Development of a quantification method for up to 50 pesticides in honey and beeswax by LC-MS/MS and GC-MS detection

Joëlle Widart[1], Bach K. Nguyen[2], Christelle Deleuze[1], Laetitia Heudt[1], Gauthier Eppe[1], Jean-François Focant[1], Eric Haubruge[2], Edwin De Pauw[1]

[1]CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège
[2]Pure and applied Zoology Unit, Gembloux Agricultural University

Introduction

In Belgium, some beekeepers were confronted to hush of their beehives. External observers did not find any classical illness in those hives. Some hypotheses were proposed to explain the withering: specific illnesses, pesticide contamination, poor beekeepers practices. Although, several projects were conducted in Europe for the study of separated factors such as imidacloprid levels [1], no satisfactory explanation could however be given. A multifactorial study of withering has thus been initiated at the Belgian Walloon region level. In a first stage, specialized observers have studied beekeeping practices, common illness in the hives and environmental conditions around hives for selected Belgian locations. Consequently, a list of pesticides including both product types used in apiculture and for the surrounding agriculture has been extracted. The second part of the project is dedicated to the development of trace (ppb) measurement methods for residues of those pesticides (about 50 analytes) in honey, beeswax and bees. The third part will consist in the monitoring of selected POP's in honey, bees and beeswax.

On the analytical aspect, the simultaneous analysis of more than 50 pesticides in apiarian products (honey, beeswax) has not been described until now. Our approach is to optimize extraction and detection to reach the ppb level. Two different kinds of extraction methods were tested: classical Liquid-Liquid Extraction (LLE) and on-column LLE. The use of disposable cartridges packed with natural diatomaceous earth showed to be an efficient approach. Indeed, simple deposition of honey (dissolved in water) and elution with organic solvent such as ethyl acetate, dichloromethane or toluene gives high recovery rates.

Material and method

LC-MS/MS			GC-MS			
Pesticides	Transition	rt	Pesticides	ions		rt
Amidosulfuron	370>218 (20) 370>261 (10)	9.81	Bifenox	341	343	13.32
Atrazine	216>174 (15) 216>146 (20)	10.34	Bifenthrin	181	166	13.06
Benfluralin	336>236 (15) 336>220 (15)	17.05	Chlorothalonil	266	268	8.75
Bitertanol	338>269 (5) 338>99 (15)	13.57	Cyfluthrin 1	163	165	14.93
Carbofuran	222>165 (10) 222>125 (15)	9.65	Cyfluthrin 2	163	165	15.00
Chlorotoluron	213>140 (20) 213>168 (15)	10.03	Cyfluthrin 3	163	165	15.09
Difenoconazole	406>337 (15) 406>251 (20)	14.52	Lambda-Cyhalothrin	208	197	13.85
Diflufenican	395>266 (25) 395>246 (30)	15.53	Alpha-Cypermethrin	208	181	15.34
Fipronil	437>290 (25) 437>368 (15)	14.43	Deltamethrin	253	255	16.54
Flufenacet	364>194 (10) 364>152 (15)	13.67	Tau-Fluvalinate 1	250	252	16.08
Flusilazole	316>247 (15) 316>165 (25)	13.34	Tau-Fluvalinate 2	250	252	16.12
Imidacloprid	256>209 (15) 256>175 (15)	6.22	Iprodione	314	316	12.90
Isoxaben	333>165 (15) 333>150 (30)	13.23	Kresoxim methyl	131	206	11.34
Isoxaflutole	360>251 (10)	12.54	Procymidone	283	285	10.57
Linuron	249>182 (15) 249>160 (15)	12.16	Tefluthrin	177	197	8.56
Metconazole	320>125 (25) 320>70 (20)	13.67	Tolyfluanid	238	240	10.39
Methiocarb	226>169 (10) 226>121 (15)	12.05	Vinclozolin	285	287	9.16
Methiocarb sulfoxide	242>185 (10) 242>170 (20)	6.08	Captan	149	79	10.49
Nicosulfuron	411>182 (15) 411>213 (15)	8.54	Fluazifop-P-butyl	282	383	11.47
Pirimicarb	239>182 (15) 239>195 (10)	8.35	Coumaphos	362	364	14.69
Rimsulfuron	432>325 (15) 432>182 (20)	10.03	Dimethenamid	154	203	9.09
Simazine	202>132 (15) 202>124 (15)	8.72	Haloxyfop-R-methyl	375	377	10.61
Terbuthylazine	230>174 (15) 230>146 (20)	12.03	Pendimethalin	281	252	10.30
Tribenuron-methyl	396>155 (10) 396>181 (15)	11.32	Phosalone	367	369	13.64
Diethofencarb	268>226 (10) 268>180 (15)	12.48	Propachlor	120	176	7.37
Metazachlor	279>211 (10) 279>135 (15)	11.21	Bromopropylate	341	343	13.10
Metosulam	419>175 (20) 419>228 (15)	10.64	Beta-endosulfan	241	243	11.78
Quizalofop-ethyl	374>300 (10) 374>272 (15)	15.89	Clorpyrifos	314	316	9.84
Rotenone	395>192 (20) 395>213 (20)	13.82				
Trifloxystrobin	409>186 (15) 409>206 (15)	15.91				
Terbuthylazine-2-OH	212>156 (15) 212>114 (20)	13.82				
Dimethoate	230>171 (15) 230>199 (10)	6.48				

(): Collision energy in eV

rt: retention time in minutes

GC-MS conditions :

GC column : ZB-5, 30m x 0.25mm x 0.25µm (Zebron, Phenomenex, Torrance, CA 90501-1430). Carrier gas : helium, constant flow 1.5mL/min, splitless injection, purge time 120 seconds, , injector temperature 275°C, transfer line 280°C, temperature gradient: 60°C for 2min, up to 180°C at 40°C/min, up to 340°C at 12°C/min, total run time 18 min.

MS : PEGASUS III (LECO, St Joseph, MI, USA) acquisition rate 10 spectra/sec, detector voltage: 1700 V, source 70V, 250°C, solvent delay : 5min.

LC-MSMS conditions :

LC column : POLARIS C18A, 2.1 x 150mm, guard column idem 2.1 x 10mm. Solvents : A : Acetonitrile 0.1% Acetic Acid, B : Water 0.1% Acetic Acid. Gradient : 10/90 A/B for 1 minute, at minute 15, 80/20 with linear gradient. Reconditioning during 10 min, flow : 0.4mL/min, split 1:1, injection volume: 20µl, column temperature: 40°C

MS : QUATTRO ULTIMA PLATINUM (Waters, Milford MA, USA), positive electrospray, desolvation temperature 250°C, source temperature 125°C, collision gas Argon, $2.1.10^{-3}$mbar.

Results and discussion

Different extraction solvents were tested (acetone, methanol, water, acetonitrile). We began with a very simple method: extraction with an organic solvent after dissolution in water. About 15 pesticides gave good results, but some of them were not recovered. We then decided to test LLE columns (ChemElut 5 or 10mL, Varian, natural diatomaceous earth) according to what had been reported by Klein et al. [2] for fruits and vegetables.

ChemElut extraction consists of dissolution of the matrix in a solvent mixture (water, ethanol, acetone, methanol), agitation, centrifugation, pouring on the column, waiting a few minutes and finally elution with an organic solvent (ethyl acetate, dichloromethane, toluene, hexane). The use of a 20% of NaCl solution in water increases efficiency of the exchange of pesticides between the two liquid phases.

Unfortunately, a common extraction solvents mixture and elution solvent is not yet optimized. The optimization of recoveries leads to water-acetone-NaCl 20% in the extraction solvent for LC-MS/MS group of pesticides and water-ethanol for GC-MS group of pesticides. The elution is conducted with ethyl acetate for the first group and with a mixture of hexane/dichoromethane 1:1 for the second. Evaporation to dryness is followed by reconstitution with water/acetonitrile 1:1 and hexane for LC and GC group, respectively.

In Table 2, recoveries are ratio between area of pesticide in blank sample spiked at the beginning of extraction and blank sample spiked at the end of extraction.

Pesticides	Conc (ppb)	Recoveries honey	Conc (ppb)	Recoveries beeswax	Pesticides	Conc (ppb)	Recoveries honey
Amidosulfuron	0.2	**87%**	0.8	**85%**	Bifenox	500	**86%**
Atrazine	0.2	**99%**	0.8	**86%**	Bifenthrin	30	**77%**
Benfluralin	2.5	**73%**	10.0	**89%**	Chlorothalonil	60	**84%**
Bitertanol	0.2	**99%**	0.8	**78%**	Cyfluthrin1	266	**77%**
Carbofuran	0.2	67%	0.8	**84%**	Cyfluthrin2	266	**71%**
Chlorotoluron	10.0	**108%**	40.0	**81%**	Cyfluthrin3	266	**72%**
Difenoconazole	0.1	**101%**	0.4	**91%**	Lambda-Cyhalothrin	80	**77%**
Diflufenican	0.5	**104%**	2.0	**85%**	Alpha-Cypermethrin	1600	**82%**
Fipronil	5.0	**98%**	20.0	68%	Deltamethrin	4000	**87%**
Flufenacet	0.2	**100%**	0.8	**73%**	Tau-Fluvalinate 1	100	**81%**
Flusilazole	0.1	**103%**	0.4	**92%**	Tau-Fluvalinate 2	100	**79%**
Imidacloprid	1.0	**93%**	4.0	**88%**	Iprodione	1600	**73%**
Isoxaben	0.2	**102%**	0.8	**98%**	Kresoxim methyl	800	**78%**
Isoxaflutole	1.0	**98%**	4.0	120%	Procymidone	300	**75%**
Linuron	1.0	**99%**	4.0	**74%**	Tefluthrin	40	**78%**
Metconazole	0.1	**102%**	0.4	**75%**	Tolyfluanid	400	**89%**
Methiocarb	5.0	**97%**	20.0	**81%**	Vinclozolin	160	**76%**
Methiocarb sulfoxide	10.0	**95%**	40.0	112%	Captan	400	112%
Nicosulfuron	0.2	8%	0.8	**75%**	Fluazifop-P-butyl	100	**74%**
Pirimicarb	0.2	**94%**	0.8	**78%**	Coumaphos	800	**81%**
Rimsulfuron	0.2	63%	0.8	**76%**	Dimethenamid	50	**82%**
Simazine	1.0	**99%**	4.0	**83%**	Haloxyfop-R-methyl	360	**77%**
Terbuthylazine	0.2	**101%**	0.8	**73%**	Pendimethalin	100	**83%**
Tribenuron-methyl	1.0	**103%**	4.0	**83%**	Phosalone	1600	**76%**
Diethofencarb	1.0	**108%**	4.0	**99%**	Propachlor	30	**84%**
Metazachlor	0.5	**110%**	2.0	**99%**	Bromopropylate	200	**72%**
Metosulam	1.0	**100%**	4.0	**89%**	Beta-endosulfan	340	**80%**
Quizalofop-ethyl	1.0	117%	4.0	**89%**	Clorpyrifos	200	**74%**
Rotenone	1.0	**105%**	4.0	114%			
Trifloxystrobin	1.0	**102%**	4.0	**72%**			
Terbuthylazine-2-OH	0.5	**91%**	2.0	**77%**			
Dimethoate	1.0	**89%**	4.0	**100%**			

Conc (ppb) : spiked concentration

Bold : recovery in between 70 and 110%

Real sample (taken in hives included in the study) were analyzed following the new methods. A concentration of more than 500ppb of rotenone was found.

In parallel, organochlorinated POP's were tested among which were seven indicator PCB's. According to their level, dioxins and dioxin-like PCB's will be analyzed to study potential correlations.

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References

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