Supercritical Fluid Extraction of Pesticide Residues from Strawberries†

Karma L. Pearce,* V. Craige Trenerry, and Stephen Were

Australian Government Analytical Laboratories, 338-340 Tapleys Hill Road, Seaton 5023, South Australia, Australia

A supercritical fluid extraction (SFE) method for the determination in strawberries of a number of commonly used organochlorine insecticides, organophosphorus pesticides, and dichloroanilide fungicides is described. Gas chromatography was used to examine the extracts, with both electron-capture detection and either flame photometric or nitrogen phosphorus detection. Confirmation was performed by gas chromatography-mass spectrometry. The analytes were extracted with supercritical carbon dioxide at 4000 psi and 50 °C with a run time of 20 min, which corresponded to an extraction volume of 20−30 mL of carbon dioxide. In developing the method, the operating parameters of temperature, pressure, extraction time, collection solvent, and sample preparation were optimized. Recoveries ranged from 74 to 126% (with the exception of iprodione), and a method precision of ±3−18% was obtained for spiking levels of 0.08−3.7 mg/kg. The method was validated for the following organochlorine insecticides and organophosphorus pesticides: aldrin, dichloran, dieldrin, *p*,*p*′-DDE(TDE), *p*,*p*′-DDD, *p*,*p*′-DDT, α-endosulfan, *β*-endosulfan, endosulfan sulfate, diazinon, dichlorvos, ethion, malathion, methyl parathion, methyl pirimiphos, mevinphos E+Z, and parathion; and the following dichloroanilide fungicides: iprodione, procymidone, and vinclozolin. The SFE procedure was quicker, more environmentally friendly, and more cost effective than the traditional solvent extraction methods.

Keywords: *Supercritical fluid extraction (SFE); residue analysis; organochlorine insecticides; organophosphorus pesticides; dichloroanilide fungicides; strawberries*

INTRODUCTION

The application of supercritical fluid extraction (SFE) to pesticide residue analysis has recently been demonstrated for a number of sample matrices, in particular biological and clinical applications (Hawthorne, 1990), soil (Snyder *et al.*, 1993), grain (King *et al*., 1993; Skopec *et al*., 1993), and food (King and Hopper, 1991). SFE is gradually becoming recognized as a potential alternative to the more traditional extraction and cleanup methods for organic residues in foods (Gurkin *et al.*, 1991; King, 1989; King and Hooper, 1992). Most of the SFE work to date has focused on relatively nonpolar analytes that are more soluble in carbon dioxide. Less research has been done in analyses of fruit and vegetables (Nelson and Abdelmessah, 1992; Myer *et al.*, 1992; Lehotay *et al.*, 1994). SFE promises a number of advantages over traditional solvent-based methods. These advantages include greater potential for automation and minimal use of organic solvents and glassware with significant savings in analysis time.

The SFE method described in this paper has been applied to the extraction of a range of organochlorine (OC) insecticides, organophosphorus (OP) pesticides, and dichloroanilide fungicides required for a routine pesticide screen on strawberries. It was anticipated that the SFE method would lower the limit of reporting for OCs (generally 0.02 mg/kg), OPs (0.1 mg/kg), and fungicides (0.1 mg/kg), improve the coefficient of variation (%CV) for the extraction of each of the pesticides (the solvent extraction method has %CV ranging from 10 to 23%), improve the speed of the analysis, reduce the amount of solvent required for the analysis, and reduce the cost of the analysis.

* Author to whom correspondence should be addressed (fax 61 8 3539800).

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MATERIALS AND METHODS

Supercritical Fluid Extraction. The extraction system used for this work consisted of two Isco model 260D syringe pumps connected to an Isco model SFX-210 supercritical fluid extractor (Lincoln, NE). One pump delivered carbon dioxide and the other pump delivered the solvent modifier. The extractor had two independent extraction chambers. The volume of the vertically orientated extraction vessel was 10 mL, and its dimensions were 6.6 cm × 15 mm i.d. Dynamic flow of the supercritical fluid was controlled by 50-*μ*m i.d. polyimide-coated fused silica restrictors that were 27 cm in length, fitted to the outlets of the extractor, and heated to 75 °C by an Isco restrictor heater. The flow rate varied between 1 and 1.5 mL/min. Sample collection was achieved by inserting the capillary outlets into 15 × 140-mm glass test tubes containing ∼10 mL of acetone.

Reagents. Extrelut was obtained from BDH Chemicals Australia Pty. Ltd., Port Fairy, Australia (catalog no. 11738). Standard material for the oganochlorine insecticides, organophosphorus pesticides, and dichloroanilide fungicides, along with Dibromo DDE and EPN (*O*-ethyl-*O*-4-nitrophenyl phenyl phosphonothioate) that were used as internal standards, were obtained from the Curator of Standards, Australian Government Analytical Laboratories, NSW, Australia. The purity of the solvents used was Chromar HPLC or Nanograde. Carbon dioxide pressurized with helium was purchased from Linde Gas Pty. Ltd., Fairfield NSW, Australia (catalog no. Un 1956), as compressed carbon dioxide pressurized to 12 000 kPa with helium.

Standard Solutions. Individual stock standard solutions were prepared by dissolving the standard material in acetone at concentrations ranging from 100 to 200 *μ*g/mL. Mixed

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working standards were prepared by diluting the stock standards with 35% acetone in hexane to a level of ~0.05−1 μg/mL.

Gas Chromatography. The levels of organochlorine insecticides and dichloroanilide fungicides were determined with a Varian 3400 gas chromatograph fitted with J&W (Folsom, CA) DB1 (100% methyl; 30 m × 0.32 mm × 0.25-μm film; catalog no. 123-1032) and J&W DB17 (50% phenyl/50% methyl; 30 m × 0.32 mm × 0.25-μm film; catalog no. 123-1732) columns operating in the split mode. Hydrogen was used as the carrier gas at a pressure of <10 psi. The injector temperature was maintained at 220 °C. The column oven was temperature programmed from an initial temperature of 170 °C (2-min hold) to 240 °C at a rate of 40 °C/min, then to 280 °C at 10 °C/min. The oven was held at this temperature for 5 min. The compounds were detected with dual electron-capture detectors at 330 °C.

The levels of organophosphorus pesticides were determined using a Varian 3400 gas chromatograph fitted with either a flame photometric- or a thermionic-specific detector. The instrument equipped with a flame photometric detector was fitted with a J&W DB1 (100% methyl; 30 m × 0.32 mm × 0.25-μm film; catalog no. 123-1032) capillary column and was operated in the splitless mode. Helium was used as the carrier gas at a pressure of 10 psi. The injector and detector temperatures were maintained at 240 and 300 °C respectively, and the column oven was programmed from an initial temperature of 55 °C (0.5-min hold) to 160 °C at 30 °C/min, then to 260 °C at 5 °C/min (0.5-min hold), and then to 280 °C at 25 °C/min (1.5-min hold).

The instrument fitted with a nitrogen phosphorus detector (NPD) had a J&W DB1 (100% methyl; 30 m × 0.32 mm × 0.25-μm film, catalog no. 123-1032) column operating in the splitless mode. Helium was used as the carrier gas at a pressure of 10 psi. The injector and detector temperatures were maintained at 280 and 300 °C, respectively. The NPD bead current was 3.10 amps. The column oven was programmed from an initial temperature of 170 °C (2-min hold) to 240 °C at 40 °C/min (0-min hold), then to 280 °C at 10 °C/min, and held at this temperature for 5 min.

Gas Chromatography−Mass Spectrometry. Where necessary, confirmation of residues was achieved by gas chromatography−mass spectrometry (GC-MS). This system consisted of a Hewlett-Packard (HP) 5890 gas chromatograph fitted with an HP5 (5% biphenyl/95% dimethylpolysiloxane; 25 m × 0.2 mm × 0.33-μm film; catalog no. 19091 J102) operating in the splitless mode; which was interfaced to a HP 5971 mass selective detector (MSD) used in the selective-ion-monitoring mode. Helium was used as the carrier gas. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The column oven was programmed from an initial temperature of 70 °C (2-min hold) to a final temperature of 250 °C at 20 °C/min (8-min hold).

Sample Preparation. Samples of strawberries were homogenized in a commercial food processor and mixed with Extrelut (60 + 40) by weight. Spiked samples were prepared by spiking 50-g aliquots of the strawberry puree with standard solutions prior to mixing with Extrelut.

Supercritical Fluid Extraction. One gram of anhydrous magnesium sulfate was placed in the SFE thimble and then filled with an accurately weighed amount of the fruit/Extrelut (60 + 40) mixture. The thimble was placed in the heated extraction chamber and allowed to come to equilibrium for 5 min at 50 °C. The extraction conditions for the supercritical carbon dioxide were a temperature of 50 °C and a pressure of 4000 psi. The dynamic extraction was performed for 20 min, which corresponded to an extraction volume of 20−30 mL of carbon dioxide. The flow rate varied between 1.0 and 1.5 mL/min. The extract was collected in 10 mL of acetone. This volume was later reduced to 5 mL under a stream of nitrogen and then used to determine the OC insecticides and dichloroanilide fungicides. One milliliter of this extract was further diluted to 5 mL and used to determine the OP pesticides. Further dilutions were sometimes necessary depending on the amount of analyte present.

RESULTS AND DISCUSSION

The current procedure used in this laboratory for the extraction for a multiresidue pesticide screen on fruit and vegetables is based on the method described in the book edited by Thier (Thier and Zeumer, 1992). This solvent extraction method involves the use of copious amounts of solvent and glassware and is time consuming. Previous work reported by other laboratories (Nelson and Abdelmessah, 1992; Meyer *et al.*, 1992) as well as our work on the extraction of captan from strawberries by SFE (Pearce *et al.*, 1994) indicated that SFE may be suitable for a multiresidue pesticide screen on strawberries.

The initial work reported was performed with a strawberry puree fruit matrix. Sample preparation required mixing an aliquot of strawberry puree with Extrelut (1+1). An amount of 1 g of anhydrous magnesium sulfate was added to the SFE thimble, which was then filled with the strawberry/Extrelut (1+1) mixture. The level of spiking used for the preliminary investigations ranged from 1.6 to 3.0 mg/kg for the OP pesticides, from 0.8 to 1.1 mg/kg for the OC insecticides, and from 1.7 to 3.7 mg/kg for the fungicides.

Two sets of preliminary extraction conditions were used: the first with a temperature of 50 °C and a pressure of 4000 psi, and the second with a temperature of 60 °C and a pressure of 3500 psi. The run time was lengthened to 60 min to ensure adequate time for all the pesticides to be recovered. The first set of conditions yielded recoveries ranging from 71 to 89% for the OP pesticides, from 74 to 121% for the OC insecticides, and from 98 to 119% for the dichloroanilide fungicides. The second set of operating conditions yielded results ranging from 74 to 85% for the OP pesticides, from 58 to 121% for the OC insecticides, and from 65 to 109% for the dichloroanilide fungicides. Even though the initial work was spiked at a high level, the results were encouraging.

The critical parameters considered in optimizing the extraction conditions for the SFE operation were solvent strength, run time, collection solvent, and sample preparation. These parameters were systematically altered to give optimum recovery of the target analytes (Hawthorne, 1990). The spiking levels used for this work were the same as those used for the initial investigations.

Optimizing the Solvent Strength of the Supercritical Carbon Dioxide. The solvent strength of supercritical carbon dioxide can be increased or decreased by varying temperature and pressure. Supercritical fluids at higher temperatures and pressures have higher solvating powers. Increasing the temperature and pressure as well as the addition of a modifier all enable supercritical carbon dioxide to increase the solubility of polar compounds. As a consequence, there could be a larger proportion of unwanted compounds extracted. It was extremely important to optimize these parameters (Gurkin *et al.*, 1991). Extractions were performed at 50, 55, and 60 °C over a pressure range of 3500 to 6000 psi. Extractions at each pressure/temperature combination were performed in duplicate and averaged. A total of 40 extractions were performed in all. The results are summarized in Figure 1.

Three general trends were observed: similar recoveries were obtained when the extractions were performed at 50, 55, and 60 °C; recoveries performed at a pressure

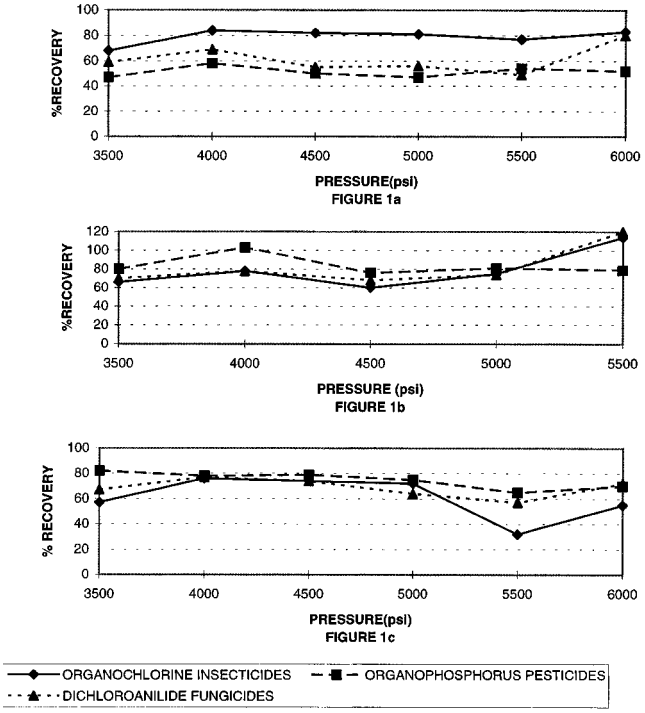

Figure 1. Optimizing the conditions for a pressure gradient of 3500−6000 psi, at temperatures of (a) 50 °C, (b) 55 °C, and (c) 60 °C for the SFE of a number of OC insecticides, OP pesticides, and dichloroanilide fungicides from strawberries. The level of spiking ranged from 0.8 to 1 mg/kg for the OC insecticides, from 1.6 to 3.0 mg/kg for the OP pesticides, and from 1.7 to 3.7 mg/kg for the dichloroanilide fungicides. The results for each group have been averaged.

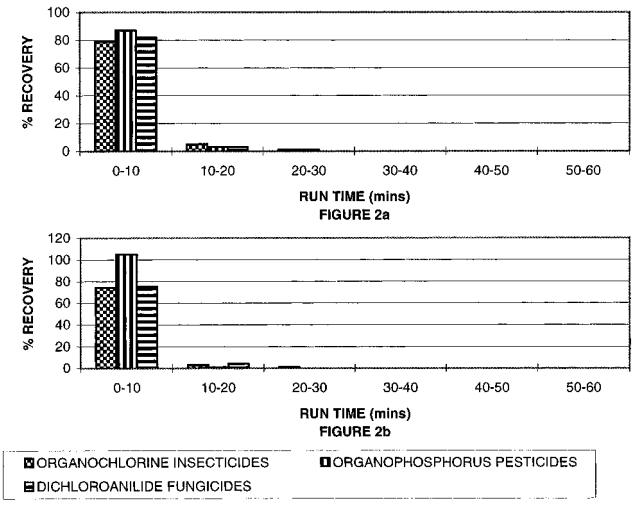

Figure 2. Optimizing the run time for a pressure of 4000 psi and temperatures of (a) 50 °C and (b) 55 °C for the SFE of a number of OC insecticides, OP pesticides, and dichloroanilide fungicides from strawberries. The level of spiking ranged from 0.8 to 1. mg/kg for the OC insecticides, from 1.6 to 3.0 mg/kg for the OP pesticides, and from 1.7 to 3.7 mg/kg for the dichloroanilide fungicides. The results for each group have been averaged.

of 3500 psi were generally lower than those performed at 4000 psi or above; and at pressures of 4500 psi, there were no significant increases in recoveries.

Optimizing the Run Time. The run time was optimized for a pressure of 4000 psi at both 50 and 55 °C. Separate fractions were collected every 10 min for a run time of 60 min/sample. The data are presented in Figure 2. In both experiments, most of pesticides were recovered in the first 10 min. There were no

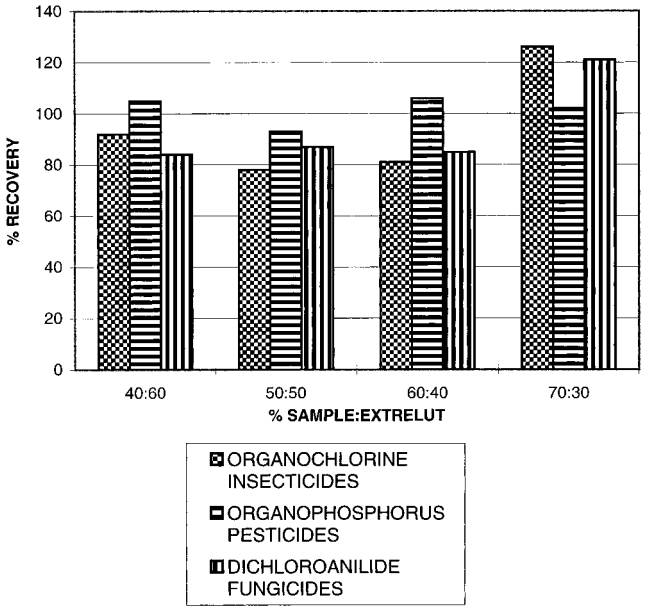

Figure 3. Optimizing the sample preparation of the proportion of sample-to-Extrelut at a pressure of 4000 psi, temperature of 50 °C, and a run time of 20 min for the SFE of a number of OC insecticides, OP pesticides, and dichloroanilide fungicides from strawberries. The level of spiking ranged from 0.8 to 1.0 mg/kg for the OC insecticides, from 1.6 to 3.0 mg/kg for the OP pesticides, and from 1.7 to 3.7 mg/kg for the fungicides. The results for each group have been averaged.

significant differences in the recovery of the analytes of interest at each of the temperatures examined.

A pressure of 4000 psi, temperature of 50 °C and run time of 20 min were considered optimal. Previous experience has shown that with all other parameters constant, lower temperatures should reduce the risk of extracting interfering compounds (Hawthorne, 1990).

Sample Preparation. The sample preparation was optimized by varying the amount of sample mixed with Extrelut. Amounts of 40, 50, 60, and 70% sample-to-Extrelut were tested. There were no significant differences in the recoveries as the relative proportion of sample-to-Extrelut was varied, except for excessively high recoveries for the endosulfan group of pesticides and iprodione when the sample-to-Extrelut ratio was 70%:30%. There were no apparent reasons for these anomalies; therefore, the ratio of sample-to-Extrelut of 70%:30% was avoided. For the extracts derived from 40, 50, and 60 sample-to-Extrelut, the recoveries for the OP pesticides were 103, 92, and 105%, the recoveries for the dichloroanilide fungicides were 85, 88, and 87%, and the recoveries for the OC insecticides, which varied the most, were 91, 78, and 81%. The ratio of 60% sample-to-40% Extrelut was chosen as being optimum because it allowed a higher proportion of sample to be loaded into the SFE cartridge, ultimately permitting a lower limit of reporting (Figure 3).

Collecting Solvent. Acetone, hexane, and 2,2,4-trimethylpentane, with an extraction solvent of supercritical carbon dioxide, and acetone, with an extraction solvent of supercritical carbon dioxide modified with 5% methanol, were examined as collection solvents (Figure 4). The acetone collection solvent produced slightly better recoveries for the target analytes. However, there did not appear to be any advantage in using supercritical carbon dioxide modified with 5% methanol compared with supercritical carbon dioxide alone as the extraction solvent.

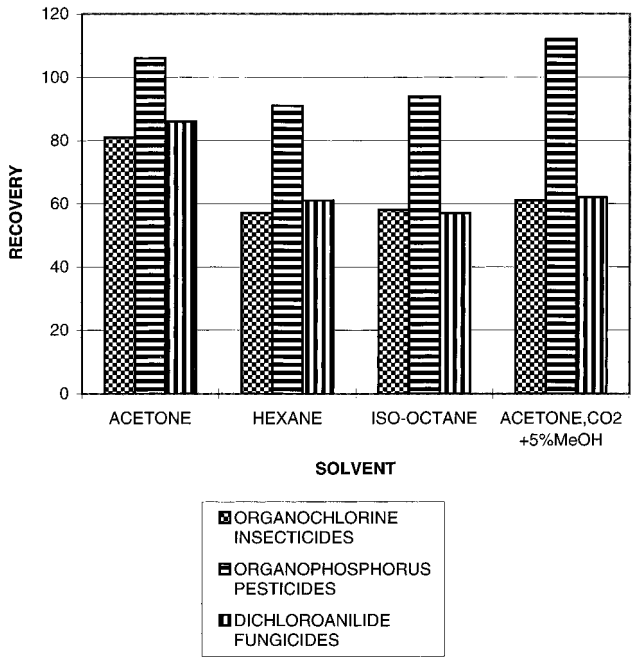

Figure 4. Optimizing the collection solvents of acetone, hexane, and 2,2,4-trimethylpentane with an extraction solvent of supercritical carbon dioxide, and acetone with an extraction solvent of supercritical carbon dioxide modified with 5% methanol for the SFE of a number of OC insecticides, OP pesticides, and dichloroanilide fungicides from strawberries. The level of spiking ranged from 0.8 to 1.0 mg/kg for the OC insecticides, from 1.6 to 3.0 mg/kg for the OP pesticides, and from 1.7 to 3.7 mg/kg for the fungicides. The results for each group have been averaged.

After establishing the optimum conditions, extractions were repeated on strawberry samples that had been spiked at four different levels: level I, maximum residue limit (MRL); level II, half the MRL; level III, limit of reporting (LOR); and level IV, half the LOR. A minimum of eight replicate samples were analyzed at each level. All of the analytes were readily recovered with acceptable relative standard deviations (RSDs). For OC insecticides at a spiking level of 1/2 LOR−1/2 MRL, the average recovery was 74−108%; and the RSD was 4.6−13.7%; at a spiking level of MRL, the average recovery was 96−110%, and the RSD was 3.2−6.6%. For OP pesticides at a spiking level of 1/2 LOR-1/2MRL, the average recovery was 78−115%, and the RSD was 4.5−17.9%; at a spiking level of MRL, the average recovery was 82−117%, and the RSD was 3.4−11.5%. For dichloroanilide fungicides at a spiking level of 1/2 LOR-1/2 MRL, the average recovery was 83−136%, and the RSD was 3.4−13.4%; at a spiking level of MRL, the average recovery was 97−103%, and the RSD was 4.5−5.7%. Iprodione recoveries at 136 and 132%, corresponding to levels of spiking of 0.37 and 1.45 mg/kg, respectively, were higher than anticipated; however, there were no apparent reasons for this anomaly. As expected, spiking at the higher level, near the MRL, resulted in an improvement in the average range of recoveries and the RSDs.

When recoveries spiked at around the MRL were performed by solvent extraction techniques, the OC insecticides were recovered with mean recoveries of 85−98% and RSDs of 10−17%. The OP pesticides had average mean recoveries in the range 78%−100%, corresponding to RSDs of 13−23%. Similarly, the dichloroanilide fungicides were recovered with an average mean recovery of 91−95%, with RSDs of 11−16%.

Table 1. Statistical Evaluation of the SFE and Solvent Extraction Methods[a]

pesticide	parameter (%)	SFE[b]	solvent extraction[c]
OC insecticide	mean recovery	96−110	85−98
	RSD	3−7	10−17
OP pesticide	mean recovery	82−117	78−100
	RSD	3−12	13−23
dichloroanilide fungicide	mean recovery	97−103	91−95
	RSD	5−6	11−16

[a] Analyzed at a level of spiking corresponding to the maximum residue limit (MRL) for a number of OC insecticides, OP pesticides, and dichloroanilide fungicides from strawberries; the results for each group have been averaged. [b] $n = 10$. [c] $n = 250$.

Table 2. Comparison of Extraction Time and Solvent Consumption for Solvent Extraction and SFE Methods[a]

extraction type	manipulative time (h)	elapsed time (h)	total extraction time (h)	solvent required (mL/sample)
SFE	3	2	5	15−16
solvent	5	9	14	530

[a] For a set of seven samples, the results for each group have been averaged.

The mean recovery range for both techniques was similar, whereas the RSDs for SFE were somewhat better. (These data, however, reflect the small number of samples analyzed; i.e., 10 samples for SFE as opposed to 250 samples with solvent extraction techniques.) The results are summarized in Table 1.

The solvent extraction method presently in use has a limit of reporting for the OC insecticides of 0.02 mg/kg, for the OP pesticides of 0.1 mg/kg, and for the dichloroanilide fungicides of 0.1 mg/kg. These reporting levels were also achieved by SFE. Improvements to the LOR could be achieved by concentrating the SFE extract before GC analysis, combining the extracts of two SFE extractions run in parallel, and altering the configuration of the GC to increase sensitivity. The SFE method will be further tested in a collaborative study against our existing solvent extraction method.

To fully evaluate both extraction techniques it was necessary to compare the analysis time and the amount of solvent required for each technique (Table 2). For a set of seven samples and a spiked sample, the manipulation time of sample preparation for the solvent extraction/gel permeation cleanup for the pesticide screen was 5 h. The elapsed time was 9 h, bringing the total analysis time to 14 h. This method used 530 mL of solvent/sample and much more glassware. In contrast, the sample manipulation time for the SFE procedure on the same number of samples was 3 h. The elapsed time incurred an additional 2 h, bringing the total analysis time to 5 h. This SFE method used 15−16 mL of solvent/sample and 30 mL of carbon dioxide, which represents a considerable saving in operator time, solvent usage and minimizes the use of glassware. The SFE method does, however, require a significant investment in capital equipment that impacts on the analysis costs.

Conclusion. SFE offers a quicker, more environmentally friendly, and more cost-effective analysis than classical solvent extraction methods for multiresidue pesticide screening on strawberries. SFE has the potential to reduce the exposure of laboratory personnel to hazardous chemicals and reduces the more tedious operation of sample cleanup with solvent extraction.

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