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Multi heart-cutting two-dimensional liquid chromatography–atmospheric pressure photoionization-tandem mass spectrometry method for the determination of endocrine disrupting compounds in water

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ABSTRACT

The multi-heart-cutting two-dimensional liquid chromatography–tandem mass spectrometry method using atmospheric pressure photoionization has been developed and successfully validated for the determination of nine endocrine disrupting compounds in river water. The method is based on the use of two different reverse-phase columns connected through a six-port two-position switching valve equipped with a 200 μl loop. An orthogonal separation was achieved by proper selection of stationary phases, mobile phases, and the use of a gradient elution in both dimensions. The method shows excellent performance in terms of accuracy (86.2–111.1%), precision (intra-batch: 6.7–11.2%, inter-batch: 7.2–13.5%), and sensitivity (1.2–7.1 ng l^{-1}). Twenty real samples collected from the Loučka and the Svratka rivers were analyzed, the studied compounds were found in all Svratka samples (9.7–11.2 ng l^{-1} for β-estradiol, 7.6–9.3 ng l^{-1} for estrone, and 24.6–38.7 ng l^{-1} for bisphenol A).

1. Introduction

Endocrine disrupting compounds (EDCs) have become crucial emerging contaminants due to their presence in environmental waters giving rise to concerns over possible adverse effects to wildlife and humans. Various groups of chemicals, natural or man-made, have endocrine-disrupting activity and hence they are of environmental relevance. These chemicals include estrogens, pesticides, dioxins, certain polychlorinated biphenyls, and plasticizers such as bisphenol A or alkylphenol ethoxylate surfactants and their degradation products.

In 2009, the U.S. EPA published the final contaminant candidate list-3 (CCL-3) which specifies the priorities for regulatory decision making and information collection. The final CCL-3 now includes several natural and synthetic estrogens such as α-estradiol, β-estradiol, 17α-ethinylestradiol, estriol, and estrone [1]. Consequently, a new EPA method was introduced in 2010 for measuring 7 hormones in drinking water (estriol, β-estradiol, 17α-ethinylestradiol, estrone, testosterone, 4-androstene-3,17-dione, and equilin): EPA Method 539, determination of hormones in drinking water by solid phase extraction (SPE) and liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) [2].

The off-line SPE and liquid chromatography–tandem mass spectrometry represent the gold standard for analysis of estrogens and xenoestrogens in various matrices, as can be extracted from the reading of some revision articles on this subject [3–6]. Substantial improvements in EDCs analysis were achieved by using on-line connection of SPE to liquid chromatography. Petrovic et al. successfully used column switching LC–MS for an integrated sample clean-up and analysis of EDCs in sediment samples. A restricted access material (RAM) pre-column with various modifications (C_4, C_8 or C_{18}) was employed [7]. In contrast, Watanabe et al. successfully integrated a surface modified MIP pre-column as a pretreatment device with the on-line LC–MS detection of E2 in water samples [8]. Salvator et al. explored the possibility of on-line sample cleanup with derivatization (utilizing dansyl chloride) directly on the solid-bed of a pre-column. In the optimized protocol, LOQs of 1 ng l^{-1} were achieved with only 1 ml of sample. Estradiol, estrone, and 17α-ethynylestradiol were under study [9]. Instead of commonly used ESI-MS, Viglino et al. employed atmospheric pressure photoionization-mass spectrometry (APPI-MS) for the detection of estrogens and progestogens during a fully automated on-line SPE method development. Surprisingly, no dopant was used and the authors state that comparative APPI experiments with a dopant (toluene and acetone) showed no improvement in sensitivity [10]. The extensive study of both separation systems (ultra-performance LC with or without post-column split, employing a mixed-mode column and on-line SPE) and MS ionization modes (ESI, APCI, APPI, and APCI/APPI) were performed by Lien et al. The best

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combination of LC systems and ion sources was UPLC coupled with an ESI source operated in positive mode when analytes were detected as appropriate dansylated derivatives (unfortunately, an ionization efficiency of native estrogens in positive and negative mode was not compared) [11].

In the solid-phase extraction, in either on-line or off-line mode, the first column serves only as a trap; therefore, the method suffers from several drawbacks, such as poor selectivity/efficiency of separation, resulting in co-extraction of interfering matrix components which can negatively affect the final qualitative and quantitative analysis. Consequently, higher demands are placed on the separation efficiency of HPLC. The lack of selectivity can sometimes be improved by using an analyte-selective sorbent based on immunoaffinity extraction [12,13] or a molecularly imprinted polymer [14,15]. An alternative approach is represented by the use of the high resolving power of multidimensional chromatography, preferably in on-line connection, which effectively utilizes the selectivity and separation efficiency available from both chromatographic columns.

In my previous work [16], the heart-cutting 2D-chromatography–tandem mass spectrometry method was developed to improve separation of four estrogens from the sediment matrix. The method was based on the direct transfer of only one fraction from the first dimension to the second. Pascoe et al. used a similar configuration of 2D-LC and demonstrated that 2D-LC–MS single-compound analysis (i.e. one compound per injection) can be more effective in reducing matrix-related signal suppression than multiple-compound analysis [17]. The aim of this work is to develop a method for the determination of several endocrine disrupting compounds in water based on multi-heart-cutting two-dimensional liquid chromatography–tandem mass spectrometry using atmospheric pressure photoionization source. Four natural or synthetic estrogens (β-estradiol, 17α-ethinylestradiol, estriol, and estrone), plastics-derived xenoestrogen (bisphenol A), and degradation products of alkylphenol ethoxylate surfactants (linear and branched octyl- and nonylphenol) were under study.

2. Experimental

2.1. Chemicals

Estriol (E3) (min. 99.7%), estrone (E1) (min. 99.3%), β-estradiol (β-E2) (min. 98.9%), 17α-ethynylestradiol (EE2) (min. 99.3%), and 4-*n*-nonylphenol (*n*-NP) (99.9%) were purchased from Fluka (Steinheim, Germany). 4-*tert*-Octylphenol (*t*-OP) (99%) was obtained from Supelco (Bellefonte, PA, USA). Bisphenol A (BPA) (99%), 4-octylphenol (OP) (min. 99%), and nonylphenol (NP) (analytical standard, technical mixture of ring and chain isomers) were obtained from Aldrich (Steinheim, Germany). Each compound was dissolved in MeOH to make a stock solution at a concentration of 50 μg ml^{-1} and was stored at −10 °C. Working solutions were prepared by mixing and diluting the stock solutions with methanol prior to use.

Acetonitrile (ACN) and methanol (MeOH) were obtained from Riedel-de Haën (LC/MS CHROMASOLV® grade, Seelze, Germany). Anisole (purum, 99%) was purchased from Fluka (Steinheim, Germany). Formic acid and ammonium hydroxide solution were purchased from Fluka Chemie (puriss p.a., eluent additive for LC–MS grade, Buchs, Switzerland). Demineralized water was purified using a Milli-Q-RG (Millipore, Bedford, MA, USA).

2.2. Sample collection and preparation

Water samples were collected at 20 randomly chosen sites along the river Svratka (Brno, Czech Republic) and a relatively unspoiled area of the river Loučka (the Bohemian-Moravian Highlands, Czech Republic) in September of 2011. The samples were preserved with formaldehyde (1%, v/v) to prevent biological degradation and stored in amber glass bottles at 4 °C. The Loučka samples were used in all SPE and 2D-LC preliminary experiments.

Oasis WAX (150 mg, weak anion exchanger) cartridges (Waters, Milford, MA, USA) were used for a concentration and a clean-up step of the water samples using an Alltech (Deerfield, IL, USA) manifold system. The cartridges were conditioned with 5 ml of MeOH and 5 ml of water prior to use. Thereafter, the samples (500 ml) were passed through the cartridge at a rate of 10 ml min^{-1}. The cartridge was rinsed with 5 ml of 5% formic acid in water:MeOH (80:20, v/v), followed by 5 ml of 5% ammonium hydroxide in water:MeOH (80:20, v/v). All retained analytes were eluted with 5 ml of MeOH. After SPE, solvents were evaporated under a gentle stream of nitrogen, the residue was dissolved in 75 μl of MeOH, filtered through a 0.45 μm nylon membrane filter and a 25 μl aliquot together with 10 μl of water was injected into the chromatographic system.

2.3. Heart-cutting-2D-HPLC

A schematic of the column configuration and valve switching is shown in Fig. 1. After injection of the sample onto the column 1, the eluted fraction containing analyte(s) is trapped in the loop. By switching the valve, this fraction is then transferred onto the column 2 and separated. The chromatographic separation was performed using an Agilent 1100 chromatographic system (Agilent, Waldbronn, Germany, equipped with a vacuum degasser, a quaternary pump, an autosampler, a column thermostat, and a diode-array detector). The second mobile phase pump was a Knauer HPLC 100 pump (Dr. Ing. Herbert Knauer GmbH Berlin, Germany, equipped with a vacuum degasser). Two analytical columns were connected via an electronically controlled six-port two-position switching valve equipped with a 200 μl loop.

A reverse-phase Luna C5 (100 mm × 2.0 mm, 5 μm particle size, column 1) (Phenomenex, Torrance, CA, USA) column equipped with a HPLC Ultra Column in-line filter (porosity 0.5 μm, Phenomenex,

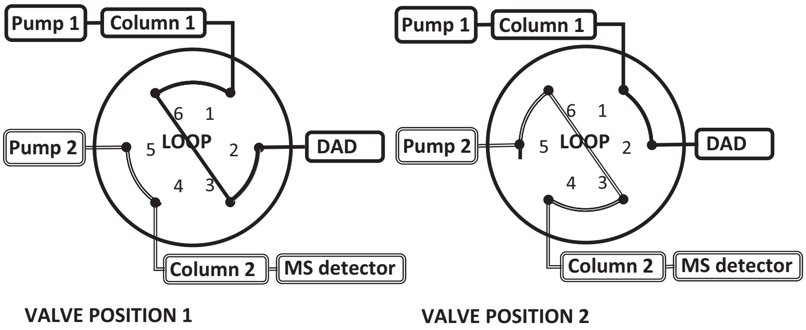

Fig. 1. Schematic of the heart-cutting 2D-HPLC-MS/MS system.