LC-MS/MS method combining the analysis of some Avermectins, QACs and Chloridazon in fruits, vegetables and grain

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[LC-Xtra]

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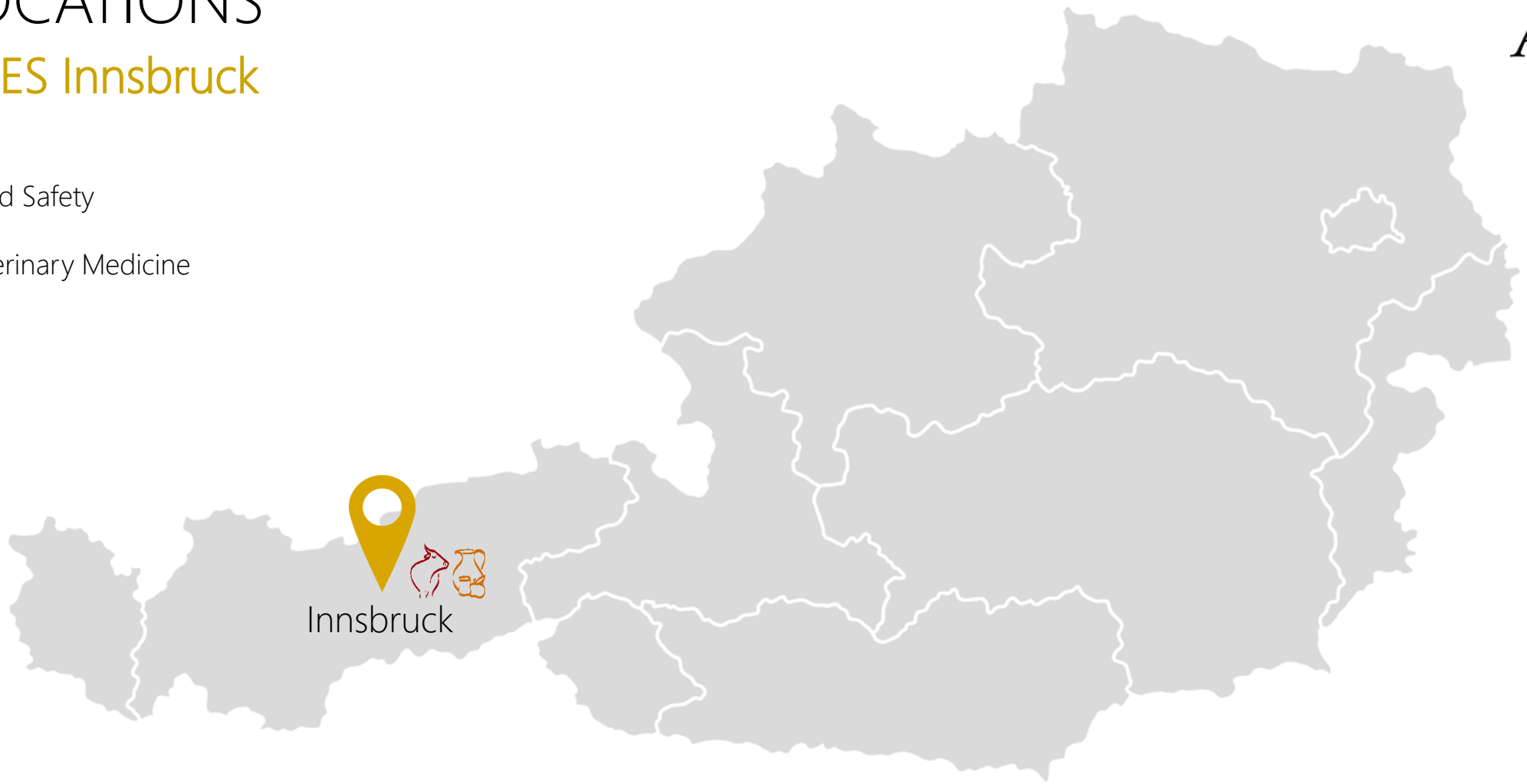
LOCATIONS

AGES Innsbruck

Food Safety

Veterinary Medicine



Innsbruck
Technikerstr. 70, 6020 Innsbruck

LOCATIONS

AGES Innsbruck

Institute for Food Safety (LSI)

National Reference Laboratory
for Pesticide Residues

Institute for Veterinary Medicine

National Reference Laboratory
for *Trichinella*

LOCATIONS

AGES Innsbruck

INSTITUTE FOR FOOD SAFETY INNSBRUCK (LSI)

- National Reference Laboratory (NRL)

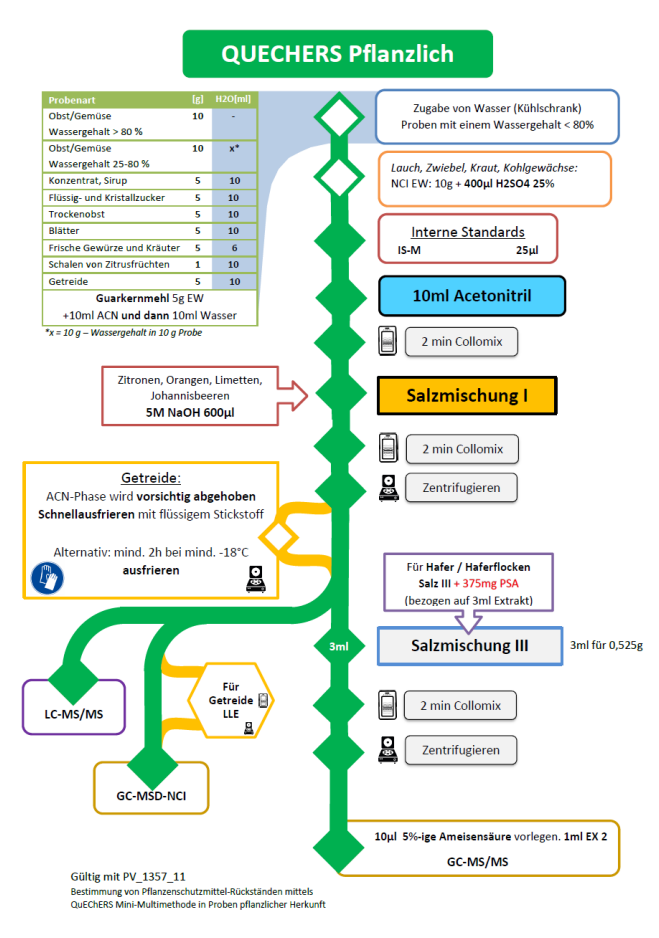
1. Residues of pesticides in fruit and vegetables – multi methods
2. Residues of plant protection products – single methods
3. Residues of plant protection products in Cereals and feedstuff
4. Residues of plant protection products in Food of animal origin and commodities with high fat content
5. Residues of plant protection products in products of organic farming

WHY?

EURL SRM – Method

Abamectin

Sample Preparation (QuEChERS)



Community Reference Laboratories for Residues of Pesticides

Single Residue Methods
Version: Abamectin-V1

Analysis of Abamectin via QuEChERS and LC-MS/MS
- Brief Description -

Standards:

Abamectin: for example from Dr. Ehrenstorfer GmbH, Germany, Purity 95 % (91.5 % Avermectin B1a and 3.5 % Avermectin B1b)

Sample Preparation (QuEChERS):

- Weigh 10 g of the homogeneous, frozen sample into a 50 ml centrifugation tube
- Add 10 ml acetonitrile and 100 µl internal standard solution (e.g. triphenylphosphate c = 20 µg/ml); close the tube and shake vigorously for 1 min.
- Add 4 g Magnesium sulphate anhydrous, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate and shake for 1 min; centrifuge for 5 min at 3000 rpm
- Transfer an aliquot of the extract into a centrifugation tube which contains 25 mg PSA and 150 mg magnesium sulphate per mL extract; shake the tube vigorously for 30 s and centrifuge for 5 min at 3000 rpm.
- Fill an aliquot of the extract into a vial and employ for LC-MS/MS analysis

Analysis by LC-MS/MS *(Please consider: the LC-MS/MS data is exemplary)*

Instrument: API 3000, Applied Biosystems
Mode: ESI positive
The mass-transitions used are as follows:

- **Avermectin B1a:** 890.5 → 567.4; 890.5 → 305.1; 891.5 → 568.1
- **Avermectin B1b:** 876.6 → 553.3; 876.6 → 291.2;
- **Z-isomer of avermectin B1a:** 890.5→ 305.1; 890.5 → 567.3; 891.5 → 568.3

Column: Zorbax XDB Eclipse 150 x 2.1 mm; 3.5µ
Eluents:

- **A:** 950 mL water, 50 mL ACN, 10mmol ammonium formate brought to pH 4 with formic acid;
- **B:** 920 mL ACN and 80 mL water, 10 mmol ammonium formate (should be solved in water before diluting with ACN).

Gradient: The gradient starts with 30% B and goes to 100% B.
Flow: 0.3 ml/min
Injection volume: 10 µl

Notes:
High source temprature may reduce signals (better set 300 °C or below)
With the ammonium adduct being used as the mother ion for the MRM-transition the buffer concentration, as well as the pH, are of high importance.

Performance:
Validation data: see www.crl-pesticides-datapool.eu:
Pesticide names: Avermectin B1a, Avermectin B1b and Avermectin B1a, 8,9-Z
LOQ: 0,005 mg/kg for Avermectin B1a and B1b (using the instrument above)

Community Reference Laboratory for Single Residue Methods
CVUA Stuttgart, Schaflandstr. 3/2, 70736 Fellbach, Germany
CRL@cvuas.bwl.de

EURL SRM – Method

Abamectin

LC-MS/MS Analysis

Column: Zorbax XDB Eclipse 150 x 2.1 mm; 3.5µm

Eluents:

- A: 950 mL water, 50 mL ACN, 10mmol ammonium formate brought to pH 4 with formic acid;
- B: 920 mL ACN and 80 mL water, 10 mmol ammonium formate (should be solved in water before diluting with ACN)

Gradient:

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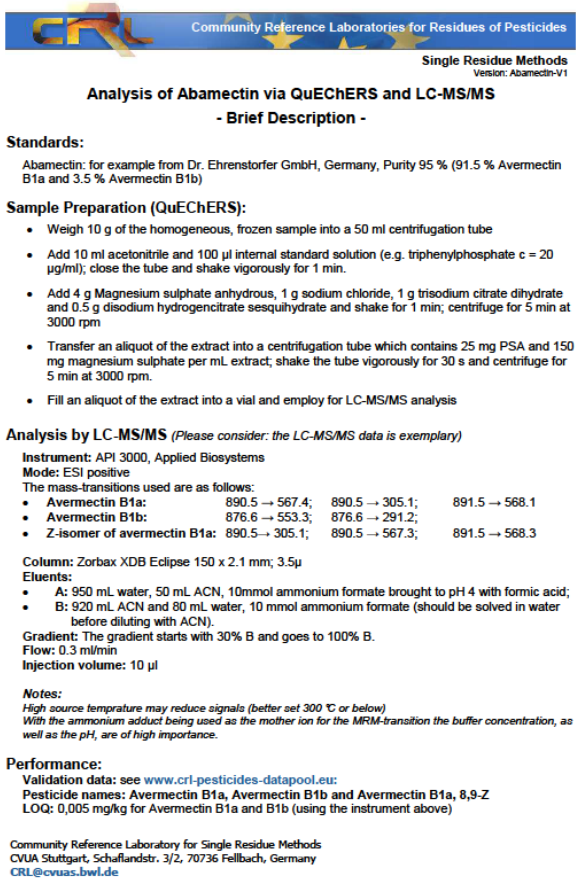
Flow: 0.3 ml/min

Injection volume: 10µl



Community Reference Laboratories for Residues of Pesticides

Single Residue Methods
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Eluents:

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Notes:
High source temprature may reduce signals (better set 300 °C or below)
With the ammonium adduct being used as the mother ion for the MRM-transition the buffer concentration, as well as the pH, are of high importance.

Performance:
Validation data: see www.crl-pesticides-datapool.eu:
Pesticide names: Avermectin B1a, Avermectin B1b and Avermectin B1a, 8,9-Z
LOQ: 0,005 mg/kg for Avermectin B1a and B1b (using the instrument above)

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EURL SRM – Method

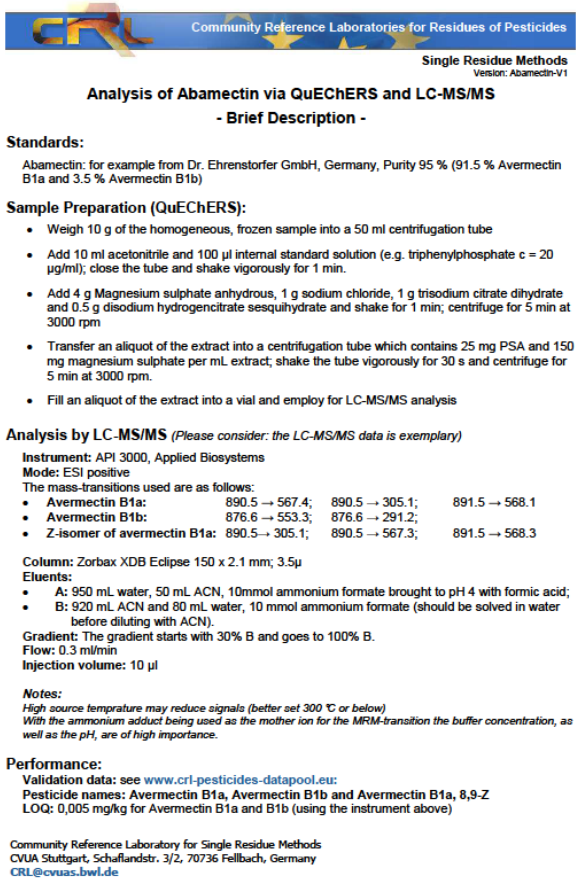
Abamectin

SRM Comment

High source temperature may reduce signals (better set 300 °C or below)

With the ammonium adduct being used as the mother ion for the MRM-transition the buffer concentration, as

well as the pH, are of high importance.



Community Reference Laboratories for Residues of Pesticides

Single Residue Methods
Version: Abamectin-V1

Analysis of Abamectin via QuEChERS and LC-MS/MS
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LOQ: 0,005 mg/kg for Avermectin B1a and B1b (using the instrument above)

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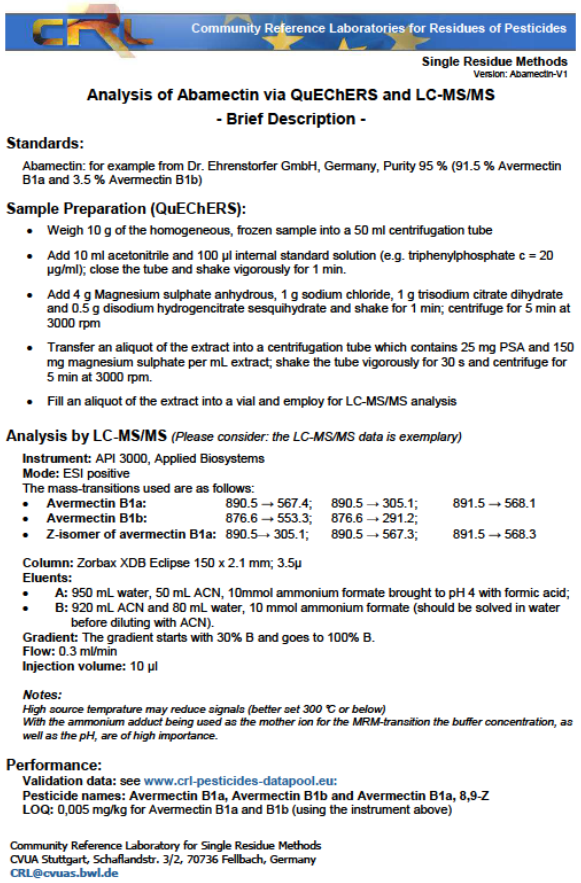
EURL SRM – Method

Abamectin

https://www.eurl-pesticides.eu/library/docs/srm/meth_Abamectin_CrlSrm.PDF



Community Reference Laboratories for Residues of Pesticides

Single Residue Methods
Version: Abamectin-V1

Analysis of Abamectin via QuEChERS and LC-MS/MS
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Eluents:

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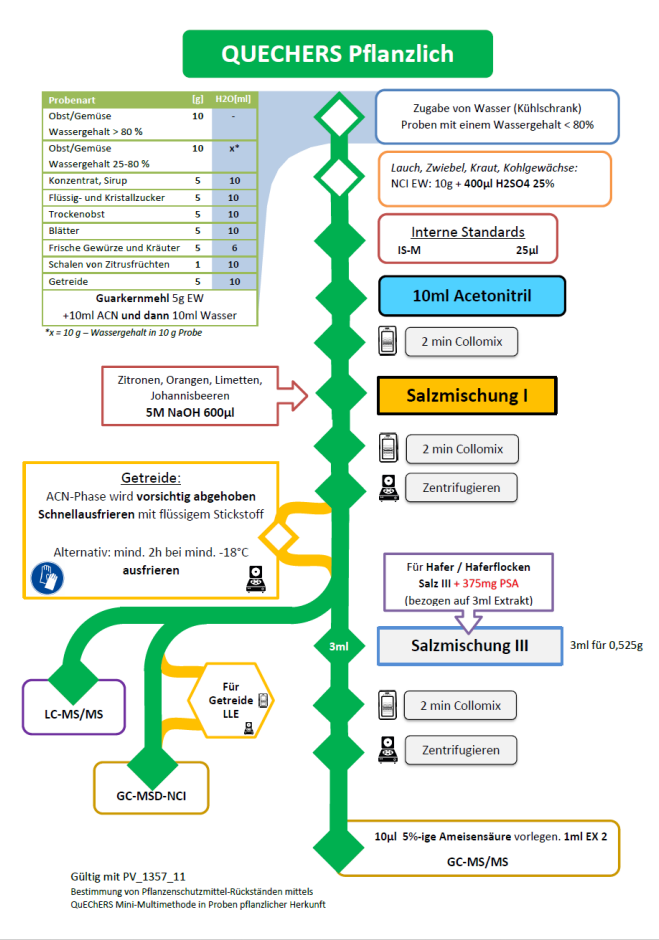
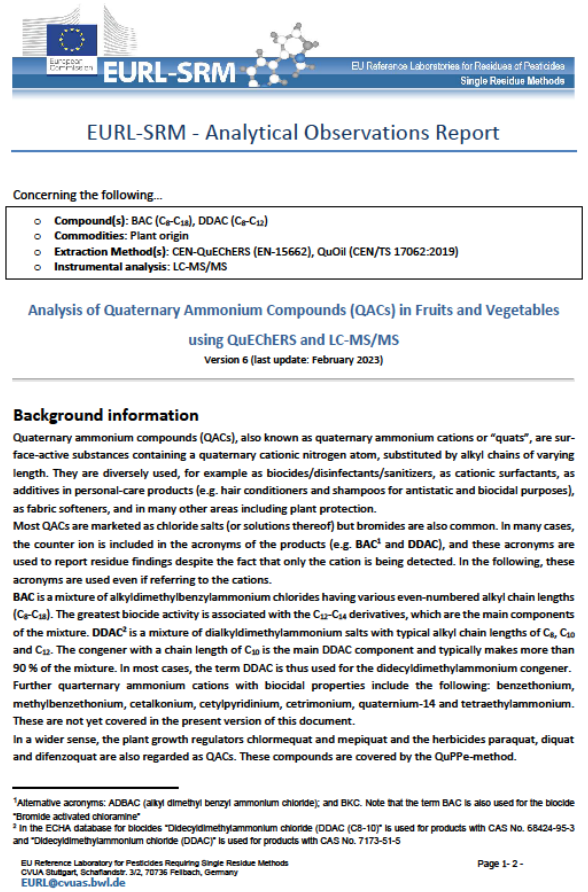
EURL SRM – Method

Quaternary Ammonium Compounds (QACs)

Sample Preparation (QuEChERS)





EURL-SRM – Analytical Observations Report

Concerning the following…

- **Compound(s)**: BAC (C_8-C_{18}), DDAC (C_8-C_{12})
- **Commodities**: Plant origin
- **Extraction Method(s)**: CEN-QuEChERS (EN-15662), QuOil (CEN/TS 17062:2019)
- **Instrumental analysis**: LC-MS/MS

Analysis of Quaternary Ammonium Compounds (QACs) in Fruits and Vegetables using QuEChERS and LC-MS/MS

Version 6 (last update: February 2023)

Background information

Quaternary ammonium compounds (QACs), also known as quaternary ammonium cations or "quats", are surface-active substances containing a quaternary cationic nitrogen atom, substituted by alkyl chains of varying length. They are diversely used, for example as biocides/disinfectants/sanitizers, as cationic surfactants, as additives in personal-care products (e.g. hair conditioners and shampoos for antistatic and biocidal purposes), as fabric softeners, and in many other areas including plant protection.

Most QACs are marketed as chloride salts (or solutions thereof) but bromides are also common. In many cases, the counter ion is included in the acronyms of the products (e.g. **BAC**[1] and **DDAC**), and these acronyms are used to report residue findings despite the fact that only the cation is being detected. In the following, these acronyms are used even if referring to the cations.

BAC is a mixture of alkyldimethylbenzylammonium chlorides having various even-numbered alkyl chain lengths (C_8-C_{18}). The greatest biocide activity is associated with the C_{12}-C_{14} derivatives, which are the main components of the mixture. **DDAC**[2] is a mixture of dialkyldimethylammonium salts with typical alkyl chain lengths of C_8, C_{10} and C_{12}. The congener with a chain length of C_{10} is the main DDAC component and typically makes more than 90 % of the mixture. In most cases, the term DDAC is thus used for the didecyldimethylammonium congener. Further quarternary ammonium cations with biocidal properties include the following: benzethonium, methylbenzethonium, cetalkonium, cetylpyridinium, cetrimonium, quaternium-14 and tetraethylammonium. These are not yet covered in the present version of this document.

In a wider sense, the plant growth regulators chlormequat and mepiquat and the herbicides paraquat, diquat and difenzoquat are also regarded as QACs. These compounds are covered by the QuPPe-method.

[1] Alternative acronyms: ADBAC (alkyl dimethyl benzyl ammonium chloride); and BKC. Note that the term BAC is also used for the biocide "Bromide activated chloramine"

[2] In the ECHA database for biocides "Didecyldimethylammonium chloride (DDAC (C8-10)" is used for products with CAS No. 68424-95-3 and "Didecyldimethylammonium chloride (DDAC)" is used for products with CAS No. 7173-51-5

EURL SRM – Method

Quaternary Ammonium Compounds (QACs)

LC-MS/MS Analysis

Column: Phenomenex Aqua C18, 20x50 mm, 5 µm, 125 A at 40 °C

Eluents:

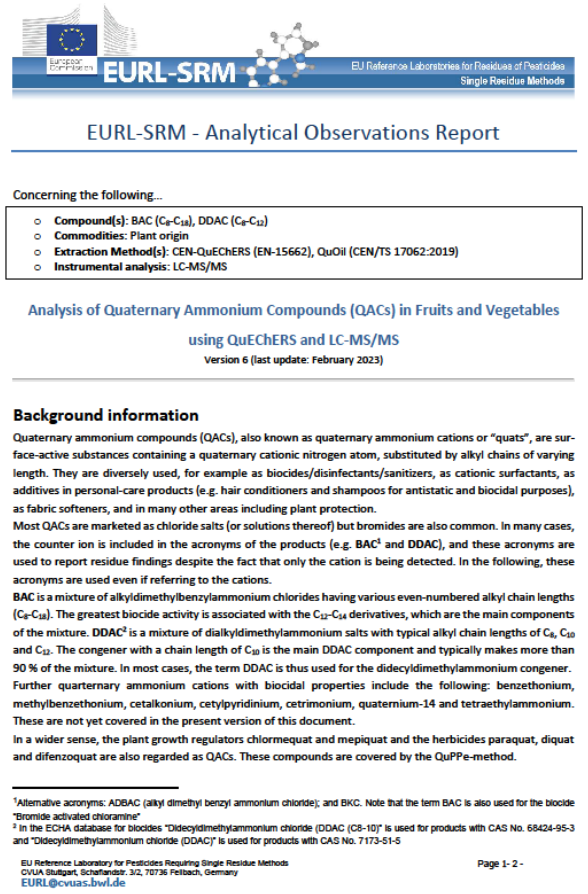
- A: 5 mmol NH4-formiate in H2O + 0,01 % formic acid;
- B: 95 mmol NH4-formiate in methanol (MeOH) + 0,01 % formic acid

Gradient:

The gradient starts with 5% B and goes to 90% B

Flow: 0.4 ml/min

Injection volume: 5µl



EURL-SRM – EU Reference Laboratories for Residues of Pesticides – Single Residue Methods

EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compound(s)**: BAC (C_8-C_{18}), DDAC (C_8-C_{12})
- **Commodities**: Plant origin
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- **Instrumental analysis**: LC-MS/MS

Analysis of Quaternary Ammonium Compounds (QACs) in Fruits and Vegetables using QuEChERS and LC-MS/MS

Version 6 (last update: February 2023)

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Most QACs are marketed as chloride salts (or solutions thereof) but bromides are also common. In many cases, the counter ion is included in the acronyms of the products (e.g. **BAC**[1] and **DDAC**), and these acronyms are used to report residue findings despite the fact that only the cation is being detected. In the following, these acronyms are used even if referring to the cations.

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Further quarternary ammonium cations with biocidal properties include the following: benzethonium, methylbenzethonium, cetalkonium, cetylpyridinium, cetrimonium, quaternium-14 and tetraethylammonium. These are not yet covered in the present version of this document.

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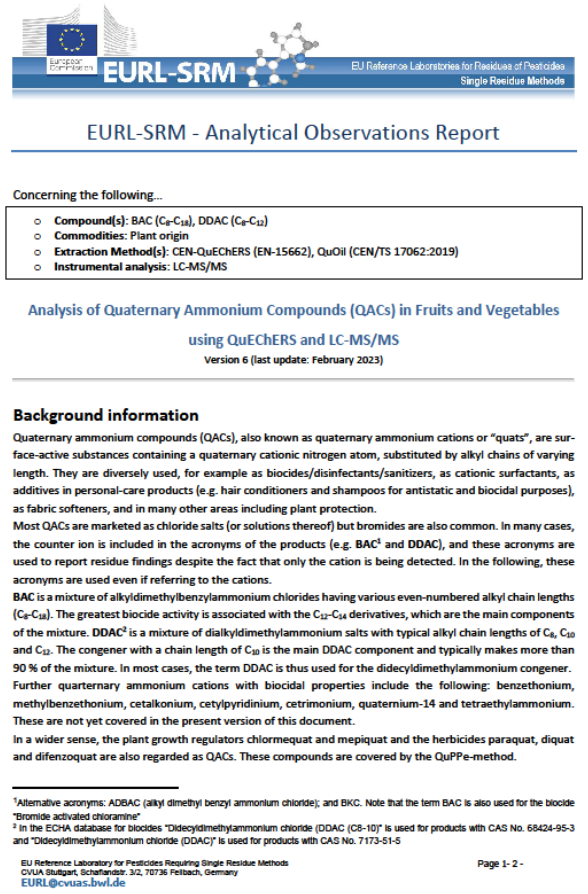
EURL SRM – Method

Quaternary Ammonium Compounds (QACs)

SRM Comment

QAC background contaminations of variable intensity and composition are often reported. These background levels seriously compromise their ability of the labs to analyze those compounds at low levels and to control MRL compliance. Background contaminations deriving from the LC system in front of the autosampler, e.g. from contaminated eluents and tubing appear to be the most crucial to address. A simple and practical way of separating the background contamination deriving from the LC-system is the use of the trap column, between the pump and the autosampler of the LC-system. Any QAVs contaminants eluting from the system in-between chromatographic runs (e.g. during column equilibration), are trapped onto this trap column, and prevented from accumulating at the beginning of the analytical column or pre-column. During the chromatographic run, these contaminants will eventually also elute through the analytical column, but they will experience additional retention (in the trap column), and will thus separate from their analogues introduced through the injection of sample extracts.



EURL-SRM

EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

EURL-SRM - Analytical Observations Report

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Version 6 (last update: February 2023)

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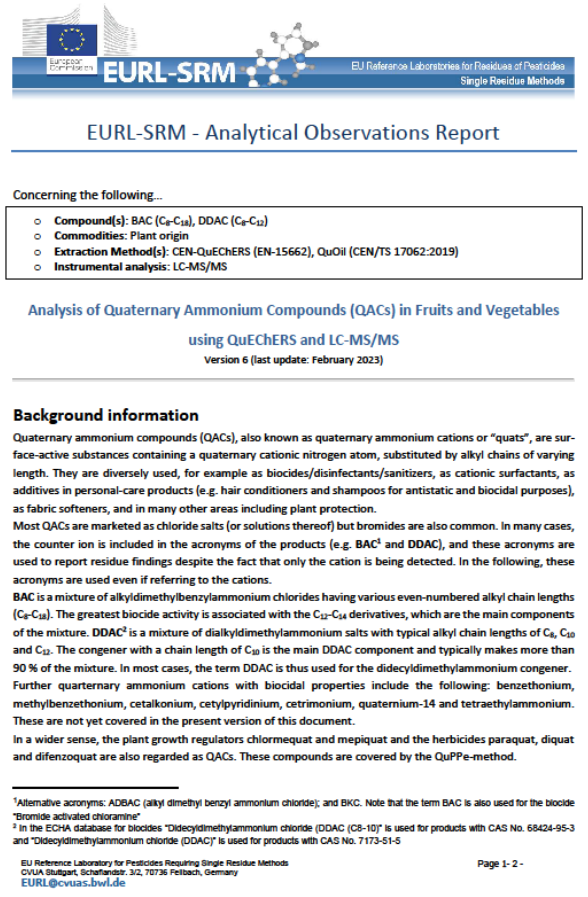
EURL SRM – Method

Quaternary Ammonium Compounds (QACs)

https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_Observation_QAC_V6.pdf



EURL-SRM

EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

EURL-SRM - Analytical Observations Report

Concerning the following…

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- **Commodities**: Plant origin
- **Extraction Method(s)**: CEN-QuEChERS (EN-15662), QuOil (CEN/TS 17062:2019)
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Analysis of Quaternary Ammonium Compounds (QACs) in Fruits and Vegetables using QuEChERS and LC-MS/MS

Version 6 (last update: February 2023)

Background information

Quaternary ammonium compounds (QACs), also known as quaternary ammonium cations or "quats", are surface-active substances containing a quaternary cationic nitrogen atom, substituted by alkyl chains of varying length. They are diversely used, for example as biocides/disinfectants/sanitizers, as cationic surfactants, as additives in personal-care products (e.g. hair conditioners and shampoos for antistatic and biocidal purposes), as fabric softeners, and in many other areas including plant protection.

Most QACs are marketed as chloride salts (or solutions thereof) but bromides are also common. In many cases, the counter ion is included in the acronyms of the products (e.g. **BAC**[1] and **DDAC**), and these acronyms are used to report residue findings despite the fact that only the cation is being detected. In the following, these acronyms are used even if referring to the cations.

BAC is a mixture of alkyldimethylbenzylammonium chlorides having various even-numbered alkyl chain lengths (C_8-C_{18}). The greatest biocide activity is associated with the C_{12}-C_{14} derivatives, which are the main components of the mixture. **DDAC**[2] is a mixture of dialkyldimethylammonium salts with typical alkyl chain lengths of C_8, C_{10} and C_{12}. The congener with a chain length of C_{10} is the main DDAC component and typically makes more than 90 % of the mixture. In most cases, the term DDAC is thus used for the didecyldimethylammonium congener.

Further quarternary ammonium cations with biocidal properties include the following: benzethonium, methylbenzethonium, cetalkonium, cetylpyridinium, cetrimonium, quaternium-14 and tetraethylammonium. These are not yet covered in the present version of this document.

In a wider sense, the plant growth regulators chlormequat and mepiquat and the herbicides paraquat, diquat and difenzoquat are also regarded as QACs. These compounds are covered by the QuPPe-method.

[1] Alternative acronyms: ADBAC (alkyl dimethyl benzyl ammonium chloride); and BKC. Note that the term BAC is also used for the biocide "Bromide activated chloramine"

[2] In the ECHA database for biocides "Didecyldimethylammonium chloride (DDAC (C8-10)" is used for products with CAS No. 68424-95-3 and "Didecyldimethylammonium chloride (DDAC)" is used for products with CAS No. 7173-51-5

EURL SRM – Method

Chloridazon-desphenyl

- Sample Preparation (QuEPPe Methode)

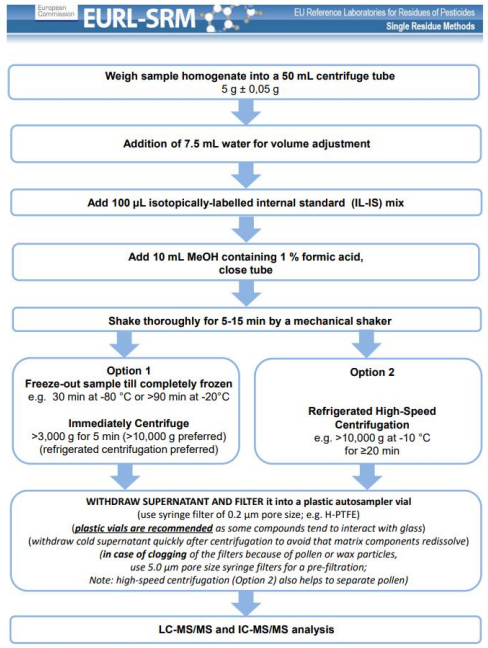
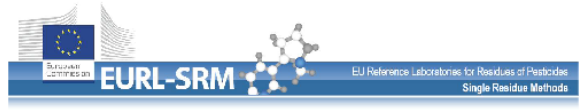


Figure 1: QuPPe method for honey samples at a glance.



EURL-SRM – Analytical Observations Report

concerning the following…

- **Compound(s)**: Chloridazon-Desphenyl
- **Commodities**: Honey
- **Extraction Method(s)**: QuPPe
- **Instrumental analysis**: LC-MS/MS; SFC-MS/MS, LC-QToF

Risk of False Positives of Chloridazon-Desphenyl in Honey by LC-MS/MS

Version 1 (last update: 14.04.2023)

Background information / Initial Observations:

Within the frame of an EURL-SRM pilot monitoring study on pesticide residues in honey, it was noticed, that a remarkably large share of samples analyzed by QuPPe and LC-MS/MS (via method M4.2), showed signals faking the presence of chloridazon-desphenyl.

While measuring honey samples, it was noticed, that the three acquired MRM-traces of chloridazon-desphenyl showed peaks at the expected retention time with two of them being within the expected ion-ratio. The third trace, however, showed a very large signal, that was initially interpreted as originating from the matrix. This led to a large number of fake chloridazon-desphenyl "findings", mostly at "levels" exceeding the MRLs for chloridazon (sum). The unusually high frequency of apparent findings and apparent MRL-violations (despite chloridazon being phased out, at least in the EU), together with the strongly interfered MRM-transition, raised the need to run confirmatory analyses by other techniques to check the presence of this compound.

Chloridazon used to be a popular herbicide and was used e.g. in beet cultivation. However, due to massive findings of its degradation products in underground water, its use was eventually restricted. Thereafter the EU-approval was not renewed and expired by the end of 2018, with the period of grace for sales ending in mid-2020, and the end of use of stocks terminating by the end of 2020.

Chloridazon degrades into two main metabolites, chloridazon-desphenyl and chloridazon-methyl-desphenyl. For chloridazon-desphenyl, the half-life in soil is reported at 235.5 days.

Due to the persistence and leachability of chloridazon-desphenyl, findings are still reported in surface and underground water[1]. Findings in food of plant origin are also still occasionally reported, and findings in organic products were also initially of concern[2].

[1] Jan Chabera et al.; The effect of chronic exposure to chloridazon and its degradation product chloridazon-desphenyl on signal crayfish Pacifastacus leniusculus; Ecotoxicology and Environmental Safety Volume 208, 15 January 2021, 111645; https://doi.org/10.1016/j.ecoenv.2020.111645

[2] Labor Friedle / Reiana (June 2020): file:///C:/Users/anastasiadee/Downloads/KundenInformation%20Chloridazon.pdf

EURL SRM – Method

Chloridazon-desphenyl

LC-MS/MS Analysis

Column: Acquity BEH Amide, 2x100 mm, 1.7 µm

Eluents:

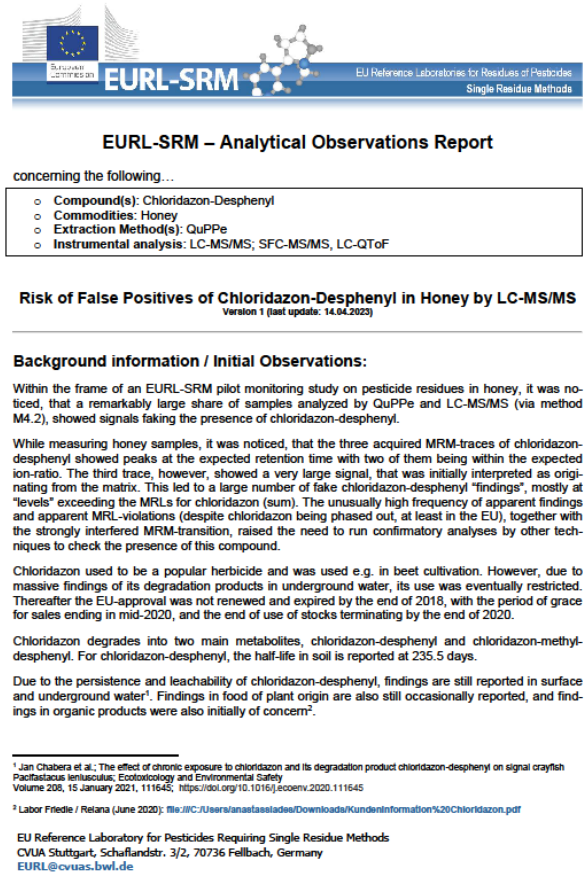
- A: 50 mmol NH4formate in purified water
- B: Acetonitrile

Gradient:

The gradient starts with 97% B and goes to 40% B

Flow: 0.5 ml/min

Injection volume: 2µl



EURL-SRM – Analytical Observations Report

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- **Commodities**: Honey
- **Extraction Method(s)**: QuPPe
- **Instrumental analysis**: LC-MS/MS; SFC-MS/MS, LC-QToF

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[2] Labor Friedle / Reiana (June 2020): file:///C:/Users/anastaeslades/Downloads/KundenInformation%20Chloridazon.pdf

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EURL SRM – Method

Chloridazon-desphenyl

LC-MS/MS Analysis

Or

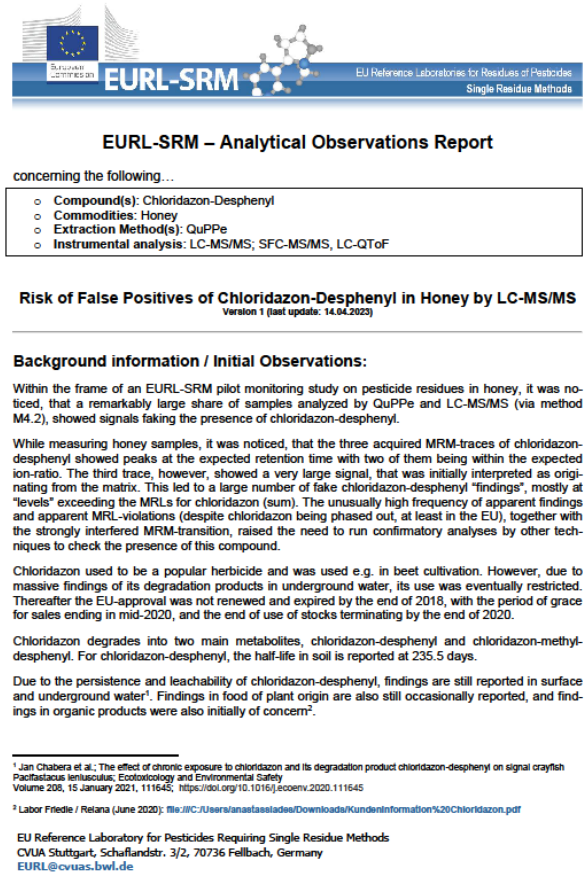
SFC-MS/MS

Or

A different HILIC column

Or

The same column and High Mass Resolution



EURL-SRM – Analytical Observations Report

concerning the following…

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- **Commodities**: Honey
- **Extraction Method(s)**: QuPPe
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Risk of False Positives of Chloridazon-Desphenyl in Honey by LC-MS/MS

Version 1 (last update: 14.04.2023)

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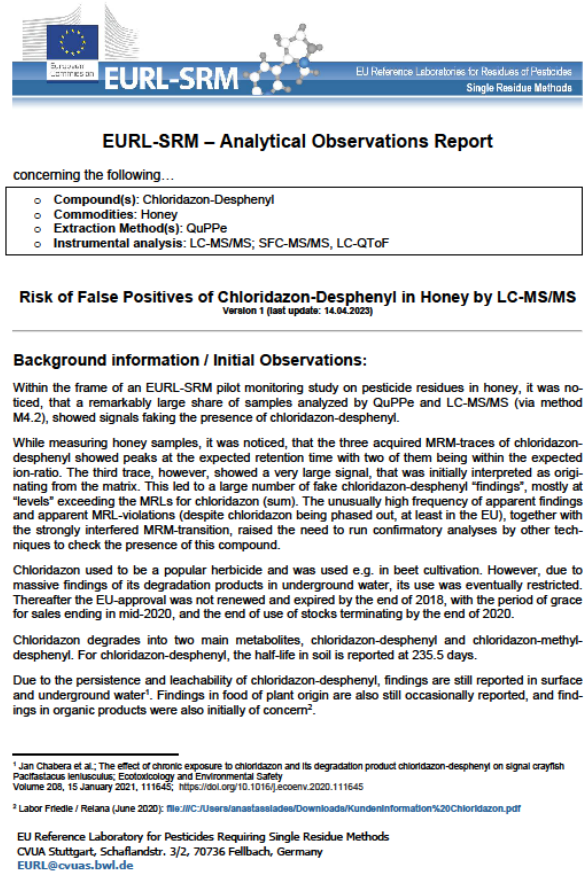
EURL SRM – Method

Chloridazon-desphenyl

https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_chloridazon-desphenyl_honey_V1.pdf



EURL-SRM – EU Reference Laboratories for Residues of Pesticides – Single Residue Methods

EURL-SRM – Analytical Observations Report

concerning the following…

- **Compound(s)**: Chloridazon-Desphenyl
- **Commodities**: Honey
- **Extraction Method(s)**: QuPPe
- **Instrumental analysis**: LC-MS/MS; SFC-MS/MS, LC-QToF

Risk of False Positives of Chloridazon-Desphenyl in Honey by LC-MS/MS

Version 1 (last update: 14.04.2023)

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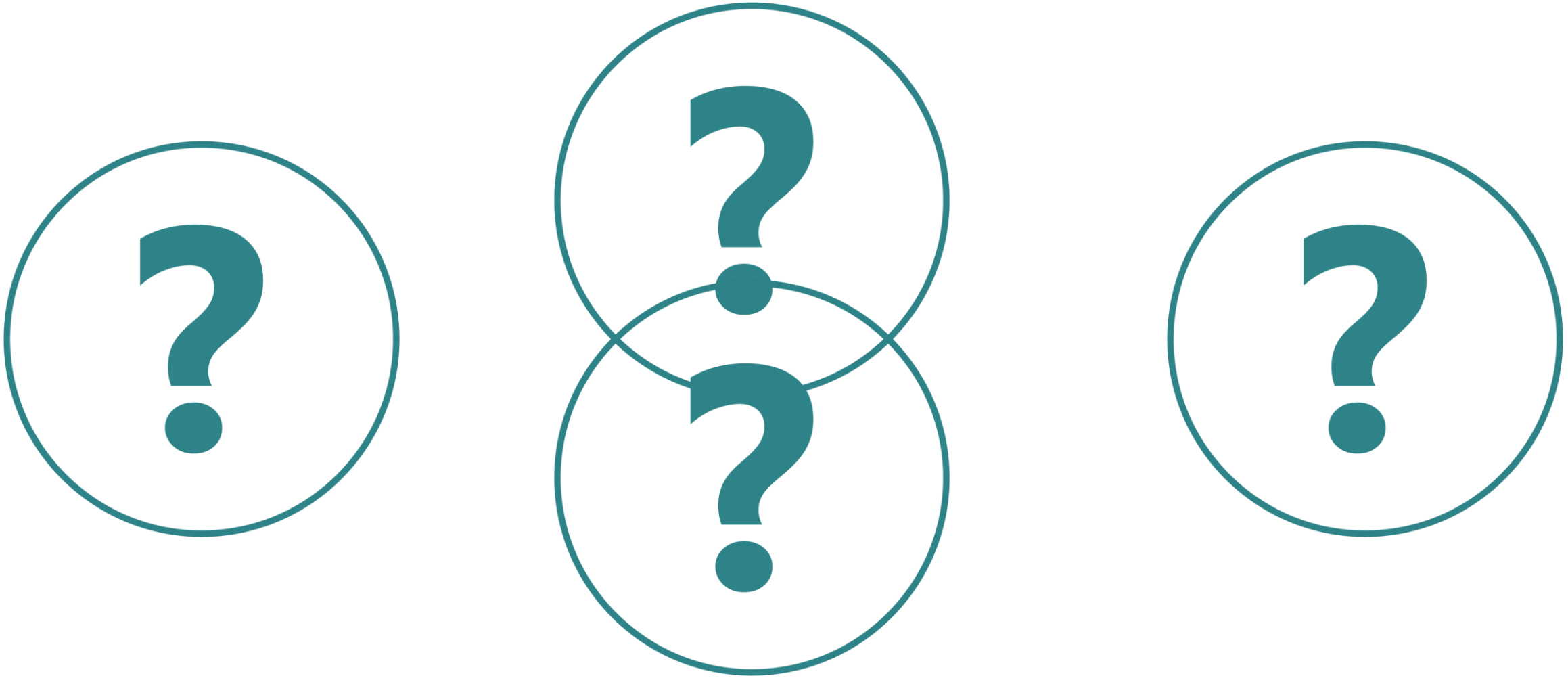

Method Development

3 different Methods

Community Reference Laboratories for Residues of Pesticides

Single Residue Methods
Version: Abamectin-V1

Analysis of Abamectin via QuEChERS and LC-MS/MS
- Brief Description -

Standards:

Abamectin: for example from Dr. Ehrenstorfer GmbH, Germany, Purity 95 % (91.5 % Avermectin B1a and 3.5 % Avermectin B1b)

Sample Preparation (QuEChERS):

- Weigh 10 g of the homogeneous, frozen sample into a 50 ml centrifugation tube
- Add 10 ml acetonitrile and 100 µl internal standard solution (e.g. triphenylphosphate c = 20 µg/ml); close the tube and shake vigorously for 1 min.
- Add 4 g Magnesium sulphate anhydrous, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate and shake for 1 min; centrifuge for 5 min at 3000 rpm
- Transfer an aliquot of the extract into a centrifugation tube which contains 25 mg PSA and 150 mg magnesium sulphate per mL extract; shake the tube vigorously for 30 s and centrifuge for 5 min at 3000 rpm.
- Fill an aliquot of the extract into a vial and employ for LC-MS/MS analysis

Analysis by LC-MS/MS *(Please consider: the LC-MS/MS data is exemplary)*

Instrument: API 3000, Applied Biosystems
Mode: ESI positive
The mass-transitions used are as follows:

- **Avermectin B1a:** 890.5 → 567.4; 890.5 → 305.1; 891.5 → 568.1
- **Avermectin B1b:** 876.6 → 553.3; 876.6 → 291.2;
- **Z-isomer of avermectin B1a:** 890.5→ 305.1; 890.5 → 567.3; 891.5 → 568.3

Column: Zorbax XDB Eclipse 150 x 2.1 mm; 3.5µ
Eluents:

- **A:** 950 mL water, 50 mL ACN, 10mmol ammonium formate brought to pH 4 with formic acid;
- **B:** 920 mL ACN and 80 mL water, 10 mmol ammonium formate (should be solved in water before diluting with ACN).

Gradient: The gradient starts with 30% B and goes to 100% B.
Flow: 0.3 ml/min
Injection volume: 10 µl

Notes:
High source temprature may reduce signals (better set 300 °C or below)
With the ammonium adduct being used as the mother ion for the MRM-transition the buffer concentration, as well as the pH, are of high importance.

Performance:
Validation data: see www.crl-pesticides-datapool.eu:
Pesticide names: Avermectin B1a, Avermectin B1b and Avermectin B1a, 8,9-Z
LOQ: 0,005 mg/kg for Avermectin B1a and B1b (using the instrument above)

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EURL-SRM EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

The extract is directly subjected to LC-MS/MS separation and measurement. Exemplary LC-MS/MS conditions are given in **Table 6**.

Table 6: Instrumentation and method details (LC: Agilent 1290 Infinity II; MS: Sciex QTrap 6500+)

Instrument parameters	Conditions					
Column/temperature	Phenomenex Aqua C18, 20x50 mm, 5 µm, 125 A at 40 °C					
Pre-column	Aqua C18 125A 4mm x 2mm (Phenomenex AJO-7510)					
OPTIONAL: Trap column *(in case of background contamination deriving from contaminated eluent components or other LC-consumables)*	Waters XBridge BEH C18, 2.1 mm X 50 mm, 5 µm, 130Å *Please be aware, that the column should also be compatible with the eluent conditions used for possible other methods run on this system!*					
Eluent A	5 mmol NH4-formiate in H2O + 0,01 % formic acid					
Eluent B	5 mmol NH4-formiate in methanol (MeOH) + 0,01 % formic acid					
Gradient	%A	Flow [mL/min]	Time [min]			
	95	0.4	0			
	50	0.4	2			
	40	0.4	8			
	10	0.4	12			
	10	0.4	14			
	95	0.4	14.5			
	95	0.4	21			
Injection volume	5 µL					
Acquired mass transitions (m/z)	Compound	Mass transitions and their MS-parameters				
		Q1 (m/z)	Q3 (m/z)	DP[1] (V)	CE[1] (V)	CXP[1] (V)
	BAC-C_8	248	156	36	25	25
		248	91	36	41	8
	BAC-C_{10}	276	184	55	27	10
		276	91	55	37	36
	BAC-C_{12}	304	212	91	29	10
		304	91	91	37	16
	BAC-C_{14}	332	240	83	31	10
		332	91	83	59	8
	BAC-C_{16}	360	268	78	33	12
		360	91	78	67	10
	BAC-C_{18}	388	296	31	33	8
		388	91	31	111	26
	DDAC-C_8	270	158	46	35	6
		270	43	46	61	2
	DDAC-C_{10}	326	186	61	39	12
		326	41	61	93	6
	DDAC-C_{12}	382	214	121	43	6
		382	58	121	67	4
	Chlorpyrifos-D_{10} (internal standard)	360	199	95	23	12
	Propyzamid-D_3 (internal standard)	259	193	61	21	10
	Optional:					
	BAC-C_{10} D_7	283	98	81	51	4
	BAC-C_{12} D_6	310	218	91	37	16
	BAC-C_{14} D_7	339	98	96	61	4
	DDAC-C_{10} D_6	332	192	96	41	10

EURL-SRM EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

EURL-SRM – Analytical Observations Report

concerning the following…

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- **Extraction Method(s)**: QuPPe
- **Instrumental analysis**: LC-MS/MS; SFC-MS/MS, LC-QToF

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Version 1 (last update: 14.04.2023)

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Method Development

- 3 very different Methods
- Not very much in common at the beginning

- Lots of different injections for a handful of analytes

Method Development

What does the methods have in common?

- 2 of 3 methods have a Quechers based extraction approach
- 3 of 3 methods should have some ammonium in the solvents
- 2 of 3 methods have analytes which do not have very much sensitivity with Sciex LC-MS/MS

Method Development

LC-MS/MS Instruments

- 3 Instruments at the lab [at that point]

- 1x Sciex 6500+ for pesticides in fruit + vegetables + feed + animal origin
- 1x Sciex QTrap 5500 for QuEPPe

- **1x Sciex QTrap 5500 for the rest**
  Sudan Dyes, Tropane alkaloids, QACs, Avermectins, Chloridazin + Chloridazon-desmethyl

Method Development

LC-MS/MS Instruments

- **1x Sciex QTrap 5500 for the rest**
  Sudan Dyes, Tropane alkaloids, QACs, Avermectins, Chloridazin + Chloridazon-desmethyl

- Every method needs a different solvent

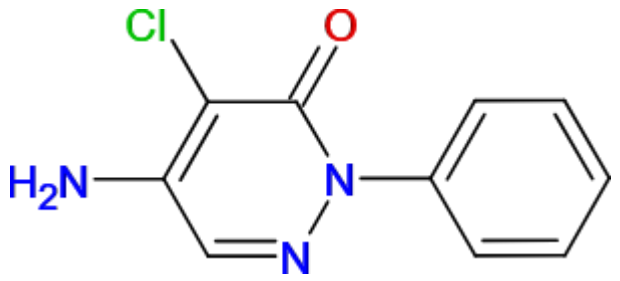
- Quit often to measure something of these samples

- Quit often to change the solvent

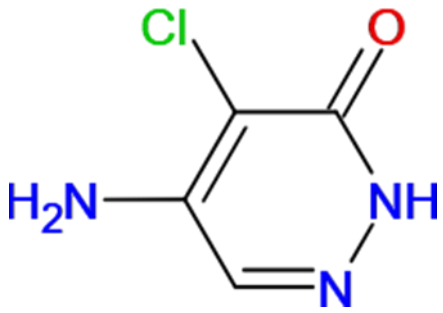
Method Development

- Starting with Chloridazon and Chloridazon-desmethyl

- Chloridazon



- Chloridazon-desmethyl



Method Development

Starting with Chloridazin-desmethyl

LC	Agilent 1290 Infinity II UHPLC						
MS/MS	SCIEX API 5500 Q-Trap, run in ESI positive mode						
Column	Acquity BEH Amide, 2x100 mm, 1.7 µm						
Pre-column	Acquity Van Guard BEH C_{18}, 2x5 mm, 1.7 µm						
Mobile Phase	A: 50 mmol NH_4formate in purified water (pH 3) B: acetonitrile						
Gradient	Time (min)		Mobile Phase A (%)		Mobile Phase B (%)		
	0		3		97		
	0.5		3		97		
	4.0		30		70		
	5.0		60		40		
	6.0		60		40		
	6.1		3		97		
	10		3		97		
Flow	0.5 mL min^{-1}						
Injection volume	2 µL						
Column temperature	40°C						
MRM-Transitions	Intensity ranking	Parent (m/z)	Daughter (m/z)	DP (V)	CE (V)	CXP (V)	
Chloridazon-desphenyl	1	146	117	120	31	6	
	2	146	66	120	51	2	
	3	148	119	120	31	6	
Chloridazon-desphenyl $^{15}N_2$ (IL-IS)	1	148	117	120	31	6	
	2	148	102	120	35	6	

Method Development

- Chloridazin-desmethyl – applying the same column

Method Development

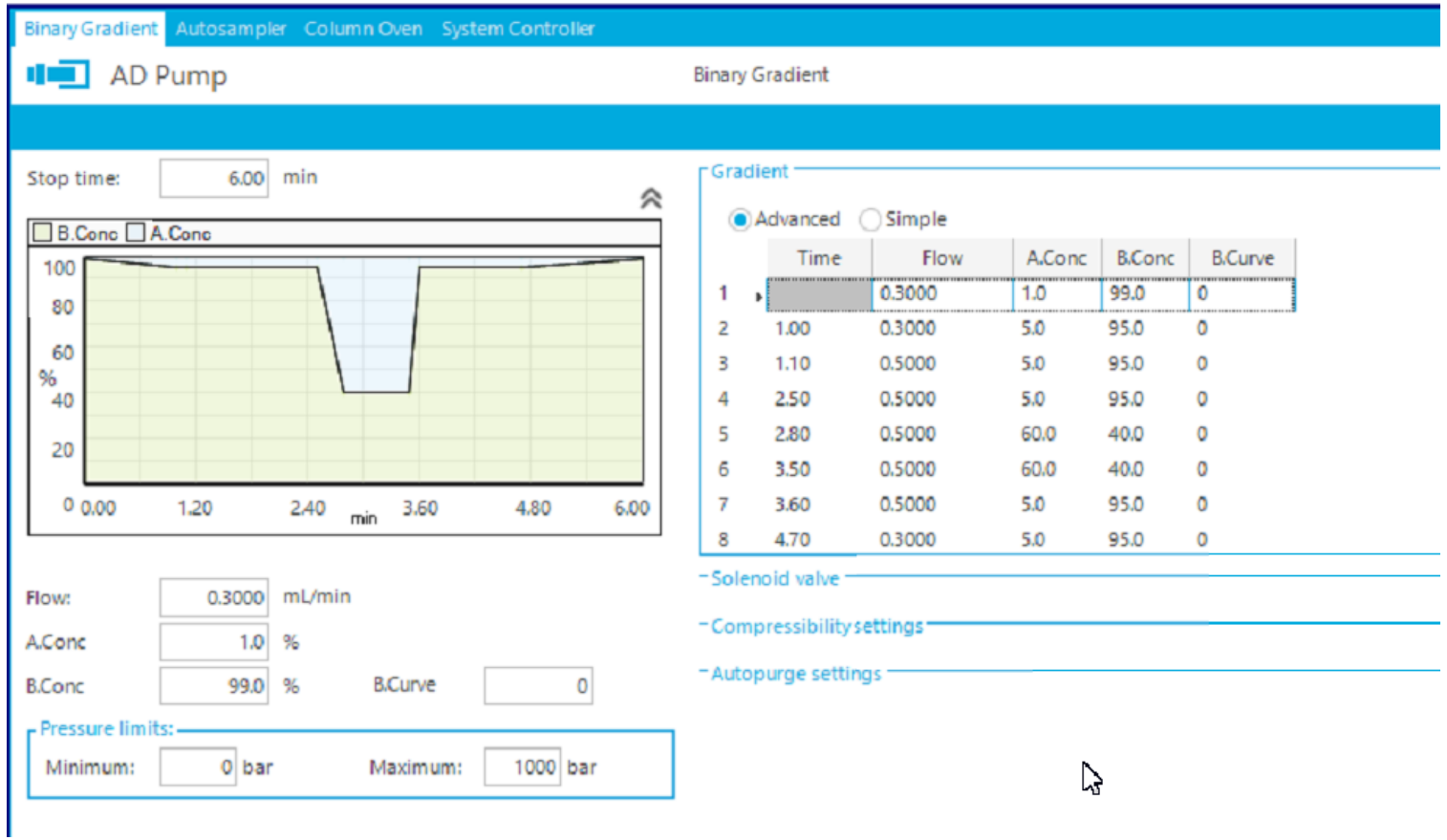
- Chloridazin-desmethyl – and change the solvents a little

Solvent A:
97,3%Water + 1,9% Acetonitril
0,4% Ammonium acetate solution + 0,4% formic acid

Solvent B:
99,8% Acetonitril + 0,2% formic acid

Method Development

- Chloridazin-desmethyl – and speed up a little



Method Development

- Chloridazon + Chloridazin-desmethyl

9	222.000	104.000	0.00	Chloridazon 1	Chloridazon		1	100000000.00	5.00	11.000	31.000
10	222.000	92.000	0.00	Chloridazon 2	Chloridazon		1	100000000.00	5.00	121.000	35.000
11	146.100	66.100	0.00	Chloridazon-Des	Chloridazon-Des		1	100000000.00	10.00	42.000	47.000
12	146.000	88.000	0.00	Chloridazon-Des	Chloridazon-Des		1	100000000.00	10.00	40.000	41.000

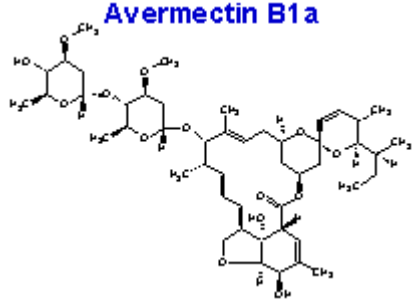
Method Development

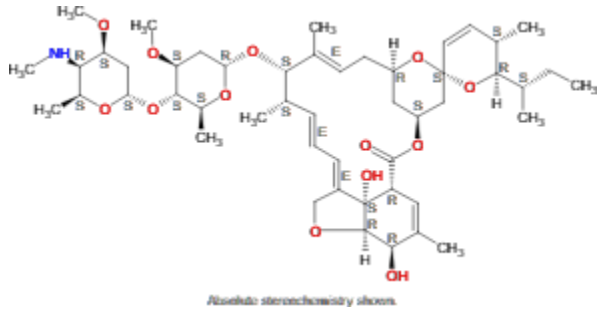
- Avermectins – adding some Avermectins because they need ammonia in the solvents
- Avermectin B1a

  Emamectin B1a

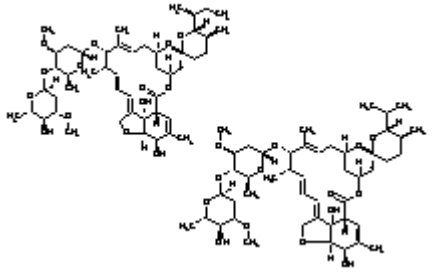
  Ivermectin B1a
- Azadirachtin

Avermectin B1a

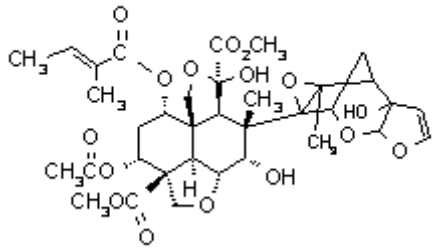




Ivermectin



Azadirachtin



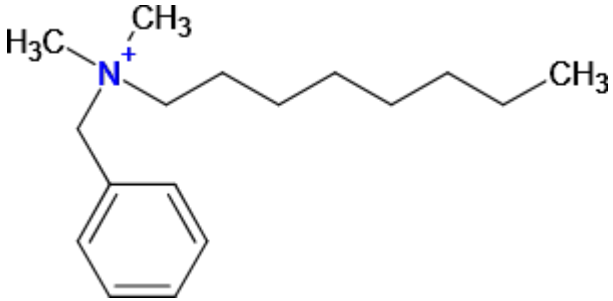
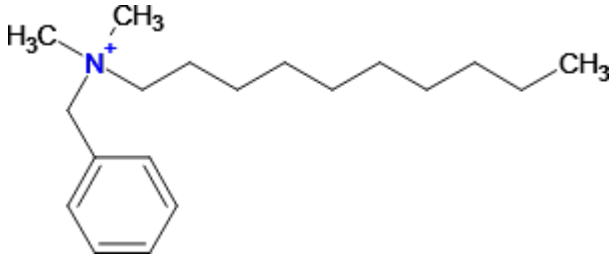
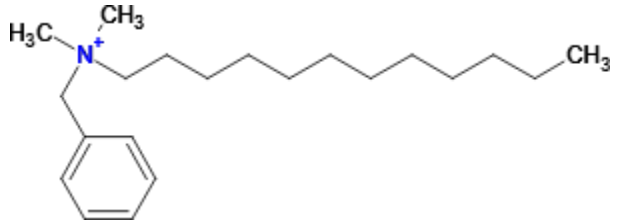
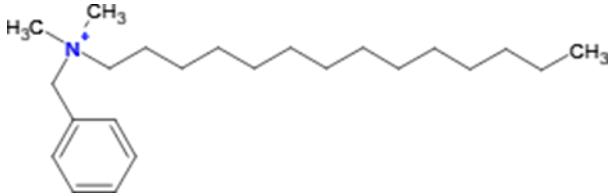
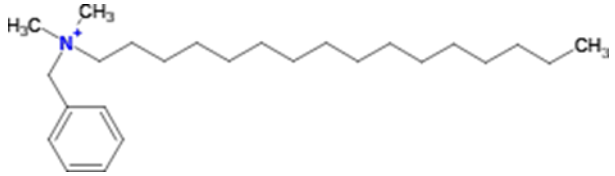
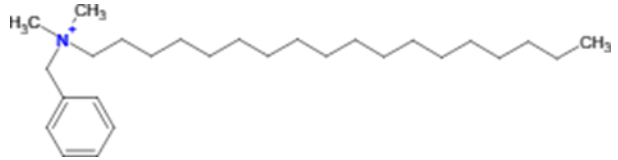
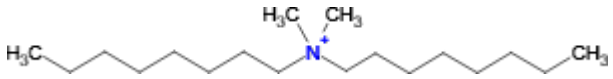
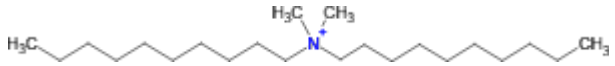
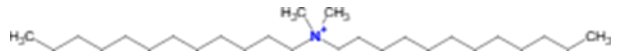
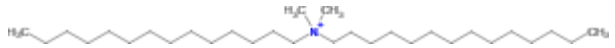
Method Development

- Avermectins – adding some Avermectins because they need ammonia in the solvents

	Q1 Mass (Da)	Q3 Mass (Da)	Retention Time (min)	ID	Group	MRM Window (sec)	Primary / Secondary	Trigger Threshold	Dwell Weight	DP (volts)	CE (volts)
1	890.500	305.200	0.00	Avermectin B1a	Avermectin B1a		1	100000000.00	10.00	81.000	37.000
2	890.600	567.300	0.00	Avermectin B1a	Avermectin B1a		1	100000000.00	10.00	61.000	19.000
3	738.300	567.100	0.00	Azadirachtin 1	Azadirachtin		1	100000000.00	10.00	41.000	23.000
4	738.300	585.100	0.00	Azadirachtin 2	Azadirachtin		1	100000000.00	10.00	41.000	19.000
5	886.300	82.100	0.00	Emamectin B1a 1	Emamectin		1	100000000.00	5.00	21.000	111.000
6	886.300	158.100	0.00	Emamectin B1a 2	Emamectin		1	100000000.00	5.00	21.000	41.000
7	892.400	569.400	0.00	Ivermectin B1a 1	Ivermectin B1a		1	100000000.00	10.00	61.000	23.000
8	892.600	307.000	0.00	Ivermectin B1a 2	Ivermectin B1a		1	100000000.00	10.00	71.000	37.000

Method Development

- And finally adding the QACs in the method
- BAC 8 – BAC 18 + DDAC 8 – DDAC 14

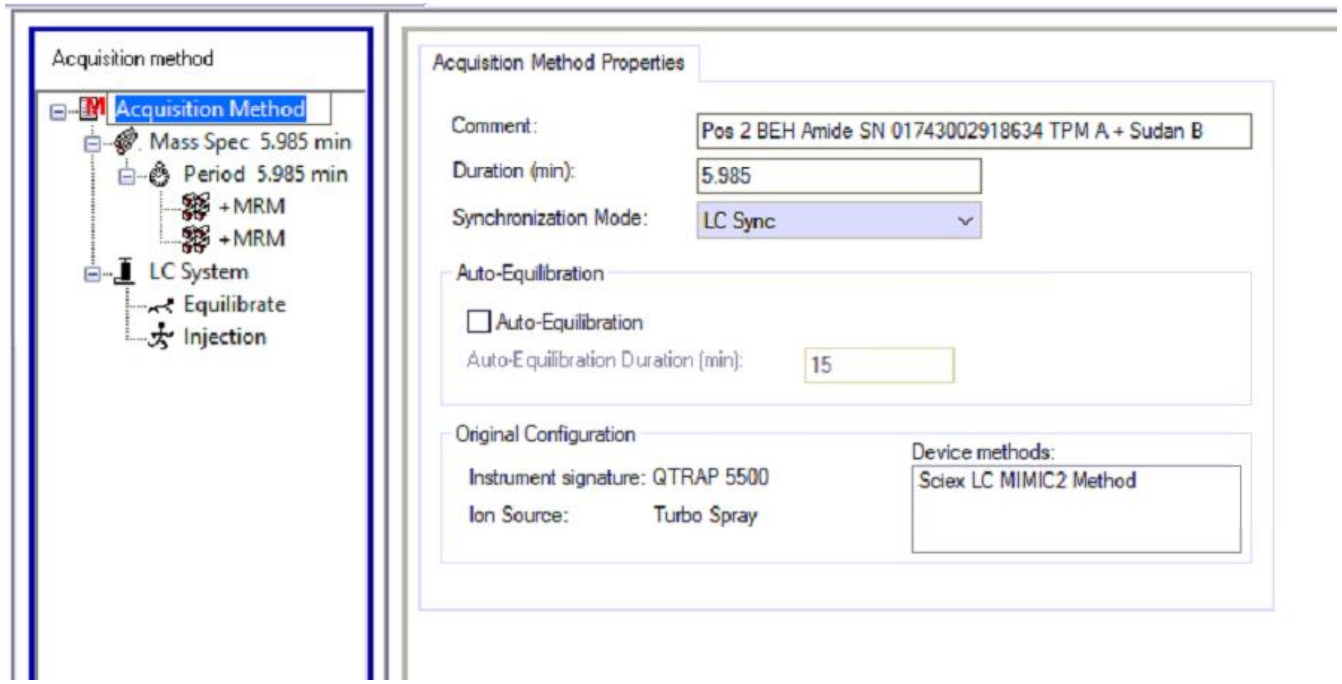
Method Development

QACs

6	248.000	156.000	0.00	CVUA BAC 8 1	CVUA BAC 8		1	100000000.00	1.00	83.000	31.000	10.000
7	248.000	91.000	0.00	CVUA BAC 8 2	CVUA BAC 8		1	100000000.00	1.00	83.000	59.000	8.000
8	276.000	184.000	0.00	CVUA BAC 10	CVUA BAC 10		1	100000000.00	1.00	78.000	33.000	12.000
9	276.000	91.000	0.00	CVUA BAC 10	CVUA BAC 10		1	100000000.00	1.00	78.000	67.000	10.000
10	304.000	212.000	0.00	CVUA BAC 12	CVUA BAC 12		1	100000000.00	5.00	31.000	33.000	8.000
11	304.000	91.000	0.00	CVUA BAC 12	CVUA BAC 12		1	100000000.00	5.00	31.000	111.000	26.000
12	332.000	240.000	0.00	CVUA BAC 14	CVUA BAC 14		1	100000000.00	5.00	46.000	35.000	6.000
13	332.000	91.000	0.00	CVUA BAC 14	CVUA BAC 14		1	100000000.00	5.00	46.000	61.000	2.000
14	360.000	268.000	0.00	CVUA BAC 16	CVUA BAC 16		1	100000000.00	5.00	61.000	39.000	12.000
15	360.000	91.000	0.00	CVUA BAC 16	CVUA BAC 16		1	100000000.00	5.00	61.000	93.000	6.000
16	388.000	296.000	0.00	CVUA BAC 18	CVUA BAC 18		1	100000000.00	3.00	121.000	43.000	6.000
17	388.000	91.000	0.00	CVUA BAC 18	CVUA BAC 18		1	100000000.00	3.00	110.000	47.000	13.000
18	270.000	158.000	0.00	CVUA DDAC 8	CVUA DDAC 8		1	100000000.00	1.00	110.000	47.000	13.000
19	270.000	43.000	0.00	CVUA DDAC 8	CVUA DDAC 8		1	100000000.00	1.00	110.000	47.000	13.000
20	326.000	186.000	0.00	CVUA DDAC 10	CVUA DDAC 10		1	100000000.00	7.00	110.000	47.000	13.000
21	326.000	41.000	0.00	CVUA DDAC 10	CVUA DDAC 10		1	100000000.00	7.00	110.000	47.000	13.000
22	382.000	214.000	0.00	CVUA DDAC 12	CVUA DDAC 12		1	100000000.00	1.00	110.000	47.000	13.000
23	382.000	58.000	0.00	CVUA DDAC 12	CVUA DDAC 12		1	100000000.00	1.00	110.000	47.000	13.000

Method Development

- Because of the differences in the sensitivity in the analytes there are 2 Experiments applied



Method Development

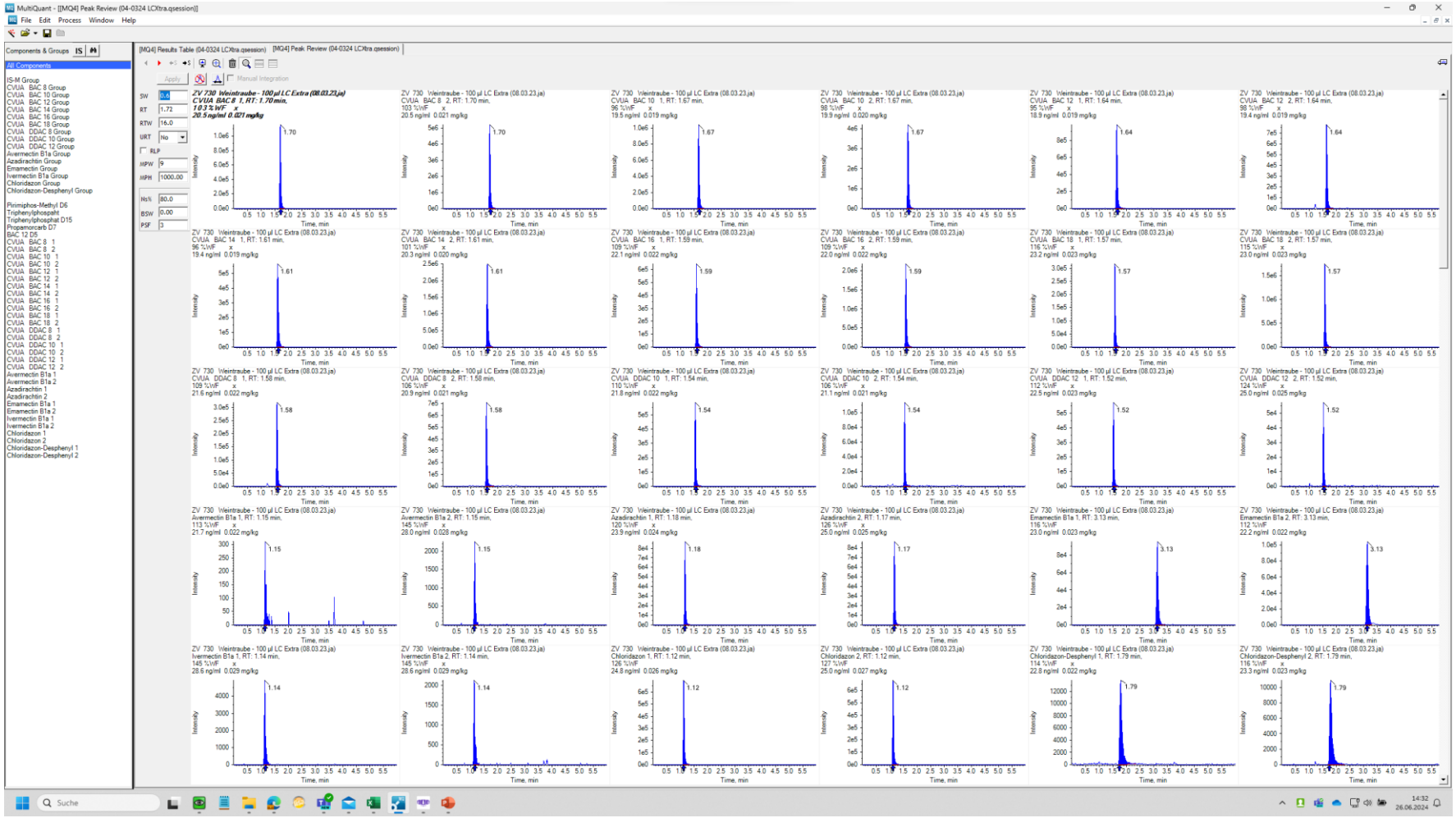
- And everything is working great

Method - Discussion

- The method is fully validated and accredited in fruits, vegetables and grain
- The MRRL is validated at 0,010 mg/kg
- Except Abamectin @ Apples and Pears
  Even the low MRRL of 0,006 mg/kg is applied
- We are allowed to report the results

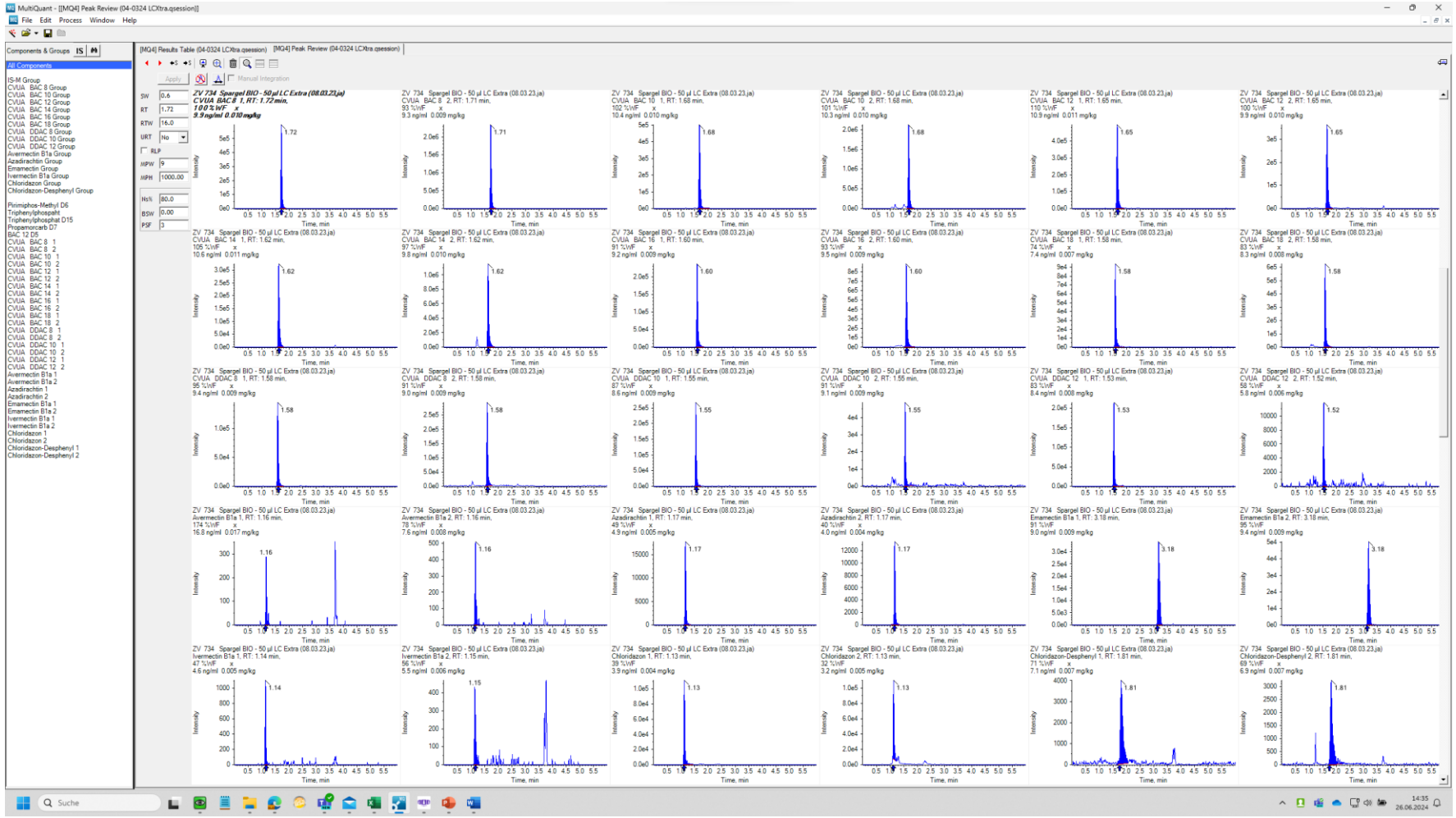
Method - Discussion

- Some Chromatograms: Grapes QC @ 0,020 mg/kg



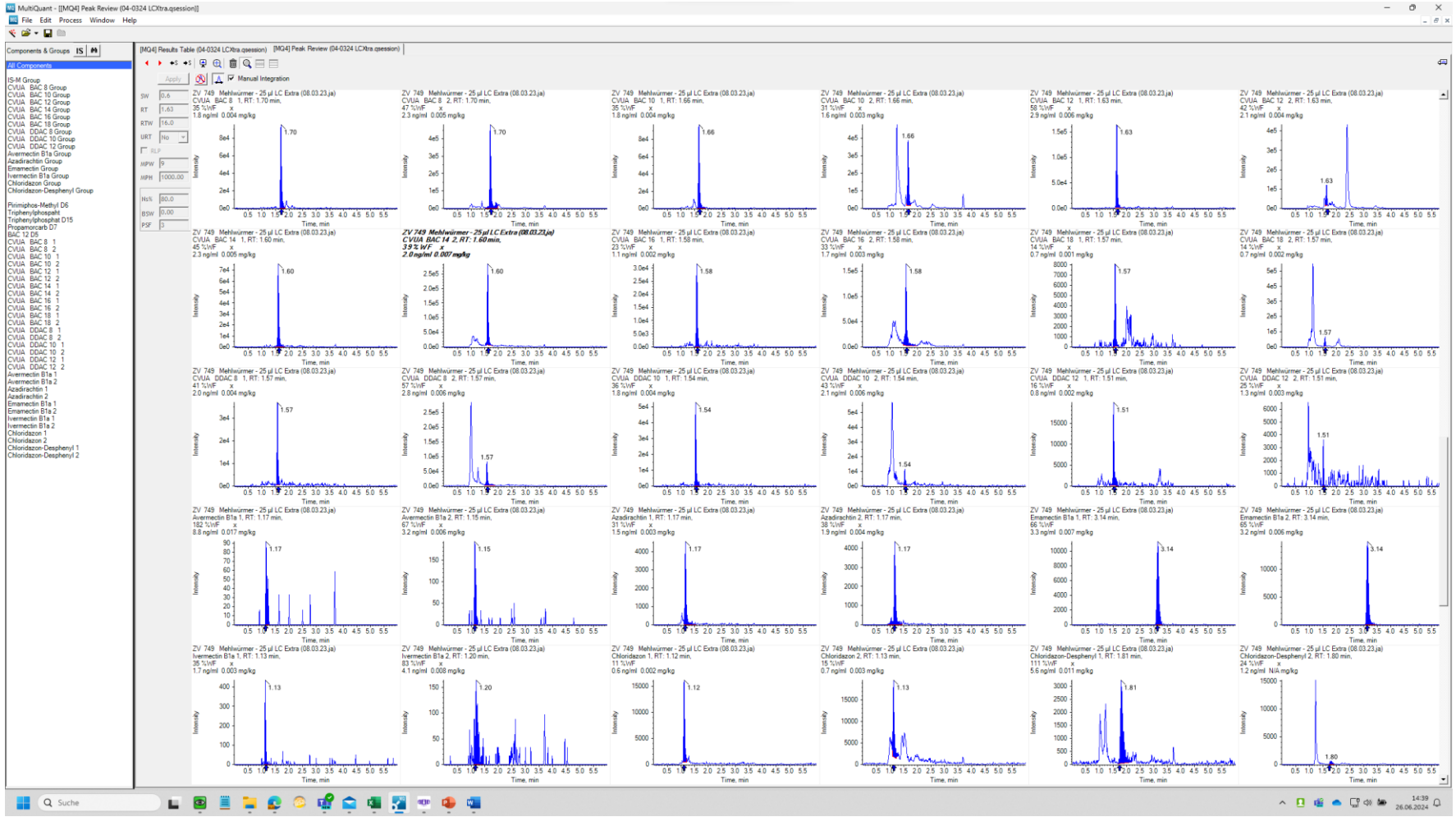
Method - Discussion

- Some Chromatograms: Aspargus QC @ 0,010 mg/kg



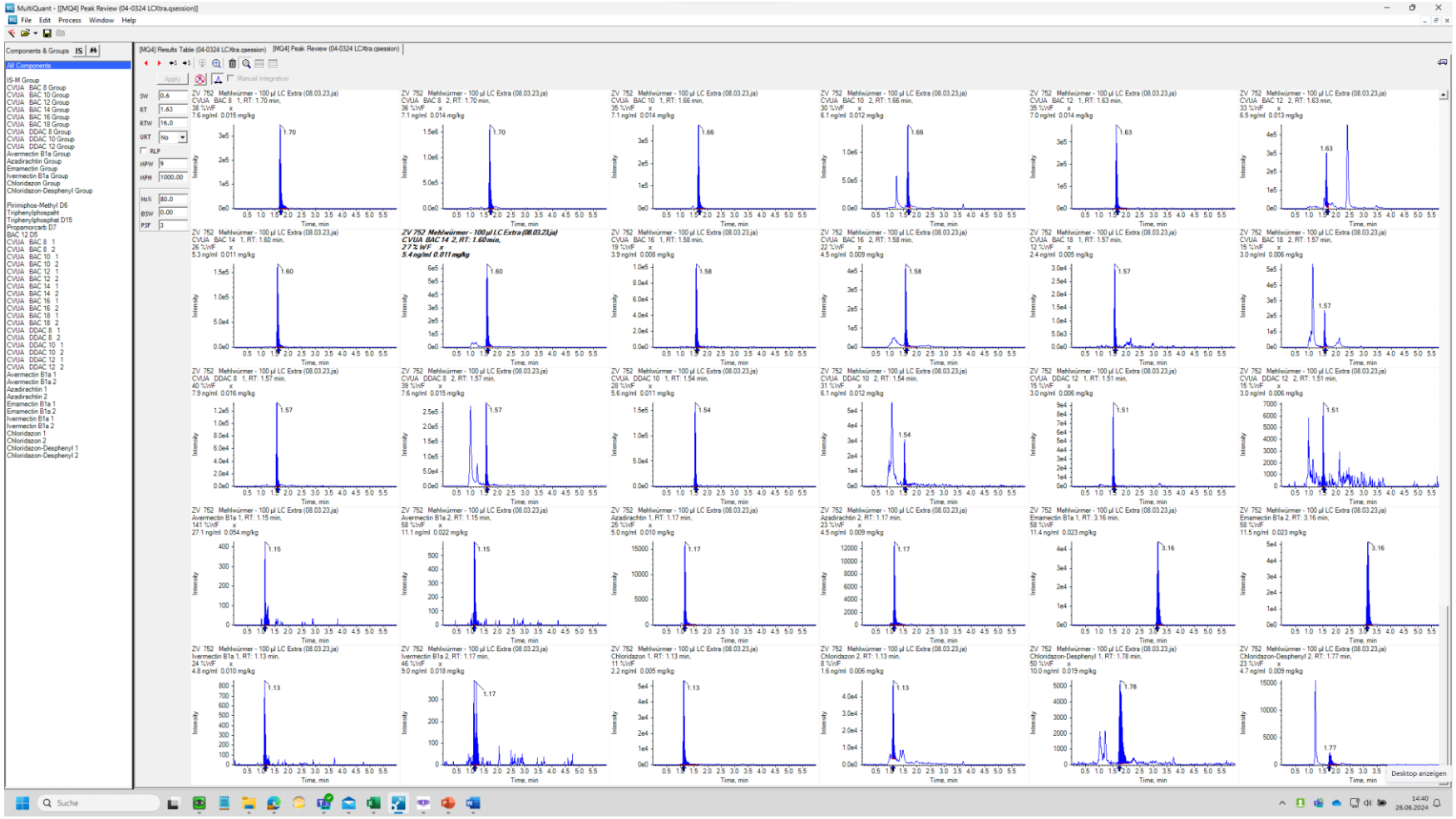
Method - Discussion

- Some Chromatograms: Worms QC at 0,010 mg/kg



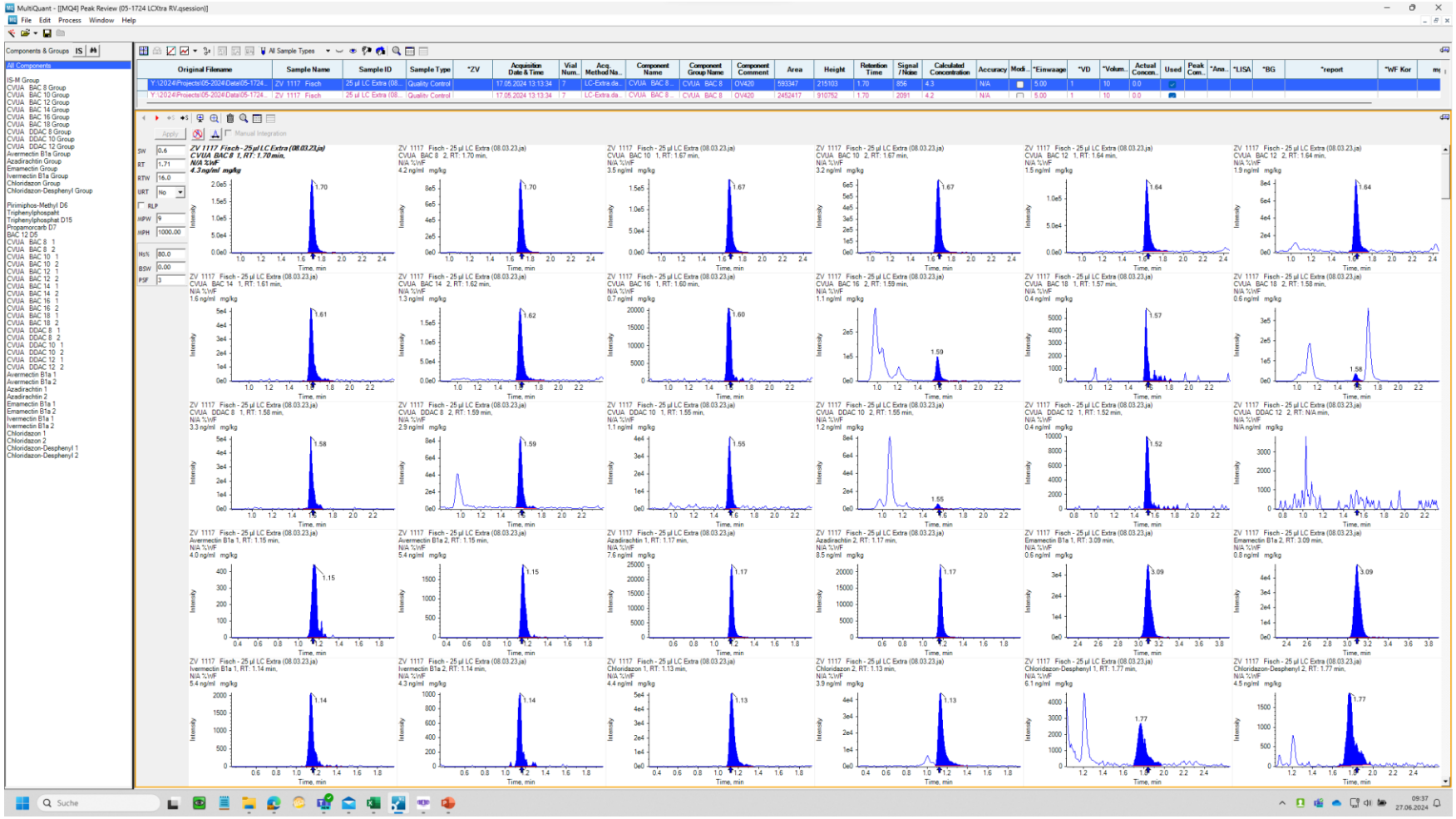
Method - Discussion

- Some Chromatograms: Worms QC at 0,040 mg/kg



Method - Discussion

- Some Chromatograms: some Fish QC at 0,010 mg/kg – for the PT

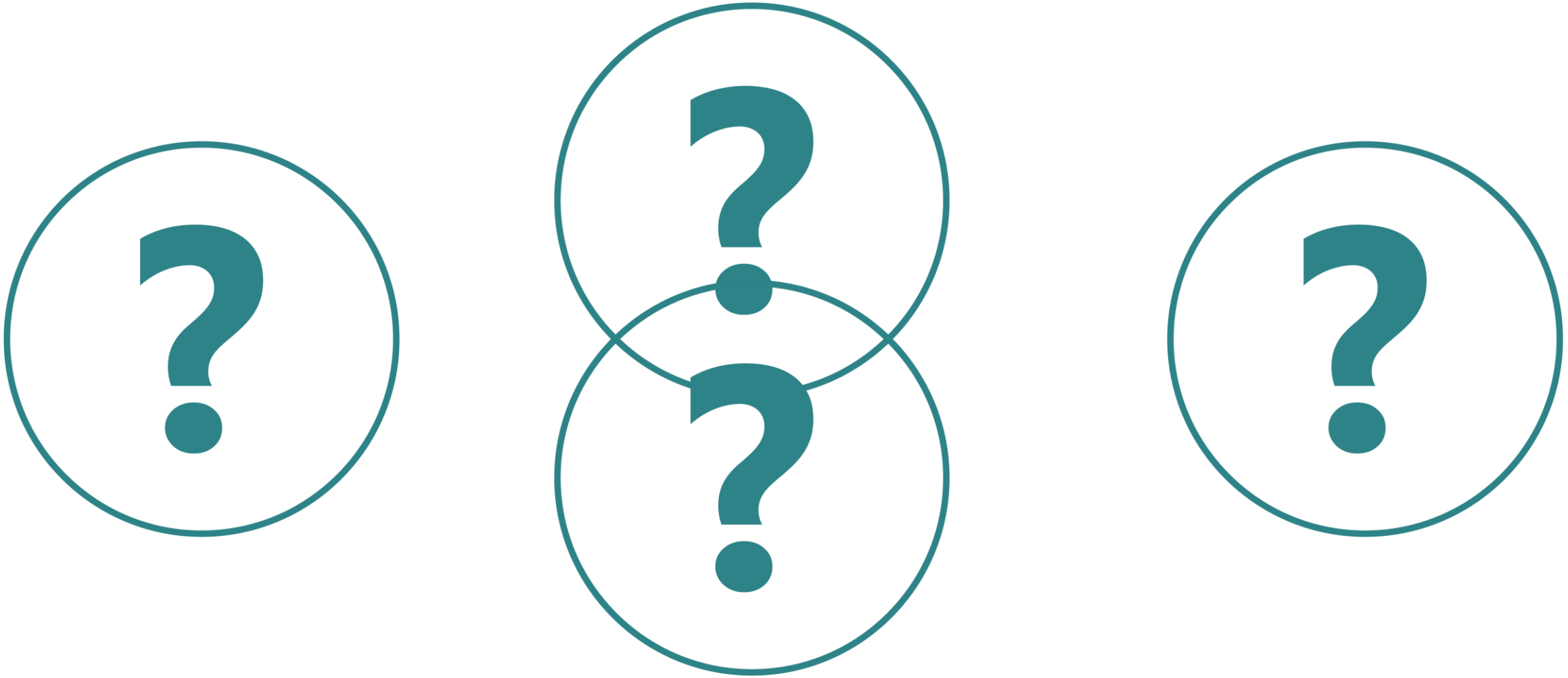


Method - Discussion

- And everything is working great

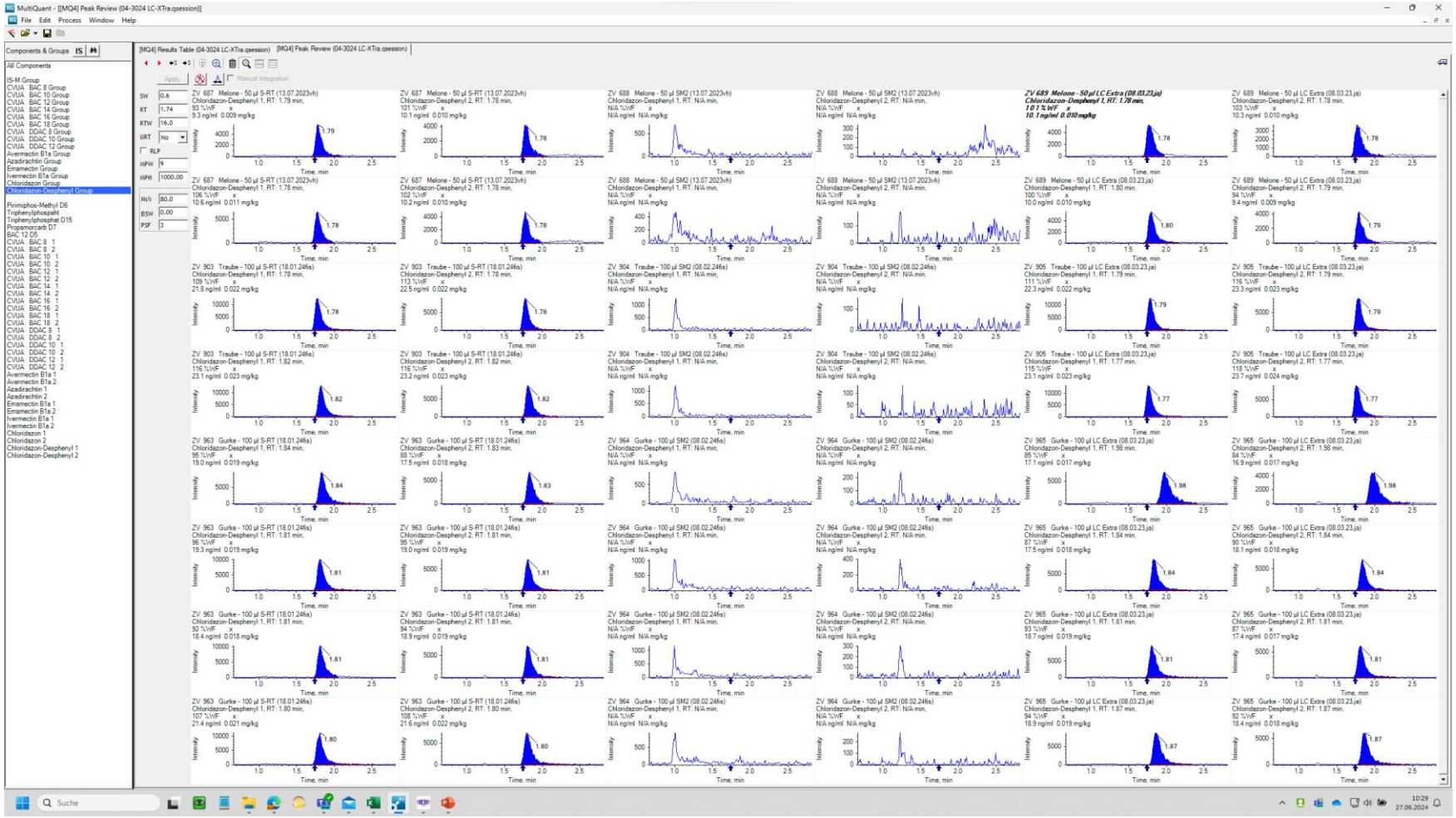
Method - Discussion

And everything is working great



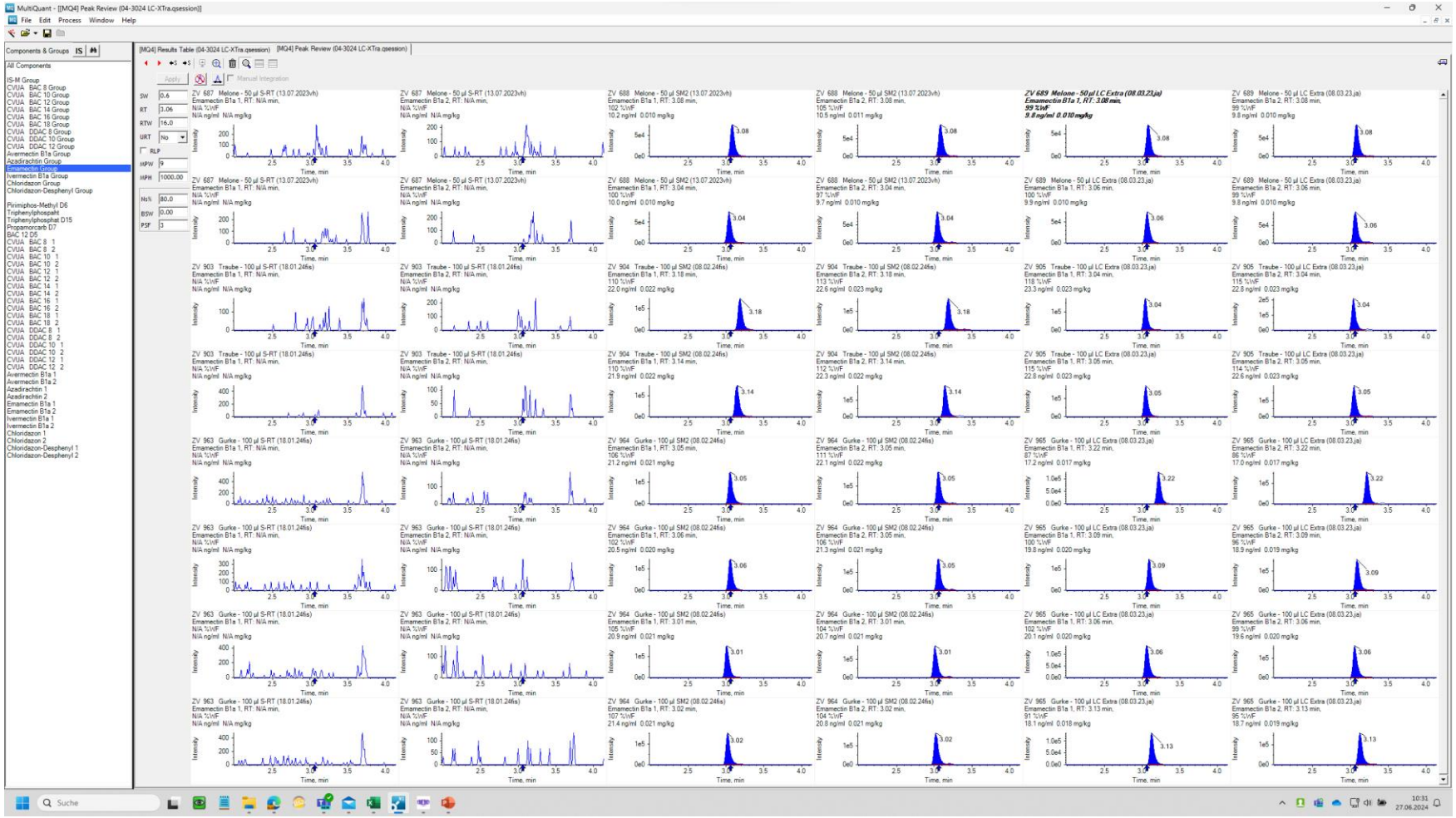
My already known issues

- Shifting Retention Time for some analytes in different matrices



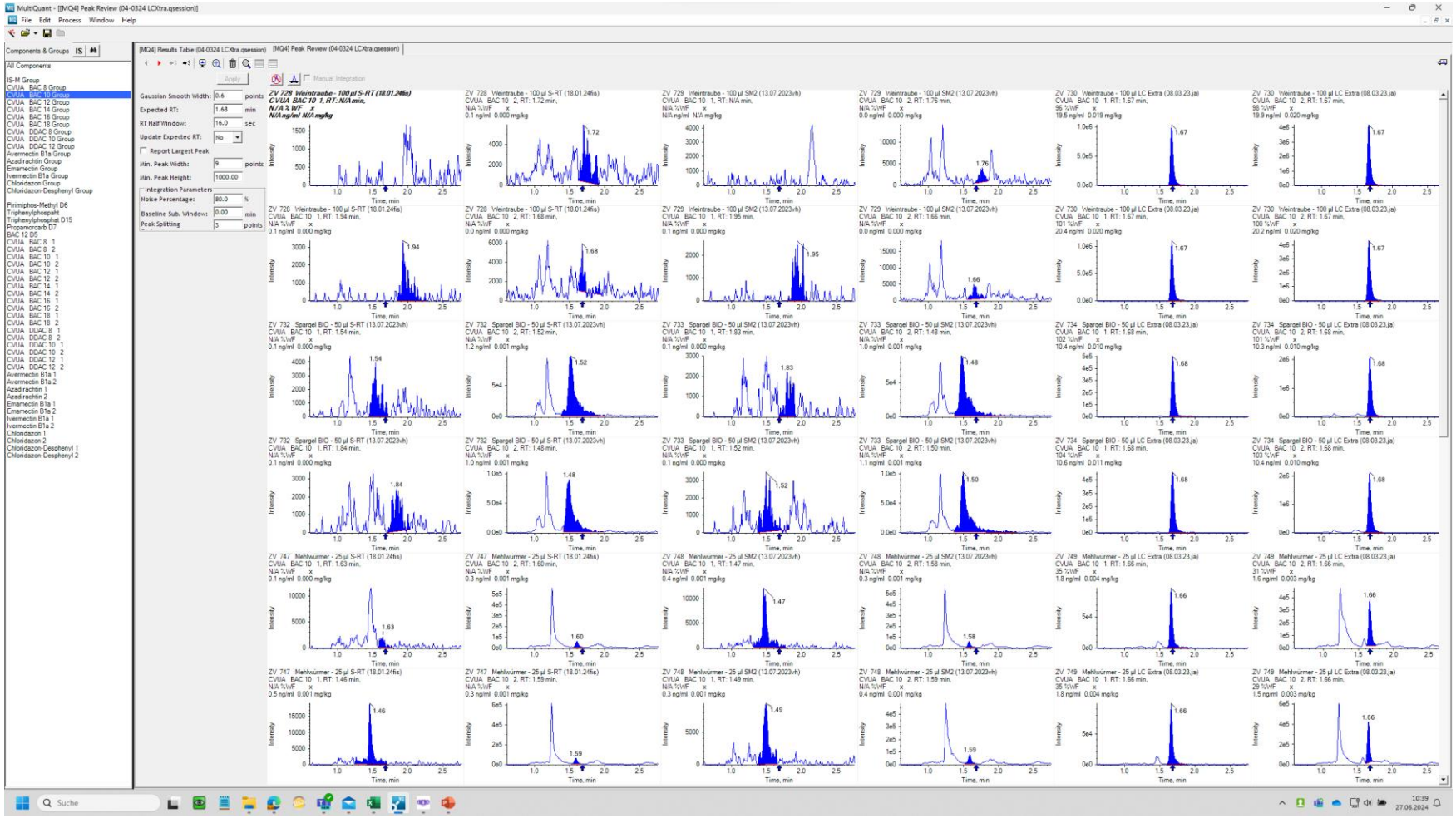
My already known issues

- Shifting Retention Time for some analytes in different matrices



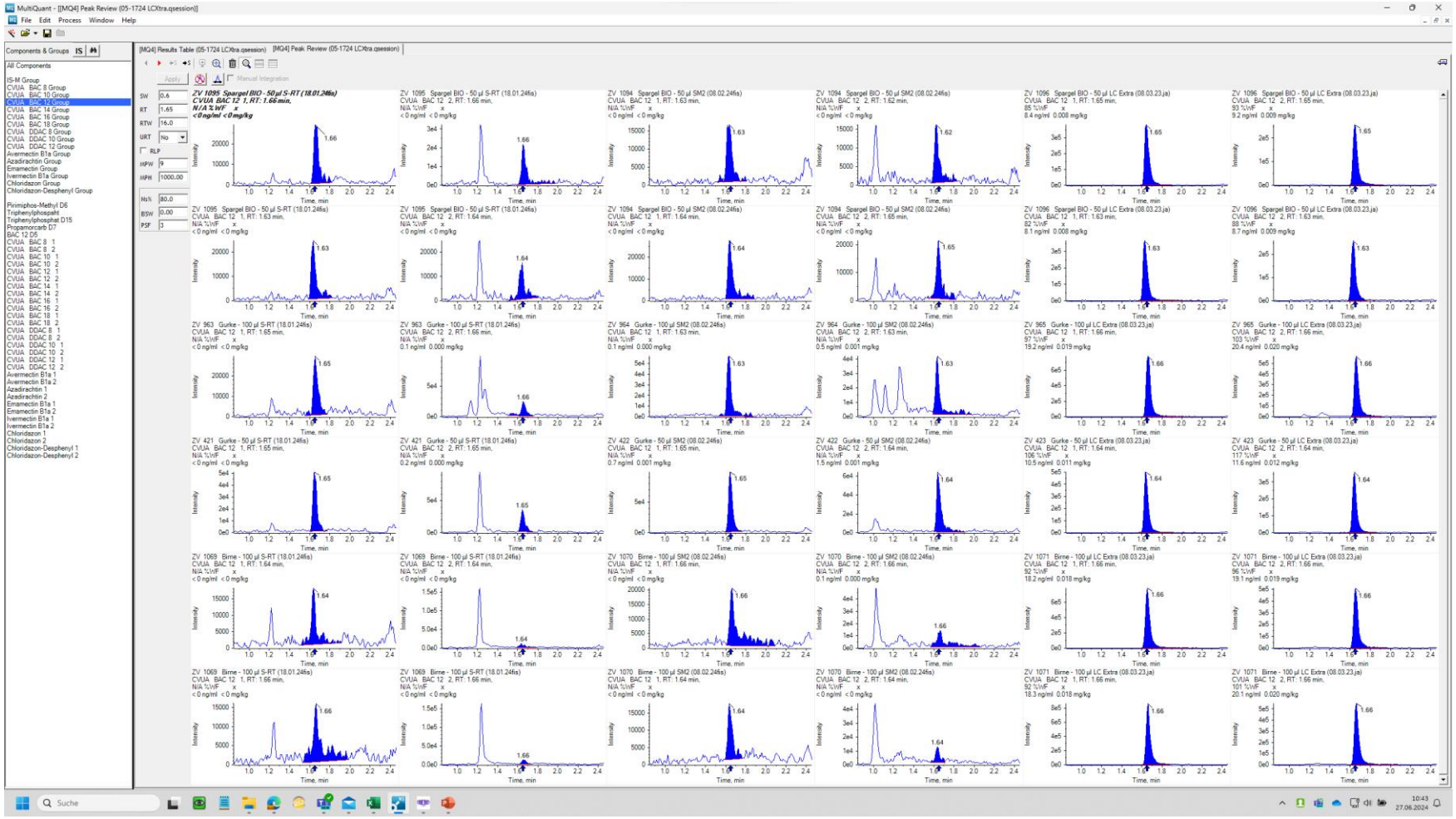
My already known issues

- Different responses in different matrices



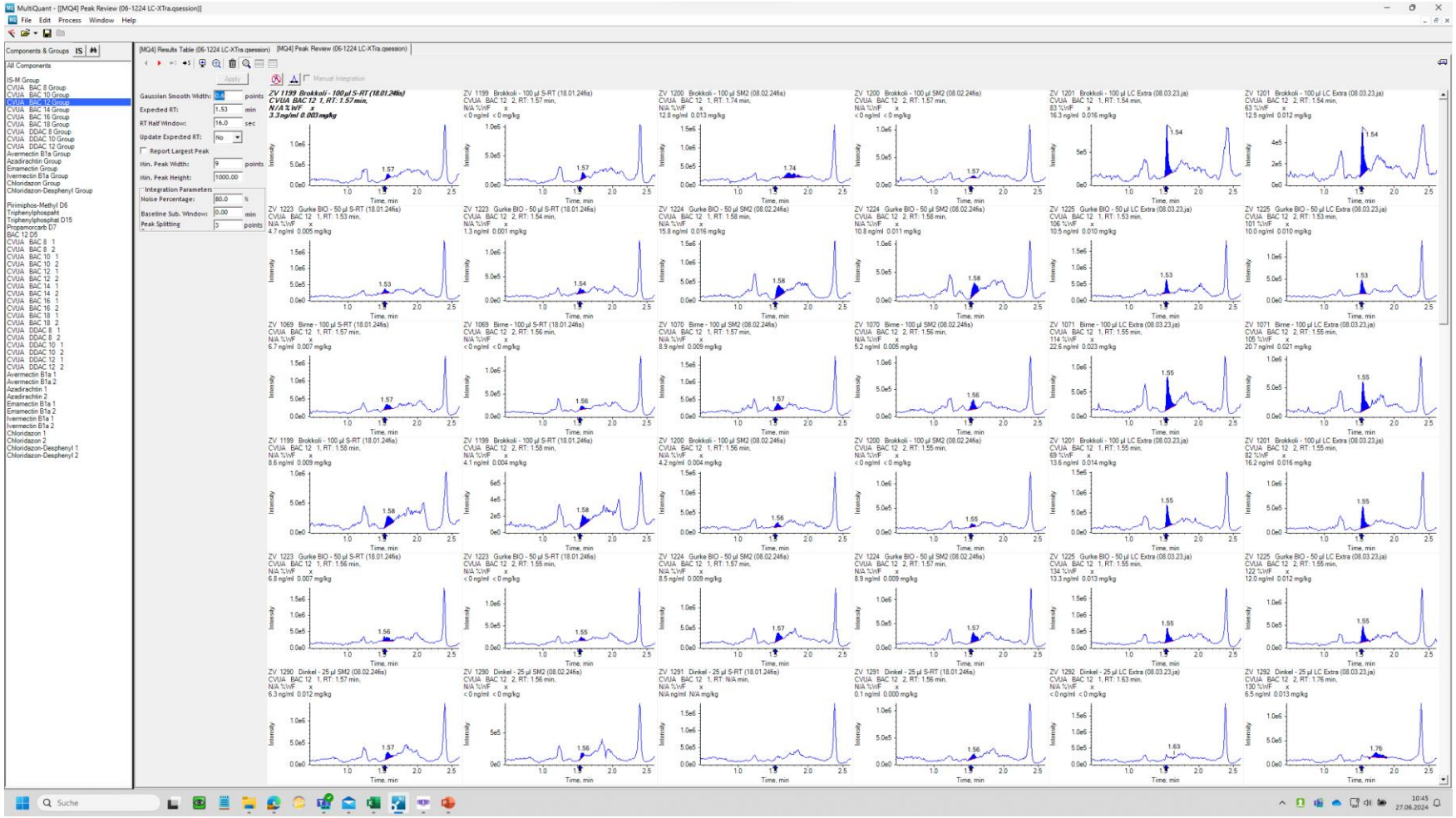
My already known issues

- And suddenly some surprise



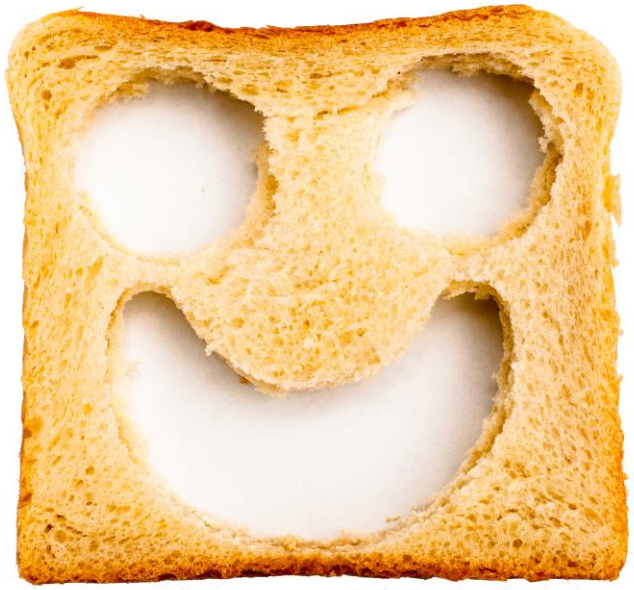
My already known issues

- And suddenly some surprise



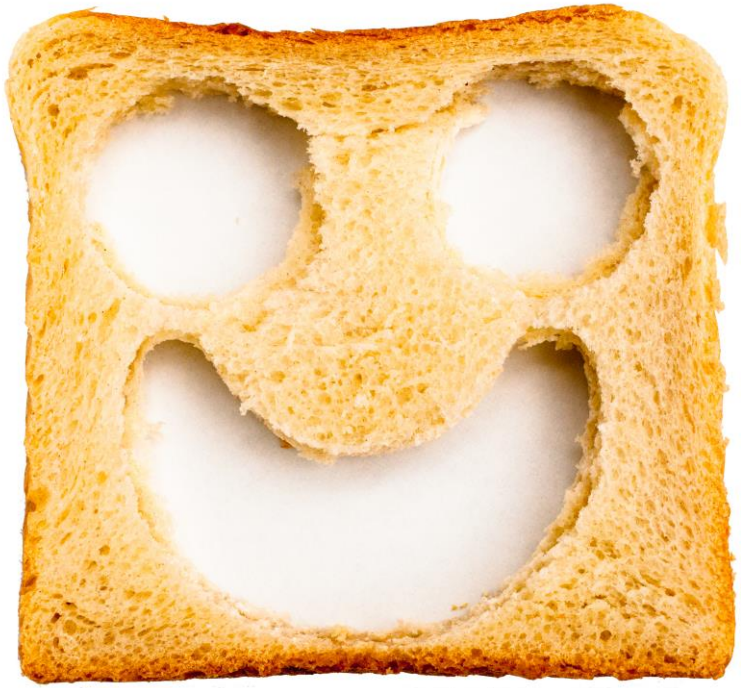
My Summary

- ~~And everything is working great~~



My Summary

- It is a good method to work with
- With the known limitation



Thank You

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