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Simultaneous screening of estrogens, progestogens, and phenols and their metabolites in potable water and river water by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

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ABSTRACT

In this study, a method employing ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) was developed to simultaneously screen for 36 endocrine-disrupting chemicals (EDCs; e.g., estrogens, progestogens, phenols, and their metabolites) both in potable and river water. From the selected compounds, 21 target compounds, for which reference standards were available, were used as model compounds for method development and optimization. The other target compounds, for which reference standards were unavailable, were investigated in post-target analysis on the basis of their theoretical molecular masses. The solid-phase extraction and chromatographic separation steps were optimized. For this method, limits of detection for the target compounds were less than 0.72 ng L^{-1}, and the overall recoveries varied between 46% and 134% with relative standard deviations ranging from 7% to 35%. The mass errors between theoretical and experimental mass for all resulting precursor and characteristic fragment ions ranged from −1.9 to 2.8 mDa. The method developed was successfully used to analyze the composition of potable and river water in Shanghai City; in addition, some compounds of interest (estriol, estrone, and bisphenol A) were identified accurately. Further, a post-target analysis was performed and an estrogen metabolite was hypothesized in the water samples due to the excellent sensitivity of the method in full-spectrum acquisition mode and the valuable accurate mass information in MS and tandem MS mode. Therefore, UPLC-Q-TOF-MS has proven to be a powerful technique for wide-scope screening and identification of relevant EDCs in environmental water sources.

1. Introduction

Endocrine-disrupting chemicals (EDCs) are an extensive group of natural and anthropogenic compounds that can act as hormone-like substances to influence the regulation of development and growth in animals and humans. In addition, EDCs have been associated with prostate cancer, reproductive tract disorders, low sperm counts, and breast cancer [1–5]. In the past twenty years, various types of EDCs have been found in the environment [6–10]. Therefore, the effect of environmental exposure of EDCs on population health has raised concerns, and a comprehensive investigation of the environmental exposure level of EDCs became a pressing issue [11,12].

Estrogenic steroid compounds are of particular interest because they possess the greatest potency of all estrogenic compounds and also occur ubiquitously in the environment [13,14]. It has been well documented that both estrogen and progestogen can modulate hormonal effects at concentrations measured in nano- or picograms per liter [15–18]. These compounds, predominantly derived from human or livestock sources [19–21], enter the environment directly or through effluents from wastewater treatment plants (WTPs). These compounds have been detected in the environment in concentrations that were sufficient to induce active hormonal effects [22,23]. Alkylphenols, such as 4-nonylphenol (4-NP) and 4-octylphenol (4-OP), and bisphenol-A (BPA) are typical examples of EDCs [24]. Although their estrogenic potency is three orders of magnitude lower than that of a steroid hormone [25], they still garner significant attention due to their widespread use in domestic products [26]. Alkylphenols and BPA in the environment are mainly derived from the degradation or direct release of the corresponding products, such as alkylphenol ethoxylates (APEOs), polycarbonate plastic resin, and epoxy resin [27].

Identification and determination of EDCs is a challenging task because of the extremely low levels at which they are present in the environment (nano- or picograms per liter). Previously, gas chromatography with mass spectrometric detection (GC–MS) was most commonly used for analysis of EDCs [28,29]. However, due to the need for derivatization and in consideration of the lower sensitivity of GC–MS when compared with liquid chromatography coupled with triple quadrupole mass spectrometry (LC–QqQ MS), LC–QqQ MS has gradually replaced GC–MS for

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analysis of EDCs in environmental samples [30–34]. However, confirmation and sensitivity are compromised when using LC–QqQ MS because the qualitative information required to support the structural elucidation of analytes is lost in the multiple reaction monitoring (MRM) mode and, in the full-scan mode, the qualitative information can be obtained, but with a loss of sensitivity. Hybrid quadrupole time-of-flight mass spectrometry (Q-TOF-MS) can resolve this limitation by its ability to provide accurate mass measurements of full-product ions, thus assuring accurate identification of analytes, and the sensitivity obtained is better than that of QqQ MS in the full-scan mode. UPLC overrides conventional liquid chromatography as it generates narrow peaks, facilitates resolution of analytes and matrix interference, and shortens chromatographic runs [35]. Combining UPLC with Q-TOF-MS offers a method with high chromatographic resolution and exact mass measurement for MS and MS/MS; therefore, it provides significant advantages with regard to selectivity, sensitivity, accuracy, and speed for rapid screening for organic contaminants in complicated environmental samples. However, only a few reports in the literature have focused on the screening of organic contaminants in the water environment by using UPLC-Q-TOF-MS [36–40].

The objective of this study was to develop a sensitive and accurate screening method for estrogens, progestogens, and phenols in potable and river water by using UPLC-Q-TOF-MS and to demonstrate its reliability in identifying target compounds at low levels in complicated water samples. To these authors' knowledge, the analytical method described in this study is the first to use UPLC-Q-TOF-MS to simultaneously screen for estrogens, progestogens, and phenols in environmental water samples. This method was successfully applied to screen for all the three groups of estrogens, progestogens, and phenols in potable and river water in Shanghai City (China).

2. Materials and methods

2.1. Chemicals

The standard reference samples (>95% in purity) of nine estrogens, seven progestogens, and five phenols were purchased from Sigma-Aldrich (Shanghai, China). Individual stock solutions of these reference standards were prepared to a concentration of 1 mg mL^{-1} in methanol and stored at −20 °C until further use. Working standard mixtures of the test compounds were prepared in a solution of acetonitrile (ACN) and water (1:9, v/v) at different concentrations by appropriate dilution of the individual stock solutions. Some characteristics of the 21 target compounds are listed in Table 1.

The LC–MS grade reagents used in this study include: water and ethyl acetate from J.T. Baker (Phillisburg, NJ, USA); methanol (MeOH) and ACN from Fisher (Fair Lawn, NJ, USA); and acetic acid, ammonium hydroxide solution, and ammonium formate from Sigma-Aldrich. Deionized water was obtained from Milli-Q-Plus (Millipore, Bedford, MA, USA).

2.2. Sample collection and preservation

Potable water samples were collected from four local residential areas, representative of four different potable water-supply plants in Shanghai City, located in the Yangtze River Delta along China's eastern coast. River water samples were collected from four different sampling sites along the Huangpu River, which flows across Shanghai City into the Yangtze River. All the water samplings were performed in March 2011.

Table 1
Characteristics of 21 target compounds and 15 post-target compounds.

Group of compounds	Name	Abbreviation	CAS registry number	Production way	Molecular formula	Molecular weight
Target estrogens	Estriol	E_3	50-27-1	Natural	$C_{18}H_{24}O_3$	288.1725
	β-Estradiol	β-E_2	50-28-2	Natural	$C_{18}H_{24}O2$	272.1776
	α-Estradiol	α-E_2	57-91-0	Natural	$C_{18}H_{24}O_2$	272.1776
	Equilin	EQ	474-86-2	Natural	$C_{18}H_{20}O_2$	268.1463
	17α-Ethinylestradiol	EE_2	57-63-6	Synthetic	$C_{20}H_{24}O_2$	296.1776
	Estrone	E_1	53-16-7	Natural	$C_{18}H_{22}O_2$	270.1620
	Diethylstilbestrol	DES	56-53-1	Synthetic	$C_{18}H_{20}O_2$	268.1463
	Dienestrol	DE	84-17-3	Synthetic	$C_{18}H_{18}O_2$	266.1307
	Hexestrol	HES	84-16-2	Synthetic	$C_{18}H_{22}O_2$	270.1620
Target phenols	Bisphenol A	BPA	80-05-7	Synthetic	$C_{15}H_{16}O_2$	228.1150
	4-tert-Butylphenol	4-t-BP	98-54-4	Synthetic	$C_{10}H_{14}O$	150.1045
	4-tert-Octylphenol	4-t-OP	140-66-9	Synthetic	$C_{14}H_{22}O$	206.1671
	4-n-Octylphenol	4-n-OP	1806-26-4	Synthetic	$C_{14}H_{22}O$	206.1671
	4-n-Nonylphenol	4-n-NP	25154-52-3	Synthetic	$C_{15}H_{24}O$	220.1827
Target progestogens	Norethindrone	NTD	68-22-4	Synthetic	$C_{20}H_{26}O_2$	298.1933
	17-Hydroxyprogesterone	17-HPT	68-96-2	Natural	$C_{21}H_{30}O_3$	330.2195
	21-Hydroxyprogesterone	21-HPT	64-85-7	Natural	$C_{21}H_{30}O_3$	330.2195
	D(−)-Norgestrel	NGT	797-63-7	Synthetic	$C_{21}H_{28}O_2$	312.2089
	Chlormadinone-17-acetate	CMA	302-22-7	Synthetic	$C_{23}H_{29}ClO_4$	404.1754
	Megestrol-17-acetate	MTA	595-33-5	Synthetic	$C_{24}H_{32}O_4$	384.2301
	Progesterone	PGT	57-83-0	Natural	$C_{21}H_{30}O_2$	314.2246
Post-target estrogens	16-Epiestriol	16-epiE_3	547-81-9	Natural	$C_{18}H_{24}O_3$	288.1725
	17-Epiestriol	17-epiE_3	1228-72-4	Natural	$C_{18}H_{24}O_3$	288.1725
	16α-Hydroxyestrone	16α-OHE_1	566-76-7	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Methoxyestrone	2-$MeOE_1$	362-08-3	Natural	$C_{19}H_{24}O_3$	300.1725
	4-Methoxyestrone	4-$MeOE_1$	58562-33-7	Natural	$C_{19}H_{24}O_3$	300.1725
	3-Methoxyestrone	3-$MeOE_1$	5976-63-6	Natural	$C_{19}H_{24}O_3$	300.1725
	2-Hydroxyestrone	2-OHE_1	362-06-1	Natural	$C_{18}H_{22}O_3$	286.1569
	4-Hydroxyestrone	4-OHE_1	3131-23-5	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Methoxyestradiol	2-$MeOE_2$	362-07-2	Natural	$C_{19}H_{26}O_3$	302.1882
	4-Methoxyestradiol	4-$MeOE_2$	26788-23-8	Natural	$C_{19}H_{26}O_3$	302.1882
	16-Ketoestradiol	16-ketoE$_2$	566-75-6	Natural	$C_{18}H_{22}O_3$	286.1569
	2-Hydroxyestradiol	2-OHE_2	362-05-0	Natural	$C_{18}H_{24}O_3$	288.1725
Post-target progestogens	Medroxyprogesterone acetate	MPA	71-58-9	Synthetic	$C_{24}H_{34}O_4$	386.2457
	6α-Methyl-11β-hydroxyprogesterone	MHPT	2668-66-8	Natural	$C_{22}H_{32}O_3$	344.2351
	17α,20β-Dihydroxy-4-pregnene-3-one	DPO	1662-06-2	Natural	$C_{21}H_{32}O_3$	332.2351