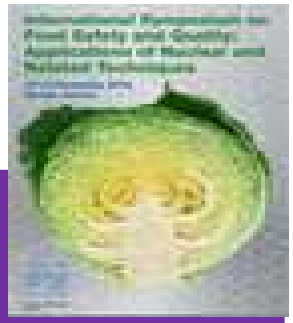


Pesticide residues analysis in Bee products: a useful tool to assess food safety and agro ecosystems sustainability

Silvina Niell, Natalia Gérez, Andres Perez, Rosana Diaz, Yamandu Mendoza
Horacio Heinzen, María Verónica Cesio*

*GACT: Pharmacognosy & Natural Products, Fac. de Quimica, UdelaR
PAAP; Litoral Chemistry Department, CENUR, UdelaR

Apiculture in Uruguay

- 3.000 beekeepers
- 500.000 beehives
- 12.000 ton honey/ year
- 90% of the production is exported
- U$S 35.000.000
- High social impact

Toxic substances bees faced nowadays

- **Increased use of herbicides & agrochemicals in agriculture activities.**
- **Increased use of "bee chemicals"**

	2000	2005	2010
Herbicides (kg active principle)	2.079.534	4.946.492	11.856.155
Insecticides (kg active principle)	503.356	1.057.895	1.732.906

Changes in soil use

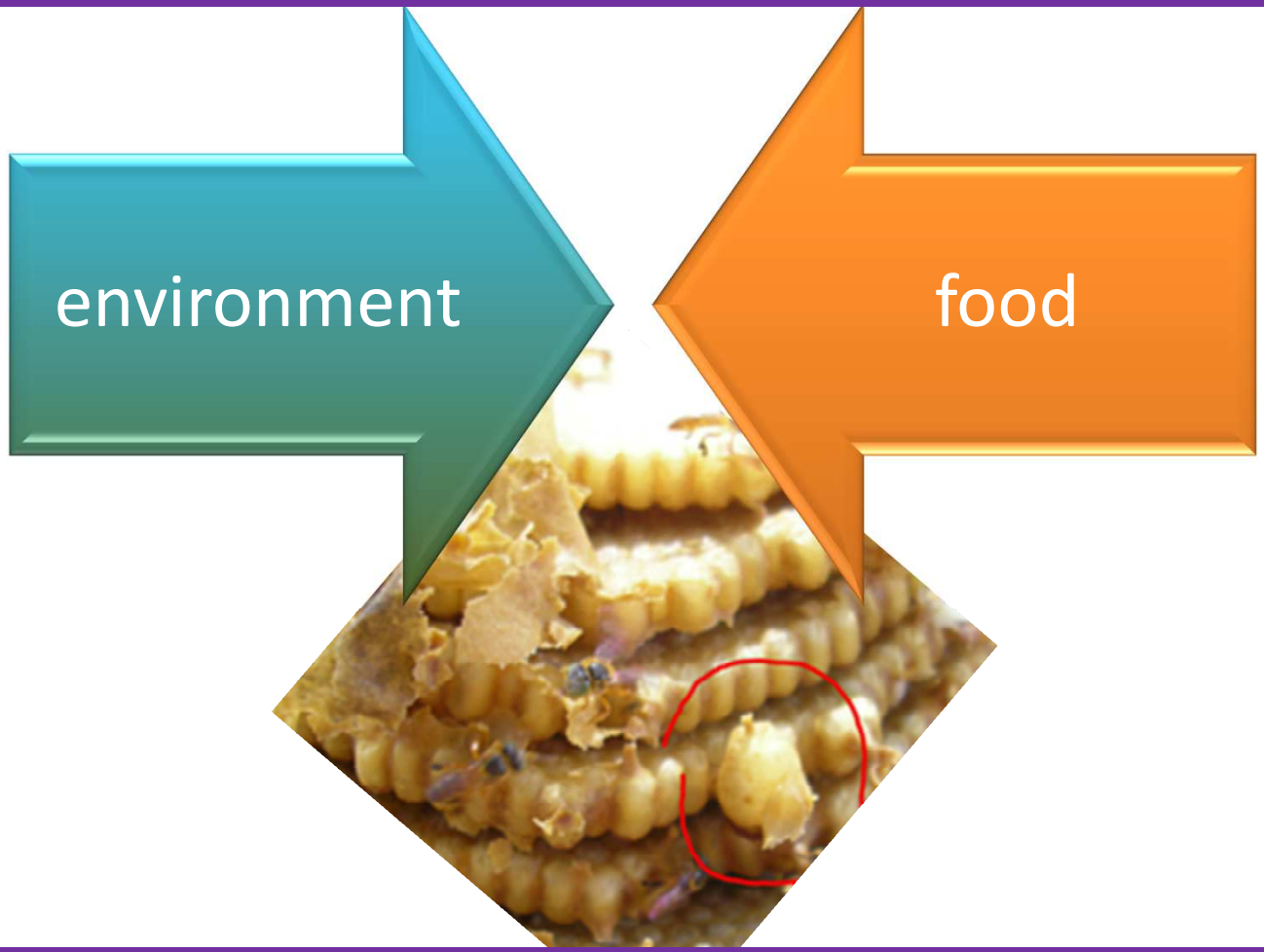
Disminishing of polen & néctar sources.

Beehive products important food components

Bee and bee products as markers of the environmental status of an agroecosystem


why?
Bees fly up to 2000m looking for food!!!

The hive is not only a source of food but also could give environmental information

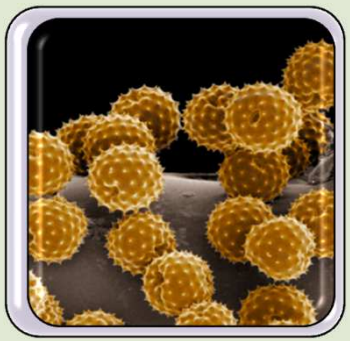


THE ANALYSIS FOR PESTICEDES RESIDUES OF THE BEE PRODUCTS IS OF PARAMOUNT IMPORTANCE

Beehive representative matrices



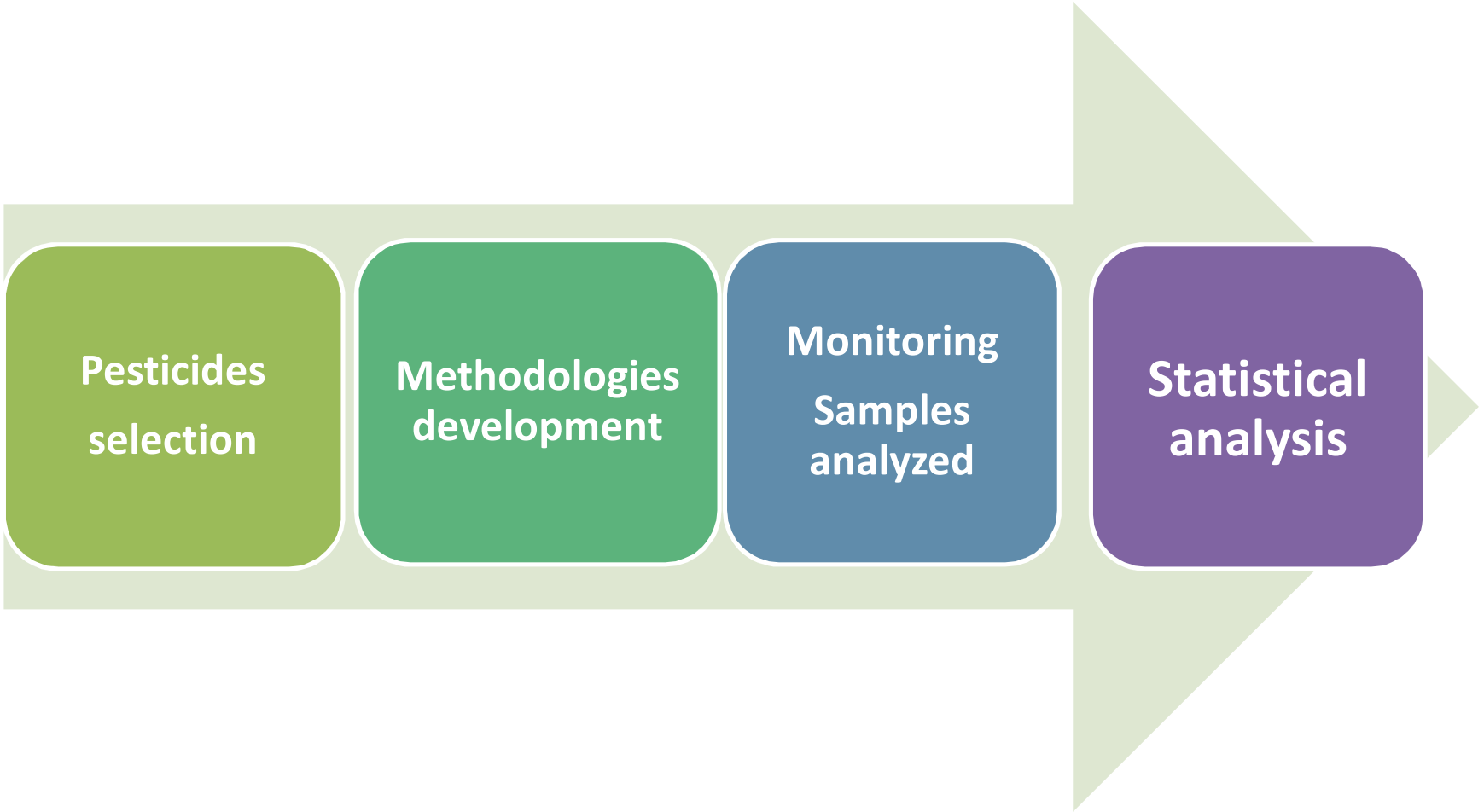




Propolis	Honey	Wax	Royal jellly	Pollen

From the analysis of hive matrices, information for both, food safety & environmental pollution can be gathered

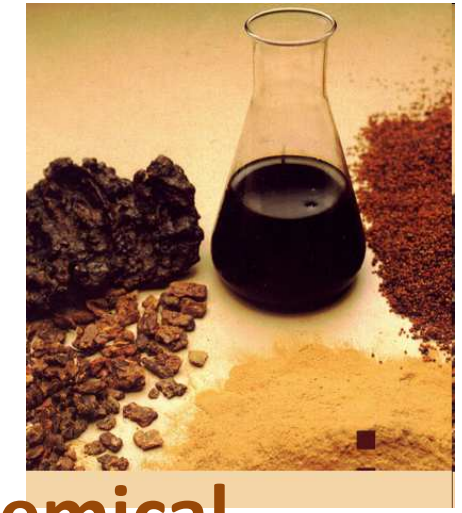
Strategy of the work



Validation parameters

- Lineality
- % Recovery
- Repeteability (RSD_r)
- Intermediate precision (RSD_{wR})
- Reporting Límit
- Matrix effect

Method validation and quality control procedures for pesticide residues analysis in food and feed. Document No. SANCO/12571/2013

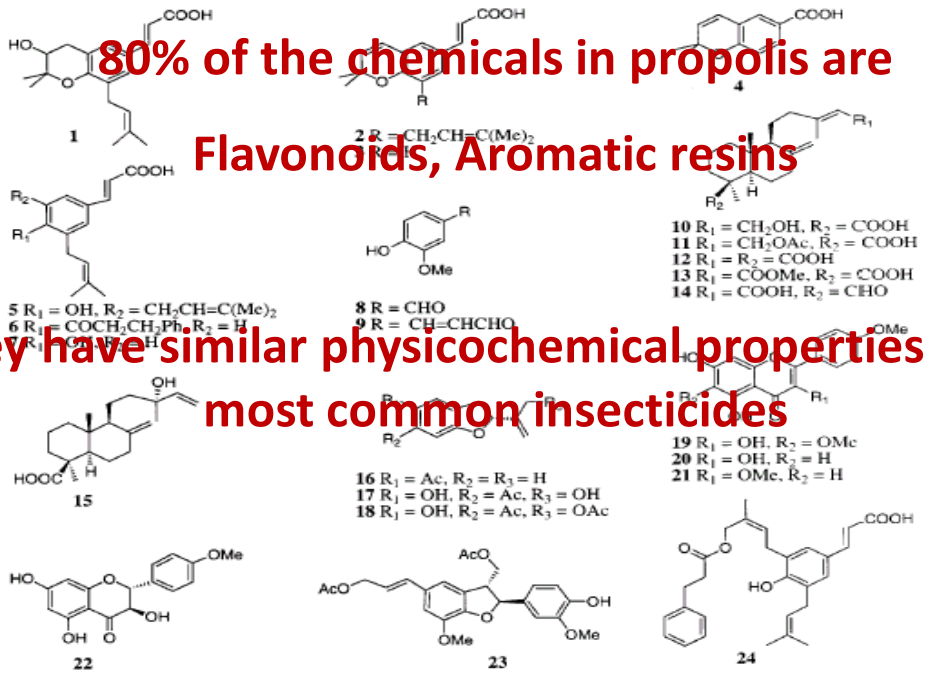


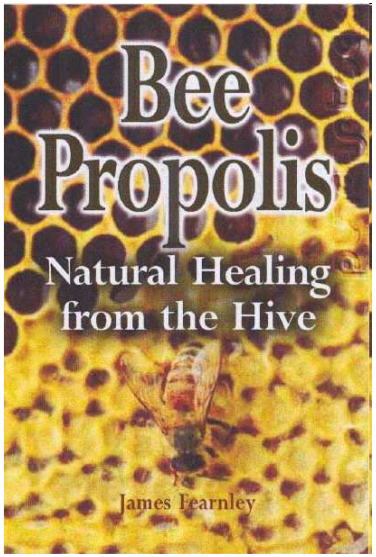
Propolis has a complex chemical composition

80% of the chemicals in propolis are

Flavonoids, Aromatic resins

They have similar physicochemical properties to the most common insecticides

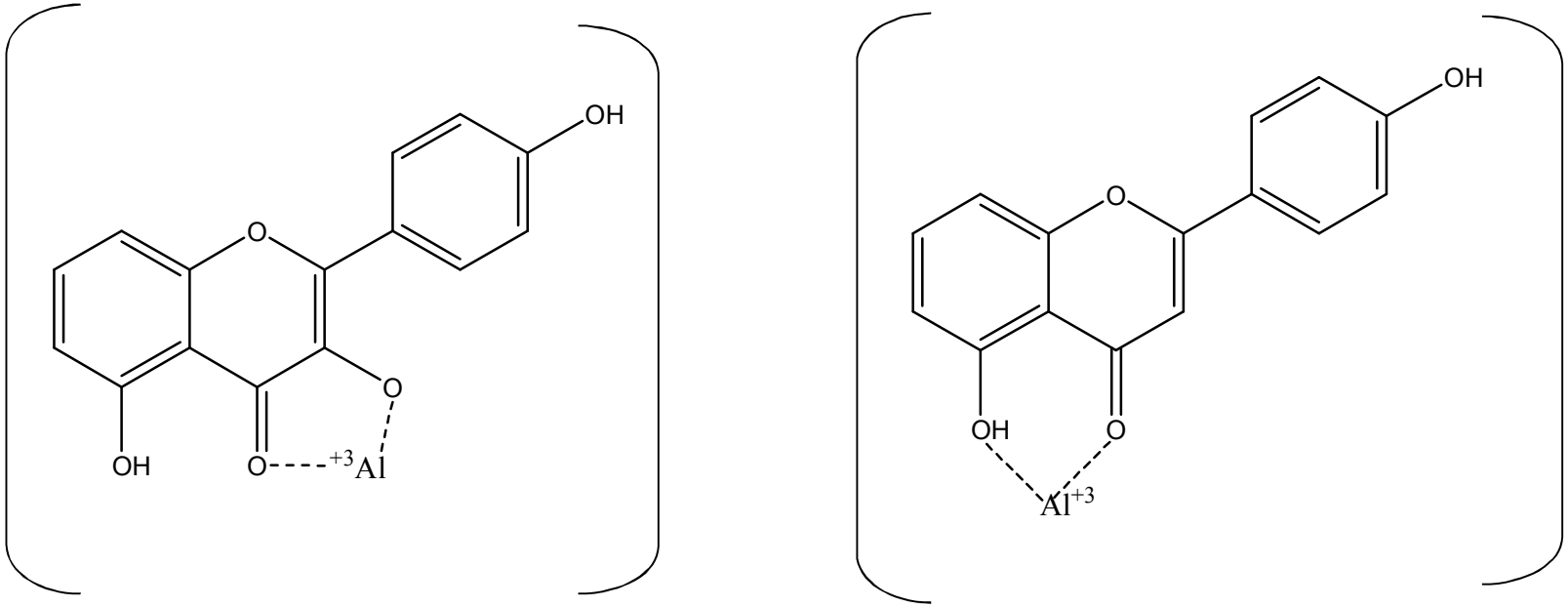




Clean-up through MSPD

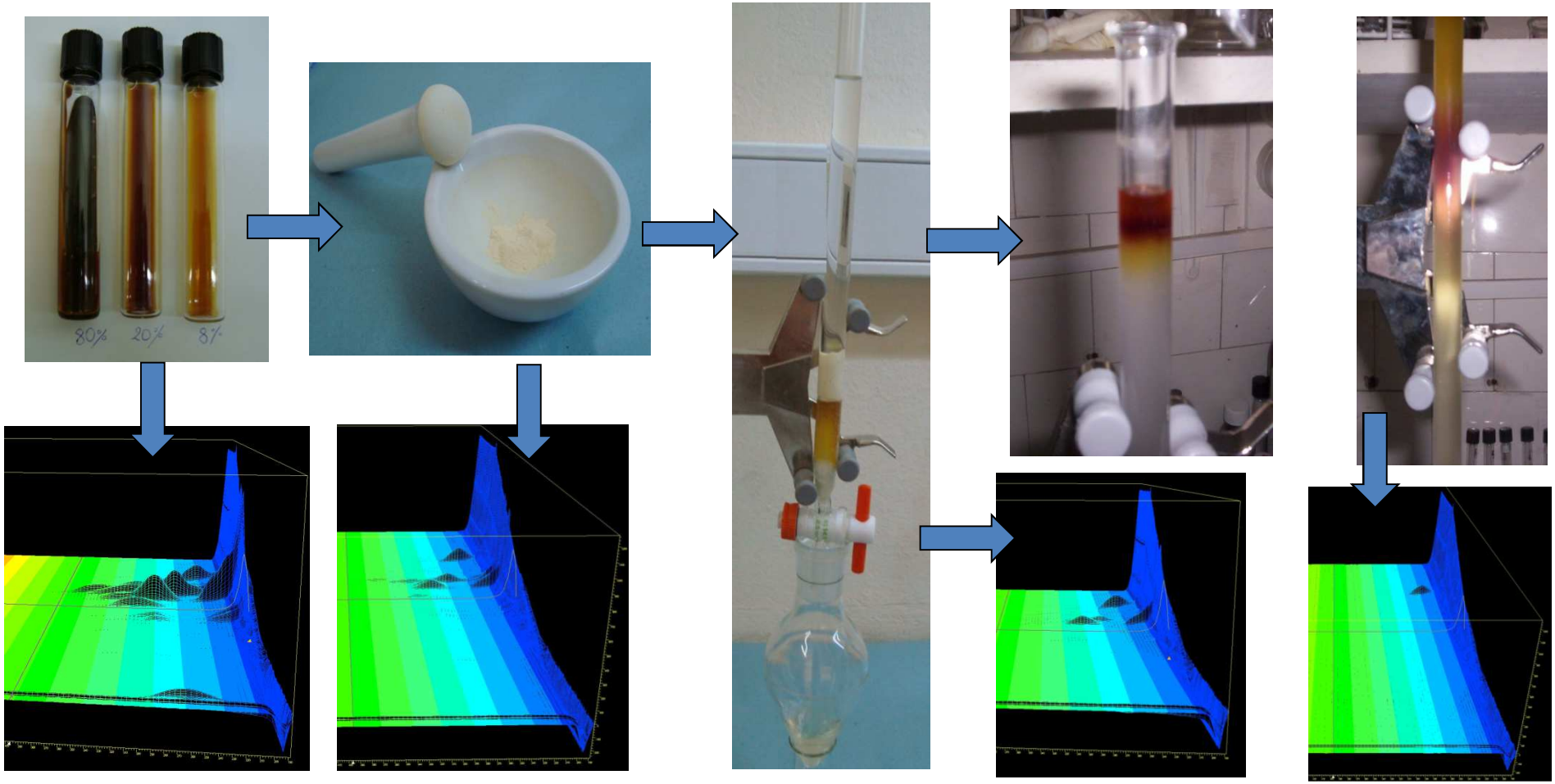
Dispersant for propolis: aluminium salt

$Al_2(SO_4)_3$



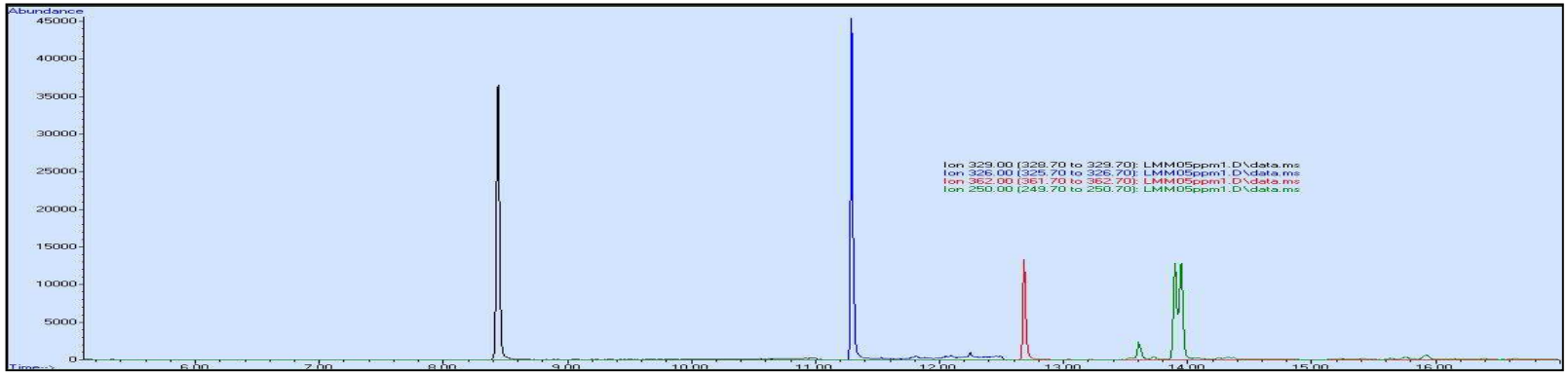
Complexation reaction: Sequesters interferring compounds from the matrix changing its polarity, allowing a better chromatographic separation

Pesticides in propolis: following the clean up process



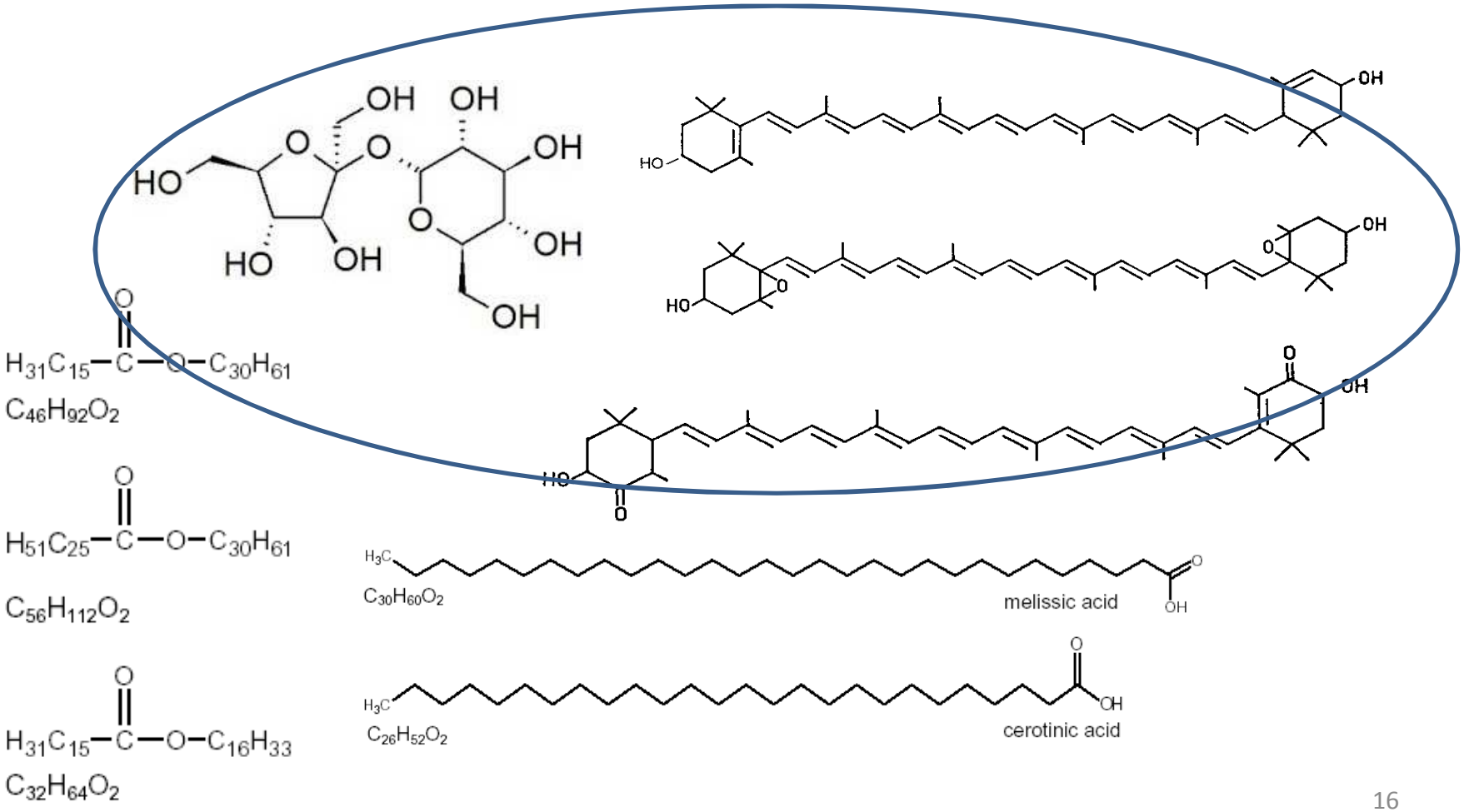
PEREZ-PARADA ANDRÉS; COLAZZO M.; N. BESIL; GEIS ASTEGIANTE, L; REY F; HEINZEN, H Determination of coumaphos, chlorpyrifos and ethion residues in propolis... Journal of Chromatography - A, v.: 1218 34, p.: 5852 - 5857, 2011

Simultaneous determination of fluvalinate & coumaphos using GC-MS(SIM)



Pesticide	t_R	m/z	rec.(%)	RSD(%)
Coumaphos	12.68	334	106	1
		362		
		364		
τ-Fluvalinate	13.9	181	96	1
		250		
		252		

Chemical complexity of the different matrices




A FIGUEIRDA

Honey QuEChERS citrate

Sample+H_2O+MeCN+ CEN

↓

Dispersive Clean-up ($MgSO_4$, PSA); Freeze out

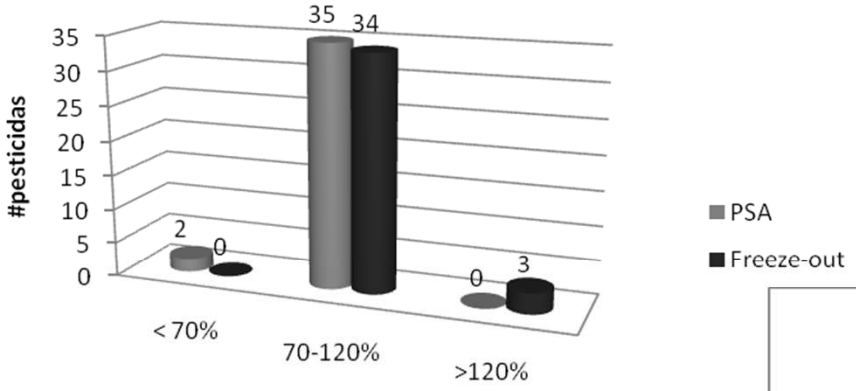
↓

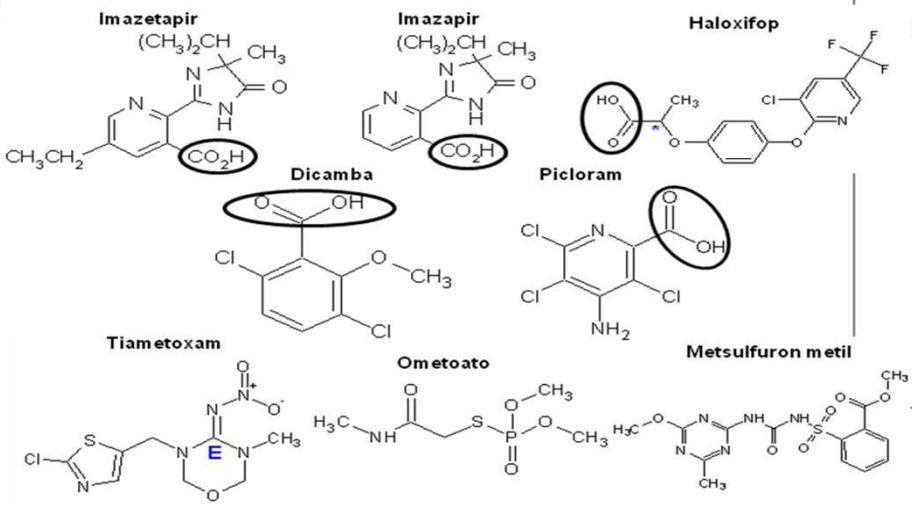
Instrumental Analysis

NEILL, S.; M.V. CESIO; HEPPERLE J; ROUX D; KOBERG D; KEISH L; ANASTASIADES, M; HEINZEN, H . Analisis multiresiduo de 41 pesticidas en miel por LC-MS/MS: evaluación de dos métodos de Clean-up. Agrociencia (Uruguay), v.: 17 1, p.: 101 - 107, 2013

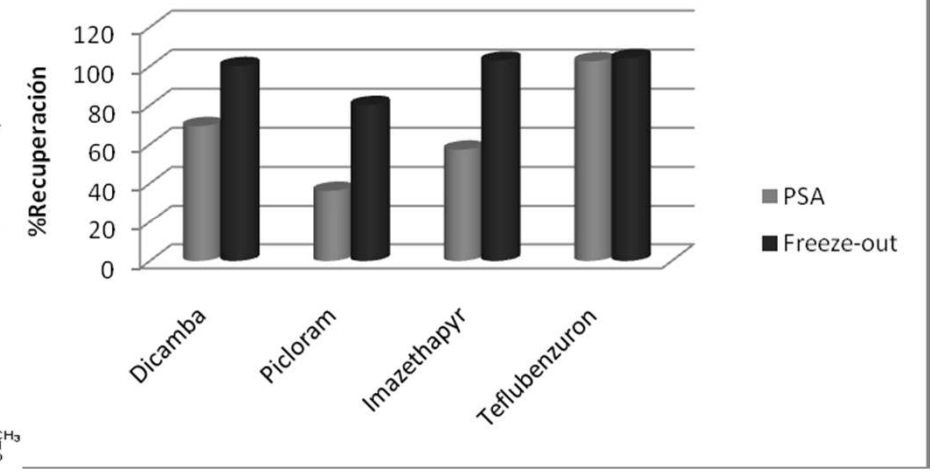
Honey QuEChERS citrate
LC-MS/MS

Pesticides analyzed in (+) mode





Pesticides analyzed in (-) mode



QuEChERS citrate validation

GC-MS	0,1 mg/kg	
	%rec	RSD
Trifluralin	89	19
Atrazina	87	11
Clorotalonil	78	10
Diazinon	95	11
Clorpirifos-metil	58	5
Malation	73	7
Clorpirifos	61	11
Clorfenvinfos	81	13
Fipronil	82	4
Metidation	93	11
Endosulfan	nd	nd
p,p-DDE	69	12
Etion	81	11
Endosulfan sulfato	81	6
p,p-DDT	76	13
Acetamiprid	75	4
Bromopropilato	78	8
Azinfos-metil	80	11
Cumafos	83	7
Ciflutrina	97	14
Cipermetrina	nd	nd
Tau-Fluvalinato	82	14
Deltametrina	84	13

GC-ECD

	0,1 mg/kg		0,05 mg/kg		0,01 mg/kg	
	% rec	RSD	% rec	RSD	% rec	RSD
Endosulfan a	131	3	109	14	85	11
Endosulfan b	81	4	97	3	54	10
Ciflutrina	77	5	96	3	76	6
Cipermetrina	215	5	301	4	127	3
Tau-Fluvalinato	87	4	74	4	92	5
Deltametrina	79	5	93	6	79	7


A FIGUEIRDA

Analytical Method for candies with honey and propolis

Extraction

- 10 g crushed candies + 1.5g NaCl + 8g $MgSO_4$ + 10 mL ethyl acetate + 10mL distilled water.
- 5 minutes vigorous manual agitation
- 15 minutes of centrifugation at 3000 rpm.

d-SPE Clean-up

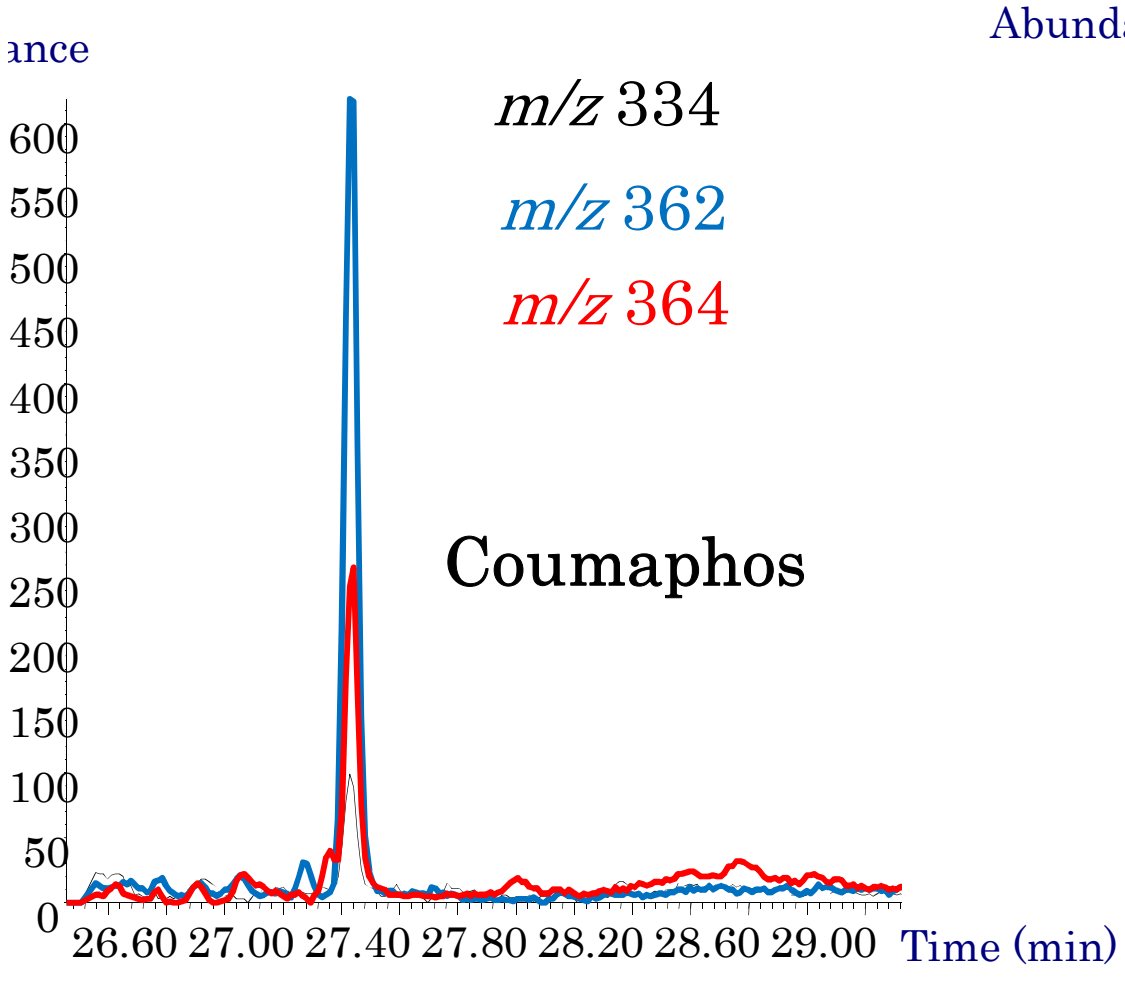
- 5.0 mL supernatant + 750 mg $MgSO_4$
- 150 mg PSA + 100 mg GCB
- Vortex and centrifugation
- 4.0mL to dryness under reduced pressure.

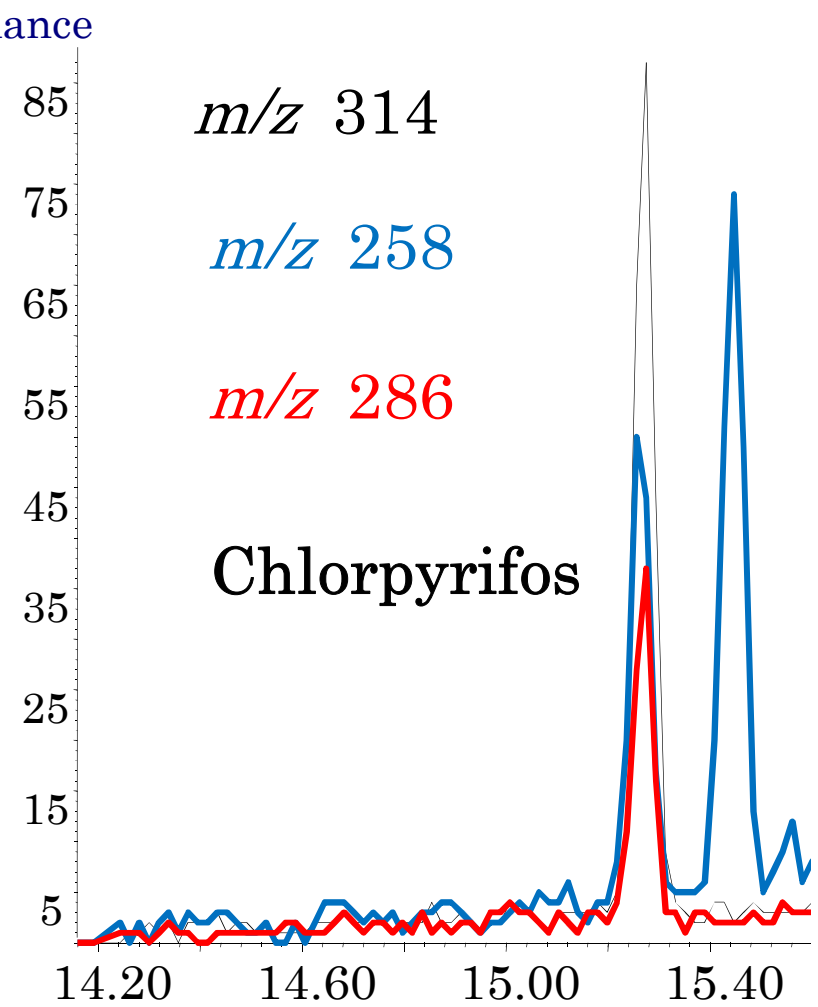
GC-MS

- 1,00 mL of TPP (Internal Standard) in EtAc is added and analized GC-MS.

Some analytical parameters of the validated methodology

		Honey Candies 0.10 mg/L		Propolis Candies 0.10 mg/L	
Pesticide	***m/z***	**% Rec (100 µg/kg)**	**% RSD**	**% Rec (100 µg/kg)**	**% RSD**
Bifenthrin	**181** 165 166	137	16	134	11
Bromopropilate	**341** 339 183	121	14	121	8
Chlorpyrifos	258 286 **314**	103	17	83	13
Coumaphos	334 **362** 364	55	19	78	4
Deltamethrin	**181** 251 253	126	15	139	8
Dibromobenzophenone	**185** 340 183	53	3	131	9
DMPF (amitraz metabolite)	120 132 106	135	23	NA	NA
Ethion	**231** 384 153	113	19	150	11
Fenvalerate	**125** 209 181	110	17	129	2
Fipronil	**367** 213 369	109	16	133	13
Malathion	125 158 **173**	80	9	126	7
Tau-fluvalinate	**250** 252 181	94	19	146	3
Tetradifon	356 **159** 229	124	19	102	9
Vinclozolin	**212** 287 198	106	18	150	6



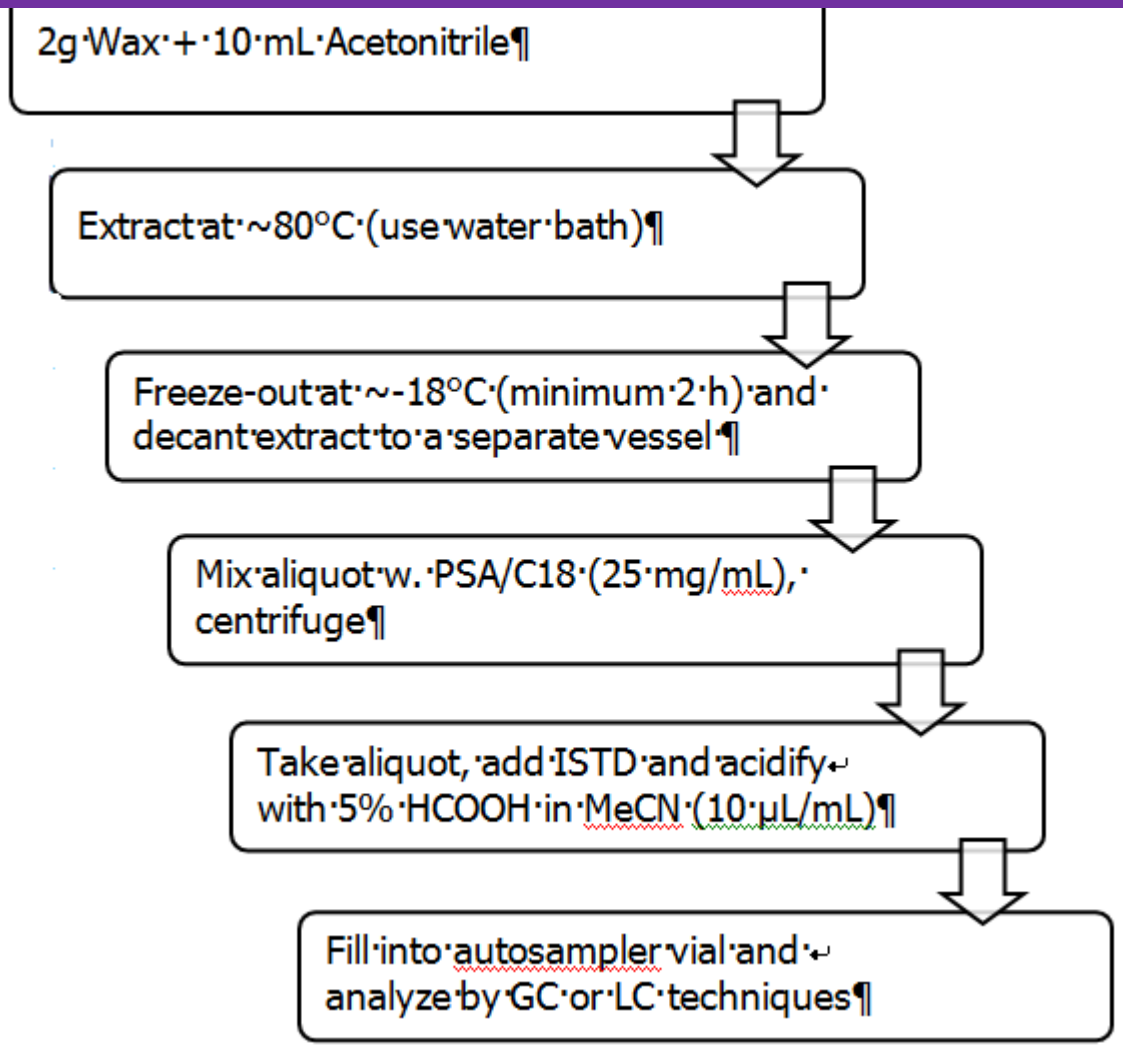


✓ samples of both honey and propolis cointaining candies were analyzed.
✓Coumaphos and chlorpyrifos residues were found in propolis candies.
✓No pesticides were detected in honey candies.

Pesticide residues in propolis and honey candies


APIGUARDA

Final methodology for beeswax



Niell S, Cesio M.V, Hepperle J, Doerk D, Kirsch L, Kolberg D, Scherbaum E, Anastassiades M, Heinzen H.
J Agric Food Chem. 2014 Apr 30;62(17):3675-83.

LC-MS/MS

GC/MS

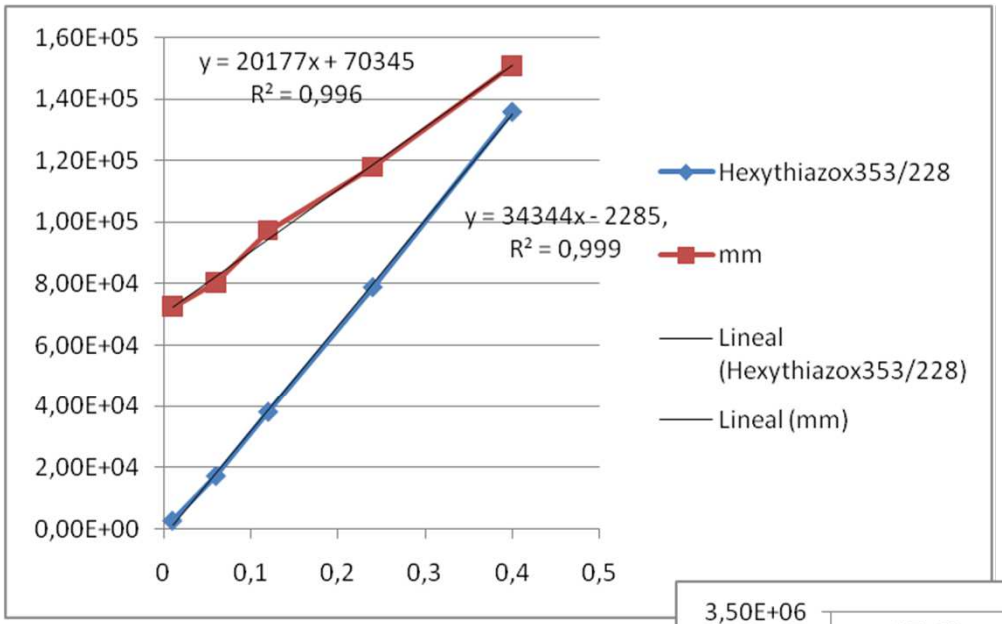
Pesticide	Selected ions (m/z)	Confirmation Ion (m/z)	Quantification ion (m/z)
Atrazina	200, 215,217	215	200
Azinfos-metil	160, 132,125	125	160
Bromopropilato	341, 343, 339, 183	343	183
Cipermetrina	163, 181, 165, 209	181	163
Clorfenvinfos	267, 269, 323	323	267
Clorotalonil	264, 266, 268, 231	264	266
Clorpirifos-metil	286, 288, 125	125	286
Ciflutrina	163, 165, 206, 226	226	163
Deltametrina	181, 253, 251, 255	251	181
Diazinon	179, 137, 304, 152	304	179
Endosulfan alfa	241, 237, 195, 239	237	195
Endosulfan beta	195, 237, 241, 207	241	195
Endosulfan sulfato	272, 274, 387, 237	387	272
Etion	231, 153, 384	384	231
Fipronil	367, 369, 213	213	367
Lambda Cialotrina	181, 197, 208	208	181
Malation	173, 125, 127, 93	127	173
Metidation	145, 85, 125, 302	302	145
p,p-DDE	246, 248, 318	318	246
p,p-DDT	235, 237, 165	165	235
Tau-Fluvalinato	250, 252, 181, 251	181	250
Trifluralin	306, 290, 335	335	306

QuEChERS LC-MS/MS /VALIDATION

	0.2 mg/kg		0.1 mg/kg		0.01 mg/kg		RL
	Rec(%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	(mg/kg)
Amitraz	73	2	77	4	85	13	0,01
Atrazina	89	4	94	4	97	13	0,01
Azoxistrobin							
Boscalid							
Carbarilo							
Carbendazim							
Carboxin							
Clorpirifos-eti							
Clodinafop-propa							
Cumafos*							
Cihalotrina-laml							
Dimetoato							
Epoxiconazol							
Fenpropatrin							
Flutriafol							
Hextiazox							
Imidacloprid							

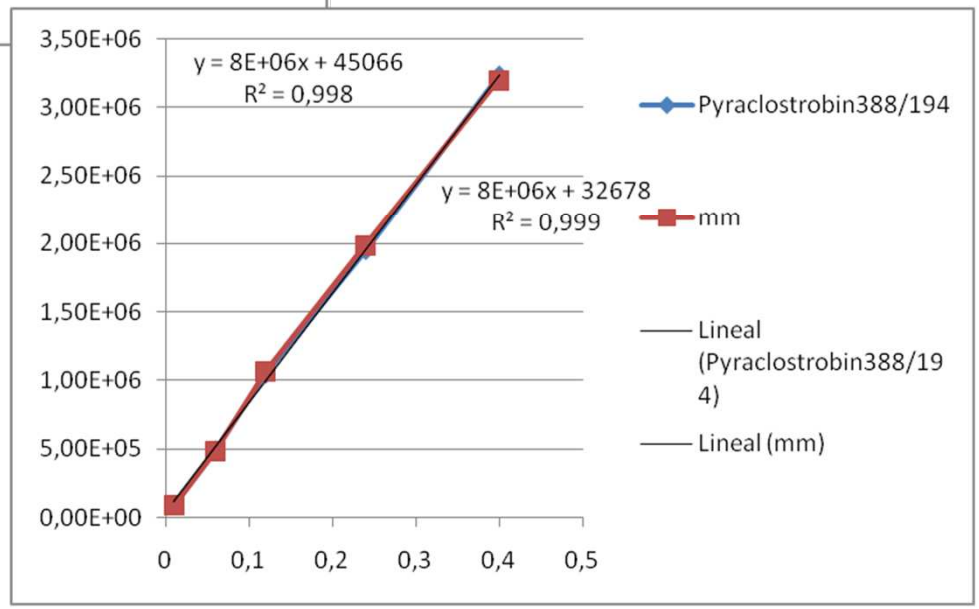
	0.2 mg/kg		0.1 mg/kg		0,.01 mg/kg		RL
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	(mg/kg)
Iprodione	108	27	123	24	nd		0,2
Linuron	96	6	99	5	107	15	0,01
Metalaxil	107	3	109	5	113	15	0,01
Metomilo	111	8	127	4	113	12	0,01
Metoxifenozide	107	4	111	5	146	7	0,1
Metolaclor	96	3	103	2	120	17	0,01
Metribuzin	102	7	111	7	nd		0,1
Ometoato	96	11	104	8	111	6	0,01
Pendimetalin	81	19	83	19	nd		0,1
Pirimicarb	108	3	114	4	111	11	0,01
Profenofos	89	6	94	6	92	18	0,01
Piraclostrobin	102	4	106	5	112	14	0,01
Tebuconazol	98	11	99	5	103	27	0,1
Tebufenozide	96	4	99	5	104	15	0,01
Tetraconazol	103	7	104	6	111	23	0,1
Tiacloprid	113	7	120	5	108	18	0,01
Tiametoxam	103	14	103	7	106	15	0,01
Tiodicarb	90	5	85	7	107	12	0,01
Tiofanato-metil	94	5	91	7	97	15	0,01
Triflumuron	91	5	98	10	104	12	0,01

QuEChERS Matrix Effect

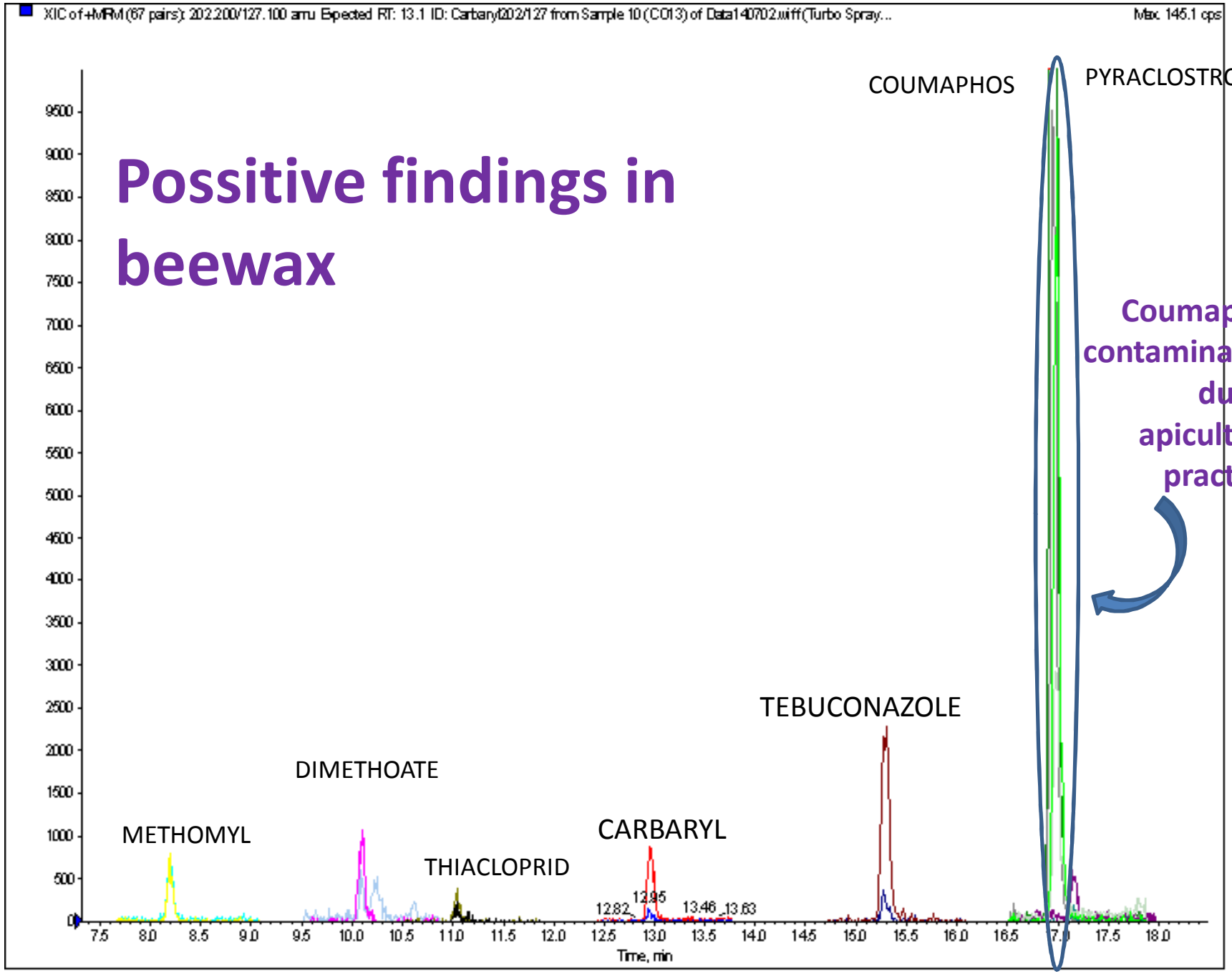


lineal range:
0.005-0.25 mg/kg LC
0.012-0.24 mg/kg GC

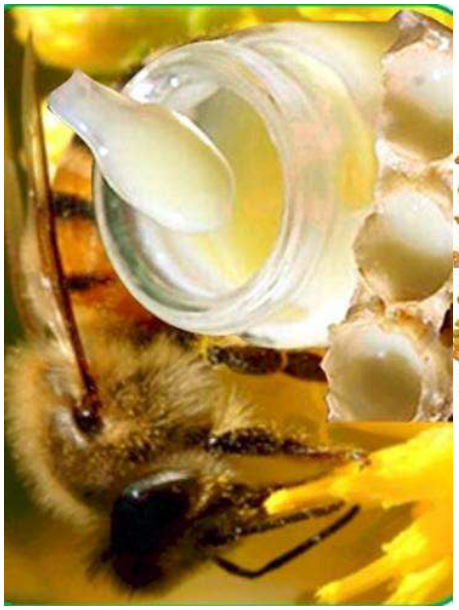
Moderate Matrix effect (<13%) for most of the pesticides
- azoxistrobin (24%)
- carbendazim (32%)
- imazapir (-55%)
- cumafos (24%)
- hexitiazox (-41%).



Possitive findings in beewax



Coumaphos contamination due to apicultural practices

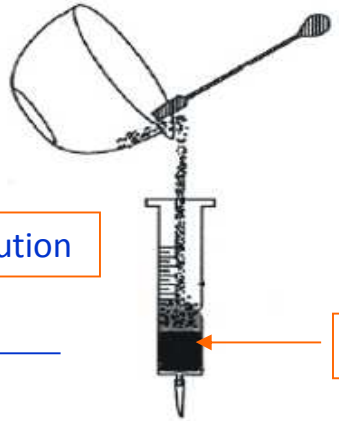



APIGUARDA

Royal Jelly used as nutricional supplement
MSPD method optimization

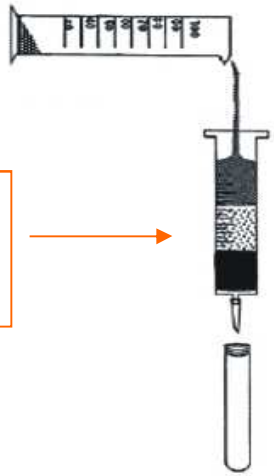
0.5 g of royal jelly adsorbed

- **Al_2O_3(N)/ $MgSO_4$**
- MgSO4 / Al_2O_3(N)
- Al_2O_3(N)/ Florisil
- Al_2O_3(N) + MgSO4/Florisil



Pesticide Elution

1.5g florisil



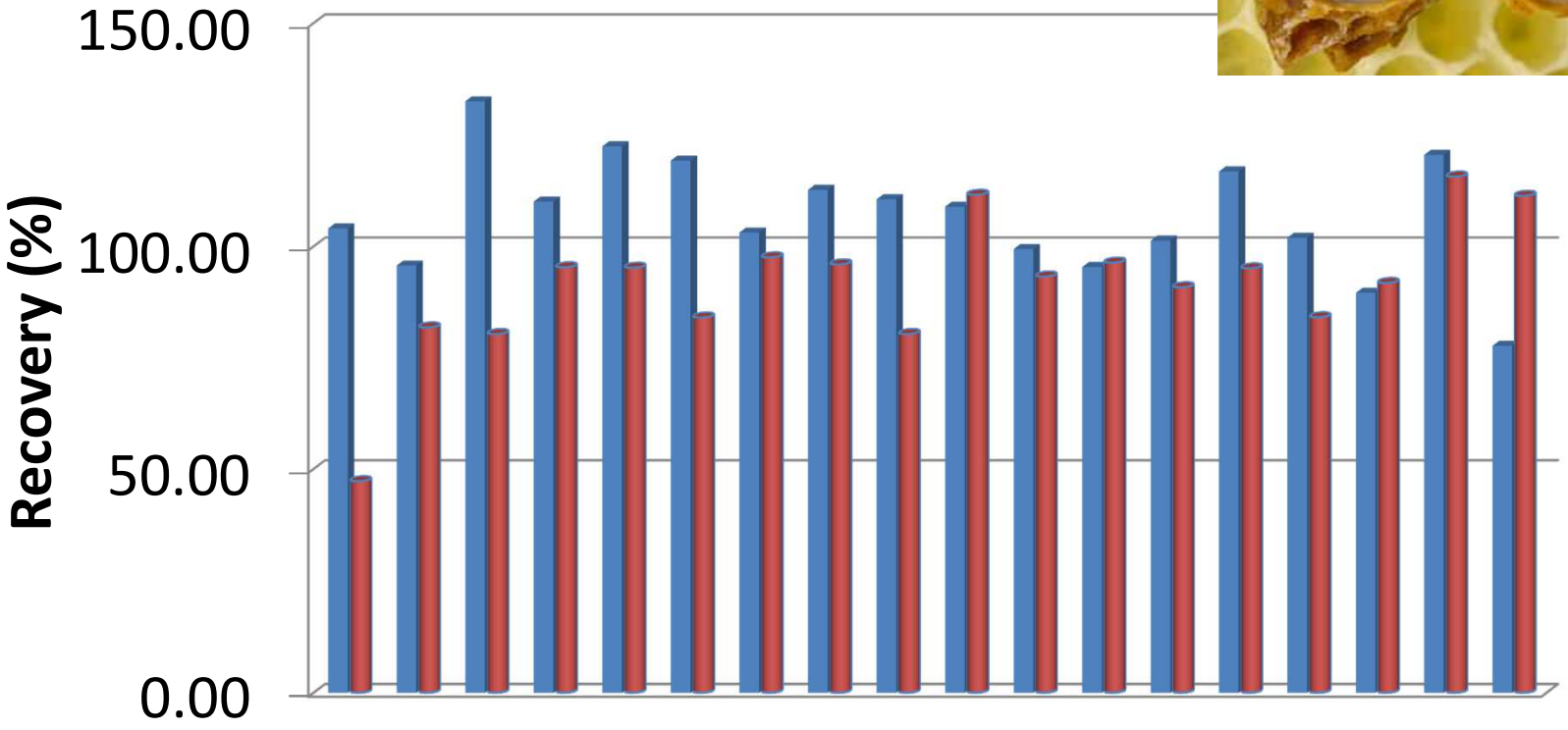
15mL - CH_2Cl_2 / AcOEt (9:1)
- **Ethyl Acetate**

Evaporate

Redissolve 1.0 mL Bromofos metihy 1 mg/L en AcOEt

GC-ECD

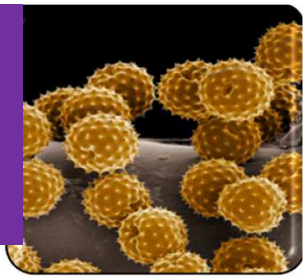
GC-MS



Pesticide recoveries at 0.10 and 0.25 mg/kg


APIGUARDA

Pollen used as a health promoter agent

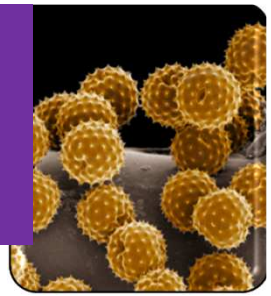


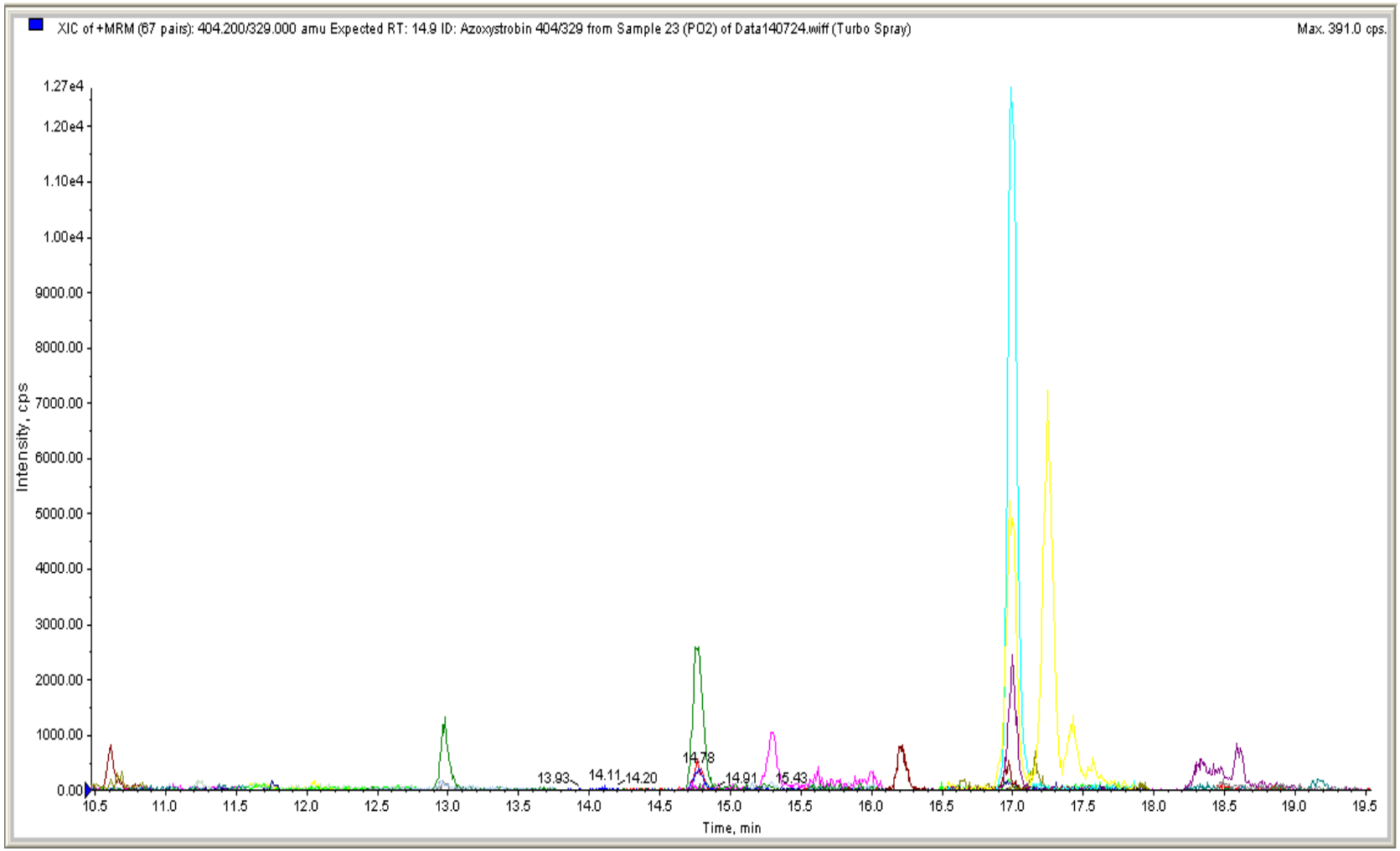
- 5g homogenized polen
- 5 mL H2O and 10 mL ACN

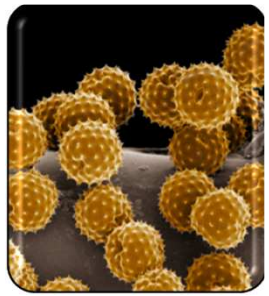
- 4g of MgSO4, 1g ACONA and 100uL Acetic Acid
- Agitation and centrifugation

- 6mL supernatant
- 150mg PSA and 900 mg MgSO4
- Vortex and centrifugation and 1 mL to LC-MS/MS

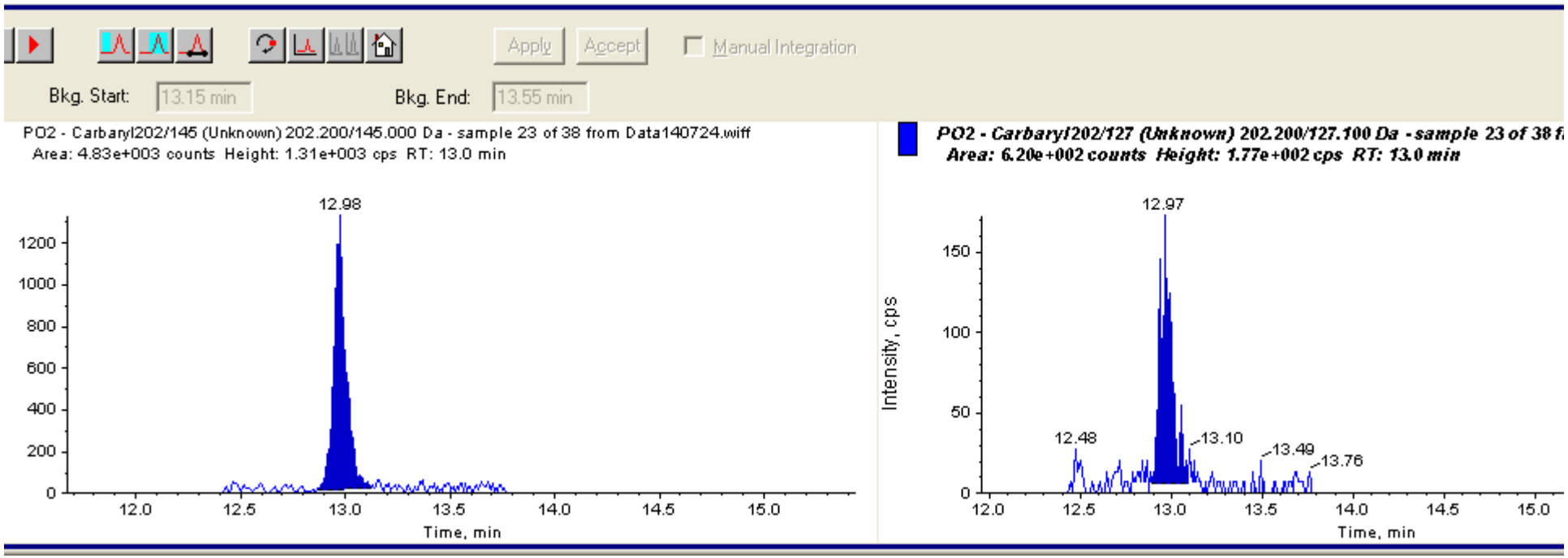
Real sample findings

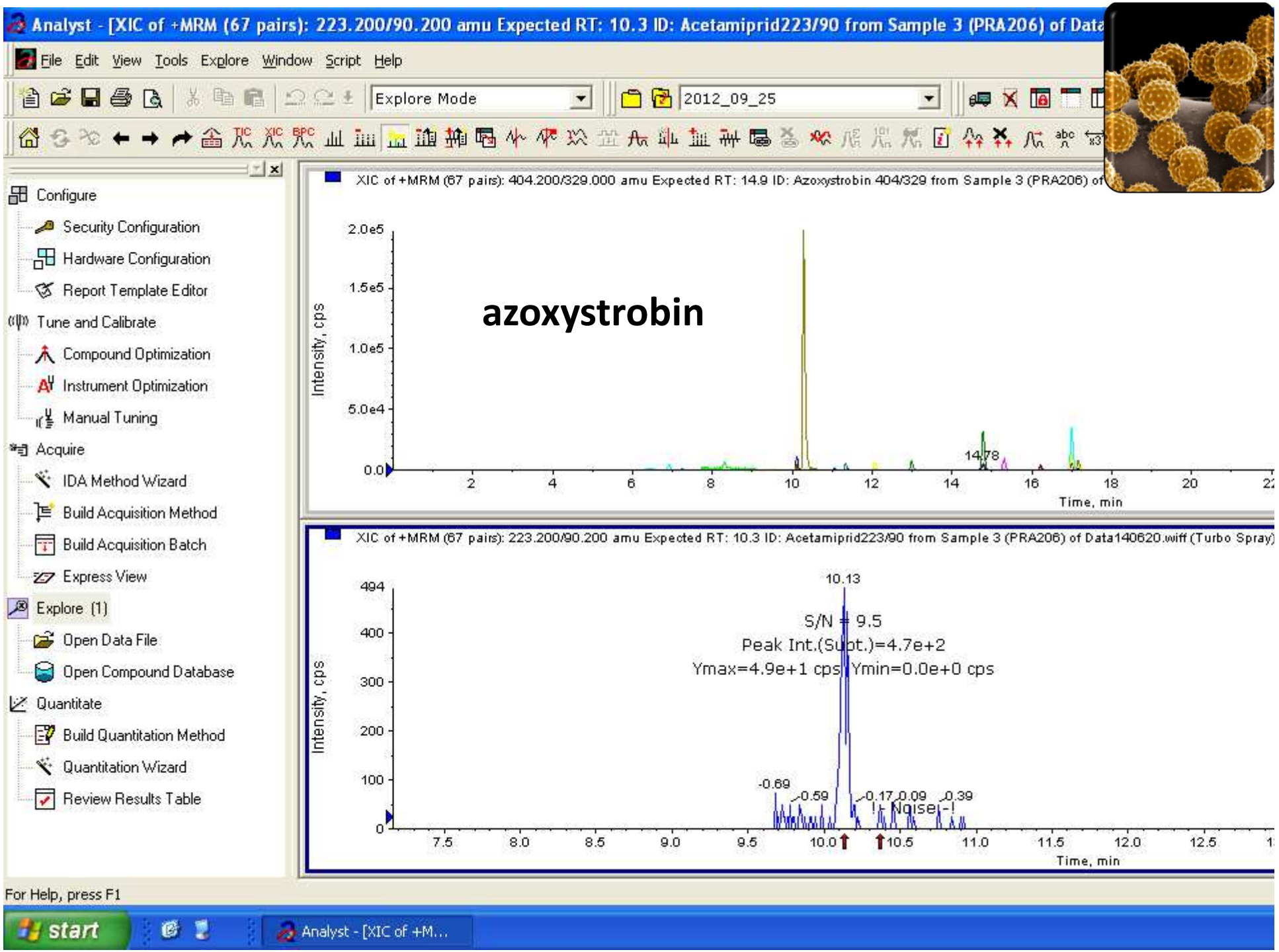


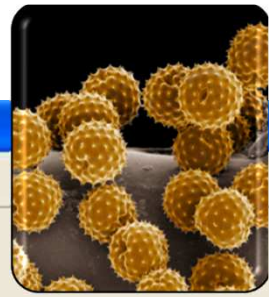




- Carbaryl


Apply
Accept
Manual Integration
Bkg. Start:
13.15 min
Bkg. End:
13.55 min
PO2 - Carbaryl202/145 (Unknown) 202.200/145.000 Da - sample 23 of 38 from Data140724.wiff
Area: 4.83e+003 counts Height: 1.31e+003 cps RT: 13.0 min
12.98
Time, min
PO2 - Carbaryl202/127 (Unknown) 202.200/127.100 Da - sample 23 of 38 f
Area: 6.20e+002 counts Height: 1.77e+002 cps RT: 13.0 min
12.97
12.48
13.10
13.49
13.76
Intensity, cps
Time, min


Analyst - [XIC of +MRM (67 pairs): 223.200/90.200 amu Expected RT: 10.3 ID: Acetamiprid223/90 from Sample 3 (PRA206) of Data
File Edit View Tools Explore Window Script Help
Explore Mode
2012_09_25
Configure
Security Configuration
Hardware Configuration
Report Template Editor
Tune and Calibrate
Compound Optimization
Instrument Optimization
Manual Tuning
Acquire
IDA Method Wizard
Build Acquisition Method
Build Acquisition Batch
Express View
Explore (1)
Open Data File
Open Compound Database
Quantitate
Build Quantitation Method
Quantitation Wizard
Review Results Table
XIC of +MRM (67 pairs): 404.200/329.000 amu Expected RT: 14.9 ID: Azoxystrobin 404/329 from Sample 3 (PRA206) of
azoxystrobin
Intensity, cps
Time, min
14.78
XIC of +MRM (67 pairs): 223.200/90.200 amu Expected RT: 10.3 ID: Acetamiprid223/90 from Sample 3 (PRA206) of Data140620.wiff (Turbo Spray)
10.13
S/N = 9.5
Peak Int.(Subt.)=4.7e+2
Ymax=4.9e+1 cps Ymin=0.0e+0 cps
-0.69
-0.59
-0.17
0.09
0.39
Noise
For Help, press F1
start
Analyst - [XIC of +M...



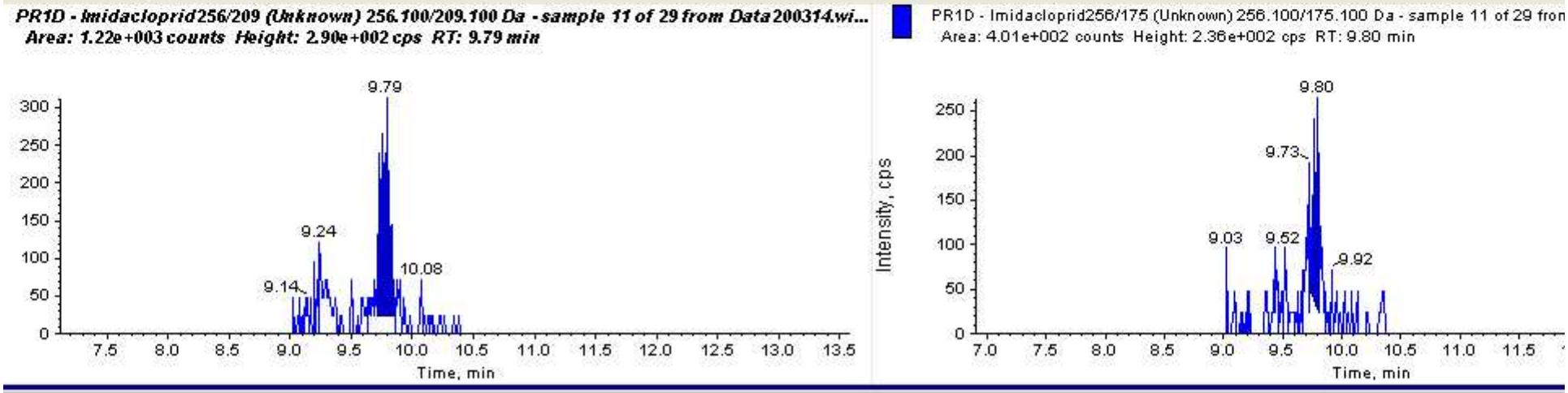
Quantitate Mode | 2012_09_25

Full Layout
Query: None
Idle
Sort : Unsorted

Sample Name	Sample ID	Sample Type	File Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (ng/ml)	Standard Query Status	Use Recor
PR1D		Unknown	200314\Data200314	1.22e+003	2.90e+002	N/A	N/A	

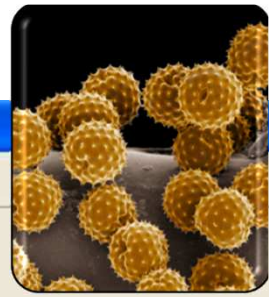
Apply | Accept | Manual Integration

Bkg. Start: 10.33 min Bkg. End: 10.57 min



User Name: LABORATO-9C50

- [Peak Revie... | Data



itled 2"]

pt Help

Quantitate Mode | 2012_09_25

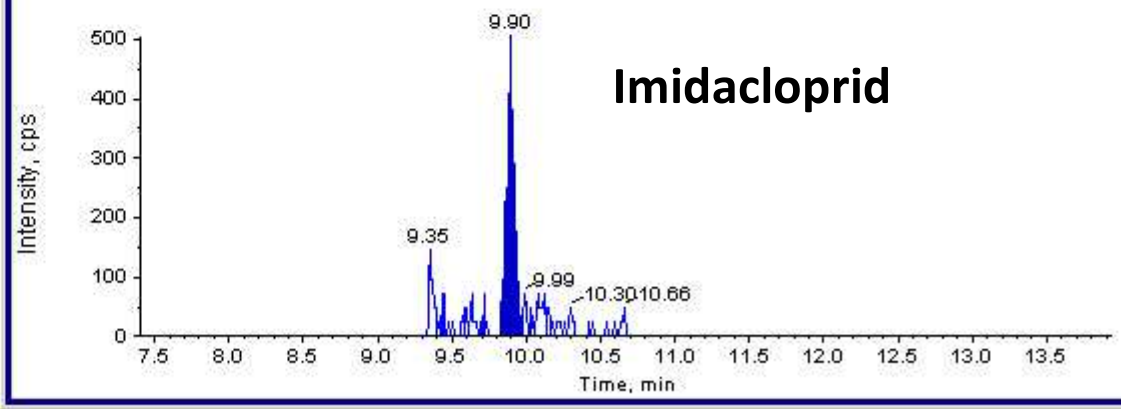
Full Layout
Query: None
Idle
Sort : Unsorted

	Sample Name	Sample ID	Sample Type	File Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (ng/mL)	Standard Query Status	Use
16	PRA206		Unknown	140620\Data140620	8.29e+002	2.82e+002	N/A	N/A	

Apply | Accept | Manual Integration

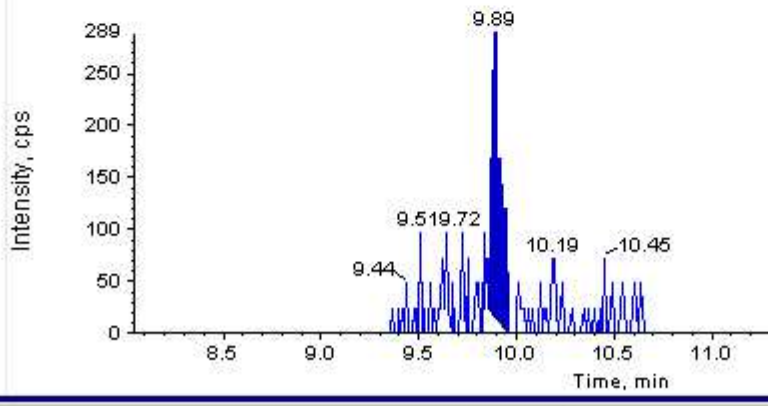
Bkg. Start: 10.31 min Bkg. End: 10.64 min

PRA206 - Imidacloprid256/209 (Unknown) 256.100/209.100 Da - sample 3 of 7 from Data140620.wiff
Area: 1.54e+003 counts Height: 5.07e+002 cps RT: 9.90 min



Imidacloprid

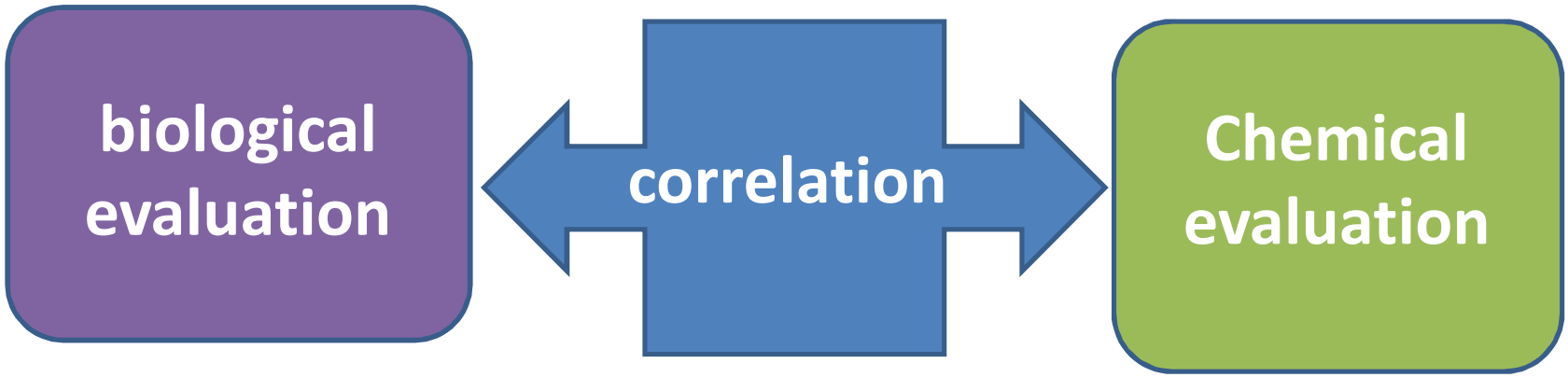
PRA206 - Imidacloprid256/175 (Unknown) 256.100/175.100 Da - sa
Area: 8.29e+002 counts Height: 2.82e+002 cps RT: 9.89 min



User Name: LABORA

Analyst - [Peak Revie... | Data

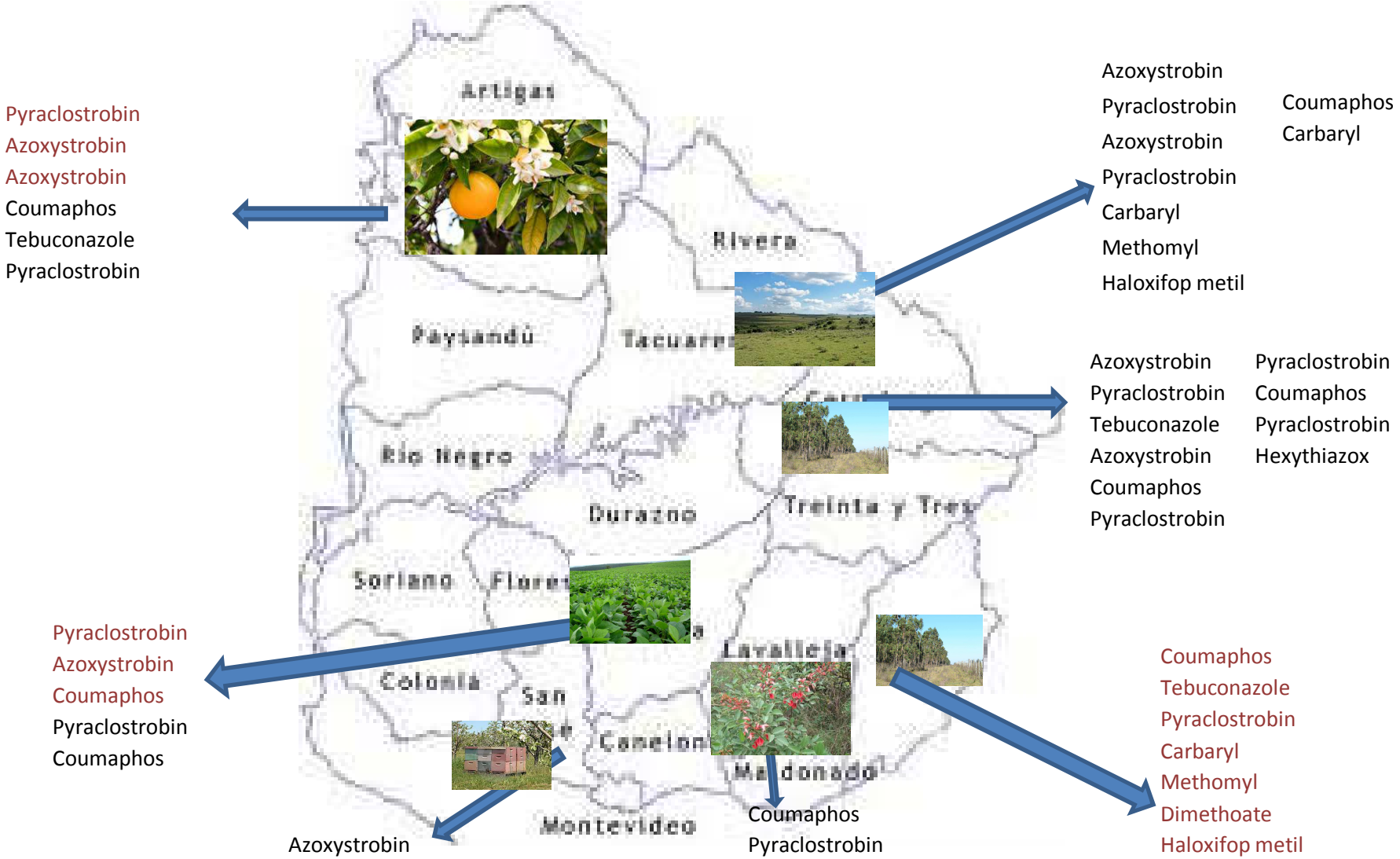
Beehive as biomonitor



Experimental design of the monitoring program

- Apiaries with >20 beehives
- 5 beehives randomly sampled
- The 5 beehives samples for each matrix are mixed and subsmpled for the analysis
- Two campaigns were evaluated 2012 & 2013

Analytical results of the monitoring program



Conclusions

- Analytical methodologies were developed and validated for 5 complex matrices from beehives.
- They can be a useful tool for the routine analysis of pesticide residues in those matrices.
- Pollen is the most contaminated matrix
- Trace amounts of imidacloprid, chlorpyrifos, azoxystrobin, tebuconazole, pyraclostrobin, iprodione, thiachloprid and haloxyfop methyl were detected in centinel hives placed in different agroecosystems.
- The presence of coumaphos and chlorpyrifos in honey and propolis was confirmed.
- Pesticides residues were found in bee products based foods.
- The results show the plasticity of the developed methods that can be applied to either food or environmental analysis.

Bees make blue honey after eating M&Ms

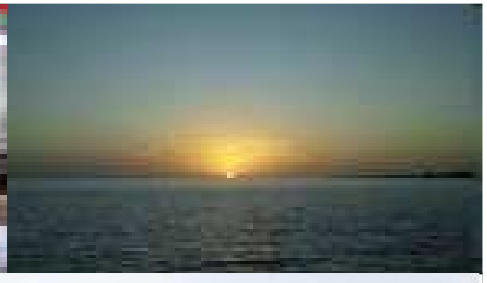
SCIENCEALERT STAFF
SATURDAY, 24 MAY 2014

We couldn't make this stuff up if we tried.

Image: Science Dump

French beekeepers were recently shocked when their bees started **producing thick, blue and**








TEATRO SOLIS


Thank you !!!

cs@fq.edu.uy