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Analytical Method Development and Validation

CBDA Grant Study Goal:

Develop and validate sample extraction and analysis procedures that give accurate and reproducible results for environmentally relevant concentrations of pyrethroid insecticides (home and commercial use)

Analytical Method Development and Validation

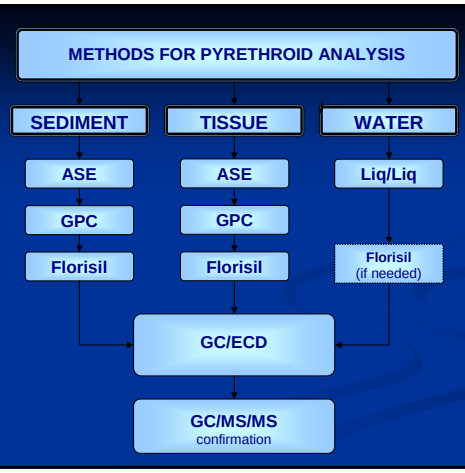
Methods

- Compatible with GC and HPLC analyses
- Applicable to water, colloids, sediment and biota
- Applicable at environmentally relevant concentrations
- Amenable to automation for high sample throughput
- Cost effective

Analytical Method Development and Validation

Development of Analytical Methods

- Optimization (sensitivity and selectivity) of instrument methods used for analysis and confirmation (GC-ECD, GC-MS, LC-MS, GC-MS/MS and LC-MS/MS)
- Isomer, enantiomer and stereoisomer identification and stability (deltamethrin, tralomethrin)
- Evaluation of water extraction methods (liq-liq, SPE, SPME)
- Evaluation of sediment and tissue extraction and matrix separation methods (PFE, GPC, Florisil)



Water Sample Extraction

Liquid-liquid Extraction

- **Sample Prep: 1 liter unfiltered water sample**
  2 liter separatory funnel
  Surrogate/Spike solution
- **Solvent: Methylene chloride (DCM), 120 mL (2x)**
- **Concentrate and solvent exchange:**
  Evaporate using K-D and µK-D (75-80˚C),
  Exchange solvent with petroleum ether
  (~ 50 mL, 3x)
- **Reconstitute: 2 mL iso-octane**
- **Analysis: GC/ECD and/or GC/MS/MS**

Pyrethroids in American River Water

Method Validation: Spike Level 2-10 ng/L

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	Standard Deviation (n=8)	MDL=t*Stdev (ng/L)	RL (ng/L)
Bifenthrin	88.1	1.76	0.31	0.98	2.00
Cyfluthrin	103	4.13	2.19	6.88	12.0
Cypermethrin	101	4.04	1.40	4.39	8.00
Deltamethrin	106	2.12	0.51	1.61	4.00
Es/Fenvalerate	101	2.02	0.55	1.73	4.00
Lambda-cyhalothrin	96.2	1.93	0.51	1.61	4.00
Permethrin	102	6.12	0.69	2.17	4.00

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Sediment/Tissue Sample Extraction

Pressurized Fluid Extraction

Instrument: Automated Dionex ASE 200

ASE Conditions

Solvent: Acetone/Dichloromethane (DCM) 50/50

Program: 100˚C, 1500 psi

Sample Prep: 10 g homogenized sediment/tissue

7 g Hydromatrix®

33 mL pre-rinsed extractor cell

Surrogate/spike solution

Extract Collection: Two 60 mL VOA vials per sample

Evaporate and solvent exchange: K-D and µK-D (75-80˚C),

Exchange solvent with petroleum ether (~ 50 mL, 3x)

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Extract Cleanup

Gel Permeation Chromatography

Instrument: J2 Scientific AccuPrep 170, Autoinject 110

GPC Conditions

Solvent: Dichloromethane (DCM)

Column: 60 x 2.5 cm

Column Packing: 65 g BioBeads, 200-400 mesh

Flow: 5 mL/min

Program: Dump-19 min, Collect-24 min, Rinse-10 min

Sample size: 10 mL DCM extract, 5 mL sample loop

Evaporate and solvent exchange: K-D and µK-D (75˚C),

Exchange solvent with petroleum ether (~ 50 mL, 3 times)

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Florisil Column Fractionation

Column Prep: 25 g Preactivated Florisil (130ºC, 24 hours) embedded between sodium sulfate with glass wool plug, 11 mm x 300 mm glass column

Reagents:

0 % ethyl ether in petroleum ether (Fraction 1)

6 % ethyl ether in petroleum ether (Fraction 2)

15 % ethyl ether in petroleum ether (Fraction 3)

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Distribution of Pyrethroids Among Florisil Fractions

(0%) Fraction 1	(6%) Fraction 2	(15%) Fraction 3
Do Not Collect (PCBs and other pesticides)	Bifenthrin Permethrin	Cyfluthrin Cypermethrin Es-fenvalerate Lambda-cyhalothrin other pyrethroids

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Pyrethroids in American River Sediment

Method Validation: Spike Level 5-20 ng/g

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	STDEV (n=8)	MDL=t*Stdev (ng/g)	RL (ng/g)
Bifenthrin	106	5.30	0.14	0.43	1.00
Cyfluthrin	108	10.4	0.85	2.55	4.00
Cypermethrin	108	11.1	0.81	2.44	4.00
Deltamethrin	62.0	3.10	0.69	2.08	4.00
Es/Fenvalerate	107	5.39	0.27	0.80	4.00
Lambda-cyhalothrin	104	5.17	0.38	1.15	4.00
Permethrin	99	16.2	1.27	3.80	8.00

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Round Robin Sediment Results

Sample 1: Results in Triplicate

Sample Identification	GC/ECD			Average	STDev
	SED PY 005	SED PY 005	SED PY 005	SED PY 005	SED PY 005
Pyrethroids by GC/ECD	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	
Bifenthrin	11.9	12.3	15.8	13.3	2.19
Cyfluthrin	2.54	1.53	2.76	2.27	0.66
Cypermethrin	3.23	1.63	3.95	2.93	1.19
Delta/Tralo-methrin	ND	ND	ND	ND	NA
Esfen/Fen-valerate	12.2	11.8	12.0	12.0	0.18
Fenpropathrin*	3.75	4.55	4.29	4.20	0.41
Lambda-Cyhalothrin	3.80	3.74	3.63	3.72	0.08
Permethrin	34.2	36.2	38.6	36.3	2.24
Surrogate (% Recovery)					
Dibromooctafluorobiphenyl	79.2	78.6	106	88.1	NA
Dibromochlorendated	82.6	78.4	79.6	80.2	NA

*Estimated MDL

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Round Robin Sediment Results

GC/ECD vs GC/MS/MS (TSQ)

Sample Identification	SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)	
Pyrethroid Pesticides	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)
Bifenthrin	11.9	10.2	12.3	13.7	15.8	17.4
Cyfluthrin	2.54	1.53	1.53	2.10	2.76	1.91
Cypermethrin	3.23	2.56	1.63	3.07	3.95	6.91
Delta/Tralo-methrin	ND	ND	ND	ND	ND	ND
Esfen/Fen-valerate	12.2	14.4	11.8	16.0	12.0	17.4
Fenpropathrin*	3.75	8.05	4.55	10.40	4.29	9.69
Lambda-Cyhalothrin	3.80	3.20	3.74	4.17	3.63	4.86
Permethrin	34.2	43.2	36.2	51.1	38.6	67.6

*Estimated MDL

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Operating Parameters for Agilent 6890 GC/ECD

- **Gases**
  - **Carrier: Helium, 1 mL/min;**
  - **Makeup: Nitrogen, 1 mL/min**
- **Columns**
  - **DB-5, 60 m x 0.25 mm I.D. x 0.25 µm film thickness**
  - **DB-17-MS, 60 m x 0.25 mm I.D. x 0.25 µm film thickness**
- **Inlet: Isothermal @ 240 °C**
- **Oven Program**
  - **Initial temperature: 80 °C, hold for 1.00 min**
  - **Ramp 1: 15 deg/min, final temp 210°C, hold time 10.00 min**
  - **Ramp 2: 2 deg/min, final temp 290°C, hold time 14.00 min**
- **Detectors: 63Ni µECD**
- **Temperature: 310 °C**
- **Combined Flow: 31 mL/min (column + make-up flow)**
- **Injection Volume:3 µL**

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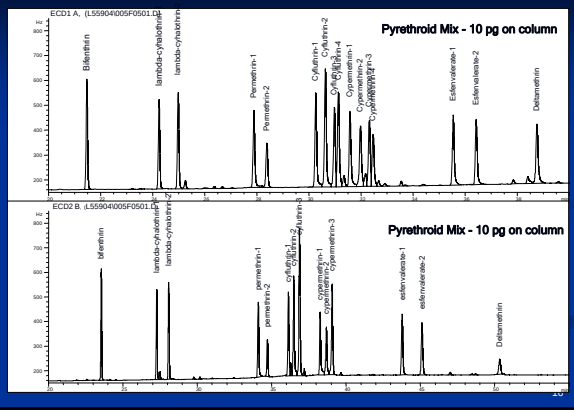
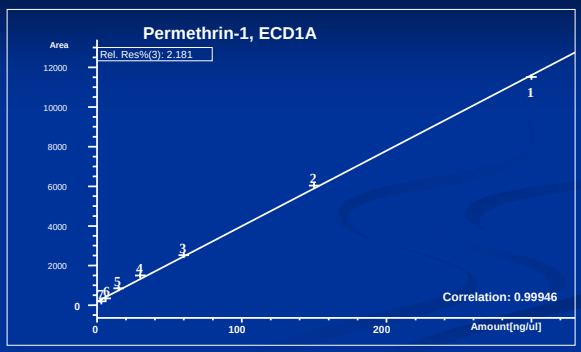
GC/ECD Chromatograms



GC-ECD Calibration Curve



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Pyrethroid Confirmation by GC-MSMS Operating Parameters

Instrument: Varian GC-MS-Ion Trap Detector, Saturn 2000

Carrier gas: Helium

Column: DB5MS, 30 m x 0.25 mm I.D. x 0.25 µm film thickness
Varian 1078 Inlet: Isocratic temp @ 200 °C
Injection Volume: 2 µL
Oven: Initial temperature: 80 °C Initial time: 1.00 min
Ramp 1: 15.0 deg/min, final temp 210 °C, hold time 10.00 min
Ramp 2: 2.0 deg/min, final temp 280 °C, hold time 0.50 min

MS-MS Operating Conditions

Trap Temperature: 240 °C
Manifold Temperature: 80 °C
Transferline Temperature: 280 °C
Ionization mode: EI Auto
Ion preparation: MS/MS
Scan time: 3µ scan/sec
Emission current: 50 µAmps
Waveform type: Non-resonant
Filament delay: 5 minute

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Confirmation by GC-MSMS Ion Trap Operating Parameters

MS-MS Conditions

Segment description	Low Mass	High Mass	Parent Ion	Daughter Ion	Excitation Storage m/z	Excitation Amplitude volts
Acenaphthene-d_8 (IS)	156	169	NA	NA	NA	NA
Resmethrin	52	125	123	81,91	54	45
Bifenthrin	77	183	181	166	79.7	80
Lambda-cyhalothrin	77	183	181	141,161	79.7	90
Permethrin	78	185	183	127,163	80.5	90
Cyfluthrin	69	165	163	127,163	71.7	63
Cypermethrin	69	165	163	127,163	71.7	72
Es-fenvalerate	97	227	225	125,167	99.1	70
Deltamethrin	77	183	181	141,152	79.7	90

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Confirmation by GC-MSMS TSQ Operating Parameters

Analyte	Q1	Q3	Collision Energy (-volts)
Bifenthrin	181	166	15
Lambda-cyhalothrin	181	152	25
Permethrin	183	153+163	10
Cyfluthrin	163	127	10
Cypermethrin	163	127	10
Es/fenvalerate	167	125	15
Deltamethrin	181	152	20

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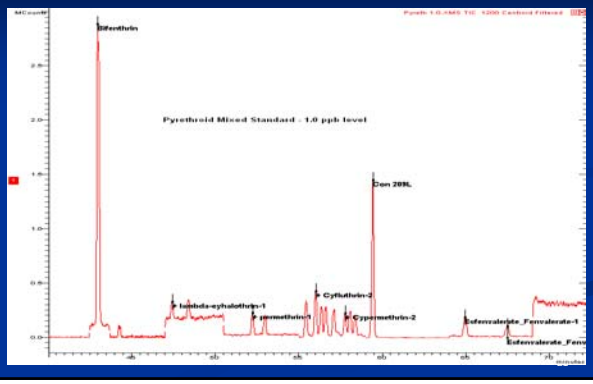
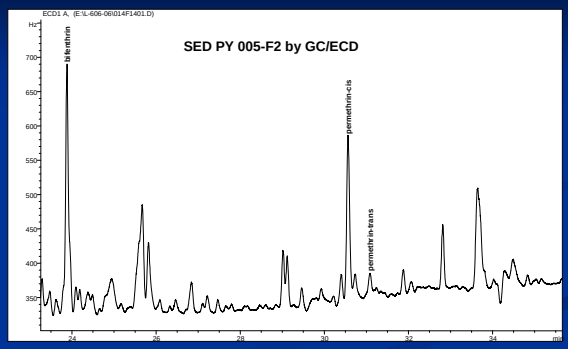
Pyrethroid Mixed Standard TSQ MS-MS 1.0 ppb



Round Robin Sediment Extract



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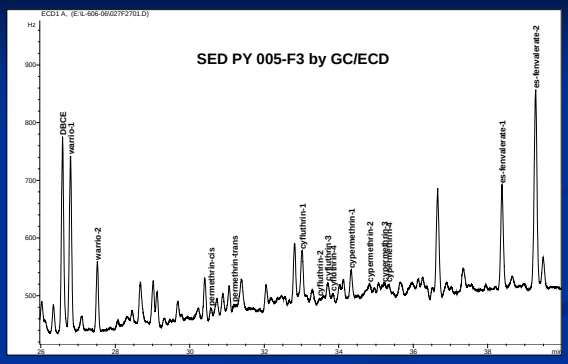
Round Robin Sediment Extract



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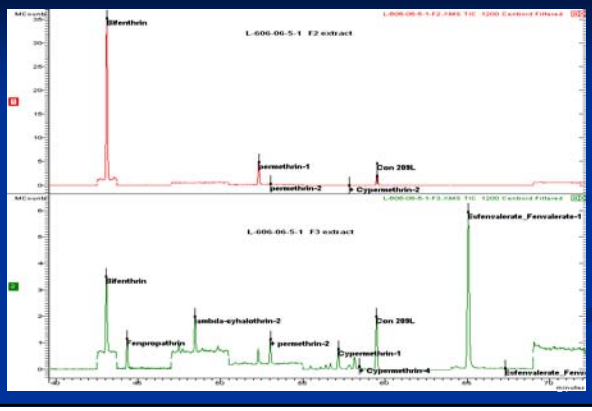
Round Robin Sediment Extract

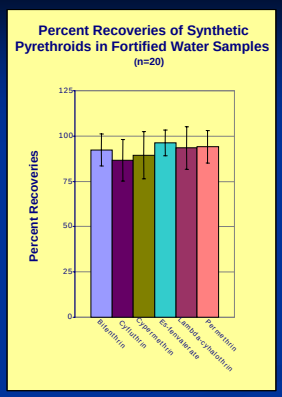


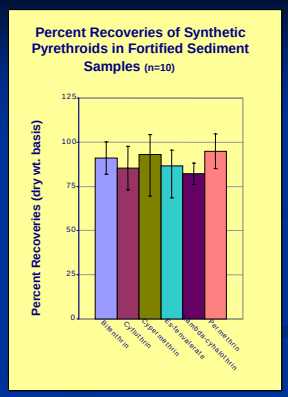
Pyrethroid Tissue Validation

American River Hatchery Trout, Spike Level 4-16 ng/g

Pyrethroid Pesticides	AVERAGE % Recovery	AVERAGE Amount Recovered	STD DEV (n=7) Amount Recovered	MDL=t*Stdev Fresh Wt. ppb (ng/g)	RL Fresh Wt. ppb (ng/g)
Bifenthrin	88.1	3.52	0.21	0.65	2.00
Cyfluthrin	98.7	15.8	0.86	2.72	6.00
Cyhalothrin-Lambda	89.1	3.56	0.47	1.49	4.00
Cypermethrin	74.3	5.94	0.53	1.660	4.00
Deltamethrin	41.8	1.67	0.19	0.60	2.00
Esfenvalerate	52.8	2.11	0.53	1.68	4.00
Fenpropathrin*	85.0	3.40	0.43	1.34	4.00
Permethrin	97.2	11.7	0.71	2.23	6.00

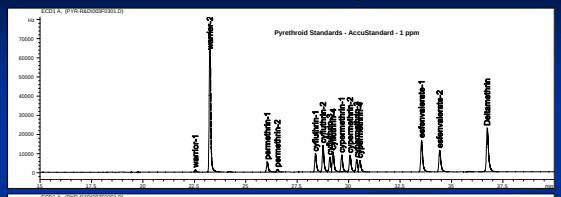
*Estimated MDL

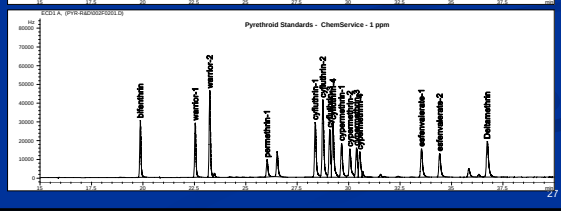




Pyrethroid Analytical Standards

Difference in Isomer Ratio by Manufacturer





Additional Pyrethroid Analytes

Fortified American River Water

Spike Level: 20 ng/L

Extraction Method	AR 1 liq/liq	AR 2 liq/liq	AR 3 liq/liq		
Pyrethroids by GC/ECD	% Recovery	% Recovery	% Recovery	AVERAGE % Recovery	STD DEV
Allethrin	109	98.4	108	105	5.85
Flucythrinate	88.3	83.3	84.3	85.3	2.65
Phenothrin	87.5	88.5	95.5	90.5	4.36
Prallethrin	94.7	86.8	92.6	91.4	4.09
Tetramethin	112	114	116	114	2.00
Tralomethrin	43.5	51.3	56.2	50.3	6.40
Surrogate (%Rec)					
DBOB	90.0	98.3	112	100	11.1

Additional Pyrethroid Analytes

Fortified American River Water

Spike Level: 40 ng/L

Extraction Method	AR 1 SPE-Acidified	AR 2 SPE-Acidified	AR 3 SPE-Acidified		
Pyrethroids by GC/ECD	% Recovery	% Recovery	% Recovery	AVERAGE % Recovery	STD DEV
Allethrin	113	101	105	106	6.11
Flucythrinate	92.5	91.8	88.8	91.0	1.97
Phenothrin	97.2	94.0	97.3	96.2	1.88
Prallethrin	118	107	109	111	5.86
Tetramethin	128	113	119	120	7.55
Tralomethrin	85.6	80.2	77.0	80.9	4.35
Surrogate					
DBOB	95.2	86.1	85.3	88.9	5.50

Additional Pyrethroid Analytes

Fortified American River Sediment

Spike Level: 4 ng/g

Extraction Method	AR 1 ASE	AR 2 ASE	AR 3 ASE		
Pyrethroids by GC/ECD	% Recovery	% Recovery	% Recovery	AVERAGE % Recovery	STD DEV
Allethrin	126	101	110	112	12.7
Flucythrinate	113	105	108	109	4.04
Phenothrin	101	99.2	77.7	92.6	13.0
Prallethrin	122	103	103	109	11.0
Tetramethin	120	106	127	118	10.7
Tralomethrin	93.0	91.6	94.5	93.0	1.45
Surrogate (%Rec)					
DBOB	126	104	100	110	14.0

Summary

- Methods developed and validated for water, sediment and tissue
- Method of quantitation - total vs. isomers
- Method extends to other pyrethroids – allethrin, flucythrinate, phenothrin, prallethrin, tetramethin and tralomethrin
- GC/MS/MS (TSQ): Very sensitive – at least 2 – 10 times lower than GC/ECD; best tool to obtain lower reporting limit by reducing background interference

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Future Method Development

- Optimize GC/MS/MS analysis by using appropriate internal standards and surrogates
- Complete validation of GC-MS/MS-TSQ
- Complete validation of GC-MS/MS-Ion Trap
- SPME Method Development using MS/MS
- Investigate LC-MS and LC-MS/MS

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