Study on the performance condition optimization of analytic method for p-chlorophenol compounds by high performance liquid chromatography conditions

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ABSTRACT

Little universal method is monitored p-chlorophenols compounds in environmental media. In the paper, analytic method of p-chlorophenols compound by high performance liquid chromatography (HPLC) was optimized. The result were that the mobile phase was 0.5% phosphoric acid and acetonitrile, and flow rate was 1.5mL/min. The column temperature of HPLC was 30 ℃, and the injection volume was 10 μL, detection wavelength was 218nm. In monitoring process, the gradient elution mode determination of pentachlorophenol was used. In this condition, the pentachlorophenol, o-chlorophenol, m-chlorophenol and phenol could be seperated very well, the standard cures were Y=68.523x+360.08, Y=18.392x+17.575, Y=20.09x+16.319, and Y=26.379x+17.061, respectively. And the relative standard deviation (RSD) was 0.83%, 1.04%, 0.91%, 0.40%, respectively. The average recovery of the added index were from 89.17~100.58%.At last, sample of pentachlorophenol wastewater after biodegradation was monitored by HPLC in accordance with the optimization of the chromatographic conditions.. The result indicated that there was pentachlorophenol 12.35mg/L, p-chlorophenol 6.75mg/L, o-chlorophenol 3.24 mg/L, phenol 1.79mg /L.

Key words: chlorophenols; HPLC; gradient elution

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INTRODUCTION

Since 1930s, chlorophenols (CPs) have been widely used as herbicides, textiles, leather, preservatives [1-2]. However, CPs is high toxicity compounds, and easily absorbed into body. Therefore, CPs could be human health, after CPs was into the environment [3-5]. For example, CPs could be harm for the skin, lungs, etc. Especially, 2-2, 4-two chlorophenols, chlorophenols, 2,4,6-three Chlorophenol and pentachlorophenol are highly toxic substances, so that these compounds have been priority control pollutants in American Environmental Protection Agency (USEPA) in 1977.

Therefore, many researches have focused on distribution characteristics of all kinds CPs in environmental media, such as soil, water. But there is still little universal method to determine CPs in every environmental media. Especially, in biodegradation process, every type of CPs could be determined in one sample, directly related to indicate degradation pathway of CPs [6-7].

In order to monitor every CPs in one sample, some effective method should be selected, and the condition is also optimized. In recently, in analytic chemistry, High-performance liquid chromatography (HPLC), is considered as to separate and identify the components in a mixture, and, the method could accurately quantify each component [8-9]. The principle of HPLC is that the sample dissolved pressurized liquid pass column filled with a solid adsorbent material by pumps [10-11]. At present, HPLC is important technology, and widely used in many field, e.g, chemical

and biochemical analysis, pharmaceutical research, chemical, environmental, life sciences, and food industry. Therefore, CPs could be monitored by HPLC.

In this paper, an analytic method was used by high performance liquid chromatograph, and the conditions, e.g. wavelength, kinds and proportion of mobile phase etc., were optimized. The study cloud be provide the technical means for the qualitative and quantitative of chlorophenols in water and monitoring degradation pathway of biodegradation.

EXPERIMENTAL SECTION

INSTRUMENTS AND REAGENTS
Main equipment: Agilent 1100 high performance liquid chromatography with diode array detector, reversed phase C18 column; Eclipse XDB-C18 (4.6×250mm, 5μm).

Main reagents: methanol, acetonitrile (all HPLC); phosphoric acid (HPLC); pentachlorophenol (GR); phenol, p-chlorophenol and o-chlorophenol (all chemically pure).

ANALYSIS OF CHROMATOGRAPHIC CONDITIONS
The flow rate of mobile phase was 1.5mL/min; column temperature of HPLC was 30℃; the injection volume was 10 μL; detector wavelength was 218nm.The mobile phase is compose of 0.5% phosphoric acid and acetonitrile or 0.5% phosphoric acid and methyl alcohol. The proportion of 0.5% phosphoric acid and acetonitrile or 0.5% phosphoric acid and methyl alcohol were shown in Table.1

Table.1   proportion of mobile phase

composition		composition	
0.5% phosphoric acid(%)	methyl alcohol(%)	0.5% phosphoric acid(%)	acetonitrile(%)
10	90	80	20
20	80	55	45
50	50	20	80
90	10	20	80

PREPARATION OF STANDARD STOCK SOLUTION
The CPs were accurately weighed and dissolved in methanol. And, the CPs were prepared three different concentrations of liquid reserves, 1000mg/L, 800mg/L and 600mg/L, respectively.

SAMPLE PREPARATION.
Monitoring the CPs of wastewater after biodegradation could be used to analyze the degradation way of biodegrading pentachlorophenol. Thus, the sample of pentachlorophenol wastewater after biodegradation was monitored. And, the sample was firstly centrifugal separation after 4000r/min for 5 min. Then, 1.5mL supernatant was centrifugal separation by 10000r/min for 20 minutes. At last, the supernatant water was filtered after 0.45 micron membrane.

RESULTS AND DISCUSSION

DETECTION WAVELENGTH SELECTION
According to the benzene ring structure which have strong absorption UV in the ultraviolet band, four kinds of phenolic compounds were firstly scanned in the range of 190nm ~ 400nm. The result showed that the maximum absorption peak of were in 198nm, 202nm and 218nm. In order to avoid the interference of impurities, the detection wavelength is determinated in 218nm.

THE SELECTION OF MOBILE PHASE
The selection of mobile phase is in accordance with some views:

Firstly, due to increasing number of halogen, the time of peak in HPLC was prolonged, so selection of mobile phase should consider the cost.

Additionally, considering the similar character of o-chloro phenol and p-chlorophenol, these also are not easily discriminated.

Therefore, the mobile phase is composing of 0.5% phosphoric acid and methyl alcohol or acetonitrile, and gradient

elution mode is used. The optimal gradient elution mode was studied as Table 1, table 2.

Table.1   gradient elution mode of 0.5% phosphoric acid and methyl alcohol

	Flow rate	Time of elution(min)	composition		curve
			0.5% phosphoric acid (%)	methyl alcohol(%)	
1	1.0	0	50	50	linear
		20	10	90	linear
		22	50	50	linear
		0	50	50	linear
2	1.0	15	10	90	linear
		18	50	50	linear
		22	50	50	linear
		30	50	50	linear
3	1.0	0	80	20	linear
		10	20	80	linear
		20	80	20	linear
4	1.0	0	80	20	linear
		20	20	80	linear
		22	80	20	linear
5	1.5	0	80	20	linear
		20	20	80	linear
		22	80	20	linear

Table.2   gradient elution mode of 0.5% phosphoric acid and acetonitrile

time(min)	composition		curve
	0.5% phosphoric acid(%)	acetonitrile(%)	
0	80	20	linear
7.5	55	45	linear
9.5	20	80	linear
12	20	80	linear

Through the investigation of the elution gradient, the baseline was stable, no drift, and peak shape and separation effect of CPs was very good, with the 0.5% phosphoric acid -acetonitrile as the mobile phase. At the same time, the separation process could be completed in 12min.

Therefore, acetonitrile -0.5% phosphate was identified as mobile phase of high performance liquid. And, pentachlorophenol, o-chlorophenol, m-chlorophenol and phenol were separated shown in Fig.1.

1-phenol; 2-o-chlorophenol;3-m-chlorophenol,;4-pentachlorophenol
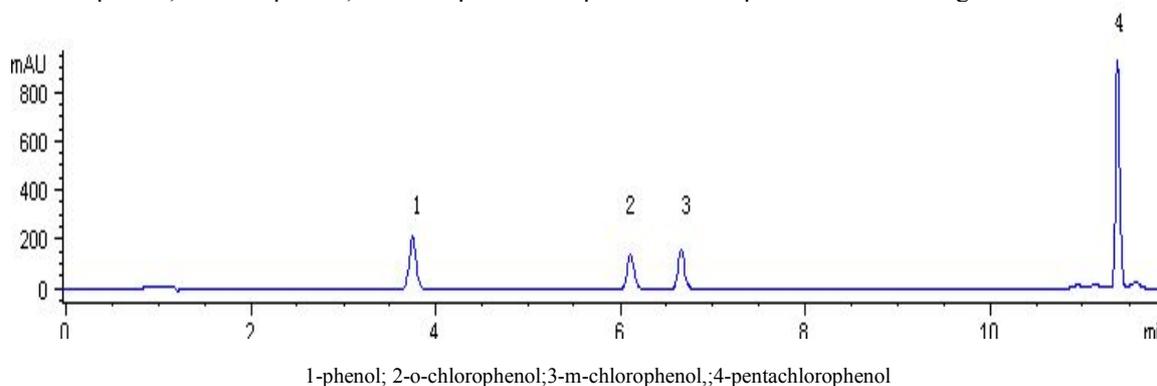
Fig.1   gradient elution mode in acetonitrile -0.5% phosphate condition

STANDARD CURVE
Mixed chlorophenols of pentachlorophenol, o-chlorophenol, m-chlorophenol and phenol were diluted mixed 10, 20, 40, 60, 80mg/L. And analyzing sample of 10 μL, the standard curves were determined, and   shown in Table 3.

Table.3   standard curves and detection limit

chlorophenol	standard curve	correlation coefficient	detection limit ng/mL
pentachlorophenol	Y=68.523x+360.08	R^2=0.9942	0.13
m-chlorophenol,	Y=20.09x+16.319	R^2=1	0.52
o-chlorophenol	Y=18.392x+17.575	R^2=0.9999	0.81
phenol	Y=26.379x+17.061	R^2=1	0.77

As shown in table 3, correlation coefficients were all high($R^2 > 0.9942$), and the linearity were good. And, the detection limits were lower. For example, the detection limit of pentachlorophenol for the detection of 0.13ng/mL with UV spectrophotometry lower limit [12-13].

PRECISION AND RECOVERY EXPERIMENTS
At last, the precision and recovery experiments were studied. The result was shown in Table 4.

Table.4   Results of precision and recovery experiments

	RSD	average recovery of the added index (%)
pentachlorophenol	0.83%	95.24
o-chlorophenol	1.04%	96.48
m-chlorophenol	0.91%	89.17
phenol	0.40%	100.58

The relative standard deviation (RSD) of pentachlorophenol, o-chlorophenol, m-chlorophenol and phenol were 0.83%, 1.04%, 0.91%, 0.40%, respectively. And, the average recovery of the added index were from 89.17~100.58%.

2.5 PRACTICAL APPLICATION
The sample of pentachlorophenol wastewater treatment anaerobic sludge was taking an example. And, the sample was moditored by HPLC in accordance with the optimization of the chromatographic conditions. The result indicated that pentachlorophenol 12.35mg/L, p-chlorophenol 6.75mg /L, o-chlorophenol 3.24 mg/L, phenol 1.79mg /L. The result could be used to analyze the degradation way of pentachlorophenol.

CONCLUSION

In the paper, the condition of CPs of high performance liquid chromatograph, were optimized. The result were that the mobile phase was 0.5% phosphoric acid and acetonitrile, and flow rate was 1.5mL/min. The column temperature of HPLC was 30℃, and the injection volume was 10 μL, detection wavelength was 218nm. In monitoring process, the gradient elution mode determination of pentachlorophenol was used. In this condition, the pentachlorophenol, o-chlorophenol, m-chlorophenol and phenol could be separated very well and the relative standard deviation (RSD) were 0.83%, 1.04%, 0.91%, 0.40%, respectively. The average recovery of the added index were from 89.17~100.58%. At last, the sample of pentachlorophenol wastewater treatment anaerobic sludge was monitored by HPLC in accordance with the optimization of the chromatographic conditions.

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