New Sample Preparation Methodology to Enable Higher Recovery and Minimize Loss of Difficult Analytes in Food and Natural Products by LC/MS or GC/MS

Authors: Lisa Wanders, Sam Ellis, Joe Machamer, Thomson Instrument Company, Oceanside, CA

Introduction

The most critical aspects of reliable food contamination analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, Solid Phase Extraction *(SPE)*, Supported Liquid Extraction *(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 which then need to be concentrated. Several studies comparing these techniques to Thomson eXtreme® Filter Vials *(patented)* for contaminant analysis were conducted in orange juice, soil, milk, shellfish and water.

SPE, SLE, liquid-liquid, syringe filtration and centrifugation are common sample preparation techniques prior to GC or LC analysis of pesticides in food and natural products. Typically, these techniques are used to concentrate analytes and to reduce interference from co-eluting compounds. These techniques are also commonly used to clean-up/filter particulates following the extraction of particulate laden samples. Drawbacks to the use of these techniques include cost, sample preparation time, use and disposal of organic solvents, and in some instances, poor recoveries due to incomplete extraction of the liquid layer, loss of analytes during wash steps, or analytes remaining bound to packing materials.

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.

Time = Money

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	1
Solvent used (mL)	90	10-15	5	0.5
Chlorinated waste (mL)	30	none	none	none
Specialized equipment	Separatory funnels, water bath, evaporator, etc.	Vacuum pump, vacuum manifold	none	none

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

Materials and Methods I – *Improved Analysis of Pesticides and Environmental Pollutants in Shrimp*

Streamlined sample cleanup using combined dispersive solid-phase extraction and in-vial filtration for analysis of pesticides and environmental pollutants in shrimp

L. Han, Y. Sapozhnikova, S.J. Lehotay, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.04.005

This process examines ways to enhance the overall method improvement of the analysis of pesticides and environmental pollutants in shrimp. Method improvements for streamlining sample clean-up using dispersive and solid phase extraction in the Thomson eXtractor3D|FV will be compared to the existing traditional QuEChERS methodology. An effective way to reduce time and cost is to eliminate the centrifugation step and combine the SPE step with in-vial filtration using the Thomson eXtractor3D|FV.

The sample matrix consisted of 42 diverse pesticides and 17 environmental contaminants in shrimp. Extracts were analyzed by both low pressure GC/MS/MS and LC/MS/MS/MS.

Sample Preparation:

Extraction:

1. **10g of spiked homogenized shrimp tissue, moisture content 86%. Atrazine-d5 was used as an Internal Standard. Samples were spiked at 10ng/g, 50ng/g and 100ng/g.**
2. **Add standards.**
3. **Vortex and allow to stand for at least 15 minutes.**
4. **Add 10mL of Acetonitrile to each sample.**
5. **Shake vigorously for 5 minutes.**
6. **Add 5g ammonium formate to each sample to induce phase separation.**
7. **Shake vigorously for 1 minute.**
8. **Centrifuge at room temperature for 2 minutes @ 4150rpm (3711rcf).**

Clean-up:

1. **75mg of sorbent + 0.5mL of extract is added to the Thomson eXtractor3D|FV shell.**
2. **Partially depress the Thomson eXtractor3D|FV plunger with .2 µm membrane into the shell.**
3. **Shake for 30 seconds.**
4. **Completely depress the plunger into the shell to filter the sample and analyze.**

eXTRACTOR3D|FV

PVDF .2µm
95531

Equipment Conditions:

LPGC/MS/MS *(low pressure GC)* – Agilent 7890A

- **220V fast oven heating upgrade**
- **Column – Restek non-coated restrictor column & a Supelco SLBTM-5ms, 15m x 0.53mm x 1µm film thickness**
- **Vacuum outlet – 5.5m x 0.18mm i.d.**
- **Constant Flow – 2mL/min**
- **Carrier Gas – He**
- **Oven Temperature – 70°C for 1.5 minutes**
- **Injection – 5µL**

LC/MS/MS – Agilent 1100 HPLC coupled to a Applied Biosystems API 3000 MS/MS

- **Electrospray ionization in positive mode**
- **Source temperature: was set to 525°C**
- **Column: Phenomenex Reverse Phase Prodigy ODS3 column, 150mm x 3.0mm x 5µm particle size**
- **Column Temperature: maintained @ 30°C**
- **Flow rate: 0.3mL/min**
- **Mobile Phase:**

A: 0.1% aqueous formic acid

B: 100% Acetonitrile

Time (min)	%A	%B
Initial	70	30
8.0	70	30
12.6	0	100

Results:

This approach to streamlining the QuEChERS protocol for the analysis of shellfish by combining the dispersive, sorbent and sample filtration into one vial, Thomson eXtractor3D|FV 0.2µm PVDF membrane, saves time, uses less solvent, and does not require special equipment. Table 1 shows the overall average recoveries of the 59 analytes in shrimp using different sorbents (n = 9 from triplicate spikes each at 10, 50, and 100 ng/g). Atrazine-d5 was used as the internal standard in both LPGC-MS/MS and HPLC-MS/MS. Recoveries of 13 of the 59 analytes were recovered at 100% while 42 of the 59 pesticides and contaminants tested were >70% with < 20% RSD independent of the sorbent used. The following pesticides were partially recovered depending on degradation, extraction partitioning factors, and specific sorbent used. Detection limits were < 5ng/g (with the exception of PCB's)

Table 1: Overall average recoveries (and RSD) of the 59 analytes in shrimp using the 3D eXtractor filter vial d-SPE approach with different sorbents.

#	Analyte	$MgSO_4$ + filter %Rec. (%RSD)	$MgSO_4$ + PSA + C18 + Z-Sep + filter	$MgSO_4$ + PSA + C18 + Z-Sep + CarbonX* + filter
1	Aldicarb*	99 (9)	104 (11)	103 (2)
2	Aldicarb sulfone*	97 (6)	96 (9)	97 (6)
3	Atrazine	103 (3)	103 (4)	104 (4)
3	Atrazine*	102 (4)	103 (4)	101 (6)
4	Azoxystrobin	106 (9)	105 (14)	97 (12)
4	Azoxystrobin*	110 (4)	115 (3)	113 (3)
5	Carbaryl*	103 (4)	106 (4)	85 (7)
6	Carbofuran	109 (12)	94 (17)	99 (12)
6	Carbofuran*	104 (2)	109 (5)	109 (4)
7	Chlordane	108 (6)	102 (12)	104 (10)
8	Chlorpropham	102 (11)	97 (14)	95 (14)
9	Chlorpyrifos	110 (7)	105 (11)	101 (11)
10	Cyazofamid*	102 (5)	108 (5)	111 (4)
11	Cypermethrin	113 (7)	107 (14)	102 (9)
12	o,p'-DDE	102 (7)	99 (11)	101 (11)
13	Deltamethrin	111 (10)	107 (15)	100 (16)
14	Diazinon	106 (9)	102 (12)	103 (11)
15	Dimethoate*	104 (6)	106 (7)	104 (7)
16	Endosulfan sulfate	112 (8)	113 (6)	115 (8)
17	Ethoprophos	103 (11)	91 (15)	91 (14)
18	Ethoprophos*	102 (5)	101 (4)	101 (8)
19	Fenthion	108 (4)	108 (6)	100 (5)
20	Fenthion sulfone	109 (7)	111 (8)	103 (5)
21	Imidacloprid*	100 (9)	106 (7)	98 (6)
22	Lindane(γ-HCH)	87 (7)	82 (15)	83 (13)
23	Linuron*	99 (7)	106 (6)	87 (9)
24	Methidathion	107 (3)	106 (6)	106 (6)
25	Methidathion*	107 (4)	112 (4)	111 (3)
26	Phosmet	118 (4)	106 (7)	91 (5)
27	Phosmet*	107 (5)	111 (6)	99 (7)
28	Pirimiphos-methyl	111 (7)	106 (11)	105 (11)
29	Pyriproxyfen	104 (6)	104 (6)	95 (3)
30	Pyriproxyfen*	106 (10)	114 (4)	98 (4)
31	Tetrahydrophthalimide	99 (15)	90 (12)	93 (11)
32	Tolclofos-methyl	101 (10)	98 (11)	90 (14)
33	Triflumizole	94 (4)	95 (6)	90 (6)
34	Triflumizole*	107 (5)	103 (6)	99 (5)
	Environmental Contaminants			
35	PBDE 47	108 (10)	102 (17)	93 (10)
36	PBDE 99	103 (9)	94 (11)	70 (9)
37	PBDE 100	110 (10)	102 (8)	89 (8)
38	PCB 105	101 (8)	92 (15)	79 (7)
39	PCB114	99 (11)	98 (12)	82 (9)
40,41	PCB118+123	98 (8)	91 (11)	80 (9)
42	PCB156	105 (8)	97 (14)	78 (14)
43	PCB157	100 (12)	96 (12)	76 (8)
44	PCB167	99 (11)	95 (12)	77 (8)
	Internal Standards			
45	Atrazine-d5	84 (5)	80 (10)	76 (6)
46	Atrazine-d5*	84 (9)	80 (5)	80 (6)
47	Fenthion-d6	86 (7)	81 (11)	72 (8)

***a* HPLC-MS/MS results.**

Streamlined sample cleanup using combined dispersive solid-phase extraction and in-vial filtration for analysis of pesticides and environmental pollutants in shrimp L. Han, Y. Sapozhnikova, S.J. Lehotay, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.04.005

Conclusions:

The results clearly show the Thomson eXtractor3D|FV 0.2µm PVDF membrane approach to sample preparation using QuEChERS in an autosampler ready vial for partitioning, clean-up and filtration of shellfish is a fast and convenient method. This method lowers cost, solvent usage, and time. The Thomson eXtractor3D|FV yielded recoveries of 42 of the 59 pesticides and contaminants tested with >70% and < 20% RSD in shrimp. Future experiments will include optimization of dispersive and sorbent concentrations.

Filter Vial

eXTREME|FV

eXTRACTOR3D|FV

THOMSON MADE IN USA

Materials and Methods II - *Improved Pesticide Recovery in Juice and Tea*

Provided by : Micro Quality Labs Inc, Burbank, CA - Uday Sathe & Karine Aylozyan

This method investigates whether SPE is required for the analysis of pesticides in high pulp orange juice and green tea. 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 Orange Juice and Green Tea:

Extraction:

1. **A. Spike 10mL of commercially available High Pulp Orange Juice with 1mL of 1 ppm pesticide standard mix containing 87 pesticides in a 40mL vial for a final concentration of 0.100 ppm.**

   B. Spike 2.0g of commercially available Green Tea 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.

2. **Add one pack *(approximately 6g)* of Restek Extraction Salts *(Restek catalog # 26236)* to the spiked orange juice.**
3. **Extract the spike 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 10 mL 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".**

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.

Clean-up:

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

1. **Wash one Restek 6 mL Combo SPE Cartridge (packed with 200 mg CarboPrep 200 and 400mg PSA Resek 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.**
5. **Filter sample with a syringe and syringe filter, PTFE 0.45µm and elute into autosampler vial.**

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.**

Results:

The results for the orange juice can be seen in Table 2, Pesticides in Orange Juice Comparison of SPE to eXtreme Filter Vials, and Table 3, Pesticides in Green Tea Comparison of SPE to eXtreme Filter Vials, 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 juices and tea leaves, specifically orange juice and green tea, for the clean-up of samples prior to pesticide analysis.

Fig 2, Pesticides in Green Tea Comparison of SPE to eXtreme Filter Vials.

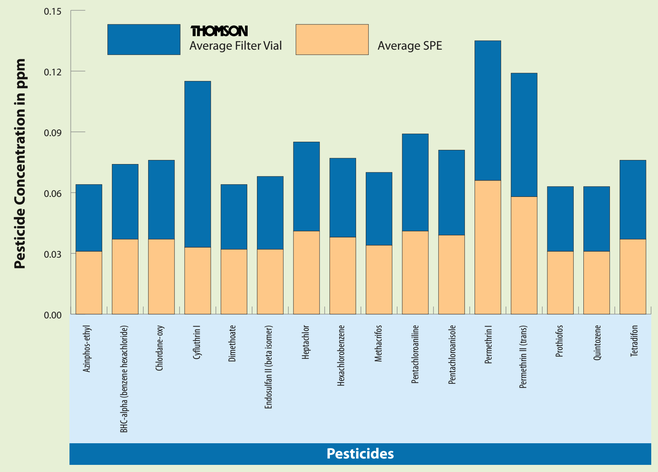


Table 2: *Pesticides in Green Tea Comparison of SPE to eXtreme Filter Vials.*

Compound/Sample Name	SPE Clean-up Average ppm	eXtreme|FV® 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

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

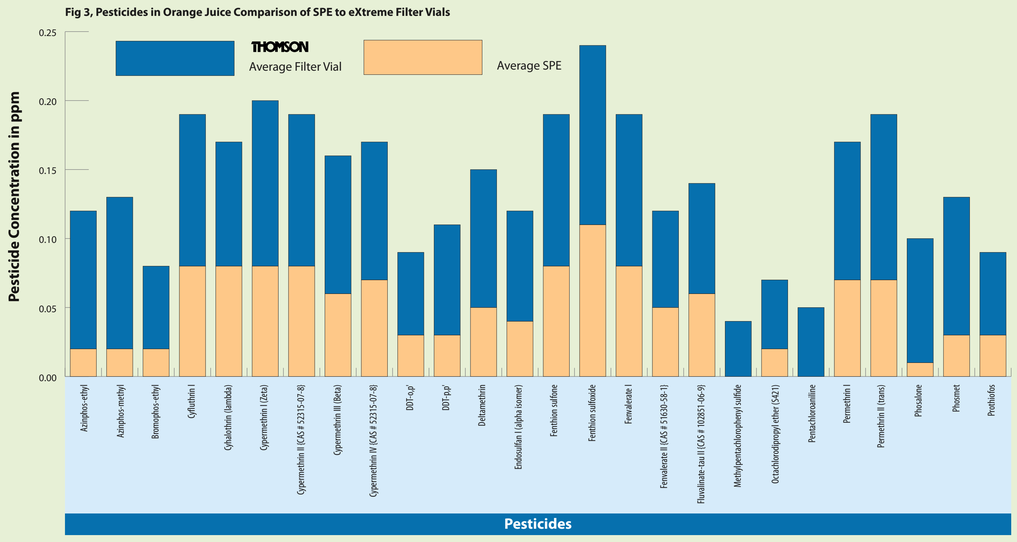


Table 3: *Pesticides in Orange Juice Comparison of SPE to eXtreme Filter Vials*

Compound/Sample name	SPE+ Routine Syringe Filter PPM	Only Extreme| FV w/o SPE 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 (CAS # 52315-07-8)	0.08	0.113
Cypermethrin III (Beta)	0.058	0.104
Cypermethrin IV (CAS # 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 (5421)	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

Conclusions:

The results clearly show Thomson eXtreme Filter Vials offer a viable alternative with higher recovery and less preparation time compared to sample clean-up with SPE for the preparation of juices and tea leaves, specifically orange juice and green tea samples prior to pesticide analysis. The Thomson eXtreme 0.45µm, PTFE Filter Vials patented (Thomson # 85540-500) yielded 26% higher recoveries on average when tested with 87 different common pesticides (Table 1). In the cases highlighted in the results table, greater than 428% increases in recovery was 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. Future testing is required to further streamline this method by re-evaluating the extraction procedure specifically the need for the concentration/re-suspension steps.

eXTREME|FV

PTFE .45µm
85540

Materials and Methods III – *Improved Method for the Analysis of Hexavalent Chromium in Water*

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

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

Filter Vial

PTFE .45µm
35540

Equipment Conditions:

- **Varian ProStar 210 HPLC**
- **Varian 820MS ICP-MS**
- **Pump Rate (rpm): 20**
- **Stablization delay(s): 0**
- **Skimmer Gas Source: H_2**
- **Skimmer Flow: 30**

Column:

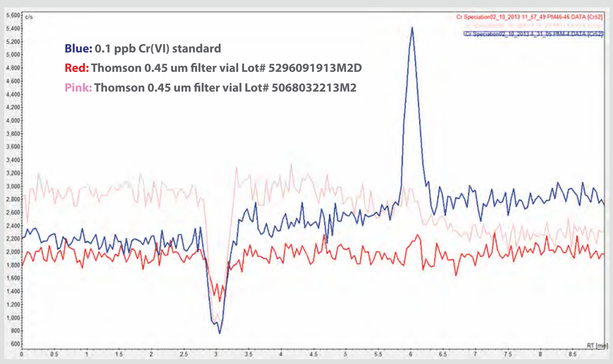
- **Hamilton PRP-X100 Anion Exchange Column & Guard Column**
- **Mobile Phase:**
- **Mobile Phase A: 100mM/L Ammonium Nitrate, pH ≥ 9, pH adjust with 16N Nitric Acid**
- **Mobile Phase B: DI Water, pH ≥ 9, pH adjust with Ultra Pure Ammonium Hydroxide**

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

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.

Conclusion

The methods presented here for the analysis of contaminants in food sources show the Thomson Filter Vials compared to traditional methods of clean-up, including SPE, liquid-liquid extraction and syringe filtration. The results of simplifying the traditional QuEChERS Method for pesticide analysis in shrimp clearly show the Thomson eXtractor3D (patented) PVDF 0.2 µm (95531-500) is a fast and convenient approach to sample preparation in an autosampler ready vial for partitioning, clean-up and filtration of shellfish. Simply add the sample, dispersive salt, and sorbent to the outer shell of the Thomson eXtractor3D, vortex, filter and analyze. No vacuums or centrifuges.

For sample clean-up, post extraction, in the analysis of pesticides in both orange juice and green tea, the Thomson eXtreme Filter Vials (patented) PTFE 0.45 µm (85540-500) showed improved recovery of many of the pesticides. 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 in both orange juice and green tea. Simply add the extracted sample to the eXtreme Filter Vial, filter and analyze.

In the analysis of Hexavalent Chromium, the Thomson Standard Filter Vials (patented) PTFE 0.45 µm (35540-500) showed no chromium contamination in the vial or membrane materials and reproducible analysis at 0.1 ppb. Thomson Standard Filter Vials replace 4 part numbers: syringe, syringe filter, autosampler vial and cap.

What do all these methods have in common? **All of the Thomson Filter Vials (patented) formats keep in the QuEChERS theme of simplifying processes, lowering costs, and time savings.**

TIC-PL-082-266 Rev. A