1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin (Figures 1-8) in sediment using an external standardisation procedure. The limit of quantitation of the method is 0.1 $\mu g\ kg^{-1}$ for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 $\mu g\ kg^{-1}$ for permethrin.

Figure 1

Compound : Bifenthrin

IUPAC Name : 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 82657-04-3

CAS Name : (2-methyl[1,1'-biphenyl]-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

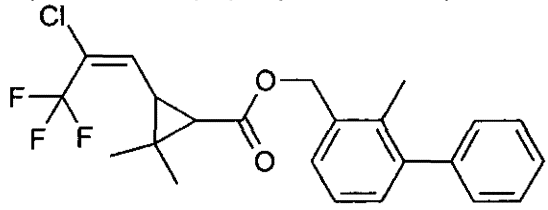


Figure 2

Compound : Cypermethrin

IUPAC Name : (*RS*)-α-cyano-3-phenoxybenxyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 52315-07-8

CAS Name : Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

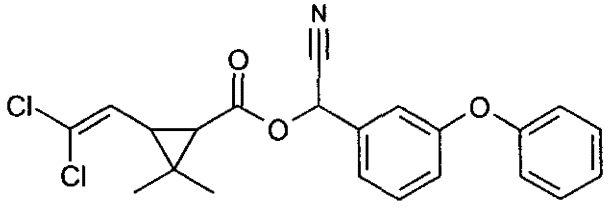


Figure 3

Compound : Cyfluthrin

IUPAC Name : *RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 68359-37-5

CAS Name : Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

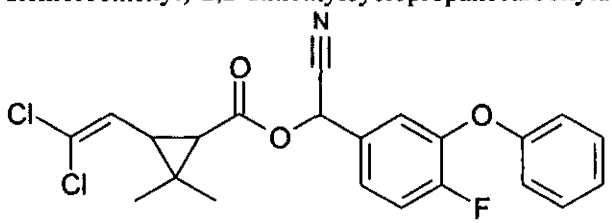


Figure 4

Compound : Deltamethrin

IUPAC Name : (*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 52918-63-5

CAS Name : 1-[*R*-[1-α(*S**),3α]]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

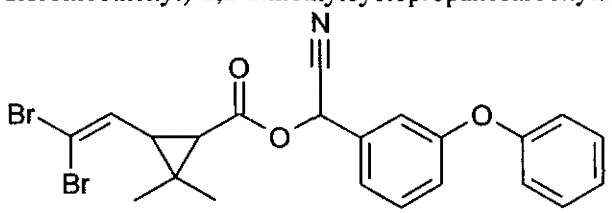


Figure 5

Compound	:	Esfenvalerate
IUPAC Name	:	(*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate
CAS Number	:	66230-04-4
CAS Name	:	[*S*-(*R**,*R**)]-cyano(3-phenoxyphenyl)methyl 4-chloro-2-(1-methylethyl)benzeneacetate

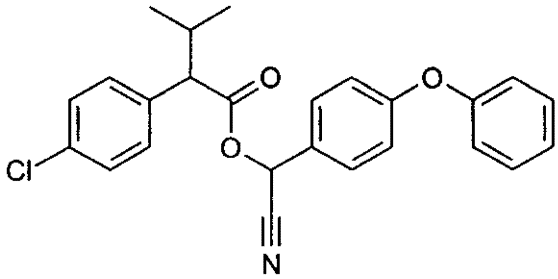


Figure 6

Compound	:	Fenpropathrin
IUPAC Name	:	(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
CAS Number	:	64257-84-7
CAS Name	:	Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate

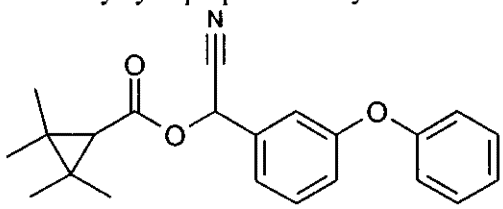


Figure 7

Compound : Lambda-cyhalothrin

IUPAC Name : A reaction product containing equal quantities of (*S*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 91465-08-6

CAS Name : [1α(*S**),3α(*Z*)]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate

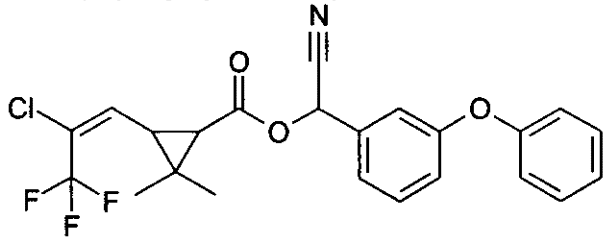


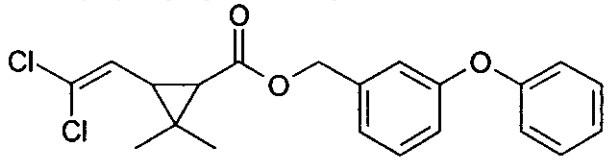
Figure 8

Compound : Permethrin

IUPAC Name : 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 52645-53-1

CAS Name : (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate



1.2 Method Summary

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues are extracted from sediment by shaking with methanol/water mixture and hexane for one hour. The sample is centrifuged and an aliquot of the upper hexane layer evaporated to dryness and re-dissolved in a small volume of hexane. The hexane sample is then subjected to a silica solid phase extraction (SPE) procedure prior to residue determination by gas chromatography with mass selective detection using negative ion chemical

ionisation (GC-MS/NICI). The limit of quantitation of the method is 0.1 μg kg^{-1} for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 μg kg^{-1} for permethrin.

2. Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

2.1 Apparatus

See Appendix 1 for a list of apparatus used during this method.

2.2 Reagents

All solvents and other reagents must be of high purity, i.e. pesticide grade solvents and analytical grade reagents. Extreme care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

2.3 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a 5 figure balance, sufficient bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin analytical standards to allow dilution in acetone to give a 200 μg mL^{-1} stock solutions in a volumetric flasks. Make serial dilutions of these stock solutions to give 100 μg mL^{-1}, 10 μg mL^{-1}, 1.0 μg mL^{-1}, 0.1 μg mL^{-1} and 0.01 μg mL^{-1} standards in acetone. If required, mixed standards can also be prepared to 0.01 μg mL^{-1} in acetone.

When not in use, always store the standard solutions in a refrigerator at ≤7°C to prevent decomposition and/or concentration of the standard. Analytical standards should be replaced with freshly prepared standards after six months.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Reference 1).

Reagent Hazards

	Acetone	Hexane	Methanol	Diethyl Ether
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	✓	✓	✓
Harmful by Skin Absorption	x	✓	x	x
OES Short Term (mg m^{-3})	3560	3600	310	1500

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

2.5 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples over the course of 1 day.

2.6 Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure. Acceptable external standard recoveries will validate the work stoppages. Samples should be stored in sealed vessels at a temperature of ≤7°C.

3. Analytical Procedure

3.1 Sample Preparation

Sediment samples should be thoroughly mixed prior to taking an aliquot for analysis to ensure sample homogeneity.

3.2 Extraction

a) Weigh a representative amount of aquatic sediment (50 g) into a plastic screw capped centrifuge bottle (250 ml size). At least one untreated control and two control samples fortified with known amounts of bifenthrin, cypermethrin, cyfluthrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin in acetone should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

Note : To avoid sample cross contamination, extraction vessels should be used only once and discarded after use.

b) Add methanol/water solution [1:1 v/v, 75 mL] and hexane (50 mL) to the sample and shake the sample using a mechanical shaker for 60 minutes.

c) Centrifuge the sample at a speed that disperses any emulsions formed on shaking e.g. 4000 rpm for five minutes.

3.3 Solid Phase Extraction

a) Transfer an aliquot of the upper hexane layer from the sediment extract equivalent to 10 g of sediment (10 mL) into a test tube (10 mL size). Evaporate the sample to dryness under a stream of clean, dry air in a heating block set to 40°C. Re-dissolve the sample in hexane (2 mL), with ultrasonication.

b) Place a Varian Silica Bond Elut™ solid phase extraction cartridge (500 mg, 3 mL size) on a suitable vacuum manifold. Add hexane (3 mL) and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL min^{-1}, discarding the eluate.

c) Transfer the sample aliquot from section 3.3 (a) onto the cartridge and allow to percolate through under gravity or low vacuum (approx. 200 mbar). Discard the eluate.

d) Add hexane (1 mL) to the cartridge and allow to percolate through under gravity or low vacuum. Discard the eluate.

e) Place a collection tube (10 mL) in the manifold rack. Elute the analytes from the column with hexane/diethyl ether solution [9:1 v/v, 6 mL] drawing through under gravity or low vacuum at a rate of approximately 2 mL min^{-1}, collecting the eluate in the tube.

f) Evaporate the column eluate to dryness under a stream of clean, dry air in a heating block with the temperature set to 40 °C. Re-dissolve the sample in acetone

+ 0.1% (v/v) peanut oil solution (1 mL) with ultrasonication. Transfer the sample to an autosampler vial ready for final determination by GC-MS/NICI.

Note : The 0.1% peanut oil in acetone solution is used to minimise the effect of matrix related GC-MSD response enhancement and to minimise possible peak tailing due to adsorption.

3.4 Preparation of GC-MSD Calibration Standards

GC-MSD calibration standards should be prepared in acetone + 0.1% (v/v) peanut oil solution. The 0.1% peanut oil in acetone solution is used to minimise the effect of matrix related GC-MSD response enhancement and to minimise possible peak tailing due to adsorption.

For example, to prepare a 1.0 ng mL^{-1} calibration standard, transfer 1 mL of a 0.01 μg mL^{-1} bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin mixed standard in acetone to a volumetric flask (10 mL) and dilute to 10 mL volume with acetone.

4. Final Determination by GC-MSD

The following instrumentation and conditions have been found to be suitable for this analysis in this laboratory. Other instrumentation can also be used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

Instrument Conditions

GC system	:	Agilent 6890 with split/splitless injector
MSD system	:	Agilent 5973 with negative ion chemical ionization
Injection temperature	:	275°C
Injection liner	:	4 mm i.d. double gooseneck splitless liner (unpacked)
Column	:	Varian CPSil 8 30 m × 0.25 mm, 0.25 μm film thickness (5% diphenyl, 95% dimethylpolysiloxane)
Column flow rate	:	0.9 mL min^{-1} constant flow
Injection mode	:	Pulsed splitless, 30 psi for 1 min, purge flow to split vent 50 psi @2 min
Injection volume	:	2 μL
Column temperature program	:	80°C for 1 min then program at 40°C/min to 180°C, hold for 0 min then program at 5 °C/min to 305 °C, hold for 0 min.
MS transfer line temp	:	280°C

Ionization mode	:	Negative ion CI
Reagent gas	:	Methane
System calibration	:	Autotune
System resolution	:	Low
Acquisition type	:	Selected Ion Monitoring (SIM)

	Target Ion	**Qualifier 1**	**Qualifier 2**
Bifenthrin	$m/z = 386$	$m/z = 387$	$m/z = 241$
Fenpropathrin	$m/z = 141$	-	-
Lambda-cyhalothrin	$m/z = 205$	$m/z = 241$	$m/z = 243$
Permethrin	$m/z = 207$	$m/z = 209$	-
Cyfluthrin	$m/z = 207$	$m/z = 209$	$m/z = 171$
Cypermethrin	$m/z = 207$	$m/z = 209$	$m/z = 171$
Esfenvalerate	$m/z = 211$	$m/z = 213$	-
Deltamethrin	$m/z = 297$	$m/z = 81$	$m/z = 296$

Retention Times

Compound Name	Peak	Retention Time (min)
Bifenthrin	1	18.1
Fenpropathrin	1	18.5
Lambda-cyhalothrin	1 2	19.6 19.9
Permethrin	1 2	21.5 21.8
Cyfluthrin	1 2 3 4	22.5 22.7 22.8 22.9
Cypermethrin	1 2 3 4	23.2 23.4 23.5 23.6
Esfenvalerate	1 2	24.9 25.3
Deltamethrin	1 2	25.9 26.3

Typical chromatograms are shown in Appendix 4.

5. Calculation of Results

Residues may be calculated using an external standardisation procedure. For lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate and deltamethrin, isomer peaks are resolved in the GC chromatogram. The peak areas of the individual isomer peaks for each pyrethroid should be summed together and a total residue value calculated for each compound.

5.1 Using Mean Bracketed Single-Point Calibration

a) Make repeated injections of a standard containing bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin at an appropriate concentration into the GC-MSD operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for each peak.

b) Make an injection of each sample solution and measure the peak areas of the peaks corresponding to the each analyte.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the residues in the sample, expressed as $\mu g\ kg^{-1}$, using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards

Standard Conc. = Concentration of standard ($ng\ mL^{-1}$)
Sample Conc. = Sample concentration ($g\ mL^{-1}$)

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} (\mu g\ kg^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

5.2 Using Multi-Point Calibration

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues may be calculated in $\mu g\ kg^{-1}$ for each sample as follows.

a) Prepare standard solutions containing bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four). Injections of these standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

b) Make an injection of each standard and sample solution into the GC-MSD operated under conditions as described in Section 4 and measure the peak areas of the peaks corresponding to each analyte.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Square" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where y is the instrument response value, x is the standard concentration and a, b, c are constants.

g) Calculate the residues in the sample, expressed as $\mu g\ kg^{-1}$, as follows

$$\text{Residue}\ (\mu g\ kg^{-1}) = \frac{\text{Analyte found}\ (ng\ mL^{-1})}{\text{Sample conc.}\ (g\ mL^{-1})}$$

Where analyte found ($ng\ mL^{-1}$) is calculated from the standard calibration curve and sample conc. is the final sample concentration in $g\ mL^{-1}$.

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}}\ (\mu g\ kg^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

6. Control and Recovery Experiments

Control experiments should be completed as Section 3 for each set of samples analysed to verify that samples are free from bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery experiments (untreated samples accurately fortified with a known amount of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin prior to extraction) should also be completed alongside each batch of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The recovery levels should be appropriate to the residue levels expected.

Recovery data is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of $\leq$20%.

7. Interference

7.1 Matrix

Due to the high selectivity of the detection technique, no interference arising from the sediment matrix has been observed.

7.2 Reagent and Solvent Interference

Using high purity solvents and reagents no interference has been found, however it is recommended that each batch of reagents or solvent is checked for contamination prior to use.

7.3 Labware Interference

The method uses disposable labware which minimises the possibility of cross contamination. All labware should be discarded after use and not re-used.

9. Modifications and Potential Problems

Isomerisation of pyrethroids has been observed with certain GC instrumentation which can reduce instrument sensitivity. This effect appears to be dependant upon the condition of the instrument used i.e. the age of the column and GC inlet liner, and the nature of previous samples injected. Addition of 0.1% (v/v) acetic acid to all samples and standards has been found to prevent pyrethroid isomerisation taking place (Reference 3). If isomerism is observed, it is recommended that 0.1% (v/v) acetic acid is added to all samples and standards. It should be noted however, that the results presented in this method have not been generated with the addition of 0.1% acetic acid.

10. Limitations

The method has been tested on representative sediment types. It can reasonably be assumed that the method can be applied for other sediment types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

11. Conclusions

The method described is suitable for the analysis of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues in aquatic sediment. Only commercially available laboratory equipment and reagents are required. The analysis of a batch of 20 samples can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.1 $\mu g\ kg^{-1}$ for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 $\mu g\ kg^{-1}$ for permethrin.

12. References

1. Luxon, S. G. (1992): Hazards in the Chemical Laboratory 5th Edition. The Royal Society of Chemistry. Thomas Graham House, The Science Park, Cambridge CB4 4WF, UK. ISBN 0-85186-229-2.

2. Reed, R. L. II (2006): Laboratory Validation: Validation of the Residue Analytical Method: 'Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin And Permethrin in Sediment. Morse Laboratories report number ML06-1286-PWG.

3. You, J.; Lydy, M. J. (2006): Abstracts of Papers, 232nd ACS National Meeting, San Francisco, CA, United States, Sept. 10-14, 2006 , AGRO-159.

Appendix 1 : Apparatus

General laboratory glassware e.g. volumetric flasks, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

250 ml screw capped polypropylene centrifuge bottles, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

10 mL disposable glass test tubes, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA

Varian Bond Elut™ Silica solid phase extraction columns 500 mg, 3 mL size, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

SPE sample processing station, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Gas chromatograph fitted with a mass selective detector e.g. Agilent 6890 GC fitted with a 5973 series mass selective detector, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

CP SIL-8 CB Low bleed MS capillary column 30 m x 0.25 mm id, 0.25 µm film thickness, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Double gooseneck injection liner for Agilent splitless injectors (4 mm i.d.), available from Restek Corporation, 110 Benner Circle, Bellefonte, PA 168230-8812, USA.

Crimp cap autosampler vials and caps available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Appendix 2 : Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

Hexane, methanol, acetone and diethyl ether, pesticide grade, available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

Peanut oil, cooking grade, available from Planters Company, East Hanover, NJ 07936.

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin analytical standards, available from Dr. Ehrenstorfer GmbH, Bgm.-Schlosser-Str. 6 A, 86199 Augsburg, Germany.