

# Analysis of Pesticide Residues in Fruit and Vegetables after Cleanup with Solid-Phase Extraction Using ENV+ (Polystyrene–Divinylbenzene) Cartridges

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A rapid and simple cleanup procedure for the existing multimethod using solid-phase extraction columns to measure pesticide concentrations in fruit and vegetables is presented. After extraction with ethyl acetate, the sample is passed through ENV+, polystyrene–divinylbenzene extraction column, and eluted with ethyl acetate and injected on to capillary GC columns connected to various detectors. The extraction column has the capacity to retain a broad range of pesticides and is widely used in environmental water samples. In this paper, the sample is extracted in an organic solvent. In contrast to what could be expected, it has been found that the column has the capacity to retain pesticides when used as the normal phase.

**Keywords:** Pesticides; solid-phase extraction; ENV+; food

## INTRODUCTION

The objective of the present study was to develop a rapid and simple cleanup step to detect and quantify pesticide residues in fruit and vegetables. The multimethod (Andersson and Pålsheden, 1991, 1998) used in Swedish monitoring of pesticide residues in fruit and vegetables is based on extraction with ethyl acetate followed by cleanup with gel permeation chromatography (GPC) including slightly more than 200 pesticides and their metabolites. Despite automation of GPC, the multimethod is still time-consuming due to the concentration and redissolving steps (Table 1). The early-eluting analytes on the GPC cause quantification problems. New break points for early-eluting pesticides are obtained at the expense of more interfering peaks in the chromatograms. Fatty acids and enhanced matrix-induced effects make quantification difficult. There is a need for a rapid, cost-effective, and efficient cleanup step that could replace GPC in the existing multiresidue method. This paper shows the first results obtained with solid-phase extraction (SPE) using polystyrene–divinylbenzene sorbent, ENV+ extraction columns. Polymeric sorbents have been applied for the analysis of pesticides in water and have proved to be highly efficient in retaining pesticides with a wide range of polarities. The present approach is unique in the sense that the column is used for samples in more nonpolar conditions. The sample is extracted in ethyl acetate, and after adjustment of the extraction solvent, the sample is applied to the extraction column. The elution is then performed with ethyl acetate. The retention characteristics of pesticides in organic solvent were similar to those exhibited in water (Pihlström et al., 1997).

## EXPERIMENTAL PROCEDURES

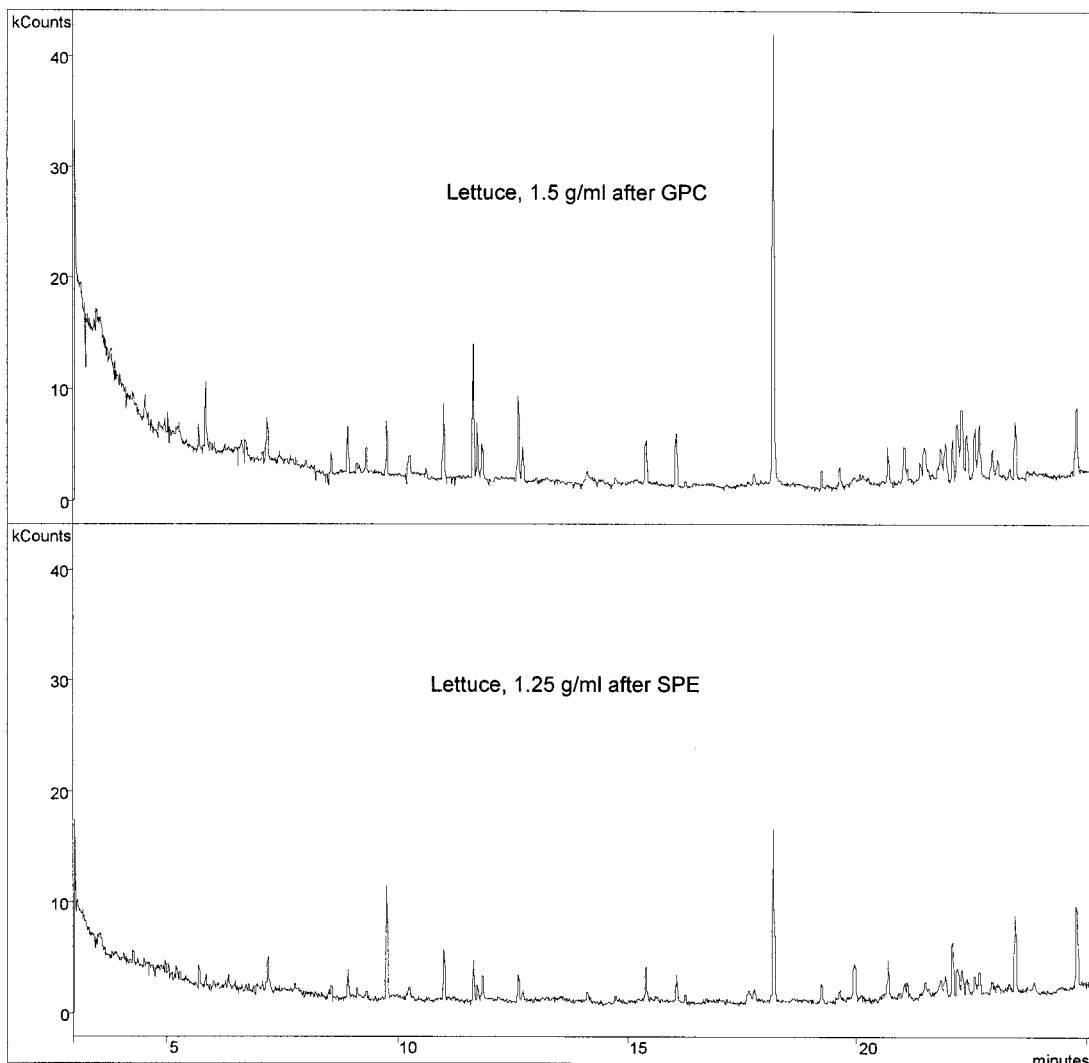
Isolute SPE columns containing 200 mg of ENV+ were purchased from International Sorbent Technology (IST). A VacMaster sample processing station was used to maintain the columns during extraction.

To evaluate the suitability of ENV+ for extracting pesticides in fruits and vegetables, the retention of analytes was of interest. In the first assay, the appropriate retention solvent and the minimum sample volume for the pesticides were determined. A mixture of pesticides with different polarities and partition coefficients in various organic solvent compositions was tested to evaluate the capacity of the column to retain pesticides. One milliliter of standard mixture dissolved in various retention solvents was passed through the column and collected. Furthermore, three fractions of 1 mL were eluted using the same retention solvent in order to estimate the breakthrough volume of analytes. The most appropriate mixed solvent was found to be 25/75 ethyl acetate/cyclohexane, which resulted in no breakthrough of the pesticides studied when 1 mL of standard mixture dissolved in the corresponding solvent was passed through the column. In accordance with previous studies (Pihlström et al., 1997), the sample was then eluted with 3 mL of ethyl acetate, thus maintaining recoveries for the pesticides studied. No drying was needed due to the compatibility of the extraction solvent with the eluent.

## RECOVERY STUDIES

The homogenized matrix samples were fortified with pesticide mixtures of different concentrations. The analytes were mainly chosen because of their different chemical properties and in order to check the analytical response of various detectors. The extraction method used was the GC-based multiresidue method from the National Food Administration (Andersson and Pålsheden, 1991, 1998) (Table 1). A 75-g sample was blended for 3 min with 200 mL of ethyl acetate and then filtered. Then an aliquot of 100 mL was evaporated and redissolved in 5 mL (1 + 1) of ethyl acetate/cyclohexane.

Instead of GPC, the cleanup step with ENV+ was performed. Pretreatment of the sample extract to 25/75 in ethyl acetate/cyclohexane was done by dissolving 500  $\mu$ L of the extract (7.5 g/mL) in 500  $\mu$ L of cyclohexane. Then the sample was put on the cartridge and passed through the preconditioned column, eluted by gravity flow with 3 mL of ethyl acetate, and then 2  $\mu$ L of the extract was injected on a capillary column and analyzed by means of various detectors. The quantifica-



**Figure 1.** GC/ITD chromatograms obtained after GPC and SPE in lettuce showing matrix peaks.

tion was done against one point calibration using matrix-matched standards to avoid too high recoveries deriving from matrix effects (Andersson and Pålsheden, 1998; Andersson et al., 1996, 1998).

**Standards and Solvents.** Pesticide standards of analytical grade were used. Stock solutions of standards were prepared in acetone and used in recovery tests. The stock solutions and all standard solutions were stored at 4 °C. Acetone, cyclohexane, and ethyl acetate of pesticide quality were used.

**Chromatographic Conditions.** *Gas Chromatography (TSD/ECD).* Samples were analyzed according to the NFA's GC multiresidue method (Andersson and Pålsheden, 1998) used for monitoring pesticide residues in fruits and vegetables. A Varian 3800 gas chromatograph equipped with a nitrogen–phosphorus detector (thermionic specific detector, TSD) connected to an OV-1701 fused silica capillary column and a Hewlett-Packard HP 5890 gas chromatograph equipped with two electron capture detectors (ECD) and two columns of SE-54 fused silica and an OV-1701 fitted to the same injector were used for the analysis.

The dimensions of all the columns were 25 m × 0.32 mm i.d. OV-1701 had a phase thickness of 0.20 μm, and SE-54 had a phase thickness of 0.32 μm.

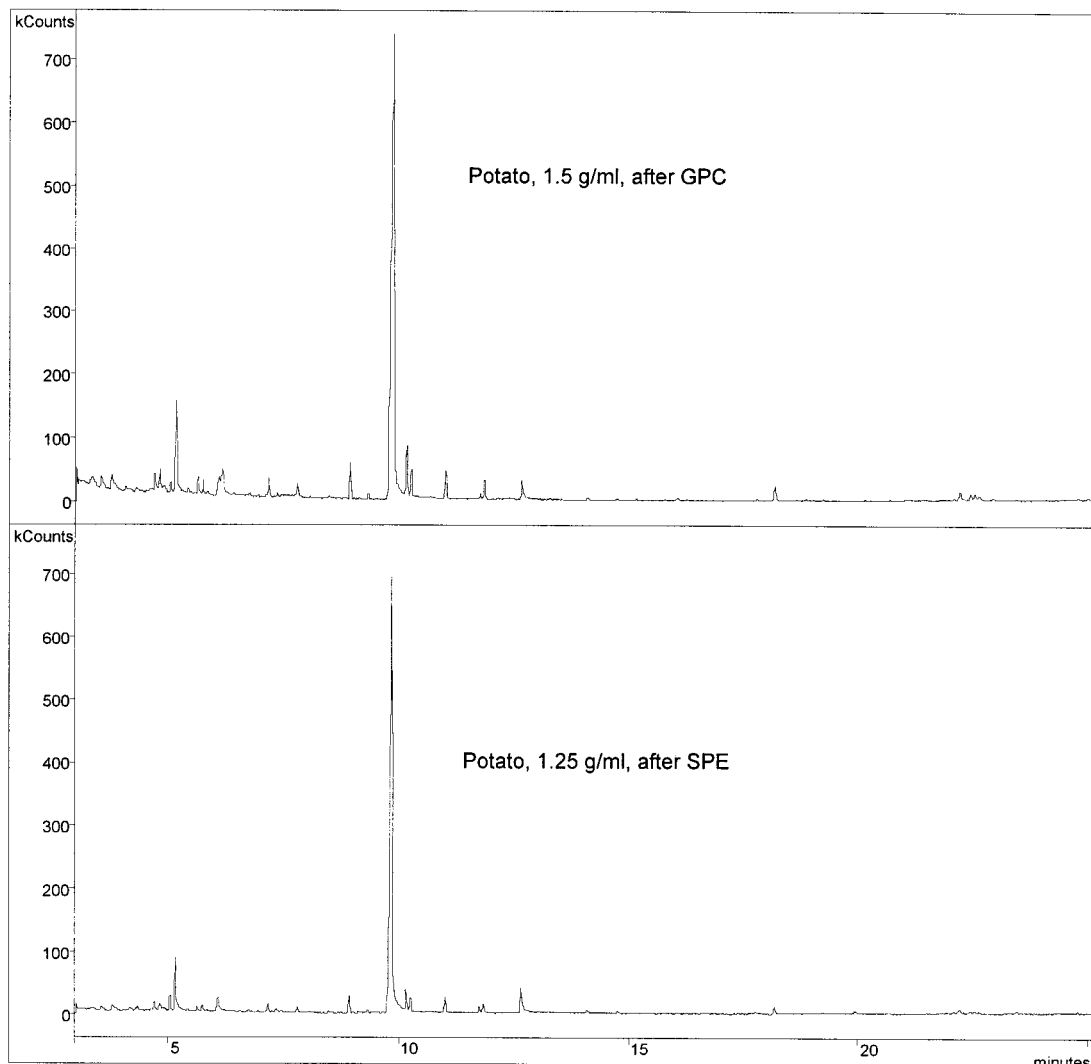
The oven temperature of 90 °C (4 min) increased at 30 °C/min to 180 °C, then increased at 4 °C/min to 260

°C, and was finally held for 6 (TSD) and 12 min (ECD), respectively. The injection volume was 2 μL.

*Gas Chromatography/Mass Spectrometry (GC/MS).* A Varian 3400 gas chromatograph combined with a Varian Saturn 2000 ion trap detector with DB 5 MS 30 m × 25 mm, 0.25 μm was used. The initial injector temperature was 170 °C/min. The column temperature started at 90 °C (1 min), increased at 30 °C/min to 180 °C (0.5 min), then at 5 °C/min to 280 °C (5 min), and finally at 30 °C/min to 320 °C. The injection volume was 2 μL, and the flow rate was 1 mL/min.

## RESULTS AND DISCUSSION

The preliminary results, presented in Tables 2 and 3, are generated by ECD, TSD, or GC/MS ITD. In the majority of cases, the recoveries were within acceptable levels (70–110%), but in an apple sample, spiked with 0.2 mg/kg of pirimicarb, no pirimicarb was recovered due to strong retention on the column. When the column was eluted with a more powerful eluent (pure acetone), this gave no improvement concerning pirimicarb, which was still retained. Nevertheless of the 172 pesticide standards screened (not reported), 150 have achieved recoveries of between 70% and 110% in preliminary studies. More studies are necessary to investigate the retention mechanism of pesticides with poor or no recoveries.



**Figure 2.** GC/ITD chromatograms obtained after GPC and SPE in potato showing matrix peaks.

**Table 1. Scheme of NFA's GC Multiresidue Method Completed with SPE Cleanup**

Extraction	
75 g of sample 200 mL of ethyl acetate, 40 g of Na <sub>2</sub> SO <sub>4</sub> decant and filtrate through 20 g of Na <sub>2</sub> SO <sub>4</sub> 100-mL aliquot of ethyl acetate concentrate and redissolve in 5 mL of ethyl acetate/cyclohexane (1+1), 7.5 g/mL filtrate the crude extract through a 0.45- $\mu$ m Teflon filter	
Cleanup	
GPC on SX-3 column 1 mL extract (7.5 g/mL)  elute with ethyl acetate/cyclohexane (1 + 1)  concentrate and redissolve in 5 mL of ethyl acetate/cyclohexane sample concentration, 1.5 g/mL	SPE on ENV+ column column conditioning with 10 mL of 25/75 ethyl acetate/cyclohexane 1 mL of sample, 500 $\mu$ L of extract + 500 $\mu$ L of cyclohexane (3.75 g/mL) elute with 3 mL of ethyl acetate  sample concentration, 1.25 g/mL

Some experiments were also done in order to compare GPC and SPE cleanup procedures. After extraction with ethyl acetate, the lettuce and potato samples were cleaned up using GPC and SPE. As shown in Table 4, there was no considerable change in pesticide recoveries. In both cleanup methods, matrix-matched standards were used due to an enhanced response to pesticides in pure solvent. A comparison of the cleanup capabilities of the GPC and ENV+ column shows few differences in the pattern of impurities in the chromatogram. (Figures 1 and 2).

## CONCLUSIONS

The present study indicates that the ENV+ extraction column could in fact be applied as a normal phase and still provide sufficient cleanup for fruit extracts. The studied pesticides are adsorbed efficiently onto the solid phase. The modified cleanup step simplifies the analysis and reduces the analysis time considerably, while still preserving acceptable recoveries for most of the pesticides. The solvent composition that was decided on can be conveniently applied to the existing multimethod

**Table 2. Mean Recoveries Using ENV+ Solid-Phase Extraction Columns with GC/TSD or GC/ECD Determination**

pesticide	fortified (mg/kg)	matrix	n	recovery (%)	detector/column
dimethoate	0.6	lettuce	2	108	TSD/OV-1701
	0.6	potato	3	88	TSD/OV-1701
fenthion	0.7	lettuce	2	87	TSD/OV-1701
	0.7	potato	3	70	TSD/OV-1701
parathion-methyl	0.5	lettuce	2	92	TSD/OV-1701
	0.5	potato	3	73	TSD/OV-1701
methidathion	0.7	lettuce	2	97	TSD/OV-1701
	0.7	potato	3	77	TSD/OV-1701
vinclozoline	0.5	lettuce	1	86	ECD/SE-54
	0.5	potato	2	74	ECD/SE-54
methazachlor	0.5	strawberry	2	112	ECD/SE-54
	0.5	lettuce	1	83	ECD/SE-54
cypermethrin	0.5	potato	2	67	ECD/SE-54
	0.4	lettuce	1	81	ECD/SE-54
delthamethrin	0.4	potato	2	56	ECD/SE-54
	0.4	lettuce	1	122	ECD/SE-54
captan	0.4	potato	2	84	ECD/SE-54
	0.2	apple	2	64	ECD/SE-54
pholpet	0.2	strawberry	2	111	ECD/SE-54
	0.2	apple	2	60	ECD/SE-54
chlormephos	0.3	strawberry	2	114	ECD/SE-54
	0.5	strawberry	2	87	ECD/SE-54
dicloran	0.05	strawberry	2	101	ECD/SE-54
HCB	0.03	strawberry	2	97	ECD/SE-54
quintozene	0.04	strawberry	2	111	ECD/SE-54
pentachloro-aniline	0.05	strawberry	2	101	ECD/SE-54
vinchlozoline	0.12	strawberry	2	112	ECD/SE-54
fenson	0.09	strawberry	2	126	ECD/SE-54
ditalimphos	0.40	strawberry	2	107	ECD/SE-54
p,p'-DDE	0.08	strawberry	2	99	ECD/SE-54
p,p'-DDD	0.13	strawberry	2	103	ECD/SE-54
o,p'-DDT	0.13	strawberry	2	82	ECD/SE-54
p,p'-DDT	0.13	strawberry	2	90	ECD/SE-54
iprodione	0.93	strawberry	2	105	ECD/SE-54
bromopropylate	0.27	strawberry	2	86	ECD/SE-54
leptophos	0.11	strawberry	2	104	ECD/SE-54
coumaphos	0.53	strawberry	2	113	ECD/SE-54

**Table 3. Mean Recoveries Using ENV+ Solid-Phase Extraction Columns with GC/MS Ion Trap Determination**

pesticide	fortified (mg/kg)	matrix	n	recovery <sup>a</sup> (%)	recovery <sup>b</sup> (%)
mevinphos	0.2	apple	2	117	89
tecnazene	0.2	apple	2	89	71
pirimicarb	0.2	apple	2	0	0
chlorpyrifos-methyl	0.2	apple	2	120	116
vinklozoline	0.2	apple	2	91	76
parathion-methyl	0.2	apple	2	107	83
malathion	0.2	apple	2	84	73
chlorpyrifos-ethyl	0.2	apple	2	73	68
chlorfenvinphos1	0.2	apple	2	169	100
chlozolate	0.2	apple	2	73	69
chlorfenvinphos2	0.2	apple	2	113	81
azinphos-methyl	0.2	apple	2	101	88
fenarimol	0.2	apple	2	109	81
fenthion sulfoxide	0.2	apple	2	155	77

<sup>a</sup> Standard in pure solvent. <sup>b</sup> Matrix matched standard.

before or after GPC. The technique can also be used for the screening of pesticides in monitoring programs, especially when a selected group of pesticides is to be

**Table 4. Comparison of Cleanup Procedures between GPC and SPE/ENV+ Columns**

pesticide	fortified (mg/kg)	matrix	n	recovery <sup>a</sup> (%)	
				ENV+ (a/b)	GPC (a/b)
dimethoate	0.6	lettuce	1	85/96	85/75
	0.6	potato	2	96/83	90/78
fenthion	0.7	lettuce	1	78/85	74/68
	0.7	potato	2	102/70	115/79
parathion-methyl	0.5	lettuce	1	71/85	80/67
	0.5	potato	2	82/74	78/71
methidathion	0.7	lettuce	1	69/90	86/66
	0.7	potato	2	84/75	82/76
vinchlozoline	0.5	lettuce	1	106/86	134/98
	0.5	potato	2	114/74	128/82
metazachlor	0.5	lettuce	1	140/83	146/87
	0.5	potato	2	109/67	128/78
cypermethrin	0.4	lettuce	1	122/81	136/91
	0.4	potato	2	105/56	120/64
deltamethrin	0.4	lettuce	1	130/122	137/129
	0.4	potato	2	174/84	190/93

<sup>a</sup> a/b = standard in pure solvent/matrix-matched standard.

determined. The technique is easy to automate, and the use of organic solvents is reduced. Finally, the possibility of concentrating the samples, if residue levels are very low, makes this technique useful for screening pesticide residues in fruits and vegetables.

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