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APPLICATION NOTE

Challenges for Analysis of Pyrethroid Residues: Overall Strategies and Solid Phase Micro-Extraction (SPME) Case Study

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INTRODUCTION

Pyrethroid insecticides play an important role controlling insect pests in agriculture and home and lawn care, and also in protecting humans and animals from parasites. They thus can sometimes be found at low levels in environmental samples; therefore accurate and highly sensitive analysis of pyrethroids in various matrices is important. Analysis of pyrethroids has some challenging aspects; strategies for meeting these challenges, with an emphasis on SPME analysis, will be presented.

ANALYTICAL CHALLENGES AND RESPONSES

Challenge: Pyrethroids have extremely low water solubility values and tend to sorb onto particulates and container walls, requiring special care for handling/preservation of aqueous samples.

Response: Immediately after collection, methanol and hexane are added to the aqueous sample to reduce (and possibly reverse) adsorption to container walls. When ready for analysis the entire container is shaken and the hexane layer is removed, followed by a final hexane rinse of the entire container after sample transfer.

Challenge: The instrument of choice for multi-analyte pyrethroid methods is negative chemical ionization gas chromatography with mass spec detection (NCI-GC-MS), which is prone to variability due to differential matrix effects (i.e., sample extracts vs. solvent standards) in the inlet, as well as detector drift.

Response: The addition of 0.1% peanut oil as an “analyte protectant” has helped to smooth out inlet eects and maintain sharper peak shapes. More signicantly, deuterium (d6) stable isotope analogues were custom synthesized and are being used as internal standards to normalize instrumental response.

Challenge: The multi-analyte method has been applied to complex matrices such as wastewaters and biosolids from Publicly Owned Treatment Works (POTWs). The evaluation of method performance is complicated by the lack of analyte-free materials for fortification purposes and the sample-to-sample variability that implies that recoveries measured from a given biosolids sample may not represent the method performance for a biosolids sample from another POTW source.

Response: It has been empirically determined that two of the eight pyrethroids (d6-esfenvalerate and d6-fenpropathrin) are suitable as surrogates that are added to all samples for evaluation of method performance. Because these d6 materials could not be used as instrumental internal standards to accurately and precisely quantify native esfenvalerate and fenpropathrin, respectively, experimentation indicated that deltamethrin-d6 and bifenthrin-d6, respectively, served adequately as “replacement” internal standards for the quantification of native esfenvalerate and fenpropathrin, respectively.

Challenge: The conventional approach to analyzing water samples measures total pyrethroid content, including any residues binding to non-filterable (or non-filtered) particulates or complexing with dissolved organic carbon – this approach does not determine the bioavailable concentrations.

Response: Use of an alternate technique – Solid Phase Micro-Extraction (SPME) – has been applied to the immediate (without storage) analysis of aqueous fractions, allowing accurate measurement of the bioavailable pyrethroid component of the sample, as discussed in more detail on the following page.

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ANALYTICAL CHALLENGES AND RESPONSES

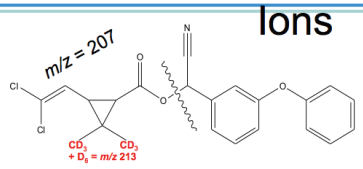
Pyrethroids are notable for low water solubility

Compound	Water Solubility (µg/L)
Bifenthrin	0.014
Deltamethrin	0.20
Cyfluthrin	2.3
Cypermethrin	4.0
Lambda-Cyhalothrin	5.0
Permethrin	5.5
Esfenvalerate	6.0
Fenpropathrin	10.3
Diflubenzuron	140
Chlorpyrifos	2,000
Diazinon	40,000
Carbaryl	120,000
Oxamyl	280,000,000

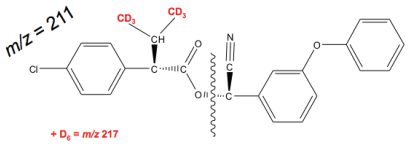
Sources: (Pyrethroids) Laskowski, D.A., Res Environ Contam Toxicol 174:49-170, 2002 (Others) The Agrochemicals Handbook, Royal Society of Chemistry, 2nd Ed.

Compound A	Background, ppb	Fortified, ppb	Total Measured, ppb	Fortified ppb, corrected for "True" background	Corrected recovery, %
"True" value	500	200	700	500	100
Measured low end	400 (+/- 20%)	180 (+/- 10%)	580	80	40
Measured high end	600 (+/- 20%)	220 (+/- 10%)	820	320	160

Examples of NCI-GC-MS Target Ions



Cypermethrin
Target Ion = *m/z* 207, Qualifier = *m/z* 209



Esfenvalerate
Target Ion = *m/z* 211, Qualifier = *m/z* 213

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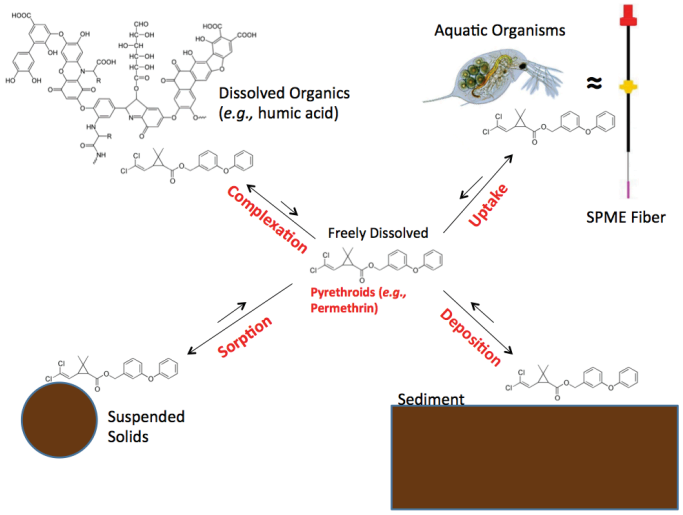
When applied to the immediate (without storage) analysis of aqueous fractions, Solid Phase Micro-Extraction (SPME) allows for the accurate measurement of the bioavailable pyrethroid component of the sample. This measurement of the bioavailable pyrethroid componentis not possible using conventional approach to analyzing water samples.

SPME CASE STUDY

Pyrethroids in aqueous solution equilibrate among a freely-dissolved phase (see below) and various noncovalently-bound phases. These equilibria strongly favor bound states over the freely-dissolved state. Only the freely-dissolved phase is bioavailable to organisms in the water column, and dissolved and suspended organic compounds can reduce pyrethroid bioavailability by competing with organisms for the freely-dissolved phase.

This model is supported by experiments showing that pyrethroid uptake and toxicity in Daphnia and Cereodaphnia species are correlated with the activity (freely dissolved concentration), rather than the total concentration, of pyrethroid. Similar to aquatic organisms, a PDMS-coated SPME fiber introduced into the water absorbs an amount of pyrethroid that is directly proportional to the pyrethroid activity. SPME thus provides an attractive tool for measuring an endpoint that is both ecotoxicologically relevant and otherwise difficult to measure.[1]

Stable isotope-labeled internal standards enable simultaneous determination of activity and total concentration. In our experiments with sediment pore water, deuterated analogs of the pyrethroids under study were added to pore water samples in known amounts. Samples were then vortexed and sonicated to ensure equilibration of the added internal standard. Since the deuterated compound binds to every system component to the same extent as the analyte, quantitation based on the internal standard-corrected instrument response yields the total analyte concentration, while quantitation based on uncorrected instrument response (external standard calculation) yields the activity, both from the same GC-MS injection.[2]



SPME provides an attractive tool for measuring an endpoint that is both ecotoxicologically relevant and otherwise difficult to measure.

At EAG Laboratories, SPME measurements were applied to the analyses of "pore water" samples (generated via the centrifugation of wet sediment), with the goal of determining the ratio of the pyrethroid concentrations in the sediment to the freely dissolved

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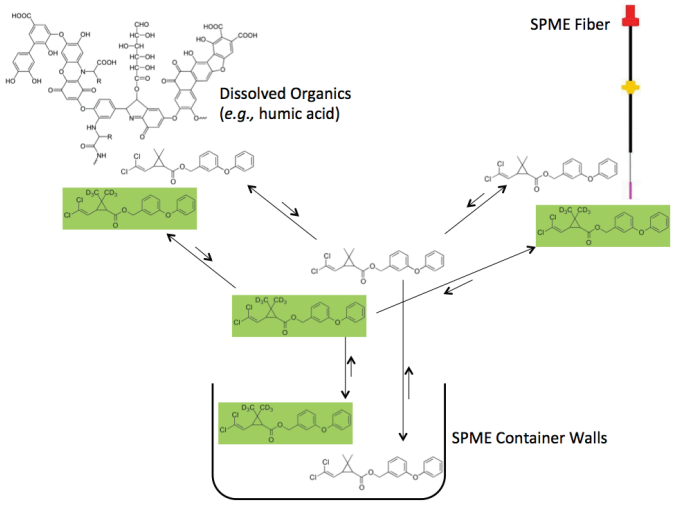


Figure 2. SPME measurements are applied to the analyses of "pore water" samples, with the goal of determining the ratio of the pyrethroid concentrations in the sediment to the freely dissolved concentrations in the pore water.

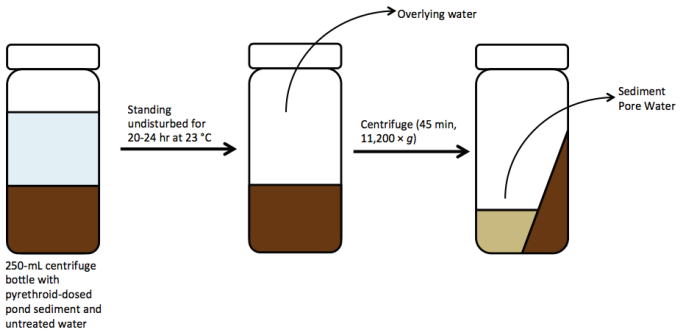


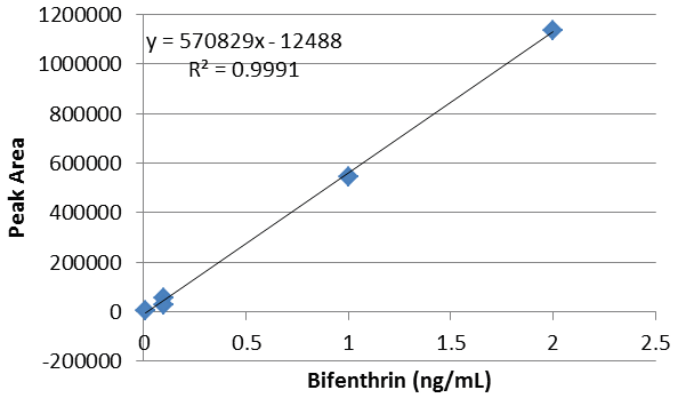
Figure 3. Preparation of Pond Sediment Pore Water.

(and thus bioavailable) concentrations in the pore water.

Photo above shows three pore water samples in SPME analysis vials. In order to minimize the adsorption onto the walls of these SPME sample vials, analysis of the generated pore water samples was performed as quickly as feasible following the generation of samples via centrifugation. Also, the calibration standards (in water) were prepared one at a time, each immediately before use.

In order to facilitate the SPME measurements within a reasonable analysis time, a non-equilibrium (and thus non-depletive) approach was used. This technique requires strict control of the analysis parameters. The same fiber was used for a given batch of analyses, and a Gerstel autosampler/autoinjection system was used to maintain a constant sampling time and a consistent injection technique. In addition the autosampler swirled the contents of the standard and sample vials during the SPME sampling process.

The pyrethroid activity is known only when the activity is the same as the total concentration. This condition is met in pure water (in the absence of dissolved organic compounds). Pure water was therefore used to prepare calibration curves.



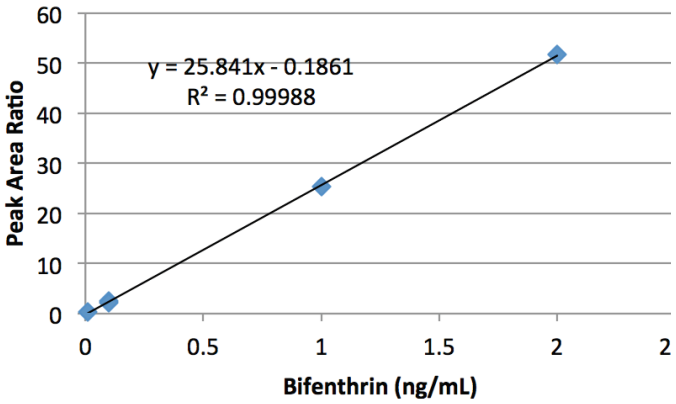


Table 1. Bifenthrin External Standard (top) and Internal Standrd (bottom).

Curves were plotted based upon raw peak areas *and* analyte-internal standard response ratios. The freely-dissolved analyte values were calculated from the raw peak area curves, and the total analyte values were calculated from the analyte-internal standard response ratio curves, using the approach described by Bondarenko and Gan. An example curve of each type, generated from the same experiment, is shown below.[2]

POSSIBLE FURTHER APPLICATIONS OF SPME

The selectivity of SPME in measuring the bioavailable portion of pyrethroids in aqueous systems has potential applications for passive sampling of streams and other natural water systems. Analysis of the adsorbed pyrethroids could be accomplished either by transfer of the adsorbed pyrethroids into the GC inlet via specialized equipment, or extraction of the sorbed component via organic solvent, followed by NCI-GC-MSD analysis.

ACKNOWLEDGEMENTS

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REFERENCES

[1]Yang, W., et al., J Agric Food Chem 2006, 54, 3967-3972.

[2]Bondarenko, S., and Gan, J. Environ Sci Technol 2009, 43, 3772-3777.