Screening and Quantitation of About 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 an integrated On-Line Extraction (OLE)-UHPLC-MS/MS system for analysis of pesticides in honey.

A study using the EVOQ analyzed about 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. The honey was diluted 10-fold and filtered before 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 and then the valve switched to couple the trap column with analytical column for separation and detection. The linear range was about 1 to 1000 ng/g and the linear regression co-efficiency R^2 was >0.99.

Sample Preparation

- Weigh about 50 mg of honey in the filter vial (Part number 85531-5, Thomson Instrument Company).
- Add solvent (MeOH/water, 50/50, v/v) or standard solution to make 100 mg/mL solution.
- Mix and press filter plunger (0.2 µm PVDF) to filter and ready for injection.

Fig. 1 EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker integrated On-Line Extraction-UHPLC and CTC Autosampler

Methods

Instruments:

EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker UHPLC and CTC Autosampler (see Fig. 1)

LC Parameters:

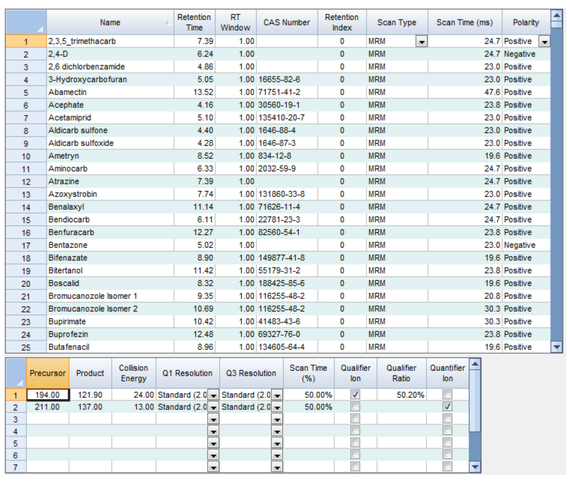
Trap Column: YMC-Pack ODS-AQ, 10 µm, 10 mm x 3.0 mm I.D.
Mobile Phase C: 5 mM ammonium fluoride (AF) in water
Equilibration flow: 1000µL (3.0 min)
Loading Flow: 600 µL
Analytical Column: YMC-Pack ODS AQ, 3 µm, 150 mm × 3.0 mm (I.D.)
Column Temperature: 40 °C
Injection Volume: 50 µL
Mobile Phase A: 5 mM AF in water
Mobile Phase B: MeOH

Gradient:

Time	%A	%B	Flow (µL/min)
0.0	90	10	400
0.2	90	10	400
1.5	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

MS Parameters:

Source: HESI
Spray Voltage (Positive):4000V
Spray Voltage (Negative):4000V
Cone Gas Flow:20-unit
Cone Temperature:250° C
Heated Probe Gas Flow:45-unit
Heated Probe Temperature:350°C
Nebulizer Gas Flow:65-unit
Exhaust Gas:On
Q2 pressure (Argon): 2.0 mTorr



	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	13.52	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	Bromucanozole Isomer 1	9.35	1.00	116255-48-2	0	MRM	20.8	Positive
22	Bromucanozole 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%	✓	50.20%	
2	211.00	137.00	13.00	Standard (2.0	Standard (2.0	50.00%			✓
3									
4									
5									
6									
7									

Fig. 2. MRM method for pesticides

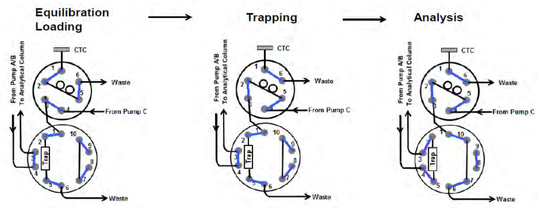


Fig. 3. OLE Valves configuration

Honey Source=>	India	Canada	China	USA-1	USA-2	USA-3
Pesticide				**ng/g**		
Acetamiprid	ND	ND	0.6	ND	ND	ND
Boscalid	ND	17.5	ND	ND	0.2	3.4
Carbaryl	ND	0.7	ND	ND	ND	ND
Dioxacarb	ND	ND	ND	ND	1.4	2
Fenpyroximate	ND	ND	ND	ND	0.3	55
Fludioxinil	ND	1.5	ND	ND	ND	ND
Fluometuron	ND	ND	ND	ND	ND	2.8
Hexaythiazox	ND	ND	0.2	ND	ND	ND
MCPA	ND	0.7	ND	ND	ND	ND
Metalaxyl	ND	0.1	ND	ND	ND	ND
Methoxyfenozide	ND	ND	ND	ND	ND	0.9
Picoxystrobin	ND	4.2	ND	ND	ND	ND
Piperonyl butoxide	ND	0.3	ND	0.6	0.8	0.2
Propargite	ND	0.3	ND	0.1	ND	ND
Thiamethoxam	ND	4.9	ND	ND	ND	ND

Table 1. Test result (ND= not detected or <0.1ppb)

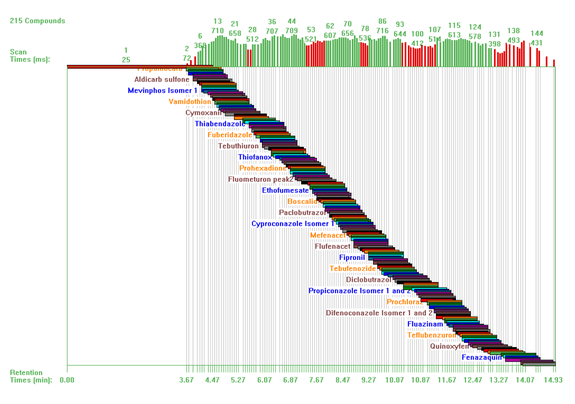


Fig. 4. Timed MRM windows for 215 pesticides

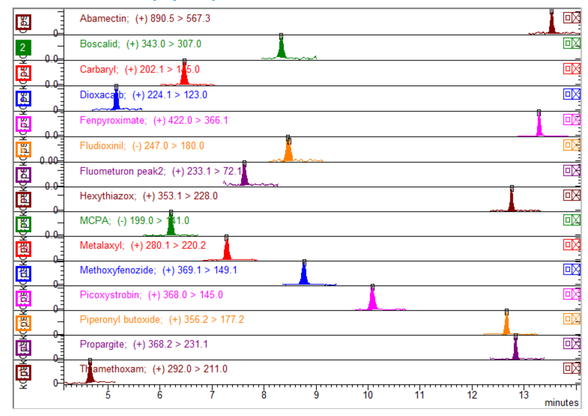


Fig. 6. Chromatograms of standard solution of the compounds listed in table 1 at 0.05 ng/mL (equivalent to 0.5 ng/g in Honey).

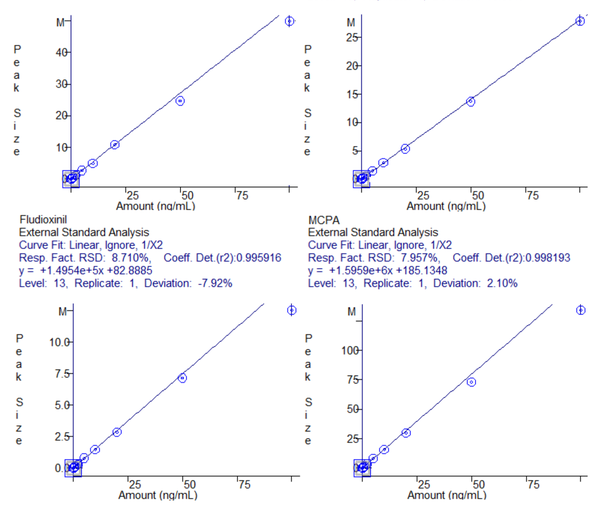


Fig. 5. Selected calibration curves for pesticides in table 1. Top and bottom curves are positive and negative pesticides, respectively. Standard solution linear range was 0.01 ng/mL to 100 ng/mL.

Results & Discussion

- Simple
  - dilute-filter-shoot.
- Sensitive
  - LOQ at 0.01 ng/mL for 158 pesticides <0.1 ng/mL
  - LOQ <0.1 ng/mL for others.
- Good retention time distribution and autocalculating scan time for each pesticide (fig 4).
- Single run for positive and negative pesticides with hundreds of MRM transitions.
- High concentration of sugar washed off from the trap column without getting into MS system.
- No peak shape change by injecting 50 µL solution containing 50% MeOH.
- High organic in sample solution helps to reduce pesticides binding to the plastic vial.
- Detected fifteen pesticides in honeys from different sources (table 1.).
- No detectable level of pesticides by the method in honey from India (table 1.).
- High level of Fenpyroximate detected in US source honey.

Conclusions

- Method is simple, sensitive, easy of use and single run for positive and negative pesticides.
- Bruker Advance UHPLC with OLE coupled to EVOQ LC-QQQ provides a more convenient and simpler approach than the SPE to analyze pesticides in honey.