Analysis of Pesticides in Honey Using QuEChERS Extraction and Triple-Quadrupole GC-MS/MS Analysis

1 Introduction

The introduction of pesticides to improve agricultural productivity has led to significant changes in how crops are grown. However, heavy pesticide use has also had some unintended consequences, including to a number of health issues that can have severe consequences for both humans and the environment in general. One particular area of concern is the effect of pesticides on pollinators, such as honeybees. Research suggests that pesticides are adversely affecting honeybee populations and contributing to colony collapse disorder [1, 2]. Honeybees and other pollinators are also an important part of the global economy. According to the American Beekeeping Federation, honeybees contribute approximately $20 billion to crop production in the USA alone [3]. With these concerns in mind, regulatory agencies all over the world have placed restrictions on the types and levels of pesticides that can be used for agriculture to not only address health concerns for humans, but also for pollinators [4, 5]. In order to monitor pesticide uptake by honeybees, honey should be tested regularly for pesticide content.

Triple-quadrupole gas chromatograph tandem mass spectrometry (GC-MS/MS) is widely used for pesticide analysis due to the sensitivity and specificity of the technique. In particular, the specificity offered by GC-MS/MS is excellent for handling the analysis of samples with complex matrices like honey. In this app note, we demonstrate the ability of the JMS-TQ4000GC to measure the pesticide content of honey samples purchased from local grocers and apiaries. The high sensitivity and specificity offered by selected reaction monitoring (SRM) will be used against a complex honey extract matrix, including matrix-matched standard samples.

The JEOL JMS-TQ4000GC.

2 Experimental

All honey samples were purchased from local grocers and apiaries. Due to their low water content, each sample was prepared by adding 10 grams of water to 5 grams of honey in a 50 mL plastic tube, and heated gently to promote dissolution of the honey into the water prior to extraction [6]. The AOAC 2007.01 QuEChERS method [7] was used as follows to extract the pesticides from the sample and remove interferences: 15 mL of acetonitrile (HPLC grade, Fisher Scientific) was added to each tube. Samples were spiked with 3.75 μL of 100 ppm chlorpyrifos-d10 (Cambridge Isotope Laboratories, PN# DLM-4360-1.2) as an internal standard to monitor recovery and then extracted using Restek Q-sep QuEChERS extraction salts (PN# 25851). Samples were then centrifuged for 10 minutes.

Eight mL of the supernatant was then extracted by dSPE using Restek Q-sep QuEChERS dSPE Tubes (PN# 26222) by following the provided dSPE instructions. One mL of the resulting supernatant was put through another dSPE cleanup step using Restek (PN# 26125). The dSPE supernatant was either used to create matrix-matched standards, or injected directly into the GC as a sample. For standard samples, the extracted matrix was spiked with stock pesticide standards (Restek PN# 34124 - 34129) to concentrations ranging from to 0.1 to 100 ppb.

Samples were analyzed on the JMS-TQ4000GC using the parameters in Table 1. Transitions and collision energies used for each compound are listed in supplementary Table S1. Optimal product- and precursor-ion pairs and optimized collision energies for each pesticide were determined using built-in SRM optimization tools. Each standard and sample were run in triplicate with the exception of the one ppb standard samples, for which ten replicates were done to calculate the instrument detection limit (IDL) where possible.

Table 1: GC-MS parameters.

Agilent 7890B GC		**JMS-TQ4000GC**	
Column	ZB5-MSplus 30.0 m, 0.25 mm i.d., 0.20 μm (Phenomenex, Torrence, CA)	Ion Source Temp.	250 ℃
		Interface Temp.	300 ℃
		Ionization Mode	EI+, 70 eV, 100 μA
Inlet Liner	4 mm single taper w/wool on bottom (Phenomenex, Torrence, CA)	Measurement Mode	SRM, High
		Target Cycle Time	$\approx$ 330 ms
		Channel Time	20 - 100 ms
Inlet Temp.	250 ℃	Relative EM Voltage	900 V
Carrier Gas Type, Flow	He, 1.000 mL/min	Collision Gas	N_2, 10 %
Mode	Pulsed Splitless	**Oven Program**	
Pulsed Press., Time	206.84 kP, 0.55 min	80 ℃ (0.75 min) $\rightarrow$	
Purge Flow	30 mL/min, 1.0 min	35 ℃/min $\rightarrow$ 190 ℃ $\rightarrow$	
Septum Purge Flow	3.0 mL/min	5 ℃/min $\rightarrow$ 240 ℃ $\rightarrow$	
Injection Volume	2.0 μL	20 ℃/min $\rightarrow$ 330 ℃ (4 min)	

3 Results and Discussion

Figure 1 shows the total ion chromatogram for all measured SRMs at 25 ppb, while Figure 2 shows SRM chromatograms for select pesticides at 5 ppb. Of the 54 pesticides measured, 48 were detected at 5 ppb or less, and instrument detection limits (IDL) of 40 pesticides were less than 1 ppb (Table 2). Calibration curves for selected pesticides are shown in Figure 3. Good linearity (R2 > 0.99) was observed for every pesticide within its dynamic range except for azoxystrobin (R2=0.9844) (Table 2). Chlorinated pesticides, such as cypermethrin and chlordane, which are difficult to measure using LC-MS/MS, were easily measured using the TQ4000GC.

Data for honey samples purchased from local grocers and apiaries are shown in Figure 4 and Table 3. Generally, recoveries were poor compared to other applications of QuEChERS to food products, but it is likely that adding the second dSPE step significantly affected the recoveries. The appearance of clofentezine in every sample is interesting; even honey from a national distributer contained approximately 2 ppb of clofentezine (Figure 4). Research by Bahreini et al. suggests it could be used to control Varroa mite populations in beehives [8]; however, clofentezine can also kill bees in high enough concentrations. More concerning is that carbaril, which is highly toxic to bees [9, 5], was also found in all samples.

Small amount of the fungicides myclobutanil, paclobutrazole, and tebuconazole were found in several samples. Due to such low concentrations of these analytes, poor peak shape and IQ ratios prevented the quantitation of these pesticides. A few other pesticides were also detected in some of the honey samples, but most of them were at concentrations that could not be quantified accurately. Very little difference was found between the nationally distributed honey (Brands E & F), and honey purchased from local apiaries. Both Brands E & F did contain detectable amounts of carbofuran as well as select samples from other apiaries. Carbofuran is also highly toxic to bees [9].

4 Conclusions

The performance of the JMS-TQ4000GC triple quadrupole mass spectrometer was tested for measuring pesticides in extracted honey samples. Of the 54 pesticides measured, 48 were observed at 5 ppb or less in matrix-matched standard samples, and 40 had IDLs < 1 ppb. Calculated linearity and IDLs also showed good performance. Chlorinated pesticides, which can be difficult to measure using LC-MS/MS, were easily measured using GC-MS/MS.

Additionally, honey samples were purchased from local grocers and apiaries, and analyzed using methods mentioned above. Clofentezine was detected in every sample, though for honeybees, its toxicity has been rated as low. Carbaril, which is considered highly toxic to honeybees, was also found in all samples and is a matter of concern. Very few differences were observed between nationally distributed honey and honey purchased from local apiaries. The results above show that the JMS-TQ4000GC can perform at the low ppb level for many pesticides when analyzing complex, real-world samples such as honey. Due to its sensitivity and selectivity, the JMS-TQ4000GC could be a valuable addition to any food lab conducting pesticide testing.

References

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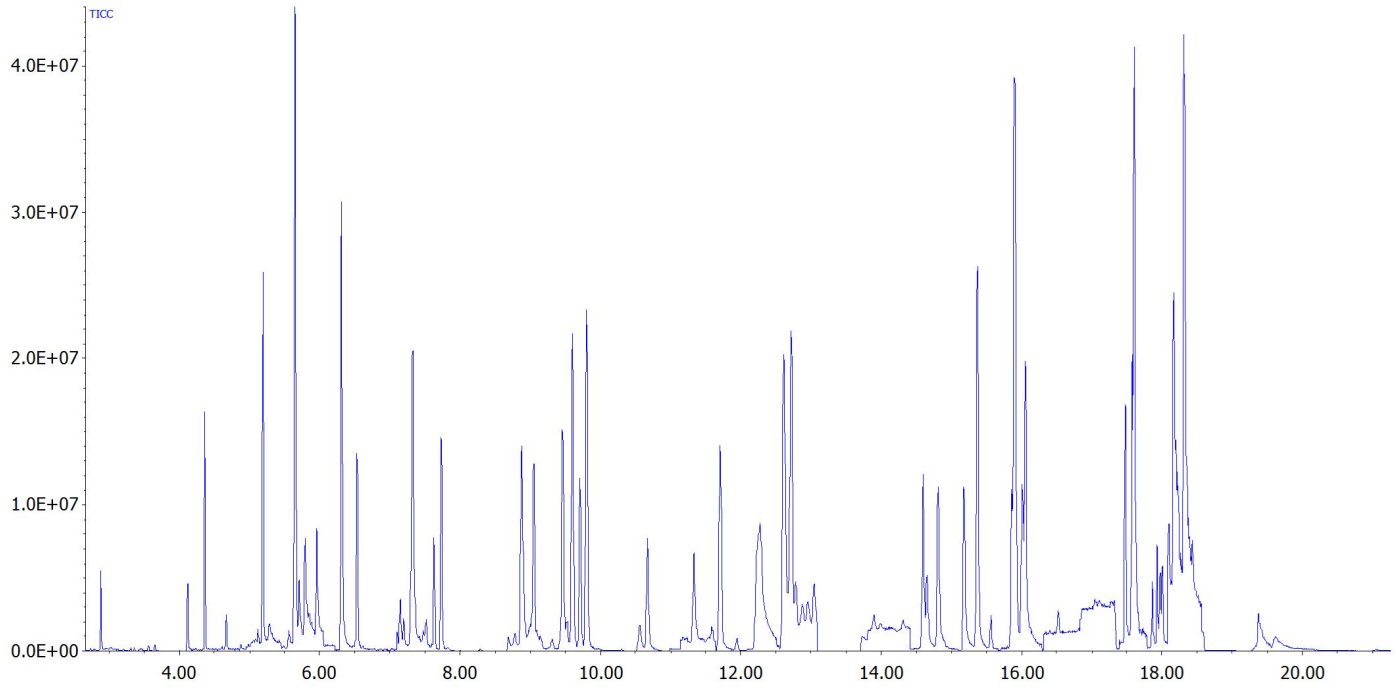

Figure 1: Total ion chromatogram of honey matrix spiked with 25 ppb pesticide standards.

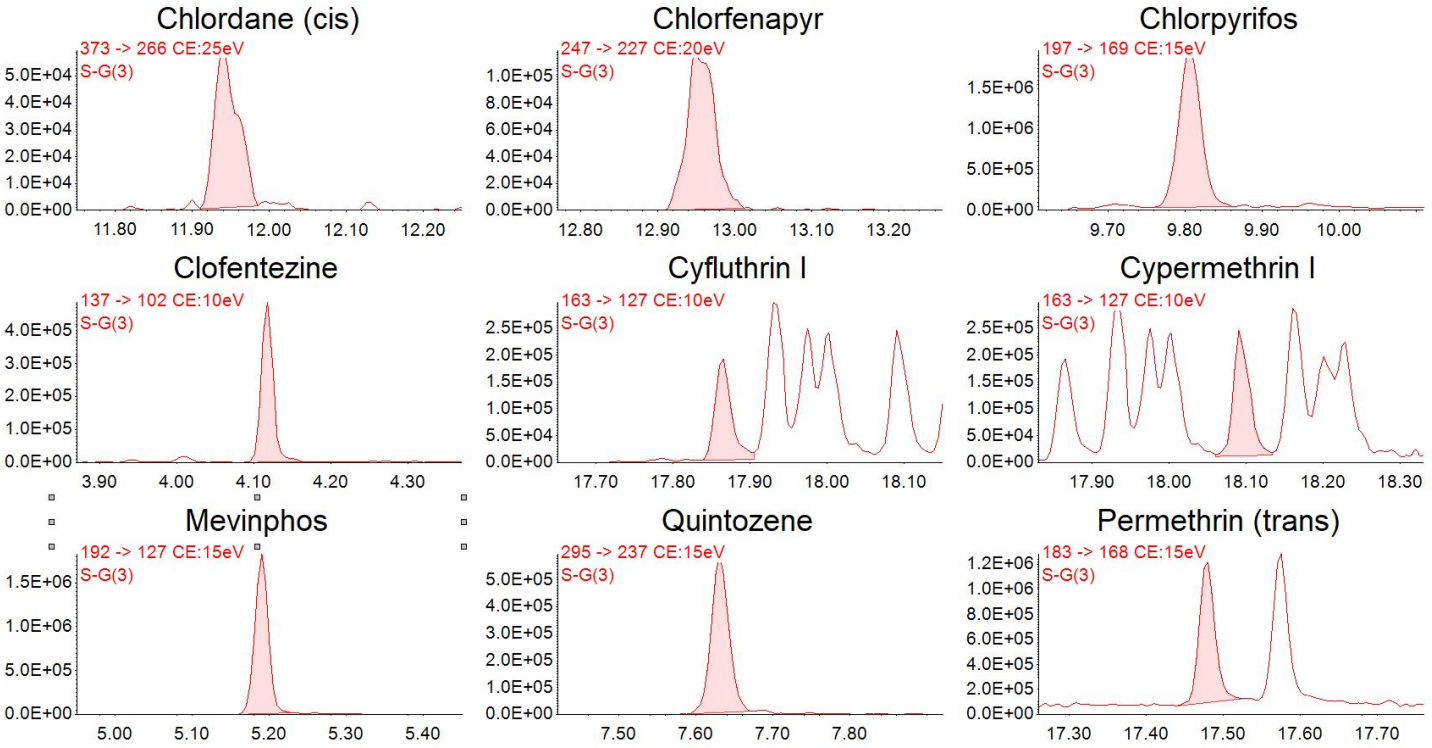

Figure 2: Selected SRM Chromatograms of a 1 ppb matrix-matched standard sample.

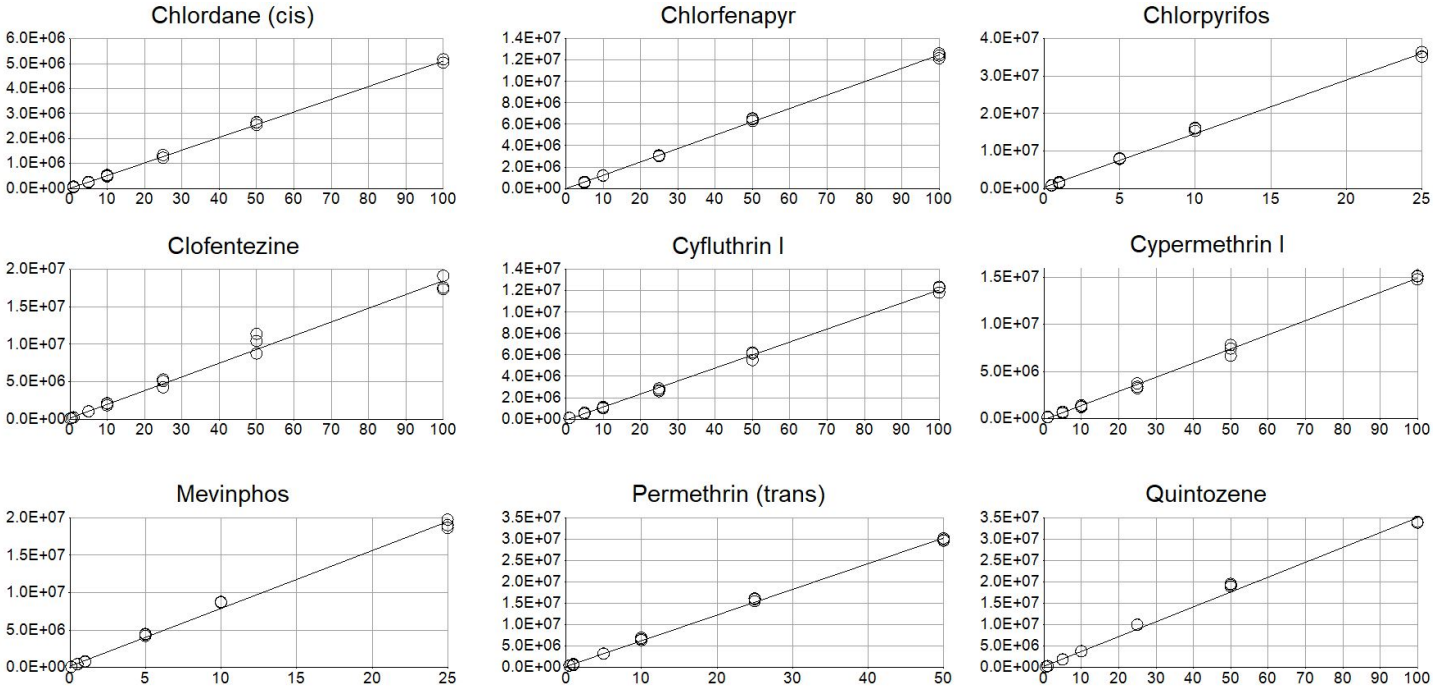

Figure 3: Selected calibrations curves for matrix-matched standard samples.

Table 2: Range, linearity, instrument detection limit (IDL), and CV of pesticide standards measured in spinach matrix.

Compound	Range (ppb)	Linearity R^2	IDL (ppb)	CV (%)
Acephate	25-100	0.9984	N/A	N/A
Aldicarb (decomp)	0.5-100	0.9994	0.46	16.17
Azoxystrobin	25-100	0.9844	N/A	N/A
Bifenazate	1-100	0.9985	0.46	16.38
Bifenthrin	0.1-100	0.9982	0.11	3.76
Boscalid	1-100	0.9982	0.38	13.58
Carbaril	5-100	0.9928	N/A	N/A
Carbofuran	1-100	0.9975	0.19	6.90
Chlorantraniliprole	10-100	0.9910	N/A	N/A
Chlordane	1-100	0.9997	0.69	24.28
Chlorfenapyr	1-100	0.9996	0.37	13.19
Chlorpyrifos	0.5-100	0.9987	0.17	6.03
Chlorpyrifos-d10	0.5-100	0.9995	0.11	3.91
Cinerin	5-100	0.9991	N/A	N/A
Clofentezine	0.1-100	0.9954	0.11	3.80
Coumaphos	5-100	0.9941	N/A	N/A
Cyfluthrin	1-100	0.9990	0.24	8.51
Cypermethrin	1-100	0.9990	0.38	13.42
Diazinone	0.5-100	0.9997	0.10	3.68
Dichlorvos	0.5-100	0.9982	0.13	4.67
Dimethoate	1-100	0.9989	0.29	10.17
Dimethomorph	5-100	0.9967	N/A	N/A
Ethoprophos	0.5-100	0.9998	0.11	3.91
Etofenprox	0.5-100	0.9978	0.15	5.41
Etoxazole	0.5-100	0.9990	0.18	6.40
Fenoxycarb	5-100	0.9963	N/A	N/A
Fipronil	0.5-100	0.9974	0.12	4.37
Fludioxonil	1-100	0.9992	0.16	5.71
Imazalil	5-100	0.9982	N/A	N/A
Jasmolin	10-100	0.9988	N/A	N/A
Kresoxim-methyl	0.5-100	0.9998	0.16	5.77
Malathion	0.5-100	0.9985	0.09	3.33
Metalaxyl	1-100	0.9990	0.20	7.12
Methiocarb	50-100	0.9946	N/A	N/A
Methomyl	5-100	0.9920	N/A	N/A
Methyl Parathion	1-100	0.9990	0.35	12.55
Mevinphos	0.5-100	0.9981	0.08	2.73
Myclobutanil	0.5-100	0.9981	0.16	5.50
Oxamyl	N/A	N/A	N/A	N/A
Paclobutrazol	0.5-100	0.9988	0.15	5.25
Permethrin (trans)	0.5-100	0.9994	0.21	7.52
Phosmet	1-100	0.9966	0.20	7.15
Piperonyl butoxide	0.5-100	0.9983	0.16	5.81
Prallethrin	1-100	0.9991	0.20	6.93
Propiconazole	1-100	0.9996	0.19	6.73
Propoxur	0.5 -100	0.9996	0.14	4.85
Pyrethrin	25-100	0.9840	N/A	N/A
Pyridaben	0.5-100	0.9994	0.14	4.84
Quintozene	0.5-100	0.9997	0.21	7.44
Spiromesifen	1-100	0.9983	0.45	15.82
Spiroxamine	0.5-100	0.9993	0.16	5.58
Tebuconazole	0.5-100	0.9992	0.10	3.51
Thiamethoxam	1-100	0.9943	0.51	18.04
Trifloxystrobin	1-100	0.9992	0.13	4.62

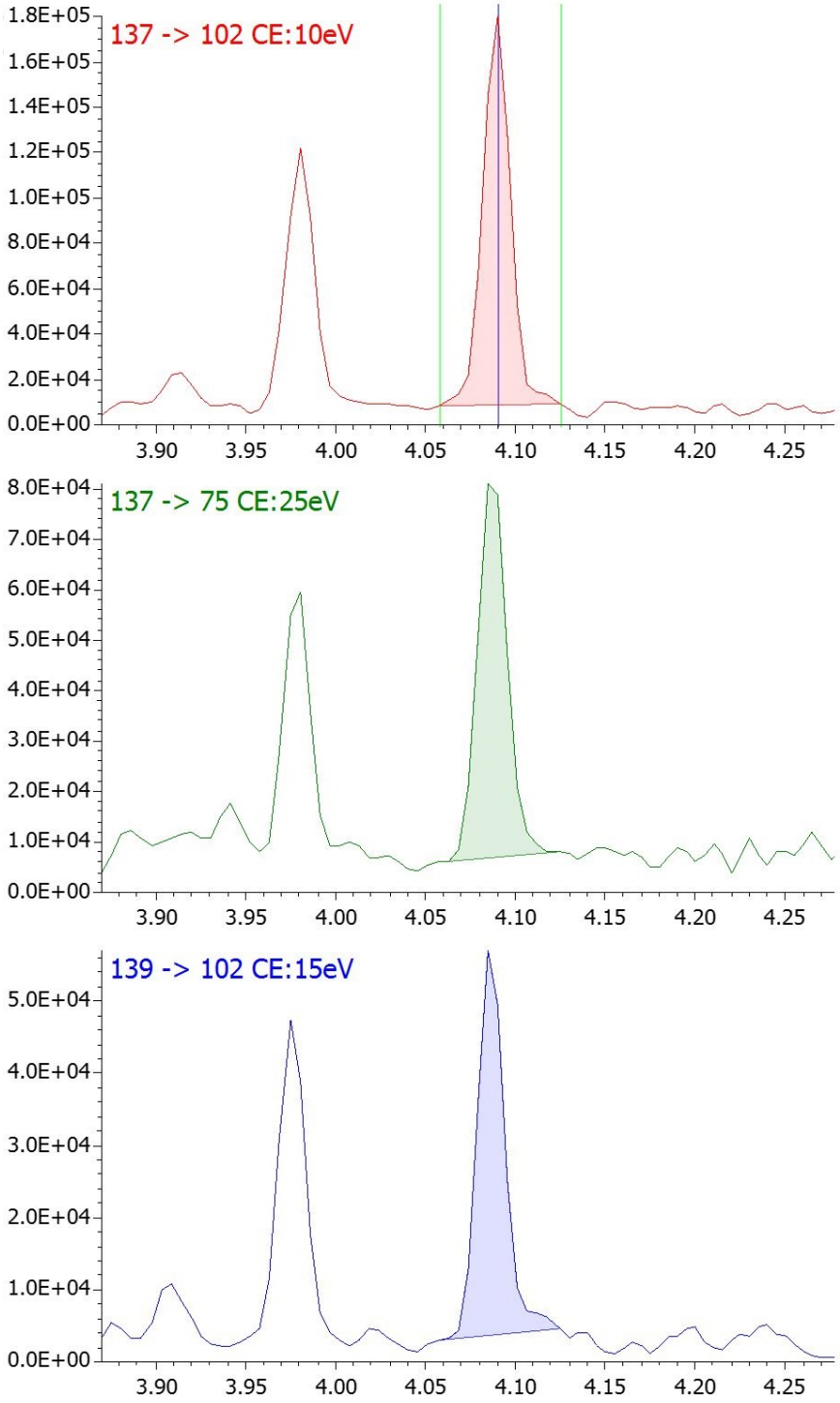

Figure 4: SRM Chromatogram of clofentezine found in Brand E honey. The top chromatogram is the quantitative ion; qualifier ions are shown in the middle and bottom chromatograms.

Table 3: Pesticides detected (ppb) in honey samples and sample recoveries.

	Brand A					
Compound	**Mountain 1**	**Mountain 2**	**Huckleberry**	**Valley**	**Clover**	**Pure comb**
Acephate						
Bifenthrin						
Carbaril	5.75	6.68	5.98	5.19	3.98	4.38
Carbofuran						< 0.5
Cinerin II			1.12			
Clofentezine	< 0.5	< 0.5	< 0.1	< 0.1	< 0.1	2.77
Dimethomorph	t	t				
Myclobutanil	< 0.5	< 0.5	< 0.1	< 0.1	< 0.1	< 0.1
Paclobutrazol	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.5
Piperonyl butoxide	t	t		1.16		< 0.1
Tebuconazole	0.108	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Recoveries (%)	31.7	32.5	32.3	31.4	34.0	71.2

	Brand B				Brand C		
Compound	**Wildflower**	**Bamboo**	**Blueberry**	**Spring**	**Idaho**	**Orange**	**Clover**
Acephate					< 0.5		
Bifenthrin					< 0.1	t	
Carbaril	6.63	5.74	6.30	6.63	6.78	4.67	4.68
Carbofuran			t				< 0.5
Cinerin II							
Clofentezine	< 0.1	< 0.1	< 0.5	< 0.1	< 0.1	< 0.1	2.32
Dimethomorph	6.51		t				t
Myclobutanil	< 0.1	< 0.1	< 0.5	< 0.1	< 0.5	< 0.1	< 0.5
Paclobutrazol	< 0.1	< 0.1	0.705	< 0.1	< 0.1	< 0.1	< 0.5
Piperonyl butoxide							
Tebuconazole	< 0.1	< 0.1	0.188	< 0.1	< 0.1	< 0.5	< 0.5
Recoveries (%)	33.5	35.4	31.9	33.0	35.5	31.5	59.0

	Brand D		Brand E	Brand F
Compound	**Agave**	**Organic Raw**	**Distrib**	**Distrib**
Acephate				
Bifenthrin				
Carbaril	4.61	4.47	2.58	3.50
Carbofuran	< 0.5	< 0.5	< 0.5	< 0.5
Cinerin II				
Clofentezine	2.69	2.56	2.21	2.07
Dimethomorph				
Myclobutanil	< 0.5	< 0.5	< 0.5	< 0.5
Paclobutrazol	< 0.1	< 0.5	< 0.5	< 0.5
Piperonyl butoxide			2.79	0.478
Tebuconazole	< 0.5	< 0.5	< 0.5	< 0.5
Recoveries (%)	60.5	68.4	67.0	67.8

Table S1: SRM transitions and collision energies for each pesticide.

	Quantitative Ion		Qualifier Ion 1		Qualifier Ion 2	
	Transition	**CE (eV)**	**Transition**	**CE (eV)**	**Transition**	**CE (eV)**
Acephate	136 → 94	15	125 → 79	10	125 → 47	20
Aldicarb (decomposed)	115 → 68	5	115 → 100	5	115 → 69	5
Azoxystrobin	344 → 156	30	388 → 345	20	388 → 300	20
Bifenazate	258 → 196	15	300 → 196	20	300 → 258	10
Bifenthrin	181 → 165	30	181 → 166	10	181 → 164	40
Boscalid	140 → 112	10	140 → 76	25	142 → 114	15
Captan	149 → 105	5	149 → 79	15	149 → 77	25
Carbaril	144 → 115	20	144 → 116	15	115 → 89	20
Carbofuran	164 → 103	30	164 → 149	10	149 → 77	25
Chlorantraniliprole	384 → 357	20	386 → 359	15	112 → 76	15
Chlordane (cis)	373 → 266	25	375 → 266	25	371 → 264	30
Chlordane (trans)	373 → 266	25	375 → 266	25	371 → 264	30
Chlorfenapyr	247 → 227	20	247 → 200	30	328 → 247	25
Chlorpyrifos	197 → 169	15	199 → 171	20	314 → 258	15
Chlorpyrifos-d10	200 → 172	20	260 → 167	25	260 → 139	30
Cinerin I	123 → 81	10	107 → 91	10	123 → 79	20
Cinerin II	107 → 91	10	121 → 93	5	167 → 107	5
Clofentezine	137 → 102	10	137 → 75	25	139 → 102	15
Coumaphos	226 → 163	15	226 → 198	10	362 → 226	15
Cyfluthrin I	163 → 127	10	163 → 91	15	165 → 91	15
Cyfluthrin II	163 → 127	10	163 → 91	15	165 → 91	15
Cyfluthrin III	163 → 127	10	163 → 91	15	165 → 91	15
Cyfluthrin IV	163 → 127	5	163 → 91	15	165 → 91	15
Cypermethrin I	163 → 127	10	163 → 91	15	165 → 91	15
Cypermethrin II	163 → 127	10	163 → 91	15	165 → 91	15
Cypermethrin III	163 → 127	10	163 → 91	15	165 → 91	15
Cypermethrin IV	163 → 127	10	163 → 91	15	165 → 91	15
Diazinone	304 → 179	15	179 → 121	35	179 → 137	20
Dichlorvos	109 → 79	5	220 → 185	5	145 → 109	10
Dimethoate	93 → 63	10	125 → 79	10	87 → 42	10
Dimethomorph I	301 → 165	10	303 → 165	10	301 → 273	10
Dimethomorph II	301 → 165	10	303 → 165	10	301 → 273	10
Ethoprophos	158 → 97	15	158 → 81	15	158 → 114	10
Etofenprox	163 → 107	25	163 → 135	10	163 → 77	35
Etoxazole	141 → 113	15	300 → 270	25	204 → 176	10
Fenoxycarb	116 → 88	5	255 → 186	15	186 → 157	15
Fipronil	367 → 213	30	351 → 255	15	369 → 215	35
Fludioxonil	248 → 182	15	248 → 154	15	154 → 127	10
Imazalil	215 → 173	10	173 → 145	20	173 → 109	30
Jasmolin I	123 → 81	10	123 → 79	20	164 → 109	10
Jasmolin II	107 → 91	10	121 → 77	20	121 → 93	10
Kresoxim-methyl	116 → 89	15	131 → 89	25	116 → 63	25
Malathion	127 → 99	10	173 → 99	10	173 → 117	10
Metalaxyl	160 → 130	20	160 → 144	20	160 → 145	10
Methiocarb	168 → 109	15	168 → 153	10	153 → 109	10
Methomyl	105 → 88	5	105 → 58	10	88 → 44	5
Methyl Parathion	263 → 109	15	109 → 79	10	125 → 79	10
Mevinphos	192 → 127	15	127 → 109	10	127 → 79	20
Myclobutanil	179 → 125	15	150 → 123	20	181 → 127	15
Naled	145 → 109	10	109 → 79	5	147 → 109	10

Table S1: (continued)

	Quantitative Ion		Qualifier Ion 1		Qualifier Ion 2	
	Transition	**CE (eV)**	**Transition**	**CE (eV)**	**Transition**	**CE (eV)**
Oxamyl	162 → 115	10	162 → 145	5	145 → 111	10
Paclobutrazol	236 → 125	10	125 → 89	25	236 → 132	20
Permethrin (cis)	183 → 168	15	183 → 153	15	183 → 165	10
Permethrin (trans)	183 → 168	15	183 → 153	15	183 → 165	10
Phosmet	160 → 133	15	160 → 77	20	160 → 105	20
Piperonyl butoxide	176 → 131	10	176 → 145	15	176 → 161	10
Prallethrin	123 → 81	5	134 → 91	10	123 → 95	5
Propiconazole I	173 → 145	20	173 → 109	30	175 → 147	20
Propiconazole II	173 → 145	20	173 → 109	30	175 → 147	20
Propoxur	110 → 63	25	152 → 110	15	110 → 64	20
Pyrethrin I	123 → 81	10	105 → 77	20	123 → 79	15
Pyrethrin II	160 → 117	15	107 → 91	10	133 → 105	10
Pyridaben	147 → 117	20	147 → 115	35	147 → 119	10
Quintozene	295 → 237	15	249 → 214	15	249 → 179	35
Spiromesifen	272 → 254	5	272 → 209	15	272 → 231	15
Spiroxamine I	100 → 72	10	100 → 58	10	100 → 41	20
Spiroxamine II	100 → 72	10	100 → 58	10	100 → 41	20
Tebuconazole	250 → 125	20	125 → 89	20	125 → 99	20
Thiamethoxam	212 → 139	10	247 → 212	5	247 → 182	10
Trifloxystrobin	116 → 89	15	131 → 89	30	131 → 116	20