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**ON-LINE DERIVATIZATION GAS CHROMATOGRAPHY
ION TRAP MASS SPECTROMETRY FOR DETERMINATION
OF ENDOCRINE DISRUPTORS IN SURFACE WATER**

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INTRODUCTION

Endocrine disruptors (EDs) have raised great concerns as aquatic pollutants owing to their ability of disrupting the normal endocrine and reproductive functions of animals and humans.¹ EDs include natural hormone 17- β -estradiol (estradiol) and synthetic hormones such as diethylstilbestrol (DES), bisphenol A (BPA) and nonylphenol (NP) are reported to mimic estrogenic activity and have serious health effects.^{2,3} EDs owing to their polarity, persistence and water solubility can pass through wastewater treatment plants and enter the aquatic environment causing increased concern about their impact on wildlife and human health. They may even be related to increase hormone-dependent cancers and decrease in sperm quality and quantity in humans.⁴ Determining the EDs at low concentration with less sample preparation steps is important and thus a method is developed in which online derivatization and GC-MS detection have been used to simultaneously determine the presence of EDs in surface water.

EXPERIMENTAL

The surface water samples and sewage effluents were collected from three different sources including dormitory, lake water and the river water. These water samples were extracted using RP-18 SPE cartridges and clean-up was using aluminum oxide column. The extracted analytes were online derivatized using tetramethylammonium hydroxide (TMAOH) reagent and the derivatized products were analyzed by GC-MS and GC-

MS/MS. For CI MS analysis, CH₄, CH₃OH, CS₂ and CH₂Cl₂ were introduced using a laboratory-built multiple CI reagent introduction system to select the reagent.

RESULTS AND DISCUSSION

On-line derivatization of EDs

On-line derivatization of the hydroxyl (-OH) groups in EDs was achieved using direct sample injection as shown in Fig. 1 and TMA-OH as derivatization reagent. TMA-OH was observed to react readily with each -OH group in the EDs and yielded completely derivatized products. On the contrary, in absence of TMA-OH ion-pair reagent, only one peak corresponding to NP was observed and other EDs were not detected.

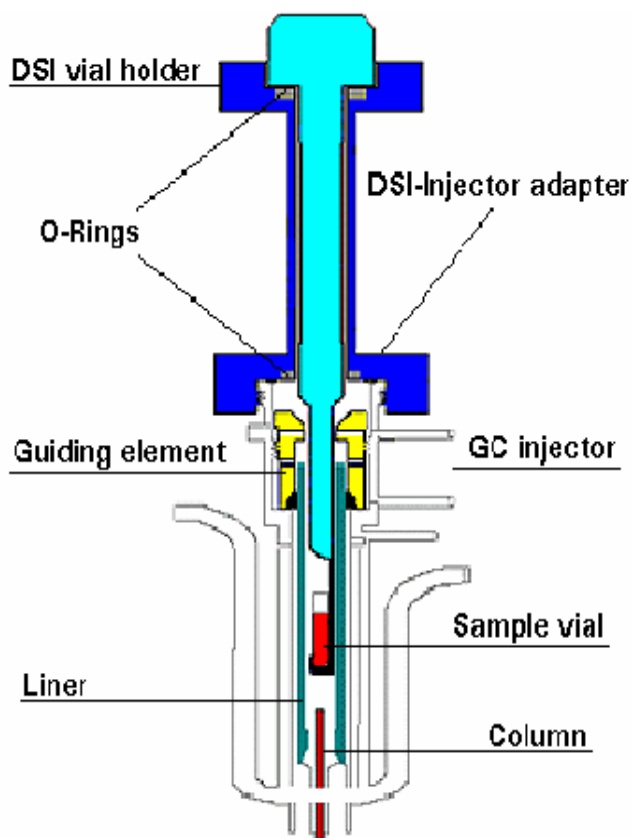


Figure 1. Direct sample injection design

Sensitivity evaluation

EI MS is generally used for identification of the EDs, but usually failed at low analyte concentration owing to inadequate ion intensities. Thus CI reagent like CH₄, CH₃OH, CS₂ and CH₂Cl₂ are used in this work. Experimental results showed that using CH₂Cl₂ CI reagent, ion currents of base peak ions apparently increased 3-10 times compared with those observed in EI-MS, CH₄, CH₃OH and CS₂ CI-MS. Furthermore, because of extremely low concentrations of the estradiol^{5,6} and the complexity of the environmental matrices, tandem mass spectrometry (MS/MS) is used to improve performance, reducing the matrices interference and quantification limits and enhancing detection sensitivity of analytes.

Linearity, precision, detection limits, and recovery

The linearity of the EDs was examined between 0.5 and 5000 µg/L. All analytes show good linearity with correlation coefficients of $r^2 \geq 0.99$ (Table 1). The recovery from RP-18 SPE cartridge and Al₂O₃ clean-up is evaluated using DI water and blank surface water spiked with a known amount of Chrysene-D₁₂ (100 g/L). Precision is measured by a 3-fold SPE procedure at a concentration level of 1, 1, 0.01 and 0.01 (g/L for NP, BPA, DES and estradiol, respectively). Good recovery is obtained for four spiked EDs between 74 and 112 %. The RSDs ranged from 5 to 17 %, indicating good repeatability of the method developed in this work. The values of method detection limits are estimated from 0.3 to 1 ng/L in blank surface water, defined at a signal-to-noise ratio (S/N) of > 10.

Table 1. Linear range, precision, detection limits and extract recovery of NP, BPA, DES and estradiol.

Compound	Linear Range Studied (µg/L)	Corr. Coef. (r^2)	Extract Recovery (% RSD) ⁿ⁼³		LOQ ^c (ng/L)	
			C ₁₈ +Al ₂ O ₃ (Pure water)	C ₁₈ +Al ₂ O ₃ (Blank surface water)	Pure water	Blank surface water ^c
NP ^a	1-5000	0.9980	112 (11 %)	103 (15 %)	0.4	1
BPA ^a	0.5-5000	0.9917	94 (9 %)	89 (17 %)	0.4	1
DES ^a	0.1-100	0.9981	86 (8 %)	74 (12 %)	0.1	0.3
estradiol ^b	0.5-50	0.9971	84 (5 %)	88 (14 %)	0.4	0.5

^aCH₂Cl₂ CI MS

^bCH₂Cl₂ CI MS/MS

^crepresents the limits of quantification for EDs

Application to environmental samples

Table 2 shows the detectable concentrations of each EDs and spike recovery of each EDs, in the real water samples. The recovery of spiked samples in matrix ranges from 69 to 128% indicates the method is suitable for analyzing EDs in real environmental samples. Fig. 2 shows the CH₂Cl₂ CI-MS and CI-MS/MS selection ion chromatogram and their corresponding mass spectrum from Chengkung lake sample. For application the occurrence of selected ED compounds in three different real water samples were investigated. NP and BPA are common aquatic contaminants and were detected in all water samples. On the other hand DES is rarely present and thus was not detected in all the samples. The detection of types of EDs in different sources of sample was in agreement with our expectation. Thus it can be concluded that use of CH₂Cl₂ CI-MS is effective method and can be used for the analysis of trace levels of estradiol in complex matrices of the real environmental water samples.

CONCLUSION

The analytical method developed herein shows that it is reliable, sensitive and convenient for trace determination of EDs with -OH functional group in the real water samples using TMA-OH derivatization agent. Large-volume (30 L) DSI sample introduction is an attractive method to decrease detection limits and to prevent contamination of injector and liner. The method significantly cuts the solvent waste and simplifies sample preparation, typically avoiding derivatization with hazardous reagents.

Using CH₂Cl₂ as the reagent gas in CI MS provided strong signals of molecules ion improving the sensitivity and thus can be used for detection of trace levels of estradiol in complex matrices of the real environmental water samples.

Table 2. Estimated average concentration and recovery results of NP, BPA, DES and estradiol in different real water samples.

Sample	NP	BPA	DES	estradiol
Student dormitory sewage effluent				
Average concentration (ng/L) (Spiked recovery, %)	5.8 (75 %)	0.5 (69 %)	nd ^a	2.1×10 ⁻³ (83 %)
Chengkung Lake				
Average concentration (ng/L) (Spiked recovery, %)	4.2 (113 %)	1.1 (72 %)	nd ^a	1.3×10 ⁻³ (128 %)
Touqian River				
Average concentration (ng/L) (Spiked recovery, %)	0.9 (81 %)	2.9 (86 %)	nd ^a	nd ^b

nd^a= Not detected at method quantitation limit of 0.3 ng/L for DES.

nd^b= Not detected at method quantitation limit of 0.5 ng/L for estradiol.

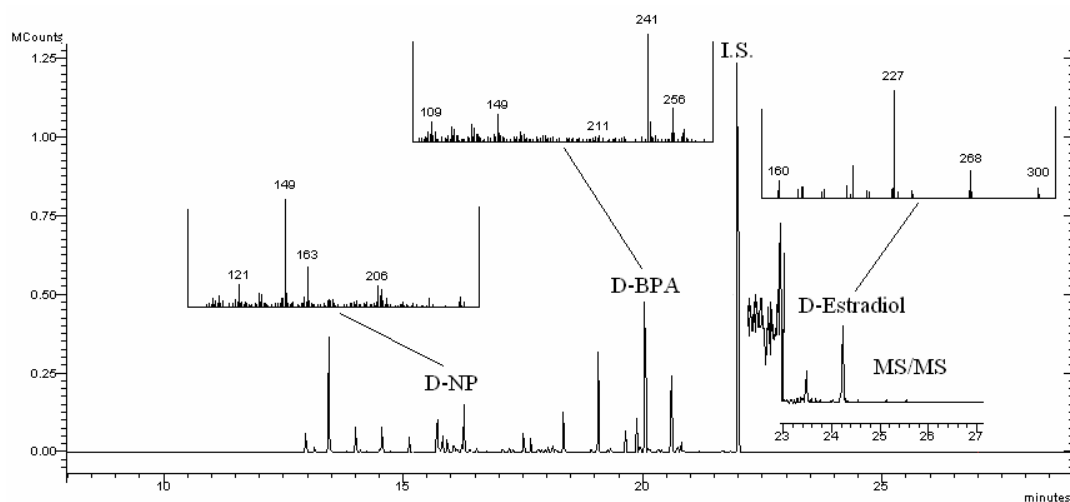


Figure 2. CH₂Cl₂ CI MS and CI MS/MS selection ion chromatogram of NP, BPA, DES, I.S. and Estradiol and the the corresponding mass spectra from Chengkung Lake sample. (I.S. is internal standard, 100 µg/L Chrysene-D₁₂)

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