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Development of Techniques for Measuring the Quality of Groundwaters – Analysis of Pesticides and Metabolites etc.

I.Harrison

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Foreword

This report is the published product of a study by the British Geological Survey (BGS) on measurement of the quality of groundwaters. It focuses on the determination of a variety of pesticides and some of their metabolites etc. in groundwaters by chromatographic methods. Details of the analytical procedures are outlined.

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Contents

Summary

An attempt has been made to develop simple and effective analyses for a range of pesticides, pesticide mixtures, pesticide TEAPs and pesticide metabolites and to detail the practical aspects required for their accomplishment. All the methods are based essentially on two fundamental chromatographic techniques, *i.e.* high performance liquid chromatography (HPLC) and solid phase extraction (SPE). The former affords separation and quantification of the analytes, the latter provides pre-concentration so that trace levels of analytes may be determined.

The aim is to provide sufficient information to permit a competent chromatographer to be able to generate analytical data on the concentrations of pesticides and their metabolites for use in studies and investigations that concern these environmentally significant species.

1 Introduction

During the past sixty years the use of synthetic organic chemicals as pesticides has contributed substantially towards increasing global food production. It is acknowledged that modern agricultural systems rely heavily on pesticides as a cost- and time-effective measure for the control of weeds, insects and other assorted pests. However, the widespread use of pesticides, both agriculturally and domestically, carries an associated risk of pollution of subsoils and groundwaters and ultimately the possible contamination of drinking water resources. Pesticide leaching has been studied extensively over the past decade to characterise the potential for pollution and attenuation. Underpinning such investigations is the ability to identify and quantify the compounds of interest. Until recently the focus had been on the active pesticides themselves. However it is now appreciated that biotic and abiotic soil processes can transform pesticides into other substances. The incorporation of metabolite behaviour is fundamental to understanding the fate of pesticides in groundwaters particularly as these substances can, in some instances, be more toxic than the parent pesticide.

BGS has been actively involved in a variety of pesticide/metabolite studies aimed at assessing their impact on groundwater (Chilton et al., 2000; Gooddy et al., 2002a/b; Gooddy et al., 2003a/b; Williams et al., 2003; Harrison et al., 2003) and methods have been, and are continuing to be, developed to assist in and enhance our analytical capabilities in this area.

The development of analytical capability described in this report, has taken three directions:

- Development of solid phase extraction (SPE) techniques for the pre-concentration of groundwaters containing low levels of pesticides.
- Development of single-run chromatographic methods for the analysis of mixtures of pesticides and their associated metabolites.
- Refinement of existing techniques of pesticide analysis.

In the following report the aim has been to:

- Clearly and succinctly delineate the methods developed so that they may easily set up and run.
- Highlight important and significant caveats that aid in their performance.
- Provide an insight into the process of development so that should any modification or departures be required then the underlying logic will be available for consideration.

2 Developments in Pesticide Analysis

2.1 DIURON AND ITS METABOLITES

At BGS Keyworth a method had been developed for the determination of diuron and its metabolites in connection with a study of pesticide degradation in unsaturated chalk (Gooddy et al., 2002a/b; Gooddy et al., 2003a). In this study, relatively high concentrations of diuron and its three principal metabolites, DCPMU, DCPU and DCA were encountered. Thus, in porewaters and sediments maximum concentrations arising during the investigation were approximately:

	Systematic Chemical Name	Max. Porewater Conc. (µg/l)	Max. Sediment Conc.(µg/kg)
Diuron	N′-[3,4-dichlorophenyl]-N,N-dimethylurea	2300	9000
DCPMU	N′-[3,4-dichlorophenyl]-N-methylurea	400	2500
DCPU	N′-3,4-dichlorophenylurea	60	400
DCA	3,4-dichloroaniline	100	300

Under these conditions, analysis based on modification of a reported method (Field et al., 1997) and employing HPLC/UV, could be conducted without the need for sample pre-concentration. A linear calibration range of 0 – 2500 µg/l and a limit of detection of *ca*. 0.1 µg/l was obtainable with the following procedure:

1). Sample Preparation - Aqueous Sample

No preparation of aqueous samples is necessary other than allowing them to stand until clarification of the supernatant occurs. A minimum of 1.5 ml of clarified supernatant then needs to be withdrawn into an HPLC syringe for injection into, and analysis by, the HPLC system. Two aqueous calibration standards containing diuron and the three metabolites, one at 100 µg/l each and the other at 1 µg/l each, showed no propensity for deterioration over a 6 month period when stored in a refrigerator. Accordingly, no preservative was added to samples if only short term refrigerated storage before analysis was undertaken (up to one week). Storage for longer periods (> 1 week) was accompanied by preservation and it was found that either sodium azide or mercury(II) chloride, at their effective concentrations of 0.2% w/v and 25 mg/l respectively, had no deleterious effect on the subsequent chromatography.

2). Sample Preparation - Soil sample

Approximately 200 g of soil is weighed accurately into a tared conical beaker. To this is added twice as much methanol (HPLC grade) by weight (density *ca*. 0.8 g/ml). The mixture is carefully stirred with a large palette knife-type spatula which is also used to break down any large soil aggregates. The beaker is then placed in a heated ultrasonic bath (40ºC) and sonicated for 15 mins. Stirring followed by sonication is repeated a further two times at intervals over a 24 h period. The contents of the beaker are then transferred to a Buchner funnel and filtered through Whatman No. 41 filter paper. The residue is rinsed with methanol and the filtrate reduced in volume on a rotary evaporator until nearly all the methanol has been removed (if most of the methanol is not removed 'strong solvent' effects may be observed during subsequent chromatography). The resulting, predominantly, aqueous concentrate (*ca*. 10 - 20 ml) is then made to an appropriate volume (*e.g*. 1 L for samples >1000 µg/kg diuron or 100 ml for samples <1000 µg/kg diuron) with pure water (*e.g*. MilliQ), then stoppered and shaken. A sample of *ca*. 2 ml (1.5 ml minimum) is withdrawn into an HPLC syringe.

Note – either a dried or moist soil sample may be used. If using a moist sample a weighed aliquot may be dried at 105ºC then re-weighed so that the % moisture may be calculated. If using a dry sample *ca.* 50 ml pure water should be added to the filtrate before the rotary evaporation.

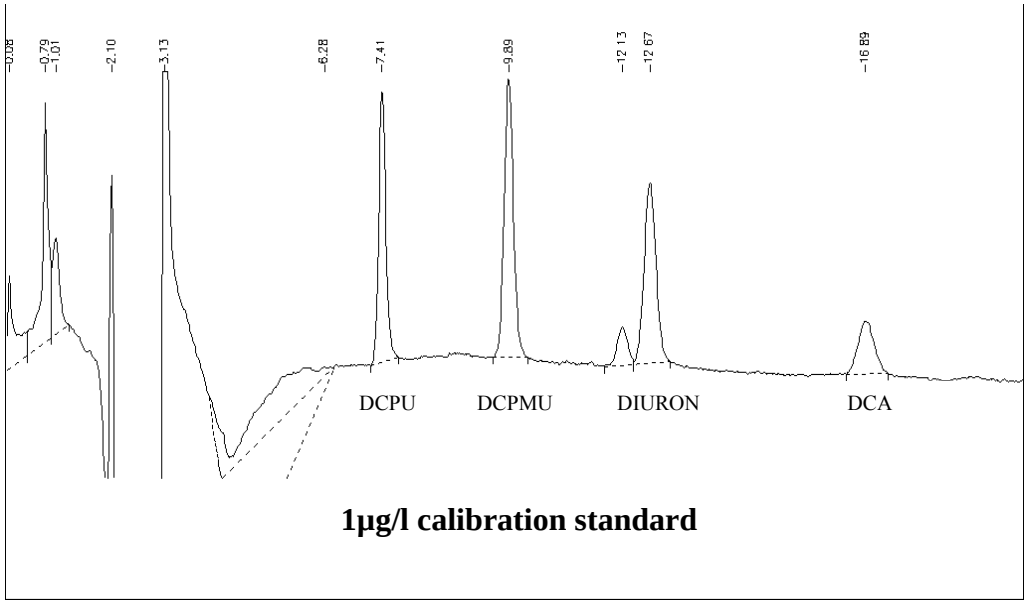
3). HPLC Conditions for diuron and metabolites

Mobile phase	- 35% acetonitrile (HPLC grade) : 65% MilliQ (18MΩ) water
Pump	- Isocratic HPLC pump (*e.g*. Merck-Hitachi L6000)
Flowrate	- 1 ml/min (Back pressure *ca*. 80-90 bar).
Injection Volume	- 1 ml sample loop on a Rheodyne 7125 Injector
Analytical column	- Supelcosil LC-18-DB (150 mm x 4.6 mm ID) 5 µm
Guard column	- Supelguard LC-18-DB (20 mm cartridge) 5 µm
Detection	- UV detector (*e.g*. Waters 2487) set to 252 nm.
Integration Software	- *e.g*. Perkin Elmer Turbochrom 4.2

Notes on Sample Loading and Column Back-Flushing

Before loading a sample into the 1 ml sample loop (installed on the Rheodyne valve), the loop is first rinsed with 5 ml pure water from another HPLC syringe and then flushed through with air from this same syringe. To clean and then fill the loop with air is important if consistent and complete filling with such a relatively small volume of sample (*i.e.* 1.5 to 2 times the loop volume) is to be achieved.

After each sample run the column and guard are disconnected, reversed and back-flushed with *ca*. 20 ml of acetonitrile HPLC grade, then re-connected and equilibrated with mobile phase before injecting the next sample. If this precaution is not undertaken then other slowly eluting compounds present in the sample that remain in the column at the end of the sample run may elute and interfere with the target compounds as they elute on following runs. Backflushing also has the advantage, of dislodging at least some of the particulate material that can collect on the frit at the head of the guard column reducing the need for its over-frequent replacement. Typical chromatograms for a standard solution and an extracted soil sample are shown in Figure 1.



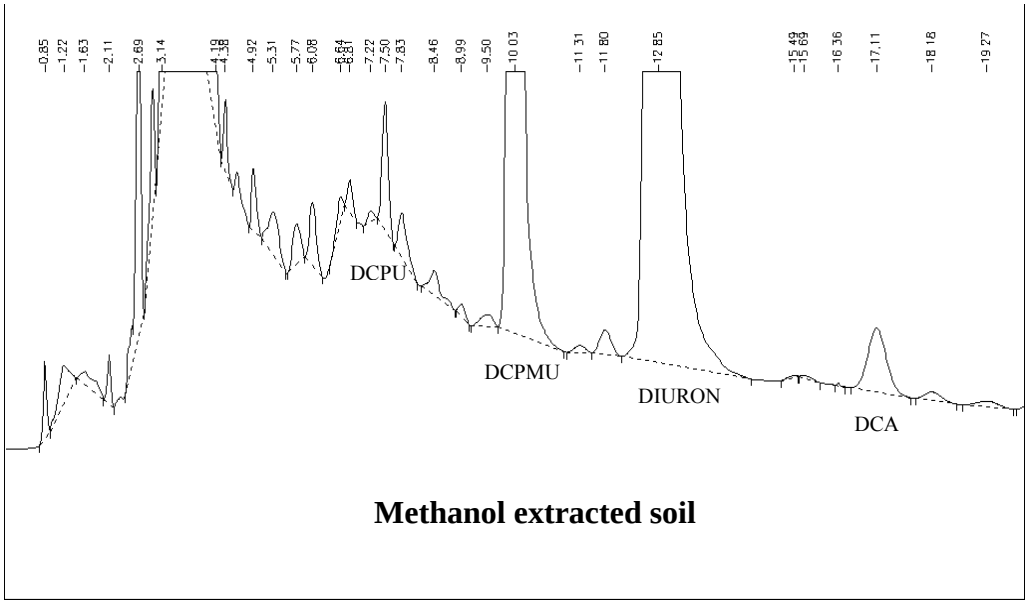


Figure 1 Typical chromatograms for diuron and three metabolites.

4). Sample Pre-concentration

As mentioned, the initial study for which the method was developed involved porewater analysis. Since the porewaters were obtained from the interstices of moist soil samples by centrifugation only comparatively limited volumes of this aqueous material were available for analysis (*ca*. 10 ml). Even though the limit of detection without pre-concentration of the porewaters ultimately proved adequate for the study, nevertheless, at the outset an embryonic SPE method based on the use of a polymeric phase was developed in case pre-concentration was required. Because it was designed for 10 ml of porewater, it had only a modest capacity to pre-concentrate (*i.e*. x 5 pre-concentration) *cf.* Field et al., 1997 where a sample of 100 ml was utilised and a x 50 pre-concentration was afforded. That said, the Field et al. method though it offered excellent recoveries for diuron (99%), DCPMU (98%) and DCPU (98%) the recovery for DCA (78%) was somewhat low. This was attributed to slow and incomplete elution for DCA that was bound to residual silanols in the Empore disks (a base-deactivated variety of which is not currently manufactured). It was felt that perhaps this problem could be overcome by the use of a non silica-based SPE sorbent and so polymer-based SPE cartridges were examined. By using Varian Nexus cartridges for the pre-concentration of 10 ml of aqueous diuron and its metabolites each at a concentration of 100 µg/l recoveries of diuron (94%), DCPMU (94%), DCPU (98%) and DCA (95%) were obtained.

Varian Nexus SPE method (for small aqueous samples, *e.g.* porewaters *ca*. 10 ml):

- Precondition a Varian Nexus LRC 30 mg SPE cartridge by passing 5 ml methanol (HPLC grade) followed by 5 ml pure water.
- Pass the 10 ml of aqueous sample through the conditioned cartridge at *ca.* 5 ml /min. using controlled vacuum.
- Rinse cartridge with 1 ml pure water.
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 5 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 4 x 500 µl of methanol (HPLC grade) into a 5 ml graduated glass centrifuge tube.
- Blow down the methanol extract with nitrogen to *ca*. 0.2 ml then make to 2 ml with pure water.
- Inject the resulting sample into the HPLC system and analyse for diuron and the three metabolites.
- Limit of detection *ca*. 20 ng/L

However, in a later study undertaken with the Environment Agency (Gooddy et al., 2004), where diuron and its metabolites were present at the nanogram per litre level it became necessary for a much greater pre-concentration to be obtained (*i.e*. x 1000 pre-concentration). When using the method above with Varian Nexus 60 mg cartridges and a 200 ml sample containing diuron and metabolites, each at a concentration of 0.5µg/l in 0.2 % w/v sodium azide, a x100 pre-concentration gave the following recoveries *i.e.* diuron (97%), DCPMU (95%), DCPU (88%) and DCA (17%). The cause of the low recovery for DCA was assumed to be due some strong form of interaction of it with the Nexus sorbent (*i.e.* a divinylbenzene/methylmethacrylate co-polymer). So to retain the benefits of using polymeric phases (high capacity, fast flowrates and tolerance to small amounts of air being drawn through the bed), experiments were conducted with Waters Oasis HLB cartridges. The Oasis sorbent bed is based on a divinylbenzene/N-vinyl pyrrolidone co-polymer and so some differences in retention may be anticipated.

Repeating the method but with a Waters Oasis HLB 60 mg cartridge to pre-concentrate the 200 ml sample produced a better recovery of DCA, *i.e.* diuron (93%), DCPMU (90%), DCPU (94%) and DCA (70%). When acetone (Romil UpS grade) was substituted for methanol to increase the eluotropic strength of the eluting solvent much better recovery of DCA was apparent *i.e.* diuron (94%), DCPMU (90%), DCPU (99%) and DCA (92%).

Waters Oasis SPE method (for aqueous samples, *ca.* 200 ml):

- Precondition a Waters Oasis HLB 60 mg SPE cartridge by passing 5 ml acetone (Romil UpS grade) followed by 5 ml pure water.
- Pass the 200 ml of aqueous sample (containing 0.2 % w/v sodium azide) through the conditioned cartridge at *ca.* 5 ml /min. using controlled vacuum.
- Rinse cartridge with 1 ml pure water.
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 5 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 4 x 500 µl of acetone (Romil UpS grade) into a 5 ml graduated glass centrifuge tube.
- Blow down the acetone extract with nitrogen to *ca*. 0.2 ml then make to 2 ml with pure water.
- Inject the resulting sample into the HPLC system and analyse for diuron and the three metabolites.
- Limit of detection *ca.* 1 ng/L

An attempt was made to extend the procedure to larger volumes of sample by processing 2 L of water spiked with diuron and metabolites to a concentration of 0.05 µg/l of each. To compensate for the increased volume two Waters Oasis cartridges in series were used initially each with a 200 mg sorbent bed. This afforded recoveries of diuron (90%), DCPMU (85%), DCPU (90%) and DCA (77%). The lower recoveries were not due to breakthrough since negligible amounts of the analytes were found to be present on the lower of the two cartridges. This also made it clear that only one 200 mg cartridge would be required. However, the SPE process appeared to yield reproducible recoveries and so it was used to analyse trace (ng/L) levels of diuron and the three metabolites in the samples of groundwater in the EA study (Gooddy et al., 2004).

Waters Oasis SPE method (for aqueous samples, *ca.* 2000 ml):

- Precondition a Waters Oasis HLB 200 mg SPE cartridge by passing 5 ml acetone (Romil UpS grade) followed by 5 ml pure water.
- Pass the 2000 ml of aqueous sample (containing 0.2 % w/v sodium azide) through the conditioned cartridge at *ca.* 10 ml /min. using controlled vacuum.
- Rinse cartridge with 1 ml pure water.
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 5 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 3 x 5 ml of acetone (Romil UpS grade) into a 15 ml graduated glass centrifuge tube.
- Blow down the acetone extract with nitrogen to *ca*. 0.8 ml then make to 2 ml with pure water.
- Inject the resulting sample into the HPLC system and analyse for diuron and the three metabolites.
- Limit of detection *ca*. 0.1 ng/L except DCA 5 ng/L (see text)

The samples were provided in 2.5 L amber glass Winchesters and upon receipt each was preserved by the addition of 5 g of sodium azide before storage under refrigerated conditions until the supernatant appeared visibly to have clarified. It had been found that sample turbidity can result in clogging of the frit used to contain the sorbent bed and subsequently an increasingly reduced flowrate through the cartridge. If settling is insufficient to provide supernatant clarity the sample can be filtered through a Whatman GFC glass-fibre disc filter prior to SPE.

The volume to be concentrated by SPE was ascertained by carefully decanting settled groundwater from the Winchester into a clean 1 L measure and pouring the remainder into a clean 2 L beaker without disturbing the sediment and discarding the turbid last few hundred mls. Measured aliquots from the 1 L measure were transferred into a clean empty Winchester so that

the volume for pre-concentration was known. Sample identity and volume was recorded before passage of groundwater through the SPE system. The apparatus for the simultaneous extraction of two groundwater samples is as illustrated by the diagram in Figure 2.

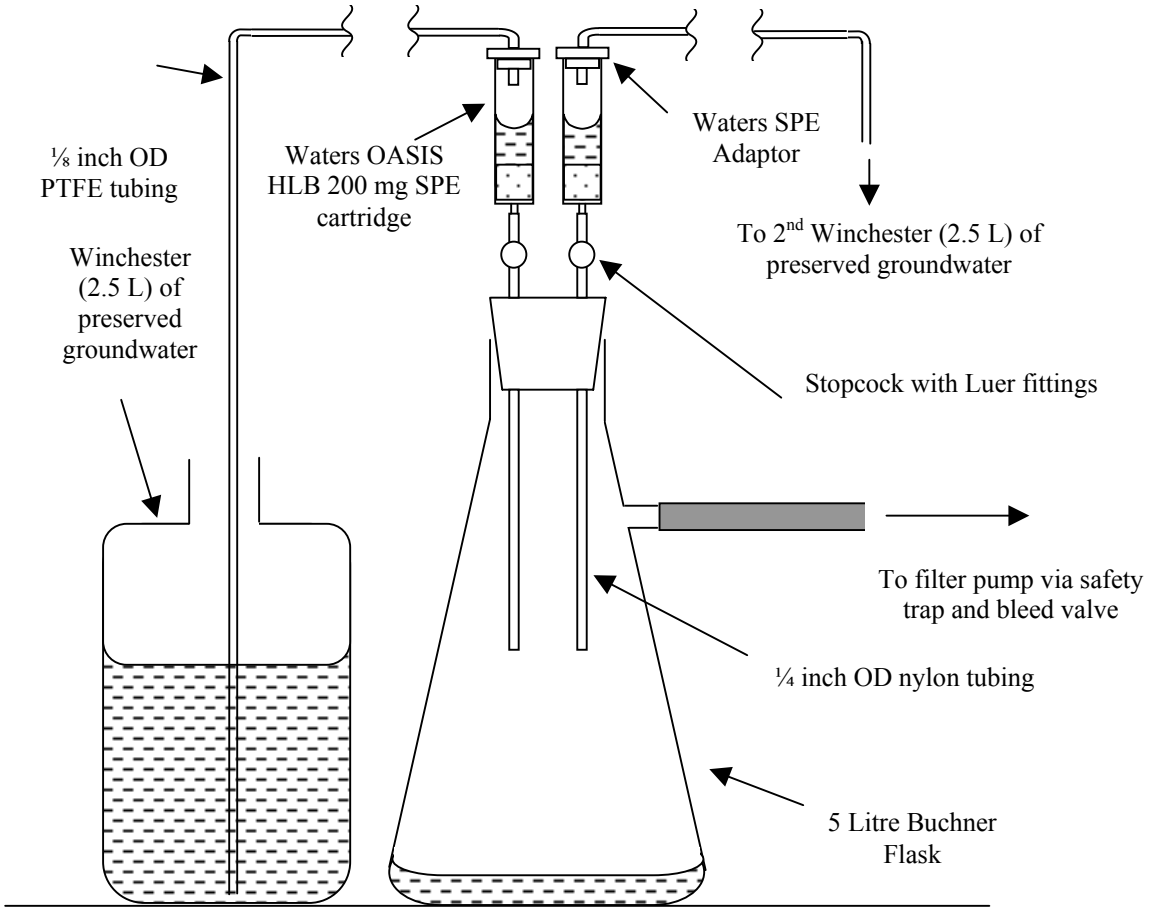


Figure 2 – Vacuum apparatus for SPE of 2 L volumes of groundwaters

Limits of detection were found to be diuron (0.1 ng/l), DCPMU (0.1 ng/l), DCPU (0.1 ng/l) and DCA (5 ng/l). The elevated limit for DCA was due to an interfering peak, that eluted with the same retention time as DCA, detected when procedural blanks (*i.e.* 2 L of pure water substituted for groundwater in the SPE procedure) were run. Accordingly, procedural blank subtractions were applied to all analyses of real samples.

2.2 ATRAZINE, DIURON AND METABOLITES

An investigation into pesticides in groundwaters from the Triassic Sandstone in south Yorkshire (Buckley, 2003) required that groundwaters be analysed for diuron and its three principal metabolites (see Section 2.1) and also for atrazine (AT) and its three main metabolites, *i.e*. DIPA (deisopropylatrazine), DETA (desethylatrazine) and 2HAT (2-hydroxyatrazine). Development work was undertaken to produce a chromatographic method of analysis for all the above compounds preferably within a single run and to generate a method for pre-concentration by SPE. Given that diuron and its three major metabolites had been successfully analysed using a Supelcosil column it was thought that this column should be investigated initially for the separation of the required eight analytes.

During the preparation of standard solutions limited solubility problems were encountered in preparing a 2HAT concentrate (500 mg/l). Unlike atrazine, DIPA and DETA which dissolved readily in 50% aqueous methanol, 2HAT appeared to be insoluble in both water and the usual HPLC organic solvents. Review of the literature concerning this compound made it clear that the addition of hydrochloric acid was necessary for its dissolution in water (Steinheimer, 1993). To prepare a 500 mg/l standard 50 mg of 2HAT was rinsed into a 100 ml volumetric flask with pure water until about 50 ml had been added. To this concentrated hydrochloric acid AR was added dropwise with swirling until a clear solution was obtained. The aqueous solution was then made to volume with methanol (HPLC grade). The solution pH was found to be 1.8 and even after dilution to 100µg/l in a mixed standard this had consequences for the chromatography in that if the eluent was unbuffered (as with the diuron and its metabolites) then there was still sufficient acidity for 2HAT, with a pKa of 5.2 (Coquart et al., 1993) to remain in its ionised form and exit the column with the solvent front. This did not occur for atrazine, DIPA and DETA since their pKa values are 1.7, 1.3 and 1.3 respectively. However, use of a mobile phase consisting of a mixture of acetonitrile and phosphate buffer pH 6.6 in place of acetonitrile and water overcame this problem (see HPLC Conditions). Once a satisfactory gradient programme had been experimentally derived and the wavelength for detection optimised then HPLC as illustrated in the chromatogram in Figure 3 was obtained. Concentrations in the range 0-2500 µg/l were found to produce linear calibrations.

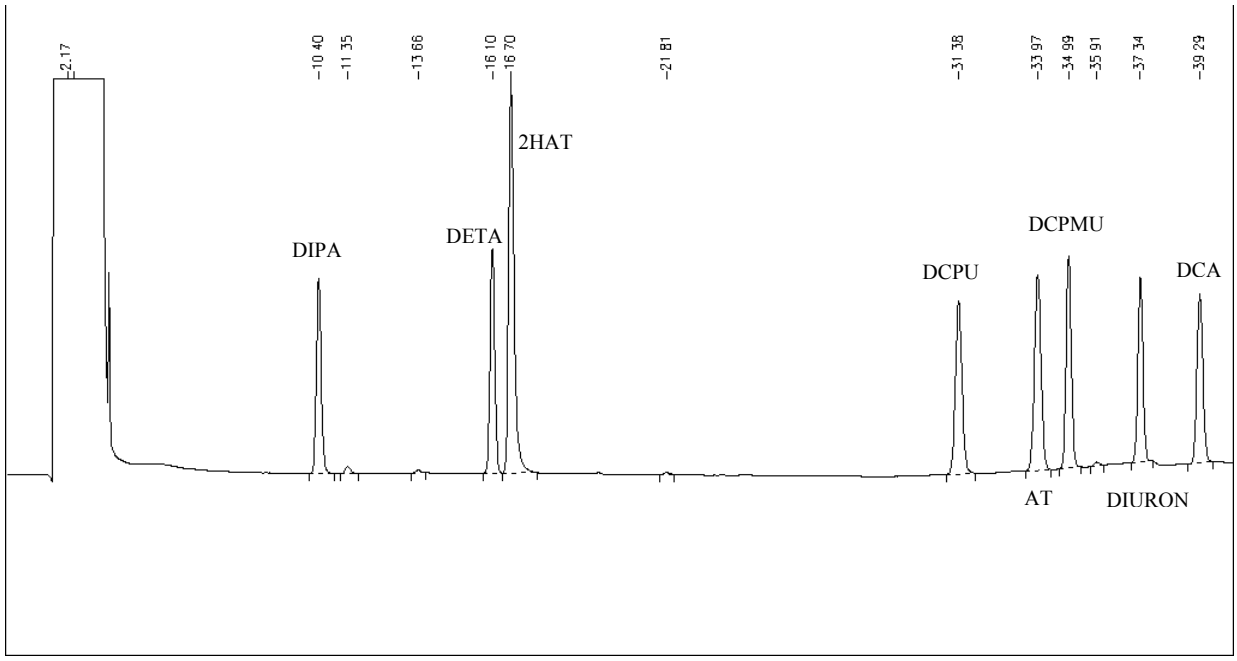


Figure 3 Chromatogram of atrazine, diuron and their metabolites (100 µg/l each)

1). HPLC Conditions for diuron, atrazine and metabolites

Mobile phase	- Acetonitrile (HPLC grade): 20 mM disodium hydrogen phosphate buffer (*i.e.* 5.68 g Na_2HPO_4 AR to 2 L with pure water – adjust to pH 6.6 with H_3PO_4 AR - vacuum filter 0.45µm before use)
Pump	- Gradient HPLC pump (*e.g.* Waters 600E)
Flowrate	- 1 ml/min (Back pressure *ca.* 80-90 bar)
Gradient programme	- 10% acetonitrile linearly to 25% acetonitrile after 15 mins. hold 25% acetonitrile until 22 mins. then 25% acetonitrile linearly to 40% acetonitrile after 32 mins. hold 40% acetonitrile to end of run (40 mins.)

Injection Volume	- 1 ml sample loop on a Rheodyne 7125 Injector
Analytical column	- Supelcosil LC-18-DB (150 mm x 4.6 mm ID) 5 µm
Guard column	- Supelguard LC-18-DB (20 mm cartridge) 5 µm
Detection	- UV detector (*e.g.* Waters 2487) set to 235 nm.
Integration Software	- *e.g.* Perkin Elmer Turbochrom 4.2

2). Sample Pre-concentration

As with diuron and its metabolites, development work on the SPE was conducted with polymeric phase cartridges. A variety of polymeric SPE phases were tested for their ability to pre-concentrate 200 ml of pure water containing each component at 0.5 µg/l. Methanol (HPLC grade) was used as the eluting solvent and in each case this was blown-down under nitrogen to *ca*. 0.5 ml and then made to 2 ml with pure water so that a pre-concentration of x100 was achieved. The following recoveries were obtained:

		Varian Nexus	**IST ENV+**	**Phenomenex Strata X**	**Waters Oasis HLB**
Sorbent bed weight (mg)		30	200	60	60
Eluting Volume (ml)		1.5	5	6	6
% Recovery	DIPA	20	131	94	98
	DETA	66	117	98	100
	2HAT	2	89	95	104
	DCPU	88	101	100	102
	AT	90	101	96	101
	DCPMU	95	105	98	97
	Diuron	97	102	106	101
	DCA	17	69	88	94

On the basis of these results Waters Oasis HLB (60 mg) were chosen as the preferred SPE cartridges for the x100 pre-concentration of 200 ml of groundwater.

Waters Oasis SPE method (for aqueous samples, *ca.* 200 ml):

- Precondition a Waters Oasis HLB 60 mg SPE cartridge by passing 5 ml methanol (HPLC grade) followed by 5 ml pure water.
- Pass the 200 ml of aqueous sample through the conditioned cartridge at *ca.* 5 ml /min. using controlled vacuum.
- Rinse cartridge with 1 ml pure water.
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 5 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 2 x 3 ml of methanol into a 10 ml graduated glass centrifuge tube.
- Blow down the acetone extract with nitrogen to *ca*. 0.5 ml then make to 2 ml with pure water.
- Inject the resulting sample into the HPLC system and analyse for diuron, atrazine and their six metabolites.
- Limit of detection *ca.* 1 ng/L

N.B. It is important during the analysis that the advice offered in the 'Notes on Sample Loading and Column Backflushing' (p.3) be followed.

2.3 MECOPROP ENANTIOMERS, A METABOLITE AND TEAPs

For some time BGS Keyworth has, in collaboration with the EA, been involved with the determination by HPLC of mecoprop enantiomers and their breakdown product 4-chloro-2-methylphenol (4-CMP) in groundwaters emanating from a group of leaking landfill sites at Helpston, Cambs. (Williams et al., 2000; Williams et al., 2003; Harrison et al., 2003). The method utilises chiral HPLC in which a chiral selector, (*i.e.* a cyclodextrin derivative covalently bonded to the 5µm silica particles of the column) is employed to separate the stereoisomeric mecoprop enantiomers. Because, relatively high mecoprop concentrations, up to 10 mg/l, were encountered in the vicinity of the landfills, the following HPLC conditions provided satisfactory data for groundwater samples without the need for pre-concentration. A typical chromatogram for a standard solution containing 1 mg/l of racemic mecoprop (*i.e.* 0.5 mg/l each of *(R)*- and *(S)*-mecoprop) and 0.5 mg/l 4-CMP is depicted in Figure 4. The limit of detection for either enantiomer was *ca.* 0.5µg/l and that for 4-CMP was *ca.* 0.3µg/l. The responses of the two enantiomers and 4-CMP were found to be linear in the range 0 – 50 mg/l. The addition of a preservative (*i.e.* sodium azide to a give a concentration of 0.2% w/v) prevented microbial degradation and no perceptible sample deterioration was found to have occurred in preserved samples that had been stored in a refrigerator for 3 months.

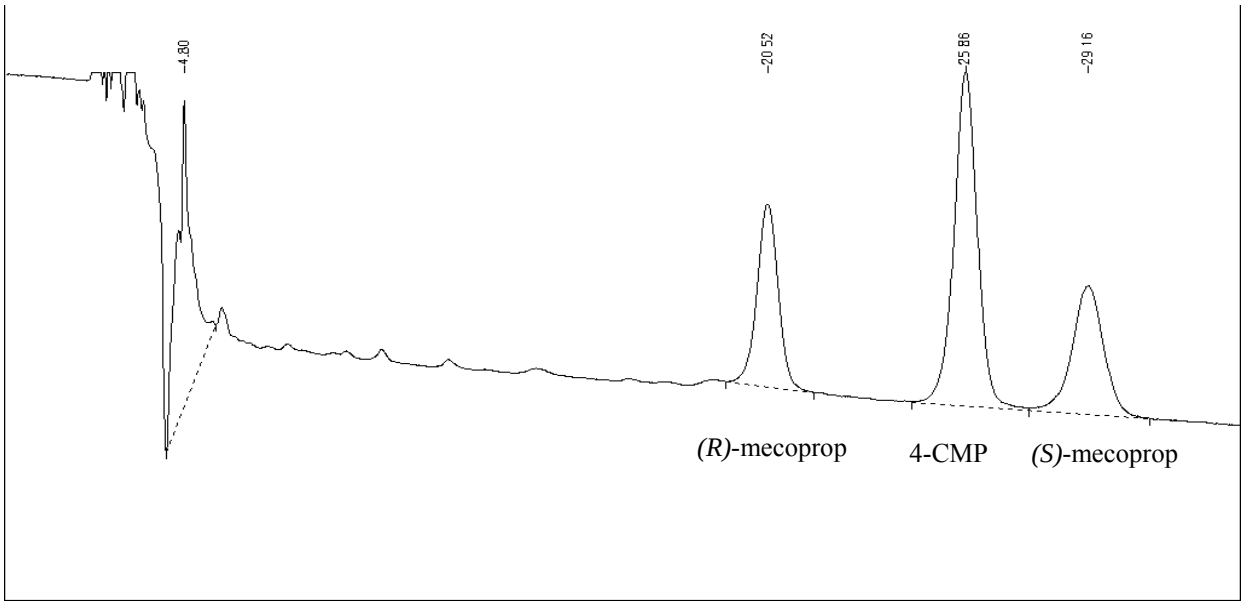


Figure 4 Typical chromatogram of mecoprop enantiomers and metabolite

1). HPLC Conditions for mecoprop enantiomers and metabolite

Mobile phase	- 65% methanol (HPLC grade) : 35% 25 mM disodium hydrogen phosphate buffer (*i.e.* 7.1 g Na_2HPO_4 AR to 2 L with pure water adjust to pH 3 with H_3PO_4 AR - vacuum filter 0.45µm before use)
Pump	- Isocratic HPLC pump (*e.g.* Merck-Hitachi L6000)
Flowrate	- 0.7 ml/min (Back pressure *ca.* 180 bar).
Injection Volume	- 1 ml sample loop on a Rheodyne 7125 Injector
Analytical column	- Macherey-Nagel Nucleodex α-PM (200 mm x 4 mm ID) 5 µm
Guard column	- Macherey-Nagel Nucleodex α-PM (8 mm x 4 mm ID) 5 µm
Detection	- UV detector (*e.g.* Waters 2487) set to 280 nm
Integration Software	- *e.g.* Perkin Elmer Turbochrom 4.2

N.B. Sometimes groundwater samples of *ca.* pH 7 with a high ionic strength, from dissolved salts, tended to produce very tailing peaks (almost amounting to peak splitting in certain cases). This effect was due to the essentially buffered sample raising the pH of the mobile phase upon injection and so altering the pH within the column. It was found to be overcome by the addition of 1 drop of 3M hydrochloric acid (AR) to a 2 ml sample (the volume normally taken by syringe for injection into the HPLC system).

The cyclodextrin-based stationary phase of the chiral column is not as robust as typical HPLC column packings (*e.g.* C18) and will not tolerate back pressures greater than 250 bar. This why it is necessary to use a flowrate of 0.7 ml/min (Zipper et al., 1996). In some investigations into method refinement the use of acetonitrile in place of methanol in the mobile phase was studied. The benefit here was that acetonitrile/aqueous buffer mixtures have a much lower viscosity than comparable methanol/aqueous buffer mixtures and so generate much lower back pressures when pumped through HPLC columns. Use of an optimised acetonitrile-based mobile phase gave chromatography as illustrated by Figure 5. Note the reduced retention times but also some loss of resolution when compared with the Figure 4 chromatogram. A considerable reduction in back pressure was achieved.

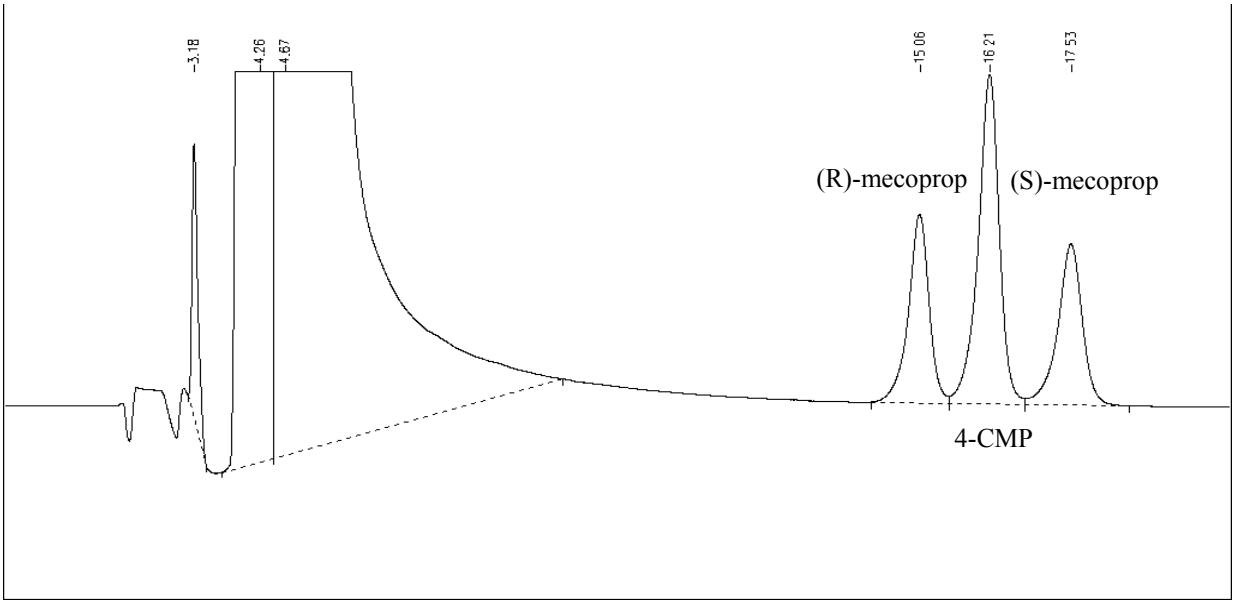


Figure 5 Chiral mecoprop and 4-CMP with acetonitrile-based eluant

2). Alternative HPLC Conditions for mecoprop enantiomers and metabolite

Mobile phase	- 40% acetonitrile (HPLC grade) : 60% 25 mM disodium hydrogen phosphate buffer (*i.e.* 7.1 g Na_2HPO_4 AR to 2 L with pure water adjust to pH 3 with H_3PO_4 AR - vacuum filter 0.45µm before use)
Pump	- Isocratic HPLC pump (*e.g.* Merck-Hitachi L6000)
Flowrate	- 0.7 ml/min (Back pressure *ca*. 105 bar).
Injection Volume	- 1 ml sample loop on a Rheodyne 7125 Injector
Analytical column	- Macherey-Nagel Nucleodex α-PM (200 mm x 4 mm ID) 5 µm
Guard column	- Macherey-Nagel Nucleodex α-PM (8 mm x 4 mm ID) 5 µm
Detection	- UV detector (*e.g.* Waters 2487) set to 280 nm
Integration Software	- *e.g.* Perkin Elmer Turbochrom 4.2

N.B. (i). Once again it is important during the analysis that the advice offered in the 'Notes on Sample Loading and Column Backflushing' (p.3) be followed.

(ii). As previously noted preservation of samples with sodium azide was found to be very effective and had no effect whatever upon the chiral HPLC. However, this situation did not pertain when some other biocides were investigated. Thus, use of mercuric chloride solutions should be avoided since Hg(II) is known to bind strongly to the cyclodextrin-based stationary phase, causing profoundly adverse effects upon the chromatography. Prolonged flushing with aqueous 0.1 M EDTA was required to restore a column that had experienced passage of this biocide. When the use of another popular biocide, chlorhexidine diacetate 'Hibitane', was attempted its later elution in the form of broad and unpredictable peaks had a deleterious effect on the chromatography of the mecoprop enantiomers. Therefore, the use of any biocide (organic or inorganic), other than sodium azide, is not recommended.

At distances > 1 km from the landfills mecoprop concentrations were much lower <1 μg/l and it was necessary to devise an SPE pre-concentration procedure before such samples could be analysed. Because of the polar nature of mecoprop it was thought that a polymeric sorbent would probably afford maximal recoveries. Experiments with Varian Nexus yielded essentially quantitative recovery (*i.e.* 100%) when the following optimised SPE method was used:

3). Varian Nexus SPE method (for aqueous samples, *ca.* 200 ml):

- No cartridge preconditioning is necessary.
- Adjust the pH of 200 ml of aqueous sample to pH 2.2 ± 0.2 with concentrated hydrochloric acid AR.
- Pass the 200 ml of aqueous sample through the Varian Nexus 3 ml/60 mg cartridge at *ca.* 5 ml /min. using controlled vacuum.
- Rinse cartridge with 5 ml acidified pure water (adjusted to pH 2 with concentrated hydrochloric acid AR).
- Dry the cartridge bed by allowing the vacuum to pull air through the cartridge for about 20 mins.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 3 x 1 ml of acetone AR into a 5 ml graduated glass centrifuge tube.
- Blow down the acetone extract to dryness with nitrogen.
- Add ca. 2 mg of sodium azide to the tube.
- Add a weighed 2 g of pure water to the tared tube, stopper and shake vigorously.
- Inject the resulting sample into the HPLC system and analyse for mecoprop (this protocol was only used for enantiomeric analysis and as such was not tested on 4-CMP).
- Limit of detection *ca.* 5 ng/L

In a later study of pesticide contaminated groundwaters from Boston Park Farm in the Triassic Sandstone aquifer of South Yorkshire (Gooddy et al., 2003b) a greater degree of pre-concentration of mecoprop was required. To achieve this Varian Nexus 200 mg cartridges were substituted for the 60 mg cartridges and *ca*. 6 litres of acidified groundwater were drawn through by vacuum. By this means a pre-concentration of approximately x 3,000 was achieved.

One facet of the Helpston study employed laboratory microcosms to aid in identifying the terminal electron accepting processes (TEAPs) used by microorganisms in their biodegradation of mecoprop. In order to accomplish this it was necessary to determine the nitrate, nitrite and sulphate concentrations in both sterile and non-sterile microcosms since all these species can be involved in anaerobic TEAPs. Unfortunately, using the existing BGS ion chromatographic facility (Dionex) for the analysis of standard aqueous anions it was found that the azide (0.2% w/v sodium azide) used as a biocide in the sterile microcosms interfered with nitrate and nitrite analysis.

An alternative HPLC method for nitrate and nitrite analysis was sought which permitted their separation from azide. Some experiments with a strong anion exchanging (SAX) HPLC column eventually led to a satisfactory method for the determination of mg/l levels of nitrate and nitrite in groundwaters as shown in Figure 6. Since azide, nitrate and nitrite are all UV absorbers it was possible to use UV detection for their quantification. The calibration was linear for nitrate in the range 0-10 mg/l and for nitrite in the range 0-5 mg/l. The limit of detection for both nitrate and nitrite was *ca.* 10 µg/l. An added benefit was that the bromide, a constituent of both natural and artificial groundwaters used in the construction of the microcosms, could be determined in the range 0-10 mg/l with a limit of detection of *ca.* 20µg/l.

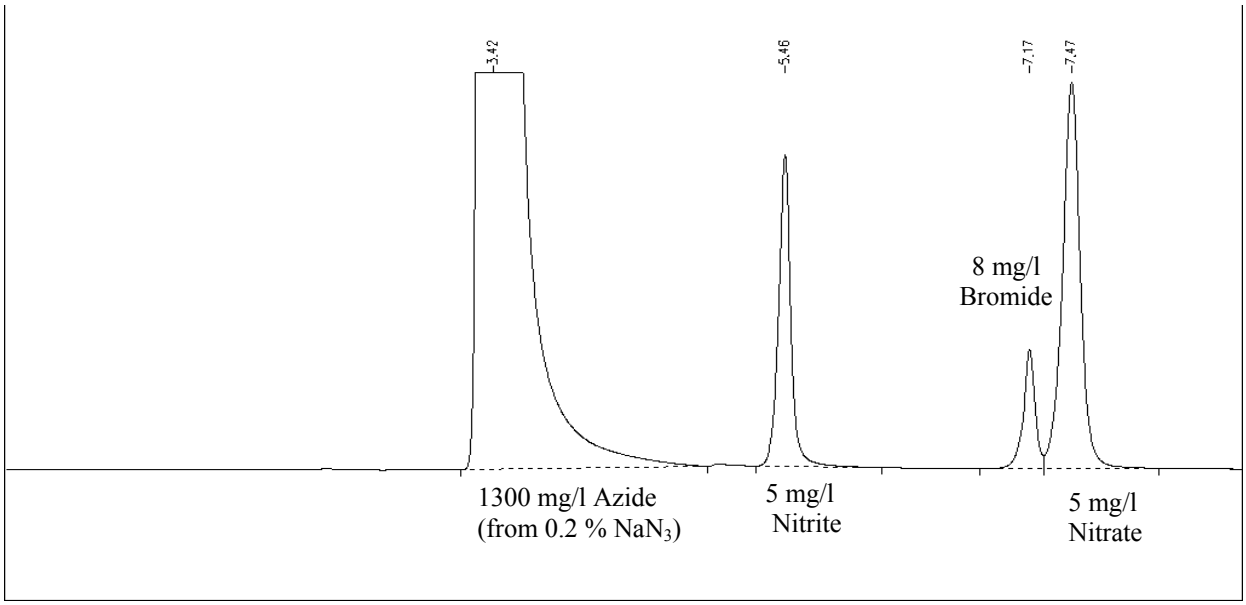


Figure 6 Chromatogram for nitrate, nitrite and bromide in the presence of azide

4). HPLC Conditions for nitrate, nitrite and bromide in sterile mecoprop microcosms

Mobile phase	- 25 mM disodium hydrogen phosphate (*i.e.* 7.1 g Na_2HPO_4 AR to 2 L with pure water adjust to pH 3 with H_3PO_4 AR - vacuum filter 0.45µm before use)
Pump	- Isocratic HPLC pump (*e.g.* Merck-Hitachi L6000)
Flowrate	- 1.0 ml/min (Back pressure *ca.* 105 bar).
Injection Volume	- 50 µl sample loop on a Rheodyne 7125 Injector
Analytical column	- Spherisorb SAX (250 mm x 4.6 mm ID) 5 µm
Guard column	- Spherisorb SAX (10 mm x 4.6 mm ID) 5 µm
Detection	- UV detector (*e.g.* Waters 2487) set to 215 nm
Integration Software	- *e.g.* Perkin Elmer Turbochrom 4.2

2.4 BENTAZONE, ATRAZINE, ISOPROTURON AND MECOPROP ENANTIOMERS

As part of the investigation conducted for Yorkshire Water in 2003, the analysis of porewaters and groundwaters for bentazone, atrazine, isoproturon and mecoprop was required. Since there was the capability to determine the enantiomers of the latter the possibility of developing a method to analyse for these and the remaining other three pesticides in a single chromatographic run was considered. Injecting separately bentazone, atrazine and isoproturon solutions through the chiral HPLC column, under the conditions employed for the separation of mecoprop

enantiomers, (*i.e.* as presented in section 2.3), revealed that all three pesticides were poorly retained and eluted at, or very close to, the solvent front. It was conjectured that if a column capable of separating these three was coupled before the chiral column then a satisfactory separation might result. Because column coupling greatly increases the overall back-pressure of the HPLC system a short column, (with a correspondingly low operating back-pressure), was desirable. Since bentazone, atrazine and isoproturon are all relatively polar compounds a 50 mm x 4.6 mm ID reversed-phase column with a stationary phase specifically designed for the retention of polar compounds (Phenomenex Synergi™ RP-Polar) represented a suitable candidate for further examination. After coupling, and in the isocratic mode with the usual mecoprop enantiomers mobile phase (65% methanol:35% pH3 phosphate buffer), the following retention times were noted:-

Bentazone	Atrazine	Isoproturon	*(R)*-Mecoprop	*(S)*-Mecoprop
6.9 mins	*9.2 mins*	*9.7 mins*	*18.3 mins*	*24.9 mins*

Unfortunately, the bentazone peak was interfered with by solvent front disturbance peaks and so the solvent strength was reduced to 60% methanol: 40% buffer. This produced the following:-

Bentazone	Atrazine	Isoproturon	*(R)*-Mecoprop	*(S)*-Mecoprop
8.2 mins	*11.0 mins*	*12.3 mins*	*28.2 mins*	*40.1 mins*

Plainly, while this had a beneficial effect on the early eluters, *(S)*-mecoprop was taking a long time to elute. Accordingly, gradient programming seemed to be indicated and after some experimentation a programme was devised that yielded the following retention behaviour:-

Bentazone	Atrazine	Isoproturon	*(R)*-Mecoprop	*(S)*-Mecoprop
12.8 mins	*14.9 mins*	*15.8 mins*	*21.5 mins*	*25.4 mins*

The resulting chromatogram for a mixed standard containing 100µg/l of each pesticide is shown below in Figure 7. It should be borne in mind that since the mecoprop used was racemic (*i.e.* contained equal amounts of each enantiomer) the individual *(R)*- and *(S)*-mecoprop concentrations are 50µg/l.

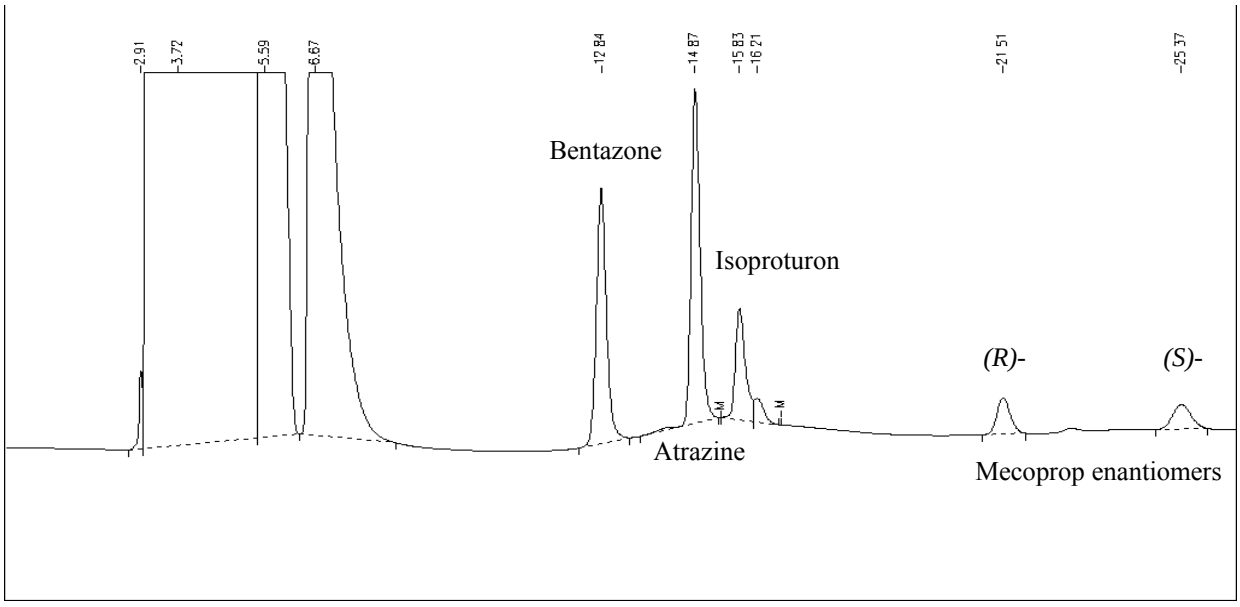


Figure 7 Chromatogram of bentazone, atrazine, isoproturon and mecoprop (100 µg/l each)

1). HPLC Conditions for bentazone, atrazine, isoproturon and mecoprop enantiomers

Mobile phase	- Methanol (HPLC grade) : 25 mM disodium hydrogen phosphate buffer (*i.e.* 7.1 g Na_2HPO_4 AR to 2 L with pure water adjust to pH 3 with H_3PO_4 AR - vacuum filter 0.45µm before use)
Pump	- Gradient HPLC pump (*e.g.* Waters 600E)
Flowrate	- 0.7 ml/min (Back pressure *ca*.250 - 300 bar)
Gradient programme	- 50% methanol held for 1 min then linearly to 70% methanol after 5 mins. hold 70% methanol to end of run (30 mins.)
Injection Volume	- 1 ml sample loop on a Rheodyne 7125 Injector
Analytical column	- Phenomenex Synergi RP-Polar (50 mm x 4.6 mm ID) 4 µm *coupled before* Macherey-Nagel Nucleodex α-PM (200 mm x 4 mm ID) 5 µm
Guard column	- Phenomenex SecurityGuard C-18 (2 mm cartridge) 5 µm
Detection	- UV detector (*e.g.* Waters 2487) set to 226 nm.
Integration Software	- *e.g.* Perkin Elmer Turbochrom 4.2

Use of a one ml sampling loop and UV detection at 226 nm yielded a detection limit of approximately 1 µg/l for each pesticide. Linear calibrations in the range 0 – 500 µg/l were obtained for each pesticide with linear regression coefficients *ca.* 0.998.

Pre-concentration by SPE, so that lower limits of detection could be achieved, was necessary because anticipated sample pesticide concentrations were in the main <1 µg/l. The constraints on the amount of pre-concentration that could be achieved were that ca. 2 mls of extract would be required (to rinse and fill the 1 ml sampling loop) and that for the porewaters only a limited amount of sample (20 - 30 ml) was available. Accordingly, in most cases 25 ml of porewater were used to prepare 2 ml of concentrate, *i.e.* a 12.5x pre-concentration factor. For the three pumped groundwaters, where much larger volumes of sample were available (ca. 2 litres each) greater pre-concentrations were possible. However, when pre-concentration factors greater than 500x were attempted co-extracted natural organics produced detector overload and since some of the pesticide peaks appeared superimposed on these broad peaks it was necessary to avoid such overloading. Because all the analytes were relatively polar molecules with poor retention characteristics on the typical C18 SPE cartridges, the use of polymeric sorbent SPE cartridges as an alternative was examined, on the basis that such cartridges tend to be much more retentive for polar aromatic compounds. Some commercially available polymeric cartridges were assessed *viz.* Varian Nexus, Phenomemex Strata X and Waters Oasis HLB. All gave high recoveries of each pesticide (i.e. >90%), however optimum performance, in terms of lack of interference by materials extractable from the cartridge components, was afforded by Waters Oasis HLB.

The following SPE procedures were devised :-

Waters Oasis SPE method for Porewater Samples, *ca.* 25 ml:

- Precondition a Waters Oasis HLB 60 mg SPE cartridge by passing 3 ml acetone (Romil UpS grade) followed by 2 x 1 ml pure water (Rathburn).
- Pass the 25 ml of aqueous sample through the conditioned cartridge at *ca.* 5 ml /min. using controlled vacuum.
- Rinse cartridge with 1 ml acidified pure water (USF Elga Maxima water) adjusted to pH3 with orthophosphoric acid (Hypersolv).
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 30 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 2 x 1 ml of acetone (Romil UpS grade) into a pre-weighed 50 ml rotary evaporation flask.

- Rotary evaporate for 5 mins at 55°C then make the contents to 2.00 g with pure water (Rathburn).
- Inject the resulting sample into the HPLC system and analyse.
- Limit of detection *ca.* 0.1 µg/L

Waters Oasis SPE method for Groundwater Samples, *ca.* 1 litre:

- Precondition a Waters Oasis HLB 200 mg SPE cartridge by passing 6 ml acetone (Romil UpS grade) followed by 2 x 3 ml pure water (Rathburn).
- Pass the litre of aqueous sample through the conditioned cartridge at *ca.* 10 ml /min. using controlled vacuum.
- Rinse cartridge with 3 ml acidified pure water (USF Elga Maxima water) adjusted to pH3 with orthophosphoric acid (Hypersolv).
- Remove excess water from cartridge bed by allowing the vacuum to pull air through the cartridge for about 30 secs.
- Elute the cartridge under gravity (*i.e.* vacuum removed) with 3 x 2 ml of acetone (Romil UpS grade) into a pre-weighed 50 ml rotary evaporation flask.
- Rotary evaporate for 10 mins at 55°C then make the contents to 2.00 g with pure water (Rathburn).
- Inject the resulting sample into the HPLC system and analyse.
- Limit of detection *ca.* 2 ng/L

Figure 8 shows a typical chromatogram obtained with a porewater sample from the Yorkshire Water investigation – Sample BPF 3/9 12m. Shoe Sample (from Boston Park Farm).

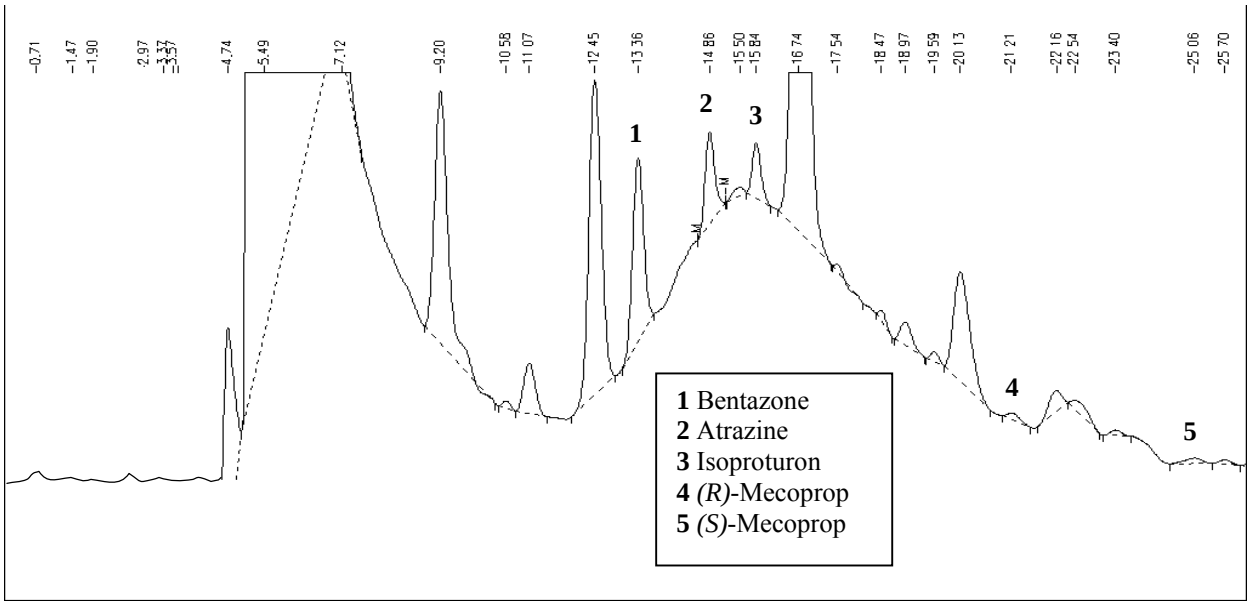


Figure 8 Chromatogram of BPF 3/9 12 m. shoe sample (after SPE)

The sample had been pre-concentrated (12.5x) by solid-phase extraction (SPE) prior to the acquisition of the chromatogram from which the concentrations of pesticides in the original sample were found to be 1.61 µg/l bentazone, 0.59 µg/l atrazine, 0.88 µg/l isoproturon, 0.35 µg/l *(R)*-mecoprop and 0.30µg/l *(S)*-mecoprop, yielding an enantiomeric ratio (i.e.[*R*]/[*S*]) for mecoprop of 1.17.

It should be noted that despite the use of a short column, (for the separation of bentazone, atrazine and isoproturon), the coupling of columns nevertheless resulted in high back pressures

near to the operating limits for the columns. This is not particularly good practice, and so alternative lower viscosity mobile phases need to be evaluated. The replacement of methanol with acetonitrile and some readjustment of proportions within the mobile phase has produced good chromatography on the chiral column (*i.e*. for mecoprop enantiomers and 4-chloro-2-methylphenol). It remains to be seen whether this can be extended to the columns in their coupled configuration.

3 Conclusions

It has proved possible, with the aid of some development of capability, to provide analyses for a range of pesticides, pesticide mixtures, pesticide TEAPs and pesticide metabolites using comparatively basic HPLC equipment combined with SPE for the pre-concentration of aqueous samples. This report has aimed to deliver the salient pragmatic features of the methodologies, to the extent that a competent chromatographer should be able to undertake any of the analyses outlined without encountering undue difficulty.

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Most of the references listed below are held in the Library of the British Geological Survey at Keyworth, Nottingham. Copies of the references may be purchased from the Library subject to the current copyright legislation.

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