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Multi-residue analysis of pharmaceutical compounds in aqueous samples☆

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Abstract

Pharmaceutical compounds are nowadays an emerging group of organic pollutants in aquatic systems. Several methodologies have already been published to measure these pollutants in the environment, showing the difficulties to take into account the various compounds belonging to numerous therapeutical and chemical groups. In order to develop environmental monitoring, there is a need for a less costly and time-consuming multi-component procedure. The work presented here deals with the development of an extraction procedure which enables the measurement of a wide spectrum of pharmaceuticals at trace levels ($ng\,l^{-1}$) with quite simple equipment (i.e. GC–MS with single quadruple as analyzer). The analyzed compounds comprise anti-inflammatories, antidepressants and hypolipidic drugs. The reliability and sensitivity have been tested on 18 different compounds (7 basic compounds and 11 acidic drugs) extracted simultaneously and analyzed by GC–MS. The optimized procedure has been successfully applied to the analysis of wastewaters, surface waters and drinking waters from the following areas: first the Cortiou rocky inlet, in the Mediterranean Sea (South coast of France), highly impacted by the Marseilles wastewater treatment plant effluent and secondly the Hérault watershed by studying drinking water, surface water and wastewater. In both cases, the level of pharmaceuticals was totally unknown. Results obtained have demonstrated the suitability of the method for multi-residue analysis of different types of water matrices.

Keywords: Gas chromatography–mass spectrometry (GC–MS); Pharmaceuticals; Multi-residue analysis; Urban wastewaters; Marine waters; Drinking waters; Surface waters; Solid-phase extraction (SPE)

1. Introduction

The presence of pharmaceuticals in the environment, classified as the so-called emerging contaminants, has raised great concern among the scientific community during the last few years. Unfortunately, as a result of their growing use, these compounds have been found in aquatic systems, in sewage treatment plant effluents [1,2] as well as in surface waters [3] even detected in drinking waters [4].

Their ubiquity in the environment has prompted researchers to identify the effects that these compounds could have on non-target species [5,6] and to develop chronic exposure risk assessment on aquatic organisms [7] as well as on human beings [8,9].

Their monitoring is necessary to provide wider knowledge about their occurrence in the environment, to understand their fate, partition and organism exposure levels [10].

The quantification of pharmaceuticals in human biological matrix such as blood, plasma or urine [11] has been developed for a long time. But similar developments concerning pharmaceuticals in natural waters present more difficulties: these compounds are present at low levels and as very complex mixtures of dozens of different molecules. Simultaneous extraction of different therapeutic groups is particularly focused on antibiotics and steroids with HPLC–MS–MS or GC–MS analyses [12].

Some studies have already presented very efficient analytical procedures designed for specific pharmaceutical classes [13]. Analyzing simultaneously a wide spectrum of pharmaceuticals with different physico-chemical properties is rather difficult and requires a compromise, sometimes not resulting in the obtention of the best conditions for all analytes. Some procedures have allowed to measure pharmaceuticals at trace levels but

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with very tedious extraction procedures [14] and large extraction volumes, reducing the number [8] of analyzed samples which makes the environmental screening difficult to achieve [15]. Nowadays, multi-residue analytical methods are required in order to provide wider knowledge about the presence of pharmaceuticals in the environment. The work presented here deals with the development of an extraction procedure that enables the measurement of a wide spectrum of pharmaceuticals at trace level (ng l^{-1}), including anti-inflammatory d rugs, antidepressants, hypolipidic drugs, etc. The reliability and sensitivity have been tested on 18 different compounds (7 basic compounds and 11 acidic drugs) extracted simultaneously by off-line SPE and analyzed with GC–MS. In order to validate the applicability of the method, it has been applied to the analysis of wastewaters, surface waters and drinking waters from the Hérault watershed and the Cortiou rocky inlet. First, the Hérault watershed has been studied including wastewaters as well as surface waters and spring waters dedicated to human consumption, with low pharmaceutical levels. On the other hand, seawaters, highly contaminated by the Marseilles wastewater treatment plant effluent [16] and with a high organic matter content, have been monitored in the Cortiou rocky inlet, in the Mediterranean Sea (Marseilles area, South coast of France).

2. Materials and methods

2.1. Chemicals and reagents

Pharmaceutical products (presented in Table 1) as well as pyrene and 1-hydroxypyrene used as recovery determination standards were purchased from Sigma–Aldrich (St. Quentin Fallavier, France; purity >98%). Deuterated products (diazepam *d*5, amitryptiline *d*6 and nordiazepam *d*5) were purchased from Euriso-Top (St. Aubin, France, purity >98%). Acetone, ethyl acetate and methanol (HPLC reagent grade, Scharlau) were purchased from ICS (Belin-Beliet, France). Hydrochloric acid 37% (reagent grade) and phosphoric acid 85% (reagent grade) were obtained from Atlantic Labo (Eysines, France). Ultrapure water was obtained with a Milli-Q system (Millipore, Molsheim, France). Sixty-milligram Oasis MCX cartridges were purchased from Waters (St. Quentin en Yvelines, France).

MSTFA (*N*-methyl-*N*-(trimethylsylil)trifluoroacetamide, purity >97% from Acros Organics (Noisy-le-Grand, France)) was used as the derivatizing reagent for GC–MS analyses. Whatman GFF glass fibre filters (pore size 0.7 μm) were purchased from VWR International (Fontenay-sous-Bois, France) and Atlantic Labo (Eysines, France).

2.2. Sample pretreatment and solid-phase extraction optimization

Water samples have been collected in amber glass bottles, previously detergent washed, acid rinsed and heated at 450 °C for 6 h. The samples were filtered on GFF fibre filters immediately after collection and pharmaceutical extraction was done during the day of sampling.

Raw water was filtered on GFF filters to separate dissolved phase and particles. For natural waters, 1 l was filtered whereas for Wastewater Treatment Plant Effluent (WWTP effluent), 500 ml was used for each extraction. Sample pH was adjusted prior to extraction at a value of 2 with HCl (3.5 M). Moreover, internal standards (25–50 μl of a methanolic mixture containing 1 μg g^{-1} of each standard depending on the type of waters: the least for surface waters, the most for wastewaters) were added to the samples.

Before sample loading, SPE cartridges were conditioned with 3 ml of ethyl acetate and 3 ml of Milli-Q-water at adjusted pH 2. Water was percolated under vacuum onto the cartridges at a flow rate of 12–15 ml min^{-1} and afterwards dried for 1 h under vacuum. After elution with three successive solvents, 3 ml of ethyl acetate, 3 ml of ethyl acetate/acetone (50/50; v/v) and 3 ml of ethyl acetate/acetone/ammonium hydroxide (48/48/2; v/v/v), respectively, the samples were completely evaporated under nitrogen and transferred into GC injection vials in 50–100 μl of ethyl acetate. For recovery control, pyrene was added to the final extracts for basic compounds before GC–MS analysis and 1-hydroxypyrene was added to the final extracts for acidic compounds before the derivatization step, consisting in adding 30 μl of MSTFA before incubation at 65 °C for 35 min.

All additions of matrix, standards, solvents or reagents were gravimetrically controlled. Blanks were performed for each batch experiment in order to prevent any contamination. No compounds have been found in blank samples.

2.3. GC–MS analysis

GC–MS analyses were carried out using an HP 6890 gas chromatograph from Agilent Technologies (Palo Alto, CA, USA). The capillary column was an HP5/MS (30 m × 0.25 mm × 0.25 μm film thickness; phase: 5% diphenyl, 95% dimethylsiloxane) from Bios Analytique (L'Union, France). Samples were injected (1 μl) into the GC in splitless mode at 250 °C using an HP 6890 series injector. The carrier gas was ultrapure helium (99.99990%, Linde Gas, Bassens, France) set at constant flow mode (1.3 ml/min). For GC separation, the temperature program started at 70 °C (held for 2 min), set at 10 °C/min to 250 °C and then was held isothermally at 250 °C for 5 min. The gas chromatograph was coupled to an HP 5973N mass selective detector (LMSD, Agilent Technologies, Palo Alto, CA, USA), operated under electronic impact (EI) mode at 70 eV using scan mode (from 50 to 600 amu, 2.69 scan s^{-1}) and single ion monitoring mode at 1.67 scan s^{-1} (dwell time 70 ms). The transfer line, source and quadruple temperatures were 280, 230 and 150 °C, respectively.

Each compound has been first characterized individually in scan mode in order to identify the main ions (*m*/*z* ratio) constituting the mass spectrum and to choose the ions for quantification and for confirmation (Table 1). For acidic compounds, detection has also been investigated after the derivatization step with *N*-methyl-*N*-(trimethylsylil)trifluoroacetamide (MSTFA). For this purpose, the solution has been kept for 35 min in an oven at 65 °C after adding 30 μl of MSTFA.