METHOD DEVELOPMENT FOR ANALYSIS PENTACHLOROBENZENE AND HEXACHLOROBUTADIENE IN WASTEWATER BY GC/MS

PHÁT TRIỂN QUY TRÌNH PHÂN TÍCH PENTACLOBENZEN VÀ HEXACLOBUTADIEN TRONG NƯỚC THẢI BẰNG SẮC KÝ KHÍ KHỐI PHỔ

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ABSTRACT

This research presents the optimization of Pentachlorobenzene (PeCB) and Hexachlorobutadiene (HCBD) analysis in surface water. The study investigates the operating parameters of the GC/MS system and the clean-up, enrichment procedures based on liquid-liquid extraction using surrogate standard (Phenanthrene-D10). The calibration curve demonstrated a strong linear correlation (~0.994) within the concentration range of 25 - 600μg/L. The method detection limits were 0.008μg/L for PeCB and 0.004μg/L for HCBD. The recovery of the analytical procedure ranged from 85% to 114%. The method was applied to waste water samples showed good repeatability.

Keywords: *PentachloroBenzene (PeCB), HexachloroButadiene (HCBD); GC/MS.*

TÓM TẮT

Nghiên cứu này trình bày quá trình tối ưu hóa phân tích Pentachlorobenzene (PeCB) và Hexachlorobutadiene (HCBD) trong nước mặt. Nghiên cứu tập trung vào các thông số vận hành của hệ thống GC/MS và quy trình làm sạch, làm giàu mẫu bằng phương pháp chiết lỏng-lỏng có sử dụng chất chuẩn đồng hành (Phenanthrene-D10). Đường chuẩn cho thấy mối tương quan tuyến tính cao (~0,994) trong khoảng nồng độ từ 25 - 600μg/L. Giới hạn phát hiện của phương pháp là 0,008μg/L đối với PeCB và 0,004μg/L đối với HCBD. Hiệu suất thu hồi của quy trình phân tích dao động từ 85% đến 114%. Phương pháp này đã được áp dụng để phân tích mẫu nước thải và cho kết quả lặp lại tốt.

Từ khóa: *PentachloroBenzene (PeCB), HexachloroButadiene (HCBD); GC/MS.*

1. INTRODUCTION

The Stockholm Convention on Persistent Organic Pollutants (POPs) is an international environmental treaty, signed in 2001 and effective since May 2004. Its primary objective is to eliminate or restrict the production and use of POPs. The Convention raises global awareness of the health and environmental risks associated with POPs, especially in developing countries.

Currently, the Convention lists 36 POP groups, including Hexachlorobutadiene (HCBD) and Pentachlorobenzene (PeCB), categorized as Unintentionally Produced POPs (UPOPs) [1]. UPOPs are unintentionally formed and released from various industrial activities, and are known for their high toxicity, widespread dispersal, and difficult-to-control impacts.

As a signatory to the Stockholm Convention, Vietnam has both obligations and entitlements in implementing the Convention's provisions [2]. This includes developing legal regulations, policies, and management strategies aimed at restricting, banning, and ultimately eliminating POPs. Consequently, the development of accurate analytical methods for quantifying PeCB and HCBD in the environment, particularly in water, is essential. Such methods support environmental authorities in monitoring and assessing pollution levels and implementing mitigation measures.

Common techniques for determining HCBD and PeCB concentrations include Gas Chromatography with Electron Capture Detection (GC-ECD) and Gas Chromatography-Mass Spectrometry (GC-MS). GC-ECD offers high sensitivity and lower cost, particularly for

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chlorinated compounds like HCBD and PeCB. However, its selectivity is limited and subject to interference from other halogenated substances, requiring extensive sample clean-up. Additionally, GC-ECD only provides signal detection without structural identification. In contrast, GC-MS addresses these limitations by offering high sensitivity, selectivity, and accuracy, with less stringent requirements for sample preparation [3-6].

This study focuses on optimizing a simultaneous analytical procedure for HCBD and PeCB in wastewater. The method development was based on standard protocols (US.EPA Methods 3510C, 35353A, 8270D) to identify optimal experimental conditions. In addition, the development and application of all three US EPA standard methods have helped to specify every step of the extraction, cleanup, enrichment, and analysis of HCBD and PeCB in wastewater samples. The validated procedure was then applied to real wastewater samples, yielding highly repeatable and reliable results.

2. EXPERIMENTS

2.1. Chemicals and standards

All solvents and chemicals must be of analytical purity, by Merck or Fisher including: n-hexane, dichloromethane, acetone, sodium sulfate granules, glass wool, silica gel 60-130 Mesh (Merck).

Standards: PeCB, HCBD 100 µg/ml and Phenanthrene D10 200µg/ml from CIL.

2.2. Instrument

- GC-MS analysis was performed using an Agilent GC7890A coupled with an MSD5975 (USA).
- DB-5MS chromatographic column (30m × 0.25mm ID, 0.25µm film thickness)
- SPE Vacuum Manifold (12-port visiprep, Supelco, USA).

2.3. Analysis of HCBD and PeCB

2.3.1. Method for sample preparation and clean up

Several constituents commonly present in wastewater such as dissolved organic matter, suspended solids, and pH can significantly influence the analytical performance in the determination of HCBD and PeCB. These components may interact with the target analytes, thereby decreasing their recoveries during liquid-liquid extraction with dichloromethane. Furthermore, matrix interferences, including grease and surfactants, can impair the efficiency of cleanup procedures using florisil-based SPE cartridges. To minimize these effects, pre-treatment steps such as sample filtration and pH adjustment should be conducted prior to extraction.

Transfer 1.0L of the sample into a separatory funnel and add 20µL of a 10pg/µL surrogate standard. Add 60mL dichloromethane, shake for 1 - 2 minutes, and allow phase separation for 10 minutes. Extract the solvent layer, repeat twice with fresh solvent, and combine all extracts. Concentrate the extract to 2 - 3mL by using a rotary evaporator.

Activate florisil before using at 650^{0}C in 2 hours. Prepare to stuff the florisil column into a 5mL plastic syringe, inner diameter 1cm in the following: glass wool, Na_2SO_4, 1g florisil, Na_2SO_4. Put the column to SPE vacuum manifold. Rinse the column with n-hexane, remove the solvent. Put 5mL mixture solvents n-Hexane:acetone = 90:10 (v/v) through the column with flow rate about 2mL/min. Transfer the sample extract to the column. After that, control the pressure about 224mmHg and elute the column with 10 mL mixture solvents n-Hexane:acetone = 90:10 (v/v). Collect 5mL of final elution solution. Concentrate with N_2 gas to 1mL, transfer to vial, seal with a PTFE septum, vortex, and inject the sample into the GC/MS for analysis.

2.3.2. Method for quantification

GC-MS analysis was performed using an Agilent GC7890A coupled with an MSD5975 (USA). The system utilized an DB-5MS chromatographic column (30m × 0.25mm ID, 0.25µm film thickness).

Optimize the conditions of gas chromatography and mass spectrometry: optimize the conditions of GC such as Helium flow, temperature program, mass segment.

2.3.3. Method validation

Validate method through determine method detetion limit (MDL), limit of quantification (LOQ), linear range, repeatability, reproducibility, trueness (recovery).

The MDL and LOQ is calculated using the following equation:

$MDL = t_{(n-1,1-\alpha=0.99)} \times SD$

Where: t = Student's t-value for n−1 degrees of freedom at the 99% confidence level (e.g., t = 3.143 for n = 7).

SD = Standard deviation of replicate measurements (n ≥ 7) of a sample spiked at a low concentration.

3. RESULTS AND DISCUSSION

3.1. Optimize the conditions for the simultaneous determination of HCBD and PeCB by GC-MS

Using automatic scan mode to fragment the molecular ion into product ions, and choose the most intense product ion for quantification. Optimize the collision energy. The optimized results are presented in Table 1.

Table 1. The conditions for analysis

He flow	1.0mL/min, splitless mode	
Column	DB-5MS 30m x 250µm x 0,25µm	
Oven	Maximum oven temp 320^{0}C Initial 40^{0}C. Hold time 2 min Ramp 1: 300^{0}C. Rate 20^{0}C/min. Hold time 2 min	
Aux temp	280^{0}C	
Scan time segment	m/z	label
	188, 94	Phenanthrene D10
	225, 227	HCBD
	250, 252	PeCB

3.2. Optimize the volume mixture solvents to elute HCBD and PeCB from Florisil SPE cartridges

The mixture of n-hexane:acetone was selected as the elution solvent for the solid phase extraction of simultaneous determination of HCBD and PeCB due to its balanced polarity and strong elution capability for moderately non-polar compounds. n-hexane is effectively dissolves hydrophobic analytes such as HCBD and PeCB. However, when used alone, it may not sufficiently disrupt the weak polar interactions between the analytes and Florisil – the sorbent in SPE cartridges. The addition of a little acetone, a moderately polar solvent, enhances the elution power by slightly increasing the overall polarity of the mixture, facilitating the desorption of the analytes from the sorbent without co-eluting excessive matrix interferences. Thus, this study was optimized ratio of n-hexane:acetone and elution volume to elute HCBD and PeCB from Florisl SPE cartridges.

To determine the optimal ratio between n-hexane and acetone to elute HCBD and PeCB from the Florisil SPE cartridges, various ratios of n-hexane:acetone (v/v) were tested, including 95:5, 90:10, 85:15, 80:20 (v/v). The results are showed in Figure 1.

The research results show that when the ratio of n-hexane:acetone increases from 5% to 10%, the recovery increases. However, when the ratio is further increased to 15% - 20%, the recovery tends to decrease. This is because increasing the acetone content raises the polarity of the solvent mixture, reducing its desorption capacity for non-polar compounds such as HCBD and PeCB. Additionally, matrices interference appears due to some co-eluting substances. Therefore, the most suitable solvent ratio of n-hexane:acetone is 90:10 (v/v).

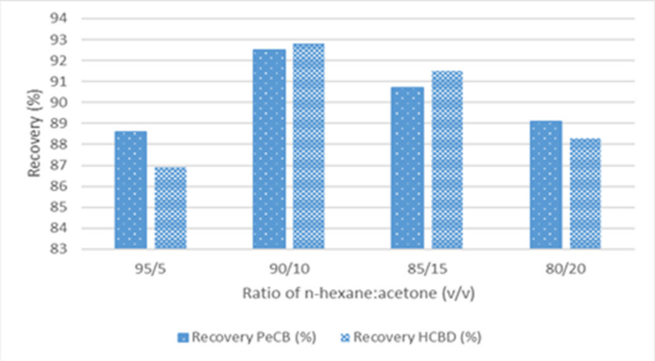

Figure 1. Relative between recovery and ratio of n-hexane:acetone

To determine the optimal elution volume for effective recovery of HCBD and PeCB from the Florisil SPE cartridges, various volumes of n-hexane:acetone (9:1, v/v) were tested, including 2.5, 5, 7.5, 10, 12.5 and 15mL. The results are showed in Figure 2.

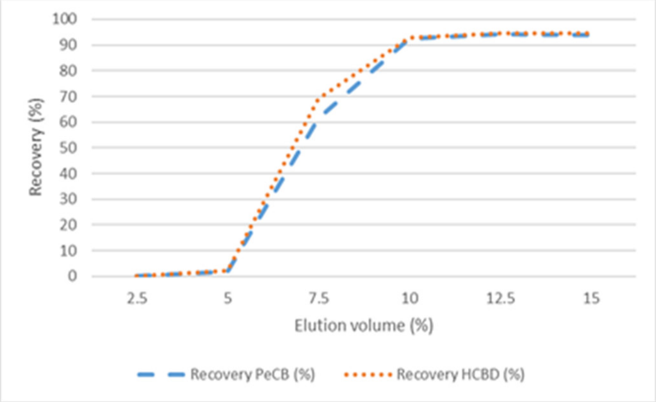

Figure 2. Relative between recovery and elution volume

The results indicate that the recovery increased a lot with elution volume from 5mL to 10mL (1.72% to 92.4% for PeCB and 0.87% to 93.70% for HCBD), after that, when increasing volume of elution, the recovery change insignificant. Thus, 10mL of n-hexane:acetone (9:1) was selected as the optimal elution volume.

3.3. Method validation

3.3.1. Determine method detection limit (MDL) and limit of quantification (LOQ)

MDL was determined for wastewater matrix by conducting spiking experiments. A concentration of 0.025µg/L of PeCB and HCBD was spiked into 1 liter of wastewater by adding 1µL of a 25µg/L standard solution of each compound. The spiked samples were then extracted using the established solid-phase extraction procedure. The final extracts were concentrated and reconstituted to 1mL and transferred to autosampler vials for analysis by gas chromatography–mass spectrometry (GC-MS).

The procedure was repeated seven times to evaluate repeatability and to calculate MDL and LOQ based on statistical analysis of the results. The calculated results indicated that the method achieved MDL value of 0.008 and 0.004µg/L and LOQ value of 0.027 and 0.011µg/L for PeCB and HCBD respectively.

The obtained values are better than those reported by Shimadzu (MDL of 0.5 and 0.2µg/L, LOQ of 2.5 and 1.0µg/L for HCBD and PeCB, respectively) [11]. However, these results are 10 - 40 times higher than those reported in some studies using GC/MS/MS, which achieved MDLs of 0.3 and 0.2ng/L, and LOQs of 1.0 and 0.5ng/L for HCBD and PeCB, respectively, due to the higher sensitivity of the GC/MS/MS technique [12, 13].

3.3.2. Determine linear range

A calibration curve was constructed at the concentration levels of 25, 50, 100, 200, and 500µg/L. The relationship between peak area and corresponding concentration was established, resulting in the calibration curve and correlation coefficient as shown in Table 2, The chromatogram at the concentration of 50µg/L is shown in Figure 3.

Table 2. The calibration curve of HCBD and PeCB

Analyte	Calibration curve formula	Correlation coefficient (R^2)
HCBD	y = 0.284173 * x – 0.026129	0.997
PeCB	y = 0.658265 * x – 0.11779	0.995

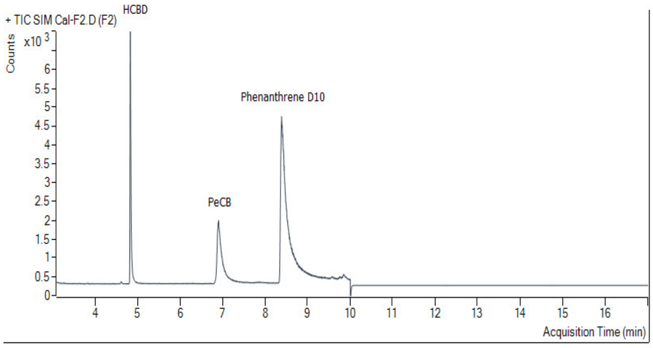

Figure 3. Chromatogram of analysis

3.3.3. Determine repeatability, reproducibility and recovery

Repeatability and reproducibility testing was performed 10 times of each on spiked wastewater samples at three different concentration levels: 0.025, 0.10, and 0.400µg/L of HCBD and PeCB. The coefficient of variation and recovery efficiency are presented in Table 3.

Table 3. Results of repeatability, reproducibility and recovery

Analyte	C_{spiked} (µg/L)	Average (µg/L)	RSD (%)	Recovery (%)
Repeatability				
PeCB	0.023	0.022	5.82	95.65
	0.099	0.098	2.66	98.99
	0.446	0.429	2.29	96.19
HCBD	0.023	0.021	3.99	91.30
	0.092	0.091	3.20	98.91
	0.422	0.413	1.78	97.87
Reproducibility				
PeCB	0.023	0.022	7.90	95.65
	0.099	0.097	5.78	97.98
	0.446	0.413	3.89	92.60
HCBD	0.023	0.021	8.08	91.30
	0.092	0.094	4.98	102.17
	0.422	0.396	3.16	93.84

The results indicate that the method meets the AOAC requirements for accuracy, with recoveries ranging from 40 to 120% and relative standard deviation (RSD) below 30% at concentration level 1µg/L [9].

3.3.4. Determine the concentration of HCBD and PeCB in wastewater

This study evaluated the concentrations of HCBD and PeCB in 10 wastewater samples collected from factories under the Vietnam Defense Industry. These facilities operate various technological lines specializing in the production and processing of weapons, ammunition, propellants, explosives, chemicals, explosive materials, chemical defense materials, smoke-generating substances, and decontamination agents for both defense and economic purposes. The analysis results are presented in Table 4.

Table 4. Concentrations of HCBD and PeCB in selected wastewater samples

No	Analyte	Number of samples	Concentration range		Average	Median
			min	max		
1	HCBD (µg/L)	10	0.040	0.100	0.052	0.040
2	PeCB (µg/L)	10	0.200	8.400	1.260	0.500

In the wastewater samples collected from factories, both HCBD and PeCB were detected, with concentrations ranging from 0.04 to < 0.1µg/L for HCBD and from 0.2 to 8.4µg/L for PeCB. This indicates the widespread presence of HCBD and PeCB in industrial wastewater sources, particularly at facilities that may use or generate chlorinated organic compounds during production processes. Although Vietnam has not yet issued specific regulatory limits for HCBD and PeCB in wastewater, the detection of PeCB concentrations as high as 8.4µg/L is a matter of concern when compared to international standards, such as the EU limit of 0.007µg/L [10].

4. CONCLUSION

This study successfully demonstrated the simultaneous determination of HCBD and PeCB in wastewater using the GC-MS method. The analytical procedure is simple and has been validated in terms of linearity, repeatability, recovery, method detection limit (MDL), and limit of quantification (LOQ). The validation results confirm that the developed method is suitable for determining the concentrations of HCBD and PeCB in wastewater. The analysis results of wastewater samples from several factories under the Vietnam Defense Industry indicate a potential risk of HCBD and PeCB emissions from production activities. These findings highlight the necessity for further research into the sources and environmental accumulation of these substances, as well as the development of an appropriate legal framework to effectively control persistent organic pollutants like HCBD and PeCB in Vietnam.

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THÔNG TIN TÁC GIẢ

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