

APPLICATION OF LC-MS/MS FOR MONITORING PESTICIDES IN BEES



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Abstract

Honey bees (*Apis mellifera* L.) often pollinate agricultural crops, which are exposed to chemicals that are dangerous to bees, belong to the group of pesticides, which is closely related to environmental pollution. Bees are a suitable local bioindicator, so early identification of factors leading to their death allows faster initiation of possible decontamination of the environment and the associated rescue of other animals or prevention of the spread of contaminated bee products among the population.



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.

The aim of this work is to develop a fast and user-friendly colorimetric sensor for the determination of bee poisoning by pesticides from the group of organophosphates and carbamates, which act as blockers of acetylcholinesterase (AChE).

We present here an analytical method for monitoring of organophosphates based on UPLC-MS/MS. Molecules of interest (acephate, dimethoate, diazinon, chlorphenvinphos, chlorpyrifos) 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.

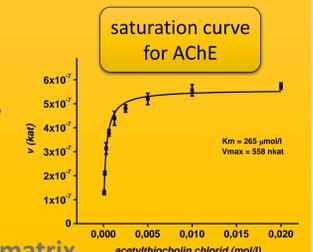
Methods - QUEChERS



- 1) Crushing a dead bee
- 2) Extraction solvent addition - acetonitrile:hexane (7:2, v:v)
- 3) Shaking on a minishaker - 6 min, 600 RPM
- 4) Buffering salts addition: 200 mg MgSO₄, 50 mg NaCl, 25 mg C₆H₆Na₂O₇ • 1,5 H₂O, 50 mg C₆H₅Na₃O₇ • 2 H₂O
- 5) Shaking on a minishaker - 5 min, 600 RPM
- 6) Centrifugation - 5 min, 10 000 RPM
- 7) Sorbent addition to acetonitrile layer - C18/PSA, 60 mg
- 8) Shaking on a minishaker - 2 min, 600 RPM
- 9) Centrifugation - 5 min, 11 000 RPM
- 10) Supernatant filtration - nylon, 0.22 µm

Colorimetric sensor

- Preparation of base matrices by 3D printing
- Reference spectrophotometric method: Ellman's method is used to determine AChE activity
- Optimization of colorimetric reaction on printed matrix



UPLC-MS/MS

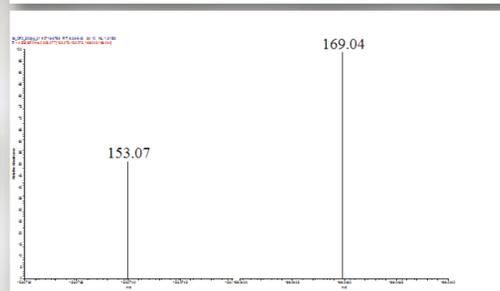
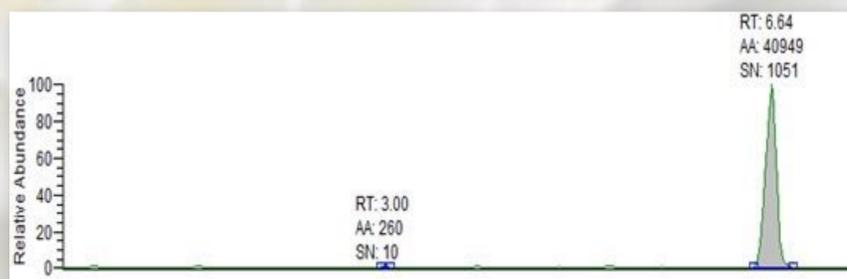
Instrumentation:
UHPLC-QqQ analyses were carried out in Ultimate 3000 system combined with TSQ Acces Max.

HPLC Conditions:
Column: Ascentis Expres C18 (5 cm x 2.1mm, 2µm, Supelco Analytical)
Mobile phase:
Solvent A: Ammonium formate (25 mM water solution)
Solvent B: Ammonium formate (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

Results

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	10.02
	184.116	49.171	20	51	
Diazinon	305.077	153.074	19	61	5.13
	305.077	169.043	19	61	
Dimethoate	230.002	170.948	15	49	6.64
	230.022	124.975	20	49	
Chlorphenvinphos	358.941	99.007	28	56	10.78
	358.941	169.906	36	56	
Chlorpyrifos	351.927	199.861	17	56	11.23
	351.927	99.007	28	56	



UPLC-MS/MS method for monitoring of organophosphates was developed
 Separation of analytes from bees was optimized



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