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Liquid chromatography with diode array detection and multivariate curve resolution for the selective and sensitive quantification of estrogens in natural waters

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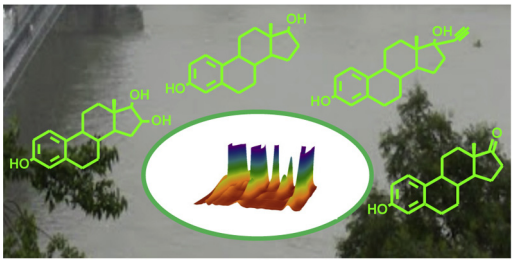
HIGHLIGHTS

- Potent endocrine disruptors are easily analyzed using non-sophisticated instrumental.
- Selectivity is successfully achieved by applying multivariate curve resolution.
- Quantification in real samples is accomplished using green-chemistry principles.

GRAPHICAL ABSTRACT



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ABSTRACT

Following the green analytical chemistry principles, an efficient strategy involving second-order data provided by liquid chromatography (LC) with diode array detection (DAD) was applied for the simultaneous determination of estriol, 17β-estradiol, 17α-ethinylestradiol and estrone in natural water samples. After a simple pre-concentration step, LC–DAD matrix data were rapidly obtained (in less than 5 min) with a chromatographic system operating isocratically. Applying a second-order calibration algorithm based on multivariate curve resolution with alternating least-squares (MCR-ALS), successful resolution was achieved in the presence of sample constituents that strongly coelute with the analytes. The flexibility of this multivariate model allowed the quantification of the four estrogens in tap, mineral, underground and river water samples. Limits of detection in the range between 3 and 13 ng L^{-1}, and relative prediction errors from 2 to 11% were achieved.

Abbreviations: ANDR, Androgens; BPA, Bisphenol A; CF, Cigarette filter; CORT, Corticosteroids; CP-CPE, Co-precipitation assisted cloud point extraction; DAD, Diode array detector; DERIV, Derivatization; DES, Diethylstilbestrol; DHS, Dihydrostilbestrol; DIS, Dienestrol; DSW, Discharging sites water; DLLME, Dispersive liquid–liquid microextraction; ECF, Estrogen conjugated forms; ENNFM, Electrospun nylon6 nanofibrous membrane; FD, Fluorescence detector; FW, Fishpond water; GC, Gas chromatography; HF-MMLLE, Hollow-fiber microporous membrana liquid–liquid extraction; LC, Liquid chromatography; LVI, Large volume injection; LOD, Limit of detection; LOQ, Limit of quantification; LW, Lake water; MES, Mestranol; MIP, Molecularly imprinted polymer; MM-SPE-MPS, Magnetic-mediated solid-phase extraction micro-particle sorbent; MS, Mass spectrometry; MS/MS, Tandem mass spectrometry; MW, Mineral water; OP, Octylphenol; PPs, Pharmaceutical products; PROG, Progestagens; RW, River water; SBSE, Stir bar sorptive extraction; SPE, Solid-phase extraction; SPW, Spring water; SW, Surface water; SWW, Sewage water; TW, Tap water; UPLC, Ultra performance liquid chromatography; US, Ultrasonication; UW, Underground water; W, Water; WW, Wastewater; WWTPI, Wastewater treatment plant influent; WWTPE, Wastewater treatment plant effluent.

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1. Introduction

Estrogens are steroidal hormones which play an important role in human physiology, including, among others, reproductive female functions, modulation of tissues growth and bone integrity [1]. The three major naturally occurring estrogens 17β-estradiol (E2), estriol (E3), estrone (E1), and the synthetic estrogen 17α-ethynylestradiol (EE2), widely used in contraceptive pills, are the main contributors to the total estrogenicity in waterways [2]. In fact, active estrogen forms are constantly excreted into the aquatic environment and may cause serious health effects in animals and humans, especially in regards to reproduction [3,4]. Since estrogens are the most potent endocrine disrupting compounds (EDCs) which, in turn, are defined as chemicals that may negatively interfere with the endocrine system of humans and wildlife [5], it is not surprising that continuous efforts are devoted to find sensitive and selective methods for their quantification in natural samples.

Complete overviews on the development of the analysis of steroidal hormones in environmental matrices can be found in the literature [2,6–9]. As indicated in the latter works, liquid chromatography (LC) and gas chromatography (GC) followed by detection with mass spectrometry (MS) or tandem MS are the most employed analytical tools to determine estrogens and other EDCs in many different water sources. However, this instrumental is sophisticated and usually requires important capital investment and personnel training. In addition, because of the complexity of certain environmental matrices, a great effort must be devoted to sample preparation, with the additional risk of loss of analytes during extensive extraction and clean up steps [2].

In such situations, multivariate data analysis can be used for improving the selectivity of data collected in less expensive equipment by mathematical means. Specifically, multi-way calibration based on higher-order data (e.g., second-order LC-diode array detection or LC–DAD data) allows the prediction of analyte concentrations in samples containing potential interferences. This useful property, named the "second-order advantage" [10,11], avoids the requirement of interference removal, with the concomitant saving of experimental work and analysis time. Further, toxic organic solvents frequently used for clean up procedures are prevented.

As part of a program devoted to the development of high performance methods within the framework of green chemistry principles [12,13], the use of isocratic LC–DAD data coupled to second-order multivariate calibration, was proposed as a useful approach for rapid and selective detection of estrogens. The LC–DAD matrix data were obtained in short times and using minimal solvent volumes. In the first phase, determinations were carried out in solutions containing the studied estrogens and additional compounds selected as potential interferences. In the second step, the proposed methodology was applied to real samples.

Two issues had to be taken into account when choosing the appropriate algorithm to process the present data: (1) component profiles in the elution time mode usually change in shape and/or position from sample to sample, and (2) the absorption spectra of the studied analytes are very similar. These problems were overcome applying the so-called extended multivariate curve resolution-alternating least-squares (MCR-ALS) algorithm [14], using specific strategies which will be discussed below. It is important to remark that this algorithm has been proposed for handling different types of chromatographic challenges [15,16] and, in the present report, it was successfully used for improving both the sensitivity and selectivity of the applied chromatographic method.

2. Experimental

2.1. Instrumentation

Chromatographic runs were performed on an HP 1200 liquid chromatography (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump, a manual injector fitted with a 50 μL loop and a diode array UV–visible detector set at a wavelength range from 200 to 330 nm. Three C18 chromatographic columns provided by Agilent Technologies (Santa Clara, CS, USA) were checked: Zorbax Eclipse XDB (4.6 mm × 150 mm, 5 μm particle size), Poroshell 120 EC (4.6 mm × 100 mm, 2.7 μm particle size), and Poroshell 120 EC (4.6 mm × 50 mm, 2.7 μm particle size). The data were collected using the software HP ChemStation for LC Rev.HP 1990–1997.

2.2. Reagents and solutions

All reagents were of high-purity grade and used as received. Estriol, 17β-estradiol, 17α-ethynylestradiol, estrone, naproxen (NX), drospirenone (DRSP), norethisterone acetate (NETA), androstenedione (AED), and diazepam (DZM) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, USA).

Methanol stock solutions of estrogens and potential interferents were prepared and stored in dark flasks at 4 °C. A set of five calibration solutions by duplicate (10 samples) containing E3, E2, EE2 and E1, each equally spaced in the range 0–110 ng mL^{-1}, were prepared by measuring appropriate aliquots of standard solutions, placing them in 2.00 mL volumetric flasks, evaporating the solvent with a nitrogen stream, and completing to the mark with the solvent mixture used as mobile phase. A test set of additional 19 samples, containing the four analytes and also NX, DRSP, NETA, AED, and DZM, were similarly prepared. The concentrations of each potential interferent ranged between 70 and 340 ng mL^{-1}, and were randomly selected.

2.3. Real samples

Because the evaluated water samples (tap, mineral, underground and river waters) did not contain the studied estrogens at levels higher than the attained detection limits, a recovery study was carried out by spiking them with standard solutions of E3, E2, EE2 and E1, obtaining concentration levels in the range 10–100 ng L^{-1}. These water samples were prepared in duplicate and, with the exception of river water, they underwent no previous treatment. River water was collected from Paraná River (Rosario, Argentina) in a 4 L amber glass bottle rinsed with methanol and Milli-Q water, stored at 4 °C immediately after sampling, and analyzed as soon as possible (within 48 h after collection) in order to avoid addition of chemical preservatives. River samples were filtered twice prior to injection: first through a paper filter and then through a cellulose acetate 0.2 μm pore size filter.

The solid-phase extraction (SPE) procedure was carried out using SPE disks Empore Octadecyl C18 (Supelco, Bellefonte, PA, USA). The membrane was conditioned with 1 mL of methanol and then the extraction of 250 mL of the sample was carried out in approximately 12 min per sample. This flow rate is in the optimum range for maximum breakthrough volume (10–30 mL min^{-1}) [17]. The retained estrogens were eluted with methanol, and this solvent was evaporated with a nitrogen stream. Then, the solutions were reconstituted with 0.200 mL of mobile phase and subjected to the same chromatographic analysis as the test samples. In this way, the preconcentration factor was 2500.