

# Potential of a Simple Mechanical Extraction System for the Rapid Extraction of Pesticides from Foodstuffs. Application to the Determination of Organochlorine Pesticides in Lettuce

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The efficiency of a simple mechanical extraction system as applied in veterinary drug analysis has been tested in the field of pesticide residue analysis. As a first application, the system was used for the extraction of organochlorine pesticides from vegetables. The convenience of this simple extraction system consists of performing mechanical extraction in disposable polyethylene-based extraction bags, reducing considerably manual operation and cross-contamination. For the tested compounds and matrix (lettuce), recoveries of the 10 organochlorine pesticides performed at two spiking levels ( $n = 4$  each level and compound) ranging between 0.06 and 3.3 mg/kg were between 60 and 80%, with most standard deviations  $< 5\%$ . The extraction method appeared to be simple and fast with a great potential for the analysis of many pesticide–matrix combinations.

**Keywords:** *Simple extraction method; organochlorine pesticides; vegetables; gas chromatography; electron capture detection*

## INTRODUCTION

Analysis of trace levels of pesticides in foodstuff frequently requires extensive extraction and purification procedures to remove high molecular weight interferences such as lipids and natural resins before analysis by gas chromatography or high-performance liquid chromatography. The extraction process is the first and a major limiting step in pesticide residue analysis, often involving sample preparation such as chopping and maceration followed by solvent extraction. Various extraction techniques have been employed ranging from the existing blender liquid/liquid extraction method (Jones and Riddick, 1952; Faubert et al., 1964) and Soxhlet extraction method (Synder et al., 1992; Ministry of Public Health, Welfare and Sports, The Netherlands, 1996) to the newer supercritical fluid extraction (SFE) (France et al., 1991; Nam and King, 1994), matrix solid-phase dispersion (MSPD) (Di Muccio et al., 1991; Torres et al., 1995), and microwave extraction procedures (Hummert et al., 1996; Chee et al., 1996). Conventional methods often involve laborious blending and vigorous processes that consume large volumes of solvent and are prone to the risk of cross-contamination. Newer and more efficient extraction methods, on the other hand, involve higher costs of investment and maintenance.

The use of a simple mechanical extraction system in pesticide residue analysis was first suggested by Tuinstra (1995). It was based on use in the food industry and hospital laboratories, where it is routinely used in microbiological analysis (Aerts et al., 1988; Keukens et al., 1993; Ackermans et al., 1992). Aerts et al. (1988) reported the use of polyethylene bags in the extraction of residues of Carbadox and its metabolites from swine tissues using organic solvents of various polarities in combination with HPLC analysis. They also reported

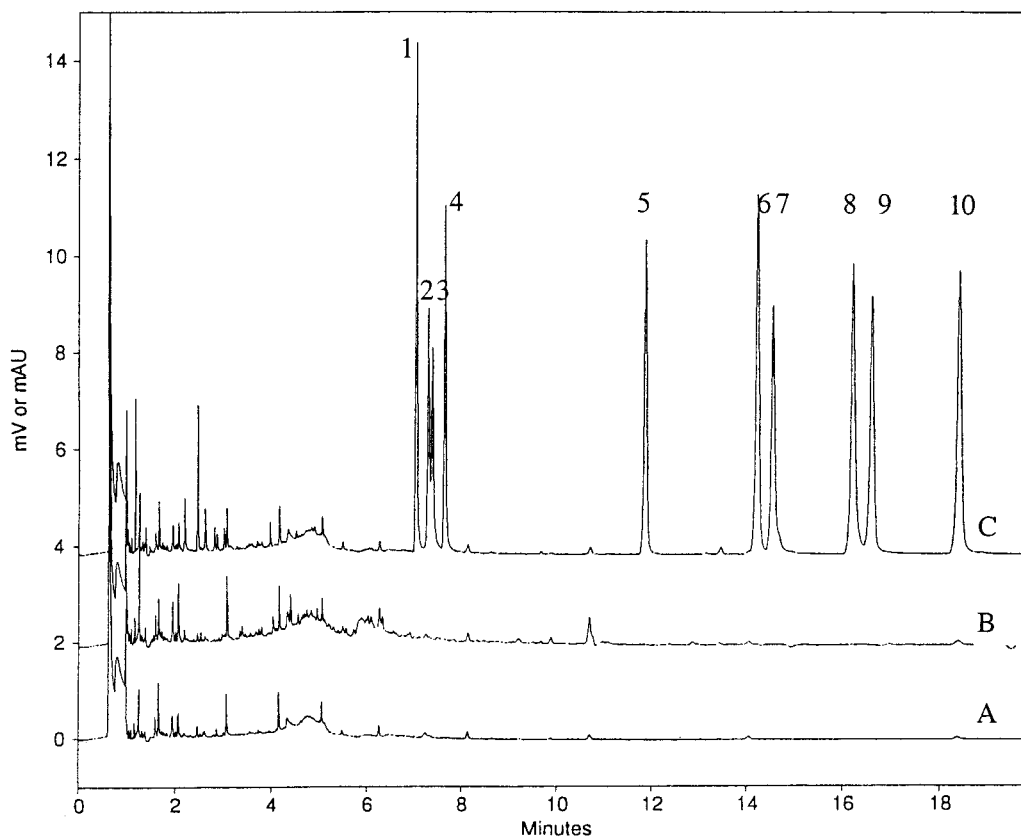


**Figure 1.** Stomacher 400 Lab System.

that there was less risk of cross-contamination of samples using this simple mechanical extraction system than in conventional blending in an Ultra-Turrax. Keukens et al. (1993) successfully employed this system for the rapid extraction of nitroimidazole drugs from poultry meat with water followed by fully automated sample processing involving dialysis coupled on-line with trace enrichment by column switching HPLC. Ackermans et al. (1992) used the simple mechanical extraction system for the extraction of sulfonamides from pork meat using capillary zone electrophoresis.

The use of this system has not been reported in the field of pesticide residue analysis, although Baudys and Tuinstra (1995) used it for the extraction of acid herbicides in apples in their unpublished work.

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**Figure 2.** GC/ECD chromatograms of (A) solvent blank (ethyl acetate), (B) blank lettuce extract (B), and (C) spiked lettuce extract. Peak identification:  $\alpha$ -HCH (1),  $\beta$ -HCH (2), HCB (3),  $\gamma$ -HCH (4),  $\beta$ -Hepo (5), dieldrin (6), *p,p'*-DDE (7), *p,p'*-TDE (8), *o,p'*-DDT (9), and *p,p'*-DDT (10).

The efficacy, combined with the easy mode of operation of this system in the veterinary field for the extraction of veterinary drugs from animal tissues, prompted the investigation into the potential of this extraction technique in pesticide residue analysis. In this study applicability for the extraction of 10 organochlorine pesticides from lettuce prior to the instrumental analysis using GC-ECD has been examined.

#### EXPERIMENTAL PROCEDURES

**Apparatus.** The extraction procedure was carried out in a Stomacher 400 Lab System (Seward, London, U.K.) of 80–400 mL capacity and sample bags of the type Stomacher 400 filter bags and Stomacher 400 nonfilter bags.

GC analysis was performed in a Varian 3500 equipped with a  $^{63}\text{Ni}$  electron capture detector (ECD) set at 300 °C and a Varian 8200 autosampler; a dual GC column system has been applied, which consisted of an HP1-25 m  $\times$  0.32 mm  $\times$  0.17  $\mu\text{m}$  (column A) and an HP 17-30m  $\times$  0.32 mm  $\times$  0.15  $\mu\text{m}$  (column B) (Hewlett-Packard, Avondale, PA), both fitted with a twin-hole ferrule in one injector. The second column was used for confirmation of data obtained from column A. A volume of 2  $\mu\text{L}$  was injected using the splitless mode into each column with an injector temperature set at 220 °C. Helium was used as the carrier gas at a pressure of 112 kPa, and the column temperature programming was as follows: 70 °C for 2.0 min, raised at 30 °C/min to 150 °C, raised at 4 °C/min to 220 °C, raised at 30 °C/min to 275 °C, with a final hold of 3 min.

**Chemicals.** Organochlorine pesticide standards included HCB,  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH,  $\beta$ -heptachlorepoxide ( $\beta$ -hepo), dieldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-TDE, and *p,p'*-DDT with a purity of >99% obtained from Dr. S. Ehrenstorfer (Promochem, Wesel, Germany). A calibration mixture of concentration 3.1–16.5 ng/mL was prepared in acetone.

Ethyl acetate and acetone, both of pesticide grade, were purchased from J. T. Baker (Deventer, The Netherlands).

Sodium sulfate anhydrous, analytical grade (Baker), was dried at 500 °C for 4 h before use.

**Sample Preparation.** Lettuce samples were bought from a local supermarket, chopped without washing, and portioned into 50 g representative subsamples, which were stored at –20 °C. These portions of 50 g were taken as required for every analysis.

Frozen subsamples were transferred to doubled filter extraction bags, and, after thawing, 100 mL of ethyl acetate and 50 g of sodium sulfate were added. The samples were blended in the Stomacher apparatus at high speed during 5 min. The extracts were filtered over sodium sulfate, and 2  $\mu\text{L}$  was injected in the GC-ECD for analysis after appropriate dilution.

Defrosted subsamples were also spiked overnight in doubled Stomacher filter bags with a mixture of the pesticide standards at levels of 0.1 and 1.0 mg/kg of sample. Extraction was performed according to the same procedure described for unspiked samples. A volume of 2  $\mu\text{L}$  was injected in the GC-ECD for analysis after appropriate dilution.

**Blender Extraction Procedure.** The standard extraction procedure in the *Manual of Analytical Methods for Pesticide Residues in Foodstuffs* (Ministry of Public Health, Welfare and Sports, The Netherlands, 1996) was used. Aliquots of 50 g of sample were transferred into an Ultra-Turrax blender cup, and 50 g of sodium sulfate and 100 mL of ethyl acetate were added. The mixture was homogenized for 2–3 min, and the extract was filtered over sodium sulfate. Spiked samples were extracted in a similar manner, and a volume of 2  $\mu\text{L}$  was injected on the GC-ECD for analysis after appropriate dilution.

#### RESULTS AND DISCUSSION

The Stomacher System displayed in Figure 1 involves mechanical actions in which paddles apply pressure to a sample bag, thereby homogenizing the sample and effecting extraction of the analytes by the added extraction solvent. The disposable sample bag is of polyeth-

**Table 1. Recoveries of Organochlorine Pesticides from Lettuce Using the Simple Mechanical Extraction Procedure and Existing Blender Extraction Procedure<sup>a</sup>**

compound	spiking level (mg/kg)	average recovery <sup>b,c</sup> (%)	RSD (%)	average recovery <sup>b,d</sup> (%)	RSD (%)	spiking level (mg/kg)	average recovery <sup>b,d</sup> (%)	RSD (%)
$\alpha$ -HCH	0.1	81	1	61	3	1.2	53	4
$\beta$ -HCH	0.2	94	1	78	2	2.0	61	3
HCB	0.1	74	2	50	8	0.6	49	5
$\gamma$ -HCH	0.1	81	1	58	3	1.0	54	6
$\beta$ -Hepo	0.2	80	0	48	5	1.6	46	3
dieldrin	0.2	80	0	47	5	2.1	48	2
<i>p,p'</i> -DDE	0.2	84	2	62	6	1.8	59	4
<i>p,p'</i> -TDE	0.3	86	3	65	4	3.2	62	1
<i>o,p'</i> -DDT	0.3	91	0	61	5	3.3	62	2
<i>p,p'</i> -DDT	0.3	101	0	68	6	3.3	65	2

<sup>a</sup> Sodium sulfate was not added to the extraction mixture in the simple mechanical extraction procedure. <sup>b</sup> Samples were spiked overnight with the pesticide mixture. <sup>c</sup> Extraction by conventional blender procedure. <sup>d</sup> Extraction by the simple mechanical extraction procedure.

**Table 2. Organochlorine Pesticide Recoveries from Spiked Filter Bags<sup>a</sup>**

compound	spiking level (mg/kg)	average recovery <sup>b</sup> (%)	RSD (%)	average recovery <sup>c</sup> (%)	RSD (%)	spiking level (mg/kg)	average recovery <sup>b</sup> (%)	RSD (%)	average recovery <sup>c</sup> (%)	RSD (%)
$\alpha$ -HCH	0.1	66	3	105	0	1.2	67	5	100	1
$\beta$ -HCH	0.2	79	1	115	1	2.0	72	11	103	0
HCB	0.1	70	0	105	1	0.6	72	3	101	0
$\gamma$ -HCH	0.1	69	3	107	1	1.0	71	9	101	0
$\beta$ -Hepo	0.2	69	1	101	1	1.6	73	7	101	1
dieldrin	0.2	70	2	105	1	2.1	71	5	99	1
<i>p,p'</i> -DDE	0.2	84	3	104	1	1.8	88	3	103	0
<i>p,p'</i> -TDE	0.3	82	1	108	0	3.2	86	1	103	2
<i>o,p'</i> -DDT	0.3	79	1	108	1	3.3	78	1	102	1
<i>p,p'</i> -DDT	0.3	83	1	112	0	3.3	82	1	101	1

<sup>a</sup> Sodium sulfate was not added to the extraction mixtures. <sup>b</sup> Average recovery from empty filter bags spiked overnight with the pesticide mixture. <sup>c</sup> Average recovery from empty filter bags spiked just prior to extraction.

ylene material and contains a filter bag that holds the sample, thereby excluding particulate parts from the extract and offering an extra advantage of a very clean working environment, which excludes dangers of cross-contamination.

Ethyl acetate, selected as a suitable extraction solvent in both the simple mechanical extraction procedure and the existing blender procedure, is a relatively strong organic solvent capable of coextracting other organic nonpolar and polar compounds present in the polyethylene matrix of the filter bags. Therefore, a crucial step to be considered is whether the extraction system results in an increase in background signals with the ECD in order to be of significance in the analytical application of GC-ECD for the detection of the organochlorine pesticides.

The chromatograms of a solvent blank and extract from lettuce in the extraction bag are shown in parts A and B, respectively, of Figure 2. Rather surprisingly, minimal background interferences were encountered, indicating that these bags can be applied during sample pretreatment preceding a GC-ECD analysis. A typical chromatogram of the 10 organochlorine pesticides in a spiked sample that has been extracted using the simple mechanical extraction procedure is shown in chromatogram C of Figure 2.

With the assurance of a minimal background interference from the filter bags, the efficiency of the method was tested in comparison with the conventional extraction procedure of the 10 organochlorine pesticides from spiked lettuce samples. Recoveries of the 10 organochlorine pesticides from lettuce samples in the filter bags were low compared to those obtained with the conventional blender extraction method. Table 1 shows a recovery range of 47–78% for the organochlorine pesticides at a level of 0.1 mg/kg using the simple mechan-

ical extraction system, in comparison with recoveries of >70% for the pesticides in the same spike range using the conventional blender method. As shown in Table 1, recoveries at higher spiked levels (>1 mg/kg) using the simple mechanical extraction system were equally low (46–65%).

In verification of possible adsorption of the pesticides onto the matrix of the filter bag, recoveries were determined in extracts from empty (without lettuce) bags spiked overnight and in combination with bags just prior to extraction. The results given in Table 2 show that when spiking was done just prior to extraction, high recoveries were obtained in comparison to spiking by overnight standing in the filter bag. The good recoveries obtained when spiking is done just prior to extraction indicate that the extraction step itself does not contribute to a decrease in recovery. However, the low recovery during an overnight standing indicates an irreversible adsorption effect to the Stomacher bag for these types of very nonpolar pesticides. This effect was not observed before when using the Stomacher for the extraction of rather polar analytes such as veterinary drugs in animal tissues (Aerts et al., 1988; Keukens et al., 1993; Ackermans et al., 1992) and chlorophenoxy acid herbicides in vegetables (Baudys and Tuinstra, 1995).

Initially, extraction with the simple mechanical extraction system was done in single bags and did not include sodium sulfate in the extraction mixture. To improve recovery and to be consistent with the conventional blender procedure, 50 g of sodium sulfate was added to the sample mixture in the filter bag before extraction. However, rupturing of the bag resulted due to the additional coarse effect of the sodium sulfate on the sample and bag. The latter effect was easily alleviated by doubling the bag.

**Table 3. Organochlorine Pesticide Recoveries Using the Simple Mechanical Extraction Procedure with Sodium Sulfate in the Extraction Mixture**

compound	spiking level (mg/kg)	average recovery <sup>a</sup> (%)	RSD (%)
α-HCH	1.2	65	4
β-HCH	2.0	72	10
HCB	0.6	66	4
γ-HCH	1.0	63	5
β-Hepo	1.6	61	3
dieldrin	2.1	62	5
<i>p,p'</i> -DDE	1.8	74	2
<i>p,p'</i> -TDE	3.2	77	2
<i>o,p'</i> -DDT	3.3	72	5
<i>p,p'</i> -DDT	3.3	78	2

<sup>a</sup> Overnight spiking of lettuce with the pesticide mixture.

As shown in Table 3, the addition of sodium sulfate to the extraction mixture improved recoveries of the organochlorine pesticides by ~20%. Though recoveries were still lower than recoveries obtained by the conventional blender procedure, the results were remarkably reproducible. This improvement in recoveries is ascribed to the abrasive, macerating action of the coarse sodium sulfate crystals on the lettuce leaves, resulting in an increased surface area for extraction.

#### CONCLUSIONS

The efficiency of the simple mechanical extraction system for the determination of organochlorine pesticides in lettuce in combination with GC-ECD determination was investigated. For this pesticide/matrix combination the procedure yielded recoveries of ~70%. The use of disposable filter bags for extraction is more convenient and less prone to cross-contamination in comparison with conventional blender extraction procedures.

The good repeatability and reproducibility of the recovery experiments indicate the usefulness of the simple mechanical extraction system in the field of pesticide residue analysis.

This extraction method has a great potential for the analysis of more polar pesticides in other vegetable and animal materials, as has been demonstrated in the field of veterinary drug analysis.

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