

Experimental - Antibiotics

Instrument Parameters

UHPLC
 Trap Column: YMC-Pack ODS-AQ, 10µm, 10mm x 3.0mm I.D.
 Mobile Phase C: 0.1% FA in water
 Equilibration flow: 1000µL (4.0 min)
 Loading Flow: 500µL
 Analytical Column: YMC- UltraHT Pto C18 , 2 µm, 100mm x 2.0mm I.D.
 Column Temperature: 40 °C
 Injection Volume: 10µL (100 µL Loop)
 Mobile Phase A: 0.1% FA in water
 Mobile Phase B: MeOH
Gradient:

UPLC Gradient			
Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate µL/min
0.0	80	20	200
0.2	80	20	200
4.0	0	100	200
6.0	0	100	200
6.1	80	20	200
8.0	80	20	200

EVOQ MS Conditions

Source: HESI
 Spray Voltage: ±4000 V
 Cone Gas Flow: 20
 Cone Temperature: 350 °C
 Heated Probe Gas Flow: 15
 Heated Probe Temperature: 400 °C
 Nebulizer Gas Flow: 55
 Exhaust Gas: On

Sample Preparation

1. Weigh approximately 50mg of honey into the outer shell of the eXtreme|FV® (p/n 85531, Thomson Instrument Company).
2. Add solvent (MeOH/water, 50/50, v/v) to make 100 mg/mL solution.
3. Mix by pipet and press the filter plunger, 0.2 µm PVDF, of the eXtreme|FV® (p/n 85531) completely to filter.
4. Solution is ready for injection.

Fig 1. Antibiotics

Compound Name	Retention Time	Q1 First Mass	Q3 First Mass	Structure	Tolerance Limit (ug/kg, ppb)
Ciprofloxacin	3.168	332.2	314		5
			230.9		
			245		
Enrofloxacin	3.201	360.3	342		5
			286		
			316		
Tetracycline	3.169	445.2	410		5
			154		
			427.1		
Erythromycin	4.370	734	158.1		5
			576.3		

Results

Fig 2. Results for store bought honey from various countries.

Antibiotics	Ciprofloxacin	Enrofloxacin	Erythromycin	Tetracycline
Honey Source	ng/g			
USA-1	ND	ND	ND	ND
USA-2	ND	ND	ND	ND
USA-3	ND	ND	ND	ND
Canada	ND	ND	ND	ND
China	ND	ND	ND	ND
India	ND	ND	ND	3.8

ND: Not Detected or <0.5ng/g. test result based on calibration curve of antibiotics in honey. The antibiotics was spiked in Honey USA-1. Tolerance Limit for all four antibiotics is 5µg/kg.

Fig 3. Calibration Curves -Antibiotics in Honey

Calibration Level	ng/g	ng/mL
1	0.5	0.05
2	1	0.1
3	2	0.2
4	5	0.5
5	10	1
6	20	2
7	50	5
8	100	10
9	200	20

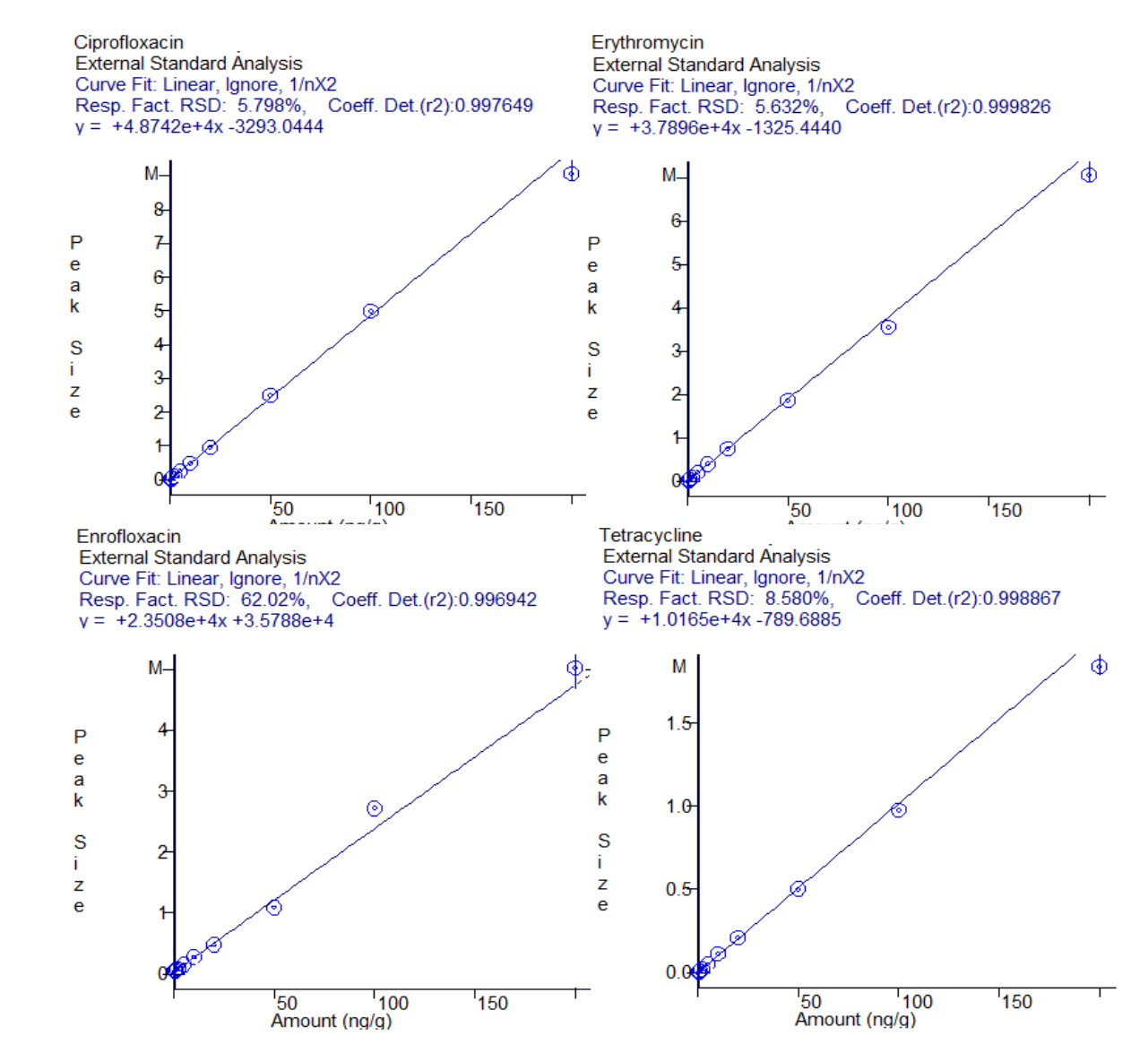
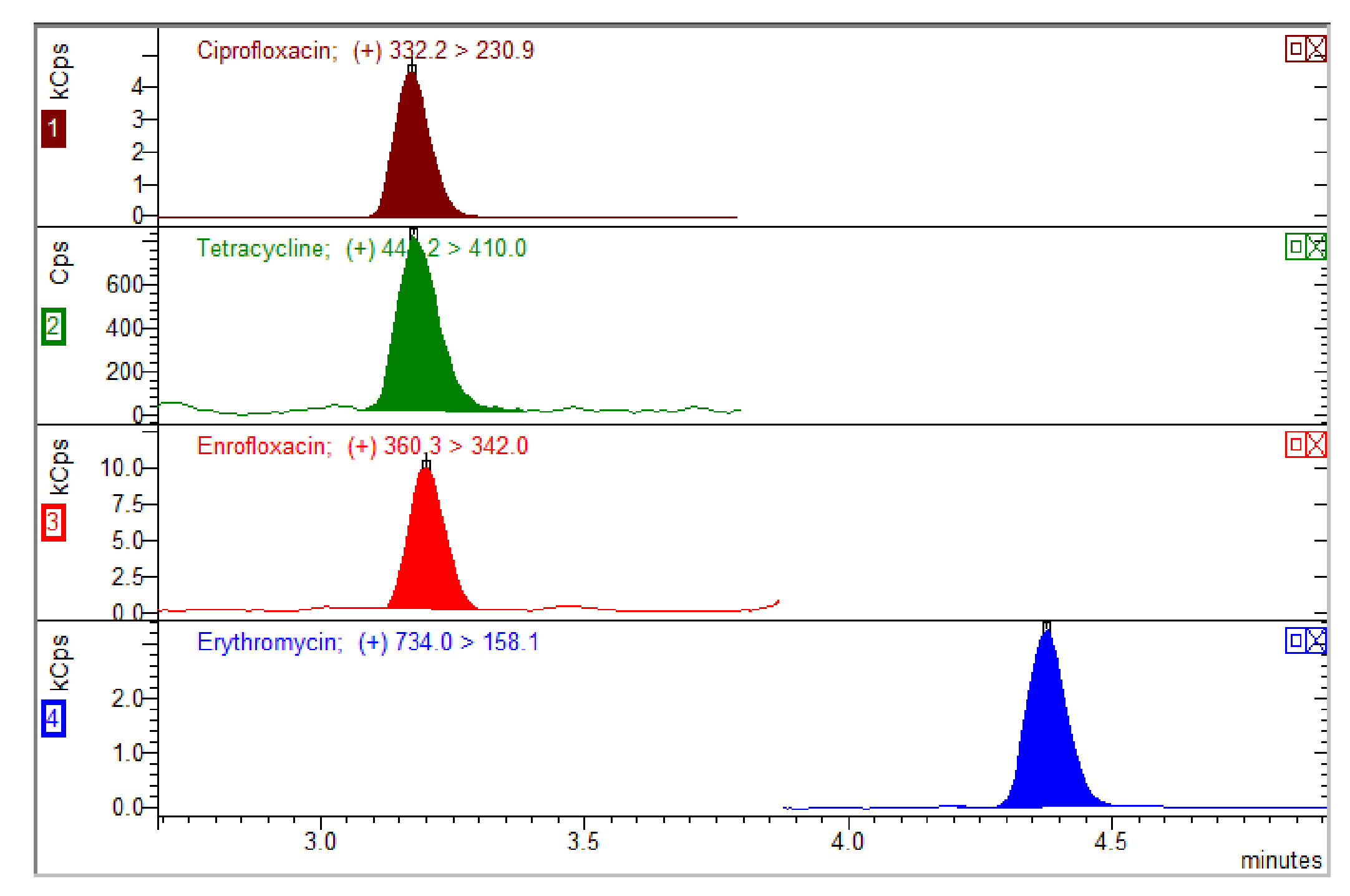


Fig 4. Chromatograms - 0.5ng antibiotics spiked in 1g honey (concentration: 0.05ng/mL)



Recovery

- Calculations are based on matrix matched calibration curves = 100/(detected amount/spiked amount)
- The recovery for Ciprofloxacin and Erythromycin looks consistent across all levels. The Enrofloxacin signal is enhanced in matrix and Tetracycline signal is enhanced at low concentration.

Standard(ng/g)	Recovery			
	Ciprofloxacin	Enrofloxacin	Erythromycin	Tetracycline
0.5	109.6	-	85.5	191
1	121.1	-	85.7	278
2	114.2	6666.7	88.2	233
5	158.9	511.8	83.8	335
10	93.2	207.4	86.1	116
20	111.3	202.8	88.9	169
50	103.3	180.7	93.4	132
100	109.8	179.1	96.2	127
200	133.4	190.5	99.3	120

Conclusion

- Simple: Dilute-Filter-Shoot.
- Good recovery.
- Excellent linearity and retention time distribution and auto calculating scan time for each antibiotic. Single run for positive and negative antibiotics
- High concentration of sugar washed off from the trap column without getting into MS system.



Experimental - Pesticides

Instrument Parameters

UHPLC
 Trap Column: YMC-Pack ODS-AQ, 10µm, 10mm x 3.0mm I.D.
 Mobile Phase C: 0.1% FA in water
 Equilibration flow: 1000µL (4.0 min)
 Loading Flow: 500µL
 Analytical Column: YMC- UltraHT Pto C18, 2µm, 100mm x 2.0mm I.D.
 Column Temperature: 40 °C
 Injection Volume: 10µL (100µL Loop)
 Mobile Phase A: 0.1% FA in water
 Mobile Phase B: MeOH
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

EVOQ MS Conditions

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

1. Weigh approximately 50mg of honey into the outer shell of the eXtreme|FV® (p/n 85531, Thomson Instrument Company).
2. Add solvent (MeOH/water, 50/50, v/v) to make 100 mg/mL solution.
3. Mix and press filter plunger (0.2 µm PVDF) to filter and ready for injection

Results

Fig 1. Calibration Curves -Antibiotics in Honey

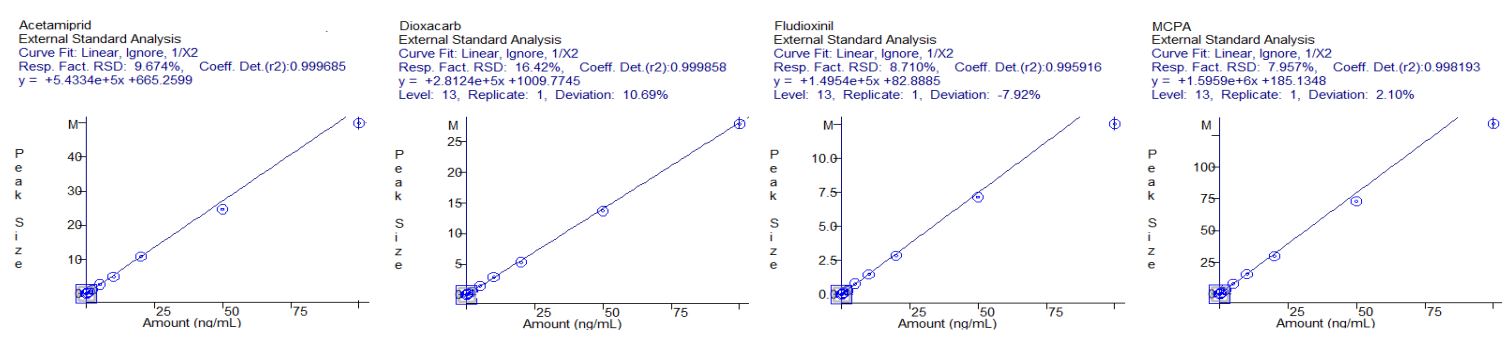


Fig 2. Timed MRM windows for 215 pesticides

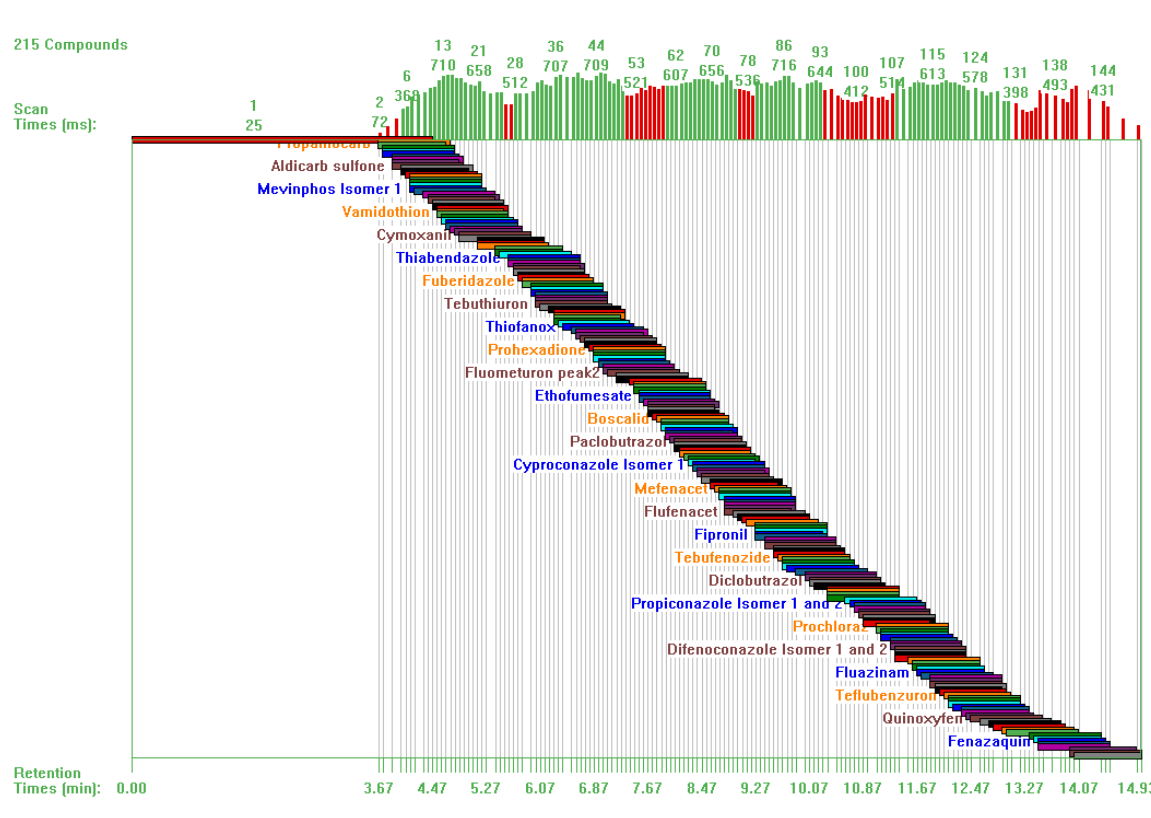


Fig 3. Chromatograms - 0.5ng pesticides spiked in 0.5g/mL honey concentration

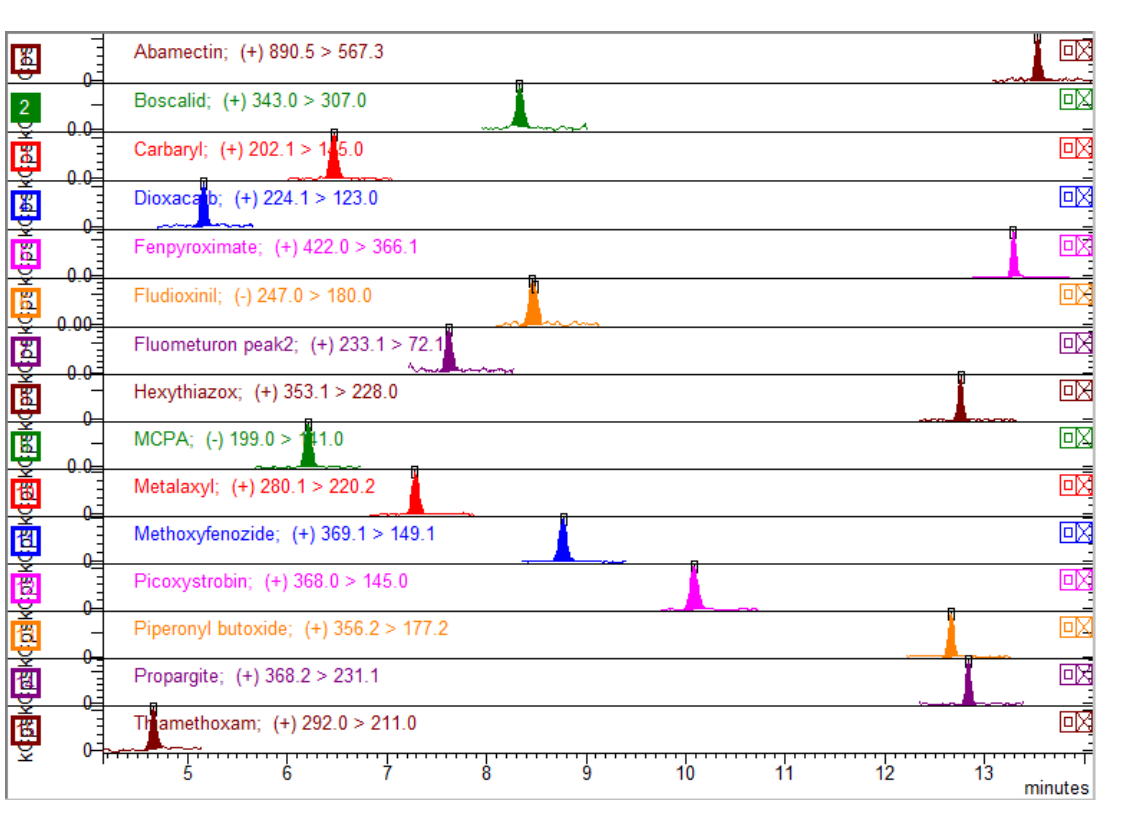


Fig 4. Results for store bought honey from various countries.

Honey Source=>	India	Canada	China	US-1	US-2	US-3
Pesticide				ng/g		
Acetamidiprid	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
Dioxcarb	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
Hexythiazox	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

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

Conclusion

- Simple:
- Dilute-Filter-Shoot.
- Sensitive:
- LOQ at 0.01ng/mL for 158 pesticides <0.1ng/mL
- LOQ <0.1ng/mL for others.
- Good retention time distribution and auto calculating scan time for each pesticide (fig 1).
- 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 50µL 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.

