THOMSON Solutions ™ At Work
INSTRUMENT COMPANY

series	cap color	membrane	pore size	part #
eXtreme|FV®	●	PVDF	0.2µm	85531

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

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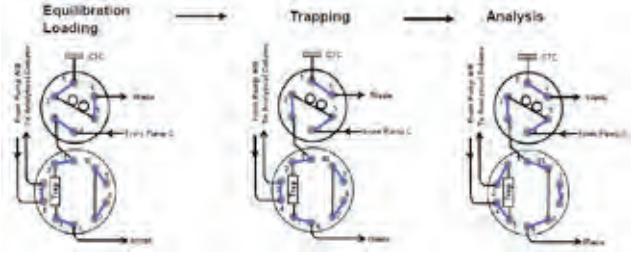
Introduction

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 eXtreme|Filter Vials 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 eXtreme|FV. The honey was diluted 10-fold and filtered with 0.2um PVDF eXtreme|FV 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

LC Gradient			
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate (µL/min)
0.0	90	10	400
0.2	90	10	400
2.0	30	70	400
6.5	20	80	400
8.0	0	100	400
15.0	0	100	400
15.1	90	10	400
18.0	90	10	400

- OLE Valves Configuration
- EVOQ Conditions

Source parameters	
Source:	HESI
Spray Voltage (+/-)	4000V
Cone Gas Flow	20
Cone Temperature	250˚C
Heated Probe Gas Flow	45
Heated Probe Temperature	400˚C
Nebulizer Gas Flow	65
Exhaust Gas	On

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 1. Example of Mass Spec Pesticide MRM Set-up

	Name	Retention Time	RT Window	CAS Number	Retention Index	Scan Type	Scan Time (ms)	Polarity
1	2,3,5_trimethacarb	7.39	1.00		0	MRM	24.7	Positive
2	2,4-D	6.24	1.00		0	MRM	24.7	Negative
3	2,6 dichlorbenzamide	4.86	1.00		0	MRM	23.0	Positive
4	3-Hydroxycarbofuran	5.05	1.00	16655-82-6	0	MRM	23.0	Positive
5	Abamectin	13.62	1.00	71751-41-2	0	MRM	47.6	Positive
6	Acephate	4.16	1.00	30560-19-1	0	MRM	23.8	Positive
7	Acetamiprid	5.10	1.00	135410-20-7	0	MRM	23.0	Positive
8	Aldicarb sulfone	4.40	1.00	1646-88-4	0	MRM	23.0	Positive
9	Aldicarb sulfoxide	4.28	1.00	1646-87-3	0	MRM	23.0	Positive
10	Ametryn	8.52	1.00	834-12-8	0	MRM	19.6	Positive
11	Aminocarb	6.33	1.00	2032-59-9	0	MRM	24.7	Positive
12	Atrazine	7.39	1.00		0	MRM	24.7	Positive
13	Azoxystrobin	7.74	1.00	131860-33-8	0	MRM	23.0	Positive
14	Benalaxyl	11.14	1.00	71626-11-4	0	MRM	24.7	Positive
15	Bendiocarb	6.11	1.00	22781-23-3	0	MRM	24.7	Positive
16	Benfuracarb	12.27	1.00	82560-54-1	0	MRM	23.8	Positive
17	Bentazone	5.02	1.00		0	MRM	23.0	Negative
18	Bifenazate	8.90	1.00	149877-41-8	0	MRM	19.6	Positive
19	Bitertanol	11.42	1.00	55179-31-2	0	MRM	23.8	Positive
20	Boscalid	8.32	1.00	188425-85-6	0	MRM	19.6	Positive
21	Bromuconazole isomer 1	9.35	1.00	116255-48-2	0	MRM	20.8	Positive
22	Bromuconazole isomer 2	10.69	1.00	116255-48-2	0	MRM	30.3	Positive
23	Bupirimate	10.42	1.00	41483-43-6	0	MRM	30.3	Positive
24	Buprofezin	12.48	1.00	69327-76-0	0	MRM	23.8	Positive
25	Butafenacil	8.96	1.00	134605-64-4	0	MRM	19.6	Positive

	Precursor	Product	Collision Energy	Q1 Resolution	Q3 Resolution	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	194.00	121.90	24.00	Standard (2.0	Standard (2.0	50.00%	✓	56.20%	
2	211.00	137.00	13.00	Standard (2.0	Standard (2.0	50.00%			✓
3									
4									
5									
6									
7									

Fig 1. Timed MRM Windows for 215 Pesticides

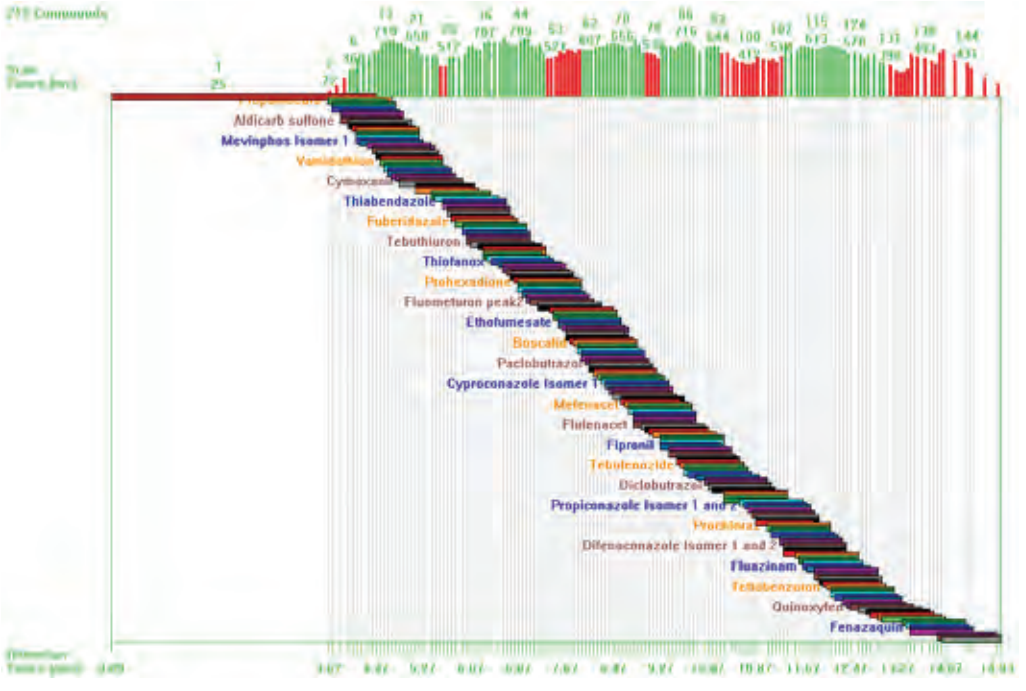


Table 2. Store bought honey from the US (3 different brands), Canada, China and India were analyzed for Pesticides analyzed in this method. Test result (ND= not detected or <0.1ppb).

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.

Fig 1. Examples of 9 point calibration curves.

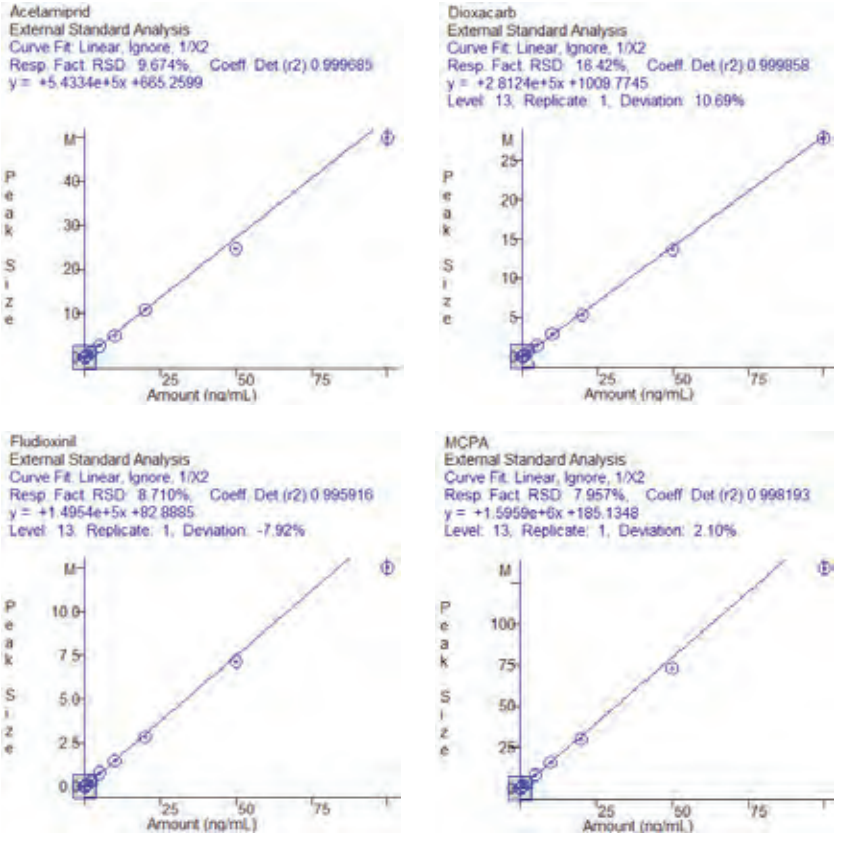


Fig 2. Chromatograms of pesticides spiked into honey, USA-1.