

# Optimization of enrichment processes of pentachlorophenol (PCP) from water samples

LI Ping, LIU Jun-xin

(Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085. E-mail: liping15cn@yahoo.com.cn)

**Abstract:** The method of enriching PCP (pentachlorophenol) from aquatic environment by solid phase extraction (SPE) was studied. Several factors affecting the recoveries of PCP, including sample pH, eluting solvent, eluting volume and flow rate of water sample, were optimized by orthogonal array design (OAD). The optimized results were sample pH 4; eluting solvent, 100% methanol; eluting solvent volume, 2 ml and flow rate of water sample, 4 ml/min. A comparison is made between SPE and liquid-liquid extraction (LLE) method. The recoveries of PCP were in the range of 87.6%–133.6% and 79%–120.3% for SPE and LLE, respectively. Important advantages of the SPE compared with the LLE include the short extraction time and reduced consumption of organic solvents. SPE can replace LLE for isolating and concentrating PCP from water samples.

**Keywords:** pentachlorophenol (PCP); solid-phase extraction; liquid-liquid extraction

## Introduction

PCP is acutely toxic to variety of microorganism and mammals as it is an inhibitor of oxidative phosphorylation and is thought to be mutagenic or at least comutagenic. Human exposure to PCP is considered to pose significant health hazards (Volker, 2001). In China, sodium pentachlorophenate (Na-PCP) has been sprayed widely since the 1960s to control the spread of snailborne schistosomiasis. Although Na-PCP has been replaced by some new molluscicides since 1990s, the persistence of PCP resulted in the accumulation in soil and sediments. The remains of PCP also provide a source leading to continuous contamination in aquatic environment (Zheng, 2000). PCP is also one of by-products generated from a number of industrial manufacturing processes, such as polymeric resin production, oil refining, pesticide, paint, solvent, wood preserving chemicals, and paper and pulp industries. Most of them flow into aquatic environment with industrial effluents (Wan, 2000).

To assess the possible impact of PCP on ecosystems and support the improvement of remediation techniques, sufficiently selective and highly sensitive analytical procedures are highly desirable for the routine monitor of PCP in water. Due to the complexity of wastewater, especially in sewage system, for routine monitoring of PCP in wastewater, the gas chromatography and high performance liquid chromatography are important and effective methods (Vriendt, 2001; Michela, 2000). During the analysis procedures, PCP must be isolated and preconcentrated from the water sample before chromatographic measurements. In general, LLE and SPE are adopted. The former is based on solvent partitioning in separating funnels. LLE is frequently used for the isolation of pesticides from water sample (Christian, 2000; Parrilla, 1997). Dichloromethane is the commonest solvent because it is capable of extracting compounds with a wide range of polarities and easy to evaporate. However, LLE produces emulsion compounds, requires large amounts of solvent, and is laborious and difficult to automate.

SPE has been widely used to isolate the analytes from the water sample in recent decade. To perform this technique either on-line or off-line, there are many factors, which an analyst has to take into consideration in extraction process. Aquatic environment is a complex

and holistic system. For each individual compound, the efficiency of the recovery differs from each other, which depends on its chemical structure and the condition of SPE. A detailed optimization of SPE condition would, therefore, help to search for the optimum condition to get the highest recovery percentage for the special constituents of the sample.

For PCP, the development of SPE method involves the investigation of many variables influencing the efficiency of extraction. Therefore, how to decide these variables and their levels is critical. In this research, the selection of the variable scopes was based on the previous information from SPE (Giuseppina, 2001; Curini, 2001; Silvia, 1997). In this procedure, the scopes should be broad enough to include the possible optimum, but not to cause inconvenience in the experiment. The levels were set close to the possible optimum to get the most accurate information.

The theory and methodology of orthogonal array design (OAD), as a chemometric method for the optimization of the analytical procedure, has been described in detail. In this paper, several factors, which have effects on the recovery of the PCP, were studied and optimized by OAD. The final results were employed for the SPE of PCP from aquatic environment.

A comparative study between LLE and SPE is also carried out in the research.

## 1 Materials and methods

### 1.1 Chemicals

All chemicals were analytical-grade or the best quality available. Distilled deionized water was used throughout the experiment. Potassium hydrogenophosphate and phosphoric acid with analytical grade were used for the preparation of buffer solutions and as part of the mobile phase. Stock solution was prepared by dissolving PCP 10 mg in 10 ml methanol. Working standards solutions were prepared by appropriate dilution of stock solution with deionized water. When not in use, all the solutions were stored at 4°C in a refrigerator.

### 1.2 SPE procedure

Water samples were filtered through a 0.45 µm porous nylon

membrane filter of 50 mm diameter to remove all the particles that would obstruct the cartridge. The samples were driven through the cartridges by means of negative pressure from water pump. For the recovery researches, water samples were fortified with known amounts of the standard solution and agitated vigorously for approximate 1 min, then poured into a glass reservoir, respectively, which connected to the adsorbent cartridge. The sample pH was adjusted to corresponding value with phosphoric acid or potassium hydroxide. The extraction cartridges were rinsed by driving 5 ml of methanol through the cartridge, and then, followed by 10 ml of water adjusted to the same pH as the sample. Teflon tubes were connected between sample reservoir and cartridges. Sample loading was performed at a designation flow rate under vacuum. The sorbent was never allowed to dry during the rinsing and sample loading procedures. After extraction, most of the water was removed from the cartridges by forcing the room air through them for 10 min. Elution was performed by gravity, but a little pressure was put on at the end of elution. Different elution solvents and elution volumes were tested. The eluate was analyzed by HPLC.

### 1.3 LLE procedure

Water samples were filtered through millipore membrane filter(0.45  $\mu$ m) before this procedure, and then fortified with known amounts of the standard solution. After agitation, 200 ml of water sample was acidified to pH = 4 by adding of phosphoric acid and extracted twice with dichloromethane(50 ml + 25 ml) in a 500 ml separatory funnel and vigorous shaking. The combined extractes were concentrated to approximately 2 ml by K-D concentrator, and then evaporated to dryness under the condition of gentle nitrogen stream. The residue was dissolved in 2 ml methanol and analyzed by HPLC.

### 1.4 Mensuration procedure

The concentration of PCP was analyzed by HPLC system. The analytical column: irregular-HC18 micron 4.6 id  $\times$  25 cm; column temperature: 40 $^{\circ}$ C, the UV detector operated at 248 nm, mobile phase component: methanol : buffer = 85 : 15 (v/v). Buffer of 0.01 mol/L potassium hydrogenophosphate was adjusted to pH = 4 with phosphoric acid, flow rate, 1 ml/min.

The concentrations of the PCP in the water samples were calculated by measuring the PCP peak and compared with those obtained from standard solutions. All solvents and samples were filtered through millipore membrane filters before injection into the column.

## 2 Results and discussion

### 2.1 Optimization of SPE procedure

In this experiment, four major factors that may effect the SPE efficiency were studied. These factors are as follows: the pH of sample, 7, 5 and 3 (factor A), eluting solvents, methanol, 100%, 90% and 80% (factor B), eluting solvent volume, 4 ml, 2 ml and 1 ml (factor C) and sample flow-rate, 12 ml/min, 8 ml/min and 4 ml/min (factor D). In this work, because a three-level and four-factor variable will be optimized, an  $\text{OAD}_3(3^4)$  matrix is employed to assign the factors. The assignment of the factors and the levels in this design and the results of the experiment are summarized in Table 1.

The average level means of the recoveries of each factor were calculated according to the assignment of the experiments. For example, to obtain the level mean of factor B at level 2 in this experiment, the recovery data of three trials in level 2 of B (trials 2, 5, and 8, Table 1)

were pooled and divided by the number of the trials:  $B_2 = (79.5 + 73.7 + 91.3)/3 = 81.5\%$ , similar,  $B_1 = (76.3 + 89.5 + 82.5)/3 = 82.7\%$ . The different means at the two levels of a factor reveal how the recovery change with the change of the factor level. The average recovery of each factor at different levels was calculated and is summarized in Table 2 and Fig. 1.

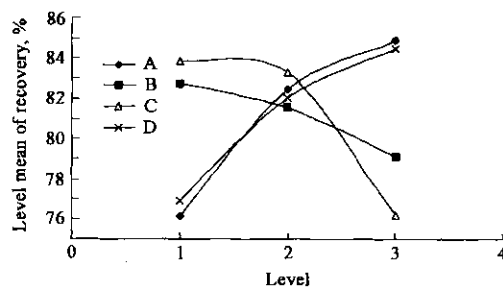
**Table 1** Assignment of the factors and levels of the optimize experiment by using an  $\text{OAD}_3(3^4)$  matrix and the results of the effects of selected variables on the recovery

Trial number	Factor				Result
	(A)Sample, pH	(B)Eluting solvent, methanol, %	(C)Eluting volume, ml	(D)Flow rate of water sample, ml/min	
1	(1) 7	(1) 100	(1) 4	(1) 12	76.3
2	(1) 7	(2) 90	(2) 2	(2) 8	79.5
3	(1) 7	(3) 80	(3) 1	(3) 4	72.5
4	(2) 5	(1) 100	(2) 2	(3) 4	89.5
5	(2) 5	(2) 90	(3) 1	(1) 12	73.7
6	(2) 5	(3) 80	(1) 4	(2) 8	84.0
7	(3) 3	(1) 100	(3) 1	(2) 8	82.5
8	(3) 3	(2) 90	(1) 4	(3) 4	91.3
9	(3) 3	(3) 80	(2) 2	(1) 12	80.8

**Table 2** The average recovery of every level and the rang of each variables (%)

	(A) Sample pH	(B) Eluting solvent, %	(C) Eluting volume, ml	(D) Sample water flow rate, ml/min
Level 1	76.1	82.7	83.8	76.9
Level 2	82.4	81.5	83.2	82.0
Level 3	84.8	79.1	76.2	84.4
Range	8.7	3.6	7.6	7.5

The importance of each variable on the recovery was presented by range( $R$ ). The  $R$  equal to the difference between the maximum and minimum level mean of recovery in the same factor. The result is shown in Table 2. Visual relationship between the level mean of average recoveries and the level of different factors are shown in Fig. 1.



**Fig. 1** Relationship between the level means of average recoveries and the factor level

Table 2 and Fig. 1 show that water sample pH have significant effects on the average recovery of PCP (range = 8.7, the largest in four factors). PCP is a hydrophobic ionizable organic compound with a  $\text{pK}_a$  of 4.75. When the pH value of water sample was higher than 5 most of PCP exists in ionized form, on contrary most PCP exist in neutral form. The recovery increase from 76.1% to 82.4%, when the pH was lowered from 7 to 5 (from level 1 to level 2), however, increase from 82.4% to

84.8%, when the pH was lowered from 5 to 3 (from level 2 to level 3). The different recoveries for PCP at various pH water samples perhaps have directly relations with the existing form of PCP. Subsequent experiments were carried out, the sample pH was designed by 4, 3, 2, and 1, and other conditions are similar to trial 8. The curves were made for sample pH and the recovery of PCP. The result is that the range of them was not more than 1. Because pH = 4 are more closed to normal pH value of aquatic environment. So pH = 4 was chosen for further experiments.

In this research, three elution solvents chosen were tested, 100% methanol, 90% methanol, and 80% methanol. The average recovery decreased from 82.7% to 79.1%, for different elution solvents. The range of elution solvents was 3.6, lower than other variables. Therefore, the elution solvent was not a major factor that affects the recovery of PCP. PCP could almost be eluted by either of these elution solvents, but using 100% methanol can obtain a little higher recovery for PCP.

The variation in PCP recovery as a function of elution solvent volume was researched in this work. The range of factor(C) was 7.6. So elution solvent volume had also significant effect on the recovery of PCP. However, Fig. 2 shows that the recovery of the factor(C) between level 1 and level 2 approached a plateau region of the curve. It showed that 2 ml and 4 ml elution solvent has almost the similar efficiency of elution. But for the same water samples, the limit of detection for 2 ml elution solvent was 1/2 for 4 ml, and the methanol used for elution is also reduced. Therefore, 2 ml methanol was chosen for further experiments.

The effect of flow rate on recovery was investigated at three different flow rates 4, 8 and 12 ml/min. The average recovery for PCP drop from 84.4% to 76.7% when the flow rates increase to 12 ml/min from 4 ml/min, and the range = 7.5. The experiment results showed that the flow rate is also the major variable effecting the recovery for PCP. The lower flow rate is, the higher recovery will get. However, lower flow rates will increase the time needed for sample preparation, especially when large sample volumes are used. So in this experiment 4 ml/min was chosen as optimum sample flow rate.

## 2.2 Validation and comparison of SPE and LLE

The SPE method was applied in routine analysis and compared with the LLE method. The comparison was performed with three different water sample at two PCP concentrations, which were divided in 200 ml portions and extracted by SPE and LLE methods, respectively. The results are summarized as Table 3.

**Table 3 The recovery of different water sample in comparison study of SPE and LLE (%)**

Water sample	Concn. = 20 µg/L		Concn. = 200 µg/L	
	SPE	LLE	SPE	LLE
Distilled water	87.6	79	93.8	94.7
Municipal wastewater	119.5	88	100.4	107.8
Industrial wastewater	133.6	120.3	117.6	98.5

The results showed that there was no significant difference between the two extraction methods. At the concentration level of 200 µg/L the recoveries were of 93.8%—117.6% and 94.7%—107.8%, respectively, by the SPE and LLE methods. At the concentration level of 20 µg/L the recoveries of the SPE and the LLE were 87.6%—133.6% and 79%—120.3%, respectively.

Compared with LLE, SPE has several advantages. First, the

velocity of SPE is faster than LLE, especially when water samples comprise high concentration of dissolved organic carbon, which the formation of emulsion can cause some problems during LLE. Second, it is more practical to extract many samples using SPE at the same time. Third, less time and less organic solvents are needed for cleaning of glass equipment, because disposable SPE cartridges are used for extraction.

## 3 Conclusions

A SPE method to analyze the concentration of PCP in various water samples have been developed and validated in this experimental research. Compared with LLE, SPE has advantages, e.g. rapid, less labor intensity and reducing the need of solvent, which is hazardous and expensive. So SPE can replace LLE for isolating and concentrating PCP from aquatic environment.

## References:

- Christian W T, Olav L, Agnethe L, 2000. Development of a solid-phase extraction method for phenoxy acids and bentazone in water and comparison to a liquid-liquid extraction method[J]. *J Agric Food Chem*, 48: 5829—5833.
- Curini R, Gentili A, Marchese S *et al.*, 2001. Monitoring of pesticides in surface water: off-line SPE followed by HPLC with UV detection and confirmatory analysis by mass spectrometry[J]. *Chromatographia*, 53: 244—250.
- Divincenzo J P, Sparks D L, 2001. Sorption of the neutral and charged forms of pentachlorophenol on soil: evidence for different mechanisms [J]. *Arch Environ Contam Toxicol*, 40: 445—450.
- Divincenzo J P, Sparks D L, 2001. Sorption of the neutral and charged forms of pentachlorophenol on soil: evidence for different mechanisms [J]. *Arch Environ Contam Toxicol*, 43: 445—450.
- Giuseppina M, Roberta T, Marta A *et al.*, 2001. Determination of 5-fluorouracil in environmental samples by solid-phase extraction and high-performance liquid chromatography with ultraviolet detection[J]. *Journal of Chromatography B*, 750: 25—32.
- Lawrence M, 1999. Can adsorption isotherms predict sediment bioavailability? *Chemosphere*, 41: 1091—1100.
- Michael G S, Donald L S, Steven K D, 1994. Sorption of pentachlorophenol to HDTMA-clay as a function of ionic matter[J]. *Environ Sci Technol*, 28: 2330—2335.
- Michela P, Gianluca B, Fabio C, 2000. Determination of phenoxyalkanoic acids and other herbicides at the ng/ml level in water by solid-phase extraction with poly (divinylbenzene-co-N-vinylpyrrolidone) sorbent and high-performance liquid chromatography-diode-array detection[J]. *Journal of Chromatography A*, 867: 169—175.
- Parrilla P, Martinez J L V, 1997. Determination of pesticide residues in water using LLE or SPE and HPLC/DAD detection[J]. *Analytical letters*, 30: 1719—1738.
- Radke C J, Prausnitz J M, 1972. Adsorption of organic solutes from dilute aqueous solution on GAC[J]. *Ind Engin Chem Fund*, 11: 445—451.
- Silvia L, Daniel F, Damia B, 1999. Efficient solid-phase extraction procedures for trace enrichment of priority phenols from industrial effluents with high total organic carbon content[J]. *Journal of Chromatography A*, 857: 97—106.
- Vriendt C A de, Sassenbroeck D K van, Rosseel M T *et al.*, 2001. Development and validation of a high-performance liquid chromatographic method for the determination of  $\gamma$ -hydroxybutyric acid in rat plasma [J]. *Journal of Chromatography B*, 752: 85—90.
- Voluker D, Wolfgang H, Klausdieter B *et al.*, 2001. Association of elevated blood levels of pentachlorophenol(PCP) with cellular and humoral immunodeficiencies[J]. *Archives of Environmental Health*, 56: 77—83.
- Wang J L, Qian Y, Nigel H *et al.*, 2000. Bioadsorption of pentachlorophenol

- from aqueous solution by activated sludge biomass[J]. *Bioresource Technology*, 75: 157—161.
- Zheng M H, Zhang B, Bao Z C *et al.*, 2000. Analysis of pentachlorophenol from water, sediments, and fish bile of Dongting Lake in China[J]. *Bull Environ*

*Contam Toxicol*, 64: 16—19.

(Received for review September 24, 2002. Accepted October 11, 2002)

## The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences

The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (RCEES/CAS), formerly Institute of Environmental Chemistry, Chinese Academy of Sciences, was founded in 1975. In 1986, it was amalgamated with Research Center of Ecology, CAS, and was renamed as Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Since 1996, RCEES was under the jurisdiction of both Chinese Academy of Sciences and State Environmental Protection Administration. The main research areas of RCEES include environmental chemistry, environmental engineering, and systems ecology. Aiming to realize the interpenetration of environmental chemistry, environmental engineering, ecology, geoscience and other disciplines, RCEES is taking the advantage of the multidisciplinary research to solve the major environmental and ecological problems at the local, regional, national as well as global levels.

Since 1975, RCEES has carried out a large number of research projects granted by the central government, CAS, and local governments as well as many international collaborative projects, obtaining both nationally and internationally recognized achievements. RCEES has also acted as a training center in environmental sciences and ecology in China. RCEES has significantly contributed to the establishment and development of eco-environmental sciences and technology, especially the environmental chemistry and systems ecology in China, and to environmental pollution control and eco-environmental protection and sustainable development in China and become a nationally leading and internationally recognized research institute in the field of eco-environmental sciences and technology.

There are 308 staffs in RCEES, including 3 academicians, 48 research professors. There are 8 research laboratories and one center for information and publication, in which there are one state key laboratory

(State Key Laboratory of Environmental Aquatic Quality) and two CAS key laboratories (Key Laboratory of Environmental Chemistry and Ecotoxicology, and Key Laboratory of Systems Ecology). Research Center for National Status, CAS is also at RCEES.

RCEES offers three year M. Sc. programs in environmental sciences, ecology, environmental engineering, organic chemistry and analytical chemistry. Three year Ph. D programs are offered in environmental sciences, environmental engineering, and ecology, and two year postdoctoral programs are offered in environmental sciences.

RCEES has established academic exchanges, cooperation and joint student training initiatives with Germany, USA, Japan, UK, Canada, Switzerland, Sweden, the Netherlands, Australia and other countries. RCEES has cooperated with national universities in offering joint postgraduate programs and carrying out joint research projects. The joint organizations established by RCEES and other units are Sino-Australia Soil Environment Joint Laboratory, Joint Institute of Environmental Sciences of RCEES and Hong Kong Baptist University, and RCEES-Aglient Environmental Analytical Lab of Asian-Pacific Region. RCEES has organized many important international conferences on eco-environmental sciences and promoted the cooperation and exchange among foreign and Chinese scientists.

Add: 18 Shuangqing Road, Haidian District

P.O. Box 2871

Beijing 100085, China

Tel: 86-10-62923549

Fax: 86-10-62923563

E-mail: std@mail.rcees.ac.cn

Website: <http://www.rcees.ac.cn>