1

of/de

1

PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

**SUPERCRITICAL FLUID EXTRACTION OF PRIORITY POLLUTANTS
FROM SOIL MATRICES**

by Marc Lafontaine

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of**

Master of Science

Department of Chemistry

**Carleton University
Ottawa, Ontario
24 november 1993**

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Votre référence*

Our file *Notre référence*

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-92953-7

Canada

Nom Marc Lefontaine

Dissertation Abstracts International est organisé en catégories de sujets. Veuillez s.v.p. choisir le sujet qui décrit le mieux votre thèse et inscrivez le code numérique approprié dans l'espace réservé ci-dessous.

Sciences Pures, Chimie Analytique

SUJET

0496

U-M-I

CODE DE SUJET

Catégories par sujets

HUMANITÉS ET SCIENCES SOCIALES

COMMUNICATIONS ET LES ARTS

Architecture	0729
Beaux-arts	0357
Bibliothéconomie	0399
Cinéma	0900
Communication verbale	0459
Communications	0708
Danse	0378
Histoire de l'art	0377
Journalisme	0391
Musique	0413
Sciences de l'information	0723
Théâtre	0465

ÉDUCATION

Généralités	515
Administration	0514
Art	0273
Collèges communautaires	0275
Commerce	0688
Économie domestique	0278
Éducation permanente	0516
Éducation préscolaire	0518
Éducation sanitaire	0680
Enseignement agricole	0517
Enseignement bilingue et multiculturel	0282
Enseignement industriel	0521
Enseignement primaire	0524
Enseignement professionnel	0747
Enseignement religieux	0527
Enseignement secondaire	0533
Enseignement spécial	0529
Enseignement supérieur	0745
Évaluation	0288
Finances	0277
Formation des enseignants	0530
Histoire de l'éducation	0520
Langues et littérature	0279

Lecture	0535
Mathématiques	0280
Musique	0522
Orientation et consultation	0519
Philosophie de l'éducation	0998
Physique	0523
Programmes d'études et enseignement	0727
Psychologie	0525
Sciences	0714
Sciences sociales	0534
Sociologie de l'éducation	0340
Technologie	0710

LANGUE, LITTÉRATURE ET LINGUISTIQUE

Langues	
Généralités	0679
Anciennes	0289
Linguistique	0290
Moderne	0291
Littérature	
Généralités	0401
Anciennes	0294
Comparée	0295
Médiévale	0297
Moderne	0298
Africaine	0316
Américaine	0591
Anglaise	0593
Asiatique	0305
Canadienne (Anglaise)	0352
Canadienne (Française)	0355
Germanique	0311
Latino-américaine	0312
Moyen-orientale	0315
Romane	0313
Slave et est-européenne	0314

PHILOSOPHIE, RELIGION ET

THÉOLOGIE	
Philosophie	0422
Religion	
Généralités	0318
Clergé	0319
Études bibliques	0321
Histoire des religions	0320
Philosophie de la religion	0322
Théologie	0469

SCIENCES SOCIALES

Anthropologie	
Archéologie	0324
Culturelle	0326
Physique	0327
Droit	0398
Économie	
Généralités	0501
Commerce Affaires	0505
Économie agricole	0503
Économie du travail	0510
Finances	0508
Histoire	0509
Théorie	0511
Études américaines	0323
Études canadiennes	0385
Études féministes	0453
Folklore	0358
Géographie	0366
Gérontologie	0351
Gestion des affaires	
Généralités	0310
Administration	0454
Banques	0770
Comptabilité	0272
Marketing	0338
Histoire	
Histoire générale	0578

Ancienne	0579
Médiévale	0581
Moderne	0582
Histoire des noirs	0328
Africaine	0331
Canadienne	0334
États-Unis	0337
Européenne	0335
Moyen-orientale	0333
Latino-américaine	0336
Asie, Australie et Océanie	0332
Histoire des sciences	0585
Loisirs	0814
Planification urbaine et régionale	0999
Science politique	
Généralités	0615
Administration publique	0617
Droit et relations internationales	0616
Sociologie	
Généralités	0626
Aide et bien-être social	0630
Criminologie et établissements pénitentiaires	0627
Démographie	0938
Études de l'individu et de la famille	0628
Études des relations interethniques et des relations raciales	0631
Structure et développement social	0700
Théorie et méthodes	0344
Travail et relations industrielles	0629
Transports	0709
Travail social	0452

SCIENCES ET INGÉNIERIE

SCIENCES BIOLOGIQUES

Agriculture	
Généralités	0473
Agronomie	0285
Alimentation et technologie alimentaire	0359
Culture	0479
Élevage et alimentation	0475
Exploitation des pâturages	0777
Pathologie animale	0476
Pathologie végétale	0480
Physiologie végétale	0817
Sylviculture et faune	0478
Technologie du bois	0746
Biologie	
Généralités	0306
Anatomie	0287
Biologie (Statistiques)	0308
Biologie moléculaire	0307
Botanique	0309
Cellule	0379
Écologie	0329
Entomologie	0353
Génétique	0369
Limnologie	0793
Microbiologie	0410
Neurologie	0317
Océanographie	0416
Physiologie	0433
Radiation	0821
Science vétérinaire	0778
Zoologie	0472
Biophysique	
Généralités	0786
Médicale	0760

SCIENCES DE LA TERRE

Biogéochimie	0425
Géochimie	0996
Géodésie	0370
Géographie physique	0368

Géologie	0372
Géophysique	0373
Hydrologie	0388
Minéralogie	0411
Océanographie physique	0415
Palaéobotanique	0345
Palaéocologie	0426
Palaéontologie	0418
Palaéozoologie	0985
Palynologie	0427

SCIENCES DE LA SANTÉ ET DE L'ENVIRONNEMENT

Économie domestique	0386
Sciences de l'environnement	0768
Sciences de la santé	
Généralités	0566
Administration des hôpitaux	0769
Alimentation et nutrition	0570
Audiologie	0300
Chimiothérapie	0992
Dentisterie	0567
Développement humain	0758
Enseignement	0350
Immunologie	0982
Loisirs	0575
Médecine du travail et thérapie	0354
Médecine et chirurgie	0564
Obstétrique et gynécologie	0380
Ophthalmologie	0381
Orthophonie	0460
Pathologie	0571
Pharmacie	0572
Pharmacologie	0419
Physiothérapie	0382
Radiologie	0574
Santé mentale	0347
Santé publique	0573
Soins infirmiers	0569
Toxicologie	0383

SCIENCES PHYSIQUES

Sciences Pures	
Chimie	
Généralités	0485
Biochimie	487
Chimie agricole	0749
Chimie analytique	0486
Chimie minérale	0488
Chimie nucléaire	0738
Chimie organique	0490
Chimie pharmaceutique	0491
Physique	0494
Polymères	0495
Radiation	0754
Mathématiques	0405
Physique	
Généralités	0605
Acoustique	0986
Astronomie et astrophysique	0606
Électronique et électricité	0607
Fluides et plasma	0759
Météorologie	0608
Optique	0752
Particules (Physique nucléaire)	0793
Physique atomique	0748
Physique de l'état solide	0611
Physique moléculaire	0609
Physique nucléaire	0610
Radiation	0756
Statistiques	0463

Sciences Appliquées Et Technologie

Informatique	0984
Ingénierie	
Généralités	0537
Agricole	0539
Automobile	0540

Biomédicale	0541
Chaleur et thermodynamique	0348
Conditionnement (Emballage)	0549
Génie aérospatial	0538
Génie chimique	0542
Génie civil	0543
Génie électronique et électrique	0544
Génie industriel	0546
Génie mécanique	0548
Génie nucléaire	0552
Ingénierie des systèmes	0790
Mécanique navale	0547
Métallurgie	0743
Science des matériaux	0794
Technique du pétrole	0765
Technique minière	0551
Techniques sanitaires et municipales	0554
Technologie hydraulique	0545
Mécanique appliquée	0346
Géotechnologie	0428
Matériaux plastiques (Technologie)	0795
Recherche opérationnelle	0796
Textiles et tissus (Technologie)	0794

PSYCHOLOGIE

Généralités	0621
Personnalité	0625
Psychobiologie	0349
Psychologie clinique	0622
Psychologie du comportement	0384
Psychologie du développement	0620
Psychologie expérimentale	0623
Psychologie industrielle	0624
Psychologie physiologique	0989
Psychologie sociale	0451
Psychométrie	0632

The undersigned hereby recommend to
the Faculty of Graduate Studies and Research
acceptance of this thesis,
"Supercritical Fluid Extraction of Priority Pollutants from Soil Matrices",
submitted by Marc Lafontaine, B.Sc.,
in partial fulfilment of the requirements of
the Master of Science degree

Chair, Department of Chemistry

Thesis Supervisor

Carleton University,

13 January 1994

ABSTRACT

Supercritical fluid extraction has been performed on four reference soil samples spiked with priority pollutants. These soils were provided by Agriculture Canada. The pollutants chosen were: benzo(a)pyrene, pentachlorophenol, heptachlor and 2,2',6,6'-tetrachlorobiphenyl. They were added to the soils at a level of 10 ppm. The surface area of the soils has been measured.

Supercritical fluid extraction using CO₂ modified with methanol provided the best overall recoveries compared to Soxhlet extraction. Supercritical CO₂ modified with benzene increased the recoveries but not as much as the CO₂ modified with methanol. Pure CO₂ gave poor recoveries at the conditions used.

An enhancement in the recoveries was also noted when the temperature was increased from 60° C to 100° C. The time needed to extract 85% of the analytes, using CO₂ modified with methanol was in the order of ten minutes. No correlations relating the amounts extracted and the soil characteristics, such as % of organic content and surface area, could be identified.

REMERCIEMENTS

J'aimerais remercier sincèrement Merv Fingas qui a bien voulu m'accepter dans son laboratoire équipé à la fine pointe de la technologie. La liberté qu'il m'a conféré devait sûrement découler d'une dose de confiance et j'en lui suis fort reconnaissant. Bien que nos discussions furent plutôt rares, il n'en demeure pas moins qu'il était en tout temps disponible. La présence d'équipements informatiques et d'un centre de documentation exceptionnel m'ont fortement aidé tout au long de ma maîtrise. Son ardeur au travail fut, tout au long de ces deux années, un exemple à suivre pour moi.

Tout le personnel du laboratoire m'a toujours supporté lorsque nécessaire, particulièrement Dr. Zhendi Wang qui m'a divulgué plusieurs de ses secrets concernant l'analyse par chromatographie haute pression en phase liquide. Sans ces précieuses informations, je serais probablement devenu maboule... De plus, les nombreuses heures du midi passées à jouer au ping-pong, on fait de moi un athlète plus complet!

Mon partenaire de maîtrise, Andrew Zlotorzinski, figure maintenant parmi mes amis. Nos nombreuses discussions acharnées (parlées en "Anglais International...") nous ont permis tous les deux (je l'espère), une meilleure compréhension de la science des sols et de la chimie analytique.

Mes remerciements les plus profonds, je les dois à Dr. Kruus. Sa patience, son sens critique, ses connaissances et son implication sont autant de qualités incommensurables qui, en le cotoyant, m'ont permis de "survivre" face au défi que représentait l'obtention d'une maîtrise en chimie. J'ai rarement rencontré un individu aussi sincère et dévoué.

Un gros merci à toute son équipe, Sharon, Marc, Peter et Mohamed et spécialement Bob Burk. Leur soutien et leur intérêt a toujours été fortement appréciés. Enfin, ma compagne Christiane Aubry, m'a toujours appuyé et surtout aidé lorsque je me trouvais englouti par une mer de travail... Je lui dois un immense merci et j'espère pouvoir un jour lui rendre la pareil

TABLE OF CONTENTS

ABSTRACT	i
REMERCIEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES.	vii
1 INTRODUCTION.	1
1.1 Implication of the research	1
1.2 Choice of contaminants	2
1.3 Choice of soils	3
2 APPLICATIONS OF SUPERCRITICAL FLUIDS	4
2.1 Properties of supercritical fluids	5
2.2 Choice of supercritical fluid	10
2.3 Extraction of polar compounds	15
2.3.1 Addition of modifiers	15
2.3.2 In situ chemical derivatization.	16
2.4 Technical aspects	18
2.4.1 Extract collection	18
2.4.1.1 Off-line collection	19
2.4.1.2 On-line collection	20
2.4.2 Control of pressure	21
2.4.3 Mode of extraction	22

3 REVIEW OF SFE APPLIED TO SOILS	23
3.1 State of the research	23
3.2 Key parameters	24
3.2.1 Solubility	25
3.2.2 Kinetics of extraction	26
3.2.3 Matrix effects	28
4 EQUIPMENT, MATERIALS AND METHODS	29
4.1 Specific surface area	29
4.2 Spiking procedures	29
4.3 Analysis	30
4.4 Soxhlet extractions	33
4.5 Supercritical fluid extraction	33
4.6 Solvent extraction	36
5 RESULTS AND DISCUSSION	37
5.1 Specific surface area	37
5.2 Supercritical fluid extraction	39
5.2.1 Effect of modifiers	39
5.2.2 Effect of temperature	47
5.2.3 Time dependence of SFE	47
5.2.4 Effects of soil characteristics	52
5.3 Soxhlet extraction	55
5.4 Solvent extraction	59
6 CONCLUSION	62
Appendix	63

REFERENCES	69
----------------------	----

LIST OF TABLES

Table 1.2.1: Physicochemical properties of target molecules.	3
Table 1.3.1: Characteristics of the soils studied.	4
Table 2.1.1: Physical properties of supercritical fluids, liquids and gases. . .	6
Table 2.2.1: Solubility parameter of selected pure compounds at $p/p_c=2$, $T/T_c=1.02$	11
Table 2.2.2: Critical parameters of various supercritical fluids.	13
Table 2.2.3: Examples of supercritical fluid extraction involving fluids other than CO_2	14
Table 2.3.1: Environmental applications of SFE with in situ chemical derivatization.	17
Table 2.4.1: Comparison of off-line and on-line collection.	18
Table 3.1.1: Data published before 1990 on the use of SFE for the extraction of contaminants from environmental samples.	23
Table 5.1.1: Specific surface area and water content of the soils ($n=4$). . . .	37

LIST OF FIGURES

Figure 2.1.1:	Phase diagram of carbon dioxide.	5
Figure 2.1.2:	Effect of pressure and temperature on the viscosity of CO ₂ . .	7
Figure 2.1.3:	Comparison of the diffusion coefficient in supercritical fluids and in liquids.	7
Figure 2.1.4:	Variation of the reduced density with the reduced pressure and reduced temperature.	8
Figure 2.2.1:	Effect of temperature and pressure on the solubility parameter	12
Figure 2.4.1:	A) linear restrictor (fused silica) B) Tescom valve.	21
Figure 3.2.1:	SFE Triangle.	25
Figure 3.2.2:	Time dependence of SFE.	26
Figure 3.2.3:	Solubility-limited hot-ball model plots.. . . .	27
Figure 4.3.1:	Variation of the retention times with % of acetonitrile for the compounds investigated.	31
Figure 4.3.2:	Elution program for the HPLC analysis.. . . .	32
Figure 4.5.1:	Schematic diagram of the supercritical fluid extractor.	35
Figure 5.1.1:	Specific surface area of the soils measured by BET vs clay content (n=4).. . . .	38
Figure 5.2.1:	SFE extraction of the four soils using CO ₂ (p=254 atm, T=80°C, ρ=0.7 g mL ⁻¹)	40
Figure 5.2.2:	SFE extraction of soils using CO ₂ / MeOH (p=254 atm, T=80°C)	41
Figure 5.2.3:	SFE extraction of soils using CO ₂ / Benzene (p=254 atm, T=80°C)	42
Figure 5.2.4:	Increase in recoveries using CO ₂ /MeOH compared with CO ₂ . (p=254 atm, T=80°C).	44
Figure 5.2.5:	Increase in recoveries using CO ₂ /Bz compared with CO ₂ . (p=254 atm, T=80°C)	45

Figure 5.2.6:	Effect of the addition of modifier to CO_2 on the recoveries. . .	46
Figure 5.2.7:	Variation of the amounts extracted with the density of the fluids. ($p=254 \text{ atm}$, $T=80^\circ\text{C}$)	48
Figure 5.2.8:	Variation of the amounts extracted with calculated values of the Hildebrand solubility parameter. ($p=254\text{atm}$, $T=80^\circ\text{C}$)	48
Figure 5.2.9:	Effect of the temperature on the extraction recovery ($p=0.7 \text{ g mL}^{-1}$).	49
Figure 5.2.10:	HPLC chromatograms of SFE extracts after SFE at: A) 60°C and B) 80°C	50
Figure 5.2.11:	Time profile of extraction.	51
Figure 5.2.12:	Variation of recoveries with % of organic carbon of the soils using SFE with CO_2/MeOH	53
Figure 5.2.13:	Variation of the recoveries with the specific surface areas of the soils using SFE with CO_2/MeOH	54
Figure 5.3.1:	Soxhlet extraction recoveries for the four soils.	56
Figure 5.3.2:	HPLC chromatogram of a Soxhlet extract of a non-spiked soil (Humo-clay).	57
Figure 5.3.3:	HPLC chromatogram of soxhlet extract (soil: Humo-clay). . .	57
Figure 5.4.1:	Recoveries from solvent extraction of the four spiked soils. . .	59
Figure 5.4.2:	HPLC chromatogram of a solvent extract (soil: Humo-clay). .	60
Figure 5.4.3:	Comparison of recoveries of solvent extraction over SFE using CO_2/MeOH	62

1 INTRODUCTION

1.1 Implication of the research

The Emergencies Sciences Division (ESD), a branch of Environment Canada, has to provide rapid and efficient analytical intervention in case of chemical spills. They are therefore interested in fast, efficient and versatile methods for the analysis of freshly contaminated soils. In most analyses, the extraction of the toxics from the matrix is usually the time-consuming step.

Soxhlet extraction is the standard method of extraction for most organic contaminants but it has major drawbacks. The time needed to perform a quantitative extraction can vary from eight to eighteen hours. It also needs a large amount of solvent, which must be evaporated and disposed of. The extracts usually need an extra clean-up step before the analysis. This technique also requires the use of a Kuderna-Danish evaporator. This adds another time consuming step before the analysis, and the chance for analyte loss because of evaporation, breakdown, or reaction of the compound is greatly increased. Such equipment is relatively large for a mobile unit; it is labour intensive as it needs assembling and clean-up; and it is therefore not ideal for rapid and efficient analysis in emergency situation.

Supercritical Fluid Extraction (SFE) appears to be a promising alternative. It provides quantitative recoveries for almost all kinds of organic contaminants when used under the appropriate conditions. The extraction is usually done in less than an hour. It can use CO₂ as the solvent, avoiding the use of toxic and/or expensive solvents. The extracts are also contained in only a few millilitres, eliminating the use of a Kuderna-Danish evaporator. The extracts are cleaner than Soxhlet extracts and the extractor is ready for use from extraction to extraction without any intensive clean-up and assembling. Numerous excellent reviews concerned with SFE have recently been published^(1,2,3,4).

This thesis extends the knowledge of SFE of contaminants from soils relevant to the aims of ESD.

1.2 Choice of contaminants

In 1980, the environmental emergency branch of Environment Canada developed a priority spill list as a tool to improve the response and countermeasures technology for spills of materials other than oil. The ranking of the compounds was based on supply volume, reported spill frequency and historical spill volume and toxicity⁽⁵⁾. Pentachlorophenol (PCP) and a congener of chlorinated biphenyl, 2,2',6,6'-tetrachlorobiphenyl (PCB) were chosen as target molecules since they appear in the top ten materials in this list. Polyaromatic hydrocarbons (PAHs) represent a family of environmental concern. They are suspected to be carcinogenic and they are produced in large quantity from the incomplete combustion of fossil fuel. Benzo(a)pyrene (BaP), a five ring PAH was chosen since it has been reported to be difficult to extract⁽⁶⁾. Finally, heptachlor (Hepta), a non-planar chlorinated pesticide was also chosen. Table 1.2.1 lists some useful physicochemical properties of the target molecules. K_{ow} is the octanol-water partition coefficient and p_{vap} is the vapour pressure at 25° C.

Table 1.2.1: Physicochemical properties of target molecules⁽⁷⁾.

		M.W./ g/mol	M.P./ °C	Log K _{ow}	P _{ow} /atm at 25°C
Benzo(a)pyrene	252	177	6.5	10 ⁻¹¹
Pentachlorophenol	266	188	3.2	10 ⁻⁴ -10 ⁻⁵
2,2',6,6'-tetrachlorobiphenyl	290	198	5.9	10 ⁻⁴ -10 ⁻⁵
Heptachlor	373	95	5.5	10 ⁻⁴

1.3 Choice of soils

In 1987, Agriculture Canada made a round robin analysis of soils to estimate the extent of soil data variability in Canada and to provide a reference base for soil laboratories⁽⁸⁾. Eight reference soil samples were analyzed by the participants and a report, as well as soil samples, are available upon request. Within these soils, four have been chosen for this present work. The choice was driven by the need to have soils with wide range of characteristics and properties. Table 1.3.1 lists the characteristics of the four selected soils. The names chosen are based on personal choice and do not reflect any official classification system. These names will be used in this work.

Table 1.3.1: Characteristics of the soils studied.

Soil #	1	2	3	5
Origin	P.E.I.	Quebec	Quebec	Ontario
Great group	Humo-Ferric Podzol	Ferro-Humic Podzol	Humic Gleysol	Melanic Brunisol
% organic carbon	0.06	5.32	0.21	2.9
% clay	6.3	2.5	52.8	26.2
% sand	62.6	84.9	1.7	28.7
C.E.C./ (meq 100g ⁻¹)	3.1	33.6	23.2	24.2
pH in water (1:1 soil:water)	5.4	4.4	7.3	6.6
Soil horizon	C	bf/f	C	Ah
Name	Ferric	Humic	Clay	Humo-clay

C.E.C.: Cation exchange capacity.

2 APPLICATIONS OF SUPERCRITICAL FLUIDS

2.1 Properties of supercritical fluids

A fluid at a temperature T and pressure p over its critical point is said to be in the supercritical state. If only one of T or p exceeds its critical value, the fluid is in the subcritical state⁽⁹⁾. Figure 2.1.1 shows the pressure-temperature diagram for CO_2 . Note that as the triple point of CO_2 is at more than the ambient pressure, CO_2 has no liquid state at 1atm pressure.

Figure 2.1.1: Phase diagram of carbon dioxide⁽⁹⁾.

Supercritical fluids (SF) possess specific properties intermediate between a gas and a liquid⁽⁹⁾. These properties are summarized in Table 2.1.1.

Table 2.1.1: Physical properties of supercritical fluids, liquids and gases.

	Supercritical fluid	Liquid	Gas
Density/ g cm ⁻³	0.2-0.9	0.8-1.0	(0.6-2.0)x10 ⁻³
Diffusivity/ cm ² s ⁻¹	(0.5-3.3)x10 ⁻⁴	(0.5-2.0)x10 ⁻⁵	0.01-1.0
Viscosity/ g cm ⁻¹ s ⁻¹	(2.0-9.9)x10 ⁻⁴	(0.3-2.4)x10 ⁻²	(0.5-3.5)x10 ⁻⁴

From a kinetic point of view, the viscosity of supercritical fluids is five to twenty times lower than of liquids. The pressure and temperature dependence of viscosity for CO₂ is presented in Figure 2.1.2 . Note that at higher p, the viscosity decreases as T increases, in agreement with normal liquid behaviour. If p is sufficiently low, then viscosity increases with T as for normal gas.

This low viscosity makes for easy solvent flow. It also results in solute diffusion coefficients higher than those measured in liquids, giving rapid mass transfer. Figure 2.1.3 shows typical solute diffusion coefficient in supercritical fluids and in liquids.

A better penetration of the fluid into porous matrices is also obtained because supercritical fluids completely wet solids.

Figure 2.1.2: Effect of pressure and temperature on the viscosity of CO₂.

Figure 2.1.3: Comparison of the diffusion coefficient in supercritical fluids and in liquids.

From a thermodynamic point of view, supercritical fluids have a density a hundred to a thousand times higher than gases, providing a solvating power similar to liquids. Moreover, the density is tunable simply by changing the pressure and/or the temperature, allowing the possibility of selectively extracting families of compounds. This behaviour is illustrated in Figure 2.1.4 where the variation of the reduced density (ρ/ρ_c) vs reduced pressure (p/p_c) is presented. Note that density, pressure and temperature are expressed in reduced units.

Figure 2.1.4: Variation of the reduced density with the reduced pressure and reduced temperature.

Most of the useful supercritical fluids are gases at room temperature and atmospheric pressure, so that the fluid is eliminated after the extraction simply by expansion. It is therefore easy to recover solutes, eliminating the problem of concentrating the samples.

2.2 Choice of supercritical fluid

The solvating power of a supercritical fluid can be described by the Hildebrand solubility parameter introduced by Giddings⁽¹¹⁾.

$$\delta = \frac{1.25 p_c^2 \rho}{\rho_{liq}}$$

δ = Hildebrand solubility parameter

p_c = critical pressure of the fluid

ρ = density of the fluid

ρ_{liq} = density of the liquid

If the solubility parameter of the fluid and solute are similar, sufficient solubility in the SF can be expected. The Hildebrand solubility parameter for supercritical CO₂ and some common solvents are presented in Table 2.2.1. The solubility parameter δ is given in the traditional units of cal^{1/2} cm^{-2/3}. Figure 2.2.1 shows the variation of the solubility parameter vs temperature at different pressures for carbon dioxide.

Carbon dioxide is by far the most commonly used supercritical fluid. Its critical temperature of 31.1°C allows extraction of thermolabile compounds. It is chemically inert, non flammable, available in high purity at relatively low cost,

and miscible with almost all solvents. The major disadvantage of CO₂ is its low polarity, which varies between that of pentane and chloroform depending on the density. Polar compounds will thus be extracted quantitatively only if modifiers are added to the fluid. Modifiers (co-solvents or entrainers) are usually a liquid organic solvent added to the supercritical fluid. In general, compounds that can be eluted by gas chromatography can be extracted with supercritical CO₂⁽³⁾.

Table 2.2.1: Solubility parameter of selected pure compounds at $p/p_c=2$, $T/T_c=1.02^{(11)}$.

Compound	$\delta/\text{cal}^{1/2}\text{cm}^{-2/3}$	Compound	$\delta/\text{cal}^{1/2}\text{cm}^{-2/3}$
Helium	1.0	Ethane	5.8
Hydrogen	2.6	Ethene	5.8
Neon	4.2	Krypton	5.9
Nitrogen	4.7	Xenon	6.1
n-Hexane	4.9	Tetrahydrofuran	6.2
n-Pentane	5.1	Acetonitrile	6.3
n-Butene	5.2	Nitrous oxide	7.2
n-Butane	5.3	2-Propanol	7.4
Diethyl ether	5.4	Carbon dioxide	7.5
Argon	5.5	Methanol	8.9
Sulfur hexafluoride	5.5	Ammonia	9.3
Ethyl acetate	5.7	Water	13.5

Figure 2.2.1: Effect of temperature and pressure on the solubility parameter.

Other fluids have been used with success but the number of applications is limited because some are flammable (alkanes, N_2O), some present environmental concerns (CHClF_2), some are reactive (MeOH, ammonia), and some have too high a critical temperature. The critical parameters of these fluids and their dipole moment are listed in Table 2.2.2. Applications involving other supercritical fluids are listed in Table 2.2.3.

Table 2.2.2: Critical parameters of various supercritical fluids⁽²⁾.

Solvents	Critical temperature /°C	Critical pressure /atm	Dipole moment /Debye
CO ₂	31.1	72.8	0.0
N ₂ O	36.5	72.5	0.1
CH ₃ OH	239.5	81.0	1.7
C ₂ H ₆	32.3	48.2	0.0
C ₄ H ₁₀	96.7	41.9	0.1
H ₂ O	374.2	217.6	1.8
CHClF ₂	96.1	37.1	1.4
SF ₆	45.5	37.1	0.0
NH ₃	132.5	111.3	1.5

Table 2.2.3: Examples of supercritical fluid extraction involving fluids other than CO₂.

Fluid	Solutes extracted	Matrix	References
N ₂ O	PAHs, alkanes	River sediments, particulate matter, fly ash, soot, alumina.	6,12,13,14,15
	Polychlorinated dioxins and furans	Marine sediments, fly ash, various sorbents.	15,17,18
	PCBs	River sediments	19
CH ₃ OH	PAHs	Coal	20
	Pesticides	Plant tissues	21
C ₂ H ₆	PAHs	River sediments, particulate matter, fly ash	6
C ₄ H ₁₀	Condensed polyaromatics	Spherocarb	22
H ₂ O	Hydrocarbons	Polluted soils	23
CHClF ₂	PAHs	Sediments, sludge	28
	PCBs	Contaminated soils	
SF ₆	PAHs, alkanes	Alumina	12,16

2.3 Extraction of polar compounds

Since CO₂ has a limited solvent power, the extraction of polar and high molecular weight compounds is difficult due to lack of solubility. Other fluids have been used but they present major inconveniences. Two approaches can be envisaged. The first is to add modifier to the CO₂. The second approach, which can be combined with the first one, is derivatize the target compound in situ to make it more soluble in the fluid.

2.3.1 Addition of modifiers

A small amount of organic solvent, a modifier, can be added to the primary fluid to make a binary system. The effect would be to increase the density and/or the polarity of the fluid. Also, the modifier can act as a displacement agent, releasing compounds adsorbed on the matrix⁽³⁾.

The mechanism in SFE is poorly understood. It seems to depend mainly on the solubility mechanism, the diffusion mechanism and the adsorption mechanism⁽²⁾. All these depend upon the sample matrix and the target analyte. The selection of modifier is largely empirical because very little solubility data exists for modified supercritical fluids.

From a practical point of view, the modifier can be already pre-mixed in the cylinder of CO₂ or mixed in the syringe pump. These options have the main disadvantage of contaminating the whole system. If one needs to perform extractions with other modifiers or pure CO₂, it is advisable to flush out all the system, a time-consuming step. On the other hand, the amount of modifier delivered to the cell will be constant. The modifier can be added directly to the cell, offering a quick alternative, especially for method development. The modifier concentration needs to be optimized and will change during the run, starting at a very high concentration to none of it, depending on the duration of

extraction. The best alternative is to add another pumping system connected with a "T" to the main stream just before the cell. The concentration and flow of modifier can then be adjusted and maintained constant during the extraction. Care must be taken when using a modifier, as the working condition must be that the system forms a one-phase system.

2.3.2 In situ chemical derivatization

The other alternative for extracting polar compounds is to perform in situ chemical derivatization. The technique consists of adding a chemical agent directly into the cell, pressurizing, allowing a period of time to let the reaction go to completeness, and then opening the flow outlet and collecting the extract. The derivatized compound will be more soluble in the fluid. Moreover, since compounds difficult to extract with CO₂ are also difficult to analyze by gas chromatography, the derivatized compound will be amenable to GC. It is even possible and desirable to add functional groups that would ease the analyses, like adding a fluorine atom to enable the use of an electron capture detector.

The reaction has been found to be reagent limited: the derivatizing agent reacts with interferences present in the matrix such as biological carboxylic acids in soils or phospholipids in biological material. Matrices with high organic content would need a higher concentration of derivatization agent and higher derivatizing time. Therefore, multi-step derivatization may be necessary to complete the extraction and a more selective derivatizing agent may be needed. Table 2.3.1 lists some environmental applications of in situ chemical derivatization.

Table 2.3.1: Environmental applications of SFE with in situ chemical derivatization.

Target compound	Derivatizing agent	Matrix	References
2,4-D, Dicamba	TPMA, BF_3/MeOH	Spiked sand, spiked river sediment, agricultural soil	24
Phenolic compounds	TPMA, TPMA+trifluoroethanol	Coal gasification waste water deposited on sand, wood soot leachate on c-18 disk	25
Chlorinated phenolics	Triethylamine/ acetic anhydride	Sediments of pulp mill origin	26
PAHs	Tri-Sil reagent	Standard urban dust, Standard marine sediment	27
2,4-D	Tri-Sil reagent, BF_3/MeOH , TFTMFA/MeOH	Spiked soils	28

2,4-D: 2,4-dichlorophenoxy acetic acid

TPMA: trimethylphenylammonium hydroxide

Tri-Sil reagent: hexamethyldisilane+trimethylchlorosilane (2:1, v/v)

TFTMFA: m-trifluoromethylphenyl trimethylammonium hydroxide

2.4 Technical aspects

2.4.1 Extract collection

Two approaches are possible: off-line and on-line collection. The former allows the collection and differential analysis of the extract while the on-line collection is a direct coupling of the extractor with another instrumental method. Table 2.4.1 shows the advantages and disadvantages of both techniques.

Table 2.4.1: Comparison of off-line and on-line collection.

	Off-line collection	On-line collection
Advantages	<ul style="list-style-type: none">- Simple and cheap set-up- Flexibility for subsequent analysis	<ul style="list-style-type: none">- Increase in sensitivity and reproducibility- Loss and degradation minimized- Easy to automate- Faster information
Disadvantages	<ul style="list-style-type: none">- Loss, contamination or degradation possible- More manipulations	<ul style="list-style-type: none">- Apparatus more complicated- Sample size must be adapted- Coextractants might interfere with the analysis

2.4.1.1 Off-line collection

There are different methods for the off-line collection of the extracts:

- 1) expansion of the fluid and precipitation of the solutes in the gas;
- 2) after expansion, bubbling of the gas through a liquid solvent;
- 3) adsorption on a stationary phase;
- 4) cryogenic trapping.

Expansion of the fluid: The fluid is expanded in a glass tube which is cooled by the expansion of the gas via the Joule-Thompson effect. This technique has been successfully applied for relatively large sample (10 to 20 g) but it is not applicable to trace analysis, since significant loss occurs because of aerosol formation. Adding polar modifier decreases the loss since the solutes will dissolve in droplets of solvent and deposit in the receptacle.

Solvent trapping: The restrictor is inserted in a solvent which is cooled by the expansion. The solvent must solubilise the analyte and can not be too volatile. After collection, it can be evaporated to concentrate the sample and ease the subsequent analysis. This technique is limited to low flow. A flow of only 1 mL min^{-1} of supercritical fluid already corresponds to a gaseous flow of about 500 mL min^{-1} , which can cause loss, specially for volatile species, as well as partial evaporation of the solvent.

Sorbent trapping: The extracts precipitate at the exit of the restrictor and are deposit on a stationary phase. After the collection, the solutes can be recovered by elution of an organic solvent with proper solvent strength or by thermal desorption. The flow can be much higher than in solvent trapping, eg up to 4 mL min^{-1} , which will give a faster extraction. Quantitative retention is particularly difficult with organically-modified CO_2 because the modifier becomes a liquid solvent upon depressurization and can elute the analytes themselves

from the stationary phase.

Cryogenic trapping: This technique seems the most appropriate for trapping of volatile species. The temperature of the trap must be between -30 and +15°C. The higher the flow of the fluid, the lower must be the temperature. Unfortunately, the temperature can not go below -50° C because of formation of solid CO₂, which causes blocking of the restrictor. The device should include solid beads (glass, stainless steel *etc.*) that will increase the total surface area available for precipitation.

2.4.1.2 On-line collection

It is possible to directly couple the SFE apparatus with different chromatographic techniques: gas chromatography, liquid chromatography and supercritical fluid chromatography. The latest allows the use of various detectors like the ones used in liquid chromatography (UV-Vis, fluorescence spectroscopy, *etc.*) and those used in gas chromatography (flame ionization, mass spectrometry, *etc.*). It is specially suitable where the sample is available in very small quantities and when maximum sensitivity is desired. It is composed of four successive steps:

- 1) Extraction with the supercritical fluid.
- 2) Depressurization, evacuation of the gaseous CO₂ and recovery the solute extracted with the smallest deposit possible.
- 3) Quantitative transfer of the solutes in the chromatographic column.
- 4) Separation and analysis of the extract.

The second step is the most difficult to achieve.

2.4.2 Control of pressure

There are different techniques for maintaining the pressure in the extraction cell. They are illustrated in the following figure.

Figure 2.4.1: A) linear restrictor (fused silica) B) Tescom valve.

The simplest way is to use a linear restrictor made of capillary fused silica. The loss of pressure is given by the Darcy law⁽²⁹⁾.

$$\Delta p = \Phi \eta L u / d_o^2$$

where Δp is the loss of pressure, Φ the flow resistance factor, η the viscosity of the mobile phase, L the length of the restrictor, u the linear velocity of the mobile phase and d_o the inside diameter of the restrictor.

Even if the flow depends on the applied pressure, this technique is still used because the restrictor presents a low dead volume. This limits the risk of remixing selective extracts.

The pressure can also be regulated using a Tescom valve⁽³⁰⁾ (figure 2.4.1). This system will deliver a flow that will be independent of the pressure usually if the flow is higher than 1 mL min^{-1} . Nonetheless, the dead volumes are quite large and this can cause partial mixing of the fractions.

All these devices need to be heated if the sample contains water or a high content of extractable material. Ice formation or precipitation of the extract may occur during the decompression causing partial and even complete blocking of the restrictor and therefore change the flow.

2.4.3 Mode of extraction

The extraction can be done using static or dynamic mode. During the static mode the fluid is introduced into the cell and maintained. In general, agitation will increase the contact between the sample and the fluid. During the dynamic mode, a continuous flow of fluid is passed through the sample. The static mode allows a better penetration of the fluid into porous material⁽⁹⁾ and a longer contact time. Therefore, a longer static period is appropriate for analytes where solubility can be a limiting factor.

3 REVIEW OF SFE APPLIED TO SOILS

3.1 State of the research

SFE of organic chemical substances has been widely used in industrial processes such as the separation of caffeine from coffee beans and oil from natural products. SFE was however developed for analytical work only recently. Before 1990, only a limited number of applications dealing with SFE of contaminants from environmental samples have been published. Table 3.1.1 lists the analytical SFE published before 1990.

Table 3.1.1: Data published before 1990 on the use of SFE for the extraction of contaminants from environmental samples.

Year	Matrix	Contaminants	References
1986	River sediment. Standard urban dust.	PCBs PAHs	31
1987	Standard urban dust, fly ash, river sediment	PAHs	6
1989	River sediment	Triazine herbicides	32
1989	Sand	Phenolic compounds	33

In 1986, Schantz and Chesler⁽³¹⁾ used SFE to extract PCBs from sediment and PAHs from standard urban dust. The SFE efficiency was compared with Soxhlet extraction using dichloromethane as the solvent. The recovery was equal or better with SFE and required much less time.

In 1987, Hawthorne and Miller⁽⁶⁾ used SFE with different supercritical fluids (CO₂, N₂O, ethane, CO₂ + 5% MeOH, N₂O + 5% MeOH) to extract PAHs from standard urban dust, spiked fly ash and river sediment. The best overall

recoveries were obtained using N_2O + 5% MeOH. The time taken for SFE (30 to 60 min) was much less than 8 hrs of Soxhlet or 4 hrs sonication. A smaller sample size could be used and much less solvent was required, minimizing concentration steps.

In 1989, Janda et al.⁽³²⁾, used SFE to extract s-triazine herbicides from spiked river sediment. The recoveries were high for all compounds except one because of the poor solubility in the non polar CO_2 . Adding a small amount of MeOH directly to the cell prior to the extraction gave complete recoveries of all compounds.

In 1989, Akgerman et al.⁽³³⁾, used SFE to extract phenol from spiked water and soil. They compared the recovery using CO_2 , CO_2 +benzene and CO_2 +MeOH from a dry soil and a wet soil. The presence of moisture was found to have no effect on the recovery using pure CO_2 . The CO_2 modified with MeOH gave complete recovery from the dry soil but poor recovery from the wet soil. The CO_2 modified with benzene increased the recovery from the dry soil and gave complete recovery from the wet soil. Starting from the 90's, an increasing number of published SFE work has emerged.

3.2 Key parameters

There are three main interrelated factors to consider when performing SFE. This is schematically shown in figure by the "SFE triangle".

Figure 3.2.1: SFE Triangle.

3.2.1 Solubility

First, the solute must be sufficiently soluble in the supercritical fluid. The pressure at which the solute starts to dissolve (at constant T) is called the threshold pressure. The control of that parameter during extraction will allow selective extraction. Another useful parameter is the pressure at which a solute reaches a maximum solubility in the compressed fluid.

The solubility of a compound in supercritical fluids depends on two factors: the volatility of the substance, and the solvating effect of the compressed fluid^(11,34). Knowing the melting point of the substance is particularly useful since most solutes dissolve to a greater extent in the SF when in their liquid state.

A great compilation of solubility data for supercritical fluids has been recently published⁽³⁵⁾ from which the threshold pressure can be determined. Such data can be obtained by gravimetric or chromatographic techniques. However, solubility is not as important for extraction as first thought, since trace analysis is concerned with less than micrograms of material, and solubility is seldom the limiting factor.

3.2.2 Kinetics of extraction

The time dependence of SFE is usually as plotted in figure 3.2.2.

Figure 3.2.2: Time dependence of SFE.

If there is no solubility limitation, the rate determining process of the removal of an analyte in a continuous flow is diffusion out of the matrix. If a spherical geometry for the matrix and an effective diffusion coefficient are assumed, the "hot - ball" model can describe the process⁽²⁾.

$$\frac{m}{m_0} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left(-\frac{n^2 \pi^2 D t}{r^2} \right)$$

D = diffusion coefficient

r = particle radius

m_0 = initial mass of solute

m = mass of solute after a time t

n = integer

The ratio of mass of solute m after time t over initial mass m_0 is represented by a sum of exponential decays. At long times, the latter term becomes less important than the first term, so that a graph of $\ln (m/m_0)$ vs t becomes linear.

The dependence on r is as $1/r^2$. The speed of extraction will therefore be increased by crushing or grinding solids. Real samples are not spherical, so that the curve has a larger intercept compared with a value of -0.5 for spheres. This equation has been verified experimentally⁽³⁶⁾. Extrapolation after an initial period can be estimated without exhaustive extraction. If m_1 is the mass extracted in the initial non-exponential period, and m_2 and m_3 are the masses extracted in two subsequent equal time periods, the total mass $\hat{m}_0$ is given by this equation:

$$\hat{m}_0 = m_1 + \frac{m_2^2}{m_2 - m_3}$$

As stated before, the "hot-ball" model implies that there is no solubility limitation. If this is the case the plot will look like figure 3.2.3 where the solubility increases from curve 1 to curve 6.

Figure 3.2.3: Solubility-limited hot-ball model plots.

3.2.3 Matrix effects

The other important factor, and probably the least understood, is the matrix effect. This is specially important for environmental heterogeneous solids like soil and sediment. Soils are very complicated systems comprised of various organic and inorganic species.

Unfortunately, the nature of analyte-matrix interactions between organic pollutants and heterogeneous environmental samples is difficult to estimate⁽³⁷⁾. The composition of soils is very complicated. For example, pollutants in a soil sample may have competitive interactions with inorganic species such as aluminum and iron oxides, clays and organic species like humic and fulvic acids. Extraction of the pollutants may be inhibited by physical barriers like micropores and interstitial space between clay plates. These micropores may be covered by bulk organic material or coated with water. Therefore, no clear picture of the problem has been provided yet.

Naturally aged samples are much more difficult to extract than spiked ones. For example, PAHs were much more easier to extract from freshly spiked soils than native PAHs (real contaminated samples)⁽³⁸⁾. After less than five minutes of SFE, over 95 % of the spiked amount was recovered using pure CO₂, while sixty minutes with stronger conditions (CO₂ modified with methanol) was necessary to extract most of the native PAHs (as compared with sonication in dichloromethane).

In a paper published recently⁽³⁹⁾, different type of soils (sand, furnace treated, river sediment, clay and top soil) have been spiked with different organochlorine and organophosphate pesticides. There were no correlations between the amounts extracted and the type of soils except for the spiked sand which always gave high recoveries.

4 EQUIPMENT, MATERIALS AND METHODS

4.1 Specific surface area

The specific surface area of the four soils was determined using a commercial apparatus (Micromeritics model 2200). The samples were weighed and then degassed under nitrogen for twelve hours at 200° C. Samples were then equilibrated at liquid nitrogen temperature and gaseous nitrogen was admitted until the pressure stabilized. The instrument converts the amount of gas adsorbed into equivalent surface area in units of m^2 . Dividing that value by the weight gives the specific surface area of the sample. Before doing the soil samples, the instrument was checked by measuring the specific surface area of characterized material. The surface areas of silica gel samples were measured and the final results agreed with the known values.

4.2 Spiking procedures

Weighed amounts of each soils (100 g) were placed in 250 mL Nalgene erlenmeyers equipped with tight screw caps. The soils were covered with acetonitrile. The desired amount of each of the four contaminants were dissolved in acetonitrile: pentachlorophenol, 2,2',6,6'-tetrachlorobiphenyl, heptachlor and benzo(a)pyrene (PolySciences, purities >99%) . The quantity added was calculated to give a final concentration of 10 ppm for each contaminant. These solutions were then added to the soils in three steps , each one, followed by manual shaking. The four containers were then shaken sixteen hours with a tumble shaker (Cafarmo). The solvent was then evaporated under a gentle stream of N_2 for a period of 36 hrs. The gas stream was delivered via a Pasteur pipette inserted in the soil/solution system. The containers were agitated slowly using a Gyrotory Shaker model G2 (New Brunswick Scientific). The final weight

differed from the initial weight by a factor no more than $\pm 0.4\%$. The soils were aged 4 months in closed vessels at room temperature protected from the light.

4.3 Analysis

High pressure liquid chromatography (HPLC) was used to analyze the extracts instead of gas chromatography (GC) for several reasons: PCP analysis with GC requires a derivitization step to increase volatility which would add an extra step. The extracts might contain high molecular weight compounds (humic species) and water which are difficult to handle with GC. Nonetheless, HPLC has also drawbacks. There is less resolution, and the one used was not equipped with a computer and did not have an autosampler. This caused much longer analysis compared to fully equipped GCs.

The apparatus consisted of two pumps (Shimadzu, LC610), which allows gradient mobile phase programming, a system controller (Shimadzu, SCL-68), a chart recorder/integrator (Shimadzu, CR501), and a UV-VIS spectrophotometric detector (Shimadzu, SPD-6AV). The column used was a C-18 of 4.6 mm by 15 cm with particles size of 5 μm (Zorbax) equipped with a guard-column of the same packing. The injection valve (Rheodyne) was equipped with a 50 μL sample loop. The mobile phase used was a gradient of acetonitrile (HPLC grade, BDH) and 1% acetic acid (AnalaR, BDH) in water (HPLC grade, BDH). The mobile phases were degassed by bubbling helium through them. A gradient elution program was developed since isocratic flow was unsuccessful in separating the compounds under an acceptable time period. Figure 4.3.1 shows the retention time of the compounds with a flow of 1 mL min^{-1} vs the % of organic mobile phase (CH_3CN). The chart speed was 10 mm min^{-1} .

Figure 4.3.1: Variation of the retention times with % of acetonitrile for the compounds investigated.

The final program used is schematically shown in figure 4.3.2. It should be mentioned that the wavelength was set at 254 nm except for a short period of time corresponding to the elution of heptachlor. The wavelength was lowered to 235 nm to allow better detection of heptachlor since its extinction coefficient at 254 nm is much less than that of the other compounds. The UV-VIS spectra of the compounds are presented in appendix I.

A five level calibration curve was used to quantify the extracts. No internal standard nor surrogate were added to the extracts, but the calibration curve was checked each day by injecting a standard. The calibration curves are presented in appendix II.

Figure 4.3.2: Elution program for the HPLC analysis.

4.4 Soxhlet extractions

Approximately 2.0 g of soil were placed in 25 mm x 80 mm cellulose extraction thimbles (Whatman). The Soxhlet extractors used a volume of 150 mL of dichloromethane (BDH). The extractions were run 12 hrs at approximately 0.1 cycle min⁻¹. The extracts were then evaporated to ca 0.2 mL using a Kuderna-Danish evaporator and the final volumes were adjusted precisely to 1 mL using acetonitrile. Before each run, the extractors and the thimbles were cleaned 8 hrs by running them with dichloromethane.

4.5 Supercritical fluid extraction

Supercritical fluid extractions were performed using a commercial apparatus (SFE-150, Suprex) which was slightly modified. The selecting valve inside the oven was changed to a 4 ports / 2 positions injection valve (Valco) outside the oven. This change was been done because leaking occurred with the original valve. The apparatus consists of three main modules: the syringe pump, the controller and the extraction chamber (oven). Liquid CO₂ (SFC/SFE grade, Air Liquids) was used as supercritical fluid. When used, the modified CO₂ were prepared by injecting the desired amount of solvent via the injection valve while the syringe pump was filling. The modified CO₂ was equilibrated 2 hrs in the syringe pump at pressure of 200 atm to allow uniform mixing of the supercritical fluid.

The dimension of the extraction cell was 10 cm x 4.6 mm ID (V=1.67 mL) and the sample was retained by frits of 5µm pore diameter (Keystone Scientific). The flow restrictor used was a crimped tubing of stainless steel (ID 1/16"). The end of the tubing was squeezed with a pair of pliers until it gave the desired flow. Once calibrated, over 200 extractions were performed without any noticeable flow changes.

The restrictor was connected to a S.S. tubing of 1/8" ID which was submerged in acetonitrile contained in a 10 mL Reacti-Vial (Pierce). The connection was by an internal reducing union fitting (Supelco) which was covered with heating-tape heated at 120° C (Supelco).

After the extraction, the 1/8" tubing was disconnected and rinsed with ca 2 mL of CH₃CN. The rinse was combined with the solvent contained in the vial and then evaporated under N₂ to ca 0.1 mL. The final volume was adjusted precisely to 1 mL. The extract was then analyzed as is. For all C-E extractions, the cell was first put in the oven, heated to 80° C, and then, the cell was pressurized to the selected pressure. A 2 min static period was applied and then the valve was open.

The trapping efficiency was checked by putting 10 ppm of the standards in acetonitrile on a filter paper. After a short period of solvent evaporation, the filter paper was extracted at 254 atm, 80° C for 30 min. Over 98% of all the contaminants were recovered with a relative standard deviation less than 5% (n=3).

The apparatus is schematically shown in figure 4.5 1. All SFE results and other extractions results are presented in appendix IV.

extraction vessel:

heated flow restrictor:

Figure 4.5.1: Schematic diagram of the supercritical fluid extractor.

4.6 Solvent extraction

Solvent extraction, using the solvent used for the spiking, has been performed on the spiked soils. Two grams of soils were placed in a 10 mL Reacti-Vials. About 10 mL of acetonitrile was added. The vials were then shaken 6 hrs through the tumble shaker. The soils/solution were filtered through 0.2 μ m filter (Nalgene) and rinsed with 2 mL of acetonitrile. The solutions were then centrifuged 6 hrs in order to sediment the suspended particles. The solutions were evaporated to 1 mL using N₂.

5 RESULTS AND DISCUSSION

5.1 Specific surface area

The results of the specific surface area and the water content for each soil are presented in table 5.1.1. The relative standard deviations of four determinations are shown in parentheses. A vacuum at 200° C was applied during the experiment.

Table 5.1.1: Specific surface area and water content of the soils (n=4).

Soils	Specific surface area/ (m ² g ⁻¹)
Ferric	3.9 (25%)
Humic	0.2 (141%)
Clay	27.6 (16%)
Humo-clay	5.2 (9.6%)

It should be mentioned that the method used (BET) measures external surfaces only. Therefore, the surface available within the interlayer of phyllosilicates will not be included. Nonetheless, the results are proportional to the clay content, as shown in figure 5.1.1.

Figure 5.1.1: Specific surface area of the soils measured by BET vs clay content (n=4).

5.2 Supercritical fluid extraction

All the SFE results are those obtained at a flow rate between 0.45 to 0.75 mL min⁻¹ for a period of 30 min. The pressure was always set at 254 atm. All results are the average of a minimum of three to a maximum of five repetitions. Extractions with flow rates different from that range have been discarded. The initial spiking level for each soil was 10 ppm for each contaminants. In all extractions (SFE, Soxhlet, acetonitrile), 10 ppm recovery was never reached; it seems that the initial amount adsorbed on the soils is different for both the compounds and the soils.

As previously mentioned, trapping efficiency has been checked. Therefore, the apparatus can not be responsible for the poor recoveries. Blank extractions (with an empty cell) have been performed between extractions to check whether there were any accumulations in the system. All graphs show amount extracted and the standard deviation is represented as the error bar.

5.2.1 Effect of modifiers

SFE has been performed on the four spiked soils using different fluids: CO₂, CO₂ + methanol (CO₂/MeOH) and CO₂ + benzene (CO₂/Bz). Modifier concentrations were 10% on a molar basis. These results are plotted in figures 5.2.1, 5.2.2 and 5.2.3 respectively. With CO₂ alone, we can observe that the overall recoveries are highest with the humic soil: the one having the highest organic content, and also the highest sand content. High organic content soils should be more difficult to extract since adsorption of the organic species onto the organic fraction will be favoured. Nonetheless, the organic content, on a weight basis, is only 5.3% while the sand content is 85%. Sand is known to be a weakly adsorptive matrix. It is often used as an inert matrix to verify trapping efficiency for SFE. PCP is not extracted from the clay soil. That soil possesses

Figure 5.2.1: SFE extraction of the four soils using CO₂ ($p=254$ atm, $T=80^{\circ}\text{C}$, $\rho = 0.7$ g mL⁻¹) .

Figure 5.2.2: SFE extraction of soils using CO₂/ MeOH (p=254 atm, T=80°C)

Figure 5.2.3: SFE extraction of soils using CO₂/ Benzene (p=254 atm, T=80°C)

a high clay content of 53% and a low organic content of 0.2%.

As illustrated in the previous three figures, 10 ppm recoveries, corresponding to the initial spiking, is never reached, whatever the conditions. Loss of compounds may have occurred during the evaporation stage of the spiking procedure. The period needed to evaporated the solvent was long (36 hrs). Therefore, even if the compounds have relatively low vapor pressure (10^{-4} to 10^{-11} atm), chances are that some might have evaporate.

The solvent used for the spiking was not necessarily the best. Using acetone or dichloromethane, which have much lower boiling points, would have reduced the evaporation period.

These compounds are supposed to be fairly stable. PCP can be decomposed via biological processes⁽⁴⁰⁾ while Hepta is also sensitive to epoxidation via biological activity⁽⁴¹⁾. It should be mentioned that before spiking, the purity of the compounds has been verified with GC with mass spectrometric detection. The only detectable signals present were those of the standards. Strong or irreversible adsorption may also have occurred. Therefore, there is no clue concerning the loss of the analytes.

The increase in recoveries while using CO_2 /MeOH and CO_2 /Bz compared with pure CO_2 is shown in figures 5.2.4 and 5.2.5, respectively. There is a significant increase of recoveries for all matrices. BaP is the compound most affected by the presence of MeOH, while Hepta shows the least increase in recovery. CO_2 / Bz shows similar increase, but not as great as CO_2 /MeOH. There is an overall increase in recoveries and again, the extraction of BaP is the most affected by the modifier.

Figure 5.2.6 presents the recoveries of the compounds vs the type of modifiers for the humo-clay soil. We can see that acetonitrile is not a very good modifier. Benzene is a better modifier, even if it is much less polar than acetonitrile.

Figure 5.2.4: Increase in recoveries using CO₂/MeOH compared with CO₂. (p=254 atm, T=80°C).

Figure 5.2.5: Increase in recoveries using CO_2/Bz compared with CO_2 . ($p=254$ atm, $T=80^\circ\text{C}$)

Figure 5.2.6: Effect of the addition of modifier to CO_2 on the recoveries for the humo-clay soil.

At constant pressure and temperature, the resulting densities would be different depending on the modifier used. The densities of the modified CO₂ have been calculated using a commercial software⁽⁴²⁾. The equations used are presented in appendix III. Figure 5.2.7 shows that better recoveries are not obtained by higher densities. The Hildebrand solubility parameters have been calculated using the same program. The recoveries against that parameter are presented in figure 5.2.8. Again, there seems to be no correlation.

5.2.2 Effect of temperature

It has been reported that an increase in temperature increased extraction efficiencies⁽⁴³⁾. SFE with pure CO₂ at constant density (0.7 g mL⁻¹) and different temperatures (60, 80, 100 °C) has been performed on the humic soil. These results are presented in figure 5.2.9. It seems that there is an overall increase of recoveries with increased temperature, especially from 60 °C to 80 °C. It would have been interesting to try higher temperatures but there are technical constraints. Note that the low recovery of PCB at 80 °C seems to come from poor resolution of the chromatograms rather than inefficient extraction as shown in figure 5.2.10 where chromatograms of the extract obtained at different temperature are presented.

5.2.3 Time dependence of SFE

One of the major advantages of SFE is speed; this advantage has to be kept in mind when considering the use of SFE to provide fast responses in case of chemical spills. Figure 5.2.11 shows the amounts extracted vs time for the ferric soil using CO₂/MeOH as the supercritical fluid. After ten minutes, more than 85% of what can be extracted has come out;

Figure 5.2.7: Variation of the amounts extracted with the density of the fluids. (p=254 atm, T=80°C)

Figure 5.2.8: Variation of the amounts extracted with calculated values of the Hildebrand solubility parameter. (p=254atm, T=80°C)

Figure 5.2.9: Effect of the temperature on the recoveries using SFE with CO_2 ($\rho = 0.7 \text{ g mL}^{-1}$).

Figure 5.2.10: HPLC chromatograms of SFE extracts after SFE at: A) 60°C and B) 80°C.

Figure 5.2.11: Time profile of SFE using CO₂/MeOH (Soil: Humic).

after 20 min, the extraction is completed. It should be mentioned that even if the profile becomes level off, it does not mean that the extraction is completed. Stronger conditions might be necessary to extract more strongly adsorbed molecules. Nevertheless, SFE is of value for emergency response, at least to provide a fast screening.

5.2.4 Effects of soil characteristics

As previously discussed, there seems to be no obvious correlation between SFE effectiveness and soil characteristics, such as organic content and clay content. This is confirmed in figures 5.2.12. and 5.2.13, where the recoveries using SFE with CO₂ /MeOH vs organic content and specific surface area, respectively, are presented. No direct correlations are evident, exist except for BaP, where there seem to be an increase in recovery with an increase in organic content and specific surface area.

The opposite behaviour would have been expected. It may not be reliable to correlate the amount recovered with soil characteristics, since there are no confident values of the final amounts adsorbed on the soils. The fact that BaP is extracted more from higher organic content soils and higher specific surface area soils might simply indicate that there was more BaP adsorbed (less loss during spiking and aging).

Figure 5.2.12: Variation of recoveries with % of organic carbon of the soils using SFE with CO₂/MeOH.

5.2.13: Variation of the recoveries with the specific surface areas of the soils using SFE with CO₂/MeOH.

5.3 Soxhlet extraction

To compare techniques, Soxhlet extraction with dichloromethane (DCM) as the solvent was performed on the four spiked soils. DCM was chosen since it is frequently used for extraction of PAHs, PCBs and chlorinated organic compounds. Figure 5.3.1 shows the recoveries for the four spiked soils. Results for PCP are not presented because of poor analysis.

Figure 5.3.2 presents chromatograms of a Soxhlet extraction of a blank soil (non-spiked). As seen, there are not too many signals observed, only at the beginning of the chromatogram where polar compounds elute. Figure 5.3.3 presents a chromatogram of a Soxhlet extract of the same soil (humo-clay), but a spiked one. There are much more interferences present, indicating that the spiking procedures may have modified the integrity of the soil or that some decomposition of the spiking material has occurred. This was the case for all four soils. It has been observed that after spiking, the aggregate sizes were much smaller than before.

Figure 5.3.1: Soxhlet extraction recoveries for the four soils.

Figure 5.3.2: HPLC chromatogram of a Soxhlet extract of a non-spiked soil (Humo-clay).

Figure 5.3.3: HPLC chromatogram of soxhlet extract (soil: Humo-clay).

5.4 Solvent extraction

Extraction of the spiked soils with the same solvent used for spiking (acetonitrile), have been performed. Figure 5.4.1 shows the recoveries for the four soils. It should be mentioned that it was experimentally difficult to extract using that method. The soil particles were so small that they pass through filter of pore size of 0.2 μm . The extracts had to be centrifuged for a long period of time (6 hrs) before being suitable for analysis.

Figure 5.4.2 shows a chromatogram of a solvent extract. There are much more interferences compared with SFE or Soxhlet extraction using DCM. A chromatogram of the solvent did not show any interferences. Figure 5.4.3 presents the amount extracted by such extraction compared with SFE using CO_2/MeOH .

Figure 5.4.1: Recoveries from solvent extraction of the four spiked soils.

Figure 5.4.2: HPLC chromatogram of a solvent extract (soil: Humo-clay).

Figure 5.4.3: Comparison of recoveries of solvent extraction over SFE using CO_2/MeOH .

6 CONCLUSION

The overall recoveries using SFE with CO₂ at p=254 atm, T=80° C (density of 0.7 g mL⁻¹) were poor compared with the best conditions found, SFE using CO₂/MeOH at the same pressure and temperature. SFE using CO₂/Bz increased the recoveries, but not as much as CO₂/MeOH. There was no increase of recoveries with increased densities or Hildebrand solubility parameters of the extracting fluid. This seems to confirm that the solubility is not the limiting factor when extracting organic pollutants from environmental heterogeneous samples using SFE.

No correlations have been observed between the quantities extracted and soils characteristics such as the amount of organic carbon or the specific surface area. It was difficult to find correlations since the real amount initially adsorbed was not known. Significant losses seem to have occurred since the initial spiked amount (10 ppm) was never reached during extractions. Evaporation during the spiking procedures should be partially responsible for the loss but, decomposition, strong or irreversible adsorption can not be discarded as source of potential loss. No attempts have been made to characterize co-extractants.

Increasing temperature from 60° C to 80° C (while staying at constant density) slightly enhanced the overall recoveries using CO₂.

The time needed to extract over 85% of the contaminants, using CO₂/MeOH, was only 10 min. Therefore, SFE used under proper conditions, appears to be a suitable tool for emergency analytical responses. Moreover, SFE used much less solvent, the extracts are relatively clean and do not need much manipulations before the analysis. No filtration, centrifugation or long period of solvent evaporation are necessary.

Appendix I: UV-VIS spectra of target molecules

Appendix II: Calibration curves for the standards

Appendix III: Equations used by the Isco program

Pressure to density conversion-binary fluid:

The SF-Solver software uses a modified Handinson-Brobst-Thompson (HBT) method to calculate the critical temperature, critical pressure, acentric factor, and average molecular weight for a mixture.

Mixing rules:

$$\omega_m = \sum_i x_i \omega_{mi}$$

$$P_{cm} = \frac{(0.291 - 0.080\omega_m)RT_{cm}}{V_m^*}$$

$$T_{cm} = \frac{\sum_i \sum_j x_i x_j V_{ij}^* T_{cij}}{V_m^*}$$

$$V_m^* = \frac{1}{4} \left(\sum_i x_i V_i^* + 3 \left(\sum_i x_i V_i^* \right)^{\frac{2}{3}} \left(\sum_i x_i V_i^* \right)^{\frac{1}{3}} \right)$$

ω = acentric factor

P_{cm} = critical pressure

T_{cm} = critical temperature

V^* = characteristic volume

R = gas constant

x_i = mole fraction

These parameters are then used to relate pressure to density of binary fluids at a given temperature:

$$\omega_b = x_1\omega_{a1} + x_2\omega_{a2}$$

$$P_{cb} = \frac{(0.291 - 0.080\omega_b)RT_{cb}}{V_b^*}$$

$$T_{cb} = x_1^2 V_1^* T_{c1} + 2x_1 x_2 (V_1^* V_2^* T_{c1} T_{c2})^{\frac{1}{2}} + x_2^2 V_2^* T_{c2}$$

$$MW_b = x_1 MW_1 + x_2 MW_2$$

Appendix IV: Results of extraction

All results are expressed in ppm and the standard deviation is presented in parentheses. Three to five experiments were performed.

SFE: CO₂ at 80°C

	PCP	PCB	Hepta	BaP
Ferric	2.2(0.1)	2.7(0.1)	3.3(0.1)	0.34(0.02)
Humic	5.4(0.4)	4.7(0.1)	6.0(0.2)	6.1(0.3)
Clay	0(0)	3.6(0.9)	2.9(0.01)	0.73(0.2)
Humo-Clay	2.6(0.1)	0.6(0.1)	4.3(0.6)	1.6(0.1)

SFE: CO₂/MeOH

	PCP	PCB	Hepta	BaP
Ferric	8.6(1.0)	6.8(0.6)	2.2(1.2)	2.9(0.6)
Humic	7.4(0.4)	6.0(0.4)	4.2(1.8)	8.8(0.3)
Clay	5.8(1.0)	5.6(0.2)	4.5(2.6)	7.1(0.2)
Humo-Clay	5.2(1.5)	4.6(0.7)	5.2(0.1)	7.4(1.5)

SFE: CO₂/Bz

	PCP	PCB	Hepta	BaP
Ferric	5.4(0.1)	4.7(0.3)	5.8(0.01)	0.9(0.2)
Humic	4.7(1.4)	4.6(0.1)	6.7(0.2)	7.4(0.03)
Clay	0.8(0.4)	3.7(0.4)	6.8(0.5)	2.8(0.1)
Humo-Clay	5.3(1.1)	4.4(1.0)	4.0(0.5)	5.8(0.2)

Solvent (CH₃CN)

	PCP	PCB	Hepta	BaP
Ferric	2.8(0.2)	6.8(0.3)	2.7(0.3)	0.6(0.2)
Humic	7.1(0.2)	3.7(0.5)	5.6(0.5)	5.4(0.3)
Clay	0.01(0.001)	4.4(0.1)	2.1(0.7)	3.2(0.1)
Humo-Clay	3.7(1.2)	3.5(0.2)	3.1(0.3)	4.3(0.3)

Soxhlet

	PCP	PCB	Hepta	BaP
Ferric	—	6.9(0.3)	3.6(0.2)	1.6(0.3)
Humic	—	7.6(0.5)	6.9(2.1)	8.1(1.4)
Clay	—	5.9(0.4)	3.8(0.1)	6.0(0.4)
Humo-Clay	—	7.0(0.2)	4.3(0.8)	6.2(0.5)

SFE: CO₂ at different temperatures ($\rho=0.7 \text{ g mL}^{-1}$) for the humic soil

	PCP	PCB	Hepta	BaP
60° C	0.25(0.07)	1.4(0.2)	1.0(0.1)	0.8(0.1)
80° C	2.6(0.09)	0.6(0.07)	2.27(0.3)	1.6(0.1)
100° C	2.9(0.5)	1.3(0.5)	2.0(0.3)	1.8(0.4)

REFERENCES

1. Camel V., Tambute A., Caude M., *J Chromatogr*, **642** (1993) 263.
2. Janda V, Bartle K.D., Clifford A.A., *J Chromatogr*, **642** (1993) 283.
3. Hawthorne S.B., Miller D.J., Burford M.D., Langenfeld J.J., Eckert-Tilotta S., Louie P.K., *J Chromatogr*, **642** (1993) 301.
4. Chester T.L., Pinkston J.D., Raynie D.E., *Anal Chem*, **64** (1992) 153R.
5. Fingas M., Stone N., Leblanc D., Sergy S., Mansfield B., Cloutier G., Mazerolle P., *Development of a new chemical spill priority list*, Environment Canada, 1980.
6. Hawthorne S.B., Miller D.J., *Anal Chem*, **59** (1987) 1705.
7. Schwarzenbach R.P., Gschwend P.M., Imboden D. M., *Environmental organic chemistry*, John Wiley & Sons, NY, 1993.
8. Sheldrick, B.H., Wang C., *Compilation of data for ECSS Reference soil samples*, Land Resource Research Centre, 1987.
9. McHugh M.A., Krukonis V.J., *Supercritical Fluid Extraction, principles and practice*, Butterworths, MA, 1986.
10. Hildebrand J.H., Scott R.L., *The solutions of nonelectrolytes*, Reinhold, NY, 1950.
11. Giddings J.C., Myers M.N., McLaren L., Keller R.A , *Science*, **162** (1968) 67.
12. Levy J.M., Cavalier R.A., Bosh T N., Rynaski A.F., Hulak W.E., *J. Chromatogr Sci*, **27** (1989) 341.
13. Hawthorne S.B., Miller D.J., Kriegers M.S., Freseniu's Z, *Anal Chem*, **330** (1988) 211.

14. Hawthorne S.B., Miller D.J., Langenfeld J.J., *J Chromatogr Sci*, **28** (1990) 2.
15. Hawthorne S.B., Miller D.J., *J Chromatogr*, **403** (1987) 63.
16. Levy J.M., Storozinsky E., Ravey R.M., *J High Resolut Chromatogr*, **14** (1991) 661
17. Onuska F.I., Terry K.A., *J High Resolut Chromatogr*, **12** (1989) 357.
18. Pawliszyn A.N., *Anal Chem*, **61** (1989), 2770.
19. Ashraf-Khorassani M., Taylor L.T., Zimmerman F., *Anal Chem*, **62** (1990) 1177.
20. Capriel P., Haish P., Khan S.U., *J Agric Food Chem*, **34** (1986) 70.
21. Lancas F.M., De Martinis B.S., Da Matta M.H.R., *J High Resolut Chromatogr*, **13** (1990) 838.
22. Wright B.W., Wright C.W., Gale R.W., Smith R.D., *Anal Chem*, **59** (1987) 38.
23. Nowak K., Brunner G., *Actes du 2^e symposium international sur les fluides supercritiques*, Boston (1991) 165.
24. Hawthorne S.B., Miller D.J., Nivens D. E., White D.C., *Anal Chem*, **64** (1992) 405.
25. Hills J.W., Hill H.H., Tsuneaka M., *Anal Chem*, **63** (1991) 2152.
26. Lee H.B., Peart T.E., Hong-You R.L., *J Chromatogr*, **636** (1993) 263.
27. Hills J.W., Hill H.H., *J Chromatogr Sci*, **31** (1993) 6.
28. Rochette E.A., Harsh J.B., Hill H.H., *Talanta*, **2** (1993) 147.
29. Rosset R.C., Jardy A., *Manuel pratique de chromatographie en phase liquid*, Masson, Paris, 1982.
30. Andersen M.R., King J.W., Hawthorne S.B., *Analytical Supercritical Fluid Extraction*, Chromatography conferences, Utah, 1990.

31. Schantz M.M., Chesler S.N., *J Chromatogr*, **363** (1986) 397.
32. Janda V., Steenbeke G., Sandra P., *J Chromatogr*, **479** (1989) 200.
33. Akgerman A., Roop K.R., R.K. Hess, *Supercritical fluid science and technology*, ACS # 496, 1988.
34. King J.W., *J Chromatogr Sci*, **27** (1989) 355.
35. Bartle K.D., Clifford A.A., Jafar S.A., Shilstone G.F. *J Phys Chem Ref Data*, **20** (1991) 713.
36. Bartle K.D., Clifford A.A., Hawthorne S.B., Langenfeld D.J., Miller D.J., Robinson R.E., *J Supercrit Fluids*, **3** (1990) 143.
37. Dragun J., *The soil chemistry of hazardous materials*, Hazardous Materials Control Research Institute, Maryland, 1988.
38. Hawthorne S.B., Burford M.D., Miller D.J., *Anal Chem*, **65** (1993) 1497.
39. Snyder J.L., Grob R.L., McNally M.E., Oostyk T.S., *J Chromatogr Sci*, **31** (1993) 183.
40. World Health Organization, *Environmental Health Criteria 71*, Pentachlorophenol, Geneva, 1984.
41. World Health Organization, *Environmental Health Criteria 38*, Heptachlor, Geneva, 1984.
42. ISCO, SF-Solver, *Software for Supercritical Fluid Analysis*, Lincoln, 1991.
43. Levy J.M., Dolata L.A., Ravey R.M., *J Chromatogr Sci*, **31**(1993) 349.

END

0 8-1 1-94

FIN