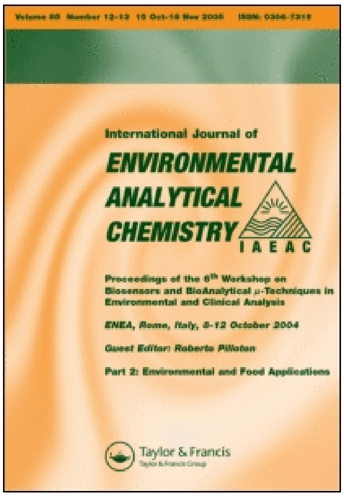

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Optimization of High Quality Analytical Methods for the Monitoring of Pyrethroid Mothproofing Agents by Gas Chromatography-Mass Spectrometry Operated in Negative-Ion Chemical Ionization Mode

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To cite this Article Hancock, P. M. , Yasin, M. , Baugh, P. J. , Bonwick, G. A. and Davies, D. H.(1997) 'Optimization of High Quality Analytical Methods for the Monitoring of Pyrethroid Mothproofing Agents by Gas Chromatography-Mass Spectrometry Operated in Negative-Ion Chemical Ionization Mode', International Journal of Environmental Analytical Chemistry, 67: 1, 81 — 95

To link to this Article: DOI: 10.1080/03067319708031396

URL: http://dx.doi.org/10.1080/03067319708031396

Intern. J. Environ. Anal. Chem., Vol. 67, pp. 81-95
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OPTIMIZATION OF HIGH QUALITY ANALYTICAL METHODS FOR THE MONITORING OF PYRETHROID MOTHPROOFING AGENTS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY OPERATED IN NEGATIVE-ION CHEMICAL IONIZATION MODE

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(Received 12 April 1996; In final form 23 July 1996)

An analytical method based on gas chromatography combined with negative-ion chemical ionization mass spectrometry in selected ion monitoring mode has been optimized for the simultaneous determination of five synthetic pyrethroid insecticides in water, sediment, moss and fish tissue. The pyrethroids were originally extracted from the environmental matrices by steam distillation extraction but this has since been superseded by liquid-liquid extraction for water samples and ultrasonic extraction for solid matrices with particular attention for solvent selectivity. Recovery studies were performed on all the environmental matrices for each pyrethroid and the internal standard, mirex. The percentage recoveries ranged from 80.6 ± 5.6 to 116.2 ± 4.3, where n = 4. The limit of quantitation for the method was determined statistically from the linear calibration curve, giving values in the range 0.01 to 4.4pg of material introduced into the system. depending on the pyrethroid in question. The method was applied to environmental samples collected from a contaminated river ecosystem currently being monitored for the target analytes.

Keywords: GC-NICI-MS; pyrethroids; environmental matrices; ultrasonic extraction; liquid-liquid extractions; steam distillation extraction

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INTRODUCTION

The effectiveness of the natural pyrethrins as insecticides instigated a search for more persistent synthetic analogues with increased potency and/or more specific toxicity. Several synthetic pyrethroids have been developed which are characterised by both high insecticide activity and greater stability in light and air. The pyrethroids are now broadly recognised as the fourth major class of synthetic organic insecticide.

The correlation between stereo chemical structure and insecticide activity was pioneered by Elliott[1], leading to the discovery of many important pyrethroids which are insecticide analogues of cyclopropanecarboxylic acid esters, of which permethrin, cyfluthrin, cypermethrin and deltamethrin are examples. Fenvalerate, the other pyrethroid in this study, is a carboxylic acid ester but is without the cyclopropane ring.

Contamination of fresh water ecosystems by pyrethroids occurs either due to the direct discharge of industrial and agricultural effluents or indirectly through the discharge from sewage treatment works where it is accumulated by the surrounding biosphere. Although pyrethroids have relatively low toxicity towards mammals and are of limited persistence in the environment compared with many other pesticide classes, they are extremely toxic to fish and aquatic organisms, reflected by the corresponding environmental quality standards (EQS), for example, for permethrin and cyfluthrin, 0.01 $\mu g\ l^{-1}$ and 0.001 $\mu g\ l^{-1}$, respectively[2]. Pyrethroids may degrade to a variety of hydrolysis and oxidation products following their application but the major component of any residue has been the applied pyrethroid[3]. For these reasons the different methods developed for pyrethroid residue analysis have dealt primarily with the detection of the parent compound at the sub-microgram level.

Several methods have been previously reported for monitoring pyrethroid residues employing gas-liquid chromatography with electron capture detection (GC-ECD)[4–9] or high-performance liquid chromatography (HPLC)[8,9]. Most of these methods are either inefficient or meet with some difficulty when dealing with trace residues in field samples due to poor detection limits, the presence of background contamination and non-specific chemical interference.

The present study has focused on pyrethroid analysis in the components of a river ecosystem (water, sediment, moss and fish) by gas chromatography combined with negative-ion chemical ionization mass spectrometry (GC-NICI-MS). In this paper, we review the development and application of GC-NICI-MS in conjunction with various extraction techniques, for example, steam distillation extraction (SDE)[10] and ultrasonic extraction (USE)[10–13]. We also introduce pyrethroid extraction from water samples using liquid-liquid extraction. The methods can be successfully applied for the simultaneous determination of permethrin, cyfluthrin, cypermethrin, deltamethrin and fenvalerate in samples obtained from a contaminated ecosystem and conclude with a study of isomer ratios in environmental matrices.

EXPERIMENTAL

The extraction procedures used were based on United States Environmental Protection Agency (USEPA) method 625[14] and Methods for the Examination of Waters and Associated Materials[15] for analysing the target analyte in spiked and real samples.

Materials

Permethrin [3-phenoxybenzyl-(1R,S)–3-(2,2-dichlorovinyl)–2,2-dimethylcyclopropane carboxylate], cyfluthrin [(R,S)-α -cyano–4-fluoro–3-phenoxybenzyl-(1 R,S)–3-(2,2-dichlorovinyl)–2,2-dimethylcyclopropanecarboxylate], cypermethrin [(R,S)-α-cyano–3-phenoxybenzyl-(1 R,S)–3-(2,2-dichlorovinyl)–2,2-dim-ethylcyclopropanecarboxylate], deltamethrin [(S)-α -cyano–3-phenoxyben-zyl-(1R)–3-(2,2-dibromovinyl)–2,2-dimethyl cyclopropanecarboxylate] and fenvalerate [(R,S)-α -cyano–3-phenoxybenzyl-(R,S)–2-(4-chlorophenyl)–3-methylbutyrate] were all purchased from Promochem UK. Mirex and decachlorobiphenyl (DCBP) were obtained from British Greyhound. HPLC grade hexane, dichloromethane and iso-octane, analytical grade acetone and diethyl ether and anhydrous sodium sulfate from Fisons were used. The clean-up materials were alumina powder, Merck 1097 (70–230 mesh ASTM), and florisil, Kodak (20–100 mesh).

Column Clean-up Materials

Florisil was activated by heating at 300°C for 4h and then deactivated prior to use by mixing with distilled water (8% w/v) and agitating well to get a free-flowing powder. The florisil column (30cm × 2cm i.d. glass column fitted with a PTFE stopcock) was prepared by placing a small plug of glass wool at the bottom of the column. 5g florisil was introduced to give a homogenous packing and capped with $2cm^3$ anhydrous sodium sulfate. The column was prewashed with 10ml hexane.

Basic alumina was prepared with 50g Merck 1097, activated by heating in a muffle furnace at 800°C for 4h, cooled to 200°C and then to room temperature in a dessicator. The alumina was deactivated prior to use by mixing with distilled water (4% w/v) and agitating well to get a free-flowing powder. Acidic alumina was prepared by washing 50g Merck 1097 with 1 M HCl and filtered through a sintered glass funnel. It was then dried at 150°C for 4h, cooled down to room temperature, deactivated with distilled water (4% w/v), agitated and stored. These alumina preparations were discarded after seven days and fresh material produced[16]. The mixed acid-base alumina column was prepared by placing a small plug of glass wool at the bottom of the column. Acidic alumina (2g) followed by basic alumina (2g) was introduced and capped with 2cm^3 anhydrous sodium sulfate. The column was prewashed with 10ml hexane.

Sample Collection and Processing

Clean materials used for recovery were previously determined to be free of any pesticide in the study. Uncontaminated stream sediment and moss was obtained from a tributary of the River Tame, Uppermill, Lancashire, UK. Contaminated water, sediment and moss samples were collected from seven sites in the Meltham area of the River Calder catchment, Yorkshire, UK, approximately 80 miles West of the Humber estuary. The River Calder is itself a tributary of the River Aire and, ultimately, the River Humber. Water samples were collected such that a head space was avoided. Sediment and moss samples were obtained manually from the surface of the river bed as the rocky substrate prevented use of standard sampling apparatus. Untreated, clean glass sample bottles and jars fitted with PTFE-lined caps were used throughout. After collection, samples were stored at 4°C. Waters contained minimal suspended solids and colloids, they were not filtered. Sediments were air-dried at room temperature, ground and graded through a metal sieve (20 mesh) prior to extraction. Moss samples were also air-dried at room temperature but were ground with a pestle and mortar to produce a fine powder. Brown trout *(Salmo trutta)* and gudgeon were captured from one site by electro-fishing and were killed *in situ.* Fish were wrapped in silver foil then placed in crushed ice during transport to the laboratory where they were stored at −20°C. Individual whole fish were homogenized in a Waring Blender while still frozen and then freeze-dried

Instrumentation

A HP5890 Series II gas chromatograph with split/splitless capillary injection and an HP7673A auto sampler (Hewlett-Packard. Cheadle Heath. Manchester, UK) coupled to a VG Trio 1000 quadrupole mass spectrometer with EI and PICI/

NICI capability (Fisons Instruments, Wythenshaw, Manchester, UK) was employed. Control of the instrument and data manipulation was performed by a VG LabBase data system.

GC Conditions

A fused-silica DB5-MS capillary column, 15m × 0.32mm i.d., 0.25μm film thickness (Jones Chromatography, Hengoed, UK) was used with helium (CP grade) as the carrier gas (BOC, Eccles, Manchester, UK) with a head pressure of 5psi. A 1μl volume was injected by the auto sampler applying a hot splitless injection technique with a venting time of 1.5min. The temperature programme of the oven started at 100°C (for 1min) and increased at the rate of 20°C/min to 220°C, then 10°C/min to a final temperature of 300°C where it was held for 3min. The injector and interface temperatures were maintained at 270°C.

MS Acquisition Parameters

Negative-ion chemical ionization mode with methane (CP grade, BOC, Eccles, Manchester, UK) as the reagent gas was used. The general mass spectrometer conditions were; ion source 250°C; electron voltage 70eV; and source current 350μA. When operating in scanning mode, the scanned mass range was 50–550u in 0.9s while in SIM mode, the mass range was 0.02u in 0.02s. The voltages of the ion repeller, ion focus, ion and electron energies, and the quadrupole mass filter parameters were periodically optimized using the ion at *m/z* 452 generated from the calibration compound, perfluorotributylamine (PFTBA).

Calibration

Long-term storage stock solutions of the reference compounds and the volumetric (DCBP) and internal (mirex) standards were prepared at concentrations of 100mg l^{-1}. The stock solutions were serially diluted to prepare mixed working standards in the required concentration range (0.5μg l^{-1} to 500μg l^{-1}) of the internal standard and each pyrethroid together with 50μg l^{-1} of the volumetric standard in hexane. These standard solutions were subsequently analysed by GC-NICI-MS operated in SIM mode. Peak areas were obtained from the mass chromatograms generated for the quantitation ions of each analyte (Table I). Calibration curves were obtained from plots of response factor (pyrethroid peak area/DCBP peak area) against analyte concentration. DCBP was selected as the volumetric standard because of its wide employment and high reliability for sam-

TABLE I Characteristic fragmentation ions of the pyrethroids, mirex and DCBP

Compound	*Characteristic ions*	*Quantification ion*
Permethrin	171, 173, 207, 209, 211	207
Cyfluthrin	171, 173, 207, 209, 211	207
Cypermethrin	171, 173, 207, 209, 211	207
Deltamethrin	79, 81, 137	79
Fenvalerate	167, 169, 211, 213	167
Mirex	366, 368, 370, 402, 404, 406	368 or 404
DCBP	496, 498, 500	498

ple analysis using GC-ECD. Furthermore, it elutes in a narrow retention window adjacent to the targets, has maximum response in a high mass region (m/z 498) and exhibits a wide linear dynamic range in response.

Limit of Detection (LOD)[17]

In general terms, the limit of detection of an analyte may be described as that concentration which gives an instrumental signal (y) significantly different from the "blank" or "background" signal. This description is vague giving a good deal of freedom to decide the exact definition of the limit of detection. A more useful definition is that the limit of detection is the analyte concentration giving a signal equal to the blank signal, y_B, plus three standard deviations of the blank, s_B.

$$y - y_B = 3s_B \tag{1}$$

A fundamental assumption of the unweighted least-squares method for the calculation of a regression equation is that each point on the plot has a normally distributed variation (in the y-direction only) with the standard deviation estimated by $s_{y/x}$, It is therefore appropriate to use $s_{y/x}$, in place of s_B in the estimation of the limit of detection. The value a, the calculated intercept, can be used as an estimate of y_B, the blank itself.

$$LOD,\ y = a + 3_s = a + 3\left[\frac{\Sigma(y_i - \gamma_i)^2}{n-2}\right]^{\frac{1}{2}} \tag{2}$$

This equation utilises the y-residuals, $y_i - \text{-}\gamma_i$, where the γ_i values are the points on the calculated regression line corresponding to the individual x-values.

Recovery Studies

Recovery studies were performed at three fortification levels of each pyrethroid and internal standard, in contamination-free water, sediment, moss and fish tissue. These samples were prepared by spiking with 1 ml of pyrethroid composite solution in acetone. The samples were placed on a shaker (6h) and left overnight to ensure homogeneity and then extracted and analysed as environmental samples.

Steam Distillation Extraction-Water and Sediment[18–19]

Water (1000 ± 10ml) was transferred to a round-bottomed flask. Sediment (25.0g) was placed in a round-bottomed flask with distilled water (1000 ± 10ml). The sample was spiked with 1ml of 50ppb mirex in acetone, as the internal standard, and the flask shaken vigorously to ensure homogeneity. The sample was acidified (pH 4.0) by the addition of sulfuric acid. Sample acidification was introduced following a period of method optimization with the maximum efficiency of extraction found to be pH 4.0. The flask was attached to a modified Nielsen-Kryger apparatus and the extraction solvent, iso-octane, added (20 ± 2ml). The system was steam-distilled for 4h over a heating mantle. The extract was evaporated to incipient dryness under a gentle stream of clean, dry nitrogen. The residue was re-suspended in 1ml of 50μg l^{-1} DCBP in hexane, as the volumetric standard.

Liquid-liquid Extraction-Water

Water (1000 ± 10ml) was transferred to a separating funnel. The sample was spiked with 1ml of 50μg l^{-1} mirex in acetone, as the internal standard. and the funnel shaken vigorously to ensure homogeneity. Sodium chloride (30g) was added and the sample acidified (pH 4.0) by the addition of sulfuric acid. Dichloromethane (50 ± 5ml) was added and the funnel shaken vigorously for 5min. The phases were allowed to separate and the lower dichloromethane fraction transferred to a flask containing anhydrous sodium sulfate (5 ± 1g). This procedure was repeated twice and the extracts combined. The extract was evaporated to approximately 5ml by rotary evaporation under reduced pressure at 40°C. Further concentration of the samples was performed as for the steam-distilled extracts (above).

Ultrasonic Extraction-Sediment, Moss and Fish Tissue

Sediment (10.0 g), moss (1.0 g) and fish tissue (1.0 g) extraction all follow the same procedure. The sample was spiked with 1 ml of 50 μg l^{-1} mirex in acetone, as the internal standard, and placed on a shaker (1 h) to ensure homogeneity.

Hexane:dichloromethane (20 ± 2 ml) solvent mixture was added. The flask was then submerged in an ultrasonic bath (Dawe Sonicleaner, type 644) and sonicated for 30 min. The supernatant was decanted through a glass sinter (porosity 4) capped with anhydrous sodium sulfate (5 ± 1 g). The extraction procedure was repeated twice and the extracts combined. The extract was evaporated to 5 ml by rotary evaporation under reduced pressure at 40°C. Subsequent concentration of sediment samples was performed as for the steam-distilled extracts (above) while moss and fish tissue had to undergo clean-up.

Column Clean-up-Moss and Fish Tissue

The concentrated moss extract, with additional washings, was transferred to a previously packed Florisil column while the concentrated fish tissue extract was transferred to a previously packed mixed acid-base alumina column. The solution was allowed to percolate into the column. Pyrethroid residues were eluted with 6 ml of hexane:diethyl ether (7:3 v/v). The total eluate was collected and concentrated to 1 ml as described for the steam-distilled extracts (above).

RESULTS AND DISCUSSION

Representative NICI mass spectra and chromatograms for the pyrethroids, mirex and DCBP have previously been reported[10–13] Permethrin, fenvalerate and deltamethrin were totally resolved into two diastereoisomers while cyfluthrin and cypermethrin, which contain a third asymmetric centre, were partially resolved into envelopes of four and three diastereoisomers, respectively, *cis* and *trans* isomers of permethrin and fenvalerate were quantified separately while the other pyrethroids were quantified using their base peak.

In negative ion chemical ionization mode using methane as the reagent gas, permethrin, cyfluthrin and cypermethrin being esters of dichlorovinylcyclopropane carboxylic acid (CPA) undergo dissociation by electron capture to yield CPA^- anions, where m/z 207 is the primary quantitation ion and m/z 209 and 211 are used for diagnostic purposes. In spite of deltamethrin having a similar pyrethroid structure, the base ion is in the low mass region at m/z 79 (81) resulting from the favoured fragmentation yielding Br^- ions. where the ion background interference is greatest. The compound also has the characteristic ion at m/z 137 from the cyclopropane carboxylic acid unit after the loss of the two bromide ions but the intensity ratio of m/z 79 (quantitation ion) to m/z 137 (diagnostic ion) is approximately 10:1. Fenvalerate undergoes similar dissociation to ion fragments at m/z 167 (169), the quantitation ion, and m/z 211 (213) the diagnostic ions, which correspond to the portion of the molecule containing the isopropyl group.

Linearity was observed for the pyrethroids and the internal standard over the concentration range 0.5 µg l^{-1} to 500 µg l^{-1} with correlation coefficients of 0.999 obtained routinely. The absolute detection limits in SIR mode were calculated (Equation 2) using the quantification ions (Table I) for each analyte and are reported in Table II. Also shown are the reliable routine quantification limits presented on the basis of statistical considerations in order to create confidence in the analytical procedure at low concentration levels.

TABLE II Instrumental detection limits of the pyrethroids and mirex

Compound	*Instrumental limit of detection (LOD) pg*
cis-Permethrin	0.001 (0.01)
trans-Permethrin	0.022 (0.2)
Cyfluthrin	0.14 (1.4)
Cypermethrin	0.069 (0.7)
Deltamethrin	0.43 (4.3)
cis-Fenvalerate	0.44 (4.4)
trans-Fenvalerate	0.085 (0.9)
Mirex	0.085 (0.9)

Values in parentheses denote 10 x LOD = limit of quantification.

Preliminary work on the recovery of permethrin, cyfluthrin and mirex from water and sediment was conducted using steam distillation extraction (SDE). The percentage recoveries are compared to those obtained using liquid-liquid extraction (LLE) for water and ultrasonic extraction (USE) for sediment in Table III.

TABLE III Recovery of permethrin, cyfluthrin and mirex from water and sediment employing various extraction techniques

Compound	*Average recovery (%) ± sd*			
	LLE	*SDE*		*USE*
	Water $10 \mu g\ l^{-1}$ *level*	*Water* $10 \mu g\ Kg^{-1}$ *level*	*Sediment* $100 \mu g\ Kg^{-1}$ *level*	*Sediment* $100 \mu g\ Kg^{-1}$ *level*
Permethrin	98.5 ± 3.0(4)	93.9 ± na (2)	90.9 ± 14.5 (4)	92.7 ± 11.9 (4)
Cyfluthrin	97.3 ± 16.0(4)	72.8 ± na (2)	63.9 ± 12.8 (4)	102.0 ± 10.1 (4)
Mirex	99.2 ± 2.4 (4)	74.0 ± na (2)	82.2 ± 12.5 (4)	98.8 ± 4.4 (4)

Values in parentheses denote number of observations, na = not available.

Overall, SDE proved to be a suitable method for the extraction of permethrin while cyfluthrin and mirex were both poorly recovered. For example, SDE gives a cyfluthrin recovery of 63.9 ± 12.8 from sediment compared to the more acceptable USE recovery of 102.0 ± 10.1. The poor recoveries of cyfluthrin and mirex, together with a need of a more rapid alternative to SDE led to the complete implementation of USE for solid matrices and LLE for water samples.

Initial USE experiments were carried out on soil employing different extraction solvents, this work has previously been reported[12,13]. Hexane gave good recoveries for the less polar analytes, permethrin and mirex, while cyfluthrin, cypermethrin, deltamethrin and fenvalerate were very poorly recovered. The hexane:dichloromethane (1:1 v/v) solvent system gave the best recoveries for all five pyrethroids and was employed in all subsequent studies.

The percentage recoveries of the pyrethroids and mirex from environmental matrices, water, sediment, moss and fish tissue are summarized in Table IV. Other data has previously been reported to show the effect of varying the concentration of the analytes[12,13]. The recovery results show no significant variation between the high and low spiking levels. The fortifications levels were chosen to give an accurate representation of levels that have been observed in the environment. For example, the water sample was fortified at 10 μg l^{-1}, which gives a matrix level of 10 ng l^{-1}as 1000 ml of water is analysed, a value typically observed in environmental water. Four fortified samples and one procedural blank were analysed simultaneously. The procedural blanks gave no response above the detection limit for the ions used in quantification of the target compounds analysed. The reproducibility of an analytical method is characterized by its standard deviation, here, most of the standard deviations are below 12%. The

TABLE IV Recovery of pyrethroids from water, sediment, moss and fish tissue at realistic fortification levels using ultrasonic extraction

Compound	*Average recovery (%) ± sd, n = 4*			
	*Water** *10μg l^{-1}*	*Sediment* *100μg kg^{-1}*	*Moss* *100μg kg^{-1}*	*Fish tissue* *100μg kg^{-1}*
cis-Permethrin	98,5 ± 3.0	92.7 ± 11.9	98.1 ± 11.6	114.5 ± 2.1
trans-Permethrin	97.6 ± 8.0	98.6 ± 10.9	107.9 ± 11.2	84.5 ± 2,8
Cyfluothrin	97.3 ± 16.0	102.0 ± 10.1	87.6 ± 8.9	86.6 ± 6.9
Cypermethrin	106.7 ± 6.6	108.2 ± 2.6	98.5 ± 8.5	86.6 ± 5.7
Deltamethrin	94.0 ± 3.9	84.8 ± 6.3	84.3 ± 8.2	96.9 ± 4.5
cis-Fenvalerate	116.2 ± 4.3	98.4 ± 2.4	94.5 ± 10.0	100.4 ± 3.7
Trans-Fenvalerate	95.5 ± 6.6	94.6 ± 4.6	80.6 ± 5.6	98.3 ± 6.8
Mirex	99.2 ± 2.4	98.8 ± 4.4	100.6 ± 6.2	84.8 ± 5.1

*Water samples employ liquid-liquid extraction.

means of the standard deviations in each environmental matrix are; water with ± 6.4, sediment with ± 6.7, moss with ± 8.8 and fish tissue with ± 4.7. The recovery data illustrate the reliability of the methods for the routine analysis of pyre throids in four environmental matrices at low concentrations

Due to functionality differences between the pyrethroids. lack of availability and cost, stable isotope analogues have not yet been employed as internal standards while mirex has been shown to be useful over the entire range. Mirex was selected in this study as an internal standard because of its elution in close proximity to the target analytes, the low probability of its environmental occurrence, optimal high mass response (m/z 368) and wide dynamic range. Mirex was used to check the recovery efficiency of the method but was not used to correct pyrethroid data. Mirex shows high percentage recoveries in both selective and non-selective solvents indicating good reliability for different polarity targets. The mean recovery of mirex from the spiked samples was 95.9 ± 4.5 which compares favourably with a recovery of 78.5 ± 6.6 from the environmental samples. The spiking procedure, although used as a quality control monitor, may not accurately assess the ability of an organic solvent to extract the pesticides incorporated into an environmental sample[20].

During this study, clean stream sediments, moss and fish tissue were used to examine the effectiveness of the extraction procedure as standard materials were not available. Although it may be argued that spiking and extraction should have been conducted using a standard matrix, it is considered that the use of a material which more closely simulates the pesticide-retaining properties of the target matrix, as in this study, ultimately provides for greater accuracy.

Field samples from a contaminated freshwater ecosystem were analysed to assess the performance of the method when extended to real environmental samples. They were collected from seven sites (Figure 1): BDI and HR are environmental blanks, BDII, BDIII and BR are downstream from an active textile mill, while STW and HHB are downstream of the effluent discharge from a sewage treatment works. These samples were processed in an identical fashion to the spiked materials and the concentrations of the pyrethroids in the environmental matrices are recorded in Tables V, VI and VII.

Permethrin was detected in all the environmental matrices while cyfluthrin was only detected in water and fish tissue. The other pyrethroids were not detected at these sites, however, this is considered to reflect the pattern of mothproofing agent usage by the local industry rather than a failure of the quantitative technique employed. The not detected results from BDI and HR helped to confirm that the analytical method was not subject to interference as these sites are not known to have a history of contamination and were therefore expected to be clean sites.

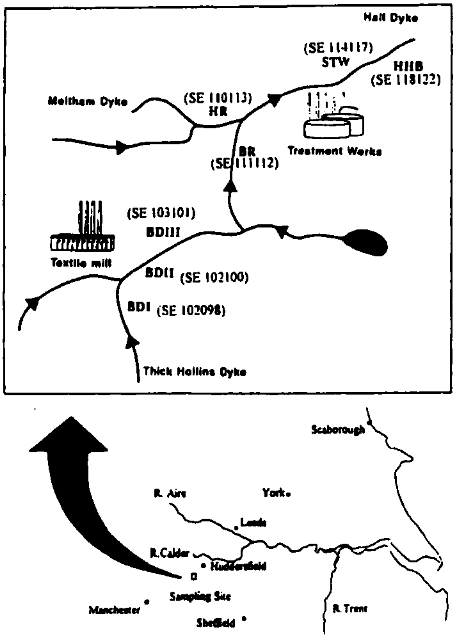

FIGURE 1 Location of the sampling sites in the Meltham Catchment area West Yorkshire, UK - (values in parentheses denote the National Grid Reference of the sampling sites)

The concentration of permethrin in the water (Table V) at sites BDII, BDIII, BR, STW and HHB was in excess of the EQS value (0.01 μg l^{-1}) while the cyfluthrin concentration was in excess (0.001 μg l^{-1}) at sites BDIII and HHB. The highest concentration observed for *cis*-permethrin in environmental water was 0.0865 μg l^{-1} and for cyfluthrin, 0,0123 μg l^{-1}.

TABLE V Permethrin and cyfluthrin levels (μg l^{-1}) in environmental water from the Meltham Catchment area

Sampling site	*Pyrethroid concentration (μg l^{-1})in water*		
	cis-Permethrin	*trans-Permethrin*	*Cyfluthrin*
BDI	0.0012	0.0006	nd
BDII	0.0825	0.0552	nd
BDIII	0.0865	0.0650	0.0123
BR	0.0469	0.0442	nd
HR	0.0029	0.0017	nd
STW	0.0470	0.0298	nd
HHB	0.0604	0.0322	0.0016

nd = not detected (lower than the analytical method quantitation limit).

Sediment and moss values (Table VI) were considerably higher than those recorded for the water samples and indicated that accumulation may have occurred. Large concentrations of permethrin were observed at all the active sites, BDII, BDIII, BR, STW and HHB, indicating the major sources of permethrin contamination in the Meltham Catchment area are the final effluent from the sewage treatment works (STW) and a source in the proximity of the textile mill (BDII and BDIII). The highest concentration of *cis*-permethrin in environmental sediment was 606.9 μg kg^{-1} and in environmental moss was 440.3 μg kg^{-1}.

TABLE VI Permethrin levels (μg kg^{-1}) in environmental sediment and moss from the Meltham Catchment area

Sampling site	*Permethrin concentration (μg kg^{-1})*			
	Sediment		*Moss*	
	cis	*trans*	*cis*	*trans*
BDI	nd	nd	nd	nd
BDII	101.1	40.1	440.3	362.9
BDIII	606.9	228.4	421.9	271.9
BR	603.0	208.2	112.5	89.7
HR	6.1	1.7	13.5	7.3
STW	214.7	66.0	165.5	89.9
HHB	210.5	233.8	247.3	161.7

nd = not detected (lower than the analytical method quantitation limit).

TABLE VII Permethrin and cyfluthrin levels (μg kg^{-1} in environmental fish from site HHB in the Meltham Catchment area

Sample	*Weight (g)*	*cis-Permethrin*	*trans-Permethrin*	*Cyfluthrin*
1	11.58	27.5	25.6	44,4
2	29.6	5769.0	357.0	30.0
3	40.4	1709.0	597.0	109.7
4	41.4	577.0	858.0	3.3
5	49.4	55.0	22.0	2.6
6	70.3	794.0	1.2	3.0
7	100.8	334.0	172.0	nd
8	116.9	118.2	10.7	nd

nd = not detected (lower than the analytical method quantitation limit).

Brown trout *(Salmo trutta)* and gudgeon captured during the study from site HHB have also shown evidence of pyrethroid accumulation (Table VII).

Although EQS levels have not been set for aquatic solid matrices, recent EC directives (93/57–58/EEC) have defined a maximum residue level (MRL) in many foods and in most cases it has been set at 0.05 μg kg^{-1}. On this basis the fish from the Meltham catchment would be considered unsuitable for human consumption. The highest concentration observed in environmental fish of *cis*-permethrin was 5769 μg kg^{-1} and for cyfluthrin, 109.7 μg kg^{-1}. The *cis/trans* isomer ratios for permethrin in environmental matrices give more evidence that the laboratory was not a source of contamination. The ratio determined for the laboratory standards (0.34) is significantly different from those observed in the environment (Table VIII). The water samples have a higher isomer ratio than would be expected, this is probably due to a different ratio being employed in the mothproofing formulations compared to our reference compound. The greater abundance of the *cis* isomer in comparison to the *trans* isomer observed in all the matrices is comparable to that observed in samples from both terrestrial and aquatic ecosystems. This phenomenon is due to the reported preferential biodegradation and faster metabolism of *trans*-permethrin compared to *cis*-permethrin[21–23]. This would appear to be supported by the larger ratios in all matrices for site *STW*, where the analyte would have passed through the treatment works and hence experienced a much greater influence prior to discharge into the aquatic environment.

TABLE VIII *cis/trans* Isomer ratio of permethrin in environmental matrices from selected sampling sites

Matrix	*cis/trans ratio*			
	STW	*BDII*	*BDIII*	*BR*
Water	1.58	1.30	1.33	1.06
Sediment	3.26	2.52	2.66	2.90
Moss	1.84	1.21	1.55	1.25
Reference Compound	0.34			
Fish	Site HHB-Ratio varies from 16.16 to 0.67			

In conclusion, it has been shown that GC/NICI-MS in SIM mode is capable of monitoring below the EQS levels for the pyrethroids in water. These analytes can also be detected in complex matrices such as stream sediments, moss and fish tissue, however, clean-up procedures are sometimes required. Ultrasonic extraction of solid matrices was not only effective and inexpensive, but also simple and rapid. The time required to extract the pesticides from solid matrices was 90 minutes compared to the 4h using steam distillation extraction. It has been demonstrated that methods developed for the analysis of spiked samples are also applicable to environmental samples with similar matrix compositions.

Acknowledgements

We would like to thank R. Armitage (NRA, West Yorkshire Division, UK) for permission to collect samples from the Meltham Catchment area. We also thank the University of Salford SRI Chem. Sci. Division for the HP7673A auto sampler. P. Hancock would like to thank EPSRC with reference to their financial support for the duration of his PhD.

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