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Simultaneous Supercritical Fluid Derivatization and Extraction

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Simultaneous supercritical fluid derivatization and extraction (SFDE) was investigated as a rapid alternative to liquid solvent extraction followed by derivatization for analysis by gas chromatography (GC). Addition of a commercially available silylation reagent, tri-sil concentrate, directly to the sample matrix, enables extraction of analytes from samples that were previously exhaustively extracted with conventional supercritical fluid extraction (SFE). The SFDE extracts were collected, ready for GC analysis without additional derivatization, not only resulting in time savings but also in improved extraction yield of both derivatized and underivatized species. In addition to making polar compounds more soluble in the supercritical extracting fluid through derivatization, the derivatization reagent is thought to compete with the analyte for active sites of the matrix, thus displacing compounds from the matrix. Application of SFDE for the extraction and analysis of roasted coffee beans, roasted Japanese tea, and marine sediment is demonstrated.

INTRODUCTION

The unique chemical and physical properties of supercritical fluids have attracted considerable attention during the past decade for use in analytical chemistry. Supercritical fluid chromatographic methods have extended the high-resolution separation of semivolatile and thermally labile compounds beyond the limit of gas chromatography, entering the domain of liquid chromatography (1). In addition, analytical-scale supercritical fluid extraction (SFE) has emerged as a rapid and efficient method for extracting organic compounds for separation and detection by gas or supercritical fluid chromatography (1-4). The primary advantage of SFE over liquid-phase extractions is the lower viscosity and variable density of the supercritical fluid (SF). Mass transfer occurs more quickly and efficiently in supercritical fluids than in liquids. Driven by the desire to limit toxic solvent waste, reduce analysis times, and increase extraction efficiencies, the practice of SFE is growing at such a rapid rate that within several years the use of traditional liquid-phase extractions may be the exception rather than the norm.

Although carbon dioxide SFE is rapidly becoming recognized as an efficient and time-saving alternative to Soxhlet extraction for trace organic analysis, carbon dioxide is limited as an extracting solvent by its nonpolar character. CO₂ SFE extraction efficiency of polar and ionic organic species can be poor. More polar supercritical fluids require critical conditions that are too extreme for practical utility. For example, water with a critical pressure of 226.3 atm and a critical temperature of 374.4 °C has proved technically too difficult for practical analytical-scale application.

Generally, SFE conditions have been empirically determined with emphasis placed on the widely variable solvent strength of supercritical fluids (3-6). In contrast, a recent review by Hawthorne (1) and results reported by Alexandrou and Pawliszyn (7) call attention to the matrix from which the extraction is being made. Alexandrou and Pawliszyn's extractions of tetrachlorodibenzo-*p*-dioxins (TCDDs) were made from fly ash with SF CO₂ and with SF CO₂ after treatment of the fly ash with acid. The acid treatment displaced adsorbed TCDD from polar active sites on the fly ash, resulting in improved extraction efficiencies (9% without acid treatment, 100% with acid treatment). Some compounds in the study could not be extracted at pressures of 400 atm before treatment with acid. They concluded that the extraction was limited, not by the solubility of the solute in supercritical CO₂ but rather by the desorption process of the compound from the active site.

Improved solvent characteristics and extraction efficiencies can be obtained if polar solvents such as methanol are added to the supercritical carbon dioxide (8). Several investigators have used polar modifiers (most commonly methanol) mixed in the supercritical fluid, increasing the solvent polarity and extraction efficiency. Modifiers are thought to aid extraction in two ways: increasing the solvent strength of the fluid as well as competing with adsorbed compounds for active sites of the matrix.

Extraction is a partitioning of the compound between the matrix and the solvent. Clearly the equilibrium is driven to favor solvation of the compound either if the compound is made more soluble in a relatively nonpolar supercritical fluid or if the compound is driven off the matrix. Like adding modifiers, addition of derivatizing reagents may drive the equilibrium in both of these ways. Covering polar moieties by derivatization will make the compound more soluble in SF

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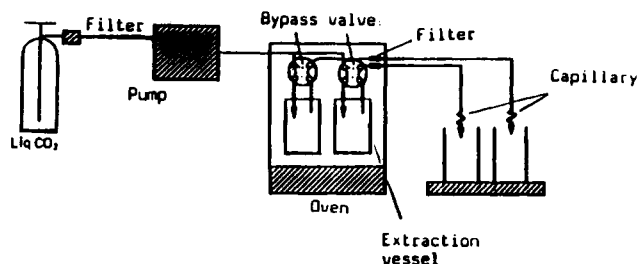


Figure 1. Supercritical fluid derivatization and extraction apparatus.

CO₂. Also, derivatization of the matrix active sites will prevent resorption of the analyte.

In liquids, our research group has shown that extraction efficiency is improved when derivatizing reagents are added to the extraction solvent (9). In this previous work, derivatization of the sample during ultrasonic agitation to form trimethylsilyl derivatives increased the recovery of organic acids, alcohols, and phenols from airborne particulate matter. Derivatization in liquids has also served to impart detection and separation characteristics to the analyte of interest.

Unfortunately, little information about chemical reactions in supercritical fluids exists, and no investigation has been published concerning analytical derivatizations in supercritical fluids. However, investigations of chemical reactions in supercritical fluid, not used in analytical applications, have been reviewed recently (13). Supercritical conditions were reported to improve yield, selectivity, and product separation while permitting reactions to proceed at lower temperatures. Also, supercritical conditions were reported to achieve enhanced reaction rates and product selectivities for a variety of reaction mechanisms including free-radical reactions.

At the recent International Symposium on Supercritical Fluid Chromatography and Extraction where we presented our initial investigations (10), preliminary information was also presented by Levy on the *in situ* trimethylsilylation of sucrose esters (11). In addition, White *et al.* reported fatty acid methyl ester (FAME) formation under supercritical fluid conditions (12). The successful extraction and methylation of 2,4-dichlorophenoxyacetic acid from stream sediment samples using supercritical fluid derivatization and extraction procedures was reported as well (12). Thus, it appears that the use of supercritical fluids as solvents for analytical derivatizations is beginning to generate considerable interest, although much more information needs to be gathered before its full potential is achieved.

The objective of this work was to provide evidence that the direct application of a silylating reagent to complex samples had the power to serve both as a polar modifier and a derivatizing reagent under supercritical conditions.

EXPERIMENTAL SECTION

These initial investigations of simultaneous supercritical fluid derivatization and extraction experiments were performed with the extraction and derivatization apparatus shown in Figure 1. The apparatus consisted of a pump able to produce pressures up to 400 atm CO₂, two four-port switching valves, heated reaction/extraction cells, flow restrictors, and solvent traps to collect the products. The pump was connected to a reaction/extraction cell through a pressure meter by 1/16-in. stainless steel tubing and drew liquid CO₂ from a standard gas cylinder. Two cells in parallel allowed two reactions to be performed simultaneously. The extraction cells were constructed of 9-mm-i.d. stainless steel tubing with bottoms and caps drilled for inlet and outlet fittings. The size of the cell could be varied for smaller samples by changing the length of the 9-mm-i.d. stainless steel tubing. Cell volumes range from 2 to 5 mL. The supercritical fluid inlet (1/16-in. stainless steel tubing) extended to the bottom of the extraction cell so that the fluid must pass the entire length of the reaction/extraction cell before exiting through the outlet to the re-

Table I

sample	mass	pressure, atm	temp, °C	vol of reagent
roasted coffee beans	0.5 g	200	80	1 mL
roasted tea	0.5 g	200	80	1 mL
roasted tea	0.5 g	400	80	1 mL
marine sediment	10 mg	300	60	250 µL

strictor and the solvent trap. Supercritical temperature was maintained in the cells by an oven that also enclosed two four-port switching valves and frit filters placed before and after the cell. The frits protected the switching valves and the capillary restrictor from sample particles entrained in the fluid. Four-port rotating valves located above each cell allowed the fluid to either flow through the cell or bypass the cell and flow directly to the outlet restrictor. To perform static reaction/extraction, the pressurized cell was bypassed; for dynamic extractions, the flow was directed through the cell. The cell pressure was maintained by a length of 50-µm-i.d. capillary silica tubing 50–80 cm long. The low-pressure end of the restrictor was placed in the solvent trap, which was a small vial (2–5 mL) containing 1–4 mL of solvent.

After the extract was collected in the solvent trap, the excess solvent was evaporated under a gentle nitrogen stream to dryness. The sample extract was then brought up in 0.50 mL of solvent. Chromatographic injections were made from this solution.

Test samples for these experiments included roasted coffee beans, Japanese tea leaves, and ocean marine sediment. After exhaustive extraction with pure carbon dioxide, tri-sil concentrate (Pierce, Rockford, IL), a mixture of hexamethyldisilane (HMDS) and trimethylchlorosilane (TMCS) containing no solvent, was added directly to the sample followed by normal supercritical fluid extraction. Extracts were evaluated for organic content by using capillary gas chromatography with flame ionization detection. One-microliter injections were made on a 30-m DB-225 column (J&W Scientific) with He carrier gas at an initial temperature of 50 °C ramped at 2 °C/min to 235 °C.

The flow rate of the supercritical fluid is between 450 and 500 mL/min at ambient pressure and temperature. This corresponds to a flow rate of supercritical fluid through the extraction cell of about 2–4 mL/min. Table I summarizes the various parameters used in the SFE and SFDE experiments.

RESULTS AND DISCUSSION

Figure 2 shows our initial results with a sample of roasted coffee beans. The top chromatogram is a tracing of the first SFE extract with pure supercritical carbon dioxide at 200 atm and 80 °C. The extraction was performed in a static mode for 10 min followed by a dynamic mode while the extract was collected by bubbling through 1 mL of acetone. During the collection process, the capillary restrictor and transfer tube were heated to 100 °C. As expected, the gas chromatogram of the extract showed numerous peaks representing organic components extracted from the roasted coffee beans.

This pressure, temperature, and extraction time were not sufficient to extract all of the components from the coffee beans, and the process was repeated five times with the same sample until chromatogram B, shown in Figure 2, was obtained. As expected, these successive extractions yielded fewer compounds in lesser amounts. To the exhaustively extracted sample, 1 mL of tri-sil concentrate reagent was added and the extraction process repeated a sixth time. With the reagent present under these conditions, however, the 10-min static period was not only an extraction but also a derivatization reaction step. As can be seen from chromatogram C, many additional components were extracted from the coffee beans when the derivatizing reagent was added to the matrix during the extraction. Some of the peaks were identified by GC/MS to be the trimethylsilyl esters and ethers of oxalic acid (ethanedioic acid), 2-hexenedioic acid, 2-propyldecan-1-ol. Compounds extracted and identified by GC/MS not as the silyl derivative but rather as the native compounds include

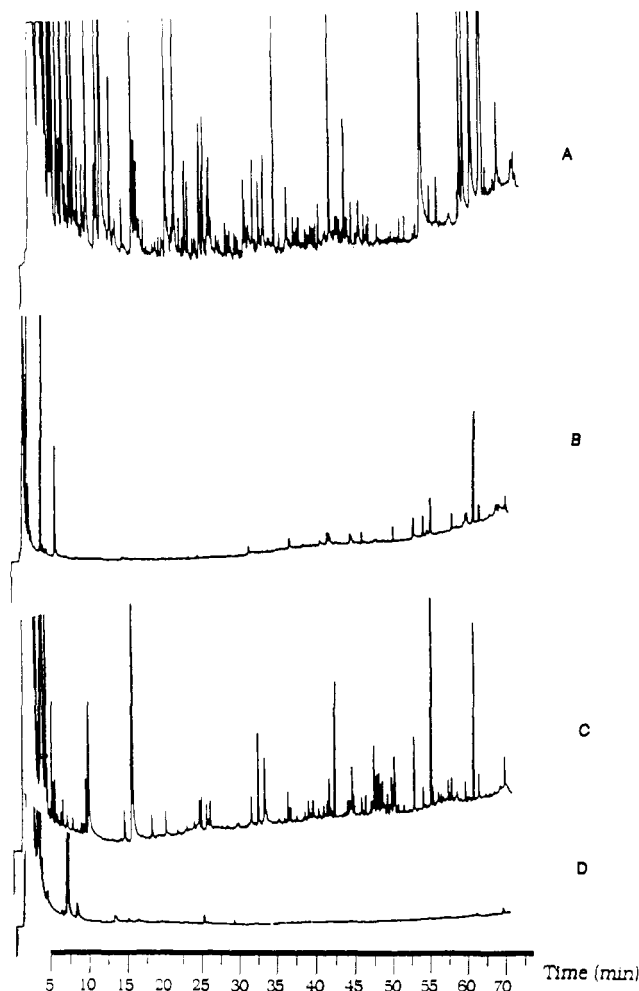


Figure 2. Gas chromatograms of extracts from roasted coffee beans.

mesitylene (1,3,5-trimethylbenzene), 1-ethyl-3-methylbenzene, 1-ethyl-4-methylbenzene, 2-propionophenone, tetrahydro-2-furanmethanol, dihydro-2-furanone, benzenedicarboxylic acid bis(2-methoxyethyl) ester, nonacosane, and caffeine. The extraction of these nonderivatized components was clearly aided by the presence of the derivatizing reagent. The last chromatogram, chromatogram D, is a procedural blank to illustrate that the peaks shown in chromatogram C result from the sample, not contaminants in the derivatizing reagent.

Figure 3 shows the results of a similar experiment using tea leaves rather than coffee beans. The first three chromatograms are of sequential extractions performed with pure carbon dioxide under conditions identical with those described for Figure 2. Chromatogram D represents the extract after tri-sil concentrate was added to the sample, and chromatogram E is the procedural blank. The major peak found at an elution time of 60 min was determined to be caffeine from mass spectral information and by matching the retention time with that of the standard. From the sequential extractions with pure carbon dioxide, clearly this compound is not completely extracted under these conditions. This was expected since in the commercial decaffeination process water must be used as a modifier to achieve sufficient extraction efficiencies. Using caffeine as an example of a polar but nonderivatizable compound, it can be seen that adding tri-sil concentrate not only served as a derivatizing reagent but also served the function of a coextracting reagent, which benefits the extraction of nonderivatizable polar compounds. By summing the area counts for caffeine in the three SFE extracts and comparing with the area counts produced by caffeine from the SFDE extract, the caffeine extracted by the SFDE method

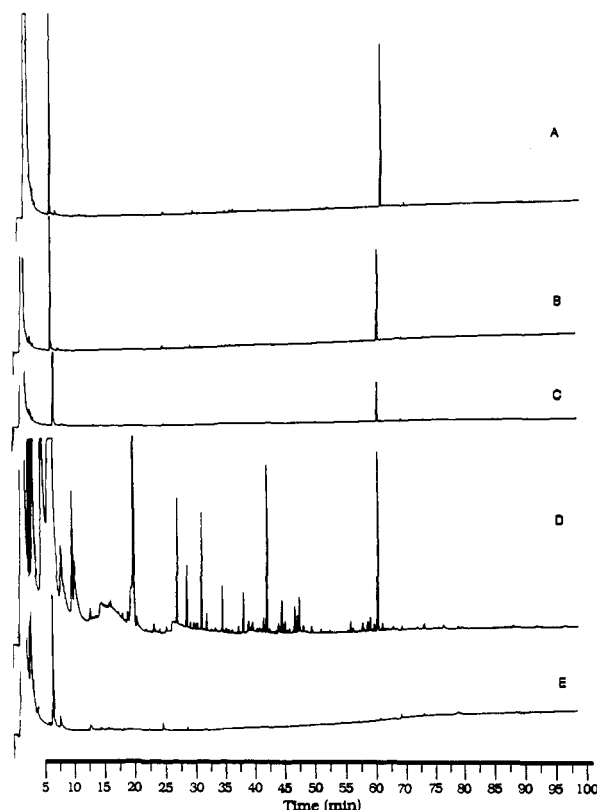


Figure 3. Gas chromatograms of tea extracts obtained at 200 atm.

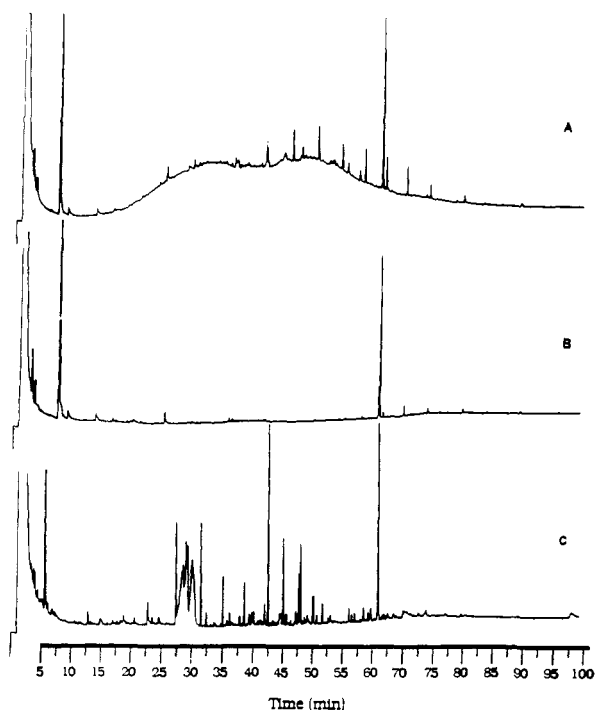


Figure 4. Gas chromatograms of tea extracts obtained at 400 atm.

was found to be 27% of the total caffeine extracted from the four extractions. Other compounds extracted from the tea samples after SFDE were shown to contain trimethylsilyl groups, but complete identities and peak matching with standards have not been completed.

Somewhat more efficient extraction with pure carbon dioxide is shown in Figure 4 when 400 atm, 80 °C conditions were used, instead of 200 atm, 80 °C, to extract the tea sample. As can be seen from the top tracing, considerably more unresolved organic matter was initially extracted at the higher pressure. After successive extractions, however, chromatogram

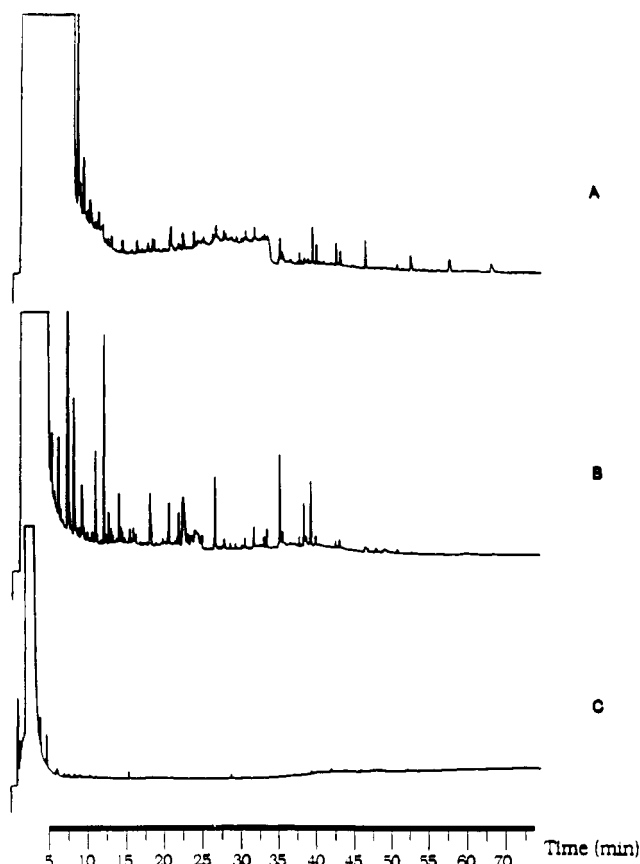


Figure 5. Gas chromatograms of extracts of 10 mg of marine sediment.

4B looks similar to the first three chromatograms of Figure 3, and the chromatogram after tri-sil concentrate shows additional peaks caused by extracted TMS derivatives and more efficiently extracted nonderivatized polar compounds such as caffeine. Except for two broad peaks eluting after about 30 min, all of the major peaks observed in chromatogram 4C match those seen in chromatogram 3D. Low molecular weight compounds observed in 3D but not in 4C may have been extracted in pure CO₂ at the higher pressure.

Since we were not limited in the quantity available to us, relatively large quantities (0.5-g samples) were used for the initial experiments with coffee and tea. For environmental and biological samples, however, this is often not the case. To determine if the SFDE approach was feasible for small sample sizes, we investigated a marine sediment sample that was collected as organic "rain" in the ocean. Collection and identification of the organic material contained in these sediments are important for determining the organic mass balance of the oceans. This sediment was collected by density-balanced filters floating beneath the ocean surface. Sediment material deposited on the filters as they settled down toward the ocean floor. Sediment samples collected in this manner are costly, and only small quantities can be obtained for analysis. After modifying our extraction cell to a smaller volume, we extracted the 10 mg of sediment in the same manner as the coffee and tea samples.

Figure 5A shows the results of standard SFE of the 10 mg of marine sediment in which pure carbon dioxide at 300 atm of pressure and a temperature of 60 °C was used to extract the lipophilic portion of this sample. This extraction was followed by our SFDE approach, Figure 5B, in which both the silyl derivatives and the native molecules of fatty acids

and terpenes were identified by MS, such as palmitic acid (hexadecanoic acid), vaccenic acid (octadecanoic acid), Nernol (3,7-dimethyl-2,6-octadin-1-ol), and isocitronellene. Figure 5C is a procedural blank.

CONCLUSIONS

Enhanced extraction efficiency for the nonreactive compounds was presumably due to a displacement mechanism similar to that observed when modifiers are used with carbon dioxide. The active site is the recipient of the derivatizing reagent, displacing the analyte into the supercritical fluid as the native compound. In this scheme, derivatization offers an advantage over polar modifiers in that derivatization is irreversible. The analyte is driven into solution without the opportunity to readsorb.

Enhanced extraction efficiency for reactive compounds can occur by either dissociative or associative derivatization mechanisms. In a dissociative mechanism, the adsorbed analyte must first desorb from a matrix active site and be solvated in the supercritical fluid and then react to form the less polar derivative, which favors solvation in the supercritical carbon dioxide. In an associative mechanism, derivatization occurs while the analyte is adsorbed on the active site of the matrix. Reaction with the adsorbed analyte results in the desorption of the nonpolar derivative into the supercritical fluid.

It is obvious that these experiments are only preliminary in nature and more investigations are needed to select the best derivatizing reagents, optimize conditions, and quantify results. Nevertheless, many new peaks, both silylated and unsilylated, were observed in the gas chromatographic separation of the SFDE extracts even after the samples had been exhaustively extracted with pure supercritical carbon dioxide. Whatever mechanism prevails, the SFDE approach appears to offer increased extraction efficiencies over standard SFE approaches, especially for polar and reactive compounds. Investigations of different types of reagents, conditions, and matrices will lead to more efficient and selective SFDE methods.

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