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ANALYSIS OF ILLICIT DRUGS IN MUNICIPAL WASTEWATER USING LC-MS/MS: A METHOD VALIDATION STUDY

(Analisa Dadah Berbahaya dalam Air Sisa Perbandaran Menggunakan LC-MS/MS: Satu Kajian Pengesahan Kaedah)

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Abstract

An analytical method based on solid-phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of five illicit drugs, namely amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), and morphine (MOR) in municipal wastewater has been optimized and validated. Sample preparation was performed using Oasis MCX SPE cartridges. LC separation was performed using a Zorbax Eclipse Plus C_{18} RRHD column. The linearity of the calibration curve was between 5 ng/mL and 250 ng/mL, with determination coefficient (R^2) greater than 0.99, except for morphine. The mean recoveries of target analytes ranged from 91.6 to 112%, and the method demonstrated good inter-day repeatability (coefficient of variation, CV ranged from 2 to 19%). The limit of detection (LOD) for AM, MA, MDMA, MDA, and MOR was 0.29, 0.37, 0.86, 1.09 and 7.56 ng/mL, respectively. The method was applied to municipal wastewater samples collected from sewage treatment plants in Kuantan, Pahang, in which AM, MA and MDA were detected in all 3 samples.

Keywords: amphetamine, illicit drugs, LC-MS/MS, methamphetamine, municipal wastewater

Abstrak

Kaedah analisis berdasarkan spektrometri jisim kromatografi-tandem cecair (LC-MS/MS) untuk penentuan lima dadah berbahaya, iaitu amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4methylenedioxyamphetamine (MDA), dan morfin (MOR) dalam air sisa perbandaran telah dioptimumkan dan disahkan.

Penyediaan sampel dilakukan menggunakan kartrij SPE Oasis MCX. Pemisahan LC dilakukan dengan menggunakan turus Zorbax Eclipse Plus C_{18} RRHD. Kelinearan keluk tentukuran bagi semua analit sasaran berada di antara 5 ng/mL dan 250 ng/mL, kecuali morfin. Putara pemulihan analit sasaran berjulat antara 91.6 hingga 112%, dan kaedah ini menunjukkan kebolehulangan antara hari yang baik (pekali variasi berkisar antara 2 hingga 19%). Had pengesanan (LOD) bagi AM, MA, MDMA, MDA dan MOR adalah 0.29, 0.37, 0.86, 1.09 and 7.56 ng/mL. Kaedah ini digunakan untuk sampel air sisa perbandaran yang dikumpulkan dari loji rawatan kumbahan di Kuantan, Pahang, di mana AM, MA dan MDA telah ditemui dalam ketiga-tiga sampel.

Kata kunci: amphetamine, dadah berbahaya, LC-MS/MS, methamphetamine, air sisa perbandaran

Introduction

Drug abuse has been identified as a serious public health issue in Malaysia. Despite strict drug trafficking and possession laws, the Malaysian National Anti-drug Agency (AADK) reported 128,325 drug users and addicts in 2020, with Amphetamine-Type Stimulants (ATS) recorded the highest consumption (65.2%), followed by Opiates (30.9%) and Cannabis (2.7%) [1]. These official statistics, which are compiled from drug sweep operations conducted by The Royal Malaysia Police (PDRM) in cooperation with AADK, numerous ministries and governmental agencies as well as the Private Drug Rehabilitation Centers (PDRC), can provide a valuable overall picture of drug use in this country. However, since no extensive surveillance test is done, the actual consumption rates and drug use prevalence at the community, district or national level may be underestimated because these official figures are derived directly from consumers. Administered drugs are excreted as either unchanged parent drugs, conjugated forms or metabolites, flushed down the drain and eventually reach sewage treatment plants (STPs) [2]. By collecting STPs influent samples and quantifying the concentration of drug residues or metabolites in the samples, the population-normalized total consumption of a given drug within the population of a sewage treatment service area could be estimated. This wastewater-based epidemiology (WBE) method has been widely used to objectively monitor population-level drug usage, notably in the Western countries [3-6]. In a recent study conducted in Kuala Lumpur, Malaysia, 14 illicit drug residues with concentrations up to 1640 ng/L were found in municipal sewage samples [7]. This finding suggests that WBE could be used as a complementary monitoring approach in our country to estimate drug consumption.

Current analytical methods for quantifying illicit drugs in sewage commonly include a sample concentration step, such as solid-phase extraction (SPE) with various sorbent materials, followed by instrumental analysis with liquid chromatography-tandem mass spectrometry (LS-MS/MS) [8]. Direct injection and/or large-volume injection to LC-MS/MS (without pre-concentration) are also reported in the literature [9]. Before applying an analytical method to real samples, it is essential to determine whether it is suitable for the intended use. Therefore, in this study, an analytical method for the determination of ATS drugs, namely AM, MA, MDMA and MDA, as well as MOR (one type of opiates drugs) in sewage based on SPE and LC-MS/MS was validated. The method was tested on sewage samples taken from STPs in the Kuantan area to check if it was sensitive enough to detect these substances. The validated method will be used in the future to measure the usage of these drugs by utilizing the WBE approach.

Materials and Methods

Reagents and materials

The reference standards for the target compounds (AM, MA, MDMA, MDA and MOR) (Figure 1) as well as the deuterated internal reference standards used (AM-d_6, MA-d_5, MDMA-d_5, MDA-d_5 and MOR-d_3) were purchased from Lipomed AG (Switzerland) at concentrations of 1 mg/mL and 0.1 mg/mL in methanol (MeOH), respectively. Working solutions with concentrations ranging from 5 ng/mL to 250 ng/mL were prepared in MeOH. LC-grade MeOH and acetonitrile were purchased from Merck (Germany). Analytical grade ammonium hydroxide (NH_4OH) and hydrochloric acid (HCl) were obtained from Merck (Germany). Formic acid (Optima LC/MS) was obtained from Fisher Chemical (UK). Oasis MCX (60 mg, 3 mL) SPE cartridges were purchased from Waters Corporation (Milford, MA, USA). Glass fiber filters (0.7 µm) were obtained from Whatman (UK).

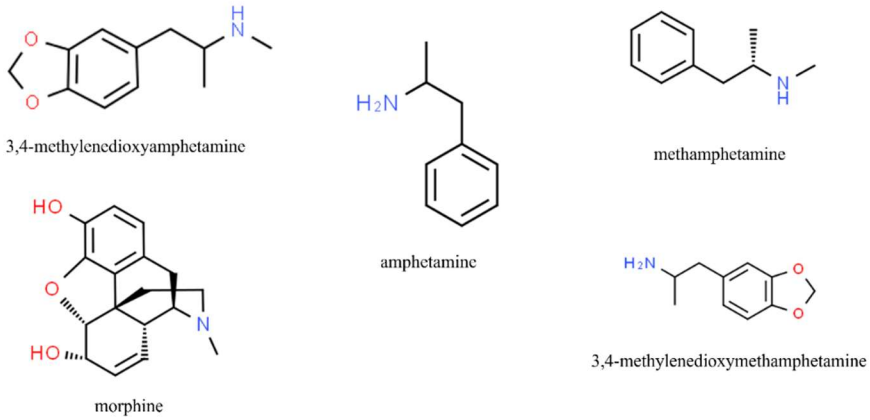

Figure 1. Chemical structures of the investigated of illicit drugs

Sample preparation, extraction, and sampling

A spiking test was used to validate the sample preparation and extraction method used by Kinyua et al. [10]. In brief, 500 mL of deionized (DI) water was spiked with reference standards (both native and deuterated internal standards at three concentration levels: 12.5, 50 and 100 ng/mL) and then analyzed to determine the extraction recovery, inter-day, and intra-day repeatability. For MOR, only 2 levels of spiking (50 and 100 ng/mL) were performed due to its high limit of detection (LOD). The samples were loaded onto Oasis MCX SPE cartridges preconditioned with 6 mL of methanol (MeOH), 4 mL DI water, and 4 mL of DI water at pH 2. After that, the cartridges were washed with 3 mL DI water before drying. The target analytes were eluted with 2 mL MeOH and 2 mL 5% NH_4OH in MeOH, evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 100 µL of DI water containing 5% of acetonitrile and 0.1% formic acid. For each batch of extraction, one procedural blank (solvent blank spiked with internal standards) was included.

Three sewage samples were taken on August 10th, 17th and 24th, 2021, respectively, at the inlet of three STPs in Kuantan (designated STP-A, STP-B and STP-C). One 24-hour composite influent sample was collected at each station using a Watson-Marlow qdos 20 peristaltic pump at a flowrate of 5 mL/min. The samples were brought to pH 2 by adding 2M HCl, and they were then kept at -20 °C until analysis. Prior to extraction, the sewage samples were thawed and filtered through a 0.7 µm glass fiber filter to remove solid particles. For each sample, a 500-mL aliquot of the filtrates was spiked with internal standards at a concentration of 50 ng/mL and subjected to the previously described SPE procedure.

LC-MS/MS analysis

An Agilent 6410B Triple Quadrupole LC-MS/MS with an electrospray ionization (ESI) technique was used to detect and quantify target analytes. LC-MS/MS method used by Lai et al. [11] was adopted and optimized accordingly. In short, the chromatographic separation was performed using a Zorbax Eclipse Plus C18 RRHD column (2.1 × 50mm, 1.8µm). A mobile phase composed of A: 0.1% formic acid in water, and B: 0.1% formic acid in acetonitrile, was applied at a flow rate of 0.35 mL/min. The gradient was as follows: 0-2 min: 92% A, 2-2.1 min: 75% A, 2.1-3 min: 100% B. The total run time, which included column equilibration, was 5 minutes. The column and autosampler temperatures were set to 45°C and 4°C, respectively. The injection volume was set to 1 µL. Other MS parameters included gas temperature of 300°C, gas flow of 11 L/min, nebulizer pressure of 45 psi, and capillary voltage of 4000 V. Multiple reaction monitoring (MRM) was used to quantify the analytes. The mass spectrometer compound dependent parameters are listed in Table 1.

Table 1. LCMS/MS parameters for target drugs for MRM acquisition in positive mode

Drugs	Parent Ion (m/z)	Fragmentor Voltage (V)	Dwell (sec)	Collision Energy (V)	Product Ion (m/z)	Retention Time (min)
AM	136.1	60	160	23, 13	91, 119	1.18
MA	150.1	70	160	29, 17	91, 119	1.33
MDA	180.1	60	160	15, 33	163.1, 105	1.30
MDMA	194.1	80	120	17, 35	163, 105.1	1.40
MOR	286.1	150	160	80, 80	152, 128	0.55

Results and Discussion

LC optimization

During LC optimization, one mix native standard solutions (containing all targeted native compounds at a concentration of 500 ng/mL) and one mix internal standards (IS, deuterated standards at 500 ng/mL) were prepared and analyzed. A longer LC column (Zorbax RRHD Eclipse Plus C18, 2.1 × 150mm, 1.8µm) was initially tested with the LC method used by Lai et al. [11]; however, not all of the target compounds were well retained. A shorter column with the same stationary phase (2.1 × 50mm, 1.8µm) was then chosen due to its good retention and separation capacity for the target compounds. Different injection volumes (1 and 3 µL) were tested. Results showed that injection volume of 1 µL gave the best peak shape for the compounds compared to 3 µL (Figure 2). As all the target compounds eluted within 0.2 to 2 minutes, the gradient of the mobile phase was then modified to shorten the total run time to 5 minutes. An example of a chromatogram of a spiked sample is shown in Figure 3.

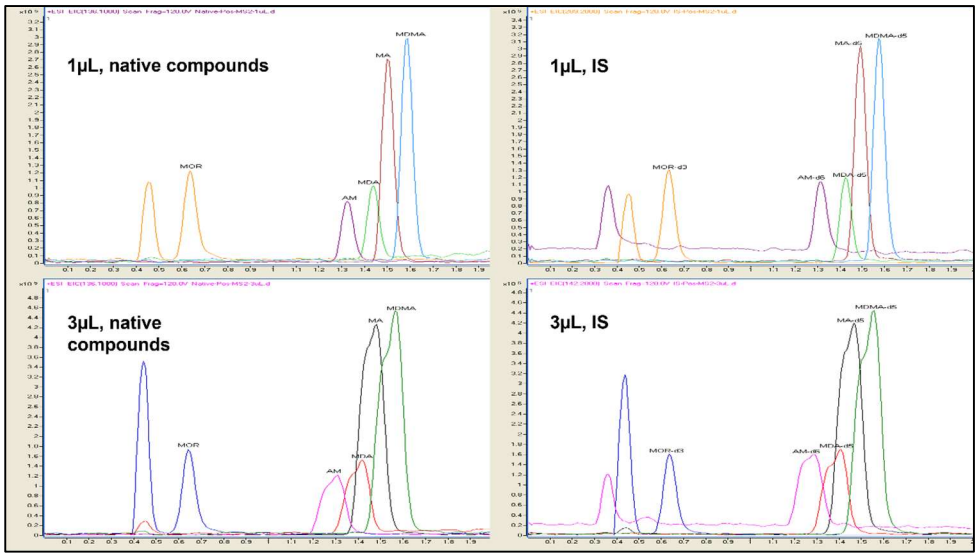

Figure 2. Chromatograms showing the peak shapes for different injection volumes

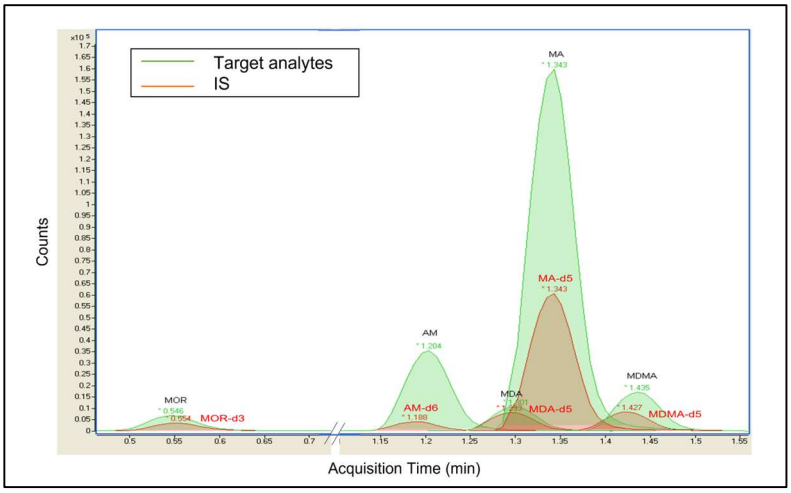

Figure 3. Typical LC-MS/MS chromatogram of a spiked sample

Method validation

The analytical method was validated in terms of linearity, recoveries, inter- and intra-day repeatability, and lower limit of quantification (LLOQ) according to the Bioanalytical Method Validation guidelines provided by the European Medicines Agency (EMEA) [12]. The method validation results were summarized in Table 2 and Table 3. The calibration curves were linear between 1 ng/mL and 250 ng/mL for AM and MA, 5 – 250 ng/mL for MDMA and MDA, and 10 – 250 ng/mL for MOR. The determination coefficients (R^2) for all calibration curves were higher than 0.99, except for MOR. The LOD and LLOQ were calculated based on the lowest concentration of standards with signal-to-noise ratio of 3 and 10, respectively. The mean recovery of spiked standards ranged between 91.6 and 112%, with the coefficient of variation (CV) between 2 and 19%, which was within the acceptable criteria of the EMEA guidelines. Target illicit drugs in all the procedural blanks were below LOD in all batches of extractions.

Table 2. Linear ranges, detection limits and calibration equations for the determination of illicit drugs

Drugs	Linear Range (ng/mL)	LOD (ng/mL)	LLOQ (ng/mL)	Calibration Equation	R^2
AM	1-250	0.29	0.95	y = 10.962427x -0.144109	0.9999
MA	1-250	0.37	1.22	y = 1.773839x + 0.029082	0.9999
MDA	5-250	0.86	2.86	y = 0.925804x + 0.026275	0.9999
MDMA	5-250	1.09	3.64	y = 1.361590x -0.021144	0.9997
MOR	10-250	7.56	25.2	y = 0.949434x + 0.031253	0.9494

Table 3. SPE-LCMS/MS method intra-day and inter-day precision at three spiking concentrations (low = 12.5 ng/mL, medium = 50 ng/mL, high = 100 ng/mL)

Drugs	Intra-day (n=3, 1 day) (mean ±SD) (CV) (%)			Inter-day (n=9, 3 days) (mean ±SD) (CV) (%)		
	Low	Medium	High	Low	Medium	High
AM	99.7±9.10 (9)	100 ±9.97 (10)	99.3±10.6 (11)	91.6±14.0 (15)	97.3 ±13.0 (13)	97.9±9.07 (9)
MA	97.5±17.5 (18)	111 ±6.27 (6)	107±11.1 (10)	98.3±15.1 (15)	106 ±13.0 (12)	105±14.7 (14)
MDA	101±18.8 (19)	97.6 ±5.62 (6)	96.3±12.2 (13)	94.1±18.1 (19)	109 ±10.2 (9)	108±15.8 (15)
MDMA	93.3±9.26 (8)	112 ±9.87 (9)	107±15.3 (14)	106±7.84 (9)	95.5 ±7.68 (8)	107±12.6 (12)
MOR	*	102 ±2.29 (2)	93.6±14.0 (15)	*	99.9 ±5.39 (5)	103±15.6 (15)

*: No data as spiking test for MOR was performed on 2 levels (50 and 100 ng/mL)

Sewage samples

The validated procedure was applied to 3 sewage samples collected from STPs in Kuantan. Results showed that AM, MA, and MDA were detected in all samples at concentrations ranging from 5.27 to 82.2 ng/L (Table 3). MDMA and MOR were below LOD in all sewage samples. This finding is consistent with the AADK official numbers, which show that ATS including AM, MA, MDMA and MDA, are the most commonly used illicit drugs in Malaysia [1]. The absence of MDMA in sewage samples could be explained by MDMA degradation in sewage [13-14]. The levels of AM, MA, MDA detected in this study were within the range of that reported by Du et al. [7] in 2 STPs at Kuala Lumpur. The absence of MOR in this study could indicate a different drug use patterns in different communities. However, due to the limited number of samples, the findings in this preliminary study require further research involving more communities.

Table 3. Concentrations (ng/L) of the target analytes in influent sewage from STPs in Kuantan

STP	Date of sampling	AM	MA	MDMA	MDA	MOR
STP-A	10/8/2021	5.28	82.2	ND	7.21	ND
STP-B	17/8/2021	10.1	423	ND	11.6	ND
STP-C	24/8/2021	11.7	443	ND	20.0	ND

ND: Below LOD

Conclusion

In this study, an analytical method based on SPE–LC-MS/MS was validated for the detection of five illicit drugs (AM, MA, MDA, MDMA and MOR) in municipal wastewater. Results from spiking test indicated satisfactory recoveries and good inter- and intraday repeatability. The validation demonstrates that the established analytical method is applicable for detecting the presence of illegal substances in STPs by providing good LODs, linearities, and recoveries. In the future, this analytical procedure will be applied in a large monitoring program in Kuantan, which aims at calculating the drug consumption from their concentrations in sewage.

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