Improved Sample Clean-up Options For Contaminant Analysis For Juices And Water By GC/MS And LC/MS

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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 MS analysis. However, these techniques are time consuming, adversely impact recovery, require expensive consumables, and use large amounts of solvent (which then need to be concentrated). Several studies were undertaken to investigate different designs of filter vials to improve sample clean-up methods in orange juice, apple juice, grape juice, vegetable juice and water.

Thomson eXtreme® Filter Vials (patented) offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates. 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. The filtration process from sample pipetting to autosampler ready only requires 15 seconds. Benefits to the use of Thomson eXtreme® Filter Vials include lower cost, faster sample preparation time, less use and disposal of organic solvents, and in some instances improved recoveries.

Thomson eXtractor3D|FV® (patented) offer filtration with increased volume, enabling multiple extraction techniques with different resins/sorbents or solids/large particulates (greater than 35%) to autosampler ready vials. eXtractor3D|FV® is a product uniquely designed for the addition of resins/sorbents, QuEChERS dispersive salts, pills, or special resins in the standard autosampler ready vial. The filter vial consists of two parts: a filter vial shell and a plunger which includes a multi-layer filter on one end and a screw cap on the other end. Large solids/large particulates can be placed within the eXtractor3D|FV® where multiple extraction techniques occur. Prior to the introduction of the eXtractor3D|FV®, samples required multiple steps using SPE, or other methods to remove interfering analytes and co-eluting compounds. SPE or QuEChERS can now be completed with multi-depth filtration without risk of solids compromising the autosampler. Pills and other large solids can be broken down for complete testing using the eXtractor3D|FV®. The eXtractor3D|FV® allows for compounds to be separated from the matrix with the addition of resins/sorbents, resulting in both a higher signal to noise ratio and peaks that are more differentiated.

Method I- eXtreme Filter Vials® vs SPE for the analysis of Pesticides in Orange Juice by GC/MS

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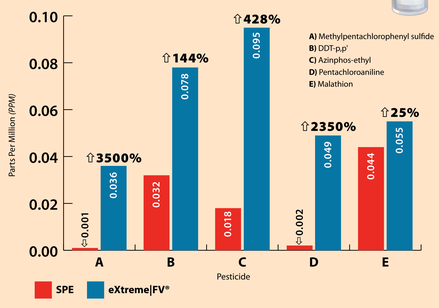
Comparison of Pesticide Recoveries



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. 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 eXtreme Filter Vials, 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 eXtreme Filter Vial step.

Experiment

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

Sample Extraction:

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 eXtreme Filter Vial".

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

1.) Wash one Restek 6mL Combo SPE Cartridge (packed with 200mg CarboPrep 200 and 400mg PSA Restek catalog #26127) 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.

Thomson eXtreme Filter Vial Cleanup Prior to Analysis

1.) Add 400µL of the re-suspended residue from the flask labeled "for Thomson eXtreme Filter Vial" to the shell of one Thomson eXtreme Filter Vial 0.45µm, PTFE *(Thomson Part Number 85540-500)*.

2.) Insert plunger completely.

Compound/Sample name	SPE+ Routine Syringe Filtration ppm	eXtreme| FV® ppm
Azinphos-ethyl	0.018	0.095
Azinphos-methyl	0.023	0.115
Bromophos-ethyl	0.025	0.057
Cyfluthrin I	0.082	0.113
Cyhalothrin (lambda)	0.076	0.091
Cypermethrin I (Zeta)	0.082	0.117
Cypermethrin II ICAS # 52315-07-8]	0.08	0.113
Cypermethrin III (Beta)	0.058	0.104
Cypermethrin IV ICAS # 52315-07-8]	0.07	0.097
DDT-o,p'	0.035	0.065
DDT-p,p'	0.032	0.078
Deltamethrin	0.053	0.102
Endosulfan I (alpha isomer)	0.041	0.076
Fenthion sulfone	0.081	0.107
Fenvalerate I	0.076	0.106
Fenvalerate II [CAS # 51630-58-1]	0.055	0.073
Fluvalinate-tau II [CAS # 102851-06-9]	0.058	0.084
Methylpentachlorophenyl sulfide	0.001	0.036
Octachlorodipropyl ether (S421)	0.021	0.047
Pentachloroaniline	0.002	0.049
Permethrin I	0.068	0.097
Permethrin II (trans)	0.071	0.115
Phosalone	0.005	0.089
Phosmet	0.031	0.104
Prothiofos	0.033	0.06

Table 3: Pesticides in Orange Juice Comparison of SPE to eXtreme Filter Vials

Analysis

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

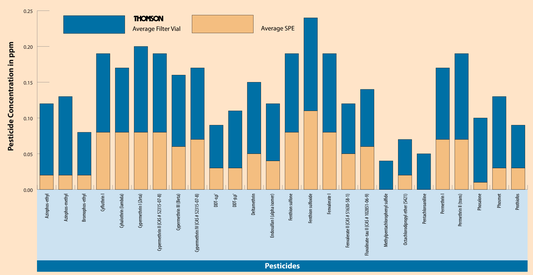
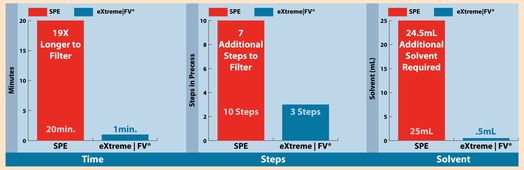


Fig 3, Pesticides in Orange Juice Comparison of SPE to eXtreme Filter Vials

What did we learn?

SPE -vs- eXtreme|Filter Vials®



Conclusions

The Thomson eXtreme 0.45µm, PTFE Filter Vials patented *(Thomson #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 eXtreme Filter Vial. The use of Thomson eXtreme 0.45µm, PTFE Filter Vials as a substitute for SPE conforms to USP Method 561.

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

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

Meets the requirements for Method HEXCR-E3510 Canadian Ministry of Environment Laboratory Services Branch

This method utilizes a hyphenated technique, i.e. ion exchange chromatography (IC) coupled to an inductively couple 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.

Previous 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.

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

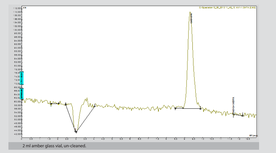
Note: $r^2 > 0.995$ for the calibration curve

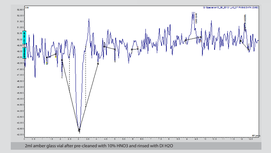
Equipment:

- **Varian ProStar 210 HPLC**
- **Varian 820MS ICP-MS**
- **Pump Rate (rpm): 20**
- **Stabilization delay(s): 0**
- **Skimmer Gas Source: H_2**
- **Skimmer Flow: 30**
- **Column: Hamilton PRP-X100 Anion Exchange Column & Guard Column**
- **Mobile Phase:**
  - **Mobile Phase A: 100mM/L Ammonium Nitrate, pH ≥ 9, pH adjust with 16N Nitric Acid**
  - **Mobile Phase 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

Existing Canadian HEXCR-E3510 Procedure used a Syringe & Filter, amber glass vial and cap to filter water samples prior to analysis. This required pre-cleaning (10% HNO_3 , rinse with DI-H2O and dried) of the amber glass vials

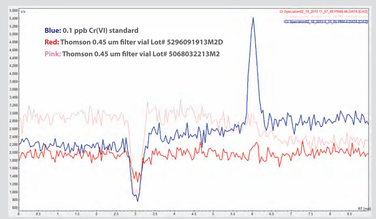




Results:

Results of spiked hexavalent chromium calibration standard in the concentrations of 0.05µg/L, 0.1µg/L, 0.5µg/L, 1.0µg/L yielded $r^2 > 0.995$. Figure 1 shows a chromatogram of a 0.1ppb Cr(VI) standard overlayed with 2 different lots of blank samples.

Figure 1: Chromatogram of a 0.1ppb Cr(VI) standard overlayed with 2 different lots of blank samples



Conclusions:

The Thomson Standard Filter Vials showed no chromium contamination in either the filter vial materials or the filter membrane allowing for their use in the analysis of hexavalent chromium in water. The validation of the Thomson Standard Filter Vials into a validated method for the analysis of hexavalent chromium reduces both time and waste compared to the previous filtration method.

Filter Vial
PTFE .45µm
35540

TIC-PL-082-257 Rev. A

Method III- Screening and Quantitation of 250 Pesticides in Fruit Juices with Positive/Negative Switching LC/MS/MS

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Introduction:

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. Many labs currently perform multi-residue analysis of pesticides using separate positive and negative methods due to instrument limitations. This requires twice the sample and twice the analysis time. 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. A study using the EVOQ for the analysis of 250 pesticides in apple juice, cranberry juice, grape juice, orange juice and vegetable juice using only one method with positive negative switching for over 500 MRM transitions. The measurements were conducted using store bought juices with a dilute-and-shoot approach without sample enrichment.

Sample Prepration:

1. Mix 50µL fruit juice with 450µL of solvent (MeOH/water, 10/90, v/v) directly into the outer shell Thomson eXtreme Filter Vial, 0.2µm PVDF and press filter plunger to filter

   * Store bought Fruit include: Apple Juice, Cranberry Juice, Grape Juice, Orange Juice and Vegetable Juice

2. Load the Thomson eXtreme Filter Vials into the autosampler

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 3 µm
- Column Temperature: 40 °C
- Injection Volume: 30 µL
- Mobile Phase Gradient: 5 mM Ammonium Fluoride in Water/Methanol
- Flow rate: 400 µL/min

Results:

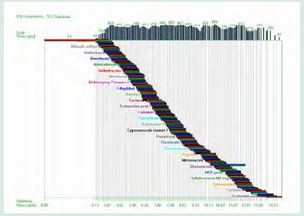

Fig.1. The compound based scanning (CBS) of 250 pesticides can automatically compute and assign the scan (dwell) time for each MRM for timed MRM, based on peak width and data points required.

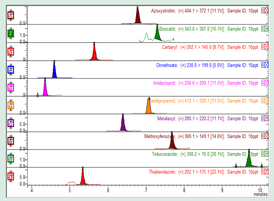

Fig 2. Chromatograms of standard solution of the compounds listed in table 1 at 0.01 ppb (equivalent to 0.1ppb in juice).

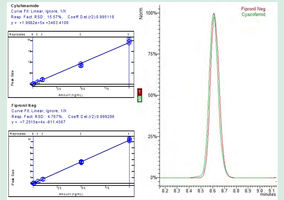

Fig 3. Calibration curve of negative pesticide Fipronil (left top) and positive pesticide Cyazofamid (left bottom), and their co-eluting plots (right).

Fruit 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	ND	ND
Dimethoate	ND	0.30	ND	ND	ND
Imidacloprid	ND	ND	0.60	ND	0.20
Mandipropamid	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
Thiabendazole	1.8	ND	ND	ND	ND

Table 1. Test results for store bought juices. ND is not detected <0.1ppb

Conclusion:

- The calibration on triplicate injections showed excellent linearity and response factor RSD over 3 orders range using a simple sample prep of adding juice and water directly into the Thomson eXtreme Filter Vial and then to the autosampler.
- Good linearity, sensitivity and response factor RSD for positive and negative co-eluting pesticides.
- A total of twelve pesticides were detected in apple juice, orange juice, cranberry juice or vegetable juice
- The multiple pesticides detected in orange juice, cranberry juice and juice may suggest that juice comes from multiple sources of raw materials or pooled juices.

TIC Chromatograms

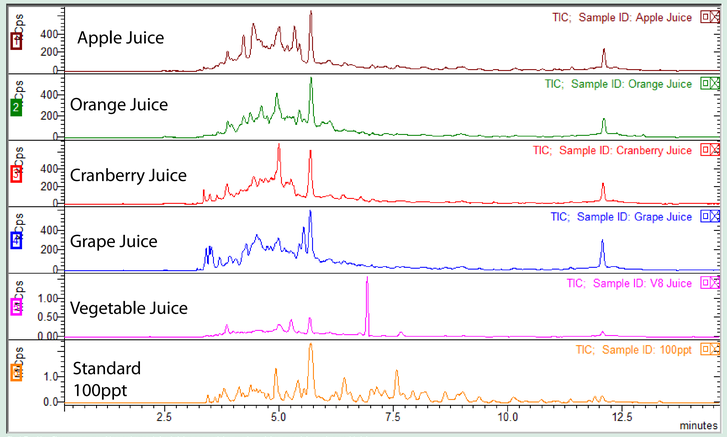

Fig 4. Total Ion Chromatograms of (top to bottom): Apple juice, orange juice, cranberry juice, grape juice and vegetable juice.

Overall Conclusion

To process 6 samples	Traditional SPE or GPC	QuEChERS With SPE clean-up	QuEChERS with Thomson Filter Vial clean-up*	Thomson Filter Vial Benefits*
Estimated (minutes)	120	20	10	5
Solvent used (mL)	90	10-15	5	0.5
Chlorinated waste (mL)	30	none	none	none
Specialized equipment	Separatory funnels, water baths, evaporator, etc.	Vacuum pump, vacuum manifold	none	none

* Significant time & money savings because lengthy wash steps are eliminated

Final Conclusion

The methods presented here for the analysis of contaminants in juices and water show the Thomson Filter Vials compared to traditional methods of clean-up, including SPE, liquid-liquid extraction and syringe filtration.

For sample clean-up, post extraction, in the analysis of pesticides of orange juice by GC/MS, the Thomson eXtreme Filter Vials, showed improved recovery of many of the pesticides by as much as 428%. The improved method yielded higher recovery and, used less solvents and less sample preparation time by eliminating the SPE step for clean-up for the analysis of pesticides of orange juice. Simply add the extracted sample to the Thomson eXtreme Filter Vials, PTFE 0.45µm (P/N 85540-500), depress the plunger to filter and load onto the autosampler.

Analyzing 250 pesticides in store bought apple, grape, orange and vegetable juices by LC/MS sample preparation was streamlined to diluting the juice directly into the eXtreme Filter Vials. 0.2µm (P/N 85531-500) and placing the filter vial onto the HPLC. This method has excellent linearity and sensitivity down to < 0.1ppb in real life samples.

In the analysis of Hexavalent Chromium, the Thomson Standard Filter Vials, PTFE 0.45 µm (P/N 35540-500) showed no chromium contamination in the vial or membrane materials and reproducible analysis at 0.1ppb. Thomson Standard Filter Vials replaced 4 part numbers: syringe, syringe filter, autosampler vial and cap and saves hours of technician time pre-cleaning vials.

What do all these methods have in common? All of the Thomson Filter Vials simplify sample preparation, lower costs and save time.