Optimization of a Fast-Analytical Method for the Determination of Selected Agricultural Pesticides Using Supercritical Fluid Chromatography

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INTRODUCTION & AIM

- In apiculture, **pesticides,** and especially acaricides, are closely linked to controlling ***Varroa destructor***, a parasitic mite harmful to honeybees [1].

- Resistance and overuse have resulted in **residues accumulating** in hive products, which poses risks to human health and the environment [2].

Fig. 1. *Varroa destructor* mite.

atrazine   coumaphos   fluvalinate

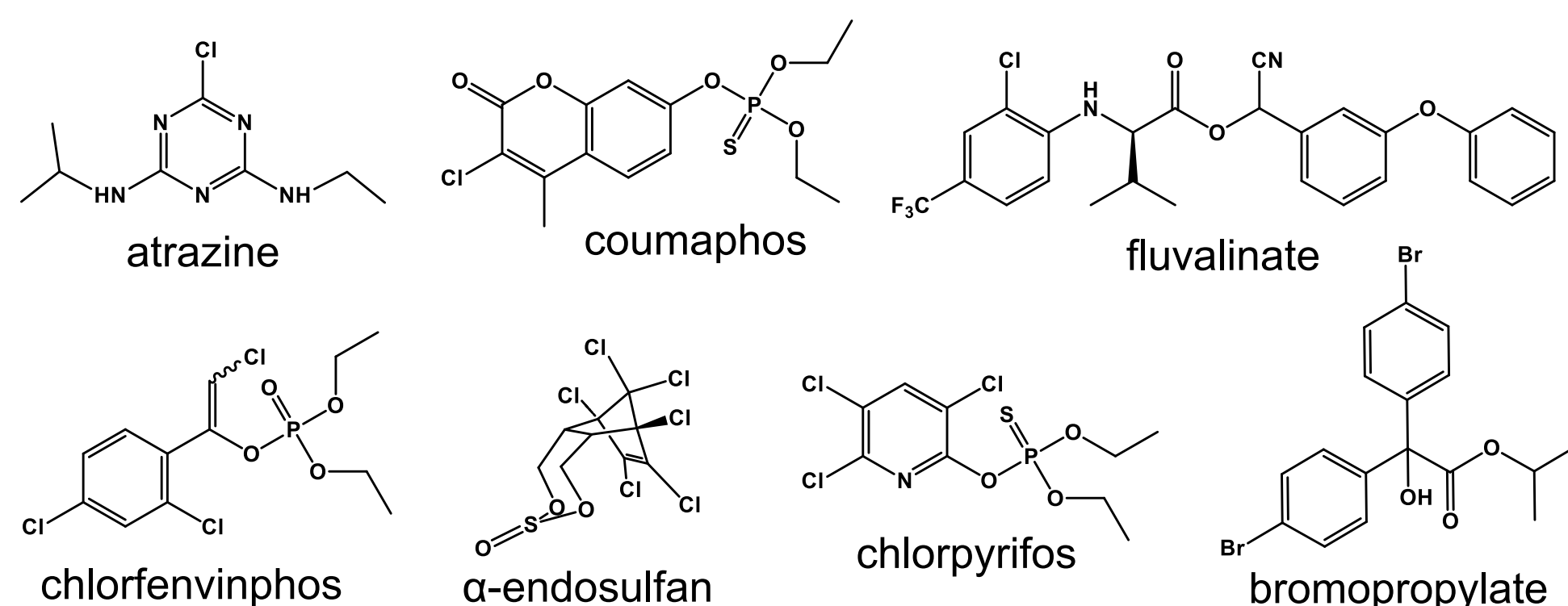chlorfenvinphos   α-endosulfan   chlorpyrifos   bromopropylate

Fig. 2. Pesticides analysed in the proposed method.

The **objective** of this work is to develop an analytical method for the determination of seven of the most employed pesticides in apiculture (see **Fig. 2.**) using supercritical fluid chromatography (SFC), for its possible application to the analysis of residues of these compounds in honey samples.

METHOD

① Columns (stationary phases)

- DCpak PBT (250 mm × Ø 4.6 mm, 5 µm): polybutylene terephthalate on silica.

- **DCpak P4VP (250 mm × Ø 4.6 mm, 5 µm): poly(4-vinylpyridine) on silica.**

- Hypersil-NH2 (250 mm × Ø 4.6 mm, 5 µm): aminopropyl silica.

- Lichrosphere CN (250 mm × Ø 4.6 mm, 5 µm): cyanopropyl silica.

- Hypersil silica (250 mm × Ø 4.6 mm, 5 µm): silicagel.

- **Lichrosphere 100 diol (250 mm × Ø 4.6 mm, 5 µm): propanediol silica.**

② Wavelengths

- **200 nm**
- **220 nm**
- **230 nm**
- **240 nm**
- **250 nm**

③ Temperatures

- 20 ºC
- 22 ºC
- 25 ºC
- 27 ºC
- 30 ºC
- 32 ºC
- **35 ºC**
- 37 ºC

④ Additives

- **No**
- FA
- TFA
- TEA

⑤ Modifiers

- **MeOH**
- EtOH
- ⁱPrOH

⑥ Pressures

- **150 bar**
- 200 bar

⑦ Elution mode

- **Isocratic**
- Gradient

RESULTS & DISCUSSION

Table 1. Conditions for the final method.

Column	Lichrosphere 100 Diol		
Injection volume (µL)	10		
Flow (mL min⁻¹)	3		
Gradient	**Time (min)**	**% MeOH**	**Increment**
	0	2	-
	2	2	0
	5	5	1
Analysis time (min)	4		
Temperature (ºC)	35		
Initial pressure (bar)	150		

1.Chlorpyrifos
2.Endosulfan
3.Fluvalinate
4.Fluvalinate
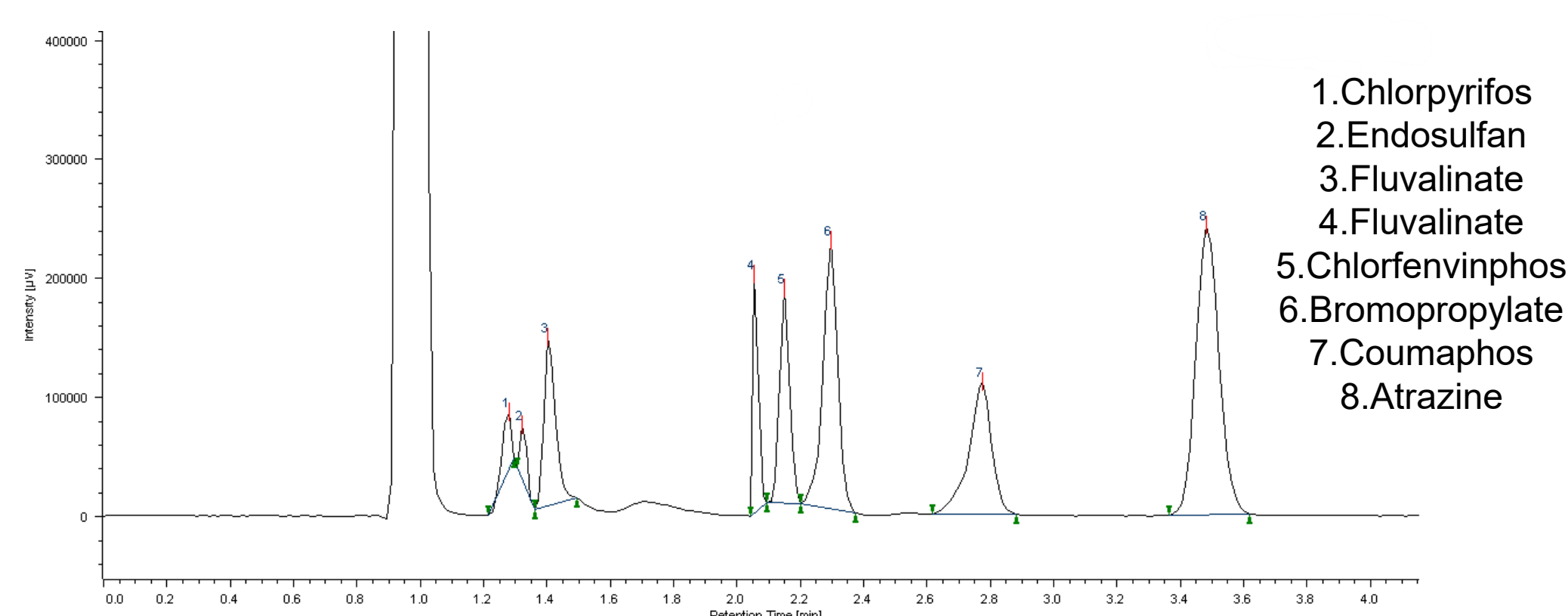5.Chlorfenvinphos
6.Bromopropylate
7.Coumaphos
8.Atrazine

Fig. 3. SFC-DAD Chromatogram obtained using the optimized conditions.

Table 2. Retention times of the studied pesticides.

Analyte	t_R (min)
Chlorpyrifos	1.33
Endosulfan	1.55
Fluvalinate	2.15
Chlorfenvinphos	2.28
Bromopropylate	2.48
Coumaphos	2.99
Atrazine	3.90

CONCLUSIONS

- **Seven pesticides** were separated by SFC using six stationary phases.

- Best results with Lichrospher 100 Diol and DCpak P4VP columns.

- **DCpak P4VP** shows good resolution but longer retention (up to 30% modifier).

- **Lichrospher 100 Diol** provides full separation in under 4 min with a maximun modifier percentage of 5%, being faster than GC (~20 min).

- PDA detection showed limited sensitivity so **MS detection is required**.

ACKNOWLEDGEMENTS

FUTURE WORK / REFERENCES

[1] M. Murcia-Morales, H. Heinzen, P. Parrilla-Vázquez, M. del M. Gómez-Ramos, A.R. Fernández-Alba, Presence and distribution of pesticides in apicultural products: A critical appraisal, TrAC - Trends in Analytical Chemistry 146 (2022) 116506. https://doi.org/10.1016/j.trac.2021.116506.

[2] A. Fuente-Ballesteros, A. Jano, J. Bernal, A.M. Ares, Development and validation of an analytical methodology based on solvent extraction and gas chromatography for determining pesticides in royal jelly and propolis, Food Chem 437 (2024) 137911. https://doi.org/10.1016/j.foodchem.2023.137911.