

SUPPLEMENTARY MATERIALS

Development and Validation of a Gas Chromatography-Mass Spectrometry Method for Determining Acaricides in Bee Pollen

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Table S1. Evaluation of the extraction efficiency (recovery percentages) and the matrix effect when employing 10 mL of different solvent mixtures with spiked blank bee pollen samples at medium level ($100 \mu\text{g kg}^{-1}$) (mean \pm %RSD; three replicates that were injected in triplicate).

Acaricide	ACN/AcOH (99:1 ; v/v)		ACN/TEA (98:2 ; v/v)		ACN/Hx (50:50 ; v/v)		AcOEt/Cx (20:80 ; v/v)		AcOEt/Cx (50:50 ; v/v)	
	EE	ME	EE	ME	EE	ME	EE	ME	EE	ME
Atrazine	10	16	111	48	98	1	80	-2	85	-2
Chlorpyrifos	-	-	51	-21	35	-32	-	-	-	-
Chlorfenvinphos	11	3	56	-2	64	-11	99	-10	108	-8
α-Endosulfan	7	-4	53	-29	33	-28	89	-30	90	-15
Bromopropylate	15	5	54	50	35	31	85	15	104	7
Coumaphos	12	15	86	77	44	83	92	20	98	10
τ-Fluvalinate	15	1	38	43	45	-13	101	14	109	16

ACN, acetonitrile; AcOEt, ethyl acetate; AcOH, acetic acid; Cx, cyclohexane; EE, extraction efficiency; Hx, hexane; ME, matrix effect; TEA, tryethylamine.

Table S2. Summary of precision studies (minimum and maximum %RSD values) for the determination of acaricides in spiked blank bee pollen samples.

	Spiking Level	Multifloral Bee Pollen	
		Min.	Max.
Intraday precision	Low	2	5
	Medium	4	8
	High	3	7
Interday precision	Low	4	7
	Medium	4	7
	High	2	5

Low level, LOQ, (see Table 3); **Medium level**, 100 µg kg⁻¹; **High level**, 700 µg kg⁻¹.

Table S3. Chemical structure of the studied acaricides.

Atrazine	Chlorpyrifos
CAS number: 1912-24-9	CAS number: 2921-88-2
<p>Atrazine is a triazine herbicide. It consists of a central nitrogen atom bonded to two methyl groups and two chlorine atoms. Each chlorine atom is also bonded to a nitrogen atom, which is part of a methylene group (CH2-). This group is further bonded to a methyl group (CH3).</p>	<p>Chlorpyrifos is an organophosphate insecticide. It features a 4-chloro-2,6-dimethylphenyl ring attached to a phosphorus atom. The phosphorus is bonded to a methyl group, a dimethylaminomethyl group (-CH2-CH2-N(CH3)2), and two ethyl groups (-CH2-CH3).</p>
α -Endosulfan	τ -Fluvalinate
CAS number: 959-98-8	CAS number: 102851-06-9
<p>α-Endosulfan is a chiral organochloride. It has a cyclohexane-like core with four chlorine atoms and a propylsulfone side chain (-CH2-CH2-SO2-CH3).</p>	<p>τ-Fluvalinate is a nitrile-based insecticide. It contains a cyclohexane ring with a methyl group, a cyano group (-C#N), and a phenyl ring substituted with a 4-chlorophenyl group.</p>
Chlorfenvinphos	Chlorfenvinphos-d ₁₀
CAS number: 470-90-6	CAS number: 1346606-54-9
<p>Chlorfenvinphos is an organophosphate insecticide. It has a 4-chlorophenyl ring attached to a phosphorus atom, which is bonded to a methyl group, a dimethylaminomethyl group, and a propylsulfone group.</p>	<p>Chlorfenvinphos-d₁₀ is a deuterated derivative of Chlorfenvinphos. The propylsulfone group is replaced by a deuterium-labeled propylsulfone group (-CH2-CH2-SO2-D3).</p>
Bromopropylate	Coumaphos
CAS number: 18181-80-1	CAS number: 56-72-4
<p>Bromopropylate is an organophosphate insecticide. It has a 4-bromo-2-hydroxyphenyl ring attached to a propyl ester group (-CH2-CH2-COOCH2CH3).</p>	<p>Coumaphos is a cyclic organophosphate. It features a five-membered ring containing phosphorus, sulfur, and two oxygen atoms. The ring is substituted with a 4-chlorophenyl group and a 4-methyl-2-oxo-2H-chromene-3-carboxylate group.</p>

Figure S1. MS spectra of chlorfenvinphos in standard in (A) solvent and (B) in matrix at the same concentration (0.5 mg L⁻¹). The GC-MS conditions are summarized in subsection 3.4 and **Table 1**.

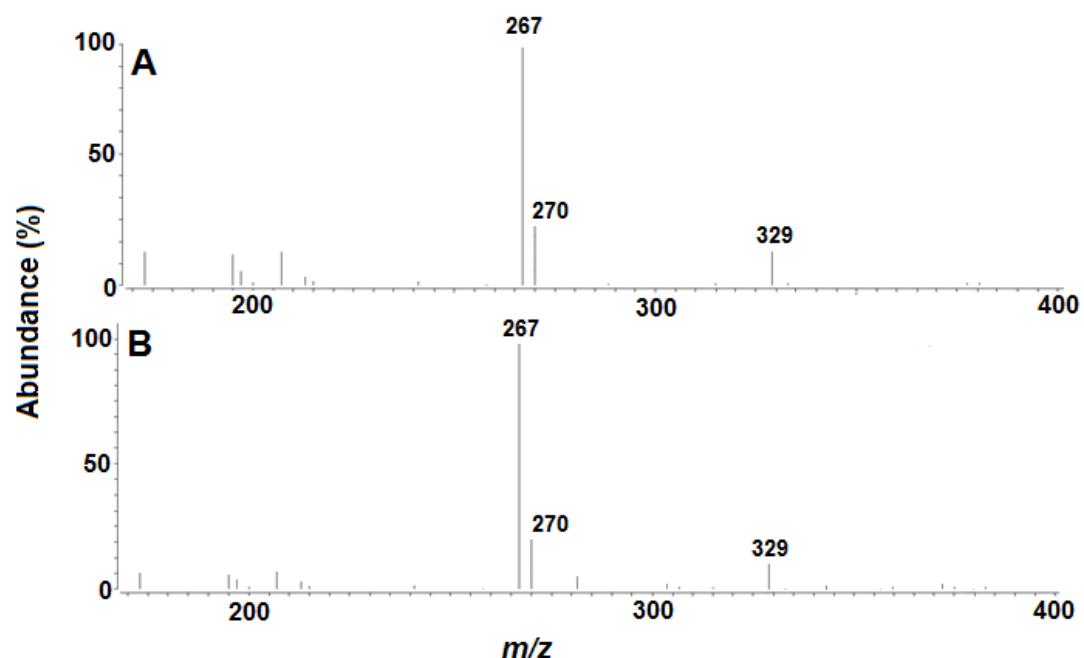


Figure S2. Representative GC-MS chromatogram (SIM mode using the quantification/target ions; see **Table 1**) obtained from multifloral bee pollen sample (E3; 97 $\mu\text{g kg}^{-1}$) with endogenous τ -fluvalinate content over LOQ. GC-MS conditions are summarized in Subsection 3.4 and **Table 1**.

