Supercritical Fluid Extraction and Enzyme Immunoassay for Pesticide Detection in Meat Products

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Two techniques supercritical fluid extraction (SFE) and enzyme immunoassay (EIA) were integrated into an analytical method for the rapid detection of pesticide residues in meat samples. The pesticides of interest were extracted from meats using supercritical CO_2. A pumpless SFE system, which was designed in our laboratory, and commercial equipment were used in SFE experiments. The presence of pesticide residues in the extract was quantitatively determined using the magnetic bead-based EIA kits. Several types of pesticides (alachlor, carbofuran, atrazine, benomyl, and 2,4-D), spiked in the meat samples (bovine liver, ground beef, and lard), were extracted and analyzed. Interferences caused by the coextracted substances from these complex sample matrices required the use of a cleanup step prior to the EIA test. The described techniques are potentially portable and could be used for the rapid screening of meat samples in plant environments.

Keywords: *Pesticide, extraction, supercritical fluid, immunoassay, meats*

INTRODUCTION

As part of its regulatory responsibility, the Food Safety and Inspection Service (FSIS) agency of the U.S. Department of Agriculture regularly analyzes a large number of meat products for pesticide residues. These analyses are traditionally performed in laboratories utilizing techniques, such as extraction with organic solvents and sample cleanup by column chromatography, followed by final chromatographic separation and detection of the analyte. However, due to growing demands for increased analytical capability and capacity, there is a need for faster analytical methods that are simple and also suitable for use in nonlaboratory environments (Ellis, 1989).

An alternative technique to the conventional solvent extraction is supercritical fluid extraction (SFE). This extraction method using supercritical fluids, such as carbon dioxide, is a faster and more cost-effective technique. SFE has already been shown to be an efficient technique for the extraction of a wide variety of organic analytes (Hawthorne, 1990). It has also been successfully utilized for the extraction of pesticide residues from meat samples (Hopper and King, 1991; Snyder et al., 1993).

As a rapid testing method, the immunochemical assay technique has seen a steady increase in use for the determination of pesticide residues. Some of the potential aspects and advantages of enzyme immunoassay (EIA), which employs enzymes as markers, are described in recent reviews (Vanderlaan et al., 1988; Van Emon and Lopez-Avila, 1992). The cost savings of EIA over the traditional chromatographic techniques have been shown to be substantial (Kaufman and Clower, 1991). As an alternative tool for pesticide residue analysis, EIA has been extensively used for water and soil samples (Jung et al., 1989; Van Emon et al., 1989; Hall et al., 1990). Assay kits are now commercially available for many pesticides and can be used as rapid and easy-to-use screening tests that are suitable for both field and laboratory use. However, only a limited number of EIAs have been applied to samples such as meats (Ellis, 1989). Detection of pesticide residues in such samples are complicated by interfering materials that have to be reduced or eliminated prior to conducting an assay.

When combined with the SFE technique, EIA can be an effective method to screen the sample for the potential presence or absence of a target pesticide and to determine the concentration of the analyte. The incorporation of these two techniques into a tandem analytical method offers many advantages (e.g., cost and speed) and has the potential to complement, or possibly replace, in some instances, the traditional methods for monitoring the pesticide residue content of foods. Due to higher selectivity achieved by both SFE and immunoassay, laborious and time-consuming cleanup steps necessary in the classical methodology can be reduced or eliminated. This will contribute significantly to increasing the speed of the analysis, especially for complex sample matrices like meats. Both techniques have an added benefit of requiring only small amount of liquid–organic solvent, since SFE generally uses carbon dioxide and the EIA test is performed in aqueous media. Coupled with the development of a simple and portable SFE system, EIA kits could further satisfy the need for on-site screening of meat samples for pesticide residues and allow detection of analytes that are unstable and easily degradable.

Potential benefits of the SFE and EIA methods combined for use in the pesticide residue analysis have been shown in a preliminary study conducted at our laboratory (France and King, 1991). Similar techniques have also been applied in the field of environmental analysis (Wong et al., 1991; Lopez-Avila et al., 1993). In this current study, more in-depth investigations were completed, with goals of continued improvement of the SFE–EIA method and the use in quantitative extraction and analysis. SFE was initially evaluated with respect to the quantitative recovery of five widely used pesticides (alachlor, carbofuran, atrazine, benomyl, and 2,4-D) from various meat products. SFE was performed with a pumpless system of our own design, as well as with commercial SFE equipment. EIA was then evaluated for its compatibility with SFE and meat sample matrices for the accurate quantitation of selected pesticide residues.

MATERIALS AND METHODS

Samples and Reagents. Beef liver and ground beef were purchased from a local meat packing house and homogenized in

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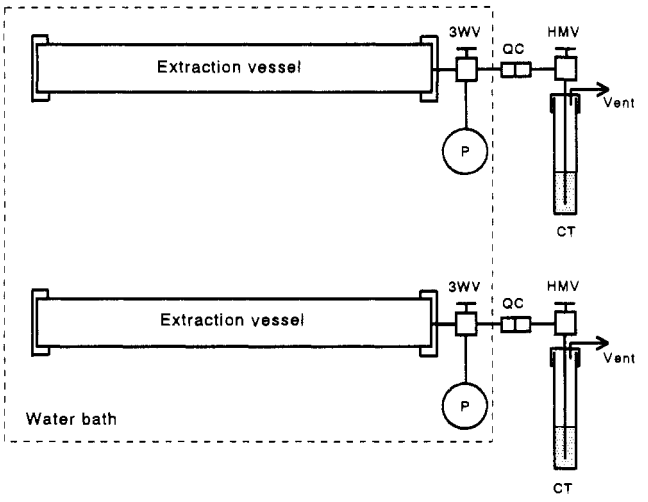

Figure 1. Schematic diagram of the pumpless SFE system utilized for static extraction. 3WV = 3-way valve; QC = quick-connect fittings; P = pressure gauge; HMV = heated micrometering valve; CT = collection tube.

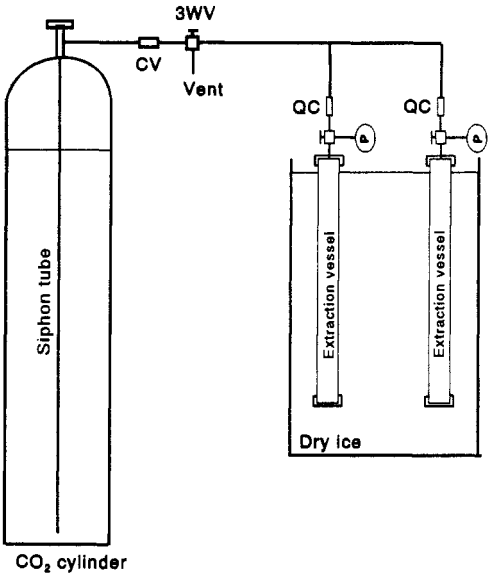

Figure 2. Schematic diagram showing SFE vessels in the pumpless system being charged with CO_2 prior to the extraction. CV = check valve; 3WV = 3-way valve; QC = quick-connect fittings; P = pressure gauge.

a food chopper. These samples were divided into 5-g portions and stored in a freezer until the extraction step. A lard sample was also obtained from the same source and used as supplied. Samples requiring fortification were spiked with alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide], carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate), atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine], benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], and/or 2,4-D (2,4-dichlorophenoxyacetic acid) standard solutions. Individual stock solutions of these standards were prepared in methanol, at 1 mg/mL concentration, with pure (>97%) analytical standards purchased from Chem Service, Inc. (West Chester, PA). Standard solutions for the spiking purpose were then prepared by serial dilution of these solutions. Frozen samples were thawed and mixed with 3–5 g of Hydromatrix (pelletized diatomaceous earth; Analytichem International, Harbor City, CA) before placement in the extraction vessel.

Supercritical CO_2 Extraction. Prior to performing the EIA test for pesticide residues, the meat samples were extracted with supercritical CO_2, modified with methanol. SFE was performed by two different methods: static extraction with a pumpless system designed in our laboratory and dynamic extraction using a commercial apparatus.

(*A*) *Construction of a Pumpless SFE System.* The experimental apparatus for the pumpless SFE was designed and constructed similarly to the previously described system (France and King, 1991) with the following modifications (Figure 1). Two sets of extraction vessels and collection systems were used simultaneously, thereby doubling sample throughput. The extraction vessels were heated by immersion in a thermostated water bath (Model 8854; Cole-Parmer Instrument Co., Niles, IL). This water bath was equipped with an ultrasonic generator, which was used to sonicate the sample and the extraction fluid inside the vessel. A micrometering valve (Model 10VRMM-2812, Autoclave Engineers, Inc., Erie, PA), which was heated with hot air from an air gun, was used to regulate the CO_2 gas flow during the decompression step. This arrangement was less prone to plug during venting of the extraction fluid and was also more practical than the widely used linear restrictor, especially for the extraction of meat products containing high moisture (e.g., liver) and fat (e.g., lard). Quick-connect fittings (Model SS-QM2-200; Swagelok Co., Solon, OH) were utilized to couple the extraction vessel to the micrometering valve. The collection vessel consisted of a 50-mL (25- × 150-mm) glass tube and a screw-top cap with two holes. These modifications proved advantageous in reducing the extraction time of the sample and improving extraction efficiency.

(*B*) *Static Extraction by a Pumpless SFE System.* Two physically different sources of CO_2 were tested as an extraction medium with this pumpless SFE device: dry ice and liquefied CO_2. Dry ice was prepared in the laboratory by using a portable dry ice maker (Lux Scientific Instrument Corp., Tucson, AZ). The dry ice powder was formed in this case, directly from the liquefied CO_2 in a welding grade cylinder (National Welding Supply Co., Inc., Bloomington, IL) equipped with a dip tube. A sample (5 g of meat mixed with approximately 5 g of Hydromatrix) was then blended with the dry ice (approximately 40–44 g) and put into the precooled 70-mL extraction vessel. When a cosolvent was required, a small amount (0.5–1 mL) of liquid methanol was added to the 70-mL extraction vessel before adding the sample mixture. The contents of the extraction vessel were held in place with glass wool plugs. The vessel was then capped and placed in the water bath, which was heated to the extraction temperature (40–60 °C). This technique generated approximately 95–110 atm.

In the second option, a substantially higher extraction pressure was obtained, when the liquefied CO_2, instead of dry ice, was transferred to the precooled extraction vessel (Figure 2), which was then disconnected from the supply cylinder and heated by immersion in the water bath. Approximately 1–1.5 mL of methanol cosolvent was added along with the sample mixture in the vessel prior to adding CO_2. The head pressure (63 atm) of the CO_2 cylinder (equipped with a siphon tube) and the dry ice bath was used to fill the 70-mL extraction vessel with more than 70 g of CO_2. Depending on the length (0–25 min) of the filling period, up to 600 atm of pressure was produced when the extraction vessel was heated to 50 °C.

In both cases, after an appropriate equilibration period (30–60 min) for the static extraction, the exit valve was opened to permit the supercritical CO_2 extract to pass through the manually controlled micrometering valve and vent into a collection tube containing approximately 8 mL of liquid solvent (a pure or aqueous solution of methanol). Complete depressurization of the vessel was accomplished in 5–10 min by venting CO_2 gas at approximately 2 L/min. After the SFE vessel was completely depressurized, a small amount (1–2 mL) of the liquid solvent was used to rinse the trace of analyte residues that had precipitated inside the micrometering valve and adjoining transfer line. The collection solvent was adjusted to 10 mL and subsequently filtered. The extract was then diluted 10–25-fold with a buffered diluent before running the EIA test. The diluent was either supplied with the assay kit or prepared from a Tris-buffered (2.5 mM, pH 7.6) saline preparation (Part No. 28376-G; Pierce, Rockford, IL).

(*C*) *Dynamic Extraction by a Commercial SFE System.* A commercial SFE system (Model 723; Dionex Corp, Salt Lake City, UT), which can simultaneously extract up to eight samples, was used for the dynamic extraction of pesticides from meat samples. To extract a 5-g portion of the meat sample mixed with approximately 3 g of Hydromatrix, an extraction vessel of 10-mL

volume was utilized. All extractions were carried out with approximately 70 g of SFC/SFE grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) at conditions of 400–450 atm and 50 °C for 60 min. For a cosolvent, 5 mol % of methanol was added via the auxiliary pump in the Model 723. The linear flow restrictors (500 mL/min of expanded CO_2) were maintained at 150 °C. The extracted materials were collected into 10 mL of liquid solvent (methanol) cooled to approximately 5 °C. This collection solvent was filtered and then diluted 10–25-fold with the Tris-buffered diluent prior to the EIA test.

Cleanup Procedures To Remove Interfering Materials. Initial investigation of the cleanup methods to remove interfering substances from the dynamic SFE-extracted meats prior to conducting EIA included solid phase extraction (SPE) and liquid/liquid partitioning. SPE was performed with a C-18 Sep-Pak cartridge (Part No. WAT051910; Millipore Corp., Milford, MA), which was prewashed with 3 mL each of methanol and deionized water using a vacuum manifold. Five milliliters of the sample extract after 10–25-fold dilution with deionized water was then passed through the cartridge. The adsorbed pesticides were eluted with 3 mL of methanol. This methanol eluate was dried under a nitrogen stream to approximately 0.5 mL and reconstituted to 5 mL with the Tris-buffered diluent for EIA tests.

For the alternative cleanup technique of liquid/liquid partitioning of the pesticides of interest, a binary solvent system of acetonitrile/hexane was used. For this study, extracts of meat samples were collected in acetonitrile, instead of methanol, and partitioned successively with three equal amounts (10 mL) of hexane. The acetonitrile layer was dried under a nitrogen stream to about 1 mL and made to 10 mL with the Tris-buffered diluent for EIA tests.

The third option for the cleanup was an additional filtration of the dynamic SFE extracts after the final dilution step just prior to conducting EIA. Approximately 3 mL of diluted SFE extracts was filtered through a 0.5-μm Millex-LCR membrane filter (Part No. SLCR-013-NS; Millipore Corp., Bedford, MA) to remove any precipitates. The presence of the target analytes in this final filtrate was determined by independently testing with five separate EIA kits for the different analytes.

Enzyme Immunoassay Procedure. Immunoassays for alachlor, carbofuran, atrazine, benomyl, and 2,4-D pesticides were accomplished using magnetic bead-based EIA (enzyme-linked immunosorbent assay) kits, obtained from Ohmicron (Newtown, PA). The benomyl assay kit detected both benomyl and its hydrolysis product, carbendazim (methyl 2-benzimidazolecarbamate). A magnetic rack, with a capacity for 60 sample tubes, and a portable spectrophotometer (Model RPA-III) were also purchased from the same company for the EIA tests.

The assay procedure for each pesticide was followed as described in the test kit instructions. Specified amounts of sample extract, enzyme conjugate, and pesticide-specific antibody coated on magnetic particles were added to a test tube and incubated at room temperature for 20–30 min. A series of pesticide calibration solutions, provided in each assay kit, were prepared similarly. These assay tubes were then placed in the magnetic rack and inverted to decant the reaction mixture. The magnetic particles trapped inside the tube were washed twice with washing solution. A mixture of the enzyme substrate (hydrogen peroxide) and the chromogen (3,3′,5,5′-tetramethylbenzidine) was added to the tube and allowed to develop color for 20–30 min at room temperature. The color reaction was then stopped and stabilized by the addition of 2 M sulfuric acid. Measurement of the final color density in the assay tube was made using the spectrophotometer at a 450-nm wavelength.

The intensity of the color was inversely proportional to the amount of pesticide present. This color inhibition was quantitated in terms of B/B_0, where the absorbance value (B) observed for a sample or standard was divided by the absorbance (B_0) at zero analyte concentration. The concentration of pesticide in the sample was determined by comparing the observed values to a calibration curve prepared from the pesticide standards, which were supplied with the test kit and had concentration ranges of 0.1–5.0 μg/L for alachlor, carbofuran, and atrazine, 1–50 μg/L for 2,4-D, and 0.25–5.0 μg/L for benomyl, which was measured as carbendazim. A linear calibration curve was obtained when the mean value of B/B_0 for each standard was plotted against the corresponding concentrations on a logit vs logarithmic graph scale.

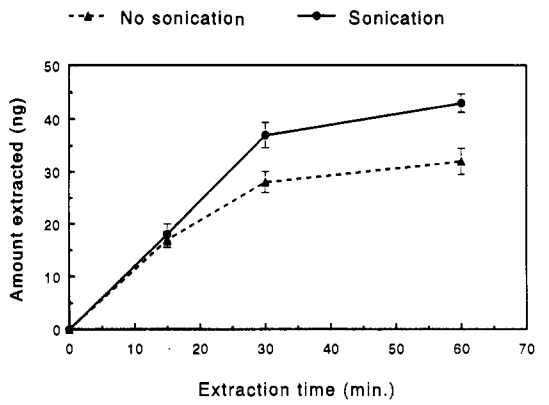

Figure 3. Extraction efficiency of alachlor from bovine liver with the pumpless SFE system. The amount of pesticide was determined by an EIA kit.

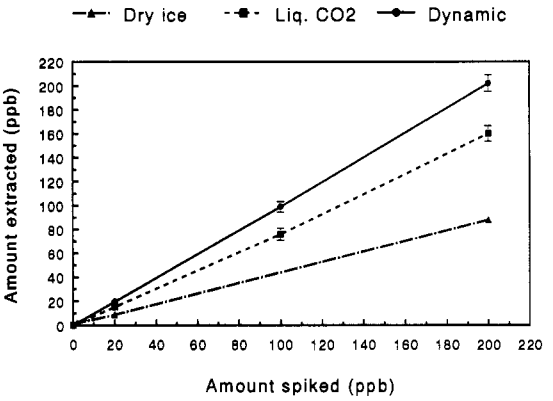

Figure 4. SFE efficiency of alachlor from bovine liver. The amount of pesticide was determined by an EIA kit. Dry ice = static extraction by pumpless system, 100 atm. Liq. CO_2 = static extraction by pumpless system, 500 atm. Dynamic = dynamic extraction by commercial system, 450 atm.

RESULTS AND DISCUSSION

Supercritical CO_2 Extraction. Alachlor and bovine liver were chosen as the initial test analyte and matrix, respectively, for evaluating the efficiency of the new pumpless SFE. First, the potential loss of analytes due to the relatively fast rate (ca. 2 L/min) of CO_2 decompression during the collection step was examined by running a number of blank extractions with collection solvents containing a known concentration of alachlor. Subsequent assays revealed that all of the spiked analytes remained in the collection solvents having up to 80% of water in methanol. Short depressurization and extraction periods will be beneficial, if a number of static extractions are required on the same sample to achieve maximum analyte recovery.

The effect of the equilibration time on the transfer of alachlor analyte from liver matrix to the supercritical CO_2 is shown in Figure 3. The plateau in recovery was reached after 30 min from the start of the static extraction. It was also noted that sonicating the extraction vessel during this extraction period enhanced the extraction efficiency. Applying ultrasound during SFE is known to be an efficient mechanism of mixing, thus decreasing the external mass transfer resistance of the sample particles (Wright et al., 1988). Therefore, all subsequent static extractions using the pumpless SFE apparatus were performed by equilibrating the cell for 60 min in a sonicating water bath.

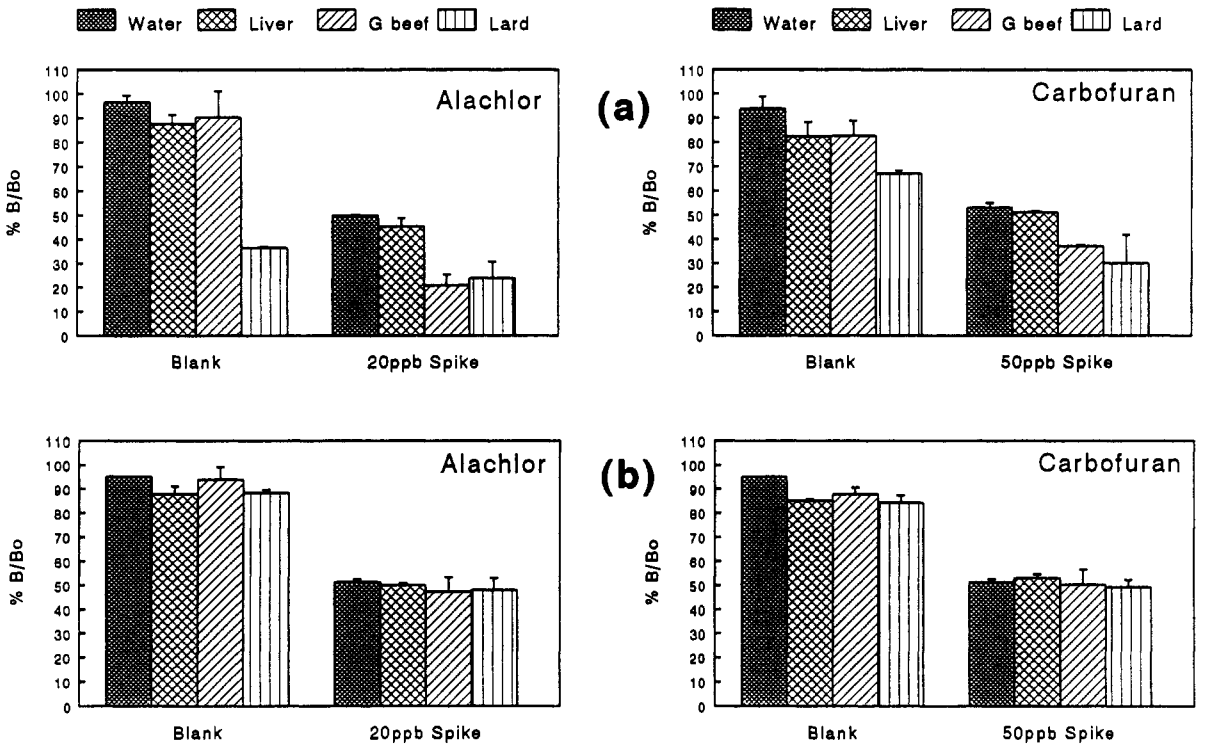

Figure 5. Alachlor and carbofuran EIAs of meat samples prepared by dynamic SFE: (a) interferences caused by coextracted materials and (b) removal of interfering materials from the buffer-diluted extract by microfiltration (n = 4).

Up to 20% enhancement in the recovery of a test analyte was achieved by adding a small amount (0.5–1 mL) of methanol as a modifier directly to the extraction vessel before commencing the extraction. A linear relationship between the amount of alachlor extracted and the spike level ranging from 2 to 200 ppb is shown in Figure 4 (dry ice). The average recovery values were 50%. No discernible improvement in recovery was observed when other extraction conditions (temperature and depressurization speed) were varied. The main factor limiting the analyte recovery was the insufficient CO_2 extraction pressure that could be generated with dry ice medium. Increasing the amount of CO_2 by using a larger vessel or by performing multiple extractions on a sample could yield higher recoveries.

As a second option of the extraction fluid source, a liquid CO_2 fill generated substantially higher extraction pressure (maximum of 600 vs 110 atm by dry ice source). Accordingly, the average recovery of alachlor analyte extracted by CO_2 and 1 mL of methanol at 400–500 atm, increased to 80% (liq CO_2 in Figure 4). Overall, the pumpless apparatus showed similar capability and advantages noted in previous studies which utilized a pump for the static SFE of environmental pollutants, such as pesticides, PCBs, and PAHs, etc., from animal tissues (Nam et al., 1990) and soils (Miller et al., 1993).

Sixty minutes of dynamic extraction of alachlor-fortified liver using a commercial SFE system with a mixture of CO_2 and 5 mol % methanol at the pressures of 400–450 atm yielded complete (100%) recovery of the analyte (dynamic in Figure 4).

EIA of Meat Extracts Prepared by SFE. Since the materials extracted by SFE were captured in a solution containing a varying amount (20–100%) of methanol; it was essential to check the effect of this organic solvent on the subsequent EIA quantitation of target analytes. Since the EIA kits used in this study can tolerate greater than 10% organic solvent, no significant interference was observed during the assays of the aqueous SFE extracts containing a maximum of 10% organic solvent after 10–25-fold dilution. No extraneous matter was introduced from the CO_2 sources (both welding grade and SFC/SFE grade CO_2 cylinders) that may have contributed to the EIA response above the minimum detectable concentration of 0.05 μg/L (in water) for alachlor. Background responses from the materials extracted from the meat matrices (liver, ground beef, and lard) by dry ice plus 0.5–1 mL of methanol cosolvent were also below the minimum detection limit (1 μg/kg for 5-g meat samples diluted 100-fold) for alachlor. Furthermore, pesticide-fortified meats extracted in the static mode under similarly benign conditions using the dry ice source had low variability in the EIA quantitation.

When the meat samples were extracted in the static mode at high pressures (367–626 atm at 50 °C) using a liquid CO_2 source, a significant amount of coextracted substances (mainly fats) were visible in the collection tube. These lipid coextractives caused higher artifact EIA responses for the neat meats; however, they were still below the detection limit of the method as specified by FSIS (Brown, 1991). Therefore, the possibility of seeing any false-positive signal above the FSIS residue tolerance limit of 20 ppb alachlor during the detection of analyte concentration in the meat samples is remote. Similar results were observed when a carbofuran EIA was employed on the same meat samples, which had a method detection limit of 3 μg/kg (for 5-g meat samples diluted 250-fold).

However, EIA measurement for both alachlor and carbofuran in the fortified samples extracted at similarly high pressures using the pumpless SFE and the liquid CO_2 source produced a large variance (up to 33% standard deviation). The main cause of this variance is attributed to the increase in the interferences from coextracted substances (i.e., fats). Of the three meat products, the lard sample produced the highest background response in the EIA test, especially when extracted at increased pressures.

When the pesticide-fortified meats were rigorously

extracted in the dynamic mode by a commercial SFE to recover all of the spiked pesticides, a large amount of substrates was coextracted along with the analytes of interest and caused a significant level of interferences during the final EIA determination. Accordingly, the extracts of the neat meat samples consistently exhibited high EIA response for alachlor and carbofuran. The difference in the absorbance (% B/B_0) between the meat matrices and the water blank is shown in Figure 5a (blank). The lard sample, in particular, had the largest amount of coextracted fat and yielded very high background signals, which were equivalent to alachlor and carbofuran concentrations of 46 and 13 ppb, respectively.

The difference in the absorbance between the water and meat samples, which were spiked with same concentration of alachlor and carbofuran, is illustrated in Figure 5a (spike). Ground beef and lard, in particular, had large influences from their matrix composition and produced inaccurate EIA determinations of analyte recovery, which were substantially higher than the expected 100%.

The static SFE methods minimized interference with the subsequent immunoassay from coextracted substances, at least to the extent that it could be used as a semiquantitative survey method for the presence of pesticides in meat samples (Figures 3 and 4). However, the inaccurate assay results on meat matrices containing high levels of coextracted substances indicated that some degree of extract cleanup was needed to remove the interfering materials. Although selective, EIA methodologies are known to be susceptible to sample matrix effects. Similar types of interferences for bovine liver and meat samples, when extraction was performed with a liquid solvent, have been noted in the literature (Stanker et al., 1989; Lehotay et al., 1993). EIA quantitation, based on the calibration curve derived by adding pesticide standard to a negative control sample extract in place of the normal blank solution, is noted as a possible remedy, if the level of interferences is relatively small and constant between samples of the same matrix (Bushway et al., 1989).

Removal of Interferences from Sample Extract Prior to EIA. Sample cleanup steps, such as solvent partitioning and chromatographic fractionation, have been suggested to remove the above background interferences (Newsome, 1986). Accordingly, cleanup of pesticide-fortified meat samples after dynamic SFE, by C-18 solid-phase cartridge or acetonitrile/hexane partitioning techniques, allowed accurate EIA quantitation of alachlor and carbofuran recoveries with low variability. Following the C-18 SPE cleanup, average recoveries of alachlor and carbofuran determined by assay were 103 ± 8% and 97 ± 8%, respectively. The cleanup by the acetonitrile/hexane partitioning scheme yielded 109 ± 6% and 93 ± 8% recoveries for alachlor and carbofuran, respectively.

Alternatively, removal of the precipitates (mainly fat globules) formed in the meat extract when diluted with the Tris-buffered diluent following the dynamic SFE, by the use of a microfiber (0.5-μm membrane) filter, also reduced the interference level and allowed accurate EIA measurements of pesticide content. For example, 100 ± 7% and 100 ± 4% of the fortified alachlor and carbofuran, respectively, were recovered from meat extracts after the filtration step. EIA responses from all three pesticide-free meat extracts, which previously had high background signals, fell below the analyte detection limits (blank in Figure 5b). Consequently, EIA absorbance between the spiked meats and the blank samples was clearly distinguishable at the residue tolerance limits. Furthermore, reproducibility of EIA quantitation of the recovered

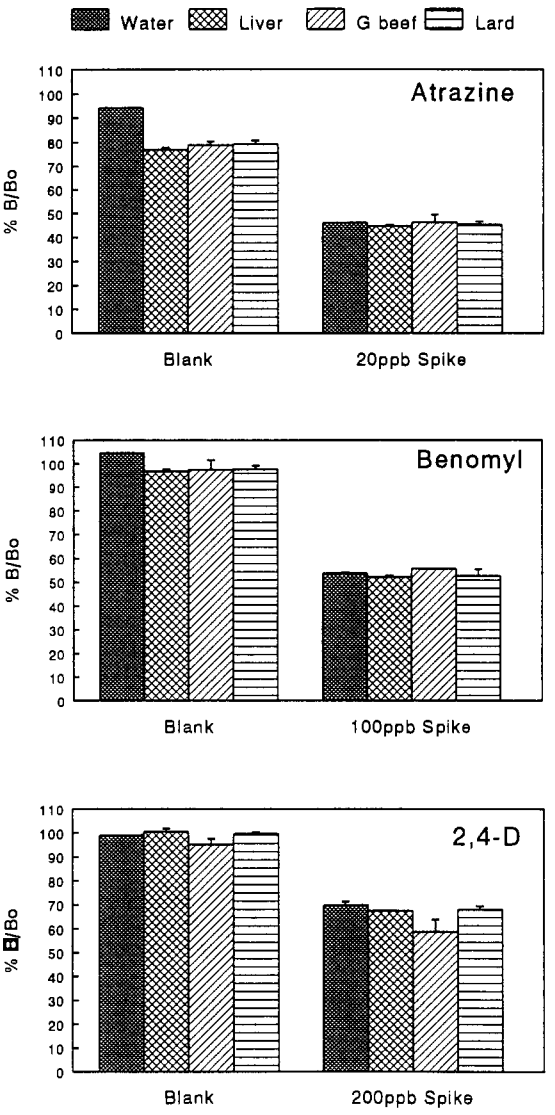

Figure 6. Atrazine, benomyl, and 2,4-D EIAs of meat samples prepared by dynamic SFE. Interfering materials were removed by microfiltration of buffer-diluted extract (n = 4).

alachlor and carbofuran from the meat matrices has drastically improved. Similar improvement in the accuracy and precision of EIA measurement was observed for the meats fortified with atrazine, benomyl, and 2,4-D (Figure 6).

Hence, with an extra filtration step, the integrated SFE–EIA technique allowed quantitation of the pesticide residues in all three meat matrices without the need for additional sample cleanup steps, as shown in Table 1. In addition, the minimum detectable concentrations by the SFE–EIA method were well below the levels that are specified in the FSIS Residue Program (Table 1), allowing successful screening of meat products.

Conclusions. A simple pumpless SFE system was successfully used for static extraction of pesticides from meat products by utilizing alternative sources of extraction medium (either dry ice or liquid CO_2) and small amounts of methanol as a modifier. Coupled with EIA kits for subsequent analyses, it allowed fast screening of alachlor, carbofuran, atrazine, benomyl, and 2,4-D fortified in meat matrices. When the meat sample was exhaustively extracted using a commercial SFE system, the level of interferences on the EIA from the coextracted substances increased substantially. An extra filtration of the diluted sample extract using a microfiber membrane filter prior

Table 1. SFE–EIA Results for Dynamic Extraction of Pesticide-Fortified Meat Products

	SFE–EIA		FSIS residue program	
compd	recovery[a] ± SD (%)	MDL[b] (ppb)	LDL[c] (ppb)	residue limit[d] (ppb)
alachlor	118 ± 13	1		20
carbofuran	93 ± 10	3	5	50
atrazine	98 ± 2	1	5	20
benomyl	101 ± 7	5	50	100
2,4-D	140 ± 35	14	200	200

[a] Mean of liver, ground beef, and lard. SD = standard deviation (n = 6). Amount of spiked pesticides was the same as the residue limits listed in d. [b] SFE–EIA method minimum detectable level for meat sample, calculated on the basis of a 5-g sample size, 100–250-fold dilution, and the EIA kit's minimum detectable concentration (in H_2O). [c] Lowest detectable limit of the current appropriate methodology used by FSIS (Brown, 1991). [d] Residue limit (tolerance/action level) for meat products, established by FSIS (Brown, 1991).

to conducting the EIA alleviated this interference problem and yielded accurate results.

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