Selective Extraction of Pesticides from Lipid-Containing Matrixes Using Supercritical Binary Gas Mixtures

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When supercritical carbon dioxide is used for extraction of trace compounds from lipid-rich samples, the resulting extract can contain a substantial amount of lipid coextractive which interferes in the subsequent chromatographic analysis. In this case, a cleanup step, which is time-consuming, is required in order to remove or reduce the lipid content in the extract. In this study, we report on a new approach for the extraction of trace compounds which significantly reduces the quantity of lipid coextractives, thereby eliminating the need for a sample cleanup step. An autoclave high-pressure reactor equipped with a mechanical stirring device was used for mixing gases, such as carbon dioxide and nitrogen, to generate the desired fluid mixtures. The composition of the mixtures could be adjusted by two mass flow controllers prior to the autoclave mixing device. The generated carbon dioxide/nitrogen binary mixtures were then employed to facilitate supercritical fluid extraction (SFE) at high pressures and elevated temperatures. The pressurized CO_2/nitrogen binary fluid mixture demonstrated sufficient solvation power for quantitative recoveries of trace fortified organophosphorus and incurred organochlorine pesticides from poultry fat while significantly reducing lipid solubility in the fluid. This allowed the development of a method that produced an extract with minimal lipid content that could be used directly for gas chromatographic analysis, thereby eliminating the need to cleanup the extract.

Supercritical fluid extraction (SFE) has become an increasingly important technique in recent years for extraction of trace compounds from food and agricultural products.[1−3] Because of its low critical temperature and pressure and relative nontoxic nature, carbon dioxide (CO_2) remains the fluid of choice for SFE. When SFE is used for analysis of lipid-containing samples, large quantities of lipid coextractives can often accompany the target analytes of interest due to the substantially high solubility of lipids in supercritical carbon dioxide (SC-CO_2). Because of their high boiling points and molecular weights, many lipid species are difficult to elute under conventional gas chromatographic (GC) conditions. Hence, the lipid moieties tend to accumulate in a GC injector port, resulting in highly irregular chromatographic profiles. Various approaches have been developed to minimize the interference of these lipid coextractives in GC assays. These have included off-line cleanup via size-exclusion chromatography (SEC), alumina, and other sorbents. Using SFE, cleanup has been effected by packing the extraction cell with sorbents such as alumina, silica, or Florisil.[4−6] More sophisticated cleanup systems, such as an integrated SFE−SFC−GC system, have also been reported.[7] Other methods, such as preextraction with acetonitrile, followed by cleanup via SFE,[8] as well as the use of solid-phase extraction columns after SFE, have also found application.[9] However, all of the above extract cleanup techniques could be avoided if the SFE step could be made more selective for the target analyte.

Gahrs has reported that the solubility of caffeine in SC-CO_2 can be reduced by 50% when 5% of nitrogen is mixed with CO_2.[10] Such a result has been rationalized according to the rules suggested by Brunner[11] governing the effect of "cosolvent" fluids on suppressing or increasing solute solubility in supercritical fluid mixtures. Additional evidence for the effect of a fluid cosolvent (having a critical temperature less than carbon dioxide) is provided by studies on the effect of helium on the solvation power of SC-CO_2, which can be reduced quite significantly if the carbon dioxide is mixed with gases such as helium and nitrogen in delivery cylinders.[12−14] Recently, while studying the solvation power of the SC-CO_2 generated from a helium-headspace CO_2 cylinder, we

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(1) Lee, M. L.; Markides, K. E. *Analytical Supercritical Fluid Chromatography and Extraction*; Chromatography Conferences: Provo, UT 1990; Chapters 5 and 7.

(2) Hopper, M. L.; King, J. W. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 661−665.

(3) King, J. W. *J. Assoc. Off. Anal. Chem.* **1993**, *76*, 857−862.

(4) France, J. E.; King, J. W.; Snyder, J. M. *J. Agric. Food Chem.* **1991**, *39*, 1871−1874.

(5) Murugaverl, B.; Voorheers, K. J. *J. Microcolumn Sep.* **1991**, *3*, 11−15.

(6) Maxwell, R. J.; Lightfield, A. R.; Stolker, A. A. M. *J. High Resolut. Chromatogr.* **1995**, *18*, 231−234.

(7) Nam, K. S.; King, J. W. *J. High Resolut. Chromatogr.* **1994**, *17*, 577−582.

(8) Argauer, R. J.; Eller, K. I.; Ibrahim, M. A.; Brown, R. T. *J. Agric. Food Chem.* **1995**, *43*, 2774−2778.

(9) Pensabene, J. W.; Fiddler, W.; Maxwell, R. J.; Lightfield, A. R.; Hampson, J. W. *J. Assoc. Off. Anal. Chem. Int.* **1995**, *78*, 744−748.

(10) Gahrs, H. J. *Ber. Bunsen-Ges. Phys. Chem.* **1984**, *88*, 894−897.

(11) Brunner, G. In *Ion Exchange and Solvent Extraction;* Marinsky, J. A., Marcus, Y., Eds.; Marcel Dekker, Inc.: New York, 1988; Vol. 10, pp 132−134.

(12) Gorner, T.; Dellacherie, J.; Perrut, M. *J. Chromatogr.* **1990**, *514*, 309−316.

(13) Raynie, D. E.; Delaney, T. E. *J. Chromatogr. Sci.* **1994**, *32*, 298−300.

(14) King, J. W.; Johnson, J. H.; Eller, F. J. *Anal. Chem.* **1995**, *67*, 2288−2291.

(15) Zhang, Z.; King, J. W. *J. Chromatogr. Sci.* **1997**, *35*, 483−488.

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found that, as the helium content in the fluid increased, the solubility of soybean oil in the fluid decreased significantly.[15] Noting these trends, we deduced that mixing helium or nitrogen with CO_2 could potentially reduce the lipid solubility during SFE, thereby suggesting a potential route to the selective extraction of trace compounds, devoid of lipid interferences.

To illustrate how this can be achieved, we can invoke the solubility parameter concept, which can be used as a measure the solvation power of the supercritical fluid. According to Giddings et al., the solubility parameter of a supercritical fluid is calculated by eq 1 as[16]

$$\delta = 1.25 P_c^{1/2} \frac{\rho_r}{\rho_r(\mathrm{liq})} \tag{1}$$

where P_c is the critical pressure of the supercritical fluid, ρ_r is the reduced density of the fluid, and $\rho_r(\mathrm{liq})$ is assumed to be 2.66, a typical reduced density of the fluid in the liquid state. When the solubility parameter of the fluid is calculated as a function of the applied pressure on the system, it will initially increase with pressure, as shown in Figure 1, but to a significantly different degree depending on the identity of the fluid. For example, the increase in the solubility parameter for CO_2 below 4000 psi is quite large, while a more gradually change in δ occurs in the case of nitrogen or helium. Therefore, if mixtures of two gases are used, i.e., CO_2 and N_2, the fluid having a lower critical temperature (N_2) will moderate the increase in the solubility parameter with pressure of the binary mixture. This is particularly significant in the lower pressure regime, where the moderating fluids such as helium or nitrogen have only gradual, approximately linear changes of δ with pressure.

The solubility of a solute in a supercritical fluid can be calculated by knowing the solubility parameters of both the dissolved solute and fluid by the Hildebrand–Scatchard equation,[17]

$$\ln x_s = \frac{\Delta H_f}{R}\left(\frac{1}{T_f} - \frac{1}{T}\right) - \frac{V_s \phi^2}{RT}(\delta - \delta_s)^2 \tag{2}$$

where x_s is the solute molar fraction, ΔH_f is the fusion energy of the solute and T_f the solute melting point, R is the gas constant and T the temperature, V_s is the molar volume of the solute, ϕ is the volume fraction of the solvent, and δ and δ_s are the solubility parameters of solvent and solute, respectively. Hence, eq 2 can be used to estimate an analyte's solubility in the supercritical fluid at a given temperature and pressure, since the solubility parameter of the supercritical fluid can be obtained from eq 1.

An example of this calculation is shown in Figure 2, where the molar fraction solubility of naphthalene in CO_2 is plotted against the solublity parameter of CO_2 at 60 °C. Therefore, to dissolve a ppm level of naphthalene at 60 °C, Figure 2 suggests that the solubility parameter of CO_2 should be about 1.5 $(\mathrm{cal/cm^3})^{1/2}$, which translates into an extraction pressure of less than 1500 psi at 60 °C. Such a condition indicates that SFE can be used for extraction of trace compounds from sample matrixes at conditions where the solubility parameter of the extracting fluid is quite low, i.e., under conditions where the solubility parameter of the fluid does not correspond to the value required for maximizing the solubility of the solute (analyte) in the extraction fluid (i.e., conditions where the solubility parameters of the solute and solvent are equal). These experimental conditions are more than sufficient to provide the required solubility for dissolving trace compounds. However, it should be recognized that, for the SFE of some sample matrixes, a stronger fluid solvation power will be required in order to overcome analyte–matrix interactions.

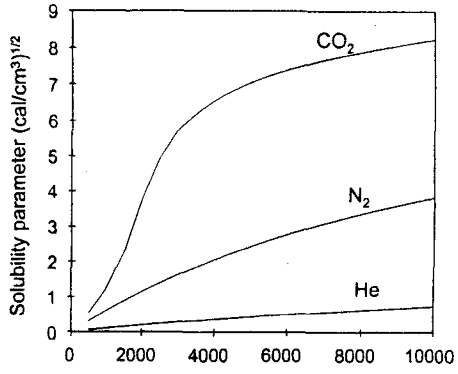

Figure 1. Calculated solubility parameters vs fluid pressure for carbon dioxide, nitrogen, and helium at 70 °C.

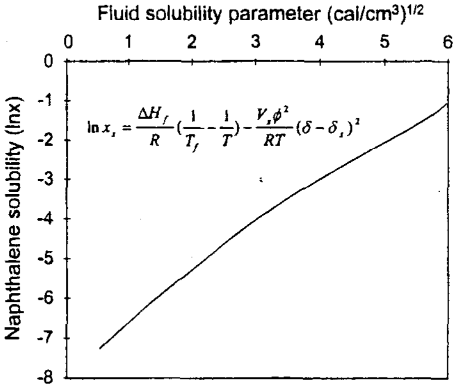

Figure 2. Molar fraction solubility of naphthalene in CO_2 at 60 °C vs the solubility parameter of CO_2.

As noted previously, in our prior studies of helium-headspace CO_2, the solubility of triglyceride-based oils at a given temperature is determined by the solubility parameter of the supercritical fluid. Hence, the larger the solubility parameter of the fluid, the larger the amount of lipid material that can be dissolved in the extracting fluid. A reduction in the solvating power of SC-CO_2 can be affected by changing either or both its temperature and pressure. Although higher extraction temperatures will reduce the magnitude of the δ as a function of pressure, leading to a more linear-like dependence of δ with pressure, it also inceases the vapor pressure of the solutes (analytes), leading to higher solute solubilities in pressurized fluid. Therefore, this is not always an experimental parameter adjustment that will lead to a more discriminant extraction.

Lowering the extraction pressures can also reduce the solvating power of the fluid, but again this does not necessarily lead to selective extraction of one type of solute over another. The use

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(16) Giddings, J. C.; Myers, M. N.; McLaren, L.; Keller, R. A. *Science* **1968**, *162*, 67–73.

(17) Hildebrand, J. H.; Scott, R. L. *The Solubility of Nonelectrolytes*, 3rd ed.; Dover Publications: New York, 1964.

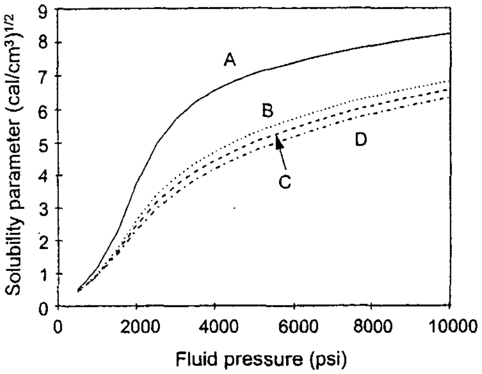

Figure 3. Solubility parameters of CO_2 and CO_2/nitrogen gas mixtures (in mol %) at 70 °C and different pressures: (A) pure CO_2; (B) 75% CO_2 and 25% N_2; (C) 70% CO_2 and 30% N_2; (D) 65% CO_2 and 25% N_2.

of the "threshold pressure" concept,[18] in which one uses selected pressures consistent with minimal solute solubility in the fluid phase, to selectively extract one solute with respect to another is difficult to affect, since many solutes have similar threshold pressures.[19] These problems are compounded in the case of CO_2 as the extraction fluid, since its density and, hence, solubility parameter increase exponentially with pressure in the lower pressure region.

One way of overcoming these problems is to use binary gas mixtures as extraction fluids for the reasons stated previously. In this case, the solvation power of the major extraction fluid (CO_2) can be moderated by introducing a fluid having weaker solvation properties then CO_2. This principle is illustrated in Figure 3, where the reduction in solvent strength relative to that of neat CO_2 (curve A) is shown for mixtures of nitrogen with carbon dioxide versus pressure at 70 °C. Clearly, this shows that the solubility parameter of composite supercritical fluid can be lowered by mixing CO_2 with an inert gas, such as nitrogen. Curves B, C, and D in Figure 3 show the solubility parameters of CO_2/nitrogen binary gas mixtures containing 75, 70, and 65 mol % of CO_2, respectively, as a function of pressure. The recorded reduction in the solubility parameter is quite significant. Further adjustment of the binary mixture's solubility parameter can be made by adjusting the extraction temperature and pressure in order to fine-tune the SFE for optimal results. In this study, we have made use of the above principle to selectively extract trace levels of pesticides from several lipid-laden mixtures, thereby minimizing the extraction of unwanted components (lipids, fat) in the resultant extract.

EXPERIMENTAL SECTION

Note: Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Reagents and Samples. Individual organochlorine and organophosphorus pesticides were purchased from Chem Service Inc. (West Chester, PA). Stock solutions of organochlorine pesticides were made up in isooctane, while organophosphorus pesticides were constituted in acetone, both at the 100 μg/mL level in their respective solvents. Standard solutions for the calibration of the GC/electron capture detector (ECD) were prepared by serial dilution from these two stock solutions. Chicken adipose tissue containing incurred organochlorine pesticides[20] was used for one set of extraction experiments. Chicken fat fortified with organophosphorus pesticides was prepared by spiking known amounts of pesticides into a known weight of the rendered fat. Neutral alumina, Brockman activity 1 (Fisher Scientific, Pittsburgh, PA), was used for the alumina cleanup method. It was heated at 800 °C for 4 h and cooled to room temperature, and then its surface activity was moderated by mixing with 5% of distilled water by weight.

Several premixed CO_2/nitrogen gas cylinders of known composition were provided by Scott Specialty Gases (Troy, MI). The error of gas composition is within 1% of the stated value. High-purity nitrogen was from R & R Welding Supply Co. (East Peoria, IL). SFE-grade CO_2 (without diptube and helium headspace) was purchased from Scott Speciality Gases.

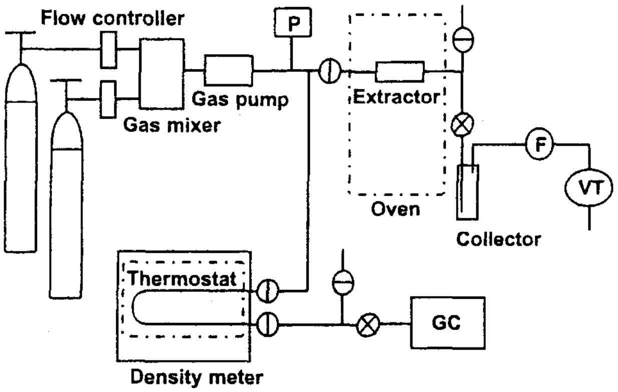

Figure 4. Schematic of the binary gas mixing device and extraction apparatus.

Apparatus. Figure 4 illustrates the apparatus used for generating the CO_2/nitrogen binary gas mixtures and includes the system for conducting the SFEs and measuring both the density and gas composition of the fluid phase. An autoclave-type pressure reactor (item no. 4501, Parr Instruments Co., Moline, IL) of 1-L capacity, equipped with a mechanical stirring device, was used to mix the CO_2 and N_2 streams. The composition of the binary gas mixtures was adjusted by utilizing two mass flow controllers (part no. 5236A1V-5K, Scott Specialty Gases, Plumsteadville, PA). The mechanically mixed CO_2/nitrogen gas mixtures were pressurized by a Haskel gas booster pump (model no. AGT62-152, Haskel, Inc., Burbank, CA). The fluid was then delivered to a Spe-ed SFE unit (Applied Separations, Inc., Allentown, PA), which was used without the provided pump, for the SFE experiments. The composition, density, and homogeneity of the CO_2/nitrogen mixtures were monitored by a GC equipped with a thermal conductivity detector (model 69-400-TCD-1, GOW-MAC, Lehigh Valley, PA) as well as a high-precision density meter equipped with a high-pressure measuring cell (model DMA 48

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[18] McHugh, M. A.; Krukonis, V. J. *Supercritical Fluid Extraction—Principles and Practice*, 2nd ed.; Butterworth-Heinemann: Boston, MA, 1993; pp 367–370.

[19] King, J. W. *J. Chromatogr. Sci.* **1989**, *27*, 355–364.

[20] Snyder, J. M.; King, J. W.; Rowe, L. D.; Woerner, J. A. *J. Assoc. Off. Anal. Chem.* **1993**, *76*, 888–892.

and 512P, respectively, Anton Parr, Graz, Austria). The relative standard deviation (RSD) of the gas mixtures generated by the gas mixer was about 2–3%. Gas flow rates were measured under ambient conditions using a dry test meter (model DTM 200A, American Meter Co., Philadelphia, PA), designated VT in Figure 4.

The GC used for the analysis of pesticides in this study was a model HP 5890 (Hewlett Packard Co, Wilmington, DE) equipped with an ECD. The GC's injector system was modified specifically for injection of lipid-containing extracts using the procedure described by Hopper.[21]

Extraction and Analysis. For the SFE experiments, about 0.2 g of poultry fat containing either the organochlorine or organophosphorus pesticides was packed inside a 10-mL extraction cell along with glass wool plugs. The extraction was carried out at selected pressures and temperatures under using a continuous flow of CO_2. The extraction flow rate was regulated by the micrometering valve on the Spe-ed unit. Typically, extraction flow rates were set at approximately 0.5 L/min (expanded gas), with extraction times running about 60 min. The amount of fat that was coextracted was determined by gravimetry. The collection solvent was isooctane for the organochlorine pesticides and acetone for the organophosphorus pesticides. The collection was carried out by letting depressurized gases bubble through a selected solvent (15 mL) placed in a collection tube (25 mL capacity).

In the GC/ECD analysis, aldrin was used as an internal standard for quantitation of the organochlorine pesticides. For organophosphorus pesticide analysis, quantitation was performed using external calibration. The conventional alumina cleanup method used in the pesticide residue analysis was that described in the procedures of the Food Safety and Inspection Service, Method No. 5.002.[22]

RESULTS AND DISCUSSION

Initial experiments were designed to find the appropriate composition of a CO_2/nitrogen binary gas mixture which would allow two goals to be achieved simultaneously. One objective was to reduce the amount of lipid coextractives to an acceptable level in order to avoid the need for a sample cleanup step prior to GC analysis. Second, conditions needed to be optimized so as to yield quantitative recovery of the target analytes. In these early experiments, the described chicken fat samples containing incurred organochlorine pesticides were utilized. Initially, extractions were performed using the binary gas mixtures delivered from cylinders containing premixed compositions of CO_2 and nitrogen. These cylinders had the following compositions (in molar percentage of CO_2): 95%, 75%, 50%, and 20%, respectively. After a series of experiments, it was found that none of the four had the right composition to achieve our goal of quantitative extraction of the target pesticides, devoid of lipid coextractives. More than 50 mg of lipid was extracted with the cylinders containing 95 and 75 mol % CO_2 at extraction temperatures between 60 and 90 °C and extraction pressures between 5000 and 10 000 psi.

Since the lipid solubility in the fluid at a given temperature increases with pressure and CO_2 content of the fluid, it became apparent that it was not necessary to try every combination of experimental conditions coupled with a change in the extracting fluid composition. For example, the level of lipid coextractives was about 60 mg at 60 °C and 5000 psi, using a mixture consisting of 75 mol % CO_2/25 mol % N_2. Therefore, it was apparent that more lipid matter would be extracted at pressures greater than 5000 psi and/or at CO_2 compositions higher than 75 mol % at 60 °C.

To confirm the above conclusions, additional SFEs were run at the above conditions. Because the effect of temperature on lipid solubility is more ambiguous, four different temperatures, 60, 70, 80, and 90 °C, were utilized. For gas mixtures containing 50 and 20 mol % of CO_2, little or no fat was extracted. However, the recoveries for the organochlorine pesticides from chicken fat were only 30% or lower at the temperatures and pressures noted above (60–90 °C and 5000–10 000 psi). Since the level of lipid coextractives was low under these conditions, only the temperature and pressure that produced the maximum recovery of pesticides were investigated.

For example, since the recovery of pesticides was approximately 30% at 70 °C and 10 000 psi using a 50 mol % CO_2 mixture, it followed that analyte recoveries at 70 °C would probably be lower when the extraction pressure was less than 10 000 psi using a fluid mixture containing less than 50 mol % CO_2. Again, due to the ambiguous effect of temperature, SFEs at four different extraction temperatures (60, 70, 80, and 90 °C) under the above conditions were examined. Unfortunately, these experiments did not succeed in achieving the selective extraction of the target pesticides as desired; however, these results further suggested that there was an optimal CO_2/nitrogen composition between 50 and 75 mol % CO_2 that could yield the desired result. Since this would require the generation of a number of different binary gas compositions, it became apparent that construction of a binary fluid mixing system would be highly desirous. The system described in the Experimental Section proved very facile for this purpose and allowed any binary fluid composition to be generated in a highly reproducible manner, as judged by the gas chromatographic and high-pressure density measurements.

Using the above apparatus, gas mixtures having molar percentages of CO_2 of 63, 67, 70, and 73% were generated. Extractions were then performed over the range of temperatures 60–90 °C and at pressures between 5000 and 10 000 psi. The systematic search for the optimal composition of the gas mixture was simplified on the basis of the initial extractions. For example, at a CO_2 composition of 63 mol %, the pesticides recoveries tended to be below 70% at 10 000 psi at all four temperatures (60, 70, 80, and 90 °C) that were utilized. Consequently, extraction at other pressures was not tried since it was unlikely that the pesticide recoveries would improve. Similarly, due to the high lipid content in the extract at 5000 or 6000 psi, at all four of the above temperatures, additional extractions at higher pressures were not performed (at a CO_2 level of 73 mol %). It was eventually found that a SFE carried out at 60 °C and 8000 psi, using a fluid composition of 70 mol % CO_2/30 mol % N_2, yielded the best result in terms of reducing the level of lipid coextractives and yielding quantitative recoveries of the incurred organochlorine pesticides.

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(21) Hopper, M. L. *J. High Resolut. Chromatogr.* **1987**, *10*, 620–622.

(22) *Revised Basic Chemistry Laboratory Guidebook*; Section 5.002, Micro Alumina Screening Analysis for Chlorinated Hydrocarbons and PCB Residues in Fatty Foods; U.S. Department of Agriculture, Food Safety and Inspection Service: Washington, DC, June 1987; pp 5–9.

Table 1. Concentration of Incurred Organochlorine Pesticides in Chicken Fat, Obtained Utilizing Extraction with a Binary Gas Mixture (70 mol % CO_2 and 30 mol % Nitrogen) at 60 °C and 8000 psi Versus Cleanup with Alumina[a]

pesticide	concentration ($\mu g/g$)[a] alumina cleanup	binary gas
heptachlor epoxide	1.13 ± 0.10	0.86 ± 0.15
dieldrin	3.08 ± 0.15	3.07 ± 0.15
endrin	2.75 ± 0.05	2.91 ± 0.15

[a] Standard deviations are based on three replicates.

It should be emphasized that most of the currently used cleanup procedures do not reduce the level of lipid contaminants to zero. However, what is of importance is to achieve a reduction in the level of lipid content in the resultant extract that will no longer interfere in the final analysis method (GC/ECD in our case) and will allow repeated analyses to be preformed without any adverse effect. For example, Hopper[21] performed systematic studies on the effect of co-injected lipid matter on the performance of GC analysis of pesticides. He concluded that the performance of GC assay did not suffer as long as the lipid concentration in the injected solution was at a 10 mg/mL level or below. A similar criterion was used in this study. For example, when a sample size of 2 mL was used, the extract was considered ready for GC analysis if the amount of lipid coextractives were less than 20 mg. At 60 °C and 8000 psi, using a fluid composition of 70 mol % CO_2 and 30 mol % nitrogen, the amount of lipid coextractives was found to be about 12 mg, while the recoveries of organochlorine pesticides were 80% or higher.

To verify that the binary fluid extraction was capable of yielding quantitative extraction, parallel experiments were carried out to determine the concentration of incurred organochlorine pesticides in chicken fat by binary gas extraction and via the traditional alumina cleanup method mentioned in the Experimental Section. Table 1 summarizes these results for three extraction replicates for each cleanup method. Although it would appear, in Table 1, that the concentration of heptachlor epoxide is low in the case of binary fluid extraction, statistical evaluation revealed that, at a 95% confidence level, all of the concentrations for the three incurred organochlorine pesticides determined using the two different cleanup methods were statistically equivalent. Further, Figure 5 compares the GC/ECD chromatograms of the extracted organochlorine pesticides from the adipose tissue extract using either binary fluid gas extraction or alumina column cleanup. The resultant two chromatographic profiles are almost identical except for a few small, extra peaks in the beginning of the chromatogram, representing the extract from the binary gas extraction. We believe this is due to the presence of trace contaminants in one of the fluids.

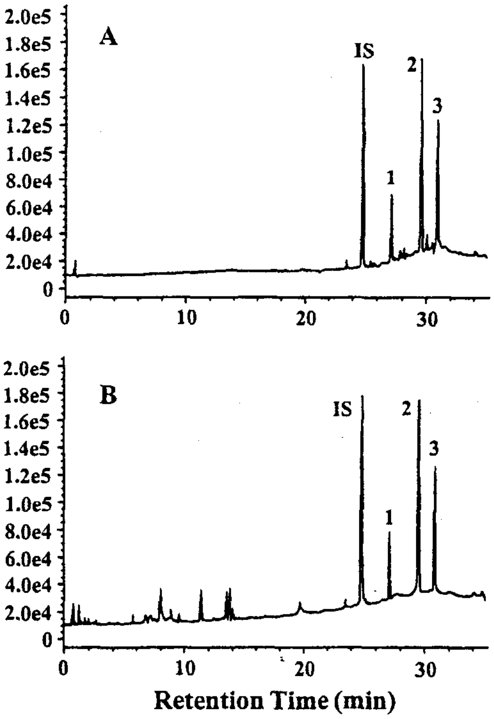

Figure 5. Chromatograms of organochlorine pesticides extracted from chicken fat: (a) cleanup using alumina, (b) by binary gas extraction (70 mol % CO_2 and 30 mol % nitrogen) at 60 °C and 8000 psi. IS was internal standard aldrin; 1, heptachlor epoxide; 2, dieldrin; and 3, endrin.

Table 2. Recoveries of Spiked Organophosphorus Pesticides in Chicken Fat Extracted by a Binary Gas Mixture (70 mol % CO_2 and 30 mol % Nitrogen) at 80 °C and 8000 psi[a]

pesticides	spiked concn ($\mu g/g$)	extracted concn ($\mu g/g$)	recovery (%)
methyl parathion	1.1	0.88 ± 0.05	80
fenitrothion	1.1	1.16 ± 0.09	105
chlorpyrifos	1.1	1.16 ± 0.09	105
ethion	1.1	1.15 ± 0.05	105
carbophenothion	1.1	1.09 ± 0.03	99

[a] Standard deviations are based on three replicates.

To illustrate the general applicability of binary fluid extraction, the technique was applied for the selective extraction of organophosphorus pesticides from a fat-containing matrix. Once again, chicken adipose tissue was used and fortified with five organophosphorus pesticides before commencing with extraction using a binary carbon dioxide/nitrogen mixture. Because organophosphorus pesticides are more polar than the previously extracted organochlorine moieties, initial extraction conditions were attempted at slightly higher pressures and CO_2 content in the binary fluid. However, extractions conducted at these adjusted conditions showed that more lipid matter was extracted. Consequently, extraction conditions were adjusted by using a 70% CO_2/30% N_2 mixture at 8000 psi and a temperature 20 °C higher (80 °C) than that used in the extraction of the organochlorine pesticides. This yielded quantitative recovery of the organophosphorus pesticides and an even lower level of lipid coextractives than in the case of the organochlorine pesticide extraction, approximately 5 mg.

Table 2 lists the recoveries of organophosphorus pesticides from the chicken fat matrix. At the 1.1 $\mu g/g$ spiking level, recoveries ranged from 80 to 105%, with RSDs ranging from 2.8 to 5.7%. Figure 6 shows a typical chromatogram of the organophosphorus pesticides extracted using the binary gas mixture. Note that the resultant chromatographic profile is sufficiently clear of any interferences. It should be acknowledged that the ECD

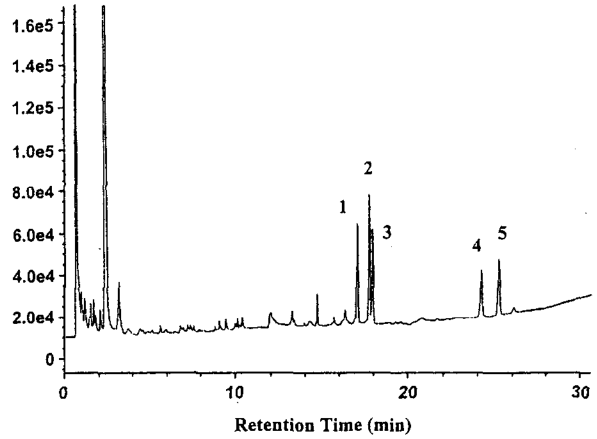

Figure 6. Chromatogram of organophosphorus pesticides extracted by a binary gas mixture (70 mol % CO_2 and 30 mol % nitrogen) at 80 °C and 8000 psi; 1, methyl parathion; 2, fenitrothion; 3, chlorpyrifos; 4, ethion; and 5, carbophenothion.

detector is not the most the sensitive heteroelement detector for the analysis of organophosphorus pesticides, but the results given in Figure 2 show that a reasonable level of detection is feasible.

It should be noted that the GC performance in terms of baseline stability, resolution, and retention time did not deteriorate after more than 50 sample injections of samples containing lipid concentration levels between 3 and 8 mg/mL, indicating that the lipid coextractives from the binary gas extraction did not interfere with the GC analysis. Therefore, it would appear that, at this level of lipid contamination, cleanup of lipid from the extract was not needed.

These results clearly demonstrated that, by mixing nitrogen with CO_2 to reduce the solvation power of CO_2, trace levels of analytes can be selectively extracted from lipid-rich samples with very little lipid coextraction. This new approach completely eliminates the need for cleanup of the resultant extract, thus saving time and reducing the potential loss of analytes in the cleanup step. It should also be noted that, once a particular composition of CO_2 and nitrogen has been demonstrated to be effective in yielding the desired selective extraction, then a premixed gas cylinder of equivalent composition can be designated and purchased for continuous use. It would also appear from the results presented here that the extraction temperature and pressure can then be fine-tuned for optimizing the recovery of the desired trace level components in the sample matrix.

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