Talanta 115 (2013) 367–373

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of steroids in the dissolved and in the suspended phases of wastewater and Danube River samples by gas chromatography, tandem mass spectrometry

Nóra Andrási, Borbála Molnár, Bernadett Dobos, Anikó Vasanits-Zsigrai, Gyula Záray, Ibolya Molnár-Perl *

Institute of Chemistry, Department of Analytical Chemistry, L. Eötvös University, H-1518, P.O. Box 32, Budapest 112, Hungary

ARTICLE INFO

Article history:
Received 5 March 2013
Received in revised form
13 May 2013
Accepted 14 May 2013
Available online 20 May 2013

Keywords:
Steroids
Dissolved
Suspended solids
GC–MS/MS
Wastewater
Danube River

ABSTRACT

In this paper, a new working approach is described for the analysis of steroids as environmental water pollutants. As novelty to the field, steroids were identified and quantified both in the dissolved and in the suspended phases, as their trimethylsilyl-(oxime)-ether derivatives, applying a recently developed tandem gas chromatographic mass spectrometric (GC–MS/MS) method, applying multiple reaction monitoring (MRM) acquisition, suitable for their quantitation in the low ng/L level, in wastewater and in Danube River samples.

In addition to the analysis of filtrates obtained by the common solid phase extraction (SPE) enrichment, even the insoluble, isolated by filtration prior to the SPE, and usually discarded part of steroids were identified and quantified, simultaneously, for the first time. For this purpose a new, time, labor, cost efficient and quantitative, ultrasound assisted extraction process was developed.

Reproducibility, reliability and practical utility of the ultrasound assisted extraction process were proved by the proportionality of the extracted suspended steroids obtained from different sample volumes: prepared from 0.5 L and 1.0 L influent wastewater, as well as from 3 L, 5 L and 10 L Danube River water samples. Steroids' concentrations, identified and quantified in suspended conditions, showed proportionality, characterized with the relative standard deviation percentages (RSD%) of analyses: varying in case of Danube River water in the range of 0.92–6.0%, with an average of 4.10% RSD, while in the case of influent wastewater in the range of 1.59–5.8%, with an average of 4.03% RSD.

Partition of steroids, between the dissolved and suspended phases of influent and effluent wastewaters and river water samples, meaning, the total amounts of steroids that the ecosystem is liable to, were defined in river water samples for the first time.

Distribution of found steroids revealed that their considerable and/or overwhelming part (relating to their total amounts), are present in suspended phases: in average, 71% from wastewater and 64% from Danube River samples.

1. Introduction

The presence of steroids in environmental waters, their hazardous effect on aquatic ecosystems is widely known; summed up also in our recent papers [1,2] related to their identification and quantification, as their trimethylsilyl (oxime) ethers, by GC–MS/MS. In the knowledge of steroids' limited solubility we extended our attention from 2010 on to the analysis of the insoluble (suspended) parts of water samples, isolated by filtration and usually, discarded, except to a very recent, excellent two dimensional gas chromatography–time-of-flight mass spectrometry based analysis of wastewater samples, only [3]. Our decision was also inspired by the European Water Framework Directive (EWFD) [4] addressed to the Member States of the European Union, i.e., "As sediments appropriate for monitoring contaminants may not always be available…" consequently, "…suspended particulate matter as an alternative to sediments …" should be monitored [5].

Steroids' analysis in the suspended phases of environmental water samples is of primary importance since even under suspended conditions they are biologically available, as proven by bioassays [6,7], by in vitro [8] and by in vivo tests [9]. Suspended steroids contribute to the total estrogenic activities of waters [6], consequently, they should be considered as an integral part of environmental samples.

Based on the overview of recent, relevant papers it turned out that distribution studies of steroids, performed simultaneously, between the dissolved and suspended phases, considering influent and effluent wastewater and river water samples are not available.

Extraction approaches proposed for the analysis of the suspended phases proved to be labor-intensive and time-consuming.

* Corresponding author. Tel.: +36 1 372 26 16, fax: +36 1 372 25 92.
E-mail addresses: perlne@chem.elte.hu, molnar.perl@gmail.com (I. Molnár-Perl).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.05.029

They involved the costly, pressurized liquid extraction (PLE) for drugs of abuse [10,11] for selected pharmaceuticals [12] and for the analysis of steroid hormone runoff from agricultural tests obtained from municipal biosolids [13]. Enrichment of antidepressant and non-steroidal anti-inflammatory drugs was performed via ultrasonic extraction, freeze-drying of samples followed by SPE cleanup [14]. Polyfluoro alkyl compounds were filtered on syringe nylon membrane polypropylene filters [15], while alkylphenol derivatives, ß-estradiol and ethinylestradiol were isolated on membrane filters [16], followed membrane filters' immersion into ultrasonic bath. Steroidal hormone profile of particular matter from Jalle d'Eysines River and from wastewater effluent was isolated applying three consecutive steps [17] (1: 0.7 μm pore size filter, 2: filters' focused microwave extraction, and 3: residue's purification with HLB and NH_2 cartridges). Estrogens of suspended materials from Scheldt estuary (Belgium – The Netherlands) were collected using flow through centrifuge followed residue's treating with accelerated solvent extraction (ASE) [18]. Xenobiotics of suspended materials – from maximum 1 L surface water – were collected on SPE disks: preventing plugging of SPE cartridges and permitting the analysis of suspended residues, dissolving them from the disks, separately [19]. The analysis of fecal sterols, one by one, from catchment waters' suspended parts consisted of a 2 h long Soxhlet extraction followed by saponification, continued by ethanol extraction and freeze-drying [20].

Concerning chromatographic methods applied for the analysis of suspended steroids liquid chromatography tandem mass spectrometry (LC–MS/MS) [10–12,14–16], GC–MS [17,20] GC–MS/MS [13,18,21] and GC × GC–TOFMS [3] were performed). (Note: due to the sample preparation of suspended steroids, performed usually with organic solvents it is obvious that this is one of the reasons why GC–MS/MS proved to be the preferable chromatographic technique compared to LC–MS/MS).

Out of the above detailed approaches, identification and quantification of suspended steroids were planned in six cases [13,16–19,21] and found effectively in four cases, only [3,13,16,18]. The amounts of steroids in suspended phases, with two exceptions [3,13], were defined as μg/g dried suspended material, without any correlation with the volume of water samples they have been prepared from.

Based on our recent experiences [1,2,22], optimum conditions for the derivatization, mass fragmentation, gradient elution and tandem mass spectrometric acquisitions for steroids, together with cholic acids, were available [23].

This work was undertaken in order

(1) to develop a simple, time, cost, labor efficient and quantitative method for the analysis of steroids existing in the suspended phases of target waters;
(2) to identify and quantify steroids in their counterpart phases of water samples at the same time (omitting storage steps), under the same analytical conditions, simultaneously;
(3) to define their distribution between the dissolved and suspended phases of water samples, expressed in ng/L or μg/L values to be comparable with their corresponding parts in dissolved condition;
(4) to convince analytical chemists that pollutants' analysis of low solubility, like steroids, is obligatory, both in suspended and dissolved phases, as they are available for living organisms, as a whole, and
(5) to prove the analytical performance and the practical utility of the optimized technique by the analysis of suspended steroids isolated from the influent and effluent samples of wastewater treatment plants (WWTPs) and from the Danube River water.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical reagent grade. Pyridine, hydroxylamine · HCl and concentrated hydrochloric acid were purchased from Reanal (Budapest, Hungary). Hexane, methanol, ethyl acetate, hexamethyldisilazane (HMDS), trifluoroacetic acid (TFA) and model compounds like, steroids were the same described in our previous papers [1,2]. Glass micro-fiber filters (GF/A 125 mm, ∅, Cat no. 1820-125) were from Whatman (Maidstone, UK). Cartridges (Oasis, HLB 6cc), for solid phase extraction (SPE), were from Waters (Milford, MA, USA). SPE extractions were performed on the Visiprep DL Vacuum Manifold for 12 samples (Cat no. 57044) from Supelco (Bellefonte, PA, USA). Ultrasonic extraction were performed on the Bandelin Sonorex (RK 52 H) apparatus (Bandelin electronic), Berlin, Germany.

2.2. Sample preparation

To separate steroids present in the suspended and in the dissolved phases of environmental waters, samples were collected in amber bottles with glass stopcocks, stored in the dark at 4 °C for maximum 18 h, before being analyzed. Appropriate aliquots (0.5 L or 1.0 L wastewater, 3 L, 5 L or 10 L of Danube River water), were taken from the (by shaking) homogenized samples and filtered on glass microfiber filter papers previously weighed with analytical precision.

2.2.1. SPE extraction of dissolved phase

Cartridges, prior to extraction were conditioned with 5 mL hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL distilled water. Filtered aliquots were adjusted to pH 4 with hydrochloric acid. Extractions were followed with a flow rate of 4–5 mL/min. Cartridges have been dried by vacuum and elutions were performed, in order of listing with 5 mL hexane, 5 mL ethyl acetate and 10 mL methanol.

2.2.2. Ultrasound assisted solvent extraction of suspended solid phase

Glass microfiber filter papers were dried overnight at ambient temperature (prior to extraction, until constant weight) then cut to 5 × 5 mm pieces and put in glass beakers (150 mL). Extractions were made with a solvent mixture of hexane/ethyl acetate/methanol 1/1/2 (v/v/v%) (applying the same solvent ratios as used for the SPE process). At first, 40 mL of solvent mixture was added to the glass beakers and sonicated for 20 min. This step was repeated two times with 20 mL solvent mixture. Solvent portions were filtered on glass micro-fibre paper, unified and treated the same way as the eluents of the SPE process.

The unified eluents of both the SPE and the ultrasonic extraction process were reduced in volume, evaporated to dryness by means of a rotary evaporator {Büchi Rotavapor R-200 and Büchi Vacuum pump V-700, both from Büchi (Flawil, Switzerland)} at 30–40 °C (further on: extract). Blank tests (reagent blanks, SPE blanks and glass filter paper blanks) were carried out with each series.

2.3. Preparation of the TMS and TMS (oxime) ether/ester derivatives

Model compounds (20–25 mg/100 mL), weighed with analytical precision were dissolved in ethanol, or in water/ethanol = 1/1 (v/v) solution and further diluted for 10 ×, 100 ×, and 1000 ×. Model solutions and the extracts were rotary evaporated to dryness at 30–40 °C. The residues were treated with 125 μL