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Project Summary

Method for the Supercritical Fluid Extraction of Soils/ Sediments

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The Environmental Protection Agency is interested in new and improved analytical methods that are faster, better, and cheaper than the present methods, and that, at the same time, are safe and generate little or no waste. For a number of years, supercritical fluid extraction has been publicized as a new and promising technique for the extraction of organic compounds from solid matrices; however, applications of supercritical fluid extraction techniques to the extraction of compounds regulated by the Environmental Protection Agency from matrices of concern to the Agency have been rather limited. In late 1988, we began a study to develop a supercritical fluid extraction technique for environmental matrices such as soil, sediment, and fly ash. The approach taken in this study was carried out in various phases. In the first phase, we reviewed the literature published on the supercritical fluid extraction technique. While the literature gathering and review took place, we contacted manufacturers of supercritical fluid extraction instrumentation to identify the most suitable equipment for this study. In the second phase of the study, we purchased a supercritical fluid extractor system and conducted a series of experiments to familiarize ourselves with the instrumentation and to determine the feasibility of extracting various classes of organic compounds from solid matrices. Sand was the primary matrix selected for investigation, although soils and sediments have also been tested. Upon completion of the second phase, we prepared a protocol that describes in detail how a solid sample should be extracted under supercritical conditions with carbon dioxide or carbon dioxide containing modifiers, the instrumentation requirements, etc. The third phase of the study was devoted primarily to method optimization, application of the supercritical fluid extraction technique to various environmental matrices (mostly standard reference materials certified for certain organic compounds), and instrument modification to increase sample throughput (e.g., installation of two and four extraction vessels for parallel extractions).

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

An important step in the analysis sequence for organic pollutants is extraction of analytes from the samples, that means, their separation from the matrices. The sample extraction technique should, to the extent possible, yield quantitative recoveries of the target analytes from the matrices, be selective so that extraction of interferants is minimized, not generate large volumes of waste solvents, require little sample and extract handling to minimize analyte losses and contamination, and be fast and inexpensive. For the extraction of organic pollutants from solid samples (soil, sediment, tissue, fly ash, etc.), two methods are currently included

in the SW-846 methods manual (1): Soxhlet extraction (Method 3540) and sonication extraction (Method 3550).

For a number of years, a new and promising technique for the extraction of organic compounds from solid matrices has been publicized: supercritical fluid extraction (SFE). The actual, or claimed, advantages of SFE over conventional extraction methods include:

- SFE is much faster than Soxhlet extraction; extraction times of as little as 1 min can result in quantitative recoveries.
- No toxic and expensive solvents are required; this results in reduced materials and waste disposal costs and in reduced environmental pollution. No solvent removal or glassware cleaning is required.
- SFE conditions can easily be optimized by just varying pressure and temperature and by using modified supercritical fluids (SFs).
- Overall, the use of SFE techniques in place of conventional methods results in substantial cost and labor savings.

The most commonly used SF is carbon dioxide; it is so popular because of its low critical temperature (31.3°C) and pressure (72.9 atm), and because it is nontoxic, nonflammable, and its use does not result in a waste disposal problem.

Utilization of the unique extraction properties of SFs for the preparation of analytical samples has been reported in a number of publications in the recent technical literature. However, applications involving extraction of compounds regulated by the Environmental Protection Agency have been quite limited. A more detailed discussion of such applications reported in the literature can be found in Appendix A of this report.

The study described in this report was carried out in various phases. In the first phase, we reviewed the literature published on the SFE technique. While the literature gathering and review took place, we contacted manufacturers of SFE instrumentation to identify the most suitable equipment for this study. In the second phase, we purchased an off-line supercritical fluid extractor system from Suprex Corporation and conducted a series of experiments to familiarize ourselves with the instrumentation and to determine the feasibility of extracting various classes of organic compounds from solid matrices. Sand was the primary matrix selected for investigation, although soils and sediments have also been tested. Upon completion of the second phase, we prepared a protocol that describes in detail how a solid sample should be extracted by SFE, the instrumentation requirements, etc. This protocol is included as Appendix B of this report. The third phase was devoted primarily to method optimization, application of the SFE technique to various environmental matrices, and instrument modification to increase sample throughput (e.g., installation of two and four extraction vessels for parallel extractions). The fourth phase which will not be discussed in this report will address the evaluation of various SFE systems that are available commercially.

Experimental

Apparatus

Supercritical fluid extraction system— Suprex Model SE-50. The system was set up either with one, two, or four extraction vessels for parallel extractions. Supercritical pressures were maintained inside the extraction vessels by using uncoated fused-silica tubing as restrictor. Figure 1 shows a schematic diagram of the four-vessel-extraction setup. Eight restrictors were mounted in the 12-port valve to allow collection of two fractions per sample. Fractions 1A, 2A, 3A, and 4A were collected when the 12-port valve was in the load position. Fractions 1B, 2B, 3B, and 4B were collected when the 12-port valve was in the inject position. Collection of the extracted material was performed by inserting the outlet restrictor into a 15-mm × 60-mm glass vial containing an organic solvent spiked with a known concentration of an internal standard (terphenyl-d_{14}).

Hexane was used as collection medium for the experiments performed with carbon dioxide, and methylene chloride or methanol was used as collection medium for the experiments performed with carbon dioxide modified with 10 percent methanol.

Procedure

A known amount of sample (typically 1 to 10 g) was weighed out in an aluminum cup; if the sample had to be spiked with the target analytes, then spiking was performed directly in the aluminum cup by using 100 to 1000 μL volumes of concentrated stock solutions of the target analytes. After the solvent had evaporated completely (approximately 15 min), the spiked sample was transferred to the extraction vessel. Surrogates were spiked directly into those samples already placed in the extraction vessels and that were to be analyzed by GC/MS. Details of sample extraction are given in the protocol included as Appendix B of this report. Extractions were performed with both carbon dioxide and carbon dioxide containing 10 percent methanol at constant temperature and pressure or under conditions in which both the temperature and pressure were varied. Furthermore, several fractions were collected per sample to verify the completeness of the extraction. For example, a standard reference soil contaminated with polynuclear aromatic hydrocarbons (PAHs) was extracted with carbon dioxide with 10 percent methanol as follows: to collect Fraction 1, the extraction was started at 150 atm and 50°C; after pressurizing the vessel for 10 min, the pressure was increased to 200 atm and the temperature to 60°C; after another 10 min, the pressure was increased to 250 atm and the temperature to 70°C and maintained for 10 min. Fraction 2 was collected at 250 atm and 70°C (30 min), Fraction 3 at 300 atm and 70°C (30 min), and Fractions 4 through 9 at 350 atm and 70°C (30 min each).

Method Optimization

The experimental design for method optimization focused on seven variables, each chosen at two levels (high and low). Two separate sets of tests were performed. The seven variables were pressure (P), temperature (D), moisture (M), cell volume (V), sample size (S), time (T), and modifier volume (C) for Test 1, and pressure (P), temperature (D), volume of toluene (F), collection volume (G), moisture (M), glass beads (B), and static extraction time (E) for Test 2. The group differences for Test 1 (V_P through V_C) were calculated using Equations 1 through 7; those for Test 2 were calculated in a similar manner.

$$V_P = 1/4(w + x + y + z) - 1/4(s + t + u + v) = p - P \quad (1)$$

$$V_D = 1/4(u + v + y + z) - 1/4(s + t + w + x) = d - D \quad (2)$$

$$V_M = 1/4(t + v + x + z) - 1/4(s + u + w + y) = m - M \quad (3)$$
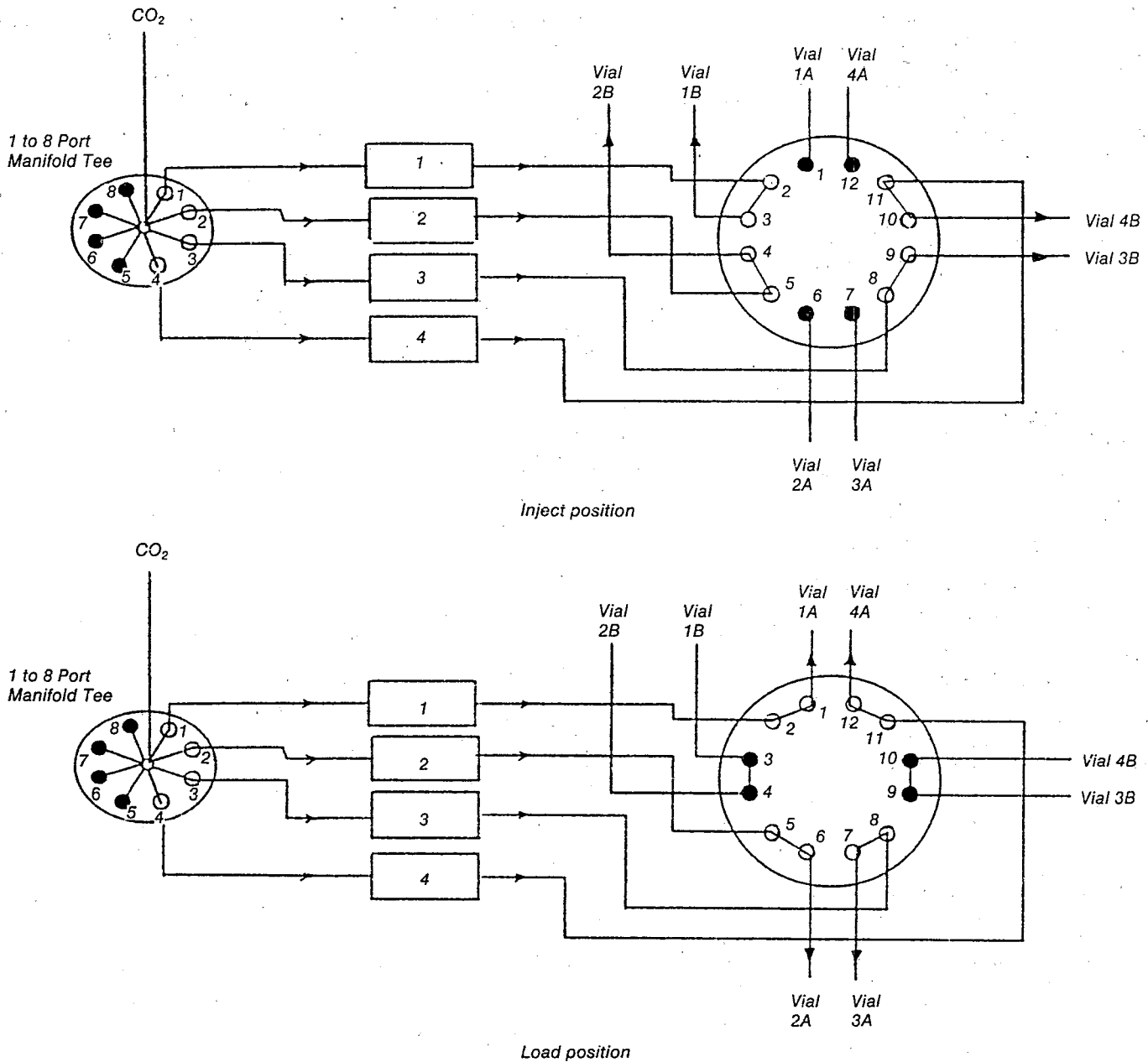
$$V_V = 1/4(u + v + w + x) - 1/4(s + t + y + z) = v - V \quad (4)$$

Figure 1. Schematic representation of four-vessel-extraction setup.

$$V_S = 1/4(t + v + w + y) - 1/4(s + u + x + z) = s - S \quad (5)$$

$$V_T = 1/4(t + u + x + y) - 1/4(s + v + w + z) = t - T \quad (6)$$

$$V_C = 1/4(t + u + w + z) - 1/4(s + v + x + y) = c - C \quad (7)$$

The relative changes as $100 \times$ change/average recovery at low level were calculated and the data were sorted by variable and compound. The variables were ranked in increasing order of absolute relative change for each compound and the sum of ranks was calculated.

A full factorial design experiment was conducted for four variables (time, pressure, moisture, and sample size). All experiments were performed in duplicate using the standard reference soil contaminated with PAHs (SRS103-100 soil). The values for the four variables were as follows: time (1 hr and 2 hrs), pressure (250 atm and 350 atm), moisture content (10 percent and 25 percent), and sample size (1 g and 2.5 g). The duplicate experiments were performed in parallel by splitting off the incoming carbon dioxide to two extraction vessels. All other

variables were kept constant as follows: temperature 70°C, collection solvent—hexane 5 mL, restrictor 50 μm ID, no static extraction, extraction vessel 2 mL.

From the recovery data that we obtained for the 32 experiments, we established the effect of the individual variables, the interaction between the two variables, and the interaction between three variables. Analysis of variance was performed using the general linear model procedure of the Statistical Analysis System.

Results and Discussion

Summary of the SFE Preliminary Work

The extraction efficiencies we achieved for sand samples spiked with Aroclor 1232 and 1260, organochlorine pesticides, organophosphorus pesticides, PAHs, nitroaromatic compounds, haloethers, and some of the base/neutral/acidic compounds targeted by the U.S. Environmental Protection Agency were quite good (an example is shown in Table 1 for the organochlorine pesticides). However, the extraction efficiencies we achieved with the standard reference materials (Tables 2 and 3) varied considerably. Some of our recoveries were much lower than the certified values or the values published by others. However, on closer examination, it turns out that many of the studies reported so far in the literature were more of a qualitative nature, were often conducted with homemade equipment, focused mostly on PAHs, and used small sample sizes (as small as a few milligrams). To develop an SFE method that can successfully be applied to samples of interest to the EPA, we have to use commercially available equipment, consider a wide variety of sample matrices and groups of pollutants, and use sample sizes large enough to compensate for the inevitable inhomogeneities of most real environmental and hazardous waste samples. We, therefore, reassessed our approach to SFE evaluation and first looked more closely at the many variables that affect SFE efficiency, as summarized in the following paragraphs.

Small sample sizes (as small as 20 mg or even less) are adequate when homogeneous material is extracted, e.g., for the extraction of stabilizers and plasticizers from polymers, extraction of waxes, of many pharmaceutical materials, etc.

Table 1. Percent Recoveries and Percent RSDs of 41 Organochlorine Pesticides from Spiked Sand Using Supercritical Carbon Dioxide Modified with 10 Percent Methanol[a]

Compound No.	Compound	Spike Level (ng/g)	Percent Average Recovery	Percent RSD
1	alpha-BHC	62.5	93.6	16.8
2	gamma-BHC	62.5	121	10.8
3	beta-BHC	62.5	93.6	25.2
4	Heptachlor	62.5	91.7	23.0
5	delta-BHC	62.5	103	19.9
6	Aldrin	62.5	98.7	15.9
7	Heptachlor epoxide	62.5	88.6	12.2
8	Endosulfan I	62.5	88.2	10.3
9	4,4'-DDE	62.5	95.4	10.6
10	Dieldrin	62.5	103	19.6
11	Endrin	62.5	92.8	13.6
12	4,4'-DDD	125	120	10.1
13	Endosulfan II	62.5	89.6	6.9
14	4,4'-DDT	62.5	99.9	4.9
15	Endrin aldehyde	125	120	10.8
16	Endosulfan sulfate	62.5	125	8.8
17	Methoxychlor	62.5	107	2.4
18	Endrin ketone	62.5	98.1	6.7
19	Hexachlorocyclopentadiene	62.5	26.1	13.7
20	Etridiazole	62.5	87.9	14.5
21	Chloroneb	1,250	92.5	20.8
22	Propachlor	1,250	95.8	22.1
23	Hexachlorobenzene	62.5	114	7.3
24	Trifluralin	125	94.5	19.7
25	Diallate	1,875	109	9.9
26	Pentachloronitrobenzene	62.5	97.8	14.8
27	Chlorothalonil	125	93.4	9.9
28	Alachlor	625	103	20.5
29	DCPA	62.5	107	5.9
30	Isodrin	62.5	98.6	17.9
31	trans-Permethrin	625	102	17.9
32	gamma-Chlordane	62.5	95.8	18.5
33	alpha-Chlordane	62.5	99.8	11.4
34	trans-Nonachlor	62.5	108	11.4
35	Captan	125	95.0	6.5
36	Perthane	1,875	79.0	8.9
37	Chlorobenzilate	125	28.4	12.6
38	Chloropropylate	62.5	109	10.3
39	Kepone	62.5	106	19.5
40	Mirex	125	96.4	3.7
41	DBCP	62.5	35.4	29.2

[a] The number of determinations was three. The sample size was 8 g. Each extraction was performed with carbon dioxide modified with 10 percent methanol at 150 atm/50°C/10 min static, 200 atm/60°C/10 min dynamic, 250 atm/70°C/10 min dynamic.

However, most environmental and waste samples are inhomogeneous even after mixing. Therefore, the sample sizes specified in SW-846 are 10 g (Soxhlet) and 30 g or 2 g (sonication), depending on the concentrations of the analytes in the sample. For this reason, samples for SFE in the environmental field should be in the 1- to 10-g range, preferably at least 5 g.

There is a lack of standard reference materials that cover the matrices and pollutants of environmental concern. The materials that are available were certified for only a very limited number of compounds, e.g., PAHs. It is therefore difficult, even currently impossible, to determine absolute extraction efficiencies for most analytes because in most cases, removal of a spike from a sample matrix is much easier than removal of "incorporated" or "native" compounds. This, however, is a problem that hampers the evaluation of all extraction methods, not just SFE, and one is usually confined to comparing relative extraction efficiencies.

Table 2. Comparison of Recoveries Obtained by SFE with Certified Values Reported for SRS 103-100[a]

Compound	Certified Value (μg/g)			Percent Average Recovery by SFE[b]	Percent RSD
Naphthalene	32.4	±	8.2	63.8	11.1
2-Methylnaphthalene	62.1	±	11.5	82.6	2.2
Acenaphthylene	19.1	±	4.4	64.6	9.4
Acenaphthene	632	±	105	98.2	4.2
Dibenzofuran	307	±	49	92.9	3.5
Fluorene	492	±	78	80.4	4.3
Phenanthrene	1,618	±	348	124	9.8
Anthracene	422	±	49	78.4	14.7
Fluoranthene	1,280	±	220	92.3	6.5
Pyrene	1,033	±	289	78.2	7.0
Benzo(a)anthracene	252	±	38	67.6	4.2
Chrysene	297	±	26	68.4	10.2
Benzo(b+k)fluoranthene	152	±	22	53.3	14.3
Benzo(a)pyrene	97.2	±	17.1	32.2	19.3
Pentachlorophenol	965	±	374	141	11.1

[a] The number of determinations was three. The sample size was 2.5 g. Each extraction was performed with supercritical carbon dioxide at 300 atm and 70° C for 60 min; 10 percent moisture was added to each sample prior to extraction.
[b] As percent of certified values.

Table 3. Comparison of Recoveries Obtained by SFE with Certified Values Reported for NIST SRM 1941[a]

Compound	Certified Value (μg/g)			Concentration Found (μg/g)	Percent Recovery
Phenanthrene	0.577	±	0.059	0.25	43.3
Anthracene	0.202	±	0.042	ND[b]	–
Pyrene	1.08	±	0.20	0.70	64.8
Fluoranthene	1.22	±	0.24	0.70	57.4
Benzo(a)anthracene	0.550	±	0.079	0.20	36.4
Benzo(b)anthracene	0.78	±	0.19	0.10	12.8
Benzo(k)anthracene	0.444	±	0.049	ND	–
Benzo(a)pyrene	0.67	±	0.13	ND	–
Perylene	0.422	±	0.033	–	–
Benzo(g,h,i)perylene	0.516	±	0.083	ND	–
Indeno(1,2,3-c,d)pyrene	0.569	±	0.040	ND	–
Surrogate recovery					
2-Fluorophenol					64.1
Phenol-d_5					71.5
Nitrobenzene-d_5					86.1
2-Fluorobiphenyl					85.1
2,4,6-Tribromophenol					68.9

[a] The experiments were performed with supercritical carbon dioxide at 350 atm/60° C for 20 min. The sample size was 2 g.
[b] ND–not detected; estimated detection limit 0.1 μg/g.

Temperature and pressure changes affect the density and viscosity of an SF and therefore its solubilizing ability. However, little is understood about what happens on the surfaces of the solid matrices during the extraction process, and what the desorption, solvation, and transport mechanisms are, and little is known about how to optimize pressure and temperature for specific matrices and analyte groups. An understanding of the desorption and transport mechanisms of a solute under supercritical conditions would provide clues if the use of static or dynamic extraction conditions, or a combination of the two, would be advantageous; whether rapid pressure fluctuations would improve extraction rates and, maybe, extraction efficiencies and selectivities.

Modifiers are liquids that can be added to the SF or directly to the sample. They usually are more polar than the basic SF; for example, benzene, toluene, methanol, and acetone have been added to carbon dioxide. Their use improves solvent strength and affects selectivity. However, we are mostly confined to the trial-and-error approach. We do not know to what extent, and in what way, the modifier changes the properties of the supercritical system and in which way solubility is enhanced; the selection of modifiers for specific applications is still largely guesswork.

Draft Protocol

Based on our results from the extraction of relatively simple matrices, we developed a draft protocol in the SW-846 format, "Extraction Procedure Using Supercritical Fluids." Our goal was to write a generic protocol that is applicable to as many different SFE systems as possible. This protocol was written for solid matrices like soils and sediments; the target analytes include organochlorine pesticides, organophosphorus pesticides, nitroaromatics, haloethers, base/neutral/acidic compounds, and PAHs. The protocol addresses interferences, apparatus and materials, sample preparation (including extraction), and quality assurance; recovery data for the above groups of compounds are included. The protocol will be refined and updated as additional data become available.

Method Optimization

The experimental design of our method optimization study focused on two sets of seven variables, each chosen at two levels. A full factorial design would have required 128 experiments. Instead, this design required us to perform only eight experiments in order to estimate the main effects of the two sets of seven variables; however, we could not test for statistical significance of any of these effects because there were no degrees of freedom for the error terms. From these data, one can calculate the effect on the recovery of each variable at its low and its high value using Equations 1 through 7. To get an overall picture of which variables affect

the recovery of the highest number of compounds, the group differences were ranked and the ranks across the 15 compounds were summed up.

From these sums, we concluded that in Test 1 the recovery was most affected by time (sum of ranks 88) and least affected by the volume of the modifier (sum of ranks 31). Pressure and moisture ranked second (sum of ranks 70) and third (sum of ranks 69) in importance after time. The sum of ranks for sample size, temperature, and cell volume are 65, 50, and 47, respectively.

When the experiments were performed at the same pressure, temperature, and moisture conditions but toluene was used as modifier and the extraction vessel was pressurized for 15 or 30 min during the 60-min extraction, then recovery was most affected by moisture (sum of ranks 88) followed by pressure (sum of ranks 70) and volume of toluene (sum of ranks 68). The fourth variable to influence was the static extraction time (sum of ranks 57). Temperature, volume of collection solvent, and the presence/absence of glass beads were the least important.

From the experiments in Test 1, we concluded that the four variables time, pressure, moisture, and sample size should be further investigated. Pressure and moisture were also identified as being important variables in the experiments conducted in Test 2. Therefore, we designed a full factorial experiment in which the four variables were investigated at two levels in 16 experiments. The sample used was again SRS 103-100. From the recovery data, we established the effect of the individual variables, the interaction between two variables, and the interaction between three variables. The significance levels from the analysis of variance (ANOVA) for each of the 15 compounds known to be present in the SRS 103-100 are included in the report. The four-way interaction (time $\times$ pressure $\times$ moisture $\times$ sample size) was not considered in ANOVA.

CONCLUSION

The results of this study indicate that SFE with carbon dioxide or carbon dioxide containing modifiers is an attractive method for the extraction of organic contaminants from environmental solid matrices. Potential advantages of the method include less solvent use and disposal, reduced manpower requirements, and increased speed and selectivity (in combination with modifiers). However, more developmental work has to be done before SFE becomes an easy-to-use, off-the-shelf method.

References

1. Test Methods for Evaluating Solid Waste (1986), 3rd Ed., SW-846, U.S. Environmental Protection Agency, Washington, DC.
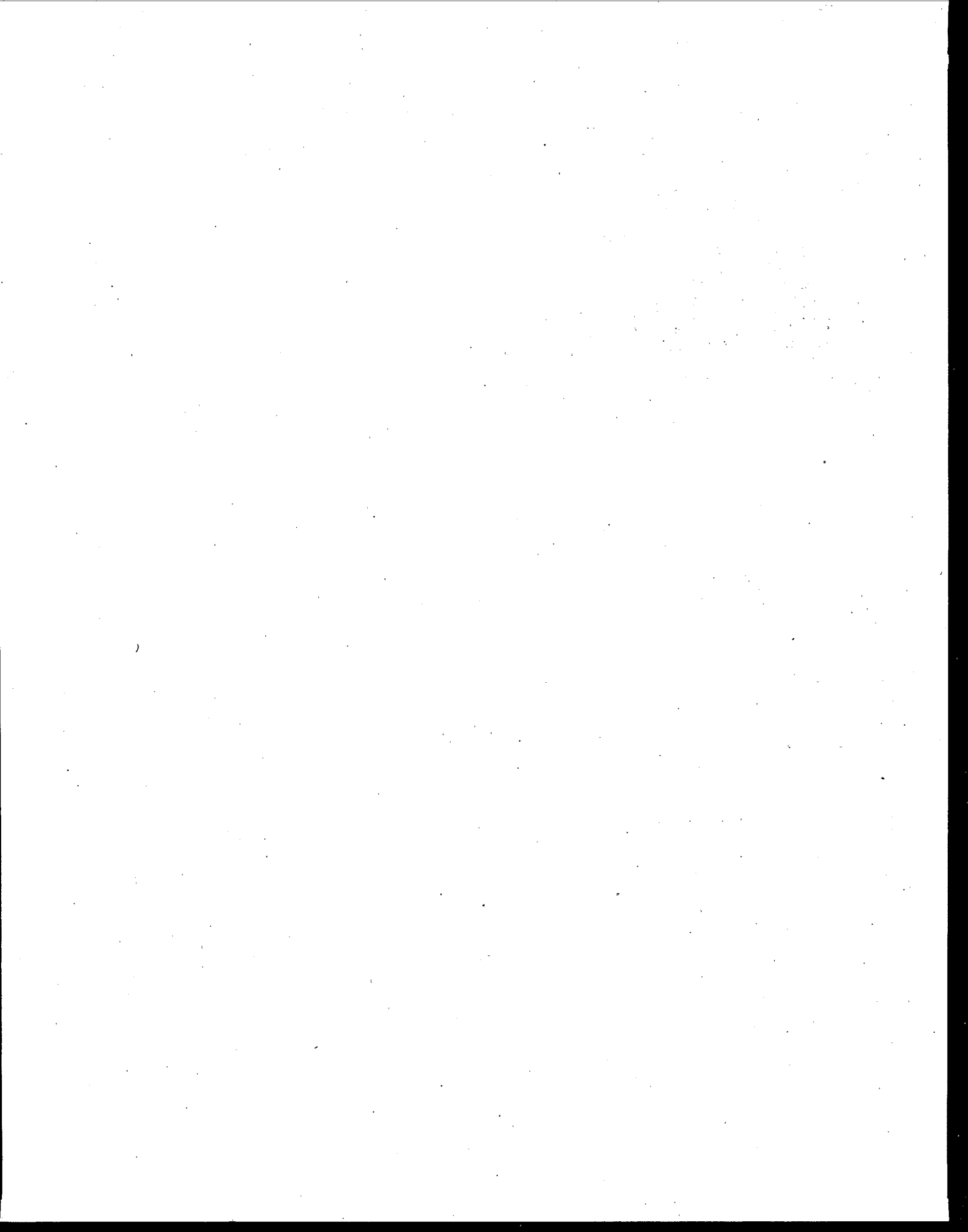
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The complete report, entitled "Method for the Supercritical Fluid Extraction of Soils/ Sediments," (Order No. PB91-127 803/AS; Cost: $31.00, subject to change) will be available only from:

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