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Coupling of headspace solid phase microextraction with ultrasonic extraction for the determination of chlorinated pesticides in bird livers using gas chromatography

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Abstract

In the present study a combined analytical method involving ultrasonic extraction (USE), sulfuric acid clean-up and headspace solid-phase microextraction (HS-SPME) was developed for the determination of chlorinated pesticides (CPs) in bird livers. Extraction of CPs from 1 g of liver was performed by ultrasonication for 30 min using 20 mL of solvent mixture (n-hexane:acetone (4:1, v/v)). The extract was subsequently subjected to a clean-up step for lipid removal. A comparative study on several clean-up procedures prior to the HS-SPME enrichment step was performed in order to achieve maximum recovery and optimal clean-up efficiency, which would provide suitable limits of detection in the gas chromatographic analysis. For this purpose, destructive (sulfuric acid or sodium hydroxide treatment) and non-destructive (alumina column) clean-up procedures has been assayed. The treatment of the extract with 40% (v/v) H_2SO_4 prior to HS-SPME process showed the best performance since lower detection limits and higher extraction efficiencies were obtained. The method detection limit ranged from 0.5 to 1.0 ng g^{-1} wet weight and peak areas were proportional to analyte concentrations ($r^2 > 0.990$) in the range of 5–500 ng g^{-1} wet wt. The method was found to be reproducible (R.S.D. < 10%) and effective under the operational conditions proposed and was applied successfully to the analysis of CPs in liver tissues of various bird species from Greece.

Keywords: Chlorinated pesticides; Headspace solid phase microextraction; Ultrasonic extraction; Livers; Gas chromatography

1. Introduction

Chlorinated pesticides (CPs) are persistent ubiquitous environmental contaminants. Although the use of most of these chemicals have been banned or restricted in industrialized countries, they are still detected in the environment because of high persistence and low biodegradility. Biological samples such as liver can be used as indicators for environmental pollution in biota since usually accumulate CPs to relatively high concentrations. Hence, there is a clear need for simple, accurate and cost effective tools to determine their concentration levels in biological samples in order to provide essential data to assess health risk on both human and wildlife.

CPs are among the most difficult organic contaminants to measure in biological media and analytical problems associated with their analysis in these kinds of samples are well known, especially when common GC analysis is applied. Thus, proper pre-treatment of contaminated biological samples including clean-up procedure is crucial since even small amount of lipids can harm columns, detectors or cause signal suppression. Furthermore, the lipid removal is considered to be the most laborious step of the analytical process.

As a consequence, a variety of extraction procedures employing different clean-up techniques and a variety of detection methods have been developed over the past years in order to improve the extraction performance as well as to reduce overall analysis time and costs. The most commonly used methodology is based on Soxhlet extraction after a comprehensive clean-up step [1–3], but it is time-consuming and requires a large amount of solvent. Latest developments include supercritical fluid extraction (SFE) [4–7], subcritical water extraction

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[6,8,9], pressurized liquid extraction [5–7,10–12], accelerated solvent extraction (ASE) [13,14] and microwave assisted solvent extraction (MASE) [5,7,14,15]. These techniques have allowed sample size and solvent volume to be reduced. However, the selective extraction of trace analytes from complex matrices with high fat content such as animal tissues is difficult because of the coextraction of matrix components (proteins, lipids and other endogenous compounds) and most of these techniques still require an extensive clean-up step, with the extent of sample pre-treatment being dependent upon the analytical goal as well as the selectivity and sensitivity of the detection system.

In view of the fundamental problems described above, new sample preparation techniques are being developed in order to reduce the analysis step, enhance compound extraction efficiency from complex matrices, obtain better resolution and protect expensive instruments.

SPME [16] constitutes a good alternative to other commonly used extraction methods as sampling can be done rapidly and directly, without solvent, and can be easily automated. In this way, the extraction of the sample with a solvent followed by lipid removal with sulfuric acid (H_2SO_4) treatment and enrichment using the headspace solid phase microextraction (HS-SPME) technique would be an attractive approach. The ultrasonic extraction (USE) followed by SPME is a newly developed combination for sample preparation procedures and has been successfully applied for the determination of different organic chemicals in different solid matrices [17–22]. In all these studies, the coupling of USE and SPME proved to be more efficient compared to other traditional extraction methods such as Soxhlet and sonication alone, shortening sample preparation, reducing solvent use and obtaining enrichment and clean-up in a single step. However, most of these studies demonstrate the applicability of USE/SPME in sediments and soils and to the best of our knowledge, no previous study has ever been targeted in the investigation of CPs in biological samples.

In this respect, the specific objectives of the present study were as follows: (1) to evaluate the potential of combining ultrasonic extraction (USE) with headspace solid-phase microextraction (HS-SPME) for the determination of CPs in bird livers; (2) to compare the influence of different sample treatments for lipid removal, previously to the HS-SPME step, in order to study differences in terms of selectivity and sensitivity of the combined analytical method; (3) to apply the optimized USE/SPME method for the trace determination of CPs in liver tissues of various birds species from Greece.

2. Experimental

2.1. Collection of samples

The samples from five bird species were provided from the Hellenic Wildlife Hospital (Aigina Island, Greece). The birds have been brought to the center dead or alive but injured or debilitated for various reasons. Liver samples were taken from each corpse of dead birds or those died naturally later. The samples were wrapped in aluminum foil, placed individually in plastic bags and they were stored frozen at −20 °C until analysis. The birds were collected from various regions of Greece.

2.2. Reagents and materials

Organochlorine pesticide standard solutions for α-HCH, β-HCH, lindane (γ-HCH), δ-HCH, heptachlor, endosulfan I, II and endosulfan sulfate, γ-chlordane, heptachlor epoxide, aldrin, dieldrin, endrin, endrin ketone, endrin aldeyde, methoxychlor, *p*,*p*′-DDT, *p*,*p*′-DDD and *p*,*p*′-DDE were obtained from Supelco (Bellefonte, PA, USA). All solvents used (n-hexane, acetone, dichloromethane (DCM)), were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd., Dublin, Ireland). Alumina, and sodium sulfate (pro-analysis) were from Merck (Darmstadt, Germany). 2,4,5,6-Tetrachloro-*m*-xylene used as internal standard (IS) was obtained from Supelco (Bellefonte, PA, USA). Glassware was soaked, cleaned with chromic solution, thoroughly rinsed with distilled water and acetone and heated at 150 °C for 12 h.

Fused-silica fibers coated with 100 μm of poly(dimethylsiloxane) were selected for this study taking into account the data available in the open literature [23,24]. Fibers were handled in SPME manual holders supplied by Supelco and conditioned before use as recommended by the manufacturer.

2.3. Fortification procedure

Chicken liver cut into small pieces and then was thoroughly homogenized in a grinder before processing. Optimization experiments were carried out on 1 g homogenate of liver by spiking appropriate amounts of the diluted working standards solutions to get final concentrations of 5–500 ng g^{-1} wet wt. The spiking of the liver samples was performed with 250 μL of n-hexane containing the above-mentioned amounts of pesticides. The samples were left at least 4 h at room temperature to fully evaporate the solvent. To prepare pesticide-free samples a portion of the livers were extracted and analyzed prior to the recovery experiments. Finally, it was determined that there were no detectable levels of target analytes in the liver samples before spiking by using the conventional Soxhlet method described by Sakellarides et al. [2]. Here after, this sample was referred as blank liver sample.

2.4. Ultrasonic extraction

For sample pretreatment 1 g of sample was mixed and ground in a porcelain mortar with anhydrous sodium sulfate (Na_2SO_4:liver; 4:1, w/w) in triplicate. Then, the mixture was transferred in a 50 mL glass tube extracted with 20 mL of n-hexane:acetone (4:1, v/v) mixture by ultrasonication for 30 min and then centrifuged at 4500 rpm for 5 min. The supernatant liquid was concentrated to 2.0 mL using a rotary evaporator at 40 °C and was subjected to the following clean-up approaches.