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Environment Canada

Water Science and
Technology Directorate

Direction générale des sciences
et de la technologie, eau
Environnement Canada

Evaluation of the Zymark Autotrace (Disk Version) Solid
Phase Extraction System

By:

C. Sullivan, M. Fox, R. Maguire

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**Evaluation of the Zymark Autotrace™ (Disk Version)
Solid Phase Extraction System**

1. Preliminary Studies with Model Compounds in Laboratory Pure Water

Cheryl A. Sullivan¹, Michael E. Fox² and R. James Maguire¹

¹Aquatic Ecosystem Protection Branch
²Aquatic Ecosystem Restoration Branch
National Water Research Institute
867 Lakeshore Road
Burlington, Ontario, L7R 4A6

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MANAGEMENT PERSPECTIVE

This report represents the first stage of the implementation of a new technology, automated solid phase extraction (SPE), for the extraction and concentration of organic contaminants from natural waters, by the Lakes Remediation Project of the Aquatic Ecosystems Restoration Branch at NWRI. The collaboration of staff members from the Aquatic Ecosystems Protection Branch is indicative of the wider interest in this technique which holds the promise of greater efficiency and especially the near elimination of large scale use and discharge to the environment of chlorinated solvents. As with the adoption of any new technique, the testing and comparison with prior methods must be cautious and thorough. A second stage in the evaluation of automated SPE is currently in progress at a field location and will be the subject of a further report.

ABSTRACT

The Zymark Autotrace™ extractor was evaluated for the extraction and concentration of trace contaminants in water. Eight organochlorine compounds of environmental interest and covering a wide range of log k_{ow} values were dissolved in laboratory pure water and extracted with the Autotrace using a variety of eluents and conditions based on a survey of the literature. The most successful eluent was 15% diethyl ether in n-pentane. Recoveries of the six mid-range log k_{ow} analytes were very satisfactory, ranging from 72-91%. Recovery of the highest log k_{ow} analyte, mirex was only 64%. Up to about 15% of the missing mirex could be accounted for by a combination of breakthrough and retention on the SFE disk. Recovery of the lowest log k_{ow} analyte, 1,4-dichlorobenzene was a very disappointing 31%. The missing dichlorobenzene was not accounted for by breakthrough or disk retention. A possible loss route is volatilisation during the disk drying step.

INTRODUCTION

The extraction of trace organic contaminants from environmental water samples has long been accomplished by partitioning into smaller volumes of immiscible solvent(s) with a high affinity for the analyte (liquid-liquid extraction or LLE). Early techniques, still in use today, involved shaking or mixing small samples of up to 2 L with appropriate solvents in separatory funnels or capped bottles. The need to extract much larger volumes of water to analyse low levels of analytes in relatively unpolluted water bodies led to the design of rugged mechanical large volume batch extractors (McCrea and Fischer, 1985; Fox, 1986) and a large volume continuous flow extractor (Goulden and Anthony, 1985). All of these related techniques suffer from the drawbacks of excessive use of solvents, much of which is not easily recovered.

A parallel development in which the analytes are partitioned onto a particulate adsorbent (solid phase extraction or SPE) has been pursued since at least the mid 1970s but has only recently received any significant degree of acceptance. The renewed interest in SPE has been largely driven by more stringent regulations governing the use and disposal of solvents. SPE can be divided into two distinctly different categories. The first category employs a polymer bead of uniform composition wherein the analytes are adsorbed within the pore structure. Significant (but smaller than LLE) volumes of eluent are required to elute the analytes since depth penetration occurs to some extent. The second type of SPE adsorbent utilises a thin coating of a compound such as octadecyl silane (C18) bonded to a silica substrate. The thin film of relatively high capacity sorbent which, in recent developments, can be exposed to the sample in the form of a filter-like disk, has allowed the elution of many adsorbed analytes with less than 10 mL solvent; this is 100-1000 fold less than the volume used in a typical LLE extraction.

The Zymark Autotrace™ manufactured by Zymark Corporation is possibly the most advanced of several commercially available automated or semi-automated SPE extractors.

capable of the simultaneous extraction of several samples. The Autotrace can pump up to six aqueous samples simultaneously through SPE disks or cartridges at preselected constant flow rates of up to 60 mL/min. The disk pretreatment, sample loading and various rinse, pause, dry and elute conditions are chosen by the analyst and loaded into the instrument microprocessor from a method diskette created on a non-dedicated PC. The automation of each step in the extraction of samples, combined with precise microprocessor controlled flow rates leads to better reproducibility than that achieved by manual methods - typically 5x better is claimed by the manufacturer. Another advantage of the Autotrace system is the use of positive pressure for disk loading and elution processes. Many other disk or cartridge SPE systems use suction to draw fluids through the adsorbent. It has been the experience of ourselves and others that suction systems are prone to air voids, dry surfaces and variable flow rates, all of which lead to variable recoveries of analytes.

Most of the earlier SPE extraction systems utilised a disposable cartridge of glass or plastic containing typically up to 6 mL of end capped C8 or C18 reverse phase adsorbent bonded onto silica beads. The SPE disk was pioneered by 3M Corporation and consists of C8 or C18 beads bonded to a fluoropolymer fibre mat by a patented process. The product is known as Empore™ and is available in a range of sizes, adsorbents and in sheet form. The 47 mm dia. size is the most commonly used due to the widespread use of standard Millipore type suction systems. The advantages of disks over cartridges are higher flow rates and smaller elution volumes. The main drawbacks are lower capacity due to the smaller amount of sorbent and, in the case of the Empore disk, poor wettability due to the hydrophobic nature of the fluorocarbon matrix. A more recent development consists of an oriented glass fibre disc to which various reverse phase and ionic sorbents are bonded. They are marketed as SPEC™ discs by ANSYS Inc. and differ from the Empore disk in possessing greater wettability, flow rates and mechanical rigidity.

A considerable body of literature exists to ostensibly assist the reader select optimum extraction and elution conditions for the extraction of a wide variety of dissolved organic compounds from distilled water, natural waters and various effluents and special solutions. Unfortunately, the literature on the subject is frequently incomplete and sometimes even contradictory. Reported recovery efficiencies for individual analytes are often inexplicably different according to different authors. For these reasons we decided to preselect operating conditions on which a majority of authors were in agreement and perform recovery studies on a selected group of analytes dissolved in distilled water. The analytes were representative of a range of log k_{ow} values, were of environmental analytical interest, and were amenable to separation and sensitive analysis by electron capture gas chromatography. We chose not to investigate sample loading rates, disk elution rates or the effects of pH, all of which had relatively minor impact on analyte recoveries as typically documented for cartridge systems by Raisglid *et al.*, (1993) and disk systems by the disk manufacturers. Since significant differences in analyte recoveries between C8 and C18 reverse phase adsorbents have been reported (Raisglid *et al.*, 1993) we decided to investigate this aspect. Unfortunately, the SPEC disks were not available in the C8 form when the studies were carried out. Although some differences in analyte recoveries using different wetting agents and different concentrations were reported (Raisglid *et al.*, 1993), we selected methanol at 0.5% as near optimum, based on that study and our selection of analytes.

EXPERIMENTAL

Reagents and Materials:

Pesticide grade solvents were purchased from a number of suppliers and tested for purity at the approximate concentration factors that would be used in the experiments. The organochlorine compounds were obtained from Aldrich Chemical Co. Inc., Milwaukee, WI.,

Chem Service, West Chester, PA., Pestanal - Caledon Laboratories Ltd., Georgetown, Ont., Supelco Canada, Oakville, Ont., and US EPA, Research Triangle, NC., with a minimum purity of 98%. Empore C8 and C18 extraction disks were purchased from Chromatographic Specialties, Inc., Brockville, Ont., and SPEC-47-C18 AR disks were purchased from the SPEC Division of ANSYS, Inc., CA. Anhydrous sodium sulfate powder (analytical grade) was purchased from BDH, Inc., Toronto, Ont. and heated at 500°C for 24 hours and stored in a desiccator. All glassware was cleaned and solvent rinsed prior to use. Blanks and spiked solutions were made up with Milli-Q™ (Millipore - Waters, Mississauga, Ont.) water with 0.5% methanol added as wetting agent. The organochlorine stock solution (acetone) was diluted with methanol (5:1) and 0.1 mL of this solution dissolved in 500 mL Milli-Q water to produce the concentrations shown in Table 1.

Table 1 - Organochlorine Compounds in Study

Compound	log Kow	Concentration ng/L*
1,4- Dichlorobenzene (DCB)	3.60	1625.60
1,2,4-Trichlorobenzene (TCB)	3.98	211.92
1,2,3,4-Tetrachlorobenzene (TeCB)	4.55	85.60
Pentachlorobenzene (PCB)	4.90	81.68
Hexachlorobenzene (HCB)	5.47	80.72
g-Chlordane (g-CHL)	6.00	102.80
p,p-DDT (DDT)	6.36	80.48
Mirex (MIR)	6.90	80.72

* Concentration in Milli-Q water

The samples were analyzed on a Hewlett Packard 5890 Series II gas chromatograph with dual electron capture detectors (300°C), single splitless injector (250°C) and dual columns. The front column was a Hewlett Packard HP 50 and the rear column, a J & W Scientific DB 5-MS. Both columns were 30m x 0.25 mm i.d. with a film thickness of 0.25 µm. The initial column temperature was 90°C for one minute and heated to 280°C at a rate of 20°C/min with a final hold @ 280°C of 5 minutes. Hydrogen carrier gas flowed at rate of 1.1 and 1.15 mL/min for front and rear column, respectively and nitrogen auxiliary gas flowed at 38.5 and 36.2 mL/min for front and rear column, respectively. Flows were held constant at all temperatures by electronic flow programming.

Drying Experiment:

Preliminary tests of the Autotrace with a simple loading and elution program quickly revealed the existence of variable amounts of water in the collected eluant, even when an air or gas drying step was included between the loading and elution steps. A drying experiment was conducted to ensure dryness of the disks prior to elution. Two of each type of extraction disk (Empore C18, Empore C8 and SPEC C18) were weighed, placed in the disk holders of the Autotrace Workstation, conditioned, and loaded with a blank on one and a spike on the other. After the disks were rinsed with Milli-Q water and excess water removed with nitrogen gas, the instrument was paused, and the disks removed, weighed and placed into a desiccator. The disks were removed from desiccator and reweighed every 15 minutes for two hours to remove all residual water (Fig. 1). The disks were then replaced in the disk holders and soaked then eluted with 5 then 3 mL ethyl acetate followed by elution with 3 mL dichloromethane. Despite thorough drying of the disks, the eluents still contained enough water to preclude analysis. A test using a coloured aqueous dye solution revealed that a significant volume of water remained in the dead volume of the disk holders both above and below the surface where the SPE disk rests, as well as coating the solvent lines through surface tension. Virtually all of the water

from the disks, disk holders and lines was found to be removed by inserting a rinse disk with methanol step followed by a rinse disk with eluting solvent step after the disk had been removed to dry in the desiccator. An intermediate rinse was added if the first eluting solvent was not miscible with methanol. These steps were then incorporated into all future Autotrace methods.

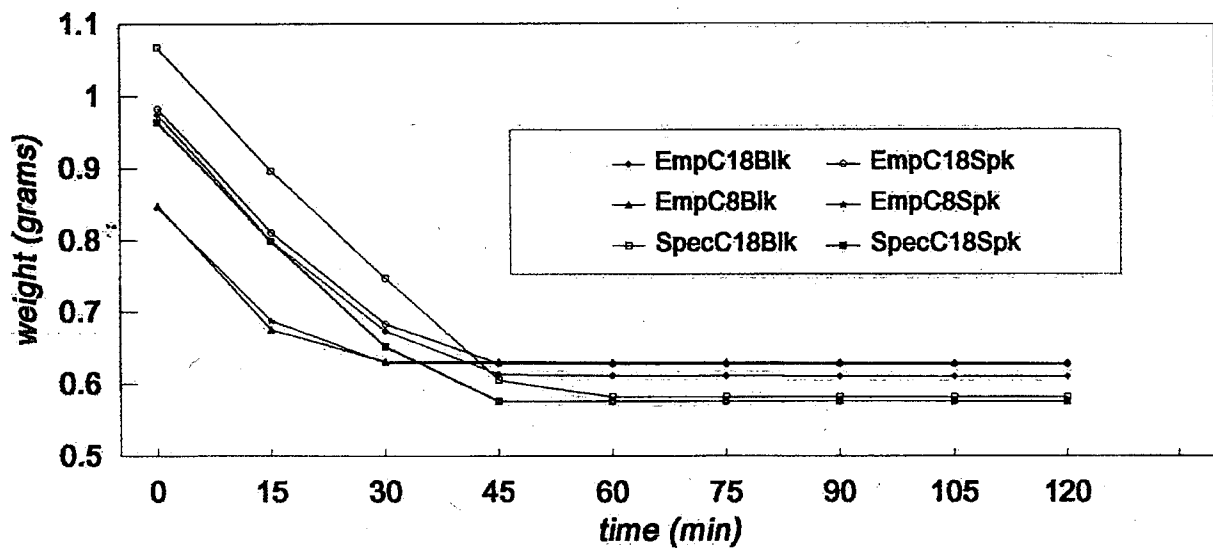


Figure 1: Drying Times for SPE Disks

Recovery Study

Evaluation of available proprietary SFE disks

Individual methods were created for Empore disks (Appendix A) to allow for soaking of eluting solvents, and SPEC disks (Appendix B) which did not require presoaking. A blank and three spiked solutions were loaded onto each of Empore C18, Empore C8 and SPEC

C18 SPE disks. The spiked solutions and blanks to be extracted on Empore disks were chilled to 4°C prior to loading. All blanks and spiked solutions contained 0.5% methanol as wetting agent. The combined eluents (5 mL ethyl acetate, 5 mL dichloromethane, and 5 mL 1:1 ethyl acetate:dichloromethane) were dried by passing through microcolumns containing sodium sulfate. Iso-octane (2 mL) was added and the extract was concentrated to a final volume of 1 mL under a stream of dry nitrogen.

Pentane/ether elution:

Elution experiments were conducted using 100% pentane, 10%, 15%, 20% and 25% ether in pentane respectively and SPEC C18 disks. The eluents were divided into two 5 mL fractions. The Autotrace method was the same as that developed for the SPEC disks in the ethyl acetate/dichloromethane elution studies with the exception of the solvent type and fractions. Loading flow rates were decreased to 50 mL/min due to pump failure at 60 mL/min. Additionally, in the first two experiments (100% pentane and 10% ether in pentane), the water from the blank and spiked solutions was collected directly from the discharge pipe into 1 L separatory funnels after passing through the SPE disks in order to test for analyte break through. The discharge samples were extracted with 3 x 30 mL dichloromethane (the first two 30 mL fractions were used to rinse the sample container and then added to separatory funnel for extraction). The extracts were dried and concentrated to 1 mL in iso-octane as described earlier. Also, the eluted extraction disks from the 100% pentane and 10% ether in pentane studies were placed in a 100 mL beaker containing 50 mL dichloromethane and allowed to leach for 18 hours. The dichloromethane was then transferred to 250 mL round bottomed flasks and 3 x 10 mL dichloromethane was rinsed through the disks and added to the flasks. Extracts were then concentrated to 1 mL in iso-octane as described earlier. The SPE disk extracts were treated as described earlier.

RESULTS AND DISCUSSION:

Drying Experiment:

Many of our early experiments were unable to yield results due to an excess of water in the eluent. Eluents with a small enough volume of water to dry with sodium sulfate yielded poor recoveries. By applying nitrogen gas at 15 psi for a period of time (1-10 minutes) the water could be driven from the SPE disks but also caused large losses of the more volatile analytes. McDonnell *et al* (1993), showed that drying the loaded disks in an atmospheric pressure desiccator was a superior technique. In order to determine how much time the disks should remain in the desiccator, the disks were weighed every 15 minutes to a constant weight (Fig. 1). The C18 Empore disks reached a constant weight in 45-60 minutes, the C8 Empore disk reached a constant weight after 30-45 minutes and C18 SPEC disks reached a constant weight in 45-60 minutes. The final dry weight of the disks was less than the weight of fresh, dry disks from the box, which suggests that there may be some material washing away in the conditioning steps and/or the disks were already partially hydrated as purchased. This procedure worked well to remove water from the disk, and the addition of a methanol rinse step to the method before the elution solvent rinse step provided a temporary solution to water in the final extract. A better solution to the problem of residual water in the system would be a redesign of the disk holders. The manufacturer of the Autotrace was asked to undertake a redesign of the disk holders for a future version of the system.

Recovery Study - based on different bonded phases:

Three types of SPE disks; Empore C18, Empore C8 and Spec C18 were tested in separate experiments using loading and elution procedures suited to the differing physical properties of the disk substrates. The results (Fig. 2), show no one type of disk to produce better

results than another for the chosen analytes. On this basis we selected the SPEC C18 disks for further use since the cost is significantly lower than the PTFE based disks. In addition, the glass fibre composition allows for greater flow-through porosity than the PTFE based disks and is rigid and easier to handle. A further significant consideration was the cleaner blank chromatograms produced by the SPEC Disks.

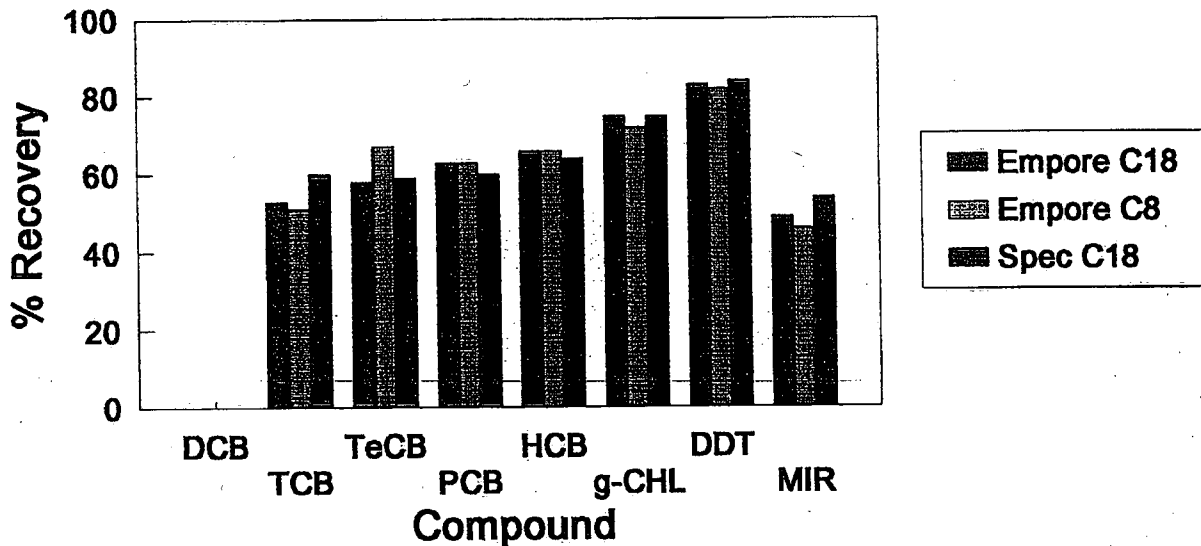


Figure 2: Recovery Study - SPE Disk Type

Pentane/Ether Recovery Study

Figure 3 summarizes the efficiency of extraction using different pentane/ether mixtures. Although the lowest relative standard deviations (2.2 - 7.4%) were obtained in the 10% ether in pentane experiment, the best recoveries were obtained in the 15% ether in pentane experiment. The recoveries ranged from 31% recovery for 1,4-dichlorobenzene to 91% recovery for DDT with relative standard deviations ranging from 1.4 - 12.5%. When extraction was compared between eluting with 15% ether in pentane and the ethyl acetate/dichloromethane elution studies (Fig. 4), the pentane/ether system yielded higher

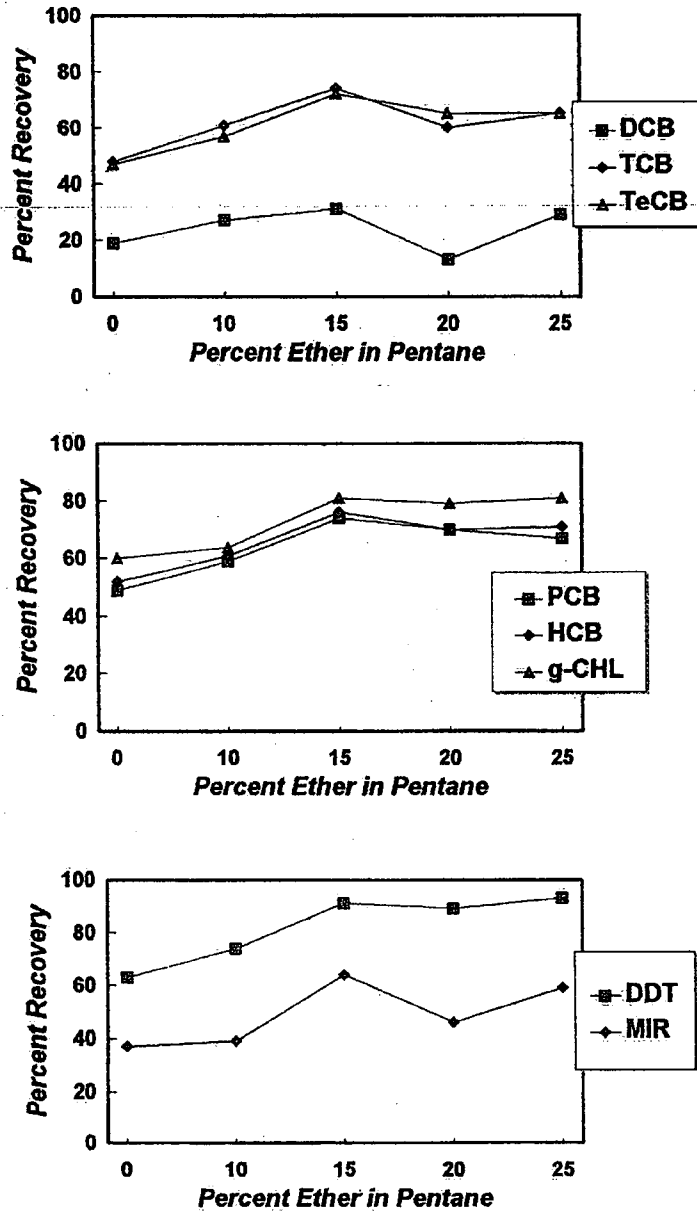


Figure 3: Summary of Recoveries Using Different Pentane/Ether Mixtures

recoveries in every case. T. McDonnell *et al* (1993) demonstrated, using Hamilton Harbour water, that although a 25% ether in pentane solution would give a better overall recovery

than a 10% solution, it would also elute a large quantity of pigment and/or non specific interference which may compromise chromatographic analysis and give rise to large relative standard deviations. Since we have shown that a 15% ether in pentane solution gives better recoveries than a 25% ether in pentane solution, it is our hope that this mixture will also be the best compromise between extraction efficiency and the elution of interfering compounds.

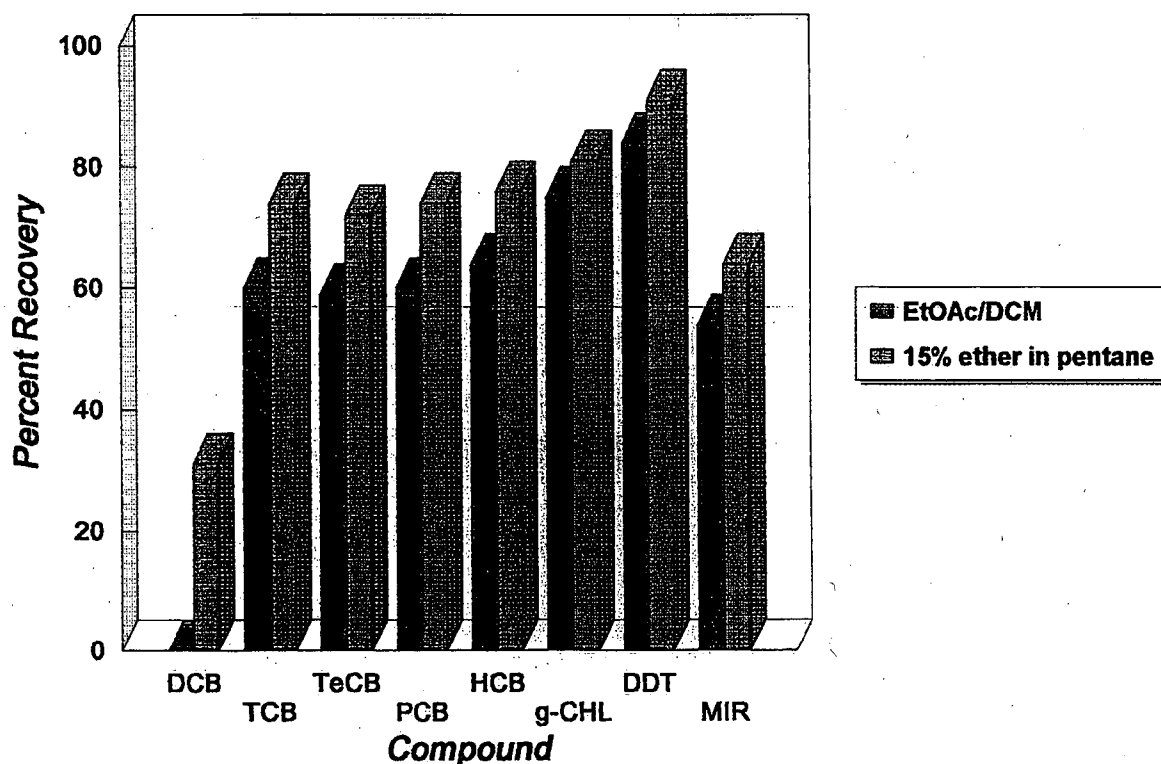


Figure 4: Comparing 15% Ether in Pentane to Ethyl Acetate/DCM Extract

In their study using Empore C8 and C18 disks, McDonnell *et al* (1993) demonstrated three classes of recoveries; high yield (70% or better), low yield due to breakthrough and those

in low yield for unknown causes. For this reason, we collected and extracted the depleted water after loading onto SPEC C18 disks and retained the eluted disks for examination for retained analytes from the first two experiments of this study as described in the experimental section. Recoveries of analytes from the depleted water were inconsistent (Table 2) but up to about 10% of the mirex appeared to have broken through the disks or remained adsorbed to the walls of the vessel containing the test solution. Since we combined the solvent rinses of this vessel with the discharge water extraction, we were unable to distinguish between the two loss routes. The study of McDonnell *et al* (1993) however, suggests that adsorption to the solution vessel walls of mirex may be discounted.

Table 2 - Breakthrough and Disk Retention Study

Liquid/liquid recoveries from disk effluent

Compound	100% Pentane			10% Ether in Pentane		
	1	2	3	1	2	3
	-----% Recovery*-----					
DCB						
TCB		6				
TeCB						
PCB			1			
HCB						
g-CHL		1			2	
DDT						
MIR		10	2	10	12	8

Disk leach recoveries

Compound	100% Pentane			10% Ether in Pentane		
	1	2	3	1	2	3
	-----% Recovery-----					
DCB						
TCB						
TeCB						
PCB			1			
HCB		2	3		1	1
g-CHL		4	4	1		
DDT						
MIR		5	5	2	1	

* recoveries corrected for interferences in the blank

The results when the eluted disk were leached were also inconsistent, possibly due to the fact that the extracts contained solid particles. The near baseline chromatography made the distinction between analyte and interfering substances tentative. Combined losses of mirex due to breakthrough and retention on the disks represent a maximum of 15% of added analyte. Since the recovery of mirex from the eluent did not exceed 64%, there is still at least 20% unaccounted for. More study is necessary to determine what is happening with this analyte. The lowest recovery was recorded for 1,4-dichlorobenzene at 31% maximum with the 15% diethyl ether/pentane system and no recovery recorded when ethylacetate/dichloromethane was the eluent. Since we failed to detect any breakthrough or disk retention of this analyte, we may tentatively conclude that the losses occurred in the vapour phase when the disk was dried and on evaporation of the higher vapour pressure ethyl acetate.

CONCLUSIONS:

- A preliminary evaluation of the Zymark Autotrace extractor and various proprietary reverse phase extraction disks was conducted on eight model organochlorine contaminants dissolved in laboratory pure water.
- The most successful recovery of analytes was achieved using an elution mixture of 15% ether in pentane.
- Satisfactory recoveries (72-91%) of the six model compounds with $\log k_{ow}$ 4 - 6.4 were obtained.
- Moderate recovery, up to 64%, of the high $\log k_{ow}$ mirex (6.9) was achieved. Up to 15% of the missing mirex could be accounted for by breakthrough and disk retention.
- Very poor recovery, maximum 31%, of the low $\log k_{ow}$ analyte, 1,4-dichlorobenzene (3) was achieved. This loss was not accounted for by breakthrough or disk retention.
- We concluded that the SPEC disks are better suited to our requirements than the Empore disks. ANSYS, Inc. now manufactures SPEC disks with a C8 bonded phase.

Technical Evaluation of the Autotrace Extractor

The Autotrace offers great advantages in the field of aqueous environmental analysis from the standpoint of time savings, environmental considerations and reproducibility. There were though, many drawbacks to this instrument that require further development. We found the overall durability of the instrument to be poor, especially when considering the simplicity of the instrument's design and the high purchase price of the instrument (>\$35K). We lost a significant amount of time in our method development with problems such as sample pumps shutting down (sometimes at flows as low as 30 mL/min), solvent lines and 12-port valve leaking and occasionally disconnecting all together. This problem seemed to be linked to the plastic fittings and rubber o-rings, which were not resistant to all solvents. The barbed fittings at the ends of each of the solvent line connections often became crimped, impeding flow of solvents and, on occasion, caused the switching valve to overheat and breakdown (i.e. overworking to push solvent through crimped line). The manufacturer now makes the barbed fittings of polyether ether ketone (PEEK™) and the o-rings of Viton™ which are more resistant to organic solvents and buffers. PEEK is not inert to dichloromethane, concentrated nitric and sulphuric acids and tetrahydrofuran. Other problems included liquid flow back into air push syringe, leaking disk holders, dispenser nozzles not lining up with collection vessels, and volume disparities (the amount dispensed by instrument was not entirely dispensed to collector even after a large air push and concentrate with gas step). The disk holders must be compressed to engage and enable the conditioning, loading and eluting of the SPE disk and, regardless of the care taken in doing this gently, a fragile plastic piece at the base of the holder may break off after very few compressions. Since the piece is integral with the main body of the disk holder, the entire disk holder must be replaced. Replacement parts for the Autotrace are more expensive than comparable items available elsewhere.

We also had some concerns regarding the systems software. We did not like the fact that each procedure printed required two pages (waste of paper, second page lists only the

solvent names which are stated clearly in the body of the procedure), only ten procedures can be archived on a hard drive file and if you did not realize that the hard drive file was full and saved another file to it the first ten files are erased and archiving restarts from file number one. Assigning a drive takes a long time since all drives are scanned first. If a mistake is made when creating a procedure or a step is missed there is no provision to insert or delete a step and the method must be recreated from scratch. Floppy disks (1 MB only) must be specially formatted through the Autotrace program and only one procedure can be saved per floppy disk.

Overall, the Autotrace has a great deal of potential but, in our opinion, a considerable amount of development is necessary to make the instrument a viable and dependable alternative to traditional liquid/liquid extraction systems.

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APPENDIX A

Zymark AutoTrace Extraction WorkStation 1.01

APPENDIX A

Zymark AutoTrace Extraction WorkStation 1.01

AutoTrace Extraction Procedure: Test # 12 (Empore)

Estimated time for samples: 60.6 min.

Date: 28 Apr 94

- Step 1 : Process 4 samples using the following procedure:
- Step 2 : Wash syringe with 10 ml of Methanol
- Step 3 : Wash syringe with 10 ml of EtOAc/DCM 1:1
- Step 4 : Condition disk with 10 ml of EtOAc 1:1 into SOLVENT WASTE
- Step 5 : Pause for 3 minutes
- Step 6 : Rinse disk with 10 mL of EtOAc/DCM 1:1 into SOLVENT WASTE
- Step 7 : Dry disk with gas for 0.5 minutes
- Step 8 : Wash syringe with 10 ml of Methanol
- Step 9 : Condition disk with 10 ml of Methanol into SOLVENT WASTE
- Step 10 : Pause for 2 minutes
- Step 11 : Wash syringe with 10 ml of DI Water
- Step 12 : Condition disk with 10 ml of DI Water into AQUEOUS WASTE
- Step 13 : Pause for 2 minutes
- Step 14 : Load 505 ml of sample onto disk
- Step 15 : Rinse disk with 10 ml of DI Water into AQUEOUS WASTE
- Step 16 : Dry disk with gas for 0.3 minutes
- Step 17 : Pause and Alert operator, resume when CONTInue is pressed
- Step 18 : Wash syringe with 10 ml of Methanol
- Step 19 : Rinse disk with 10 ml of Methanol into SOLVENT WASTE
- Step 20 : Wash syringe with 10 ml of Ethyl Acetate
- Step 21 : Rinse disk with 10 ml of Ethyl Acetate into SOLVENT WASTE
- Step 22 : Dry disk with gas for 1 minutes
- Step 23 : Pause and Alert operator, resume when CONTInue is pressed
- Step 24 : Soak and Collect 5 ml fraction using Ethyl Acetate
- Step 25 : Pause for 1 minutes
- Step 26 : Wash syringe with 5 ml of DCM
- Step 27 : Soak and Collect 5 ml fraction using DCM
- Step 28 : Pause for 1 minutes
- Step 29 : Wash syringe with 5 ml of EtOAc/DCM 1:1
- Step 30 : Collect 3 ml fraction into sample tube using EtOAc/DCM 1:1
- Step 31 : Concentrate Sample with gas for 0.5 minutes
- Step 32 : END

SETUP PARAMETERS**AutoTrace Extraction Workstation****FLOW RATES**

Cond Flow: 30.0 ml/min
Load Flow: 50.0 ml/min
Rinse Flow: 30.0 ml/min
Elute Flow: 30.0 ml/min
Cond Air Push: 30.0 ml/min
Rinse Air Push: 3 0.0 ml/min
Elute Air Push: 30.0 ml/min

SPE PARAMETERS

Push Delay: 10 sec
Air Factor: 0.0
Autowash Vol: 0.00 ml

WORKSTATION PARAMETERS

Max. Elution Vol.: 15.0 ml
Exhaust Fan on: Y Y=YES N=NO
Beeper on: N Y=YES N=NO

APPENDIX B

Zymark AutoTrace Extraction WorkStation 1.01

APPENDIX B**Zymark AutoTrace Extraction WorkStation 1.01**

AutoTrace Extraction Procedure: Test # 12 (SPEC)

Estimated time for samples: 50.1 min.

Date: 25 Apr 94

- Step 1 : Process 4 samples using the following procedure:
- Step 2 : Wash syringe with 10 ml of Methanol
- Step 3 : Wash syringe with 10 ml of Ethyl Acetate
- Step 4 : Rinse disk with 10 ml of Ethyl Acetate into SOLVENT WASTE
- Step 5 : Dry disk with gas for 0.5 minutes
- Step 6 : Wash syringe with 10 ml of Methanol
- Step 7 : Condition disk with 10 ml of Methanol into SOLVENT WASTE
- Step 8 : Wash syringe with 10 ml of DI Water
- Step 9 : Condition disk with 10 ml of DI Water into AQUEOUS WASTE
- Step 10 : Load 505 ml of sample onto disk
- Step 11 : Rinse disk with 10 ml of DI Water into AQUEOUS WASTE
- Step 12 : Dry disk with gas for 0.3 minutes
- Step 13 : Pause and Alert operator, resume when CONTInue is pressed
- Step 14 : Wash syringe with 10 ml of Methanol
- Step 15 : Rinse disk with 10 ml of Methanol into SOLVENT WASTE
- Step 16 : Wash syringe with 10 ml of Ethyl Acetate
- Step 17 : Rinse disk with 10 ml of Ethyl Acetate into SOLVENT WASTE
- Step 18 : Dry disk with gas for 1 minutes
- Step 19 : Pause and Alert operator, resume when CONTInue is pressed
- Step 20 : Collect 5 ml fraction into sample tube using Ethyl Acetate
- Step 21 : Concentrate Sample with gas for 0.25 minutes
- Step 22 : Wash syringe with 5 ml of DCM
- Step 23 : Collect 3 ml fraction into sample tube using DCM
- Step 24 : Concentrate Sample with gas for 0.25 minutes
- Step 25 : Wash syringe with 5 ml of EtOAc/DCM 1:1
- Step 26 : Collect 3 ml fraction into sample tube using EtOAc/DCM 1:1
- Step 27 : Concentrate Sample with gas for 0.5 minutes
- Step 28 : END

SETUP PARAMETERS**AutoTrace Extraction Workstation****FLOW RATES**

Cond Flow: 30.0 ml/min
Load Flow: 50.0 ml/min
Rinse Flow: 30.0 ml/min
Elute Flow: 30.0 ml/min
Cond Air Push: 30.0 ml/min
Rinse Air Push: 30.0 ml/min
Elute Air Push: 30.0 ml/min

SPE PARAMETERS

Push Delay: 10 sec
Air Factor: 0.0
Autowash Vol: 0.00 ml

WORKSTATION PARAMETERS

Max. Elution Vol.: 15.0 ml
Exhaust Fan on: Y Y=YES N=NO
Beeper on: N Y=YES N=NO

Appendix C

Technical problems encountered with the AutoTrace extractor

Several problems were identified in the operation of the AutoTrace extractor. These problems were communicated to the manufacturer, resulting in at least one re-designed feature (see below):

(1) Sample pump failures

Sample pump failures, leaking of the 12-port valve, and leaking from solvent lines occurred frequently in the initial stages of this work. These problems appeared to be linked to plastic fittings and rubber O-rings which were not resistant to some solvents used. The barbed fittings at the ends of each of the solvent line connections often became crimped, impeding flow of solvents and, on occasion, causing the switching valve to overheat and break down. The manufacturer now makes the barbed fittings of polyether ether ketone (PEEK™) and the O-rings of Viton™ which are more resistant to organic solvents and buffers. It should be noted that PEEK is not inert to dichloromethane, concentrated nitric and sulfuric acids and tetrahydrofuran.

(2) Other hardware problems

Other problems included liquid flow back into the air push syringe, leaking disk holders, dispenser nozzles not lining up with collection vessels, and volume disparities (*i.e.*, the amount dispensed by the instrument was not entirely collected even after a large air push and concentrate with gas step). The disk holders must be compressed to engage and enable the conditioning, loading and eluting of the SPE disk and, even when this compression was done gently, a fragile plastic piece at the base of the holder occasionally broke off after only a few compressions. Since the piece is integral with the main body of the disk holder, the entire disk holder had to be

replaced.

(3) Software deficiencies

Only ten procedures can be archived on a hard drive file. Addition of more procedures resulted in the erasure of the first ten files. If a mistake is made when creating a procedure, or a step is missed, there is no provision to insert or delete a step and the method must be recreated. Floppy disks (1 Mb only) must be specially formatted through the AutoTrace program and only one procedure can be saved per floppy disk. Each procedure printed required two pages, which was a waste of paper, because the second page listed only the solvent names which are stated clearly in the body of the procedure on the first page.

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National Hydrology Research Centre

11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

St. Lawrence Centre

105 McGill Street
Montreal, Quebec
H2Y 2E7 Canada

Place Vincent Massey

351 St. Joseph Boulevard
Gatineau, Quebec
K1A 0H3 Canada

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Case postale 5050
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Burlington (Ontario)
L7R 4A6 Canada

Centre national de recherche en hydrologie

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Saskatoon (Saskatchewan)
S7N 3H5 Canada

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105, rue McGill
Montréal (Québec)
H2Y 2E7 Canada

Place Vincent-Massey

351 boul. St-Joseph
Gatineau (Québec)
K1A 0H3 Canada