

# Analysis of 8 kinds of estrogens in environmental water by ultra high performance liquid chromatograph hybrid triple quadrupole mass spectrometer

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## Introduction

Water environment is the largest repository of environmental estrogens. Estrogen in the natural environment are difficult to be decomposed, but into various animal through the food chain, adverse effects on male reproductive system. Environmental estrogenic substances into the human body, which causes human hormone excess and affects human sex hormones in normal work. Environmental hormones have become the

third largest environmental. All the countries in the world are aware of environmental estrogens on environmental and human hazards, and conducted in-depth research. This article use the SHIMADZU ultra performance liquid chromatography LC-30A and three triple quadrupole mass spectrometer coupled LCMS-8030, established a rapid and accurate method for determination of estrogen.

## Results and Discussion

100  $\mu$ L of 8 kinds of Estrogens in standard solution was injected, the standard solution was separated in 5 minutes, and the MRM chromatogram was showed in Fig.1. The calibration curve information, the limits of quantification (LOQ) and the limits of detection quantification (LOD) for the method of 8 kinds of Estrogens were investigated, and the results were showed in Table 2. The sample concentration and the peak area showed excellent linear relationship, with a coefficient of

determination greater than 0.999. The repeatability 8 kinds of Estrogens in different concentration were investigated, and the area RSD and retention time RSD (%) were less than 4.843% and 0.638%, respectively, as showed in Table 3. The spiked sample (17 $\beta$ - Estradiol and Ethinyloestradiol, 2 ng/L ; Others,1 ng/L) were analyzed, and results showed in Fig. 2, which showed high sensitivity for detection of Estrogens.

## Experimental

### HPLC

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pump, CTO-30A column oven, DGU-30A3 degasser, and SIL-30AC auto injector.

Column: Shimadzu Shim-pack XR-ODS III 2.0 mm $\times$ 75 mm. 1.6  $\mu$ m

Mobile phase: A –water;B – ACN / MeOH = 1/1 (v/v)

Flow rate: 0.4 mL/min

Column oven: 40°C

Injection volume: 10  $\mu$ L

Gradient program: showed in table 1

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## Mass spectrometry

A triple quadrupole mass spectrometer (Shimadzu LCMS-8030, Kyoto, Japan) was connected to the Shimadzu fast-analytical UHPLC instrument via an ESI interface.

Interface: ESI +  
 Interface voltage: 4.5 kV  
 Nebulizing gas: N<sub>2</sub>, 3 L/min  
 Drying gas: N<sub>2</sub>, 15 L/min  
 Collision gas: Ar<sub>2</sub>  
 DL: 250°C  
 Heat block: 400°C  
 Acquisition: MRM mode  
 Pause time: 3 ms  
 Dwell time: 10 ms

Table 1 LC Gradient Program

Time (min)	B.Conc
1.50	55
4.00	60
4.50	100
5.00	100
5.10	45
7.00	

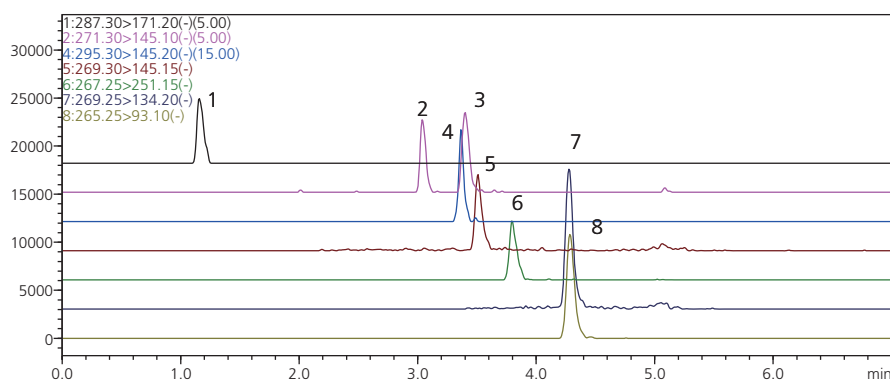


Fig. 1 Chromatogram of Estrogen Standards (100 µg/L)  
 (1-Estriol ; 2-17β-Estradiol ; 3-17α-Estradiol ; 4-Ethinylestradiol ; 5-Estrone ; 6-Diethylstilbestrol ; 7-Hexoestrol ; 8-Dienoestrol )

Table 2 Calibration curve informations of Estrogen s

No.	Name	Calibration curve	correlation coefficient r	Linearity range (µg/L)	LOD (ng/L)	LOQ (ng/L)
1	Estriol	Y = (0.120659)X	0.9998	1-100	0.05	0.15
2	17β-Estradiol	Y = (0.146104)X	0.9999	1-100	0.25	0.75
3	17β-Estradiol	Y = (0.0709176)X	0.9998	2-100	0.65	1.95
4	Ethinylestradiol	Y = (0.039812)X	0.9999	1-100	0.60	1.80
5	Estrone	Y = (0.392514)X	0.9999	1-100	0.30	0.90
6	Diethylstilbestrol	Y = (0.129798)X	0.9999	0.5-500	0.15	0.45
7	Hexoestrol	Y = (0.199119)X	0.9999	0.5-500	0.20	0.60
8	Dienoestrol	Y = (0.175211)X	0.9998	0.5-500	0.15	0.45

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Table 3 Repeatability of Estrogens with different concentration (n=6)

No.	RSD% (5 µg/L)		RSD% (20 µg/L)		RSD% (100 µg/L)	
	Area	R.T	Area	R.T	Area	R.T
1	3.160	0.638	2.448	0.135	1.801	0.122
2	4.236	0.323	1.318	0.084	1.852	0.053
3	4.843	0.240	4.354	0.080	1.816	0.057
4	4.401	0.454	4.675	0.084	2.846	0.097
5	4.007	0.241	1.818	0.051	1.146	0.051
6	4.753	0.271	0.929	0.079	0.864	0.059
7	2.372	0.034	0.868	0.080	1.463	0.057
8	1.159	0.194	1.079	0.058	0.981	0.095

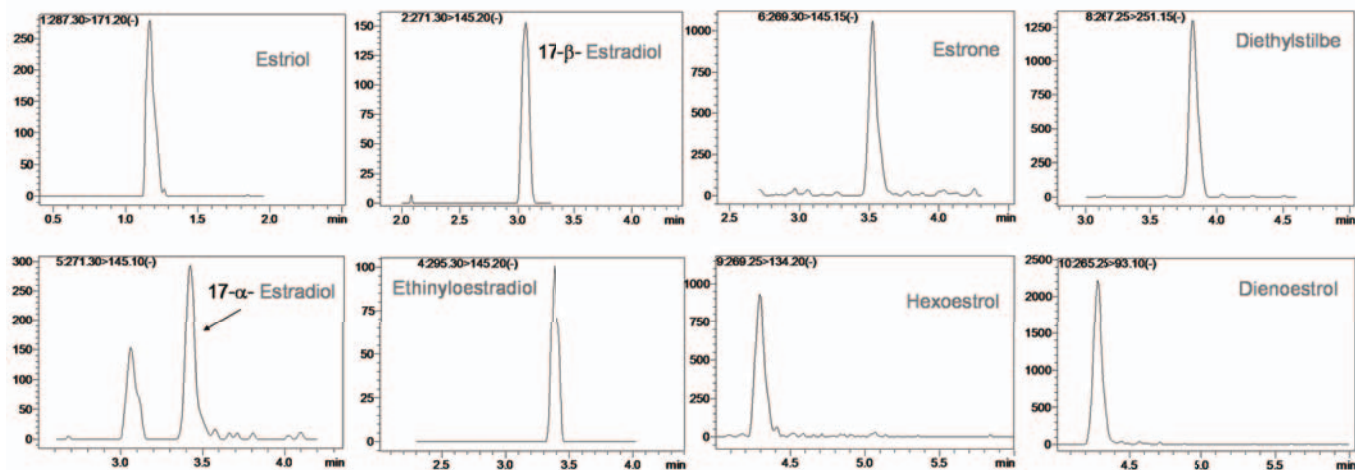


Fig. 2 Chromatogram of spiked sample (17β- Estradiol and Ethinyloestradiol, 2 ng/L ; Others,1 ng/L )

## Conclusions

A LCMS/MS method has been developed for the chemical analysis of 8 kinds of Estrogens in water were detected by Shimadzu Nexera UHPLC and LCMS-8030 triple quadrupole mass spectrometer. All of them were separated in 5 minutes. The linear range was from 0.5 to 500 µg/L with correlation coefficients (r) more than 0.999. Retention

times and peak areas results were highly reproducibility. The limit of quantification (LOQ) were less than 2 ng/L for all of Estrogens. This method is rapid, efficient and highly sensitive for quantitative analysis of 8 Estrogens in environmental water.



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