EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compounds**: Cypermethrin, Alpha-Cypermethrin (a.k.a. Alphamethrin), Beta-Cypermethrin, Zeta-Cypermethrin, Theta-Cypermethin
- **Commodities**: Plant Origin, Animal Origin
- **Extraction Methods**: CEN-QuEChERS; QuOil
- **Instrumental analysis**: LC-MS/MS

Analysis of Residues of Cypermethrin Mixtures in Food employing QuEChERS and LC-MS/MS

Version 1

1. Introduction and background information

Cypermethrin is approved as an insecticidal active substance for plant protection, biocidal applications and veterinary treatments[1, 2, 3]. Chemically, it contains three stereoisomeric centers resulting in eight possible stereoisomers. Various mixtures of these isomers are registered as pesticides, see Figure 1. Cypermethrin itself is composed of all eight isomers, whereas alpha-cypermethrin (also known as alphamethrin) is merely composed of the enantiomeric pair "1*R cis* α-*S*" and "1*S cis* α-*R*" at a racemic composition (also know as *cis*-II pair). The different isomers of cypermethrin have different toxicological potencies, with the 1*R cis* and α-*S*-configurations exhibiting the highest mammalian toxicity[4]. With the toxicologically potent isomer 1R *cis* α-S constituting 50% of alpha-cypermethrin versus only ca. 11 % in the case of cypermethrin, the former is toxicologically more critical. This is also reflected in the respective toxicological endpoints of alpha-cypermethrin and cypermethrin (ARfD: 0.00125 vs. 0.005 mg/kg body weight (bw) and ADI 0.00125 vs. 0.005 mg/kg body weight (bw)/day).

In conventional (non-enantioselective) chromatography, the eight cypermethrin isomers elute as four peaks each consisting of one pair of enantiomers. The pairs of enantiomers are also known as *cis*-I, *cis*-II, *trans*-I and *trans*-II. A chromatographic separation of the *cis*-II pair from the other three pairs of enantiomers is crucial for a separate quantification of alpha-cypermethrin, but this depends on the chromatographic conditions used. The majority of laboratories analyze pyrethroids including the various cypermethrin mixtures using GC-based methods. If the GC runtime is not too short, the peaks of the four enantiomeric pairs will typically separate sufficiently (see exemplary chromatogram in Figure 2). Separate quantification is thus potentially possible.

[1] Regulation (EU) 2021/2049 in its latest version

[2] Regulation (EU) No. 37/2010 in its latest version

[3] EFSA, 2023 (doi: 10.2903/j.efsa.2023.7800)

[4] Soderlund et al. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment, Toxicology 171, 1, pp. 3 - 59, 2002

A notorious problem when analyzing pyrethroids via GC is their tendency to isomerize under the hot conditions in the GC-injector with epimerization on the carbon containing the cyano-group (in α-position to the ring), being the most prominent.

The extent of isomerisation stongly depends on factors, such as:

a. the injection conditions, e.g. injection mode, temperature
b. the status of the liner surface
c. the amount and type of co-extractants in the injected solution (matrix-type)
d. the presence of analyte protectants ("APs")[5], see Figure 3.

Where the shift in isomeric composition is not properly addressed by calibration, it can lead to significant quantification errors, wrong risk assessment and potentially wrong risk management decisions. In GC-applications, quantification is further compromised by the fact that isomerization is bidirectional (i.e. isomerization from α-*S* to α-*R* and vice versa). Consequently, errors are introduced in quantification if the isomer pattern in the samples extracts differs from that in the calibration standard. Where the isomerization-rate is low, this problem looses significance.

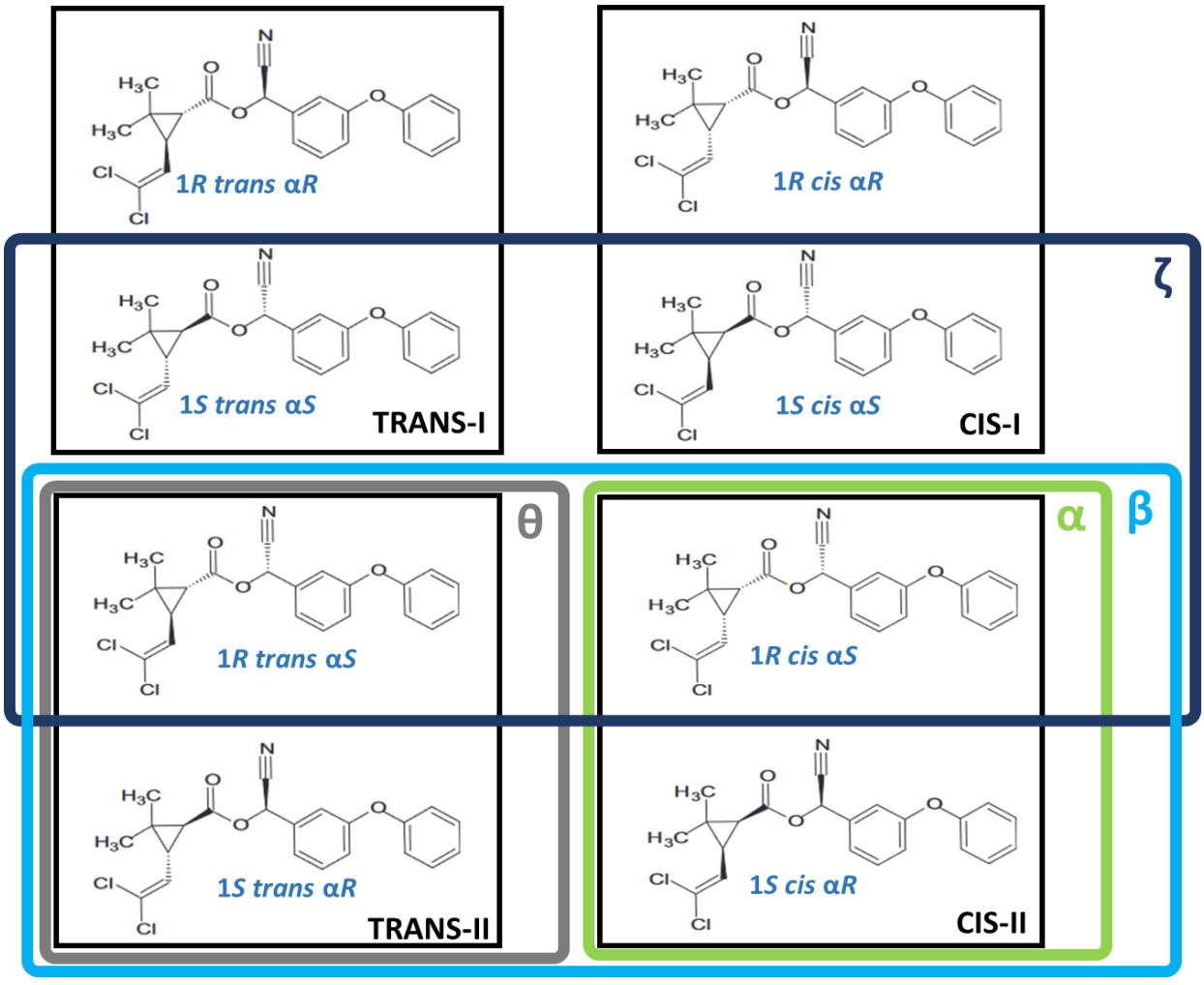


Figure 1: Overview of the eight cypermethrin isomers (four enantiomeric pairs: *cis*-I, *cis*-II, *trans*-I and *trans*-II). Different mixtures of cypermethrin isomers are registered as a.s. (i.e. alpha (α), beta (β), zeta (ζ) and theta (θ)). Differently colored frames show the main isomers of each active substance.

[5] https://www.eurl-pesticides.eu/library/docs/srm/EURL_Observation-APs.pdf

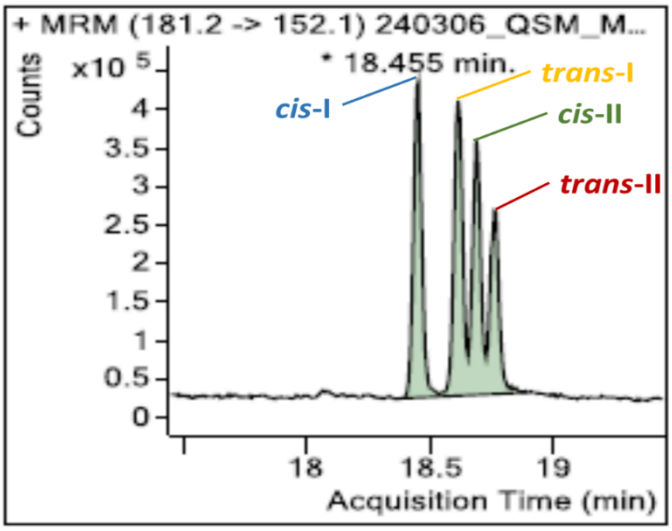


Figure 2: Typical chromatographic separation of the four enantiomeric pairs of cypermethrin in GC-applications (only the m/z 181/152 mass trace is shown (GC-MS/MS parameters see Annex).

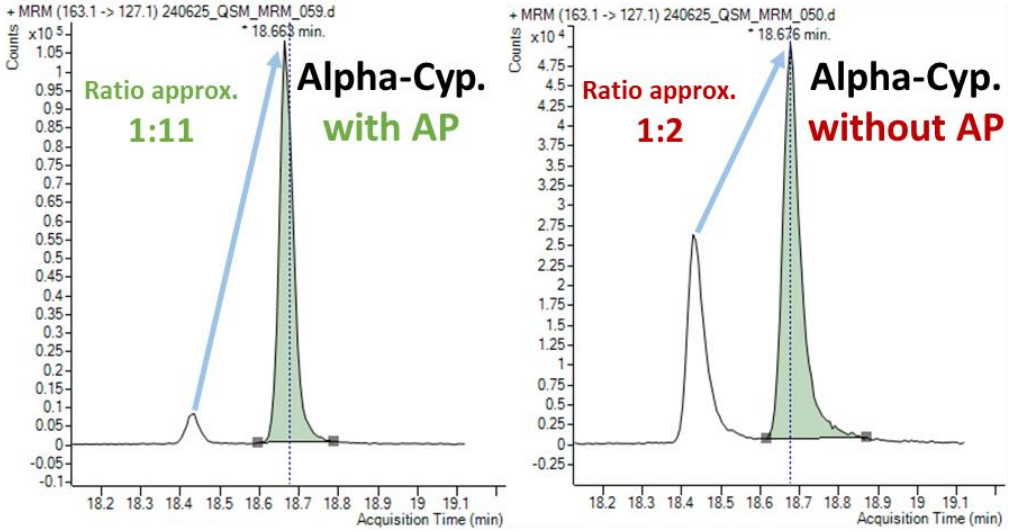


Figure 3: Extent of isomerization of alpha-cypermethrin depending on whether analyte protectants (APs) were added to the final extracts prior to GC-analysis. QuEChERS extract of banana following dSPE cleanup with PSA (and re-acidification using formic acid), with (left) and without (right) addition of APs[6].

Among the cypermethrin-based active substances, currently only cypermethrin is approved within the EU. The approval for alpha-cypermethrin terminated in June 2021, and that of zeta-cypermethrin in December 2020. For the latter, there are import tolerances according to EFSA[7]. Beta-cypermethrin was never approved within the EU, while theta-cypermethrin is not even listed within the EU pesticide database. The residue definition of cypermethrin currently entails all eight constituent isomers of cypermethrin ("*Cypermethrin including other mixtures of constituent isomers (sum of isomers)*"). This means that, as far as residue are concerned, there is no legal distinction between the various isomer mixtures circulating worldwide. As alpha-cypermethrin is known to be more toxic than other cypermethrin mixtures, EFSA screened the proposed MRLs for cypermerthin via PRIMo 3.1 using the ARfD for alpha-

[6] AP used as described in https://www.eurl-pesticides.eu/library/docs/srm/EURL_Observation-APs.pdf
[7] Review of the existing maximum residue levels for cypermethrins according to Article 12 of Regulation (EC) No 396/2005 (wiley.com)

cypermethrin to identify cases posing risk for consumers. In a worst-case scenario (assuming that the residue exhibits the toxicity of the most toxic cypermethrin mixture) several cypermethrin uses were found to be toxicologically critical. This raised the need for checking whether a more differentiated risk-assessment and management is feasible. The establishment of separate MRLs for alpha-cypermethrin in parallel to those of cypermethrin is a possible approach currently discussed. This scenario, however, requires the availability of suitable analytical methods allowing to accurately quantify alpha-cypermethrin as such in presence or absence of other enantiomeric pairs. Sufficient chromatographic separation of the alpha-cypermethrin (*cis*-II) peak and robustness as regards the impact of isomerization on quantification accuracy are important preconditions. Using GC, this undertaking is quite challenging unless isomerization is largely suppressed to become insignificant.

However, pyrethroids are also amenable to LC-(ESI-)-MS/MS, where no isomerization is promoted during injection. LC-MS/MS would circumvent the problems encountered in GC-applications, provided that sufficient peak separation and sensitivity is ensured. With this in mind, a sufficiently sensitive and selective LC-ESI-MS/MS method for the analysis of alpha-cypermethrin (the sum of its co-eluting constituent isomers, to be more precise) was developed by the EURL-SRM. The separation gradient employed was inspired by Hu *et al.* [8].

2. Analyte properties

Information about the typical compositions of cypermethrin related active substances are given in Table 1. Physicochemical properties and some additional information on cypermethrin can be found in Table 2.

Table 1: Isomeric composition of the Cypermethrin (Cyp) -related active substances based on different literature.

	EFSA 2023 [3]	BVL 2017 [9]	University of Hertfordshire [10]	JMPR 2008 [11]	
Cyp	*not available*	*cis*-isomers 40-60%	28:28:22:22 *cis*-I:*trans*-I:*cis*-II:*trans*-II	Consists of 8 isomers, four *cis* and four *trans*.	
Alpha-Cyp	racemic mix of "*cis*-II" components	racemic mix of "*cis*-II" comp.	racemic mix of "*cis*-II" comp.	racemic mix of "*cis*-II" comp.	
Beta-Cyp	2:3-mix of "*cis*-II" and "*trans*-II" comp.	2:3-mix of "*cis*-II" and "*trans*-II" comp.	2:3-mix of "*cis*-II" and "*trans*-II" comp.	-	
Zeta-Cyp	~ 1:1-mix. of *cis*:*trans*: 1S *cis* αS (of *cis*-I)+1R *cis* αS (of *cis*-II) **:** 1S *trans* αS (of *trans*-I)+ 1R *trans* αS (of trans-II)	~ 1:1-mix of *cis*:*trans*: 1S *cis* αS (of *cis*-I)+1R *cis* αS (of *cis*-II) **:** 1S *trans* αS (of *trans*-I)+ 1R *trans* αS (of *trans*-II)	~ 1:1-mix of *cis*:*trans*: 1S *cis* αS (of *cis*-I)+1R *cis* αS (of *cis*-II) **:** 1S *trans* αS (of *trans*-I)+ 1R *trans* αS (of *trans*-II)	1:1-mix of *cis*:*trans*:	
				cis-I	3% 1R *cis* αR 22% 1S *cis* αS
				cis-II	22% 1R *cis* αS 3% 1S *cis* αR
				trans-I	3% 1R *trans* αR 22% 1S *trans* αS
				trans -II	22% 1R *trans* αS 3% 1S *trans* αR
Theta-Cyp	*not available*	racemic mixture of "*trans*-II" comp.	racemic mixture of "*trans*-II" comp.	*not available*	

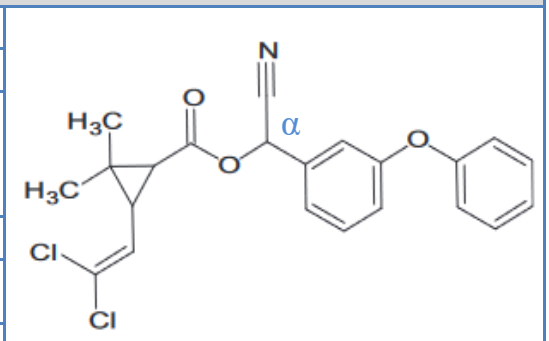
[8] Wei Hu, Wanbin Xie, Shaohua Chen, Ning Zhang, Yiping Zou, Xiaolin Dong, Muhammad Rashid, Ying Xiao, Meiying Hu and Guohua Zhong, Separation of *Cis*- and *Trans*-Cypermethrin by Reversed Phase High Performance Liquid Chromatography, Journal of Chromatographic Science, 2015; 53:612-618; **https://doi.org/10.1093/chromsci/bmu094**

[9] https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/pestizide_mit_Isomerenpeaks.pdf?__blob=publicationFile&v=5

[10] https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm

[11] https://www.fao.org/fileadmin/user_upload/IPM_Pesticide/JMPR/Evaluations/2008/Cypermethrin.pdf

Table 2: Chemical properties of Cypermethrin(s)

Cypermethrin (CAS: 52315-07-8) **/Alpha-Cypermethrin** (CAS: 67375-30-8)		
Parameter	Value/Notes	
Molecular Mass	416.3 g/mol	
Formula	$C_{22}H_{19}Cl_2NO_3$	
IUPAC name	**Cypermethrin:** (RS)-α-cyano-3phenoxybenzyl-(1RS)-*cis*, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate **Alpha-cypermethrin:** [(S)-cyano-(3-phenoxyphenyl)methyl] (1R,3R)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	
pKa	10.65 (very weakly acidic at alpha carbon)	
LogP	Cypermethrin: 5.45 (ECHA, average) 5.44 Chemicalize (computed) Alpha-Cypermethrin: 6.94 (Tomlin), 5.44 Chemicalize (computed)	
Water solubility	Practically insoluble	
Stability	Isomerisations take place in the field (e.g. sunlight induced) and at high temperatures (e.g. during GC); Thermally stable up to 220 °C.	
Residue definition (EU)	Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers)) (Fat soluble)	
Approvals within the EU:	• Cypermethrin: AT,BE,BG,CY,CZ,DE,EE,EL,ES,FI,FR,HR,HU,IE,IT,LT,LU,LV,MT,NL,PL,PT,RO,SE,SI,SK • Alpha-Cypermethrin: NOT APPROVED • Beta-Cypermethrin: NOT APPROVED • Zeta -Cypermethrin: NOT APPROVED (but import tolerances reported[12]) • Theta -Cypermethrin: *not listed as active substance within the EU Pesticide Database*	
Toxicity	• Cypermethrin: Acute Tox. 4; STOT SE 3; Aquatic Acute 1; Aquatic Chronic 1; ARfD: 0.005 mg/kg bw • Alpha-Cypermethrin: Acute Tox. 3; STOT SE 3; STOT RE 2; Aquatic Acute 1; Aquatic Chronic 1; ARfD: 0.00125 mg/kg bw	
Other sources	Cypermethrin is approved for use as a biocide in the EEA and/or Switzerland, for, e.g. wood preservation, controlling insects, ants, etc...[13]	

3. Chemicals and Consumables

The used materials and apparatuses are listed in CEN-QuEChERS (EN-15662) and QuOil (CEN/TS 17062:2019) standard procedures. The suppliers of the used analytical standards are shown in **Table 3**.

Table 3: Sources of Analytical standards (exemplary).

Compound	CAS	Company	Order No.
Cypermethrin	52315-07-8	Dr. Ehrenstorfer GmbH	DRE-C11890000
Alpha-Cypermethrin	67375-30-8	Dr. Ehrenstorfer GmbH	DRE-C11890100
Beta-Cypermethrin	86753-92-6	Chem Service Inc.	Not available
Zeta -Cypermethrin	1315501-18-8	HPC Standards	680421
Theta -Cypermethrin	71697-59-1	Dr. Ehrenstorfer GmbH	DRE-C11890300

Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Stock solutions of the substances (e.g. 1 mg/mL) are prepared in acetonitrile, taking the purity of the standard substances into account. They were stored in a refrigerator for typically up to 48 months. Working solutions, e.g. mixtures, are prepared in acetonitrile and may be stored in the refrigerator for many months.

[12] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2023.7800
[13] https://echa.europa.eu/information-on-chemicals/biocidal-active-substances/-/disas/substance/100.052.567

4. Sample Preparation and Measurement

Samples are homogenized by cryogenic milling using dry ice according to Document Nº SANTE/11312/2021(V2). The sample homogenates are extracted according to the CEN-QuEChERS (citrate-buffered) method (EN-15662) or, in case of high-oil content commodities, according to the QuOil method (CEN/TS 17062:2019) including a dispersive SPE cleanup (25 mg PSA, 25 mg ODS and 150 mg $MgSO_4$ per mL extract). As internal standards, chlorpyrifos-D_{10} and propyzamide-D_3 (e.g. 100 µL of a mixture in acetonitrile at 10 µg/mL each) may be used. The internal standards are added to the sample portion before extraction.

The extract is directly subjected to the LC-MS/MS separation and measurement. Exemplary LC-MS/MS conditions are given in Table 4.

Table 4: Instrumentation and method details (LC: Agilent 1290 Infinity II; MS: Sciex QTrap 5500+)

Instrument parameters	Conditions					
Column/temperature	Waters Cortecs UPLC C_{18}, 2.1x100 mm, 1.6 µm; 25 °C					
Pre-column	Waters Van Guard Cortecs C_{18} 1.6 µm					
Eluent A	1 mmol Ammonium formate in 60% methanol + 15% acetonitrile + 25% water					
Eluent B	1 mmol Ammonium formate in methanol					
Elution	**%A**	**Flow [mL/min]**	**Time [min]**			
	100	0.4	11			
	0	0.4	11.1			
	0	0.4	13:0			
	100	0.4	13.1			
	100	0.4	16.1			
Injection volume	2 µL					
Acquired mass transitions (m/z)	**Compound**	**Mass transitions and their MS-parameters**				
		Q 1 (m/z)	**Q 3 (m/z)**	**DP[1] (V)**	**CE[2] (V)**	**CXP[3] (V)**
	Cypermethrin isomers	433	191	1	21	22
		435	193	6	21	20
		433	127	1	41	16
		433	416	1	13	14
		435	418	6	13	16
	Chlorpyrifos-D_{10} (internal standard)	360	199	95	23	12
	Propyzamid-D_3 (internal standard)	259	193	61	21	10
Ionisation mode	ESI positive					
Ion Source Parameters	**Curtain Gas Flow**	35 psi				
	Ion Spray Voltage	5500 V				
	Temperature	500 °C				
	Nebulizer Gas Flow	70 psi				
	Heater Gas Flow	70 psi				

1) DP: Declustering Potential
2) CE: Collission Energy
3) CXP: Cell Exit Potential

5. Validation data:

Validation experiments for several dithiocarbamate active substances were conducted using matrices representing high water-content, high acid-content and dry plant commodities[14] as well as milk. The analytes were spiked in quintuplicate to the respective weighed portions of the sample homogenates. The conducted validations at 0.005 mg/kg were successful for at least four mass traces (except for milk with three successful mass traces) of all matrix-active substance-combinations according the criteria stated in Document Nº SANTE/11312/2021 V2, see Table 5 and

Table 6.

[14] According of the grouping of commodities in Document Nº SANTE/11312/2021 V2;

Table 5: *Recoveries (Rec.), relative standard deviations (RSD) for the validation of cypermethrin in cucumber, kiwi fruit and whole-fat cow's milk (each 10 g of sample weight), wheat flour and bovine liver (both with 5 g of sample weight) as well as almond butter (2 g of sample weight), each n = 5.*

Matrix	Extraction Method	Spiking level (mg/kg)	Mass Transition (m/z)	Calculation using matrix-matched calibration	
				Mean Rec. (%)	RSD (±%)
Cucumber	QuEChERS	0.02	433/191	93	5.0
			435/193	97	5.4
			433/127	100	3.2
			433/416	95	7.7
			435/418	87	4.2
Kiwi fruit		0.02	433/191	88	8.3
			435/193	96	7.9
			433/127	87	7.3
			433/416	104	6.9
			435/418	96	3.8
Wheat flour		0.04	433/191	95	6.5
			435/193	94	6.6
			433/127	99	6.9
			433/416	n.d.	n.d.
			435/418	101	13.9
Almond butter	QuOil	0.1	433/191	86	4.7
			435/193	85	10.0
			433/127	77	15.5
			433/416	89	10.4
			435/418	83	16.2
Whole-fat Cow's milk	QuEChERS	0.020	433/191	95	8.8
			435/193	99	10.8
			433/127	*Interfered by matrix*	
			433/416	5.2	9.7
			435/418	*Interfered by matrix*	
Bovine liver		0.040	433/191	5.2	6.5
			435/193	113	9.6
			433/127	108	8.4
			433/416	109	8.7
			435/418	119	13.3

Table 6: *Recoveries (Rec.), relative standard deviations (RSD) for the validation of alpha-cypermethrin* (Alpha-CP) in in cucumber, kiwi fruit and whole-fat cow's milk (each 10 g of sample weight), wheat flour and bovine liver (both with 5 g of sample weight) as well as almond butter (2 g of sample weight), each n = 5.*

Matrix	Extraction Method	Spiking level* (mg/kg)	Mass transition	Calculation using matrix-matched Alpha-CP calibration		Calculation using the Alpha-CP peak of a matrix-matched Cy-permethrin calibration	
				Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)
Cucumber	QuEChERS	0.0044	433/191	95	4.5	90	7.4
			435/193	98	7.3	100	10.8
			433/127	86	10.1	101	14.7
			433/416	84	5.7	80	7.2
			435/418	80	15.5	77	25.3
Kiwi fruit		0.0044	433/191	86	9.9	86	15.6
			435/193	86	14.2	94	17.8
			433/127	86	11.9	93	14.1
			433/416	99	11.8	108	14.6
			435/418	94	8.3	114	13.7
Wheat flour		0.0088	433/191	97	3.9	93	4.7
			435/193	100	6.0	99	6.0
			433/127	91	14.4	104	15.5
			433/416	95	11.8	101	11.2
			435/418	83	8.3	90	13.1
Almond butter	QuOil	0.022	433/191	83	4.8	84	5.3
			435/193	84	17.9	74	21.7
			433/127	n.d.	n.d.	n.d.	n.d.
			433/416	105	11.2	88	15.5
			435/418	85	15.2	80	17.8
Whole-fat Cow's milk	QuEChERS	0.0044	433/191	97	9.0	104	10.3
			435/193	92	10.5	102	10.9
			433/127	*Interfered by matrix*			
			433/416	102	8.7	105	9.3
			435/418	*Interfered by matrix*			
Bovine liver		0.0088	433/191	93	5.2	104	5.8
			435/193	100	9.3	120	11.5
			433/127	101	11.7	112	8.4
			433/416	117	12.4	113	10.5
			435/418	89	7.3	119	5.5

* As part of spiked cypermethrin assuming 22% alpha-cypermethrin (*cis*-II) content.

6. Miscellaneous hints and aspects

a. Chromatographic separation on LC-MS/MS

The elution profiles of various cypermethrin-based isomer mixtures using the above LC-MS/MS conditions are shown below. Despite their deviating composition, cypermethrin and zeta-cypermethrin are barely distinguishable.

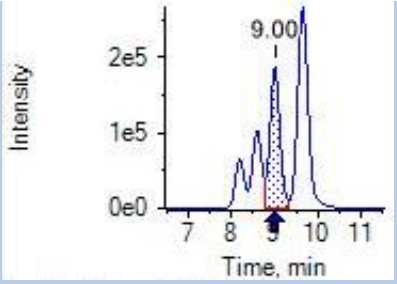
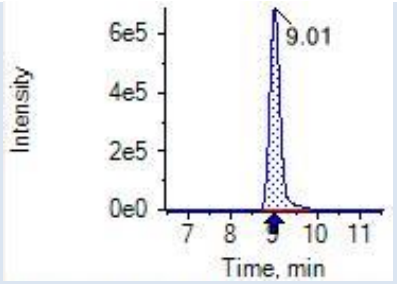
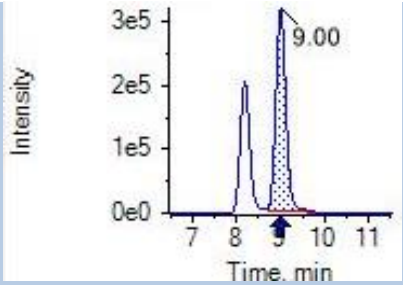
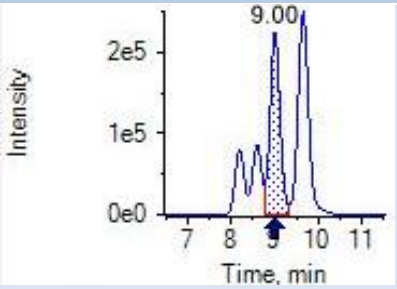
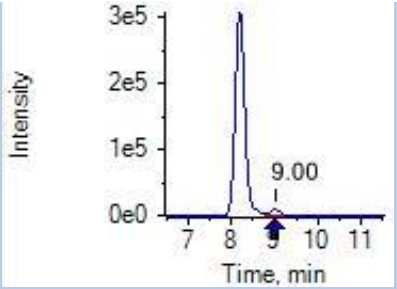
Substance (@1 µg/mL each)	Mass transition m/z 433/191
Cypermethrin	
Alpha-Cypermethrin	
Beta-Cypermethrin	
Zeta-Cypermethrin	
Theta -Cypermethrin	

Figure 4: *Chromatographic separation and behavior of Cypermethrin and its related active substances; the cis-II peak is hatched in each case.*

b. Chromatographic elution order of the four enantiomeric pairs using GC/MS and LC/MS:

The elution order of the four enantiomeric pairs of cypermethrin differs between GC- and LC-, see chromatograms in Figure 5. Interestingly, the *cis*-II pair of cypermethrin elutes third in order in both techniques. The availability of "*cis*-II" (alpha-cypermethrin pair) and "*trans*-II" (theta-cypermethrin pair) allows easy peak-allocation for these two enantiomeric pairs, see also Figure 4 and Table 1. The only standards available containing "*cis*-I" and "*trans*-I" components are cypermethrin and zeta-cypermethrin. Both mixtures, however, contain "*cis*-I" and "*trans*-I" in a 1:1 ratio, not allowing to identify which is which, based on their relative peak-intensity. The observed isomerization of alpha-cypermethrin in solution (assumedly from "*cis*-I" to "*cis*-II") points to the direction, that the fourth peak of the LC-MS/MS separation could belong to the "*cis*-I" pair and the remaining first peak consequently to the "*trans*-I" pair. If this assumption is correct, this would mean that the *cis*-isomers have stronger detector responses compared to the *trans* isomers under the measurement conditions used.

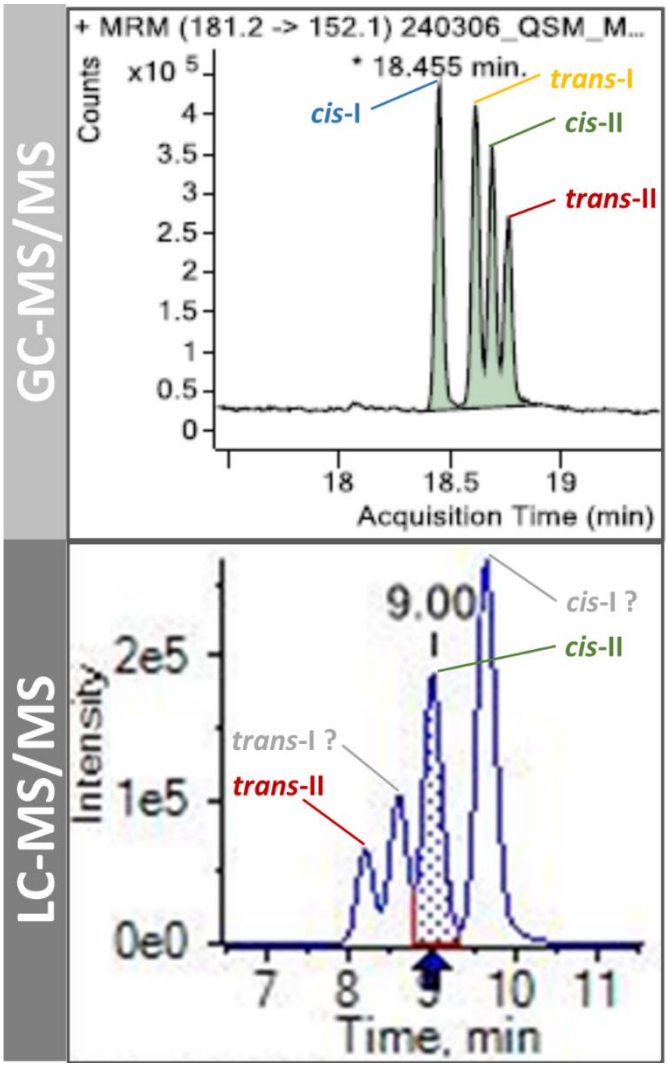


Figure 5: *Elution order of the four enantiomeric pairs (cis-I, cis-II, trans-I and trans-II) contained in cypermethrin using GC- (top) and LC-MS/MS (bottom).*

7. Conclusions

The developed LC-MS/MS method achieves a good separation of the four cypermethrin peaks and sufficient sensitivities for controlling the existing MRLs for all the Cypermethrin-related active substances. With the available standards, two of the four peaks could be unequivocally allocated to specific enantiomeric pairs of cypermethrin. The first eluting peak was linked to the *trans*-II pair of enantiomers and the third eluting peak to the *cis*-II pair. In a racemic ratio, the *cis*-II pair would correspond to alpha-cypermethrin. The second and third peak still need to be allocated. Unfortunately, not all enantiomeric pairs could be separated using a standard gradient.

It should be highlighted, however, that with the developed (non-enantioselective) separation method the two components of alpha-cypermethrin (forming the *cis*-II peak) do not separate and are thus quantified as a sum (i.e. the sum of constituent isomers of alpha-cypermethrin at any ratio). It is also assumed, that the two enantiomers exhibit the same detection response (which is typically the case). This lack of enantiomeric selectivity, should be considered in risk assessment as the isomeric ratio of the *cis*-II-components may differ depending on the active substance. While cypermethrin and alpha-cypermethrin entail the *cis*-II components (1*R cis* α-*S* and 1*S cis* α-*R*) in a racemic (50:50) ratio, zeta-cypermethrin contains them in a highly deviating ratio (22:3 = 88:12). Despite their different isomeric composition (see Table 1), cypermethrin and zeta-cypermethrin cannot be differentiated by conventional chromatography. With the 1*R cis* α-*S* being more toxic, a precautionary worst-case calculations would be indicated when it comes to risk assessment of results generated by the present method.

Isomerizations in the field, e.g. by sunlight, can also shift the isomeric ratio, but such isomerizations mainly lead to a reduction of the most toxic isomers in the case of alpha-cypermethrin (i.e. from α-*S to* α-*R* and from *cis* to *trans)*. An enantioselective (chiral) method, sufficiently separating the two *cis*-II components, both from each other and from the remaining six cypermethrin isomers would be needed for fully differentiated quantification (and risk assessment). At this stage, such an enatioselective analysis is not considered necessary for the purpose of monitoring and MRL compliance control.

It furthermore remains to be checked, whether the developed method (or a future modification of it) can be used for the analysis of a larger mixture of pesticides of a broad polarity range, as this would make the method suitable for routine MRM analysis. Another aspect that still needs further elucidation is the degree of isomerization taking place in analytical standard solutions (stock or working solutions) depending on the solvent.

8. Document History

Action	When	Changes / Actions	Document Version
Drafting the document	Q4 2024		V1
Elaboration of method	Q3 2024		

9. ANNEX

GC-MS/MS instrument parameters (Agilent 7010 GC-MS/MS with Gerstel PTV and MPS)

Ionisation mode	EI			
Column	Agilent J&W GC Column; DB-5MS + 10m Duragard (30 m; 0.25 mm ID; 0.25 µm)			
Pre-column	Integrated pre column; uncoated; 10 m; 0.25 mm ID			
Injection temperature (°C)	40			
Run time (min)	32			
Oven program		**Step**	**Temp [°C]**	**Hold Time [min]**
	Initial temperature		40	2
	Ramp 1	30°C/min	220	0
	Ramp 2	5°C/min	260	0
	Ramp 3	20°/min	280	15
Injection volume (µL)	1µl			
Injection mode	PTV solvent vent			
Dilution	no			
Carrier gas	Helium, 1.5 mL/min, constant flow			
Transferline temperature (°C)	260			
Detector	EI-MS/MS, 70eV			
Source temperature (°C)	280			