2.3	4.5	105.0	13.3
						200	108.0	2.1	2.8	103.3	3.8
NBNP	0.5	1	1–500	y = 0.00348x + 0.00336	0.9999	5	102.2	5.8	9.2	100.9	15.0
						50	101.4	5.0	8.2	104.0	−3.4
						200	108.5	8.2	10.8	111.0	−8.5
AFPM	0.2	0.5	0.5–500	y = 0.01038x + 0.000498992	0.9996	5	94.2	5.1	10.9	96.4	29.0
						50	87.2	2.2	6.6	86.2	2.3
						200	84.2	1.8	6.0	83.6	6.4
MPD	0.2	0.5	0.5–500	y = 0.02489x + 0.00879	0.9998	5	105.4	3.5	9.7	115.6	14.5
						50	107.2	2.8	4.3	113.7	25.3
						200	108.9	4.7	9.9	106.9	26.2
SC-078	0.1	0.2	0.2–500	y = 0.94981x + 0.24285	0.9993	5	109.6	2.6	9.0	99.6	13.1
						50	109.4	4.0	5.3	106.2	5.9
						200	105.0	2.7	3.2	105.0	−0.6
PTM	0.5	1	1–500	y = 0.01142x + 0.00615	0.9999	5	100.1	5.3	8.5	95.5	4.4
						50	105.0	2.9	4.1	105.6	−10.9
						200	99.2	2.5	3.8	104.4	21.5
SC-111	0.2	0.5	0.5–500	y = 0.0216x + 0.00256	0.9999	5	101.8	2.7	9.0	95.8	5.1
						50	102.6	4.1	9.7	101.8	4.2
						200	90.3	3.1	3.9	105.4	8.4

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
SC-096	0.1	0.2	0.2–500	y = 0.0064x + 0.000718749	0.9995	5	102.3	2.1	8.6	99.5	−2.3
						50	105.3	2.4	4.7	101.3	−12.3
						200	110.1	2.0	3.0	107.7	−19.1
SC-099	0.1	0.2	0.2–500	y = 0.00731x + 0.000757787	0.9976	5	108.2	3.7	9.8	109.1	0.8
						50	106.3	3.2	4.0	108.3	−12
						200	108.2	2.8	4.2	104.5	−13.3
SC-106	0.1	0.2	0.2–500	y = 0.00483x + 0.000983932	0.9959	5	98.6	5.0	10.7	86.7	−15.9
						50	105.8	3.6	7.7	97.7	−17.1
						200	111.8	5.5	7.5	104.8	−8.1
SC-107	0.1	0.2	0.2–500	y = 0.00252x + 0.0000380955	0.9971	5	87.7	2.3	7.0	87.2	−9.7
						50	104.8	4.1	4.5	100.3	−17.2
						200	107.2	2.9	3.4	106.7	−27.5
SC-110	0.1	0.2	0.2–500	y = 0.07654x + 0.0093	0.9993	5	110.0	3.6	9.0	104.0	−7.7
						50	108.5	3.4	7.2	105.7	−12.3
						200	107.7	1.2	4.4	104.3	−23.8
SC-043	0.1	0.2	0.2–500	y = 0.36224x + 0.00747	0.9994	5	100.9	2.5	9.4	108.6	23
						50	106.1	3.9	6.1	105.0	1.4
						200	106.1	3.6	7.6	106.0	−5.8
SC-095	0.1	0.2	0.2–500	y = 0.05979x + 0.00957	0.9992	5	110.7	3.8	11.4	103.4	0.1
						50	106.7	4.8	9.0	104.1	4.0
						200	101.4	2.6	5.2	104.7	−16.3
SC-113	0.1	0.2	0.2–500	y = 0.05644x + 0.00703	0.9961	5	91.3	3.9	8.8	84.6	−26.7
						50	110.0	4.9	6.7	99.5	−29.7
						200	102.0	0.6	1.7	104.9	−39.0

Table 2. *Cont.*

Target Compound	LOD/ (ng/L)	LOQ/ (ng/L)	Linearity Range/(ng/L)	Regression Equations	R^2	Added Concentration/(ng/L)	Accuracy/(%)	Precision/(%)		Filtration Recovery/(%)	Matrix Effects/(%)
								Intra-Day	Inter-Day		
SC-100	0.2	0.5	0.5–500	y = 0.01741x + 0.00503	0.9985	5	97.8	4.1	8.0	102.2	−12.2
						50	110.0	5.5	7.0	110.2	−28.1
						200	99.3	2.1	3.1	104.8	−38.5
SC-098	0.1	0.2	0.2–500	y = 0.05759x + 0.01369	0.9997	5	107.0	3.5	11.1	108.3	−1.1
						50	105.9	4.2	6.2	107.3	−3.7
						200	106.6	2.0	4.1	107.7	−19.0
SC-094	0.1	0.2	0.2–500	y = 0.00886x + 0.000102981	0.9981	5	90.5	2.1	4.1	105.3	−6.1
						50	109.3	3.5	7.2	104.2	−25.2
						200	101.9	1.0	3.4	103.9	−38.5
MDA-19	0.2	0.5	0.5–500	y = 0.02099x + 0.0061	0.9997	5	108.1	3.9	7.7	97.3	−9.0
						50	111.3	5.7	6.9	110.4	−13.8
						200	105.5	0.7	2.8	107.5	−23.9
5C-MDA-19	0.1	0.2	0.2–500	y = 0.03856x + 0.0034	0.9990	5	99.2	3.6	4.8	105.9	3.3
						50	108.3	1.6	5.5	109.2	−3.2
						200	103.6	0.5	1.6	108.4	−18.7
5F-MDA-19	0.2	0.5	0.5–500	y = 0.03642x + 0.001091	0.9985	5	102.1	5.5	10.5	96.6	1.3
						50	108.4	4.9	8.2	107.2	−6.2
						200	104.1	1.9	7.4	99.8	−18.0
THC-COOH	1	5	5–500	y = 0.00195x + 0.0012	0.9996	5	104.5	7.0	9.0	110.7	9.1
						50	105.7	9.0	10.5	105.2	−5.2
						200	101.3	6.8	7.7	107.3	−8.4

3.4. Precision and Accuracy

Accuracy and precision are important factors in ensuring the accuracy and reliability of the detection results in the methods. In the method we developed, the average recoveries of 78 target substances were 83.3~116.3%, respectively, and the intra-day and inter-day precision of 78 target substances were 0.5~11.4% at different spiked mass concentrations, which indicated a great accuracy and precision. The specific results are shown in Table 2.

3.5. Filtration Recovery and Matrix Effect

As shown in Table 2, the filtration recovery of 78 target substances ranged from 81.3% to 117.7%. This indicates that the results obtained for most compounds are satisfactory at three validation levels. The matrix effects for all target compounds indicated signal enhancement (2.2% to 39.4%) for 39 compounds and signal suppression (−88% to −2.4%) for 40 compounds.

3.6. Stability

The stability of the spiked samples showed that, after 24 and 48 h at room temperature and 4 °C, the variation range of the peak area of each target was less than 10%, which indicated that the samples were basically stable within 48 h. Therefore, the detection results are not affected throughout the entire process from sample processing to analysis completion.

3.7. Application to Wastewater Samples

The method was applied to the analysis of sewage samples at the inlet of 20 sewage treatment plants in a province, and the results are shown in Table 3. It can be seen from Table 3 that the detection rates of COD and EPD were 100%, and the highest mass concentrations of these two were 19.55 and 404.3 ng/L, respectively. The detection rates of MOR, DZ, TRD, and MA were 95%, 95%, 90%, and 80%, respectively. The highest mass concentrations of these four were 37.88, 5.84, 29.23, and 9.82 ng/L, respectively, which were lower than those reported in the relevant literature in European countries [16,26,27]. The detection rates of ET and its metabolite ETA were 20% and 60%, respectively, and the highest mass concentrations of ET and ETA were 7.81 and 71.8 ng/L, respectively. The detection rate of EDDP was 70%. The detection rate of KET was 30%, and the detection rate of other target compounds was low or not detected. The results of this study were generally not significantly different from those reported in previous literature [12,26–28]. In this study, the COD concentration ranged from 1.73 ng/L to 19.55 ng/L in all the real samples. We detected MOR in nineteen of them, at concentrations ranging from 0.79 ng/L to 37.88 ng/L. Although 6-MAM is a specific metabolite of heroin [29], its metabolic rate is low and it is unstable in wastewater [30]. MOR is the most abundant metabolite of heroin, but it also enters wastewater as medicinal MOR and COD [31]. Therefore, the MOR we detected may not represent only illicitly used drugs, but clinical ones as well.

Table 3. Concentration of target substance in influent wastewater samples from 20 sewage treatment plants in a certain province (ng/L).

No.	COD	MOR	MA	KET	ET	TRD	ETA	EDDP	DZ	CAT	EPD	TM
S1	10.48	15.46	1.05	2.35	6.49	29.23	71.8	<LOD	0.76	<LOD	291.26	3.18
S2	6.49	4.39	1.8	<LOD	<LOD	3.65	<LOD	6.61	2.26	0.61	220.33	<LOD
S3	4.27	2.73	1.07	<LOD	<LOD	<LOD	<LOD	1.41	2.81	<LOD	125.24	<LOD
S4	4.11	4.99	<LOD	<LOD	5.3	2.46	55.47	0.53	<LOD	<LOD	224.09	<LOD
S5	3.93	1.86	2.3	0.54	<LOD	6.71	4.37	1.83	3.08	<LOD	276.57	<LOD
S6	11.21	4.27	6.61	<LOD	<LOD	2.58	<LOD	4	3.8	<LOD	50.96	<LOD
S7	10.45	8.65	2.51	2.51	2.28	11.55	36.69	0.64	0.9	<LOD	320.37	<LOD
S8	11.5	<LOD	9.82	2.23	<LOD	11.93	<LOD	13.66	4.72	<LOD	157.72	7.06

Table 3. *Cont.*

No.	COD	MOR	MA	KET	ET	TRD	ETA	EDDP	DZ	CAT	EPD	TM
S9	1.73	1.76	2.34	<LOD	<LOD	2.62	<LOD	0.57	0.91	<LOD	206	<LOD
S10	3.73	3.56	<LOD	<LOD	<LOD	12.65	2.44	<LOD	1.87	<LOD	92.97	<LOD
S11	6.81	3.84	1.94	<LOD	<LOD	8.63	1.42	<LOD	0.51	0.97	375.34	<LOD
S12	4.27	2.73	1.07	<LOD	<LOD	<LOD	<LOD	1.41	2.81	<LOD	125.24	<LOD
S13	6.55	6.07	1.18	2.02	7.81	17.32	32.13	0.7	5.84	<LOD	190.88	<LOD
S14	9.84	37.88	1.1	0.83	<LOD	16.56	<LOD	<LOD	1.52	<LOD	365.28	<LOD
S15	5.9	22.29	4.51	<LOD	<LOD	18.21	<LOD	1.5	3.52	0.66	205.94	<LOD
S16	6.53	13.87	<LOD	<LOD	<LOD	2.6	0.69	<LOD	1.36	<LOD	141.55	<LOD
S17	12.45	14.59	1.25	<LOD	<LOD	6.76	9.19	7.94	2.12	<LOD	328	0.74
S18	19.55	0.79	1.17	<LOD	<LOD	18.07	3.69	<LOD	3.87	<LOD	404.3	<LOD
S19	3.6	1.85	3.54	<LOD	<LOD	6.14	23.6	1.1	2.2	<LOD	171.94	<LOD
S20	6.09	7.06	<LOD	<LOD	<LOD	8.8	62.87	0.95	1.96	<LOD	276.9	<LOD
Maximum value	19.55	37.88	9.82	2.51	7.81	29.23	71.8	13.66	5.84	0.97	404.3	7.06
Detection rate (%)	100.00	95.00	80.00	30.00	20.00	90.00	60.00	70.00	95.00	15.00	100.00	15.00

3.8. Comparison with Previous Studies

The direct-injection or Online-SPE LC-MS/MS methods reported in the literature for the determination of illicit drugs in wastewater are summarized in Table 4. Compared with the existing direct-injection methods, our study exhibited lower LOQs for almost all listed targets, some of which had over one order of magnitude in differences, such as 2-FXE, 2-MDCK, EDDP, ET, FT, KET, and MET, indicating a higher sensitivity of the present method, which could be attributed to the increased instrumental sensitivity. SPE is a high-sensitivity technique for extracting illicit drugs for domestic sewage [32]. The advantages of SPE include the enrichment of compounds in wastewater samples and ease of handling, but its deficiencies include large reagent quantities, time consumption, cumbersome operation, easily introduced external pollution, and the inconvenient application in large-scale drug-monitoring campaigns [33]. Xiang et al. has used a direct-injection method for 11 illicit drugs in sewage, which requires 30 µL of the sample and achieved LOQs from 1 ng/L to 5 ng/L [25]. Wang et al. used on-line SPE (Oasis HLB) and achieved LOQs of 0.5 ng/L for MA, AM, COC, and nine other substances [12]. Richard Bade et al. used direct injection and achieved LODs below 10 ng/L for the majority of new psychoactive substances [24]. Hue et al. used direct injection with a 1000 µL injection volume to detect nine illicit drugs in sewage, with LOQs ranging from 3 ng/L to 80 ng/L [34]. Martínez et al. developed a direct-injection detection method (injection volume was 10 µL) for 22 drugs of abuse and achieved an LOQ from 10 ng/L to 700 ng/L for wastewater and 0.5–700 ng/L for river water [35]. Although a direct-injection method can reduce the analysis time and facilitate pre-processing, the large-volume direct injection will cause instrument pollution and affect the accuracy of the quantitative result [36]. In the proposed method, the MS/MS sensitivity was improved with multiple novel hardware features [37]. Compared with these methods, our method displayed a wide linear range and high sensitivity with a low detection limit. In addition to commonly used methods, there are also capillary detection techniques used to monitor illegal substances [38].

Table 4. Summary of LC-MS/MS methods based on direct injection or online-SPE for the analysis of illicit drugs in wastewater.

Target	Pretreatment Method	Sample Volume	Injection Volume	LOQ (ng/L)	Analytical Operations Time (min)	Reference
78 illegal drugs and psychoactive substances	direct injection	4.95 mL	20 µL	0.2–5	12	This work
MA, AM and other 9 illicit drugs	direct injection	1 mL	30 µL	1–5	11.5	[25]
MA, AM and other 11 illicit drugs	Online-SPE (Oasis HLB)	5 mL	2 mL	0.5	13	[12]
32 new psychoactive substances	direct injection	3 mL	10 µL	0.5–195	18.5	[24]
MA, AM and other 9 illicit drugs	direct injection	unknown	1 mL	3–60	10	[34]
22 drugs of abuse	direct injection	unknown	10 µL	10–700	26	[35]

4. Conclusions

This study used a high-sensitivity mass spectrometer to establish a direct injection UHPLC-MS/MS analytical method, which can achieve the simultaneous and rapid detection of 78 illegal drugs and psychoactive substances in wastewater. The pre-treatment process of this method is simple, and the great selectivity, accuracy, and reproducibility of this method have been validated. All analytes achieved acceptable validation results. The LOQs for most of the analytes were <1.0 ng/L with the lowest LOQ of 0.2 ng/L. The lowest LOD was 0.1 ng/L. The method has been successfully applied to detect COD, EPD, ET, MA, MOR, TRD, KET, DZ, and other illegal drugs and psychoactive substances in domestic sewage samples from different regions, which can provide a reference for the detection of illegal drugs and psychoactive substances in the water environment in the future. Meanwhile, the straightforward and fast sample pretreatment process (only requiring filtration) made the method easier to automate, more cost-effective, require smaller volumes, reduce degradation, and more suitable for high-throughput wastewater monitoring works in the future.

Author Contributions: Conceptualization, K.L.; methodology, K.L. and Y.J.; software, X.H. and Y.J.; validation, K.L. and Y.H.; formal analysis, K.L.; investigation, K.L. and Y.H.; resources, K.L.; data curation, K.L. and X.H.; writing—original draft preparation, K.L.; writing—review and editing, K.L. and Y.H.; visualization, K.L.; supervision, X.L.; project administration, M.D. All authors have read and agreed to the published version of the manuscript.

Funding: Supported by the Science and Technology Program of the Ministry of Public Security of China (No. 2023JSM07).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. United Nations Office on Drugs and Crime (UNODC). World Drug Report 2023. Available online: https://www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2023.html (accessed on 26 June 2023).
2. Restrepo-Vieira, L.H.; Busetti, F.; Linge, K.L.; Joll, C.A. Development and validation of a direct injection liquid chromatography-tandem mass spectrometry method for the analysis of illicit drugs and psychopharmaceuticals in wastewater. *J. Chromatogr. A* **2022**, *1685*, 463562. [CrossRef] [PubMed]
3. Shafi, A.; Berry, A.J.; Sumnall, H.; Wood, D.M.; Tracy, D.K. New psychoactive substances: A review and updates. *Ther. Adv. Psychopharmacol.* **2020**, *10*, 1–20. [CrossRef] [PubMed]
4. Peacock, A.; Bruno, R.; Gisev, N.; Degenhardt, L.; Hall, W.; Sedefov, R.; White, J.; Thomas, K.V.; Farrell, M.; Griffiths, P. New psychoactive substances: Challenges for drug surveillance, control, and public health responses. *Lancet* **2019**, *394*, 1668–1684. [CrossRef] [PubMed]

5. Hall, W. The future of the international drug control system and national drug prohibitions. *Addiction* **2018**, *113*, 1210–1223. [CrossRef] [PubMed]
6. Reuter, P.; Pardo, B. Can new psychoactive substances be regulated effectively? *Assess. Br. Psychoact. Subst. Bill. Addict.* **2017**, *112*, 25–31.
7. Gravely, S.; Driezen, P.; Ouimet, J.; Quah, A.C.; Cummings, K.M.; Thompson, M.E.; Boudreau, C.; Hammond, D.; McNeill, A.; Borland, R.; et al. Prevalence of awareness, ever-use and current use of nicotine vaping products (NVPs) among adult current smokers and ex-smokers in 14 countries with differing regulations on sales and marketing of NVPs: Cross-sectional findings from the ITC Project. *Addiction* **2019**, *114*, 1060–1073. [CrossRef] [PubMed]
8. Kasprzyk-Hordern, B.; Dinsdale, R.M.; Guwy, A.J. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1161*, 132–145. [CrossRef] [PubMed]
9. Zancanaro, I.; Limberger, R.P.; Bohel, P.O.; dos Santos, M.K.; De Boni, R.B.; Pechansky, F.; Caldas, E.D. Prescription and illicit psychoactive drugs in oral fluid—LC–MS/MS method development and analysis of samples from Brazilian drivers. *Forensic Sci. Int.* **2012**, *223*, 208–216. [CrossRef]
10. López-García, E.; Mastroianni, N.; Postigo, C.; Valcárcel, Y.; González-Alonso, S.; Barceló, D.; de Alda, M.L. Simultaneous LC–MS/MS determination of 40 legal and illegal psychoactive drugs in breast and bovine milk. *Food Chem.* **2018**, *245*, 159–167. [CrossRef]
11. Christophoridis, C.; Veloutsou, S.; Mitsika, E.; Zacharis, C.K.; Christia, C.; Raikos, N.; Fytianos, K. Determination of illicit drugs and psychoactive pharmaceuticals in wastewater from the area of Thessaloniki (Greece) using LC–MS/MS: Estimation of drug consumption. *Environ. Monit. Assess.* **2021**, *193*, 249. [CrossRef]
12. Wang, J.; Qi, L.; Hou, C.; Zhang, T.; Chen, M.; Meng, H.; Su, M.; Xu, H.; Hua, Z.; Wang, Y.; et al. Automatic analytical approach for the determination of 12 illicit drugs and nicotine metabolites in wastewater using on-line SPE-UHPLC-MS/MS. *J. Pharm. Anal.* **2021**, *11*, 739–745. [CrossRef] [PubMed]
13. Hummel, D.; Löffler, D.; Fink, G.; Ternes, T.A. Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. *Environ. Sci. Technol.* **2006**, *40*, 7321–7328. [CrossRef] [PubMed]
14. Borova, V.L.; Maragou, N.C.; Gago-Ferrero, P.; Pistos, C.; Thomaidis, N.S. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography–tandem mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 4273–4285. [CrossRef] [PubMed]
15. Verlicchi, P.; Al Aukidy, M.; Zambello, E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. *Sci. Total Environ.* **2012**, *429*, 123–155. [CrossRef] [PubMed]
16. Kasprzyk-Hordern, B.; Dinsdale, R.M.; Guwy, A.J. Illicit drugs and pharmaceuticals in the environment—Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. *Environ. Pollut.* **2009**, *157*, 1773–1777. [CrossRef] [PubMed]
17. Margot, J.; Rossi, L.; Barry, D.A.; Holliger, C. A review of the fate of micropollutants in wastewater treatment plants. *Wiley Interdiscip. Rev. Water* **2015**, *2*, 457–487. [CrossRef]
18. Shao, X.T.; Yu, H.; Lin, J.G.; Kong, X.P.; Wang, Z.; Wang, D.G. Presence of the ketamine analog of 2-fluorodeschloroketamine residues in wastewater. *Drug Test. Anal.* **2021**, *13*, 1650–1657. [CrossRef]
19. Milan, S.; Lelario, F.; Scrano, L.; Ottati, C.; Bufo, S.A.; Alpendurada, M.d.F. Detection of Eight Cannabinoids and One Tracer in Wastewater and River Water by SPE-UPLC–ESI-MS/MS. *Water* **2022**, *14*, 588. [CrossRef]
20. Yuan, S.; Wang, X.; Wang, R.; Luo, R.; Shi, Y.; Shen, B.; Liu, W.; Yu, Z.; Xiang, P. Simultaneous determination of 11 illicit drugs and metabolites in wastewater by UPLC-MS/MS. *Water Sci. Technol.* **2020**, *82*, 1771–1780. [CrossRef]
21. Mercier, B.; Scala-Bertola, J.; Pape, E.; Kolodziej, A.; Gibaja, V.; Bisch, M.; Jouzeau, J.-Y.; Gambier, N. Online SPE UPLC-MS/MS method for the simultaneous determination of 33 psychoactive drugs from swab-collected human oral fluid samples. *Anal. Bioanal. Chem.* **2022**, *414*, 4203–4215. [CrossRef]
22. Sang, D.; Cimetiere, N.; Giraudet, S.; Tan, R.; Wolbert, D.; Le Cloirec, P. Online SPE-UPLC-MS/MS for herbicides and pharmaceuticals compounds' determination in water environment: A case study in France and Cambodia. *Environ. Adv.* **2022**, *8*, 100212. [CrossRef]
23. Togola, A.; Baran, N.; Coureau, C. Advantages of online SPE coupled with UPLC/MS/MS for determining the fate of pesticides and pharmaceutical compounds. *Anal. Bioanal. Chem.* **2014**, *406*, 1181–1191. [CrossRef]
24. Bade, R.; Eaglesham, G.; Shimko, K.M.; Mueller, J. Quantification of new psychoactive substances in Australian wastewater utilising direct injection liquid chromatography coupled to tandem mass spectrometry. *Talanta* **2023**, *251*, 123767. [CrossRef] [PubMed]
25. Ren, H.; Yuan, S.; Zheng, J.; Luo, R.; Qiang, H.; Duan, W.; Zhao, Y.; Xiang, P. Direct injection ultra-performance liquid chromatography-tandem mass spectrometry for the high-throughput determination of 11 illicit drugs and metabolites in wastewater. *J. Chromatogr. A* **2022**, *1685*, 463587. [CrossRef] [PubMed]
26. Baker, D.R.; Kasprzyk-Hordern, B. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 1620–1631. [CrossRef] [PubMed]

27. Postigo, C.; de Alda, M.J.; Barceló, D. Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. *Environ. Int.* **2010**, *36*, 75–84. [CrossRef] [PubMed]
28. Terzic, S.; Senta, I.; Ahel, M. Illicit drugs in wastewater of the city of Zagreb (Croatia)—Estimation of drug abuse in a transition country. *Environ. Pollut.* **2010**, *158*, 2686–2693. [CrossRef] [PubMed]
29. Tscharke, B.J.; Chen, C.; Gerber, J.P.; White, J.M. Temporal trends in drug use in Adelaide, South Australia by wastewater analysis. *Sci. Total Environ.* **2016**, *565*, 384–391. [CrossRef] [PubMed]
30. van Nuijs, A.L.; Abdellati, K.; Bervoets, L.; Blust, R.; Jorens, P.G.; Neels, H.; Covaci, A. The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? *J. Hazard. Mater.* **2012**, *239*, 19–23. [CrossRef]
31. Gracia-Lor, E.; Zuccato, E.; Castiglioni, S. Refining correction factors for back-calculation of illicit drug use. *Sci. Total Environ.* **2016**, *573*, 1648–1659. [CrossRef]
32. Fontanals, N.; Pocurull, E.; Borrull, F.; Marcé, R.M. Role of solid-phase extraction in wastewater-based epidemiology. *Curr. Opin. Environ. Sci. Health* **2019**, *9*, 26–33. [CrossRef]
33. Ng, K.T.; Rapp-Wright, H.; Egli, M.; Hartmann, A.; Steele, J.C.; Sosa-Hernández, J.E.; Melchor-Martínez, E.M.; Jacobs, M.; White, B.; Regan, F.; et al. High-throughput multi-residue quantification of contaminants of emerging concern in wastewaters enabled using direct injection liquid chromatography-tandem mass spectrometry. *J. Hazard. Mater.* **2020**, *398*, 122933. [CrossRef] [PubMed]
34. Hue, T.T.; Zheng, Q.; Anh, N.T.; Binh, V.N.; Trung, N.Q.; Trang, H.T.; Chinh, P.Q.; Minh, L.Q.; Thai, P.K. Prevalence of illicit drug consumption in a population of Hanoi: An estimation using wastewater-based epidemiology. *Sci. Total Environ.* **2022**, *815*, 152724. [CrossRef] [PubMed]
35. Martínez Bueno, M.J.; Uclés, S.; Hernando, M.D.; Fernández-Alba, A.R. Development of a solvent-free method for the simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC–MS/MS. *Talanta* **2011**, *85*, 157–166. [CrossRef] [PubMed]
36. Zheng, Q.; Eaglesham, G.; Tscharke, B.J.; O'Brien, J.W.; Li, J.; Thompson, J.; Shimko, K.M.; Reeks, T.; Gerber, C.; Thomas, K.V.; et al. Determination of anabasine, anatabine, and nicotine biomarkers in wastewater by enhanced direct injection LC-MS/MS and evaluation of their in-sewer stability. *Sci. Total Environ.* **2020**, *743*, 140551. [CrossRef]
37. Lei Xiong, I.M. Improved LC-MRM Quantification Sensitivity for Cyclic Peptides from the Natriuretic Peptide Family. Available online: https://sciex.com/tech-notes/biopharma/improved-lc-mrm-quantification-sensitivity-for-cyclic-peptides-f (accessed on 26 September 2022).
38. Wiśnik-Sawka, M.; Maziejuk, M.; Fabianowski, W.; Karpińska, U.; Szwast, M.; Weremczuk, J. Capillary sensor for detection of amphetamine precursors in sewage water. *Polymers* **2021**, *13*, 1846. [CrossRef]