The Automated SPE Assay of Fipronil and Its Metabolites from Bee Pollen.

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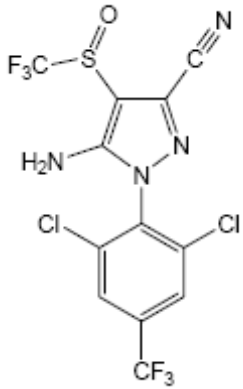
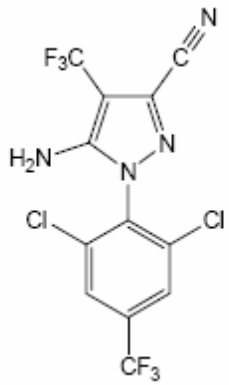
Abstract

With the recent decline in hive bee populations this year the investigation of acaricides in the environment have become more evident. One source of these analyses is the quantity of fipronil and its metabolites in bee pollen. Fipronil is used as a pesticide on crops that are pollinated by bees. Fipronil's metabolites are toxic and study of the metabolites have been increasing. Automation of this separation technique would provide laboratories with an efficient method to extract the fipronil and it's metabolites for analytical analysis. SPE is utilized for this extraction with verification via HPLC. Lower levels of detection can be achieved using LC MS or GC MS. MS can be used to determine the concentrations of the metabolites.

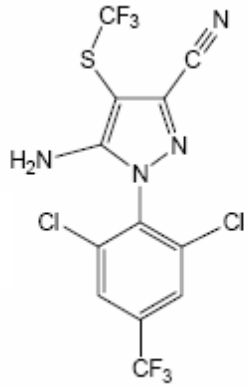
Fipronil and Its Metabolites Extracted



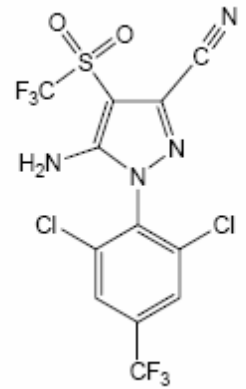
Fipronil



Fipronil Desulfinyl



Fipronil Sulfide



Fipronil Sulfone

Bee Pollen Samples

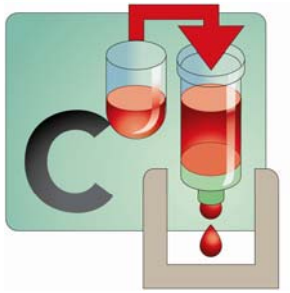
- Bee pollen samples were prepared
  - 1 gram of bee pollen was combined with with 25 ml of acetone. Mixture was shaken to extract the fipronil and its metabolites
- Prepared Samples spiked with Fipronil and metabolites
  - 0.25 ug of each was added to the sample

Instrumentation Utilized

- GX-271 Aspec
  - SPE extraction
  - Strata C18
  - Sample injection
- 305 and 306 Pumps
- 811C Mixer
- 155 UV Detector
- Waters Xterra 4.6 x 150 mm C18 column

SPE Method Optimization

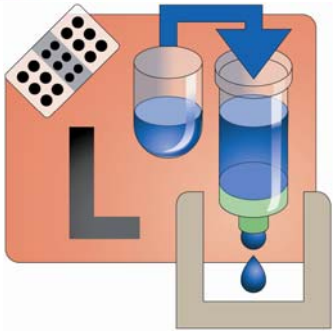
- Optimization is key to precise and accurate analytical measurements
- Sample preparation should be the most scrutinized step in any analyte quantification
- Poor sample preparation can lead to inaccuracies and imprecise measurements.
- Automation can simplify optimization procedures by testing many different sample preparation treatments and optimize the treatment that provides the most accurate and precise results



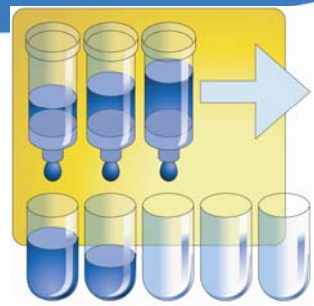
SPE Optimization
Condition

- Condition SPE Cartridge
  - Cartridges are set up with several differing volumes of conditioning agents
  - Several conditioning agents are tested
    - Methanol
    - Water
    - Ethanol
    - Acetonitrile
  - Each volume and agent is tested by Conditioning the column with different volumes and different agents then loading them with analyte. The matrix that elutes from the column is then tested for bifenthrin and fipronil by injecting onto the HPLC

Conditioning an SPE cartridge via automation is accomplished with software that allows the user to program several variables to optimize the method

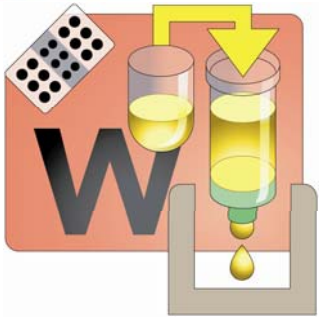


SPE Optimization
Load Sample



- Load sample on cartridge
- Differing volumes of sample are injected onto SPE cartridges
- Differing concentrations of samples are injected each onto SPE cartridges
- Differing matrices are injected each onto SPE cartridges
- Each eluent from each cartridge sample load is tested for bifenthrin and fipronil by injection onto HPLC.

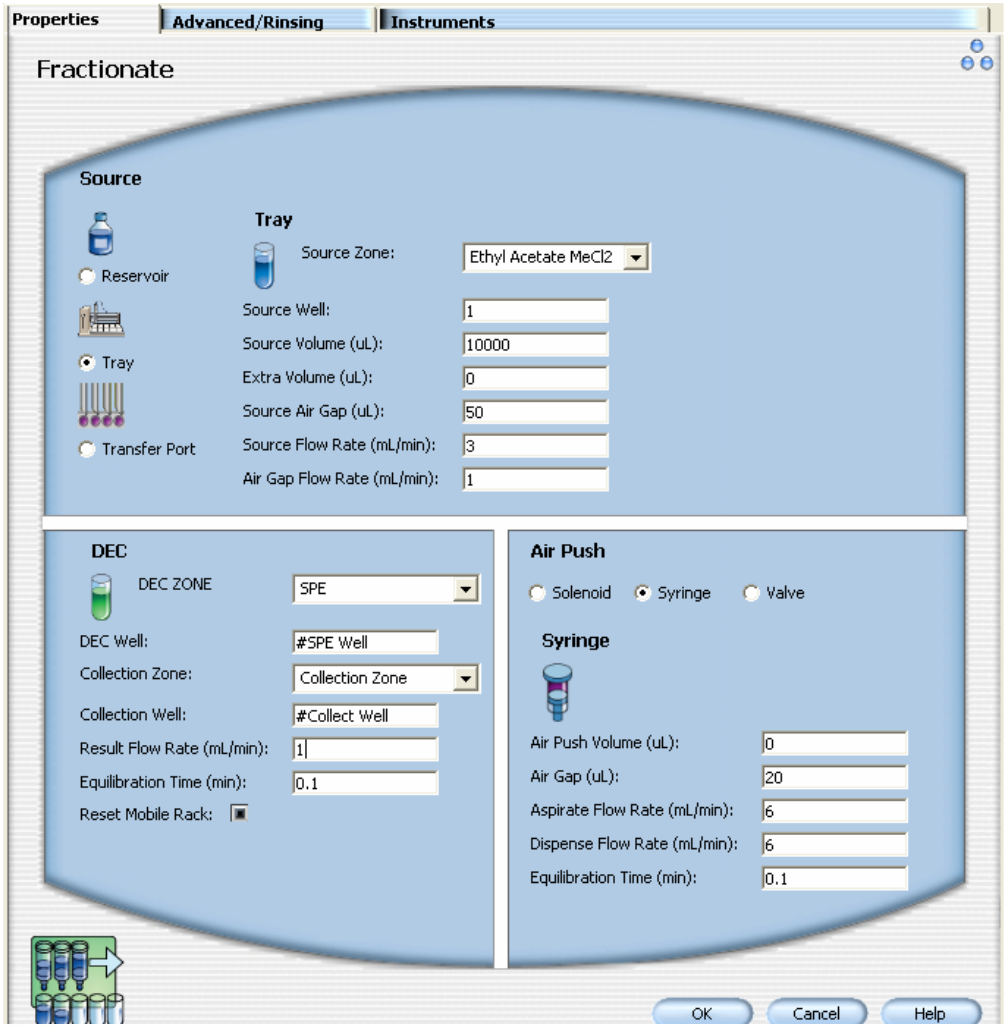
Loading conditions can be controlled easily with automation software. Each variable can be manipulated for the optimization process.



SPE Optimization
Wash Sample

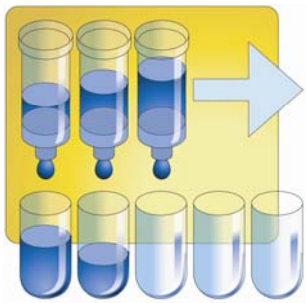
- Once the sample load and condition have been optimized the wash step is then optimized
- Optimized sample is injected onto each cartridge
- Different washes are passed through different cartridges
- Each eluent of the wash is checked for breakthrough of the acaricides.
- Each eluent is checked for interfering compounds that have been washed off.
- Those washes that elute the smallest concentration of acaricides and the greatest concentration of interfering compounds are kept and tested with the elution solvents in the next optimization

Optimization of Washing and Eluting can be accomplished within a fractionation task. The SPE cartridge is drawn across a set of test tubes and an allotted amount of wash or eluent is drained into each tube. The contents can then be analyzed for breakthrough of the analyte or elution of any interfering peaks.



SPE Optimization
Elute Acaricides

- After the wash step/steps have been optimized the Elution is then optimized
- Each SPE cartridge has sample loaded and washed
- Each SPE cartridge is then tested with differing elution solvents
- As the solvent is added the eluent is collected in a set of tubes each with the same amount of eluent (this procedure can be used to check washes and loads also)
- Each eluent is checked for breakthrough and concentration of acaricides
- The eluent and wash that delivers the greatest percent of analyte eluted and the least amount of interfering compounds is chosen



Extraction Parameters

- 5 ml methanol followed by 5 ml water to condition a 3 ml Strata C18 column
- 2 ml water followed by 3 ml water:ethanol 50:50 as a wash
- Columns were dried
- Elution with 4 ml ethyl acetate:dichloromethane 50:50
- Dry down with nitrogen and bring back up in 1 ml 80% ACN 20% water solution

HPLC Separation

- Isocratic mobile phase acetonitrile:water 85:15 v:v
- 100 ul injections
- Xterra C18 column 4.6 x 150 mm
- Mobile phase at 1.3 ml/min
- Peak retention times
  - Fipronil 3.5 minutes

HPLC Separation