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Extraction and Enrichment of Pesticides for Analysis using Binary Supercritical Fluid Mixtures

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Abstract: To improve the extraction selectivity in analytical supercritical fluid extraction (SFE), binary mixtures of supercritical fluids have been used to selectively produce lipid-free extracts of trace pesticide residues for chromatographic analysis. Mixtures of nitrogen and HC-134a have been used in conjunction with carbon dioxide to selectively extract both fortified and incurred residues from a variety of fatty foods using both experimental and commercially-available extraction instrumentation. Extraction of fortified pesticides in butter fat showed high recoveries for both organo-chlorine and -phosphorus pesticides with recoveries declining for specific pesticide moieties at the 1 ppb spiking level for CO_2/N_2 mixtures. For CO_2 and HC-134a mixtures, there was poor and little improvement in analyte recovery relative to using neat SC-CO_2 for fortified organochlorine pesticides in butter fat at the 0.5–5.0 ppm level. However using a CO_2/HC-134a mixture for organophosphorus pesticides in butter fat improved recoveries (>90%) of the analytes at the 1–3.5 ppm level relative to using neat SC-CO_2. Excellent recoveries were obtained for incurred organochlorine

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and -phosphorus pesticides from a variety of food products at ppb levels using either CO_2/N_2 or CO_2/HC-134 mixtures. Results from these and additional experiments suggest that binary fluid mixtures can significantly reduce the need for additional sample cleanup prior to chromatographic analysis, allowing in some cases, direct injection of the extract after dilution into the gas chromatograph.

Keywords: Analysis, extraction, enrichment, pesticides, supercritical fluids

INTRODUCTION

Traditionally the extraction and cleanup of samples for pesticide residue analysis has required extensive sample preparation. This time and labor consuming process is partially due to the presence of unwanted co-extractives in the initial extraction step. When using supercritical fluid extraction (SFE) on food matrices containing lipid material, supercritical carbon dioxide (SC-CO_2) has the propensity to extract an excessive amount of lipid matter in addition to the target analytes (pesticides). A variety of methods have been used to reduce the amount of lipid co-extractives when utilizing SFE, including selective density-based extractions (1), introduction of adsorbents into the extraction cell to prevent the co-extraction of lipid moieties (2, 3), and choice of the extraction agent (4–6).

In 1995, King, et al. (7) demonstrated that the addition of a fluid having a critical temperature lower than CO_2's, i.e. helium, into SC-CO_2, could substantially reduce the amount of lipid extracted at identical extraction pressures and temperatures. This verified previous observations that "inert" fluids could affect the solvent power of the fluid, as well as the solubility and/or mass transfer of solutes in these critical fluid mixtures. Zhang and King (8, 9) extended these studies for analytical purposes by using CO_2/N_2 mixtures for the selective extraction of pesticides from lipid-containing matrices, showing high recoveries of organo-chlorine or -phosphorus pesticides with minimal lipid carryover at 60–80°C and 8000 psi (55.2 MPa) and 30 mole% of N_2 in SC-CO_2.

The addition of a fluid having a critical temperature lower than CO_2's changes the critical properties (10) relative to those of the neat reference fluid, i.e. SC-CO_2. For supercritical fluid mixtures consisting of CO_2/He and CO_2/N_2, a substantial decrease also occurs in the solvent power, relative to that recorded in the neat fluid having the higher critical temperature (T_c) with increasing pressure. Such trends are also known when the temperature is increased for a neat supercritical fluid at a constant pressure, although the decrease may be less dramatic for binary fluid mixtures. However, these trends only partially explain the reduction in solute solubilities in such supercritical fluid mixtures since the fluid with the lower T_c, disrupts the solvation shell around the solute (analyte) resulting in a differential solubility effect that permits greater discrimination between solutes during SFE (11).

In this study, the above principle has been utilized for the selective extraction and enrichment of trace levels of pesticides from fatty food matrices using supercritical carbon dioxide (SC-CO_2) and other compressed fluids to eliminate the use of organic solvents for extraction of the sample. Here the emphasis has been placed on testing the above concept for trace residue analysis on complex food samples, both with experimental and commercial SFE instrumentation. In addition to N_2, the fluorocarbon HC-134a, 1,1,1,2–tetrafluoroethane, has been used in conjunction with SC-CO_2 to test the selectivity of these binary mixtures for yielding low lipid level extracts and high pesticide recoveries, suitable for direct injection after dilution, into a gas chromatograph. Comparisons have been made between the method employing binary fluids and established standard methods on butter spiked with organo-chlorine and -phosphorus pesticides as well as commercial food samples containing incurred residues.

EXPERIMENTAL

Two different experimental approaches were used to demonstrate the attributes of using the binary fluid approach using SFE. Experiments with CO_2/N_2 mixtures were conducted at the USDA laboratory in Peoria, IL, USA using a home-built apparatus to generate the desired extraction conditions. As illustrated in Fig. 1, a flow controller (Brooks Instrument, Hatfield, PA,

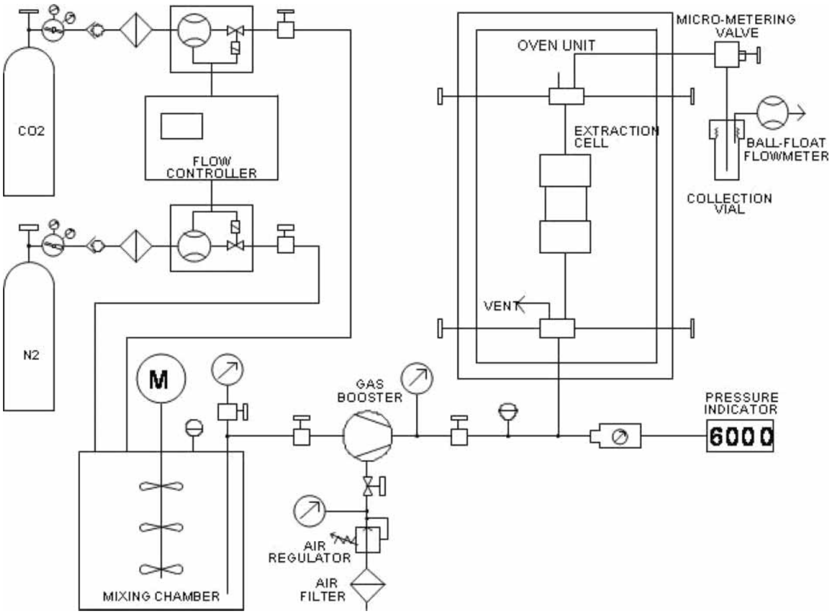

Figure 1. Experimental apparatus for binary fluid CO_2/N_2 extractions.

USA) which could regulate the composition of the resultant gas phase was placed in line with two Brooks mass flow controllers (Series 5850) for CO_2 and N_2, respectively. The resultant mixture from the flow controller module was then fed into a high pressure autoclave (1 Liter, Model 4500, Parr Instruments, Moline, IL) equipped with a stirrer before being directed to a gas booster pump (Model AGT 62/152, Haskel, Burbank, CA) which pressurized the binary fluid mixture to the desired extraction pressure. The output from the booster pump is directed into the extraction cell containing the food sample, held in thermostatted oven of a Spe-ed extractor system (Applied Separations, Allentown, PA). Collection of trace pesticide residues is accomplished in a vial after the fluid is depressurized. A detailed description of the apparatus and its operation is available in the literature (9). The extraction cell (10 mL) contained five gram samples. It was found based on the above considerations, that a 70% CO_2/30% N_2 mixture at 6000 psi (41.4 MPa) and 60°C was optimal for analyte recovery and minimizing the coextraction of lipid material. Total extraction times were 60 min in length and the flow rate of extraction fluid mixture was 0.5 L/min as measured with a volumetric test meter under ambient conditions of pressure and temperature (~60 mL of compressed fluid). The residual lipid content of resultant extracts was below 10 mg under these extraction conditions for the pesticide recoveries reported in Tables 1, 2, and 5.

Extractions using CO_2/HC-134a mixtures were conducted at the FDA's Total Diet and Pesticide Research Center in Lenexa, KS laboratory, using an Isco Model SFX 3560 commercial SFE unit (Isco, Inc., Lincoln, NE, USA). The system has been described in the literature by Hopper (12) and consisted of each extraction fluid being fed by the syringe pumps of the Model SFX 3560 unit. The microprocessor controller on Model 3560 was used to proportion the amount of each fluid to give the overall composition used in the SFE. The cell size in these experiments was again 10 mL and the sample size was 5 grams. The binary fluid composition in these experiments was 75% CO_2/25% HC-134a. An extraction pressure of 2000 psi

Table 1. Analysis of spiked amount of organochlorine pesticides using CO_2/N_2 (70:30)

	% Recovery		
Pesticide	100 ppb[a]	10 ppb[a]	1 ppb[a]
Lindane	80	50	40
Dieldrin	85	78	67
p,p-DDE	95	92	90
Endosulfan sulfate	88	84	60
p,p-Methoxychlor	100	100	100

[a] Added to 200 mg butter.

Table 2. Analysis of spiked amount of organophosphorous pesticides using CO_2 / N_2 (70:30)

	% Recovery		
Pesticide	100 ppb[a]	10 ppb[a]	1 ppb[a]
Diazinon	82	70	40
Chlorpyrifos methyl	100	98	95
Ethion	91	88	88
Malathion	88	84	60
Chlorpyrifos	93	93	90

[a] Added to 200 mg butter.

(13.8 MPa) was utilized at 100°C for both fortified and incurred samples. The flow rate during these experiments was 2 mL/min for 120 minutes, hence a total of 240 mL of fluid was used for each extraction. Extracts were trapped in acetone (8.0 mL) held at 0°C. The restrictor temperature on the Model 3560 was held at 100°C. Bone dry CO_2 (Lenwald Gas Supply, Kansas City, MO) and HC-134a from Airo Cool Company (Neodesha, KS) were used in the experiments at the FDA laboratory. Pesticide recovery results obtained using the above conditions are reported in Tables 3, 4, and 5.

Fortified samples of pesticides in butter were prepared by previously described methods (13) and then mixing the warmed butter with two grams of Hydromatrix (Varian, Inc., Harbor City, CA) (14) before insertion of the sample into the extraction cell. Food samples with the incurred residues at the stated levels given in Table 5 were obtained from the Total Diet Study program conducted by the FDA laboratory in Lenexa, KS.

To test the general applicability of this approach for multi-residue analysis by using other supercritical mixtures, additional experiments were performed using the Model 3560 extraction system. These experiments

Table 3. Analysis of spiked amount of organochlorine pesticides using CO_2/HC-134a (75:25)

		% Recovery	
Pesticide	Spike amount[a]	CO_2	CO_2/HC-134a
Lindane	0.530	68	48
p,p-DDE	0.133	44	21
p,p-Methoxychlor	0.400	28	37
Dieldrin	0.133	37	29
Endosulfan sulfate	0.200	34	28

[a] In ppm added to 750 mg butter.

Table 4. Analysis of spiked amount of organophosphorous pesticides using CO_2/HC-134a (75:25)

	% Recovery		
Pesticide	Spike amount[a]	CO_2	CO_2/HC-134a
Diazinon	0.333	93	103
Chlorpyrifos methyl	0.333	73	92
Ethion	0.133	57	79
Malathion	0.333	75	96
Chlorpyrifos	0.267	71	93

[a] In ppm added to 750 mg butter.

were similar to those reported above except that two mixtures of pesticide standards were extracted using the conditions given below. Each mixture of pesticide standards consisted of 17 individual pesticides; pesticide mixture #1 containing analytes at 0.04–0.120 μg/mL level and pesticide mixture #2 having the analytes at 0.04–0.20 μg/mL concentration. Amounts of 50 μL and 10 μL respectively of standard mixtures #1 and #2 were added to 750 mg of butter, mixed with 2 grams of Hydromatrix and 0.5 mL of water

Table 5. Analysis of pesticide residues in food products

Sample/pesticide	Standard method (ppb)	70% CO_2 30% N_2 (ppb)	75% CO_2 25%HC-134a (ppb)
Crackers (10.2% fat)			
Chorpyrifos methyl	25	22	22
p,p-Methoxychlor	0.5	0.5	0.0
Malathion	11	7	8
Pumpkin pie (9% fat)			
Chlorpyrifos methyl	13	12	15
Malathion	7	4	4
p,p-DDE	3	0.5	0.0
Tortilla chips (6.8% fat)			
Chorpyrifos methyl	3	3	
Chlorpyrifos	0.6	0.0	
p,p-Methoxychlor	0.8	0.7	
Malathion	12	9	
Fish sticks (16.4% fat)			
Chlorpyrifos	6	5	5
Malathion	6	4	6

(Tortilla Chips were not extracted with CO_2/HC-134A mixture).

(to aid in the desorption from the support), before placement into the 10 mL extraction cell.

Extractions performed on Model 3560 were similar to those previously described, except the identity of the binary fluids and their respective ratios were altered, as well as the extraction pressure and temperature and the amount of extraction fluid used. These experimental variations are summarized below, and correspond to the extraction conditions listed in Table 6:

Extraction Conditions I–3000 psi (20.7 MPa), 60°C, 240 mL total fluid used, 65% N_2/35% HC-134a

Extraction Conditions II–3000 psi (20.7 MPa), 100°C, 240 mL total fluid used, 65% N_2/35% HC-134a

Extraction Conditions III–6500 psi (44.8 MPa), 100°C, 240 mL total fluid used, 70% N_2/30% HC-134a

Extraction Conditions IV–6500 psi (44.8 MPa), 100°C, 180 mL total fluid used, 70% N_2/30% HC-134a

Extraction Conditions V–6500 psi (44.8 MPa), 100°C, 180 mL total fluid used, 70% Ar/30% HC-134a

A restrictor temperature of 100°C, collection temperature of 15°C, and flow rate of 2 mL/min was used in conjunction with the above experimental conditions. Gas percentages (%) for all of the mixtures used in these extraction studies are expressed in volume percent, including those mixtures obtained by use of the mass flow controllers.

Pesticide analysis on both the resultant extracts from the CO_2/N_2 and CO_2/HC-134a experiments were done at the FDA laboratory in Lenexa, KS. For quantitation of the organochlorine pesticides, a Varian 3600 GC (Walnut Creek, CA) was used employing a 30 m × 0.53 mm DB-1, 1.5 μm film thickness, fused silica column (J & W Scientific, Folsom, CA). The carrier gas (H_2) flow rate was 25 mL/min into a Hall 1000 electrolytic conductivity detector operating in the halogen mode. (Tremetrics, Inc., Austin, TX). The injection system incorporated a flash vaporization inlet operating at 230°C. A linear temperature program was used to affect the pesticide separation which began at 150°C for 1 minute, followed by an increase to 250°C at 7°C/min. The temperature program was then held at 250°C for 10 minutes. For organophosphorus pesticide analysis, a Hewlett Packard Model 5890 Series II GC (Hewlett-Packard, Wilmington, DE) was used in conjunction with a 30 m × 0.53 mm DB-17, 1.0 μm film thickness, fused silica column. A carrier gas flow rate of 37 mL/min was fed into a flame photometric detector (Hewlett-Packard) operating in the phosphorus mode. The injection system incorporated a direct flash vaporization inlet at 230°C. The temperature program for each GC run consisted of beginning at 150°C and

Table 6. Pesticide recoveries and coextracted lipid[a] using different extraction conditions

	Extraction conditions (% recovery)				
	I	II	III	IV	V
Pesticide std. #1					
Methamidophos	6	3	4	5	3
Acephate	5	0	4	3	0
Tributyl phosphate	104	108	127	144	123
Omethoate	26	22	22	33	19
Diazinon	110	106	112	116	112
Dimethoate	100	71	53	59	59
Chlorpyrifos methyl	81	67	93	73	93
Primiphos methyl	99	95	110	97	105
Chlorpyrifos	77	71	96	74	95
Malathion	100	84	94	106	90
Methidathion	89	64	89	65	87
Ethion OA	124	107	128	120	85
Ethion	71	66	95	57	95
Triphenyl phosphate	86	72	96	71	96
Octyl diphenyl phosphate	72	59	80	59	78
Azinphos methyl	52	44	68	37	49
Coumophos	62	54	69	45	56
[a]Lipids in mg	9.7	3.4	5.5	3.5	15.1
Pesticide std. #2					
Hexachloronorbornadiene	85	75	92	56	71
Chlorpropham	69	77	81	59	69
Dichloran	56	65	104	92	93
“Tris”	112	191	135	314	127
Tefluthrin	100	88	123	116	83
Methyl chlorpyriphos	75	80	88	78	68 (89)
Tridiphane	50	52	69	60	70
Chlorpyriphos	69	68	95	69	70 (84)
Heptachlor expoxide	53	52	63	60	52
Endosulfan I	53	44	51	70	48
Dieldrin	51	44	66	64	43
Endosulfan II	42	30	35	39	25
Endosulfan sulfate	56	42	30	59	39
p,p′-Methoxychlor	45	52	37	51	27

(*continued*)

Table 6. Continued

	Extraction conditions (% recovery)				
	I	II	III	IV	V
Iprodione isomer	69	66	42	0	0
Cis-permethrin	38	25	31	42	27
Trans-permethrin	41	26	31	39	22
[a]Lipids in mg	6.5	5.1	5.9	4.6	15.7

Extraction conditions I–3000 psi, 60ºC, 240 mL total fluid used, 65% N_2/35% HC-134a.

Extraction conditions II–3000 psi, 100ºC, 240 mL total fluid used, 65% N_2/35% HC-134a.

Extraction conditions III–6500 psi, 100ºC, 240 mL total fluid used, 70% N_2/30% HC-134a.

Extraction conditions IV–6500 psi, 100ºC, 180 mL total fluid used, 70% N_2/30% HC-134a.

Extraction conditions V–6500 psi, 100ºC, 180 mL total fluid used, 70% Ar/30% HC-134a.

[a]Lipids in mg for pesticide std. #1 are: I: 9.7, II: 3.4, III: 5.5, IV: 3.5, V: 15.1; Lipids in mg for pesticide std. #2 are: I: 6.5, II: 5.1, III: 5.9, IV: 4.6, V: 15.7.

increasing the temperature to 230°C at 5°C/min with a final hold time of 5 min at 230°C.

RESULTS AND DISCUSSION

To assure that the chosen binary fluids were in the supercritical fluid region, phase diagrams and critical loci were calculated respectively from the NIST REFPROP data base (15) for the utilized binary mixtures of CO_2/N_2 and CO_2/HC-134a. The upper critical loci curves defining the sub- and supercritical fluid regions for the binary mixtures are shown in Figs. 2a and 2b. As noted on both Figs. 2a and 2b, the terminus points of the upper critical loci occur at the respective P_c and T_c values for the individual fluids. For the CO_2/N_2 pair, this is at quite a low value for the nitrogen component, but occurs at approximately 7.2 MPa and 304°K for CO_2. As designated in Figs. 2a and 2b, the region above and to the right of the critical loci curve is the supercritical region, while the region below is subcritical, in which the potential for phase change and separation of the fluid components can occur under certain conditions. For the stated extraction conditions utilizing the CO_2/N_2 system (41.4 MPa, 333.2°K) and fluid composition, the extracting fluid system is clearly in the supercritical region with respect to both fluids (Fig. 2a).

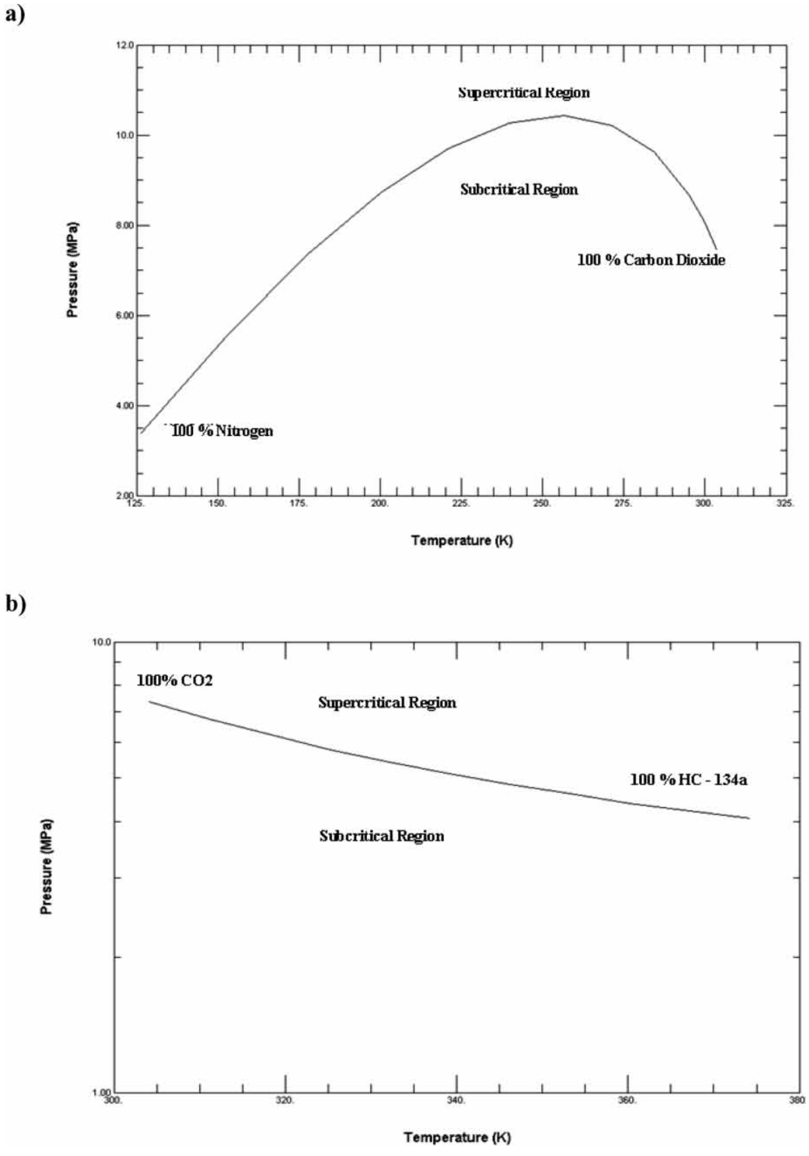

Figure 2. (a) Pressure-temperature phase diagram showing critical loci for the CO_2/N_2 system. (b) Pressure-temperature phase diagram showing critical loci for the CO_2/HC-134a system (Ref. 15).

For the CO_2/HC-134a pair, the upper critical loci are quite different in shape over the region of interest (Fig. 2b). Here the difference between the critical constants, (P_c, T_c) is substantially less, since for HC-134a they are 4.6 MPa and 374.3°K, respectively, as shown at the terminus point on the

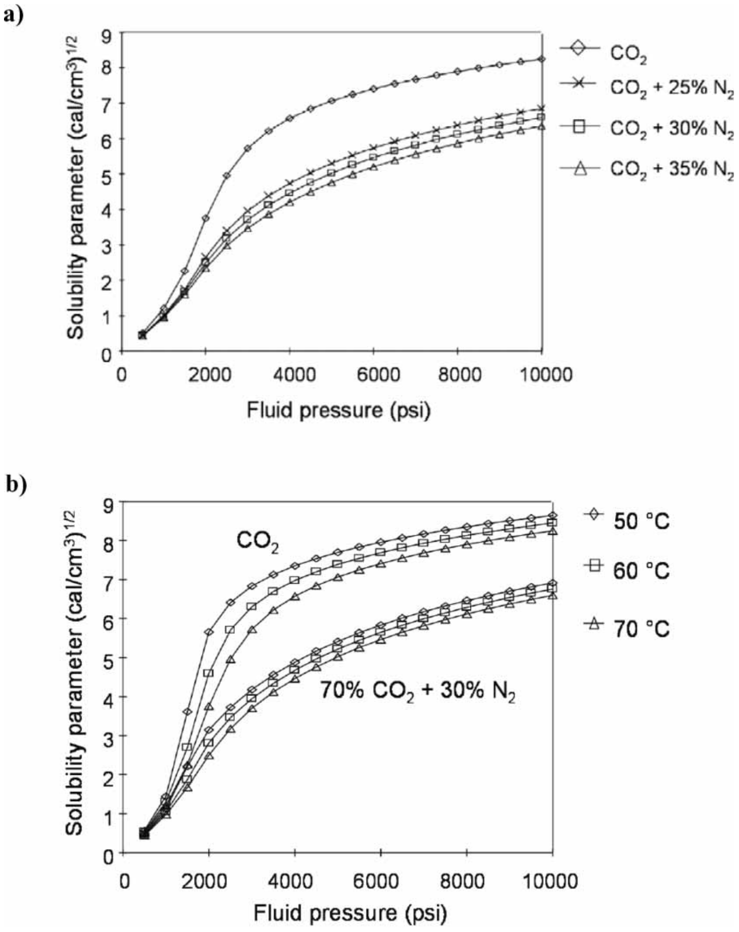

Figure 3. (a) Variation in the solubility parameter with pressure of mixtures for CO_2 and N_2 at 70°C. (b) Variation in the solubility parameter with pressure of a 70% CO_2/30% N_2 mixture as a function of temperature (Refs. 8, 9).

right side of the critical loci, and 7.2 MPa and 304°K for CO_2 on the left-hand side of Fig. 2b. From this diagram, the extraction (13.8 MPa, 373.2°K) conditions utilized for SFE in the FDA laboratory experiments are also supercritical.

Calculations of the solubility parameters for the above binary fluid mixtures (9) versus extraction fluid pressure are shown in Figs. 3a and 3b. Such data can be used to optimize extraction conditions, i.e., reduce the lipid content of the final extract. Examination of Fig. 3a indicates that mixtures of N_2 in CO_2 between 25–35% reduce the overall cohesional energy of the fluid mixture relative to pure CO_2. This trend is common when a fluid having a lower critical temperature (T_c) is added to CO_2 (10).

Similarly, variation in the extraction temperature when using such binary fluid mixtures also changes the solubility parameter (δ) as a function of pressure as shown in Fig. 3b, although the effect is less than that achieved by varying the composition of the fluid mixture. However the effect of varying the above parameters is to achieve a more selective extraction, particularly of minor trace components relative to unwanted coextractives, such as lipid moieties.

The recovery results for the specific pesticides spiked into the butter matrix at a 1–100 ppb level and extracted with the CO_2/N_2 binary fluid under the stated conditions at the USDA laboratory in Peoria, IL, are given in Table 1. The recoveries are quite respectable at the 100 and 10 ppb analyte fortification levels, with the exception of lindane. As indicated in Table 1, there is a general decrease in analyte recovery with a decreasing fortification level in the butter matrix which varies from pesticide to pesticide down to the 1 ppb level. Results for the selected organophosphorus pesticides over the same fortification range in butter (Table 2) show a similar trend when extracted under the same conditions as those used for the organochlorine pesticides (Table 1). Recorded recoveries (greater than 80%) are slightly better than those obtained for the organochlorine spiked sample, with the exception of diazinon.

SFEs performed at the FDA laboratory using the stated CO_2/HC-134a mixture are given in Tables 3 and 4. The spiked amounts in 750 mg of butter correspond to a fortification level between 0.13–0.53 ppm depending on the pesticide under consideration. Extractions were run under conditions identical using neat CO_2 and the previously cited CO_2/HC-134a composition. Organochlorine pesticides recoveries as given in Table 3 were very low for both extractions done with CO_2 and the CO_2/HC-134a mixture. The reasons for these disappointing results are not apparent, but could be due to non-optimal extraction conditions, such as using too little of the extraction fluid. On the other hand, these conditions proved suitable for the recovery of select organophosphorus pesticides (Table 4) corresponding to 0.13–0.33 ppm fortification levels in butter using either neat CO_2 or the CO_2/HC-134a mixture. In fact, recoveries were substantially improved for the five organophosphorus pesticides studied using the CO_2/HC-134a mixture relative to the recovery values using CO_2 alone. Amounts of coextracted lipid were 3.2 mg using neat CO_2 and 15.2 mg with the CO_2/HC-134a mixture for this specific case.

The amount of lipid coextracted decreases with increasing temperature using neat CO_2 or in the mixtures where CO_2 is combined with another fluid (N_2 or HC-134a). This is an example of the well known crossover solubility effect exhibited by solutes in compressed fluids as cited in the literature (16). For example, at an extraction pressure of 2000 psi (13.8 MPa), the amount of coextracted lipid at a extraction temperature of 40°C was 233 mg versus 9.2 mg when the extraction was performed at 100°C using only 120 mL of the total extraction fluid. An extraction using the 75% CO_2/25%

HC-134a fluid mixture coextracted 28.1 mg at 2000 psi and 100°C using same volume of fluid, which confirms that the binary mixture of CO_2/HC-134a at the same temperature coextracts more lipid than neat CO_2 for this specific case. However an analogous composition of CO_2/N_2 (70% CO_2/30% N_2 mixture at 6000 psi and 60°C) will extract 1/4 to 1/5 less lipid than neat CO_2. Clearly there is a tradeoff between analyte recovery and discrimination between the target analytes (pesticides) and co-extracted lipid. In general, the small amount of lipid coextracted when using the optimized SFE method, and its effect on the subsequent analysis, is minimized due to dilution of the extract prior to GC analysis.

To test the applicability of the described method on actual food samples containing confirmed incurred pesticide residues determined by standard protocols, samples of crackers, pumpkin pie, tortilla chips, and fish sticks were extracted with both the 70% CO_2/30% N_2 and 75% CO_2/25% HC-134a binary fluid mixtures. As shown in Table 5, overall the recorded incurred pesticide level results (ranging from 0.5–25 ppb) appear overall promising using either fluid mixture relative to recoveries obtained using the standard analytical protocol on food matrices ranging from 6.8–16.4% fat level. Extractions were not performed on the tortilla chips using the CO_2/HC-134a mixture. The results reported in Tables 1–5 consisted of n = 2-3 replicates; the RSDs (relative standard deviations) ranged from 2.8–10.2%, the average RSD being 5.18%.

Table 6 shows the results obtained for butter samples fortified with the two different pesticide mixtures previously described under the five extraction conditions as noted in the experimental section. In addition to the recorded pesticide recoveries, the amount of coextracted lipid is noted at the bottom of each table for the extraction conditions. For extraction conditions I–IV, the total coextracted lipid is under 10 mg–the most coextracted lipid being recorded using the Ar/HC-134a mixture (~15 mg). Efficient extraction and high recoveries of a wide range of pesticide types, even when using traditional organic solvent methodology, is difficult to achieve (17), and this fact is borne out also using the binary fluid mixtures in Table 6. The recovery pattern for an individual pesticide across Table 6 is fairly consistent, although there is some variance depending on the fluid extraction agents and conditions. Recoveries for methamidophos and acephate are traditionally very poor even when using conventional organic extraction solvents; iprodione isomer results at extraction conditions IV and V suggest a loss of this pesticide standard from the spiking solution over time, and there are high recovery values for the "tris", tris (β-chloroethyl) phosphate moiety due to analytical interferences. However, the recovery results for the other 29 pesticides is encouraging for qualitatively determining their presence, and in many cases quantitative determination. Chlorpyrifos, the only common pesticide in both pesticide mixtures gave very similar recoveries. For extraction condition V, the parenthetical value shown for methyl chlorpyriphos and heptachlor epoxide indicate that a determination was also made using flame photometric detection.

Surveying the results in Table 6 in terms of which the extraction condition gave the highest recovery for all of the 34 pesticides shows that extraction condition IV (6500 psi (44.8 MPa), 100°C, 240 mL total fluid used, 70% N_2/30% HC-134a) gave the highest recovery for 19 of the pesticides. For extraction conditions I, II, IV, and V, optimal recovery results were recorded for 9, 1, 7, and 6 of the individual pesticides, respectively. The reason that the sum of these results, 42, does not equal the total of 34 pesticides examined is that in several cases identical recovery results were obtained for the same pesticide under two of the experimental conditions. It should be noted that each recovery number in Table 6 is the average of two replicates ($n = 2$); RSDs were not given since the number of replicates is low and hence would not be statistically significant.

It should be noted that a rather unusual extraction experiment was also done using a 70% He/30% HC-134a mixture at 6500 psi, 100° utilizing 180 mL of the binary extraction fluid. The anti-solvent effect of helium on SC-CO_2 has been previously noted in the literature (8, 18) and is one way of reducing the amount of lipid coextractives present during SC-CO_2 extraction (3). In the current extraction experiment, HC-134a at a 30% level is behaving as a "cosolvent" with respect to the high pressure-temperature fluid helium and enhancing the vapor pressure effect exhibited by the individual pesticides at 100°C. The overall pesticide recovery results relative to those achieved using extraction conditions I-V are somewhat poorer making the results more of academic interest than of practical value. The level of lipid coextractives for pesticide mixtures #1 and #2 were 10.6 and 15.5 mg, respectively, under these conditions.

CONCLUSIONS

The results above illustrate the potential of using binary fluids as effective and selective extraction media that can circumvent the need for additional and laborious extract cleanup procedures. The above reported results were obtained on two types of experimental equipment from two independent laboratories using two different fluid mixtures under different conditions. SFE results for spiked standards in a butter matrix confirmed that the CO_2/HC-134a mixture was better for organophosphorus pesticide recoveries over neat fluids or the CO_2/N_2 mixture. Overall results using the CO_2/N_2 mixture gave similar results to those obtained via a standard extraction/cleanup procedure. Binary fluid extractions done on food samples actually containing incurred residues, using either CO_2/N_2 or CO_2/HC-134a, compared favorably with results from the standard analytical method procedure. Extension of this technique using other supercritical binary fluid mixtures shows the general applicability of this approach to multi-residue analysis.

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