Joint EURL Workshop, Almeria 2021

News from EURL-SRM

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21.10.2021

Overview of Current Activities

Michelangelo Anastassiades

Interlaboratory validation study round 3 - Glyphosate & Co.

Ann-Kathrin Wachtler

Facing analytical challenges DTC analysis – step by step

Hubert Zipper

Pesticides -Online

EURL-Website

EURL-DataPool

COM / EFSA
Revisions; Opinions; Meetings …

NRLs, OfLs, other labs
Workshops, Trainings, E-mails …

Data-Management + Dissemination

Technical Assistance



Proficiency Tests

Planned EUPTs

Ad-hoc PTs

Method Development/Validation

Planned + Ad-hoc Projects (Work Progr.; Art12 …)

Interlab. Validations

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations

MRL-Review (periodic): Art. 12 of Reg. 396/2005/EC

MRL-Review in case of **exposure concerns**: Art. 43 of Reg. 396/2005

Renewal Assessments: Art. 12 of Reg. 1107/2009/EC (Reg. 844/2012/EC)

Other (e.g. background levels eval., Substances not requiring MRLs …)

EURL-Input

EFSA MS-Consulation	EFSA Draft RO	COM Consulation

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations

- **Check proposed RD**
  (monitorability = analytical feasibility, specificity, plausibility (look at real residue data) -> suggest improvements if needed
- **Propose analytically feasible LOQs** for main commodity groups
- Check **commercial availability of standards**
- **Draft Evaluation Report** and submit to EFSA
- **Comment draft Reasoned Opinion** (drRO)

Technical Assistance to COM/EFSA

Pesticide (re-)evaluations

In 2019-20 alone

→ **177 requests** (COM / EFSA), concerning …

→ **124 active substances**

Requests by EFSA	Requests by COM
coordinated by	coordinated by
EURL-SRM	EURL-SRM & EURL-CF

Technical support to DG-SANTE and EFSA

Evaluation / re-evaluation of pesticides (Context: Art.12 of Reg. 396/2005 and other)

Substance	Request				Experiments conducted			
	EFSA		COM					
	ER	dRO	LOQs	other	Mthd. dev.	Metabolite(s) to consider	Standard Valid.(s)	Hydrolysis Valid./Exp.
1,2-Dibromoethane			X					
1,2-Dichloroethane			X					
1,4-Dimethylnaphtalene				X				
1,4-Dimethylnaphthalene	X				X	X	X	X
6-BAP		X						
6-Benzyladenine	X	X	X					
8-Hydroxyquinoline	X				X		X	X
Abscisic Acid				X				
Acequinocyl	X	X					X	X
Aldrin/Dieldrin			X					
Ametoctradin	X	X	X					
Aminopyralid	X	X	X		X		X	X
Amisulbrom	X	X	X				X	
BAC			X					
Benalaxyl/Benalaxyl-M		X	X					
Biphenyl				X				
Bixafen	X	X	X			X	X	
Boscalid				X				
bromide				X				
Bupirimate		X	X					
Cadusafos				X				
Captafol			X					
Carfentrazone-ethyl			X					
CCl4			X					
Chlorantraniliprole		X	X					
chlorate				X				
Chlordane			X					
Chlordecone			X					
Chromafenoxide			X					
Clethodim			X					
CS2-moieties				X				
Cyanric acid				X				
Cycloxydim		X	X			X	X	
Cyflumetofen	X						X	
Cyproconazol	X							
Dazomet			X					
DDAC			X					
DDT and isomers			X					
Demeton-S-methyl				X		X		
Dichlobenil			X					
Diclofop			X					
Diflubenzuron (a.s. that do not require an Art.12 review)	X							
Dinoseb			X					
Disodium phosphonate	X							
Emamectin		X	X				X	
Endosulfane and isomers			X					
Endrin			X					
Epoxiconazole				X				
Ethoprophos			X	X				
Ethylene	X							
Fenazaquin		X	X					
Fipronyl				X				
Fipronyl-desulfinyl				X				
Florpyrauxifen	X							
Flubendiamide	X	X	X					
Flufenoxuron	X							
Fluometuron			X					
Fluopicolide		X	X					
Fluopyram		X	X					
Fluxapyroxad		X	X					
Gibberelic acid				X				
Heptachlor			X					

Substance	Request				Experiments conducted			
	EFSA		COM					
	ER	dRO	LOQs	other	Mthd. dev.	Metabolite(s) to consider	Standard Valid.(s)	Hydrolysis Valid./Exp.
Hexythiazox			X					
Hymexazol			X					
Hymexazole		X						
Imazaquin (a.s. that do not require an Art.12 review)	X							
Imidacloprid			X					
Indolylacetic acid				X				
Indolylbutyric acid				X				
Ipconazol			X					
Ipconazole	X	X						
Isoflucypram				X			X	
Isopyrazam	X							
Lime sulfur	X							
Maltodextrin	X							
Mefentrifluconazole	X							
Meptyldinocap	X	X	X		X		X	
Metaflumizone	X	X	X			X	X	
Metam			X					
Metamitron	X	X	X					
Methiocarb			X					
Methoxychlor			X					
Metobromuron	X					X	X	
Mirex (Pentachlordecone)			X					
nicotine				X				
Nitrofen			X					
Novaluron	X							
Organochlorine Pesticides (POPs)				X				
Oxadiazon (a.s. that do not require an Art.12 review)	X							
Oxyfluorfen	X	X						
Pencycuron			X					
Penflufen		X	X					
Pentachlorbenezene			X					
Penthiopyrad	X					X	X	
Perchlorate				X				
Phopshonate				X				
Phthalimide				X				
Pinoxaden	X					X	X	X
Potassium phosphonates	X							
Propineb		X	X	X	X		X	
Propoxur				X				
Proquinazid		X	X				X	
Pyridalyl		X	X					
Pyriofenone		X	X					
Pyroxsulam	X	X					X	
Quaternary amonium compounds				X				
Quinmerac	X	X			X	X	X	X
Quinoxyfen	X	X						
Quintozene			X					
Sedaxane			X					
Spinetoram	X	X	X					
Spirotetramat		X	X			X		
Sulfuryl fluoride	X	X			X		X	
Tau-Fluvalinate			X					
Tefluthrin		X	X					
Terbutylazine	X	X	X			X	X	
Thiencarbazone	X	X	X					
Thiocyanate				X				
Thiram	X	X			X	X	X	
Triazole-derivative metabolites				X				
Triazoxide			X					
Trifluoroacetic acid				X				
Trimethylsulfonium cation				X				
Valifenalate	X					X	X	

Evaluation Report

Prepared under Article 12 of Regulation (EC) No 396/2005

30 October 2019

Example Quinmerac

Analytical validations by the EURLs and capability of official laboratories to be considered for the review of the existing MRLs for quinmerac

Draft-RO

Circulated for Consultation

EU-MSs and EURLs

REASONED·OPINION¤

APPROVED:·dd·Month·20YY¤

doi:10.2903/j.efsa.20YY.NNNN·¤

Review·of·the·existing·maximum·residue·levels·for·quinmerac·according·to·Article·12·of·Regulation·(EC)·No·396/2005¶

European·Food·Safety·Authority·(EFSA)¶

Authors'·list·¶

·¶

-DRAFT-¶

Example Quinmerac

Report on the MS Consultation for EFSA-Q-2009-00072

EFSA Question Number: EFSA-Q-2009-00072
Type of assessment: MRL Review (Reg. 396/2005 – Art. 12.1)
Active Substance: Quinmerac
Subject: Review of the existing MRLs for quinmerac
Commenting period started on: 26 June 2020
Commenting period ended on:
Comments received from:
Comments evaluated by EFSA on:
Pesticides MRL Expert Meeting on:
Document finalised on:

Example Quinmerac

No.	Reference	MS Comment		EFSA Consideration	Discussion in Pesticides MRL Expert Meeting (if applicable)	Conclusion
1.	Section Summary, Section 1.1.4, Section 1.1.6 & Conclusions	EURLS	Please take notice of the updated Evaluation Report on analytical validations by the EURLs (original report: 30 October 19; updated report: 17 July 20). It includes new validation data on quinmerac (free acid) as well as on the metabolites BH-518-2 and BH-518-4, which are included in the proposed residue definition for foods of plant origin. For these two metabolites, an SRM method with acidified extraction (QuEChERS-based) was employed.			

1,4-Dimethylnaphthalene

Sprouting inhibitor
Replacement for Chlorpropham,
that lost its approval

COMMISSION REGULATION (EU) 2015/399 of 25 February 2015:

(1) …1,4-dimethylnaphthalene … no specific MRLs were set in these Annexes, nor were the substances included in Annex IV to that Regulation, so the default value of **0,01 mg/kg** applies

Potato: 15 ppm
All other products: 0.01 ppm

0200000	2. VEGETABLES FRESH OR FROZEN	
0210000	(i) **Root and tuber vegetables**	
0211000	(a) *Potatoes*	**15**
0212000	(b) *Tropical root and tuber vegetables*	

1,4-Dimethylnaphthalene

Need to check problems with analytical selectivity:

There are 10 DMN congeners

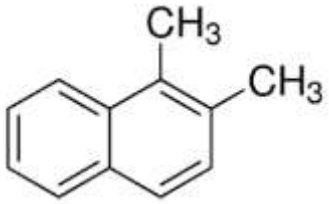
► Need to check whether any of them co-elutes with DMN

► If yes ► Limited specificity of analysis

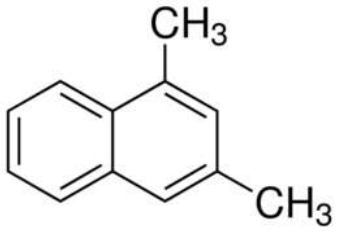
Problems with background

- **Component of petroleum (used as a solvent/adjuvant in PPPs)**
- **Component of combustion gasses**
- **Component of "Mineral Oils" ► contaminated paper (MOA-fraction)**
- **Various DMNs naturally formed in plants or during pyrolysis of carotenoids;**

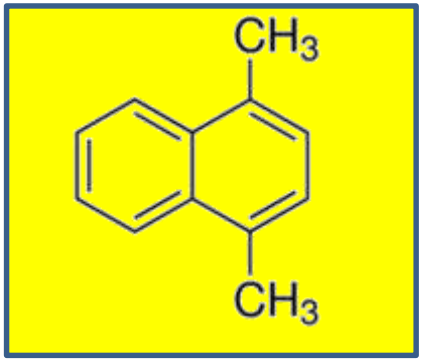
Serban C. Moldoveanu, in Pyrolysis of Organic Molecules (Second Edition), 2019
Song, J., Chen, J., Li, D. et al. ; Food Bioprocess Technol 11, 836–844 (2018).



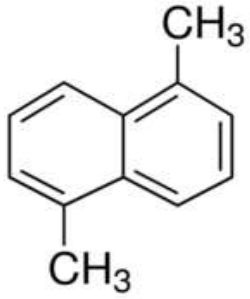
1,2-Dimethylnaphthalene



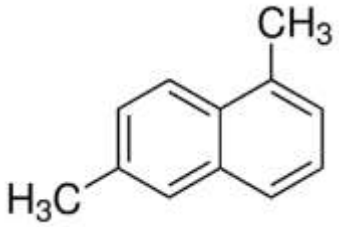
1,3-Dimethylnaphthalene



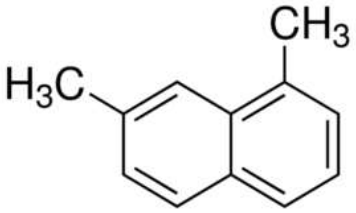
1,4-DMN



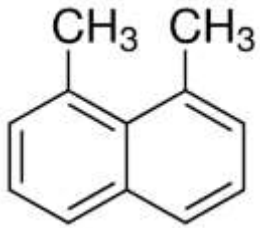
1,5-Dimethylnaphthalene



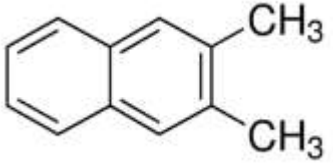
1,6-Dimethylnaphthalene



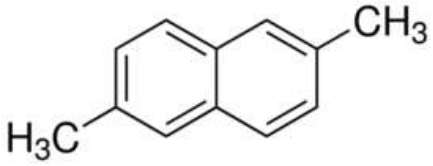
1,7-Dimethylnaphthalene



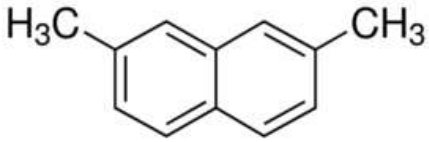
1,8-Dimethylnaphthalene



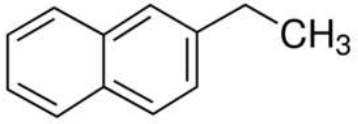
2,3-Dimethylnaphthalene



2,6-Dimethylnaphthalene

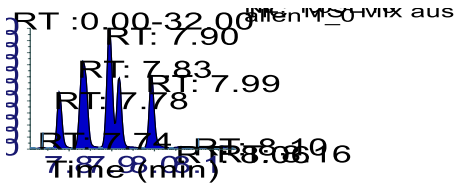


2,7-Dimethylnaphthalene



2-Ethylnaphthalene

Mix of components



1,3-DMN
1,7-DMN

Exact Mass: 156.09335

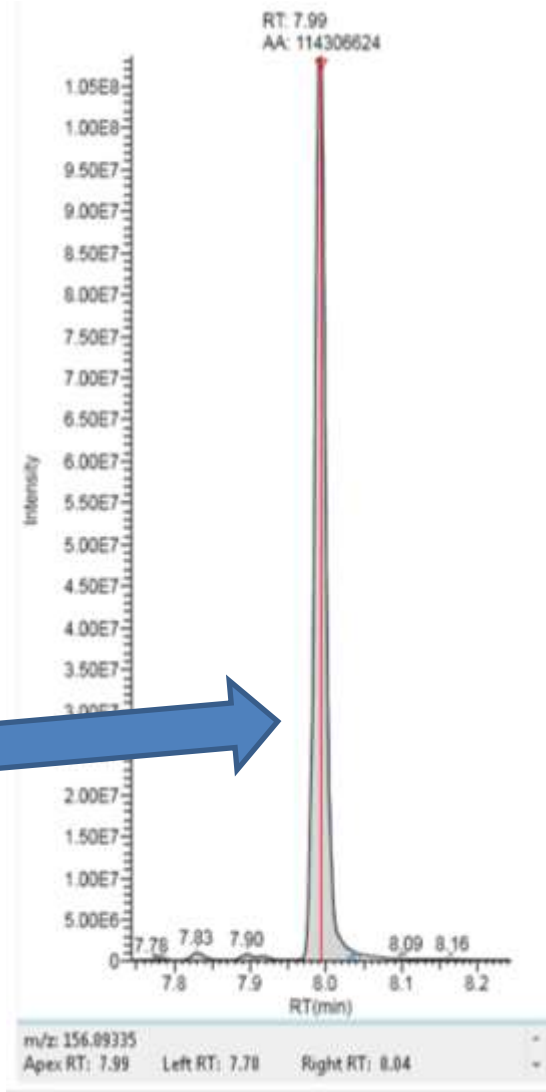
2,6-DMN
2,7-DMN

1,4-DMN
1,5-DMN
2,3-DMN

1,6-DMN

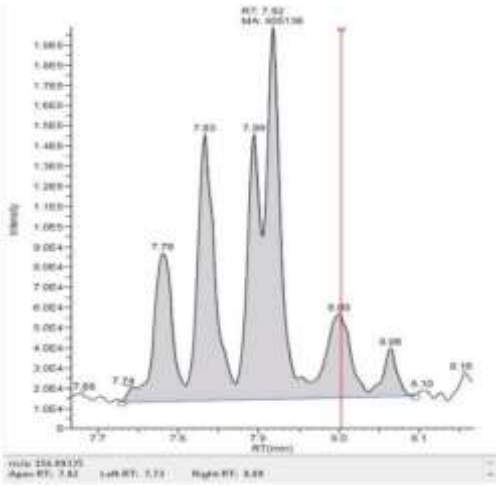
2-Ethylnaphthalene

Potato extract w. residue

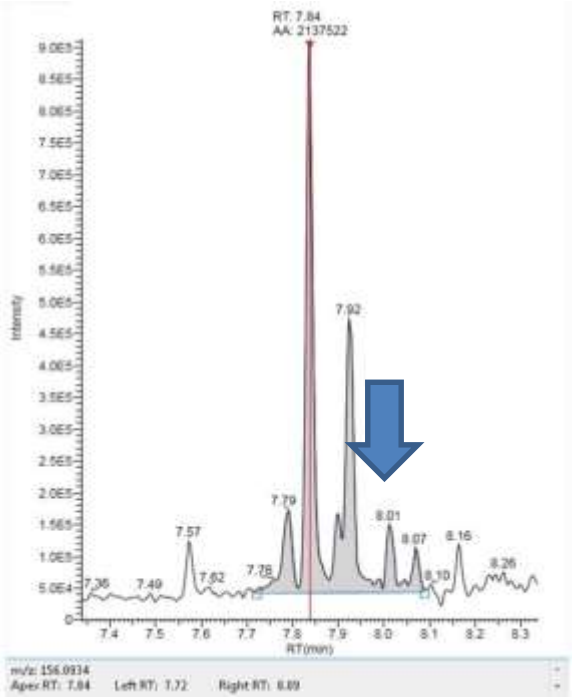


Products contaminated with Naphtha
Often used as solvents in PPP-Formulations

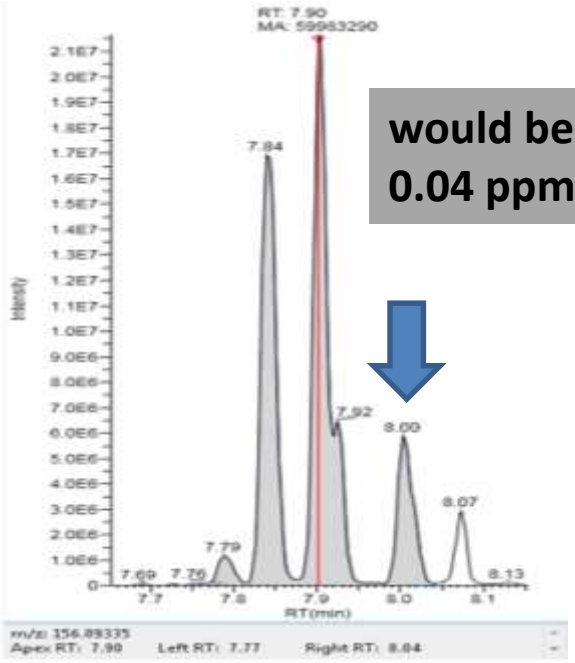
Heavy Straight Petroleum



Tomato

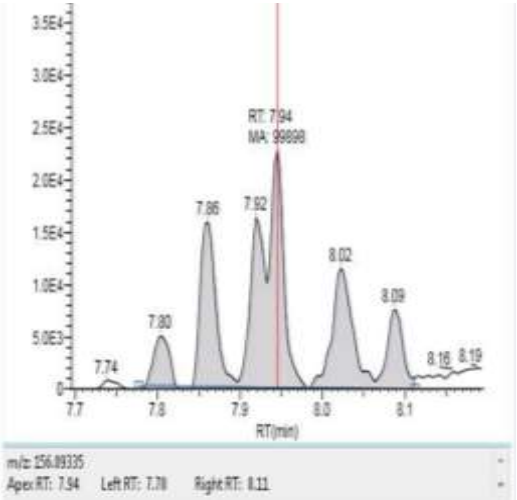


Parsley



would be 0.04 ppm

California light



Naphtha (petroleum distillate)

- Often used as solvent/adjuvant in PPP-Formulations
- Composition differs depending on the oil origin

Main DMN components: 1,3-DMN, 2,6-DMN, 2,7-DMN, 1,6-DMN, 1,7-DMN

Compound	*Peak No.*	*Alkylnaphthalene fractions*			
		1	*2*	*3*	*4*
1,2-Dimethylnaphthalene	4	3.7	3.7	3.8	3.7
1,3-Dimethylnaphthalene	5	15.4	15.6	15.5	16.0
1,4-Dimethylnaphthalene	6	3.0	2.9	2.9	3.0
1,5-Dimethylnaphthalene	7	4.0	4.0	4.0	4.0
1,6-Dimethylnaphthalene	8	21.2	21.2	21.1	21.1
1,7-Dimethylnaphthalene	9	15.6	15.3	15.4	15.0
1,8-Dimethylnaphthalene	10	0.003	n.d.	n.d.	n.d.
2,3-Dimethylnaphthalene	11	6.6	6.7	6.8	6.7
2,6-Dimethylnaphthalene	12	16.0	15.9	15.9	15.9
2,7-Dimethylnaphthalene	13	14.5	14.6	14.5	14.5

1,4-Coeluting

J. Chrom. A, Vol.267, 1983, Pages 367-372

Naphtha (petroleum distillate)

~20% of global production goes to agrochemical industry

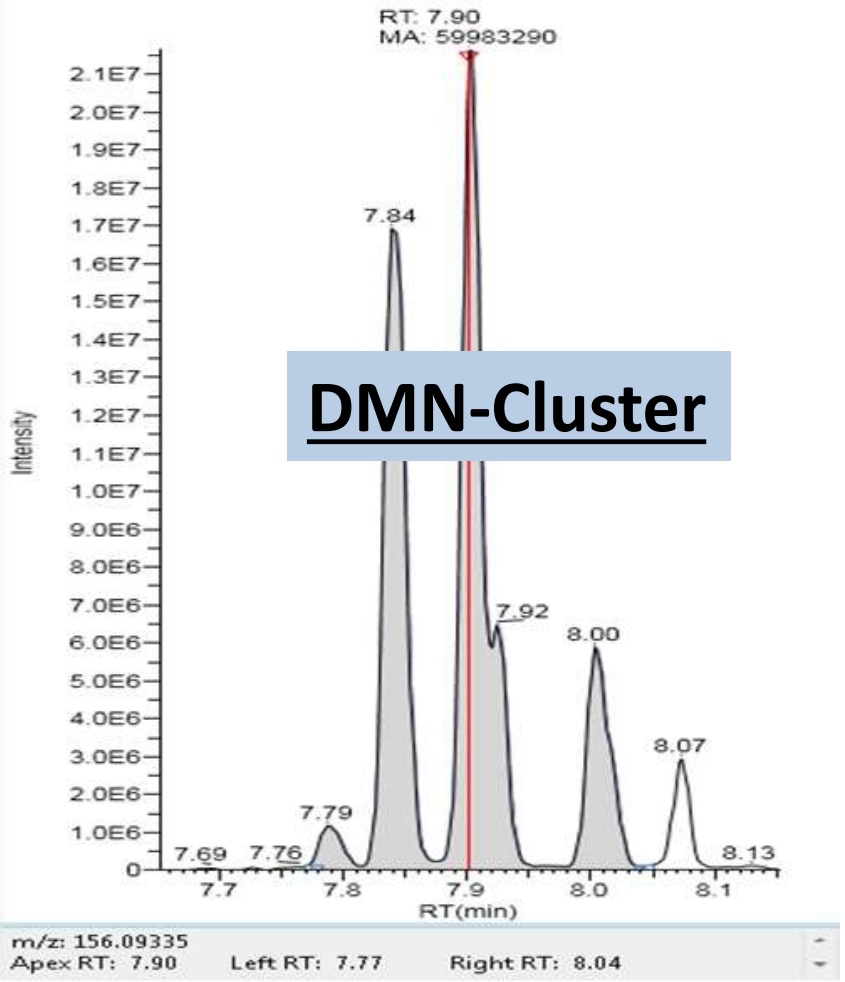
Formerly even used as active substances

Petroleum oils/(CAS 64742-55-8/64742-57-7)	NOT APPROVED
Petroleum oils/(CAS 74869-22-0))	NOT APPROVED
Petroleum oils/(CAS 92062-35-6)	NOT APPROVED

Nowadays even used as active substances

Residues in Parsley Samples

- Difenoconazole 5,6 ppm
- Azoxystrobin 0.05 ppm
- Deltamethrin 0.02 ppm
- ….



Example DIFENOCONAZOLE

SAFETY DATA SHEET according to EC directive 2001/58/EC

syngenta

SCORE 250 EC

Version 10
Revision Date 01.09.2006 Print Date 01.09.2006

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

Section 3: COMPOSITION / INFORMATION ON INGREDIENTS

Mixture:
Chemical Identity of ingredients:

Ingredient	CAS no.	Content (% w/v)
difenoconazole	119446-68-3	25
1 methyl-2-pyrrolidone	872-50-4	10-<30
Calcium alkyl benzene sulphonate	26264-06-2	<10
Solvent naphtha (petroleum) highly aromatic	64742-94-5	30-<60
Other ingredients determined not to be hazardous	-	to 100%

Example DELTAMETHRIN

SAFETY DATA SHEET

MSD Public

Deltamethrin (5%) Formulation

Version	Revision Date:	SDS Number:	Date of last issue: 09.10.2020
4.5	09.04.2021	2334789-00011	Date of first issue: 12.12.2017

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance / Mixture : Mixture

Components

Chemical name	CAS-No.	Concentration (% w/w)
Solvent naphtha (petroleum), light aromatic	64742-95-6	>= 30 -< 50
2-Methoxy-1-methylethyl acetate	108-65-6	>= 20 -< 30
Benzenesulfonic acid, C10-13-alkyl derivs., calcium salts	Not Assigned	>= 5 -< 10
2-Methyl-1-propanol	78-83-1	>= 5 -< 10
Deltamethrin (ISO)	52918-63-5	>= 5 -< 10

Example AZOXYSTROBIN

SAFETY DATA SHEET

Willowood AzoxyProp XTRA

Section 3. Composition/information on ingredients

Substance/mixture : Mixture

Chemical name : Methyl (E)-2-{2-[-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate; Azoxystrobin (13.5%); 1-[{2-92,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl}methyl]-1H-1,2,4-Triazole Propiconazole (11.7%)

Other means of identification : β-Methoxyacrylate Fungicide;Triazole Fungicide

Ingredient name	%	CAS number
Proprietary Surfactant Blend	≥10 - <20	Proprietary
Proprietary Surfactant Blend	≥10 - ≤25	Proprietary
Azoxystrobin	≥10 - <15	131860-33-8
Propiconazole (ISO)	≥10 - ≤25	60207-90-1
Solvent Naphtha (Petroleum), Heavy Arom.	≥10 - ≤25	64742-94-5

EFSA Review report on 1,4-DMN

Review of the existing MRLs for 1,4-dimethylnaphthalene

EFSA Journal

Considering the multiple sources of 1,4-dimethylnaphthalene and the lack of comprehensive data on the actual natural background levels of 1,4-dimethylnaphthalene in plants, EFSA recommends Members States to continue monitoring 1,4-dimethylnaphthalene in commodities of plant origin.

Alternative Separations for highly polar compounds

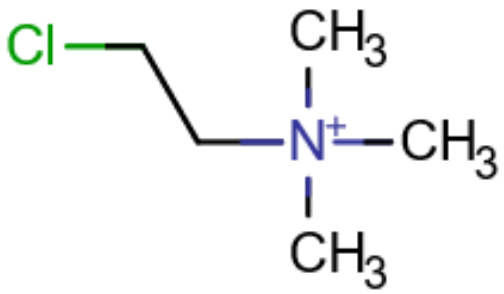
LC-MS/MS

IC-MS/MS

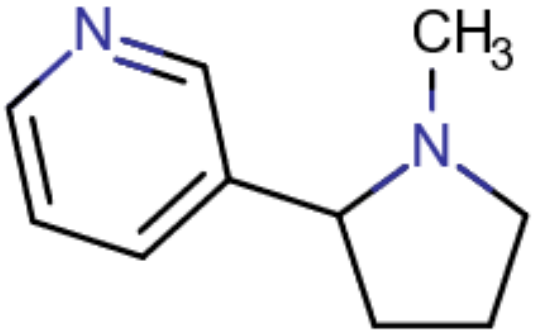
CE-MS/MS

IC-MS/MS (Cation exchange)

- **Cation exchange columns: Dionex CS 17 + Dionex CG 17**
- **Eluent: methansulfonic acid (MSA) and dynamic electrolytic suppression**
- **Highly polar ESI pos. compounds (those covered by QuPPe M4.2)**



Chlormequat

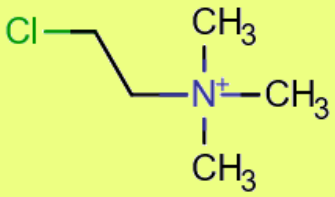


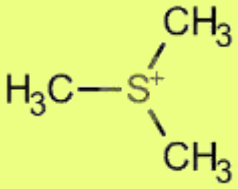
Nicotine

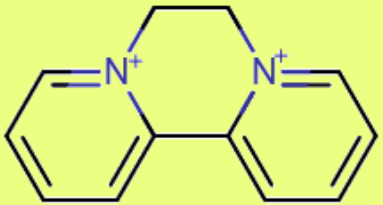
IC-MS/MS Cation Exchange

Highly polar ESI pos. compounds

Permanently Cationic!

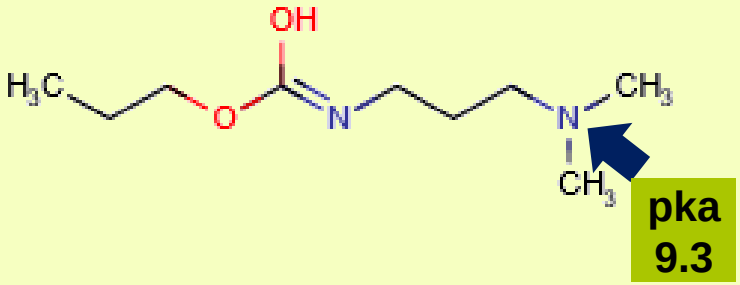


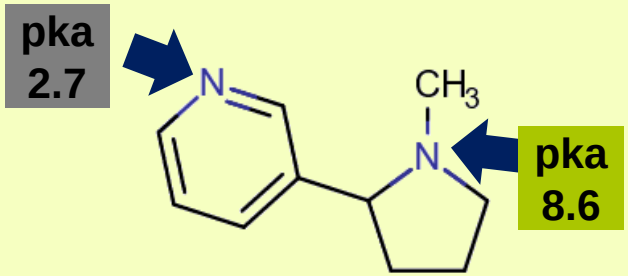




✓

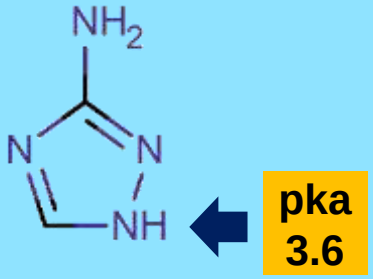
pH-Dependent Cationic through protonation

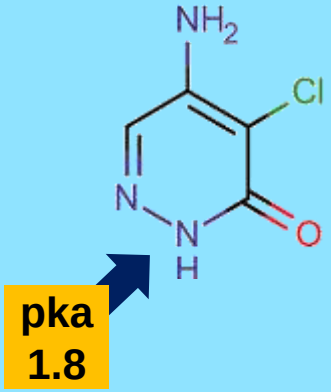




Strong / Interm. Basicity (pka > ~4-5)
(largely protonated @ pH < ~3)

✓





Weak Basicity (pKa <~4-5)
(substantially unprotonated at pH ≈ < ~ 3)

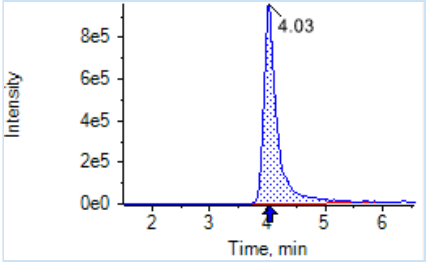
X

molecular structures and pka values by chemicalize.com

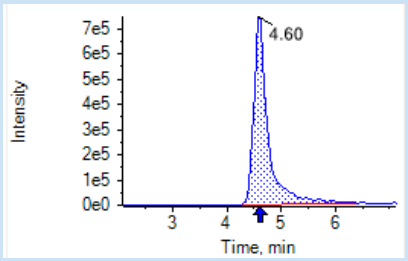
IC-MS/MS Cation Exchange

Peak shapes at 0.01 µg/mL in strawberry extract (5-fold diluted)

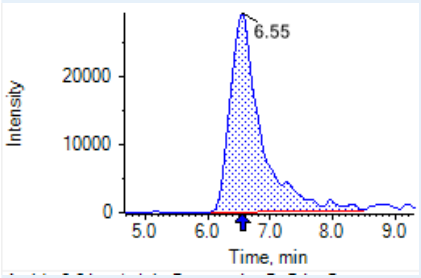
Chlormequat



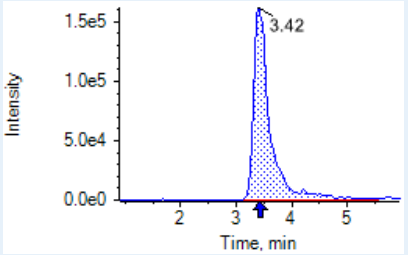
Mepiquat



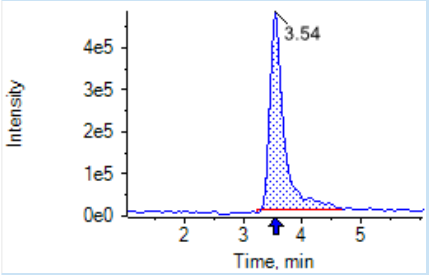
Cyromazine



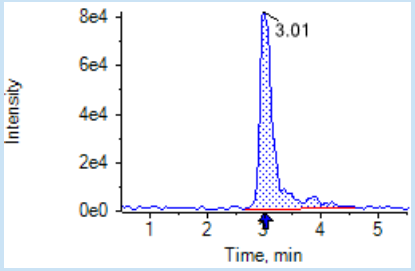
Mepiquat-4-hydroxy



Melamine*
Strong matrix effects!



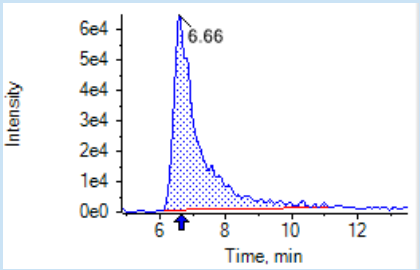
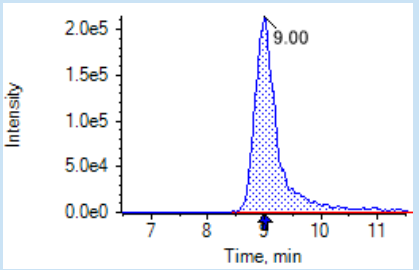
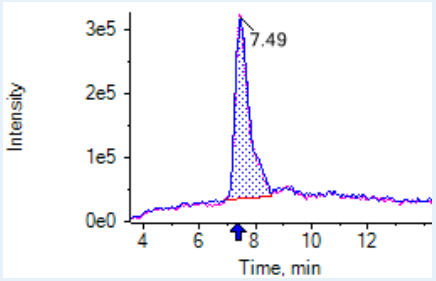
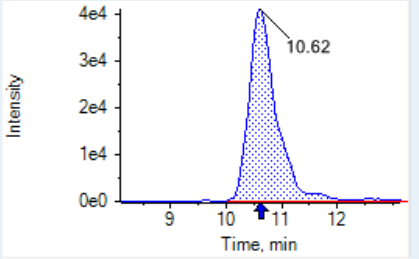
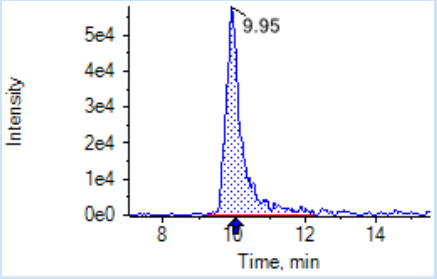
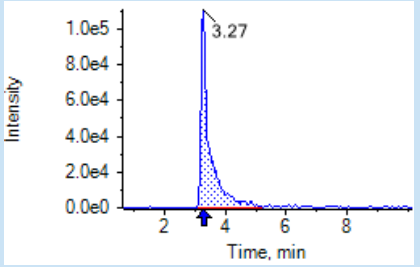
Morpholine*
Strong matrix effects!



*in solvent

IC-MS/MS Cation Exchange

Peak shapes at 0.01 µg/mL in strawberry extract (5-fold diluted)

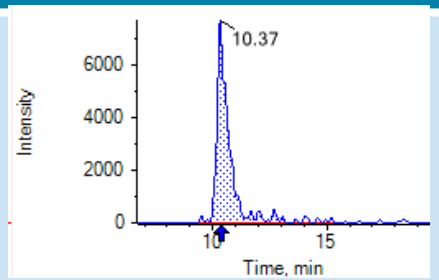
Nereistoxin		**Propamocarb-N-Desmethyl**	
Nicotine**		**Propamocarb-N-Oxide**	
Propamocarb		**Trimethylsulfonium**	

** in tomato extract in 5-fold dilution

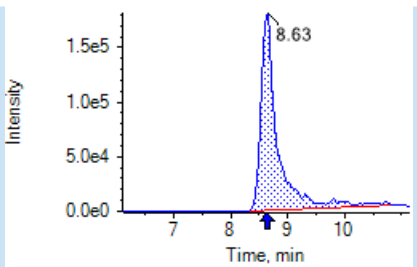
IC-MS/MS Cation Exchange

Peak shapes at 0.01 µg/mL in strawberry extract (5-fold diluted)

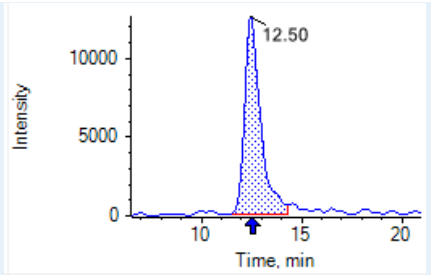
Matrine
Poor Sensitivity!



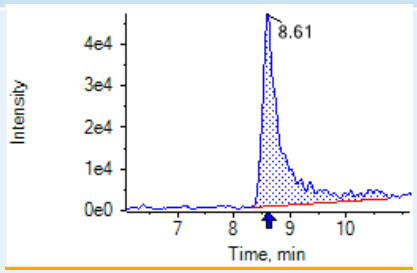
Diquat
Poor Sensitivity!



Oxymatrine
Poor Sensitivity!

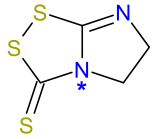
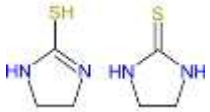
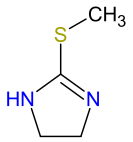
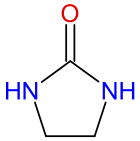
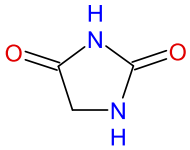
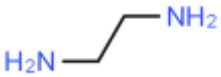


Paraquat
Poor Sensitivity!

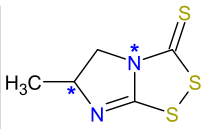
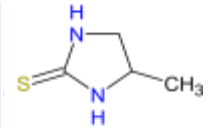
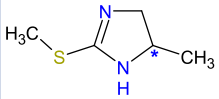
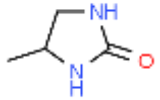
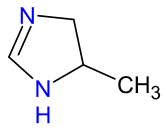
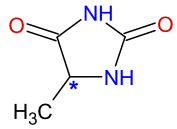
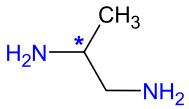


Metabolite-Screening of Dithiocarbamates via LC-MS-MS

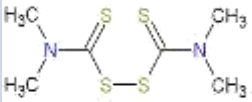
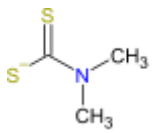
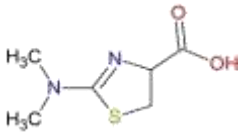
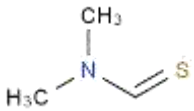
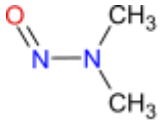
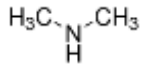
Metabolites of **ETHYLENE**-bis-DTCs – Status of Experiments

Metabolite	Structure	Recovery QuEChERS	Recovery QuPPe	ESI mode	LC- Method (used at the moment)	Comment
Etem				ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Further mth developm. needed (problems w. solubility +stability)
ETU		~ 70 %	> 80 % (ILIS avail.)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	**findings in real samples**
S-Methyl-ETU			99 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	very sensitive; **findings in real samples**
Ethyleneurea			102 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	**findings in real samples;** Target ion: sensitive; Qualifier ion: often interfered
Hydantoin			86 % (cucumber; matrix-matched; ESI-neg)	(ESI-pos) ESI-neg		poor LC-MS/MS sensitivity ESI-Neg: 1 useful MRM-trans.
Ethylenediamine				ESI-pos (only 1 MRM)		Further mth developm. needed; poor sensitivity, 1 useful MRM; background levels expected

Metabolites of **PROPINEB**

Metabolite	Structure	Recovery QuEChERS	Recovery QuPPe	ESI mode	LC-Method (used at the moment)	Comment
Propineb-DIDT		20-35 % (cucumber; matrix-matched)	14 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Further mth developm. needed (problems w. stability)
PTU		~ 70 %	> 80 % (ILIS available)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	occasional findings in samples
PTU-S-methyl						Further mth developm. needed Standard not yet availabe
Propyleneurea				ESI-pos		Very low sensitivity + matrix suppression
4-Methyl-imidazoline			95 % (grapes; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Occasional findings in samples; sensitive detection; Not stable in stock solution (switched from H2O/ACN to MeOH)
5-Methyl-Hydantoin			106 % (cucumber; matrix-matched; ESIpos)	ESI-pos (NH_4adduct) ESI-neg	QuPPe M 4.2 ("Quats&Co. BEH Amide")	ESI-pos: poor sensitivity; ESI-neg: only 1 useful MRM
Propylene-diamine				ESI-pos	C18 using ion-pairing reagent	Limited sensitivity. Analyzed as such or after CS2-cleavage

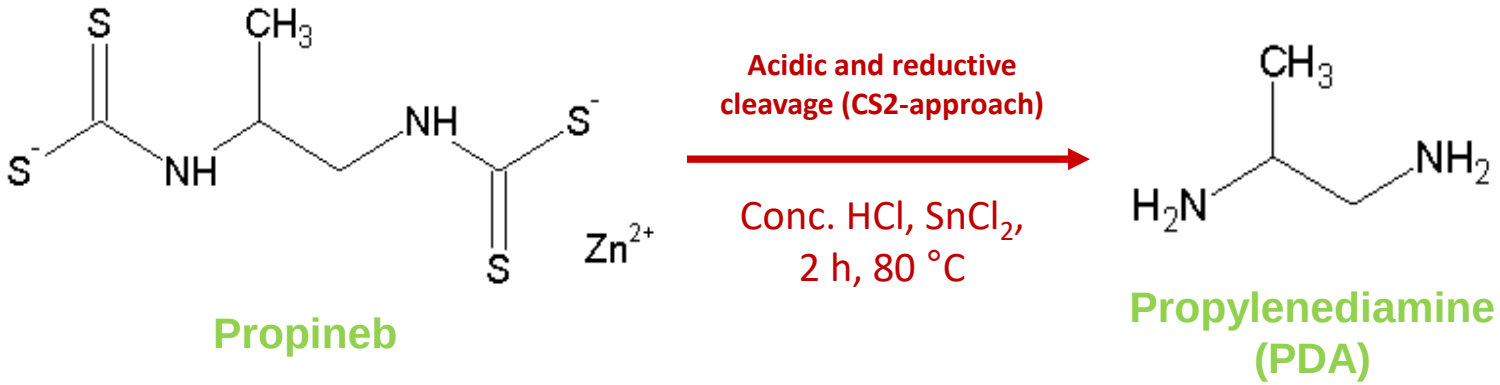
Metabolites of Thiram / Ziram

Metabolite	Structure	Recovery QuEChERS	Recovery QuPPe	ESI mode	LC-Method (used at the moment)	Comment
Thiram		98 % (beans; matrix-matched). Poor recov. in several other matrices		ESI-pos	RP (e.g. C18)	Further mth developm. needed (poor recov. in many matrices esp. when containing vit. C)
Dimethyl-DTC (Ziram monomer, Dibam)			Problems w. stability	ESI-neg		Further mth developm. needed (problems with stability)
M1		~ 1 %	95 % (cucumber; matrix-matched; ESI-pos)	ESI-pos (ESI-neg)	QuPPe M 4.2 ("Quats&Co. BEH Amide")	**Findings in samples** ESI-pos. very sensitive;
Dimethylthio-formamide (DMTF)			~ 70 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Further mth developm. needed (fluctuating recovery rates)
N,N-Dimethyl-nitrous amide		96 % (cucumber; matrix-matched)	105 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Formed during water treatment; not very sensitive
Dimethylamine			99 % (cucumber; matrix-matched)	ESI-pos	QuPPe M 4.2 ("Quats&Co. BEH Amide")	Further mth developm. needed; poor sensitivity, 1 useful MRM; background levels observed

Analysis of Propineb as Proplyenediamine (PDA)

Propineb – General Info:

- Foliar dithiocarbamate fungicide with long lasting activity
- Against various fungal infections (e.g. blight on potatoes, downy mildew on hops)
- **Not Approved** within EU
- Two Residue Definitions:
  1. Dithiocarbamates (dithiocarbamates **expressed as CS2**, including maneb, mancozeb, metiram, **propineb**, thiram and ziram)
  2. **Propineb, expressed as propilendiamine** (SANTE/12108/2020; Reg. (EC) No 149/2008)



Propineb

Propylenediamine (PDA)

Analysis of Propineb as Proplyenediamine (PDA)

PDA sample preparation in short – Traditional CS_2-method followed by modified alkaline QuEChERS

Highly acidic

Pipette 10 mL centrifugated CS_2-hydrolysate (=watery phase) in 50 mL centrifuge tube

↓

Add 100 µL isotopically-labeled internal standard (ILIS) **diaminopropane-D_6**

↓

Highly basic

Add 10 mL ACN and **3 mL sodium hydroxide solution** (25N)

↓

Shake thouroughly for 2 min.

Partitioning losses of PDA by QuEChERS (ca. 20% yield), despite basification

↓

Centrifuge

↓

Transfer 0.5 mL extract to a vial containg 1 mL of eluent A

↓

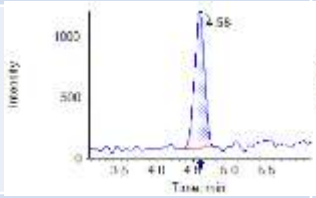
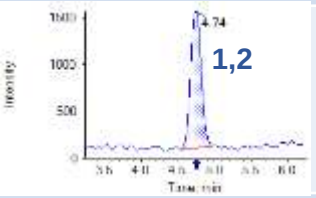
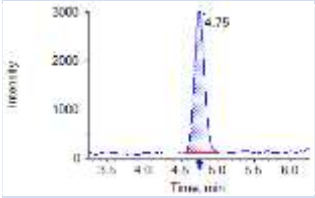
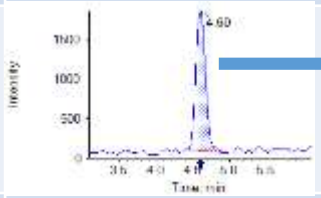
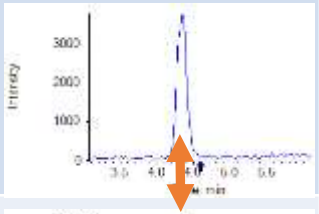
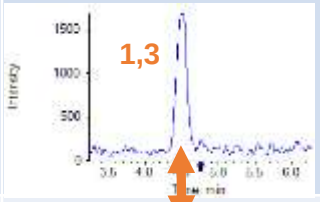
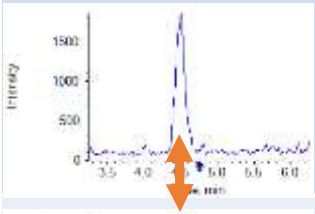
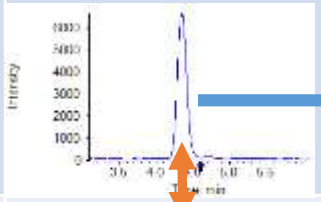
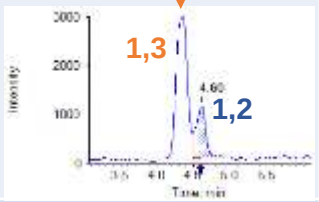
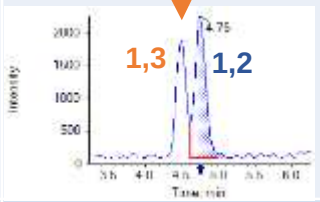
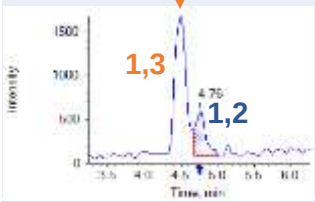
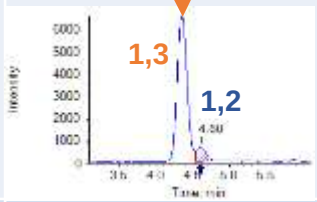
LC-MS/MS analysis by ion-pair chromatography on a C18 column

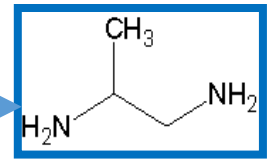
Ion-pairing LC-MS/MS Analysis of Proplyenediamine (PDA)

LC-MS/MS conditions

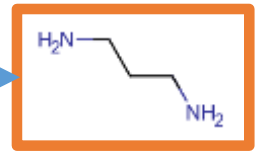
Column	Agilent Eclipse Plus C18 RRHD, 1.8 µm, 2.1x100 mm		
Mobile Phase	**Eluent A:** 4 mM heptafluorbutyric acid + 2 mM ammonium formate in water **Eluent B**: 4 mM heptafluorbutyric acid + 2 mM ammonium formate in methanol		
Gradient	**Time** (min)	**Flow** (mL/min)	**% Eluent A**
	0.0	0.2	97.5
	1.0	0.2	97.5
	7.0	0.2	60
	7.1	0.3	30
	8.0	0.3	10
	12.0	0.3	10
	12.1	0.2	97.5
	14.0	0.2	97.5
Temperature	40 °C		
Injection volume	2 µL		
Det. mass traces	75/58, 58/41, 81/64 (ILIS PDA-D_6)		
Source Parameters (Sciex QTrap 5500+)	ESI Pos, Ion spray Voltage: 5500 V, Temperature: 550 °C, Neb. Gas: 60 psi, Heater Gas: 60 psi, Curtain Gas: 25 psi		

Ion-pair LC-MS/MS Analysis of Proplyenediamine (PDA)

	Cucumber	Kiwi fruit	Avocado	Rye flour
Valid. level	0.05 ppm	0.05 ppm	0.1 ppm	0.1 ppm
Solvent Cal. (60 % Level)				
Matrix Blank				
Recovery exp. (@LSVL of Quantifier)				
	90 % ±9,7 %	**101 %** ±5,0 %	**92 %** ±10,3 %	**89 %** ±14,3 %
Remarks	Qualifier not successfully validated	Quantif. + qualif. successfully validated	Qualifier not successfully validated	Qualifier not successfully validated



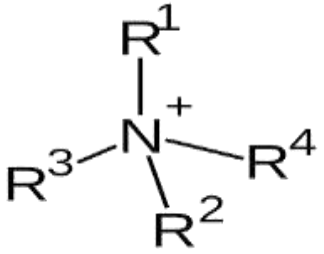
1,2-propylene diamine (PDA)



1,3-propylene diamine (naturally occuring)

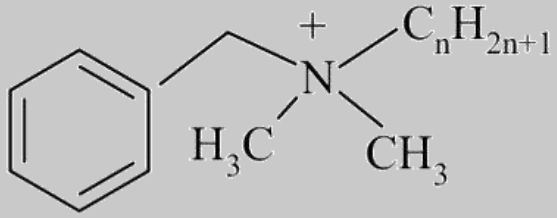
Plans: Check if better results can be obtained employing derivatization

Quaternary Ammonium Compounds - Overview

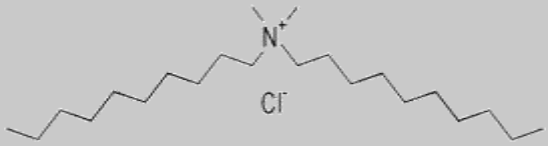


- Formerly used as pesticides, thus covered by Reg. (EC) No. 396/2005
- Nowadays widely used in
  - biocidal products and cleansing agents (e.g. surface disinfactants; air-filter sanitizers),
  - personal-care products
- Possible background contamination of samples; E.g.
  - Through contact of food with surfaces during production (e.g. pipes, belts, tanks)
  - Though touching of samples after using QAC-containing hand sanitizers
  - Through air-conditioning (?)
- Within the re-evaluation process (Art. 12 of Reg. 396/2005/EC)
- Highly sensitive analysis via LC-MS/MS (ESI-pos. Mode)

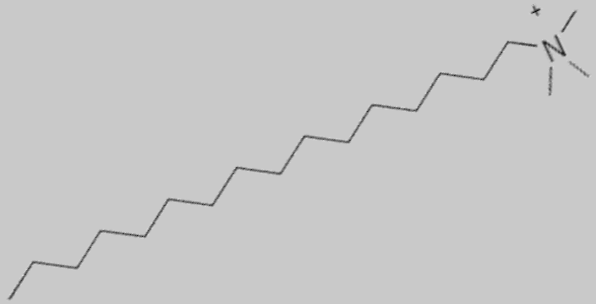
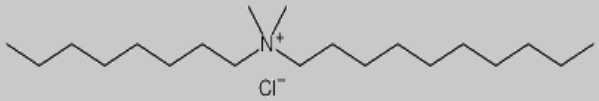
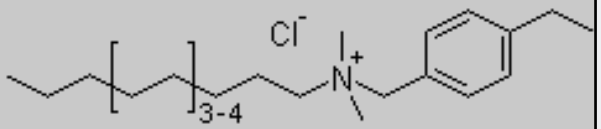
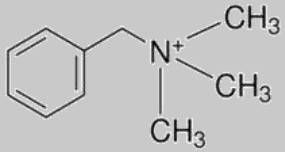
Common QACs listed in Pesticide DB

Compound listed in pesticides Database	Details	Exemplary uses
Alkyldimethylbenzyl ammonium chloride **Lauryl**dimethylbenzylammonium – a) -chloride ; b) -bromide 	*C#/C1/C1/Benzyl* = **BAC = Benzalkonium chloride** *C12/C1/C1/Benzyl* **= BAC-C12** *RD: Benzalkonium chloride (mixture of alkylbenzyldimethylammonium chlorides with alkyl chain lengths of C8, C10, C12, C14, C16 and C18*)	Detergent for surface disinfection Eye drops conservation Air filter disinfactant

Common QACs listed in Pesticide DB

Compound listed in pesticides Database	Details	Exemplary uses
Dioctyldimethyl ammonium chloride  **Didecyldimethyl ammonium chloride**	*C8/C8/C1/C1* **= DDAC–C8** *C10/C10/C1/C1* **= DDAC–C10** *RD: Didecyldimethylammonium chloride (mixture of alkyl-quaternary ammonium salts with alkyl chain lengths of C8, C10 and C12)*	Detergent for surface disinfection Eye drops conservation Air filter disinfectant

Other QACs listed in Pesticide DB

Compound listed in pest. DB	Details	Exemplary uses
Alkyltrimethyl ammonium chloride 	*C#/C1/C1/C1 (Alkyl: C10-18)* C8 = 10108-86-8 C10 = 2082-84-0 (bromide) C12= DTAC; Lauryltrimethylammonium C14 = 4574-04-3 C16= Cetrimonium-; C18= Behentrimonium-	**Detergents** **Cosmetics (e.g. hair conditioners)** **Fabric softeners**
Octyldecyldimethyl ammonium chloride 	*C8/C10/C1/C1* = Quaternium-24 = Cefazolin (CAS: 32426-11-2)	**Detergent for surface sanitation**
Alkyldimethylethyl-benzylammonium chloride 	*C#/C1/C1/Ethylbenzyl* (CAS 85409-23-0)	**Human + veterinary hygiene, disinfection/sanitation** **Washing & cleaning products**
Alkyltrimethylbenzyl ammonium chloride 	*Alkyl/C1/C1/C1/benzyl* = ? ; Could be … C1/C1/C1/Alkyl (see above) OR C1/C1/C1/benzyl (CAS 56-93-9 / 5350-41-4)	**?**
Quaternary ammonium compounds	ANY ??? !!!	

CONCLUSION ➔ Need to revice the Database entries to be more specific

Quaternary ammonium compounds – Problems with Background Contamination

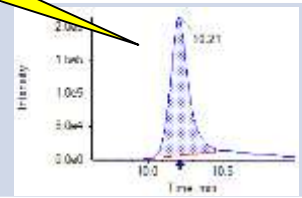
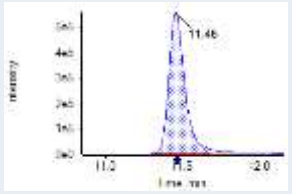
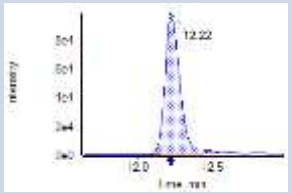
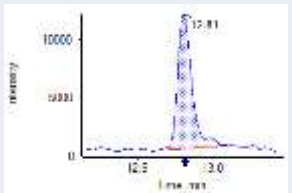
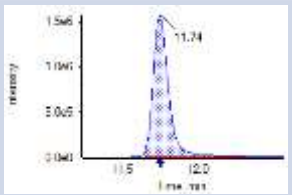
- Background levels permanently observed in routine analysis
  - ➢ in every injection (2-50 µg/kg)
  - ➢ day-to-day + periodic fluctuations of background levels
  - ➢ Mainly affected comp.: **DDAC-C10; BAC-C12; BAC-C14**
- Investigations for identifying possible sources
  - ➢ Reagents and cleaning agents
  - ➢ Equipment
    - ➢ Glasware
    - ➢ Components of analytical system (injector, column,….)
  - ➢ Personnel



→Overal inconclusive ☹ ➔ multiple sources likely

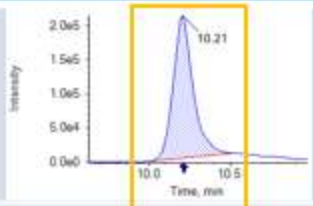
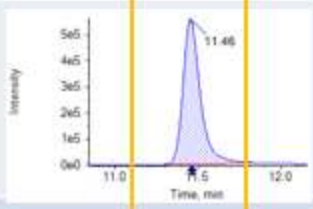
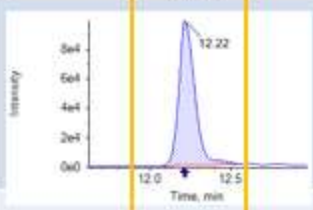
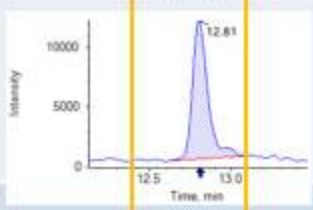
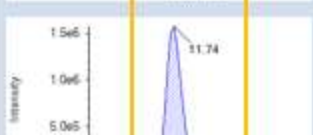
e.g. carry-over between samples, contamination of LC-System from multible sources (e.g. equipment, mobile phases ….)

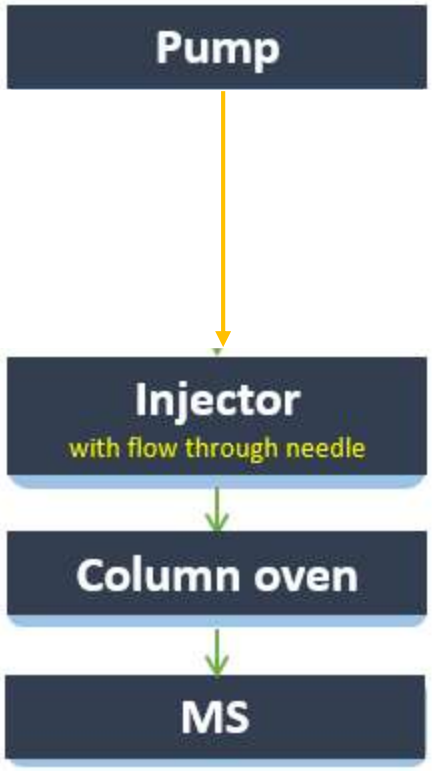
background levels

	pure ACN injected
BAC-12	
BAC-14	
BAC-16	
BAC-18	
DDAC-10	

Quaternary Ammonium Compounds – Separating Background

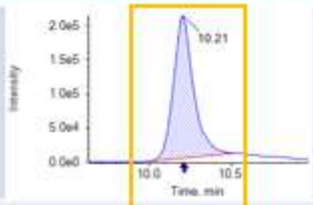
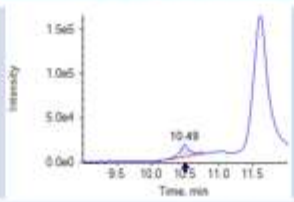
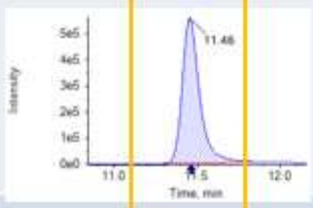
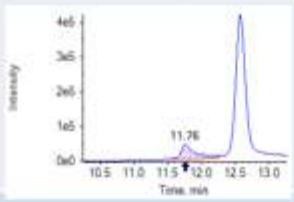
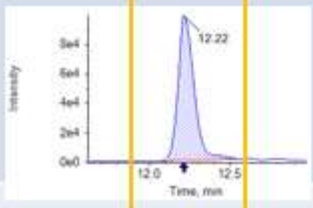
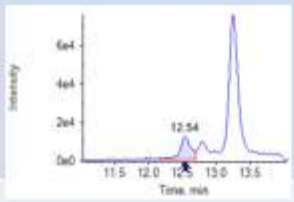
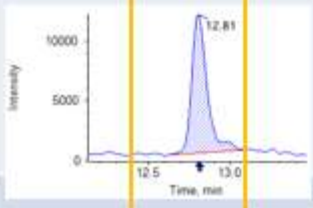
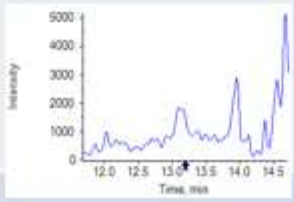
LC-MS/MS background signals when injecting pure solvent

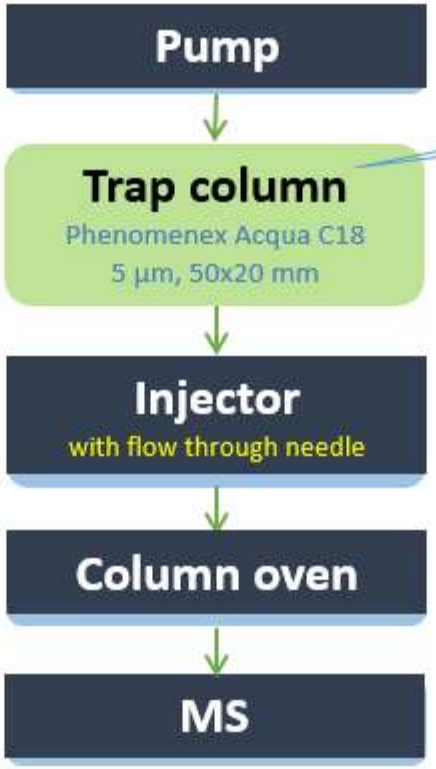
QAC	using Normal approach
BAC-12	
BAC-14	
BAC-16	
BAC-18	
DDAC-10	



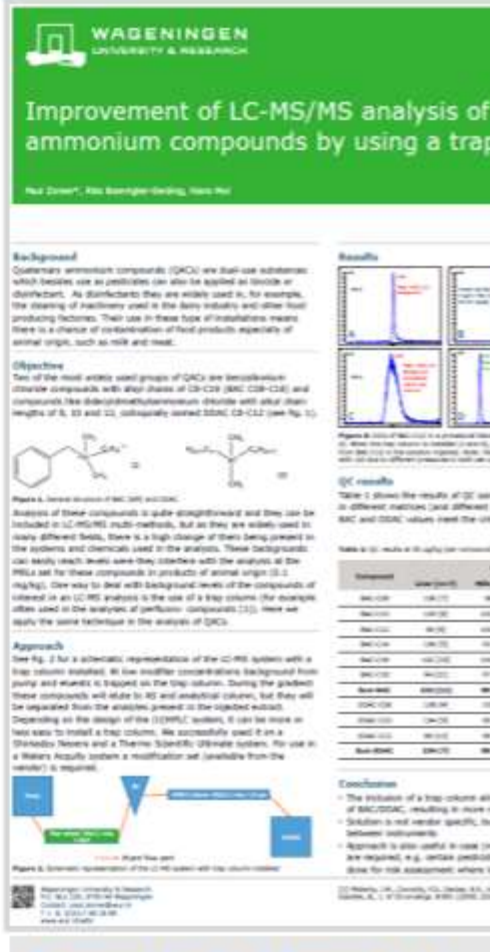
Quaternary Ammonium Compounds – Separating Background

LC-MS/MS background signals when injecting pure solvent

QAC	using Normal approach	LC-MS/MS using trap column
BAC-12		
BAC-14		
BAC-16		
BAC-18		
DDAC-10		

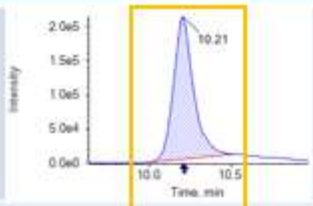
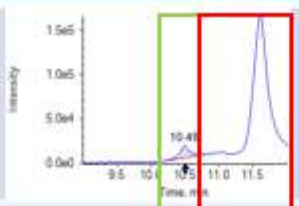
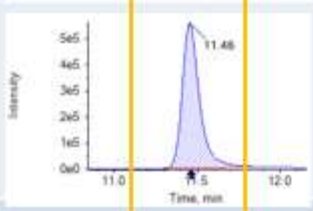
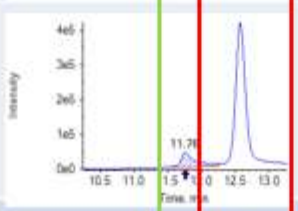
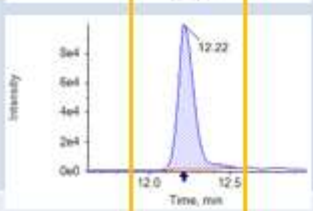
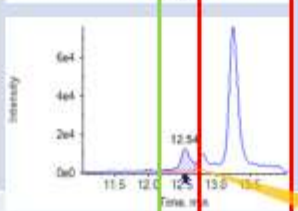
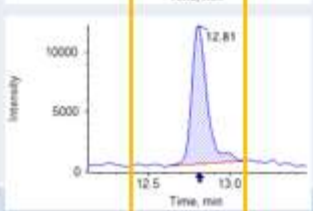
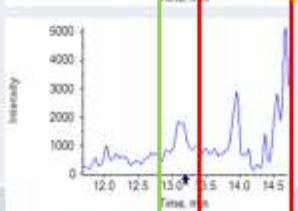
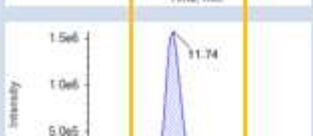
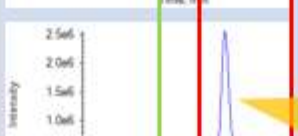


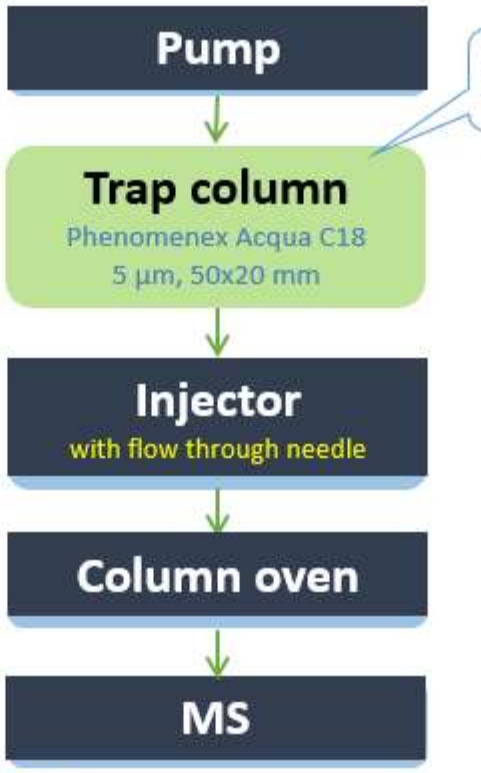
A sorted out analytical column of this method



Quaternary Ammonium Compounds – Separating Background

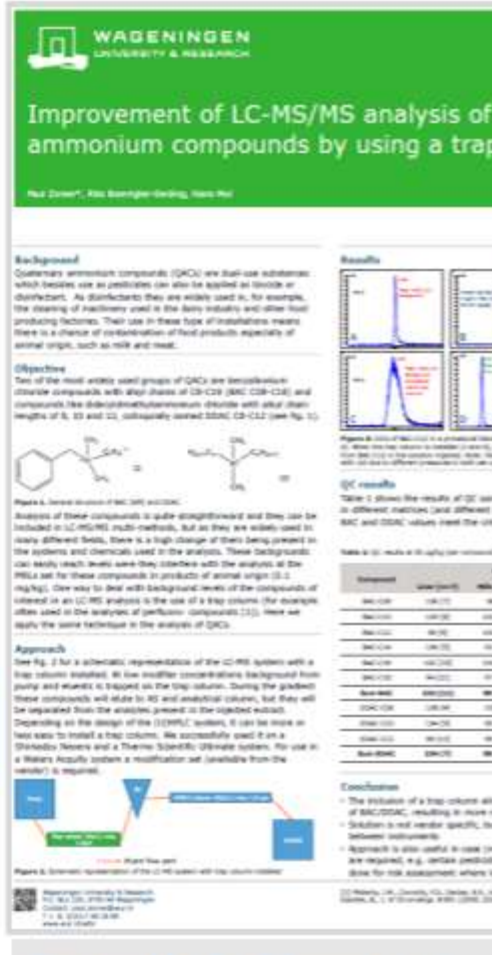
LC-MS/MS background signals when injecting pure solvent

QAC	using Normal approach	LC-MS/MS using trap column
BAC-12		
BAC-14		
BAC-16		
BAC-18		
DDAC-10		

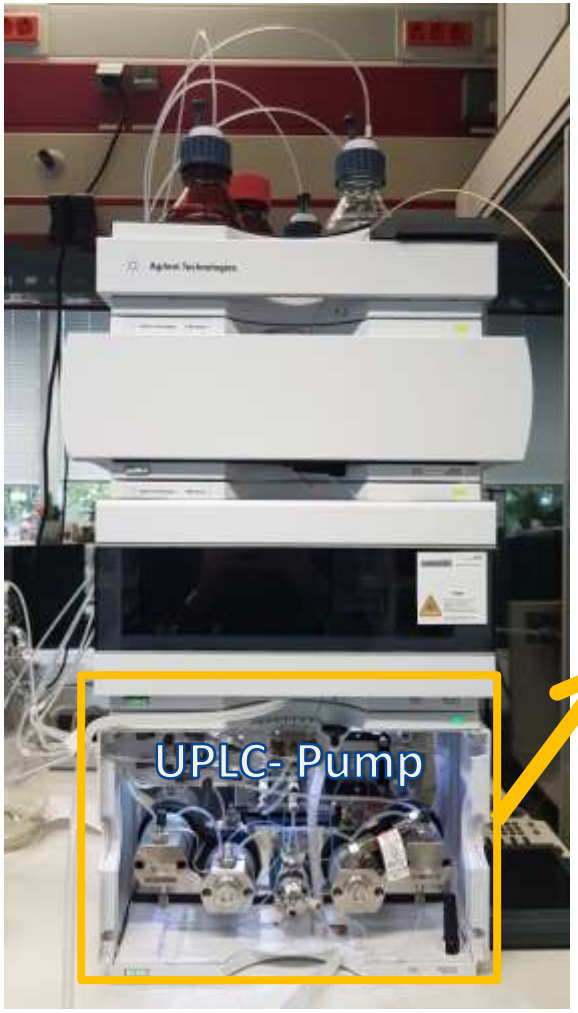


Signal originating from sample extract (~1 ppb)

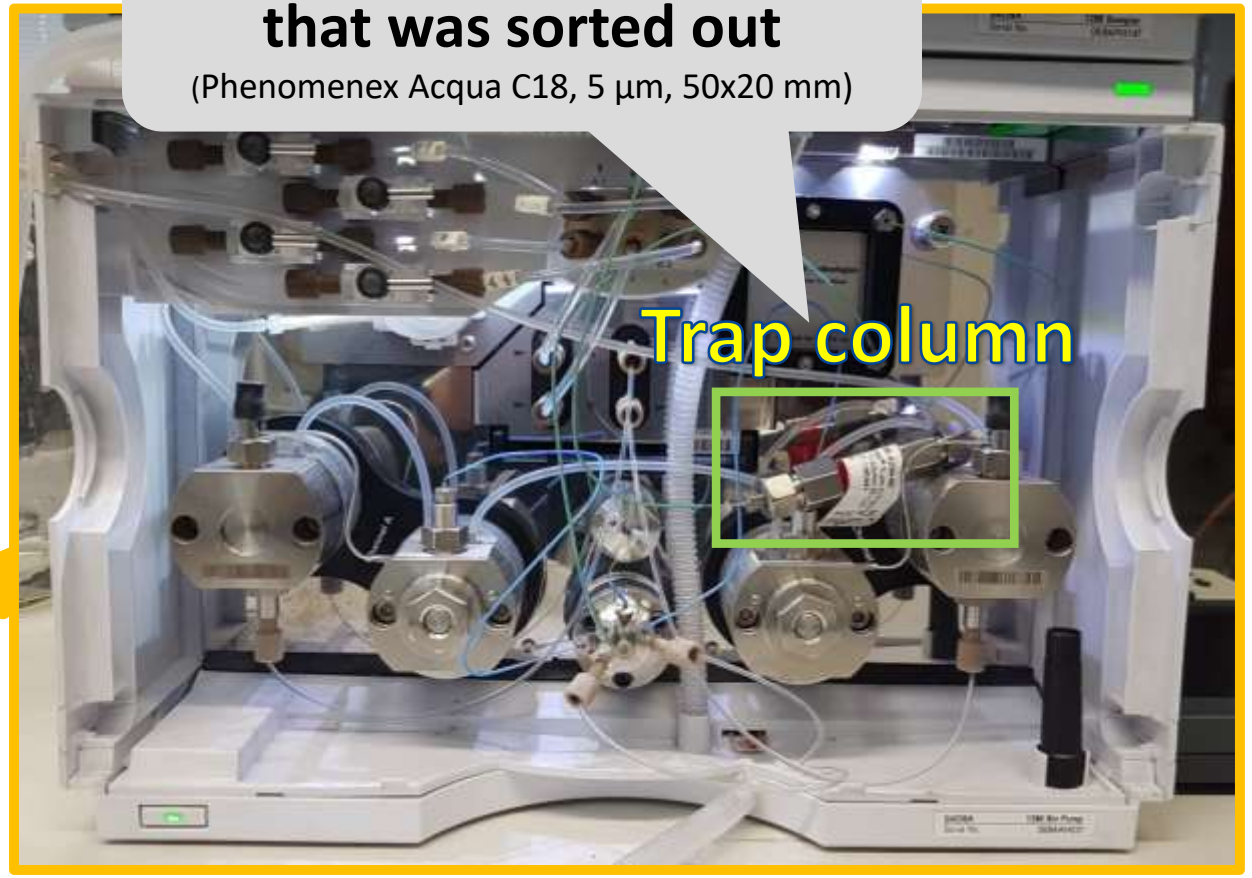
Chromatographically separated **background** (~50 ppb)



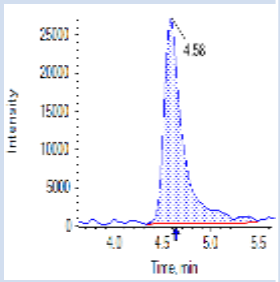
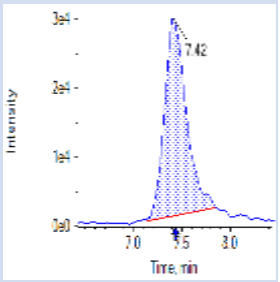
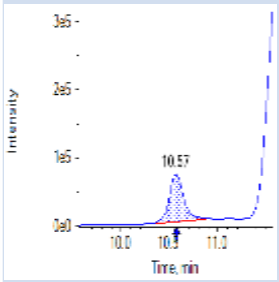
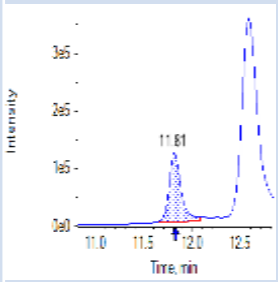
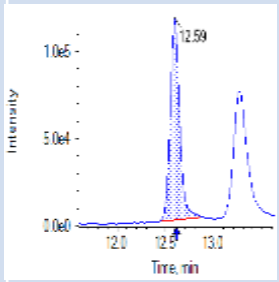
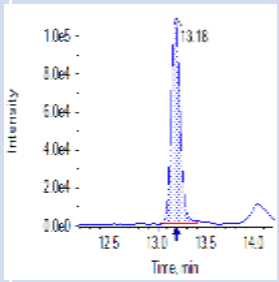
Quaternary ammonium compounds –
Chromatographic Separation of Background Levels of unknown Origin



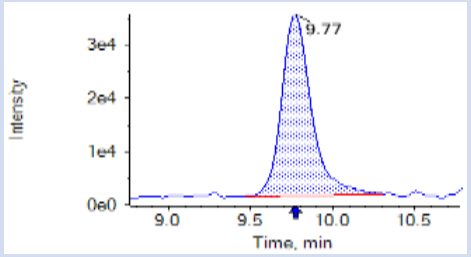
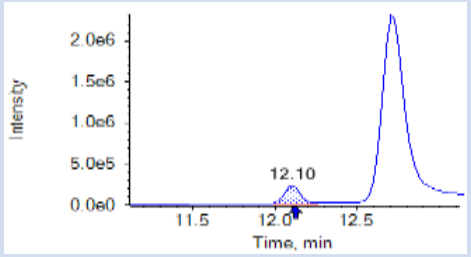
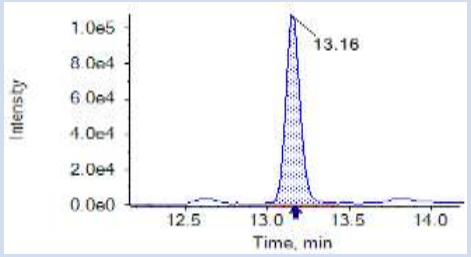
An old column
that was sorted out
(Phenomenex Acqua C18, 5 µm, 50x20 mm)



Quaternary ammonium compounds – Analytical Elimination of Background Levels of unknown Origin

Spiking level mg/kg	Validation on grapes (citrate-buffered QuEChERS, without dSPE)											
	BAC-8		BAC-10		BAC-12		BAC-14		BAC-16		BAC-18	
	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)
0.005 (LSVL)	**99**	3	**100**	3	**102**	7	**100**	3	**97**	3	**102**	3
0.010	**99**	1	**98**	2	**99**	2	**98**	2	**98**	2	**98**	2
Exemplary chromato-grams at LSVL												

Quaternary ammonium compounds – Analytical Elimination of Background Levels of unknown Origin

Spiking level	Validation on grapes (Citrate buffered QuEChERS, without dSPE)					
	DDAC-8		DDAC-10		DDAC-12	
	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)	Mean Rec. (%)	RSD (±%)
0.005 mg/kg (LSVL)	104	4,6	~~109~~	~~2,8~~	98	4,7
0.010 mg/kg	101	1,3	91	4,2	98	2,6
Exemplary chromatograms at LSVL						

DDAC10 background in "Blank" was too high for this level (34 %). Limit: 30%

Thank You
for Your Attention



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