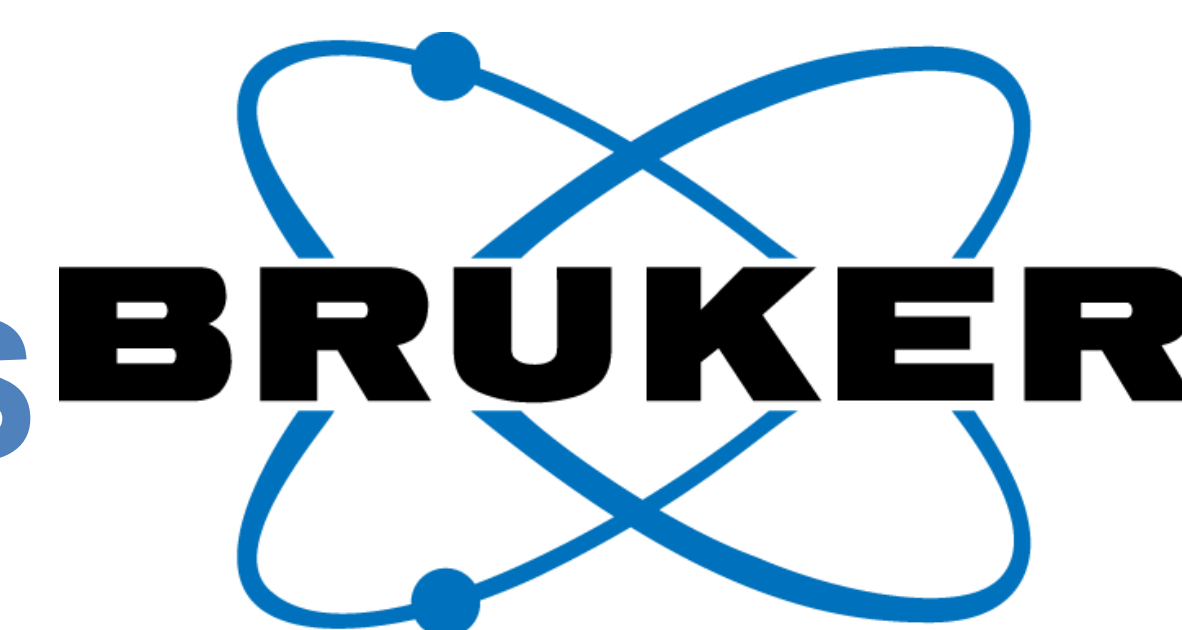


Screening and Quantitation of About 200 Pesticides in Honey by an Integrated On-Line Extraction UHPLC-MS/MS System



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Zicheng Yang and Louis Maljers

Bruker Daltonics Inc., 3500 West Warren Ave, Fremont, CA 94538

Contact: zicheng.yang@bruker.com

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 μ m, 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 coefficient 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 μ m 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 μ m, 10 mm x 3.0 mm I.D.
 Mobile Phase C: 5 mM ammonium fluoride (AF) in water
 Equilibration flow: 1000 μ L (3.0 min)
 Loading Flow: 600 μ L
 Analytical Column: YMC-Pack ODS AQ, 3 μ m, 150 mm x 3.0 mm (I.D.)
 Column Temperature: 40 $^{\circ}$ C
 Injection Volume: 50 μ L
 Mobile Phase A: 5 mM AF in water
 Mobile Phase B: MeOH

Gradient:

Time	%A	%B	Flow (μ L/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 $^{\circ}$ C
 Heated Probe Gas Flow: 45-unit
 Heated Probe Temperature: 350 $^{\circ}$ 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_3methacarb	7.30	1500	0	MS/MS	24.7	Positive	
2	2.4.0	8.24	1500	0	MS/MS	24.7	Negative	
3	2.6_dichlorbenzamide	4.80	1500	0	MS/MS	23.0	Positive	
4	3-Hydroxycarbazole	5.95	1500	63.6	0	MS/MS	23.0	Positive
5	Abamectin	13.52	1500	71751-41-2	0	MS/MS	47.6	Positive
6	Acophate	4.16	1500	39585-19-1	0	MS/MS	23.0	Positive
7	Acetamiprid	5.10	1500	135419-20-7	0	MS/MS	23.0	Positive
8	Aldicarb sulfone	4.40	1500	1648-88-4	0	MS/MS	23.0	Positive
9	Aldicarb sulfide	4.20	1500	1648-87-3	0	MS/MS	23.0	Positive
10	Azinphos	8.52	1500	824-12-8	0	MS/MS	19.6	Positive
11	Azinphosmethyl	6.33	1500	2032-59-9	0	MS/MS	24.7	Positive
12	Abamectin	7.39	1500	0	MS/MS	24.7	Positive	
13	Acetamiprid	7.74	1500	131900-33-2	0	MS/MS	23.0	Positive
14	Benflaxol	11.14	1500	71626-11-4	0	MS/MS	24.7	Positive
15	Bendocarb	6.11	1500	22781-23-3	0	MS/MS	24.7	Positive
16	Benflaxol	12.27	1500	62659-64-1	0	MS/MS	23.0	Positive
17	Benflaxol	5.02	1500	0	MS/MS	23.0	Negative	
18	Bifenoxate	8.90	1500	146977-41-5	0	MS/MS	19.6	Positive
19	Standard	11.42	1500	55179-31-2	0	MS/MS	23.0	Positive
20	Boscalid	8.32	1500	188425-85-8	0	MS/MS	19.6	Positive
21	Bromuaceton isomer 1	9.35	1500	116255-48-2	0	MS/MS	20.0	Positive
22	Bromuaceton isomer 2	10.89	1500	116255-48-2	0	MS/MS	30.3	Positive
23	Bupirimate	10.42	1500	41483-43-6	0	MS/MS	30.3	Positive
24	Buprofen	12.48	1500	69327-76-9	0	MS/MS	23.0	Positive
25	Buthoxy	8.90	1500	134605-54-4	0	MS/MS	19.6	Positive

Fig. 2. MRM method for pesticides

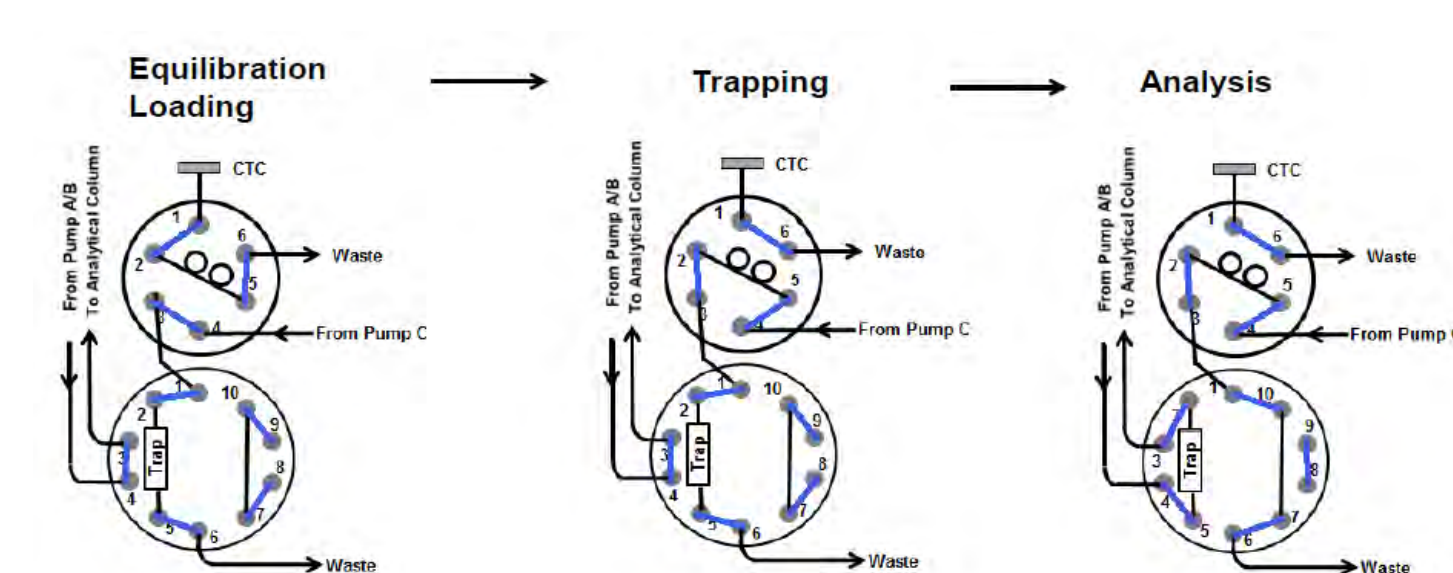


Fig. 3. OLE Valves configuration

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
Hexythiazox	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)

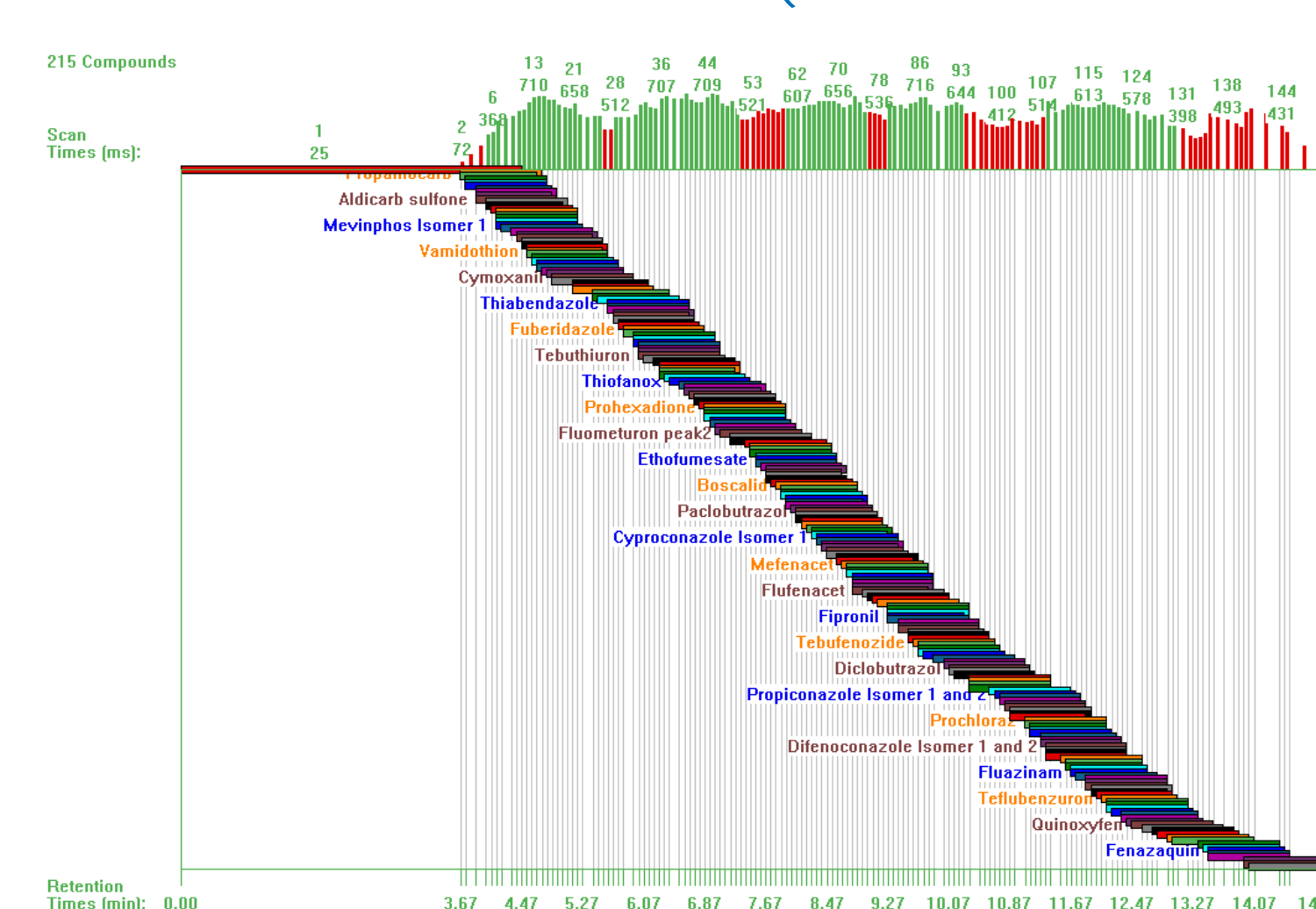


Fig. 4. Timed MRM windows for 215 pesticides

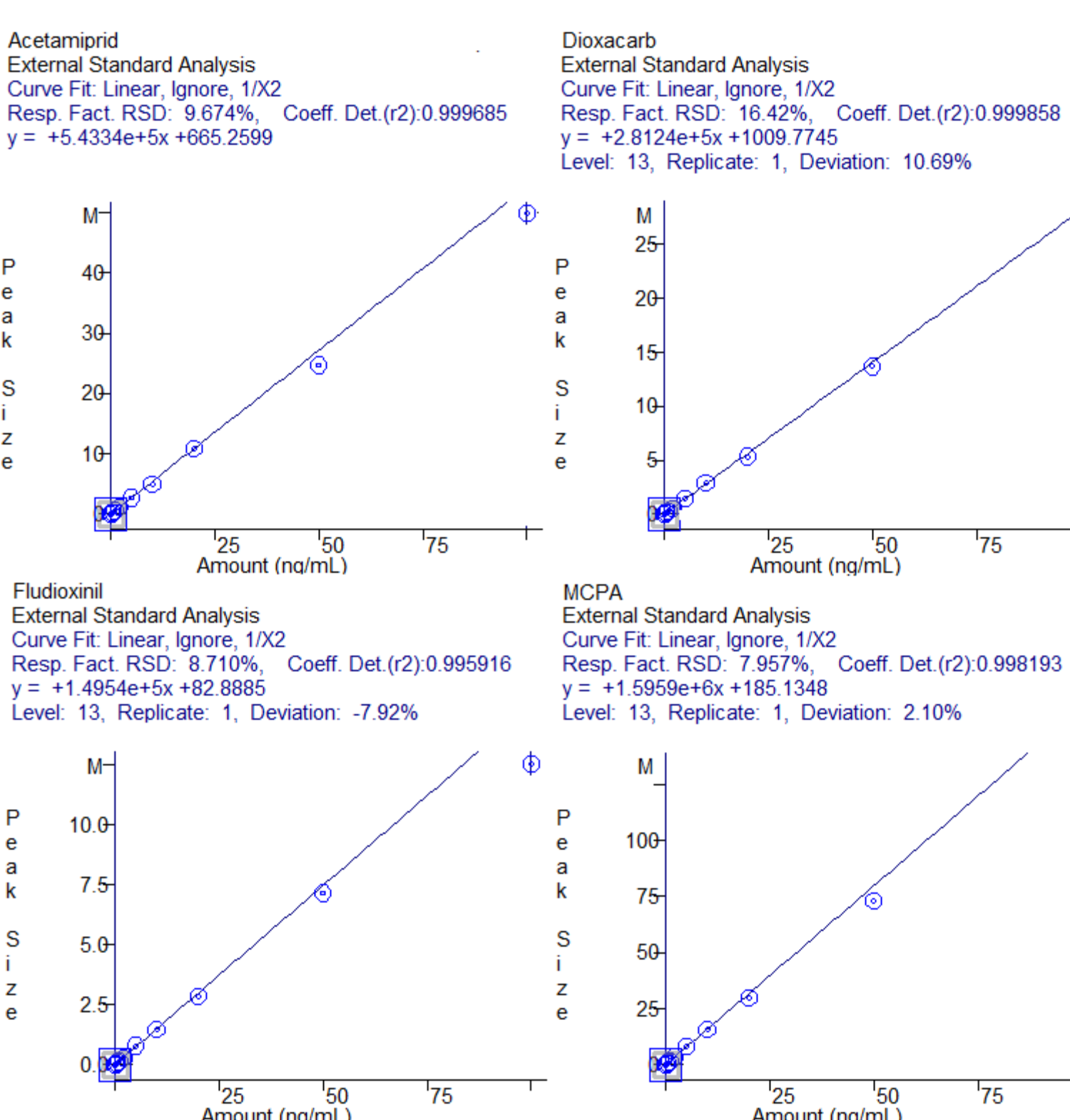


Fig. 5. Selected calibration curves for pesticides in table 1. Top and bottom curves are positive and negative pesticides, respectively. Standard solution linear range was 0.01 ng/mL to 100 ng/mL.

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.

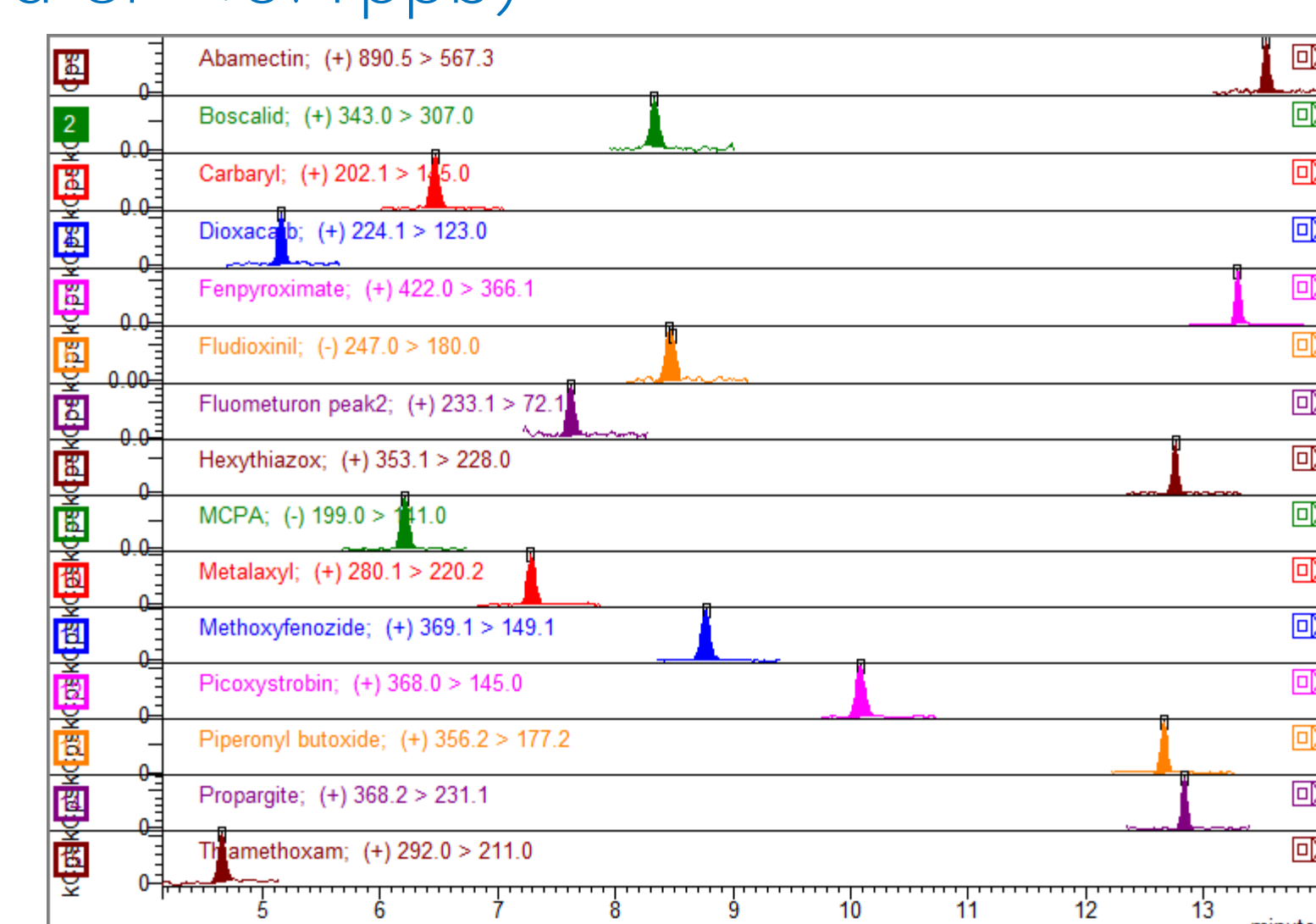


Fig. 6. Chromatograms of standard solution of the compounds listed in table 1 at 0.05 ng/mL (equivalent to 0.5 ng/g in Honey).

- High concentration of sugar washed off from the trap column without getting into MS system.
- No peak shape change by injecting 50 μ L 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.