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Determination of organochlorine compounds in fish liver by ultrasound-assisted dispersive liquid–liquid microextraction based on solidification of organic droplet coupled with gas chromatography-electron capture detection

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ABSTRACT

A simple and rapid method for the extraction of organochlorine compounds (OCs) including poly-chlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in fish liver using ultrasound assisted dispersive liquid–liquid microextraction based on the solidification of floating organic droplet (US-DLLME-SFO) was developed. For reducing the complexity of the matrix, the sample was pre-treated prior to microextraction. Factors affecting US-DLLME-SFO were optimized by using statistical design of experiment (DoE). The analysis was carried out by Gas Chromatography (GC) equipped with micro electron capture detector (μ-ECD). The optimized parameters were 4.8 min of ultrasound, 3.0 mL of Milli-Q and 24 μL of 1-undecanol as an extraction solvent as determined by DoE. US-DLLME-SFO was validated in terms of limit of detection, limit of quantification, dynamic linearity range, coefficient of determination (linearity) and extraction recovery in fish liver for OCs, and the respective values were (1.06-3.84 ng g^{-1}), (3.50-12.67 ng g^{-1}), (1.0–500 ng g^{-1}), (R^2 = 0.994-0.999), (88.5-108.4%). Interday and intraday precisions were evaluated as relative standard deviation (% RSD) and the values were ≤10%.

1. Introduction

In the past three decades, the occurrence of organochlorine compounds (OCs) such as poly-chlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) as aquatic environmental contaminants have been widespread and are a cause of concern because of their bio-accumulation, toxicity and persistence [1,2]. Organochlorine compounds move from air to soil by dry or wet deposition, then from soil to aquatic bodies by runoff due to rainfall. Due to the long-range atmospheric transport, OCs have been found in the environment [3]. As these compounds are not degradable, therefore their bio-accumulation may take place in animals and aquatic organisms [4]. Organochlorine compounds have been shown to be carcinogenic and have been reported to affect the pulmonary, reproductive, immune, nervous and endocrine systems [5,6]. Most of the developed countries have banned OCs. However, long term persistency builds up their toxicity in environment and food chains [7]. Thus, because of these facts, it is important to monitor the levels of OCs in environment and food chains. Consumption of food contaminated with OCs leads to exposure of humans to OCs [8]. Organochlorine compounds are resistant to environmental degradation and accumulate in fatty tissues due to high lipid solubility, therefore, fish is one of the main sources of these pollutants [9]. Fish is an essential source of proteins and vitamins for human beings. As the fishes accumulate organochlorine compounds, it is necessary to determine their levels in these [10]. The two major problems encountered in the determination of targeted analytes by extraction are: i) matrix interferences and ii) presence of low level of pollutants. Thus, in spite of developments in modern analytical techniques, extraction and preconcentration processes are to be evaluated to enhance the sensitivity of targeted analytes for better chromatographic response.

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Therefore, there is a need to establish a sensitive and reliable extraction method for determination of targeted analytes. Traditional extraction methods like liquid–liquid extraction (LLE) [11,12], solid phase extraction (SPE) [13,14] and soxhlet extraction [15,16] methods are still being used in many laboratories. However, the traditional methods have their limitations, namely LLE needs large quantities of organic solvents and is a time consuming process. SPE consumes less solvent than LLE, but in contrast uses expensive cartridges [17]. Soxhlet method generally needs enormous quantities of solvent and also takes more time for extraction [18,19]. QuEChERS method was also reported for the analysis of pesticides, PCBs, polycyclic aromatic hydrocarbons, and polybrominated diphenyl ethers in fish muscles [20]. The major disadvantages of QuEChERS method are that it does not use environmental friendly solvents and is mostly used for food matrices. Therefore, in recent years research has focused towards development of economically feasible, efficient, rapid micro level extraction methods. A number of microextraction techniques such as liquid phase microextraction (LPME) [21], solid phase microextraction(SPME)[22–24] and dispersive liquid–liquid microextraction (DLLME) [2,7,25–27] have been used to determine OCs in different samples.

Rezaee established the DLLME method in water samples [27]. The major advantages of DLLME are low cost, rapidity and high extraction efficiency. DLLME method has been successfully used for the determination of PCBs and OCPs at trace levels in environmental samples like water [7], soil [26] and fish tissues [10]. The major disadvantage of the DLLME method is:, it uses high density extraction solvents namely, chloroform, carbon tetrachloride, chlorobenzene which are also toxic [10]. The improved DLLME method is known as (DLLME-SFO), in which a solidified floating organic droplet is formed [28]. Regueiro et al. also made an improvement in the DLLME method by using ultrasonic radiation which replaces the dispersion solvent and named it as ultrasound-assisted emulsification microextraction method (USAEME) [29,30].

In the present study, a few microliters of extraction solvent were dispersed into aqueous sample by ultrasound radiation without using any dispersion solvent. The large surface contact between the extraction droplets and aqueous solution enhances the rapid mass transfer of analyte from the aqueous phase to the organic phase, and thus reduces the experimental time. After the selection of the most suitable extraction solvent, numerous parameters were screened out by design of experiment (DoE). Plackett Burman Design (PBD) was used for screening the significant parameters and Central Composite Design (CCD) was used to screen out the optimal values of the significant factors [31]. To the best of our knowledge, US-DLLME-SFO method for the simultaneously analysis of PCBs and OCPs in fish liver samples by using statistical design of experiment has not yet been reported.

The aim of this study is to establish US-DLLME-SFO method for the extraction of OCs from fish liver samples by design of experiment.

2. Experimental

2.1. Chemicals and solvents

Seven mixed standard solutions of PCBs (PCB No. 28, 52, 101, 138, 153, 180 and 209) and 11 standards of organochlorine pesticides were purchased from Sigma Aldrich (St. Louis, MO, USA) (Table S1). Acetone (HPLC grade solvent) and other chemicals (2-dodecanol, 1-dodecanol, 1-undecanol, 2-undecanone, and sodium chloride) were also purchased from Sigma Aldrich (St. Louis, MO, USA). Milli-Q water (18 MΩ cm) was obtained from Millipore water system (Milli-Q synthesis Elix-10, Millipore Corp., Mass., U.S.A) installed in the institutional water distillation unit.

2.2. Preparation of standard solution

The stock solutions of the PCBs and OCPs standards were prepared at 1.0 mg mL^{-1} in acetone and stored in a refrigerator at −20 °C. Mixed working standard solutions of OCs were prepared by taking each stock solution at 1.0 μg mL^{-1} and diluting it with acetone.

2.3. Sample collection

The two types of freshwater fish species Catla (*Catla catla*) and Snakehead murrel (*Channa striata*) were purchased from Kaiserbagh fish market, Lucknow, (U.P) India. The selection of the fish species was based on the consumption pattern of the fish by the general population. Fish samples were packed in polyethylene bags and transported in an ice box, as they are perishable. Individual fish liver samples of each species were chopped and homogenized, then made into a single sample, and kept at −20 °C until analysis. Lipid content of fish homogenate of both species was evaluated[32].

2.4. Instrumentation

Separation and determination of OCs were performed on an Agilent 7890A series gas chromatography equipped with a micro electron capture detector (Agilent Technologies, Palo Alto, CA, USA). In each chromatographic run, 1.0 μL of sample was injected into the GC in split mode, split ratio (20:1) at a split flow rate of 16.0 mL min^{-1}. The injection port temperature was maintained at 270 °C. The temperature of the detector was set at 300 °C and nitrogen was used as carrier gas at a flow rate of 0.8 mL min^{-1}. Chromatographic separation was achieved on DB-5 [(5%-phenyl)-95% methylpolysiloxane], 30 m × 0.32 mm ID × 0.25 m capillary column (J & W Scientific Inc., Folsom, CA, USA). The oven temperature program was as follows: initial temperature 180 °C for 2 min, raised to 200 °C at 20 °C per min for 1 min, 220 °C at 1.5 °C per min for 1.0 min and finally at 320 °C at 30 °C per min for 3 min.

2.5. Ultrasound assisted dispersive liquid–liquid microextraction based on solidification of floating organic droplet method (US-DLLME-SFO)

Fish liver of each sample was cut into small pieces, and then thoroughly homogenized with 300 mg of sodium sulphate in a grinder before processing. The entire contents were carefully transferred into a 25 mL conical flask to which 5 mL acetone was added. Optimization experiments were carried out with 1.0 g homogenate of liver by spiking appropriate amounts of the diluted working standard solutions to obtain final concentration of 50 ng g^{-1}. The contents were dynamically shaken for 20 min at 500 rpm on a horizontal automatic shaker. The supernant solution (acetone sample extract) was collected in a 10 mL glass tube. The solution was stored at −20 °C in a freezer overnight to remove the lipid content and sample solution (acetone sample extract) was completely dried in a nitrogen evaporator. Subsequently, 24 μL of 1-undecanol (as extraction solvent) and 1.0% w/v of NaCl were added to the tube. For getting appropriate medium for extraction, 3.0 mL of Milli-Q was gradually added alongside the tube wall. The sample tube was ultrasonicated at 30 °C for 4.8 min and the formation of cloudy sample solution was observed. The cloudy solution was centrifuged at 4000 rpm for 3 min and the fine droplets of 1-undecanol were found floating on the upper surface of aqueous sample solution in the conical tube. The floating organic drop was separated by cooling the tube in an ice bath till the drop solidified. The solidified floating

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