

Pesticide Residue Analysis of Honey Bee Products Using Quechers

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CCD

- ▶ Colony Collapse Disorder (CCD) - A malady affecting honey bee colonies which has caused the loss of up to 80% of commercial bee hives in some areas over the last two years.
- ▶ Research is being conducted by USDA-ARS and universities like Penn State University into the cause (or causes) of CCD.
- ▶ Three main areas of research - Parasites, Pathogens, and Pesticides



Pesticide Analysis of CCD samples

- ▶ The USDA-AMS-NSL began pesticide residue testing of CCD samples for PSU and ARS in the spring of 2007.
- ▶ Sample types include beeswax, pollen, honey, honey bees, brood, royal jelly, nectar and flowers.
- ▶ The Quechers method, with slight modifications, was utilized for pesticide extraction of all of these matrices.



Quechers Advantages

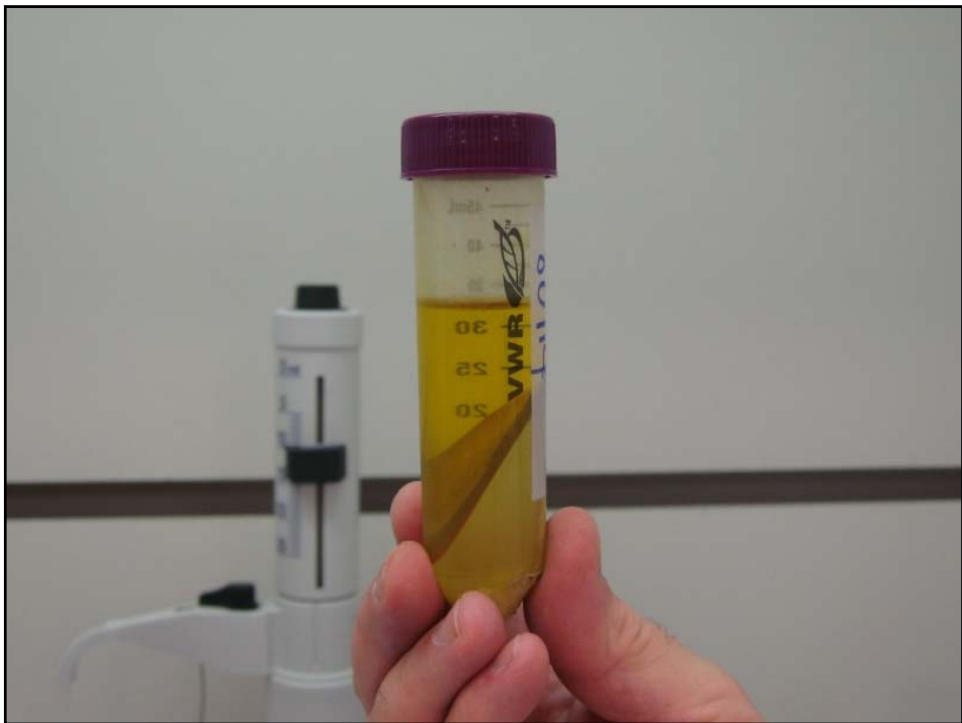
- ▶ Many CCD samples, especially pollen, are between 0.5 grams and 5 grams of sample.
- ▶ Quechers is easily adapted to variable sample sizes by proportionally adjusting reagents.
- ▶ Quechers works great for wax samples, which is the most complicated matrix to extract.
- ▶ Column SPE clean-up is utilized for GC/MS screening due to the complexity of pollen and wax matrices.

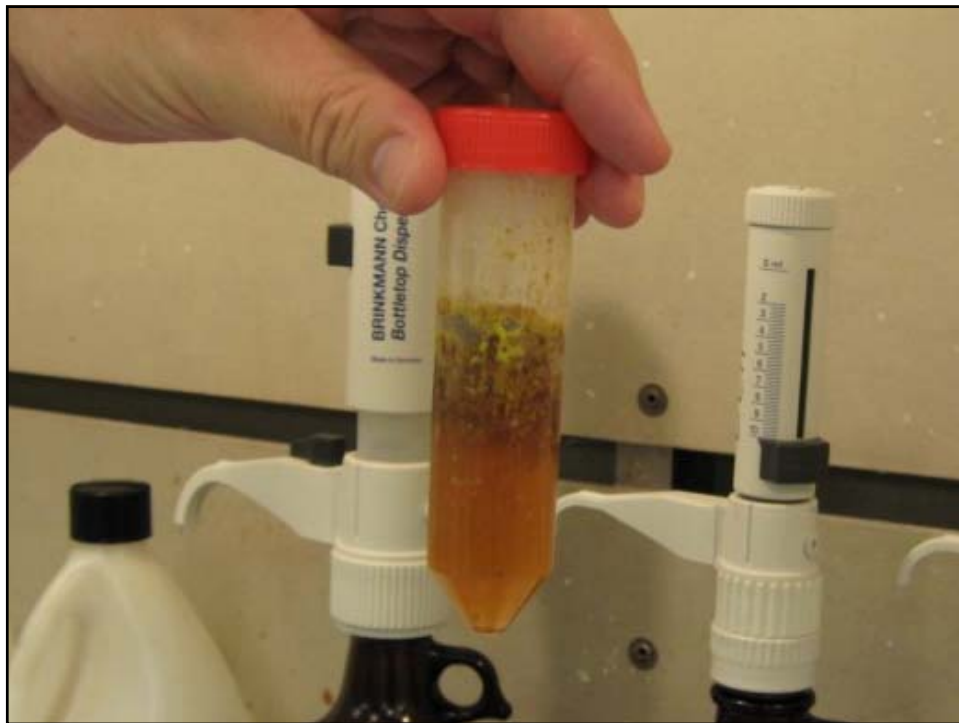


Using Quechers for CCD samples

- ▶ For CCD samples with a high water content (i.e. bees, brood, flowers, etc.), the buffered Quechers method is utilized.
- ▶ For pollen and wax, the buffered Quechers is also used with the addition of water to the sample in the following ratio - 3 grams sample, 12 mLs H₂O, and 15 mLs acidified MeCN.
- ▶ For wax, heating is required (80°C for 20 min.) after the addition of water and MeCN to dissolve the wax, then freezing (-20°C for 30 min.) is employed before centrifugation to help get the wax out of solution.







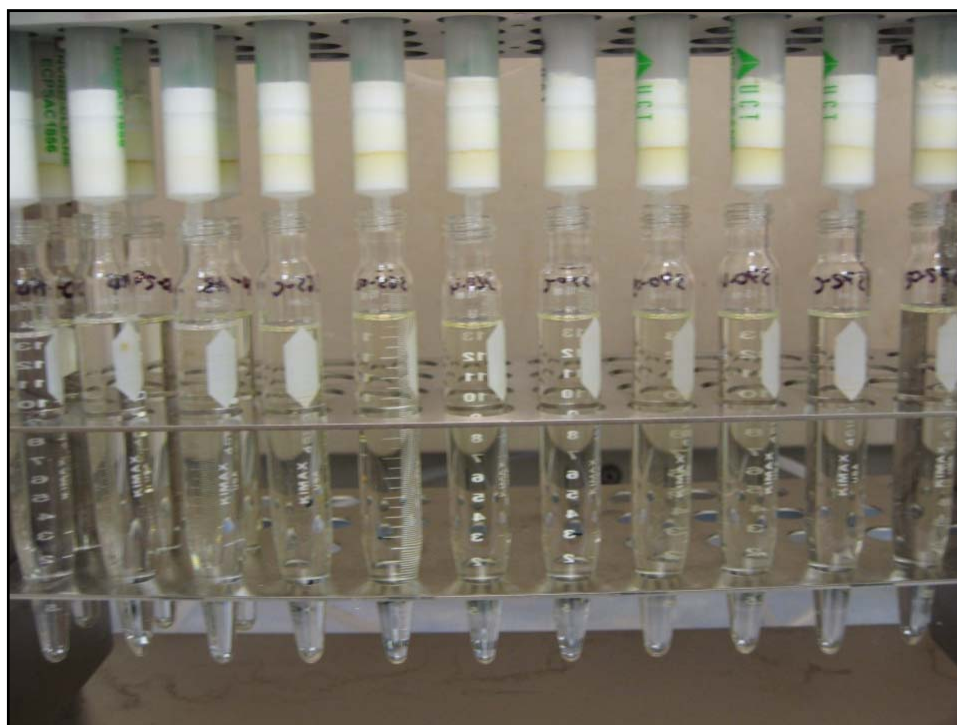






SPE Clean-up

- ▶ Pigments aren't a real issue with these samples, but lipids and wax do pose a clean-up issue for pollen and beeswax.
- ▶ C18 and PSA are the sorbents used in both column and dispersive SPE clean-up.
- ▶ Column SPE is used for GC/MS analysis and dispersive is used for LC/MS/MS analysis.
- ▶ 1 gram/mL sample concentration is obtained for high water content samples.
- ▶ For beeswax and pollen, due to the complicated matrix, 0.4 gram/mL sample concentration is the maximum concentration before chromatographic problems are encountered.



Pesticide detections in CCD samples

- ▶ 496 samples analyzed since April 2007 with 97 different pesticides detected out of 180 pesticides screened.
- ▶ One pollen sample had 35 residues detected.
- ▶ In-hive miticides (Fluvalinate & Coumaphos) for varroa mite control most prevalent and most concentrated.
- ▶ Other external pesticides of interest with high frequencies of detections are chlorpyrifos, chlorothalonil, and the pyrethroids.
- ▶ Neonicotinyls were found but not frequently.



Pesticides detected

compound	# of hits out of 496	low (PPB)	high (PPB)	compound	# of hits out of 496	low (PPB)	high (PPB)
Fluvalinate	410	1.0	204000	Captan	26	16	10000
Coumaphos	401	1.0	226000	Boscalid	25	1.4	302
Chlorpyrifos	274	1.0	890	Diazinon	25	1.0	260
Chlorothalonil	151	1.1	98900	Oxyfluorfen	25	1.0	34
Coumaphos oxon	121	1.3	1850	Bifenthrin	24	1.0	19
Endosulfan 1	106	1.0	95	Metolachlor	24	2.6	149
Esfenvalerate	97	1.0	82	Malathion	22	1.0	71
Endosulfan 2	82	1.0	39	Deltamethrin	21	9.3	193
Endosulfan sulfate	70	1.0	35	Allethrin	19	3.9	139
Cyhalothrin	59	1.0	18	Cyprodinil	18	5.3	2150
Dicofol	58	1.0	143	Vinclozolin	17	1.0	27
Fenpropathrin	58	1.0	87	Myclobutanil	16	4.4	981
Cypermethrin	57	1.5	240	Methoxyfenozide	15	3.4	650
2,4 Dimethylphenyl formamide	46	5.2	1350	Pyrimethanil	15	2.9	83
Cyfluthrin	46	1.0	19	Phosmet	14	1.6	630
Azinphos methyl	40	3.6	1240	DDE p,p'	13	2.0	31
Carbendazim	38	1.7	149	Acetamiprid	12	9.0	304
Pendimethalin	37	2.8	743	Chlorfenopyr	11	1.2	4.5
Methidathion	35	6.5	108	Diphenylamine	11	3.6	281
Trifloxystrobin	34	1.0	2610	Simazine	11	5.2	54
Permethrin	28	9.2	141	Carbaryl	10	14	1010
Atrazine	27	2.7	154	Imidacloprid	10	6.2	646
Azoxystrobin	27	2.1	107				



Pesticides detected cont.

compound	# of hits out of 496	low (PPB)	high (PPB)	compound	# of hits out of 496	low (PPB)	high (PPB)
Aldicarb sulfone	9	17	90	Acephate	2	52	163
Aldicarb sulfoxide	9	22	1146	Bendiocarb	2	5.5	22
Norflurazon	9	2.8	108	Imidacloprid 5-hydroxy	2	150	152
Thiabendazole	9	1.5	76	Imidacloprid olefin	2	554	640
Thiacloprid	9	1.7	115	Indoxacarb	2	330	531
Fenbuconazole	8	11	190	Metribuzin	2	3.4	44
Prallethrin	8	4.7	9.6	Potasan	2	101	165
Tebuthiuron	8	1.6	48	Tetradifon	2	1.6	2.7
Tetramethrin	8	6.1	23	Tribufos (DEF)	2	1.4	59
Hexachlorobenzene	7	1.0	1.0	2,4 Dimethylaniline	1	331	N/A
Parathion methyl	7	1.2	5.3	Aldicarb	1	20	N/A
Trifluralin	7	1.0	36	Amicarbazone	1	98	N/A
1-Naphthol	6	7.8	54	Carbofuran	1	32	N/A
Chlorferone	6	299	4390	Chlorpyrifos oxon	1	43	N/A
Dimethomorph	6	16	420	Clothianidin	1	35	N/A
Tebuconazole	6	18	65	Difenoconazole	1	344	N/A
Oxamyl	5	20	43	Dinotefuran	1	97	N/A
Carfentrazone ethyl	4	1.5	2.8	Etoxazole	1	1.2	N/A
Dieldrin	4	10	195	Heptachlor	1	31	N/A
Diflubenzuron	4	15	128	Malathion oxon	1	22	N/A
Fenhexamid	4	28	670	Pyraclostrobin	1	17	N/A
Pyrethrins	4	27	49	Quintozene (PCNB)	1	2.5	N/A
Tebufenozide	4	13	164	Sethoxydim	1	8.2	N/A
3-Hydroxycarbofuran	3	4.6	23	Spirodiclofen	1	6.8	N/A
4,4 Dibromobenzophenone	3	5.1	21	Thiamethoxam	1	38	N/A
Pronamide	3	68	378	THPI	1	286	N/A



PDP Honey Analysis

- ▶ Honey was added as a commodity to the Pesticide Data Program (PDP) in October 2007.
- ▶ The NSL is testing PDP honey samples using a modified Quechers method.
- ▶ For GC/MS EI and NCI analyses, ethyl acetate is used instead of acetonitrile to preserve base sensitive compounds like amitraz and chlorothalonil.
- ▶ For GC/MS–NCI, the sample extract is dried with MgSO₄ with no sorbent clean-up, while the EI extract is dried and cleaned using dispersive PSA.
- ▶ For LC/MS/MS analysis, acetonitrile is used with dispersive clean-up with PSA.



Instrumental Analysis

- ▶ LC/MS/MS
- ▶ GC/MS in EI mode
- ▶ GC/MS in NCI mode



NCI Background Information

- ▶ GC/MS Negative Chemical Ionization (NCI) utilizes a collision gas in the MS source that “softens” the fragmentation.
- ▶ NCI can be a good replacement for selective or elemental detectors like ECD, ELCD, XSD or FPD.
- ▶ NCI works well for compounds that tend to fragment excessively in EI and also compounds with electronegative properties like chlorine and sulfur containing compounds.



NCI Advantages

- ▶ Very few matrix components ionize with NCI which greatly reduces background interferences.
- ▶ Soft ionization reduces fragmentation and thus increases abundance of ions that do form versus EI, which increases MS sensitivity.
- ▶ NCI can be used as a GC/MS screening tool for compounds that are difficult to analyze by EI, like the endosulfans, and that are normally analyzed by selective detection systems.
- ▶ Provides an added MS screening tool.

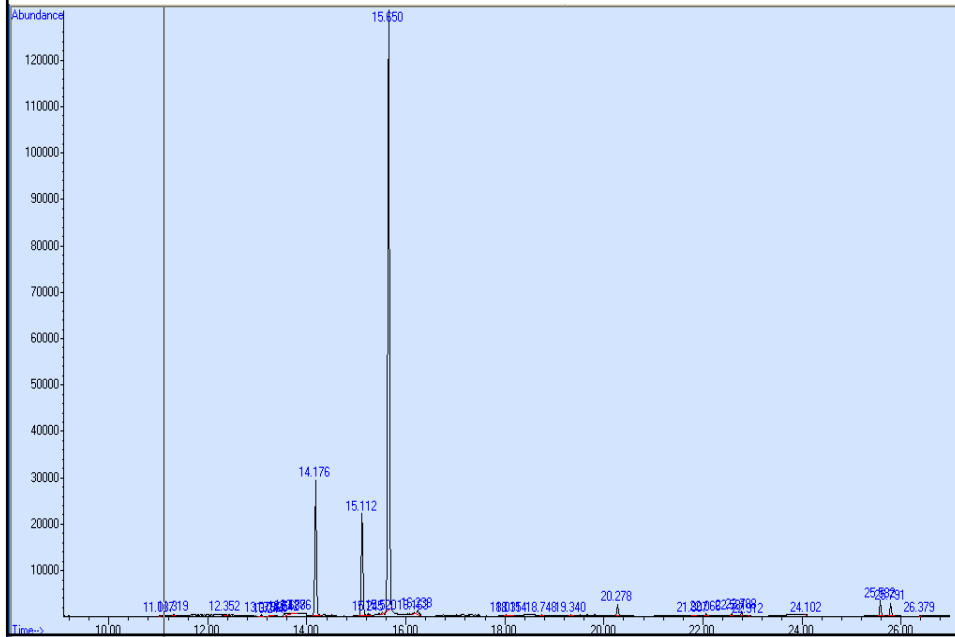


NCI Disadvantages

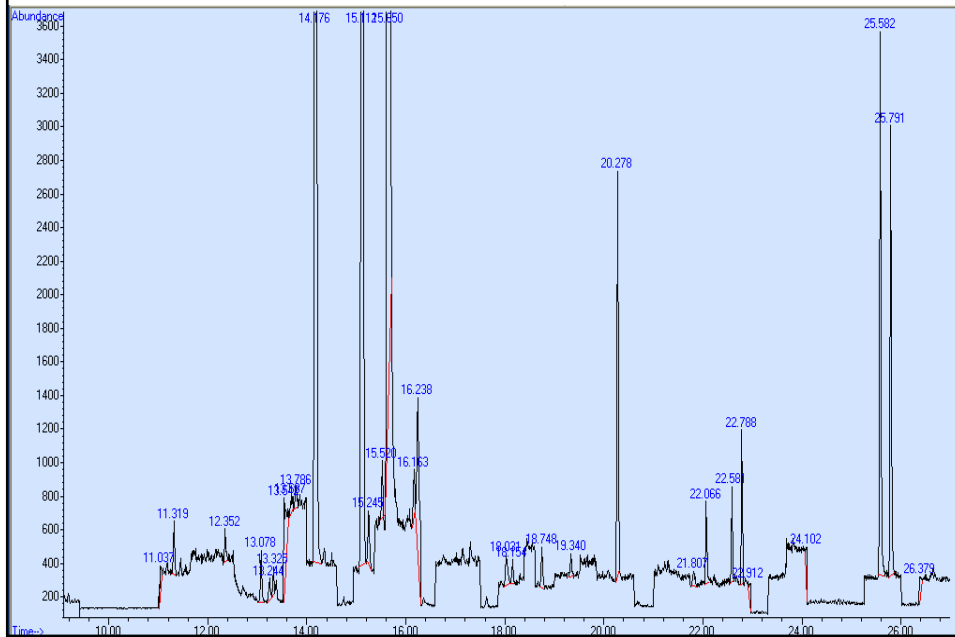
- ▶ Not all pesticides react in NCI (this can also be considered an advantage).
- ▶ For a few pesticides, only molecular or isotopic ions are formed which limits confirmation ability.
- ▶ Does not produce classic EI library spectrum.
- ▶ NCI needs more time after maintenance to stabilize.



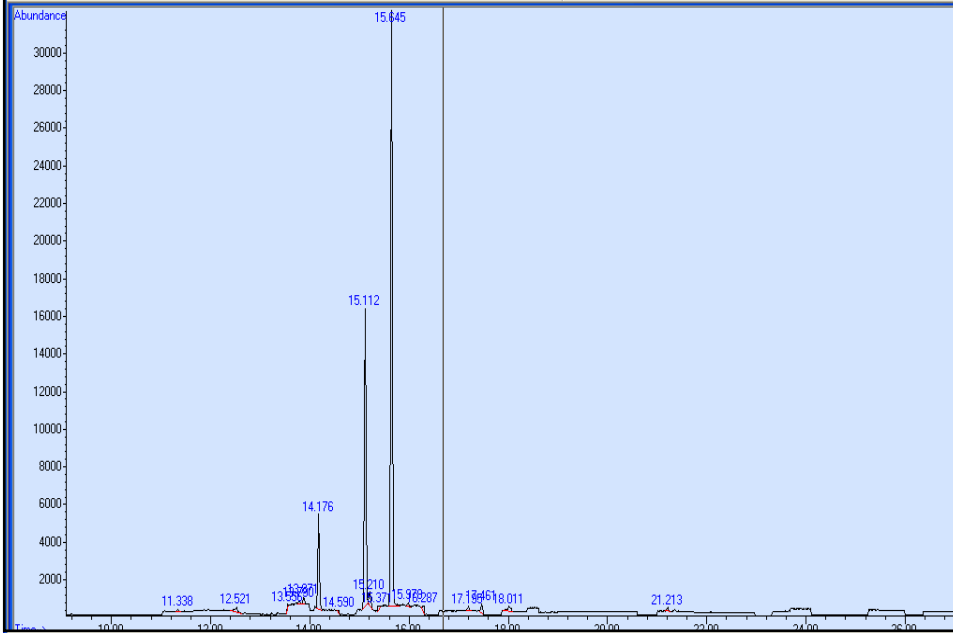
Flower TIC



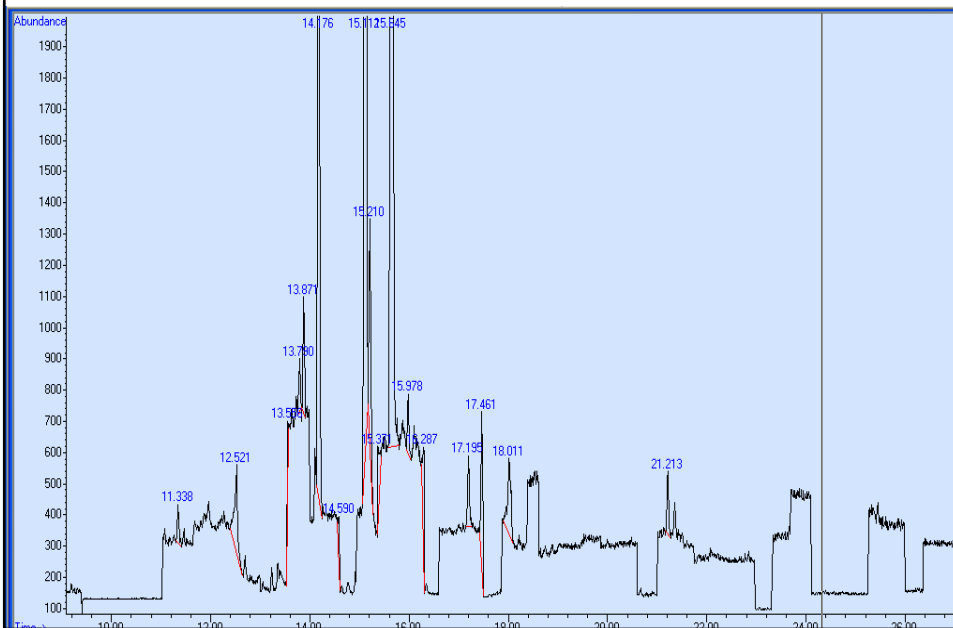
Flower TIC - zoomed



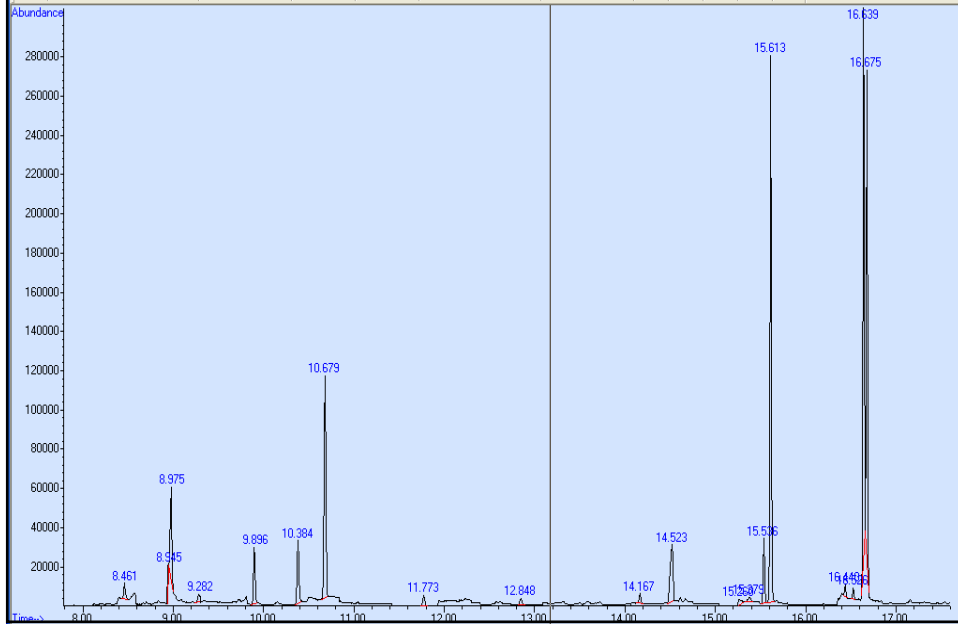
Pollen TIC



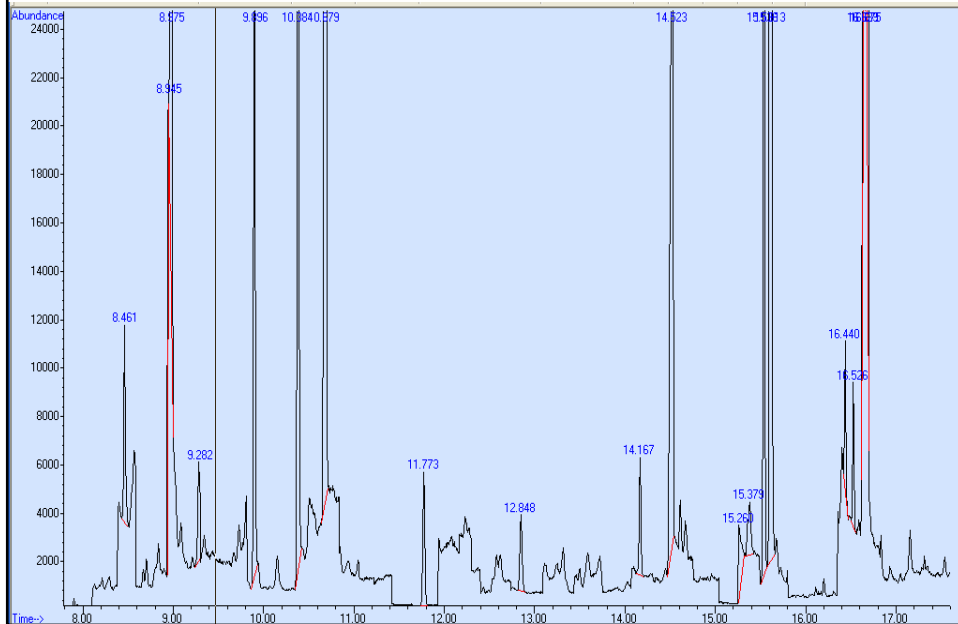
Pollen TIC – zoomed

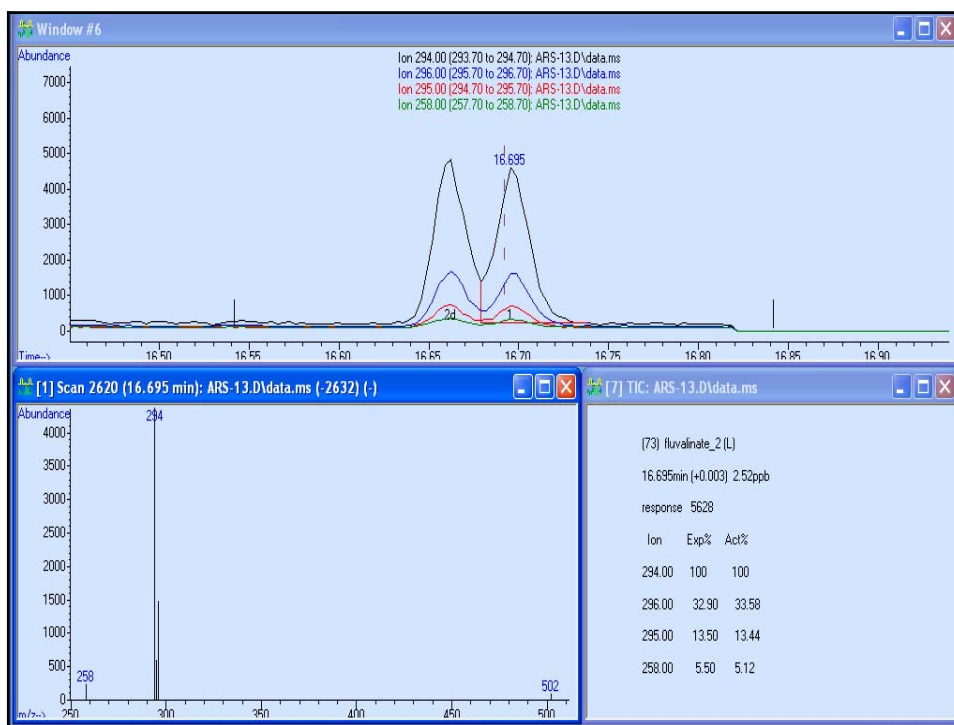
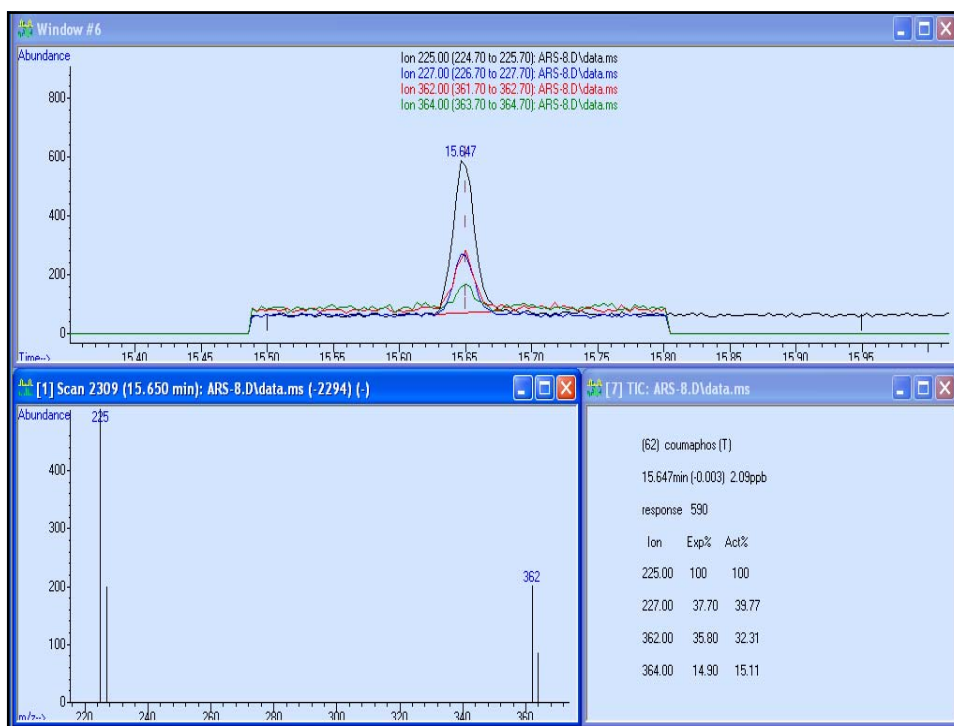


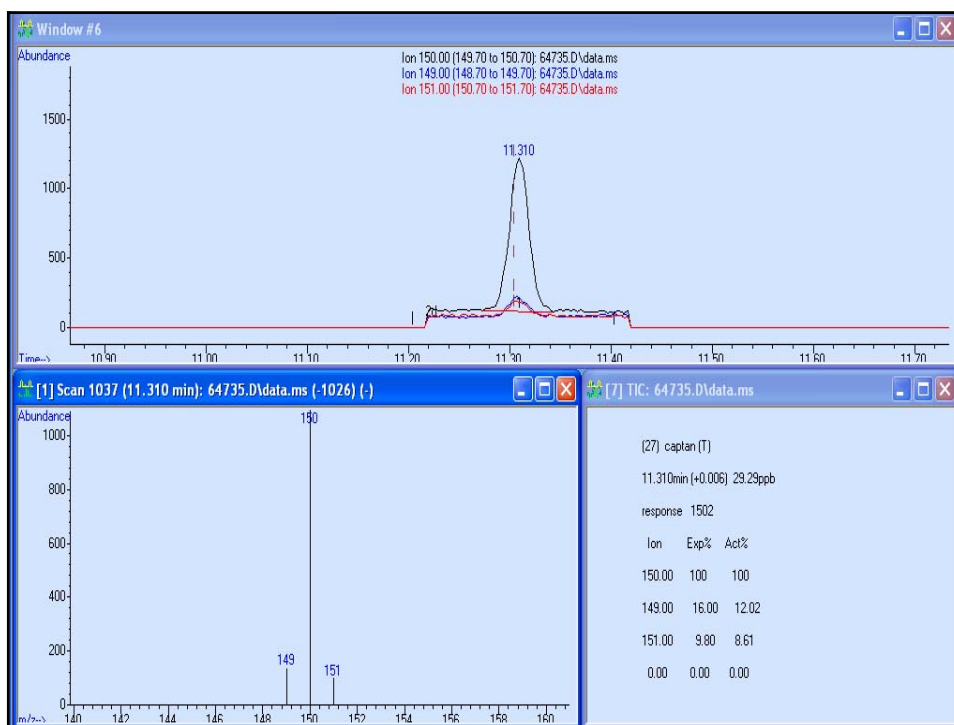
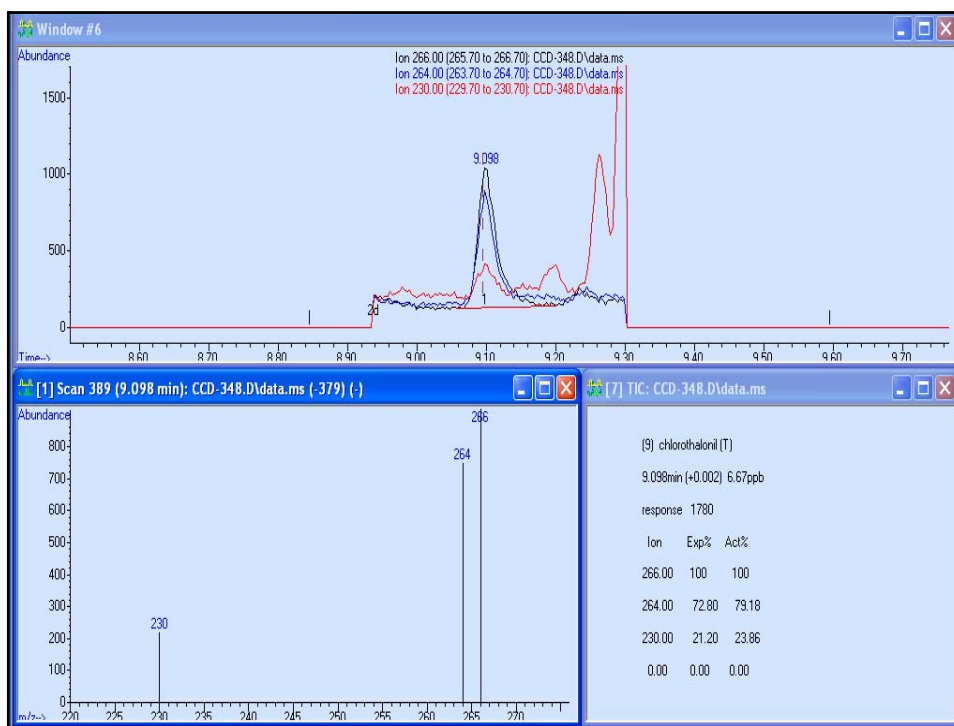
Beeswax

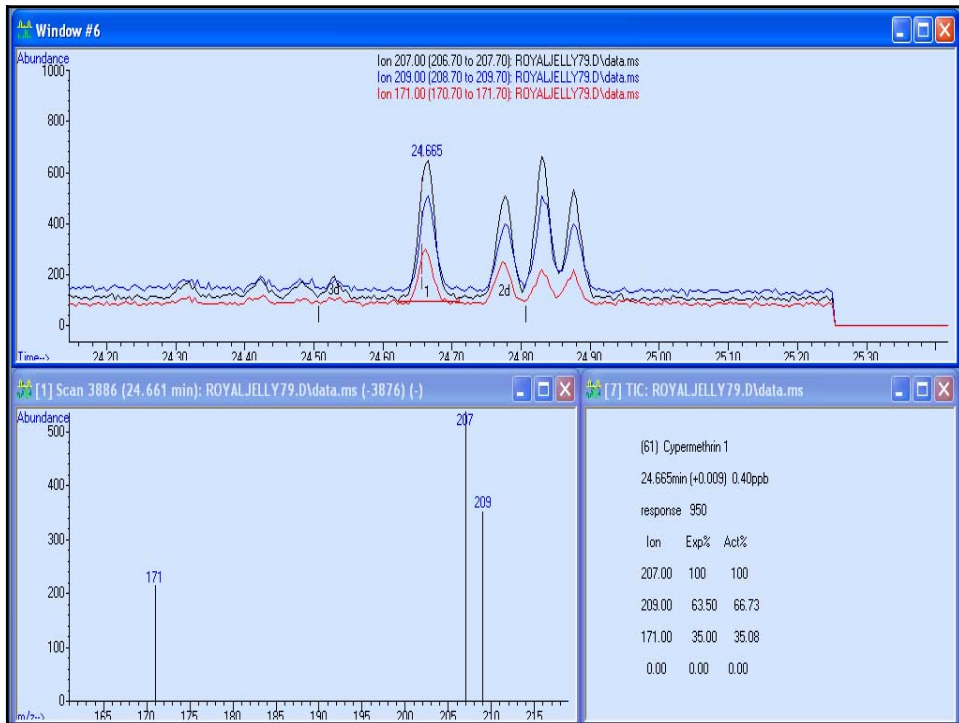
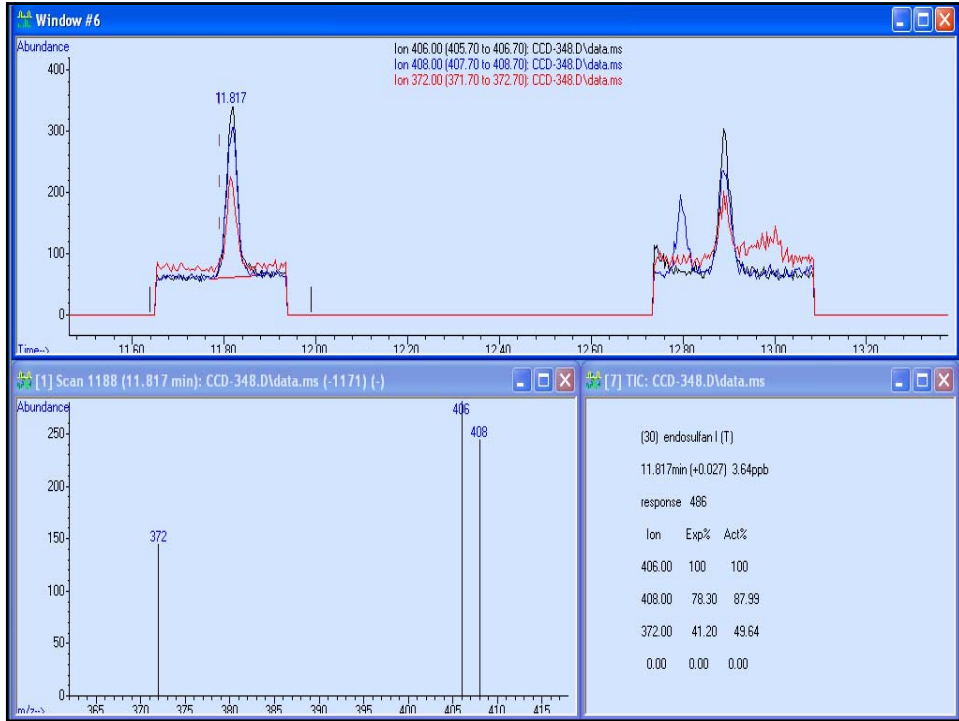


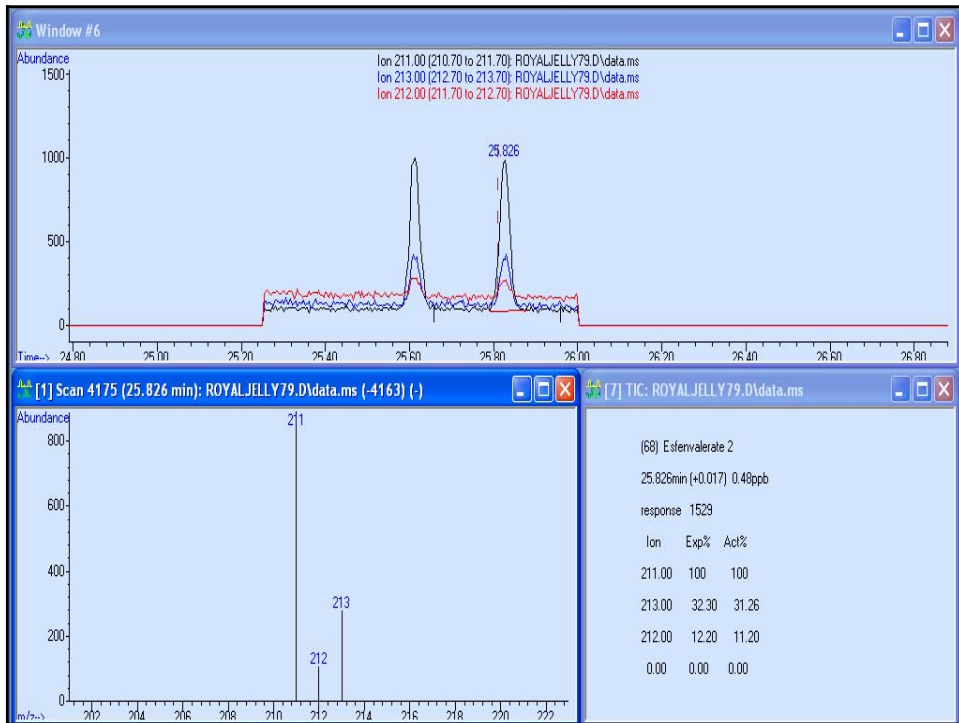
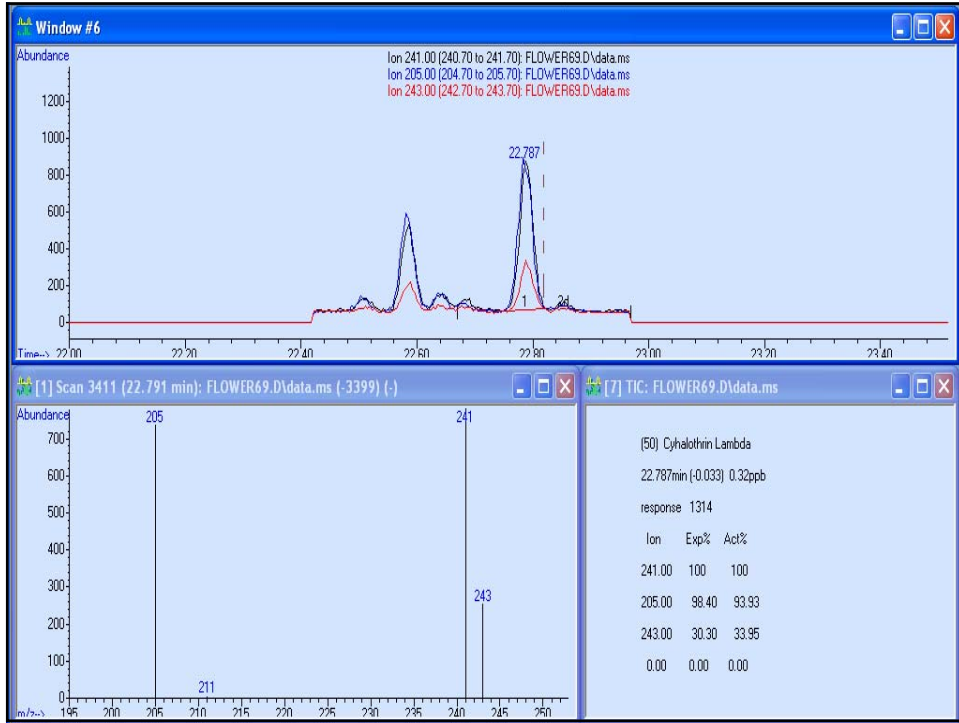
Beeswax - zoomed

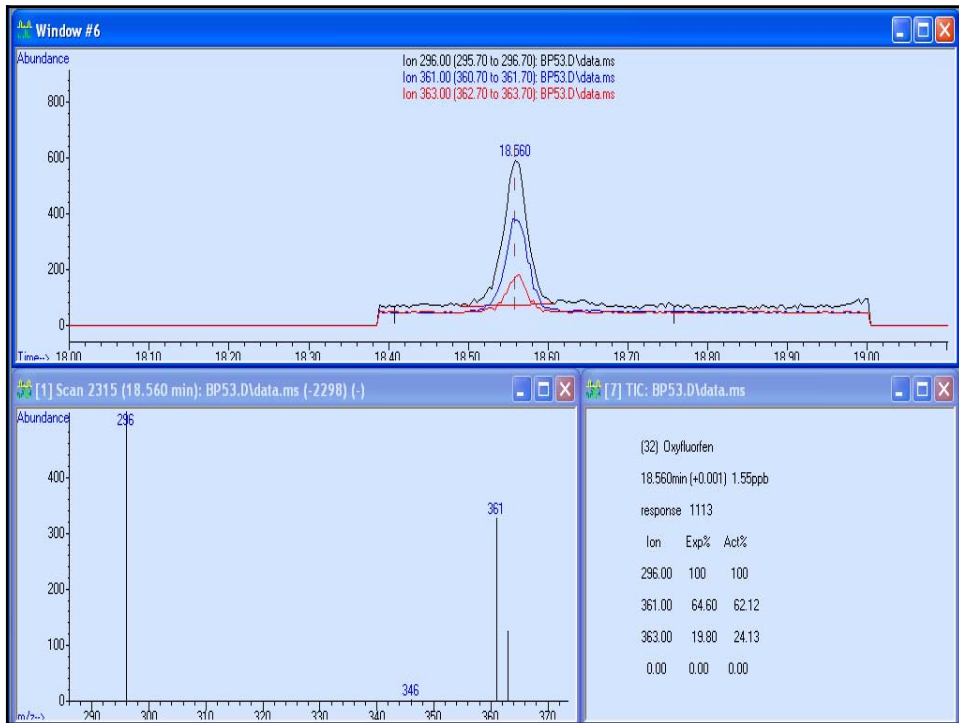
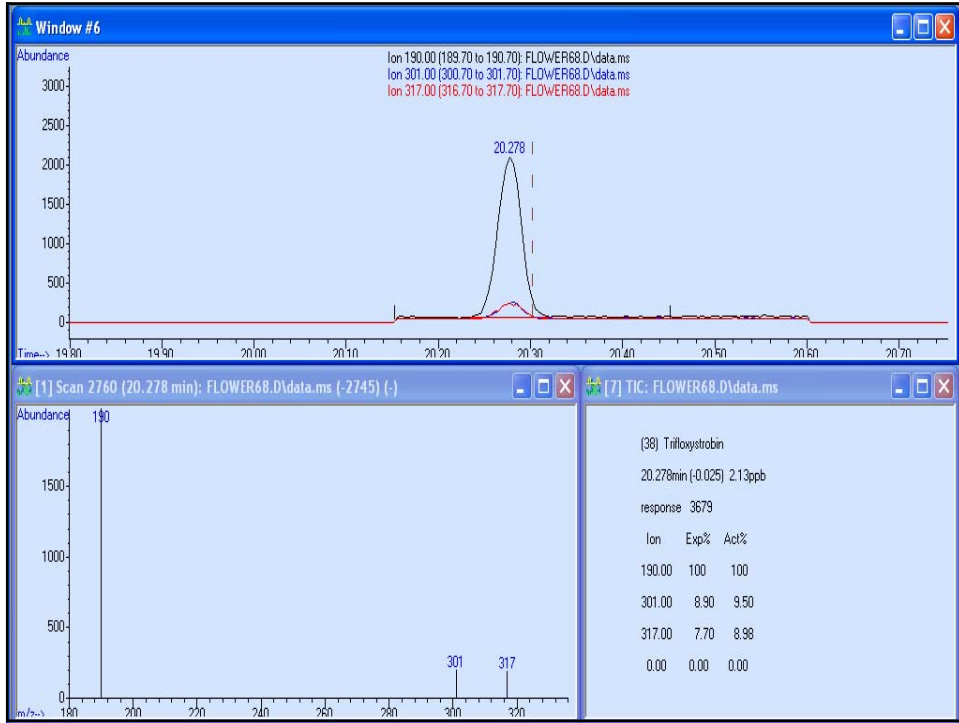


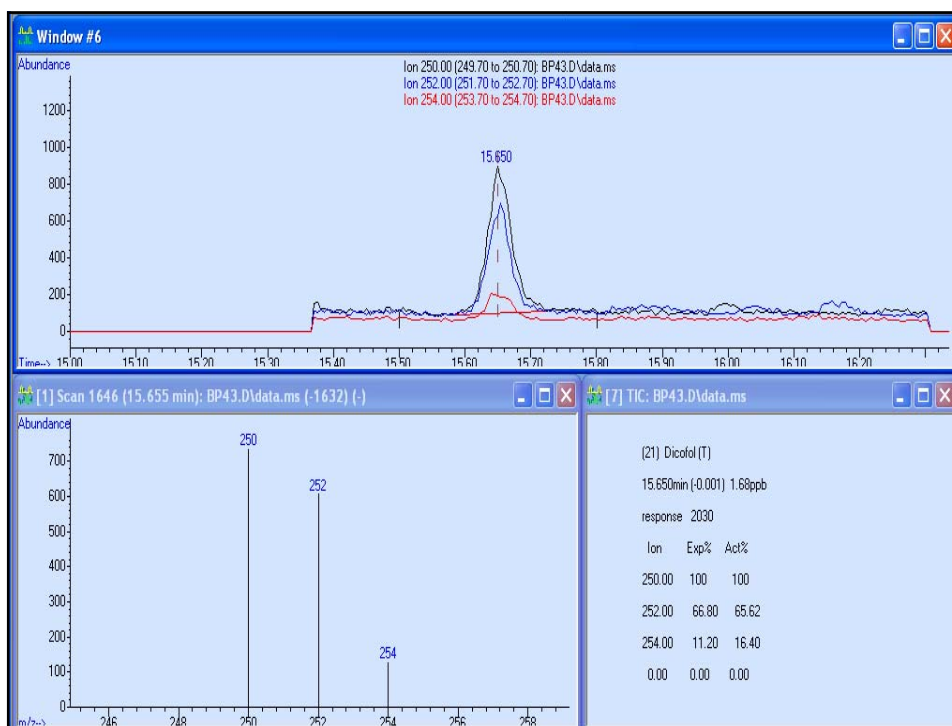












NCI pesticides

NCI compound	LOQ PPB	NCI compound	LOQ PPB	NCI compound	LOQ PPB	NCI compound	LOQ PPB
4,4 dibromobenzophenone	4	cypermethrin	2	flutolanil	4	pentachlorothioanisole (PCTA)	1
aldrin	10	DCPA	1	fluvalinate	1	permethrin total	10
allethrin	10	deltamethrin	20	heptachlor	4	phosmet	10
azinphos methyl	10	dicloran	1	heptachlor epoxide	6	prallethrin	4
azoxystrobin	2	dicolol	1	hexachlorobenzene (HCB)	1	profenofos	10
benefin	1	dieldrin	10	lprodione	10	pronamide	1
BHC alpha	4	dimethomorph	20	lindane	4	pyrethrins	40
BHC beta	4	dinitramine	1	malathion	4	pyridaben	6
BHC delta	4	disulfoton	3	methidathion	10	quintozene (PCNB)	1
bifenthrin	1	endosulfan I	1	metribuzin	1	spirodiclofen	1
captan	10	endosulfan II	1	mirex	1	tefluthrin	1
carbophenothion	1	endosulfan sulfate	1	nitrofen	1	terbufos	10
carfentrazone ethyl	1	endrin	10	nonachlor cis	1	tetraconazole	6
chlorbenzilate	2	EPN	1	nonachlor trans	1	tetradifon	1
chlordane cis	1	epoxiconazole	1	oxadiazon	1	tetramethrin	10
chlordane trans	1	esfenvalerate	1	oxychlordane	1	tolclofos methyl	1
chlorethoxyfos	1	ethalfuralin	1	oxyfluorfen	1	toxaphene	40
chlorfenopyr	1	ethion	2	parathion ethyl	2	triadimefon	2
chlorothalonil	1	fenarimol	1	parathion methyl	2	tribufos (DEF)	2
chlorpyrifos	1	fenhexamid	6	parathion methyl oxon	5	trifloxystrobin	1
chlorpyrifos methyl	1	fenitrothion	1	pendimethalin	3	triflumizole	1
coumaphos	1	fenpropathrin	10	pentachloroaniline (PCA)	1	trifluralin	1
cyfluthrin	2	flumetralin	1	pentachlorobenzene (PCB)	1	vinclozolin	1
cyhalothrin total	1	fluroxypyr methyl heptyl	2				





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