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Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

An analytical method based on pressurized liquid extraction (PLE) and liquid chromatography–(electrospray)–tandem mass spectrometry was developed for the simultaneous determination of nicotine, four drugs of abuse (opiates and alkaloids) and four of their main metabolites in sewage sludge. The optimum PLE conditions were: cell volume 11 mL, dichloromethane as extraction solvent, 5 min preheating time, 100 °C temperature, 1500 psi pressure, 60% flush volume, 1 cycle, 15 min static extraction time, 120 s purge time and sample weight 1 g. Absolute recoveries for all compounds were between 25% and 65%. Data acquisition was done by selective reaction monitoring and the two most abundant product ions were used for confirmation. Limits of detection were lower than 10 μg/kg dry weight (d.w.) and limits of quantification were between 2.5 and 25 μg/kg (d.w.).

The highest concentrations found in sludge samples from two sewage treatment plants were for nicotine and cocaine in the range of 23–173 μg/kg (d.w.) and 9–232 μg/kg (d.w.) respectively.

1. Introduction

In the last few years the determination of drugs of abuse and their metabolites in the aquatic environment has attracted considerable attention, and the measured analyte concentration has also been used to back-calculate drug usage in local communities [1]. As is well known, drugs of abuse may undergo partial degradation in the human organism and can be excreted through urine into sewage as an intact form or as metabolites. They are then released to the sewage treatment plants (STPs), where they are partially removed via degradation or sorption into the sludge.

Although there are several studies in which drugs of abuse have been determined in influent and effluent sewage [2,3], surface water [1,4] and even air samples [5–7], less attention has been devoted to their determination in sludge, which is generated during the treatment process in the STPs. The presence of these contaminants in sludge may limit its re-use due to public health and environmental protection requirements, although they are not included in current legislation.

Recent studies [8,9] related to the presence of these contaminants in sewage include their determination not only in sewage but also in sewage-suspended particulate matter (SPM), and some of these contaminants such as cocaine and its metabolite benzoylecgonine are definitively found in SPM. This confirms that some of these contaminants tend to be retained in particulate matter and hence they can also be found in sludge, although drugs of abuse are relatively polar and not expected to be much retained in the sludge.

Therefore only a few studies evaluate the presence of drugs of abuse in sludge, and most of these studies only a limited number of drugs. Kaleta et al. [10], for instance, study the presence of amphetamine in sewage sludge, Langford et al. [11] develop a multiresidue method for sludge samples in which some drugs of abuse are included although none were found in the samples, and Jones-Lepp et al. [12] determined metamphetamine in sludge. Very recently, Mastroianni et al. [13] developed a method to determine 20 drugs of abuse (cocainics, amphetamines, opioids, benzodiazepines, LSD and cannabinoids) and 16 compounds were found in sludge samples at concentrations from 0.4 to 579 ng/g of dry weight (d.w.).

Several extraction techniques have been applied to extract drugs of abuse from sludge [10–12], the most popular being pressurized liquid extraction (PLE) and ultrasonic solvent extraction (USE). In some cases solid-phase extraction (SPE) is further applied as a clean-up technique. For instance USE followed by SPE has been used to determine amphetamine [10] and PLE has been applied to determine methamphetamine [12] and a group of various drugs [11]. As regards SPM, once the sample is filtered, the suspended matter is extracted in a similar way to sludge, with PLE [8,9,14] being the most used technique.

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After extraction, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is usually applied [15,16], although gas chromatography tandem mass spectrometry (GC–MS/MS) after derivatization has also been used [17]. As regards LC–MS/MS, ultra-high performance liquid chromatography (UHPLC) is increasingly used [8,11] and different analyzers have been used to determine these compounds in water, SPM and sludge samples, with triple quadrupole (QqQ) being the most frequent [4,18,19], although quadrupole-linear trap (QTRAP) [20,21] and quadrupole-time of flight (QTOF) [22] have also been used.

The aim of this study is to develop an analytical method for the simultaneous determination of nicotine, four drugs of abuse (morphine, codeine, cocaine and methadone) and some of their main metabolites (2-ethylidene-1,5 dimethyl-3,3-diphenylpyrrolidine, dihydrocodeine, 6-acetylmorphine and benzoylecgonine) in sewage sludge. Although nicotine is a legal drug, it was included in this study due to the high consumption of tobacco worldwide. The method is based on PLE and liquid chromatography–(electrospray)−tandem mass spectrometry (LC–(ESI)MS/MS) and was applied to determine these drugs of abuse in several sludge samples from two STPs.

2. Experimental

2.1. Reagents and standards

Standards of nicotine (NIC), codeine (COD), dihydrocodeine (DIC), 6-acetylmorphine (6-AM), cocaine (COC), benzoylecgonine (BE), 2-ethylidene-1,5 dimethyl-3,3-diphenylpyrrolidine (EDDP), methadone (MTD), morphine (MOR), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and the surrogates EDDP-d_3, COD-d_6 and MOR-d_6 were acquired from Cerilliant (Round Rock, TX, USA), available as solution in 1 mL of methanol or acetonitrile at 1000 mg/L. Stock solutions of individual standards were prepared by diluting each compound in methanol at 100 mg/L and storing them at −20 °C in the dark. A mixture of all compounds in methanol:water (1:1 v/v) at 100 μg/L was prepared weekly. Working solutions were prepared daily by appropriate dilution of this solution with methanol:water (1:1 v/v).

Ultra pure water was obtained using a purelab ultra purification system (Veolia water, Sant Cugat del Vallés, Spain). The acetonitrile, acetone, dichloromethane and methanol (HPLC-grade) came from SDS (Peypin, France), the nitrogen from Carburos Metálicos (Tarragona, Spain) and the acetic acid from Prolabo (Bois, France). Diatomaceous earth was supplied by Sigma-Aldrich (St. Louis, USA).

2.2. Sample pretreatment

Sewage sludge samples were collected from two urban STPs in two cities of about 130,000 inhabitants located in southern Catalonia. They were then homogenized, frozen and lyophilized using the freeze-dry system (Labconco, Kansas City, MO, USA). The lyophilized samples were homogenized by mortar and pestle, sieved through a 125 μm screen and stored at room temperature.

To optimize the method, the pretreated sludge samples were spiked with the analytes dissolved in acetone, and then the solvent was evaporated at room temperature while submitted to vigorous shaking. The volume of acetone was enough to completely cover the sludge.

2.3. Pressurized liquid extraction

Pretreated sludge samples were extracted by PLE using an ASE 200 (Dionex, Sunnyvale, CA, USA). Deuterated compounds (MOR-d_3, COD-d_6 and EDDP-d_3) at a concentration of 125 μg/kg (d.w.) were added to 1 g of pretreated sludge. This was then thoroughly mixed with 2 g of diatomaceous earth to remove possible traces of water from the sludge and maximize effectiveness in the extraction process, especially in the case of solvents immiscible with water. The mixture was then transferred to the inside of the extraction cell and a Whatman glass fiber filter (Ahlstrom, PA, USA) was put at the top and bottom of the cell.

The extraction solvent was dichloromethane and the operating conditions were: preheating period 5 min, extraction temperature 100 °C, extraction pressure 1500 psi with a static period of 15 min in one cycle, flush volume 60% of cell volume and nitrogen purge time 120 s. The final extraction volume was approximately 15 mL, which was evaporated to dryness under a nitrogen stream and redissolved in 5 mL of methanol:water (1:1 v/v), filtered with a microfilter of 0.20 μm (Teknokroma, Barcelona, Spain), and then analyzed by LC–MS/MS.

2.4. Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

The determination was performed using an HP 1200 series liquid chromatography–triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) with electrospray ionization (ESI). The chromatographic column was a fused core Ascentis Express C_{18} (4.6 × 50 mm) with a 2.7 μm particle size (Sigma-Aldrich, Madrid, Spain) and the volume injected was 50 μL. The flow-rate was 0.4 mL/min and the column temperature was kept at 30 °C.

A binary mobile phase with a gradient elution was used. Solvent A was acidified water with acetic acid (pH 2.8) and solvent B was acetonitrile. The gradient was initially 5% B, which was increased to 15% in 3.5 min, to 50% in 2.5 min, to 100% in 6 min, kept constant for 2 min and finally returned to 5% B in 1 min. All the compounds were eluted within 13 min.

The optimized conditions for the ESI interface in positive mode were: 45 psi nebulizer pressure, 12 L/min drying gas flow-rate, 350 °C drying gas temperature and 3000 V spray potential. Cone voltage values and collision energy voltages were within 60–140 V and 15–60 V respectively. Two selective reaction monitoring (SRM) transitions for each compound were monitored and the values are shown in Table 1. The most abundant transition was used for quantification, while the other was used for confirmation. Relative ion intensities and retention time were also used as confirmation criteria.

3. Results and discussion

3.1. LC–(ESI)MS/M

Chromatographic separation was performed on a C_{18} column based on fused-core particle technology, which provides more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-μm columns [23]. The binary gradient elution enabled the ten compounds to be separated in 13 min.

LC–(ESI)MS/MS conditions were based on a previous paper by Pedrouzo [24], in which these compounds were determined in water samples. EDDP-d3, COD-d6 and MOR-d6 were used as surrogates to compensate the adverse ion suppression given in ESI. The surrogate used for each compound was chosen taking into account similar structure, proximity in retention time and similar behavior with regard to ion supression.

Due to the positive charge provided by the amino group present in the analyte's structure, the compounds were analyzed in positive ionization mode producing abundant $[M+H]^+$ ion.