

# Comparison of Two Extraction Set-ups for the Determination of Illicit Drugs from Wastewater

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**ABSTRACT Objective:** Wastewater-based epidemiology is becoming a widespread technique with the ability to fill the gaps of conventional methods such as general population surveys, drug related deaths, seizure data. Studies on analytical techniques to identify consumption rates are more of a focus of interest than extraction methods even though sample extraction techniques have a noteworthy contribution to analysis results as well. The aim of this study was to compare two vacuum pressure manifold (negative and positive) systems in off-line solid-phase extraction (SPE) method in terms of extraction efficiency in wastewater for morphine (MOR), 6-monoacetylmorphine (6-MAM), amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxyamphetamine (MDMA), benzoylecgonine (BE), cocaine (COC), 11-nor- $\Delta^9$ -THC carboxylic acid (THC-COOH). **Material and Methods:** In this study, two different extraction set-ups were used to determine illicit drugs from wastewater and analysis were carried out by using Liquid Chromatography-Mass Spectrometry. **Results:** Correlation coefficients for all substances were found as  $r > 0.999$ , LOD values were between 0.01-0.2 ng mL<sup>-1</sup>. Linear ranges of 6-MAM, METH, BE and COC were found between 0.2-100 ng mL<sup>-1</sup>, where the others' lowest calibration points were varying from 0.5 to 2 ng mL<sup>-1</sup>. Optimized SPE procedure was applied to both negative pressure manifold (NPM-SPE) and positive pressure manifold (PPM-SPE) by spiking tap water and wastewater samples separately. **Conclusion:** Efficiency differences were tested in terms of recovery, sample loading, time consumption, pressure control ability, and contamination sources. Overall recovery results revealed that there was no significant difference between PPM and NPM-SPE processes for both tap water and wastewater. Although set-ups have different superiorities to one another, it has been determined that the selection of the system should be made according to the type of targeted analytes and matrices.

**Keywords:** Sewage; forensic toxicology; solid phase extraction; waste water

**ÖZET Amaç:** Atık su-tabanlı epidemiyolojik çalışmalar; anket araştırmaları, yasadışı madde kullanımına bağlı ölümler, ele geçirme verileri gibi geleneksel yöntemlere destek olan bir araçtır. Çekitleme tekniklerinin analiz sonuçlarına önemli katkı sağladığı bilirse de, tüketim oranlarını tayin etmek için kullanılan analitik teknikler çekitleme yöntemlerine göre daha çok ilgi odağı olmaktadır. Bu çalışmanın amacı, morfin (MOR), 6-monoasetilmorfin (6-MAM), amfetamin (AMP), metamfetamin (METH), 3,4-metilendioksümetamfetamin (MDMA), benzoilekgonin (BE), kokain (COC) ve 11-nor- $\Delta^9$ -THC-karboksilik asit (THC-COOH) maddelerinin atık suda çekitleme verimi açısından, negatif ve pozitif basınçlı vakum sistemlerinin kullanıldığı manuel katı faz çekitleme (SPE) yöntemlerinin karşılaştırılmasıdır. **Gereç ve Yöntemler:** Bu çalışmada atık suda yasadışı madde tespiti için iki farklı çekitleme düzeneği kullanılmış ve tüm analizler Sıvı Kromatografi-Ardışık Kütle Spektrometresi sistemi kullanılarak yapılmıştır. **Bulgular:** Tüm maddeler için korelasyon katsayıları  $r > 0.999$ , LOD değerleri 0,01-0,2 ng mL<sup>-1</sup> arasında bulunmuştur. 6-MAM, METH, BE ve COC'nin doğrusal aralıkları 0,2-100 ng mL<sup>-1</sup> arasında bulunurken, diğer kalibrasyon noktalarının en düşükleri ise 0,5-2 ng mL<sup>-1</sup> arasında değişmektedir. Belirlenen SPE prosedürü, musluk suyu ve atık su numuneleri kirletilerek hem negatif basınç (NPM-SPE) hem de pozitif basınç (PPM-SPE) kullanılan düzeneklerde uygulanmıştır. **Sonuç:** Düzenekler arasındaki verim farklılıkları, geri kazanım, numune yükleme, zaman tüketimi, basınç kontrol yeteneği ve kontaminasyon kaynakları bakımından değerlendirilmiştir. Tüm geri-kazanım sonuçlarına bakıldığında, hem musluk suyu hem de atık su için, PPM ve NPM-SPE arasında anlamlı bir fark olmadığı bulunmuştur. Her ne kadar basınç düzeneklerinin birbirine göre üstünlükleri olsa da, sistemin seçiminin, hedeflenen analiz ve matrikslerin türüne göre yapılması gerektiği düşünülmektedir.

**Anahtar Kelimeler:** Lağım; sıvı atık; adli toksikoloji; solid faz çıkartımı; atık su

The number of abused illicit drug substances and users have been increasing day by day in all over the World. This creates a worldwide problem including public health and safety issues in every region including Turkey.<sup>1-3</sup> The latest country report released from European Center of Drugs and Drug Addiction (EMCDDA) has revealed that the number of drug related offences and overdose deaths have been raising 11.2% and 55.9 %, respectively as compare to previous year.<sup>4,5</sup> Beside these data, there are several key epidemiological indicators such as general population surveys, seizures, drug prevalence etc., however, overall interpretation of drug consumption is more complicated than adding up these values together.<sup>6,7</sup> To reach more near real-time and evidence-based results, scientists from different backgrounds have been making efforts on developing supportive tools for monitoring consumption and drug trends. Wastewater-based epidemiology (WBE) is becoming a widespread technique increasingly with the ability to fill the gaps of conventional methods. The phenomena relies on the idea that all the residues including illicit drug metabolites end up in the sewage network after individual consumptions.<sup>7,8</sup>

Whilst, WBE technique has been developing, new analytical methods to detect targeted residues collaborating with the technique have been emerging as well. Analysis have started by using High Pressure Liquid Chromatography (HPLC) and nowadays Hybrid Quadrupole-Time-of-Flight-Mass Spectrometer (QTOF-MS) and Liquid Chromatography-Linear Ion Trap FT Orbitrap Mass Spectrometer (LC-LTQ FT Orbitrap MS), techniques have also been using.<sup>9,10</sup> On the other hand, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is the most preferred instrument for the analysis of illicit drugs from wastewater. Studies on analytical techniques are more of a focus of interest than extraction methods although sample extraction techniques have significant contribution to the analysis results as well.<sup>11</sup> The reasons behind performing extraction techniques prior to analysis are mainly to concentrate analytes, obtain better detection limits, avoid interference effects and separate targeted analytes from biological ma-

trices.<sup>12-14</sup> Due to the complex nature of wastewater samples consisting of domestic wastes, extraction generally performs to isolate matrix for increasing selectivity of targeted samples. There are couple of extraction systems that are commonly used in the field such as in-house liquid-liquid extractions (LLE), commercially ready LLEs, solid-phase extraction (SPE) cartridges, fully automated SPE systems, solid-phase micro extraction (SPME) etc.<sup>11,12</sup> LLE techniques have been the conventional method for years, however, with the developing technology SPE extraction has presented better recoveries with lower matrix effects and have become the preferred step for sample preparation mostly.<sup>11</sup> On the other hand, SPME is a competitive technique with the advantage of not using solvents and skipping concentration steps with limited usage of chromatographic instruments (GC-MS mostly), yet not many studies favor the technique instead of SPE.<sup>12</sup>

In the literature review, the most common extraction technique for WBE was found as SPE which can be either off-line or online.<sup>2,14-27</sup> Fully-automated SPE systems have advantages of using less amount of sample volumes and time saving opportunities, while manual SPE cartridges have proven to perform acceptable results as well.<sup>24</sup> Nevertheless, manual SPE provides more user-friendly applications considering frequently encountered system errors of automated SPE. This makes the manual technique more preferable and practicable. Conventionally, this technique is applied with negative pressure manifold (NPM), but technological developments have led positive pressure manifolds (PPM) to develop as well. However, there is no study published comparing these two extraction processes in terms of efficiency, time consumption and contamination, yet.

In the presented study, the aim was to compare two vacuum pressure manifold (negative and positive) systems in off-line solid-phase extraction (SPE) method in terms of extraction efficiency in wastewater for morphine (MOR), 6-monoacetylmorphine (6-MAM), amphetamine (AMP), methamphetamine (METH), 3,4 methylenedioxymethamphetamine (MDMA), benzoylecgonine (BE),

cocaine (COC), 11-nor- $\Delta^9$ -THC carboxylic acid (THC-COOH) determination.

## MATERIAL AND METHODS

### CHEMICALS AND INSTRUMENTATION

MOR, 6-MAM, AMP, METH, MDMA, BE, COC, THC-COOH and their isotope labeled internal standards (ILIS) (amphetamine- $d_6$ , methamphetamine- $d_5$ , 3,4-methylenedioxymethamphetamine- $d_5$ , benzoylecgonine- $d_3$ , cocaine- $d_3$  and 11-nor- $\Delta^9$ -THC carboxylic acid- $d_3$ ) were supplied from Lipomed (Arlesheim, Switzerland).

Oasis HLB (60 mg, 3 mL) SPE cartridges were purchased from Waters (Milford, MA, USA). Glass filters (0.7  $\mu\text{m}$ ) were purchased from Merck, Millipore (Tullagreen, Carrigtwdnil, Ireland) and 0.45  $\mu\text{m}$  membrane filters were obtained from Waters (Göttingen, Germany). Formic acid, hydrochloric acid (HCl), ammonium acetate (AmOAc) and LC-grade methanol (MeOH), ethyl acetate (EA), acetonitrile (ACN) were purchased from Merck (99:8-100%, Darmstadt, Germany). All experiments were carried out using nitrogen and argon gas with >99.999% purity (Okser, Turkey).

NPM-SPE manifold with 24 ports and PPM-SPE manifold with 48 ports (Cerex, System 48 Processor, CA, USA) were used for the extraction of the samples. Evaporation under nitrogen stream at the end of sample preparation was performed with HyperVap HV-300 from Gyrozen (Daejeon, Rep. of Korea). Ultrapure water system Direct-Q UV 3 was produced from Millipore (18.2 M $\Omega$  cm) (Molsheim, France).

Analytical steps were carried out using Shimadzu 8045 LC-MS/MS (Kyoto, Japan). The positive ion mode was selected for Electrospray Ionization (ESI). Nitrogen gas was supplied from PEAK Scientific Genius 1051 (Glasgow, Scotland). Separation was performed using Raptor Biphenyl (2.7  $\mu\text{m}$ , 100 x 2.1 mm) from Restek (Bellefonte, PA, USA) with a mobile phase (A) 2 mM AmOAc in 0.1% formic acid in ultrapure water, (B) 2 mM AmOAc in 0.1% formic acid in MeOH. The injection volume was determined at 2  $\mu\text{L}$  and the total

run time was achieved in 17 min, while flow rate was arranged as 0.4 mL min<sup>-1</sup>. The interface temperature was set to 300 °C and the oven temperature was 50 °C. Heating gas flow was set to 10 L min<sup>-1</sup> and drying gas flow was set to 10 L min<sup>-1</sup>. The gradient was started by 5% increasing mobile phase B to 95% over 8 min and held for 5 min. After that solvent B was decreased to 5% and held for 4 min for equilibrium of the column. In order to achieve maximum sensitivity, dwell time was chosen as 10.0 msec for THC-COOH and 2.0 msec for other analytes.

### SAMPLE PREPARATION AND METHOD OPTIMIZATION

Stock solutions of each reference standards (100  $\mu\text{g}$  mL<sup>-1</sup>) (MOR, 6-MAM, AMP, METH, MDMA, BE, COC, THC-COOH) were mixed in a MeOH solution at a concentration of 500 ng mL<sup>-1</sup>. Calibration solutions were prepared from the aforementioned mix solution by incremental amount as blank, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, 100 ng mL<sup>-1</sup> in triplicates to obtain linear calibration curves for each analyte including 25 ng mL<sup>-1</sup> ILIS.

For sample preparation step, related literature was followed to optimize pre-extraction conditions.<sup>28</sup> 50 mL samples spiked with 25 ng mL<sup>-1</sup> ILIS at neutral pH were filtered through 0.7  $\mu\text{m}$  glass and 0.45  $\mu\text{m}$  membrane filter, respectively. In the next step, SPE procedure was performed using Oasis HLB cartridges. Prior to loading samples, cartridges were conditioned with 2 mL EA, 2 mL MeOH, and 2 mL Milli-Q water. Then, cartridges were washed by using 2 mL 5% MeOH two times (by gravity) and centrifuged for 5 min with 5000 rpm until dryness. Analytical elution step was carried out using 2 mL MeOH and 2 mL EA. The extracts were evaporated under nitrogen at 40 °C to dryness and reconstructed to 1 mL with mixture of mobile phase A and B (1:1, v/v).

The aforementioned extraction procedure was conducted for both NPM-SPE and PPM-SPE in this study. NPM-SPE process is a conventional off-line SPE technique [Figure 1](#) which enables sample loading easier since vacuum generating from a separate pump moves underneath of cartridges. Hence, vacuum control is possible with both vacuum control



FIGURE 1: The view of SPE set-up with Negative Pressure Manifold (NPM).

switches on the pump and valves on the cartridge holder. Keeping flow rate constant and making necessary pressure changes during the process is more complicated due to the structural nature of vacuum switch. As it can be seen in the Figure 1, plastic test tubes during conditioning, sample loading and washing steps, are separately positioned under each cartridge as waste containers.

In PPM-SPE, on the other hand, the vacuum moves from upper side to the flow direction through cartridges as shown in (Figure 2). Main structural difference with NPM is that PPM has embedded vacuum control switches on its body, while in NPM, vacuum generates from a distinct unit. This provides a unique pressure control mechanism for the flow rate. In PPM, all wastes coming from different vacuum channels flow into the same container Figure 2, nevertheless, in NPM each channel needs a distinct waste to use (Figure 1).

Correlation coefficients ( $r$ ) were found by applying least square regression approach with the limit of  $>0.999$ . Limit of detection (LOD) and limits of quantitation (LOQ) were calculated considering the signal to noise ratios. Furthermore, recovery percentages and relative standard deviation (RSD%) values were estimated by spiking different levels (50, 250 and 750 ng L<sup>-1</sup>) to tap water in six replicates. Additionally, in order to observe matrix effect of influent wastewater, samples were spiked at 500 and 1000 ng L<sup>-1</sup> concentrations by six

times. Satisfactory range was established as 80-120% for recovery and 15% RSD for precision.

All LC-MS/MS analysis procedures and optimizations were performed using positive ionization mode. For the optimization step, a mixture solution consisting of all targeted compounds were prepared and analyzed three times in order to achieve multiple reaction monitoring (MRM).

## RESULTS

Analytical performance characteristics of the LC-MS/MS method were shown in Table 1 detailed. In this table, retention times (Rt) and their standard deviations (SD), linearity results such as linear range, correlation coefficients (Cor. Coef), linearity equations of each substance were presented. Correlation coefficients for all substances were achieved successfully as  $r > 0.999$ . LOD values generated from three times of signal/noise ratios were between 0.01-0.2 ng mL<sup>-1</sup>. Linear ranges of 6-MAM, METH, BE and COC were found between 0.2-100 ng mL<sup>-1</sup>, where the other's lowest



FIGURE 2: The view of SPE set-up with Positive Pressure Manifold (PPM).

**TABLE 1:** Optimization results (LOD, LOQ, linearity) of LC-MS/MS method for illicit substances.

Compound Name	LOD (ng mL <sup>-1</sup> )	LOQ (ng mL <sup>-1</sup> )	Linear Range (ng mL <sup>-1</sup> )	Equation	Rt ±SD	Cor. Coef. (r)
MOR	0.20	0.66	2-100	y=0.0019x-0.00101	2.396 ± 0.006	0.9999
6-MAM	0.10	0.33	0.2-100	y=0.00493x-0.00042	3.317 ± 0.005	0.9999
AMP	0.12	0.40	2-100	y=0.02672x+0.00988	2.857 ± 0.003	0.9998
METH	0.08	0.26	0.2-100	y=0.03967x-0.00245	3.197 ± 0.004	0.9999
MDMA	0.10	0.33	0.5-100	y=0.01938x-0.00135	3.436 ± 0.005	0.9999
BE	0.01	0.03	0.2-100	y=0.06998x-0.03402	4.228 ± 0.004	0.9999
COC	0.01	0.03	0.2-100	y=0.00701x-0.00283	4.433 ± 0.006	0.9999
THC-COOH	0.20	0.66	2-100	y=0.02633x-0.0247	8.563 ± 0.005	0.9994

LOD: Limit of detection, LOQ: Limits of quantitation, LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry.

MOR: Morphine, 6-MAM: 6-monoacetylmorphine, AMP: Amphetamine, METH: Methamphetamine, MDMA: 3,4-methylenedioxyamphetamine, BE: benzoylcgonine,

COC: Cocaine, THC-COOH: 11-nor- $\Delta^9$ -THC carboxylic acid.

calibration points were varying from 0.5 to 2 ng mL<sup>-1</sup>.

Recovery studies were performed by spiking different calibration points to both tap water (50, 250 and 750 ng L<sup>-1</sup>) and real wastewater samples (500 and 1000 ng L<sup>-1</sup>) which conducted by both pressure manifolds (PPM and NPM) with the same extraction procedure.

In Table 2, extraction efficiencies were presented regarding to tap water spikes including recoveries (%) with standard deviations and RSD% values for each concentration of targeted illicit drugs. Mean recovery (%) of all substances in tap water for 50 ng L<sup>-1</sup> in PPM-SPE was found as 104.18, while NPM-SPE was 93.56. For 250 ng L<sup>-1</sup> spike concentration, average recoveries of positive and negative SPE manifolds were determined as 101.57 and 92.26 %, respectively. In PPM-SPE 96.58% mean recovery was obtained and in NPM-SPE 98.93 % mean recovery was acquired for 750 ng L<sup>-1</sup> concentration point in spiked tap water samples.

In the case of wastewater spikes, 100.29 and 98.4% mean recoveries were obtained for 500 ng L<sup>-1</sup> spikes in PPM and NPM systems respectively. Finally, for 1000 ng L<sup>-1</sup> concentration, 98.98% was found as mean recovery for positive manifold and 96.67% was calculated for negative. Aforementioned results can be found in Table 3 detailed.

For both tap water and wastewater spikes at each concentration level, all substances except

**TABLE 2:** Extraction efficiency comparisons of PPM-SPE and NPM-SPE for spiked tap water samples (50, 250 and 750 ng L<sup>-1</sup>).

Compound Name	PPM-SPE		NPM-SPE	
	Rec% (SD)	RSD%	Rec% (SD)	RSD%
<b>50 ng L<sup>-1</sup> (n=6)</b>				
MOR	119.64 (0.61)	13.59	116.28 (0.18)	5.27
6-MAM	117.04 (0.23)	6.46	112.76 (0.21)	5.834
AMP	99.36 (0.28)	11.14	87.91 (0.25)	11.53
METH	87.72 (0.41)	18.70	90.94 (0.16)	7.08
MDMA	103.53 (0.16)	6.32	81.49 (0.10)	4.90
BE	104.45 (0.22)	8.53	92.38 (0.17)	7.24
COC	99.86 (0.21)	8.33	78.13 (0.13)	6.60
THC-COOH	101.82 (0.48)	18.75	88.55 (0.25)	11.08
<b>250 ng L<sup>-1</sup> (n=6)</b>				
MOR	118.49 (0.93)	9.46	115.17 (2.25)	9.33
6-MAM	116.32 (1.24)	8.63	105.46 (2.69)	11.69
AMP	94.76 (0.31)	2.65	81.58 (0.63)	6.22
METH	92.34 (0.45)	3.87	75.31 (0.29)	3.08
MDMA	98.19 (0.83)	6.76	82.44 (0.23)	2.19
BE	106.27 (0.69)	5.19	88.44 (0.48)	4.37
COC	88.36 (0.25)	2.21	83.88 (0.26)	2.53
THC-COOH	97.79 (0.97)	7.96	105.78 (0.81)	6.11
<b>750 ng L<sup>-1</sup> (n=6)</b>				
MOR	110.88 (3.34)	4.29	110.09 (2.03)	2.82
6-MAM	109.38 (3.21)	4.88	109.30 (2.55)	2.46
AMP	90.94 (1.39)	4.07	90.77 (1.69)	4.95
METH	89.04 (0.78)	2.32	98.38 (3.42)	9.27
MDMA	94.59 (1.19)	3.37	93.58 (1.46)	4.15
BE	103.46 (0.96)	2.47	96.68 (0.69)	1.89
COC	85.22 (0.60)	1.88	92.50 (1.58)	4.55
THC-COOH	89.08 (3.28)	9.83	100.14 (4.47)	11.90

PPM-SPE: Positive pressure manifold, NPM-SPE: Negative pressure manifold,

RSD: Relative standard deviation.

MOR: Morphine, 6-MAM: 6-monoacetylmorphine, AMP: Amphetamine, METH: Methamphetamine, MDMA: 3,4-methylenedioxyamphetamine, BE: benzoylcgonine, COC: Cocaine, THC-COOH: 11-nor- $\Delta^9$ -THC carboxylic acid.

**TABLE 3:** Extraction efficiency comparisons of PPM-SPE and NPM-SPE for spiked wastewater samples (500 and 1000 ng L<sup>-1</sup>).

Compound Name	PPM-SPE		NPM-SPE	
	Rec% (SD)	RSD%	Rec% (SD)	RSD%
500 ng L <sup>-1</sup> (n=6)				
MOR	116.84 (5.93)	12.67	108.56 (5.41)	12.05
6-MAM	119.65 (3.00)	6.51	115.22 (4.88)	11.81
AMP	90.94 (1.08)	4.73	94.38 (1.85)	7.85
METH	89.72 (0.57)	2.54	88.42 (1.16)	5.27
MDMA	94.09 (1.20)	5.11	94.83 (0.71)	3.00
BE	103.37 (0.84)	3.27	100.01 (0.75)	2.99
COC	88.29 (0.34)	1.56	95.11 (0.85)	3.56
THC-COOH	99.44 (2.35)	9.47	90.70 (2.66)	11.72
1000 ng L <sup>-1</sup> (n=6)				
MOR	112.29 (4.98)	5.56	108.00 (3.62)	4.42
6-MAM	110.22 (6.63)	8.24	107.92 (3.71)	4.16
AMP	88.64 (1.54)	3.47	92.11 (1.72)	3.73
METH	92.10 (0.68)	1.47	80.79 (2.93)	7.24
MDMA	96.91 (0.72)	1.49	89.45 (1.24)	2.78
BE	87.05 (0.82)	1.88	95.01 (1.46)	3.06
COC	104.92 (0.38)	0.73	99.16 (1.18)	2.39
THC-COOH	99.69 (6.82)	13.69	100.94 (4.52)	8.95

PPM-SPE: Positive pressure manifold, NPM-SPE: Negative pressure manifold, RSD: Relative standard deviation.

MOR: Morphine, 6-MAM: 6-monoacetylmorphine, AMP: Amphetamine, METH: Methamphetamine, MDMA: 3,4-methylenedioxyamphetamine, BE: benzoylecgonine, COC: Cocaine, THC-COOH: 11-nor- $\Delta^9$ -THC carboxylic acid.

METH have RSD under 15% in PPM and NPM. Additionally, for both vacuum manifolds' recoveries were within the acceptable range of 80-120%, while RSD% of cocaine at 50 ng L<sup>-1</sup> and methamphetamine at 250 ng L<sup>-1</sup> were relatively below.

## DISCUSSION

Considering optimization parameters, all correlation coefficients were equal or higher than 0.9998, while THC-COOH found as 0.9994. The possible reasons behind THC-COOH has the lowest correlation coefficient ( $r$ ) value could be explained by the preference of analytical column and/or ionization mode. There are special efforts for the analytical improvement of THC-COOH by using negative ionization mode in MS/MS parameters, different analytical column types or extraction procedures.<sup>29-31</sup> In this manner, although most of prominent studies prefer distinct analytical methods to obtain better THC-COOH outcomes, with this presented study a simultaneous determination

method using the same extraction and analysis process for all illicit drugs was developed.

The comparison of two SPE processes was performed considering different parameters. Application efficiencies for both PPM-SPE and NPM-SPE processes were tested in terms of parameters such as recovery, sample loading, time consumption, pressure control ability, and contamination sources.

Overall recovery results revealed that there was no significant difference between PPM and NPM-SPE processes according to *Student's t-test* ( $p>0,05$ ) for both tap water and wastewater. Although wastewater is more complicated matrix than tap water, neither in recovery values nor in repeatability significant differences were observed. These findings also prove that optimized extraction method was efficient, precise and accurate apart from the matrix effect and pressure vacuum manifold preference. Beside the pressure vacuum manifold effects, sorbent suitability, elution solvent choice, evapora-

tion temperature or other instrumental parameters may also influence the recovery.<sup>20,24</sup>

In the case of sample loading evaluation, this step was found less efficient in PPM-SPE set-up due to its structural difference. For each loading cycle, since vacuum media should be provided under closed circuit, analysts should load sample and push the manifold for vacuum (Figure 2). This makes sample loading step time consuming for PPM in the case of sample volume is much higher than SPE cartridges' volume. On the other hand, in NPM-SPE, since the top of cartridges are open, this allows to add sample continuously during the extraction.

As for pressure control, this parameter was a little bit challenging for NPM, since negative manifold equipped with a separate pump to control vacuum, while PPM was embedded vacuum control switches on its body (Figure 1, Figure 2). That's why PPM-SPE set-up has the ability to control flow rate more effectively compare to NPM-SPE apparatus. This is crucial especially in the sample loading process since sorbent in the cartridge needs adequate time and volume to absorb analytes in sample and if this step occurs too fast this directly affects efficiency of the extraction undesirably.<sup>24</sup>

Contamination was another factor to determine efficiency of techniques. For PPM-SPE, no contamination sources were determined originating from its structural adjustment however, cartridge holder on the NPM-SPE was determined as

**TABLE 4:** Success rates of PPM and NPM-SPE processes applied in this study.

Parameters	PPM-SPE	NPM-SPE
Recovery%	✓ ✓ ✓	✓ ✓ ✓
Sample loading	✓	✓ ✓ ✓
Time consumption	✓ ✓	✓ ✓ ✓
Pressure control	✓ ✓ ✓	✓ ✓
Contamination risk	✓ ✓ ✓	✓

PPM-SPE: Positive pressure manifold, NPM-SPE: Negative pressure manifold.  
Success rates: ✓; Low: ✓ ✓; Medium: ✓ ✓ ✓; High.

a possible contamination source for the technique (Figure 1).

In Table 4, success rates of two set-ups were considered for this study. When comparing two extraction processes, both were efficient and satisfactory in the case of recovery and repeatability. However, in sample loading step, PPM-SPE was considered as time consuming because of high sample volume (50 mL). Another remarkable thing was sensitivity of pressure control which PPM was found user-friendly by means of embedded vacuum control panel.

Although, to the best of our knowledge, the most of off-line SPE studies in the literature were performed by negative vacuum pressure manifold system, in Table 5 both pressure manifold systems were compared with literature in terms of obtained recovery values (%).

Even though in the literature MOR illuminates a broad range of recovery percentages, the

**TABLE 5:** Comparison of average recoveries (%) from spiked wastewater samples.

Reference numbers	MOR	6-MAM	AMP	METH	MDMA	BE	COC	THC-COOH
17	102.0	103.5	95.5	95.5	102.0	114.0	106.5	113.0
31	79.0	-	-	-	-	-	-	66.5
3	101.0	-	-	-	-	-	-	61.0
21	29-83	85-90	70-96	80-96	84-125	40-100	86-121	67-96
33	-	-	-	-	-	-	-	88.0
14	100.0	106.0	85.0	87.0	94.0	96.0	93.0	43.0
25	77.0	118.0	94.0	114.0	121.0	115.0	173.0	-
<i>This study NPM</i>	108.3	111.5	93.2	84.6	92.1	97.5	97.1	95.8
<i>This study PPM</i>	114.6	114.9	89.8	90.9	95.5	95.3	96.6	99.6

MOR: Morphine, 6-MAM: 6-monoacetylmorphine, AMP: Amphetamine, METH: Methamphetamine, MDMA: 3,4-methylenedioxyamphetamine, BE: benzoylecgonine, COC: Cocaine, THC-COOH: 11-nor- $\Delta^9$ -THC carboxylic acid.

presented study for both extraction processes were found close to ideal. For 6-MAM, literature indicates similar recovery results ranging from 85 to 118% including our study as well. As for amphetamine type stimulants (AMP, METH and MDMA), comparable recovery percentages were observed within acceptable values. Only one comparative research was calculated diverse recovery results for BE, since several extraction procedures were applied.<sup>20</sup> Besides, BE results were found reasonable in the presented study. An incompatible recovery was detected among other studies listed in the table for COC, while both PPM and NPM were showed satisfactory results in our study.<sup>24</sup> Finally, THC-COOH efficiencies were varied from 43-113% in the literature, in connection with above mentioned reasons.<sup>29,30</sup>

## CONCLUSION

Solid phase extraction is the most preferred method for sample preparation process especially in forensic and environmental toxicology. Variable techniques such as on-line and off-line mechanisms are available for SPE, while in off-line SPE, two different vacuum directions exist as positive and negative vacuum pressure manifolds. In this study, a comparison of negative and positive vacuum pressure manifold processes was conducted as off-line solid phase extraction for illicit drug determination from wastewater samples. According to overall evaluation of sample loading parameter, NPM was found preferable as compare to PPM with regards to time consumption during large sample volume loading. When examining for other parameters of these two processes, PPM has more advantages with sensitive vacuum control panel and decreased

contamination sources. As a conclusion, both set-ups can be preferred depending on targeted analytes and matrixes which will be examined. The choice of extraction procedure and related set-ups should overall be considered regarding to context of the research.

**Informing:** Due to the presence of the name of the journal editor's among the authors, the assessment process of the study was conducted by the guest editor.

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### Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

### Authorship Contributions

**Idea /Concept:** Faruk Aşıcıoğlu, Selda Mercan; **Design:** Selda Mercan, Zeynep Türkmen, A. Özgür Doğru; **Supervision / Consultancy:** Müvver Açıkkol, Faruk Aşıcıoğlu; **Data Collection and /or Processing:** Tuğba Tekin, Ayşe Nur Akkuş Safran, Selda Mercan; **Analysis and / or Interpretation:** Tuğba Tekin, Merve Kuluoğlu, Selda Mercan, Zeynep Türkmen; **Source:** Selda Mercan, Zeynep Turkmen, Merve Kuluoglu; **Article Writing:** Selda Mercan, Tuğba Tekin, Merve Kuluoglu, Zeynep Turkmen; **Critical Review:** Müvver Açıkkol, Faruk Aşıcıoğlu, Özgür Doğru; **Resources and Funding:** Selda Mercan, Tuğba Tekin, Ayşe Nur Akkus Safran, Merve Kuluoglu.

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