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Determination of organophosphorus pesticides in wheat flour by supercritical fluid extraction and gas chromatography with nitrogen–phosphorus detection

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Abstract

Application of supercritical fluid extraction (SFE) for selective isolation of organophosphorus pesticides from a real-world matrix (wheat flour) has been described. The method uses extraction with supercritical carbon dioxide at 206.8 bar and 60°C, followed by quantitation by gas chromatography with nitrogen–phosphorous detection without clean-up of the extracts. Comparison of SFE with a method currently employed for sample preparation (i.e., organic solvent extraction followed by liquid–liquid extraction and gel permeation chromatography clean-up) shows that the SFE technique simplifies the sample preparation step and speeds up the determination of organophosphorus pesticides in flour. Extraction times were 60 min for a 7 g sample size. This technique was able to determine organophosphorus pesticides (ethoprophos, diazinon, chlorpyrifos methyl, fenitrothion, parathion, phenthoate, EPN) in samples at the 10 ng/g level.

Keywords: Extraction methods; Supercritical fluid extraction; Pesticides; Organophosphorus compounds

1. Introduction

The determination of pesticide residues in grains is of regulatory significance. The conventional sample preparation method for the analysis of pesticides in environmental matrices is organic solvent extraction, followed by solid-phase extraction (SPE) [1,2] or gel permeation chromatography (GPC) clean-up [19].

Supercritical fluid extraction (SFE) has become an alternative to traditional organic solvent based methods for the removal of analytes from solid matrices. The solubility of specific analytes and matrix components in a supercritical fluid can be varied by employing different extraction pressures and temperatures to effect a change in density.

Extraction of pesticides from various matrices such as soil [3,4], vegetable [5], fruits [6], grain [4,13–18], fish [7], meat [8], eggs [9], tobacco [10], and water [11] can readily be accomplished with SFE, as demonstrated by a number of researchers.

Recently, Lehotay reviewed SFE of pesticides in foods [12].

The analysis of organophosphorus pesticide residues in wheat by SFE is an important application.

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Campbell et al. reported the determination of chlorpyrifos methyl spiked on wheat by SFE followed by on-line coupled microcolumn liquid chromatography–gas chromatography (LC–GC) with electron-capture detection (ECD) [13].

King and co-workers investigated the efficiency of a supercritical carbon dioxide extraction system for spiked wheat samples containing organochlorine, organophosphorus and organonitrogen pesticides at three different pressures and temperatures [14,15]. In their work, eight pesticides were extracted, collected after decompression of supercritical CO_2 on a Florisil trap, cleaned-up by GPC and analyzed by GC with ECD or flame photometric detection (FPD) [14,15]. In addition, they analyzed incurred wheat samples containing chloropyrifos methyl at 40 ng/g. Skopec et al. reported a sensitive method for the detection of incurred pyrimiphos methyl and fenitrothion in rice. They used methanol-modified supercritical carbon dioxide for extraction followed by quantitation by GC with atomic emission detection (AED) [16].

Poustka et al. reported a method for the determination of chlorpyrifos methyl and malathion in fortified wheat. Organophosphorus pesticides were extracted and trapped on stainless steel balls and quantitated by GC with FPD [17].

Khan demonstrated that SFE could be used to extract bound pesticide residues from soil, plant and wheat samples treated with radiolabled pesticides. He used methanol-modified supercritical carbon dioxide extraction to extract deltamethrin and pirimiphos methyl in wheat which was treated for 168–196 days with pesticides, followed by quantitation by GC with thermionic detection and ECD [18].

The analysis of organophosphorus pesticide residues in wheat by SFE is an important field as evidenced above. However, the majority of early SFE methods were developed using spiked or fortified samples. Furthermore, methanol-modified carbon dioxide, or additional trapping and clean-up procedures were also used. In addition, AED or FPD was used as GC detection method instead of the more common nitrogen–phosphorus detection (NPD). The main objective of this study was to develop a simple and rapid method which can determine incurred organophosphorus pesticides in wheat flour using unmodified supercritical CO_2 and GC–NPD at concentrations as low as 10 ng/g.

2. Experimental

2.1. Chemicals

SFC-grade carbon dioxide from Scott Specialty Gases (Plumsteadville, PA, USA) was used as a supercritical fluid. Hexane was HPLC grade from Burdick & Jackson (Muskegon, MI, USA).

The pesticide standards (ethoprophos, diazinon, chlorpyrifos methyl, fenitrothion, parathion, phenthoate, EPN) were purchased from Sigma (St. Louis, MO, USA). Pesticide standard solutions of 500 mg/l (stock standard solutions) and triphenyl phosphate (TPP) internal standard (I.S.) solution of 1000 mg/l were prepared in hexane. Pesticide standard solutions for GC calibrations were prepared by suitable dilution of the stock standard solution with hexane and by addition of internal standard solution. Wheat flour was purchased from the market and a 7-g sample was extracted by SFE, and the analytes were determined by GC–NPD without further clean-up of the extracts.

2.2. Supercritical fluid extraction

An Isco 1200 (Lincoln, NE, USA) SFE instrument equipped with a Model 260D syringe pump and controller, SFX 2-10 extractor, and 10-ml extraction cell was used. A fused-silica capillary tube (30 cm×100 μm I.D.) was attached to the outlet of the extractor as a restrictor. Test tubes with PTFE caps were used as collection vessels, and the restrictor was passed through the cap and immersed in the collection solvent (about 20 ml hexane). Carbon dioxide was delivered at flow-rates between 0.7~1.5 ml/min by the syringe pump and controller to the extraction cell containing the sample.

The extraction conditions were varied from 40 to 100°C and from 72.5 to 482.6 bar, depending on the particular experiment. After the supercritical fluid was passed through the sample, extraction times were 60 min (static 20 min, dynamic 40 min) for a 7 g sample size.

One ml TPP, of 1 μg/ml for real wheat extract or 0.5 μg/ml for wheat certified reference material (CRM) extract, was added as an I.S., and the extract was evaporated in a stream of nitrogen to 1 ml. These extracts were quantitated by GC–NPD without clean-up.

2.3. Organic solvent extraction and clean-up

In preliminary experiments for selecting an organic extraction solvent, acetone–water (70:30) showed better recovery than other solvents tested, which consisted of methylene chloride, hexane, acetonitrile and acetone.

Samples of 25 g were extracted two times with 100 ml, and one time with 70 ml of acetone–water (70:30) in an ultrasonic extractor for 20 min. The extract was combined and filtered with suction through a Buchner funnel (Whatman No. 40 filterpaper, diameter 110 mm, Whatman Laboratory Division, Springfield Mill, Maidstone, Kent, UK), and the filtrate was transferred to a 500-ml round bottom flask [30 ml of acetone–water (70:30) was used as washing solvent in these steps]. The volume of this solution was reduced to 95~100 ml by rotary evaporation, then extract was transferred to a 500-ml separatory funnel and 50 ml of methanol and 30 ml of saturated sodium chloride solution was added. To this mixture, 100 ml of methylene chloride was added, followed by vigorous shaking for 5 min. The lower phase was collected in a 200-ml round bottom flask. The aqueous phase was re-extracted with an addition 50 ml of methylene chloride in the same way, and the organic phase was combined and evaporated to 2~3 ml. The concentrate was transferred to a 10-ml vial from the 200-ml round bottom flask by use of disposable pipet (the inner wall of round bottom flask was washed three times with 1 ml of methylenechloride and combined to 10 ml vial) and evaporated with nitrogen gas purging to wetness to prevent loss of pesticides.

For clean-up, 7 g of Bio-Beads S-X3 (200–400 mesh, Bio-Rad Labs., Hercules, CA, USA) was swelled in methylene chloride and slurried with elution solvent [methylene chloride–cyclohexane (1:1)]. The slurry was poured into a 30 cm×1 cm I.D. column that had a stopcock valve, and contained elution solvent, and had glass wool to hold the beads. The packed bed was washed with 10 ml of elution solvent maintaining at least a few centimeters of liquid above the resin bed. Extracted residue was dissolved in 1 ml of elution solvent and then placed on the column (before loading extract to column, elution solvent level above the resin bed was maintained at a few millimeters). Methylene chloride–cyclohexane (1:1) was used as eluent at a flow-rate of 2 ml/min. The first fraction (9 ml) containing lipid was discarded and the second fraction (11 ml) was collected and then evaporated under a nitrogen stream and ensuring that the solvent was not removed completely to prevent loss of pesticide [19,20].

One ml of TPP (0.5 μg/ml for real wheat extract or 10 μg/ml for wheat CRM extract) was added as an internal standard, and for wheat CRM extract, diluted with *n*-hexane to 5 ml, and for real wheat extract, concentrated to 1 ml. These extracts were quantitated by GC–NPD.

2.4. GC analysis

For pesticide determination, a Hewlett-Packard Model HP-5890 GC–NPD system (Palo Alto, CA, USA) and HP Ultra 2 (crosslinked 5% phenylmethylsilicone, 25 m×0.32 mm, film thickness 0.17 μm) was used. Helium was used as a carrier gas at a flow-rate of 1.5 ml/min. The sample (2 μl) was introduced in the split mode (1:10). Injector and detector temperatures were 220°C. The column temperature was programmed as follows: initial temperature 170°C retained for 6 min, then increased at a rate of 5°C/min to 225°C, then increased again at a rate of 10°C/min to 280°C, and retained for 10 min.

3. Results and discussion

3.1. Optimization of SFE conditions

An optimization study was carried out using wheat flour CRM with the aim of determining the conditions that would provide maximum recovery in SFE. The study of the influence of CO_2 pressure was carried out at 60°C, and the pressure was varied between 137.9 and 482.6 bar. As shown in Fig. 1 and Table 1, increasing pressure up to 138 bar resulted in increasing peak area (relative peak area to I.S.) and up to 483 bar there were no significant increases in peak areas. These results correspond to the results reported by Nemoto et al. [21] and Pearce et al. [22]. Nemoto et al. showed the effect of CO_2 density (from 0.30 to 0.85 g/ml, corresponding to pressure of 81~211 bar) for 88 pesticides fortified on celite. However, they did not investigate higher densities [21]. Pearce et al. showed the effect of CO_2 pressure

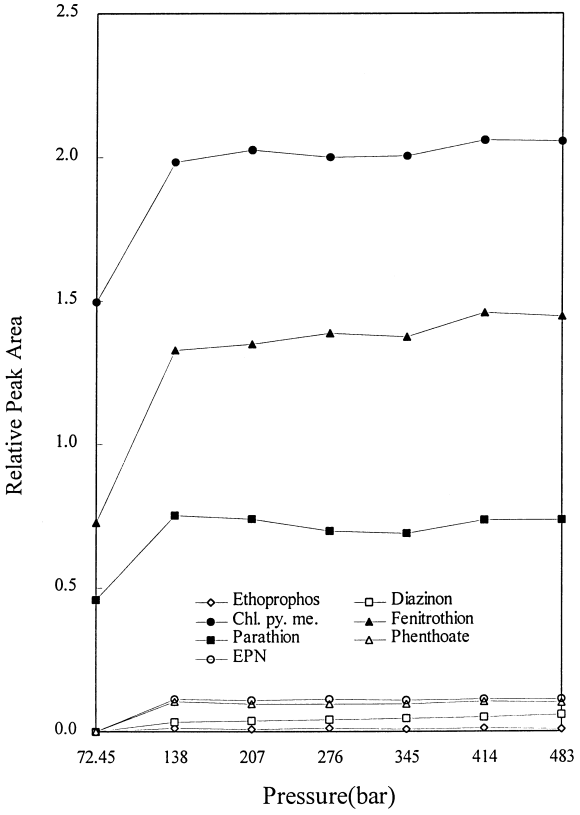

Fig. 1. Effect of pressure on the supercritical fluid extraction of OP pesticides in wheat flour CRM (temperature was fixed at 60°C).

(242~414 bar) for organophosphorus pesticides and other pesticide residues contained in strawberries [22], but they did not investigate lower pressures [21].

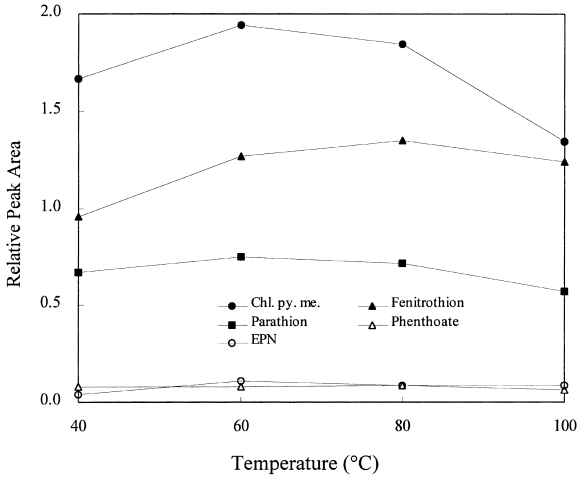

Fig. 2. Effect of temperature on the supercritical fluid extraction of OP pesticides in wheat flour CRM (pressure was fixed at 207 bar).

The effect of extraction temperature was studied at a constant pressure of 206.8 bar. Temperatures were in the range 40~100°C. As shown in Fig. 2 and Table 2, most of the pesticides examined showed the largest peak area at 60°C. Fenitrothion showed a slightly larger peak area at 80°C than at 60°C, but it did not have great effect on extraction efficiency. These results correspond to the results reported by Nemoto et al. (30~70°C) [21] and Pearce et al. (50~60°C) [22] but they did not investigate higher temperatures (80~100°C). As shown in Fig. 2 and Table 2, most of the pesticides examined showed slightly smaller peak areas at temperatures above 80°C than the peak areas at 60°C. As described in Ref. [12], we suppose this is due to thermal degra-

Table 1
Effect of pressure on the supercritical fluid extraction of OP pesticides in wheat flour CRM (temperature fixed at 60°C)

Pressure (bar)	Relative peak area±S.D.[a]						
	Ethoprophos	Diazinon	Chlorpyrifos methyl	Fenitrothion	Parathion	Phenthoate	EPN
72.5	ND[b]	ND	1.496±0.085	0.752±0.053	0.456±0.030	ND	ND
138	0.011±0.000	0.033±0.002	1.982±0.024	1.330±0.012	0.751±0.023	0.105±0.015	0.115±0.008
207	0.010±0.000	0.036±0.001	2.027±0.090	1.350±0.000	0.741±0.073	0.095±0.004	0.108±0.001
276	0.011±0.001	0.043±0.003	2.000±0.049	1.388±0.037	0.699±0.015	0.097±0.001	0.112±0.007
345	0.009±0.000	0.048±0.005	2.006±0.086	1.375±0.026	0.691±0.007	0.096±0.002	0.111±0.003
414	0.012±0.001	0.049±0.008	2.060±0.035	1.458±0.102	0.736±0.048	0.104±0.004	0.113±0.004
483	0.010±0.000	0.057±0.004	2.054±0.066	1.444±0.049	0.736±0.032	0.101±0.003	0.113±0.005

[a] Average of three.
ND=Not detected.

Table 2
Effect of temperature on the supercritical fluid extraction of OP pesticides in wheat flour CRM (pressure was fixed at 207 bar)

Temperature (°C)	Relative peak area±S.D.[a]				
	Chlorpyrifos methyl	Fenitrothion	Parathion	Phenthoate	EPN
40	1.663±0.079	0.957±0.064	0.666±0.014	0.078±0.003	0.040±0.006
60	1.940±0.048	1.268±0.034	0.750±0.056	0.081±0.003	0.108±0.017
80	1.842±0.042	1.349±0.066	0.713±0.023	0.086±0.004	0.087±0.007
100	1.342±0.044	1.240±0.074	0.57±0.026	0.061±0.011	0.084±0.009

[a] Average of three.

dation of pesticides at such high temperatures, and therefore moderate temperatures should be used. For the extraction of all the organophosphorus pesticides of interest in wheat flour, we chose 60°C and 206.8 bar as optimum conditions. The CO_2 flow-rate (0.7~1.4 ml/min) and extraction time (static extraction 20 min, dynamic extraction 40 min) were selected from the preliminary experiments and previous reports [13,18].

3.2. Comparison of results

The calibration curves from the peak area ratio of standards and I.S. to the concentration of standards were obtained. Each calibration curve had shown acceptable correlation coefficient range of $r=0.997$~0.999 in the concentration range of 0.04~2 μg/ml which were equivalent to the concentration of each pesticides in the final extractants.

Table 3
Results of pesticide analysis of the wheat flour CRM

	SFE		Solvent extraction and clean-up	
	Average[a] (μg/g)	R.S.D. (%)	Average[b] (μg/g)	R.S.D. (%)
Ethoprophos	0.021	19.7	0.026	5.9
Diazinon	0.047	10.8	0.037	20.2
Chlorpyrifos methyl	4.478	8.6	4.236	8.4
Fenitrothion	1.959	10.6	2.138	5.7
Parathion	1.030	8.6	0.932	8.8
Phenthoate	0.192	3.7	0.196	6.4
EPN	0.170	9.9	0.185	11.6

[a] Average of three.
[b] Average of 10.

Table 4
Results of pesticide analysis of the real wheat flour

	SFE		Solvent extraction and clean-up	
	Average[a] (μg/g)	R.S.D. (%)	Average[b] (μg/g)	R.S.D. (%)
Chlorpyrifos methyl	0.0040	5.856	0.0049	9.701
Fenitrothion	0.0029	9.647	0.0031	4.007
Malathion	0.0035	8.236	0.0031	6.732
Parathion	0.0021	2.689	0.0015	2.548

[a] Average of five.
[b] Average of six.

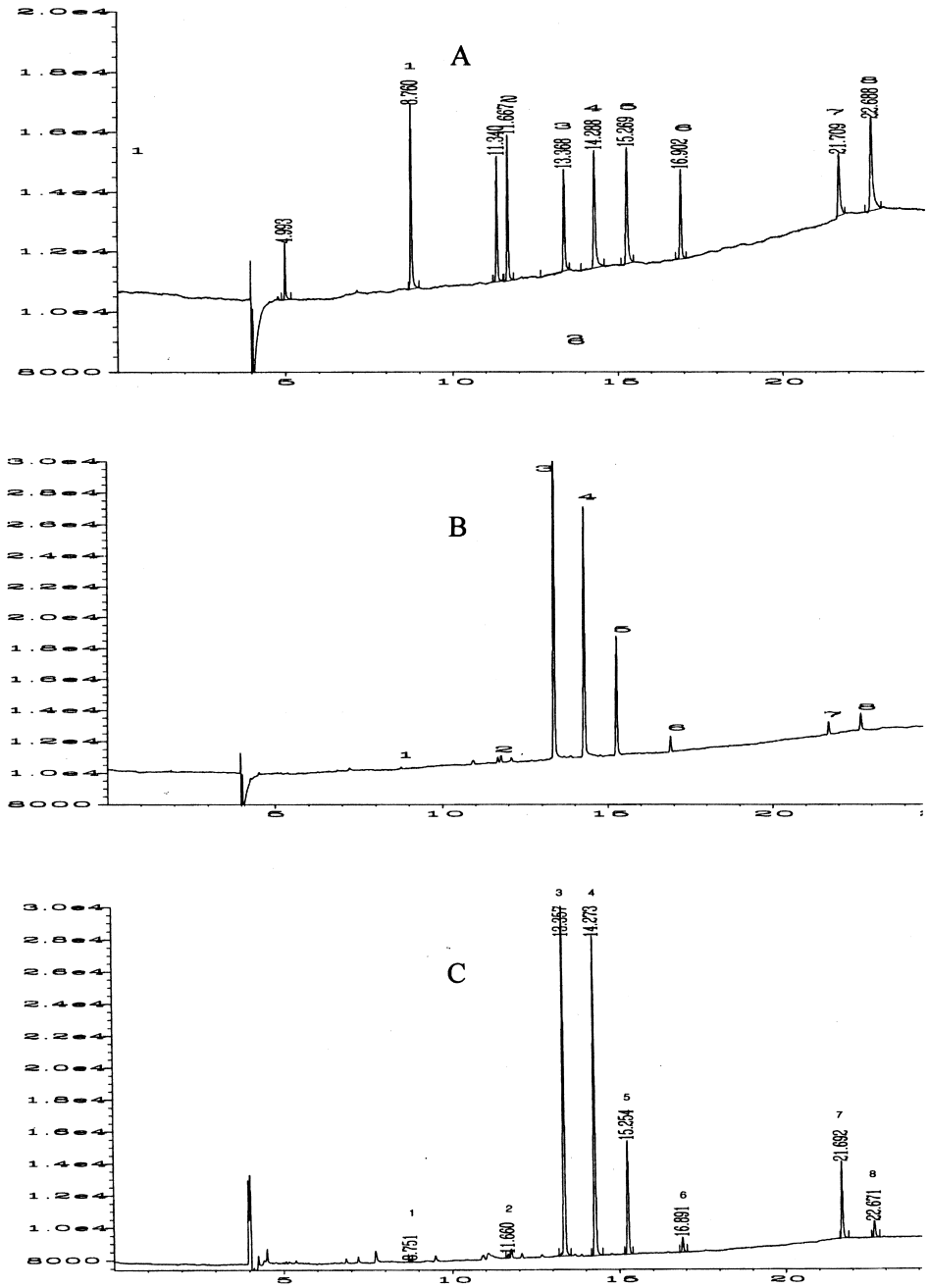

Fig. 3. GC–NPD chromatograms of standards and the wheat flour CRM (A) standards (2 μg/ml), (B) SFE, no further clean-up (a wheat flour CRM extract with supercritical CO_2 at 60°C and 206.8 bar), (C) ultrasonic extraction followed by LLE and GPC clean-up [a wheat flour CRM extract with acetone–water (70:30)]. Peaks: 1=Ethoprophos, 2=diazinon, 3=chlorpyrifos methyl, 4=fenitrothion, 5=parathion, 6=phenthoate, 7=I.S. (TPP), 8=EPN. (B) One ml of 0.5 μg/ml TPP was added to the extract and concentrated to 1 ml. (C) One ml of 10 μg/ml TPP was added to the extract and concentrated to 5 ml.

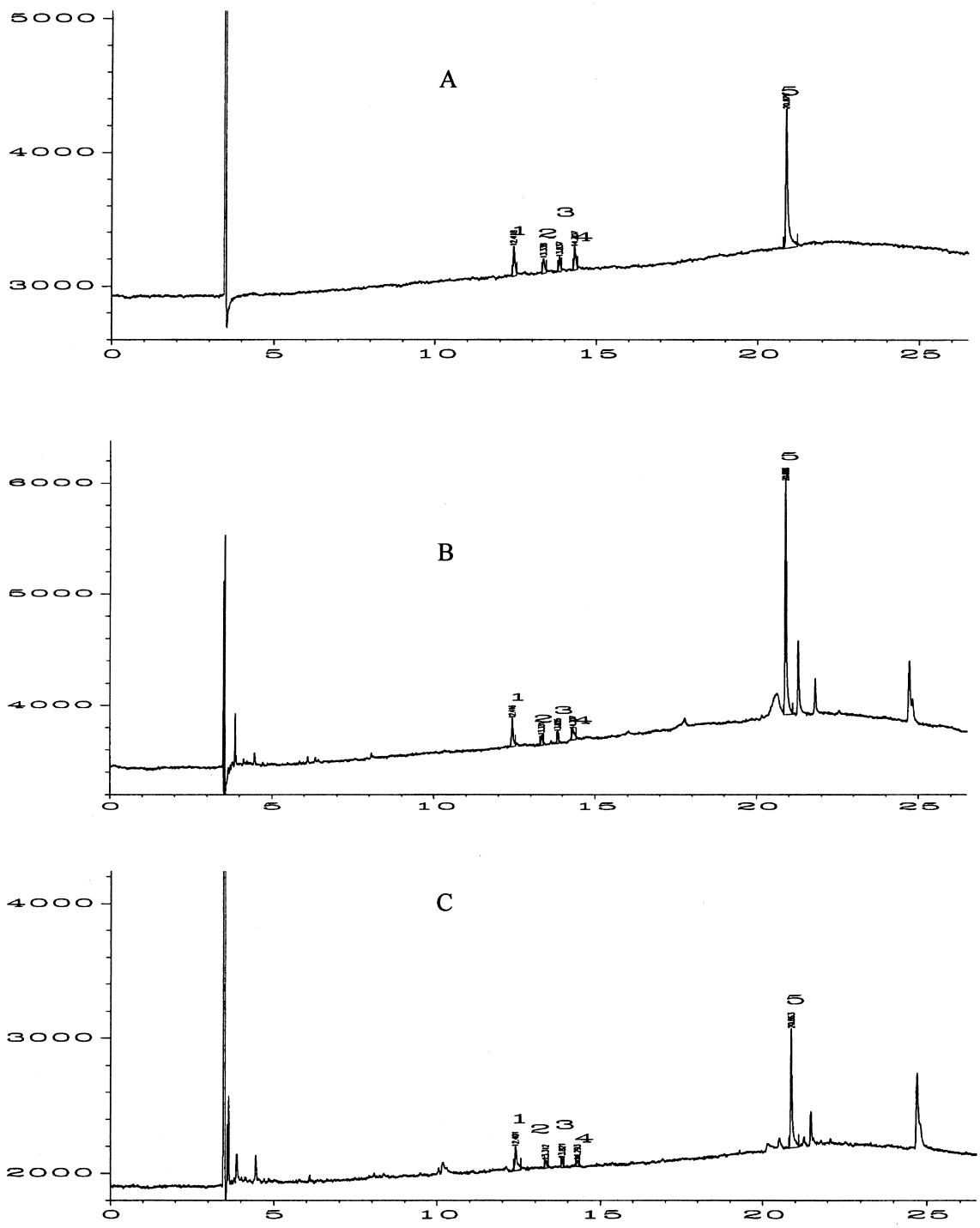

Fig. 4. GC–NPD chromatograms of standards and the real wheat flour (A) standards (0.1 μg/ml), (B) SFE (a real wheat flour extract with supercritical CO_2 at 60°C and 206.8 bar), (C) ultrasonic extraction followed by LLE and GPC clean-up [a real wheat flour extract with acetone–water (70:30)]. Peaks: 1=Chlorpyrifos methyl, 2=fenitrothion, 3=malathion, 4=parathion, 5=I.S. (TPP).

The values for the quantitation of the organophosphorus pesticides in wheat CRM and real wheat samples are shown in Tables 3 and 4. We assessed the precision of the method by repeated analysis (3–10 times). The relative standard deviations (R.S.D.s) for the supercritical extraction of wheat CRM and real sample are in the range of 2.69~10.80% (except ethoprophos, 19.7%), which were acceptable. The R.S.D.s for the organic solvent extraction of wheat CRM and real wheat sample were in the range of 2.55~11.60% (except diazinon 20.20%). Good correlation in results was obtained between SFE and organic solvent extraction, and the precision of the determined values was also comparable for both methods. GC–NPD chromatograms of the standard solution, the extracts of the wheat flour CRM and real samples by SFE (sample mass 7 g), and organic solvent extract (sample mass 25 g) are shown in Figs. 3 and 4. Pesticides of interest could be extracted selectively from the wheat matrix without clean-up. On the other hand, in organic solvent extraction, lipids were coextracted as interfering substances; accordingly extracts underwent LLE and GPC clean-up steps prior to analysis.

In a real wheat flour sample four organophosphorus pesticides were detected, which were identified by comparing retention times with those of the calibration standards. These identifications were tentatively confirmed by adding the individual standards to sample extracts and surveying the changes in area, height, width and shape of the individual peaks after analysis under the same conditions (in the future, identification by mass spectrometry, and by different column is needed). The main pesticide components in real wheat sample were chlorpyrifos methyl, fenitrothion, malathion and parathion.

4. Conclusions

These results show that, compared to current methods, SFE simplifies the sample preparation step by curtailing further trapping and clean-up procedure and by using unmodified carbon dioxide. In addition, we used GC–NPD for the pesticide analysis instead of detection methods like AED and FPD. This resulted in a fast and efficient method for analyzing organophosphorus pesticides in wheat, which allowed us to analyze a 7-g wheat flour sample containing trace levels of organophosphorus pesticide residues (2~5 ng/g) in 60 min (SFE) plus 25 min (GC–NPD).

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