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Automatic analytical approach for the determination of 12 illicit drugs and nicotine metabolites in wastewater using on-line SPE-UHPLC-MS/MS

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ABSTRACT

In this study, we developed a novel on-line solid phase extraction (SPE)-ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS)-based analytical method for simultaneously quantifying 12 illicit drugs and metabolites (methamphetamine, amphetamine, morphine, codeine, 6-monoacetylmorphine, benzoylecgonine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, cocaine, ketamine, norketamine, and methcathinone) and cotinine (COT) in wastewater samples. The analysis was performed by loading 2 mL of the sample onto an Oasis hydrophilic-lipophilic balance cartridge and using a cleanup step (5% methanol) to eliminate interference with a total run time of 13 min. The isotope-labeled internal standard method was used to quantify the target substances and correct for unavoidable losses and matrix effects during the on-line SPE process. Typical analytical characteristics used for method validation were sensitivity, linearity, precision, repeatability, recovery, and matrix effects. The limit of detection (LOD) and limit of quantification (LOQ) of each target were set at 0.20 ng/L and 0.50 ng/L, respectively. The linearity was between 0.5 ng/L and 250 ng/L, except for that of COT. The intra- and inter-day precisions were <10.45% and 25.64%, respectively, and the relative recovery ranged from 83.74% to 162.26%. The method was used to analyze various wastewater samples from 33 cities in China, and the results were compared with the experimental results of identical samples analyzed using off-line SPE. The difference rate was between 19.91% and −20.44%, and the error range could be considered acceptable. These findings showed that on-line SPE is a suitable alternative to off-line SPE for the analysis of illicit drugs in samples.

1. Introduction

Drug abuse has become a common problem in the international community. The 2019 World Drug Report [1] released by the United Nations Office on Drugs and Crime (UNODC) shows that in 2017, approximately 271 million individuals or 5.5% of the global

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population aged 15–64 years old had used drugs. Currently, the problem of drug abuse is a serious threat to human health and social security. Wastewater-based epidemiology (WBE) can be used to obtain accurate information on drug abuse and, therefore, has an important role in combating drug crime and reducing the harm caused by illicit drugs.

WBE was first proposed by Daughton [2] in 2001; it focuses on analyzing the concentrations of drug metabolites in wastewater drained from a specific city to estimate the consumption of a drug by the population of that city. Four years later, this wastewater analysis method was put into practice by Zuccato et al. [3]. They performed the first determination of cocaine (COC) and its main metabolite, benzoylecgonine (BZE) using solid phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC-MS/MS) on water samples collected from medium-sized urban wastewater treatment plants in Italy. Bones et al. [4] also supported this method and used it to evaluate the presence of drugs such as COC, BZE, and morphine (MOR) in Ireland. Since then, considerable research has led to the development of various analytical methods based on SPE for the determination of illicit drugs in wastewater samples from European countries (such as Croatia [5], France [6], Finland [7], and the Netherlands [8]) and North America (Canada [9] and the USA [10]). In China, the SPE-LC-MS/MS method was used for the first time to monitor four kinds of illicit drugs in major sewage treatment plants from Hong Kong [11]. Khan et al. [12] and Du et al. [13,14] used this method to estimate drug abuse in more major Chinese cities.

Much of the previous research on the quantification of illicit drugs in wastewater has focused on off-line SPE [15–19]. However, these methods have various acknowledged drawbacks. Off-line SPE requires large sample volumes of wastewater and a complex sample handling process. Furthermore, the multiple steps lead to a time- and organic solvent-consuming process with loss of analytes [20]. Hence, in 2008, the first fully automated method was developed by Postigo et al. [21]. The method was based on on-line SPE-LC-MS/MS for the determination of 17 compounds and metabolites in various influent and effluent samples from Spain [21]. On-line SPE has several advantages such as the reduction of sample handling and analysis time [22]. A large and growing body of literature has reported the application of on-line SPE [23,24]. In a follow-up study, Yao et al. [25] subsequently optimized this method for the detection of 10 illicit drugs in environmental samples from Chinese cities. Similarly, López-García et al. [26] studied the consumption of methamphetamine (MA), ketamine (KET), and methadone as well as their metabolites in Barcelona, Spain for one week.

Although the on-line SPE method has been relatively well established, it limits the existing research in two ways. On the one hand, the long total run time is not conducive to the rapid detection of numerous wastewater samples. On the other hand, the large matrix effects may lead to incomplete regeneration of the SPE column, which adversely affects the samples tested. In this work, these problems were resolved by reducing the analysis time and sample volume and optimizing the filtration condition for sample pre-treatment and SPE column regenerated solvent. In addition, the on-line SPE column, sample pH, and mobile phase were optimized for good sensitivity and accuracy.

The aim of this study was to develop an optimized method based on on-line SPE-LC-MS/MS for the determination of the following 13 target substances: MA, amphetamine (AM), MOR, codeine (COD), 6-monoacetylmorphine (6-MAM), BZE, 3,4-methylenedioxymethamphetamine (MDMA) 3,4-methylenedioxyamphetamine (MDA), COC, KET, norketamine (NK), and methcathinone (MC) and cotinine (COT) in various wastewater samples from 33 cities in China. In addition, we used the off-line SPE-UHLPC-MS/MS method [27] to analyze the

collected wastewater samples and compared the results of the two methods concurrently. The optimized method was shown to have minor variation, good reliability, and substitutability.

2. Materials and methods

2.1. Reagent and chemicals

All reference standards were obtained as certified solutions in methanol from Cerilliant (Round Rock, TX, USA) at a concentration of 1 mg/mL. The studied illicit drugs and some of their studied metabolites were COT, MOR, MC, COD, AM, MA, MDA, BZE, NK, 6-MAM, MDMA, KET, and COC. Deuterated compounds COT-D3, MOR-D3, MC-D3, COD-D6, AM-D5, MA-D5, MDA-D5, BZE-D3, NK-D4, 6-MAM-D3, MDMA-D5, KET-D4, and COC-D3 used as isotope-labeled internal standards (IS) were also purchased from Cerilliant as 100 μg/mL solutions in methanol.

Mixed stock solutions were prepared with methanol at concentrations of 500 and 2500 ng/mL. Working standard solutions were prepared by appropriately diluting mixed stock solutions in methanol to different concentrations. All stock and working solutions were stored in the dark at −20 °C.

HPLC-grade methanol (MeOH), acetonitrile (ACN), and isopropyl alcohol (IPA) were purchased from Tedia (Fairfield, OH, USA). Formic acid (HCOOH) was obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Sodium hydroxide (NaOH) and sodium dihydrogen phosphate dihydrate (NaH$_2$PO$_4$·2H$_2$O) were acquired from Xilong Scientific Co., Ltd. (Shantou, China). Deionized water was prepared using a Milli-Q water purification system (Nanjing Miaozhiyi Electronic Technology, Nanjing, China).

2.2. Sample collection and pre-treatment

A series of 24-h composite wastewater samples was collected from the influent of wastewater treatment plants (WWTP) in 33 different cities in China. All samples were collected in polyethylene terephthalate bottles and adjusted to pH 2 with hydrochloric acid. The samples were immediately transported to the laboratory under cool conditions and stored at −20 °C until analysis. Before extraction, the samples were thawed at room temperature and spiked with the IS. Next, the samples were centrifuged at 10,000 r/min for 5 min and filtered through 0.45-μm hydrophilic polyvinylidene fluoride (PVDF) syringe filters (Jinteng, Tianjin, China).

2.3. On-line SPE and UHPLC-MS/MS

The samples were pre-concentrated using an on-line SPE device, an automatic on-line extraction system (Shimadzu Corporation, Kyoto, Japan). The system consisted of an automated sample processor SIL-16P with a 5 mL sample loop and an LC-20AD pump unit with low-pressure gradient formation. The UHPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two LC-30AD pumps, an autosampler SIL-30ACMP, and a CTO-20AC column oven (including proportional valve FCV-36AH). The 13 target substances were separated using a Shimadzu Shim-pack GISS C$_{18}$ column (2.1 mm × 100 mm, 1.9 μm). The oven temperature was set to 40 °C.

The sample processing method comprised four steps: sample loading, cleanup, elution, and on-line SPE column regeneration, which was accomplished using a column switching operation of two on-line SPE columns [28]. All injection solutions (filtered) were adjusted to pH 5–7 with appropriate amounts of NaOH and NaH$_2$PO$_4$·2H$_2$O. Then, a small amount of the sample solution of about 5 mL was injected into an Oasis hydrophilic-lipophilic balance (HLB) cartridge (2.1 mm × 30 mm, 20 μm, Waters Corporation, Wexford, Ireland) and washed with 5% MeOH at a flow rate of 3 mL/

min to transfer all samples and remove interference such as water-soluble impurities.

After the cleanup, the analytes were eluted from the Oasis HLB into the UHPLC system using the chromatographic mobile phase (deionized water containing 0.1% formic acid/ACN) at a flow rate of 0.4 mL/min. The proportion of the organic phase was increased from 8% to 30% in the first 5 min and then to 100% in the following 13 min; subsequently, the column was washed with two different solvents (50% MeOH containing 0.1% HCOOH; MeOH:ACN:IPA (1:1:1, *V/V/V*) containing 0.1% HCOOH) to regenerate for 5 min.

The initial mobile phase conditions were maintained for 3 min to rebalance the chromatographic column between runs. During the elution and analysis of the first sample, the next sample was extracted simultaneously through another on-line SPE column. The total run time of the chromatographic analysis was 13 min per cycle. The detailed on-line SPE process is shown in Table S1, and a typical chromatogram of the sample analysis is displayed in Fig. 1.

The UHPLC system was coupled to an LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) with an electrospray ionization (ESI) source in the positive mode. The MS operating conditions were as follows: capillary voltage, 3.5 kV; nebulizer gas (N_2) flow rate and heating gas flow rate, 3 and 10 L/min, respectively; interface temperature, desolvation temperature and heated block temperature, 300, 250, and 400 °C, respectively; and drying gas (N_2) flow rate, 10 L/min.

2.4. Method validation

The method was mainly validated for the limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, repeatability, recovery, and matrix effect. All samples were quantified by peak areas and corrected using the IS method. The LOD and LOQ were defined as the concentrations in spiked blank wastewater with signal-to-noise ratios of 3 and 10, respectively. Because there were differences between the wastewater samples, they were mixed and subjected to SPE column processing, which absorbed the interfering substances to produce blank wastewater.

Linearity was established in spiked blank wastewater at different concentration levels with a seven-point calibration curve,
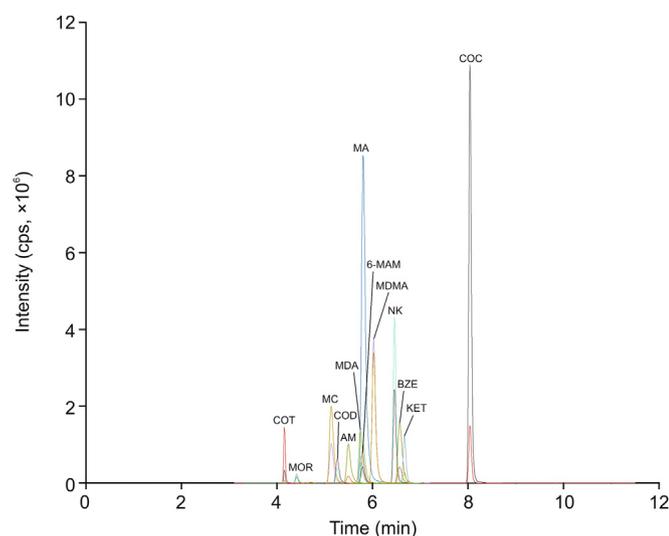
Fig. 1. Typical chromatogram in the positive electrospray ionization (ESI) mode for 13 target substances obtained using the developed on-line solid phase extraction (SPE) method. COT: cotinine; MOR: morphine; MC: methcathinone; COD: codeine; AM: amphetamine; MDA: 3,4-methylenedioxyamphetamine; MA: methamphetamine; 6-MAM: 6-monoacetylmorphine; MDMA: 3,4-methylenedioxymethamphetamine; NK: norketamine; BZE: benzoylecgonine; KET: ketamine; COC: cocaine.

using least-squares linear regression analysis. The LOQ was used as the lowest concentration point of the linear curve. The accuracy was investigated using spiked wastewater at three different concentration levels of 4, 80, and 200 ng/L, except for COT, which was used at 40, 800, and 2000 ng/L. The intra-day precision (expressed as repeatability), 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 level in 3 days. Recovery was divided into absolute recovery (AR) and relative recovery (RR), which were analyzed at the same concentration levels.

The AR was assessed by comparing the peak area of each target obtained from the on-line SPE analysis of the spiked wastewater with those obtained from samples directly injected into the UHPLC-MS/MS at equivalent amounts. The RR was calculated as the ratio between the concentration of each compound after it was corrected using the IS obtained from the on-line SPE treatment with spiked wastewater and spiked deionized water. The matrix effects were evaluated by comparing the peak area of each target obtained from the on-line SPE-UHPLC-MS/MS analysis in spiked wastewater at three concentration levels with those of the spiked deionized water.

3. Results and discussion

3.1. Method optimization

The on-line SPE and chromatographic conditions were optimized for the effective extraction and analysis of the target substances. To date, sample filtration has not been extensively discussed in the available published studies [25,26,29,30]. Optimized filtration methods play a crucial role in reducing the adsorption of particles to target substances and in protecting the instrument and reducing material losses.

In this work, the suitability of eight different 0.45-μm syringe filters was investigated: hydrophilic polytetrafluoroethylene, PVDF (both from Jinteng, Tianjin, China), mixed cellulose ester (MCE, Jinteng, Tianjin, China), regenerated cellulose (RCE), cellulose (CELL) (both from Shengze Technology Co., Ltd., Tianjin, China), polyethersulfone (PES), and nylon (both from Ameritech Scientific (Tianjin) Co., Ltd., Tianjin, China) syringe filters.

The experimental results showed that the 0.45-μm PES and nylon syringe filters adsorbed most of the tested substances compared to the unfiltered simulated samples, and the recovery of 6-MAM decreased when the 0.45-μm RCE, MCE, CELL, and nylon syringe filters were used. The results of 13 analytes were compared, and the 0.45-μm hydrophilic PVDF syringe filter was chosen as the sample filtering system. This two-stage method (centrifugation and filtration) not only removed the solid particles quickly and effectively but also exhibited a low adsorption rate, which ensured the accuracy of the target concentration.

In addition, the separation of the target compounds was investigated using three different SPE cartridges: the Oasis HLB (2.1 mm × 30 mm, 20 μm, Waters Corporation), XBridge C_{18} (2.1 mm × 30 mm, 10 μm, Waters Corporation), and XBridge C_8 (2.1 mm × 30 mm, 10 μm, Waters Corporation). The results showed that using the Oasis HLB column for the separation enabled all the analytes to be separated within a short time and their chromatographic peaks were in good shape. Different proportions of MeOH (0%, 5%, and 10%) were tested, and HPLC-grade water containing 5% MeOH was chosen as the washing solvent because it minimized the matrix effect and increased the sensitivity of the method.

Furthermore, three different compositions of the mobile phase were evaluated: 1) HPLC-grade water/ACN, 2) HPLC-grade water containing 0.1% formic acid/ACN, and 3) HPLC-grade water containing 5 mM ammonium formate/ACN. HPLC-grade water

Table 1
Validation parameters of the proposed method.

Compound	LOD (ng/L)	LOQ (ng/L)	Added concentration (ng/L)	Accuracy (RE%)	Precision (RSD%)		Absolute recovery (%)	Relative recovery (%)	Matrix effects (%)
					Intra-day	Inter-day			
COT	0.2	0.5	40	−3.8	4.69	0.77	18.75	117.88	−88.50
			800	−2.3	10.45	22.14	19.14	105.83	−92.50
			2000	3.0	6.69	22.92	21.52	132.61	−92.20
MOR	0.2	0.5	4	−7.6	3.04	5.28	10.76	90.10	−73.80
			80	6.1	7.57	15.59	10.55	89.07	−77.40
			200	5.3	4.97	25.64	11.77	89.41	−78.90
MC	0.2	0.5	4	−7.5	2.73	2.35	15.49	97.73	−63.90
			80	−3.5	2.76	7.40	18.54	99.56	−71.20
			200	−1.6	3.48	6.32	27.01	111.77	−70.90
COD	0.2	0.5	4	−3.3	4.19	0.94	18.32	126.97	−73.40
			80	2.0	4.20	0.41	21.62	135.05	−77.10
			200	3.6	4.90	5.99	22.35	140.44	−80.90
AM	0.2	0.5	4	−5.3	3.21	3.75	21.21	83.74	−66.30
			80	−8.5	2.34	19.31	20.30	84.31	−67.90
			200	−4.1	2.71	9.04	20.06	88.36	−67.50
MDA	0.2	0.5	4	−5.1	3.55	6.82	41.41	112.99	−65.20
			80	−4.0	2.81	11.7	42.10	110.79	−70.20
			200	−3.1	3.00	2.89	50.54	115.22	−72.60
6-MAM	0.2	0.5	4	−6.2	3.97	2.19	20.47	109.26	−69.90
			80	−3.8	5.09	11.20	24.83	100.82	−75.90
			200	−3.7	3.94	3.59	29.43	102.81	−80.50
MA	0.2	0.5	4	−7.9	4.25	7.10	29.13	104.90	−39.20
			80	3.3	2.67	20.04	54.82	130.62	−52.80
			200	−2.1	2.92	22.79	65.14	161.84	−64.20
MDMA	0.2	0.5	4	−7.3	2.35	3.40	63.72	100.01	−59.40
			80	−1.9	3.08	17.75	66.36	102.60	−74.50
			200	1.0	3.28	15.70	75.98	144.52	−75.80
NK	0.2	0.5	4	−6.8	3.31	1.99	29.36	97.98	−42.20
			80	−4.4	2.23	8.91	39.98	110.87	−63.70
			200	−2.9	3.11	11.65	65.28	162.26	−70.50
BZE	0.2	0.5	4	−7.9	5.57	2.04	20.39	90.49	−57.00
			80	−13.7	4.79	13.23	17.92	86.87	−60.60
			200	−2.9	3.11	11.65	18.62	84.83	−63.30
KET	0.2	0.5	4	−5.1	3.38	1.75	33.44	101.63	−76.50
			80	−0.8	2.80	13.97	34.25	101.21	−77.10
			200	1.4	2.11	7.76	52.57	104.94	−76.20
COC	0.2	0.5	4	−7.6	2.01	1.06	30.75	93.72	−26.40
			80	−2.2	2.16	11.07	59.20	140.97	−39.50
			200	−4.3	2.80	3.53	70.97	160.08	−61.10

LOD: limit of detection; LOQ: limit of quantification; RE: relative Error; RSD: relative standard deviation; COT: cotinine; MOR: morphine; MC: methcathinone; COD: codeine; AM: amphetamine; MDA: 3,4-methylenedioxyamphetamine; 6-MAM: 6-monoacetylmorphine; MA: methamphetamine; MDMA: 3,4-methylenedioxymethamphetamine; NK: norketamine; BZE: benzoylecognine; KET: ketamine; COC: cocaine.

containing 0.1% formic acid (solvent A) and ACN (solvent B) was selected as the most appropriate mobile phase because each target was adequately eluted and a low concentration of formic acid enhanced the response of the analytes. We also optimized the method using six different sample pH values (2, 3, 5, 7, 9, and 10), and three samples were examined under each condition. The results showed that the average peak areas of COT and COC decreased at pH 7−10 and the average peak areas of MOR, MC, and AM decreased at pH 2−5. The retention of COD, MDA, 6-MAM, MA, and KET on the on-line SPE was not affected by the pH of the sample solution. Based on the detection results of each target analyte, the optimal pH condition of the sample solution was 5−7.

In particular, adjusting the sample volume to 2 mL and the total run time to 13 min not only reduced the injection time and matrix effects but also resulted in sufficient sensitivity. In addition, the on-line SPE column regeneration step was re-optimized. Some previous studies only used a single organic solvent to wash the SPE column after the analysis [26,30−32], which could be a contributing factor in incomplete cleaning or even contamination of the next sample. In this experiment, different organic reagents and their mixtures were investigated depending on the properties of the target compounds. The following solvent was chosen for the SPE column regeneration: MeOH:ACN:IPA (1:1:1, *V/V/V*), containing

0.1% HCOOH, to ensure that the SPE column could be thoroughly cleaned and to guarantee the authenticity and accuracy of the sample determination.

The MS/MS conditions of the optimum performance were in the positive ESI mode. Data for each illicit drug were acquired in the multiple reaction monitoring (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. All information on the chromatographic retention time and the relevant MRM conditions for the target analytes is displayed in Table S2.

3.2. Method performance

The results of the methodological evaluation are shown in Table 1. The method was validated by examining the LOD, LOQ, linearity, intra- and inter-day precision, AR, RR, and matrix effects, which guided the determination of the wastewater samples. The sensitivity was improved using the on-line SPE because all the samples were completely transferred to the chromatographic column without analyte loss, as observed during the off-line SPE method. The LOD and LOQ of each target were obtained at 0.20 ng/L

and 0.50 ng/L, respectively. Linearity was evaluated at concentrations for all illicit drugs in the range of 0.5–250 ng/L (5–2500 ng/L for COT) using $1/y^2$ as the weighting factor.

Linearity was assumed with the correlation coefficient (r^2), which was higher than 0.99 for each substance. The intra-day precision and inter-day precision were expressed as RSD%. It can be seen that the intra-day precision was equal to or less than 10.45%, whereas the inter-day precision was consistently below 25.64%. The absolute recoveries of the 13 target compounds ranged between 10.55% and 75.98%, whereas the relative recoveries were between 83.74% and 162.26%. The results were attributed to losses in the analytical process and matrix effects, which can be corrected using the IS method. The matrix effects were evaluated using the following equation:

$$\text{Matrix effects (\%)} = [(A_{sp\ ww} - A_{ww})/A_{sp\ water} - 1] \times 100$$

where $A_{sp\ ww}$ is the analyte peak area in the spiked wastewater sample, A_{ww} is the analyte peak area in the non-spiked wastewater sample, and $A_{sp\ water}$ is the analyte concentration in the spiked deionized water [21]. Different substrates had different effects on the analytes. The positive or negative values of the dates indicated that the responses of the wastewater samples to the analytes were enhanced or inhibited. The results showed that the method was suitable for a wide range of applications and met the requirements of actual sample determination.

3.3. Analysis of wastewater samples

The validated method was used to analyze 33 wastewater samples from WWTPs in 33 cities in China. Table 2 shows the results of the analysis of selected illicit drugs in the influent wastewater samples, and most of the target analytes were detected. COT and MA were found in 100% of the wastewater samples analyzed. MOR, COD, AM, and KET were detected in 94%, 94%, 56%, and 47% of the samples analyzed, respectively, whereas 6-MAM and MDA were not detected in any sample. The average concentrations of the other illicit drugs monitored increased in the following order: COC < MDMA < NK < BZE. In addition, MC levels were measured in four wastewater samples using the on-line SPE method.

Approximately 70%–80% of the nicotine absorbed by smokers is converted to COT and excreted in the urine [33]. The high concentration of COT in the different urban wastewater samples is due to the widespread consumption of tobacco and the large population in China. 6-MAM and MC have never been studied in wastewater using on-line SPE. 6-MAM is a minor but exclusive metabolite of heroin used as a specific detection marker for heroin abuse because MOR and COD cannot be acetylated to form 6-MAM in vivo [34]. MC is a psychoactive substance of the cathinone class that acts similarly to MA and can cause acute health problems and drug dependence [35]. In all samples, the detection rate of 6-MAM was zero, and the average concentration of MC was extremely low. Generally, the levels of COC, BZE, COD, MOR, and MDMA found in this study were lower than those previously reported in the UK and Spain [36–38].

All collected wastewater samples were determined simultaneously using the off-line SPE methods described by Wang et al. [27], and the results are presented in Table 3. The difference in concentration levels detected for each substance by the two data sets was calculated to evaluate the difference between the two methods. The difference in rates was defined as the percentage of the difference between the concentration of the same target substance detected using both SPE methods and compared with the

Table 2
Concentrations (ng/L) of compounds selected by on-line SPE method in influent wastewater samples from WWTPs in 33 cities in China.

Sample No.	COT	MOR	MC	COD	AM	MDA	6-MAM	MA	MDMA	NK	BZE	KET	COC
1	851.363	ND	ND	1.732	1.274	ND	ND	29.655	ND	ND	ND	ND	ND
2	1093.667	11.687	ND	3.945	ND	ND	ND	99.308	ND	ND	ND	1.244	ND
3	958.280	22.006	ND	3.521	ND	ND	ND	15.518	ND	ND	ND	ND	ND
4	1056.664	8.336	ND	2.559	ND	ND	ND	2.808	ND	ND	ND	ND	ND
5	1527.399	24.201	ND	4.720	ND	ND	ND	8.195	ND	ND	ND	ND	ND
6	2615.712	34.465	2.590	14.443	2.904	ND	ND	47.571	ND	ND	ND	ND	ND
7	1011.889	10.318	ND	7.164	ND	ND	ND	4.060	ND	ND	ND	ND	ND
8	228.161	<LOQ	ND	ND	ND	ND	ND	2.901	ND	ND	ND	ND	ND
9	897.603	<LOQ	ND	3.135	1.315	ND	ND	4.050	ND	ND	ND	ND	ND
10	1757.184	22.572	ND	12.460	2.664	ND	ND	41.636	ND	ND	ND	0.982	ND
11	1194.711	44.974	ND	10.517	3.346	ND	ND	83.456	ND	2.221	ND	11.297	ND
12	1570.679	28.585	ND	7.895	3.723	ND	ND	70.857	ND	4.320	ND	29.117	ND
13	826.180	12.420	ND	6.125	5.907	ND	ND	26.297	ND	ND	ND	ND	ND
14	2111.022	47.923	ND	14.919	5.036	ND	ND	9.013	ND	ND	ND	ND	ND
15	1722.271	14.969	ND	2.910	ND	ND	ND	9.286	ND	ND	ND	ND	ND
16	1368.393	16.251	ND	3.203	ND	ND	ND	3.766	ND	ND	ND	ND	ND
17	572.188	<LOQ	ND	ND	ND	ND	ND	6.914	ND	ND	ND	ND	ND
18	847.909	<LOQ	ND	3.566	ND	ND	ND	3.109	ND	ND	ND	2.182	ND
19	1059.207	20.191	ND	8.674	2.892	ND	ND	20.310	2.004	1.271	ND	6.586	ND
20	954.340	14.426	3.806	4.678	1.510	ND	ND	11.922	ND	ND	ND	1.870	ND
21	673.691	18.838	ND	5.352	ND	ND	ND	12.954	1.096	ND	ND	ND	ND
22	1373.990	31.616	8.241	6.848	7.107	ND	ND	62.330	2.401	ND	ND	5.215	1.712
23	1690.936	27.105	ND	14.039	3.221	ND	ND	26.744	3.131	2.050	1.19	13.029	ND
24	922.929	62.893	ND	20.560	ND	ND	ND	36.773	ND	ND	ND	8.912	ND
25	1389.975	10.721	ND	8.650	ND	ND	ND	23.786	1.021	ND	ND	5.628	ND
26	810.938	<LOQ	ND	9.718	1.229	ND	ND	41.863	ND	ND	ND	ND	ND
27	1178.995	17.993	ND	7.309	2.909	ND	ND	28.543	1.586	ND	5.131	3.202	1.103
28	921.931	28.265	ND	13.336	2.069	ND	ND	20.213	ND	ND	3.051	0.754	ND
29	745.171	11.636	ND	4.692	ND	ND	ND	8.613	ND	ND	ND	8.881	ND
30	1020.312	15.439	ND	5.259	7.508	ND	ND	23.890	ND	ND	ND	1.308	ND
31	1451.985	21.887	ND	6.822	2.513	ND	ND	49.449	ND	ND	ND	0.708	ND
32	1189.114	31.775	5.879	10.846	17.012	ND	ND	22.318	ND	ND	ND	4.352	ND
33	634.702	8.528	ND	3.230	ND	ND	ND	6.202	ND	ND	ND	3.234	ND

WWTP: wastewater treatment plant; ND: not detected.

Table 3
Concentrations (ng/L) of compounds selected by off-line SPE method in influent wastewater samples from WWTPs in 33 cities in China.

Sample No.	COT	MOR	MC	COD	AM	MDA	6-MAM	MA	MDMA	NK	BZE	KET	COC
1	767.822	ND	ND	2.032	1.149	ND	ND	33.222	ND	ND	ND	ND	ND
2	951.895	12.737	ND	3.903	ND	ND	ND	115.818	ND	ND	ND	1.313	ND
3	812.051	19.534	ND	4.031	ND	ND	ND	14.949	ND	ND	ND	ND	ND
4	892.543	7.859	ND	2.665	ND	ND	ND	3.506	ND	ND	ND	ND	ND
5	1312.350	21.892	ND	4.401	ND	ND	ND	7.286	ND	ND	ND	ND	ND
6	2308.760	34.268	2.152	16.558	3.222	ND	ND	55.973	ND	ND	ND	ND	ND
7	840.149	9.747	ND	7.485	ND	ND	ND	4.082	ND	ND	ND	ND	ND
8	196.483	<LOQ	ND	ND	ND	ND	ND	2.604	ND	ND	ND	ND	ND
9	783.933	<LOQ	ND	3.680	1.121	ND	ND	3.990	ND	ND	ND	ND	ND
10	1528.050	22.218	ND	12.155	2.225	ND	ND	45.325	ND	ND	ND	0.975	ND
11	1015.570	49.118	ND	11.166	4.149	ND	ND	100.860	ND	1.916	ND	12.050	ND
12	1313.910	28.139	ND	7.183	3.830	ND	ND	79.278	ND	4.538	ND	30.277	ND
13	756.552	10.996	ND	5.950	6.592	ND	ND	30.953	ND	ND	ND	ND	ND
14	1805.990	45.848	ND	14.769	5.256	ND	ND	10.317	ND	ND	ND	ND	ND
15	1519.490	144.90	ND	3.249	ND	ND	ND	10.116	ND	ND	ND	ND	ND
16	1268.620	15.093	ND	3.121	ND	ND	ND	4.099	ND	ND	ND	ND	ND
17	480.057	<LOQ	ND	ND	ND	ND	ND	6.577	ND	ND	ND	ND	ND
18	764.734	<LOQ	ND	3.474	ND	ND	ND	3.373	ND	ND	ND	2.199	ND
19	920.622	19.019	ND	8.098	3.025	ND	ND	22.775	1.896	1.057	ND	6.523	ND
20	862.541	16.584	3.195	5.595	1.374	ND	ND	12.888	ND	ND	ND	1.835	ND
21	570.841	19.069	ND	4.641	ND	ND	ND	13.438	1.364	ND	ND	ND	ND
22	1208.750	30.976	7.547	6.818	6.145	ND	ND	67.218	2.966	ND	ND	4.771	1.649
23	1413.080	24.596	ND	16.033	3.103	ND	ND	30.642	3.792	1.994	1.433	13.897	ND
24	811.697	64.284	ND	20.303	ND	ND	ND	43.863	ND	ND	ND	9.741	ND
25	1161.170	11.874	ND	7.845	ND	ND	ND	26.763	0.990	ND	ND	6.297	ND
26	802.387	<LOQ	ND	11.403	1.075	ND	ND	49.020	ND	ND	ND	ND	ND
27	1027.800	18.403	ND	7.341	3.153	ND	ND	34.173	1.701	ND	6.058	3.419	1.181
28	849.668	31.941	ND	16.411	2.418	ND	ND	22.584	ND	ND	3.044	ND	ND
29	642.471	13.593	ND	4.509	ND	ND	ND	10.683	ND	ND	ND	8.908	ND
30	900.915	15.930	ND	5.650	8.546	ND	ND	26.075	ND	ND	ND	1.150	ND
31	1286.340	21.567	ND	7.149	2.413	ND	ND	57.527	ND	ND	ND	ND	ND
32	1068.510	31.899	4.970	10.207	17.618	ND	ND	25.564	ND	ND	ND	4.853	ND
33	618.671	8.342	ND	3.337	ND	ND	ND	5.441	ND	ND	ND	3.002	ND

detected concentration using the off-line SPE. The results showed that the difference in rate was between −19.91% and −20.44%, and the error range was acceptable, which proved that the optimized on-line SPE method had good applicability and could be used as an effective alternative method for the detection of illicit drugs in wastewater samples.

4. Conclusions

In this work, a novel automated analytical method based on on-line SPE-UHPLC-MS/MS was developed and validated for the simultaneous analysis of 12 illicit drugs in wastewater samples collected from 33 cities in China. The method proved to be effective for the analysis of the selected target compounds. The major advantages of this method are small sample manipulation, high sensitivity, time and cost saving, and real-time information. Drug monitoring of public security departments based on this method could accurately provide information on drug abuse that would contribute to preventing and combating drug-related crimes. Furthermore, the application of this strategy could provide important technical support for the comprehensive implementation of all narcotics control work.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.01.002.

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