



# Screening and Quantitation of 215 Pesticides in Honey by an Integrated On-Line Extraction UHPLC-MS/MS System

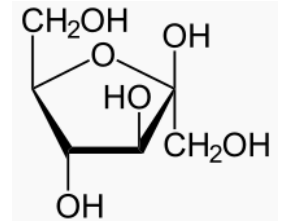
Zicheng Yang and Louis Maljers  
September 23, 2015



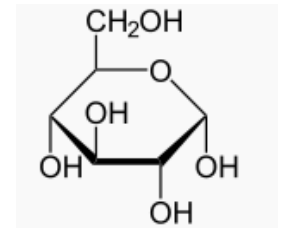
## Honey

### Nutritional value per 100 g (3.5 oz)

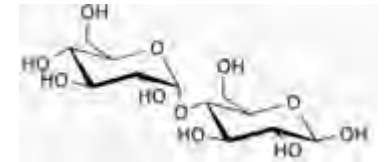
<b>Energy</b>	1,272 kJ (304 kcal)	
<b>Carbohydrates</b>	82.4 g	
Sugars	82.12 g	
Dietary fiber	0.2 g	
<b>Fat</b>	0 g	
<b>Protein</b>	0.3 g	
<b>Vitamins</b>		
Riboflavin (B <sub>2</sub> )	0.038 mg	(3%)
Niacin (B <sub>3</sub> )	0.121 mg	(1%)
Pantothenic acid (B <sub>5</sub> )	0.068 mg	(1%)
Vitamin B <sub>6</sub>	0.024 mg	(2%)
Folate (B <sub>9</sub> )	2 µg	(1%)
Vitamin C	0.5 mg	(1%)
<b>Trace minerals</b>		
Calcium	6 mg	(1%)
Iron	0.42 mg	(3%)
Magnesium	2 mg	(1%)
Phosphorus	4 mg	(1%)
Potassium	52 mg	(1%)
Sodium	4 mg	(0%)
Zinc	0.22 mg	(2%)
<b>Other constituents</b>		
Water	17.10 g	



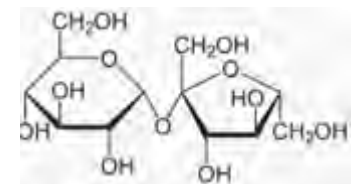
Fructose: 38.2%



Glucose: 31.3%

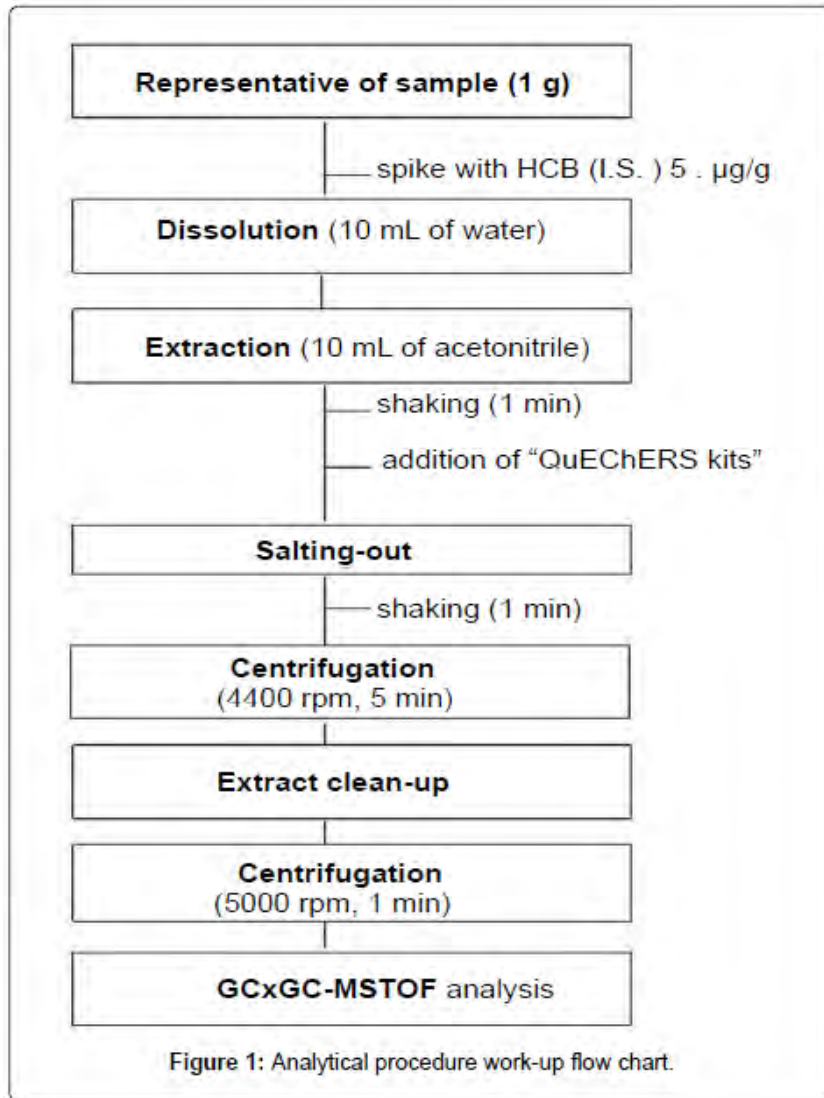


Maltose: 7.1%



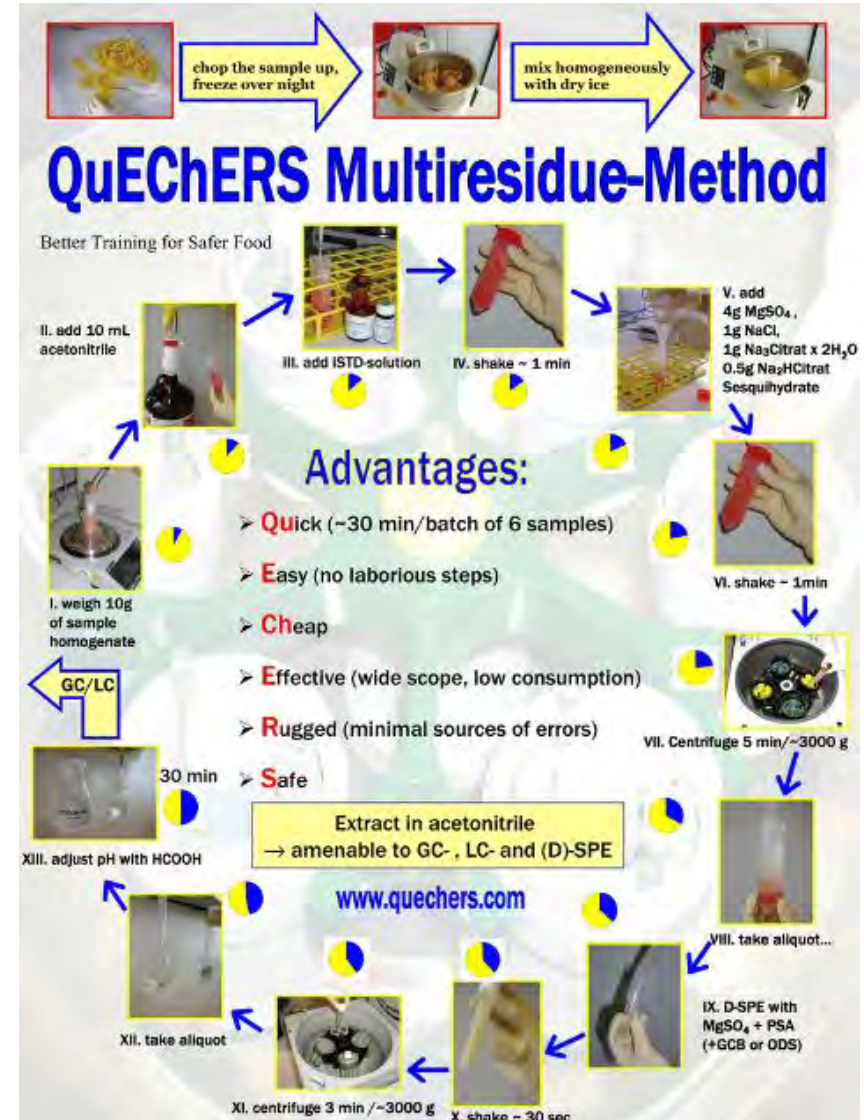
Sucrose: 1.3%

# Sample Prep Using QuEChERS



Determination of Pesticide Residues in Honey using the GCxGC-TOFMS Technique

Bargańska et al., J Bioproc Biotech 2014, 4:7  
<http://dx.doi.org/10.4172/2155-9821.1000182>

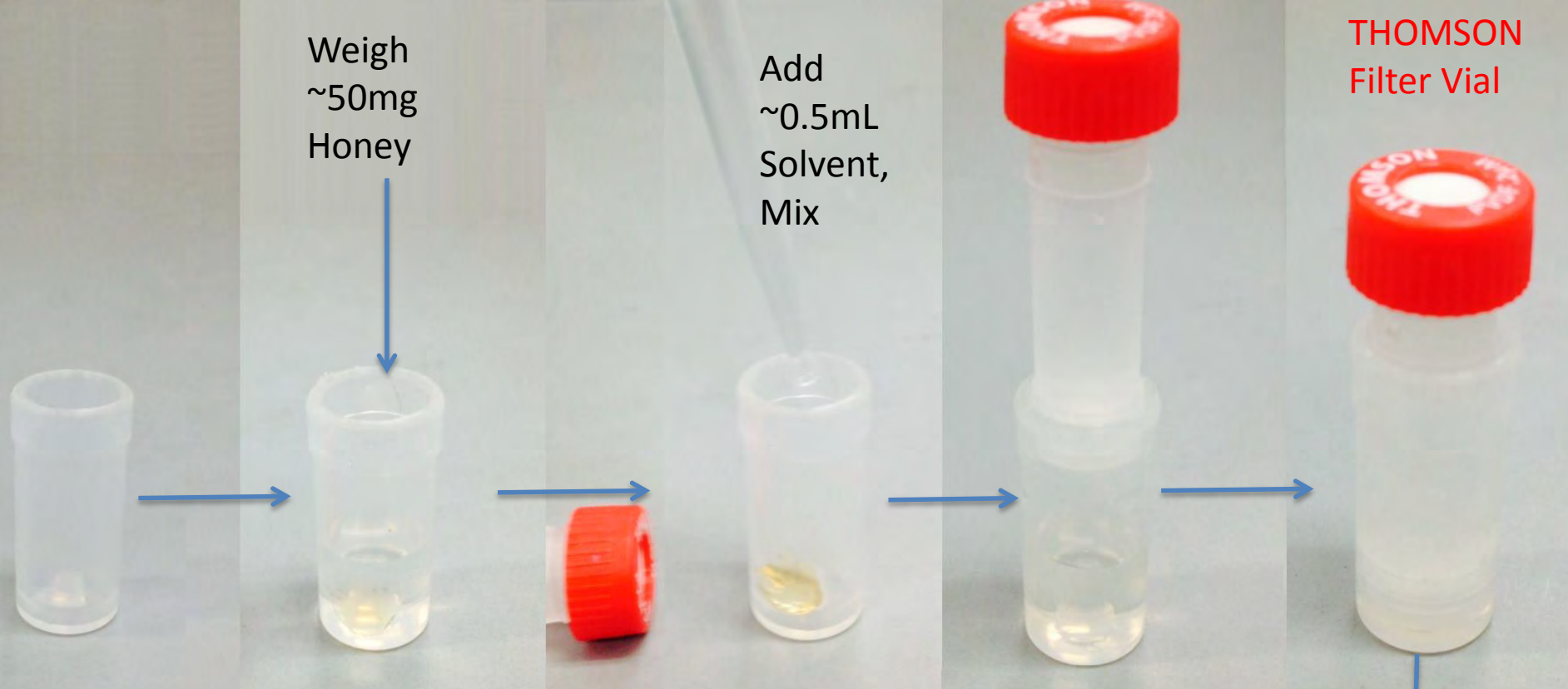


# Sample Prep Using SPE

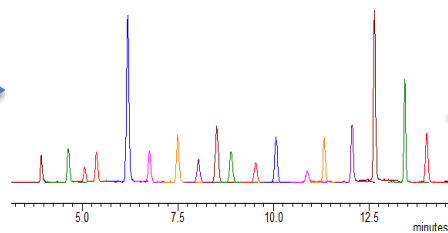


A typical solid phase extraction manifold. The cartridges drip into the chamber below, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber.

Condition SEP with MeOH	
Equilibration with water then extracting buffer	
Load sample solution	
Wash with water then Organic/water	
Dry cartridge by vacuum	
Elute with MeOH	
Evaporate to Dryness	
Reconstitute for analysis	

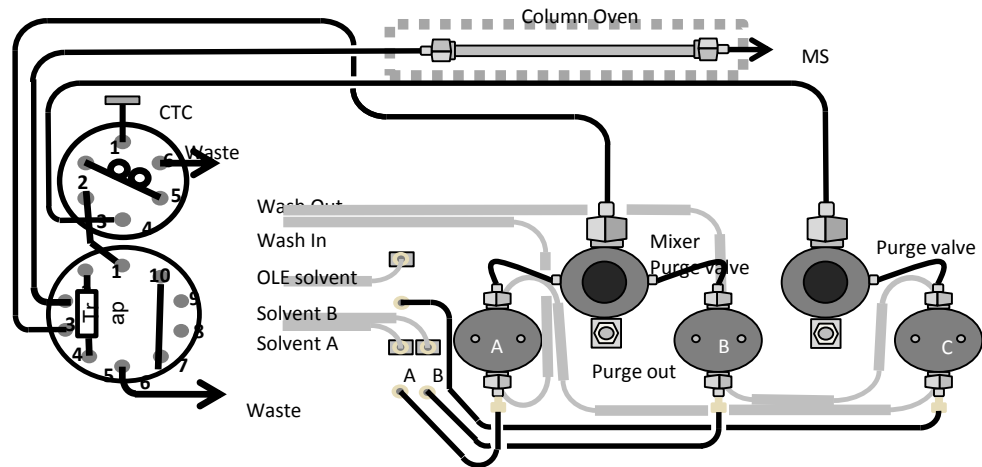


## Dilute-Filter-Shoot

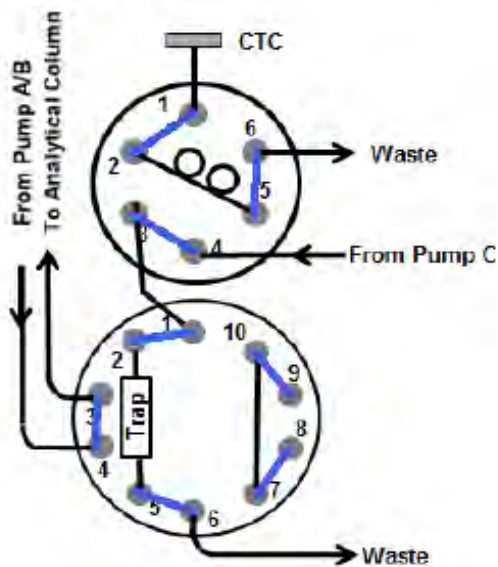


Retention Time (min)	Area	Height	Width	Signal
1.1	1000	100	0.5	1000
2.1	2000	200	0.5	2000
3.1	3000	300	0.5	3000
4.1	4000	400	0.5	4000
5.1	5000	500	0.5	5000
6.1	6000	600	0.5	6000
7.1	7000	700	0.5	7000
8.1	8000	800	0.5	8000
9.1	9000	900	0.5	9000
10.1	10000	1000	0.5	10000
11.1	11000	1100	0.5	11000
12.1	12000	1200	0.5	12000
13.1	13000	1300	0.5	13000
14.1	14000	1400	0.5	14000
15.1	15000	1500	0.5	15000
16.1	16000	1600	0.5	16000
17.1	17000	1700	0.5	17000
18.1	18000	1800	0.5	18000
19.1	19000	1900	0.5	19000
20.1	20000	2000	0.5	20000
21.1	21000	2100	0.5	21000
22.1	22000	2200	0.5	22000
23.1	23000	2300	0.5	23000
24.1	24000	2400	0.5	24000
25.1	25000	2500	0.5	25000
26.1	26000	2600	0.5	26000
27.1	27000	2700	0.5	27000
28.1	28000	2800	0.5	28000
29.1	29000	2900	0.5	29000
30.1	30000	3000	0.5	30000

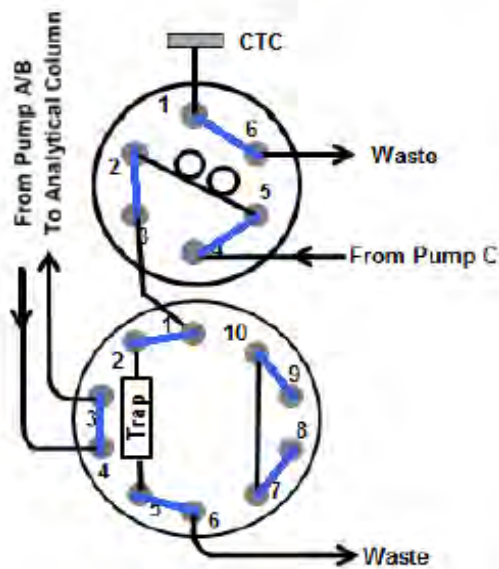
# Valves Configuration



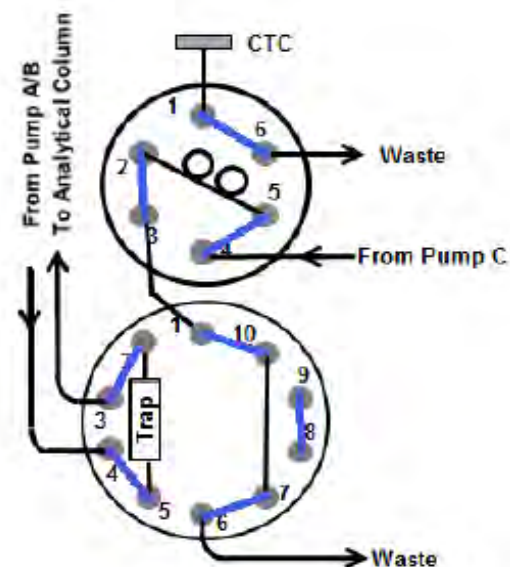
## Equilibration Loading



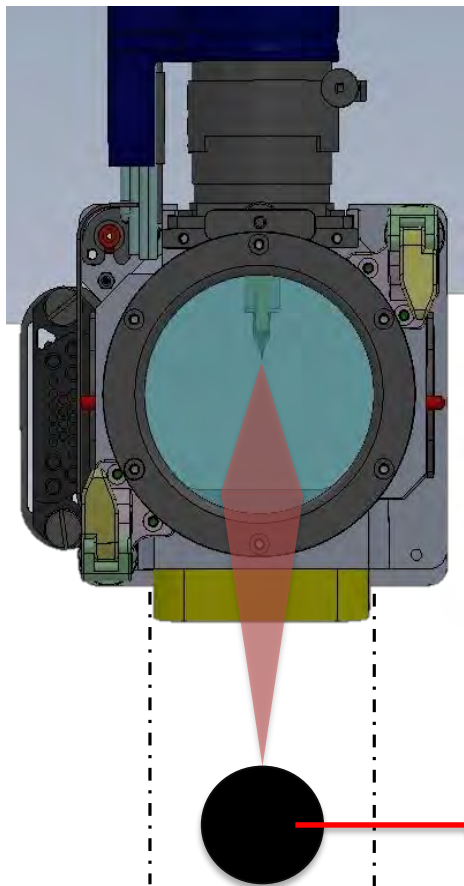
## Trapping



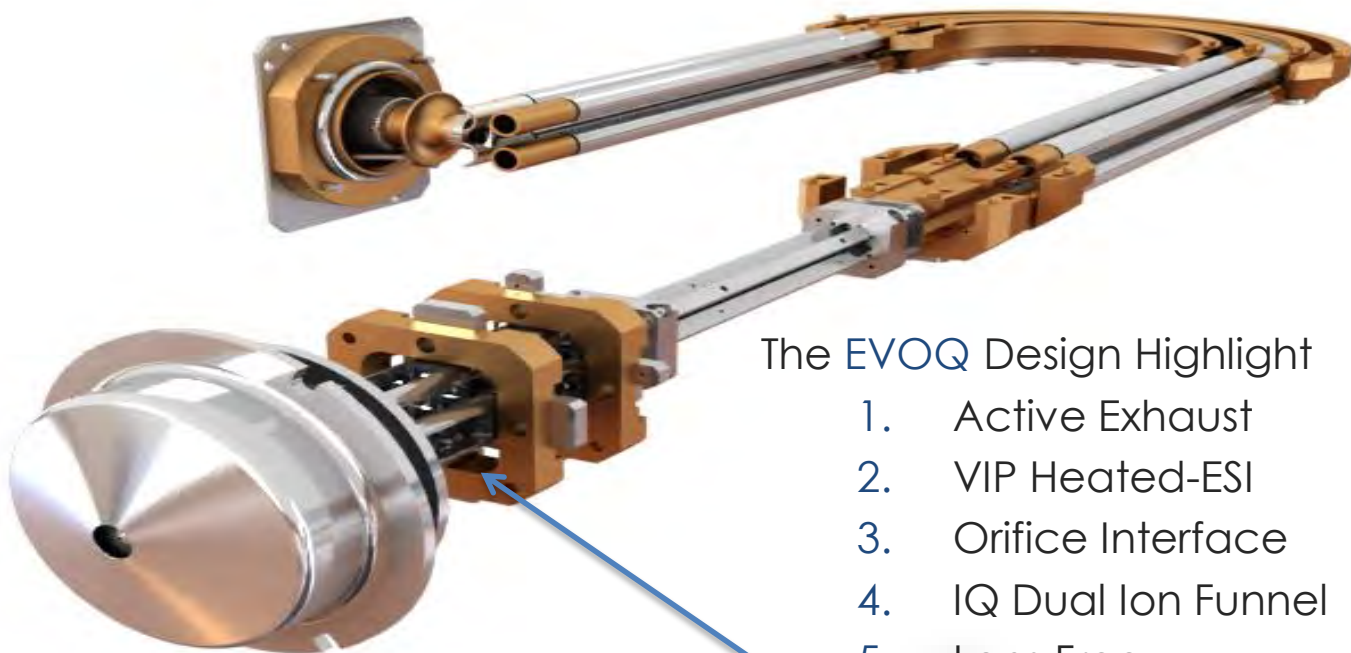
## Analysis



# MS Design Highlight

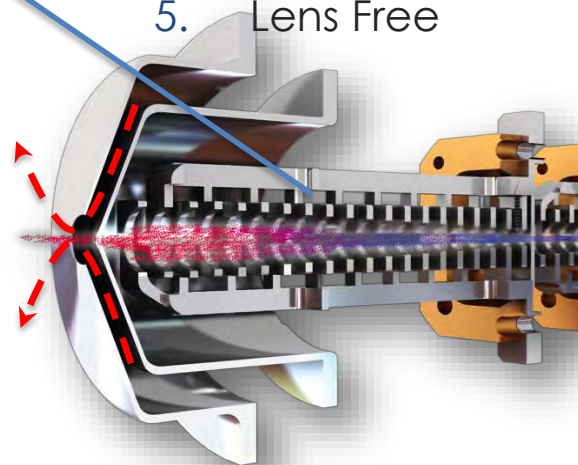


Low pressure region pulls all exhaust gases through the large exhaust opening and out of the mass spec.



## The EVOQ Design Highlight

1. Active Exhaust
2. VIP Heated-ESI
3. Orifice Interface
4. IQ Dual Ion Funnel
5. Lens Free



# LC/MS Conditions

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

LC Gradient:				MS Source Parameters	
Time min.	Mobile Phase A (%)	Mobile Phase B (%)	Flow Rate $\mu\text{L}/\text{min}$ .	Source:	HESI
0.0	90	10	400	Spray Voltage (+/-)	4000 V
0.2	90	10	400	Cone Gas Flow	20
2.0	30	70	400	Cone Temperature	250 $^{\circ}\text{C}$
6.5	20	80	400	Heated Probe Gas Flow	45
8.0	0	100	400	Heated Probe Temperature	400 $^{\circ}\text{C}$
15.0	0	100	400	Nebulizer Gas Flow	65
15.1	90	10	400	Exhaust Gas	On
18.0	90	10	400		



# MRM Transitions

	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	Bromucanazole Isomer 1	9.35	1.00	116255-48-2	0	MRM	20.8	Positive
22	Bromucanazole 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

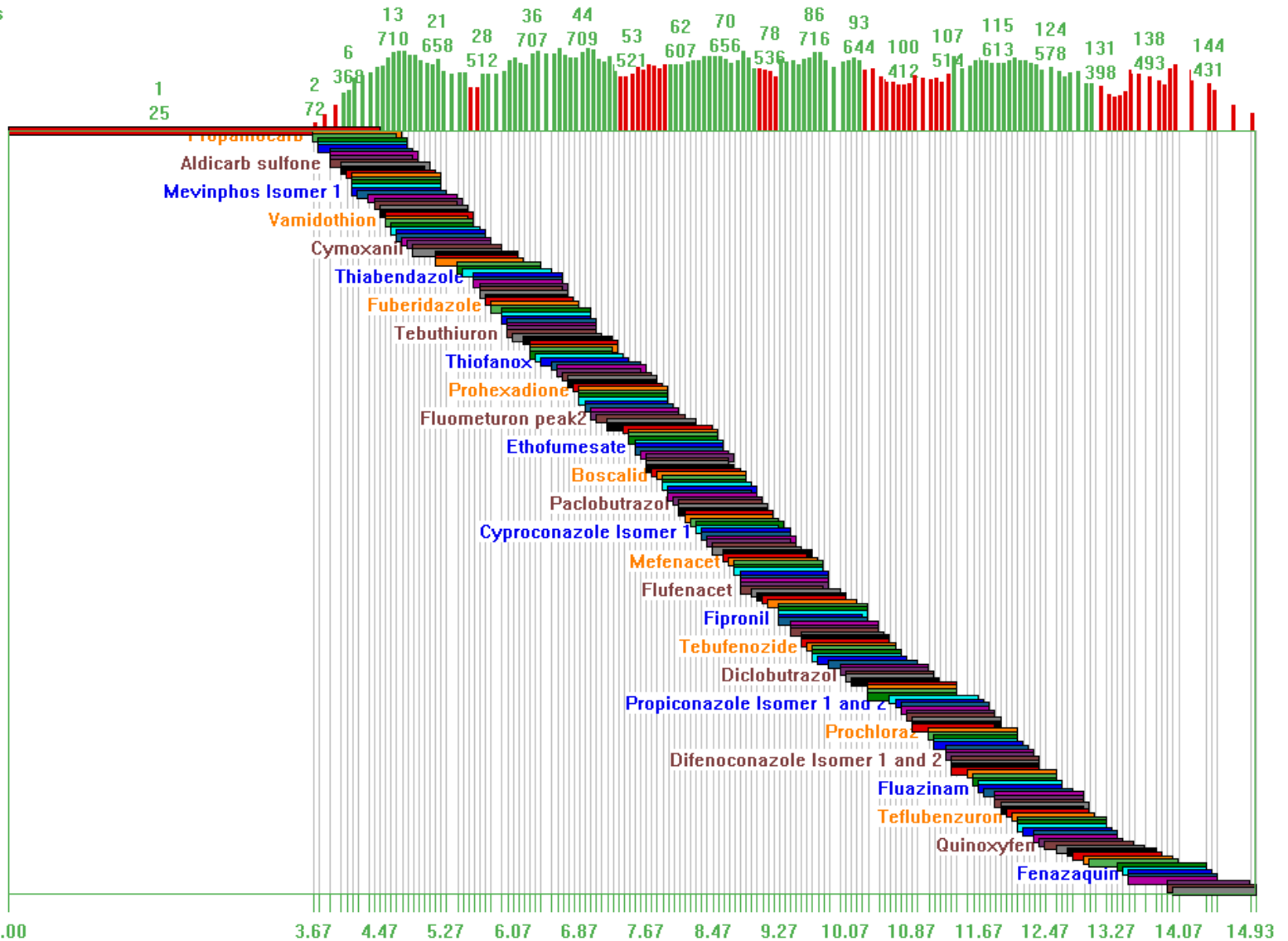
	Precursor	Product	Collision Energy	Q1 Resolution	Q3 Resolution	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	194.00	121.90	24.00	Standard (2.0)	Standard (2.0)	50.00%	<input checked="" type="checkbox"/>	50.20%	<input type="checkbox"/>
2	211.00	137.00	13.00	Standard (2.0)	Standard (2.0)	50.00%	<input type="checkbox"/>		<input checked="" type="checkbox"/>
3							<input type="checkbox"/>		<input type="checkbox"/>
4							<input type="checkbox"/>		<input type="checkbox"/>
5							<input type="checkbox"/>		<input type="checkbox"/>
6							<input type="checkbox"/>		<input type="checkbox"/>
7							<input type="checkbox"/>		<input type="checkbox"/>

# Timed MRM Windows for 215 Pesticides (430 MRM Transitions)

215 Compounds

Scan Times (ms):

Retention Times (min): 0.00

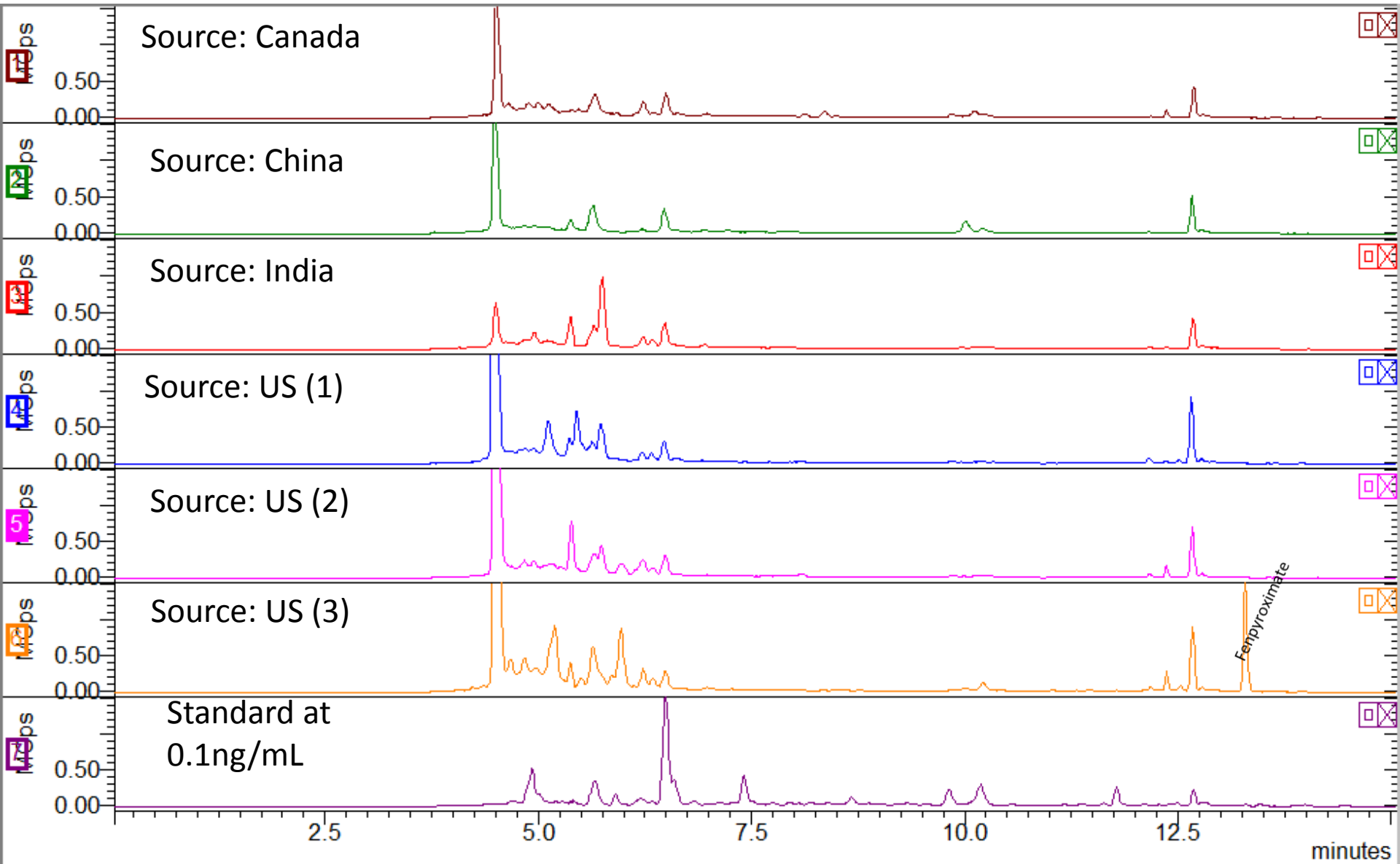


# Result

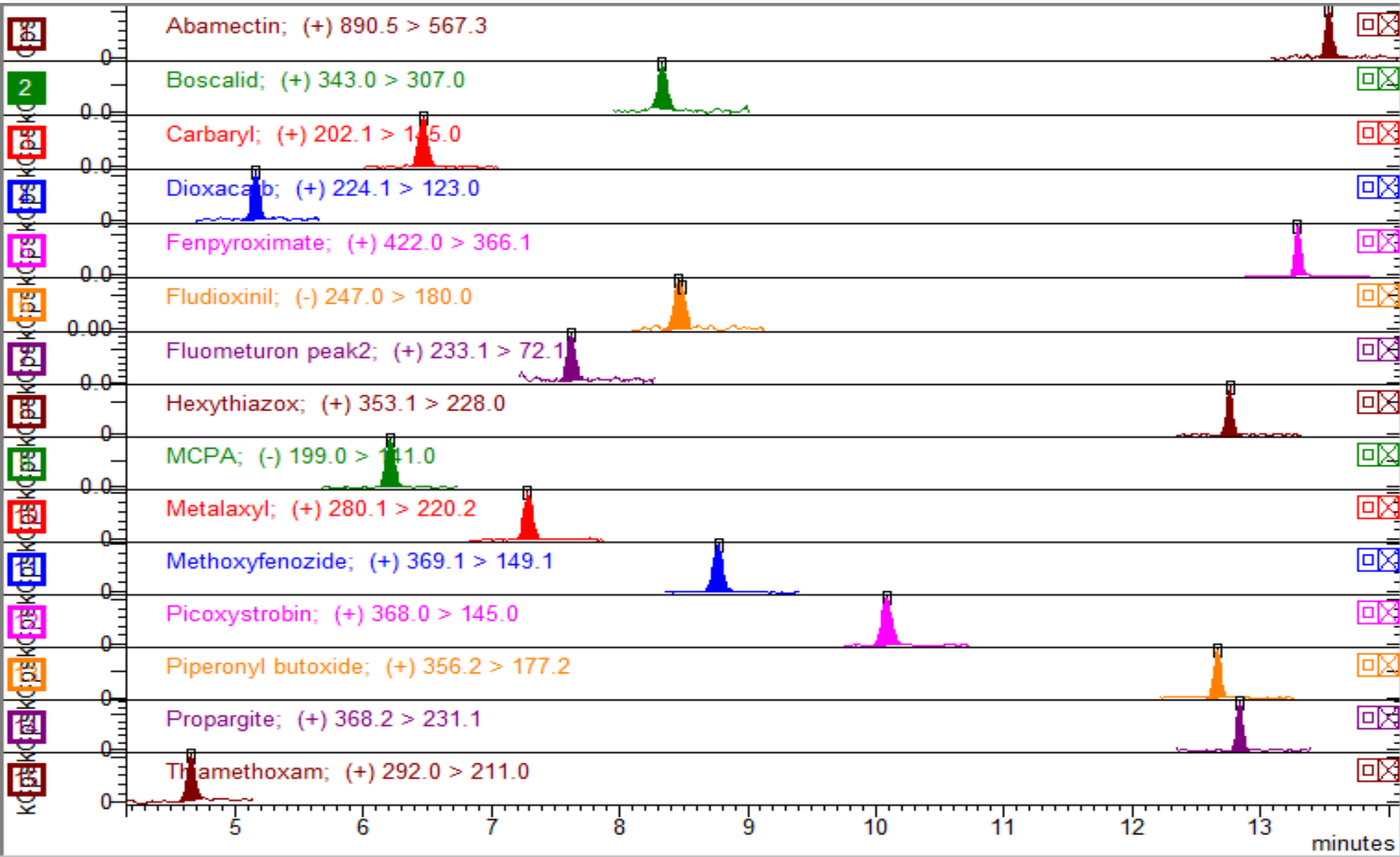
Honey Source=>	India	Canada	China	US-1	US-2	US-3
Pesticide		ng/g (ppb)				
Acetamiprid	ND	ND	0.6	ND	ND	ND
Boscalid	ND	17.5	ND	ND	0.15	3.4
Carbaryl	ND	0.71	ND	ND	ND	ND
Dioxacarb	ND	ND	ND	ND	1.35	2
Fenpyroximate	ND	ND	ND	ND	0.26	55
Fludioxinil	ND	1.5	ND	ND	ND	ND
Fluometuron	ND	ND	ND	ND	ND	2.8
Hexythiazox	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	4.23	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

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

# Honey TIC Chromatograms

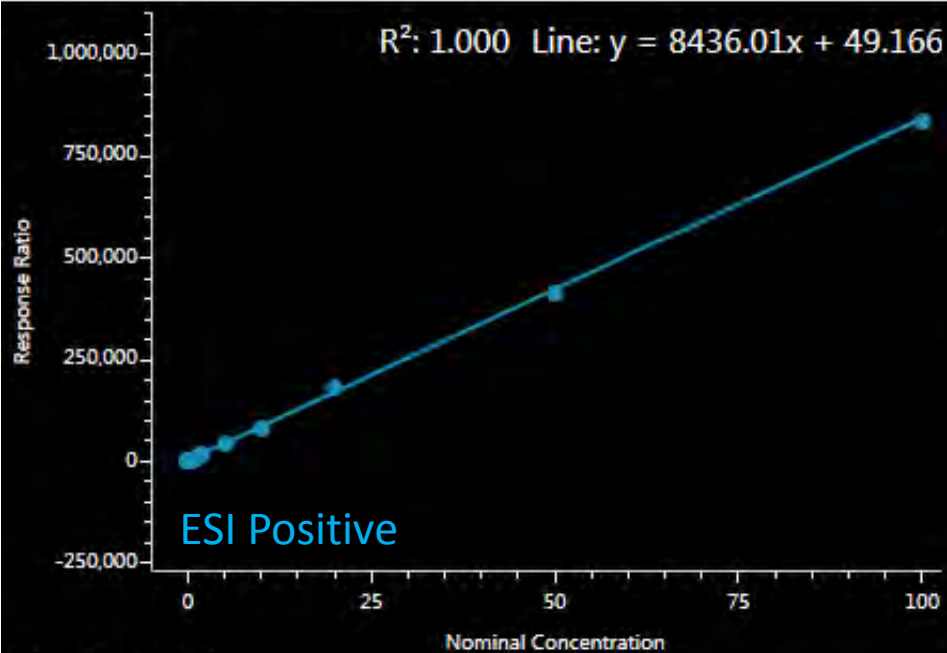


# Pesticides at 0.05 ng/mL

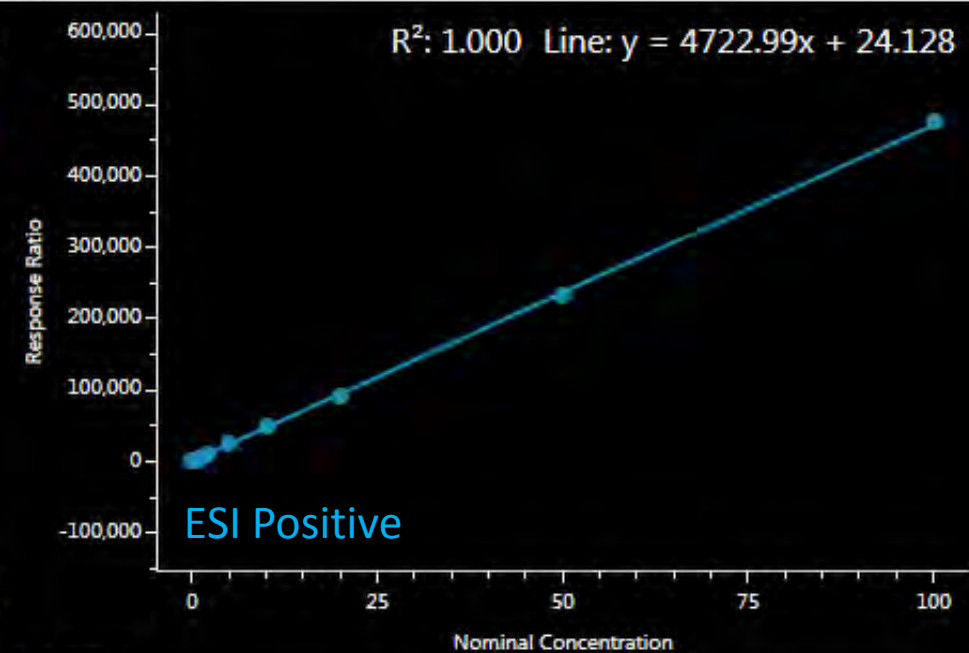


Above pesticides listed in result table

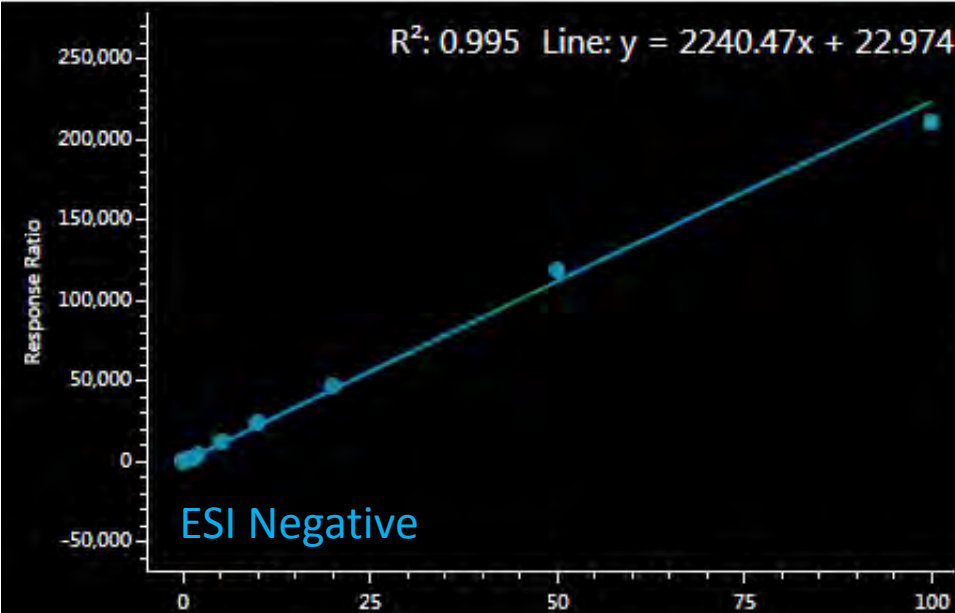
# Acetamiprid



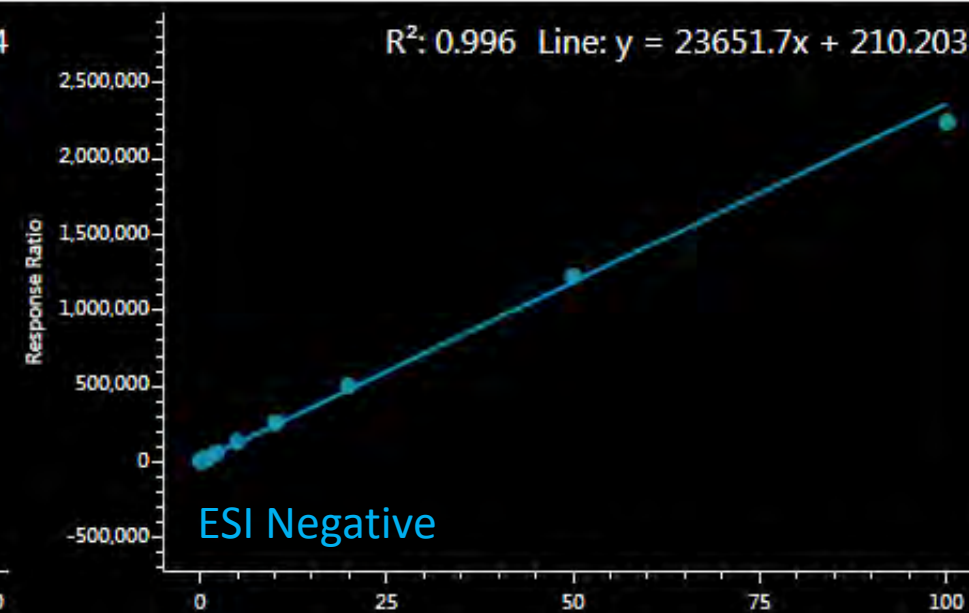
# Dioxacarb



# Fludioxinil



# MCPA





Peak Review

Fenpyroximate

Determination Review

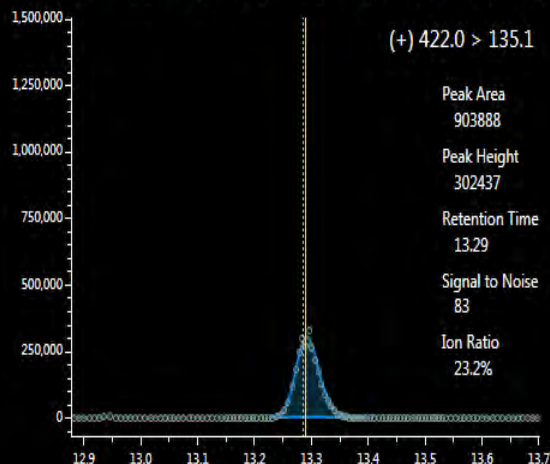
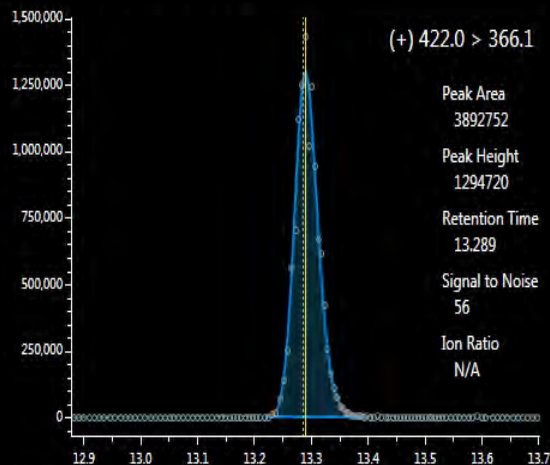
Honey\_NN3

1 of 1 Samples

Fenpyroximate

204 of 215 Compounds

Determination Chromatograms



Determination Calculation

Concentration 54.688  
Response 3892752

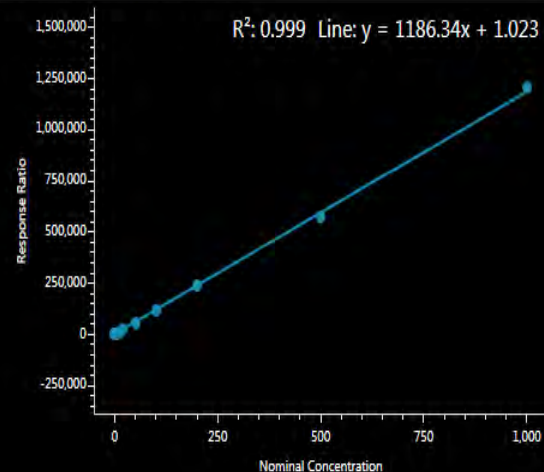
Determination Flags

There are no flags for this determination.

Determination Information

Sample Honey\_NN3  
Vial TR: CStk1-01 VL: 21  
Injection Number 0  
Sample Type Unknown  
Calibration Level 1  
Dilution 1

Determination Calibration



# Summary

- The sample prep step is quick, simple, and easy of use.
- The procedure provides a more convenient and simpler approach than QuEChERS and SPE for residue chemical analysis in honey.



# Acknowledgment

THOMSON INSTRUMENT COMPANY

