Analysis and Evaluation of 7 Indicator Polychlorinated Biphenyls (PCBs) Residues in Dried Kelp by Gas Chromatography (GC)

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Abstract. In this study, a special method was developed for the determination of 7 kinds of polychlorinated biphenyls (PCBs) residues in dried kelp by gas chromatography (GC) with electron capture detector (ECD). The PCBs were extracted with hexane/dichloromethane (1/1, v/v) by ultrasonic extraction. Clean-up methods were used by concentrated sulphuric acid, neutral alumina oxide solid phase extraction cartridge and silica solid phase extraction cartridge. The analytical compounds were quantified by an internal standard method. Under optimal experimental conditions, good linearity was observed in the range of 5~200ng/mL, and the correlation coefficients were 0.9993~0.9998. The limit of quantification (LOQ) for target analytical compounds ranged from 6.0 to 7.5µg/kg. At the spiked levels of 10, 20, 50 µg/kg, the average recoveries ranged from 81.8% to 105% with the relative standard deviations 3.05% ~ 11.2%. The result showed that the proposed method was accurate and could be used for the determination of the PCBs in dried kelp.

1 Introduction

The Polychlorinated biphenyls (PCBs) are a class of synthetic organic compounds containing carbon, hydrogen and chlorine atoms. They have been used in various industrial and commercial applications owing to their high boiling points, non-flammability, chemical stability and insulating properties. The widespread use of PCBs in different commercial and industrial applications along with their inappropriate disposal had created severe environmental contamination because of their persistence and multiple dimensions of toxicity. Many offshore waters have been contaminated by the PCBs in our country. The concentration is more than the 30 ng/L standard promulgated by the U.S. EPA [1]. They can penetrate the human body through skin contact, by inhalation of PCBs contaminated vapour and by consuming food contaminated with PCB residues [2]. The National Toxicology Program (NTP) and International Agency for Research on Cancer (IARC) have concluded that PCBs are carcinogenic to human. Polychlorinated biphenyls (PCBs), one of the earliest persistent organic pollutants (POPs), are still a global concern due to their ubiquitous and adverse effects on human health, such as endocrine disruption and carcinogenicity, even though their use has been prohibited since 1979[3].

Among the various exposure pathways, dietary intake is the major route of human exposure to PCBs [4]. Kelps are large brown seaweeds and rich in protein, vitamins and minerals which are beneficial to human body. In addition, the bioactive substances in kelp, such as

mannitol, brown algae starch, and alginic acid et al, have various medical and health functions [5]. So kelp is a kind of very nutritious food for people. Kelp is widely used to make dried kelp for long-term storage and transportation. Kelps are distributed along the world's coastlines, so it is easy to enrich organic pollutants and contaminated by the PCBs because coastal areas are easily polluted by urban waste. In order to monitor the kelp, there is a need to establish a detection method.

Sample preparation for PCBs is remarkably a judgmental process on which the accuracy of measurements depends. The reported studies [6] described the several sample extraction methods including soxhlet extraction, ultrasonic techniques, microwave extraction, supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) in the extraction of different samples. Zhao et al [7] established a method of ultrasonic extraction of polychlorinated biphenyls from kelp, but this method used a large amount of organic reagents and the detection line was high. So this paper presents a new ultrasonic extraction method. Clean-up was performed by concentrated Sulphuric acid, neutral alumina oxide solid phase extraction (SPE) and silica SPE because of the complex matrix. The most common and generally considered techniques for the identification of PCBs are gas chromatography with electron capture detector (GC-ECD) and electron ionization mass spectrometry (GC-MS). In this study, GC-ECD was selected for the applicability and operability of detection method. The established method is effective and accurate for the determination of PCBs in kelp.

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2 Materials and method

2.1 Materials and instrument

The GC system consisted of an Agilent 7890A GC (Agilent Technologies, Shanghai, China), equipped with an electron capture detector (ECD). The GC was fitted with a HP-5MS fused silica capillary column (5% Methyl Phenyl Silicone as non-polar stationary phase, 30 m, 0.25 mm i.d. and 0.25 μ m film thickness) from Agilent (J&W Scientific, Folsom, CA, USA).

PCB 28, PCB 52, PCB101, PCB 118, PCB 152, PCB 138, PCB 180, PCB 198 were purchased from Dr.Ehrenstorfer GmbH and had purities >98%. N-hexane and dichloromethane were purchased from J.T.Baker (Avantor Performance Materials, Inc.) and they were HPLC grade. Concentrated sulphuric acid were purchased from sinopharm chemical reagent Co.,Ltd and it was analytical purity. Neutral alumina oxide SPE cartridge (500mg/6mL) and silica SPE cartridge (500mg/6mL) were purchased from Agela Company. Dried kelp samples were purchased from supermarket.

Stock solutions were prepared for all standard substances were 1.0 μ g/mL in n-hexane and stored at -20°C. Calibration mixtures at various concentration levels were obtained by combining aliquots of stock solutions and internal standard with n-hexane and stored at -20°C. The concentration of internal standard in all the calibration mixtures and final sample solutions was 40ng/mL.

2.2 Method

About 2.00 g of dried kelp sample was weighed in a 50mL centrifuge tube and spiked with 40 ng PCB 198 as the internal standard. The extraction was made with 30 ml aliquots of a mixture of n-hexane/dichloromethane (1/1, v/v) ^[8] for 2 h of soaking and 30 min of ultrasonic. Then each mixture was centrifuged at 8000 r/min g for 8 min. The extraction procedure was repeated twice and the extracts from each time were combined into a pear-shaped bottle and evaporated in a rotary evaporator to dry at 40°C and weak vacuum. The residue was dissolved by 20ml n-hexane twice and then the dissolved solution was subjected to cleanup.

The dissolved solution was added 5mL concentrated sulphuric acid and fully shocked for 2 min, and then the mixture was centrifuged at 2000 r/min g for 5 min. The supernatant was normally passed through a neutral alumina oxide SPE cartridge and silica SPE cartridge conditioning with 5ml n-hexane without any pressure. The supernatant passed the SPE cartridge was collected and another 3ml n-hexane was used to elute off the analytical compounds. The elution was dried under a gentle nitrogen stream at 40°C. The residue was reconstituted with 1mL n-hexane and mixed in a vortex stirrer

2.3 GC analysis

The final samples were detected on an Agilent 7890A GC, coupled to an electron capture detector (ECD). The column temperature program was as below: 150°C hold for 1 min, ramp at 15°C/min to 200°C, hold for 5 min; ramp at 2°C/min to 270°C, hold for 2 min; ramp at 20°C/min to 280°C, and hold for 5 min. Nitrogen with a purity of 99.999% was used as the carrier gas. The carrier gas (nitrogen) flow rate was 1.2mL/min, injection port temperature was 280°C, and injection volume was 1 μ L. The injection was made in the splitless mode with purge on after 0.75 min.

3 Results and discussion

3.1 Extraction method

The methods of extracting PCBs from solid samples widely used are soxhlet extraction, supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), microwave-assisted extraction (MAE) and ultrasonic extraction (UE). The major disadvantages of the soxhlet extraction are their prolonged extraction times varying from 24 to 48 h, consumption of high organic solvent up to 500mL per sample ^[6]. Consistent extraction recoveries of ASE and MAE procedures have described that elevated pressure and temperature are appropriate to extract PCB congeners from solid matrices efficiently ^[9]. SFE is also an effective technique for the extraction of PCBs from solid materials. However, these three extraction methods need special instruments and technologies, which is not conducive to the popularization and application of detection technology. The efficiency of ultrasonic extraction is high and the method needs no special instrument, so it is easy to operate. The recovery of the method of soaking for 2h and ultrasonic for 30min could reach 90%, so this extract method was used.

3.2 Optimization of sample purification

During the analysis of PCBs in dried kelp, the recoveries might be hindered by the chromatographic interference of co-extracted lipids, protein, pigment *et al.* Therefore, it is necessary to perform cleanup procedures to remove co-extracted impurity substance from such samples prior to analysis. 7 indicator PCBs are stable in acidic environments, and therefore, it is effective to carry out cleanup processes using concentrated sulphuric acid. There was some matrix to interfere the PCB 28 and PCB 52 to detect when only concentrated sulphuric acid was used to purify the sample, and it could not reduce interference by increasing the amount of sulphuric acid. Neutral alumina oxide SPE cartridge was added to purify the sample, and it was found that some interference could be removed, but some interference still existed. So silica SPE cartridge was used to eliminate the interference, and the result showed that was effective. Due to the complex composition of the kelp, the

requirements could be met only when three purification methods are used together.

3.3 Method performance

The calibration curves for detection of the target compounds were obtained by performing a linear regression analysis on standard solution using the ratio of standard area to internal standard area (PCB 198) against analytical compounds concentrations ranging from 5 to 200ng/mL containing 40ng/mL internal standard. Good linearity was obtained for all analytical compounds, with correlation coefficients of R ranged from 0.9993 to 0.9998. The limit of detection (LOD), defined as the concentration which yield a signal-to-

noise (S/N) equal to 3, ranged from 2.0 μ g/kg to 2.5 μ g/kg (Table 1). The limit of quantification (LOQ), defined as the concentration which yield an S/N equal to 10, ranged from 6.0 μ g/kg to 7.5 μ g/kg (Table 1). The analytical compounds recovery of this procedure was evaluated by spiking 10.0, 20.0 and 50.0 μ g/kg of each analytical compounds and 40ng internal standard to sample in replicates of six. The results are listed in Tables 2. The average recoveries of each compound ranged from 81.8% to 105%. The reproducibility of this method was represented by percent relative standard deviation (R.S.D.) at each level for each compound and these values are also summarized in Table 2. The results show that the method's precision was within 15%, which is satisfactory.

Table 1. Regression equations, linear ranges, correlation coefficient, the limit of detection (LODs) and the limit of quantification (LOQs) for the 7 indicator PCBs.

Compounds	Regression equation	Linear range ρ (ng/mL)	Correlation coefficient (r)	LOD (μ g/kg)	LOQ (μ g/kg)
PCB28	$Y=0.5231X-0.02799$	5~200	0.9995	2.5	7.5
PCB52	$Y=0.3505X-0.00165$	5~200	0.9994	2.5	7.5
PCB101	$Y=0.5046X-0.01029$	5~200	0.9995	2.5	7.5
PCB118	$Y=0.6035X-0.02986$	5~200	0.9993	2.5	7.5
PCB153	$Y=0.5952X-0.01725$	5~200	0.9998	2.5	7.5
PCB138	$Y=0.8214X-0.03724$	5~200	0.9996	2.0	6.0
PCB180	$Y=1.0887X-0.04247$	5~200	0.9994	2.0	6.0

Table 2. Recoveries and R.S.D. of spiked dried kelp (n=6).

Compounds	10.0 μ g/kg			20.0 μ g/kg			50.0 μ g/kg		
	Average concentration μ g/kg	Recovery %	RSD %	Average Concentration μ g/kg	Recovery %	RSD %	Average Concentration μ g/kg	Recovery %	RSD %
PCB28	8.21	82.1	5.18	16.8	84.0	6.02	40.9	81.8	7.98
PCB52	9.05	90.5	3.42	18.7	93.5	4.25	49.1	98.2	5.21
PCB101	8.96	89.6	4.41	18.1	90.5	4.24	46.0	92.0	3.89
PCB118	8.82	88.2	7.88	17.4	87.0	6.74	44.6	89.2	11.2
PCB153	9.34	93.4	3.16	18.3	91.5	5.03	52.5	105	5.45
PCB138	9.17	91.7	3.05	18.7	93.5	3.18	46.1	92.2	4.62
PCB180	9.29	92.9	4.11	18.9	94.5	3.63	48.6	97.2	5.78

4 Results and discussion

In this investigation, a laboratory procedure was developed for simultaneous extraction and determination of seven indicator PCBs in dried kelp. The procedure includes ultrasound extraction (UE) and three steps clean up with concentrated sulphuric acid and two types of SPE cartridge. GC was used as the detector. The method had good linearity, accuracy, and precision. Good recoveries ranging from 81.8% to 105% were reached. This new method may be suitable for the surveillance of the PCBs compounds in dried kelp contaminated by the sea environment.

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