EPRW 2022 BOLOGNA

Mass spectrometry behaviour of different constituents of specific LC and GC amenable pesticides

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Co-funded by the European Union

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European Union Reference Laboratory for Pesticide Residues
Fruits and Vegetables

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Comparison of the instrumental response of different constituents of specific pesticides

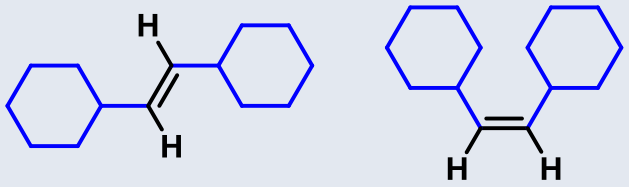
ISOMERISM

~~What are isomers?~~

Why should I care about what isomers are?

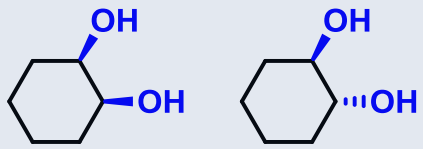
Stereoisomerism

- *E-Z* isomerism



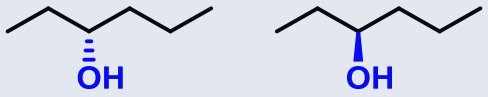
(*E*)-isomer (*Z*)-isomer

- *cis-trans* isomerism



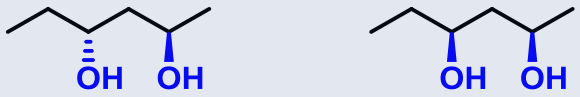
cis-isomer *trans*-isomer

- Enantiomerism



(*R*)-enantiomer (*S*)-enantiomer

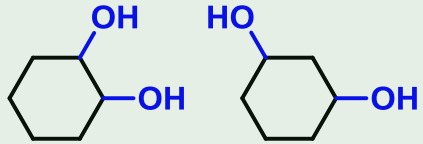
- Diastereomerism



(2*R*,4*R*)-diastereomer (2*R*,4*S*)-diastereomer

Structural isomerism

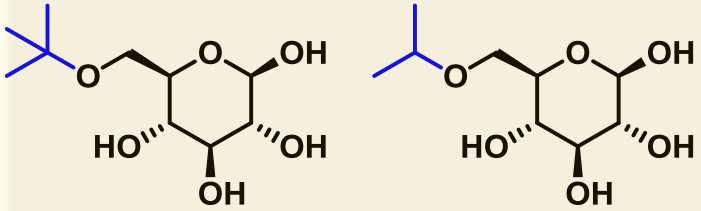
- Positional isomerism



1,2-isomer 1,3-isomer

Analogous molecules (not isomers)

- Different substituents



tert-Butyl substituent Isopropyl substituent

PRICE AND CAS NUMBERS

Compound name	Components				
Chlordane	**Mixture**	***cis*-Chlordane**		***trans*-Chlordane**	
	0.12 €/mg	**7.56** €/mg		**7.56** €/mg	
	57-74-9	5103-71-9		5103-74-2	
Cyfluthrin	**Mixture**	**Beta (β)**			
	0.18 €/mg	**0.24** €/mg			
	68359-37-5	1820573-27-0			
Cypermethrin	**Mixture**	***Alpha* (α)**	***Beta* (β)**	***Zeta* (ζ)**	***Theta* (θ)**
	0.48 €/mg	**0.64** €/mg	**0.65** €/mg	**0.83** €/mg	**17.46** €/mg
	52315-07-8	67375-30-8	1224510-29-5	1315501-18-8	71697-59-1
Fenpyroximate	**Mixture**	**(*E*)-Fenpyroximate**		**(*Z*)-Fenpyroximate**	
	1.16 €/mg	**0.95** €/mg		**16.38** €/mg	
	111812-58-9	134098-61-6		149054-57-9	
Metaflumizone	**Mixture**	**(*E*)-Metaflumizone**		**(*Z*)-Metaflumizone**	
	0.67 €/mg	**1.15** €/mg		**17.73** €/mg	
	139968-49-3	852403-68-0		139970-56-2	
Spinetoram	**Mixture**	**Spinetoram J**		**Spinetoram L**	
	2.90 €/mg	**136.90** €/mg		**408.00** €/mg	
	935545-74-7	187166-40-1		187166-15-0	
Spinosad	**Mixture**	**Spinosyn A**		**Spinosyn D**	
	1.66 €/mg	**23.67** €/mg		**175.9** €/mg	
	168316-95-8	131929-60-7		131929-63-0	

Price

Technical mixtures have a lower price.

CAS number

In the past, **wrong CAS numbers** were detected in the main companies selling pesticide standards

- Check the CAS number of the component before purchasing
- Re-check the CAS number in the certificate of analysis after arrival

PRICE AND CAS NUMBERS

Compound name	Components		
Permethrin	**Mixture**	***cis*-Permethrin**	***trans*-Permethrin**
	0.18 €/mg	**12.42** €/mg	**12.42** €/mg
	52645-53-1	61949-76-6	61949-77-7
Pyrifenox	**Mixture**	**(*E*)-Pyrifenox**	**(*Z*)-Pyrifenox**
	0.95 €/mg	**8460** €/mg	Not found
	88283-41-4	83227-22-9	83227-23-0
XMC	**Mixture**	**Pure (3,5-disubstituted)**	**Xylylcarb (3,4-disubstituted)**
	Not found	**0.54** €/mg	**18** €/mg
	Not found	2655-14-3	2425-10-7

Price

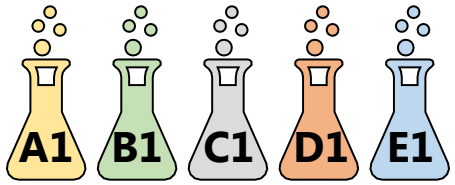
Technical mixtures have a lower price.

CAS number

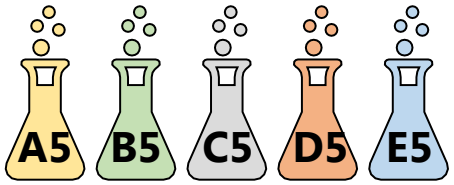
In the past, **wrong CAS numbers** were detected in the main companies selling pesticide standards

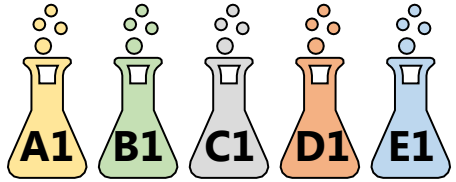
- Check the CAS number of the component before purchasing
- Re-check the CAS number in the certificate of analysis after arrival

METHODOLOGY

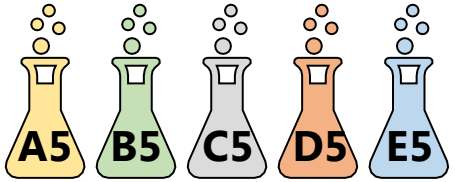


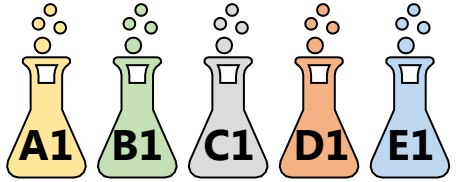
Five non-consecutive injections



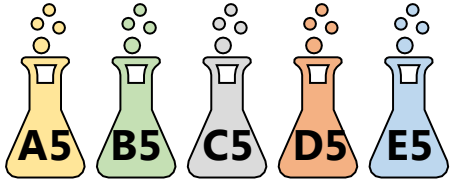


Five non-consecutive injections





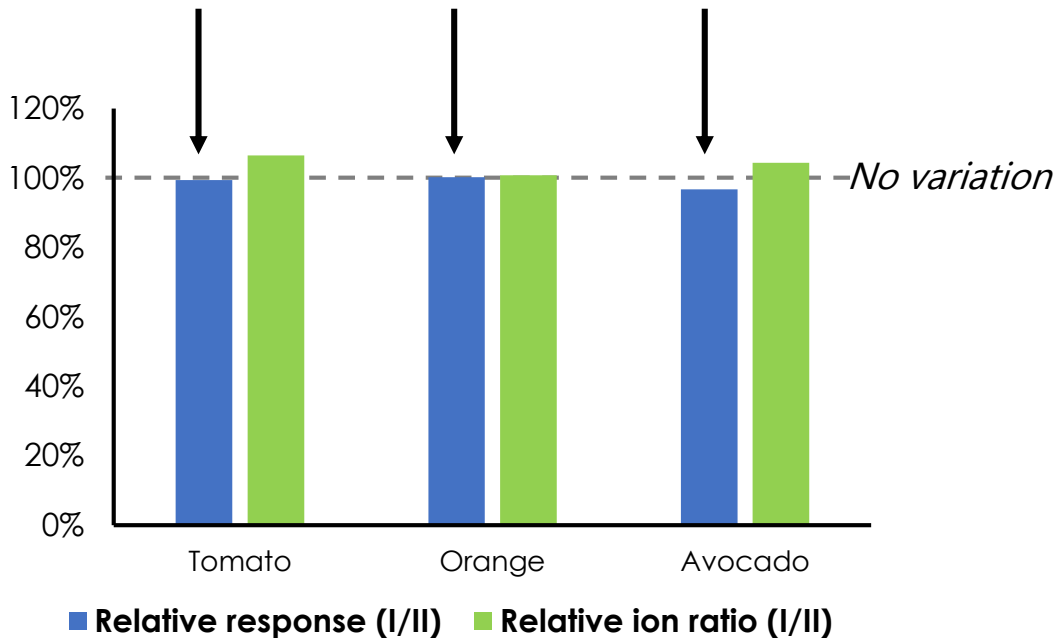
Five non-consecutive injections



METHODOLOGY

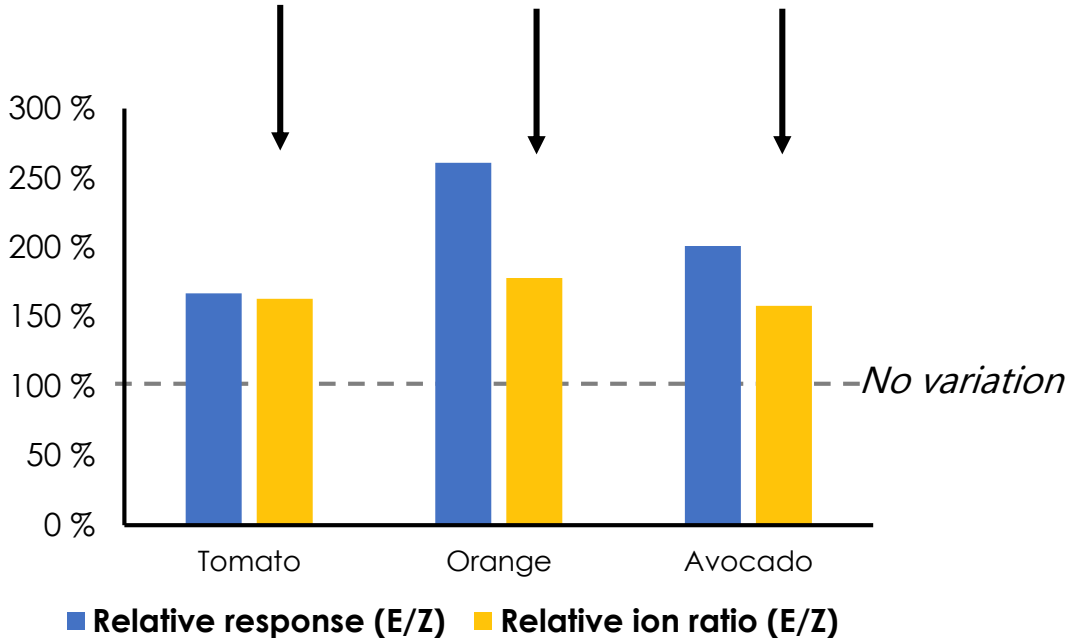
Evaluation of two parameters

Are the quantitation transitions similar?



SITUATION 1: constituents behave similarly

Are the ion ratios similar?



SITUATION 2: constituents do not behave similarly

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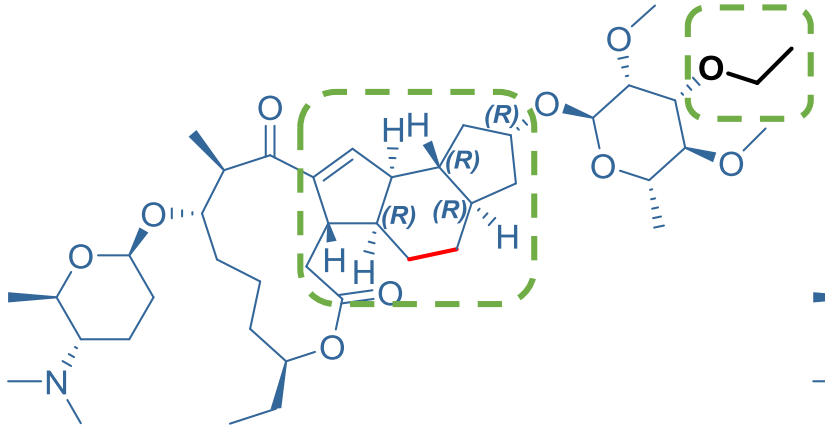
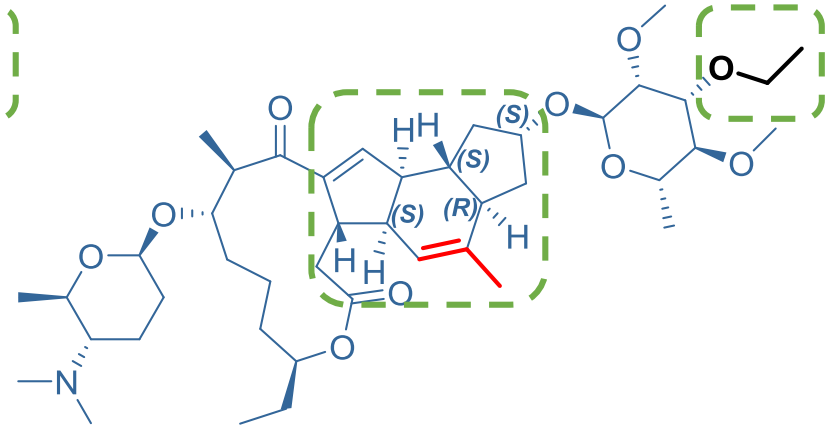
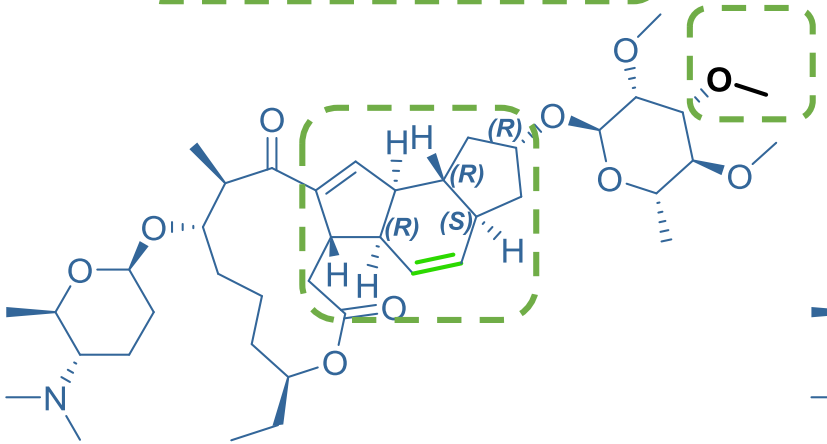


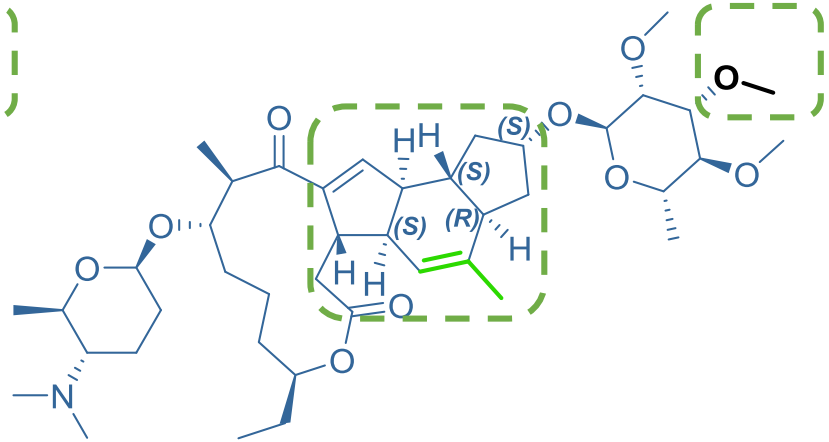
Spinetoram J
Chemical Formula: $C_{42}H_{69}NO_{10}$
Exact Mass: 747.4921 Da



Spinetoram L
Chemical Formula: $C_{43}H_{69}NO_{10}$
Exact Mass: 759.4921 Da



Spinosyn A
Chemical Formula: $C_{41}H_{65}NO_{10}$
Exact Mass: 731.4608 Da



Spinosyn D
Chemical Formula: $C_{42}H_{67}NO_{10}$
Exact Mass: 745.4765 Da

745.4765 (100.0%)
746.4799 (45.4%)
747.4832 (10.1%) ←

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SPINETORAM

LC

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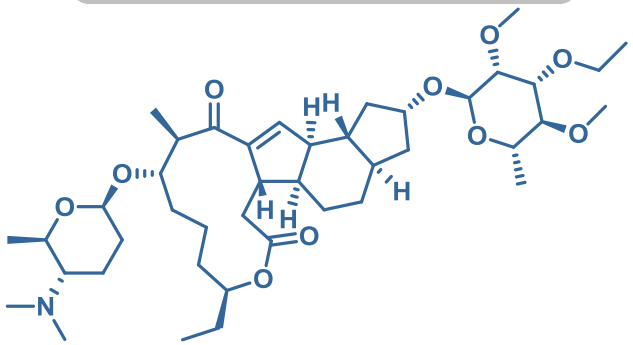
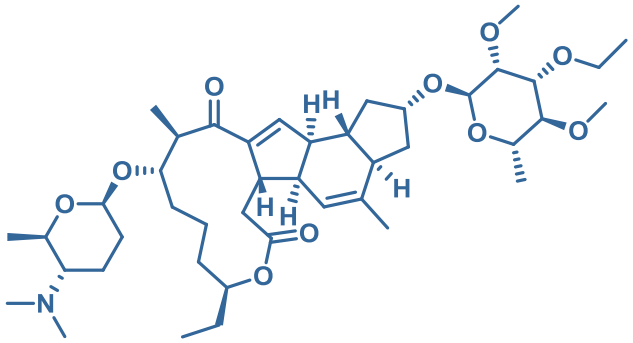
Spinetoram (sum of spinetoram-J and spinetoram-L)

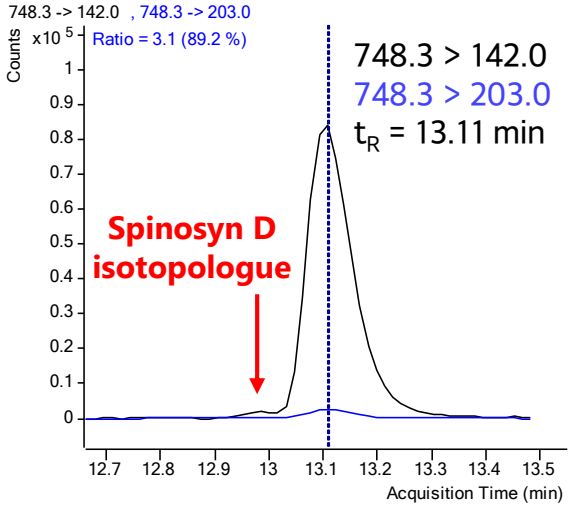


Spinetoram J

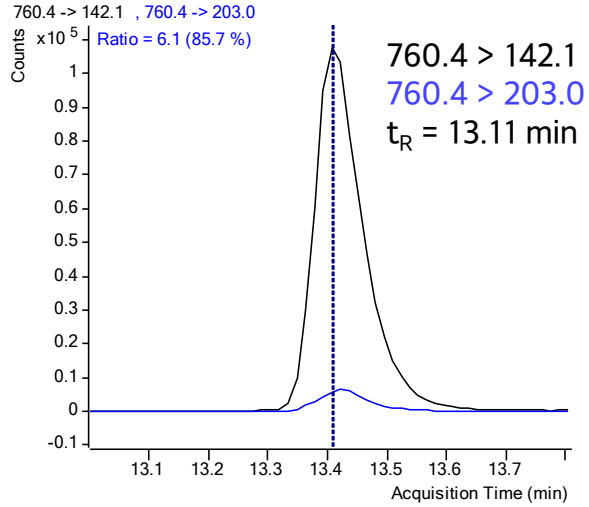


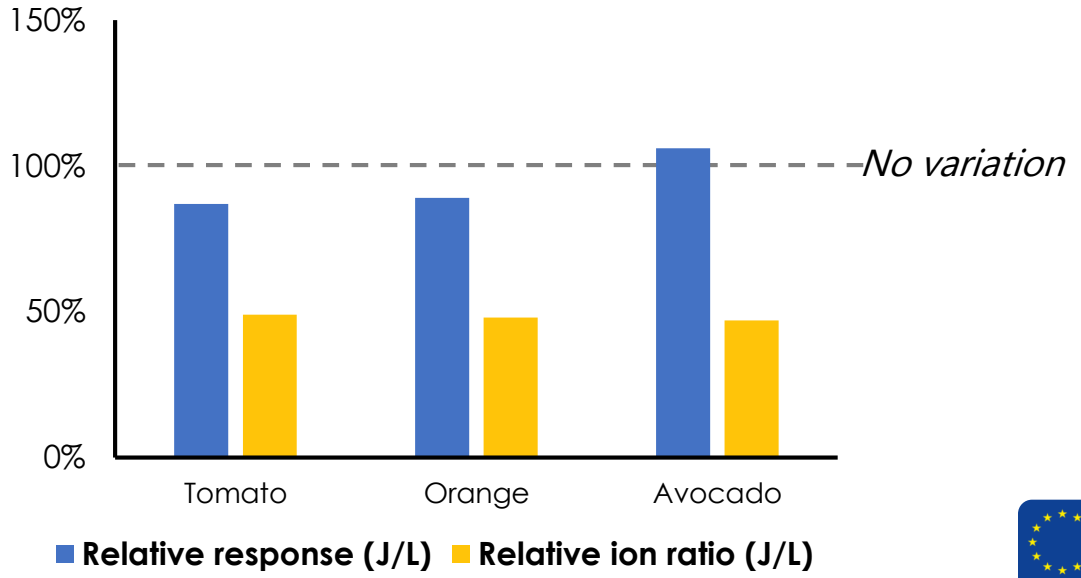
Spinetoram L

Spinetoram J



Spinetoram L





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SPINOSAD

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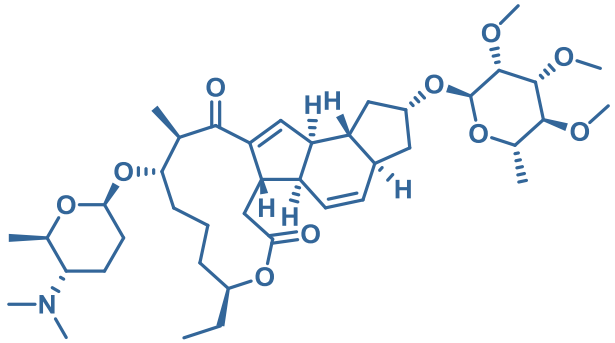
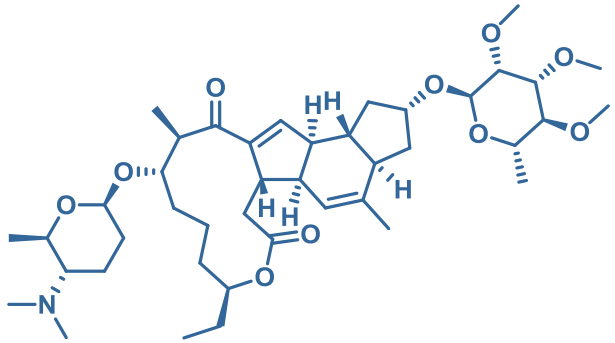
Spinosad (spinosad, sum of spinosyn A and spinosyn D)

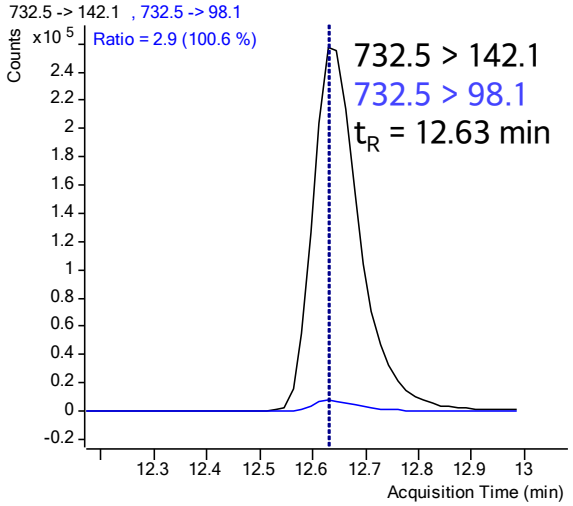


Spinosyn A

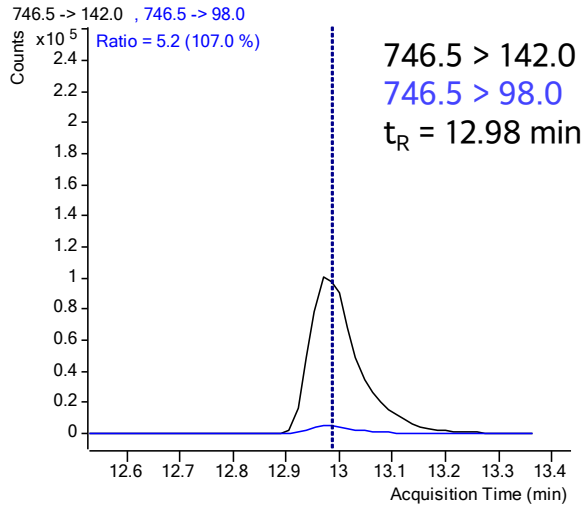


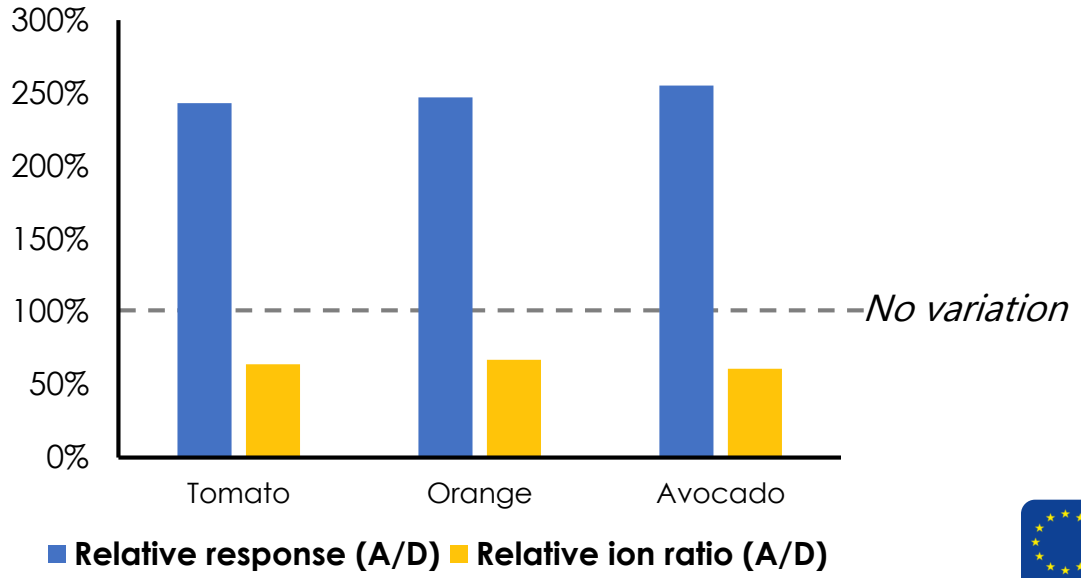
Spinosyn D

Spinosyn A



Spinosyn D





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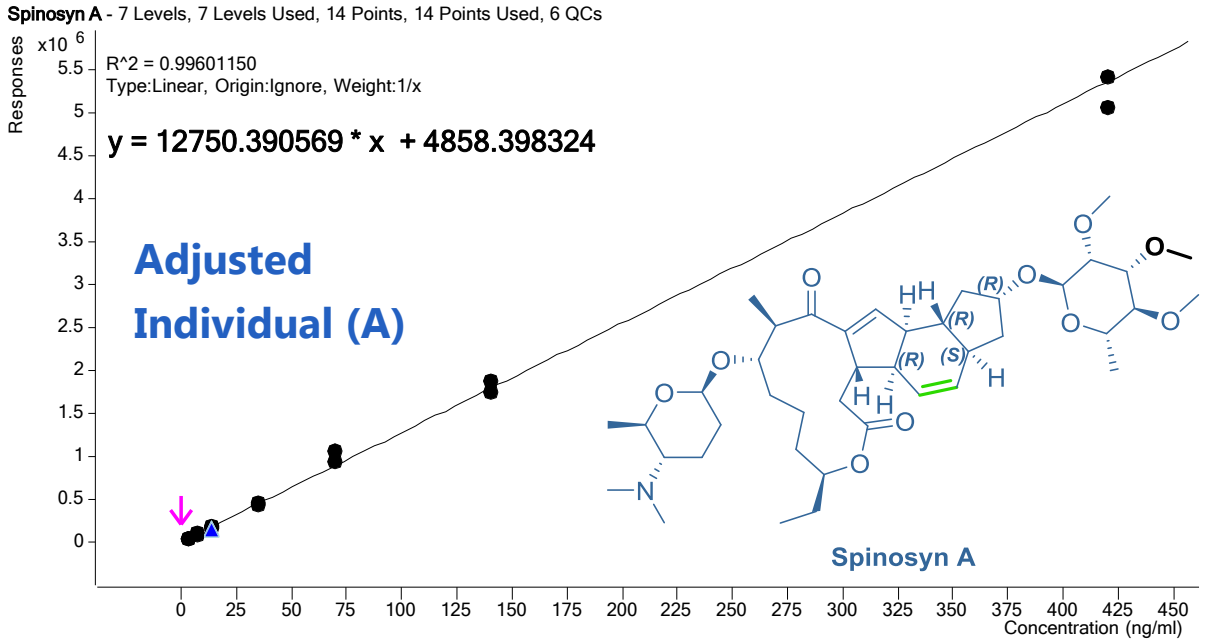
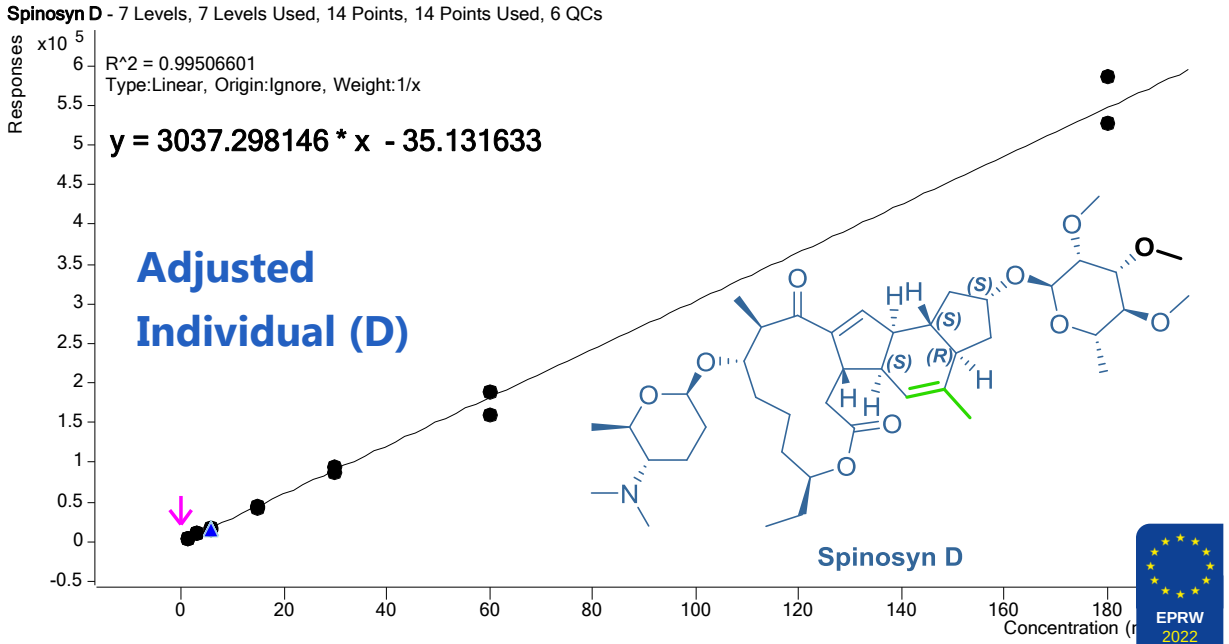
Practical examples: different quantitation approaches

1. Use of the spinosad technical mixture (e.g. 70:30, w/w, A:D)
   A. Appropriately **adjust the concentration** of each calibration point to the actual spinosyn A or D concentration.
   B. In such a 0.100 mg/L spinosad **calibration point** (here in avocado) there are 0.070 mg/L of spinosyn A and 0.030 mg/L of spinosyn D.





SPINOSAD

LC

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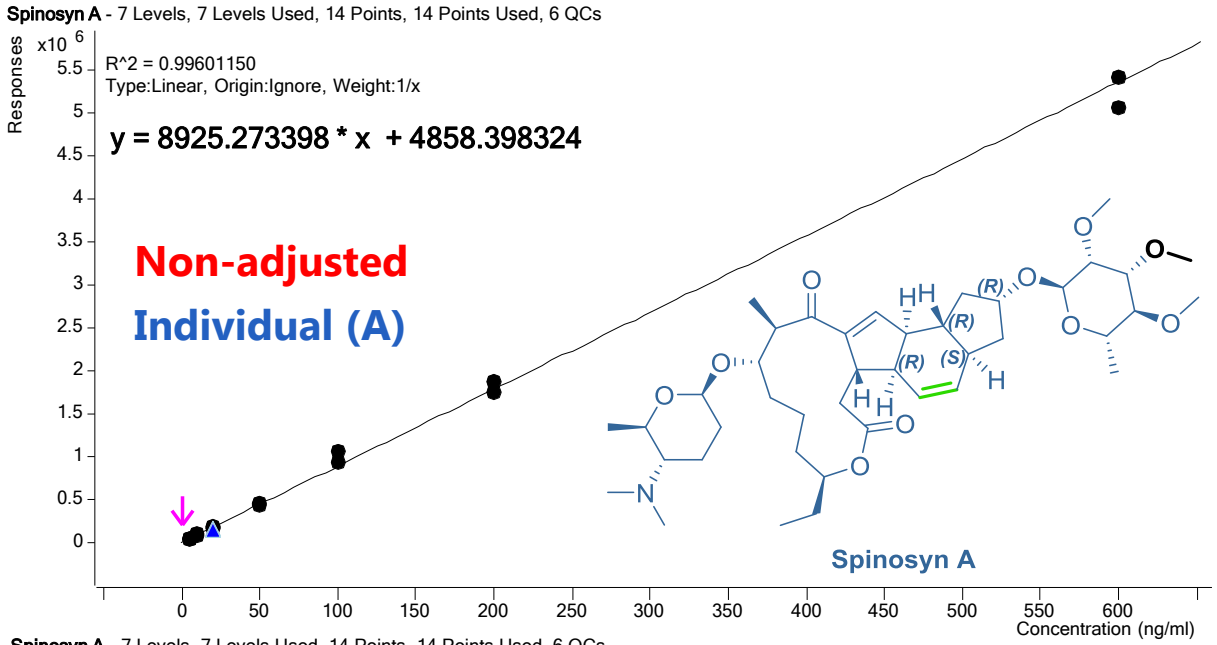

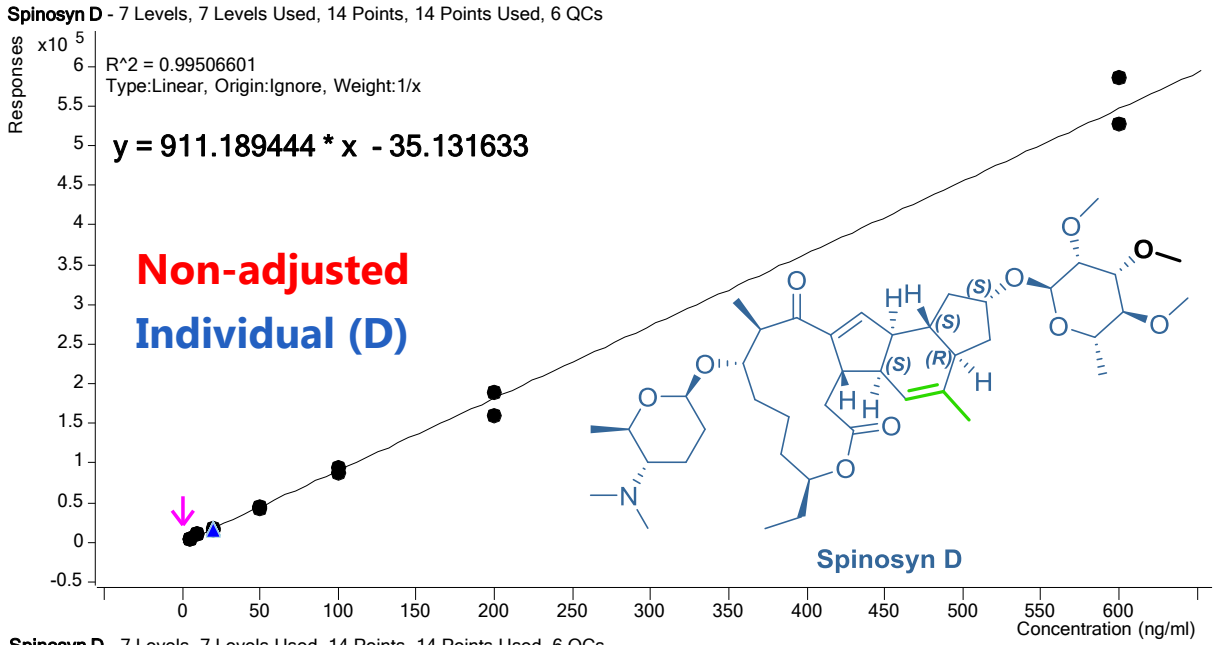

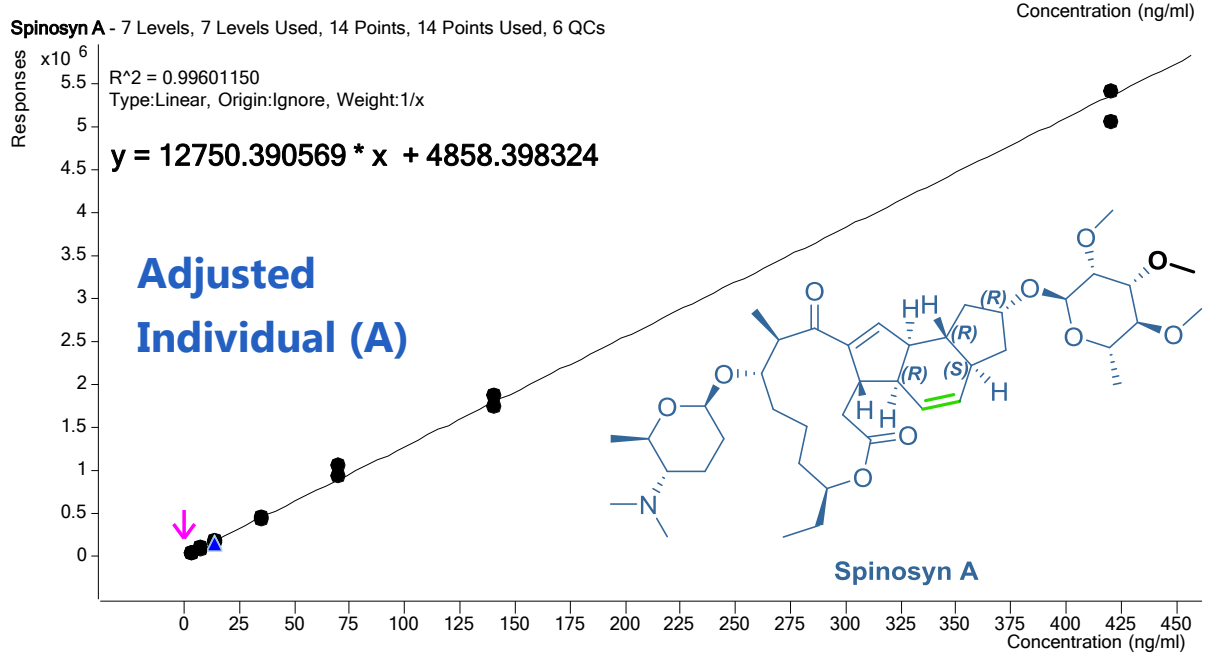

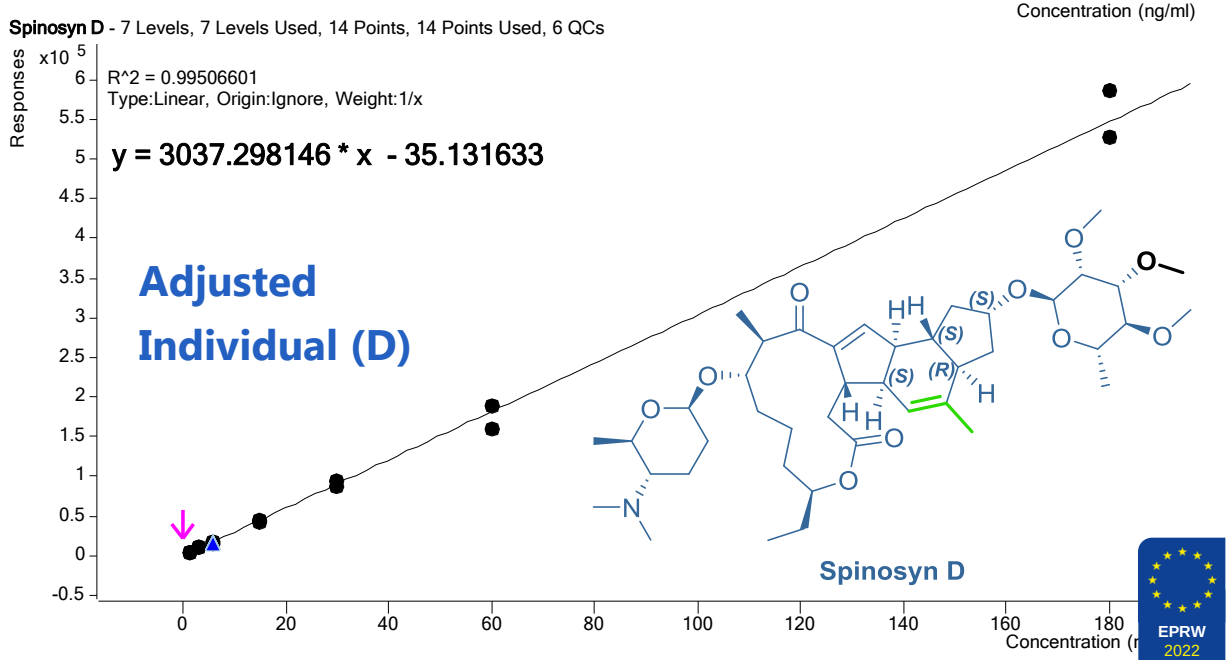

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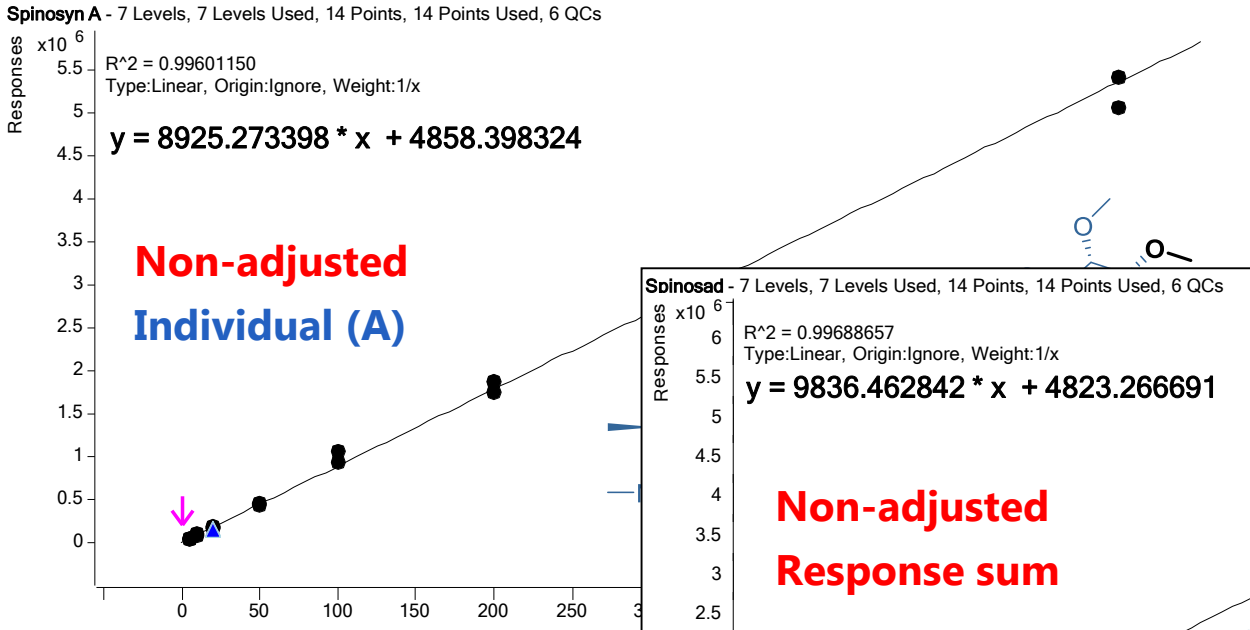

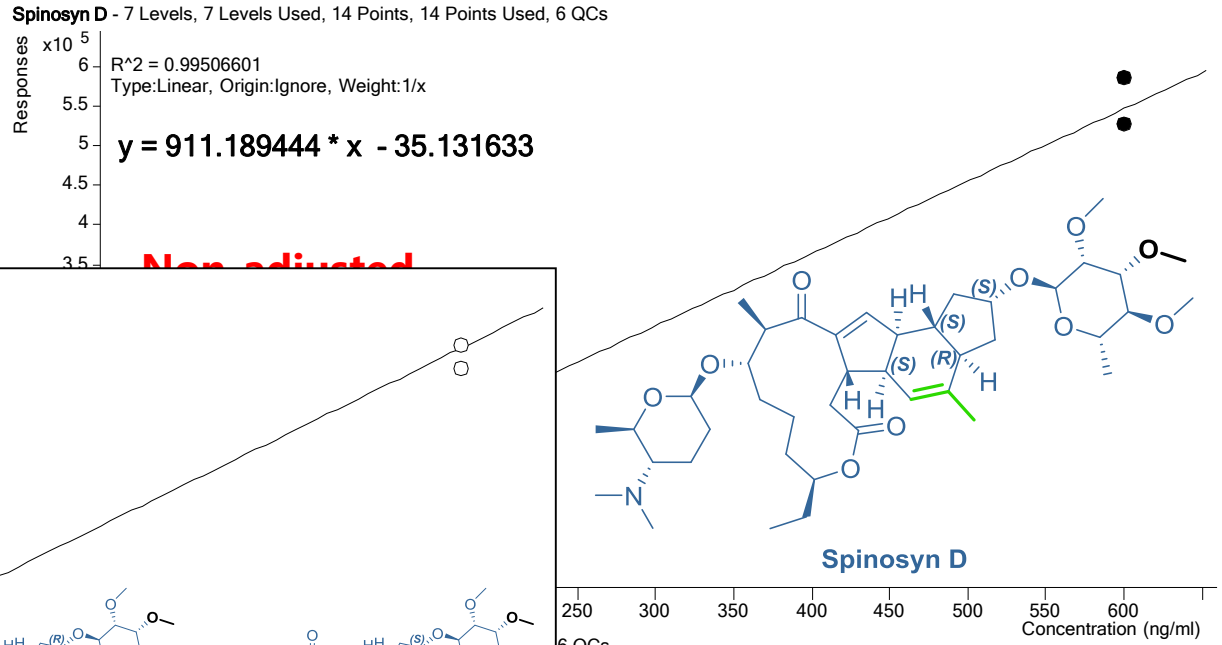

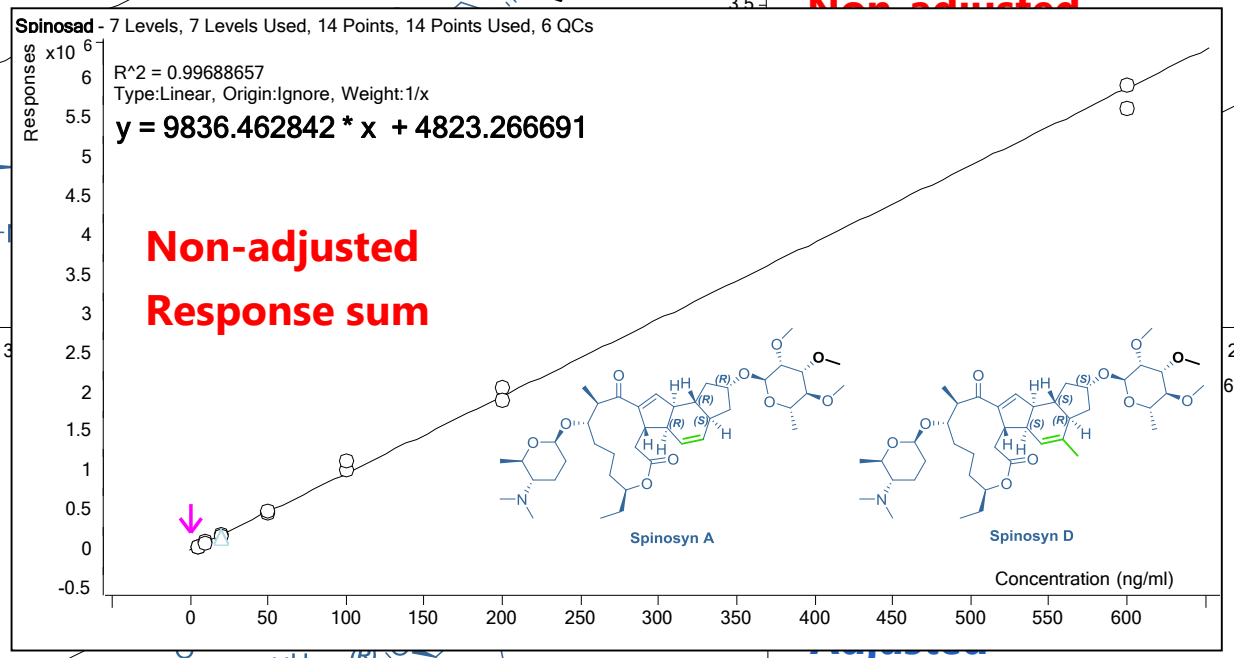

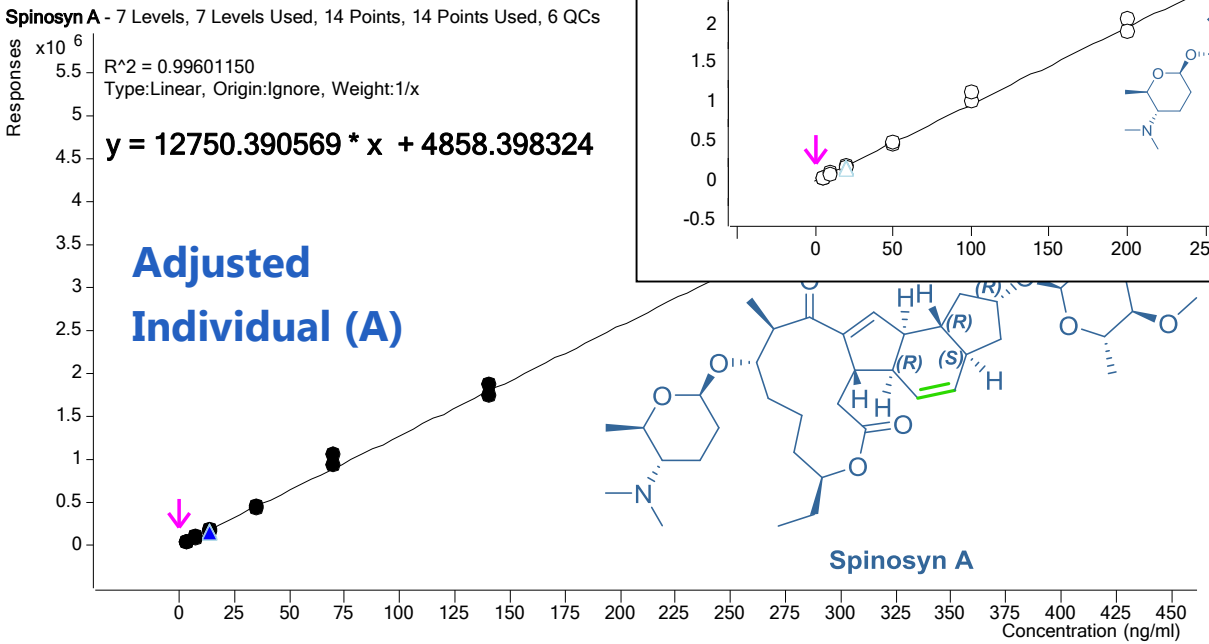

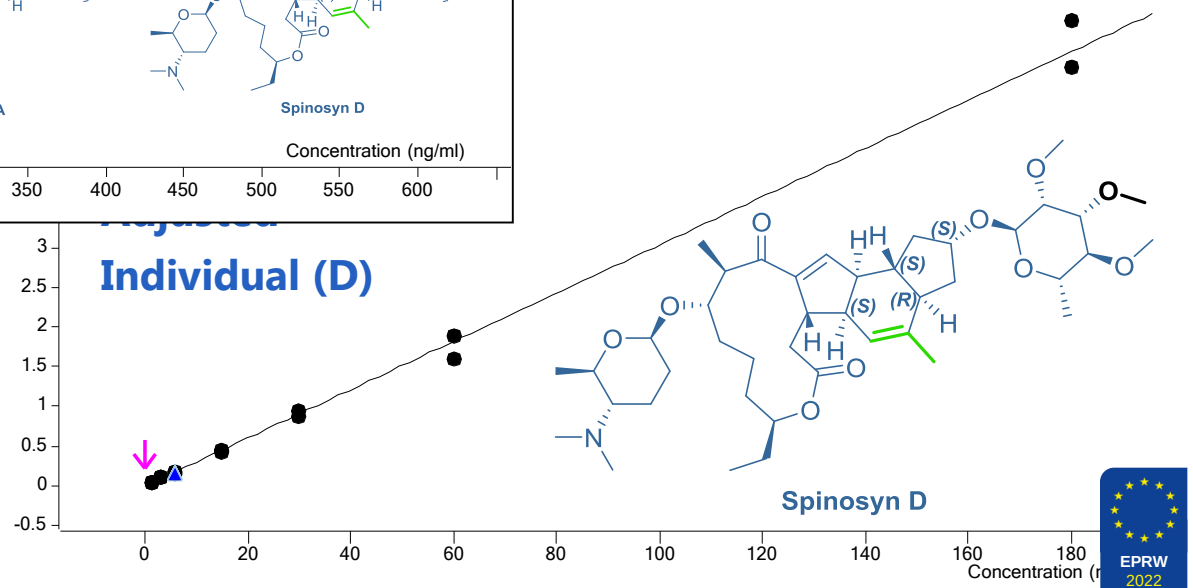

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Practical examples: different quantitation approaches

1. Use of the spinosad technical mixture (e.g. 70:30, w/w, A:D)
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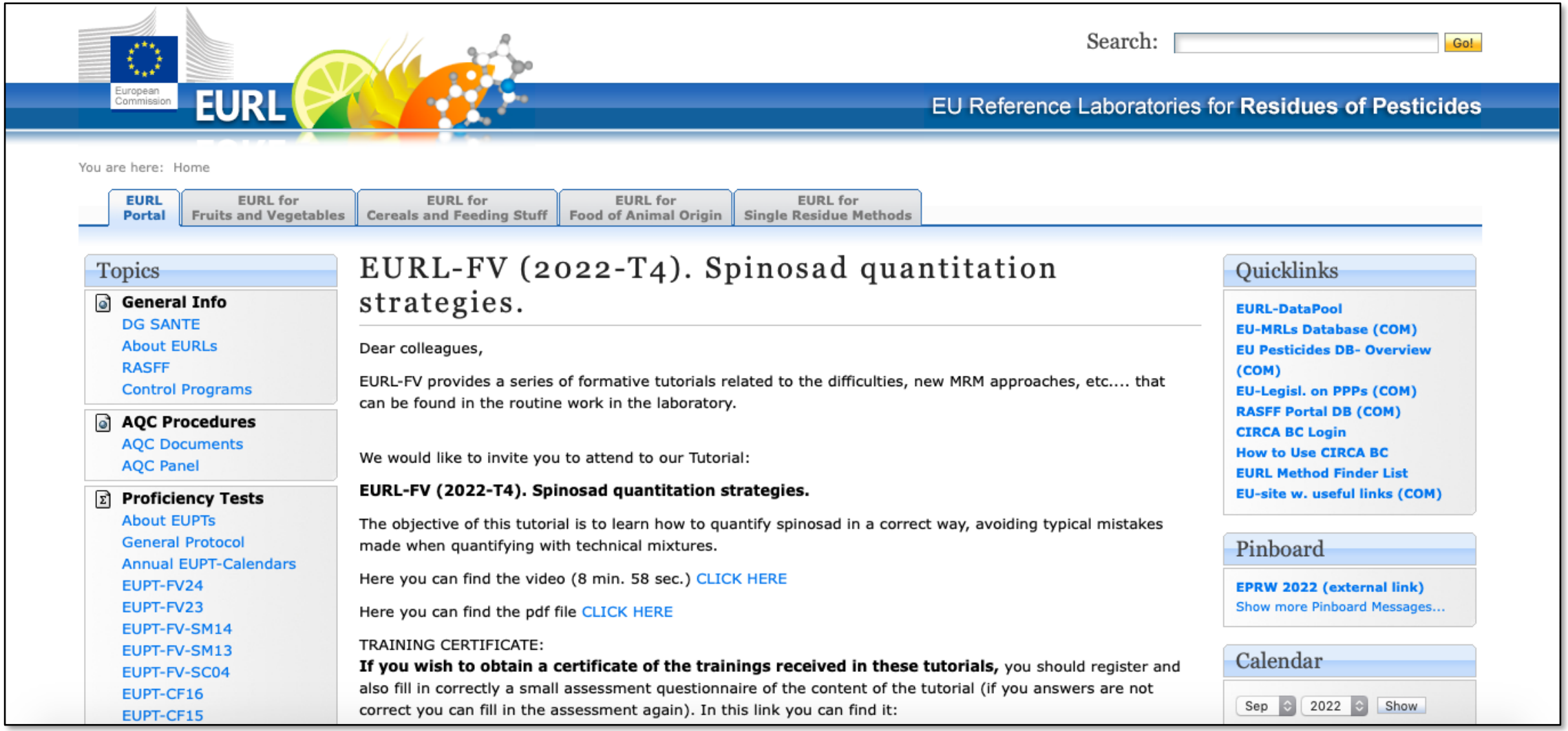
   B. In such a 0.100 mg/L spinosad **calibration point** (here in avocado) there are 0.070 mg/L of spinosyn A and 0.030 mg/L of spinosyn D.

Results for an instrumental response of 5.0E5 counts for each spinosyn A and spinosyn D			
Analyte	**Adjusted, individual calibration curves. Conc. sum of spinosyn A and D**	**Non-adjusted, individual calibration curves. Conc. sum of spinosyn A and D**	**Response sum calibration curve**
Spinosyn A	0.039 mg/kg	0.055 mg/kg	-
Spinosyn D	0.16 mg/kg	0.59 mg/kg	-
Spinosad	**0.20 mg/kg**	**0.65 mg/kg**	**0.10 mg/kg**

+325 %

-50 %



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EURL-FV (2022-T4). Spinosad quantitation strategies.

Dear colleagues,

EURL-FV provides a series of formative tutorials related to the difficulties, new MRM approaches, etc.... that can be found in the routine work in the laboratory.

We would like to invite you to attend to our Tutorial:

EURL-FV (2022-T4). Spinosad quantitation strategies.

The objective of this tutorial is to learn how to quantify spinosad in a correct way, avoiding typical mistakes made when quantifying with technical mixtures.

Here you can find the video (8 min. 58 sec.) CLICK HERE

Here you can find the pdf file CLICK HERE

TRAINING CERTIFICATE:
If you wish to obtain a certificate of the trainings received in these tutorials, you should register and also fill in correctly a small assessment questionnaire of the content of the tutorial (if you answers are not correct you can fill in the assessment again). In this link you can find it:

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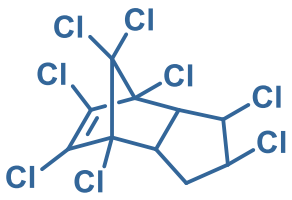
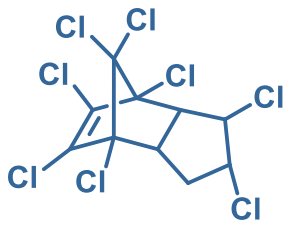
European Commission

Chlordane (sum of *cis*- and *trans*-chlordane)

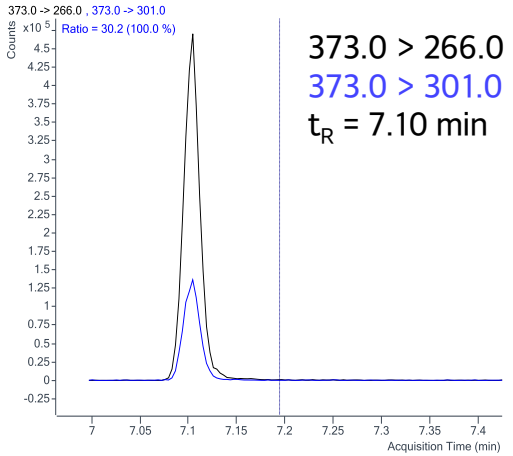


cis-Chlordane



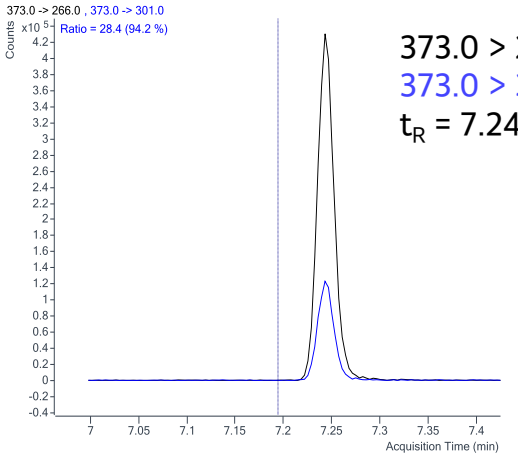
trans-Chlordane

cis-Chlordane

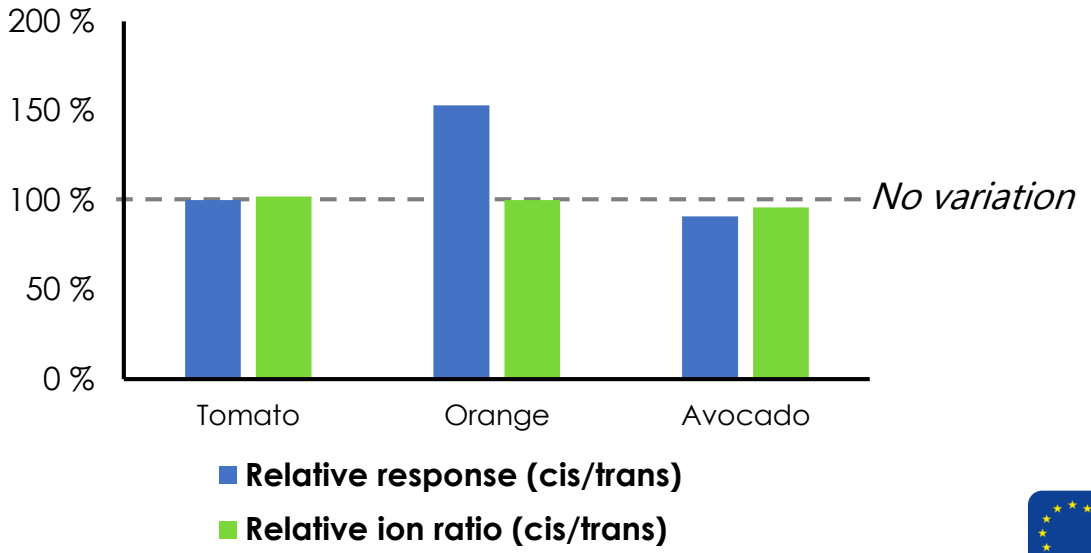


373.0 > 266.0
373.0 > 301.0
t_R = 7.10 min

trans-Chlordane



373.0 > 266.0
373.0 > 301.0
t_R = 7.24 min

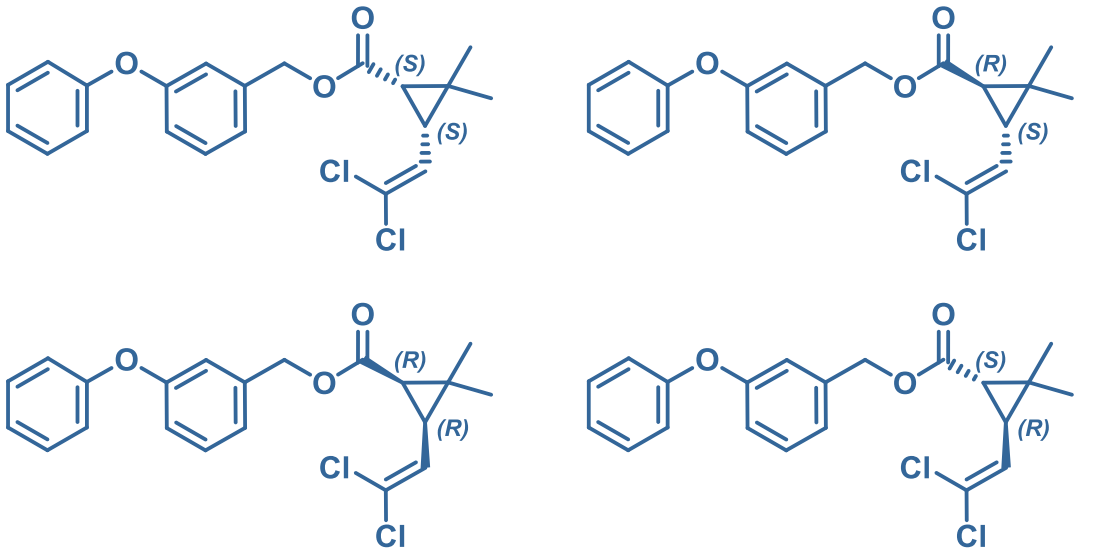


EPRW 2022 BOLOGNA

PERMETHRIN

GC

European Commission | **Permethrin (sum of isomers)**



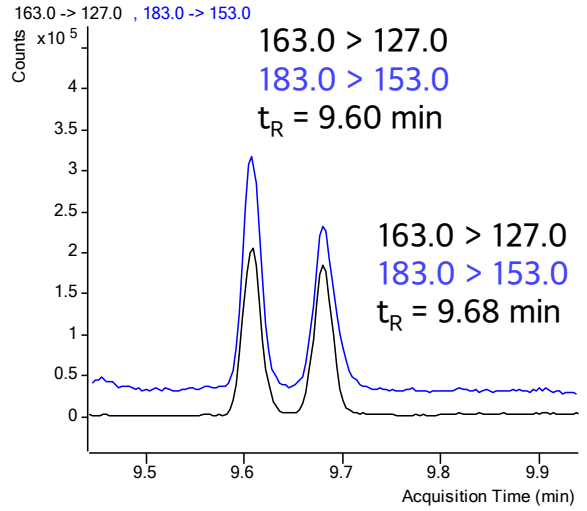
cis-Permethrin enantiomeric pair

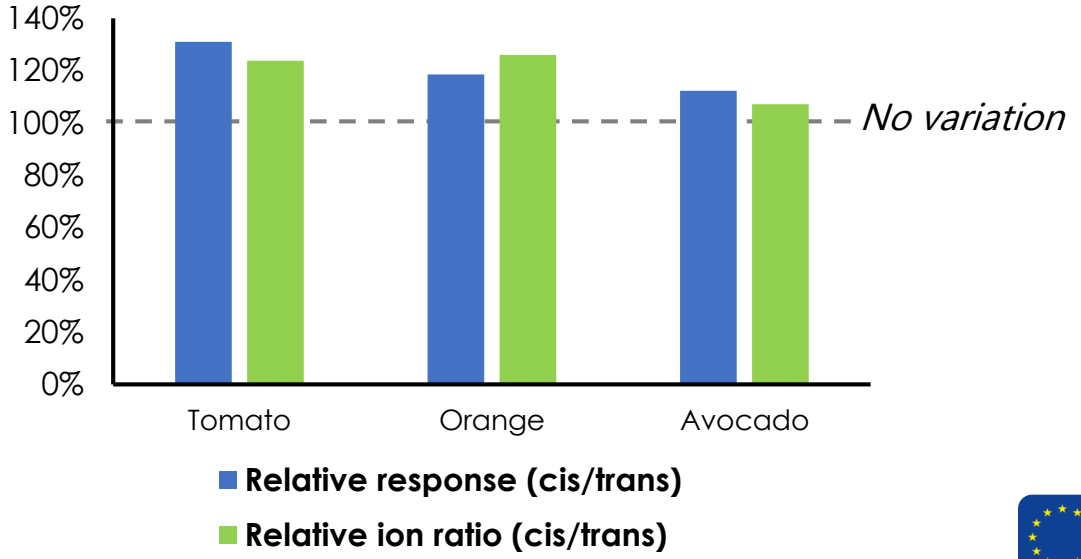
trans-Permethrin enantiomeric pair

- Currently evaluating individual *cis*-*trans* pairs
- Is the behaviour different, or is the 50:50 ratio in the standard not accurate?

cis-Permethrin

trans-Permethrin

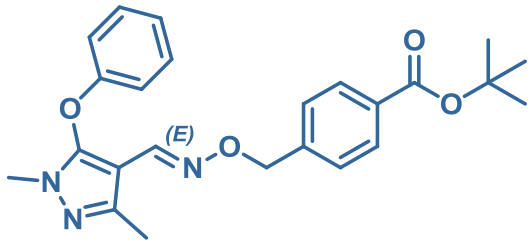




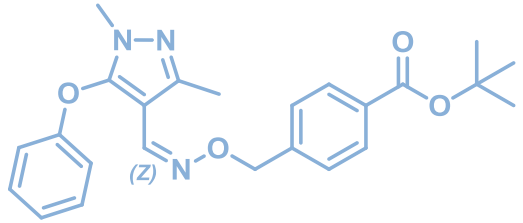
FENPYROXIMATE LC

European Commission

Fenpyroxymate

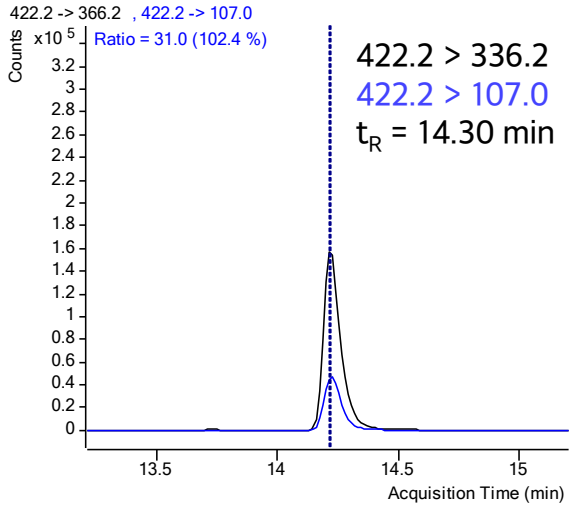


(*E*)-Fenpyroxymate

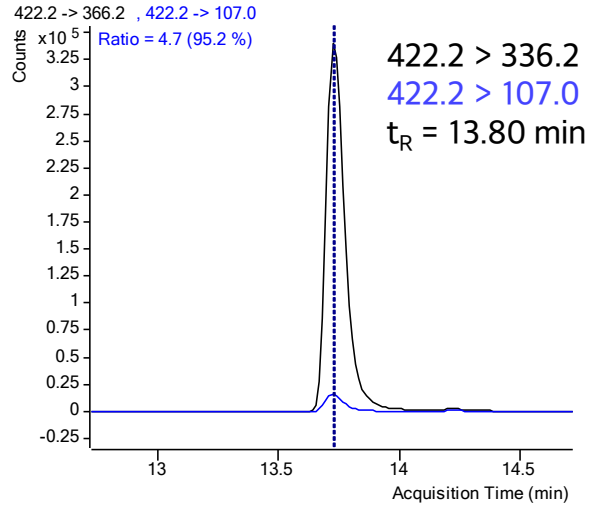


(*Z*)-Fenpyroxymate

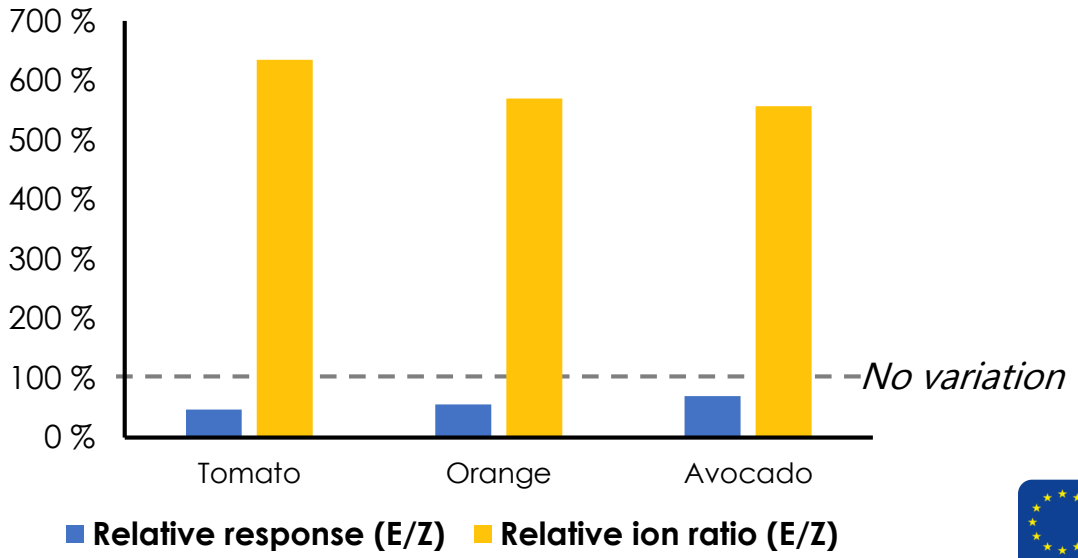
(*E*)-Fenpyroxymate



(*Z*)-Fenpyroxymate



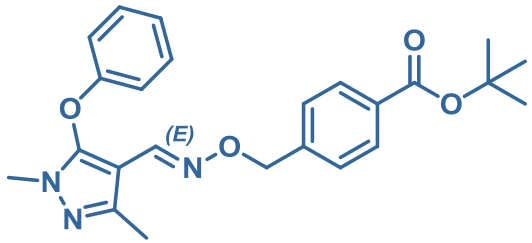
- Vendors sometimes offer **(*E*)-fenpyroximate** as "fenpyroximate"
- The (*E*)-fenpyroximate analytical standard usually contains **≤ 5 %** of the (*Z*)-isomer
- The fenpyroximate **technical mixture** is not readily available through many vendors



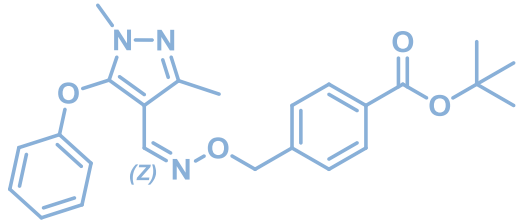
FENPYROXIMATE LC

European Commission

Fenpyroxymate

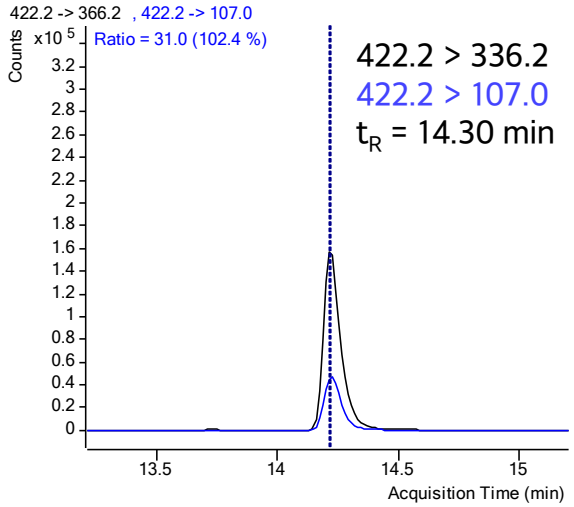


(*E*)-Fenpyroxymate

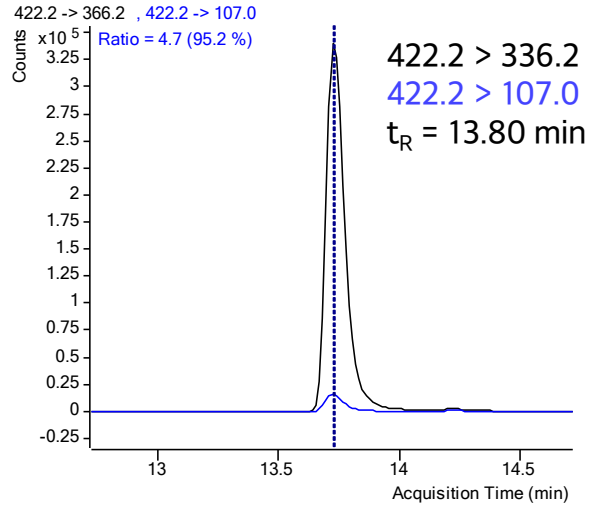


(*Z*)-Fenpyroxymate

(*E*)-Fenpyroxymate



(*Z*)-Fenpyroxymate



213	Fenpyroxymate CAS No 134098-61-6 CIPAC No 695	tert-butyl (E)-alpha-(1,3-dimethyl-5-phenoxypyrazol-4-ylmethyleneamino-oxy)-p-toluate **L 153/1 11.6.2011**

For the metabolites, the JMPR concluded that M-1, M-3, M-5, M-21, M-22 and Fen-OH would be covered by the reference values of the parent compound since these metabolites were also detected in rats at significant levels
During the EU evaluation, the metabolites M-1 and M-12 were concluded of equal or lower acute toxicity than the parent compound **10.2903/j.efsa.2019.5797**

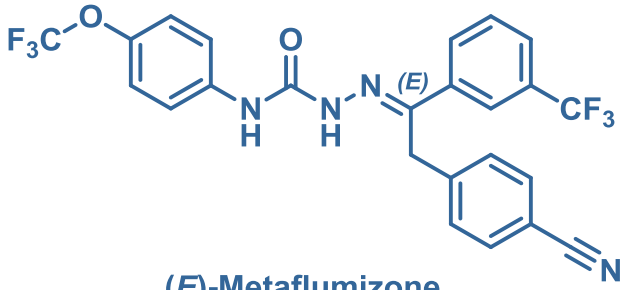
Conclusions **10.2903/j.efsa.2015.4382**

The primary crop metabolism of fenpyroxymate was investigated for foliar treatment in fruit crops (tangerine, grapes and apples) and in pulses and oilseeds (beans). However, an additional metabolism study addressing primary crop metabolism in a third crop group is still required (to cover authorisations in celeriac and hops in particular) and studies investigating the nature of the residues in rotational crops and in processed commodities were also not available. In the absence of these data, the residue definition for monitoring in plants is tentatively defined as the parent compound (E-isomer), while for risk assessment the residue definition is tentatively proposed as the sum of fenpyroxymate and its Z-isomer, expressed as fenpyroxymate. A validated analytical method for enforcement of the proposed residue definition is available for acidic and high water content commodities, but further validation in hops is still required.

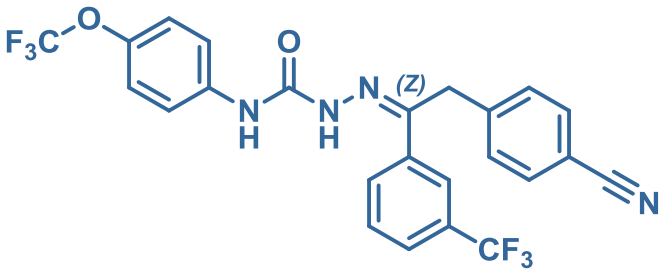
METAFLUMIZONE LC

European Commission

Metaflumizone (sum of E- and Z- isomers)

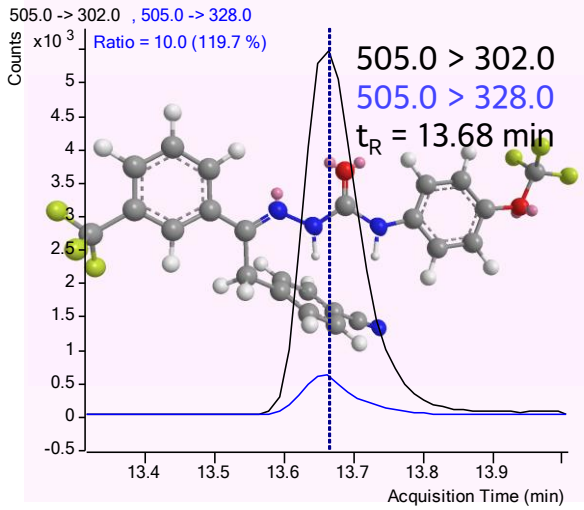


(*E*)-Metaflumizone

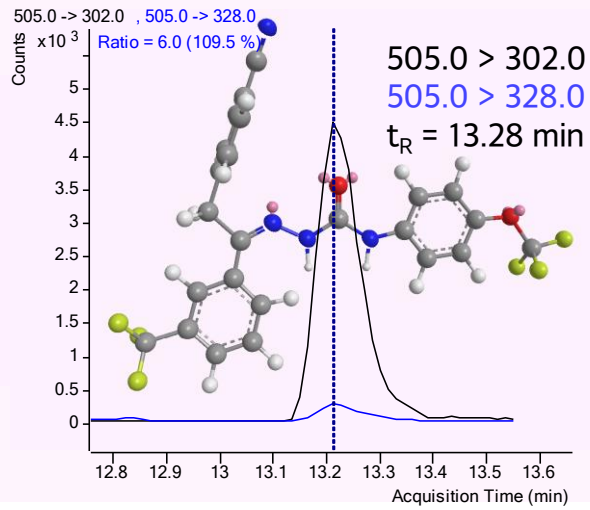


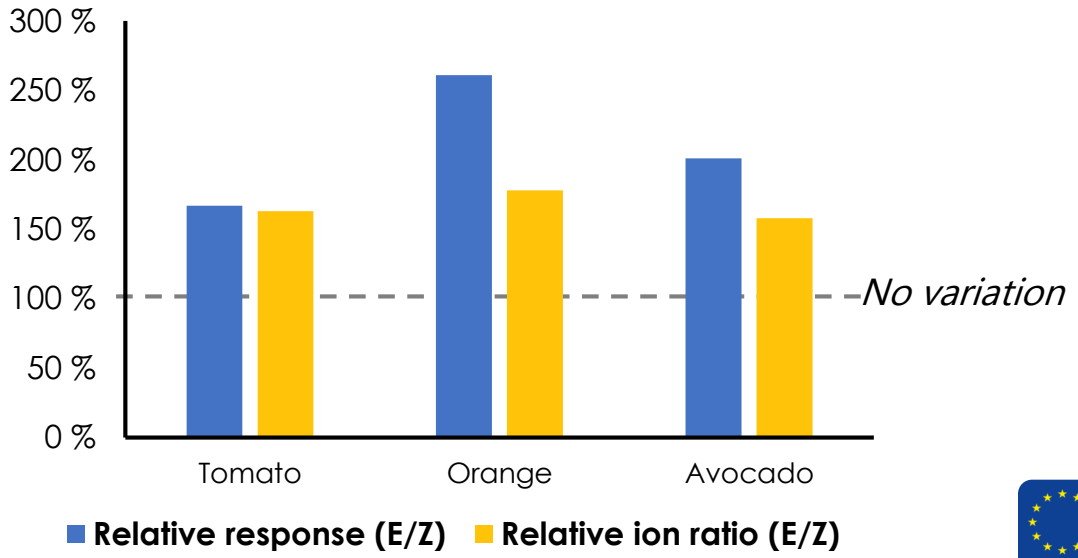
(*Z*)-Metaflumizone

(*E*)-Metaflumizone



(*Z*)-Metaflumizone





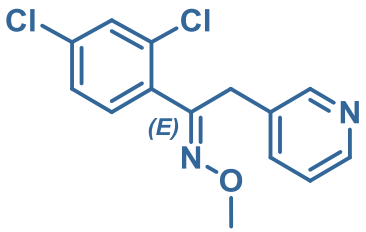
PYRIFENOX

GC

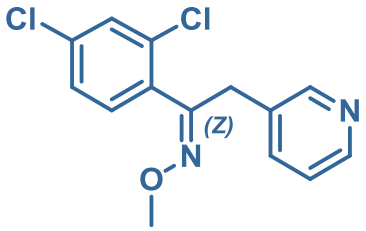
EURL-FV

European Commission

Pyrifenox



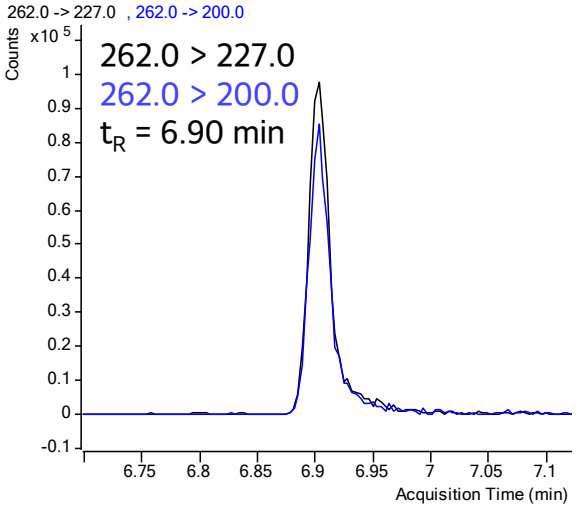
(*E*)-Pyrifenox



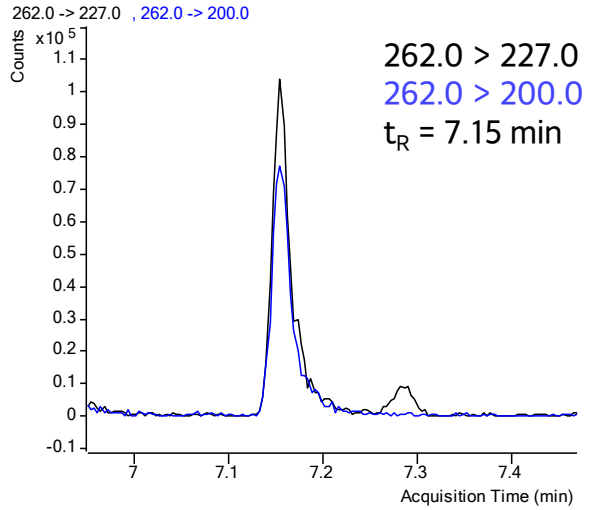
(*Z*)-Pyrifenox

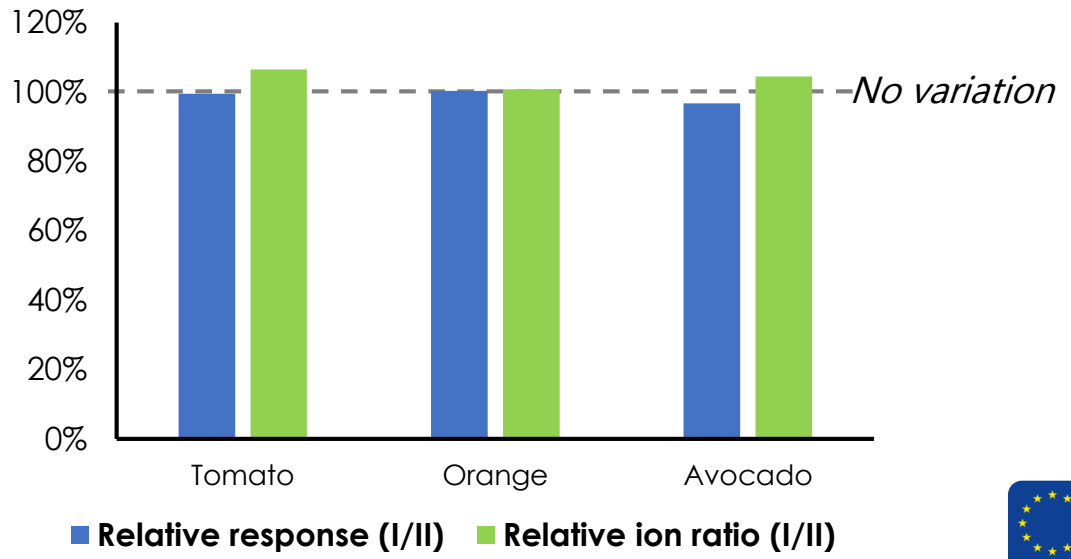
- Currently evaluating individual (*E*) & (*Z*) isomers
- Is the behaviour equivalent, or is the 50:50 ratio in the standard not accurate?

Pyrifenox (I)



Pyrifenox (II)





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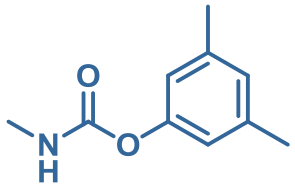
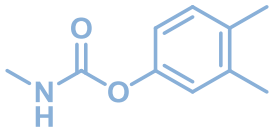
European Commission

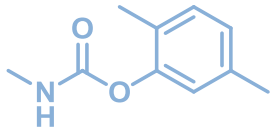
XMC



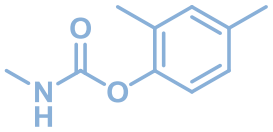
XMC
3,5-Xylyl methylcarbamate



3,4-Xylyl methylcarbamate
(Xylylcarb)



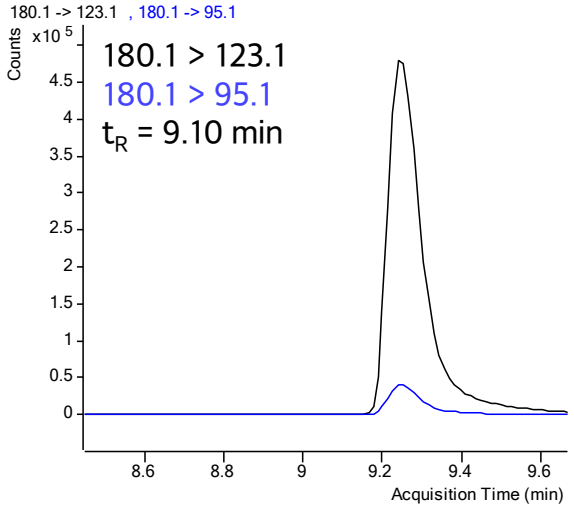
2,5-Xylyl methylcarbamate



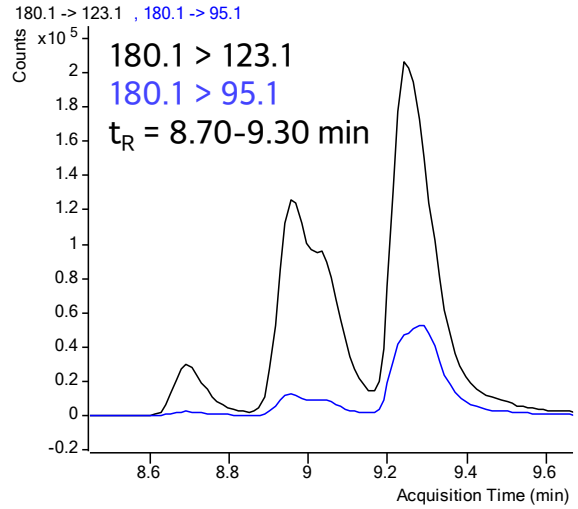
2,4-Xylyl methylcarbamate

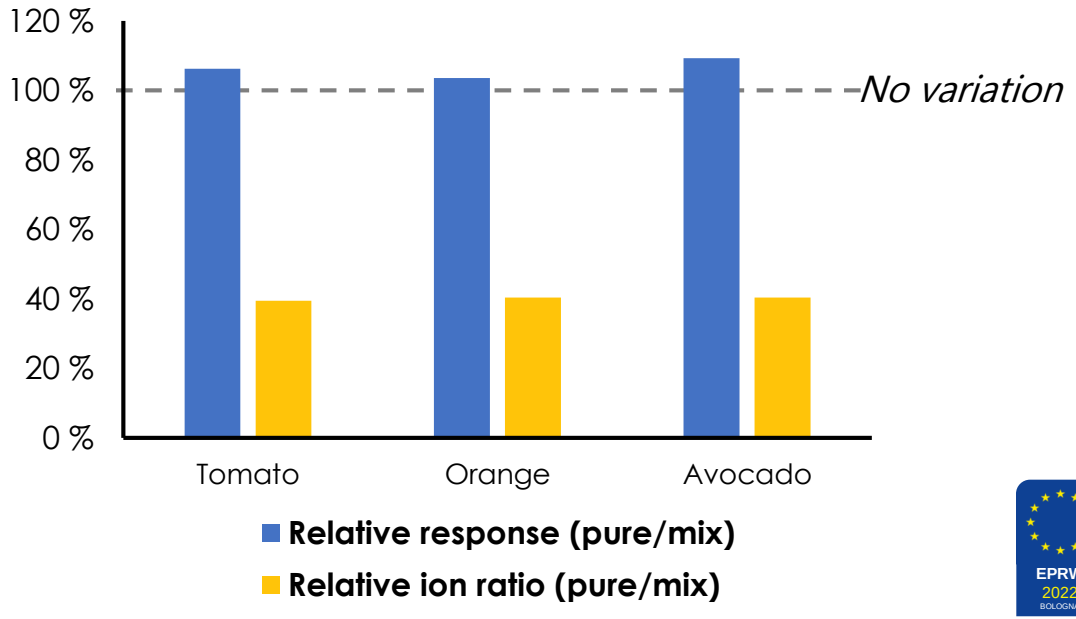
- Other examples of positional isomerism:
  - Aldicarb-sulfone & butoxycarboxim
  - Propazine & terbuthylazine

XMC (pure)



XMC (mix)



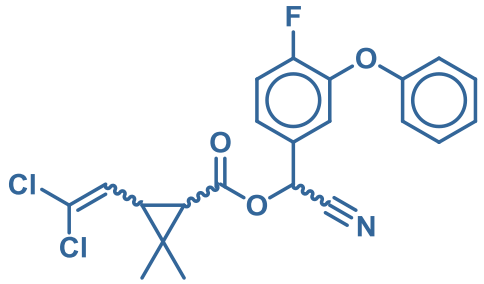


CYFLUTHRIN

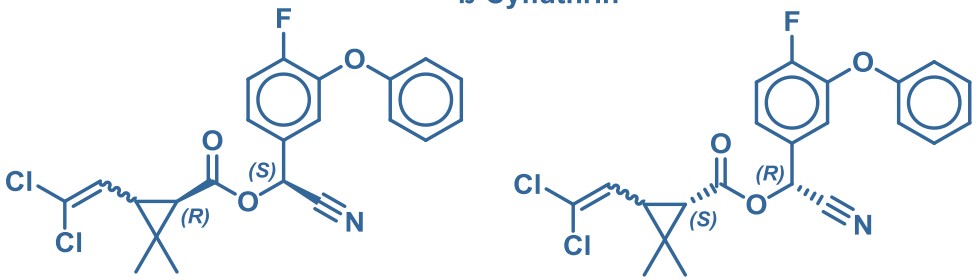
GC

European Commission

Cyfluthrin (cyfluthrin including other mixtures of constituent isomers (sum of isomers))

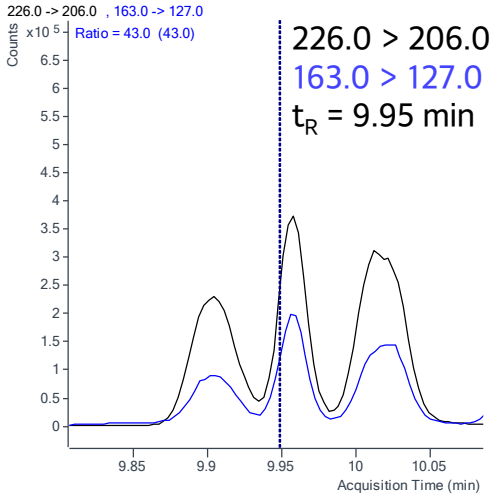


ß-Cyfluthrin

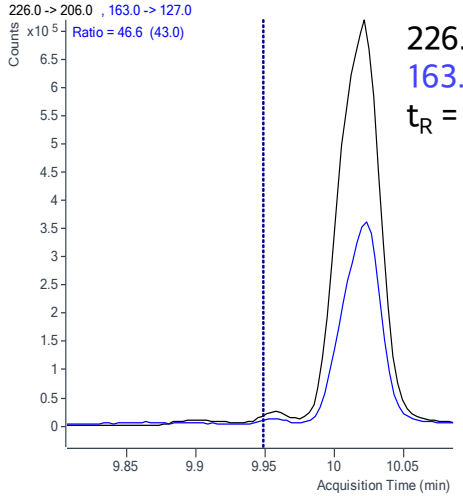


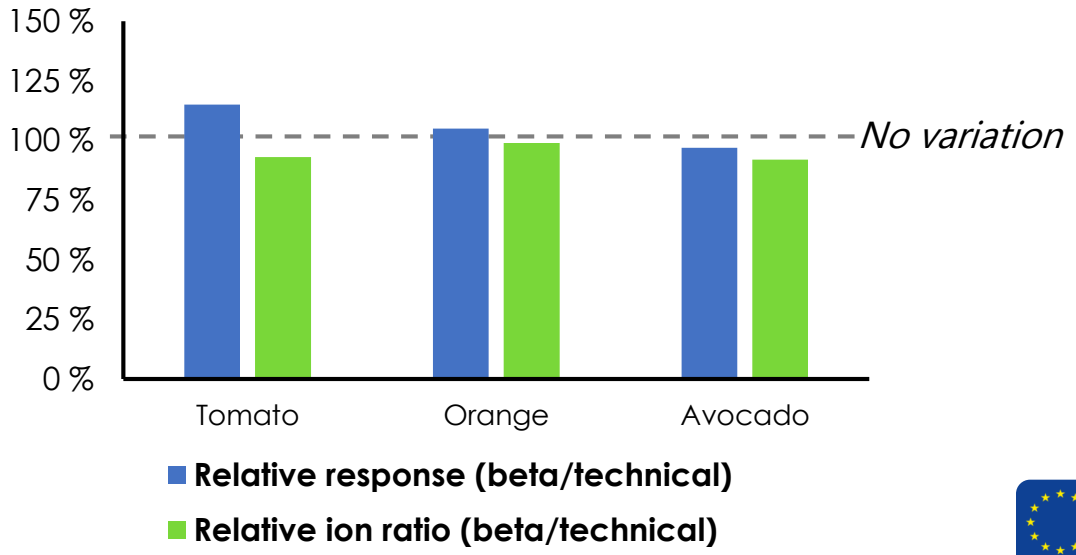
Diastereomer (enantiomer pair)	Composition (in %)	
	Cyfluthrin	β-Cyfluthrin
I (1*R*,3*R*, α*R* + 1*S*,3*S*, α*S* = 1:1; *cis*)	23-27	< 2
II (1*R*,3*R*, α*S* + 1*S*,3*S*, α*R* = 1:1; *cis*)	17-21	**30-40**
III (1*R*,3*S*, α*R* + 1*S*,3*R*, α*S* = 1:1; *trans*)	32-36	< 3
IV (1*R*,3*S*, α*S* + 1*S*,3*R*, α*R* = 1:1; *trans*)	21-25	**57-67**

Cyfluthrin



β-Cyfluthrin



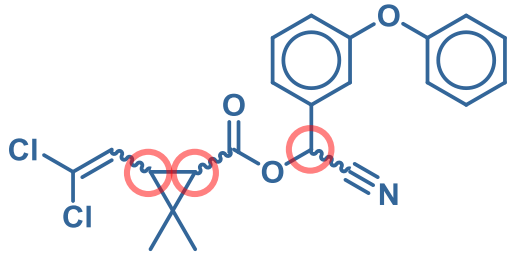


EPRW 2022 BOLOGNA

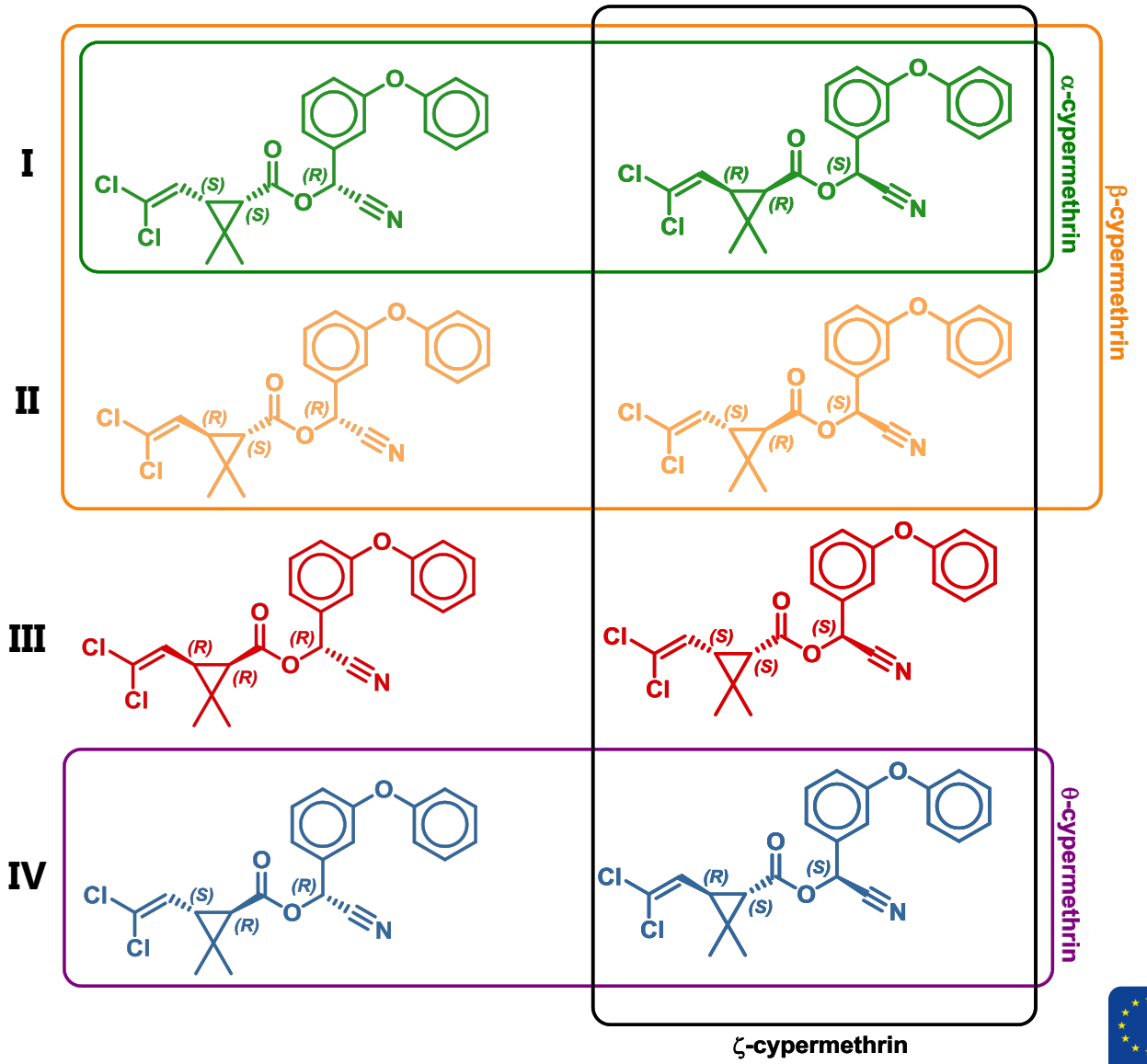
CYPERMETHRIN GC

European Commission

Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))

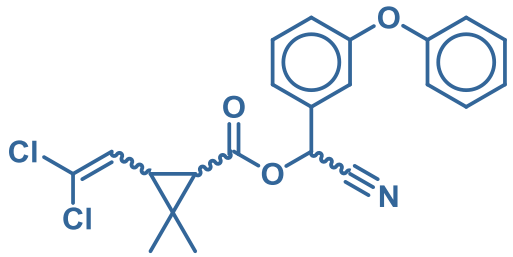


- **α-cypermethrin** (2 enantiomers): **1 peak**
- **β-cypermethrin** (2 pairs of enantiomers): **2 peaks**
- **θ-cypermethrin** (2 enantiomers): **1 peak**
- **ζ-cypermethrin** (4 isomers): **up to 4 peaks**
- **Technical mixture** (8 isomers): **up to 4 peaks**



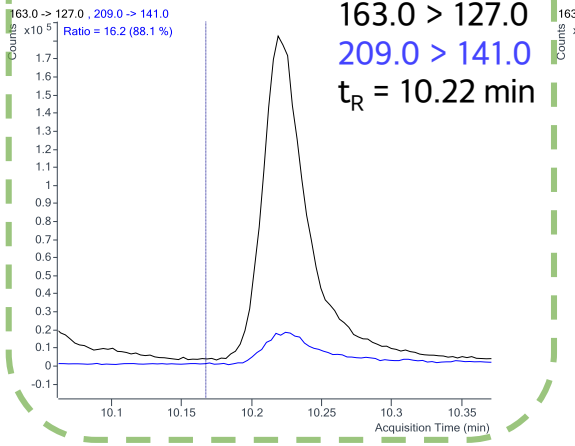
CYPERMETHRIN GC

Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))



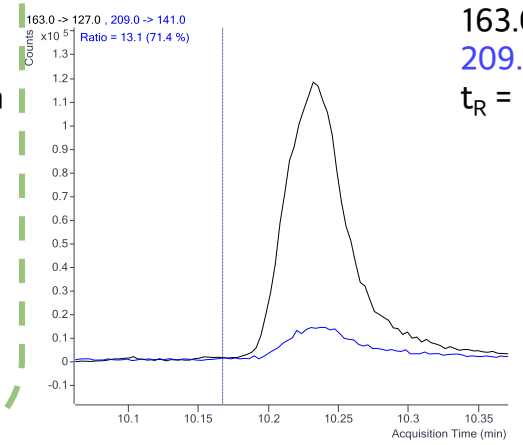
- **α-cypermethrin** (2 enantiomers): **1 peak**
- **β-cypermethrin** (2 pairs of enantiomers): **2 peaks**
- **θ-cypermethrin** (2 enantiomers): **1 peak**
- **ζ-cypermethrin** (4 isomers): **up to 4 peaks**
- **Technical mixture** (8 isomers): **up to 4 peaks**

α-Cypermethrin



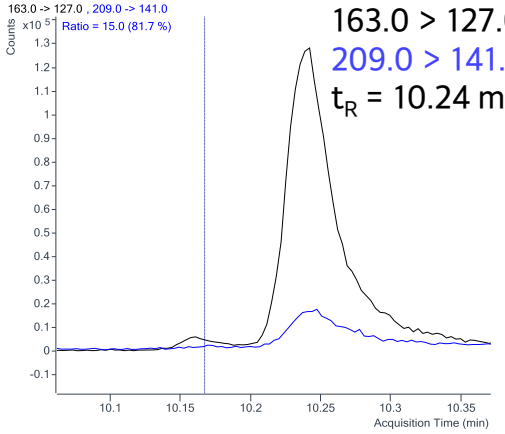
163.0 > 127.0
209.0 > 141.0
t_R = 10.22 min

β-Cypermethrin



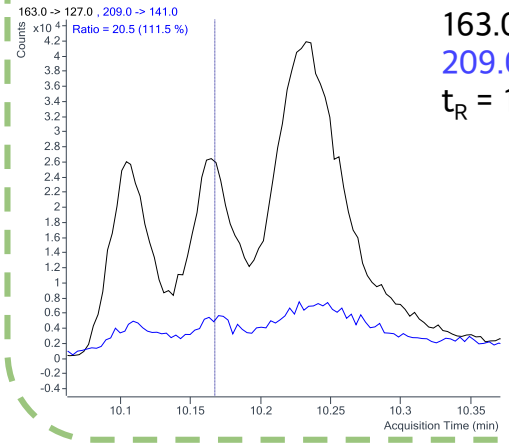
163.0 > 127.0
209.0 > 141.0
t_R = 10.24 min

θ-Cypermethrin



163.0 > 127.0
209.0 > 141.0
t_R = 10.24 min

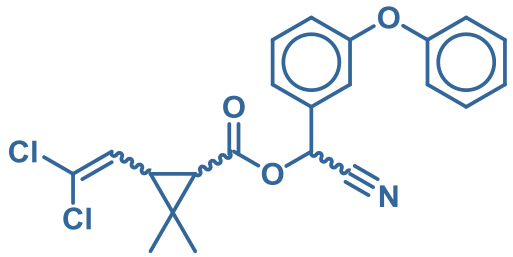
ζ-Cypermethrin



163.0 > 127.0
209.0 > 141.0
t_R = 10.17 min

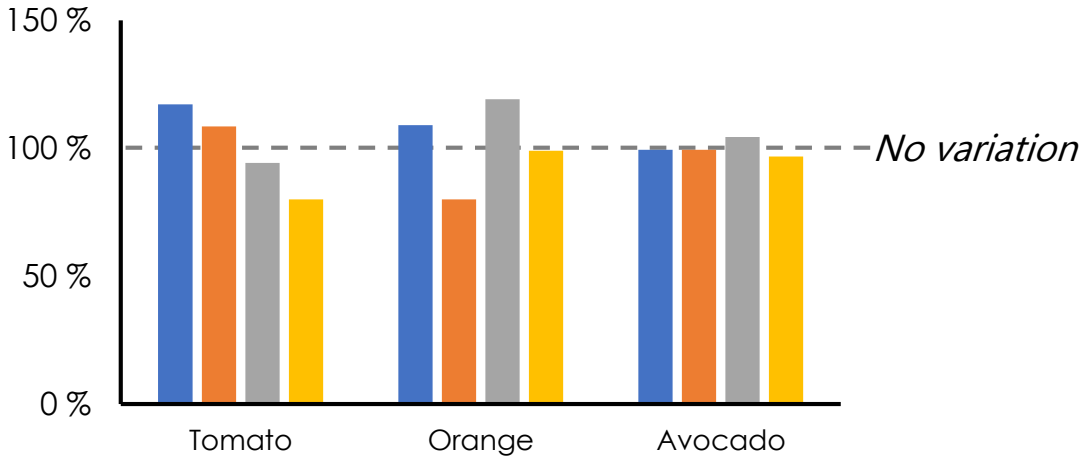
CYPERMETHRIN GC

Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))

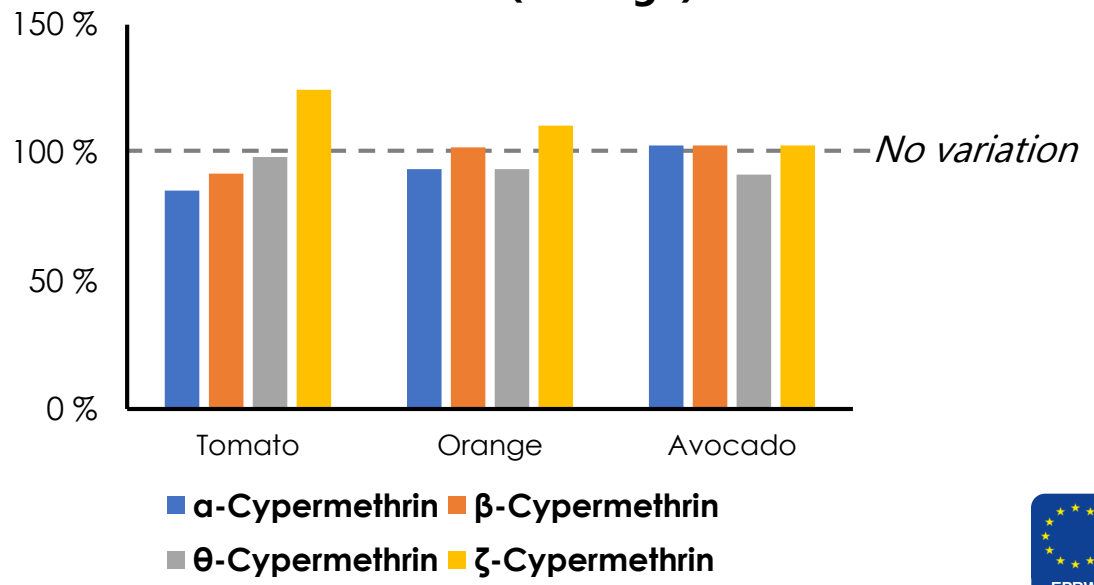


- **α-cypermethrin** (2 enantiomers): **1 peak**
- **β-cypermethrin** (2 pairs of enantiomers): **2 peaks**
- **θ-cypermethrin** (2 enantiomers): **1 peak**
- **ζ-cypermethrin** (4 isomers): **up to 4 peaks**
- **Technical mixture** (8 isomers): **up to 4 peaks**

Relative response (average)



Relative ion ratio (average)



CYPERMETHRIN GC

European Commission

Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))

CURRENT APPROVAL PERIOD 01/11/2019 - 07/06/2021 — NOT APPROVED

Alpha-Cypermethrin (aka alphamethrin)

NOT APPROVED

Beta-cypermethrin

CURRENT APPROVAL PERIOD 01/02/2022 - 31/01/2029 — APPROVED

Cypermethrin

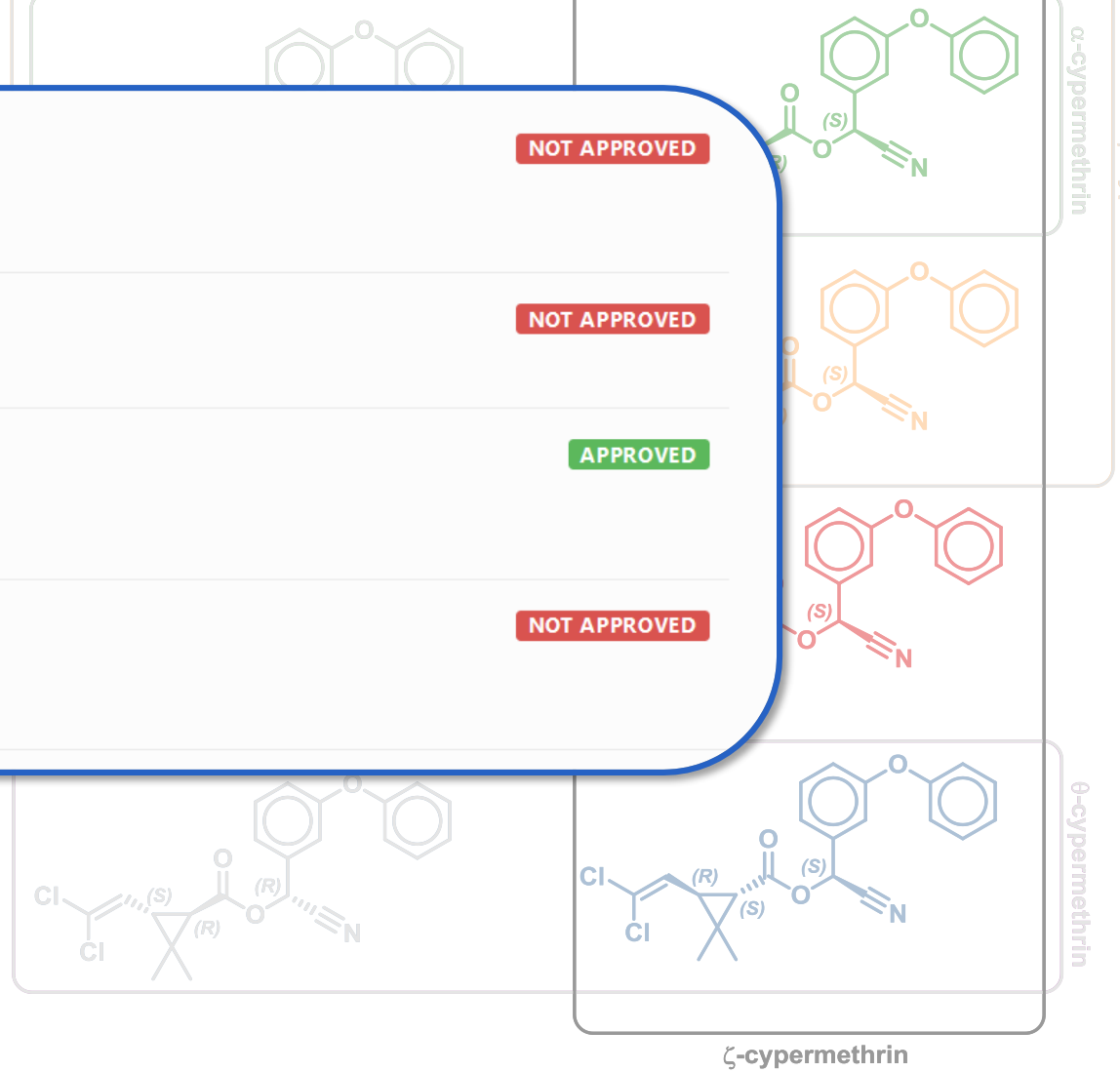
CURRENT APPROVAL PERIOD 01/12/2009 - 01/12/2020 — NOT APPROVED

zeta-Cypermethrin

- α-cypermet
- β-cypermet
- θ-cypermet
- ζ-cypermethrin

Technical mixture (8 isomers): **up to 4 peaks**

IV



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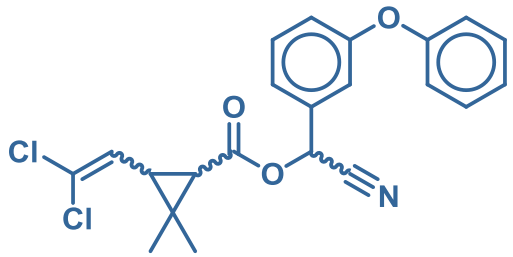
CYPERMETHRIN GC

European Commission

Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))



- **α-cypermethrin** (2 enantiomers): **1 peak**
- **β-cypermethrin** (2 pairs of enantiomers): **2 peaks**
- **θ-cypermethrin** (2 enantiomers): **1 peak**
- **ζ-cypermethrin** (4 isomers): **up to 4 peaks**
- **Technical mixture** (8 isomers): **up to 4 peaks**

Undistinguishable

Active substance	ARfD (mg/kg bw)	Source
α-Cypermethrin	0.00125	Reg (EU) 2019/160
β-Cypermethrin	0.0016	EFSA 2014
ζ-Cypermethrin	0.125	EFSA 08
Cypermethrin	0.2	Dir 05/53

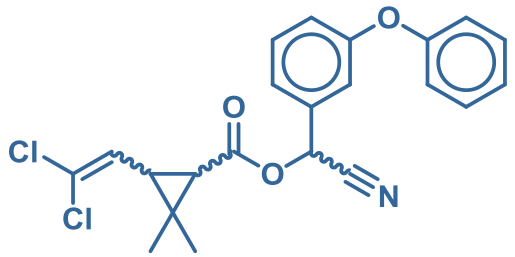
Active substance	Ecotoxicology (LD_{50})	Value
ζ-Cypermethrin	(oral) (mg/kg)	86
Cypermethrin	(oral) (mg/kg)	287
ζ-Cypermethrin	(contact) (µg/bee)	0.002
Cypermethrin	(contact) (µg/bee)	0.023
ζ-Cypermethrin	(oral) (µg/bee)	0.044
Cypermethrin	(oral) (µg/bee)	0.172
ζ-Cypermethrin	(acute 14 d) (mg/kg)	37.5
Cypermethrin	(acute 14 d) (mg/kg)	>100

Source: EU Pesticides Database and University of Hertfordshire Pesticide Properties DataBase

CYPERMETHRIN GC

European Commission

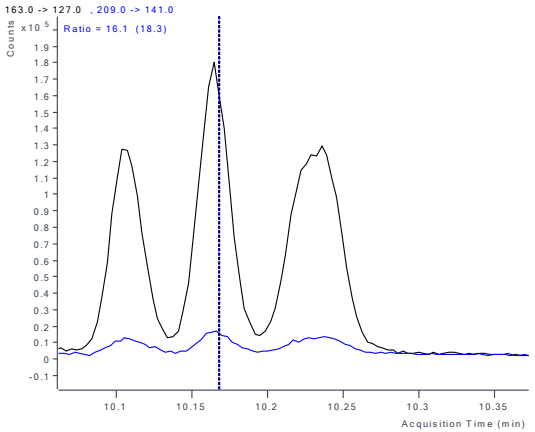
Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers))

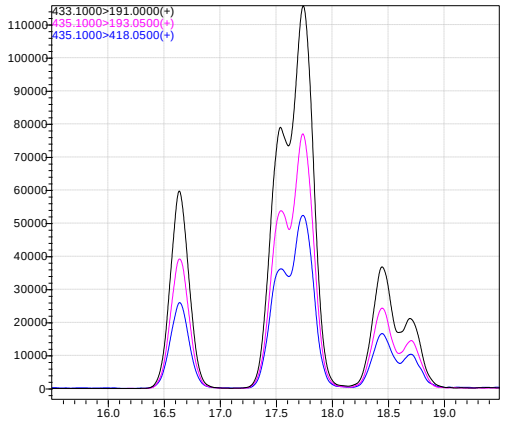


- **ζ-cypermethrin** (4 isomers): **up to 4 peaks**
- **Technical mixture** (8 isomers): **up to 4 peaks**

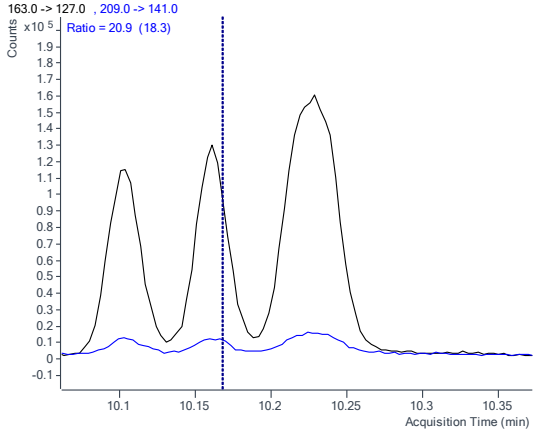
- **Undistinguishable** with standard **GC-MS/MS** techniques
- Can be **differentiated** with **chiral SFC-MS/MS**
- Positive samples in cypermethrin have been reanalysed, with **no fraudulent use detected to date**

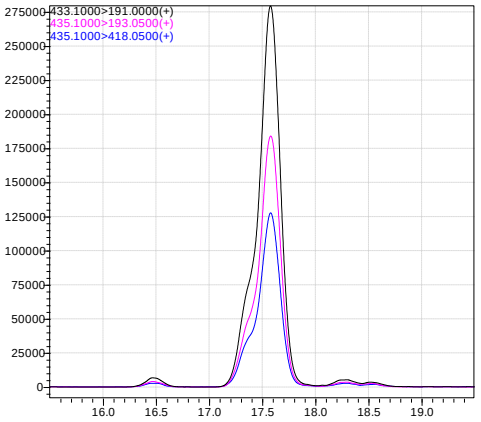
Cypermethrin (technical)





ζ-Cypermethrin





Chiral SFC-MS²

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CYPERMETHRIN GC

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European Commission

Cypermethrin (cypermethrin including

– ζ-cype

– Techn

– **Undisting**

– Can be **differ**

– Positive samples in cyper
with **no fraudulent use** d

J. Agric. Food Chem. **1990**, *38*, 1613–1617

Chemical and Photochemical Isomerization of Deltamethrin

R. James Maguire

Rivers Research Branch, National Water Research Institute, Department of Environment, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6, Canada

Chiral high-performance liquid chromatographic analyses have shown that deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] in natural water in the dark is subject to cis/trans isomerization yielding the α-*S*, 1*S* cis isomer 2′-deltamethrin, which is inactive against mice and insects. Sunlight irradiation of deltamethrin as a thin film, on potato leaves, in natural water and in hexane was, however, only a partial detoxification step. Although it produced the inactive 2′ isomer as well as the inactive α-*S*, 1*S* trans isomer 4′-deltamethrin, it did produce in addition the α-*S*, 1*R* trans isomer 3-deltamethrin, which does retain some activity toward mice and insects, albeit less than the parent deltamethrin.

"But that is another story and shall be told another time."

-Michael Ende

16.0 16.5 17.0 17.5 18.0 18.5 19.0

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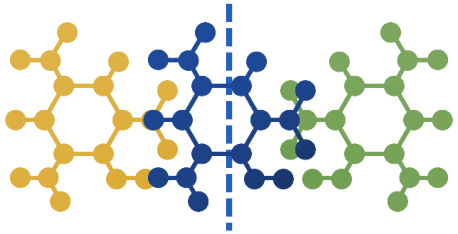
CONCLUSIONS

- Some residue definitions include **more than one analyte** (isomers of various types or other analogues)



- The mass spectrometry behaviour **cannot be predicted** *a priori*
  - Analyte behaviour has been observed to change across matrixes

- Quantitation strategies must be **studied and adapted** on a *per case* basis
  - Evaluating the equivalence of the analytical response is a good practice

- Knowledge of the residue definitions and careful evaluation of analytical standards **helps avoiding identification and/or quantitation issues**

THANK YOU

Co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA). Neither the European Union nor the granting authority can be held responsible for them.

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