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Simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormones in water and wastewater samples using gas chromatography–mass spectrometry and gas chromatography with electron capture detection

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HIGHLIGHTS

- A new SPE–GC–ECD method for the determination of pharmaceuticals in water samples.
- Application of SPE–GC–MS(SIM) method in highly complex wastewater matrices.
- Application of time windows to improve GC–MS(SIM) measurement sensitivity.
- Reliable confirmation of the presence of pharmaceuticals using ion ratio.
- Application of the proposed methods to the analysis of real samples from Poland.

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ABSTRACT

Non-steroidal anti-inflammatory drugs are the group of pharmaceuticals that is most often found in the environment, whereas estrogenic hormones are considered to be potent endocrine disruptors. However, the fate and persistence of these compounds in the environment are still unclear. In this study we propose two approaches for determining these compounds in environmental water samples: GC–MS using time windows and operating in selected ion-monitoring mode (SIM) and, for the first time, gas chromatography with electron capture detection (GC–ECD). The identification criteria of both methods fulfilled the requirements of Directive 2002/657/EC. The use of time windows improved the sensitivity of GC–MS measurements. In GC–MS analysis the pharmaceuticals were determined as trimethylsilyl, in GC–ECD as pentafluoropropionyl derivatives. The influence of such parameters as the type of reagent, type of solvent, reaction time, reaction temperature and microwave irradiation in a household microwave oven on the efficacy of silylation was investigated. Derivatization using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) in pyridine (1:1, v/v) for 30 min in 60 °C was found to be optimal. Optimization of the solid phase extraction procedure (SPE) confirmed that the application of Oasis HLB cartridges, the acidification of loading samples to pH 2 and the use of methanol as eluent gave the best absolute recoveries (ARs) of the target compounds. The following ARs of all the compounds were achieved: 58.2–106.8% in influent wastewater, 77.8–103.4% in effluent wastewater and 81.2–101.9% in surface water samples. Validation of the SPE–GC–MS method enables 13 pharmaceuticals to be determined with MDLs between 3.3 and 343.6 ng/L, depending on the analytes and matrices. GC–ECD analysis enables the determination of 6 pharmaceuticals in surface water samples with MDLs between 0.7 and 5.4 ng/L. The proposed methods were successfully used for analyzing selected pharmaceuticals in wastewaters and river waters in Poland.

Abbreviations: AR, absolute recovery; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; CF, concentration factor; EE2, 17α-ethinylestradiol; ERAs, environmental risk assessments; EtOAc, ethyl acetate; GC, gas chromatography; GC–ECD, gas chromatography with electron capture detector; GC–MS, gas chromatography–mass spectrometry; IDL, instrumental detection limit; IQL, instrumental quantification limit; IS, internal standard; LC–MS, liquid chromatography–mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MAD, microwave-assisted derivatization; MDL, method detection limit; MEC, measured environmental concentrations; MeOH, methanol; MQL, method quantification limit; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; NSAIDs, non-steroidal anti-inflammatory drugs; PFP, pentafluoropropionyl; PFPA, pentafluoropropionic anhydride; PFPOH, 2,2,3,3,3-pentafluoro-1-propanol; PNEC, predicted no effect concentration; QC, quality control; RRF, relative response factor; RSD, relative standard deviation; SIM, selected ion-monitoring; SPE, solid phase extraction; RQ, risk quotients; TGD, Technical Guidance Document; TIC, total ion chromatogram; TMCS, trimethylchlorosilane; TMS, trimethylsilyl; WWTP, wastewater treatment plant.

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1. Introduction

The pharmaceutical industry is one of the fastest growing industries; in the European Union, for example, there are over 3000 pharmaceutical products on the market (Touraud et al., 2009). Factors influencing the growth of pharmaceutical production include the aging and affluence of the population, advances in diagnosis and treatment of diseases, and the availability and advertising of non-prescription drugs. It is well known that drugs are deliberately designed to affect biochemical and physiological functions of biological systems in humans and livestock. The confirmed presence of more than 160 different pharmaceuticals in waters flowing out of sewage treatment plants (Kümmerer, 2009; Fatta-Kassinos et al., 2011; Christian et al., 2003; Aga, 2008) means they may also elicit biochemical and physiological changes in soil and aquatic organisms (Jjemba, 2006; Ding and He, 2010). For this reason recent years have witnessed increasing scientific interest in the consequences to the ecosystem and public health of the presence of such compounds in the environment (Touraud et al., 2009; Carlsson et al., 2006; Boxall et al., 2003; Fent et al., 2006; Santos et al., 2010). At present in the US and EU there are guidelines for carrying out environmental risk assessments (ERAs) of pharmaceutical compounds (EMEA, 1998; FDA-CDER, 1998; EMEA, 2005; EMEA, 2006). In particular, the study of residues of the most commonly administered pharmaceuticals and of environmental endocrine disruptors in different environmental compartments is of special concern. Non-steroidal anti-inflammatory drugs belong to the former group, estrogenic hormones to the latter one (Touraud et al., 2009; Kümmerer, 2009; Fatta-Kassinos et al., 2011; Christian et al., 2003; Aga, 2008; Carlsson et al., 2006; Fent et al., 2006). The limiting concentrations of pharmaceuticals and hormones in surface waters still await regulation by Polish and European law. On January 31st, 2012 the European Commission proposed the addition of 15 chemicals to the list of 33 pollutants that should be monitored and controlled in EU surface waters; they include diclofenac, 17α-ethinylestradiol and 17β-estradiol (COM, 2011). Although the literature describes a wide variety of analytical methods suitable for the determination of pharmaceutical residues (Kasprzyk-Hordern et al., 2007; Gómez et al., 2007; Fatta et al., 2007; Petrovic et al., 2006), there are still only a very small number of methods applicable to the simultaneous analysis of non-steroidal anti-inflammatory drugs and estrogenic hormone residues in environmental water samples (Chen et al., 2011; Yu et al., 2007; Xu et al., 2008). Moreover, the analytical procedures we are putting forward have to be very sensitive (the concentrations of target compounds in environmental complex water samples are very low, from ng to μg/L) and enable reliable quantification data to be obtained.

The methods designed for determining these pharmaceuticals in environmental samples usually employ separation techniques such as gas chromatography coupled to mass-spectrometry (GC–MS or GC–MS/MS) or liquid chromatography coupled to mass spectrometry (LC–MS or LC–MS/MS) (Kot-Wasik et al., 2007). Now that improved versions of LC–MS/MS are available, this technique is preferred for determining trace concentrations of polar pharmaceuticals in environmental analysis (Castiglioni et al., 2005; Saleha et al., 2011; Sun et al., 2009; Qin et al., 2008). Despite its many advantages, this technique has some drawbacks, for example matrix effects, especially when highly complex matrices are analyzed (Antignac et al., 2005; Kostiainen and Kauppila, 2009). Nevertheless, because of its low cost, the low limits of detection, the superior resolution, the fewer problems associated with matrix effects and the widespread availability of the relevant instruments in laboratories, GC remains an excellent tool for identifying and quantifying pharmaceuticals in environmental matrices (Kot-Wasik et al., 2007; Helenkar et al., 2010; Hu et al., 2008; Zorita et al., 2008; Nie et al., 2009; Dobor et al., 2010). GC–MS quantitative analysis generally offers better sensitivity and lower limits of detection for the investigation of complex samples (Gentili, 2007). The second inter-laboratory exercise on non-steroidal anti-inflammatory drug analysis in environmental samples (Heath et al., 2010) demonstrated that with regard to sensitivity and measurement uncertainty, GC–MS was superior to LC–MS for the analysis of drugs in matrices of greater complexity. However, there is to date no information on the application of time windows for improving the sensitivity of GC–MS measurements. It should also be noted that in almost all GC–MS methods quantification analysis is based only on retention time and the identification of two ions: the quantitative ion and the confirmation ion for each target compound. According to Directive 2002/657/EC (Commission Decision, 2002), four identification points – retention time, quantitative ion, confirmation ion, and the quantitative/confirmation ion ratio – should be applied in trace analyses to confirm the presence of the target compounds in an unknown sample. Even though electron capture detection is still frequently used in environmental laboratories, there are no reports in the literature of methods for the determination of non-steroidal anti-inflammatory drug and estrogenic hormone residues in environmental water samples using this technique. According to Directive 2002/657/EC the use of a second column with a different polarity is recommended to minimize misidentification (Commission Decision, 2002).

Taking into account all the above information the main aims of this study were:

(1) to develop SPE–GC–MS and SPE–GC–ECD methods for the simultaneous determination of non-steroidal anti-inflammatory drug and estrogenic hormone residues (Fig. 1) in environmental water samples, the identification criteria of which will fulfill the requirements of Directive 2002/657/EC;
(2) to ascertain whether the application of time windows improves the sensitivity of GC–MS measurements;
(3) to apply the proposed methods to the determination of target compounds in wastewater and river water samples collected in Poland. It should be added that although the consumption of pharmaceuticals in Poland is among the highest in the world (Willert, 2007), there is very little information on the concentrations of pharmaceutical residues in the natural environment of Poland.

2. Experimental

2.1. Chemicals and materials

Pure standards (>98%) of acetylsalicylic acid, ibuprofen, paracetamol, aminopyrine, flurbiprofen, naproxen, diflunisal, ketoprofen, diclofenac sodium salt, indomethacin, diethylstilbestrol, estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and estriol (E3), as well as 2-methylanthracene were purchased from Sigma-Aldrich (Steinheim, Germany). The derivatization reagents *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TMCS), 2,2,3,3,3-pentafluoro-1-propanol (PFPOH) and pentafluoropropionic anhydride (PFPA) were also obtained from Sigma-Aldrich. The organic solvents methanol, ethyl acetate, dichloromethane, acetone and n-hexane were supplied by Stanlab (Lublin, Poland). Freshly distilled pyridine was prepared from pyridine purchased from Lach-Ner (Neratovice, Czech Republic). 37% hydrochloric acid (HCl) of analytical grade was provided by Chempur (Piekary Śląskie, Poland). Cellulose filtration paper (0.45 μm pore size, 47 diameter) was obtained from Marchery-Nagel, Düren, Germany. The following SPE cartridges were used: LiChrolut EN, 3 mL, 200 mg (Merck, Germany); Oasis HLB, 6 mL, 200 mg (Waters, USA); Strata X, 6 mL, 200 mg and Strata C18-E 3 mL, 200 mg (both from Phenomenex, Torrance, USA). Standard stock solutions of the target compounds (0.5 mg/mL) were prepared in methanol, but 2-methylanthracene (internal standard, IS) was prepared in methylene chloride. All stock solutions were stored at −18 °C. Working calibration standard solutions were prepared by diluting standard stock solutions in appropriate amounts of methanol and stored in the dark at <4 °C.