Evaluation of alpha-cypermethrin isomerization in FV matrices

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1. Aim and scope

This study focuses on the analysis and evaluation of the alpha-cypermethrin compound, a component of the cypermethrin technical mixture (Figure 1), which comprises four pairs of enantiomers resulting in four distinct chromatographic peaks. Using GC-MS/MS, the research seeks to understand the factors contributing to isomerization, as evidenced by the appearance of an additional unexpected peak in the chromatogram. The investigation includes evaluating potential contributors to this phenomenon, such as the solvent used for injection, the type of commodity, the extraction method, and the injection mode. By addressing these factors, the study aims to provide deeper insights into the isomerization process and its implications for alpha-cypermethrin analysis.

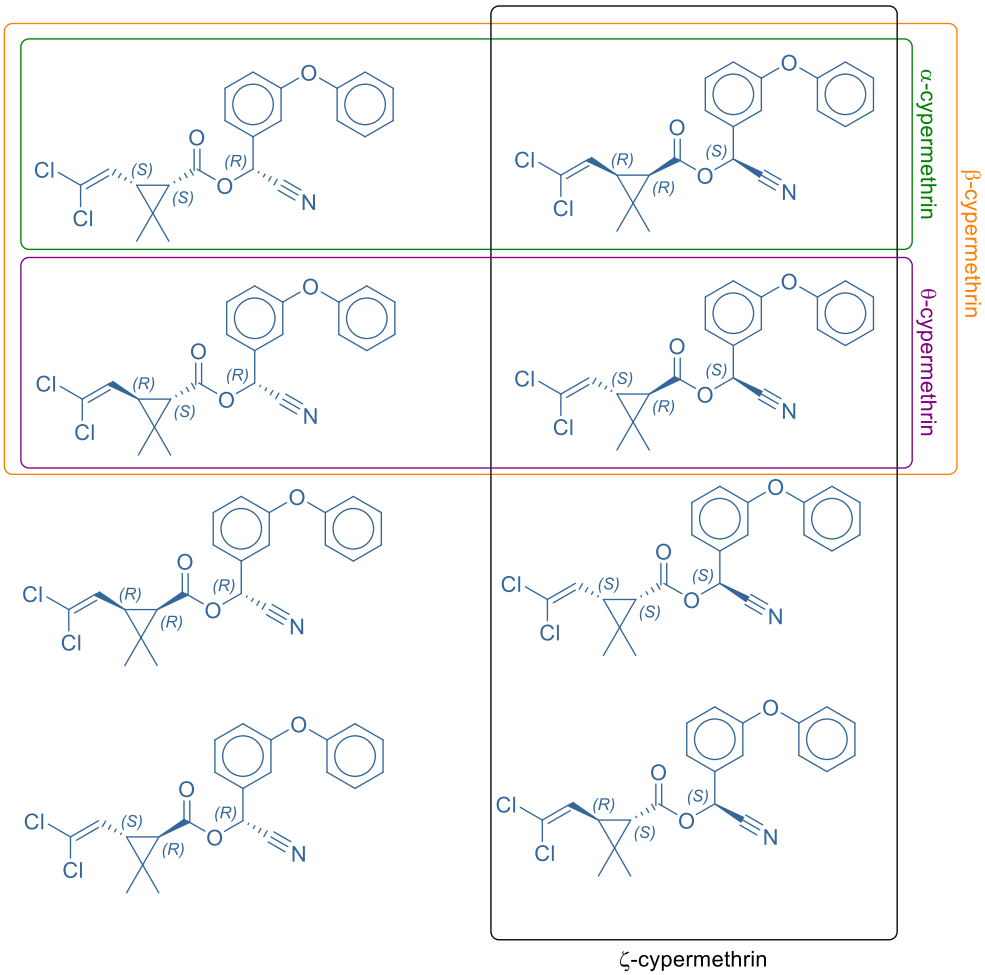

Figure 1. Representation of cypermethrin with its distinct isomers. The alpha-, beta-, theta-, and zeta-cypermethrin isomers are highlighted, emphasizing their specific structural configurations within the compound.

2. Short description

The method was optimized to achieve proper separation of the chromatographic peaks corresponding to the cypermethrin isomers, and calibration curves were injected to evaluate different peak integration methodologies. To investigate the factors influencing isomerization, seven different commodities were analyzed to assess their impact. Additionally, the effect of various GC liners on the isomerization process was evaluated. Finally, a dual-layer injection mode was tested to determine whether it could resolve the isomerization issue effectively.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 1µL to 5 Ml
- Axial shaker Agytax SR1 CP57.
- Concentration workstation.
- Injection vials, 2 mL, suitable for LC and GC auto-sampler.
- Liners:
  - Carbofrit: Single Taper Inlet Liner, 4.0 mm x 6.5 x 78.5, for Agilent GCs, Standard Deactivation, w/CarboFrit, 5-pk Catalog No. 20799-209
  - No Wool: Agilent inlet liner, Ultra Inert, splitless, single taper, Part Number: 5190-2292
  - Glass wool: Agilent inlet liner, Ultra Inert, splitless, single taper, glass wool, Part Number: 5190-2293

4. Chemicals

- Acetonitrile ultra-gradient grade
- Ethyl acetate
- Pesticide analytical standards

5. Procedure

5.1. Pesticide stock solutions and working mix solutions

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile or ethyl acetate and were stored in screw-capped glass vials in the dark at -20 °C. Working mixes were prepared in 10 mL volumetric flasks by pipetting the appropriate volume of each stock solution.

5.2. Instrumentation and analytical conditions

5.2.1. Intuvo 9000 GC system (Agilent)

- Columns: 2 planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- Injection mode: Splitless
- Sample injection volume: 1 µL
- Inlet temperature: 80 °C hold for 0.1 min, then up to 300 °C at 600 °C/min, hold for 5 min and then to 250 °C at 100 °C/min
- Carrier gas: Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2
- Carrier gas purity: 99.999 %
- Oven temperature: 60 °C for 0.5 min, up to 170 °C at 80 °C/min, and up to 310 °C at 20 °C/min (hold for 3.5 min)
- Post Run: 2.1 min, 310 °C

Injection volume	With Dual Layer	Without Dual Layer
V_{Sample} (µL)	1	1
$V_{Orange\ in\ EtAc}$ (µL)	0.5	0
V_{Total} (µL)	1.5	1

5.2.2. 7410 triple quadrupole system (Agilent)

- Ionisation mode: electron impact ionisation
- Temperature of the transfer line: 280 °C
- Temperature of ion source: 280 °C
- Collision gas: nitrogen
- Collision gas purity: 99.999 %
- Solvent delay: 2.6 minutes

6. Results

6.1 Optimization of the method

In the initial multiresidue method, the four chromatographic peaks of the cypermethrin technical mixture could not be properly resolved, as the latter two peaks exhibited coelution. One of these peaks corresponds to the alpha-

cypermethrin compound, making accurate quantification challenging. The initial method featured a total run time of 12.4 minutes, with cypermethrin eluting at a retention time of 9.65 minutes under an elution temperature of 310°C. To address this issue, the method was optimized by employing a gentler temperature ramp, which effectively resolved the two closely eluting cypermethrin peaks. The final optimized method increased the total run time to 29 minutes, with cypermethrin eluting at a retention time of 25 minutes and under a lower elution temperature of 280°C. This adjustment significantly improved peak resolution, enabling more reliable identification and quantification of the third eluting peak, corresponding to alpha-cypermethrin, in accordance with DG-SANTE criteria [1] .

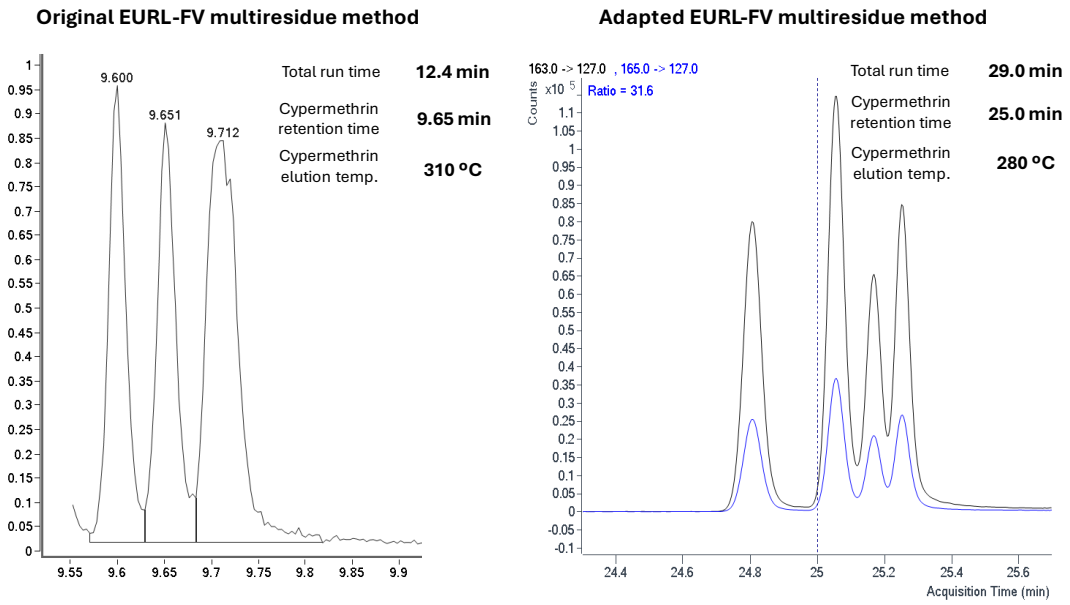

Figure 2. Chromatogram comparing the original EURL-FV multiresidue method, which does not separate the later elution peaks, and the adapted multiresidue method used for identifying the alpha-cypermethrin peak (third).

In the analysis, the third peak corresponding to alpha-cypermethrin was not fully resolved; therefore, various quantification approaches were tested to ensure its suitability for accurate measurement. A calibration curve was prepared using concentrations of 0.005, 0.010, 0.050, and 0.1 mg/kg of cypermethrin (technical). Quantification was performed using three methods: peak height, peak area limited to the protruding section, and peak area with vertical extrapolation to the baseline. In all cases, the R-squared values exceeded 0.999, demonstrating that excellent linearity was achievable with these quantification approaches.

6.2 Liners evaluation

Three different GC liners were evaluated to determine their influence on the isomerization of alpha-cypermethrin.

- Liner with glass wool: Contains a layer of tightly packed glass fibers designed to improve vaporization efficiency.
- Carbofrit liner: Features a porous carbon frit that ensures uniform vaporization and enhances sample flow.
- Liner without wool: A simple, unmodified liner that avoids potential reactive surfaces.

The results showed that the liner containing glass wool exhibited the highest isomerization rate at 7.5%, while both the carbofrit liner and the liner without wool demonstrated significantly lower isomerization rates, each at 2.5%. These findings suggest that the choice of liner material may has a considerable impact on minimizing isomerization during analysis. It was also observed that using a new, clean liner can help reduce isomerization; however, this reduction diminishes with each subsequent injection, as discussed in subsection 6.4 on reproducibility.

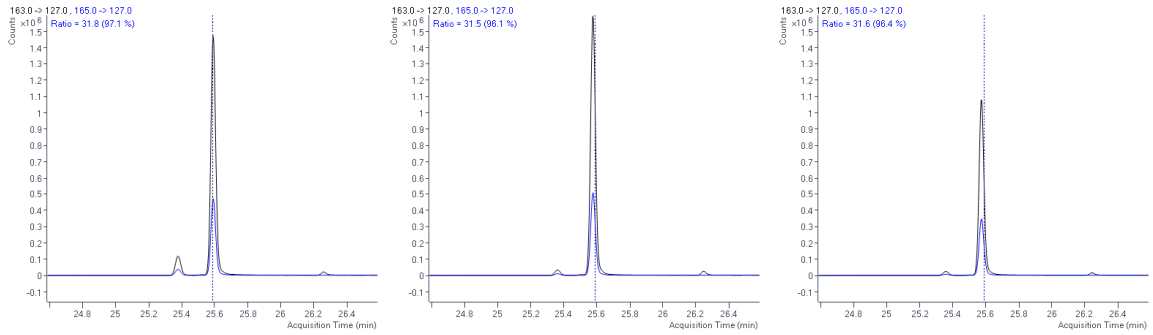

Figure 3. Chromatogram showing the isomerization rate of alpha-cypermethrin for the different liners tested.

6.3 Influence of Matrix and Solvent

The isomerization of alpha-cypermethrin was assessed across seven different matrices: tomato, banana, onion, pineapple, orange, lentils, and avocado. Additionally, the effect of solvents on isomerization was investigated by injecting these matrices with either acetonitrile or ethyl acetate as solvents. In most cases, the isomerization rate was below 10%, indicating minimal variation across the majority of matrix-solvent combinations.

However, notable deviations were observed in avocado, where isomerization reached 27% with ethyl acetate and 30% with acetonitrile. These findings suggest that certain matrices, such as avocado, can significantly influence the isomerization of alpha-cypermethrin. This pronounced effect in avocado is likely attributed to its high fat content, the highest among all the matrices studied. The elevated fat percentage in avocado may enhance interactions that promote isomerization, underscoring the importance of considering matrix composition, particularly lipid content, in analytical evaluations.

6.4 Reproducibility

The reproducibility of isomerization was evaluated through successive injections, using avocado as the matrix due to its high isomerization rate. A total of 16 successive injections were performed, alternating between ethyl acetate and acetonitrile as solvents. The results revealed a notable increase in the isomerization of the compound with successive injections, indicating that isomerization is not stable and tends to intensify as the number of injections increases.

Interestingly, ethyl acetate exhibited higher isomerization rates in the range of 11–16 injections. This may be attributed to ethyl acetate's ability to dissolve a greater amount of fats from the avocado matrix compared to acetonitrile. Given that the high fat content of avocado is likely a key factor influencing isomerization, the increased dissolution of fats by ethyl acetate could contribute to the elevated isomerization observed during these injections.

Table 1. Average isomerization in the avocado matrix during consecutive injections

Injection number	Isomerisation (%)	
	Avocado in EtAc	Avocado in ACN
1-2	16	19
3-4	22	22
5-6	25	24
7-8	27	25
9-10	31	27
11-12	33	28
13-14	34	30
15-16	37	29

6.5 Dual layer approach

The study reveals that isomerization of alpha-cypermethrin is significantly influenced by the nature of the matrix injected. Standard injection procedures demonstrate a tendency to favor isomerization, particularly in certain matrices such as avocado. However, employing a dual-layer approach, where 1 µL of the sample extract is combined with 0.5 µL of blank orange extract, drastically reduces this isomerization effect. This suggests that the dual-layer method provides a stabilizing influence, mitigating the transformation of alpha-cypermethrin under these conditions.

Additionally, solvent choice does not appear to significantly affect isomerization during standard injections, as observed when using ethyl acetate or acetonitrile. As can be observed in table 3, while acetonitrile exhibits slightly higher isomerization values in matrices such as tomato and pineapple, these values become negligible when the dual-layer method is applied. The most noticeable impact of the dual-layer approach is observed in the avocado matrix, where isomerization is reduced from 30% to 5% or less. The data further indicate that the inherent properties of the matrix in the dual-layer section play a critical role in modulating isomerization, highlighting the importance of matrix effects in minimizing this phenomenon.

Table 2. Comparison of Alpha-Cypermethrin isomerization in seven matrices with and without Dual Layer using Ethyl Acetate and Acetonitrile

	Isomerization (%)			
	EtAc		ACN	
	without DL	with DL	without DL	with DL
Tomato	2	1	9	2
Banana	5	1	5	2
Onion	2	1	2	2
Pineapple	3	1	7	1
Orange	2	1	3	2
Lentil	2	1	2	1
Avocado	27	5	30	4

7. Conclusion

The optimization of the multiresidue method addressed key challenges in the analysis of alpha-cypermethrin, particularly the coelution of chromatographic peaks. By implementing a gentler temperature ramp and extending the run time, the method achieved improved resolution of the cypermethrin peaks, enabling reliable identification and measurement of alpha-cypermethrin.

The isomerization study provided valuable insights into the complex interplay between matrix effects, solvents, and injection strategies. Experimental findings demonstrated that isomerization rates were heavily influenced by the nature of the matrix, with avocado showing the highest susceptibility, reaching 30% under standard injection conditions. This pronounced effect in avocado is likely due to its status as a fatty matrix, as its high lipid content distinguishes it from the other matrices studied and likely plays a key role in enhancing isomerization.

The dual-layer approach, which involved combining the sample extract with blank orange extract, emerged as a highly effective strategy, reducing isomerization to 5% or less, even in challenging matrices like avocado. Furthermore, the type of GC liner and its cleanliness also played a significant role, with glass wool liners exhibiting higher isomerization compared to other designs. These results emphasize the importance of carefully tailoring analytical methods to account for both matrix and equipment variables, ensuring robust, reproducible results in pesticide residue analysis and advancing the precision of multiresidue methodologies.

8. References

[1] E. Commission, SANTE 11312/2021 – Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed, 2021, pp. 1–51. https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.