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Estrogens determination in wastewater samples by automatic in-syringe dispersive liquid–liquid microextraction prior silylation and gas chromatography

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ABSTRACT

A new procedure for the extraction, preconcentration and simultaneous determination of the estrogens most used in contraception pharmaceuticals (estrone, 17β-estradiol, estriol, and 17α-ethynylestradiol), cataloged as Contaminants of Emergent Concern by the Environmental Protection Agency of the United States (US-EPA), is proposed. The developed system performs an in-syringe magnetic stirring-assisted dispersive liquid–liquid microextraction (in-syringe-MSA-DLLME) prior derivatization and gas chromatography (GC–MS). Different extraction (carbon tetrachloride, ethyl acetate, chloroform and trichloroethylene) and disperser solvents (acetone, acetonitrile and methanol) were tested. Chloroform and acetone were chosen as extraction and disperser solvent, respectively, as they provided the best extraction efficiency. Then, a multivariate optimization of the extraction conditions was carried out. Derivatization conditions were also studied to ensure the conversion of the estrogens to their respective trimethylsilyl derivatives. Low LODs and LOQs were achieved, i.e. between 11 and 82 ng L^{-1}, and 37 and 272 ng L^{-1}, respectively. Good values for intra and inter-day precision were obtained (RSDs ≤ 7.06% and RSD ≤ 7.11%, respectively). The method was successfully applied to wastewater samples.

1. Introduction

Endocrine-disrupting compounds (EDCs) were defined in 1996 by the European Commission as "exogenous substances that cause adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function" [1]. These have become of increasing health and environmental concern in recent years, as they can disturb the normal endocrine system and alter the normal reproduction and development of animals and also humans.

From the various groups of substances that have endocrine-disrupting properties, natural estrogens such as estrone (E1), 17β-estradiol (E2), estriol (E3) and the synthetic estrogen ethynylestradiol (EE2) have received special attention as they have been cataloged as Contaminants of Emergent Concern by the Environmental Protection Agency of the United States (US-EPA) [2]. These estrogens primarily released unintentionally to the terrestrial and aquatic environment arrive in the first step to sewage treatment plants (STPs) and from there into surrounding environmental water bodies such as rivers, lakes and the sea. Anthropogenic income of these estrogens into the environment is mainly through the widespread use of contraceptives or treatments for menopausal disorders formulated with these estrogens. Moreover, the fact that pharmaceuticals have been designed to have a biological effect even at low levels, combined with a continuous release from our society that makes them pseudo-persistent, has lead to research activities around the globe on the environmental occurrence of these compounds. For example, it has been demonstrated that these compounds induce estrogenic responses in fish at very low concentrations, such as feminization, decreased fertility or hermaphroditism [3].

Many techniques have been used for the determination of estrogens, including radioimmunoassay [4], enzyme-linked immunosorbent assay (ELISA) [5], liquid chromatography (LC) [6,7] and gas chromatography (GC) [8,9]. Gas chromatography-mass spectrometry (GC–MS) is one of the preferred techniques as it permits the simultaneous analysis of both synthetic and natural estrogenic steroids given its better capabilities for the separation and identification of these compounds. Nonetheless, sample clean-up and analytes preconcentration are usually required prior chromatographic separation especially when dealing with

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environmental or biological samples, in order to attain analyte enrichment and matrix removal. Traditionally, the most common preconcentration technique used for these compounds is solid phase extraction (SPE) [8,10]. However, dispersive liquid–liquid microextraction (DLLME) [11] is an attractive pretreatment technique that allows reduction of sample and solvents consumption, high preconcentration factors and simplicity of operation [12], also providing the analytes in a small organic droplet more suitable to be injected in chromatographic systems. This microextraction technique is based on the use of a disperser solvent, which is miscible in both water and extraction solvent. Both the disperser and extraction solvent are mixed with the aqueous sample producing a cloudy solution. This way the contact surface area between the organic droplets and the aqueous phase is increased, and better extraction efficiencies are achieved.

Modern analytical strategies tend toward automation and miniaturization with integration of sample pretreatment in the chromatographic systems as far as possible. In this context, the use of flow analysis techniques as front end to chromatographic systems has provided a number of enhanced analytical methods affording high throughput, decrease of the human exposure to hazardous chemical sample pretreatments and more environmentally friendly procedures obtained due to process downscaling [13,14]. DLLME can be automated in a lab-in-syringe system [15–17]. In-syringe-DLLME permits improving its reproducibility and accuracy, since it allows the precise handling of small volumes [18]. Furthermore, magnetic-stirring assisted (MSA) systems can be implemented on the syringe attaining homogeneous and fast mixing. Thus, by in-syringe-MSA-DLLME the sample pretreatment is simplified, reducing the steps of the analytical protocol and the intervention of the analyst.

Therefore, in this work we propose an in-syringe-MSA-DLLME for the extraction and preconcentration of four estrogens prior derivatization and GC–MS. Several extraction and disperser solvents have been studied. The applicability of the proposed system to wastewater samples is evaluated.

2. Experimental

2.1. Chemicals, solutions and wastewater samples

All solutions were prepared in GC-grade methanol (Scharlau, Barcelona, Spain) or in distilled water from a Milli-Q system (Millipore, Bedford, MA, USA) (resistivity > 1.8×10^5 Ω cm). Sodium chloride (Scharlau) was of analytical reagent grade. Several extraction and disperser solvents were evaluated, i.e. carbon tetrachloride, ethyl acetate, chloroform, trichloroethylene, acetone, acetonitrile, methanol (Scharlau) and pyridine (Sigma–Aldrich).

Estrone (E1), β-estradiol (E2), 17α-ethynylestradiol (EE2), estriol (E3) and estrone 3-methyl ether (internal standard, IS) were purchased from Sigma–Aldrich (Madrid, Spain). Stock solutions of estrogens were prepared by accurately weighing the appropriate mass of each compound and by dissolving it in methanol obtaining a final concentration of 500 mg L^{-1}. Stock solutions were kept in the dark at −20 °C. Working standard solutions containing either mixtures or individual compounds were prepared daily by dilution of the stock solutions in chloroform (for the direct injection of the solution into the gas chromatograph), or in water containing 3 mol L^{-1} NaCl and adjusted to pH 8 using NaOH 0.1 mol L^{-1}.

Different derivatization reagents were tested, i.e. N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), BSTFA with trimethylchlorosilane (BSTFA + TMSC, 1%) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), all purchased from Sigma–Aldrich.

Wastewater samples collected from the inlets (sample 1 and 2) and outlets (sample 3, 4 and 5) of the sewage treatment plant of Palma de Mallorca (Balearic Islands, Spain) were stored at 4 °C in plastic bottles. Due to the presence of solid particles, samples were centrifuged and the supernatant liquid was filtered through 0.22 μm nylon filters before analysis. They were adjusted to pH 8 using NaOH 0.1 mol L^{-1} and in a final solution containing 3 mol L^{-1} NaCl.

2.2. In-syringe-MSA-DLLME system and software

The basic element of the pretreatment system (Fig. 1) is an automatic burette (CRISON, Alella, Spain) coupled to a selection valve (SV). Multisyringe burettes can be equipped with four syringes that are moved simultaneously and unidirectional for either liquid delivering (dispense) or aspirating (pick up). Each syringe has a three-way solenoid valve (V) (N-Research, Caldwell, NJ, USA) placed at the head allowing multicommutation operation. The ratio of flow-rates between channels can be modified by using syringes of appropriate cross-sectional dimensions. The step motor shows a speed range of 1024–20 s for total displacement, corresponding to 40,000 steps. Thus, the multisyringe module allows precise handling of microliters and a wide flow rate range (0.057–30 mL min^{-1}, depending on the syringe volume 1–10 mL). In this work, the automatic burette is equipped with a 5 mL glass syringe (S) (Hamilton, Switzerland) where the dispersive liquid–liquid microextraction takes place. Besides, multisyringe burettes have four backside ports which enable the control of other instruments either directly or via a relay allowing remote software control, e.g. MSA system. The MSA system (Sciware Systems, Bunyola, Spain) is composed of a small magnetic bar (10 mm length, 3 mm diameter) placed inside the syringe, an external stirring support (Fig. S1) placed around the syringe's body, a motor (Fig. S1) connected with the external agitation support by a rubber band which forces the rotation of the external agitation support, and a circuit that controls both the start/stop and the motor revolutions (Fig. S1) through one of the multisyringe outputs. The external stirring support is a ring (14 mm i.d., 30 mm o.d.) with two small magnets facing each other creating a rotating magnetic field around the syringe's body. When the motor is switched on, the ring starts to rotate and with it the magnetic bar inside the syringe, mixing the phases.

The manifold is composed of poly(tetrafluoroethylene) (PTFE) tubes of 0.8 mm i.d. for reagents and sample aspiration.

A picture of the in-syringe-MSA-DLLME system is shown in Fig. S1. A multisyringe holder was used to place the burette module upside down (Fig. S1) to have the organic droplet of the extraction solvent, which is denser than water, at the head of the syringe. This way the organic droplet can be dispensed to a fraction collector while the aqueous phase stays in the syringe. The MSA system is mounted on the syringe to achieve homogeneous and rapid mixing of phases and increase the extraction efficiency.

Besides, the "off" position of the syringe is connected to the central port of a selection valve containing the reagents used to carry out the DLLME and the cleaning of the system on its peripheral ports, as follows: water (port 1), cleaning solution (acetone:H_2O 10:90, v/v) (port 2), autosampler (port 3), chloroform (extraction solvent, port 4), acetone (disperser solvent, port 7) and waste (port 8), as can be seen in Fig. 1.

A fraction collector, equipped with amber chromatographic vials containing 300 μL inserts, is connected to the syringe on its "on" position.

Instrumental control was done with the software AutoAnalysis 5.0 (Sciware Systems). This software based on DLLs (dynamic link libraries) at 32 bits is very versatile and simple permitting the implementation of whatever instrumentation needed without further modifications.