

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:

| UHPLC 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

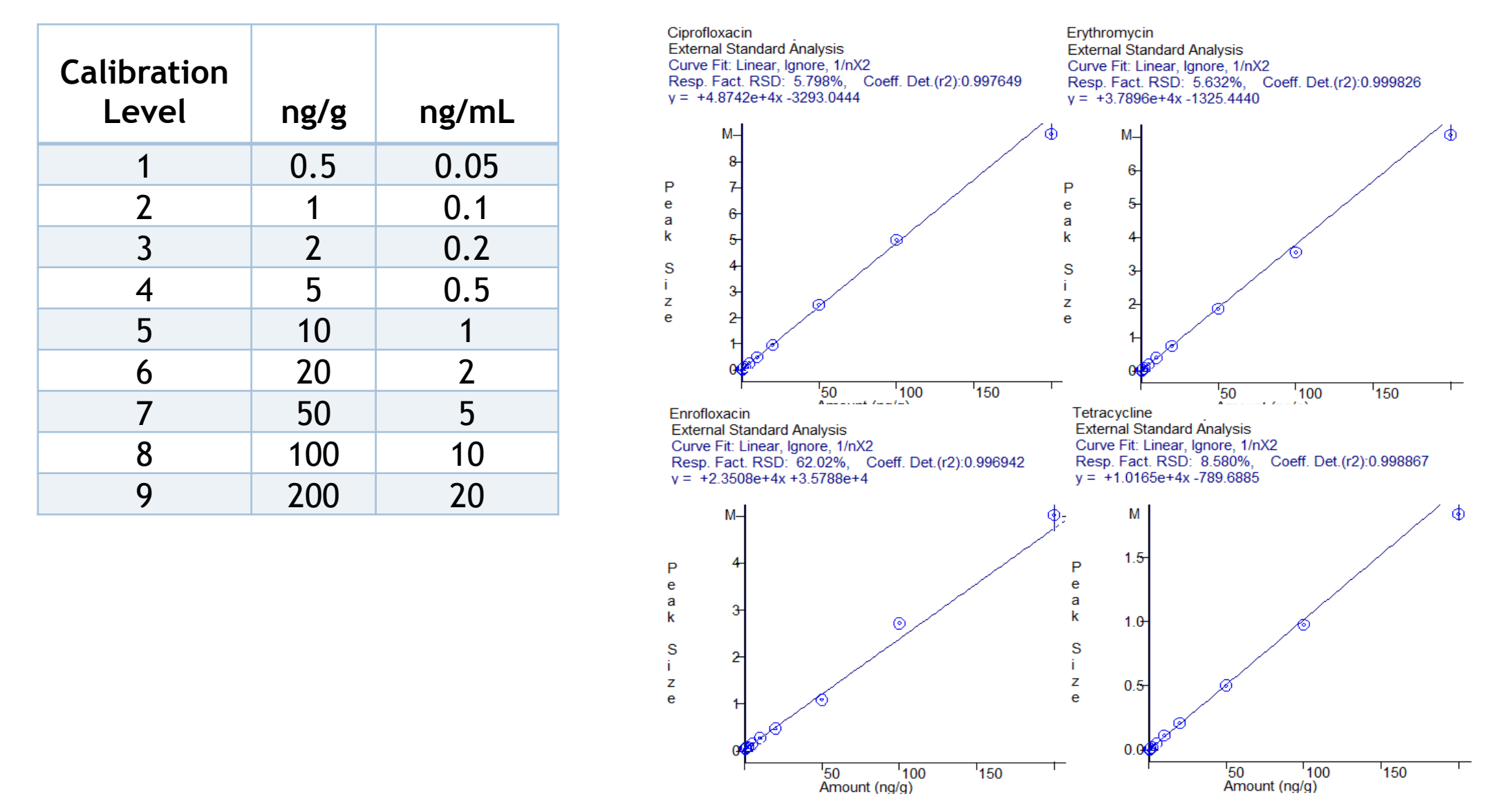
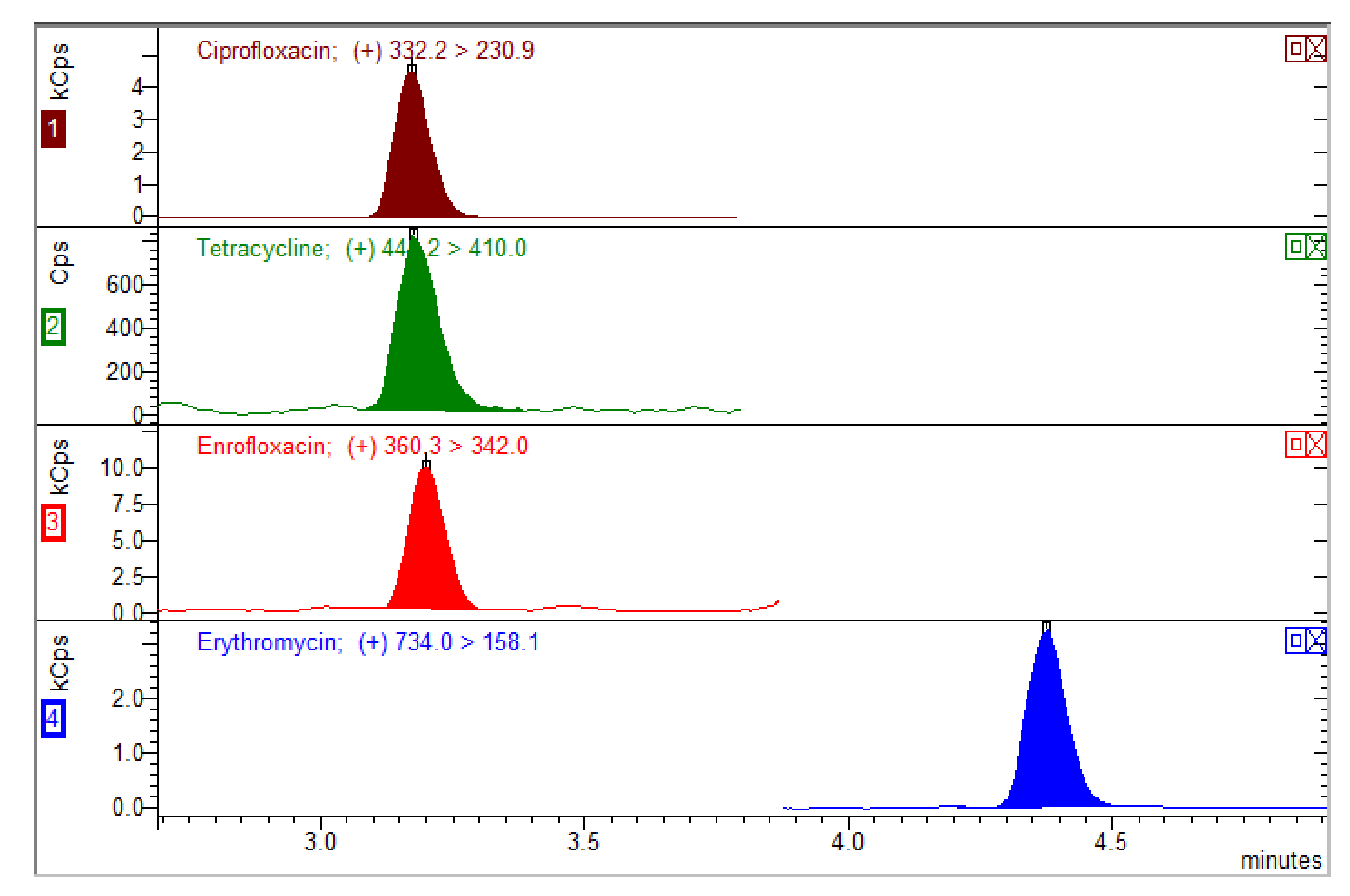


Fig 4. Chromatograms - 0.5ng antibiotics spiked in 1g honey (concentration: 0.05ng/mL)



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.

| Recovery | | | | |
|----------------|---------------|--------------|--------------|--------------|
| Standard(ng/g) | 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: 15
 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

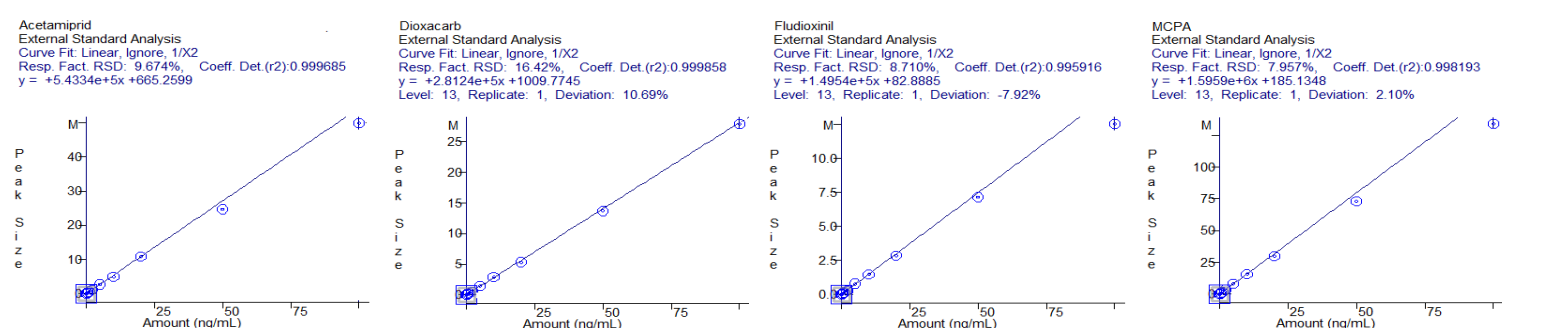


Fig 2. Timed MRM windows for 215 pesticides

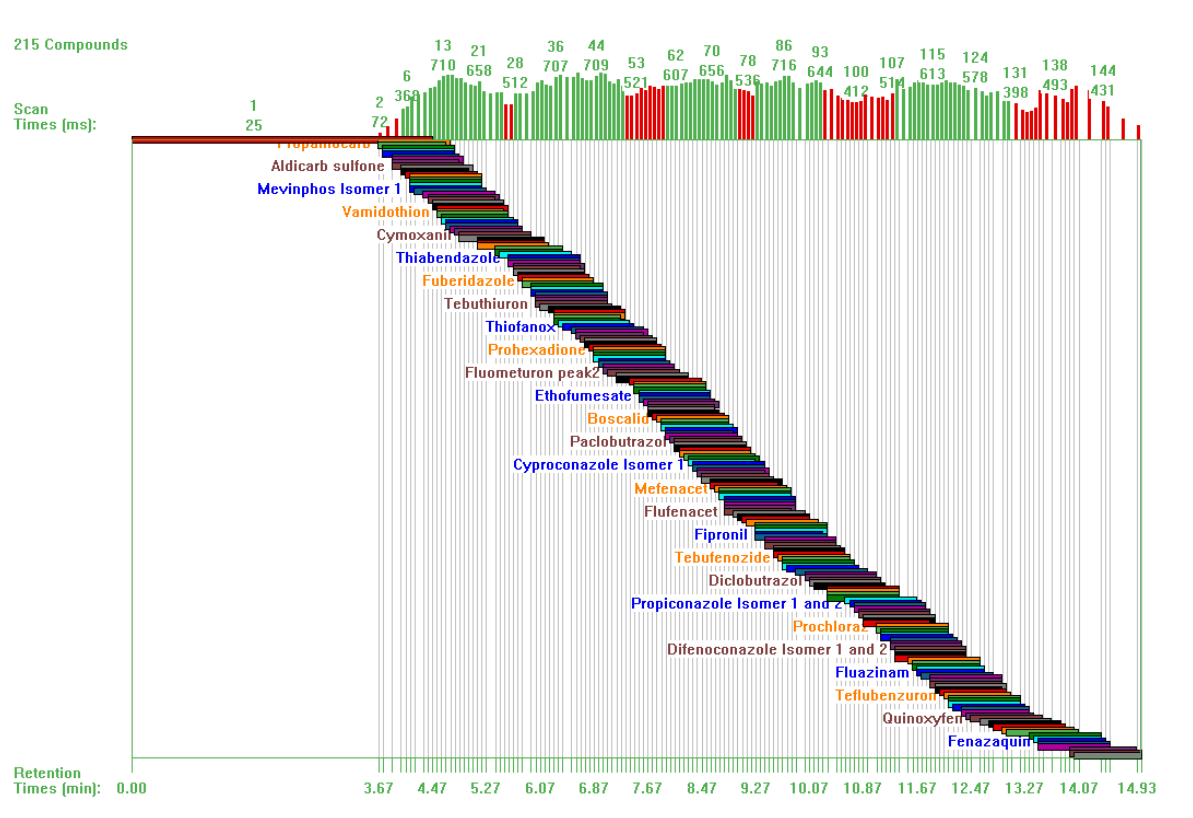


Fig 3. Chromatograms - 0.5ng pesticides spiked in 0.5g/mL honey concentration

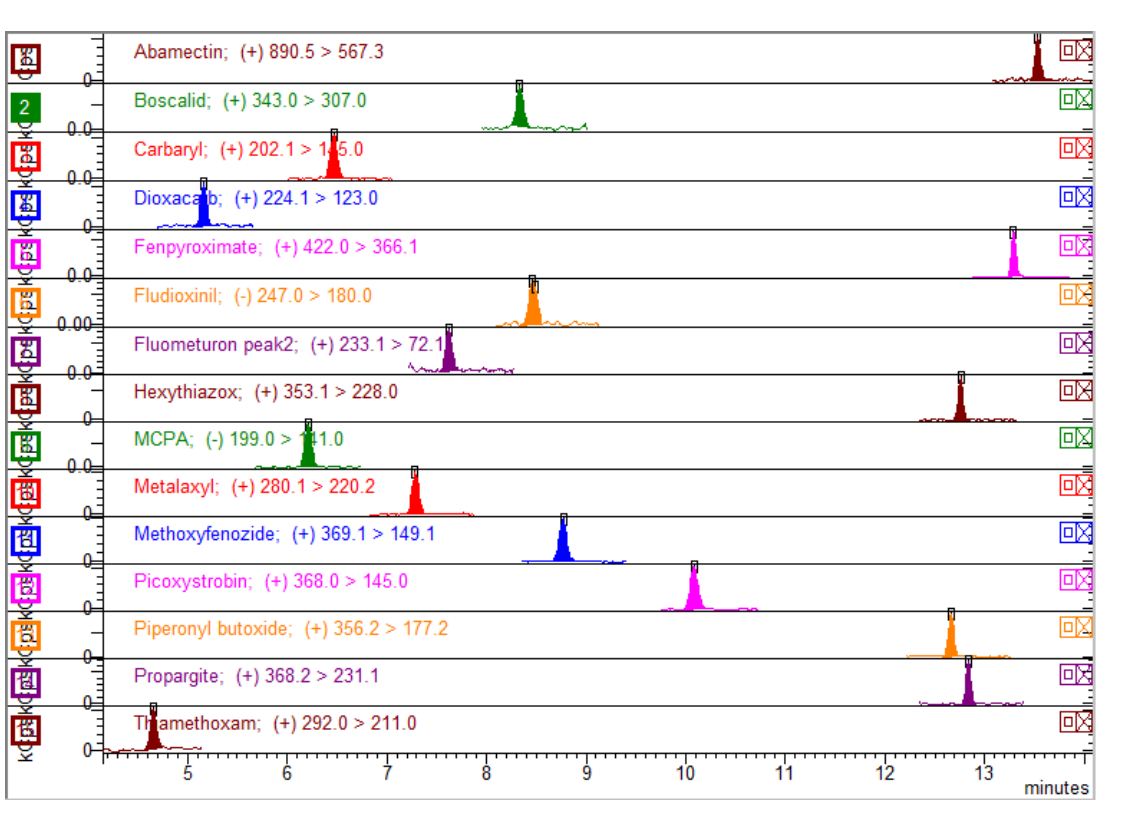


Fig 4. Results for store bought honey from various countries.

| Honey Source=> | India | Canada | China | US-1 | US-2 | US-3 |
|--------------------|-------|--------|-------|------|------|------|
| Pesticide | | | | | | |
| 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 |
| 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.

