MONITORING OF PESTICIDES CONTENT IN DEAD BEES USING UPLC-MS/MS

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INTRODUCTION

The current human population growth requires agricultural improvement to get enough food for the people. The most visible is mono-cultural agricultural areas expansion. Although, it looks as an innocent change, together with constantly evolving urbanisation and industrialization of the landscape it means an insurmountable problem to a lot of animal species, that are usually overlooked by the people.

Honey bees (Apis mellifera L.) can be mentioned as an excellent example. Increasing honey bees mortality has been observed worldwide, especially in North America and Europe. Another factor, besides those described above, is expanding utilization of chemicals, that can be subsequently found in the environment. A lot of chemicals, that are dangerous for bees, belong to the group of pesticides. Important groups of pesticides are acetylcholine inhibitors such as organophosphates and carbamates. These compounds can inhibit hydrolyzation of acetylcholine by binding to active site of enzyme Acetylcholinesterase. Following increasing of acetylcholine concentration in synapses causes increased neurotransmitter signaling, that leads to central nervous system symptoms such as hallucinations, confusion, delirium, tremor, and seizures.

We present here an analytical method for monitoring of organophosphates based on UPLC-MS/MS. Molecules of inerest (acephate, dimethoate, diazinon, chlorphenvinphos, chlorpyriphos) have been chromatographically separated using chromatographic column Ascentis® Expres C18 (5 cm x 2.1mm, 2µm, Supelco Analytical) with precolumn Ascentis® Expres C18 (0.5cm x 2.1mm, 2 µm, Supelco Analytical) and gradient elution of mobile phases (ammonium formate water solution and ammonium formate acetonitrile solution). For mass spectrometric detection an electrospray ionization and selective reaction monitoring (SRM) mode were utilized. The individual steps of developed method were optimized. All the data were statistically evaluated.

SAMPLE TREATMENT

QuEChERS:
1) **Sample homogenisation**
2) **Extraction solvent addition** - acetonitrile:hexane (7:2, v:v)
3) **Homogenisation** - 6 min, 650 RPM
4) **Buffering salts addition** - 200 mg MgS0$_4$, 50 mg NaCl,
   25 mg C$_6$H$_6$Na$_2$O$_7$·1,5 H$_2$O,
   50 mg C$_6$H$_5$Na$_3$O$_7$·2 H$_2$O
5) **Homogenisation** - 5 min, 600 RPM
6) **Sorbent addition** - C18/PSA, 60 mg
7) **Homogenisation** - 5 min, 600 RPM
8) **Centrifugation** - 5 min, 11 000 RPM
9) **Supernatant filtration** - nylon, 0.22 µm

UPLC-MS/MS

Instrumentation: UHPLC-QqQ analyses were carried out in Ultimate 3000 system combined with TSQ Acces Max.
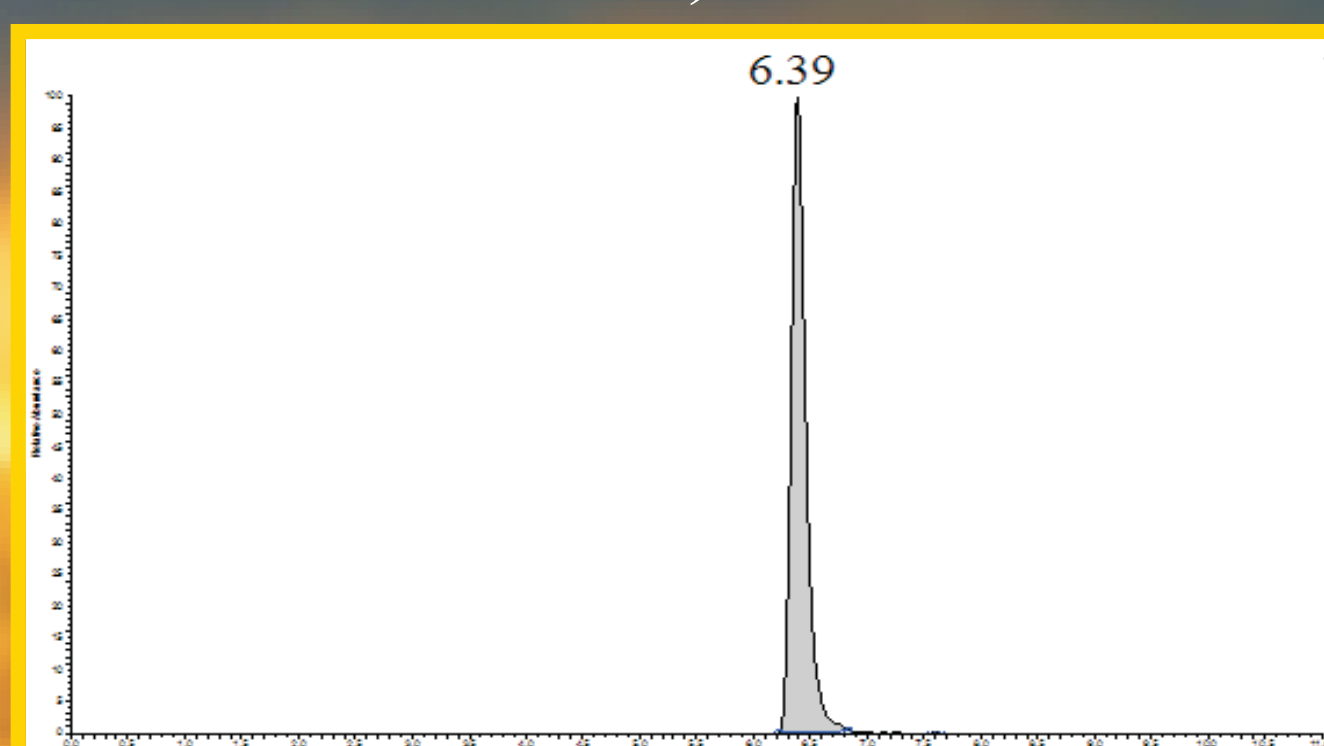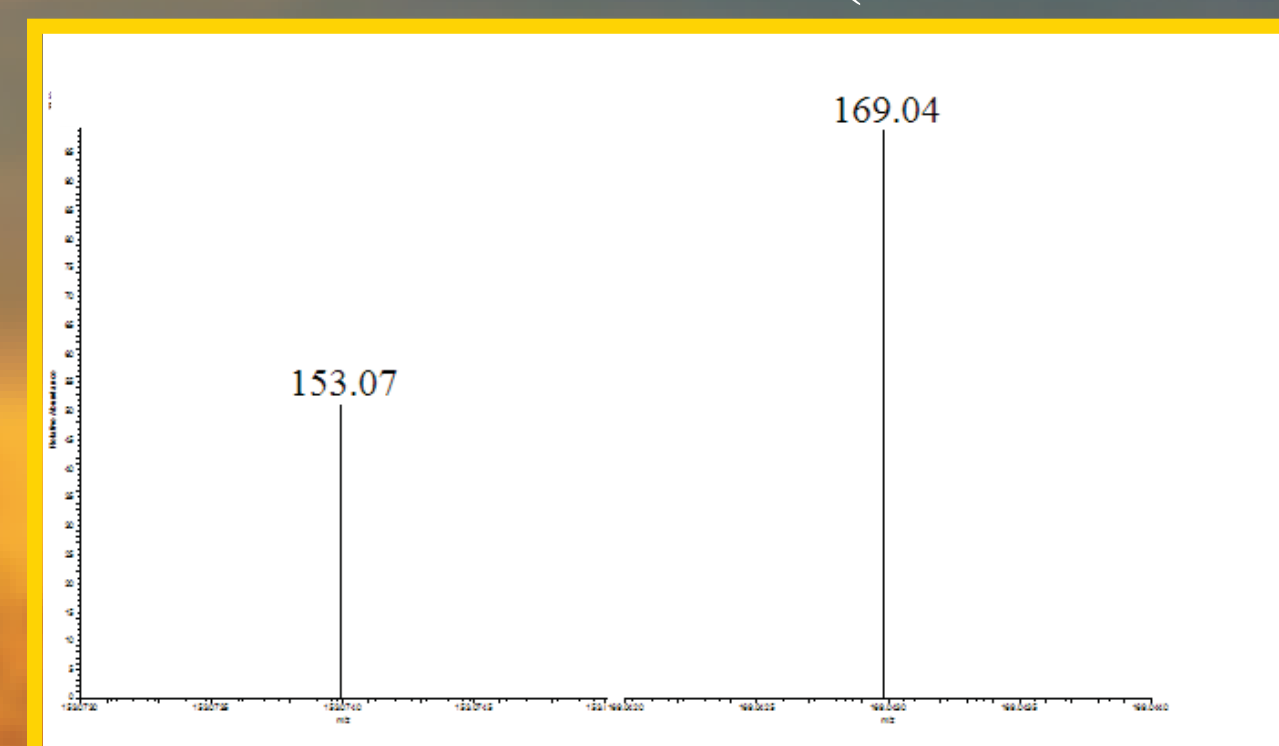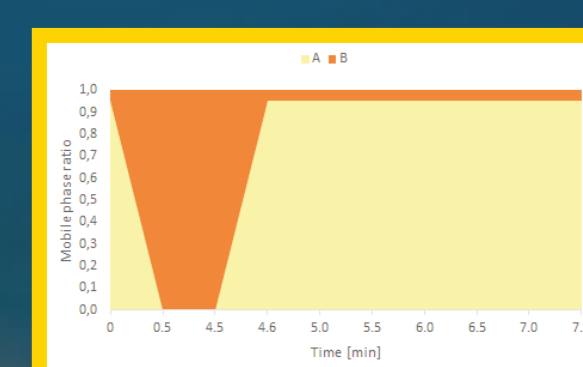
HPLC Conditions:
Column:
Ascentis® Expres C18 (5 cm x 2.1mm, 2µm, Supelco Analytical)
Mobile phase:
Solvent A:
Ammonium fomate (25 mM water solution)
Solvent B:
Ammonium fomate (25 mM solution in acetonitrile)

MS Parameters:
Ionization Mode: ESI$^+$
Scan mode: SRM
Cycle Time: 0.5 s
Collision Gas Pressure: 1.5 mTorr
Capillary Temperature in Source: 325 °C
Vaporizer Temperature: 300 °C
Auxiliary Gas Pressure: 45 arb units
Ion Sweet Gas Pressure: 2 arb units
Polarity Spray voltage: 3300 V

Analyte	Parent mass m/z [Da]	Product mass m/z [Da]	Collision energy [eV]	Tube Lens [V]	RT [min]
Acephate	184.116	143.014	5	51	7.23
	184.116	49.171	20	51	
Diazinon	305.077	153.074	19	61	6.39
	305.077	169.043	19	61	
Dimethoate	230.002	170.948	15	49	3.81
	230.002	124.975	20	49	
Chlorphenvinphos	358.941	99.007	28	56	1.61
	358.941	169.906	36	56	
Chlorpyriphos	351.927	199.861	17	56	3.96
	351.927	99.007	28	56	

CONCLUSION
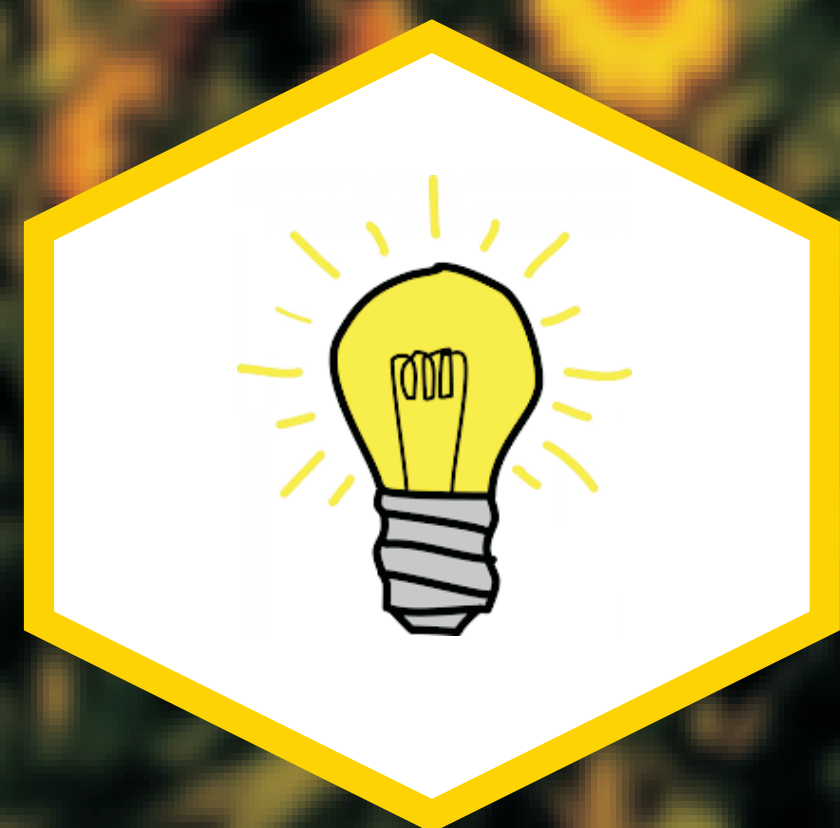
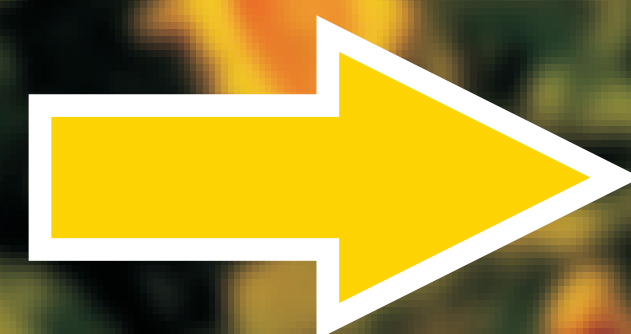• UPLC-MS/MS method for monitoring of organophosphates was devepoped
• Separation of analytes from bees was optimized

ACKNOWLEDGEMENT