

# Determination of Galaxolide (HHCB) and Tonalide (AHTN) by Solid Phase Extraction and Gas Chromatography (SPE-GC)

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**Abstract:** Galaxolide (HHCB) and tonalide (AHTN), two popular synthetic musk compounds, have been widely detected in the environment, which would pose a potential hazard to the ecosystem. To develop a robust analytical methodology for such emerging micropollutants is very important to investigate their presence and transformation in water. In this study, an analytical process of solid-phase extraction (SPE) coupled with gas chromatography (GC) was developed to determine trace levels of HHCB and AHTN in water. Specifically, the target compounds in test solutions were firstly extracted by SPE cartridges, followed by an eluting and redissolving process. Then, the enriched solutions were analyzed using a GC system with a flame ionization detector (FID). This analytical process demonstrated good recoveries for both compounds with mean recoveries of  $104.7 \pm 5.1\%$  and  $102.9 \pm 4.8\%$  for HHCB and AHTN, respectively. Above all, the SPE-GC analytical process proposed in this study provides a reliable method to assess the performance of future treatment trials.

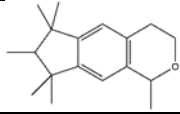
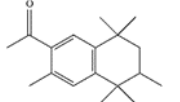
**Keywords:** galaxolide (HHCB); tonalide (AHTN); solid phase extraction (SPE); gas chromatography (GC).

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## 1. Introduction

Due to their low price and long-lasting flavor, synthetic musks have been widely used as fragrance ingredients in daily necessities, such as detergent, perfume, air freshener, and cosmetics [1-4]. Of the synthetic musk compounds, galaxolide (HHCB) and tonalide (AHTN) are the largest volume products, accounting for over 90% of the global market [5]. Due to their widespread use, synthetic musks are discharged after use via domestic wastewater and enter the wastewater treatment plants (WWTPs), and most of them are expected to be released into the environment since the elimination of synthetic musks in WWTPs is not efficient [6-9]. Therefore, research on their occurrence, transportation, toxicity, and treatment has become very popular in recent years [10-15]. In this study, HHCB and AHTN (Table 1) in simulating water samples were detected simultaneously by a simple solid-phase extraction (SPE) and gas chromatography (GC) method, which would offer a validated analytical method for the further performance assessment of HHCB and AHTN removal in treatment trials.

**Table 1.** Information about HHCB and AHTN.

Name	CAS NO	Log P	Chemical structure	Ref.
HHCB	1222-05-5	5.3		[16]
AHTN	1506-02-1	5.4		

## 2. Materials and Methods

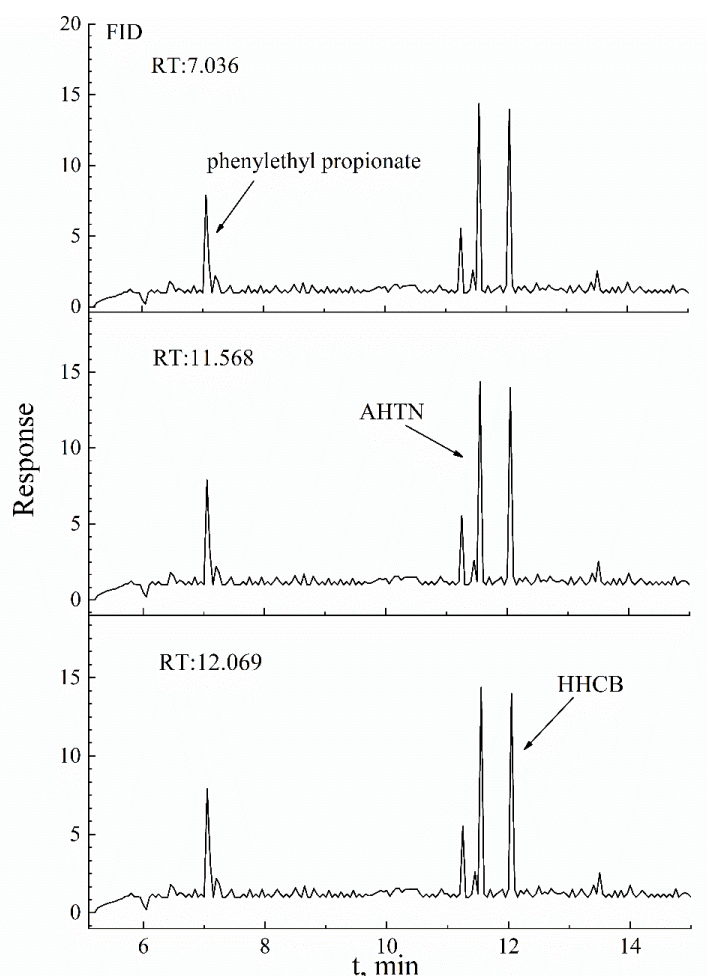
Analytical grade HHCB and AHTN standard solutions ( $10 \text{ mg L}^{-1}$ , 1 mL) were purchased from J&K Chemical (Germany). Phenylethyl propionate, which was utilized as the internal standard, was also obtained from J&K Chemical (Germany). All other chemicals and reagents were purchased from Sinopharm (China) and were applied without further purification. In order to investigate the accuracy and precision of the proposed SPE-GC analytical method, HHCB and AHTN test solutions of various concentrations were prepared and subjected to all the analytical processes. The concentrations of each target compound were set at six levels: 2, 4, 8, 10, 12, and  $16 \text{ } \mu\text{g L}^{-1}$ . The concentration of internal standard was  $200 \text{ } \mu\text{g L}^{-1}$ . Five parallel samples were prepared for each concentration.

Test solutions were filtered using  $0.45 \text{ } \mu\text{m}$  membrane filter (Anpel, China) and then subjected to SPE extraction. The SPE cartridges utilized in this study were Generik H2P 60 mg/3 mL tubes (Sepax Technologies, China). For all test solutions, 50 mL solution was used for the SPE extractions with an automatic SPE432-EVA32 SPE working station (Pretyco, China). Generally, the extraction protocol was: (1) conditioning: 10 mL methanol; (2) equilibrating: 6 mL water + 6 mL acidified water (HCl 0.1% v/v); (3) loading samples: 50 mL of test solutions were extracted at a flow rate of  $5 \text{ mL min}^{-1}$ ; (4) washing: 6 mL water at a flow rate of  $3 \text{ mL min}^{-1}$ ; and (5) elution: 5 mL methanol. The elutes were evaporated using a nitrogen blower (Pretyco, China) to dryness at  $55^\circ\text{C}$  under gentle nitrogen flow and then re-constituted to 1 mL by cyclohexane. The final enriched samples were filtered using  $0.45 \text{ } \mu\text{m}$  nylon syringe filters (Anpel, China) and then subjected to further GC analysis.

An Agilent 7820A GC system (Agilent, USA) with a flame ionization detector (FID) was employed to measure the target compounds. Target compounds were separated by an Agilent HP-88 capillary column ( $30 \text{ m} \times 0.25 \text{ mm i.d.}$ ,  $0.25 \text{ } \mu\text{m}$  film thickness, Agilent, USA) with nitrogen as carrier gas at a flow rate of  $1 \text{ mL/min}$ . The oven temperature program was as follows: initial temperature of  $120^\circ\text{C}$  (held for 4 min), then increased to  $320^\circ\text{C}$  at a rate of  $15^\circ\text{C min}^{-1}$ , and held at  $320^\circ\text{C}$  for 3 min. The injection volume was set as  $1 \text{ } \mu\text{L}$ .

## 3. Results and Discussion

HHCB, AHTN, and the standard internal chemical were separated in the GC using the HP-88 capillary column. As shown in Figure 1, the gradient increase of the column temperature led to a clear separation of three chemicals within 13 min. Phenylethyl propionate came out firstly from the column with a retention time of 7.036 min. AHTN was eluted secondly at 11.568 min and followed by HHCB at 12.069 min.



**Figure 1.** GC chromatographs of HHCB, AHTN and internal standard (HHCB, AHTN:0.8 mg L<sup>-1</sup>; internal standard: 0.2 mg L<sup>-1</sup>).

As shown in Table 2, the calibration curves of HHCB and AHTN covering 0.1~1 mg L<sup>-1</sup> demonstrated good coefficients of correlation ( $r^2 > 0.99$ ). The HHCB and AHTN spiked concentrations in test solutions were quantified according to their specific linear regression equations. The calculated recoveries of each sample were obtained based on detected and spiked quantities of target compounds in the test solutions and are presented in Table 3. The results demonstrated good recoveries of HHCB and AHTN of various initial concentrations, with overall recoveries  $104.7 \pm 5.1\%$  and  $102.9 \pm 4.8\%$ , respectively.

**Table 2.** Calibrations curves of HHCB and AHTN.

Chemical compound	Equation of linear regression	Correlation coefficient	Linear range ( mg L <sup>-1</sup> )	LOD ( mg L <sup>-1</sup> )
HHCB	$y = 0.2215x + 0.0282$	$R^2 = 0.9956$	0.1~1	0.07
AHTN	$y = 0.1759x + 0.0038$	$R^2 = 0.9991$		0.08

**Table 3.** Recoveries of HHCB and AHTN with various concentrations.

Test solutions	HHCB	AHTN
2 μg L <sup>-1</sup>	$107.1 \pm 5.3\%$	$103.1 \pm 3.8\%$
4 μg L <sup>-1</sup>	$103.4 \pm 3.7\%$	$102.2 \pm 2.1\%$
8 μg L <sup>-1</sup>	$103.3 \pm 3.8\%$	$104.1 \pm 5.3\%$
10 μg L <sup>-1</sup>	$106.0 \pm 1.8\%$	$101.8 \pm 6.1\%$
12 μg L <sup>-1</sup>	$108.9 \pm 7.9\%$	$103.3 \pm 4.9\%$
16 μg L <sup>-1</sup>	$99.3 \pm 7.8\%$	$102.7 \pm 6.7\%$
Overall recovery	$104.7 \pm 5.1\%$	$102.9 \pm 4.8\%$

**Table 4.** Comparison of recoveries with other analytical methods.

Method	Water matrix	Recovery, %		Ref.
		HHCb	AHTN	
Liquid-liquid microextraction (LLME) + GC-MS/MS	Wastewater	97±3~102±11	71±7~99±3	[17]
	Tap water	75±2~97±14	91±3~106±12	
	Sea water	96±5~107±8	92±11~101±4	
	River water	101±15~105±3	83±3~96±7	
Liquid-liquid microextraction (LLME) + GC-MS/MS	Wastewater	88±7~113±5	84±4~114±6	[18]
	Tap water	93±4~106±1	91±3~106±1	
	Sea water	91±2~115±1	102±3~112±4	
	River water	86±4~117±4	85±5~118±9	
Solid-phase microextraction (SPME) + GC-MS/MS	River water	102 ± 1	95.4 ± 7.1	[19]
SPE+GC-LID	Pure water	99.3 ± 7.8~108.9 ± 7.9	101.8 ± 6.1~104.1 ± 5.3	This study

In comparison with other studies (Table 4), the established SPE-GC analytical methods show good recoveries of HHCb and AHTN, which is reliable for determining residual concentrations of HHCb and AHTN in test solutions.

#### 4. Conclusions

An SPE-GC analytical process was established in this study to detect HHCb and AHTN in test solutions with good recoveries, which were  $104.7 \pm 5.1\%$  and  $102.9 \pm 4.8\%$ , respectively. It offers a validated analytical method for the further assessment of HHCb and AHTN removal in test solutions.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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