Improved Sample Prep of Fruit & Juices for Analysis by Mass Spectrometry Using Thomson Filter Vials

Routine Targeted Quantitation & Identification of Pesticide Residues in Spinach & Carrot using the eXtreme|FV® by LC-MS/MS

A. Schreiber, AB SCIEX, Concord, ON, Canada; J. Jasak, AB SCIEX, Darmstadt, Germany. "Routine Targeted Quantitation and Identification of Pesticide Residues using Triple Quadrupole LC-MS/MS and Advanced Scheduling of MRM Transitions" Poster presented as part of NACRW-FPRW Conference, St. Petersburg, FL., 20-23 July 2014.

Introduction

LC-MS/MS is a powerful analytical tool capable of screening samples for numerous compounds. MRM is typically used because of its excellent sensitivity, selectivity, and speed. Using QuEChERS for extraction, eXtreme|FV®s for clean-up, and UHPLC combined with core-shell particles columns provides good resolution and excellent peak shape, making it possible to detect hundreds of pesticides of a wide variety of compound classes and chemical properties in each sample. The new AB SCIEX Triple Quad™ 3500 with a Turbo V™ source and Curtain Gas™ interface supplies exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for unparalleled speed of MRM detection and fast polarity switching for comprehensive multi-component analysis. The method combines QuEChers for extraction, Thomson eXtreme|FV®s for clean-up, and the Sciex Scheduled MRM Pro Algorithm for identification of pesticides in fruit and vegetables analysis.

Equipment

- AB Sciex Triple Quad™ 3500 with Turbo V™ source and Electrospray Ionization Positive Polarity
- Column: Phenomenex Kinetex™ Biphenyl 2.6µm column
- Mobile Phase: Fast gradient of Water/Methanol with 5mM Ammonium Formate
  - Flow rate: 0.5mL/min

Step	Time(min)	A(%)	B(%)
0	0.0	90	10
1	0.5	90	10
2	2.0	70	30
3	9.0	40	60
4	11.0	20	80
5	12.0	5	95
6	15.0	5	95
7	16.0	90	10
8	20.0	90	10

Sample Preparation

1. Store-bought spinach & carrot is extracted using dispersive SPE following the European Standard Method 15662
2. SCIEX iDQuant™ standards kit are used for Pesticide Analysis
3. Extracts are diluted and filtered 5x with water in Thomson eXtreme|FV®, 0.45µm PVDF membrane

Results

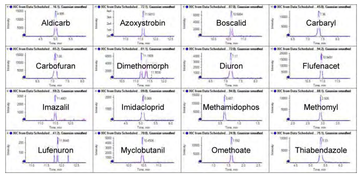


Fig 1. Sensitivity of selected pesticides detected at a concentration of 5ng/mL using the Triple Quad™ 3500 system (click image for larger)

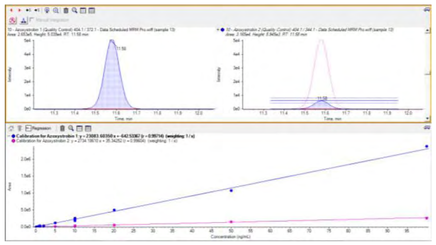


Fig 2. Calibration line of Azoxystrobin from 0.1 to 100 ng/mL (top) and quantifier-qualifier ratio for compound identification (bottom).

Sample	Pesticide	Concentration (µg/kg)	MRM Ratio (Expected Ratio)
Avocado	Azoxystrobin	55.0	0.146 (0.126)
	Imidacloprid	6.2	0.823 (0.818)
	Thiabendazole	2.9	1.035 (0.820)
Carrot	Linuron	14.3	0.613 (0.742)
	Thiabendazole	5.3	0.995 (0.820)
Grapes	Boscalid	17.3	0.240 (0.242)
	Fenhexamid	363	0.973 (1.053)
	Methamidophos	1.2	0.873 (0.698)
	Myclobutanil	14.2	0.811 (0.830)
	Pyrimethanil	687	0.482 (0.435)
	Tebuconazole	7.1	0.030 (0.261)
Orange	Imazalil	1830	0.282 (0.348)
	Thiabendazole	>3000	0.812 (0.820)
Spinach	Boscalid	12.3	0.264 (0.242)
	Dimethomorph	53.7	0.537 (0.541)
	Fenamidone	755	0.749 (0.672)
	Imidacloprid	217	0.907 (0.993)
	Propamocarb	3.1	0.260 (0.336)
	Thiabendazole	3.6	0.917 (0.820)

Conclusion

- The AB Sciex Triple Quad 3500 was used for pesticide residue identification and quantification in store-bought fruit and vegetables.
- Method combines QuEChers extraction, Thomson eXtreme|FV®s, Phenomenex Kintex Biphenyl Column, and the Sciex Scheduled MRM Pro Algorithm.
- Average gain in sensitivity of 3x was observed, with most pesticides having an LOD of < 1ng/mL.

Screening and Quantitation of 250 Pesticides in Apple & Grape Juice using the eXtreme|FV® 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 apple juice using only one method in store-bought juice and simple sample preparation using the Thomson eXtreme|FV®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 eXtreme|FV®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:

Time	%A	%B	Flow (µL/min)
0.00	90	10	400
0.20	90	10	400
2.00	30	70	400
10.0	0	100	400
15.0	0	100	400
15.1	90	10	500
18.0	90	10	400

Sample Preparation

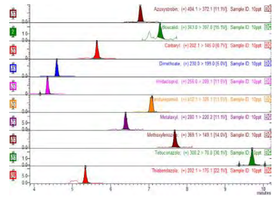
1. Pipette 50µL of store-bought apple juice and 450µL of solvent (10% Methanol/ 90% Water) directly into the outer shell of Thomson eXtreme|FV®, 0.2µm PVDF.
2. Partially depress the eXtreme|FV® plunger and vortex.
3. Depress the completely and load onto the autosampler.

Results

Table 1. Store bought fruit juice test results.

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

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



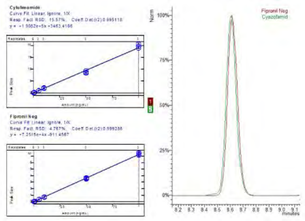
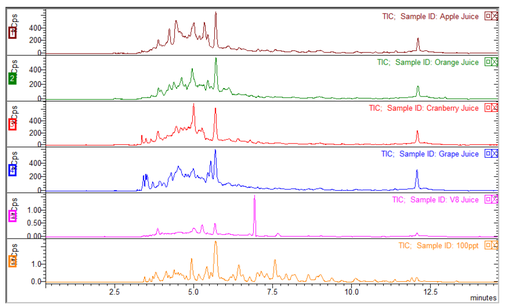


Fig 2. Calibration curve of negative pesticide Fipronil (left top) and positive pesticide Cyazofamid (left bottom), and their co-eluting plots (right).

Fig 3. Total Ion Chromatograms for all juices



Conclusion

- The calibration on triplicate injections showed excellent linearity and response factor RSD over 3 orders, range using the Thomson eXtreme|FV® for sample preparation.
- Good linearity, sensitivity and response factor, RSD for positive and negative co-eluting pesticides.
- Pesticides were detected in store-bought apple, orange, cranberry and vegetable juices.

Comparison of eXtreme Filter Vials vs SPE for Improved Pesticide Recovery in Orange Juice by GC/MS

Uday Sathe, Karin Aylozyan @ Micro Quality Labs *Poster presented as part of NACRW-FPRW Conference, St. Petersburg, FL., 20-23 July 2014.*

Abstract

Pesticides are toxic when found in sufficient quantities as pesticide residue in food. This is of particular importance for orange juice because it is consumed in high quantities by children. In order to reduce the risk to consumers, sensitive, rapid, and cost effective analytical methods are required. The pesticides in orange juice testing shown below compares recoveries for samples prepared using SPE versus Thomson eXtreme Filter Vials

Solid Phase Extraction (SPE) is a common sample preparation technique prior to GC or LC analysis of pesticides in food. Typically, SPE is used to concentrate analytes, to reduce interference from co-eluting compounds, 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, many labs are investigating 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. Prior to the introduction of eXtreme Filter Vials, some samples containing high level of particulates could only be "filtered" by SPE. 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. Since the filtration process from sample pipetting to filtered sample in an autosampler ready vial only requires 15 seconds and the multi-layered filtration membrane can filter samples with high particulate content, we have found many applications where the SPE step in a method can be replaced with a much faster and lower cost eXtreme Filter Vial step.

Sample Preparation

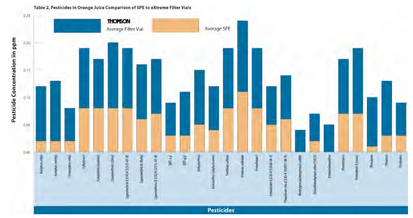
1. Spike 10 mL of commercially available High Pulp Orange Juice with 1 mL of 1 ppm pesticide standard mix containing 87 pesticides in a 40 mL vial.
2. Add one pack (approximately 6 g) of Restek Extraction Salts (Restek catalog # 26236) to the spiked orange juice.
3. Extract the spike orange juice with 4 x 25 mL 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 5 mL portions.
8. Dilute each 5 mL portion with acetonitrile to 10 mL using a volumetric flask.
9. Label one flask "for SPE" and the other "for Thomson eXtreme Filter Vial".

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

Filter Vial Cleanup Prior to Analysis - Thomson eXtreme|FV®

1.Add 400µL of the re-suspended residue from the flask labeled "for Thomson eXtreme|FV" to the shell of one Thomson eXtreme|FV® 0.45µm,
2. Insert plunger completely.



Equipment

Agilent Technologies GC/MS, 7000 Triple Quad System
Agilent 7890A GC System
Agilent 7693 Autosampler.

Results

Compound NAME	SPE+ Syringe FILTER Average ppm	EXTREME| FV Average ppm
Azinphos-ethyl	0.018	0.095
Azinphos-methyl	0.023	0.115
Bromophos-ethylBHC-beta	0.025	0.057
BromopropylateBHC-delta	0.063	0.076
Cyfluthrin	0.082	0.113
Cypermethrin	0.082	0.117
Cypermethrin III	0.058	0.104
Cypermethrin IV	0.070	0.097
DDT-o,p'DDD-p,p'Cypermethrin III (Beta)	0.035	0.065
DDT-p,p'DDE-o,p'Cypermethrin IV	0.032	0.078
Deltamethrin DDE-p,p'DCPA	0.053	0.102
Diazinon DDT-o,p'DDD-o,p'Cyhalothrin (lambda)	0.028	0.035
Dicofol DDT-p,p'DDD-p,p'Cypermethrin I (Zeta)	0.033	0.028
Dieldrin Deltamethrin DDE-o,p'Cypermethrin II	0.041	0.052
DimethoateDiazinonDDE-p,p'Cypermethrin III (Beta)	0.061	0.077
Endosulfan I (alpha isomer)	0.041	0.076
Fenvalerate I	0.076	0.106
Fenvalerate	0.055	0.073
Fluvalinate-tau II	0.058	0.084
Methylpentachlorophenyl sulfide Malathion Heptachlor endo-epoxide	0.001	0.036
Octachlorodipropyl ether (S421)Methidathion Hexachlorobenzene	0.021	0.047
Pentachloroaniline Parathion Mirex Hexachlorobenzene	0.002	0.049
Permethrin I	0.068	0.097
Phosalone Pentachloroanisole Parathion Methidathion	0.005	0.089
Phosmet Permethrin I	0.031	0.104
Prothiofos Pirimiphos	0.033	0.060

Conclusion

- The Thomson eXtreme 0.45µm, PTFE Filter Vials yielded 26% higher recoveries on average when tested with 87 different common pesticides .
- 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| FV.
- The results show Thomson eXtreme|FV offer a viable alternative with higher recovery and less preparation time compared to SPE for the preparation of juices, and specifically orange juice samples prior to pesticide analysis .
- Conforms to USP 561