Extractions of Illicit Drugs from Wastewater using Empore™ Syringe-type Membrane SPE Cartridge

Application Note
Environmental

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Abstract
This application note demonstrates the performance of the CDS Empore™ mixed-phase cation (MPC) 25mm EZ-Disk to extract 10 different species of illicit drugs from wastewater. Water samples were processed with the assistance of the EZ-Trace SPE workstation. The recovery is reported for all 10 illicit drugs for this method and demonstrates that the Empore™ MPC EZ-Disk is effective for extraction of polar molecules, such as illicit drugs, from wastewater.

Introduction
Illicit drugs and their metabolites are frequently detected in wastewater. The type of illicit drugs that are detected, along with their measured concentrations, often provides insight into drug use and trafficking patterns within a region.[1] Additionally, the presence of illicit drugs in waterways has the potential of causing adverse health effects on aquatic life. Furthermore, by leaching into soils, farm animals and humans are at greater risk of becoming negatively affected as well.[2] Consequentially, there is a need for effectively and reproducibily extracting illicit drugs from water samples.

This application note describes a novel method for reproducibily extracting illic drugs from wastewater samples with high recovery using the Empore™ mixed phase cation (MPC) syringe-type SPE cartridge, also referred to as the EZ-Disk. The EZ-Disk contains a 1.2 μm glass fiber filter, a 0.22 μm PTFE microporous filter membrane, and the Empore™ MPC SPE membrane. The diameter of all 3 is 25 mm. Recoveries and RSDs were reported for all 10 illicit drugs and compared at 3 different extractions flow rates to determine the optimum extraction conditions for this application.

Experiment Setup
SPE disk:
Solid phase extraction (SPE) was done with Empore™ mixed phase cation (MPC) 25mm EZ-Disk (CDS Analytical PN 98-0706-0102-1; Model No. 7230SD).

Extraction System:
Four extractions were performed simultaneously with the Empore™ EZ-Trace

Chemicals:
The reagents used for this application were methanol (HLPC grade), acetonitrile (HPLC grade), formic acid, 28% ammonia, and concentrated hydrochloric acid, all available from Fisher Scientific. The standard solution mix contained methamphetamine, amphetamine, morphine, O6-monoacetylmorphine, cocaine, benzoylecgonine, ketamine, non-ketamine, 3,4-methylenedioxy-methamphetamine (MDMA), and methylenedioxyamphetamine (MDA), all at 1000 ng/mL in methanol. The internal standard solution mix contained methamphetamine-d5, amphetamine-d5, morphine-d3, O6-monoacetylmorphine-d3, cocaine-d3, benzoylecgonine-d3, ketamine-d4, non-ketamine-d4, MDMA-d5, and MDA-d5, all at 1000ng/mL in methanol.

Solution Preparation
Both the standard solution mix and the internal standard mix were diluted to 25 ng/mL with 0.1% formic acid in water.

Sample Preparation
The wastewater sample was adjusted to a pH of 2 using concentrated hydrochloric acid. Prior to spiking the sample with the illicit drug mixture, the wastewater sample was filtered using a solvent filter and glass fiber membrane. A 50 mL aliquot of the wastewater sample was then obtained and spiked with 100 μL of standard illicit drug solution mix and mixed well.

Extraction and Concentration
The Empore™ MPC EZ-Disk syringe-type SPE cartridges were first conditioned with 4 mL of methanol and then washed with another 4 mL of acidified water (pH 2). Samples were loaded at a flow rate of 2 mL/min. Following sample loading, the cartridges were rinsed with 4 mL of water and then 4 mL of methanol. The cartridges were then dried under vacuum for 5 min and elution was perfomed using 2 mL of 5% NH_4OH in methanol. This procedure was then repeated using sample loading flow rates 5 and 10 mL/min.

100 μL of internal standard to the eluate and then exposed to a gently stream of nitrogen with the help of MNP automated Parallel Concentrator (Beijing Labtech Instruments Co.) until all liquid had been removed. The dried sample was then reconstituted in 250 μL of 0.1% formic acid aqueous solution. The sample was then mixed well, filtered through a microporous membrane, and then transferred to a 200 μL tube for LC-MS/MS analysis.

LC-MS/MS Analysis
Exion LC™ liquid phase system with Triple Quad™ 4500 Sciex MS

LC Parameters

Column:	Shim-pack Velox (2.7 μm, 2.1×100mm)
Column Temp:	40°C
Mobile Phase A:	0.1% formic acid
Mobile Phase B:	acetonitrile
Flow Rate:	0.3 mL/min
Injection Volume:	5 μL
Gradient Elution:	

Time	Ratio B (%)
1.0	5
6.0	5
6.2	25
8.0	100
8.2	5
10.0	5

Mass Spectrometer Parameters

Ion Source:	ESI+
Ion Source Parameters:	
IS Voltage:	5500 V
Curtain Air Pressure:	35 psi
Atomizing Gas Pressure:	55 psi
Auxiliary Gas Pressure:	60 psi
Source Temp.	40°C

Results and Discussions
The resulting LC-MS/MS chromatogram is shown in Figure 1 and the complete recovery data and RSDs (n=6) are shown in Figure 2 for all 3 sample loading flow rates. The complete data is summarized in Table 1. All 3 sample loading flow rates produced an average recovery >96% and average RSD <4%.The overall range of recoveries were between 88 and 133% while the range of RSDs were between 1 and 9%. At a smaple loading flow rate of 2 mL/min, the average recovery of 96.8% and 3.4% RSD. Flow rates of 5 and 10 mL/min produced recoveries and RSDs that were quite similar to one another. Although the differences in the recoveries and RSDs are quite minor between the 3 sample loading flow rates, higher flow rates are recommended for the prodecure to maximize effiiciency and recovery of illicit drugs.

Results and Discussions
In this experiment, the average recoveries of the 10 illicit drugs and their metabolites with the syringe-type solid phase extraction cartridge EZdisk were 88%-113%, and the relative standard deviations (RSDs) were 1%-9% (Table 1), which are satisfying for normal forensic drugs detection. The EZ-Disk requires only a small elution volume, 2 mL, to achieve this level of performance, effectively shortening the following concentration time. And when the sample loading flow rate is 10 mL/min, the RSD of six parallel experiments is still less than 10.0%. This demonstrates high reproducibility and improved efficiency provided by using the EZ-Disk. The flexibility of the EZ-Disk to different sample volumes and its universal luer lock/slip fittings make it simple to adapt the EZ-Disk to other applications.

References
1. Nefau, T.; et. al; *Sci. Tot. Environ.* **2013**
2. Deng, Y.; et. al.; *Environ. Sci.* Eur. **2020**

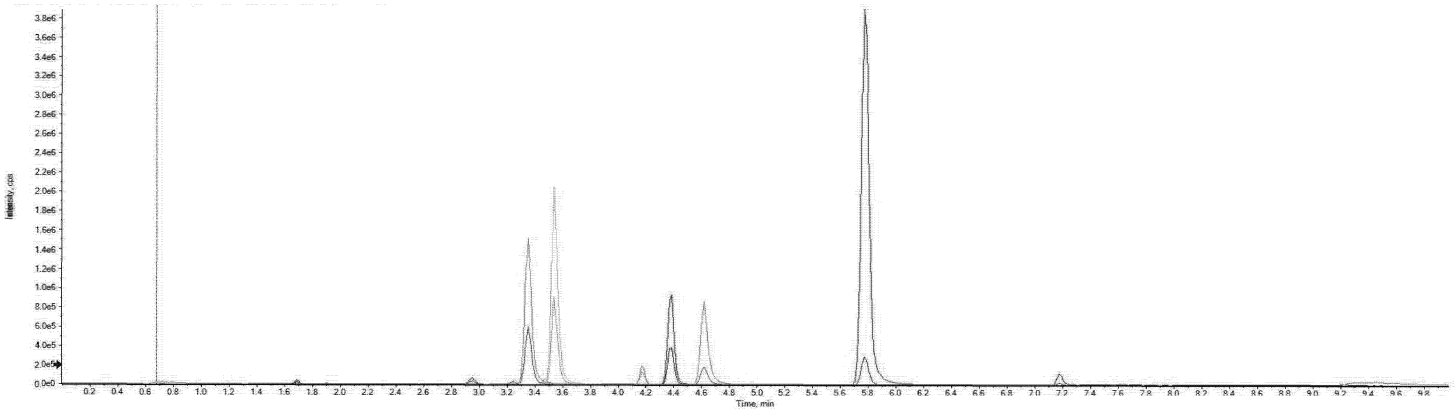

Figure 1. LC-MS/MS chromatogram of the illicit drug wastewater sample.

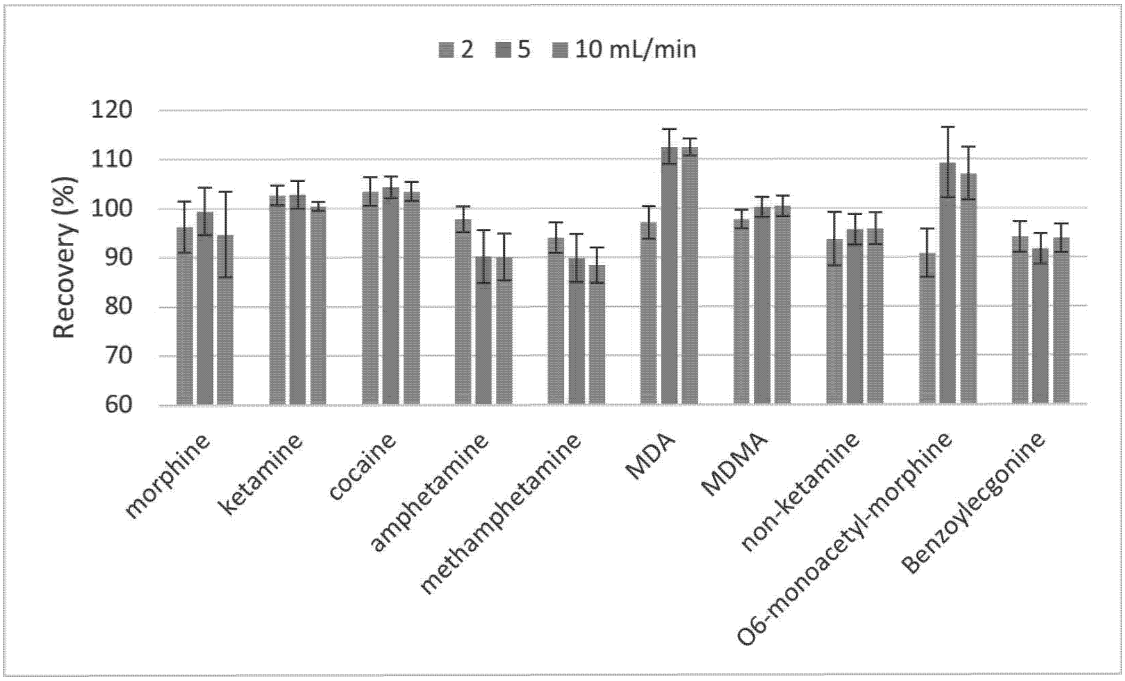

Figure 2. Recoery for all 10 illicit drugs at flow rates of 2 (blue), 5 (orange), and 10 mL/min (gray).

Table 1. Recoveries and RSD of 10 illicit drug compounds for 3 different sample loading flow rates (n=6).

	2 mL/min		5 mL/min		10 mL/min	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
morphine	96.3	5.2	99.4	4.8	94.7	8.7
ketamine	102.7	2.0	102.8	2.8	100.4	0.9
cocaine	103.4	2.9	104.3	2.2	103.4	1.9
amphetamine	97.8	2.6	90.2	5.4	90.1	4.8
methamphetamine	94.0	3.1	89.9	4.9	88.4	3.6
MDA	97.1	3.3	112.5	3.6	112.5	1.7
MDMA	97.7	1.8	100.2	2.0	100.4	2.1
non-ketamine	93.7	5.4	95.6	3.1	95.8	3.2
O6-monoacetylmorphine	90.8	4.9	109.2	7.2	107.0	5.4
benzoylegonine	94.1	3.1	91.7	3.1	93.8	2.9