Comparison of Extraction Methods by Soxhlet, Sonicator, and Microwave in the Screening of Pesticide Residues from Solid Matrices

Rashmi Sanghi* and Sasi S. Kannamkumarath**

* 302 Southern Labs, Facility for Ecological and Analytical Testing (FEAT), Indian Institute of Technology, Kanpur, 208016 India
e-mail: rsanghi@iitk.ac.in
fax: 91-512-2597866

** Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172, USA

Received February 5, 2003; in final form, July 10, 2003

Abstract—Three types of solvent extraction methods (by soxhlet, sonicator and microwave) for pesticide recoveries in solid matrices were compared and evaluated using the standard addition method. Variables (solvent and extraction time) for the optimization of microwave assisted extraction (MAE) were also studied. Three organochlorine pesticides (BHC, DDE, and Dieldrin) were chosen for this particular study because of their great presence in the soil where the samples were collected and their positive association with the risk of breast cancer. Comparison of the results obtained indicates that the efficiency of extraction varies, depending on the matrices and the pesticides analyzed. The study focused on the variation in the extraction quantities of different methods in different matrices. The extraction conditions were optimized for MAE with a single matrix (bark) and applied to the rest in order to study the variability in results. Gas chromatography with an electron capture detector (GC–ECD) was used for analysis of the extracts. The results show that even though the use of MAE improved extraction in some of the matrices studied, the extraction method must be optimized whenever a new matrix is evaluated. A statistical comparison indicated that pesticide recoveries and method reproducibility of microwave extraction compared less favorably with the conventional soxhlet method in some of the matrices, whereas the sonicator method was not found to be as efficient as the others.

INTRODUCTION

Organochlorine pesticide residues detected in penguins and seals in places as remote as Antarctica [1] and their carcinogenicity in laboratory studies [2] provide sufficient evidence of the seriousness of the problems related to the ubiquitous presence and subsequent health effects associated with their environmental persistence and bioaccumulation. Another major concern is the presence of trace levels of these pesticides in our own biological systems (in breast milk, serum, urine, and liver tissues [3]) and the potential ability of many of these chemicals at low doses to act as endocrine disrupters [4]. Regulatory authorities are increasingly aware of the harmful consequences resulting from the indiscriminate use of organochlorine pesticides and the urgent need to control their use. One way to evaluate the risk posed by these toxic chemicals is to determine the levels of these contaminants in the environment. The detection and quantification of organochlorine pesticide residues in various matrices (including biological samples) have been extensively studied using GC–ECD [3, 5, 6]. Although most of these chemicals are already banned from usage, the challenge lies in their extraction from various matrices already contaminated with these pesticide residues.

Solvant extraction of samples is often the first step in the analytical procedures following the sampling. The classical methods, soxhlet and sonicator, were until recently the most widely used techniques for the extraction of semi-volatile organic contaminants and pesticides in environmental laboratories [7]. The commonly used methods (such as soxhlet extraction and sonicator) consume time as well as rather large amounts of solvents [8]. The procedures involved in the extraction of pesticides [9] from environmental samples are usually lengthy and non-selective; they also involve a great deal of sample handling, which adds to the risk of errors [10]. The development of techniques such as supercritical fluid extraction (SFE) [7] and MAE [11–13] holds such promise, as they require less time, less solvent, and almost eliminate the concentration step, thereby enhancing the methods’ overall productivity and ease of use. In this study, the application of MAE as an alternative to the commonly used soxhlet and sonicator methods for the extraction of organochlorine pesticides is evaluated.

To study the effects of MAE and matrix variables on pesticide [14] recoveries [15], various solid matrices

1 This article was submitted by the authors in English.
fortified with a few selected organochlorine pesticides (BHC, DDE, dieldrin) were considered. Snedeker [16] recently reviewed the organochlorine pesticides DDT, DDE and dieldrin and their positive association with breast cancer risk. In the present study, the MAE method was compared with the classical soxhlet and sonication procedures for the routine determination of pesticides in different biotic and abiotic solid matrices. In each case, the extraction efficiency was examined for three series \((n = 3)\) of these three pesticides. Parameters like the extracting solvent ratio, extraction time, and microwave power were varied to optimize the MAE conditions for one matrix type (bark), and same conditions were used for the other matrices so that the results could be compared.

**EXPERIMENTAL**

**Materials and Methods**

**Samples.** The solid matrices selected for study were bark, cement, grass and vegetables. They were all spiked by different low and high concentrations of pesticides. The vegetable and grass samples were collected from an organic farm.

**Instrumentation.** MAE experiments were performed with an MDS-1200 closed microwave digestion system (Milestone Inc.) equipped with MDR hardware (a microwave digestion rotor) and having ten 100 mL Teflon vessels (the working chamber dimensions were \(15\)" \(\times 13\)" \(\times 12\)"), the power of the microwave generating magnetron was 1200 W, and the MW power frequency of 2450 MHz was equivalent to a wavelength of 1225 cm).

Sonication experiments were conducted in an ultrasonic Julabo-SR05 (Labortechnik GmBH, Germany) having a frequency of 50 Hz.

**Chemicals and standards.** The standard pesticides were obtained from Laborchemikalien GmbH (Germany) via Promochem India Pvt., Ltd (India). The pesticides chosen for study were BHC (1,2,3,4,5,6-hexachloro cyclohexane), DDE (1,1-dichloro-2,2-bis(p-chloro phenyl) ethene) and Dildrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a, octa hydro-1,4,5,8-dimethano naphthalene). The \(\beta\) BHC was 99% pure and the other pesticides were above 99.6% purity level. Standard solutions were prepared in hexane and acetone \((1 : 1)\). The chemicals used during extraction were of pesticide grade. All solutions were stored in the dark at 5°C.

**Detection of Pesticides**

The extracts were analyzed by GC–ECD using a PerkinElmer Autosystem XL (United States). This detector allows the detection of contaminants at trace level concentrations in the lower g L\(^{-1}\) range in the presence of the multitude of compounds extracted from the matrix, to which the detector may not respond. The column used was a PE-17 (length, 30 m; ID, 0.25 mm; film, 0.25 mm with a 2 mL/min flow). The carrier and makeup gases were nitrogen employing the split mode. A gradient elution program was used from 190 to 280°C with a ramp of 5 K/min. The samples were quantified (retention time and area count) against a five-point calibration standard mixed solution of all three pesticides. Each peak was characterized by its retention time and the response factors in BCD. The GC software quantified the sample results automatically. The reproducibility of the results for all three pesticides was 95.8% and above for all of the samples. However, the mean average reading of a particular type of sample analyzed in triplicate was also considered.

One GC injection (20 min) of 1 µL covered all three pesticides included in the analysis. Hamilton micro syringe injection of the pesticide dissolved in hexane and acetone \((1 : 1)\) as solvent were made directly onto the coated silanized column solid support, thereby eliminating the possibility of catalytic degradation by metallic surfaces. Pesticides were identified according to their retention times. The actual relative retention time for the different pesticides were 11.60 (\(\alpha\) BHC), 13.04 (\(\beta\) BHC), 13.09 (\(\gamma\) BHC), 14.50 (\(\beta\) BHC), 19.57 (DDE), 20.35 (Dieldrin). To obtain accurate results, the concentration of the standard sample and that of the sample to be estimated was kept the same. The multi-residue method, which can detect all three pesticides in one analytical run, was preferred. This study utilized the method developed by Sasi et al. [17] for the analysis of the pesticide residues. This method is characterized by a broad scope of application, good recoveries and sensitivity and low solvent consumption, coupled with good analytical quality control.

**Procedure**

The pre-treatment method conformed with the specifications of the US Environmental Protection Agency [18].

**Sample extraction.** Each sample of 0.5 kg was crushed or cut to the smallest possible size, out of which subsamples \((2–20\) g) in triplicate were taken, and the pesticides were extracted separately in acetone : hexane \((1 : 1)\) by soxhlet (240 min at the rate of 2–3 cycles every 5 min), sonicator \((90\) min at room temperature), and microwave \((5\) min at 50 W).

The sample amount taken for microwaving was 2 g and was spiked separately with 0.2 and 20 mg L\(^{-1}\) of pesticide mixture and quantitatively transferred to the Teflon-lined extraction vessel. Five milliliters of a \((1 : 1)\) acetone and hexane mixture was then added to each sample, and the extraction vessel was closed and programmed for 5 min at 50 W. Irradiated samples were then removed from the microwave cavity and were allowed to cool at room temperature before opening.

The sample amount taken for the sonicator was 5 g; for the soxhlet extractor, the amount was 20 g.