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Determination of benzodiazepines, related pharmaceuticals and metabolites in water by solid-phase extraction and liquid-chromatography–tandem mass spectrometry

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ABSTRACT

This work presents a method for the simultaneous determination of 23 benzodiazepines, metabolites and related pharmaceuticals (zolpidem, methylphenidate and ritalinic acid) by solid-phase extraction (SPE) followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Different SPE cartridges were considered: hydrophilic modified reversed-phase (Oasis HLB) and their modified versions containing also a cationic-exchange group (Oasis MCX) or anionic-exchange (Oasis MAX) funcionalities. Stability of analytes and the impact of the final eluate volume on the matrix effects on LC–MS/MS were also considered. In the final method, 100–200 mL of sample are extracted with Oasis MCX (60 mg), eluted with 5 mL of methanol (1.25% NH_3) and the eluate concentrated and analyzed by LC–MS/MS. Under these conditions, LOQs were established between 0.1 and 18 ng L^{-1} for influent wastewater. The use of surrogated deuterated internal standards allows obtaining recoveries in the 84–122% range. Finally the method was applied to determinate the analytes in wastewater and surface water and 10 compounds were detected in the range of 0.5–170 ng L^{-1}, being the ritalinic acid (the main metabolite of methylphenidate) the analyte detected in the highest concentrations.

1. Introduction

Benzodiazepines (BZPs) are pharmaceuticals prescribed for stress or panic situations, dream disorders, against muscular injuries and, recently, in drug-addiction detoxification programs [1]. It is well documented that one of the main disadvantages of the long-term BZPs use is the risk of dependence, accidents, faints and cognitive disturbance [2,3]. In the period 2000–2007, the Organization for Economic Co-operation and Development (OECD) Health Data [4] put the Spanish consumption of anxiolytics a 37% higher than the European average: 43 Defined Daily Doses (DDD) per 1000 inhabitants and day vs. 31.4 DDD 1000 inh.$^{-1}$ day^{-1} (European mean). Only Portugal (72 DDD inh.$^{-1}$ day^{-1}) and France (57 DDD inh.$^{-1}$ day^{-1}) were higher. In the case of sedative-hypnotics usage, Spanish prescription (21.0 DDD inh.$^{-1}$ day^{-1}) is very close to the EU average (28.9 DDD inh.$^{-1}$ day^{-1}). Anyway, the consumption of anxiolytics and sedative-hypnotics keeps raising and Spanish national surveys indicated a consumption of 89 DDD inh.$^{-1}$ day^{-1} in 2012, representing a 22% increment respecting the data of 2007 [5].

However, besides therapeutic usage, recreational and illicit use of BZPs has been recently reported by The European Monitoring Center for Drugs and Drug Addiction (EMCDDA) where BZPs have a high presence in situations of sexual assaults mediated by drugs or alcohol [6,7], or also between the younger population in order to increase the effect of illicit drugs [7]. Zolpidem (ZOLP) and methylphenidate (MPHEN) are other therapeutic drugs involved in such usage pattern. Though they are not BZPs themselves, they act over the same brain receptor. Actually, ZOLP has a very similar chemical structure to some BZPs with the presence of an imidazopyridine ring. ZOLP is prescribed against insomnia situations and because of this it is reported in the same therapeutic group (N05C-hypnotics) as some of the most commonly prescribed BZPs, e.g. bromazepam (BROM), lormetazepam (LORM) and midazolam (MID) among others [8]. MPHEN is a psycho-stimulant drug used for the treatment of narcolepsy and the attention-deficit hyperactivity disorder (ADHD). It shares with the BZP the same brain receptor for its action, although the effect is completely the opposite. The

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secondary effects of MPHEN include hallucinations, amnesia and effects similar to cocaine [9,10]. Because of this, it is a substance prone to an illicit usage. Hence, the determination of ZOLP, MPHEN and its main metabolite, ritalinic acid (RIT) are included in this work, together with BZPs and their metabolites.

Besides constituting a potential health issue, they may also be an environmental problem, since BZPs have been detected at the ng L^{-1} level in wastewater [11–14] and surface water [14–17], and can be also infiltrate into the subsoil and contaminate aquifers [18–20].

The most frequently used technique for their determination of BZPs and related compound in environmental samples is liquid chromatography–mass spectrometry (LC–MS) [11,13,14,17–21] due to the chemical structure of these target analytes (benzene diazepine fused rings), which confers them a thermolabile and a polar character. Thus, determination by gas chromatography (GC) is less frequent since a derivatization reaction has to be performed to decrease their polar character and to obtain a good sensitivity and sometimes that it is not enough to achieve the limits of quantification (LOQs) required for their determination in wastewater and environmental waters [22,23]. Enrichment is typically performed by solid-phase extraction (SPE), mostly employing Oasis MCX [11,18] and Oasis HLB [13,24,25] cartridges. However, published methods to date simply considered the determination of a few BZPs, mainly diazepam (DIA), alprazolam (ALP), lorazepam (LOR) and oxazepam (OXA), together with other pharmaceutical classes, illicit drugs and related compounds.

Hence, the goal of this work was the development of a new analytical method capable of determining a broad range of BZPs, metabolites and related drugs (ZOLP, MPHEN and RIT) in environmental waters. Thus, 23 analytes are considered here. SPE and LC–tandem MS (LC–MS/MS) determination conditions were carefully optimized to achieve the adequate limits of detection (LODs) and LOQs. Then, the method was validated and applied to the determination of these drugs in wastewater and surface water.

2. Experimental

2.1. Materials and chemicals

ALP, α-hydroxyalprazolam (AHALP), α-hydroxytriazolam (AHTRI) clonazepam (CLON), 7-aminoclonazepam (7ACLON), flunitrazepam (FLUN) 7-aminoflunitrazepam (7AFLUN), flurazepam (FLUR), 2-hydroxyethylflurazepam (2HEFLU), DIA, nordiazepam (NDIA), LOR, LORM, MID, OXA, temazepam (TEM), MPHEN HCl salt, RIT HCl salt, BROM, chlordiazepoxide (CHLOR), demoxepam (DEM), prazepam (PRAZ) and ZOLP tartrate were purchased from Cerilliant (Round Rock, TX, USA) as 1 mg mL^{-1} solutions in methanol (MeOH) or acetonitrile (ACN). All concentrations cited herein refer to the neutral species. AHALP-d_5, ALP-d_5, 7ACLON-d_4, 7AFLUN-d_7, DIA-d_5, FLUN-d_7, NDIA-d_5, OXA-d_5, 2HEFLU-d_4, LOR-d_4, ZOLP-d_6, RIT-d_{10} and MPHEN-d_9, used as surrogate internal standards (ISs), were purchased also from Cerilliant (0.1 mg mL^{-1} in MeOH or ACN). A summary of the abbreviations, structures and selected physico-chemical properties of the analytes is presented in Table 1.

LC-grade MeOH, hydrochloric acid (37%), glacial acetic acid (100%), and formic acid (98–100%) were supplied by Merck (Darmstadt, Germany) whereas methanolic ammonia solution (6 N), ammonium acetate (99.99%) and formic acid (98%) were supplied by Sigma Aldrich (Steinheim, Germany). Ultrapure water was obtained in the laboratory by purifying demineralized water in a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA).

Oasis HLB (60 mg), Oasis MCX (60 mg) and Oasis MAX SPE cartridges (60 mg) were all purchased from Waters (Milford, MA, USA). Nitrocellulose filters (size pore of 0.22 and 0.45 μm) and glass fiber pre-filters were purchased from Millipore.

2.2. Solutions

Mixed stock solution of the compounds were prepared by dilution of the individual stock solution to obtain a concentration of 10 μg mL^{-1} in methanol. This solution was further diluted with methanol/water (50:50, v/v) to obtain a solution of 1 μg mL^{-1}. Subsequent dilutions with methanol/water (50:50, v/v) were prepared to obtain the suitable concentration to spike water samples and for calibration standards. The same dilution process was performed with the deuterated standards.

All the solutions were stored at −20 °C in amber glass vials and, although no symptoms of degradation were observed, solutions were renewed after 2 months.

2.3. Samples

Grab wastewater samples of influent and effluent were obtained from a wastewater treatment plant (WWTP) serving an urban population around 130,000 inhabitants, located in the northwest of Spain. The WWTP consists of a primary and secondary activated sludge treatment. Surface water samples were collected in a river, approximately 5 km downstream the discharge point of the WWTP.

Samples were collected in 2.5 L amber glass bottles previously washed with methanol and ultrapure water and rinsed with the sample. They were filled up completely and stored at 4 °C in the fridge and analyzed prior 12 h after sampling. Before analysis, samples were firstly filtered through a glass pre-filter and finally with a 0.45 μm nitrocellulose filter (Millipore, Milford, MA, USA).

2.4. Solid-phase extraction procedure

Different experimental conditions were evaluated (pH, sample volume etc.) during SPE method development, as explained in Section 3.4. A short general description is presented, however, here of the general protocol followed with the three SPE cartridges considered.

2.4.1. Oasis HLB protocol

Oasis HLB 60 mg cartridges were sequentially conditioned with 3 mL of MeOH and 3 mL of ultrapure water. Then the sample was percolated through the cartridge assisted by a vacuum pump at ca. 5 mL min^{-1}. Subsequently, the cartridges were washed with 6 mL of ultrapure water and vacuum dried for 20 min. Finally, compounds were eluted with 5 mL of MeOH, nitrogen-stream concentrated to a final determinate volume and analyzed.

2.4.2. Oasis MAX protocol

Oasis MAX 60 mg cartridges were sequentially conditioned with 3 mL of MeOH (2% formic acid), 3 mL of MeOH and 3 mL of ultrapure water. Samples were percolated at ca. 5 mL min^{-1} and then, cartridges were washed with 6 mL of ultrapure water and vacuum dried for 20 min. Finally compounds were eluted with 5 mL of MeOH (fraction 1) and 5 mL of MeOH containing 2% formic acid (fraction 2). Fractions were collected in separate vials, nitrogen-stream concentrated to a final determinate volume and analyzed separately.

2.4.3. Oasis MCX protocol

Oasis MCX 60 mg cartridges were sequentially conditioned with 3 mL of MeOH (1.25% NH_3), 3 mL of MeOH and 3 mL of ultrapure water. Samples were percolated at ca. 5 mL min^{-1} and the cartridges were washed with 6 mL of ultrapure water and vacuum dried for 20 min. Finally compounds were eluted with 5 mL