**CHINESE JOURNAL OF ANALYTICAL CHEMISTRY**
Volume 41, Issue 2, February 2013
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

**Cite this article as:** Chin J Anal Chem, 2013, 41(2), 205–209.

RESEARCH PAPER

# Simultaneous Determination of Androgens and Progestogen in Surface Water and Sediment by Gas Chromatography-Mass Spectrometry

**JIN Wei, HUANG Bin, WANG Bin, WANG Dao-Wei, Zhao Shi-Min, PAN Xue-Jun***
Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, China

**Abstract:** A method based on gas chromatography-mass spectrometry (GC-MS) was developed for the simultaneous determination of androgens and progestogen, such as dihydrotestosterone (DIHYDRO), testosterone (TEST), androstenedione (AND) and progesterone (PROG) in surface water and sediment. The method was developed by using microwave-assisted extraction (MAE), solid phase extraction (SPE) and derivatization procedure. The MAE, SPE and derivatization procedure were investigated and optimized. The optimized conditions were as follows: sediment samples were extracted with ethyl acetate by microwave extraction system at 120 °C for 15 min; the water samples pH value were set at 4 and Oasis HLB cartridges were used with ethyl acetate as eluting solvent, trimethyliodosilane as catalytic agent and *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide as the reagent of derivatization. The hydroxyl and ketone groups of target compounds were simultaneous derivatizated at 40 °C for 20 min. The method had good repeatability and reproducibility with relative standard deviations < 9% for all target compounds in both matrices. The recoveries of spiked water and sediment samples were 89.3% –101.4% and 77.3%–92.1%, respectively. The detection limits of this method for four analytes in sediment samples and water samples were 0.1–0.5 ng·L$^{-1}$ and 0.6–0.8 ng·L$^{-1}$, respectively. The limits of quantification were 0.4–1.8 ng L$^{-1}$ and 1.9–2.6 ng g$^{-1}$ dry weight (dw) for water samples and sediment samples, respectively. The established method was successfully applied in the determination of target hormones in surface water and sediment samples collected from Erhai Lake, China.

**Key Words:** Androgens; Progestogens; Surface water; Surface sediment; Gas chromatography-mass spectrometry

## 1 Introduction

The occurrence of steroid hormones in the environment had become the focus of attention because it could interfere with reproduction and development, induce hermaphroditism of aquatic organisms, and increase the possibility of breast and testicular cancers in humans even at the level of ng L$^{-1}$ [1,2]. Up until now, most studies on the presence of steroid hormones in the environment have focused on the estrogens[3,4]. However, the environmental levels of other steroid hormones, including androgens and progestogens should be much higher, because their excretion masses in human and livestock urine are several times even hundred times higher than those of estrogens[5,6]. In addition to excretion of natural androgens and progestogens, synthetic ones have also been widely used in human and veterinary therapy, or farming practices as growth promoters. All these natural and drug using androgens and progestogens are constantly discharged into environment via wastewater treatment plants effluents or direct excretion and discharge[7,8]. Therefore, there is a need for developing a sensitive and reliable method to analyze the androgens and progestogens in surface water and sediment to assess their

Received 6 July 2012; accepted 11 October 2012
* Corresponding author. Email: xjpan@kmust.edu.cn
This work was supported by the Environmental Protection Commonweal Scientific Research Foundation of China (No. 200809136), the National Natural Science Foundation of China (No. 21267012), and the Application Fundamental Research Foundation of Yunnan Province, China (No. 2012FB124).
Copyright © 2013, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. Published by Elsevier Limited. All rights reserved.
DOI: 10.1016/S1872-2040(13)60628-3

environmental risk.

Liquid chromatography-mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS /MS) has been reported to analyze a range of steroidal androgens and progestogens in water or sediment samples[9–12]. However, matrix interference, such as ion suppression, is a significant problem for LC-MS/MS applying in more complex matrices of sediments[13,14]. In contrast, gas chromatography-mass spectrometry (GC-MS) has become a preferred technique for simultaneous analysis of steroid hormones because of its superior separation, identification capabilities, low experiment cost and suitability for complex matrices analysis. To improve the detection sensitivity and separation resolution for the analysis of steroid hormones by GC-MS, the derivatization of hydroxyl and ketone groups is required to increase the volatility and thermal stability of the analytes. In the past few years, the derivatization of hydroxyl groups was studied well. However, there is a lack of understanding simultaneous derivatization of hydroxyl and ketone groups of androgens and progestogens[15,16]. To the best of the author's knowledge, there is no published method of simultaneous determination of androgens and progestogen, such as DIHYDRO, TEST, AND and PROG in surface water and sediment by GC-MS.

The objective of this work was to develop a sensitive and reliable method based on MAE-SPE-derivatization-GC-MS for simultaneously analyzing the most common steroid androgens and progestogen, such as DIHYDRO, TEST, AND and PROG in surface water and sediment. The MAE, SPE and derivatization procedures were also discussed and optimized. Finally, this developed method was applied to the analysis of these compounds in surface water and sediment samples collected from Erhai Lake, China.

## 2 Experimental

### 2.1 Instruments and reagents

The instruments used in the experiment were as follows: Thermo Trace DSQ GC-MS (Thermo Finnigan, USA), DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, J&W Scientific, USA), ETHOS 1 advanced microwave extraction system (Milestone, Italy), freeze-dryer (Eyela FDU-1200, Japan), rotary evaporator (Buchi Rotavapor RII, Switzerland), vacuum manifold (12, Supelco, USA), SPE cartridges: Oasis HLB and Sep-Pak C18 (0.5 g, 6 mL, Waters, USA), HGC-36A nitrogen blowing meter (Heng-ao, China), GF/F filter paper (0.45 μm pore size, Millipore, MA, USA).

The targeted progestogen progesterone (PROG), internal standard (5*a*-androstane), derivatization reagents (*N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA)), dithioerythreitol (DTE), trimethyliodosilane (TMIS) were purchased from Sigma-Aldrich (St. Louis MO, USA), and androgenic hormones including androstenedione (AND), dihydrotestosterone (DIHYDRO), testosterone (TEST) from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The purities of all of the analytical standards were ≥ 99%. Ethyl acetate (EA), hexane and methanol (MeOH) were HPLC grade (Merck, Darmstadt, Germany). HPLC-grade water was prepared using a Milli-Q apparatus (Millipore, Bedford, MA, USA). A stock solution of 5*a*-androstane was prepared in hexane with a concentration of 10 ng·μL$^{-1}$. The other stock solutions (10 mg L$^{-1}$) were prepared in methanol and stored in a refrigerator at –20 °C.

### 2.2 Microwave-assisted extraction

Sludge samples were freeze-dried for 4 days, and homogenized with a stainless steel spoon. For the method development and validation experiments, 4.0 g dried samples were spiked with 100 ng of target androgens and progestogen. The spiked samples were carefully mixed with a spatula and then left for 48 h at 4 °C. 4.0 g freeze-dried sludge or spiked samples were extracted with 25 mL of ethyl acetate by a microwave-assisted extraction system (2450 MHz) at 120 °C for 15 min. The extracts were filtered through absorbent cotton into flat-bottomed flasks (300 mL). The extracts were concentrated to dryness by a rotary evaporator, redissolved in 1 mL methanol, and then diluted to a final volume of 300 mL with Milli-Q grade water, finally extracted directly according to the following water sample extraction method.

### 2.3 Solid phase extraction

Water samples (1.0 L) were filtered through Millipore 0.45 μm GF/F glass fiber paper to remove suspended matter and then adjusted pH to 4. Oasis HLB cartridges were preconditioned with 5 mL of ethyl acetate and 5 mL of methanol followed by 3 × 5 mL Milli-Q grade water. The filtered water samples passed through the cartridges for the extraction at a flow rate of less than 5 mL min$^{-1}$. Then, the cartridges were washed with 3 × 5 mL 5% MeOH in Milli-Q grade water (*V/V*) and dried under vacuum for 1 h. The target compounds were eluted from the cartridges using 3 × 5 mL ethyl acetate. The eluate was evaporated to nearly dry under a gentle stream of nitrogen. Finally, the dried residues were subjected to derivatization reaction.

### 2.4 Derivatization

The dried residues of SPE eluate or working standard solution were derivatized by adding 90 μL of MSTFA/TMIS/DTE (1000:2:5, *V*/*V*/*w*), then completely mixed using a vortex system and performed at 40 °C for 20 min. After the derivatization reaction, the derivatives were cooled to room temperature and 10 μL of internal standard (10 ng μL$^{-1}$) was added. Then, 1 μL of the mixtures was injected for GC-MS analysis.

Download English Version:

https://daneshyari.com/en/article/1182902

Download Persian Version:

https://daneshyari.com/article/1182902

Daneshyari.com