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On-line weak cationic mixed-mode solid-phase extraction coupled to liquid chromatography–mass spectrometry to determine illicit drugs at low concentration levels from environmental waters

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ABSTRACT

This study presents a fully automated method based on on-line solid-phase extraction coupled to liquid chromatography with mass spectrometry detection (on-line-SPE-LC–MS) to determine illicit drugs in environmental water samples. The mixed-mode Oasis WCX sorbent used in an optimised protocol allows the addition of an effective washing step with 0.5 mL of methanol, which washed out all the interferences retained by reversed-phase interactions and helped to reduce the matrix effect, while the cationic target analytes remained bound and could then be selectively eluted with recovery values near to 100%. This method was successfully applied to the analysis of 10 mL of environmental water (river and sewage) spiked at low ng L^{-1} levels of the analytes, with recoveries from 50 to 100% for all studied analytes. The method was also validated with river water samples with figures of merit comparable to those of the off-line SPE-LC–MS/MS method.

1. Introduction

In recent years, it has become a well-accepted fact that the use of a large number of drugs in various areas of our lives inevitably leads to the release of these drugs into the environment and their occurrence in the environment [1]. Recently, concern has grown with respect to the presence of illicit drugs and metabolites in wastewater and surface water around the world [2–6].

In view of this, in recent years efforts in analytical chemistry have focused on the development of different analytical methods to determine these drugs at the low concentration levels found in environmental samples [2]. Almost all of the published methods to determine the illicit drugs in waters include a sample pretreatment combined with liquid chromatography (LC) with mass spectrometry (MS) or tandem MS using electrospray (ESI) as an ionisation source [2,5].

Sample treatment is necessary in order to enrich the analytes of interest and obtain good analyte recoveries, and to remove matrix interferences that may compete with the target analytes in the ionisation process in LC–MS. Off-line solid-phase extraction (SPE) is the most commonly used sample preparation technique when dealing with environmental samples [2,5]. Different sorbents have been tested in illicit drug determination methods, such as Oasis HLB [7] and the mixed-mode Oasis MCX [3,6,8,9], Strata-X-C [10] and Evolute CX [11]. These mixed-mode sorbents combine ionic interactions with reversed-phase interactions, which under optimised conditions permit a washing step involving organic solvent to be introduced in the SPE process. This therefore improves the selectivity or/and removal of the interferences involved in the matrix effect.

On-line SPE is another sample treatment which, compared to the off-line mode, improves the method sensitivity and shortens the pretreatment and analysis time [12]. So far, the SPE sorbents tested in this way to determine illicit drugs are PLRP-S [13], an in-house hypercrosslinked sorbent [14], and the mixed-mode Isolute HCX [15] and Strata-X-CW [16]. However, an appropriate washing step was not included in any of these applications and the enrichment factors and/or the ion suppression might be further improved if this step were incorporated.

The present study explores a method to determine a group of illicit drugs in complex environmental water samples that is based on on-line SPE-LC–MS using a mixed-mode sorbent (Oasis WCX) as the SPE sorbent which enables an effective washing step to remove matrix interferences and overcome the ion suppression/enhancement signal effect.

2. Materials and methods

2.1. Materials

All the illicit drugs and their metabolites studied: nicotine (NIC); cocaine (COC) and benzoylecgonine (BE); morphine (MOR) and

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Table 1
Compound retention time, optimised fragmentor voltage and ions selected for quantification and confirmation in SIM mode.

Analytes	pK_a	t_R (min)	Fragmentor voltage (V)	Quantification ion (m/z)	Confirmation ions (m/z)
Nicotine	9.0	2.4	100	163	132,106
Morphine	8.3	4.5	175	286	165,153
Dihydrocodeine	8.4	7.1	150	302	324,199
Codeine	8.3	7.2	150	300	243,215
6-Acetylmorphine	8.3	8.2	150	328	268,211
BE	10.8	8.9	125	290	168,105
Cocaine	8.0	9.2	125	304	182,105
EDDP	7.7	9.9	100	278	249,234
Methadone	9.1	10.1	100	265	310,245

6-acetylmorphine (AcMOR); codeine (Cod) and dihydrocodeine (DHCod); and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidone (EDDP) and methadone (MET), were obtained from Cerilliant (Round Rock, TX, USA) as solutions at a concentration of 1000 mg L^{-1} in methanol or acetonitrile. Working solutions of a mixture of all compounds were prepared in 1:1 MeOH:H_2O (v:v). All the stock and working solutions were stored at −20 °C in the dark.

Ultrapure reagent water purified by a water purification system (Veolia, Sant Cugat del Vallés, Barcelona) was used throughout. Acetonitrile (ACN) and methanol (MeOH) (both HPLC grade) were purchased from SDS (Peypin, France). Analytical grade ammonium hydroxide (NH_4OH) and formic acid (HCOOH), which were used to adjust the pH, were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Instrumentation

The chromatographic system was an HP1100 series LC–MS selective detector (Agilent Technologies, Waldbronn, Germany) with an ESI interface. It was equipped with a degasser, a quaternary pump, a 20 μL loop injector and a column oven. The chromatographic column was a Fused-Core™ Ascentis Express C_{18} (50 mm × 4.6 mm) with a particle size of 2.7 μm (Supelco, Bellefonte, PA, USA).

The on-line solid-phase extraction system was connected to the chromatographic system by means of a six-port switching valve (Rheodyne, Cotati, CA, USA). An isocratic pump (Agilent Technologies) was used to deliver the sample through a stainless steel precolumn (dimensions 20 mm × 2 mm I.D.) fitted with 2 μm stainless steel frits, all purchased from Upchurch Scientific (Oak Harbor, WA, USA). This precolumn was packed manually using a packing funnel with ∼30 mg of the sorbent.

2.3. Chromatographic conditions

A binary mobile phase with gradient elution was used. Solvent A was ultrapure water with 0.5% HCOOH (pH 2.5) and solvent B was ACN. The gradient profile was 10% solvent B increased to 15% in 3.5 min, then increased to 50% solvent B in 2.5 min, and increased to 100% solvent B in 6 min, then held constant for 2 min, after which the mobile phase was returned to the initial conditions (10% solvent B) in 1 min (and held for 5 min to equilibrate the column for the following analysis). The flow rate was 0.4 mL min^{-1} and the temperature of the column oven was set at 30 °C.

Flow injection analysis (FIA) was carried out to find the optimum conditions for each compound in the ESI. The average conditions selected for the optimum performance of the ESI interface in the positive mode were: nebuliser pressure 40 psi, drying gas flow rate 13 L min^{-1}, drying gas temperature 350 °C, and capillary voltage 4000 V. Fragmentation voltages (optimised range was 50–250 V) were defined individually and the specific values for each compound are listed in Table 1. The ions selected for quantifying the samples are also listed in Table 1. In SIM mode, the most abundant ion, which all analytes corresponds to $[M+H]^+$ with the exception of methadone, was used for quantification and two other ions were used for confirmation.

2.4. Solid-phase extraction

The commercially available Oasis WCX (particle size of 30 μm), which is a mixed-mode polymeric sorbent based on a poly(vinylpyrrolidone-divinylbenzene) skeleton and modified with carboxylic acid moieties (that can impart weak-cationic and reversed-phase interactions), was laboratory packed (∼30 mg) into a 20 mm × 2 mm I.D. stainless-steel precolumn used for on-line trace enrichment in the SPE process.

The final protocol was as follows: the SPE precolumn was conditioned with 5 mL of MeOH and 5 mL of ultrapure water adjusted at pH 7; 10 ml of water sample adjusted to pH 7 was loaded through the conditioned precolumn. The flow-rate was 3 mL min^{-1} throughout all of these steps. The sorbent was then washed by passing 0.5 mL of MeOH through the precolumn at 1 mL min^{-1}. The retained analytes were desorbed using the mobile phase in the gradient profile of the chromatographic system, and in the back-flush mode to reduce band-broadening.

Environmental water samples (river water and effluent water from sewage treatment plant) were filtered through 0.45 μm nylon membranes (Osmonics Inc.) before the preconcentration step to eliminate the particulate matter, after which they were adjusted to pH 7 with HCOOH.

3. Results and discussion

3.1. LC–MS conditions

Chromatographic separation was performed using a Fused-Core™ Ascentis Express C_{18} column with a particle technology that enables efficiency and speed of the separation pertinent to sub-2 μm particles to be achieved while maintaining the back pressures. Two different organic solvents were tested: ACN and 1% HCOOH in ACN to establish how the ionisation is affected and hence the signal of all of the analytes. In the end, ACN on its own was selected as no significant improvement was seen with the addition of acidic solution to the organic mobile phase. Table 1 lists the retention time of the studied analytes under the optimum separation conditions. It also details both the fragmentor voltage and the ions selected for compound quantification and confirmation.

Instrumental (LC–MS) linearity was good for all the compounds when they were directly injected at low μg L^{-1} levels. The linear range was 1–500 μg L^{-1} for all of the compounds with the exception of morphine (5–1000 μg L^{-1}) and acetylmorphine (1–1000 μg L^{-1}). The limits of detection (LODs) calculated at a signal-to-noise ratio (S/N) of 3, were 0.2 μg L^{-1} for all of the compounds, except for morphine (1 μg L^{-1}).