

Extraction of Atrazine and Its Metabolites Using Supercritical Fluids and Enhanced-Fluidity Liquids

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Abstract

Supercritical fluid and enhanced-fluidity liquid extractions are performed on spiked sediment samples containing atrazine (ATRA) and five of its metabolites including desisopropyl-desethylatrazine, desethylhydroxyatrazine (DEHA), desisopropylatrazine, desethylatrazine, and hydroxyatrazine (HA). The hydroxylated metabolites are of particular interest because of their increased water solubility and the fact that their high polarity makes them difficult to analyze. Soxhlet extractions using methanol are conducted for the purpose of comparison. Results of the extractions show that the hydroxy-containing metabolites of ATRA are not effectively extracted with supercritical CO₂ alone. The solvating or desorbing power of carbon dioxide appears too low to extract HA and DEHA. The extraction recoveries of the hydroxylated metabolites increase when enhanced-fluidity liquid mixtures of methanol/CO₂ are used, and these rates increase with the methanol concentration. Enhanced-fluidity ternary liquid mixtures of H₂O/methanol/CO₂ yield the best recoveries for these compounds. ATRA recoveries are equally effective when using supercritical CO₂ or enhanced-fluidity mixtures. The other nonhydroxy-containing metabolites require the increased solvent strength of either large percentages of methanol in CO₂ or ternary mixtures of H₂O, methanol, and CO₂ for high recoveries. Recoveries with enhanced-fluidity liquid ternary mixtures are better than the recoveries from Soxhlet for all the compounds in the study.

the most widely used triazines (3,4,5). In the United States, it is used from the cornbelt to the pineapple groves of Hawaii for weed prevention, which increases crop yields (6).

In November 1994, the United States Environmental Protection Agency (EPA) initiated a special review of ATRA along with two other triazines, simazine (SIM) and cyanazine (3). The reason for this review was to investigate the growing concern that residues of ATRA and SIM in food and drinking water may increase health risks to the public. There were also health concerns for people that handle triazines. As with any herbicide, it

Table I. Compound Names, Abbreviations, Formulas, Water Solubility*, and pK_a* of ATRA and Several of Its Metabolites

Compound	Formula	Water solubility (mM)	pK _a
ATRA	C ₈ H ₁₄ ClN ₅	0.152	1.68
SIM	C ₇ H ₁₂ ClN ₅	0.025	1.65
HA	C ₈ H ₁₅ ON ₅	0.24	5.15
DEA	C ₆ H ₁₀ ClN ₅	2.0	1.3
DIA	C ₅ H ₈ ClN ₅	1.2	1.3
DIHA	C ₅ H ₉ ON ₅	> 2.0	4.65
DEHA	C ₆ H ₁₁ ON ₅	> 2.0	4.75
DEDIA	C ₃ H ₄ ClN ₅	> 2.0	1.5

*Information obtained from references 8 and 18.

Introduction

Environmental studies on pesticides continue to attract much interest in recent years. New instrumentation with the capability to detect even smaller amounts of these pollutants are constantly being developed and introduced (1). Herbicides are widely used in the agriculture of industrialized countries. Atrazine (ATRA, a member of the s-triazine group) is in wide use worldwide for both pre-emergence and post-emergence weed control (2,3). Since its initial synthesis in the 1950s, ATRA has become one of

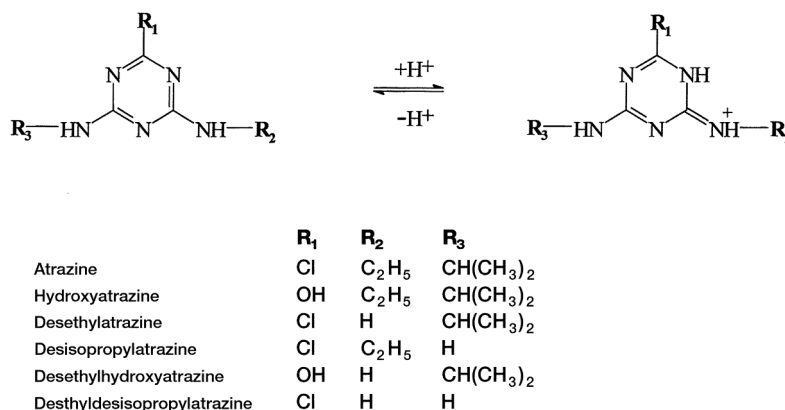


Figure 1. Structure of ATRA and its metabolites.

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must remain in the sediment long enough to eliminate selected weed species while not adversely affecting the crop.

ATRA is found in parts-per-billion levels in the drinking water of many communities in the United States, especially communities in the Midwest in which the bulk of the corn crop is grown. The maximum contaminant level allowed by the EPA for ATRA is 3 ppb (3). As of 1994, ATRA was one of 18 pesticides that the EPA regulates in drinking water (2).

Much of the concern about ATRA today is because of its breakdown from dealkylation and hydroxylation reactions into components such as hydroxyatrazine (HA) after its application (4,7,8). The formula, water solubility, and pK_a of ATRA and some of its metabolites are listed on Table I. The amount of ATRA or any of its metabolites found in a given soil is directly related to such factors as the amount of ATRA originally applied and over what period, the soil and subsoil types, the amount of precipita-

tion, sunlight, the crop grown, and organic matter. For studies in which ATRA was applied to soil over a period of years, it was discovered that significant amounts of the parent compound as well as the metabolites were present, and generally HA was present in higher concentrations than desethylatrazine (DEA) or desisopropylatrazine (DIA) (5,6,9,10). This is expected because hydroxylation is considered to be a major route of ATRA breakdown; HA also has a longer half-life and is readily adsorbed by soil organic matter (5).

Concentrations as high as 52 ppm of HA have been measured in soil (5). Although degradation of ATRA into its metabolites effectively renders it nonphytotoxic, the metabolites are more soluble in H_2O and thus more mobile than the parent compound. This makes the metabolites potentially more dangerous as water-borne contaminants than the parent until they themselves are further degraded (11).

In this study, ATRA and five of its more common metabolites including DEA, DIA, desethyldeisopropylatrazine (DEDIA), and the hydroxy-containing metabolites HA and desethylhydroxyatrazine (DEHA) were extracted from spiked sediment samples. The hydroxylated metabolites represented the most polar compounds extracted with enhanced-fluidity solvents in our laboratory to date. The chemical structure of each varies only by the substitute attached to the basic s-triazine ring (Figure 1).

Buffalo River sediment, SRM-2704 from the National Institute of Standards and Technology, was chosen to represent the natural location for these compounds if leaching from crop fields had occurred. This sediment consisted of fine clay particles and approximately 3% organic carbon. The sediment was spiked and allowed to interact with the analytes for three weeks before the solvent was evaporated. The matrix was then allowed to age for an additional three weeks to allow time for the dry sediment to equilibrate with the analytes. Greater adsorption and equilibration were expected with this longer spiking period (12,13). This would more closely mimic natural conditions (14). This also was well

Table II. Results for the Soxhlet Extractions Using Methanol as Solvent

Compound	Concentration ($\mu\text{g/g}$)	% Recovery	% Relative standard deviation
DEDIA	15 \pm 0.1	54	4
DEHA	15 \pm 0.1	66	4
DIA	15 \pm 0.1	65	3
DEA	15 \pm 0.1	55	1
HA	15 \pm 0.1	80	2
ATRA	15 \pm 0.1	67	1
DIHA (surrogate)	15 \pm 0.1	97	4

Table III. DIHA recoveries by condition

	SFE/ CO_2	BME of MeOH/ CO_2	TME of H_2O MeOH/ CO_2	BME and TME with modifiers	Soxhlet
Average	89.4	95.1	99.8	89.3	97.0
RSD	2.7	5.6	1.3	14.6	4.0

Table IV. Summary of the Recoveries for Each Compound for All SFE and Binary Extraction Mixtures Used

Condition (mol%), temperature ($^{\circ}\text{C}$), and pressure (atm)	% Recovery DEDIA	% Recovery DEHA	% Recovery DIA	% Recovery DEA	% Recovery HA	% Recovery ATRA
100% CO_2 , 50 $^{\circ}\text{C}$, 238 atm	0 (0)*	0 (0)	43 (15)	49 (10)	0 (0)	73 (3)
100% CO_2 , 50 $^{\circ}\text{C}$, 306 atm	0 (0)	1 (1)	38 (6)	49 (5)	0 (0)	73 (5)
100% CO_2 , 50 $^{\circ}\text{C}$, 408 atm	8 (3)	0 (0)	39 (1)	47 (1)	0 (0)	67 (3)
100% CO_2 , 100 $^{\circ}\text{C}$, 306 atm	10 (1)	0 (0)	41 (10)	51 (10)	0 (0)	72 (5)
100% CO_2 , 150 $^{\circ}\text{C}$, 306 atm	9 (3)	0 (0)	36 (2)	46 (1)	0 (0)	67 (3)
10% $CH_3OH/90\%$ CO_2 , 50 $^{\circ}\text{C}$, 306 atm	35 (5)	3 (2)	68 (4)	63 (3)	7 (3)	74 (5)
20% $CH_3OH/80\%$ CO_2 , 50 $^{\circ}\text{C}$, 238 atm	60 (4)	5 (1)	71 (1)	64 (1)	12 (1)	73 (1)
20% $CH_3OH/80\%$ CO_2 , 50 $^{\circ}\text{C}$, 306 atm	52 (13)	9 (1)	73 (3)	63 (3)	19 (3)	71 (3)
20% $CH_3OH/80\%$ CO_2 , 50 $^{\circ}\text{C}$, 408 atm	64 (3)	8 (2)	73 (5)	66 (2)	11 (4)	74 (3)
20% $CH_3OH/80\%$ CO_2 , 100 $^{\circ}\text{C}$, 306 atm	54 (4)	7 (2)	74 (1)	65 (1)	14 (3)	74 (2)
20% $CH_3OH/80\%$ CO_2 , 150 $^{\circ}\text{C}$, 306 atm	41 (2)	6 (2)	67 (8)	60 (6)	6 (3)	70 (5)
40% $CH_3OH/60\%$ CO_2 , 50 $^{\circ}\text{C}$, 306 atm	53 (3)	39 (5)	75 (4)	69 (5)	62 (3)	76 (3)
60% $CH_3OH/40\%$ CO_2 , 50 $^{\circ}\text{C}$, 306 atm	39 (1)	55 (3)	65 (4)	60 (1)	71 (3)	72 (0.4)
80% $CH_3OH/20\%$ CO_2 , 50 $^{\circ}\text{C}$, 306 atm	57 (5)	67 (1)	74 (4)	68 (2)	80 (1)	75 (4)

* % RSD shown in parentheses.

beyond the spiking time of 24 or less hours reported in other studies. For example, Robertson et al. (2) and Janda et al. (15) allowed the spiked solutions to interact with the matrix overnight, Ashraf et al. (16) allowed an interaction time of less than 24 h, and Papilloud et al. (6) allowed an interaction time of 2 h.

Supercritical CO₂, supercritical mixtures of methanol/CO₂, and enhanced-fluidity liquid mixtures of methanol/CO₂ and H₂O/methanol/CO₂ were compared as extraction solvents. The relative importance of pressure, temperature, and extraction solvent composition to the overall solute recovery and the extraction rate was studied. Also, the effect of the direct addition of modifiers such as HCl, NaOH, and CaCl₂ to the sediment prior to exposure to the extraction solvent was studied. Soxhlet extractions were performed for comparison.

Experimental

Materials

Analytical-grade triazine compounds including ATRA, HA, DEHA, desisopropylhydroxyatrazine (DIHA), DEA, DIA, and DEDIA were donated by Ciba-Geigy Corporation (Greensboro, NC). Compound purity exceeded 94%. Solvents used during this research included high-performance liquid chromatographic (HPLC)-grade methanol, water, and monobasic potassium phosphate (Fisher Scientific, Pittsburgh, PA).

SIM was chosen as the internal standard for the chromatographic analysis because it was readily detected at the same wavelength ($\lambda = 220$ nm) used for the other triazines. DIHA was added to the collection solvent prior to extraction in order to act as a surrogate that could provide information on the collection efficiency. This compound was chosen because of its similarity in structure to the other compounds and its being a hydroxylated triazine.

One-hundred parts-per-million standards were made first for each compound then serially diluted to 10 ppm or less as needed for analysis. ATRA, DEA, and DIA went into the methanol solution immediately; however, DEDIA went into solution only after sonication. The hydroxyatrazines (DEHA, DIHA, and HA) dissolved after adding 1% HPLC-grade acetic acid followed by sonication. All standards were refrigerated (2°C) after preparation and before and after each use.

Preparation of sediment samples

The Buffalo River sediment was spiked with 15 ppm of ATRA, HA, DIA, DEA, DEHA, and DEDIA. The spiking solvent was methanol. Sediment samples were immediately refrigerated at 2°C and shaken daily over at least a three-week period, then evaporated under a stream of dry N₂. Samples were then aged for a minimum of an additional three weeks in a refrigerator before any extractions were performed. When a sample was analyzed, the bottle was removed from the refrigerator then shaken, and a sample of sediment was obtained from the container.

Extraction system

The extraction system consisted of an ISCO (Lincoln, NE)

260-D syringe pump with a maximum operating pressure of 510 atm (442 atm, 6500 psi) and an ISCO SFX 210 supercritical fluid extractor with a maximum operating temperature of 150°C. Extraction mixtures were prepared with two ISCO 260-D syringe pumps. Pump A contained CO₂ at the desired pressure. The necessary amount of H₂O or methanol or both were then placed in a second pump (pump B), and then the correct volume of CO₂ was added from pump A to pump B. The pressure of pump B was cycled after combining the components of the mixture to provide mixing. The mixture was allowed to equilibrate at pressure for a minimum of 24 h before extractions were performed. When water was used, it was premixed with methanol before its addition to syringe pump B.

The extraction cartridges used in the supercritical fluid extractor were stainless steel 2.5-mL cells with a 0.5- μ m frit on each side to prevent solid material from escaping and going into the restrictor and collection bottle. A 30- μ m-i.d. fused-silica tube (approximately 25 cm in length) was used as a flow restrictor. The extracts were collected in 10-mL collection bottles fitted with Teflon caps. Before their use, all bottles were washed with acetone and methanol and then dried in a 100°C oven. The extraction cell, frits, and ends were sonicated in acetone and dried in an oven between extractions. The frits were also periodically cleaned with dilute HNO₃ then sonicated in acetone and dried in the oven.

Extraction procedures

A 1-g sample of sediment was used during each extraction, and these were run in triplicate. Sediment was placed into the extraction cell after the placement of a 0.5- μ m frit and a small amount of glass wool that was gently positioned using an inverted pipette. More glass wool was added on the top of the cylinder and again gently placed in position using an inverted pipette. Another 0.5- μ m frit was placed at the top of the cylinder and the endcap was firmly attached. The extraction vessel was then placed in the body of the ISCO SFX 210 Extractor and allowed to preheat for 2 min before the chamber was filled with the extraction fluid from the syringe pump.

The progress of the extractions was always measured in terms of the milliliters of fluid that had passed through the extraction cell rather than time, because the flow rate varied slightly with

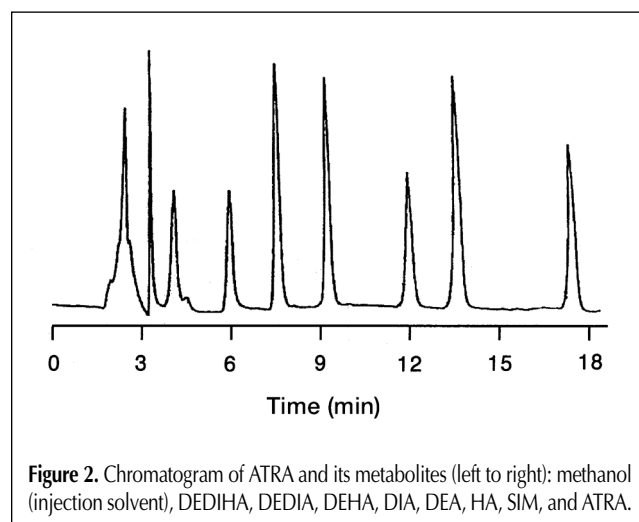


Figure 2. Chromatogram of ATRA and its metabolites (left to right): methanol (injection solvent), DEDIHA, DEDIA, DEHA, DIA, DEA, HA, SIM, and ATRA.

pressure and the partial blockage of the restriction tubing was possible. The extraction was stopped after a specified volume of fluid had passed from the pump through the extraction cell and into a collection bottle. Collection bottles were initially filled with 2 mL of methanol spiked with 10 ppm of DIHA and an additional 1 mL of HPLC-grade methanol. Collections were made after 2.5, 5.0, 7.5, 10.0, and 12.5 mL of extracting solvent. After the collection was complete, 0.5 mL of the 10-ppm internal standard (SIM) was added to each collection vial. Samples were then evaporated under a mild stream of dry N₂ to approximately 1 mL for analysis by HPLC.

Supercritical fluid extraction/CO₂ experiments

Experiments were conducted at five different conditions. The temperature varied from 50 to 150°C and the pressure from 238 atm (3500 psi) to 408 atm (6000 psi) using 10 mL of CO₂ each. Flow rates of approximately 0.4 mL/min were maintained by using a fused-silica capillary restrictor. Plugging of the restrictor during the supercritical fluid extraction (SFE) experiments was not a significant problem, although variations in the flow rate were noticed primarily during the introduction of the first 2.5 mL of the solvent to the extraction cell.

Binary mixtures of methanol/CO₂

Samples were extracted with binary mixtures of methanol and CO₂. All extractions were maintained under single-phase liquid conditions (17). Binary mixture extraction (BME) experiments were conducted at the following mole percentage (mol%) levels: 10% methanol/90% CO₂ at 50°C and 306 atm (4500 psi), 20%

methanol/80% CO₂ at 50°C and 306 atm, 20% methanol/80% CO₂ at 50°C and 238 atm, 40% methanol/60% CO₂ at 50°C and 306 atm, 60% methanol/40% CO₂ at 50°C and 306 atm, and 80% methanol/20% CO₂ at 50°C and 306 atm. Plugging of the restrictor was noticed, especially during experiments using less than 20% methanol.

In addition, to compare the relative importance of having the extraction mixtures in the supercritical or liquid state, the following binary mixtures were studied under supercritical fluid conditions (above critical point of mixture): 20% methanol/80% CO₂ at 100°C and 306 atm (4500 psi), 20% methanol/80% CO₂ at 150°C and 306 atm (4500 psi), and 20% methanol/80% CO₂ at 50°C and 408 atm (6000 psi).

Extraction with ternary mixtures (H₂O/methanol/CO₂)

Samples were extracted with ternary liquid mixtures of H₂O and methanol CO₂. All extractions were conducted under single-phase liquid conditions (18). Ternary mixture extraction (TME) experiments were run under enhanced-fluidity conditions at the following mol% compositions: 1% H₂O/9% methanol/90% CO₂ at 50°C and 306 atm (4500 psi), 2% H₂O/18% methanol/80% CO₂ at 50°C and 306 atm (4500 psi), 4% H₂O/36% methanol/60% CO₂ at 50°C and 306 atm (4500 psi), 8% H₂O/52% methanol/40% CO₂ at 50°C and 306 atm (4500 psi), and 16% H₂O/64% methanol/20% CO₂ at 50°C and 306 atm (4500 psi).

Direct addition of modifiers to matrix

The effect of directly adding modifier solvent to the sediment prior to extraction was also studied as a comparison with the

Table V. Summary of the Recoveries of Each Compound for All Ternary, Direct Modified, and Soxhlet Extractions Used

Condition (mol%), temperature (°C), and pressure (atm)	% Recovery DEDIA	% Recovery DEHA	% Recovery DIA	% Recovery DEA	% Recovery HA	% Recovery ATRA
1% H ₂ O/9% CH ₃ OH/90% CO ₂ , 50°C, 306 atm	55 (15)*	5 (3)	77 (5)	67 (4)	27 (5)	78 (5)
2% H ₂ O/18% CH ₃ OH/80% CO ₂ , 50°C, 306 atm	42 (5)	41 (5)	75 (4)	63 (3)	88 (14)	76 (2)
4% H ₂ O/32% CH ₃ OH/60% CO ₂ , 50°C, 306 atm	59 (9)	76 (5)	71 (3)	64 (2)	103 (3)	72 (2)
8% H ₂ O/52% CH ₃ OH/40% CO ₂ , 50°C, 306 atm	72 (4)	88 (4)	78 (1)	68 (1)	98 (3)	77 (2)
16% H ₂ O/64% CH ₃ OH/20% CO ₂ , 50°C, 306 atm	66 (9)	80 (3)	75 (3)	65 (2)	91 (3)	74 (2)
Direct modified HCl, 50°C, 306 atm	31 (6)	38 (11)	57 (4)	57 (2)	53 (10)	59 (1)
Direct modified NaCl, 50°C, 306 atm	31 (2)	36 (7)	62 (2)	59 (3)	49 (9)	62 (1)
Direct modified CaCl ₂ , 50°C, 306 atm	73 (9)	–(–) [†]	78 (4)	67 (2)	93 (4)	72 (4)
Soxhlet	54 (4)	66 (4)	65 (3)	55 (1)	80 (2)	67 (1)

* % RSD shown in parentheses.
[†] An unknown coeluting compound prevented quantitation of DEHA at this condition.

Table VI. Best Extraction Condition(s) Compared with Soxhlet

Compounds	Best extraction condition	% Recovery	% Soxhlet recovery
HA (5.15)*	TME 4% H ₂ O	102.5	79.7
DEHA (4.75)	TME 8% H ₂ O	88.2	65.5
ATRA (1.68)	All TME, BME, SFE, direct modified CaCl ₂ , and Soxhlet	66.7–77.9	66.8
DEDIA (1.5)	TME 8% H ₂ O and direct modified CaCl ₂	72.0–72.6	54.1
DIA (1.3)	TME *% H ₂ O and direct modified CaCl ₂	78.4–78.1	65.3
DEA (1.3)	BME 40% methanol and TME 8% H ₂ O	68.6–68.4	54.9

* The pK_a value shown in parentheses.

base condition. The extractor was then slowly filled with the solvent mixture and allowed time to equalize before starting the extraction. For these tests, 100 μL each of 0.1M HCl and 0.1M NaOH and 500 μL of 0.1M CaCl_2 were added in separate tests and run in triplicate in the same way as the other enhanced-fluidity liquid extraction experiments.

Soxhlet extractions

Soxhlet extractions were conducted using a micro Soxhlet extractor. One gram of the spiked sediment was added to a thimble and then placed in the extractor. Methanol was used as the solvent. Fifteen milliliters were added to the apparatus recovery flask. Two milliliters of methanol spiked with 10 ppm DIHA was added to the recovery flask along with a boiling stone. Extractants were recovered at intervals of 2, 4, 8, 12, 24, and 36 h and poured into collection bottles spiked with 0.5 mL of methanol containing 10 ppm SIM then evaporated using a mild stream of N_2 until approximately 2 mL remained. Samples were then analyzed using HPLC–UV.

HPLC system and analysis

HPLC analysis was chosen for this work because the non-volatile highly polar metabolites such as the hydroxy *s*-triazines could not be analyzed with gas chromatography without undergoing prior derivatization. HPLC offers direct analysis of both the parent compounds and the polar metabolites without any derivatization (19). Samples were run on a Varian (Palo Alto, CA) Gradient HPLC Pump Model 5500 connected to a Waters (Milford, MA) Model 991 Diode Array Detector. The column used in this study was an Alltech (Deerfield, IL) Platinum EPS C18 (150 \times 4.6 mm). Detection of quantities of triazines as low as 0.2 ng was possible at 220 nm.

Solvent A was mixed as 15% acetonitrile and 85% water by volume with a 5mM potassium phosphate monobasic buffer. Buffer was added to the solution and then sonicated until crystals were completely dissolved. The solution was then degassed with a vacuum pump. Solvent B was mixed as 50% acetonitrile and 50% water by volume with a 5mM potassium phosphate monobasic buffer. Buffer was added to solution and then sonicated until crystals were completely dissolved. The solution was then degassed with a vacuum pump. The study used a gradient mixture starting with 100% Solvent A and ending with 40% Solvent A and 60% solvent B. This gradient maintained peak separation while reducing the retention time for the compounds retained longer by the column. A typical chromatogram for a 2-ppm standard mixture of the triazine compounds is shown in Figure 2.

Data treatment

The extraction results were analyzed with single-factor analysis of variance (ANOVA) with replication and *t*-test comparisons using Microsoft Excel software. These were conducted to determine if the treatment differences were a result of chance or the effects themselves. The means, standard deviation, and experimental *t*

values were calculated. A resulting *p* value was obtained for multiple sets of data using ANOVA and two sets of data using *t*-test. A *p* value of 0.05 or less was deemed significant. The single-factor ANOVA investigated whether the means of the multiple sets of sample variation for a single parameter were actually statistically different or a result of chance, and the *t*-test did a similar comparison for two sets of data. Error bars on all Figures represent the standard deviation.

Results and Discussion

Soxhlet recoveries

Soxhlet recoveries versus the initial spiking level are shown in Table II. Recovery percentages ranged from 54% for DEDIA to 80% for ATRA. The surrogate DIHA that was added directly to the collection solvent bottle in the extractor setup had a mean recovery yield of 97% with 4% relative standard deviation (RSD). The recoveries from Soxhlet reached maximum yields after 8 h for ATRA and the nonhydroxy-containing metabolites including DIA, DEA, and DEDIA. The recoveries from the hydroxy metabolites (HA and DEHA) reached maximum yields in 24 h. Experiments were run for 36 h with no analytes appearing after 24 h.

Surrogate quantitation

Surrogate recoveries for the SFE/ CO_2 , BME, TME, and direct modified BME are shown in Table III. The purpose of the surrogate was to account for sample losses during the collection and concentration step of the extractions. As previously stated, DIHA was used as the surrogate during all experiments including SFE, BME, TME, direct modified extractions, and Soxhlet. DIHA was added to the methanol in the collection bottle prior to beginning the extraction. The loss of DIHA during extraction can be attributed to the loss that occurred during both the collection and concentration steps.

During Soxhlet extractions, losses from the collection should

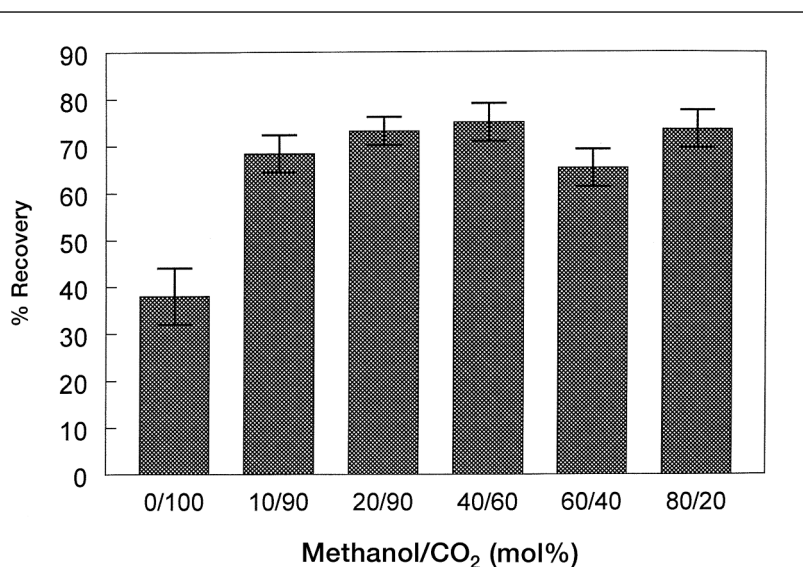


Figure 3. Recovery of DIA using SFE and BME.

not occur because a reflux mechanism was used; however, losses during the concentration were still possible because of the need to transfer the analytes to a second bottle and the evaporation of solvent prior to analysis. Surrogate recoveries ranged from 89.4% with SFE using CO₂ to 99.7% with TME using H₂O/methanol/CO₂. Surrogate recoveries were expected to be lower during SFE because the solvent quantity in the collection bottle was reduced during the extraction as a result of both solvent evaporation and aerosol formation caused by the injection of the high-pressure CO₂ into the solvent.

Supercritical CO₂ extractions

The extraction recoveries obtained for the SFE experiments using 100% supercritical CO₂ are shown in Table IV. Single-factor ANOVA was conducted for each compound to determine if changes in pressure or temperature were insignificant (because of chance) or significant (because of treatment) at a 95% confidence level. Results showed a significant relationship for both temperature and pressure for DEDIA. DEDIA recoveries increased with higher temperatures and pressures. For the

other five compounds (ATRA, HA, DEA, DIA, and DEHA), there were no significant increases shown in the percentage recovery from altering either temperature or pressure. Although the percentage recovery was significantly higher for DEDIA at higher temperatures or pressures, the overall percentage recovery was still low (less than 10% yield).

BME

The BME experiments in this study used mol% methanol/CO₂ mixtures that included 10% methanol/90% CO₂ at 50°C and 306 atm (4500 psi), 20% methanol/80% CO₂ at 50°C and 306 atm, 20% methanol/80% CO₂ at 50°C and 238 atm (3500 psi), 40% methanol/60% CO₂ at 50°C and 306 atm, 60% methanol/40% CO₂ at 50°C and 306 atm, and 80% methanol/20% CO₂ at 50°C and 306 atm. The above solutions were all liquid mixtures. The following mixtures were supercritical (above the critical point of mixture): 20% methanol/80% CO₂ at 100°C and 306 atm (4500 psi), 20% methanol/80% CO₂ at 150°C and 306 atm, and 20% methanol/80% CO₂ at 50°C and 408 atm (6000 psi). Collection volumes were 12.5 mL for each extraction.

The extraction recoveries obtained for the BME experiments using a mixture of methanol and CO₂ are shown in Table IV. Single-factor ANOVA was conducted for each compound to determine if changes in pressure or temperature were significant. There were significant differences found for the two hydroxy-containing metabolites HA and DEHA. HA showed a significant difference in recovery by changing both temperature and pressure. Increases in temperature were found to be detrimental to the percentage recovery. Thermal degradation of the hydroxy metabolites was reported in previous studies (2,20). DEHA showed a significant decrease in percentage recovery at lower pressure (238 atm). The percentage recovery also lowered with increasing temperatures, although this difference was not found to be significant. The other four compounds (ATRA, DEA, DIA, and DEDIA) showed no effect from changes in temperature or pressure.

The effect of changing the percentage of methanol in the mixture was then explored. Percentage recovery generally increased with increasing quantities of methanol, especially for the hydroxy-containing metabolites. Percentage recoveries were compared for five different methanol concentrations. Methanol concentrations of 10, 20, 40, 60, and 80 mol% were compared using single-factor ANOVA. Significant differences in the percentage recoveries were reported for five of the six compounds (DEDIA, DEHA, DIA, DEA, and HA). ATRA did not show a significant difference in percentage recovery by increasing the amount of methanol in the binary mixture. Figure 3 shows the extraction results as a function of composition for DIA and HA at 306 atm and 50°C. The trends in the extraction yields for DIA, DEA, and DEDIA were very similar. The

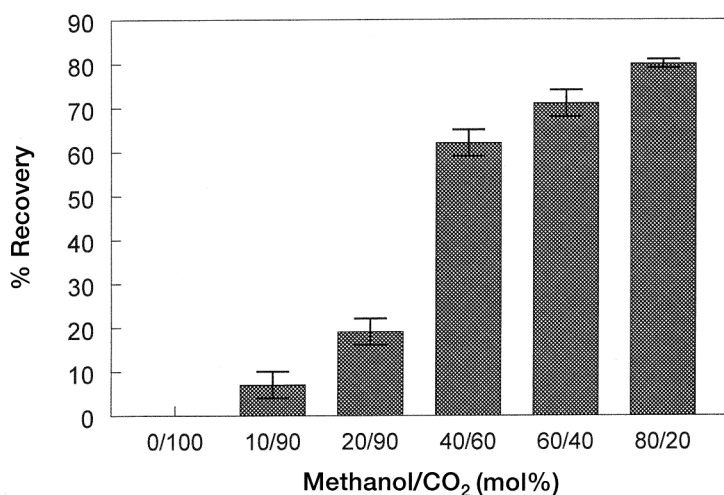


Figure 4. Recovery of HA using SFE and BME.

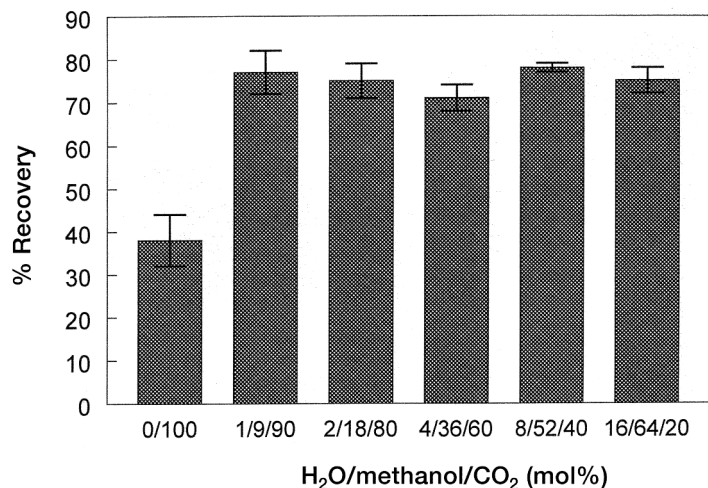


Figure 5. Recovery of DIA using TME.

addition of at least 20% methanol improved the extraction yields. However, increasing the methanol concentration further did not typically improve the yields. For some reason, the use of 60 mol% methanol/40 mol% CO₂ caused diminished extraction recovery that was improved when 80 mol% methanol/20 mol% CO₂ was used. Figure 4 shows the extraction results as a function of composition at 306 atm and 50°C for HA; similar results were obtained for DEHA. For both of these highly polar compounds, increasing the amount of methanol in the extraction solvent continued to improve the extraction yield up to a total composition of 80 mol% methanol/20 mol% CO₂.

A comparison of extraction yields was made for mixtures that were run under supercritical conditions and single-phase liquid mixtures. The following conditions were run at supercritical conditions: 20% methanol/80% CO₂ at 100°C and 306 atm, 20% methanol/80% CO₂ at 150°C and 306 atm, and 20% methanol/80% CO₂ at 50°C and 408 atm. These were compared with an enhanced-fluidity liquid mixture of 20% methanol/80% CO₂ at 50°C and 306 atm. Extraction yields were lower with increasing temperature for DEDIA and HA, although this was found not to be statistically significant.

The results from the BME experiments were then compared with the results from the Soxhlet experiments using *t*-tests. For DEDIA, significant differences in favor of Soxhlet were noted for conditions using 10% and 60% methanol with CO₂. Other conditions noted no significant differences when compared with Soxhlet. For DEHA and HA, significant differences in favor of Soxhlet were noted for conditions using less than 80% methanol. For DIA, significant differences were found in favor of BME for 20%, 40%, and 80% methanol when compared with Soxhlet. In other words, higher extraction recoveries were observed for DEA when any of the binary mixtures were used compared with the Soxhlet extraction. In regards to ATRA, significant improvement over Soxhlet was noted for 10%, 20%, and 60% BME mixtures.

Even though improvements were clearly observed by using the binary methanol/CO₂ liquid mixtures, the extraction yields for the most polar compounds (HA, DEHA, and DEDIA) remained low. Therefore, more polar ternary mixtures that included H₂O in the extraction solvent were studied.

Ternary liquid mixture extractions

Experiments were run at the following mol% compositions: 1% H₂O/9% methanol/90% CO₂ at 50°C and 306 atm, 2% H₂O/18% methanol/80% CO₂ at 50°C and 306 atm, 4% H₂O/36% methanol/60% CO₂ at 50°C and 306 atm, 8% H₂O/52% methanol/40% CO₂ at 50°C and 306 atm, and 16% H₂O/64% methanol/20% CO₂ at 50°C and 306 atm. All mixtures were enhanced-fluidity liquids, and all collection volumes were 12.5 mL. Percentage recoveries were compared for five different ternary mixtures of H₂O/methanol/CO₂ using single-factor ANOVA.

Ternary mixtures were chosen that corresponded with binary mixtures having a percentage of the methanol replaced with H₂O. This was performed by substituting 1% of the

methanol in a 10% methanol mixture for 1% H₂O and 9% methanol. This same substitution technique was used at every methanol concentration, enabling a direct comparison later between individual binary and ternary experiments using *t*-test analysis. Extraction recoveries obtained using the ternary mixtures of H₂O, methanol, and CO₂ are shown in Table V. Figure 5 compares the extraction recovery results at 306 atm and 50°C for DIA using the ternary mixture compositions. The addition of the polar cosolvent up to levels of 10% improved the recoveries. However, the further addition of cosolvent did not affect the results. Similar trends were observed for DEA and DEDIA. Figure 6 shows the extraction yields for HA (which are similar to those of DEHA) at 306 atm and 50°C as a function of ternary mixture composition. The addition of 4–8% H₂O to the ternary mixture improved the extraction yields substantially.

A *t*-test analysis was used to compare the results from TME with Soxhlet extraction. For DEDIA, the 8% H₂O mixture yielded significantly higher recoveries compared with Soxhlet. For DEHA and HA, *t*-test results comparing TME and Soxhlet showed significantly higher recoveries for 4% or more H₂O and either no difference or significantly lower recoveries for 2% or less H₂O in the mixture. For DIA, DEA, and ATRA, recovery results were significantly higher or slightly higher for all TME experiments compared with Soxhlet. In summary, ternary mixtures containing 4–8% H₂O provided optimal results.

The percentage recovery for the BME experiments was compared with the percentage recovery for the TME experiments using the *t*-test. Results were exceptionally pronounced for the hydroxy-containing metabolites. For DEHA, ternary mixtures including H₂O/methanol concentrations greater than 1% and 9% yielded significantly higher recoveries than binary mixtures containing methanol/CO₂. For HA, ternary mixtures yielded significantly higher recoveries in every concentration compared with the binary mixtures. For the other nonhydroxy-containing metabolites (DEDIA and DIA along with the ATRA parent), significant differences were only observed when comparing ternary concentrations of 8% H₂O/52% methanol in CO₂ with 60% methanol in CO₂. In these cases, the ternary mixture had significantly higher recoveries than the binary mixture. The recovery

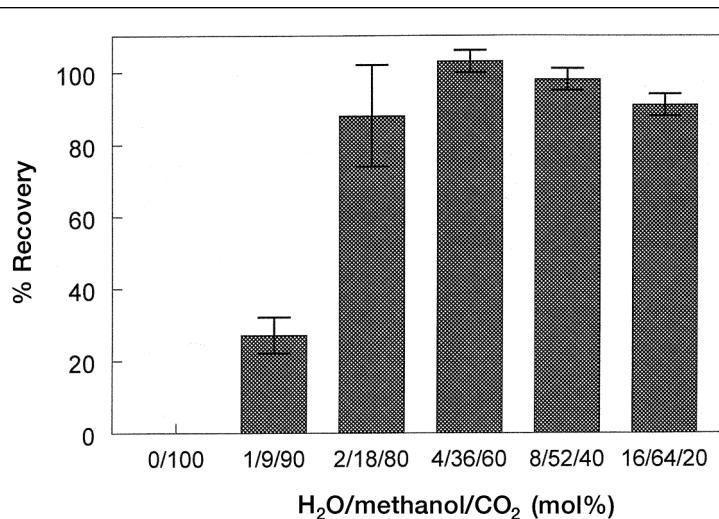


Figure 6. Recovery of HA using TME.

rates for DEA were significantly higher for concentrations of 8% H₂O/52% methanol in CO₂ with 60% methanol in CO₂ and significantly lower for concentrations of 16% H₂O/64% methanol in CO₂ with 80% methanol in CO₂.

Direct addition of modifiers

Three experiments were then conducted in which a modifier was added directly to the extraction cell. The modifiers were HCl, NaOH, and CaCl₂ (Table V). For these tests, 100 μ L each of 0.1M HCl and 0.1M NaOH and 500 μ L CaCl₂ were added in separate tests and run in triplicate similar to the other enhanced-fluidity liquid extraction experiments. HCl or NaOH was added to a 20 mol%/80 mol% mixture of methanol/CO₂, and the CaCl₂ was added to a ternary mixture of 16 mol% H₂O/64 mol% methanol/20 mol% CO₂. Extraction with an aqueous salt solution of CaCl₂ had been reported before (21). Generally, there was a significant

increase in the percentage recovery in favor of CaCl₂ over either HCl or NaOH. This was expected for the hydroxy-containing metabolites because the base solution of 16 mol% H₂O/64 mol% methanol/20 mol% CO₂ also performed better. Recovery rates for CaCl₂ were similar or slightly better than those found for the base solution without the modifier when the percentage recovery was lower for both the HCl and NaOH modifiers. This has been attributed to the salt solutions increased ability to remove both anions and cations from the sediment matrix. Because of the coelution of an unknown compound with the same retention time as DEHA, the recovery percentage was erroneous during the CaCl₂ experiment. This was the only compound and the only test affected by the impurity. The identity of the second compound was not determined.

There was also a decrease in recovery noticed for HCl and NaOH for all compounds. This could be explained that at low pH,

the hydroxy-containing analytes are protonated, the other analytes have a high fraction in protonated form, and absorption to the matrix increases despite increased solubility. At high pH, the analytes are unprotonated and their solubility decreases despite decreased adsorption.

Extraction rates

The effect of extraction solvent composition on the rate of extraction was studied. This was conducted by comparing the mean recovery percentages for each collection bottle during the extraction process. These comparisons were at 2.5, 5.0, 7.5, 10.0, and 12.5 mL. The most pronounced differences were found for the hydroxy triazines, DEHA and HA. For DEHA (Figure 7) extraction recovery was minimal for SFE/CO₂ and binary mixtures of 20% or less methanol. When using a 2% H₂O/18% methanol/80% CO₂ mixture for the extraction recovery after 12.5 mL of solvent had passed through the cell, the extraction recovery was 4 times higher using 20 mol% methanol/80 mol% CO₂. Although, it should be noted that the initial recovery using an extraction volume of 2.5 mL was approximately one half that of 20% methanol/80% CO₂ mixtures. However, when a mixture of 8 mol% H₂O/52 mol% methanol/40 mol% CO₂ was used, the rates of extraction and the overall yields for the hydroxy atrazines improved greatly. Figure 8 shows the rate of extraction for DIA. The addition of H₂O had minimal effect on the rate of extraction of DIA, DEA, DEDIA, and ATRA.

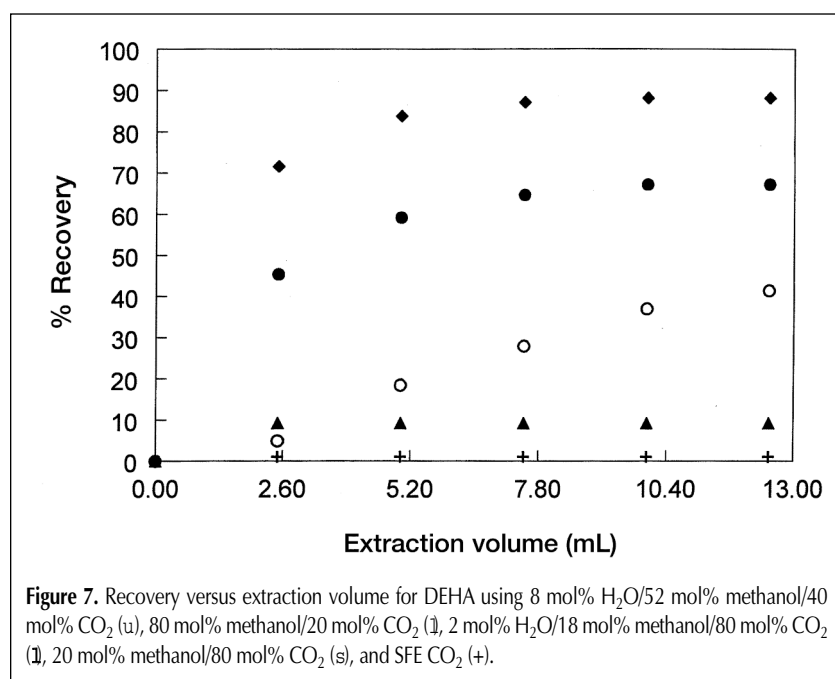


Figure 7. Recovery versus extraction volume for DEHA using 8 mol% H₂O/52 mol% methanol/40 mol% CO₂ (u), 80 mol% methanol/20 mol% CO₂ (l), 2 mol% H₂O/18 mol% methanol/80 mol% CO₂ (l), 20 mol% methanol/80 mol% CO₂ (s), and SFE CO₂ (+).

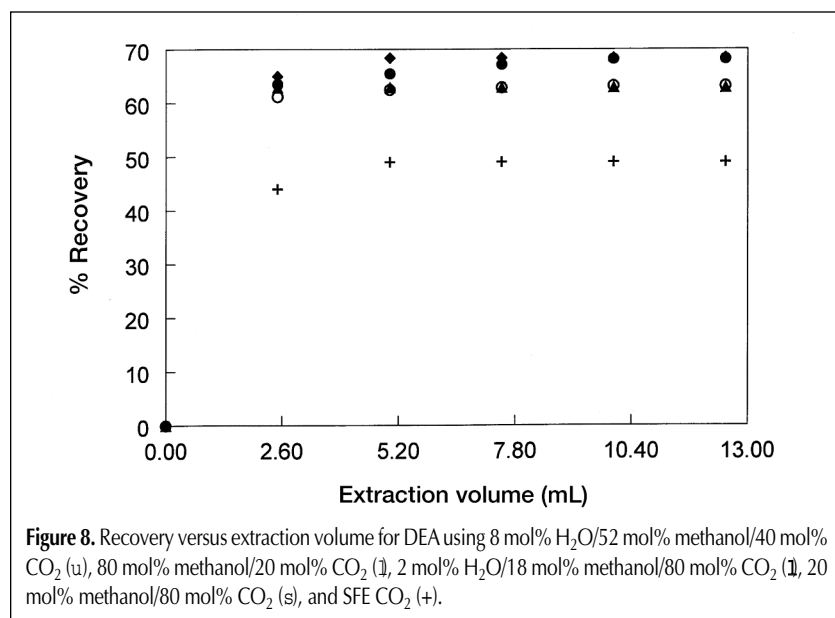


Figure 8. Recovery versus extraction volume for DEA using 8 mol% H₂O/52 mol% methanol/40 mol% CO₂ (u), 80 mol% methanol/20 mol% CO₂ (l), 2 mol% H₂O/18 mol% methanol/80 mol% CO₂ (l), 20 mol% methanol/80 mol% CO₂ (s), and SFE CO₂ (+).

Conclusion

ATRA was successfully extracted using supercritical CO₂, and most of the metabolites and especially the HAs were extracted better with enhanced-fluidity liquid mixtures. The similar-

ities between enhanced-fluidity liquids and supercritical fluids were studied by comparing the results of both supercritical and liquid binary mixtures of methanol/CO₂. Mobile phase (liquid or supercritical fluid) had minimal impact on the extraction results. However, the composition of the mixture was found to be very important.

The results showed that there was no advantage to increasing the temperature beyond 50°C or the pressure beyond 306 atm. Increasing the temperature above 50°C lowered recoveries—an indication of thermal breakdown reported by previous studies. The pressure difference between the lowest and highest pressures tested (238 and 408 atm) did not significantly improve the recoveries of any of the compounds except DEDIA. The overall recoveries for this compound were still low (approximately 10%) for the percentage of methanol/CO₂ that the recoveries were compared with.

Similarly, there was no advantage to altering the pH of the solution. By lowering the pH with HCl, ATRA and its metabolites were below or near their pK_a values. The fraction of all the compounds in cation form increased near the pK_a. As cations, the compounds will bind to the permanent negatively charged sites on the matrix, preventing their successful extraction. Raising the pH well-beyond the pK_a values should dissociate all the compounds. This will cause the hydroxy-containing metabolites to become anions. As anions, they will readily adsorb to positively charged minerals in the matrix. Raising or lowering the pH will also increase breakdown of all the compounds.

The majority of the compounds present in the samples were extracted during the first 2.5 mL of the extraction solvent used. Considering the near 0.4-mL/min flow rate, this lasts approximately 6 min. The exceptions were the hydroxy-containing metabolites, which depending on the solvent were extracted slower (approximately 30 min). This is an indication that the hydroxy-containing compounds bound more strongly to the matrix than ATRA or the dealkylated metabolites. Extraction times could be dramatically reduced if ATRA and the dealkylated metabolites were the only compounds of interest for the extraction. All recovery rates were faster than those of the Soxhlet recoveries that took 24 h to achieve complete recovery. Soxhlet recoveries were generally lower than the binary mixtures with high percentages of methanol and ternary mixtures of H₂O/methanol/CO₂. This is likely due in part to thermal degradation of the compounds when using Soxhlet extraction.

The recoveries of all of the analytes were high with the direct addition of CaCl₂ as the modifier. When CaCl₂ dissociates in solution, the Ca ion may substitute for ATRA and its metabolites at matrix sites. This is accomplished without altering the pH, which both increases breakdown and causes strong adsorption to the matrix for some compounds.

The best extraction method for each compound is listed in Table VI along with the Soxhlet results. There were marked differences in recoveries for the compounds. For ATRA, solute diffusion in the mobile phase appeared to be the limiting step in the extraction process. Accordingly, it could be extracted successfully using any of the following solvents: supercritical CO₂ and enhanced-fluidity mixtures including methanol/CO₂ and H₂O/methanol/CO₂. Soxhlet is not recommended because of the observed problem of thermal breakdown. For the metabolites using binary mixtures of

methanol/CO₂ or ternary mixtures containing H₂O/methanol/CO₂, marked differences in the recovery rates were created. DIA and DEA appeared to be best extracted using a combination of both increased solvent strength of binary and ternary mixtures (including methanol and methanol/H₂O) and maintained effective mass transport from the matrix (CO₂).

Surface desorption appeared to play a larger role in the extraction of the other metabolites. Results showed that DEDIA and DEHA can best be extracted using an enhanced-fluidity ternary mixture including methanol and greater than 4% H₂O. Similarly, HA was best extracted with ternary mixtures of greater than 2% H₂O. These compounds appeared to bind the strongest of any of the compounds to the matrix.

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