



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

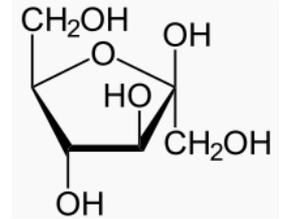
Zicheng Yang and Louis Maljers
September 23, 2015



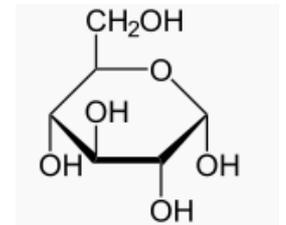
Honey

Nutritional value per 100 g (3.5 oz)

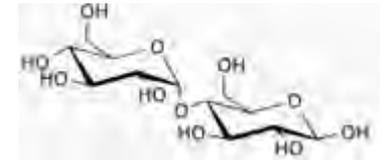
Energy	1,272 kJ (304 kcal)	
Carbohydrates	82.4 g	
Sugars	82.12 g	
Dietary fiber	0.2 g	
Fat	0 g	
Protein	0.3 g	
Vitamins		
Riboflavin (B ₂)	0.038 mg	(3%)
Niacin (B ₃)	0.121 mg	(1%)
Pantothenic acid (B ₅)	0.068 mg	(1%)
Vitamin B ₆	0.024 mg	(2%)
Folate (B ₉)	2 µg	(1%)
Vitamin C	0.5 mg	(1%)
Trace minerals		
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%)
Other constituents		
Water	17.10 g	



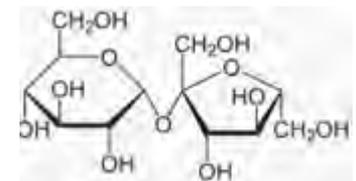
Fructose: 38.2%



Glucose: 31.3%

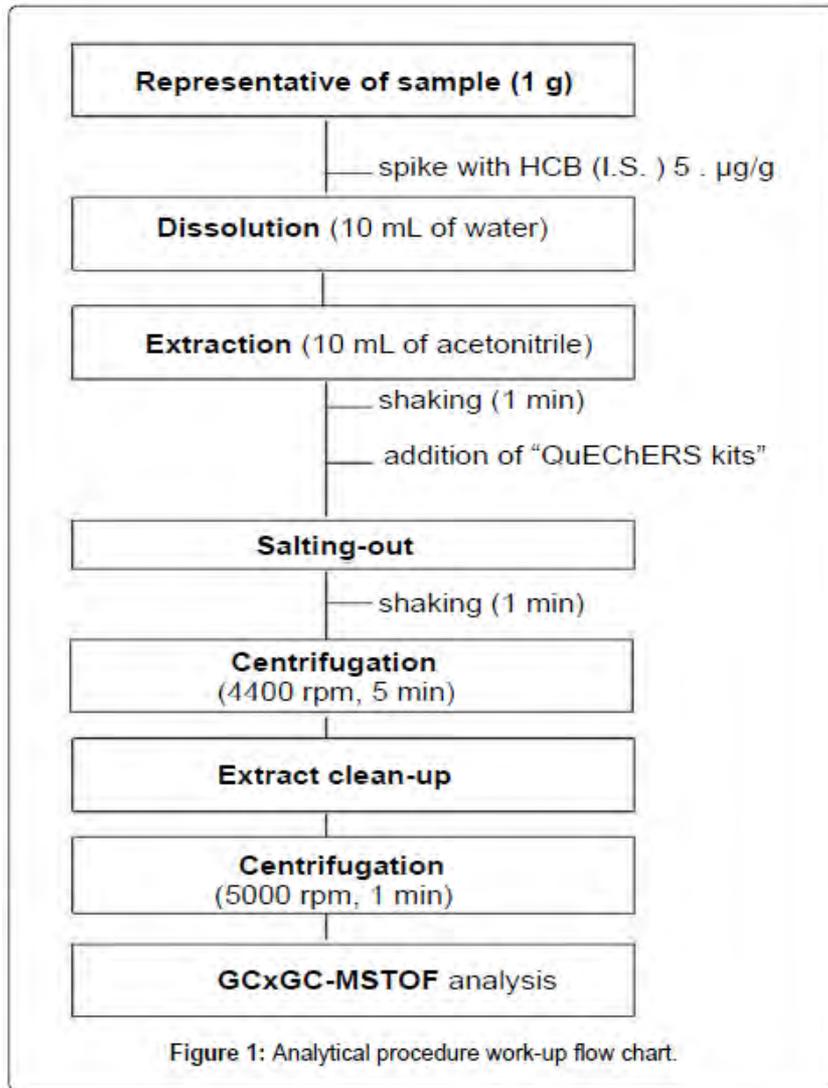


Maltose: 7.1%



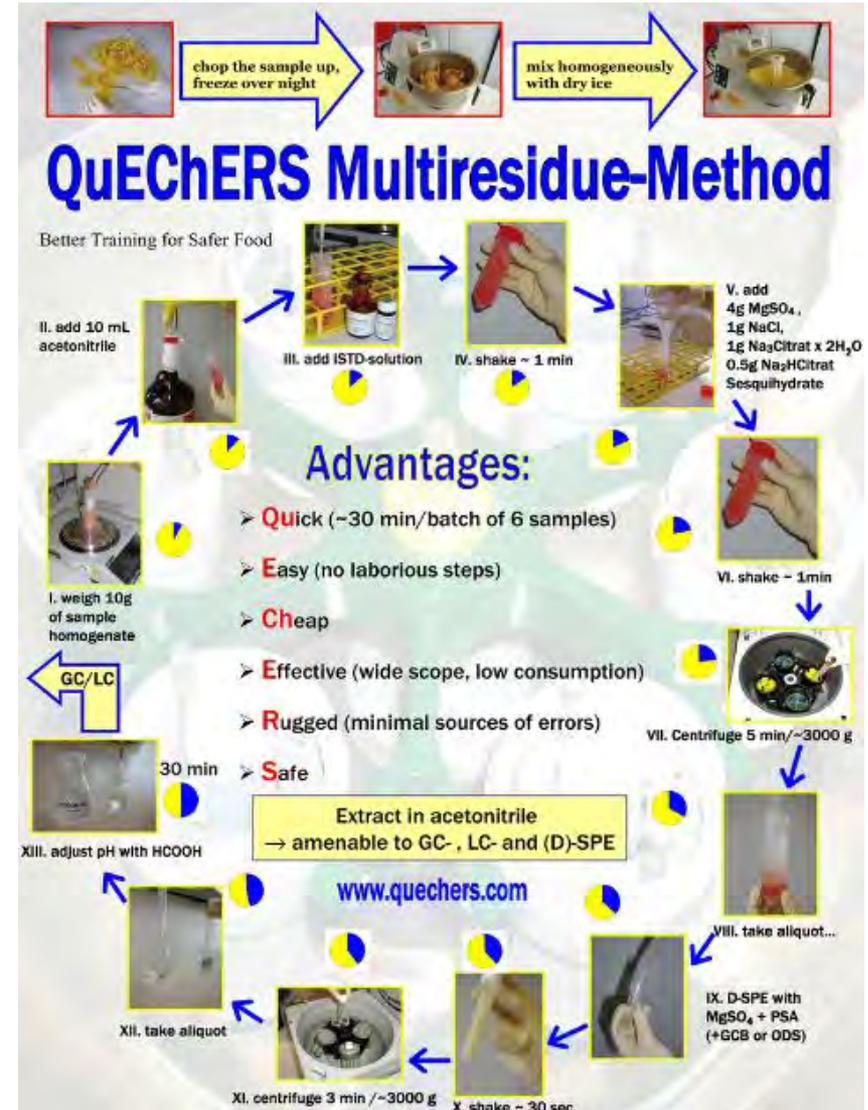
Sucrose: 1.3%

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>

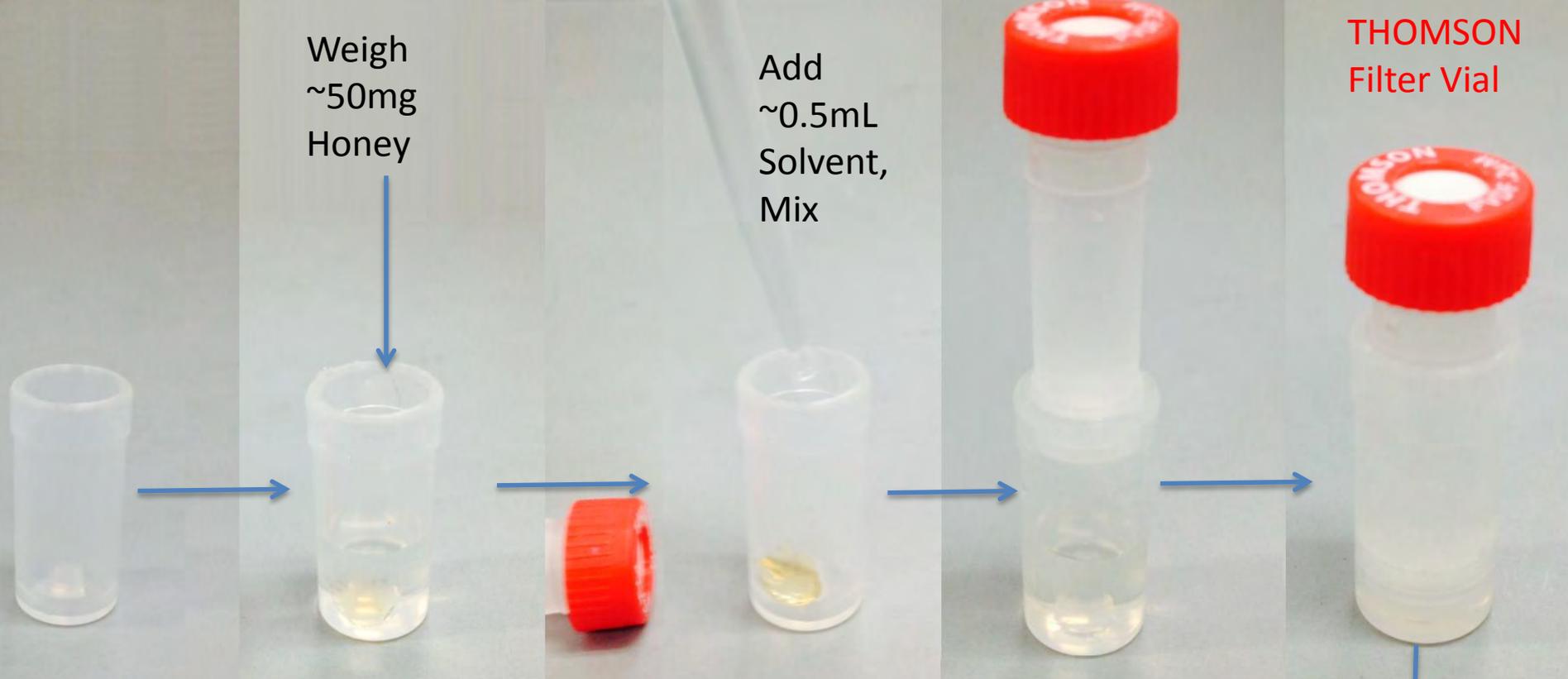


Sample Prep Using SPE

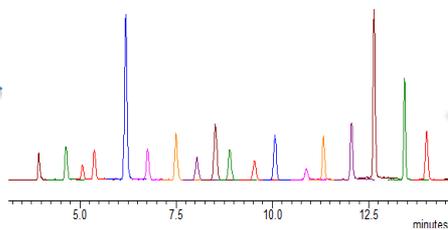


A typical solid phase extraction manifold. The cartridges drip into the chamber below, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber.

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	

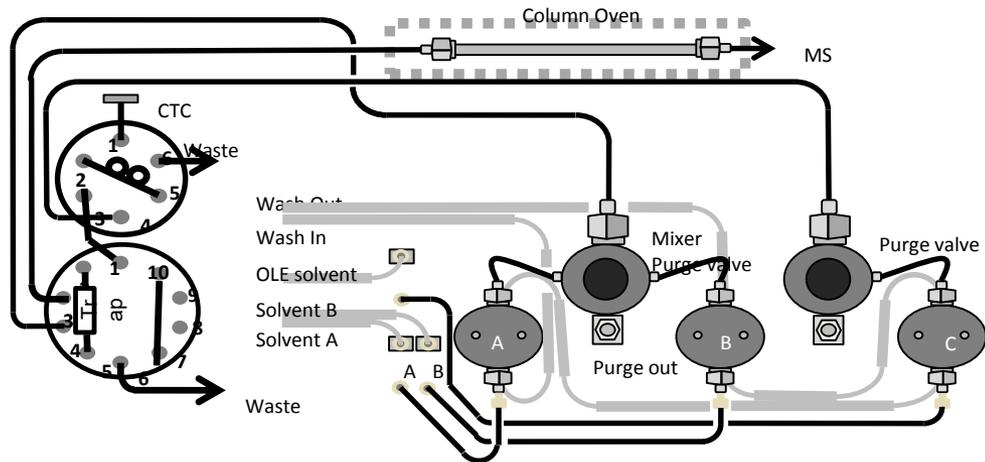


Dilute-Filter-Shoot

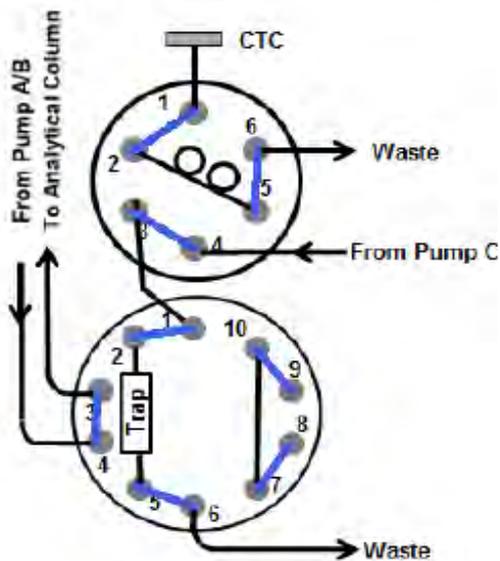


Retention Time (min)	Area	Height	Width	Height	Area	Height	Width	Height
1.1	1000	100	0.5	1000	100	0.5	1000	100
1.2	2000	200	0.5	2000	200	0.5	2000	200
1.3	3000	300	0.5	3000	300	0.5	3000	300
1.4	4000	400	0.5	4000	400	0.5	4000	400
1.5	5000	500	0.5	5000	500	0.5	5000	500
1.6	6000	600	0.5	6000	600	0.5	6000	600
1.7	7000	700	0.5	7000	700	0.5	7000	700
1.8	8000	800	0.5	8000	800	0.5	8000	800
1.9	9000	900	0.5	9000	900	0.5	9000	900
2.0	10000	1000	0.5	10000	1000	0.5	10000	1000
2.1	11000	1100	0.5	11000	1100	0.5	11000	1100
2.2	12000	1200	0.5	12000	1200	0.5	12000	1200
2.3	13000	1300	0.5	13000	1300	0.5	13000	1300
2.4	14000	1400	0.5	14000	1400	0.5	14000	1400
2.5	15000	1500	0.5	15000	1500	0.5	15000	1500
2.6	16000	1600	0.5	16000	1600	0.5	16000	1600
2.7	17000	1700	0.5	17000	1700	0.5	17000	1700
2.8	18000	1800	0.5	18000	1800	0.5	18000	1800
2.9	19000	1900	0.5	19000	1900	0.5	19000	1900
3.0	20000	2000	0.5	20000	2000	0.5	20000	2000
3.1	21000	2100	0.5	21000	2100	0.5	21000	2100
3.2	22000	2200	0.5	22000	2200	0.5	22000	2200
3.3	23000	2300	0.5	23000	2300	0.5	23000	2300
3.4	24000	2400	0.5	24000	2400	0.5	24000	2400
3.5	25000	2500	0.5	25000	2500	0.5	25000	2500
3.6	26000	2600	0.5	26000	2600	0.5	26000	2600
3.7	27000	2700	0.5	27000	2700	0.5	27000	2700
3.8	28000	2800	0.5	28000	2800	0.5	28000	2800
3.9	29000	2900	0.5	29000	2900	0.5	29000	2900
4.0	30000	3000	0.5	30000	3000	0.5	30000	3000
4.1	31000	3100	0.5	31000	3100	0.5	31000	3100
4.2	32000	3200	0.5	32000	3200	0.5	32000	3200
4.3	33000	3300	0.5	33000	3300	0.5	33000	3300
4.4	34000	3400	0.5	34000	3400	0.5	34000	3400
4.5	35000	3500	0.5	35000	3500	0.5	35000	3500
4.6	36000	3600	0.5	36000	3600	0.5	36000	3600
4.7	37000	3700	0.5	37000	3700	0.5	37000	3700
4.8	38000	3800	0.5	38000	3800	0.5	38000	3800
4.9	39000	3900	0.5	39000	3900	0.5	39000	3900
5.0	40000	4000	0.5	40000	4000	0.5	40000	4000
5.1	41000	4100	0.5	41000	4100	0.5	41000	4100
5.2	42000	4200	0.5	42000	4200	0.5	42000	4200
5.3	43000	4300	0.5	43000	4300	0.5	43000	4300
5.4	44000	4400	0.5	44000	4400	0.5	44000	4400
5.5	45000	4500	0.5	45000	4500	0.5	45000	4500
5.6	46000	4600	0.5	46000	4600	0.5	46000	4600
5.7	47000	4700	0.5	47000	4700	0.5	47000	4700
5.8	48000	4800	0.5	48000	4800	0.5	48000	4800
5.9	49000	4900	0.5	49000	4900	0.5	49000	4900
6.0	50000	5000	0.5	50000	5000	0.5	50000	5000
6.1	51000	5100	0.5	51000	5100	0.5	51000	5100
6.2	52000	5200	0.5	52000	5200	0.5	52000	5200
6.3	53000	5300	0.5	53000	5300	0.5	53000	5300
6.4	54000	5400	0.5	54000	5400	0.5	54000	5400
6.5	55000	5500	0.5	55000	5500	0.5	55000	5500
6.6	56000	5600	0.5	56000	5600	0.5	56000	5600
6.7	57000	5700	0.5	57000	5700	0.5	57000	5700
6.8	58000	5800	0.5	58000	5800	0.5	58000	5800
6.9	59000	5900	0.5	59000	5900	0.5	59000	5900
7.0	60000	6000	0.5	60000	6000	0.5	60000	6000
7.1	61000	6100	0.5	61000	6100	0.5	61000	6100
7.2	62000	6200	0.5	62000	6200	0.5	62000	6200
7.3	63000	6300	0.5	63000	6300	0.5	63000	6300
7.4	64000	6400	0.5	64000	6400	0.5	64000	6400
7.5	65000	6500	0.5	65000	6500	0.5	65000	6500
7.6	66000	6600	0.5	66000	6600	0.5	66000	6600
7.7	67000	6700	0.5	67000	6700	0.5	67000	6700
7.8	68000	6800	0.5	68000	6800	0.5	68000	6800
7.9	69000	6900	0.5	69000	6900	0.5	69000	6900
8.0	70000	7000	0.5	70000	7000	0.5	70000	7000
8.1	71000	7100	0.5	71000	7100	0.5	71000	7100
8.2	72000	7200	0.5	72000	7200	0.5	72000	7200
8.3	73000	7300	0.5	73000	7300	0.5	73000	7300
8.4	74000	7400	0.5	74000	7400	0.5	74000	7400
8.5	75000	7500	0.5	75000	7500	0.5	75000	7500
8.6	76000	7600	0.5	76000	7600	0.5	76000	7600
8.7	77000	7700	0.5	77000	7700	0.5	77000	7700
8.8	78000	7800	0.5	78000	7800	0.5	78000	7800
8.9	79000	7900	0.5	79000	7900	0.5	79000	7900
9.0	80000	8000	0.5	80000	8000	0.5	80000	8000
9.1	81000	8100	0.5	81000	8100	0.5	81000	8100
9.2	82000	8200	0.5	82000	8200	0.5	82000	8200
9.3	83000	8300	0.5	83000	8300	0.5	83000	8300
9.4	84000	8400	0.5	84000	8400	0.5	84000	8400
9.5	85000	8500	0.5	85000	8500	0.5	85000	8500
9.6	86000	8600	0.5	86000	8600	0.5	86000	8600
9.7	87000	8700	0.5	87000	8700	0.5	87000	8700
9.8	88000	8800	0.5	88000	8800	0.5	88000	8800
9.9	89000	8900	0.5	89000	8900	0.5	89000	8900
10.0	90000	9000	0.5	90000	9000	0.5	90000	9000
10.1	91000	9100	0.5	91000	9100	0.5	91000	9100
10.2	92000	9200	0.5	92000	9200	0.5	92000	9200
10.3	93000	9300	0.5	93000	9300	0.5	93000	9300
10.4	94000	9400	0.5	94000	9400	0.5	94000	9400
10.5	95000	9500	0.5	95000	9500	0.5	95000	9500
10.6	96000	9600	0.5	96000	9600	0.5	96000	9600
10.7	97000	9700	0.5	97000	9700	0.5	97000	9700
10.8	98000	9800	0.5	98000	9800	0.5	98000	9800
10.9	99000	9900	0.5	99000	9900	0.5	99000	9900
11.0	100000	10000	0.5	100000	10000	0.5	100000	10000

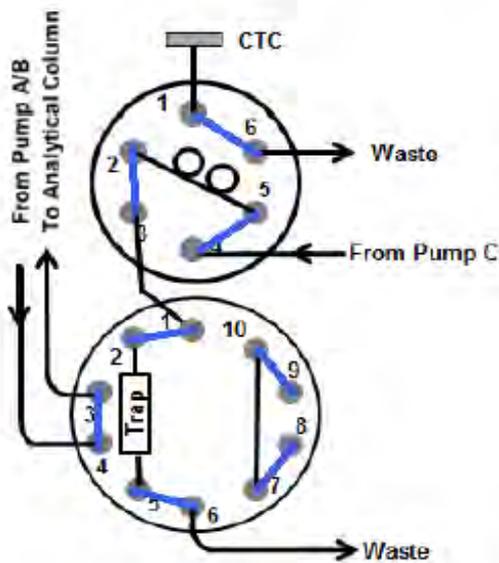
Valves Configuration



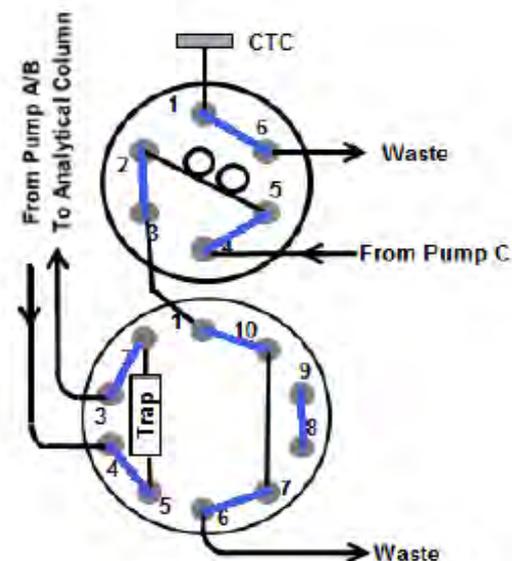
Equilibration Loading



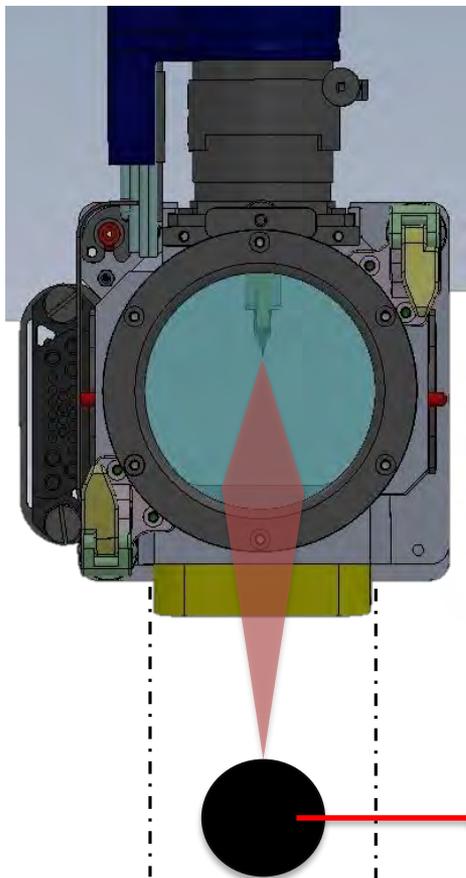
Trapping



Analysis



MS Design Highlight

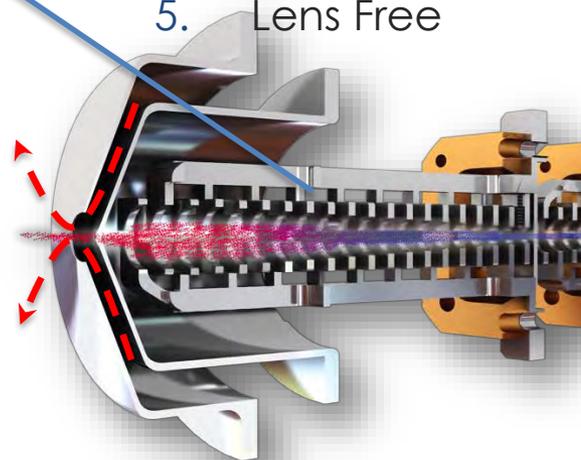


Low pressure region pulls all exhaust gases through the large exhaust opening and out of the mass spec.



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 μm , 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 μm , 150 mm \times 3.0 mm (I.D.)
- Column Temperature: 40 $^{\circ}\text{C}$
- Injection Volume: 50 μL
- 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

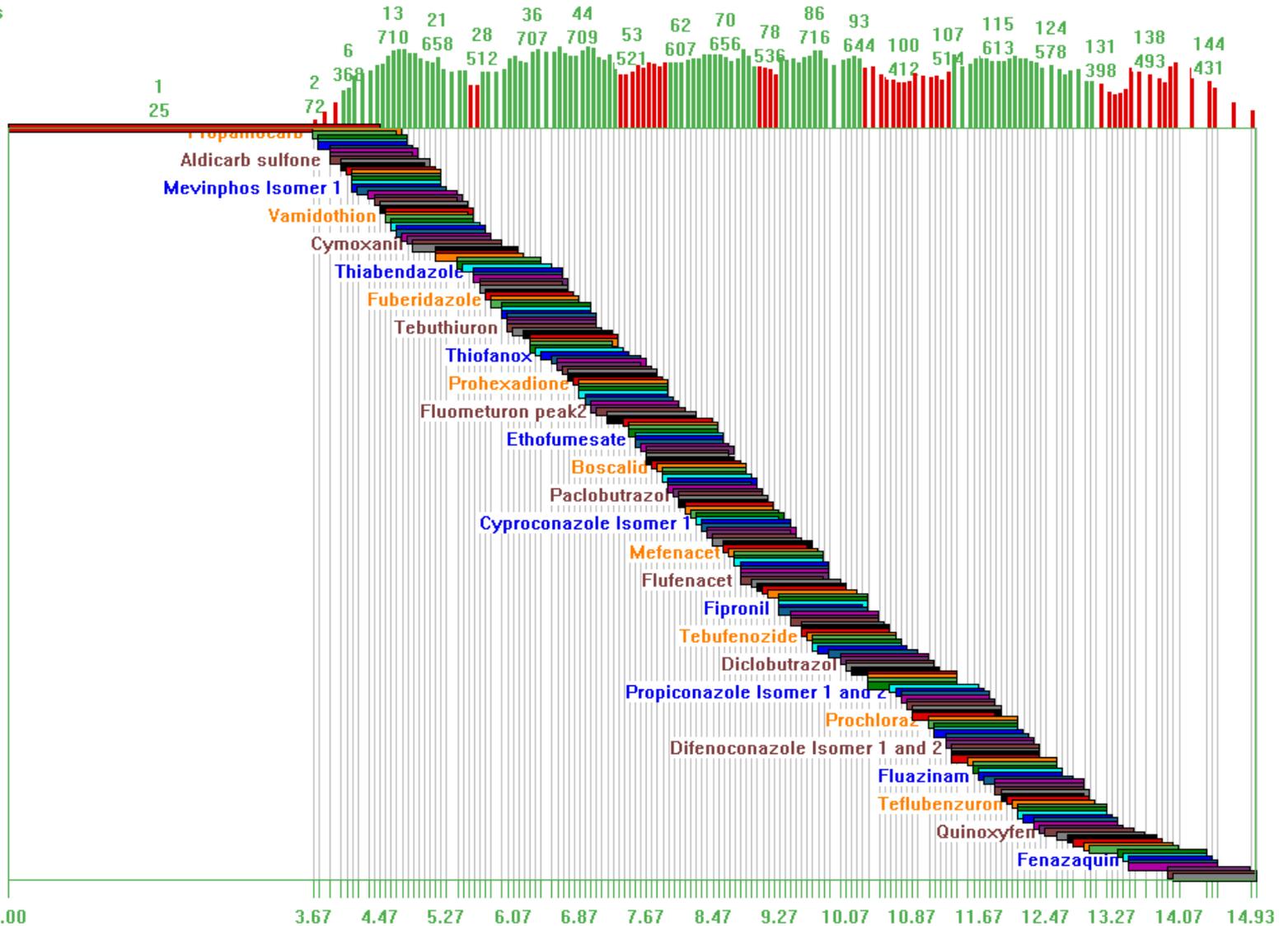
	Precursor	Product	Collision Energy	Q1 Resolution	Q3 Resolution	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	194.00	121.90	24.00	Standard (2.0)	Standard (2.0)	50.00%	<input checked="" type="checkbox"/>	50.20%	<input type="checkbox"/>
2	211.00	137.00	13.00	Standard (2.0)	Standard (2.0)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

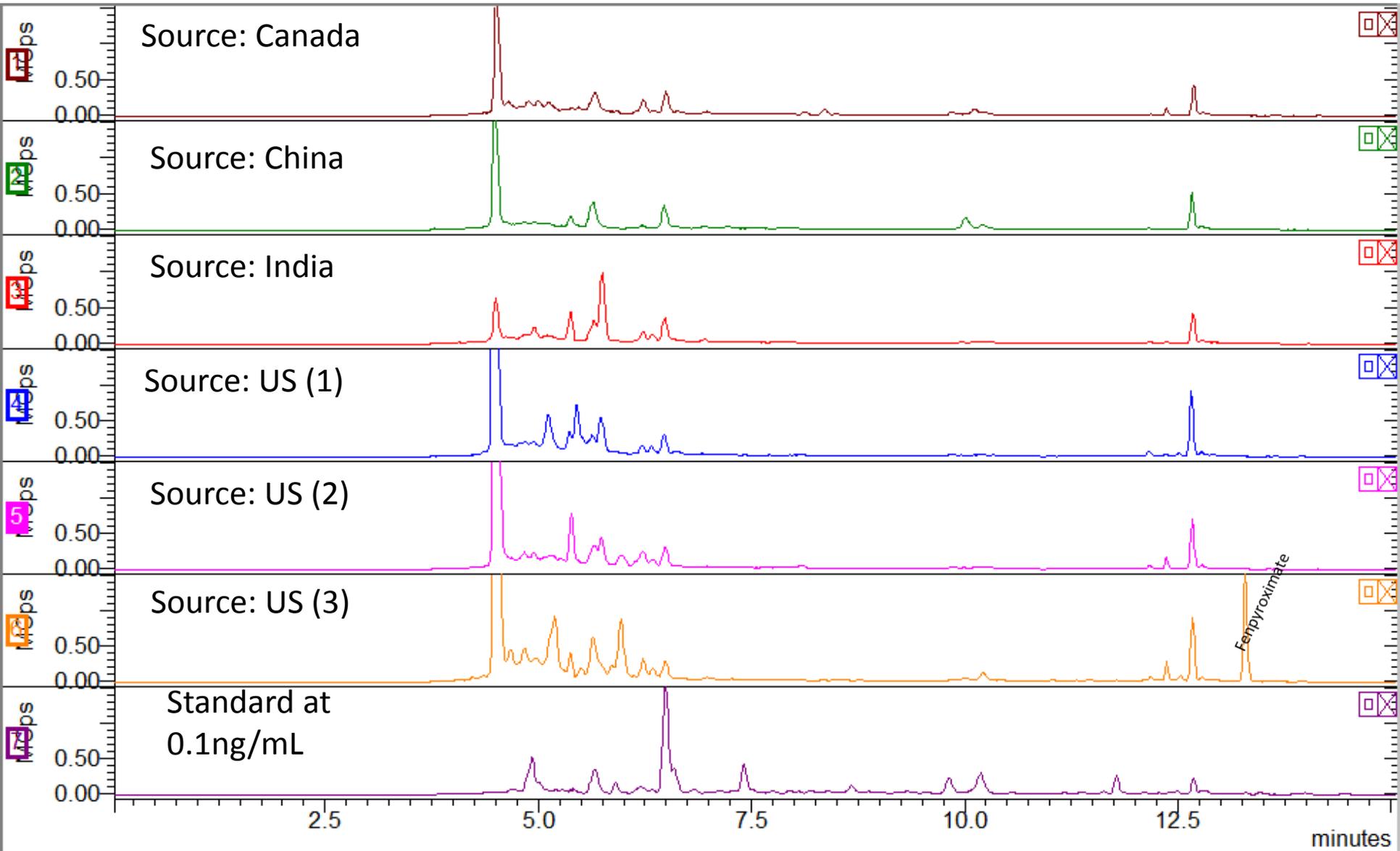


Result

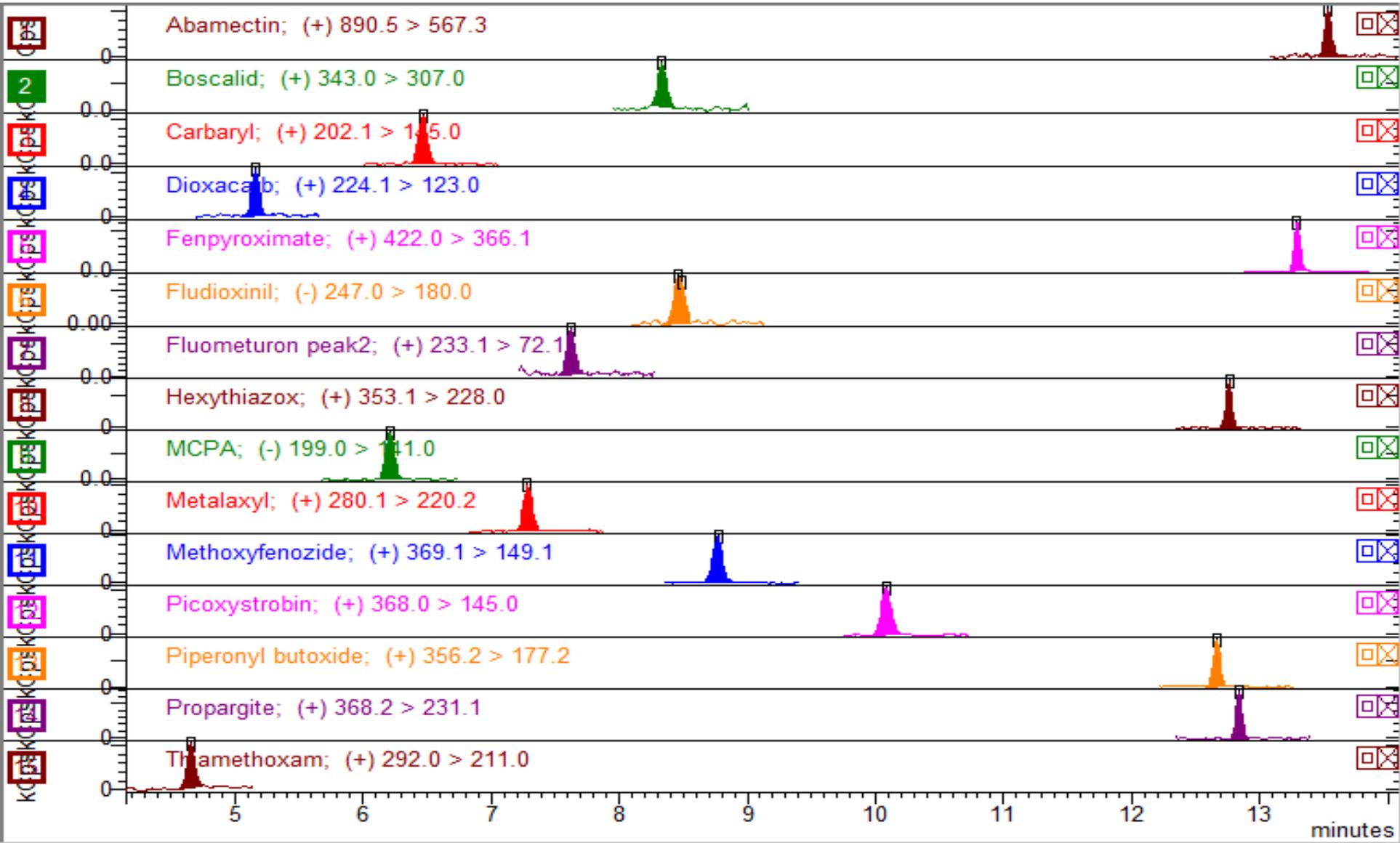
Honey Source=>	India	Canada	China	US-1	US-2	US-3
Pesticide		ng/g (ppb)				
Acetamiprid	ND	ND	0.6	ND	ND	ND
Boscalid	ND	17.5	ND	ND	0.15	3.4
Carbaryl	ND	0.71	ND	ND	ND	ND
Dioxacarb	ND	ND	ND	ND	1.35	2
Fenpyroximate	ND	ND	ND	ND	0.26	55
Fludioxinil	ND	1.5	ND	ND	ND	ND
Fluometuron	ND	ND	ND	ND	ND	2.8
Hexythiazox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

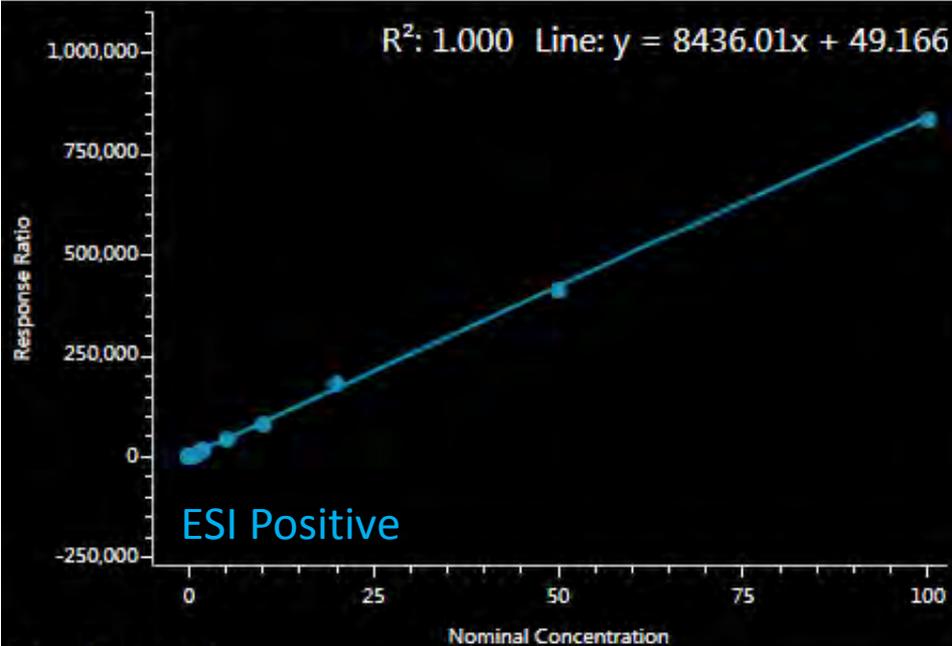


Pesticides at 0.05 ng/mL

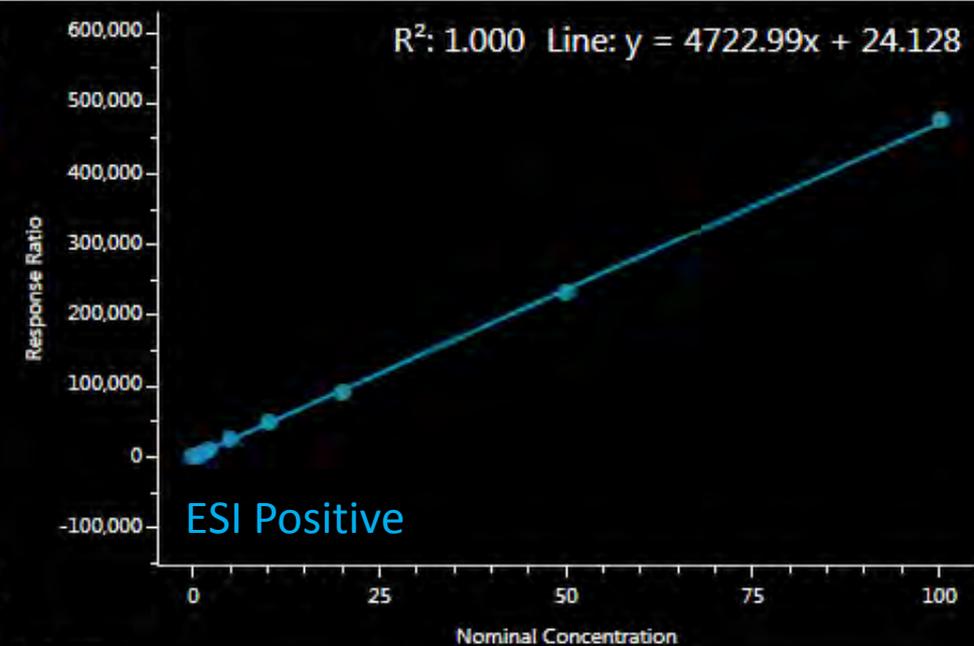


Above pesticides listed in result table

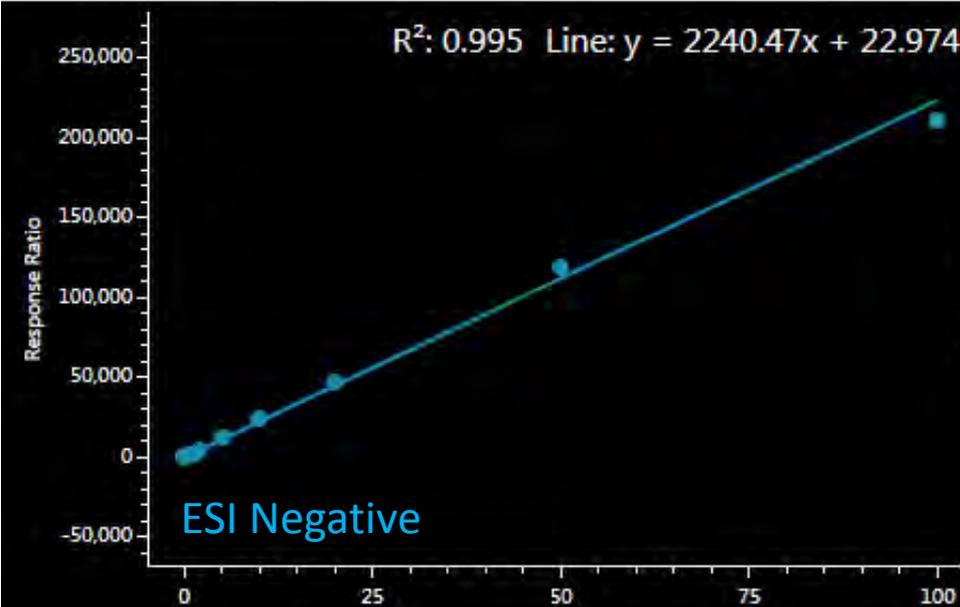
Acetamiprid



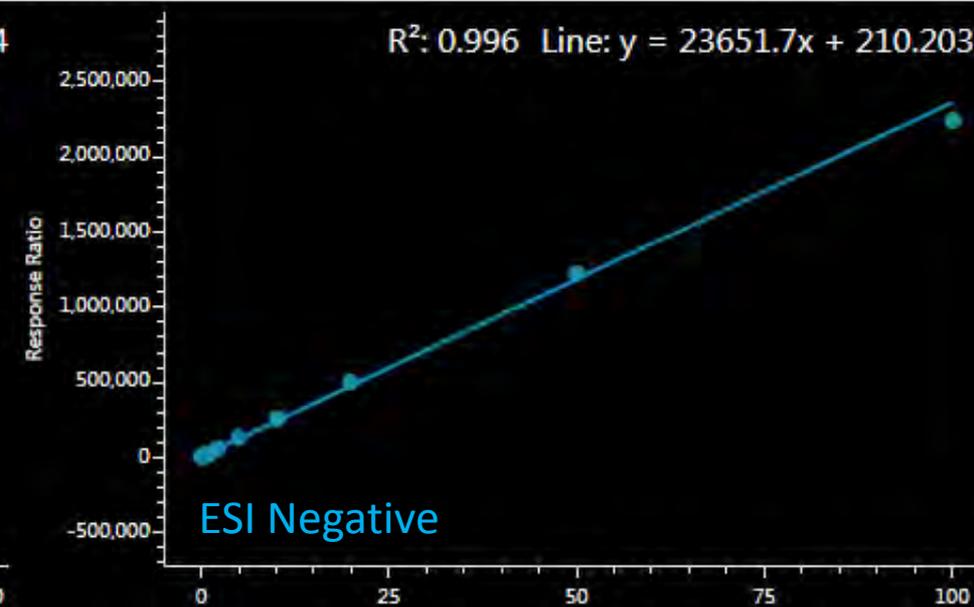
Dioxacarb



Fludioxinil



MCPA





Peak Review

Fenpyroximate

Determination Review

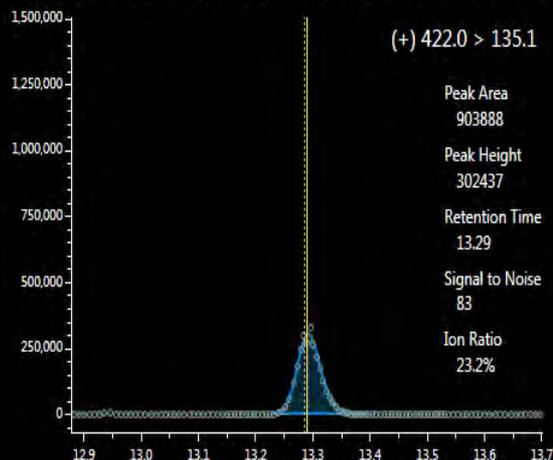
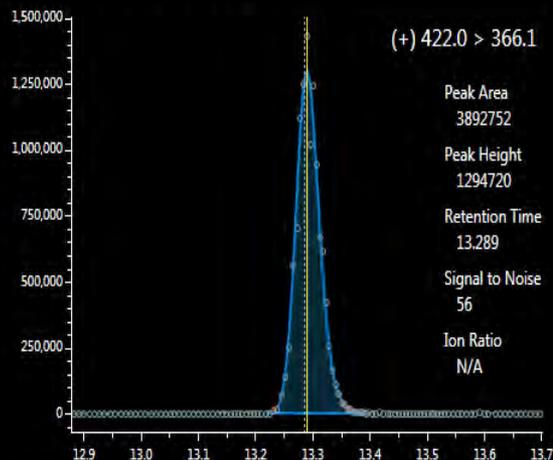
Honey_NN3

1 of 1 Samples

Fenpyroximate

204 of 215 Compounds

Determination Chromatograms



Determination Calculation

Concentration 54.688
Response 3892752

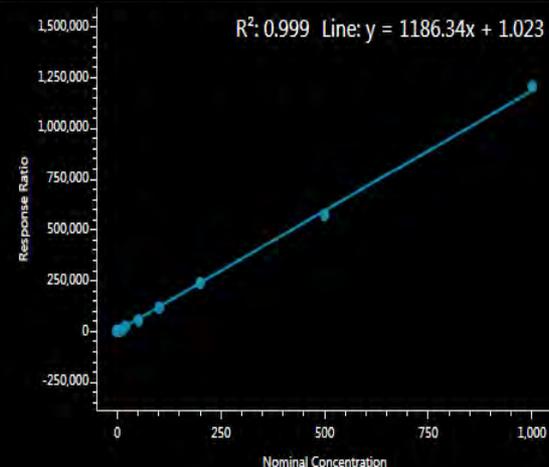
Determination Flags

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

