Improved Sample Clean-up Options for Contaminant Analysis for Vegetation, Meats & Seafood

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, 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 (which need to be concentrated). Several studies were undertaken to investigate whether four different types of filter vial designs offered improved clean-up methods.

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Method I- 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 0.2 µm PVDF membrane into the shell.**
3. **Shake for 30 seconds.**
4. **Completely depress the plunger into the shell to filter the sample and analyze.**

Equipment Conditions:

LPGC/MS/MS – 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 an 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)

#	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)

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

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.

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.

Method II- Multiclass multiresidue analysis of >100 veterinary drug residues in bovine tissues by filter-vial dispersive-SPE and LC-MS/MS

Steven J. Lehotay, Marilyn J. Schneider, and Alan R. Lightfield
U. S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center;
600 East Mermaid Lane; Wyndmoor, PA 19038; USA

High-throughput analysis is needed to meet demand for monitoring of veterinary drug residues in food animal tissues. The current veterinary drug residue monitoring method used by the USDA Food Safety and Inspection Service (FSIS) uses a combination of hexane-partitioning, dispersive-SPE, and solvent evaporation to achieve adequate cleanup for 20 mg equivalent sample injections to meet regulatory detection limit needs. This extra effort adds to the time and cost of the method and limits sample throughput. An improved method is developed and validated to streamline the sample preparation and LC-MS/MS method for identification and quantification of >120 veterinary drugs in bovine animal tissues for use in high throughput monitoring in the FSIS National Residue Program.

Experimental

Sample Preparation and Analysis

1. **Weigh 2g homogenized tissue sample into 50mL tube. Add internal standard and spike solutions as needed.**
2. **Dispense 10 mL 4/1 (v/v) MeCN/water and shake 5min on a platform shaker, then centrifuge 5min at 3700rcf.**
3. **Transfer 0.4 mL extracts to filter-vial shell containing 25 mg C18, insert plunger halfway and shake 30s, then fully depress plunger to filter final extract into autosampler vial.**
4. **Inject 1µL in LC-MS/MS.**

Equipment

LC: Agilent 1100

MS/MS: AB Sciex 6500 Q-Trap, Electrospray ionization (positive/negative switching)

Column: Phenomenex Kinetex C18 (50 x 3.0mm, 2.6µm)

Mobile phase: (A) water; (B) MeCN both with 0.1% HCO_2H

Gradient: 2% A 100% B over 8.0min, hold for 2.7min

Flow rate: 0.3 mL/min

Column temperature: 40°C

Validation and Results

- Spikes made of 18 different tissue blank samples at 0X, 0.5X, 1X, and 2X levels (n=10 each) repeated 3 days by 3 chemists
- Matrix-matched and reagent-only calibration stds prepared at equiv. tissue levels of 0X, 0.25X, 0.5X, 1X, 2X, and 3X
- Internal standards were added, but not needed nor used
- Method LOQs determined for spiked samples in matrix

Updated method logistics compared to previous

FSIS Method Logistics (UPLC-TQD)

1 chemist was able to process 60 pre-homogenized samples in an 8-hr day for an overnight sequence

(longest step was 1 hr to evaporate MeCN)

No glassware to be cleaned afterwards

Cost of materials = $3/sample (using bulk C18)

Waste = 10 mL hexane and 5 mL MeCN & two 50 mL, one 15 mL PP tubes, and AS vial

Geis-Asteggiante et al., J. Chromatogr. A, 1258 (2012) 43-54 and Lehotay et al., Drug Test. Analysis, 4 (Suppl. 1) (2012) 75-90 and USDA-FSIS Analytical Chemistry Guidebook online

New Method Logistics

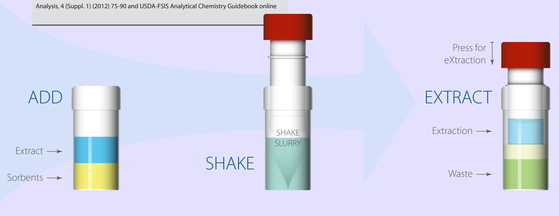
1 chemist was able to process 60 pre-homogenized samples in 3 hours

(longest steps involved labeling tubes/vials, weighing, and preparing calibration standards)

No glassware to be cleaned afterwards

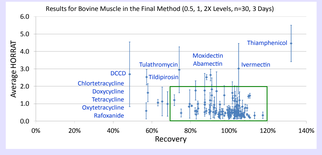
Waste = 10 mL MeCN and one 50 mL tube and an autosampler vial

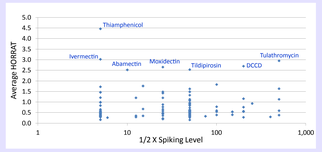
Review of results for 135 drugs x 3 transitions x 67 injections (>27,000 data points) took 8 hours



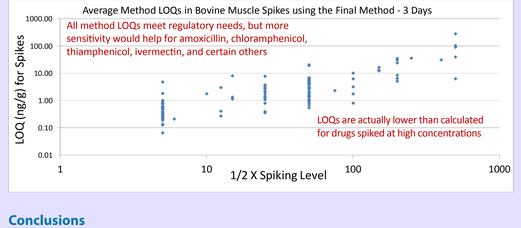
Among-Day Reproducibility of Recoveries

HorwitzRatio = HORRAT = $RSD_R/(2C^{-0.1505})$ in which RSD_R is reproducibility and C is concentration (g/g)





Limits of Quantification (LOQs) for the Final Method



Conclusions

- **Our previous MRM for vet drug residues was streamlined and improved by using modern LC-MS/MS and eliminating hexane partitioning cleanup and solvent evaporation steps, and using filter-vial d-SPE for cleanup**
- **This method is more quantitative than our previous method**
- **LOQ <10 ng/g for nearly all drug analytes tested**
- **Sample throughput was 60 samples per day per 2 chemists for both sample prep and analysis/review of results**
- **Qualitative screening and identification results still need to be evaluated for method implementation by USDA-FSIS**

Method III- Routine Targeted Quantitation and Identification of Pesticide Residues using Triple Quadrupole LC-MS/MS and Advanced Scheduling of MRM Transitions Detection of pesticides in filtered QuEChERS extracts of Avocado Carrot, Grape, and Spinach

André Schreiber1, Julia Jasak2
1 AB SCIEX, Concord, ON (Canada); 2 AB SCIEX, Darmstadt (Germany)

Experimental

Sample Prep:

1. **SCIEX iDQuant™ standards kit for Pesticide Analysis**
2. **Store bought fruit & vegetables were extracted using European Standard Method 15662**
3. **Extracts were diluted 5x with water in Thomson Filter Vials, 0.45 µm PVDF membrane**

Equipment Conditions:

- **AB Sciex Triple Quad™ 3500 with Turbo V™ source and Electrospray Ionization**
- **Positive Polarity**
- **Column:**
  - **Phenomenex Kinetex® Biphenyl 2.6 µm column**
- **Mobile Phase:**
  - **Fast gradient of water/methanol with 5mM ammonium formate**
  - **Flow rate: 0.5mL/min**
- **Injection 2 µL**

Results

- **Average gain in sensitivity of 3x was observed**
- **Most pesticides had a detection limit of < 1ng/mL**
- **All pesticides had a detection limit of < 2ng/mL**
- **Chromatograms at concentration of 5ng/mL:**

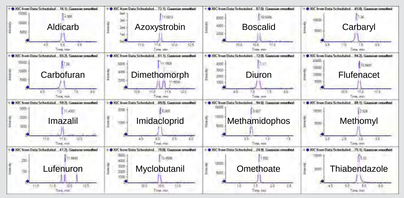


Fig 1. Sensitivity of selected pesticides detected at a concentration of 5 ng/mL using the Triple Quad™ 3500 system

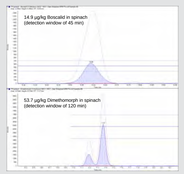


Fig. 2 Spinach sample showing 14.9 µg/kg Boscalid and 53.7 µg/kg both isomers of Dimethomorph. Examples of using the Flexible Window Width in a Scheduled MRM™ Pro method: the window for Boscalid was set to 45 sec and Dimethomorph was detected using a wider window to detect both isomers together

Examples of using the Flexible Window Width in a Scheduled MRM™ Pro method: the window for Boscalid was set to 45 sec and Dimethomorph was detected using a wider window to detect both isomers together

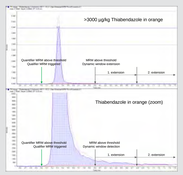


Fig. 3 Orange sample shows an example of MRM-triggered MRM and Dynamic Window Extension: the qualier MRM transition is automatically triggered when the quantier MRM transitions exceeds the threshold set in the Scheduled MRM™ Pro method, the detection window is automatically extended if the MRM signal is above the threshold at the end of the detection window

Examples of MRM-triggered MRM and Dynamic Window Extension: the qualifier MRM transition is automatically triggered when the quantifier MRM transitions exceeds the threshold set in the Scheduled MRM™ Pro method, the detection window is automatically extended if the MRM signal is above the threshold at the end of the detection window

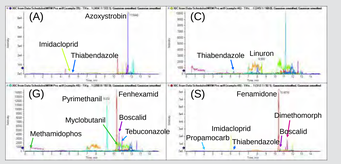


Fig 4. Detection of pesticides in filtered QuEChERS extracts of avocado (A), carrot (C), grapes (G), and spinach (S) Quantitation and identification based on MRM ratios in MultiQuant™ software, the example shows the side-by-side peak review for Boscalid with positive findings in grapes and spinach samples

Fig 5. Quantitation and identification based on MRM ratios in MultiQuant™ software, the example shows the side-by-side peak review for Boscalid with positive findings in grapes and spinach samples

Conclusions

- **The developed method was applied to the quantitation and identification of pesticides in real food extracts.**
- **Following the European Standard Method 15662, different dispersive SPE kits from Phenomenex were used for sample cleanup depending on the type of matrix.**
- **Extracts were diluted 5 times with water to minimize possible matrix effects directly in the Thompson eXtreme|FV® 0.45 µm PVDF membrane part # 85541-500 and placed into the autosampler for LC-MS/MS analysis.**