THOMSON Solutions At Work™

# Filter Vial Applications

## Food Agricultural & Environmental

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# Filter Vial Applications

## Food Agricultural & Environmental

# An Introduction to Filter Vials

Thomson Filter Vials are a single system which replaces HPLC Vials, HPLC Caps, Syringes, & Syringe Filters for the filtration of samples. In 15 seconds, Thomson Filter Vials allow for sample preparation of unfiltered samples to filtered samples in an autosampler-ready vial.

### Key Features

- Same Size as a standard HPLC Vial and will fit easily into any machine or tray available for standard HPLC vials.PTFE, PVDF, PES and Nylon membranes are available depending on the percentage of organic solvent in the sample and the amount of protein binding
- Pore sizes of either 0.2µm or 0.45µm will provide the perfect degree of filtration needed from viscous to clarified samples
- Versatility is built into Thomson's line of Filter Vials. Whether your samples are low volume or viscous or particulate-laden or contain a high volatility organic solvent Thomson has a Filter Vial to fit your needs

# Syringe Filter Built In

### Equivalent to A Syringe Filter Built Into Your HPLC Vial

Filter Vials are equivalent to a syringe filter built into your HPLC vial. Even a sample that appear clear to the eye potentially have particulates that can clog the machine and cause down time and costly maintenance. Filter Vials increase productivity by eliminating a transfer step required when using a syringe filter.



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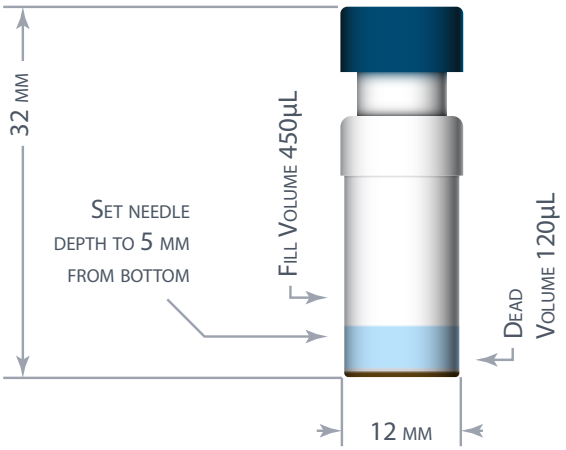
# How Filter Vials Work

### Similar to How A French Press Works...

Similar to how a french press (cafetière à piston) works, Filter Vials filter particulates out of the sample with similar membranes used in syringe filters. The pressing of the plunger into the shell vial forces the sample up through a filter to separate the particulates from the sample to be analyzed. Thomson has several filter membranes and pore sizes to choose from making the Filter Vial a versatile tool in the lab.





DEPOSIT SAMPLE

COMPRESS FILTER VIAL

READY FOR ANALYSIS

# Easy As 1, 2, ... Done!

### In Two Steps

1. Deposit 450µL of sample into shell vial
2. Insert plunger into the outer shell & press

### 15 Seconds

In two steps and 15 seconds you can have a safe and secure sample for analysis. If you need to filter more than one sample the use of a Toggle Press (up to 5) or Multi-Use Press (up to 48) can be used.

You can prepare a safe particulate free sample in less time than it takes to in the time it takes to open the syringe packaging and add the syringe filter.

# Filter Vial Membrane

## Membrane Pore Size

The recommended membrane pore size for sample filtration is based on the cell or cell debris content of the sample and the particle size of the packing material in the chromatography column used to analyze the sample. If the sample contains cells or cellular debris, then a 0.2µm pore size membrane is recommended to maintain system sterility.

### Which to use?

- **0.2µm Pore Size**
  - Cells or Cell Debris in Sample
  - Chromatography Column Particle Size <3µm
- **0.45µm Pore Size**
  - Chromatography Column Particle Size >3µm

## Membrane Material

The recommended membrane for sample filtration is based on the percentage of organic solvent in the sample and the amount of protein binding.

### Compatibility

For chemical or compound compatibility with our Filter Vials & membranes see the Chemical Compatibility Index & Compound Compatibility Index in our Technical Library

| | Aqueous | >50% Organic | Low Protein Binding |
|---|---|---|---|
| PTFE | | ● | |
| PVDF | ● | | ● |
| Nylon | ● | ● | |
| PES | ● | | ● |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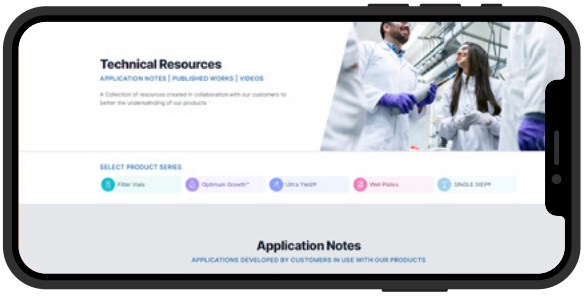
# What Applications Can the Filter Vial be Used For?

With Thomson's family of Filter Vials and membranes available to you, finding ways to replace cumbersome and expensive syringe filters in the lab is easy. Here are just some of the documented applications you can use Filter Vials for in your lab today. See our Technical Library at htslabs.com to see a full list of applications. We work hard with small and large companies to produce proven protocols and methods for our products. If you find a use for Filter Vials in your workflow we would love to hear about it.

| | nanoIFilter Vial® | Standard Filter Vial | eXtremeIFV® | eXtractor3DIFV® |
|---|---|---|---|---|
| 10µL-250µL | ● | | | |
| 450µL | | ● | ● | |
| UPLC Compatible | ● | ● | ● | ● |
| GCMS Compatible | ● | | | ● |
| 30% Particulates | | | ● | ● |
| Viscous | | | ● | ● |
| Replacement for SPE | | | ● | ● |
| General Liquids < 10% particulates | ● | ● | ● | |
| Cell Fermentation | ● | | ● | ● |
| Particulate Removal | ● | ● | ● | ● |
| Automation Compatible | ● | ● | ● | ● |
| Small Molecules | ● | ● | ● | ● |
| Food & Supplements | | ● | ● | ● |
| Toxicology | ● | ● | ● | ● |
| Pesticides | ● | | ● | ● |
| Environmental | ● | ● | ● | ● |

## Thomson's Technical Library

You can find application notes, videos and more information on our products by visiting our website at **htslabs.com**




# What do Filter Vials Replace in the Lab?

## What Do Filter Vials Replace In The Lab?

Thomson Filter Vials simplify general filtration by replacing syringes & syringe filters, microcentrifuge spin columns, and/or liquid-liquid extractions.

Applications for Thomson Filter Vials include all sample types to be analyzed by HPLC, UHPLC, LC-MS, and GC-MS.

## Optimize Your SPE, D-SPE Or QuEChERS Workflow

Thomson Filter Vials simplify general filtration by replacing syringes & syringe filters, microcentrifuge spin columns, and/or liquid-liquid extractions.

Applications for Thomson Filter Vials include all sample types to be analyzed by HPLC, UHPLC, LC-MS, and GC-MS.

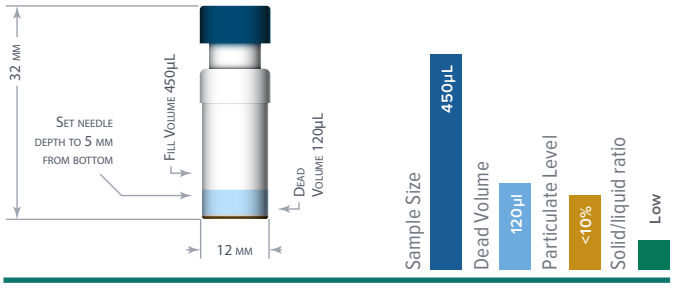

MORE
e Filters
NO MORE
HPLC Vials & Caps

# A Comparison of the Filter Vial Types

## Filter Vial

### Standard For Most Samples

**Max Fill Vol.** 450µL
**Dead Vol.** 120µL




### Key Features

- General purpose filtration
- <10% particulates

### Replaces in the lab

- Syringe Filters
- Syringes
- HPLC Vials/Caps

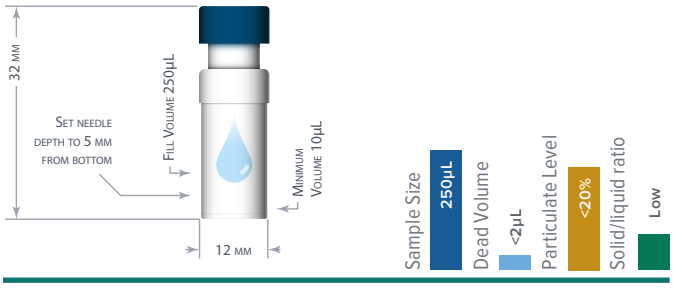
### Applications

- 120µL-450µL
- General Liquids < 10% particulates
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Environmental

## nano|Filter Vial

### When Every µL Counts

**Max Fill Vol.** 250µL
**Min Fill Vol.** 10µL (for 2µL injection)




### Key Features

- 10µL sample for 2µL injection
- Available with pre-split or non-split caps

### Replaces in the lab

- Centrifugation & Spin Filters
- Small Volume Syringe Filters
- Syringes
- High Recovery Vials/Caps
- Inserts with HPLC Vials/Caps

### Applications

- 10µL-250µL
- General Liquids < 10% particulates
- Cell Fermentation
- Particulate Removal
- Automation Compatible
- Small Molecules
- Toxicology
- Pesticides
- Environmental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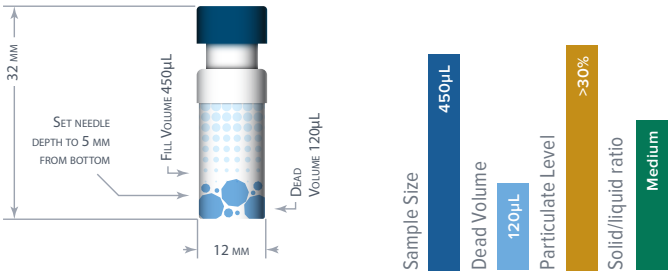
## eXtreme/FV®

### Multi-Layered Filtration

**Max Fill Vol.** 450µL
**Dead Vol.** 120µL




### Key Features

- Used for Particulate Laden Samples
- Contains a Depth Pre-Filter

### Replaces in the lab

- Syringe Filters
- Syringes
- HPLC Vials/Caps

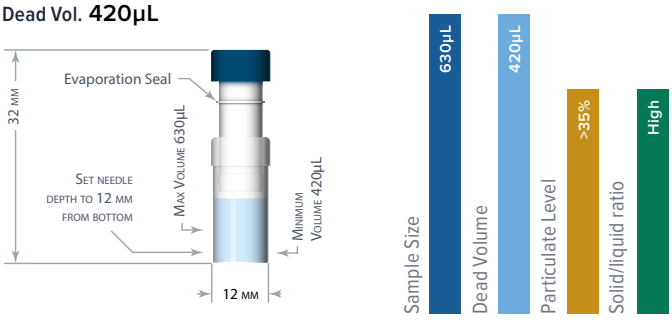
### Applications

- 120µL-450µL
- ≤ 30% Particulates
- Viscous
- Replacement for SPE
- Cell Fermentation
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Pesticides
- Environmental

## eXtractor3D|FV®

### Multi-Mode Filtration

**Max Fill Vol.** 630µL
**Dead Vol.** 420µL




### Key Features

- Minimize Matrix Effects & Ion Suppression from direct injection
- Solid/liquid ratio
- Can accept Solids or Large Particulates
- Allows for QuEChERS

### Replaces in the lab

- SPE Cartridge
- Common Syringe Filter
- Syringe
- HPLC Vial/Cap

### Applications

- ≤ 30% Particulates
- Viscous
- Replacement for SPE
- Cell Fermentation
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Pesticides
- Environmental

# Plasticizers content in Filter Vials Compared to Syringe Filters

Testing by Takeda Pharmaceutical Company Limited® UPLC - ELSD

## Introduction

Thomson Filter Vials are manufactured without the use of plasticizers or mold release agents, making them LC/MS clean. Testing with ELSD, PDA, and MS detection by Takeda Pharmaceutical showed no leaching from Thomson Standard Filter Vial with a 0.45um, PTFE membrane compared to significant leaching from Millipore Millex-FH® Filter, 0.45M, hydrophobic PTFE, 4mm. Method: A. Water B. ACN 45-90% with .05% TFA Ballistic Gradient over 1.4 minutes using Waters® Acquity® UPLC Thomson Filter Vial (patented) Part # 35540-500 Filter Vial 0.45µM hydrophobic PTFE, w/ Pre-Split Cap Millipore Syringe Filter Part #:SLFHR04NL Millex-FH® Filter, 0.45M, hydrophobic PTFE, 4mm, non-sterile.

## Method:

**A.** Water
**B.** ACN 45-90% with 0.05% TFA

Ballistic Gradient over 1.4 minutes using Waters® Acquity® UPLC

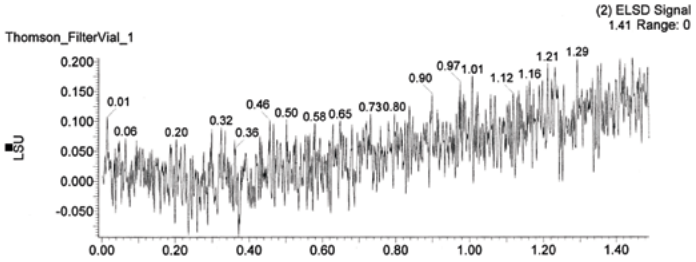
## Thomson Standard Filter Vial

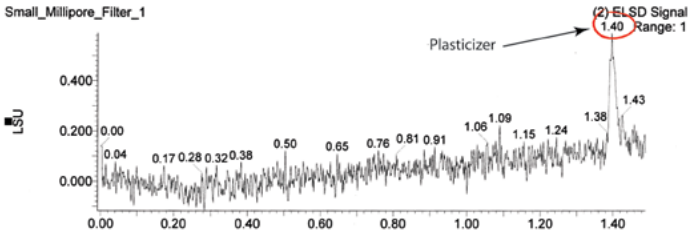
0.45µm hydrophobic PTFE, w/ Pre-Split Cap
**Part#:** 35540-500

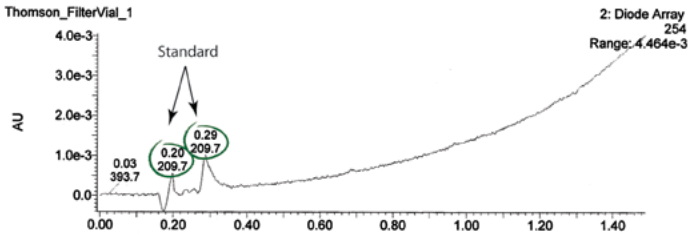
## Millipore Syringe Filter

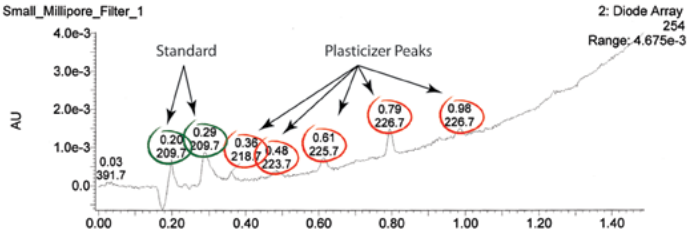
Millex-FH® Filter, 0.45µm, hydrophobic PTFE, 4mm, non-sterile.
**Part #:** SLFHR04NL

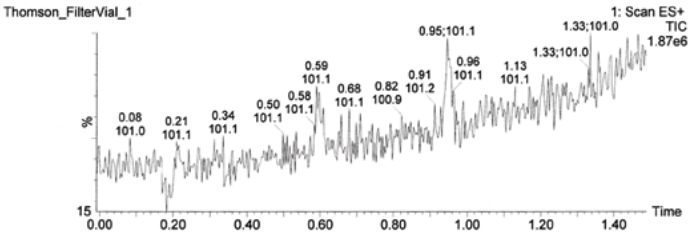
## Plasticizers

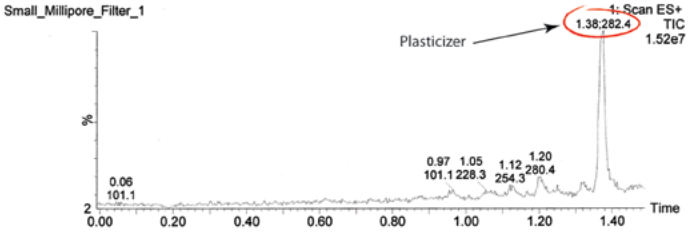



Thomson is not affiliated with Takeda Pharmaceutical Company, Millipore, Waters or their products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# Increase Signal-to-Noise Ratio with eXtractor3D|FV® for More Targeted & Accurate Peaks

### Matrix Effects & Ion Suppression:

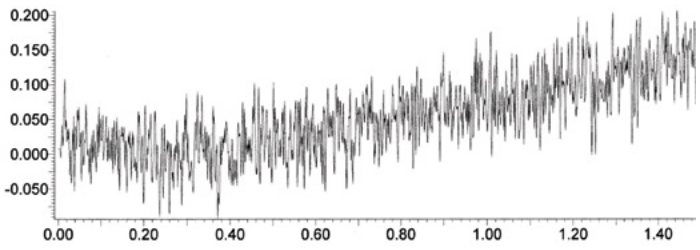
Analytes are obscured by the matrix like the octopus in this photo is difficult to find among its surroundings.

### Strong Signal; Noise Lessened:

By adding compounds to the eXtractor3D|FV® the signal to noise ratio is increased allowing you to find the analyte with ease.

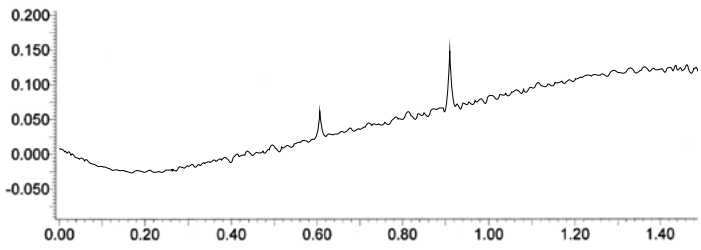
### Low Signal to Noise Ratio

Difficult to find analyte in the matrix




### High Signal to Noise Ratio

In this example the addition of C-18 to eXtraxtor3D|FV® with your sample binds excess compounds to C-18 and the Matrix clears up allowing you to see analyte peaks




Octopus images courtesy Jukin Video


# High Viscosity Presses

The Thomson Filter Vial Press enables high solid content and viscous liquids to be easily filtered through vials. Some fermentation cultures that reach 100OD or particulate laden samples may require the toggle press.

### Toggle Press

- Press up to 5 autosampler ready Filter Vials
- Allows for consistency and ergonomic concerns
- Small footprint sits on bench top
- Works with all Thomson Filter Vials

### Multi-Use Press

- 4Presses up to 48 Autosampler Ready Filter Vials at a time
- Works with 48 position block; block fits some autosamplers
- 48 position block can be transferred to a robot for automation
- Easily Automate Filter vial Pressing
- Works with all Thomson Filter Vials



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# Part Numbers

## Filter Vial

| Cap Color | Septum | Pore Size | Membrane | Part # |
|---|---|---|---|---|
| | Pre-Split | 0.2µm | PTFE | 35530 |
| | Pre-Split | 0.45µm | PTFE | 35540 |
| | Pre-Split | 0.2µm | PVDF | 35531 |
| | Pre-Split | 0.45µm | PVDF | 35541 |
| | Pre-Split | 0.2µm | NYLON | 35538 |
| | Pre-Split | 0.45µm | NYLON | 35539 |
| | Pre-Split | 0.2µm | PES | 35535 |

## Low Evap|Filter Vial

| Cap Color | Septum | Pore Size | Membrane | Part # |
|---|---|---|---|---|
| | Non-Split Crimp | 0.2µm | PTFE | 65530 |
| | Non-Split Crimp | 0.45µm | PTFE | 65540 |
| | Non-Split Crimp | 0.2µm | PVDF | 65531 |
| | Non-Split Crimp | 0.45µm | PVDF | 65541 |
| | Non-Split Crimp | 0.2µm | NYLON | 65538 |
| | Non-Split Crimp | 0.45µm | NYLON | 65539 |
| | Non-Split Screw | 0.45µm | PTFE | 64440 |
| | Non-Split Screw | 0.2µm | PTFE | 64430 |

## eXtreme|FV

| Cap Color | Septum | Pore Size | Membrane | Part # |
|---|---|---|---|---|
| | Pre-Split | 0.2µm | PTFE | 85530 |
| | Pre-Split | 0.45µm | PTFE | 85540 |
| | Pre-Split | 0.2µm | PVDF | 85531 |
| | Pre-Split | 0.45µm | PVDF | 85541 |
| | Pre-Split | 0.2µm | NYLON | 85538 |
| | Pre-Split | 0.45µm | NYLON | 85539 |
| | Pre-Split | 0.2µm | PES | 85535 |

## eXTRACTOR3D|FV

| Cap Color | Septum | Pore Size | Membrane | Part # |
|---|---|---|---|---|
| | Pre-Split | 0.2µm | PTFE | 95530 |
| | Pre-Split | 0.45µm | PTFE | 95540 |
| | Pre-Split | 0.2µm | PVDF | 95531 |
| | Pre-Split | 0.45µm | PVDF | 95541 |
| | Pre-Split | 0.2µm | NYLON | 95538 |
| | Pre-Split | 0.45µm | NYLON | 95539 |
| | Pre-Split | 0.2µm | PES | 95535 |

## nano|Filter Vial

| Cap Color | Septum | Pore Size | Membrane | Part # |
|---|---|---|---|---|
| | Non-Split | 0.2µm | PTFE | 15530 |
| | Pre-Split | 0.2µm | PTFE | 25530 |
| | Non-Split | 0.45µm | PTFE | 15540 |
| | Pre-Split | 0.45µm | PTFE | 25540 |
| | Non-Split | 0.2µm | PVDF | 15531 |
| | Pre-Split | 0.2µm | PVDF | 25531 |
| | Non-Split | 0.45µm | PVDF | 15541 |
| | Pre-Split | 0.45µm | PVDF | 25541 |
| | Non-Split | 0.2µm | NYLON | 15538 |
| | Pre-Split | 0.2µm | NYLON | 25538 |
| | Non-Split | 0.2µm | PES | 15535 |
| | Pre-Split | 0.2µm | PES | 25535 |

## High Viscosity Presses

| Press | Description | Capacity | Qty | Part # |
|---|---|---|---|---|
| Toggle Press | 5 Position for Autosampler Ready Filter Vials | 5 | 1 | 35005 |
| Multi-Use Press | 48 Position for Autosampler Ready Filter Vials | 48 | 1 | 35015 |

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| Standard | | PTFE | 0.45µm | 35540 |

# The Determination of Hexavalent Chromium in Water by Ion Exchange Chromatography Inductively Coupled Plasma Mass Spectrometry (IC-ICP-MS)

## Introduction

This method utilizes a hyphenated technique, Ion Exchange Chromatography (IC) coupled to an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine Cr(VI) in treated drinking water, surface water, and ground water. Samples are collected and preserved at a pH > 9 condition, and then injected directly into an anion exchange column. Cr(VI) is separated from other possible Cr species and other metals by the anion exchange functioning group inside the column. The column eluent is introduced directly into the sample introduction interface and the ionization source of the ICP-MS. Chromium chromatographic peak is identified and quantified by the mass spectrometry with external calibration.

## Labware Cleaning Procedure

It is critical to pre-clean and dry labware in a clean flow bench in order to minimize contamination.

- Place tubes and caps into 10% Nitric Acid (made from reagent grade) acid bath for at least 24 hours.
- Transfer tubes and caps into a DI Water bath to soak for at least 24 hours.
- Remove tubes and caps, rinse with DI Water at least three times.
- Remove as much water as possible and place inside a Class 10 Vertical Laminar Flow Metal Free Hood and let dry.

## Sample Requirements

- Sample must be preserved to achieve pH > 9 with Ultra Pure Concentrated Ammonium Hydroxide.
- Sample is collected in a 15mL amber high density polyethylene (HDPE) bottle with a plastic cap.
- Samples are stored at < 8 °C for up to 30 days, provided that the sample containers are sealed properly and stored in an acid fume free environment. However, it is recommended that samples be analyzed as soon as possible upon receipt.

## Sample Preparation

Check sample pH using a pH testing strip by transferring a small volume of sample to prevent cross contamination. If the pH is > 9, sample is ready for IC-ICP-MS analysis | Note: r2 > 0.995 for the calibration curve

- Label the Thomson 0.45µm PTFE Filter Vials (35540-500).
- Pipette 0.5mL of the sample into the filter vial shell.
- Partially insert the filter vial plunger into the filter vial shell.
- Place filter vials in the Thomson Toggle Press and press the lever to filter the samples (can press up to 5 vials each time).
- Load the filter vials into the Varian autosampler.
- Include Calibration Standards (0.05µg/L, 0.1µg/L, 0.5µg/L, 1.0µg/L) and QC Standards (DI Water Blank, Tap Water Blank, Tap Water Spiked) for every 20 samples analyzed.

## Equipment

- LC-MS:
- Varian ProStar 210 HPLC
- Varian 820MS ICP-MS
- Pump Rate (rpm): 20
- Stablization delay(s): 0
- Skimmer Gas Source: H2
- Skimmer Flow: 30

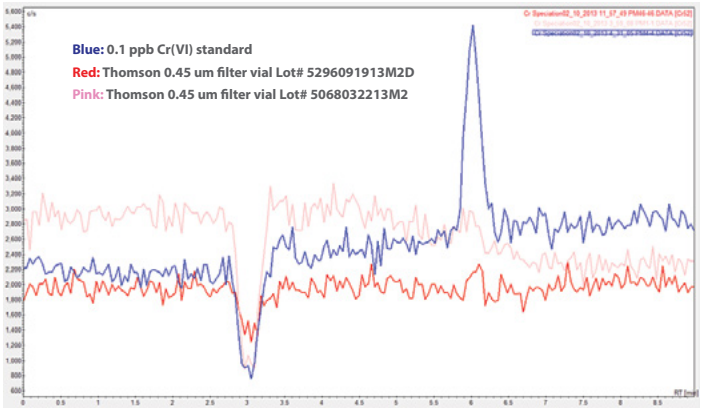
## Column

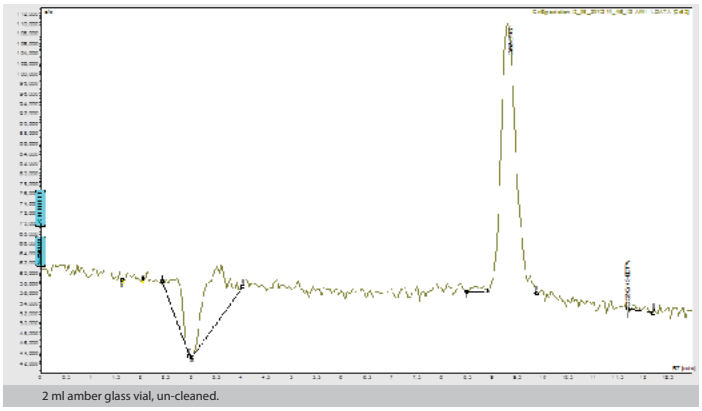
- Hamilton PRP-X100 Anion Exchange Column & Guard Column

## Mobile Phase

- A: 100mM/L Ammonium Nitrate, pH ≥ 9. pH adjust with 16N Nitric Acid
- B: DI Water, pH ≥ 9, pH adjust with Ultra Pure Ammonium Hydroxide

| Time | Flow (mL/min) | %A | %B |
|---|---|---|---|
| Pre-run | 1.0 | 80 | 20 |
| 9.0 | 1.0 | 80 | 20 |



2 ml amber glass vial, un-cleaned.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| standard | ● | PTFE | 0.2µm | 35530 |

# Pesticide Analysis in Ground Water

Olga Almaraz, Blake Gentry, Stephanie Benton, Steven Perez

Adpen Laboratories, Inc., 11757 Central Parkway, Jacksonville, Florida 32224

## Introduction

Groundwater is an important component in many industrial processes as well as irrigating our crops and recharging lakes, rivers and wetlands. Groundwater supplies drinking water for 51% of the total U.S. population and 99% of its rural population. Unfortunately, groundwater is susceptible to pollutants due to the widespread use of pesticides and fertilizers. Traditionally, syringe filtration or centrifugation have been used to remove particulates and reduce possible matrix interference prior to LC/MS analysis. However, these techniques are time consuming, adversely impact reproducibility and quantification. We investigated the potential for streamlining sample preparation method for the analysis of Prosulfuron and its metabolites in ground water using the Thomson Standard|FV compared to syringe filtration and centrifugation.

## Experimental

- Instrument Parameters:
  - HPLC: Agilent 1290 UPLC System
  - Analytical Column: Waters Acquity HSS T3, 2.1 x 150 mm, 1.8 µm
  - Column Temperature: 50 °C
  - Injection Volume: 20 µL
  - Mobile Phase A: 0.1% Formic Acid in HPLC Water
  - Mobile Phase B: 0.1% Formic Acid in CAN
  - Flow Rate: 0.6 mL/min

**Gradient:**

| Time Flow Rate | A(%) | B(%) | mL/min |
|---|---|---|---|
| 0 | 0.6 | 99 | 1 |
| 0.5 | 0.6 | 99 | 1 |
| 2.4 | 0.6 | 40 | 60 |
| 4.25 | 0.6 | 35 | 65 |
| 4.26 | 0.6 | 0 | 100 |
| 5.25 | 0.6 | 0 | 100 |
| 5.26 | 0.6 | 99 | 1 |
| 5.75 | 0.6 | 99 | 1 |

  - Mass Spectrometer: Agilent 6490 Triple Quad
  - Interface: ElectroSpray Ionization (ESI)
  - Gas Flow: 14 L/min
  - Temperature: 200 °C

## Sample Prep Procedure

### Step 1.

For recovery sample: fortify one control ground water sample at 0.1 ppb in a 10 mL volumetric flask.

Water sample with incurred residue follow directly the steps below:

### Step 2.

Prepare experimental samples in triplicate:

#### Exp. 1) Standard|FV, 0.2µm PTFE Filtration Vials:

a. Take 0.45 mL aliquots of the fortified control (step 1) and transfer into separate Thomson Filtration Vials.
b. Filter the samples by depressing the plunger completely.

#### Exp. 2) 0.2 µm PTFE syringe filter attached to a syringe:

a. Take 1.0mL aliquots of the fortified control and field sample (step 1) and transfer into separate syringe filters.
b. Pass samples through the syringe filter and into autosampler vials.

#### Exp. 3) Centrifuging:

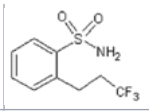
a. Centrifuge remainder of the 2 samples @ 3200 rpm for 5 minutes.
b. Transfer 400µL aliquots into autosampler vials.

### Step 3.

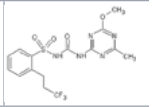
Samples ready for LCMSMS analysis.

## Pesticides Analyzed

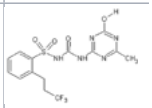
Prosulfuron (CGA152005) - 1-( 4-methoxy-6-methyl-biazin-2-yl)-3-[2-(3,3, 3-trifluoropropyl}-phenylsulfonyl]-urea, CAS #: 94125-34-5



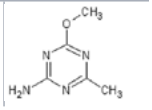
CGA300406 -1-(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl) phenylsulfonyl]-urea



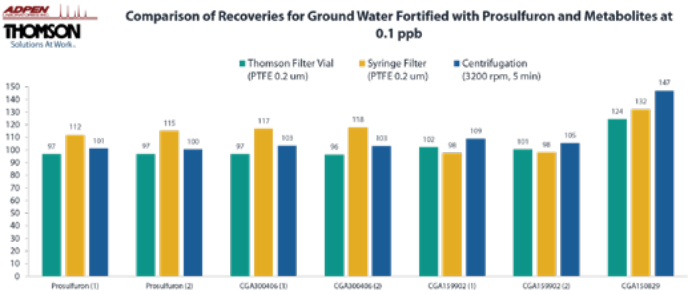
CGA159902 -2-(3,3,3-trifluoropropyl) phenylsulfonylurea, CAS #: 94125-42-5

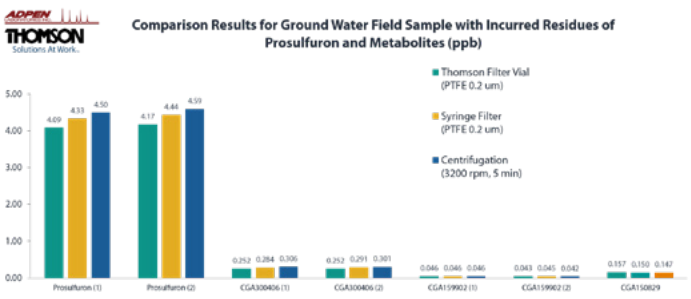


CGA150829 -2-amino-4-hydroxy-6-methyl-1,3,5-triazine, CAS #: 1668-54-8



## Results



| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PTFE | 0.2µm | 85530 |

# EPA Method 539: Determination of Hormones in Drinking Water by SPE and LC-ESI-MS/MS with eXtremeIFV®

## Introduction

Method 539, Determination of Hormones in Drinking Water by Solid Phase Extraction [SPE] and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS/MS) method for the determination of hormones in finished drinking water. Endocrine disrupting hormones in waste, surface and drinking waters have been studied extensively in the last decade. These compounds (and their metabolites) enter the environment through a variety of anthropogenic activities, with typical concentrations found in different water sources in the ng/L range. Within the scope of EPA Method 539 there are seven hormones monitored in finished drinking water. The Thomson Filter Vials were evaluated to replace the filtering step using a syringe and syringe filter.

## Analytes

- 16α-Hydroxyestradiol (Estriol)
- 17ß-Estradiol
- 17α-Ethynylestradiol
- Testosterone
- Estrone
- 4-Androstene-3, 17-dione
- Equilin

## Method

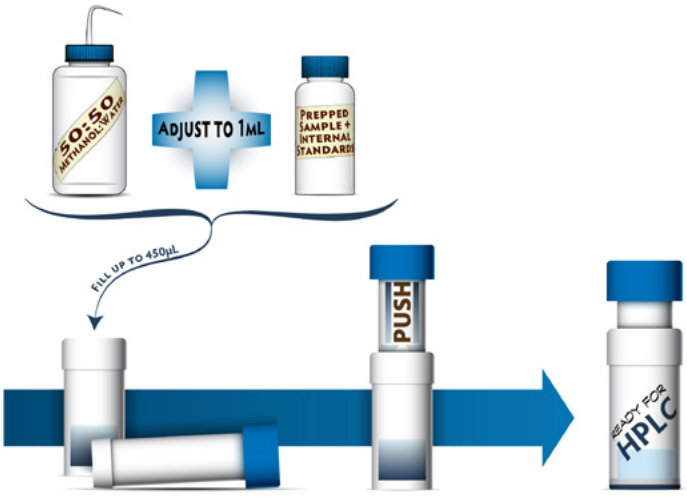
1. Prepare internal standards according to EPA Method 539
2. Prepare sample according to EPA Method 539
3. Add internal standards to sample and adjust final volume to 1 mL with 50:50 methanol:water
4. Transfer an aliquot to a Thomson eXtremeIFV
5. Press plunger
6. Load onto LCMS

## Recommended Vials

eXtremeIFV® – all membrane types, 0.2 µm.

**"I used a reporting-limit level standard and filtered it through each of the four vial types (PES, PVDF, PTFE, and Nylon). I evaluated the results against a 6-point calibration curve, and my recoveries ranged from 90.8% to 107% for all analytes and surrogates and internal standards, and filtrations of blank sample diluent revealed no interferences at my target compound retention times. … 539 requires that a standard that is filtered have no more than a 15% difference from the unfiltered standard, so any of the filter vials will meet criteria."**

Analytical Laboratory




The current EPA method recommends a 0.2 µm GHP® syringe filter. Thomson eXtremeIFV® are an efficient and cost-effective alternative that contain pre filters. Thomson Instrument Company is not affiliated with Pall® or their product the GHP-syringe filter.


htslabs.com info@htslabs.com 800 541.4792 760 757.8080

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PTFE | 0.45µm | 85540 |

# Tea Analysis with eXtremeIFV® by GC-MS

## Introduction

This method investigates whether SPE is required for the analysis of pesticides in green tea leaves using GC-MS. To simplify the comparison, the method utilizes an existing validated ISO method for the analysis of pesticides in food and natural products. The method is comprised of two sections: first, the extraction of the pesticides from the sample; second, the sample clean-up required for GC/MS.

## Experimental

## Sample Preparation for Green Tea Leaves

- Current method uses a salt extraction followed by SPE clean-up.
- Improved method uses a salt extraction followed by Thomson eXtremeIFV® clean-up.
- One large sample is extracted and then split in half. Half the sample goes through SPE and the other half through the eXtremeIFV®.
- 2.0g of commercially available Green Tea is spiked with 0.2mL of 1.0 ppm pesticide standard mix containing 87 pesticides in a 40mL vial for a final concentration of 0.050 ppm.

## SPE Cleanup Prior to Analysis - 6 mL Combo SPE Cartridge

1. Wash one 6 mL Combo SPE Cartridge (packed with 200 mg CarboPrep 200 and 400mg PSA) with acetonitrile.
2. Add the 10mL portion of the re-suspended residue from the flask labeled “for SPE” to the SPE cartridge.
3. Elute the sample from the cartridge with 50mL of acetonitrile.
4. Concentrate the eluted sample to 10mL using a Turbovap II concentrator.
5. Filter sample with a syringe and syringe filter, PTFE 0.45µm and elute into autosampler vial

## Thomson eXtremeIFV® Cleanup Prior to Analysis

1. Add 400µL of the re-suspended residue from the flask labeled “for Thomson eXtremeIFV® to the shell of one Thomson eXtremeIFV® 0.45µm.
2. Insert plunger completely.

## Equipment Conditions

Samples were analyzed utilizing an Agilent Technologies GC/MS, 7000 Triple Quad system equipped with a 7890A GC system and 7693 auto sampler.

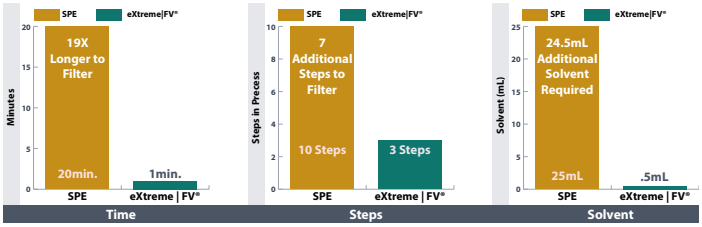
## Results

The results for the green tea can be seen in Table 1, Pesticides in Green Tea Comparison of SPE to eXtremeIFV®s and Fig. 1, Pesticides in Green Tea Comparison of SPE to eXtremeIFV®s, below, shows the recoveries for both clean-up methods: SPE and syringe filter (PTFE 0.45µm) and Thomson eXtreme® Filter Vial. The results show Thomson eXtreme® Filter Vials offer a viable alternative with higher recovery and less preparation time compared to SPE for the sample clean-up of tea leaves and for the clean-up of samples prior to pesticide analysis.

**Table 1.** Pesticides in Green Tea Comparison of SPE to eXtremeIFV®s.

| Compound/Sample Name | SPE Clean-up Average ppm | eXtremeIFV® Clean-up Average ppm |
|---|---|---|
| Azinphos-ethyl | 0.031 | 0.033 |
| BHC-alpha (benzene hexachloride) | 0.037 | 0.037 |
| Chlordane-oxy | 0.037 | 0.039 |
| Cyfluthrin I | 0.033 | 0.082 |
| Dimethoate | 0.032 | 0.032 |
| Endosulfan II (beta isomer) | 0.032 | 0.036 |
| Heptachlor | 0.041 | 0.044 |
| Hexachlorobenzene | 0.038 | 0.039 |
| Methacrifos | 0.034 | 0.036 |
| Pentachloroaniline | 0.041 | 0.048 |
| Pentachloroanisole | 0.039 | 0.042 |
| Permethrin I | 0.066 | 0.069 |
| Permethrin II (trans) | 0.058 | 0.61 |
| Prothiofos | 0.031 | 0.032 |
| Quintozene | 0.031 | 0.032 |
| Tetradifon | 0.037 | 0.039 |

## SPE -vs- eXtremeIFV®




## Conclusion

The results clearly show Thomson eXtremeIFV®s, 0.45µm, PTFE Filter Vials patented (Thomson # 85540-500) offer a viable alternative with equivalent recovery and significantly less preparation time and solvent usage compared to sample clean-up with SPE for the preparation of green tea samples prior to pesticide analysis. Future testing is required to further streamline this method by re-evaluating the extraction procedure, specifically the need for the concentration/re-suspension steps.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# Screening and Quantitation of 200+ Pesticides in Honey by an Integrated On-Line Extraction UHPLC-MS/MS System

Zicheng Yang and Louis Maljers, Bruker Daltonics Inc.

Poster presented as part of NACRW 2015 Conference, St. Petersburg, FL., 19-22 July 2015.

## Introduction

Solid Phase Extraction (SPE) is widely used for sample clean up before LC-MS/MS analysis. It is costly and time consuming. Here we present a simple, cost effective and sensitive procedure for screening and quantitation of pesticides in honey using the Thomson eXtremeIFV® for sample clean-up and the Bruker integrated On-Line Extraction (OLE)-UHPLC-MS/MS system for analysis of pesticides in honey.

A study using the EVOQ analyzed 200+ pesticides in honey using only one method with positive negative switching for 430 MRM transitions. The measurements were conducted by dilute-and-shoot without sample enrichment using the Thomson eXtremeIFV. The honey was diluted 10-fold and filtered with 0.2um PVDF eXtremeIFV prior to injection. An YMC-Pack ODS-AQ, 10 µm, 10 mm x 2 mm (I.D.) column was used as trap column. An aqueous mobile phase was used to retain the pesticides on the trap column and to elute the monosaccharides in the honey out to the waste followed by a valve switch to couple the trap column with analytical column for separation and detection. The linear range was approximately 1ng/g to 1000ng/g and the linear regression co-efficiency $R^2$ was >0.99.

## Equipment

- UHPLC Conditions
  - Trap Column: YMC-Pack ODS-AQ, 10 µm, 10 mm x 3.0 mm I.D.
    - Mobile Phase C: 5mM Ammonium Fluoride (AF) in water
  - Equilibration flow: 1000µL (3.0 min)
  - Loading Flow: 600 µL
  - Analytical Column: YMC-Pack ODS AQ, 3 µm, 150 mm × 3.0 mm (I.D.)
  - Column Temperature: 40˚C
  - Injection Volume: 50µL
  - Mobile Phase A: 5mM Ammonium Fluoride in Water
  - Mobile Phase B: 100% MeOH
- OLE Valves Configuration
- EVOQ Conditions

## Sample Preparation

- Weigh about 50mg of honey in the Thomson eXtremeIFV (p/n 85531).
- Add MeOH/Water, 50/50, v/v make 100 mg/mL solution.
- Mix by pipet and depress the filter vial plunger, 0.2 µm PVDF completely to filter.
- Solution is ready for injection.

## Results

Store bought honey samples analyzed by UHPLC-MS/MS in a 200+ pesticide panel utilizing nine point calibration curves for the individual pesticides, see Fig. 1. Simple sample prep was achieved using the Thomson eXtreme Filter Vial, 0.2um PVDF using a simple dilute – filter – shoot. High concentration of sugars were removed utilizing the trap column without getting into MS system. Excellent linearity was achieved from 0.01ng/mL to 100ng/mL. The LOQ was determined to be 0.1ng/ml for 158 pesticides and <0.01ng/mL for 57 pesticides. The LOD was determined to be <0.1ppb. Good retention time distribution and auto-calculating scan times for each individual pesticide was used for single run for both +/- pesticides with hundreds of MRM transitions. No peak shape change by injecting 50 µL solution containing 50% MeOH. High organic in sample solution helps to reduce pesticides binding to the eXtremeIFV. Fifteen pesticides were detected in store bought honey from different countries, see Table 2. High levels for Fenpyroximate was detected in US sourced honey.

**Table 2.** Store bought honey from the US (3 different brands), Canada, China and India were analyzed for Pesticides analyzed in this method. Test result (ND= not detected or <0.1ppb).

| Honey Source | India | Canada | China | US-1 | US-2 | US-3 |
|---|---|---|---|---|---|---|
| **Pesticides** | **Concentration in ng/g** | | | | | |
| Acetamiprid | ND | ND | 0.64 | ND | ND | ND |
| Boscalid | ND | 17.5 | ND | ND | 0.15 | 3.38 |
| Carbaryl | ND | 0.71 | ND | ND | ND | ND |
| Dioxacarb | ND | ND | ND | ND | 1.35 | 2 |
| Fenpyroximate | ND | ND | ND | ND | 0.26 | 55 |
| Fludioxinil | ND | 1.49 | ND | ND | ND | ND |
| Fluometuron | ND | ND | ND | ND | ND | 2.8 |
| Hexaythiazox | ND | ND | 0.16 | ND | ND | ND |
| MCPA | ND | 0.68 | ND | ND | ND | ND |
| Metalaxyl | ND | 0.1 | ND | ND | ND | ND |
| Methoxyfenozide | ND | ND | ND | ND | ND | 0.94 |
| Picoxystrobin | ND | 4.23 | ND | ND | ND | ND |
| Piperonyl butoxide | ND | 0.26 | ND | 0.57 | 0.76 | 0.21 |
| Propargite | ND | 0.32 | ND | 0.1 | ND | ND |
| Thiamethoxam | ND | 4.88 | ND | ND | ND | ND |

## Conclusion

- Bruker UHPLC combined with the EVOQ Elite Triple Quadrupole MS was used for identification and quantification of 200+ pesticides in store-bought honey sourced from different countries utilizing the Thomson eXtremeIFV, 0.2um PVDF.
- Method is simple, sensitive, and ease of use and single run for positive and negative pesticides.
- Simple sample prep consisting of diluting the sample, filtering and injecting onto the UHPLC-MS/MS achieved LOQ of < 0.01ng/mL for 158 pesticides and 0.1ng/mL for 57 pesticides.
- Bruker Advance UHPLC with OLE coupled to EVOQ LC-QQQ provides a more convenient and simpler approach than SPE to analyze pesticides in honey.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# Analysis of Antibiotics in Honey by an Integrated On-Line Extraction UHPLC-MS/MS System

Zicheng Yang and Louis Maljers, Bruker Daltonics Inc.

Poster presented as part of NACRW 2015 Conference, St. Petersburg, FL., 19-22 July 2015.

## Introduction

The most critical aspects of reliable food contamination analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, SPE, SLE, Liquid-Liquid, syringe filtration, and centrifugation have been used to reduce matrix interference prior to LC/MS analysis. However, these techniques are time consuming, adversely impact recovery, require expensive consumables, and use large amounts of solvent. Improved sample prep methods were developed using eXtremeIFV for contaminant analysis of antibiotics honey.

## Equipment

- UHLC Conditions
  - Trap Column: YMC-Pack ODS-AQ, 10 µm, 10 mm x 3.0 mm I.D.
  - Mobile Phase C: 0.1% Formic Acid in water
  - Equilibration flow: 1000 µL (4.0 min)
  - Loading Flow: 500 µL
  - Analytical Column: YMC- UltraHT Pto C18 , 2 µm, 100 mm × 2.0 mm I.D.
  - Column Temperature: 40 ˚C
  - Injection Volume: 10 µL (100 µL Loop)
  - Gradient
    - Mobile Phase A: 0.1% FA in water
    - Mobile Phase B: 100% MeOH
- EVOQ Conditions

## Sample Preparation

- Weigh about 50mg of honey in the Thomson eXtremeIFV (p/n 85531).
- Add MeOH/Water, 50/50, v/v make 100 mg/mL solution.
- Mix by pipet and depress the filter vial plunger, 0.2 µm PVDF completely to filter.
- Solution is ready for injection.

## Results

Store bought honey samples analyzed by UHPLC-MS/MS ciprofloxacin, tetracycline, enrofloxacin and erythromycin were analyzed utilizing nine point calibration curves for the individual antibiotics, see Table 3. Simple sample prep was achieved using the Thomson eXtreme Filter Vial, 0.2um PVDF. Excellent linearity was achieved from 0.05ng/mL to 20ng/mL. The LOQ was determined to be < 0.5ng/g. Chromatograms at 0.05ng/mL of spiked honey show over lapping peaks that are resolved by mass for the ciprofloxacin, tetracycline, and enrofloxacin. While the erythromycin is nicely resolved by both LC and MS. In the chromatogram s in fig.? 0.5ng antibiotics were spiked into 1.0g honey to yield a concentration of 0.05ng/mL.

Store bought honey from the US (3 different brands), Canada, China and India were analyzed for ciprofloxacin, tetracycline, enrofloxacin and erythromycin. Calculation is base on matrix calibration curve (=100/(detected amount/spiked amount).The recovery for iprofloxacin and erythromycin looks consistent across all levels. The enrofloxacin signal enhanced in matrix and tetracycline signal enhanced at low concentration. Results with an ND are < 0.05ng/mL.

**Table 1.** Calibration curve levels for antibiotics in honey.

| Calibration Level | ng/g | ng/mL |
|---|---|---|
| 1 | 0.5 | 0.05 |
| 2 | 1 | 0.1 |
| 3 | 2 | 0.2 |
| 4 | 5 | 0.5 |
| 5 | 10 | 1 |
| 6 | 20 | 2 |
| 7 | 50 | 5 |
| 8 | 100 | 10 |
| 9 | 200 | 20 |

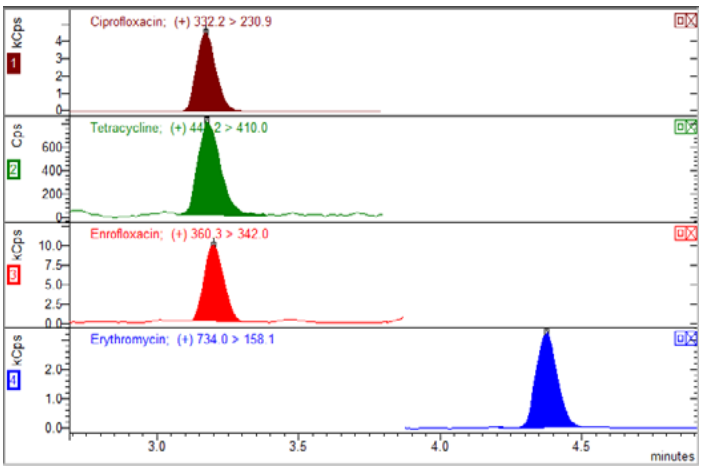



**Fig 1.** Chromatograms of antibiotics spiked into honey, USA-1.

## Conclusion

Bruker UHPLC combined with the EVOQ Elite Triple Quadrupole MS was used for identification and quantification of ciprofloxacin, tetracycline, enrofloxacin and erythromycin in store-bought honey utilizing the Thomson eXtremeIFV, 0.2um PVDF. Simple sample prep consisting of diluting the sample, filtering and injecting onto the UHPLC-MS/MS achieved LOQ of < 0.05ng/mL and LOD of 0.02ng/mL.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# High Throughput Screening and confirmation of 41 Pain Panel Drugs in Oral Fluid by an Integrated On-Line Extraction UHPLC-MS/MS System

Louis Maljers, Zicheng Yang
Bruker Daltonics Inc., 3500 West Warren Ave, Fremont, CA 94538
Presented at MSACL 2015

## Introduction

Saliva test is one of the easiest, cost-effective and most accurate ways to measure the presence of drugs in the body. Collecting saliva sample is relatively non-invasive, easier to procure and reduced risk of sample adulteration. However, saliva matrix display much lower levels of drug compounds compared to urine samples, making the need to test at lower cut-off levels more important. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a technique of choice for both screening and confirmation lower levels because it is sensitive, specific, and accurate.

Solid Phase Extraction (SPE) is widely used for sample clean up before LC-MS/MS analysis. It is costly and time consuming. Here we present a high throughput, cost effective and sensitive procedure for screening and confirmation of Pain Panel Drugs (PPDs) in Synthetic Saliva using Thomson filter vial for sample preparation and using an integrated On-Line Extraction (OLE)-UHPLC-MS/MS System for sample analysis. The lower limit of quantitation (LLOQ) was 0.01-0.2 ng/mL and upper limit of quantitation (ULOQ) was 100 ng/mL. The linearity regression coefficient R2 was >0.99. The blanks show no interference of the analysis at the LLOQ level. The sub ng/mL level PPDs detection with about three orders of dynamic detection range will cover the clinical research needs.

## Sample Preparation

- Transfer 200 µL of 60% Methanol/water containing 5 ppb internal standard into Thomson vial.
- Add 200 µL of drug standard in synthetic saliva (Immunalysis Corp., p/n NOFC-0500) to the vial and mix.
- Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials-eXtreme/FV 0.2 um PVDF, w/Pre-Slit Red Cap (p/n #85531)
- Press filter plunger down approximately ¼ of the way into each of the Thomson Vial outer shells.
- Vortex for 10 sec
- Press Filter plunger the rest of the way down using Thomson Vial Filter Press.

## Methods

### Instruments:

EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker Integrated On-Line Extraction-UHPLC and CTC Autosampler

### LC Parameters:

- Trap Column: YMC-Pack Pro ODS-AQ, 3 µm, 10 mm x 3.0 mm I.D.
- Mobile Phase C: 0.1%formic acid (FA), 0.05% TFA in water
- Equilibration flow: 600µL (3.0 min)
- Loading Flow:600 µL
- Analytical Column: YMC-Triart pfp, 1.9 µm, 50mm ×2.0 mm (I.D.)
- Column Temperature: 40 ˚C
- Injection Volume: 30 µL
- Mobile Phase A: 0.1% FA in water
- Mobile Phase B: 2 mM Ammonium formate and 0.1% FA in MeOH/ Acetonitrile=50/50

### Gradient:

| Time | %A | %B | Flow (µL/min) |
|---|---|---|---|
| 0.0 | 80 | 20 | 350 |
| 0.2 | 80 | 20 | 350 |
| 3.5 | 5 | 95 | 350 |
| 3.9 | 5 | 95 | 350 |
| 4.0 | 80 | 20 | 350 |
| 6.0 | 80 | 20 | 350 |

### MS Parameters:

- Spray Voltage(ESI positive): 4000 v
- Cone Gas Flow: 30 units
- Cone Temperature: 350 ˚C
- Heated Probe Gas Flow: 40 units
- Heated Probe Temperature: 400 ˚C
- Nebulizer Gas Flow: 65 units
- Exhaust Gas: on
- q2 pressure: 2.0 mTorr (Argon)

**Table 1.** 6MAM-$d_6$, Alprazolam-$d_5$, Buprenorphine-$d_4$, Clonazepam-$D_4$, Codeine-$d_6$, Fentanyl-$d_5$, Meperidine-$d_4$, Methadone-$d_3$, Morphine-$d_6$, Norbuprenorphine-$d_3$, Norfentanyl-$d_5$, Oxymorphone-$d_3$, Tramadol $^{13}$C-d were used as internal standard for above data.

| Name | Linear Range (ng/mL) | $R^2$ | Response Factor % RSD |
|---|---|---|---|
| 6-MAM | 0.02-100 | 0.999 | 13.3 |
| Meprobamate | 0.05-100 | 0.998 | 9.1 |
| Alprazolam | 0.01-100 | 1.000 | 3.5 |
| Methadone | 0.01-100 | 1.000 | 4.7 |
| Amphetamine | 0.02-100 | 0.999 | 7.2 |
| Methamphetamine | 0.10-100 | 1.000 | 8.0 |
| Benzoylecgonine | 0.02-100 | 1.000 | 10.3 |
| Midazolam | 0.01-100 | 0.999 | 10.0 |
| Buprenorphine | 0.02-100 | 0.999 | 8.0 |
| Morphine | 0.02-100 | 1.000 | 5.0 |
| Carisoprodol | 0.05-100 | 0.999 | 9.0 |
| Naloxone | 0.02-100 | 0.999 | 11.2 |
| Clonazepam | 0.05-100 | 1.000 | 5.7 |
| Naltrexone | 0.02-100 | 1.000 | 11.0 |
| Codeine | 0.02-100 | 1.000 | 6.6 |
| Norbuprenorphine | 0.20-100 | 1.000 | 3.6 |
| Diazepam | 0.02-100 | 0.998 | 8.1 |
| Nordiazepam | 0.02-100 | 1.000 | 9.1 |
| EDDP | 0.01-100 | 0.997 | 6.5 |
| Norfentanyl | 0.01-100 | 1.000 | 6.1 |

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PVDF | 0.2µm | 85531 |

# Screening and Quantitation of 250 Pesticides in Apple, Cranberry, Orange, Vegetable and White Grape Juices using the eXtremeIFV® by LC/MS/MS

Z.Yang, L. Maljers, Bruker, Chemical & Applied Markets (CAM) Division. "Screening and Quantitation of 250 Pesticides in Fruit Juices with Positive/Negative Switching LC/MS/MS." Poster presented as part of NACRW-FPRW Conference, St. Petersburg, FL., 20-23 July 2014.

## Abstract

A study was conducted using the Bruker EVOQ for the analysis of 250 pesticides in store-bought juice using one method and simple sample preparation using the Thomson eXtremeIFV®s in a dilute-and-shoot approach without sample enrichment. LC-MS/MS operated in Multiple Reaction Monitoring (MRM) mode with dual scan Electrospray Ionization (ESI) is widely used for polar, semi-volatile, and thermally labile pesticides in food testing. The Bruker EVOQ Elite LC-Triple Quadrupole System provides fast positive/negative switching, allowing for simultaneous determination of positive and negative co-eluting compounds numbering in the hundreds. Simple sample preparation is explored using Thomson eXtremeIFV®s for sample clean-up instead of lengthy alternatives like SPE or centrifugation followed by liquid-liquid extraction.

## Equipment

- EVOQ Elite Triple Quadrupole Mass Spectrometer
- Bruker UHPLC
- CTC Autosampler
- Source: HESI
- Spray Voltage Positive: 4000V
- Spray Voltage Negative: 4000V
- Column: YMC-Pack ODS-AQ 3µm
- Column Temperature: 40°C
- Injection Volume: 30µL
- Mobile Phase:
  - Mobile Phase A: 5mM Ammonium Fluoride in Water
  - Mobile Phase B: Methanol
    - Gradient:

## Sample Preparation

1. Pipette 50µL of store-bought apple juice and 450µL of solvent (10% Methanol/ 90% Water) directly into the outer shell of Thomson eXtremeIFV®, 0.2µm PVDF.
2. Partially depress the eXtremeIFV® plunger and vortex.
3. Depress the completely and load onto the autosampler.

## Results

**Table 1.** Store bought fruit juice test results.

| Fuit Juice | Apple Juice | Orange Juice | Cranberry Juice | White Grape Juice | Vegetable Juice |
|---|---|---|---|---|---|
| Pesticide | µg/L (ppb) | | | | |
| Azoxystrobin | ND | ND | 0.32 | ND | 0.48 |
| Boscalid | ND | ND | 0.16 | ND | ND |
| Carbaryl | ND | 0.39 | 1.47 | ND | ND |
| Carbofuran | ND | 0.14 | ND | NDd | ND |
| Dimethoate | ND | 0.30 | ND | ND | ND |
| Imidacloprid | ND | ND | 0.60 | ND | 0.20 |
| Mandipropanid | ND | ND | 0.59 | ND | ND |
| Metalaxyl | ND | ND | 0.21 | ND | ND |
| Methoxyfenozide | ND | ND | ND | ND | 0.84 |
| Tebuconazole | ND | ND | 0.32 | ND | ND |
| Thiabenazole | 1.8 | ND | ND | ND | ND |

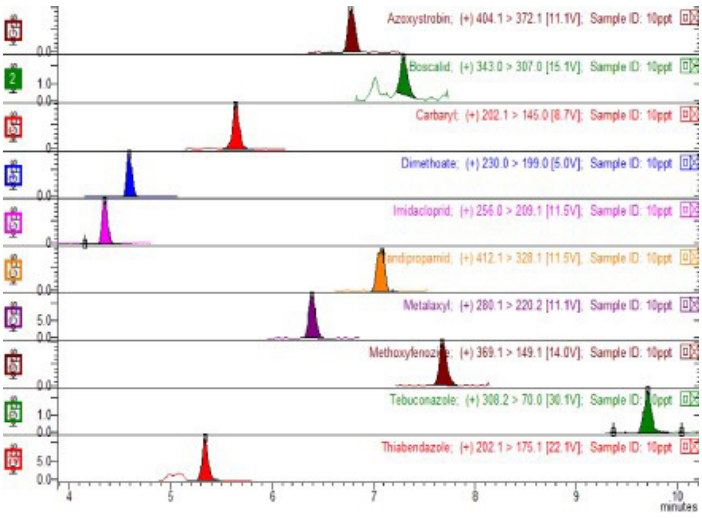



**Fig 1.** Chromatogram of a 0.01ppb standard solution containing the compounds listed in Table 1 This is equivalent to 0.1ppb in juice.

## Conclusion

The calibration on triplicate injections showed excellent linearity and response factor RSD over 3 orders, range using the Thomson eXtremeIFV® for sample preparation. Pesticides were detected in store-bought apple, orange, cranberry and vegetable juices.

- Good linearity
- sensitivity and response factor
- RSD for positive and negative co-eluting pesticides

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PTFE | 0.45µm | 85540 |

# eXtremeIFV® vs SPE for the Analysis of Pesticides in Orange Juice by GC/MS

Authors: Uday Sathe1, Karine Aylozyan1, Lisa Wanders2, Joe Machamer2, & Sam Ellis2
Micro Quality Labs1 | Thomson Instrument Company2

## Abstract

Pesticides act as toxins when found in sufficient quantities as residues in food. This is of particular importance for orange juice because it is consumed in high quantities by children. Sensitive, rapid, and cost effective analytical methods are required in order to reduce the risk to consumers.

Solid Phase Extraction (SPE) is a common sample preparation technique used prior to GC or LC analysis of pesticides in food. Typically, SPE is used to concentrate analytes, reduce interference from co-eluting molecules or to clean up/"filter" sample particulates. Drawbacks to the use of SPE include cost, sample preparation time, large sample volumes, use and disposal of organic solvents, and potentially poor recoveries. The continuing development of higher sensitivity instrumentation and improved filtration devices has led many labs to investigate whether methods can be adapted to eliminate the SPE step.

Thomson eXtreme® Filter Vials offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates. Filtration time from unfiltered sample transfer to filtered sample in an autosampler ready vial is only 15 seconds. The filter vial consists of two parts: a filter vial shell and a plunger which includes the multi-layer filter on one end and a vial cap on the other end. Samples are filtered by pipetting the sample into the filter vial shell, inserting the plunger into the shell, and then pushing the plunger into the shell.

Prior to the introduction of the eXtremeIFV®s, many samples containing high levels of particulates were only "filtered" by using an SPE step in the method. These methods are readily amendable to the replacement of the SPE step with a much faster and lower cost eXtremeIFV® step.

## Experiment

Samples were prepared and analyzed at Micro Quality Labs, Burbank, CA.

## Sample Preparation

1. Spike 10mL of commercially available High Pulp Orange Juice with 1mL of 1 ppm pesticide standard mix in a 40mL vial.
2. Add one pack (approximately 6g) of Restek Extraction Salts (Restek catalog #26236) to the spiked orange juice.
3. Extract the spiked orange juice with 4 x 25mL portions of methylene chloride.
4. Concentrate to dryness using a Turbovap II concentrator.
5. Dissolve the residue in approximately 10mL of acetonitrile.
6. Vortex and sonicate the re-suspended residue with frequent swirling.
7. Split the re-suspended residue into two 5mL portions.
8. Dilute each 5mL portion with acetonitrile to 10mL using a volumetric flask.
9. Label one flask "for SPE" and the other "for Thomson eXtremeIFV®".

## eXtremeIFV® Cleanup Prior to Analysis

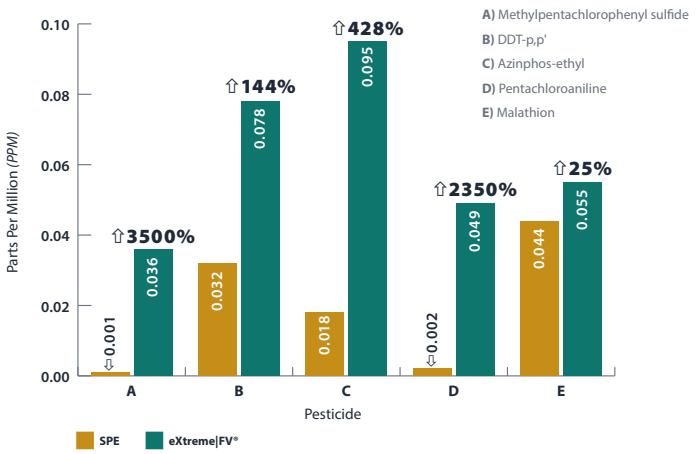
1. Add 400µL of the re-suspended residue from the flask labeled "for Thomson eXtremeIFV®" to the shell of one Thomson eXtremeIFV® 0.45µm, PTFE (Thomson Part Number 85540-500).
2. Insert plunger completely.

## Analysis

Samples were analyzed utilizing an Agilent Technologies® GC/MS, 7000 Triple Quad system equipped with a 7890A GC system and 7693 auto sampler.

## SPE -vs- eXtremeIFV®

### Comparison of spiked pesticide recoveries




## Conclusions

The Thomson eXtreme 0.45µm, PTFE Filter Vials patented (Part#: 85540-500) yielded 26% higher recoveries on average when tested with 87 common pesticides. In the cases highlighted in the results table, greater than 428% recovery increases were seen. In the case of Hexachlorobenzene, no pesticide was detected in the sample prepared by SPE and 0.019 ppm was detected in the sample prepared with the eXtremeIFV®. The use of Thomson eXtreme 0.45µm, PTFE Filter Vials as a substitute for SPE conforms to USP Method 561.

The results show Thomson eXtremeIFV®s offer a viable alternative with higher recovery and less preparation time compared to SPE for the preparation of juices prior to pesticide analysis.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PTFE | 0.2µm | 85530 |

# Time and Cost Effective Methods for Reducing Background Noise and Signal Suppression in Problem Matrices for Residue Analysis by LC-MS/MS

Presented at NACRW 2016

Joseph Kolb[1], Ariana Ramdin[1], Ryan Undeen[1]

[1]Merieux NutriSciences Corporation, Gainsville, FL 32607

## Introduction

Several clean-up methods are compared for background reduction, analyte recovery, and cost effectiveness in order to successfully analyze a wide variety of multiclass multiresidues in difficult matrices including Chili Powder and Tobacco. The most critical aspects of reliable multiresidue analysis are the reduction of interferences from the sample matrix and analyte recovery. eXtremeIFV®, were compared to an existing ISO accredited QuEChERS method, as well as a dilute and shoot approach are analyzed in conjunction with different filtration techniques for residue analysis by LC-MS/MS for minimal number of steps, speed, reduced reagent use and reduced cost.

## Experimental

In order to successfully analyze multi-residue methods on difficult matrices such as habanero flakes and tobacco, several different clean-up procedures may need to be employed. This method investigates the use of different clean-up procedures and a dilute and filter approach to successfully analyze 20 pesticide compounds facing problems from matrix effects. The cost-effectiveness of different filtering techniques was also considered.

### The following difficult to analyze compounds were tested:

| | | | |
|---|---|---|---|
| 5-OH Thiabendazole | Clofentezine | Coumaphos | Etoxazole |
| Metolachlor | Phosalone | Pirimiphos-methyl | Prallethrin |
| Prochloraz | Pymetrozine | Pyraclostrobin | Quinoxyfen |
| Simazine | Spinetoram-major | Spinetoram-minor | Thiobencarb |
| Thiophanate-methyl | Tolyfluanid | Triazophos | Trifloxystrobin |

## Equipment

- Sciex API 4000 Qtrap Mass Spectrometer
- Shimadzu LC-20AD Pumps
- Flow Rate: 0.25 mL/min
- Run Time: 20 minutes
- Injection Volume: 15µL
- Mobile Phases:
  - A: 0.1% Formic Acid and 10mM Ammonium Acetate in HPLC Water
  - B: 0.5% Formic Acid in Methanol
    - Gradient:

| Time (min.) | %A | %B |
|---|---|---|
| | 90 | 10 |
| 0.5 | 90 | 10 |
| 15 | 2 | 98 |
| 19 | 2 | 98 |
| 20 | 90 | 10 |

- Column Temperature: 40˚C
- Column: Waters Zorbax C18 3.5µm 3mm x 150mm
- Centrifuge
- Thomson eXtremeIFV® 0.2µm PTFE (p/n 85530)*
- Thomson 48 position Vial Filter Press (p/n 35015-476)

*Special Note: For some autosamplers it is important to adjust the needle depth of your autosampler when using Thomson filter vials to improve the reproducibility of injections

## Method

28 QuEChERS extracts were prepared and the filtration step was performed using two different approaches. Samples were evaluated for % recovery and timed. In both cases the samples need to be diluted with mobile phase prior to filtration in order to filter out precipates that are formed with the addition of aqueous solvent.

## Sample Preparation

### eXtremeIFV:

1. Weigh 1g sample and add internal standards and standards as appropriate.
2. Dispense 10mL water and then 15mL ACN.
3. Cap and shake for 30 seconds.
4. Centrifuge for 10 minutes at 3600 rpm.
5. Transfer 400uL and filter using Thomson eXtreme 0.2 µm PTFE Filter Vial.

### Traditional Method:

1. Weigh 5g sample and add internal standards and standards as appropriate
2. Dispense 10mL water and then 15mL 1% Acetic Acid in ACN.
3. Cap and shake
4. Add Magnesium Sulfate and Sodium Acetate QuEChERS salts to tube, vortex and then shake on Genogrinder for 1 minute.
5. Centrifuge for 10 minutes at 3600 rpm
6. Decant top layer into dispersive clean-up tubes, shake and vortex for 1 min (EMR salt clean-up requires a second dispersive SPE step)
7. Centrifuge for 5 minutes at 3600 rpm
8. Dilute 1:1 with Aqueous Mobile Phase and Filter

## Results

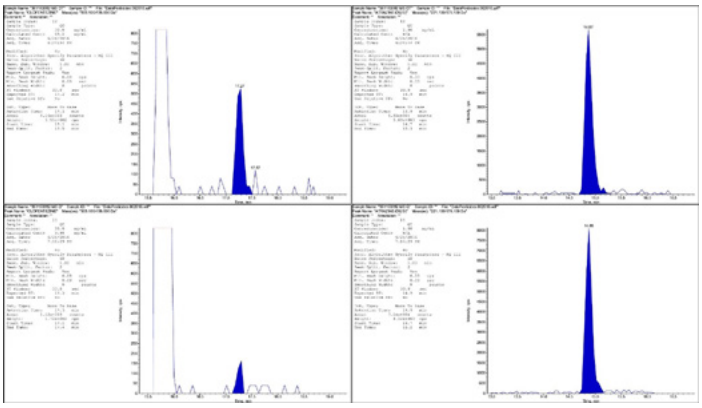
*Note: Several high recoveries (>200%) caused by matrix suppression of internal standard or matrix enhancement of analyte.

Data Comparison Table of 20 Analyte Recoveries from different extracts/matrices spiked at 30ppb. Habanero Flakes and Tobacco showed less matrix effects and increased reproducibility using the dilute and filter method and compared to the QuEChers and filter method.

| Analyte | Habanero Flakes QuEChERS +PSA % Recovery | Habanero Flakes QuEChERS + EMR % Recovery | Habanero Flakes Dilute and Filter % Recovery | Tobacco QuEChERS +PSA % Recovery | Tobacco Dilute and Filter % Recovery |
|---|---|---|---|---|---|
| 5-OH Thiabendazole | 30.9 | 41.8 | 75.6 | 35.5 | 59.3 |
| Clofentezine | 11.9 | 206 | 151 | 232 | 82.2 |
| Coumaphos | 15.3 | 107 | 87.9 | 129 | 135 |
| Etoxazole | 65 | 80.8 | 92.4 | 447 | 189 |
| Metolachlor | 32.8 | 110 | 150 | 117 | 174 |
| Phosalone | 54.7 | 121 | 86.3 | 135 | 111 |
| Pirimiphos-methyl | 192 | 409 | 262 | 267 | 264 |
| Prallethrin | 128 | 351 | 321 | 232 | 28.0 |
| Prochloraz | 75.8 | 186 | 130 | 146 | 140 |
| Pymetrozine | 136 | 129 | 328 | 449 | 319 |
| Pyraclostrobin | 28.1 | 35.6 | 77.6 | 98.7 | 103 |
| Quinoxyfen | 51.6 | 132 | 83.1 | 39.1 | 91.0 |
| Simazine | 73.6 | 117 | 186 | 112 | 97.9 |
| Spinetoram-major | 49.6 | 160 | 104 | 120 | 124 |
| Spinetoram-minor | 46.9 | 114 | 92.7 | 119 | 146 |
| Thiobencarb | 28.5 | 69.6 | 78.1 | 71.5 | 83.5 |
| Thiophanate-methyl | 18.5 | 105 | 94.7 | 314 | 128 |
| Tolyfluanid | 14.4 | 71.3 | 54.9 | 101 | 115 |
| Triazophos | 15.3 | 8.94 | 34.8 | 27.4 | 29.4 |
| Trifloxystrobin | 40.8 | 137 | 108 | 75.7 | 106 |

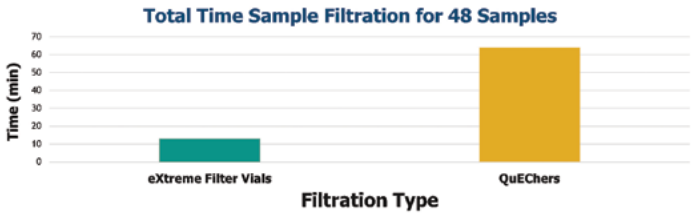
## Data

For the pesticides we compared the traditional QuEChERS method and cleaned up with PSA and syringe & filter to simply dilute and shoot with the eXtreme PTFE Thomson Vial for Chili Powder and Tobacco. Diluting the samples gives better or comparable sensitivity with several difficult analytes in which we have been experiencing matrix suppression. Here are some of the analytes where the dilute and shoot method counteracted matrix suppression: 5-Hydroxythiabendazole, Clofentezine, Coumaphos, Etoxazole, Metolachlor, Phosalone, Pirimiphos-methyl, Prallethrin, Prochloraz, Pymetrozine, Pyraclostrobin, Quinoxyfen, Simazine, Spinetoram, Thiobencarb, Thiophanate-methyl, Tolyfluanid, Triazophos, and Trifloxystrobin. The dilution extraction helped us to include these analytes in our screen despite the heavy matrix effect we saw in QuEChers extraction.



## Conclusion

The first approach was a traditional QuEChers method including filtration using a syringe, 0.2µm PTFE filter, and needle. The time taken to assemble the syringes and filter, as well as the time to mix the extract and mobile phase prior to placing in the syringe was included in the timing. The entire process took 64 minutes and 52 seconds.




With the second approach, the extract and mobile phase were placed into the bottom of a Thomson eXtreme Filter Vial together, the 0.2µm PTFE filter and cap was placed on top of the vials, and all the samples were pressed simultaneously using the Thomson Multi-Use Press. The entire process took 12 minutes and 51 seconds. Giving a time savings of 52 minutes!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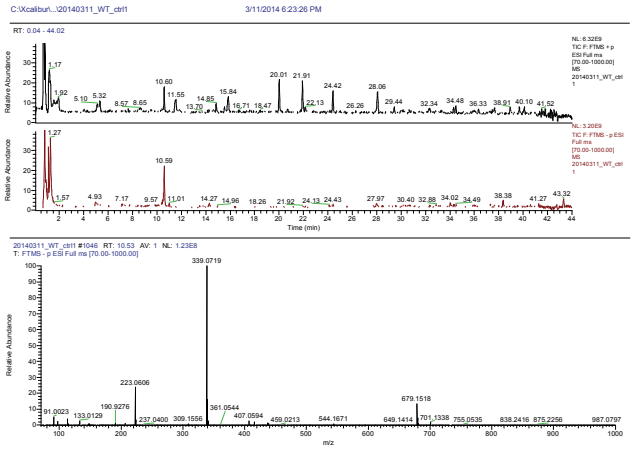
| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| nanoIFilter Vial™ | | PTFE | 0.45µm | 15540 |

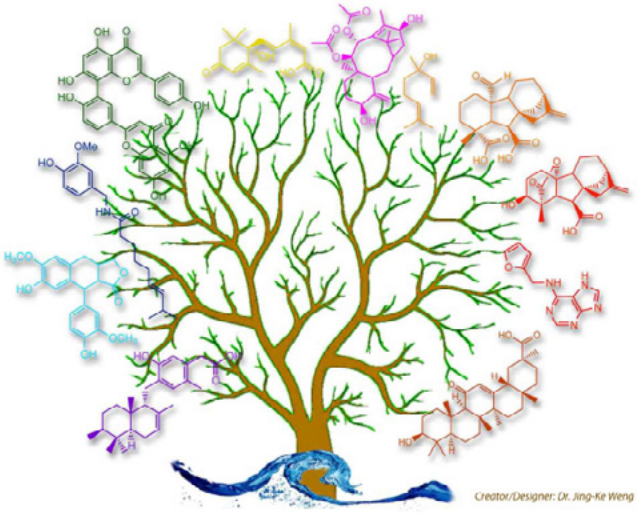
# Analysis of Sinapoylmalate in the Arabidopsis thaliana Leaf by Using the nanoIFilter Vial®: Sinapoyl Malate is a major UV protectant in *Arabidopsis thaliana*

Data provided by Jing-Ke Weng, Ph.D.
Member, Whitehead Institute for Biomedical Research
Assistant Professor of Biology, Massachusetts Institute of Technology



## Introduction

Plants contain rapidly evolving specialized metabolic system, and presumably encounter destabilized evolutionary intermediates along their mutational trajectories. Have plants evolved unique molecular mechanisms that assist folding of those destabilized proteins and/or mitigate proteotoxicity arising from protein misfolding? We use the model plant *Arabidopsis thaliana* to examine the in vivo function and behavior of mutant enzymes that exhibit broadened product promiscuity and/or decreased folding stability in vitro. We attempt to identify genetic components involved in cellular mechanisms that assist folding or alter product profile of these mutant enzymes. We designed a simple sample preparation method for the analysis of Sinapoyl Malate by LC-MS to study its mechanism in UV protection of the *Arabidopsis thaliana*.




## Method

- Grind Leaf tissue under liquid nitrogen.
- Extract with 80% MeOH (1mL MeOH to 200mg fresh weight leaves).
- Centrifuge.
- Filter with 0.2um PTFE nanoIFilter Vial™.
- Analyze by UHPLC - Orbitrap Mass Spec.

## Equipment

HPLC Column: Phenomenex Kinetex 2.6µm C18 150x3.0

## Mobile Phase

Solvent A: H2O + 0.1% Formic Acid

Solvent B: Acetonitrile + 0.1% Formic Acid

## Gradient

| time | %A | %B |
|---|---|---|
| 2min | 95 | 5% |
| 40min | 20 | 80% |
| 40.1min | 5 | 95% |
| 44min | 5 | 95% |
| 44.1min | 95 | 5% |
| 48min | 95 | 5% |

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PTFE | 0.45µm | 85540 |

# Dietary Supplement Analysis by HPLC of Vitamin $D_3$ Gel-Caps

## Introduction

Vitamin $D_3$, as Cholecalciferol, gel-caps requires a two-step process prior to HPLC. Easily dissolve the water soluble gelatin shell and then solubilize the very non polar filling Cholecalciferol, Vitamin $D_3$, has a log D (octanol/water) of ~7.

1. The gel-cap and its contents are soaked in water until the shell dissolves
2. THF is added until the oil layer mixes into solution
3. Non soluble particles (gel cap remains) are filtered out using a Thomson 0.45µm PTFE filter vial

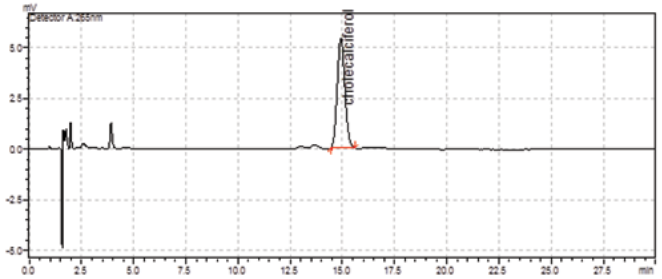
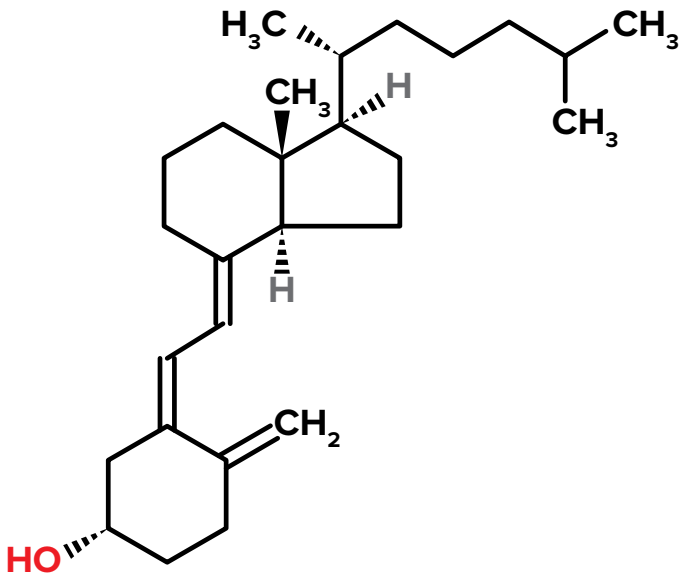



**Fig 1.** Cholecalciferol (D3) gel-capsule example.





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| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# Expedited Vitamin C Sample Preparation Through the Use of eXtremeIFV® Technology

Heidi Evenocheck, John Habel, Xun Yan

Analytical Sciences, Amway, 7575 Street E, Ada, MI 49355. Expedited Vitamin C sample preparation through the use of vial filtration technology. Poster presented as part of 128th Annual AOAC Meeting and Expo, Boca Rotan, FL, 7-10 September 2014.

## Abstract

At Amway, Vitamin C Analysis is routinely performed for large numbers of samples. With large batches of samples for preparation and processing, each step in sample prep becomes very costly in terms of analyst time. Thomson eXtremeIFV®s reduce a multi-step filtration and vial transfer process to a single step. We compared results from traditional sample preparation employing syringes, syringe filters, and HPLC autosampler vials with the results using only the Thomson Filter Vial product.

The final steps of sample prep require the analyst to filter the sample into an HPLC autosampler vial employing a syringe and syringe filter. This is a costly step in terms of time and materials that adds little value to the final result. Any gains made at this step of the procedure can reduce the time and cost associated with Vitamin C analysis. Autosampler vials with an integrated PVDF filter are now available. The use of these vials in place of the current procedure allows several samples to be filtered at one time, reducing the time required to complete this step. The vials are also cheaper than buying a syringe, filter, and vial separately, resulting in a material cost savings in addition to the time reduction.

## Method

## Instrument Method

- Isocratic 0.1% ortho-Phosphoric Acid
- Run-Time: 10 minutes
- Flow Rate: 0.6 mL/minute
- Agilent HPLC with PDA detection
- Wave Length: 245nm

## Sample Preparation

### Step 1

1. Samples are weighed into round bottom flasks.
2. Extraction solution is added to the flasks
3. Sample flasks with extraction solution are weighed again.

### Step 2

1. Chloroform is added to flask remove fats from solution.
2. Samples are then stirred for half an hour for extraction.
3. Depending on solution thickness, samples may be centrifuged to separate

### Step 3

1. 100 µL of sample is pipette into 0.2 µm filter auto sampler vial. Then 400 µL of extraction solution is added. Total volume 0.5 mL.
2. The vials are capped with filter caps and then placed in the vial press plate.
3. Once all samples have been capped they are pressed and filtered simultaneously. Once complete they are ready for analysis.



### Step 4

1. All samples are run on HPLC instrument with a set method for analysis.
2. Traditional sample prep method samples were diluted and centrifuged in 15 mL centrifuge tubes and then were filtered through syringe filters into auto sampler vials.
3. Samples were then capped and injected following a sequence on the HPLC.

## Results

Table 1 depicts a single sample processed using the original method, syringe and syringe filter, compared to the same sample diluted and filtrated using Thompson Filter Vials. Data for the two filtration methods were tested for equivalence using TOST. Analysis was performed using the rtost function of the equivalence package. For this test, samples are tested against the null hypothesis that the mean value for the filtration methods are different. Using a sigma value of 0.05 and epsilon corresponding to a 5% difference between the means gives a p-value = 0.00272. At this p-value, we conclude that the sample means are equivalent.

Based on the statistical testing, we have found there is no significant difference between the two filtration methods – syringe with filter and the Thomson Filter vial.

**Table 1.** Syringe Filtration compared to Filter Vial

| Sample Filtration | Syringe w/ Filter | Thomson Filter Vial |
|---|---|---|
| Rep 1\|2 | 67.92 \| 69.81 | 68.92 \| 68.20 |
| Rep 3\|4 | 69.93 \| 70.31 | 70.01 \| 70.79 |
| Rep 5\|6 | 69.57 \| 70.30 | 70.41 \| 71.15 |
| Mean | 69.64 | 69.91 |
| Std. Dev. | 0.89 | 1.14 |
| % RSD | 1.28 | 1.63 |

## Conclusion

- No significant difference was found in the sample results between the two filtration methods.
- The Thomson eXtremeIFV® can be used in place of traditional syringe and filter technique to save time and cost associated with sample preparation.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# THC analysis in candy using the eXtremeIFV for sample prep

## Introduction

What are the challenges faced by analytical labs working with edibles? Measuring the chemical contents and accuately labelling edible products has been a challenge to the cannabis industry. A recent study published by the Journal of the American Medical Society (JAMA) regarding cannabinoid (mis)-labeling in edible medical cannabis products, Dr. Ryan Vandrey of Johns Hopkins School of Medicine looked at 75 products from 47 separate brands purchased at medical dispensaries. Items included baked goods, beverages, and chocolate/candy. Their criteria for selection included those items with a specifically-stated cannabinoid content level. The results, indicated only 17% of edibles tested were "accurately" labeled. The results indicated a +/- 10% range of the stated THC content for beverages and baked goods while baked goods where off by +/- 25%. This could lead to over and under usage which could represent a safety concern. We looked at streamlining the sample prep and analysis of THC in candy.

## Equipment

**HPLC:** Shimadzu Prominance
**UV/VIS:** 228nm
**Column:** Raptor ARC-18, 150 mm x 4.6 mm ID
**Column Temperature:** 30 °C
**Flow Rate:** 1.0mL/min
**Mobile Phase:**
**A:** 25%: Water, 5 mM ammonium formate, 0.1% Formic Acid
**B:** 75%: Acetonitrile, 0.1% Formic Acid

## Sample Preparation

### A. Chocolate

1. 2 g of cold chocolate was weighed into a 50 mL centrifuge tube.
2. Bring up to a total volume of 40 mL with cold IPA.
3. Sonicate at 40 °C for 5 minutes followed by gentle mixing by hand
4. Allow the lipids to precipitate. If necessary, store in a -20 °C freezer for 30 minutes
5. Vortex briefly
6. Centrifuge at 3000 rpm for 5 minutes
7. Transfer the supernatant to a 20mL a graduated cylinder and diluted 10-fold in 25:75 Water:Methanol Vortex briefly
8. Filter using an eXtremeIFV, 0.2µm PVDF

### B. Hard Candy

1. 1 g of ground hard candy was weighed into a 50 mL centrifuge tube.
2. Add 5mL of HPLC grade water
3. Vortex until the candy is completely dissolved
4. Bring up to a total volume of 40 mL with cold IPA
5. Vortex for 30 seconds
6. Centrifuge at 3000rpm for 5 minutes
7. Transfer the supernatant to a 20mL a graduated cylinder and diluted 10-fold in 25:75 Water:Methanol Vortex briefly
8. Filter using an eXtremeIFV, 0.2µm PVDF

## Results

Nicely resolved THC peak allows for the simple quantification of THC in chocolate, Fig 1 and hard candy, Fig 2.

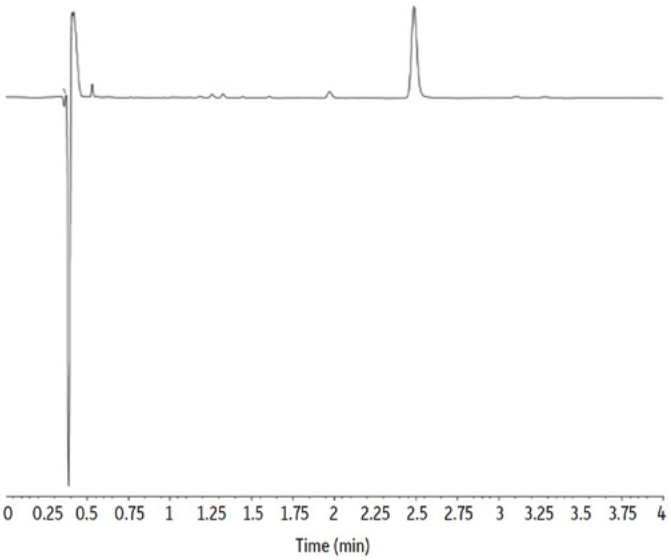


**Fig 1.** THC peak in chocolate, 2.49 minutes Δ9-Tetrahydrocannabinol (Δ9-THC)

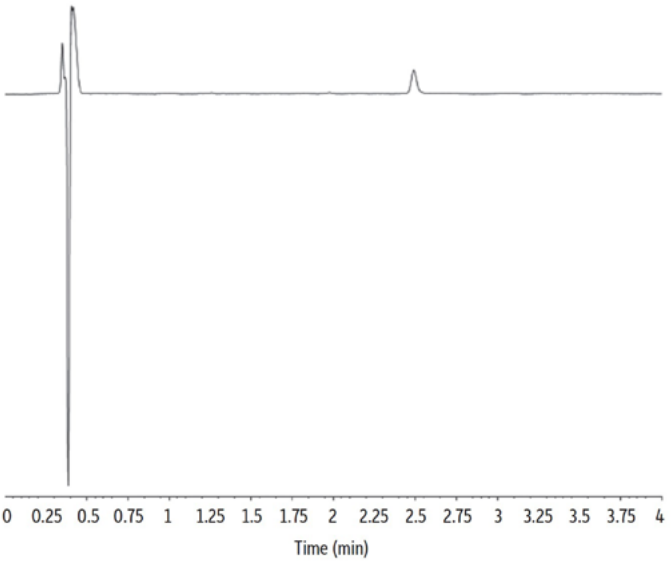


**Fig 2.** THC peak in hard candy, 2.49 minutes Δ9-Tetrahydrocannabinol (Δ9-THC)

## Conclusion

Accurate THC analysis is possible using a streasmlined approach to sample prep. Sample prep and analysis for the chocolate utilizing cold organic solvent for complete crash of the lipids and final clean-up using the eXtremeIFV is < 1 hour per sample. Sample prep and analysis for the hard crushed candy and final clean-up using the eXtremeIFV is < 20 minutes per sample.


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| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | | PVDF | 0.2µm | 85531 |

# eXtremeIFV for sample prep prior to the analysis of cannabinoids by HPLC-UV

## Introduction

Analysis of cannabinoids in marijuana flower, hemp and finished goods is becoming increasingly important as many states are legalizing it for medicinal and recreational purposes. Dosing methods include smoking/vaporizing and edibles but cannabis is still a Schedule 1 illegal drug and therefore have no FDA testing guidelines. This study evaluates streamlining the sample preparation aspect for HPLC-UV analysis of a panel of cannbinoids. The following analytes were used:

Cannabinol (CBN)
Cannabidivarinic acid (CBDVA)
Cannabinolic acid (CBNA)
Cannabidivarin (CBDV)
Δ9-Tetrahydrocannabinol (Δ9-THC)
Cannabidiolic acid (CBDA)
Δ8-Tetrahydrocannabinol (Δ8-THC)
Cannabigerolic acid (CBGA)
Cannabicyclol (CBL)
Cannabigerol (CBG)
Cannabichromene (CBC)
Cannabidiol (CBD)
Tetrahydrocannabinolic acid A (THCA-A)
Tetrahydrocannabivarin (THCV)
Cannabichromenic acid (CBCA)
Tetrahydrocannabivarinic acid (THCVA)

## Equipment

**HPLC:** Shimadzu Prominance
**UV/VIS:** 228nm

**Column:** Raptor ARC, 150 mm x 4.6 mm ID
**Column Temperature:** 30 °C
**Flow Rate:** 1.5mL/min

**Mobile Phase:**

**A:** 25%: Water, 5 mM ammonium formate, 0.1% Formic Acid
**B:** 75%: Acetonitrile, 0.1% Formic Acid

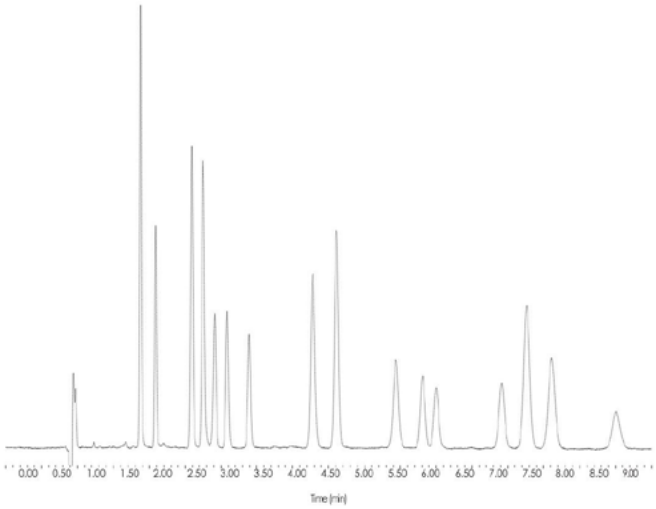
## Sample Preparation

- Place 200uL of sample into the outer shell of the eXtremeIFV, 0.2um PVDF
- Add 200uL of 25:75 (Water:Methanol)
- Partially depress the plunger
- Vortex the sample
- Depress the plunger completely

## Results

16 cannabinoids are baseline resolved using the eXtremeIFV for sample prep and a isocratic HPLC method.

| Peaks # | Analyte | Time (min) |
|---|---|---|
| 1 | Cannabidivarinic acid (CBDVA) | 1.877 |
| 2 | Cannabidivarin (CBDV) | 2.86 |
| 3 | Cannabidiolic acid (CBDA) | 2.592 |
| 4 | Cannabigerolic acid (CBGA) | 2.75 |
| 5 | Cannabigerol (CBG) | 2.912 |
| 6 | Cannabidiol (CBD) | 3.48 |
| 7 | Tetrahydrocannabivarin (THCV) | 3.391 |
| 8 | Tetrahydrocannabivarinic acid (THCVA) | 4.279 |
| 9 | Cannabinol (CBN) | 4.609 |
| 10 | Cannabinolic acid (CBNA) | 5.437 |
| 11 | Δ9-Tetrahydrocannabinol (Δ9-THC) | 5.815 |
| 12 | Δ8-Tetrahydrocannabinol (Δ8-THC) | 6.2 |
| 13 | Cannabicyclol (CBL) | 6.916 |
| 14 | Cannabichromene (CBC) | 7.263 |
| 15 | Tetrahydrocannabinolic acid A (THCA-A) | 7.612 |
| 16 | Cannabichromenic acid (CBCA) | 8.51 |




## Conclusion

The HPLC method fully resolves 16 major and minor cannabinoids. Simple quick sample prep using the eXtremeIFV allows for the baseline separation of the analytes ensuring positive identification and accurate quantitation of the cannabinoids. With <10 seconds per sample and a fast 9-minute analysis, all compounds were resolved making this method suitable for high-throughput cannabis testing labs.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| Standard Filter Vial | ● | PTFE | 0.2µm | 35530 |

# Time saving sample prep for the analysis of 54 pesticide & aflatoxin residues in Cannabis by LC-MS/MS

Presented at NACRW 2017

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## Introduction

Pesticide analysis of cannabis leaves and finished goods is becoming increasingly important as many states are legalizing it for medicinal and recreational purposes. Dosing methods include smoking/vaporizing and edibles but cannabis is still a Schedule 1 illegal drug and therefore have no FDA testing guidelines. Trace levels of pesticides can be incurred during cultivation or inhaled from dried pesticides on the cannabis. This study evaluates the sample preparation aspect for LC-MS/MS analysis of a 50+ analyte panel of pesticides, fungicides and aflatoxins. QuEChERS was used to extract the analytes from the cannabis flowers, followed by centrifugation and Thomson Standard Filter Vial for sample clean-up.

## Equipment:

- Sciex 6500 QQQ Mass Spectrometer
- Shimadzu LC-30AD Pumps
- Run Time: 15 minutes
- Flow Rate: 0.5 mL/min
- Injection Volume: 12 µL
- Column: Kinetex C18, 5µm, 3mm x 150mm
- Mobile Phase A: 0.1% FA in Water
- Mobile Phase B: 5mM Ammonium Formate, 0.1% Formic Acid in MeOH
- Centrifuge
- Thomson StandardIFV® 0.2µm PTFE (p/n 35530)*
- Thomson 48 position Vial Filter Press (p/n 35015-476)

*For some autosamplers it is important to adjust the needle depth of your autosampler when using Thomson filter vials to improve the reproducibility of injections

## Sample Preparation of Cannabis Flowers

1. Weigh out 0.25g of the flower into a 50mL conical.
2. Add 7g of QuEChERS
3. Add 15mL of 1% Acetic Acid in Acetonitrile
4. Vortex for 30 minutes
5. Centrifuge for 5 minutes
6. Transfer 400µL into the outer shell of p/n 35530
7. Add 4µL of ISTD
8. Partially depress the plunger and vortex
9. Ready to analyze

## Results

20+ compounds were extracted from cannabis flower with excellent recoveries utilizing a modified QueChERS method. The linear range for all the aflatoxins and ochratoxins are 0.5-50ng/mL; while the other analytes are 1.0-100ng/mL. Excellent linearity (see Table 2) and good recovery was achieved for all the compounds.

**Table 1.** Shows the LOQ, linear range, % CV, r2 and accuracy for each analyte

| Analyte | LOQ (ng/mL) | Linear Range (ng/mL) | % CV | r2 Value | % Accuracy |
|---|---|---|---|---|---|
| Abamectin Group 1 | 1 | 1- 100 | < 14.6 | 0.9932 | 93.4 - 105.5 |
| Abamectin Group 2 | 1 | 1- 100 | <25.4 | 0.98806 | 93.6 - 103.4 |
| AFLATOXIN B2 1 | 0.5 | 0.5 - 50 | <3.3 | 0.99837 | 93.7 - 105.7 |
| AFLATOXIN B2 2 | 0.5 | 0.5 - 50 | <4.9 | 0.99833 | 94.0 - 104.6 |
| AFLATOXIN G2 1 | 0.5 | 0.5 - 50 | <5.0 | 0.99829 | 93.1 - 105.2 |
| AFLATOXIN G2 2 | 0.5 | 0.5 - 50 | <5.4 | 0.9983 | 93.7 - 104.9 |
| AFLATOXIN B1 1 | 0.5 | 0.5 - 50 | <3.9 | 0.99805 | 92.2 - 105.9 |
| AFLATOXIN B1 2 | 0.5 | 0.5 - 50 | <4.0 | 0.99789 | 92.0 - 106.4 |
| AFLATOXIN G1 1 | 0.5 | 0.5 - 50 | <4.2 | 0.99853 | 94.1 - 104.6 |
| AFLATOXIN G1 2 | 0.5 | 0.5 - 50 | <4.5 | 0.99827 | 93.8 - 105.1 |
| Bifenthrin 1 | 1 | 1- 100 | <7.9 | 0.99699 | 92.6 - 105.6 |
| Bifenthrin 2 | 1 | 1- 100 | <6.2 | 0.99704 | 92.8 - 105.3 |
| Chlormequat 1 | 1 | 1- 100 | <1.4 | 0.99593 | 87.3 - 111.0 |
| Chlormequat 2 | 1 | 1- 100 | <4.5 | 0.99512 | 86.6 - 111.3 |
| Daminozide 1 | 1 | 1- 100 | <1.9 | 0.96303 | 66.0 - 131.6 |
| Daminozide 2 | 1 | 1- 100 | <4.5 | 0.99512 | 65.5 - 131.7 |
| Dichlorvos 1 | 1 | 1- 100 | <7.2 | 0.99369 | 86.0 - 112.4 |
| Dichlorvos 2 | 1 | 1- 100 | <7.2 | 0.99371 | 86.1 - 112.8 |
| Imidacloprid 1 | 1 | 1- 100 | <4.9 | 0.99904 | 97.4 - 101.3 |
| Imidacloprid 2 | 1 | 1- 100 | <5.5 | 0.99887 | 97.5 - 101.6 |
| Malathion A 1 | 1 | 1- 100 | <4.3 | 0.99574 | 86.9 - 108.7 |
| Malathion A 2 | 1 | 1- 100 | <3.7 | 0.99416 | 84.5 - 111.4 |

| Analyte | LOQ (ng/mL) | Linear Range (ng/mL) | % CV | r2 Value | % Accuracy |
|---|---|---|---|---|---|
| Myclobutanil 1 | 1 | 1- 100 | <3.5 | 0.99808 | 91.6 - 105.2 |
| Myclobutanil 2 | 1 | 1- 100 | <4.8 | 0.99773 | 91.0 - 106.2 |
| OCHRATOXIN A 1 | 0.5 | 0.5 - 50 | <8.6 | 0.97237 | 67.4 - 120.0 |
| OCHRATOXIN A 2 | 0.5 | 0.5 - 50 | <18.5 | 0.96764 | 67.2 - 121.2 |
| Paclobutrazol 1 | 1 | 1- 100 | <5.7 | 0.99481 | 86.6 - 109.5 |
| Paclobutrazol 2 | 1 | 1- 100 | <3.8 | 0.99469 | 85.6 - 109.6 |
| Permethrin, cis- 1 | 1 | 1- 100 | <6.6 | 0.99813 | 95.5 - 103.2 |
| Permethrin, cis- 2 | 1 | 1- 100 | <6.5 | 0.99782 | 93.6 - 102.8 |
| Permethrin, trans- 1 | 1 | 1- 100 | <8.1 | 0.99723 | 92.9 - 102.9 |
| Permethrin, trans- 2 | 1 | 1- 100 | <7.3 | 0.99694 | 91.8 - 105.2 |
| Piperonyl butoxide 1 | 1 | 1- 100 | <8.4 | 0.99523 | 93.2 - 106.3 |
| Piperonyl butoxide 2 | 1 | 1- 100 | <8.9 | 0.99526 | 93.1 - 106.3 |
| Propiconazole 1 | 1 | 1- 100 | <3.8 | 0.99759 | 90.1 - 105.4 |
| Propiconazole 2 | 1 | 1- 100 | <2.8 | 0.99722 | 89.6 - 106.7 |
| Pyrethrins Cinerin I 1 | 1 | 1- 100 | <13.0 | 0.99779 | 98.6 - 101.9 |
| Pyrethrins Cinerin I 2 | 1 | 1- 100 | <20.5 | 0.99494 | 96.4 - 103.3 |
| Pyrethrins Cinerin II 1 | 1 | 1- 100 | <8.3 | 0.99651 | 90.3 - 105.5 |
| Pyrethrins Cinerin II 2 | 1 | 1- 100 | <12.7 | 0.99351 | 88.2 - 110.2 |
| Pyrethrins Jasmolin I 1 | 1 | 1- 100 | <12.9 | 0.99702 | 94.6 - 103.7 |
| Pyrethrins Jasmolin I 2 | 1 | 1- 100 | <21.5 | 0.99449 | 96.2 - 103.5 |
| Pyrethrins Jasmolin II 1 | 1 | 1- 100 | <22.7 | 0.99355 | 93.8 - 103.3 |
| Pyrethrins Jasmolin II 2 | 1 | 1- 100 | <10.0 | 0.99751 | 94.5 - 103.7 |
| Pyrethrins Pyrethrin I 1 | 1 | 1- 100 | <17.6 | 0.99626 | 97.4 - 101.7 |
| Pyrethrins Pyrethrin I 2 | 1 | 1- 100 | <5.0 | 0.99906 | 96.4 - 102.4 |
| Pyrethrins Pyrethrin II 1 | 1 | 1- 100 | <3.2 | 0.99853 | 92.9 - 104.2 |
| Pyrethrins Pyrethrin II 2 | 1 | 1- 100 | <38.3 | 0.98319 | 91.9 - 106.9 |
| Spinosyn A 1 | 1 | 1- 100 | <4.0 | 0.99913 | 95.2 - 102 |
| Spinosyn A 2 | 1 | 1- 100 | <3.2 | 0.99931 | 96.1 - 103.0 |
| Spinosyn D 1 | 1 | 1- 100 | <3.9 | 0.99897 | 94.9 - 103.2 |
| Spinosyn D 2 | 1 | 1- 100 | <5.4 | 0.9987 | 94.8 - 103.4 |
| Spiromesifen 1 | 1 | 1- 100 | <16.6 | 0.99223 | 95.8 - 105.0 |
| Spiromesifen 2 | 1 | 1- 100 | <13.8 | 0.99457 | 95.4 - 104.1 |
| Uniconazole 1 | 1 | 1- 100 | <4.7 | 0.99774 | 91.1 - 104.8 |
| Uniconazole 2 | 1 | 1- 100 | <8.0 | 0.99667 | 89.5 - 105.5 |

## Conclusion

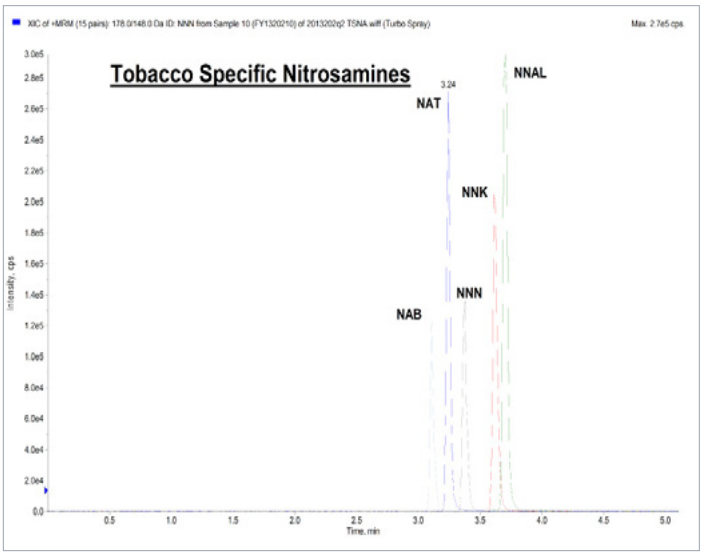
Using a modified QuEChERS approach on difficult matrices allows for many compounds to be included in multiresidue pesticide screens that would have otherwise been excluded due to matrix suppression or false negative results. This modified QuEChERS – Filter Vial method saves time, reduces solvent waste and cost over the traditional approach, QuEChERS – SPE. This validated method for the compounds in Table 2 has good linearity and recovery without having to use more expensive time consuming clean-up techniques. This approach is an extremely cost effective way to ensure problem analytes on difficult matrices can be included in a screen. The Thomson Standard Filter vials save time and money when replacing SPE and traditional syringe filtration techniques.

| series | cap color | membrane | pore size | part # |
|---|---|---|---|---|
| eXtremeIFV® | ● | PVDF | 0.45µm | 85541 |

# Analysis of Nitrosamines in Tobacco

## Introduction

Tobacco-specific nitrosamines (TSNA) are a group of carcinogens found only in tobacco products. They are formed from nicotine and related alkaloids during the production and processing of tobacco and tobacco products. In recent years due to their carcinogenic properties, efforts have been made to reduce TSNA levels in tobacco products. The desired goal of this investigation is to develop a sensitive, high-throughput method to monitor TSNA levels in tobacco and tobacco products. This method descripes a simple robust sample preparation utilizing the Thomson Filter Vials for in-vial filtration: N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB), 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).





## Prep

1. 0.25g of unburned/smokeless tobacco sample
2. Extracted with 100mM ammonium acetate solution, filtered with eXtremeIFV® PVDF 0.45 µm

## Equipment

## HPLC

**Injection Volume:** 5 µL
**Column:** Waters Xterra MS C18, 50x4.6mm, 5µm
**Aqueous phase:** 5mM ammonium acetate in HPLC water
**Organic Phase:** 5mM ammonium acetate in 95/5 acetonitrile/water.

## Gradient

| Time [min] | Organic % |
|---|---|
| 0 | 5 |
| 1 | 5 |
| 2 | 35 |
| 5 | 35 |
| 6 | 5 |
| 8 | 5 |

**Flow rate:** 1mL/min
**Temperature:** 60°C
**Detection:** MS/MS

| Analyte | Ion pair Q1/Q3 (amu) |
|---|---|
| N-Nitrosoanabasine (NAB) | 192/162 |
| N-Nitrosoanatabine (NAT) | 190/160 |
| N-Nitrosonornicotine (NNK) | 208/122 |
| 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNN) | 178/148 |
| 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) | 210/180 |

# Chemical Compatibility

| | Housing Materials | Filter Membrane | | | |
|---|---|---|---|---|---|
| | Polypropylene | PTFE | PVDF | PES | NYLON |
| **Acetic Acid (glacial)** \| *acid, organic* | TST | R | R | R | NR |
| **Acetone** \| *ketone* | R | R | NR | GNR | R |
| **Acetonitrile (ACN)** \| *nitrile* | R | R | LTD | NR | R |
| **Ammonium Hydroxide** \| *caustic* | TST | GR | R | NR | TST |
| **Ammonium Sulfate (saturated)** \| *salt, aqueous solution* | R | GR | NR | ND | R |
| **Amyl Alcohol** \| *alcohol* | R | R | R | GR | TST |
| **Benzene** \| *HC, aromatic* | NR | — | — | — | — |
| **Benzyl Alcohol** \| *HC aromatic/alcohol* | NR | — | — | — | — |
| **Butyl Alcohol** \| *alcohol* | R | GR | R | GR | R |
| **Chloroform** \| *HC, halogenated* | NR | — | — | — | — |
| **Cyclohexanone** \| *ketone* | NR | — | — | — | — |
| **Dimethyl Sulfoxide (DMSO)** \| *sulfoxide* | R | R | NR | NR | R |
| **Dimethylacetamide** \| *amide* | R | GR | NR | NR | NR |
| **Dimethylformamide** \| *amide* | R | GR | NR | ND | R |
| **Ethyl Acetate** \| *ester* | TST | R | R | GNR | R |
| **Ethyl Alcohol** \| *alcohol* | R | R | R | GR | TST |
| **Ethylene Glycol** \| *glycol* | R | R | R | GR | R |
| **Formaldehyde** \| *aldehyde* | R | R | R | ND | R |
| **Formic Acid, 50%** \| *acid, organic* | R | GR | R | ND | NR |
| **Glycerine (Glycerol)** \| *glycol* | R | GR | R | GR | R |
| **Hexane** \| *HC, aliphatic* | NR | — | — | — | — |
| **Hydrochloric Acid, 1N (HCL)** \| *acid, inorganic* | GR | R | R | GR | GR |
| **Hydrochloric Acid, 6N (HCL)** \| *acid, inorganic* | TST | R | TST | GR | TST |
| **Isobutyl Alcohol** \| *alcohol* | R | R | R | GR | TST |
| **Isopropyl Acetate** \| *ester* | TST | R | R | GNR | R |
| **Isopropyl Alcohol** \| *alcohol* | R | R | R | GR | TST |
| **Lactic Acid, 50%** \| *acid, organic/alcohol* | R | GR | TST | ND | TST |
| **Methyl Acetate** \| *ester* | TST | R | NR | GNR | R |
| **Methyl Alcohol** \| *alcohol* | R | R | R | GR | TST |
| **Methylene Chloride** \| *HC, halogenated* | NR | — | — | — | — |
| **Nitric Acid, 6N** \| *acid, inorganic* | TST | R | R | R | NR |
| **Nitrobenzene** \| *HC, aromatic* | NR | — | — | — | — |
| **Pentane** \| *HC, aliphatic* | NR | — | — | — | — |
| **Phenol (aqueous solution)** \| *phenol* | NR | — | — | — | — |
| **Potassium Hydroxide, 3N** \| *caustic* | R | R | R | ND | R |
| **Silicone Oils** \| *silicone* | R | GR | R | ND | R |
| **Sodium Carbonate (aqueous solu-tion)** \| *salt, aqueous solution* | R | R | R | ND | TST |
| **Water (Brine)** \| *salt, aqueous solution* | R | R | R | ND | R |
| **Sodium Chloride (aqueous solution)** \| *salt, aqueous solution* | R | R | R | ND | R |
| **Sodium Dodecyl Sulfate** \| *surfactant/detergent* | ND | ND | ND | ND | ND |
| **Sodium Hydroxide, 3N** \| *caustic* | R | R | R | R | R |
| **Sulfuric Acid (concentrated)** \| *acid, inorganic* | NR | — | — | — | — |
| **Tetrahydrofuran (THF)** \| *ether* | NR | — | — | — | — |
| **Toluene** \| *HC, aromatic* | NR | — | — | — | — |
| **TCA (aqueous solution)** \| *acid, organic* | R | GR | R | ND | TST |
| **Tween 20 (aqueous solution)** \| *surfactant/detergent* | ND | R | TST | ND | TST |

**R** = Recommended | **GR** = Generally Recommended | **NR** = Not Recommended | **GNR** = Generally Not Recommended
**LTD** = Limited Recommendation | **TST** = Testing Recommended | **ND** = No Data Presently Available | — = Not Recommended, polypropylene is **NR**

# Compound Compatibility

| | Recommended Filter Membrane | | | | |
|---|---|---|---|---|---|
| | PVDF | PES | PTFE | PES | PVDF |
| | .2 µm | .2 µm | .2 µm | .45 µm | .45 µm |
| 5-Fluorouracil | | | ● | | |
| (18F) Fluoromisondazole, Misiomidazole | ● | | | | |
| Acetylsalicylic acid | | ● | | | |
| Alprenolol | | ● | | | |
| Amiloride | | ● | | | |
| Atenolol | | ● | | | |
| Azathioprine | | | | ● | ● |
| Azodicarbonamide | | ● | | | |
| Bleomycin Sulfate | | | ● | | |
| Caffeine | | ● | | | |
| Cetirizine | | | | ● | ● |
| Chlorothiazide | | ● | | | |
| Chloramphenicol | | ● | | | |
| Cimetidine | | ● | | | |
| Ciprofloxacin | | ● | | | |
| Cyclosporine A | ● | | | | |
| Cytarabine | | | ● | | |
| Diclofenac | | | | | ● |
| Hydrochlorothiazide | | ● | | | |
| Ibuprofen | | | | ● | ● |
| isonicotinic acid | | | ● | | |
| Ketamine | | ● | | | |
| Levofloxacin | | | | ● | ● |
| Lomefloxacin | | | | ● | ● |
| Metoprolol | | ● | | | |
| Mitomycin | | | ● | | |
| Morphazinamide | | | ● | | |
| Nadolol | | ● | | | |
| Nicotinic acid | | | ● | | |
| Paclitaxel | ● | | | | |
| p-Aminobenzoic acid (PABA) | | | | | ● |
| p-aminosalicylic acid | | | ● | | |
| Pefloxacin | | | | ● | ● |
| Pentoxifylline (PTX) | ● | | | | |
| Phenytoin | | | | | ● |
| Ranitidine | | ● | | | |
| Rifampicin | | | | ● | ● |
| Sabeluzole | | | | | ● |
| Sulfadozine | | | | | ● |
| Sulphasalazine | | ● | | | |
| Sulpiride | | ● | | | |
| Terbutaline | | ● | | | |
| Timolol | | ● | | | |
| Tranexamic acid | | ● | | | |
| Triamcinolone Acetonide | | ● | | | |
| Tropicamide | | | | ● | |
| Vinblastine Sulfate | | | ● | | |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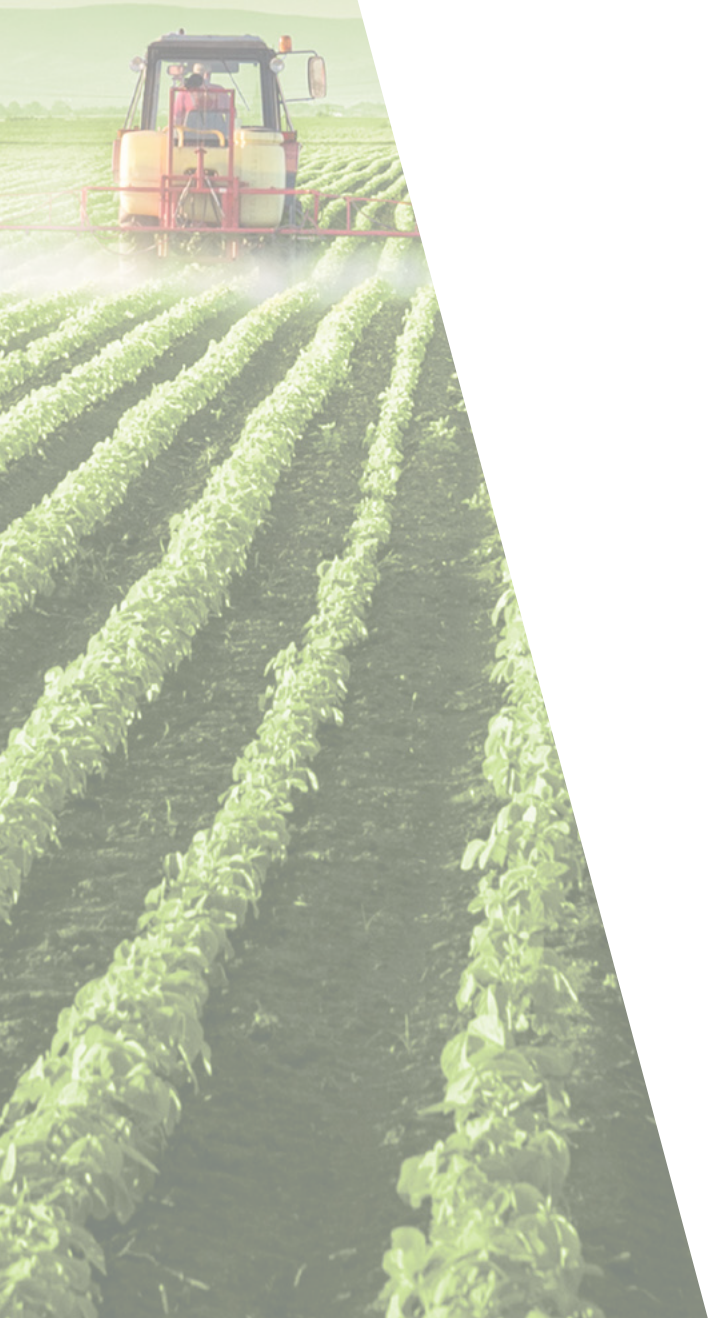

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