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INCREASING EXTRACTION EFFICIENCY IN SUPERCRITICAL FLUID EXTRACTION FROM COMPLEX MATRICES

PREDICTING EXTRACTION EFFICIENCY OF DIURON AND LINURON IN SUPERCRITICAL FLUID EXTRACTION USING SUPERCRITICAL FLUID CHROMATOGRAPHIC RETENTION

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SUMMARY

Supercritical fluid extraction (SFE) conditions have been investigated in terms of mobile phase modifier, pressure, temperature and flow-rate to improve extraction efficiency. Extraction efficiencies of up to 100% in less than 2 h have been obtained. These extraction efficiencies resulted from our extensive parameter optimization experiments.

Packed column supercritical fluid chromatography (SFC) has been used to examine the effect of these parameters on retention behavior. A relationship between chromatographic retention and extraction efficiency was sought. By determining trends of the chromatographic capacity factors using SFC conditions corresponding to SFE parameters, a prediction of extraction efficiency can be made. These predictions have shortened method development time in SFE.

INTRODUCTION

Supercritical fluid extraction (SFE) has eliminated some of the disadvantages of current extraction procedures for analysis of complex mixtures[1,2]. Packed column supercritical fluid chromatography (SFC) has been used as a technique for chromatographic elution of thermally labile agricultural compounds and their metabolites[3]. The adapted instrumentation needed to achieve the union of these two techniques has previously been demonstrated[1]. In this study, extraction conditions have been explored in terms of mobile phase modifier, pressure, temperature and flow-rate. Improved extraction efficiency in less extraction time was the goal of these optimization experiments.

Changing the mobile phase modifier, pressure, temperature and flow-rate can effect the packed column SFC capacity factor and/or limit of detection. Relationships between extraction efficiency and chromatographic retention have been proposed.

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The goal of these studies was to determine the best functional conditions which would yield high efficiencies for extracting thermally labile herbicides from soils and animal materials. Results of these studies will be discussed.

EXPERIMENTAL

Instrumentation

The Hewlett-Packard 1082 liquid chromatograph used in these studies was modified for SFC and extraction. The modifications have previously been described[1,4]. The flow-rate range for this instrument is from 0 to 10 ml/min. The collection device, previously described, is simply a glass vessel after the back pressure regulator[1].

In addition, a Varian 8500 syringe pump (250-ml syringe volume) has been coupled with a Fiatron TC-50 column oven to conduct additional extraction work. The Varian 8500 flow-rate range is from 0 to 16.5 ml/min.

A limitation to the syringe pump is the reservoir size, 250 ml. As opposed to the continuous flow from the diaphragm pumps of the HP1082B, the syringe pump must be refilled approximately every 15 min when pumping at maximum flow-rate. However, the larger flow-rates obtainable with this instrument increase the range over which the extraction efficiency as a function of flow-rate can be explored.

Species of interest

The target compounds of interest for these studies were diuron and linuron and their principle metabolite, 3,4-dichloroaniline. Structures and molecular weights can be found in Table I. The melting points of diuron and linuron are 158 and 93°C, respectively. ^{14}C-labeled diuron and linuron were used for these studies in order to easily monitor extraction efficiency.

Our studies have concentrated on the extraction of these compounds from a sandy loam soil. Soil parameters can be found in Table II.

TABLE I

COMPOUNDS OF INTEREST

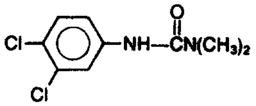
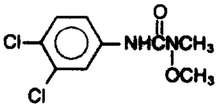
Name	*Structure*	*Mol. wt.*
Diuron		233.1
Linuron		249.1

TABLE II

CHARACTERISTICS OF SASSAFRAS SOIL

Texture	*Sandy loam*
pH (water)	6.1
P, K, Mg, Ca	150, 70, 85, 80 ppm in soil on a volume basis
Organic matter (%)	0.74

Parameters investigated

Temperature, flow-rate, pressure and mobile phase modifier type and concentration were examined for their effect on the extraction efficiency of the species of interest from the sample matrix.

Pressure and temperature were investigated because of their intrinsic influence on the behavior of supercritical fluids. Flow-rate was examined because the extraction system used is a dynamic not a static equilibrium system. Mobile phase modifier was varied based on experimental evidence of the influence of different modifiers on extraction efficiencies. It is expected that modifier effects would vary depending upon the solute and matrix of interest.

Conditions

The starting points for our SFE were determined from classical extraction conditions and SFC retention times.

Classical extraction procedures for diuron and linuron from soil used predominantly one solvent, methanol, to achieve greater than 80% recovery for both diuron and linuron[5]. This liquid–liquid classical extraction procedure is outlined in Table III. The procedure is more labor intensive and time consuming than our simple SFE scheme where the soil sample is pulverized, then packed into an empty 25 cm × 4.6 mm I.D. stainless-steel tube. The total sample handling time for this procedure is 1 h or less.

TABLE III
CLASSIC EXTRACTION METHOD OF DIURON AND LINURON FROM SASSAFRAS SOIL

(1) Pulverize soil.
(2) Extract at room temperature with three portions of methanol–water (9:1), wrist action shaker, centrifuge
(3) Extract at room temperature with three portions of 100% methanol, wrist action shaker, centrifuge

Yield room temperature extractions: 92% diuron, 78–79% linuron
Time: 1 day

(4) Soxhlet extraction with 100% methanol at 65°C

Yield: 3–4% diuron and linuron
Time: 1 day

(5) Soxhlet extraction with 100% hexane at 69°C

Yield: 1% diuron and linuron
Time: 1 day

Chromatographic experiments utilizing different mobile phase modifiers while varying temperatures and pressures showed the ability to vary the capacity factor, k', of these parent compounds. Tables IV–VI illustrate the chromatographic trends for diuron and linuron with methanol, ethanol and acetonitrile modified carbon dioxide mobile phase. The low chromatographic capacity factors and the minimal solvent variety in the classical extraction procedure suggested that diuron and linuron in soil would be ideal candidates for the evaluation of SFE parameters.

The SFE sample handling procedure is simple and requires minimal operator time[1]. Instrument time varies according to the difficulty of the extraction. Generally, the extraction efficiency was measured on a predetermined time basis. Samples were measured at 3- and 15-min intervals in preliminary extraction experiments. Larger samples or more complex matrices required longer extraction times. Extractions of 1–2 h were usually sufficient. Overnight extractions were utilized if necessary.

Efficiency evaluation

After collection of the extracted ^{14}C-labeled diuron and linuron, liquid scintillation counting permitted the extraction efficiency to be monitored during the experiments. Periodic evaluation of the extraction effluent with SFC and liquid chromatography (LC) confirmed the presence of the diuron or linuron parent compound and not the most common hydrolysis on breakdown product, dichloroaniline.

RESULTS

Diuron from Sassafras soil

Effects of temperature. Several studies were conducted to evaluate the effects of temperature. Extractions of 15 min of radiolabeled diuron from Sassafras soil using methanol-modified carbon dioxide were conducted at three different temper-

TABLE IV

CHROMATOGRAPHIC RESULTS USING ACETONITRILE-MODIFIED CARBON DIOXIDE AND VARYING PRESSURES AND TEMPERATURES

(a) Capillary SFC, 100% carbon dioxide, 20 m × 100 μm I.D. SB-cyanopropyl-25, 80°C, initial density = 0.25 g/ml for 5 min, ramp rate = 0.1 g/ml/min, final density = 0.65 g/ml. (b) Packed column SFC, acetonitrile–carbon dioxide (2:98), 4.0 ml/min, Whatman Partisil 5 ODS-3 column, 25 cm × 4.6 mm, I.D.

Conditions	*Temperature (°C)*	*Pressure (bar)*	*k'*	
			Diuron	*Linuron*
a	80	Programmed	8.7	5.7
b	55	220	4.4	0.6
b	55	330	3.0	0.3
b	75	220	3.9	0.8
b	75	330	2.2	0.4
b	100	220	4.2	2.9
b	100	290	2.4	0.8
b	100	330	2.3	0.7

TABLE V

CHROMATOGRAPHIC RESULTS USING METHANOL-MODIFIED CARBON DIOXIDE AND VARYING PRESSURES AND TEMPERATURES

Packed column SFC, methanol–carbon dioxide (4:96), 4.5 ml/min, Whatman Partisil 5 ODS-3 column, 25 cm × 4.6 mm I.D. DCA = Dichloroaniline.

Temperature (°C)	*Pressure (bar)*	*k'*		
		Diuron	*DCA*	*Linuron*
55	220	1.2	0.8	0.6
55	330	0.8	0.6	0.4
75	220	1.3	0.8	0.7
75	330	0.9	0.6	0.5
100	220	1.7	0.9	1.0
100	330	0.9	0.6	0.6

TABLE VI

CHROMTOGRAPHIC RESULTS USING ETHANOL-MODIFIED CARBON DIOXIDE AND VARYING PRESSURES AND TEMPERATURES

Packed column SFC ethanol–carbon dioxide (4:96), 4.5 ml/min, Whatman Partisil 5 ODS-3 column, 25 cm × 4.6 mm I.D.

Temperature (°C)	*Pressure (bar)*	*k'*		
		Diuron	*DCA*	*Linuron*
55	220	1.7	0.9	0.7
55	330	1.2	0.6	0.4
75	220	2.5	1.2	1.1
75	330	1.1	0.7	0.6
100	220	3.2	1.6	1.0
100	330	1.3	0.6	0.6

atures, 55, 75 and 100°C, at a constant pressure. The highest recovery was obtained at 100°C, the lowest at 55°C. A longer study was conducted at the two higher temperatures. Again the higher temperature yielded the greatest recovery: 100°C yielded 81%, 75°C yielded 48% (Fig. 1).

The first 15 min of each extraction yielded the highest recovery, 61% and 47% for the 100 and 75°C temperatures respectively. A leveling of the amount extracted was observed in less than 1 h in both cases; after this hour, the amount which continued to be extracted was reduced to less than 0.5% every 15 min.

Effects of pressure. Increasing the pressure increased the extraction efficiency of diuron from soil during the early extraction times (Fig. 2). Eventually, even though the rate of extraction was slower, the total amount extracted was statistically the same for the three pressure levels investigated, 110, 235 and 338 bar. Recoveries for these 100°C extractions averaged approximately 70%.

Effects of temperature and pressure. To see if the effects which has been exhibited in the previous experiments were additive, the pressure was increased at two

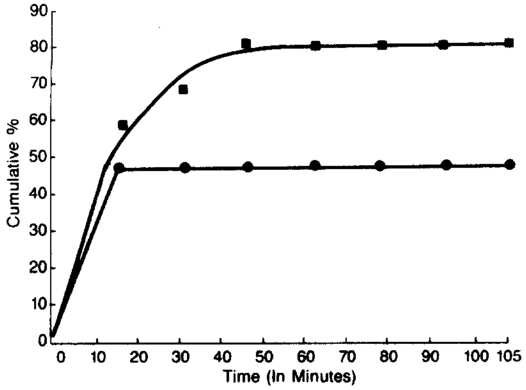


Fig. 1. Effects of increasing temperature on the extraction of diuron from Sassafras soil at constant pressure (340 bar). Flow-rate, 5 ml/min; modifier, methanol (10%). Key: (●) 75°C; (■) 100°C.

different temperatures. Increasing the pressure at 75°C increased the extraction efficiency more than at 100°C. This is not unexpected since the mobile phase density at a constant pressure is greater with lower temperature. Once again, the final extracted yield for the two pressure levels was equivalent at both temperatures.

Effects of mobile phase modifier. When experiments were conducted with no modifier in the supercritical carbon dioxide, no significant quantities of solute were extracted. Increasing the percent modifier increased the extraction efficiency. For 1.25% and 10.0% methanol, the cumulative percents extracted were 4% and 81% respectively (Fig. 3).

Methanol concentrations up to 20% were investigated at lower pressures; the

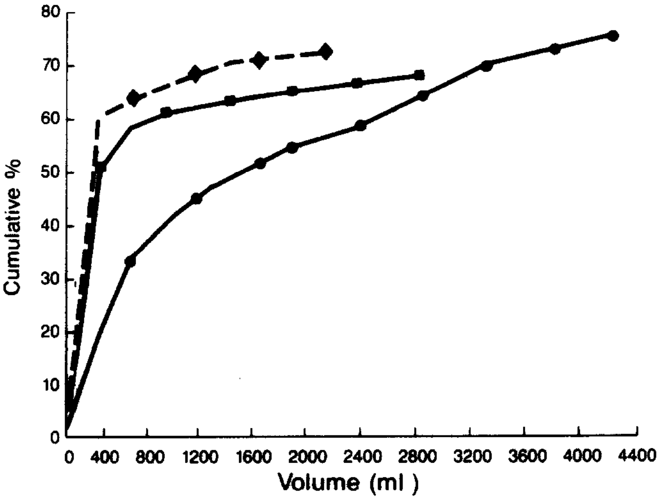


Fig. 2. Effects of increasing pressure on the extraction of diuron from Sassafras soil at constant temperature (100°C). Flow-rate, 16.5 ml/min; modifier, acetonitrile (10%). Key: (●) 110 bar; (■) 235 bar; (◆) 338 bar.

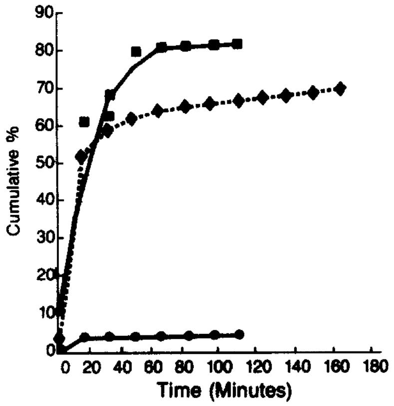


Fig. 3. Effects of modifier on the extraction of diuron from Sassafras soil at constant pressure (340 bar) and temperature (100°C). Flow-rate, 5 ml/min. Key: (●) 1.25% methanol; (■) 10% methanol; (◆) 10% acetonitrile.

general trend of greater extraction efficiency with higher mobile phase modifier concentration was obtained (Fig. 4). Irreproducibility in the amount of methanol delivered from the tank was noted with concentrations in the carbon dioxide above 10%. This was attributed to the solubility of methanol in liquid carbon dioxide as a function of temperature; homogeneity of the mixture as it leaves the tank over a period of time is suspect.

Acetonitrile was examined in addition to methanol modifier because of its slightly higher polarity. The dielectric constant of acetonitrile is 38.8 *versus* 33.6 for methanol. The extraction recoveries for acetonitrile were lower, 69% *versus* 81% for methanol (Fig. 3). The solubility of diuron in methanol is not significantly different than in acetonitrile, indicating that solubilities of solute in the liquid modifier can not necessarily predict extraction efficiency.

An investigation of solute solubility in the supercritical mobile phase was conducted where six pesticides were extracted from activated carbon using supercritical

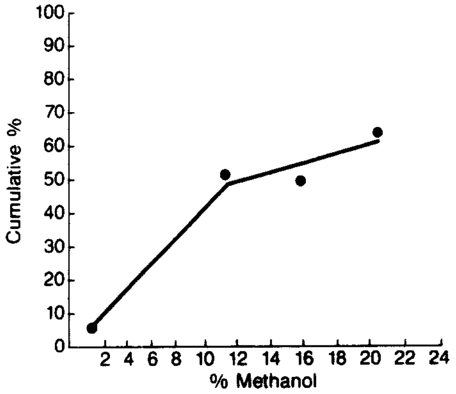


Fig. 4. Effects of increasing mobile phase modifier strength on the extraction of diuron from Sassafras soil at constant pressure (340 bar) and temperature (100°C). Flow-rate, 5 ml/min.

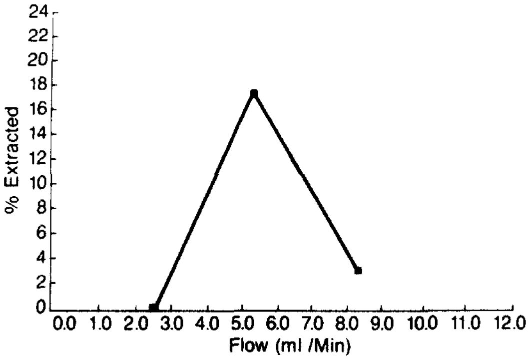


Fig. 5. Effects of increasing flow-rate on the extraction of diuron from Sassafras soil. Modifier, 10% methanol; inlet pressure, 350 bar; temperature, 75°C.

carbon dioxide[6]. In essence, their conclusions were that some sites of activated carbon were easier to regenerate than others. This was attributed to inconsistent strength of sorption forces in some positions. Higher solubilities in carbon dioxide did not yield easier removal. In addition to adsorption effects, surface chemical reactions can vary depending on the available site. These same matrix effects could be influencing our extraction experiments.

Effects of flow-rate. Experiments investigating the effects of flow variations were conducted at three rates. The percent extracted increased with a change in flow from 2.5 to 5.0 ml/min. When the flow-rate was increased from 5.0 to 7.5 ml/min, the extraction efficiency dropped dramatically (Fig. 5). Examining the pressure drop across the extraction column explained these results (Fig. 6). The pressure drop at 2.5 ml/min was 100 p.s.i., 400 p.s.i. at 5.0 ml/min, and greater than 1000 p.s.i. at 7.5 ml/min. Better control of the pressure drop across an extraction tube yields higher extraction efficiencies at higher flow-rates than 5 ml/min.

Higher pressures and temperatures were also examined at the low flow-rate of 2.5 ml/min. Recovery increases were negligible (< 1%).

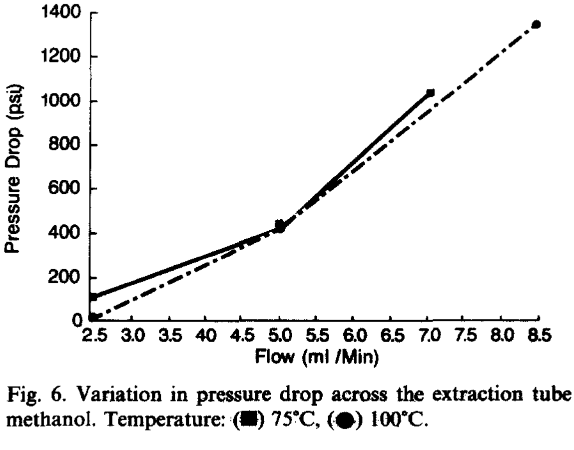


Fig. 6. Variation in pressure drop across the extraction tube with change in flow-rate. Modifier, 10% methanol. Temperature: (■) 75°C, (●) 100°C.

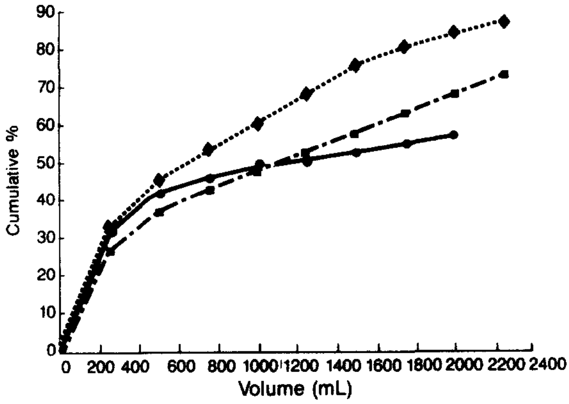


Fig. 7. Effects of increasing temperature on the extraction efficiency of linuron from Sassafras soil. Modifier, 10% acetonitrile; pressure, 290 bar; flow-rate, 16.5 ml/min. Temperature: (●) 80°C; (■) 100°C; (◆) 120°C.

Linuron from Sassafras soil

Effects of temperature and pressure. As was exhibited for diuron, increases in temperature and pressure increased the extraction efficiency of linuron from soil. Temperatures were varied up to 120°C, which significantly increased the recovery over the 100°C result from 74% to 90%. Extraction recoveries were not shown to level off with time at the lower pressures (Fig. 7 and 8).

Effects of mobile phase modifier. A drastic increase in extraction efficiency was exhibited when 10% ethanol was used as the polar modifier as opposed to methanol. The dielectric constant for ethanol is 25.0 compared to 33.6 for methanol. This seemed to suggest that lower polarity modifiers would be more conducive for these extractions; however, extractions involving more non-polar solvents as modifiers such as methylene chloride and chloroform yielded negligible recoveries. Our current hypothesis is that the polarity of the mobile phase mixture must be optimized to match the polarity of the solute.

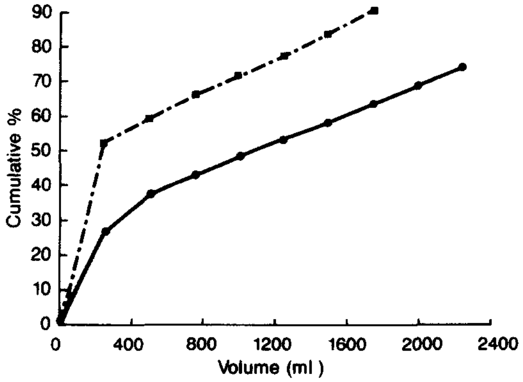


Fig. 8. Effects of increasing pressure on the extraction efficiency of linuron from Sassafras soil. Flow-rate, 16.5 ml/min; modifier, 10% acetonitrile; temperature, 100°C. Pressure: (●) 290 bar; (■) 310 bar.

Effects of flow-rate. At 75 and 100°C, the change in extraction efficiency with increasing flow followed the same trends as seen in the diuron extractions. Pressure drops across the extraction tubes were not as great; this is a function of how the individual extraction tubes were packed and is an error intrinsic to our system. Correspondingly, the percent extracted did not decrease as dramatically at the higher flow-rates.

Chromatographic correlations

The k' values (see Tables IV–VI) decreased with an increase in pressure for diuron and linuron. This supports the increased extraction efficiencies noted at higher pressures. In general, throughout all the parameters investigated linuron had lower capacity factors than diuron. Equal or higher extraction efficiencies were achieved for linuron over diuron, suggesting elution order or capacity factor can be used as an aid in predicting extraction efficiency.

Mobile phase modifier concentration is well known as a major influence in chromatographic retention. Increases in modifier concentration decreased k' values[2]. This supports the increase in extraction efficiency obtained with increased modifier in the carbon dioxide mobile phase.

Temperature trends vary both in our extraction experiments and in our chromatographic retention studies. An influence of an alternate mechanism is possible with these trends. Linuron has a lower melting point than diuron, 93°C and 158°C respectively, and a higher vapor pressure. During the extraction experiments, the volatility of the major metabolite of both linuron and diuron, dichloroanaline, could also have an influence. Further investigations are required.

CONCLUSIONS

Increasing temperature yields increased extraction efficiencies of diuron, linuron and their metabolite dichloroaniline from soil. As pressure is increased higher extraction efficiencies over shorter time periods result. Longer extraction times at lower pressures yield equivalent recoveries to those obtained with the higher pressures.

Increasing flow-rates result in higher extraction efficiencies; these effects start to diminish when larger pressure drops are obtained over the length of the sample extraction tube. An optimum flow-rate of 5.0 ml/min for our extraction experiments has been determined.

Increased concentration of polar modifier supplies higher extraction efficiencies; extraction efficiencies vary with the mobile phase modifier as shown with acetonitrile *versus* methanol and methanol *versus* ethanol. This relationship changes with solute of interest and sample matrix.

SFC retention characteristics enable some prediction of extraction efficiency. Our studies assume that the chromatographic column is behaving as a soil matrix; a more suitable stationary phase might yield more parallel results.

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