ON-LINE PRECONCENTRATION AND HIGH RESOLUTION FULL SCAN MASS SPECTROMETRY METHOD FOR THE ANALYSIS OF HORMONES AND BISPHENOL A IN SURFACE WATERS

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Introduction

Among contaminants of emerging concern (CEC) in water, hormones, nonylphenol and bisphenol A hascome under the spotlight in recent decade in terms of biological effect, occurences and persistency[1,2].The main analytical stream of this compounds are based on solid-phase extraction and liquid chromatography with tandem mass spectromatry (SPE-LC-MS/MS)[3,4]. This proven method has been provided sensitive and reliable data. However, there are needs for improvement both sample preparation and mass spectrometry. Because conventional SPE needs large sample volume, time and labors and cartridge, there is limited monitoring data for the environment. For mass spec., target monitoring prividing accurate data, but this method cannot provide full scan information. Sometimes, additional analysis is required to obtain family group of compounds[5]. For this, sample preparation of LC-MS/MS run shold be done repeatedly.

In this paper, we report a fast and accurate analytical method by online preconcentration with high resolution / accurate mass (HR/AM) spectrometry[6] for hormones, nonylphenol and bisphenol A in water.

Materials and methods

Target of this study were : 17-β-estradiol, ethinyl estradiol, estriol, equilin, estrone, bisphenol A and nonylphenol. Samples were prepared from a stock solution of hormones and phenols mixture in methanol(O2Si and BK Scientific). Calibrationsolutions were prepared from the stock solutions, resulting in10 levels of hormonesand phenolsfor analysis.The concentration range varied for eachcompound, but were in the approximate range of 10ppt to 2 ppb.For online preconcentration liquid chromatography, EQuan MAX (Thermo Scientific) model was used. This system is based on column switching technique[7,8]. Dual column are consist of loading(Hypersil GOLD aQ,20 x 2.1 mm, 12μ)and analytical (Hypersil GOLD C18, 50 x 2.1 mm, 3μ) column. Once, the first HPLC pump is use to transfer 1mL sample to the loading column for apprx. 1min, valve switching is started to back-flush the loading column onto the analytical column. Therefore, manual sample preparation is not needed. The mobile phases were water(A) and methanol (B) containing 0.1% ammonia. The gradient program for both pumps was optimized. The total run time is 8 minutes.

Orbitrap Exactive model (Thermo Scientific) with positive electrospray ionization was used in present study. Theresolution for the full scan experiment was set at 50,000 and themass range 100-350 amu was monitored. Data and spectral confirmation was analyzed using Xcalibur 2.1 software.Limits of detection(MDLs), quantitation(LOQs), precision, recovery etc. were validated for above methods.

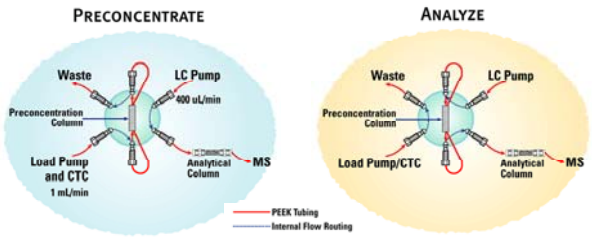

Figure 1.Column switching based online preconcentration

Results and discussion

The extracted chromatogram of target hormones and phenols are shown in Figure 1. In this experiment, the compounds tended to lose a proton to form the [M-H]- species. Due to the 2nd LC pump was consisted of ultra-pressure (18,000 psi), sharpen chromatogram and fast retention time were achieved. Unfortunately, the

sensitivity of nonylphenol was poor. HR full scan data including detected m/z and mass accuracy are summarized in Table 1. The m/z of an ion was measured to within 5ppmallows the determination of a unique elemental composition. Therefore, HR full scan data can closelymatch the expected/theoretical mass with the observed massgreatly increases the reliability of identification.

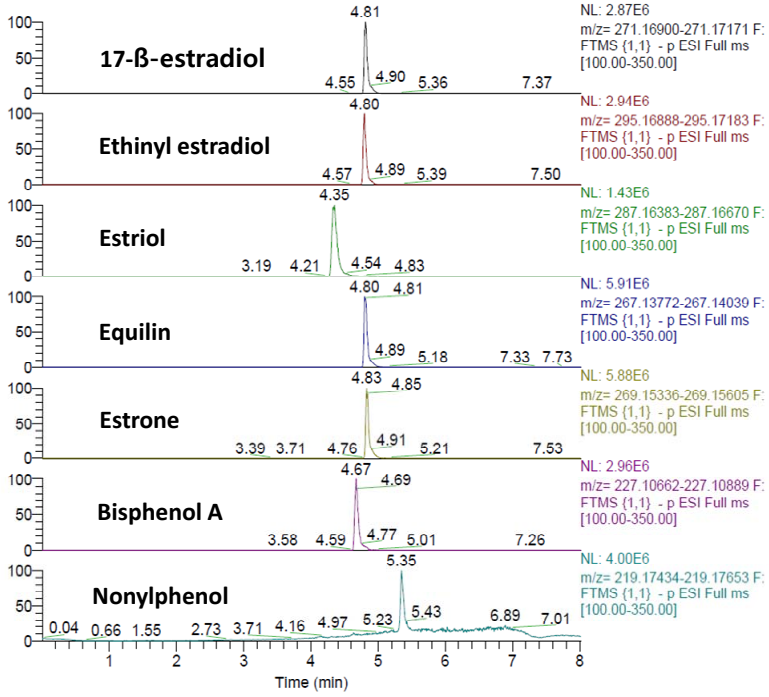

Figure 1. Extracted ion chromatogram of target hormones and phenols based on accurate mass with a mass window of 5 ppm

Table 1.Retention time, detected m/z and mass accuracy of target hormones and phenols.

Compounds	Formula	Actual RT	Detected m/z	Mass accuracy (ppm)
17-β-estradiol	C18H24O2	4.81	271.17047	0.4
Ethinyl estradiol	C20H24O2	4.80	295.17044	0.3
Estriol	C18H24O3	4.35	287.16565	1.3
Equilin	C18H20O2	4.80	267.13943	1.4
Estrone	C18H22O2	4.83	269.15488	0.6
Bisphenol A	C15H16O2	4.67	227.10744	-1.4
Nonylphenol	C15H24O	5.35	219.17493	-2.3

Calibration curves using HPLC water were tested in order to assess linearity range. Injection volume was 1 mL with 10 pg/mL to 2 ng/mL samples. For all compounds, good linearity above 0.995 was obtained except for nonylphenol, showing background noise in low concentration ranges. This shows that online preconcentration with HR full scan data providing available performance without internal calibration standard references.

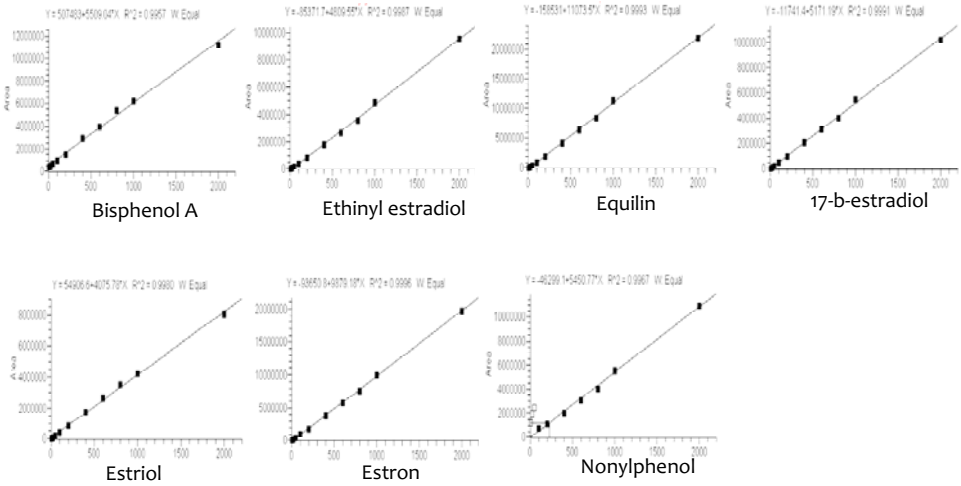

Figure 2.Linearities with 10 concentration ranges between 10 pg/mL to 2 ng/mL based on external calibration

Table 2. QA/QC result of online preconcentration with HR full scan MS method using different spiking levels.

Compound	R2	Spiking Conc.	MDL(pg/mL)	PQL(pg/mL)	Precision	Accuracy
17-b-estradiol	0.9991	25	5.5	17.5	7	100.4
		50	3.8	12	2.5	96.1
Ethinyl estradiol	0.9987	50	4.6	14.7	2.7	109.9
		100	15	47.6	4.7	100.8
Estriol	0.998	50	4.3	13.7	3.5	77.5
		100	12.9	41.2	4.2	98.2
Equilin	0.9993	50	6.9	22	4.1	106.4
		100	10.9	34.7	3.5	100
Estron	0.9996	50	5.4	17.1	3.3	104.3
		100	9	28.7	2.8	100.8
Bisphenol A	0.9957	50	14.2	45.2	9.4	95.8
		100	21.2	67.4	6.8	98.8

Method detection limits (MDLs), quantification limits (PQLs) and QC test were assessed using different two spiking samples (Table 2). Precision under 10% and accuracy of QC samples (n=10) are comparable to the reference ranges. For improvement of MDLs and PQLs for drinking water monitoring, larger volume injection test are necessary.

A surface water samples (n=40) were analyzed by 1mL injection with HR full scan MS as previously described. Among target compounds, 17-b-estradiol were frequently detected above PQLs. Distribution of this compound showed different patterns compared to pesticides and pharmaceuticals from the same area. Non point or multi input sources such as domestic or wastewater can be considered.

The online preconcentration with Orbitrap based HR full scan method provided an excellent method of detecting hormones and phenols, providing HRmass spectral information. The online preconcentration method can save more than 80-90% sample preparation time, solvent, labors etc[9,10]. Orbitrap mass spectrometry provided

high resolution with resolving power is 50,000 in present study and accurate mass datacanmatch to theoretical spectra confirming. Practical quantification limits were below 20 pg/mL ranges for most compounds. Further work to improve PQLs for drinking water using larger volume injection up to 20mL will be tested. Online preconcentration and HR full scanning mass spectrometry provides quick and accurate analysis compared to current main stream, conventional SPE-LC-MS/MS methodology.

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