Simplified analysis of pesticide residues in food using the Swedish Ethyl Acetate method (SweEt)

Susanne Ekroth
National Food Administration (NFA)
Sweden
suek@slv.se

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National Food Administration, Sweden

NFA
Located in Uppsala near Stockholm with a total of 544 employees. Sorted under Ministry for Rural Affairs.

Pesticide group
5 Chemists + 2 Technicians
2 GC-MS/MS
2 LC-MS/MS
1 HPLC-TOF

About 2 000 samples/year of which around 200 is analysed at NFA.

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Official control of pesticides at the NFA
How is it organised?

Laboratories

- National reference laboratory (NRL) and one official laboratory, both accredited

Method development

- Most of the method development at the NFA. Transfer of the methods to the official laboratory for validation and for monitoring of pesticides

Analytical methods

- Mainly multi residue methods based on ethyl acetate extraction and determination using LC-MS/MS and GC-MS/MS

Analysis

- 90% of the samples analysed by OfL

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Short history of the multi residue method for fruit and vegetables

- From 1981 to 1989 a modified Luke method (acetone extraction followed by partitioning with n-hexane/methylene chloride) and clean up with GPC was used at NFA
- Disadvantage: low recoveries for polar pesticides
- Since 1989 ethyl acetate method has been used (Andersson and Ohlin)
- At that time GC the main detection technique
- The method has continuously been improved including simplified sample preparation and faster analysis.

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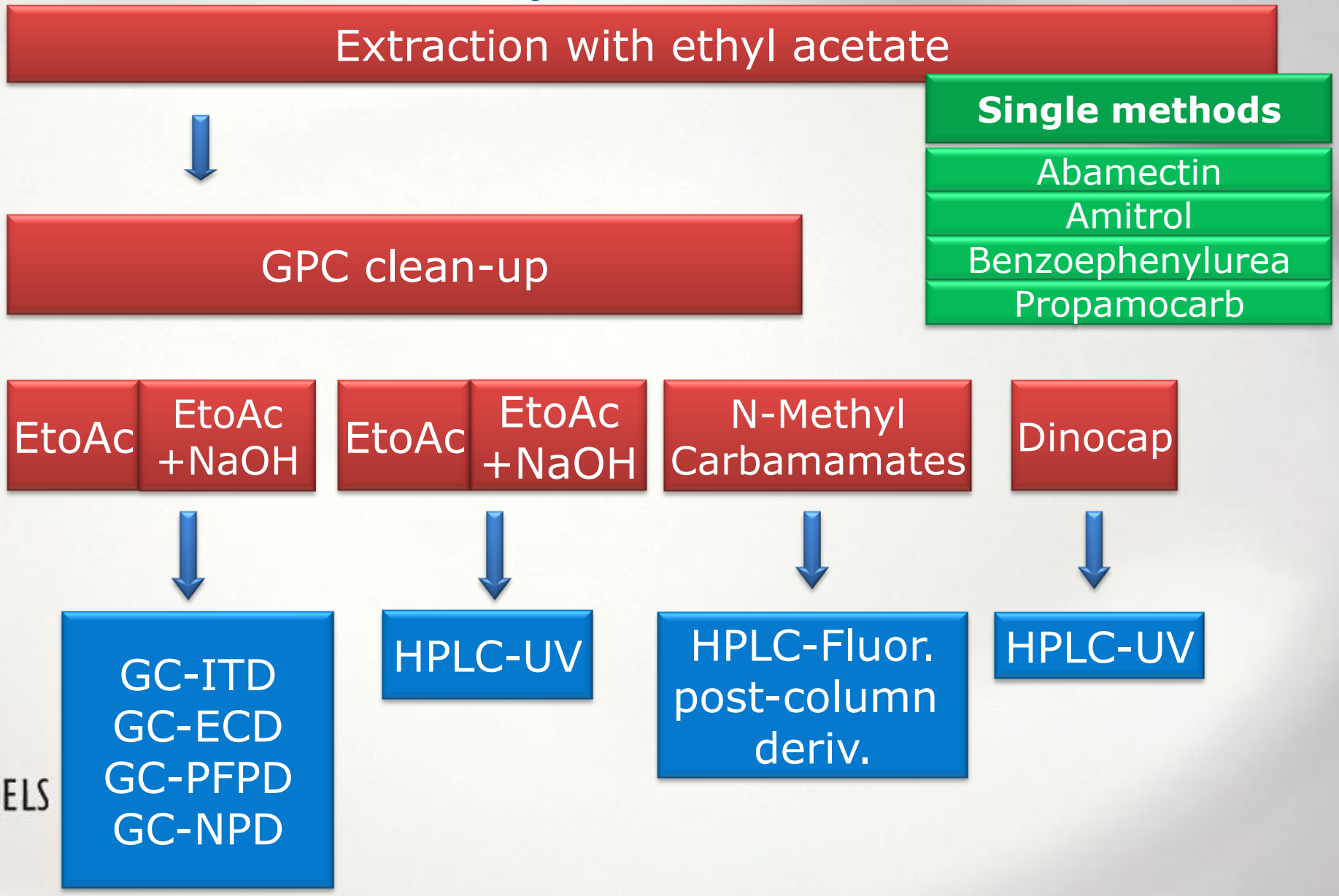
Multimethod for Fruit and Vegetables
Until year 2002:



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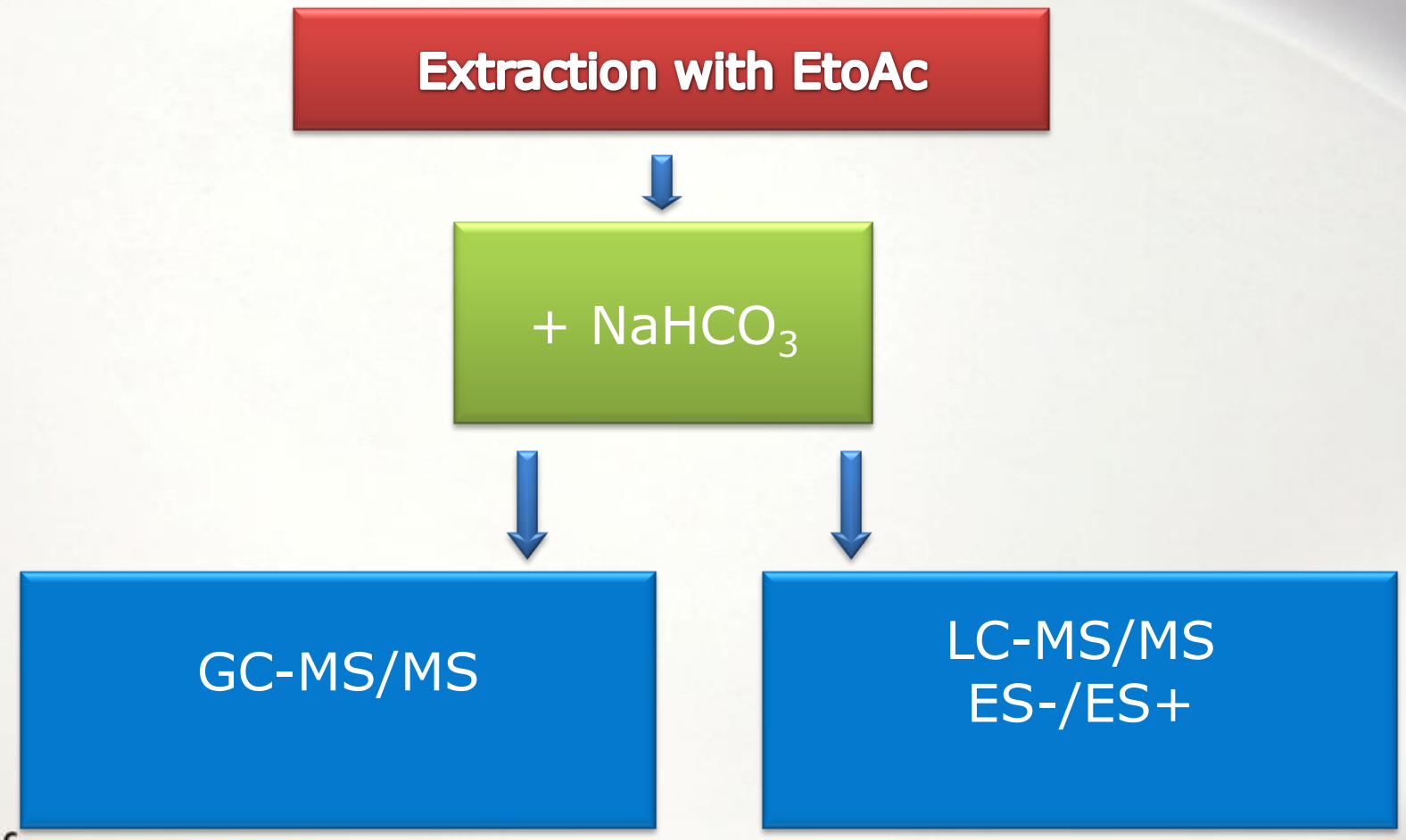
Year 2007
Introduction of GC-MS/MS



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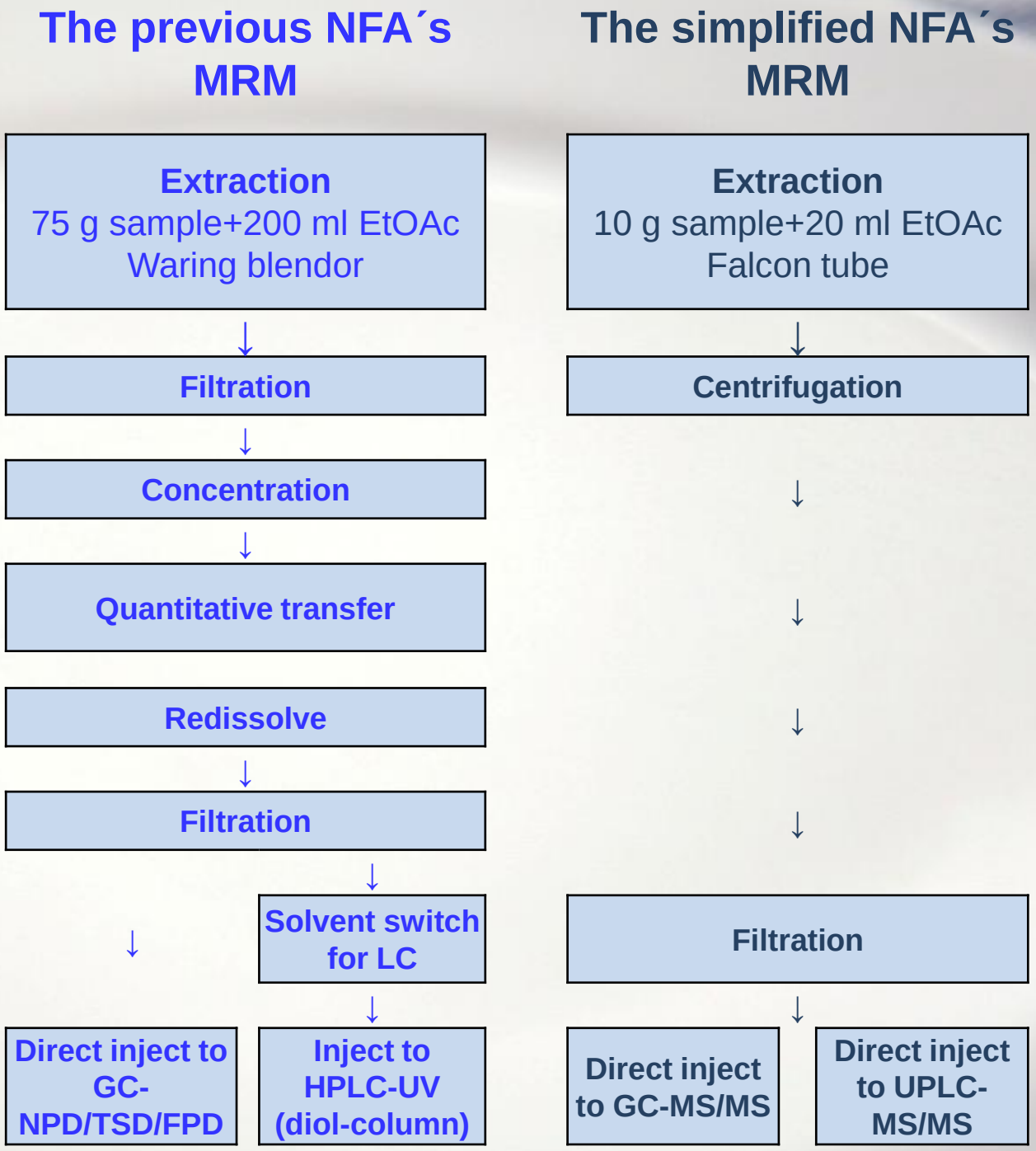
Fruit & Vegetables



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The simplified NFA´s MRM for FV (348 analytes; 129 GC, 219 LC)

Extraction

10 g sample + 3 g $NaHCO_3$

20 ml ethyl acetate and 10 g Na_2SO_4
Falcon tube in ultrasonic bath 3 minutes

Centrifugation

in 3 min. (3800 g)

Filtration

Filtrate the crude extract

0.20 µm PTFE filter

Inject to GC-MS/MS and UPLC-MS/MS

Sample conc. 0.5g/ml

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Cereals

The previous NFA´s MRM

Extraction
48 g sample+300 ml EtOAc
Waring blendor

↓

Filtration

↓

Concentration

↓

Filtration

GPC clean-up on S-X3 column

↓

Concentration

↓

Redissolve

↓ ↓

Inject to GC-NPD/TSD/FPD

Inject to HPLC-UV (diol-column)

The simplified NFA´s MRM

Extraction
5 g sample+10 ml EtOAc
Falcon tube

↓

Centrifugation

↓

↓

↓

↓

↓

Filtration

↓ ↓

Direct inject to GC-MS/MS

Direct inject to LC-MS/MS

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The simplified NFA´s MRM for cereals (250 analytes; 103 GC, 157 LC)

Extraction

5 g sample

10 ml H_2O + 20 ml ethyl acetate and 10 g Na_2SO_4
Falcon tube in ultrasonic bath 3 minutes

Centrifugation

in 3 min. (3800 g)

Filtration

Filtrate the crude extract

0.20 µm PTFE filter

Direct injection of EtOAc extract to GC-MS/MS and LC-MS/MS

Sample conc. 0.5g/ml

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Animal Origin

Method A

Extraction
5 g sample
Falcon tube

↓

Filtration

↓

Centrifugation

↓ ↓

Inject to GC-MS/MS

Inject to UPLC-MS/MS

Method B

Fat determination
Gravimetric determination after extraction with DCM

↓

Extraction

↓

Filtration

↓

Purification
GPC, SX-3

↓

Centrifugation

↓

Evaporation

↓

Reconstitution

↓ ↓

Inject to GC-MS/MS

Inject to UPLC-MS/MS

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Analysis of pesticides in products with animal products Method A (75 analytes)

Extraction

5 g sample + 0.5 g Na_2SO_4

0.2 g PSA and 0.2 g C_{18}

10 ml ethyl acetate

Centrifugation

in 5 min. (3800 g)

Filtration

Filtrate the crude extract

0.20 µm PTFE filter

Direct injection of EtOAc extract to GC-MS/MS and UPLC-MS/MS

Sample conc. 0.5g/ml

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Analysis of pesticides in products with high fat content Method B (76 analytes)

Gravimetric fat determination

Extraction

Amount of fresh sample that corresponds to 0.5 g of fat

0.5 g Na_2SO_4

10 ml ethyl acetate + cyclohexane (1+1), 10 min

Centrifugation and filtration

in 3 min. (3800 g), filtrate the crude extract with 0.20 µm PTFE filter

Clean up

5.0 ml to GPC, SX-3, EtOAc/CH (1+1)

Evaporation and Reconstitution

Parallel vacuum evaporator to dryness with N_2. Re-dissolve in 0.5 ml ethyl acetate.

Direct injection of EtOAc extract to GC-MS/MS and LC-MS/MS

Sample conc. 0.5g/ml

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Fruit & Vegetables

Extraction
Ethyl acetate
5 g sample
Falcon tube

↓

Centrifugation

↓

Filtration

↓

Direct inject to GC-MS/MS

Direct inject to UPLC-MS/MS

Cereals

Extraction
Ethyl acetate
5 g sample
Falcon tube

↓

Centrifugation

↓

Filtration

↓

Direct inject to GC-MS/MS

Direct inject to LC-MS/MS

Animal Origin, A

Extraction
Ethyl acetate
5 g sample
Falcon tube

↓

Filtration

↓

Centrifugation

↓ ↓

Inject to GC-MS/MS

Inject to UPLC-MS/MS

Animal Origin, B

Fat determination

↓

Extraction
EtoAc+CH

↓

Filtration

↓

Purification
GPC, SX-3

↓

Centrifugation

↓

Evaporation

↓

Reconstitution

↓ ↓

Inject to GC-MS/MS

Inject to UPLC-MS/MS

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Example of validation of the simplified MRM for fruit and vegetables

- Validation according to the SANCO 10684/2009 document
- Full validation in three different commodity groups (high water and high acid content, high acid content and high sugar and low water content)
- Data with more than 28 600 results stored
- The results produced by NFA and the official laboratory

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Extracted data sheet from validation data archive

Pesticide	Matrix	Detection	Spiking level mg/kg	Recovery 1 %	Recovery 2 %	Recovery 3 %	Recovery 4 %	Recovery 5 %	Mean Recovery %	RSD %
Cyprodinil	Raisins	GC-MSMS	0,01	81,55	94,61	104,50	128,40	101,50	102	16,8
Danifos	Raisins	UPLC-MSMS	0,01	87,40	93,66	90,75	89,44	92,92	91	2,8
DEET	Raisins	GC-MSMS	0,01	105,00	93,54	96,78	87,30	104,60	98	8,5
Deltamethrin	Raisins	GC-MSMS	0,01	66,52	79,44	100,60	97,24	99,56	89	17,0
Demeton	Raisins	UPLC-MSMS	0,01	92,35	89,46	97,61	95,09	98,18	95	3,9
Demeton-S-methyl	Raisins	UPLC-MSMS	0,01	94,00	89,85	99,52	95,47	100,05	96	4,4
Demeton-S-methyl-sulfone	Raisins	UPLC-MSMS	0,01	95,58	97,10	94,17	90,57	94,98	94	2,6
Demeton-S-methyl-sulfone	Raisins	UPLC-MSMS	0,01	88,33	91,33	88,23	91,09	90,35	90	1,7
Demeton-S-methyl-sulfoxid	Raisins	UPLC-MSMS	0,01	72,85	73,38	73,11	72,11	73,17	73	0,7
Desmethyl pirimicarb	Raisins	UPLC-MSMS	0,01	91,44	92,83	91,31	92,76	91,70	92	0,8
Desmetryn	Raisins	UPLC-MSMS	0,01	88,55	91,96	93,51	89,32	95,41	92	3,1
Dialifos	Raisins	UPLC-MSMS	0,01	79,28	82,71	86,97	80,09	82,83	82	3,7
Diazinon	Raisins	UPLC-MSMS	0,01	84,52	82,31	84,83	82,86	89,14	85	3,2
Dichlobenil	Raisins	GC-MSMS	0,01	94,35	93,07	101,50	84,39	86,02	92	7,5
Dichlofluanid	Raisins	GC-MSMS	0,01	90,53	83,03	87,24	115,70	95,33	94	13,5

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Results :

0.01 mg/kg and 0.05 mg/kg

Matrix		Mean%	RSD%
Orange	GC	99,2	12,8
	LC	87,8	9,5
	GC and LC	92,7	12,7
Leek	GC	102,3	19,8
	LC	93,8	7,8
	GC and LC	96,6	14,4
Raisins	GC	98,0	17,4
	LC	95,0	7,0
	GC and LC	96,1	12,3
All matrices	GC	99,8	16,6
	LC	92,1	8,8
	GC and LC	95,1	13,3

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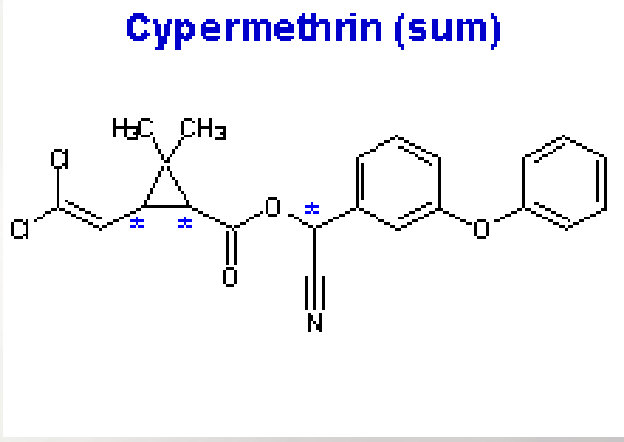
GC-MS/MS – in a real life example.

Cypermethrin was recently found in table grapes

- a pyrethroid insecticide (a synthetic pyrethrin found e.g. in Myrr C)
- 10 g sample extracted with 20 ml ethylacetate
- GC-MSMS analysis
- 2 MS/MS transitions used per analyte
  - “Quantifier”: m/z 163>127 at 5 volts fragmentation energy
  - “Qualifier”: m/z 163>91 at 11 volts fragmentation energy



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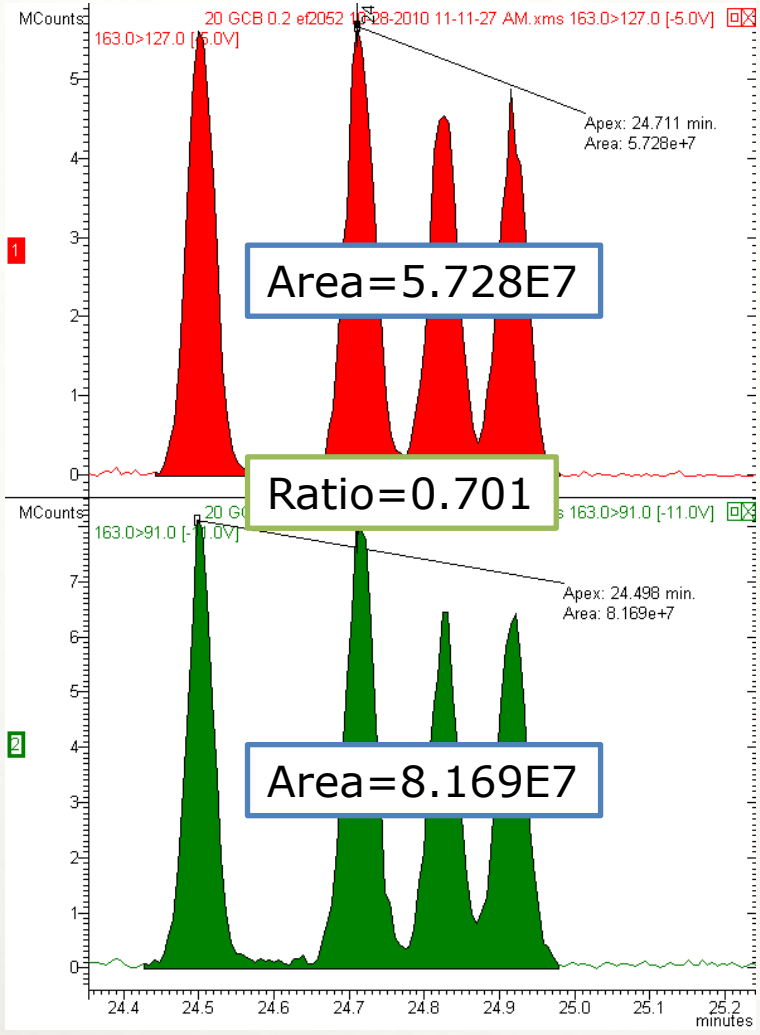
Cypermethrin in grapes

Calibration standard, known concentration, 0.4 mg/kg

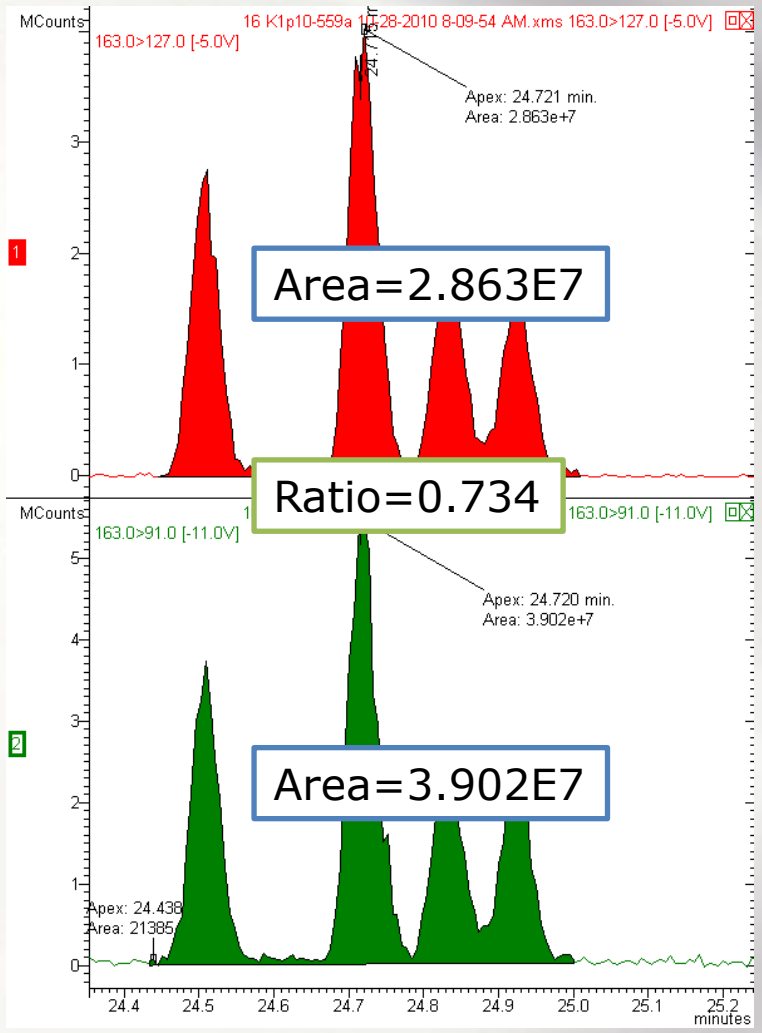
Grape sample, unknown concentration and Id

Quantifier
m/z 163>127

Qualifier
m/z 163>91



Retention time



Retention time

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Evaluation and identification

- Quantifier transition for determination of concentration
- Quantifier/Qualifier ratio to identify id
  - 1 qualifier required for pesticide identification
  - Can use several to strengthen identification
- Retention time match gives additional identification

- Cypermethrin example:
  - Identified positive as cypermethrin.
  - Residue level determined to 0.20 mg/kg
  - EU Maximum Residue level (MRL) in table grapes: 0.5 mg/kg
  - => no exceedance of MRL, no legal action

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Advantages of the new simplified method

- No evaporative concentration steps.
- The extract is injected directly into the GC and LC.
- Small volumes of solvent.
- The need of clean up has been eliminated.
- The method has been greatly simplified providing significant benefits in terms of sample throughput and performance.
- Matrix components as proteins and sugar, which often interfere with the chromatographic determination, are extracted at very low levels.

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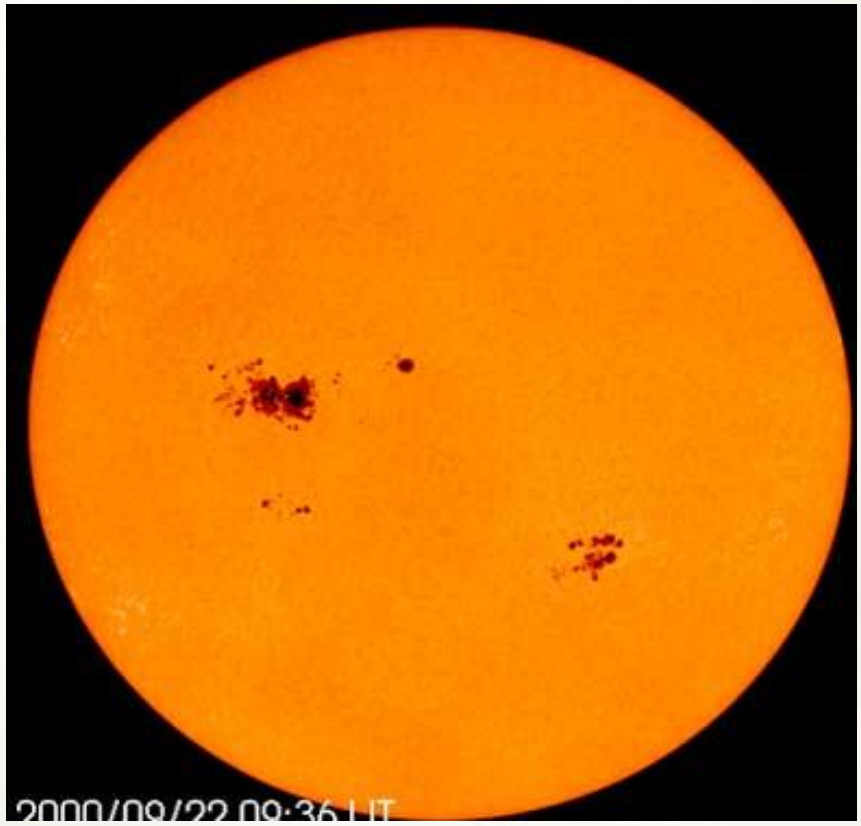
Even the sun has spots……



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The study of different extraction solvents and the amount of co extracted matrix components

J. Klein and L. Alder: Development of a Multi-Analyte / Multi-Matrix Method Based on LC-MS/MS; EPRW 2002, Rome 28.- 31.05.2002

Type of solvent

Matrix	Matrix type	Amount of extracted sample	Acetone /water 2+1	Acetonitrile /water 2+1	Methanol /water 2+1	Water	Ethyl acetate
Tomato	High water content	1.0g	38mg	36mg	34mg	33mg	8mg
Citrus	High acid and high water content	1.0g	79mg	69mg	81mg	-	3mg
Raisins	High sugar an low water content	1.0g	370mg	148mg	333mg	302mg	4mg
Avocado	High oil content	1.0g	33mg	40mg	30mg	43mg	**332mg**

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Limitations
Distorted or split peak shapes

Solvent composition ”solvent effect”

- The injection solvent is stronger than the mobile phase
- EtOAc not diluted immediately with MP, some of the analyte molecules are diluted and migrated with the MP resulting in the split peak
- Early Peaks (low k) most affected (2-5 min)

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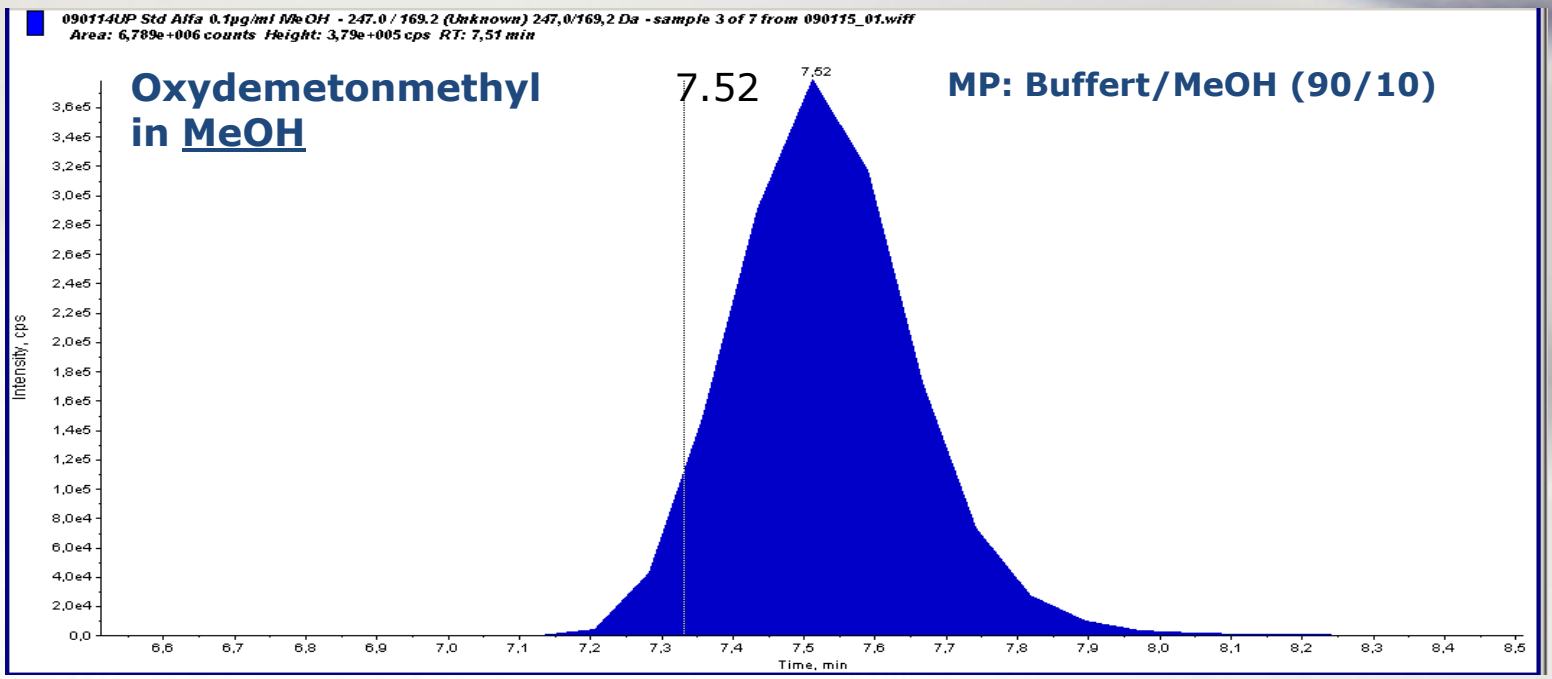

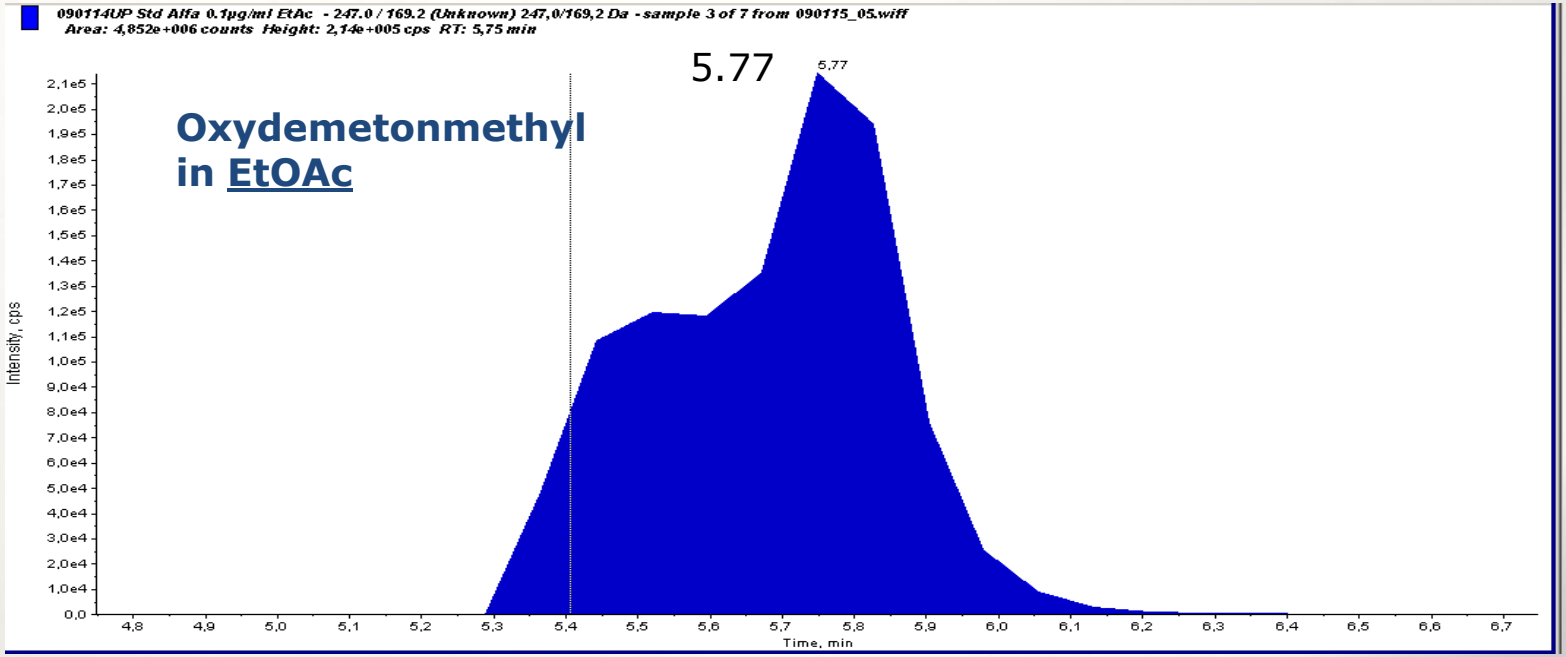

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Limitations
Distorted or split peak shapes

Volume(mass) of the injection solvent

If the injection solvent is weaker than the MP larger volumes can be injected. When the injection solvent is stronger than the MP, it is important to keep the injection volume low.

UPLC – injection volume of 2 µl is recommended to avoid the peak split.

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Figure 14: MRM chromatograms of a 100ng/ml standard in ethyl acetate using 0.5 ul injection volume.

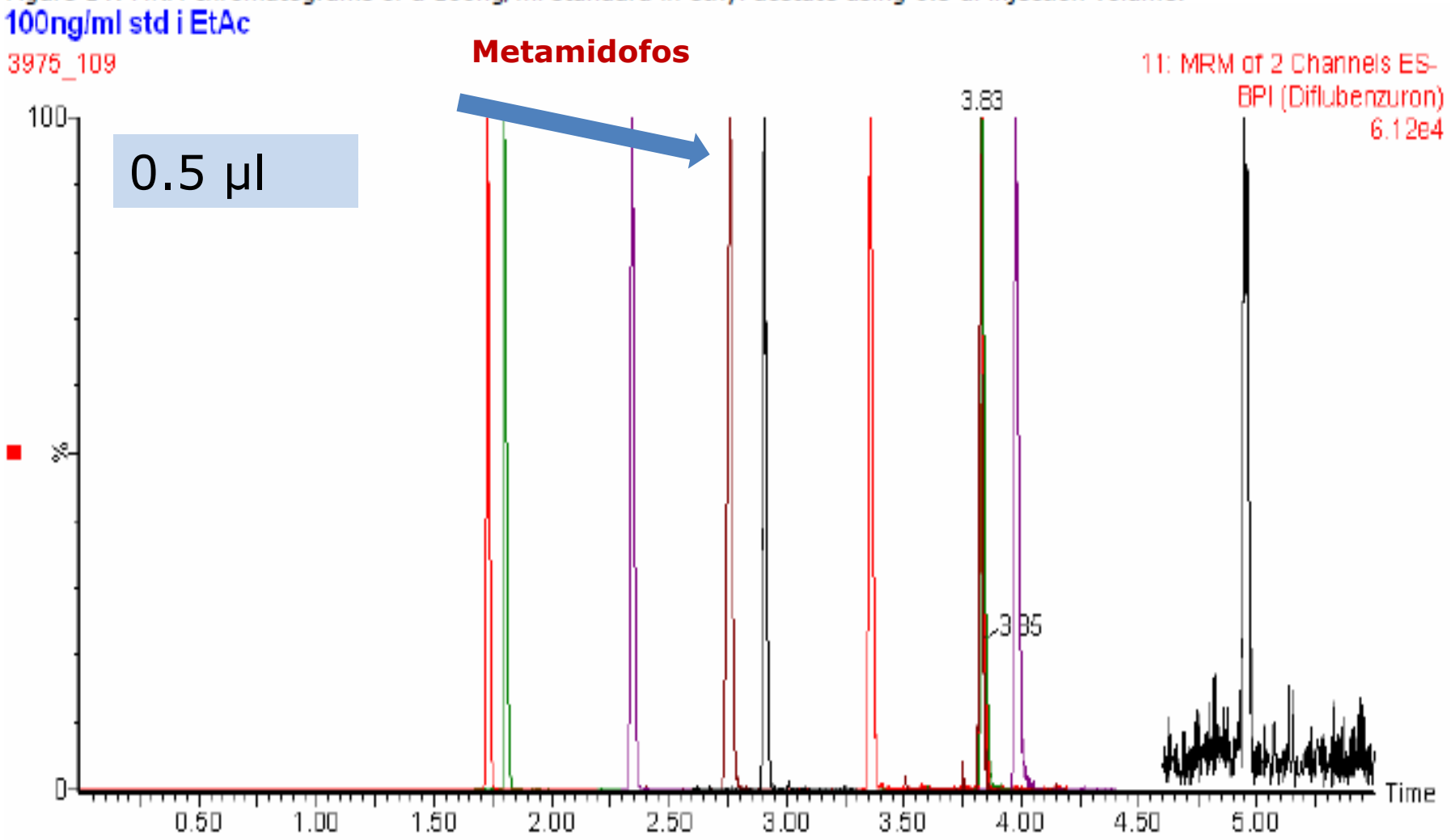
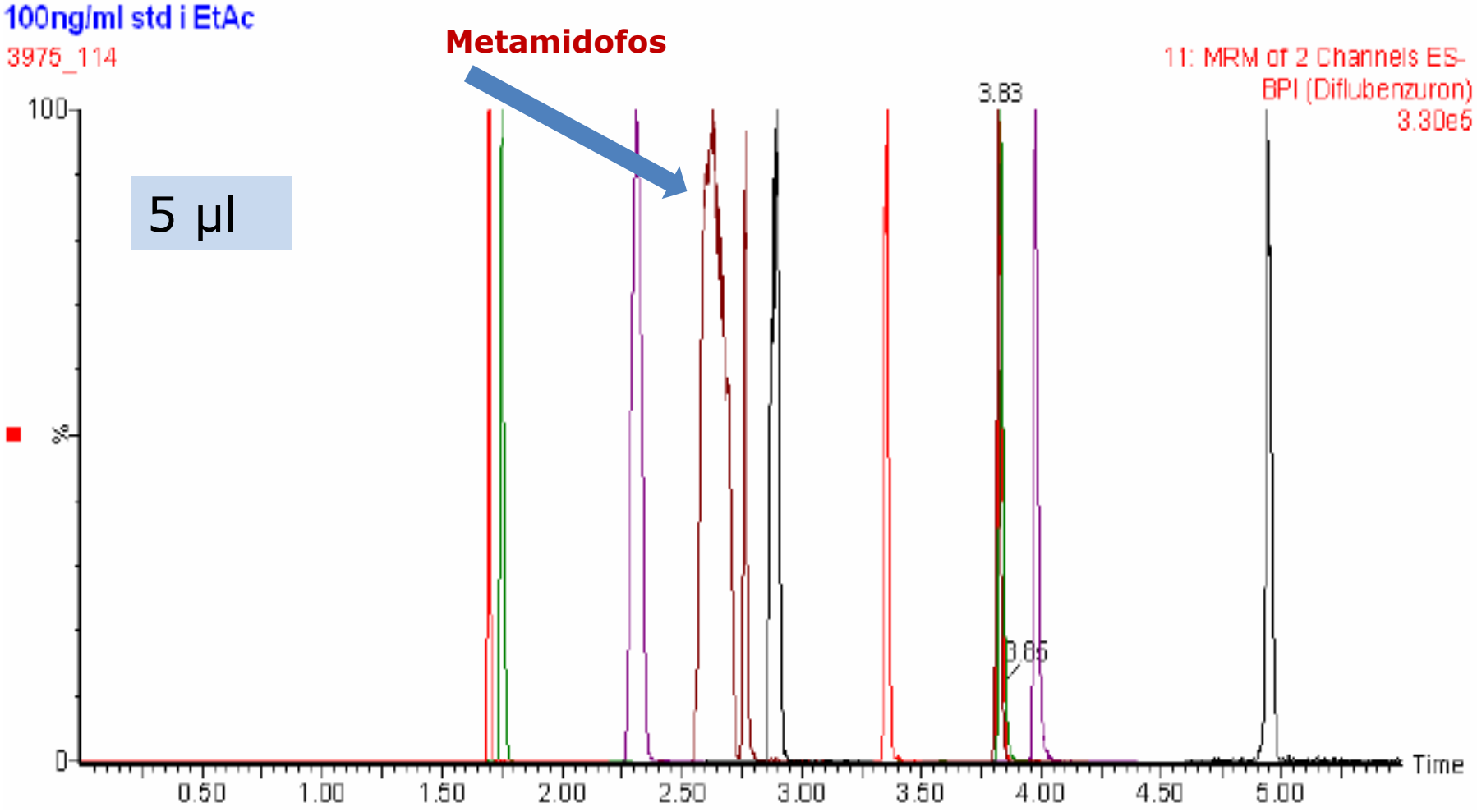


Figure 15: MRM chromatograms of a 100ng/ml standard in ethyl acetate using 5 ul injection volume.



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Preliminary results on EU RL PT 13 (mandarin)

Pesticide	NFA	Eurofins SWE
Carbendazim (sum)	0,1	0,0
Chlorpyrifos	0,4	0,3
Deltamethrin	-0,2	0,1
Diazinon	0,0	-0,6
EPN	-0,9	0,3
Imazalil	-0,3	-0,3
Indoxacarb (sum)	0,1	-0,1
Malathion (sum)	0,0	-1,4
Malathion	0,1	-1,4
Methidathion	0,0	-1,6
Methomyl (sum)	0,4	-0,1
Methomyl	0,4	-0,1
Orthophenylphenol	0,0	0,6
Oxamyl	0,4	-0,3
Pendimethalin	0,2	0,3
Phosalone	-0,2	0,7
Prochloraz	-0,7	0,0
Pyriproxyfen	-0,2	-0,2
Spinosad (sum)	-0,6	-1,3
Thiabendazole	0,4	-0,8
Tolylfluanid (only)	0,6	-1,9

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Results on EU RL PT 12 (leek)

Pesticide	NFA	Eurofins SWE
Aldicarb Sum	0,0	0,1
Azinphos-methyl	-1,3	-0,8
Carbendazim	0,0	0,1
Chlorthalonil	0,6	-3,8
Chlorpyrifos	-1,3	-0,2
Dimethoate Sum	-1,4	0,9
EPN	-1,5	-0,6
Ethion	-1,1	0,0
Fenpropathrin	-1,2	0,1
Imidacloprid	-0,4	0,3
Kresoxim-methyl	-1,7	-0,8
Methamidophos	-1,4	-0,3
Oxamyl	0,0	0,0
Prothiofos	-1,3	-0,1
Thiacloprid	-0,6	-0,5
Triflumuron	-1,5	-0,2

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EURL FV Proficiency test SM03 (qualitative screening).

- EUPT-FV-SM03 information:
  - No target list provided. Focus:
    - pesticides not included in the EU control program
  - 72 hour deadline for result submission
  - Only qualitative results requested
  - Matrix: Mandarin
  - High levels in sample, >100 ppt

- Total Number of Reporting Laboratories = 45

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Screening EUPT-FV-SM03 with the SweEt multimethod for Fruit and Vegetables

- GC-MS/MS and LC-MS/MS was used
- Two target scopes included:

Ordinary (348 pest.), used in EU control program		**Extended** (153 pest.), used only for qualitative analysis	
GC: 129	LC: 219	GC: 51	LC: 102

- Total scope: ~500 pesticides!
  - Standards available in our lab
  - Retention time and transitions known
- Two labs using SweEt participated (NFA and Eurofins Swe)

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SweEt results (preliminary)

GC-MS/MS		
Chlozolinate	Nuarimol	Quinalphos
Etrimfos	Orthophenylphenol	Quinomethionat
Mevinphos	Prothiofos	Sulfotep

LC-MS/MS		
Alachlor	Fonofos	Propoxur
Atrazine	Forchlorfenuron	Pyridaphenthion
Benalaxyl	Mecarbam	Terbufos
Carbophenothion	Ofurace	Terbuthylazine
Fenamidone	Phorate	Tolfenpyrad
Flucythrinate	Prometryn	

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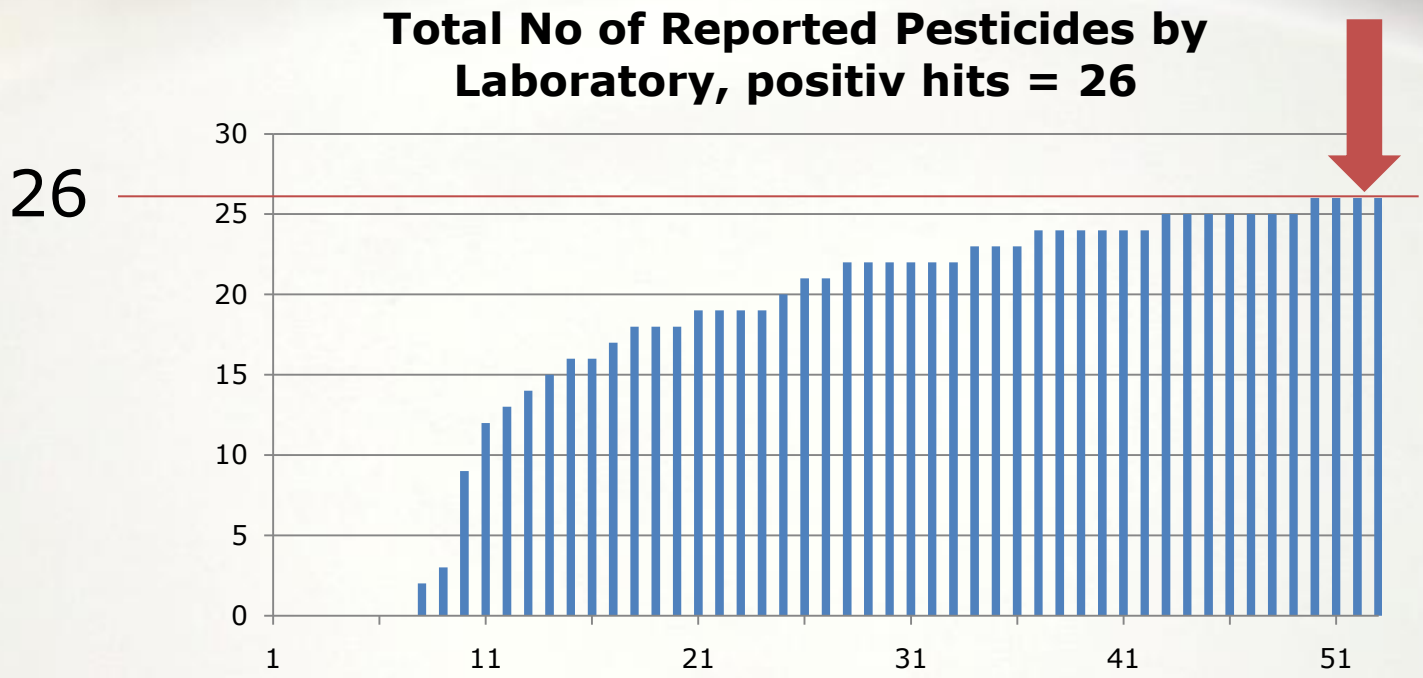
Laboratory comparison



- **100 % hit rate! - In both SweEt-labs. No false pos. No false neg.**

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SM03 and SweEt LC-TOF?

- LC-TOF-ES+, Bruker (maXis)
- LC column: C18 (Acclaim), L: 100 cm, ID 2.1 mm, 2 µm. H20/MeOH/buffer (NH_4Fo)
- Library: Retention data and molecular formula for c:a 650 compounds/adducts entries

- Setting up and running the method:
  - No method development
  - No standards was used
  - Software data processing ~30 s
  - Manual evaluation ~2-3 h

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Results using SweEt-LC-TOF

Previously found with GC-MS/MS		
Chlozolinate	Nuarimol	Quinalphos
Etrimfos	Orthophenylphenol	Quinomethionat
Mevinphos	Prothiofos	Sulfotep

Previously found with LC-MS/MS		
Alachlor	Fonofos	Propoxur
Atrazine	Forchlorfenuron	Pyridaphenthion
Benalaxyl	Mecarbam	Terbufos
Carbophenothion	Ofurace	Terbuthylazine
Fenamidone	Phorate	Tolfenpyrad
Flucythrinate	Prometryn	

Green color: direct hit

Blue: hit after including 200 molecular formulas to library (no retention data entered)

Red: Not detected

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Comments and future outlooks

- Powerful and quick tool for screening
- LC-TOF perform well at high analyte levels. At low levels, ~10 ppb, high frequency of false +/- have been reported
- Project: Run EU control prog samples using MS/MS but also test on LC-TOF
  - Performance qualification of LC-TOF method
  - Evaluate suitability to detect illegal pesticides w LC-TOF

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Proficiency Test 1-4 for cereals

EUPT-C1 2007	EtOAc z-score	Assigned value mg/kg	ACN z-score
Diazinon	-0,9	0,078	-1,0
Azoxystrobin	-0,8	0,24	-0,9
Carbendazim	-0,4	0,126	0,6

EUPT-C2 2008	EtOAc z-score	Assigned value mg/kg	ACN z-score
Pirimicarb	0,1	0,038	-1,7
Spiroxamine	0,1	0,075	-1,7
Asoxystrobin	0,3	0,239	-1,9
Prochloraz	0,0	0,239	-1,5
Epoxiconazole	-0,5	0,176	-0,4
Trifloxystrobin	-0,1	0,439	0,6

EUPT-C3 2009	EtOAc z-score	Assigned value mg/kg	ACN z-score
Asoxystrobin	-0,2	0,174	-0,1
Carbendazim	0,1	0,491	-0,5
Cyproconazole	-1,7	0,435	-1,4
Fenpropimorf	-0,2	0,121	-0,6
Tebuconazole	0,0	1,220	-0,6
Metconazole	-0,2	0,476	-0,4
Fenbuconazole	-0,7	0,503	-1,1
Flusilazole	-0,1	0,724	-0,5
Fludioxonil	-0,6	0,078	0,1
Pyrachlostrobin	-0,4	0,746	-0,3

EUPT-C4 2010 (parts)	EtOAc z-score	Assigned value mg/kg	ACN z-score
Asoxystrobin	-1,6	0,316	-0,6
Carbaryl	0,8	0,159	0,4
Carbendazim	0,5	1,27	0,3
Deltamethrin	-0,2	0,061	0,6
Fenpropimorph	0,0	2,08	1,4
Flutriafol	-0,5	2,14	0,1
Isoproturon	0,1	0,162	-0,5
2,4-D free acid	1,1	0,352	-0,2
2,4-D foll alc hydr	-0,2	0,367	-0,3

PT Results for AO

Scope: 76 pesticides
Results on PT AO5
NFA, Sweden
Screening with M501(GPC)
Quantification with M502 (PSA,C_{18})

Fat Content: 14.20%

Pesticide	Z-scores
Chlorpfenvinfos	-0,0
Cypermetrin	-0,0
Lambda Cyhalothrin	-0,1
DDT	0,3
DDE	-0,1
Diazinon	-0,1
Deltamethrin	1,3
Endosulfan	-0,0
Alfa-Endosulfan	-0,1
Beta-Endosulfan	-0,1
Alfa-HCH	-0,7
Beta-HCH	-0,1
Pirimiphos-methyl	0,6
Triazophos	-0,7

Scope: 75 pesticides
Results on PT AO5
Eurofins, Sweden
M501(GPC)

Fat Content: 18.70%

Pesticide	Z-scores
Chlorpfenvinfos	-1,5
Cypermetrin	-0,8
Lambda Cyhalothrin	-1,1
DDT	0,0
DDE	-0,2
Diazinon	-1,2
Deltamethrin	0,3
Endosulfan	-0,8
Alfa-Endosulfan	-0,7
Beta-Endosulfan	-1,1
Alfa-HCH	-0,1
Beta-HCH	-0,6
Pirimiphos-methyl	-1,2
Triazophos	-1,6

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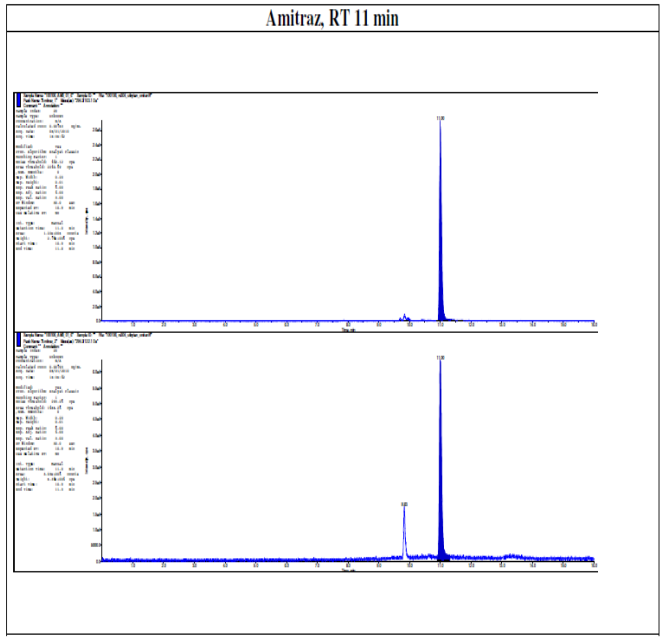
Recovery test of Amitraz, DMF and DMPF in different matrices

Pesticide	Matrix	Level mg/kg	Mean %	RSD %
Amitraz	Raisins	0.01	95 (5)	2.7
DMF	Raisins	0.01	96 (5)	1.2
DMPF	Raisins	0.01	82 (5)	5.4
Amitraz	Orange	0.01	95 (5)	2.7
DMF	Orange	0.01	92 (5)	4.0
DMPF	Orange	0.01	72 (5)	5.5
Amitraz *	Leek			
DMF	Leek	0.01	92 (5)	2.1
DMPF	Leek	0.01	94 (5)	2.7

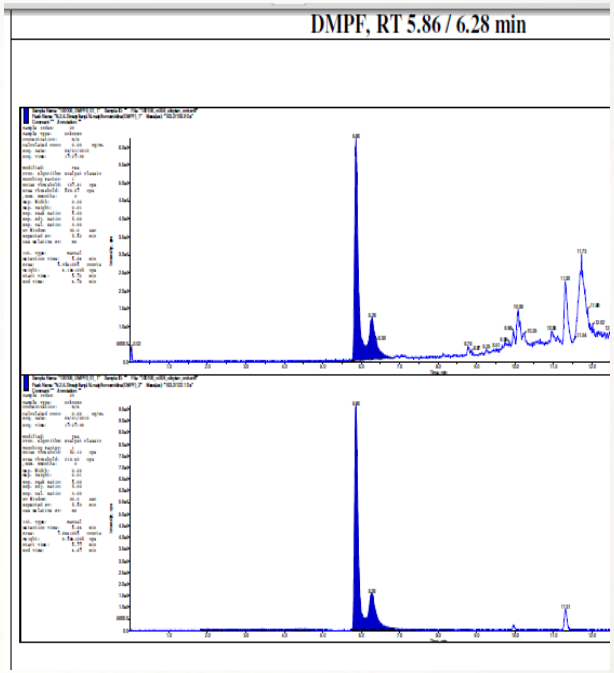
*Degradation of standard

Chromatogram of Amitraz and it metabolites in pears at 0.01 mg/kg

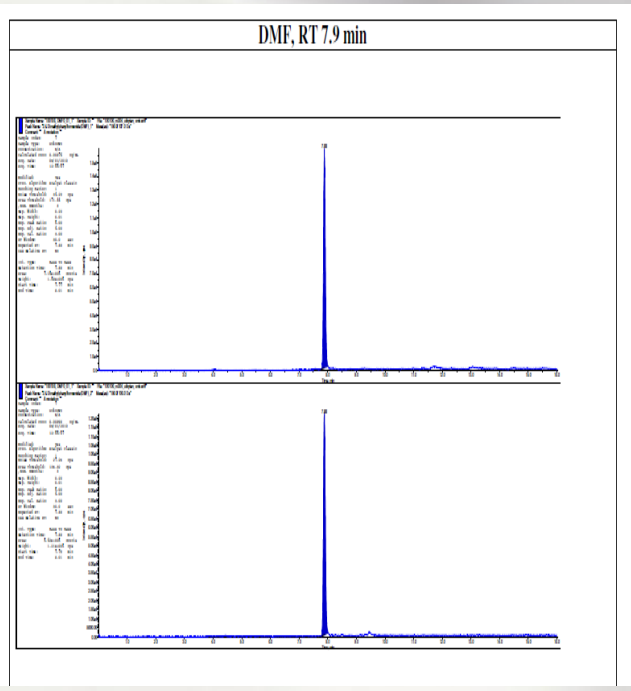
Amitraz
RT 11 min



DMPF
RT 5.8/6.3



DMF
RT 7.9



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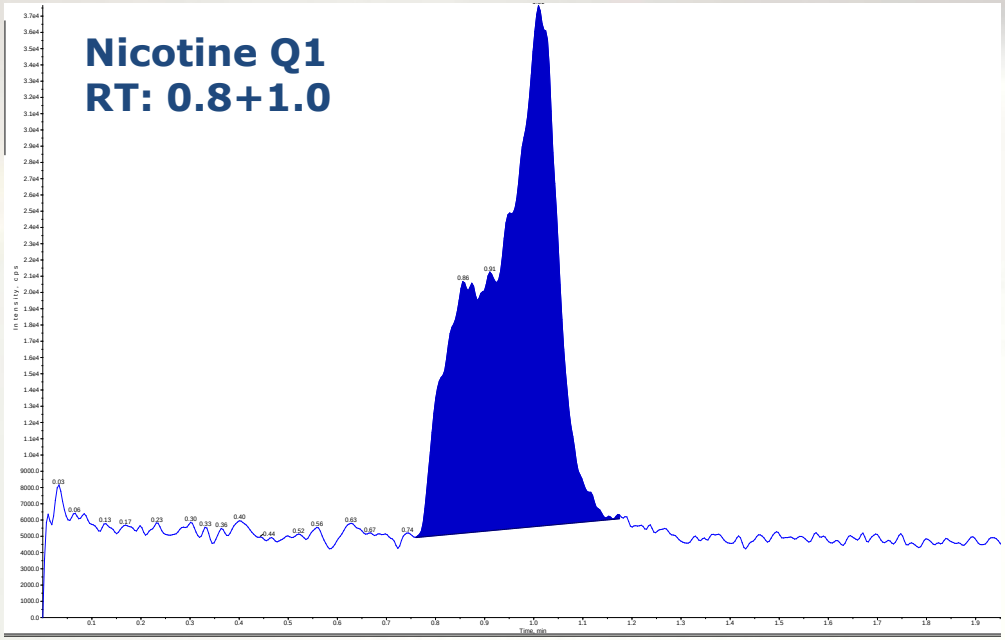
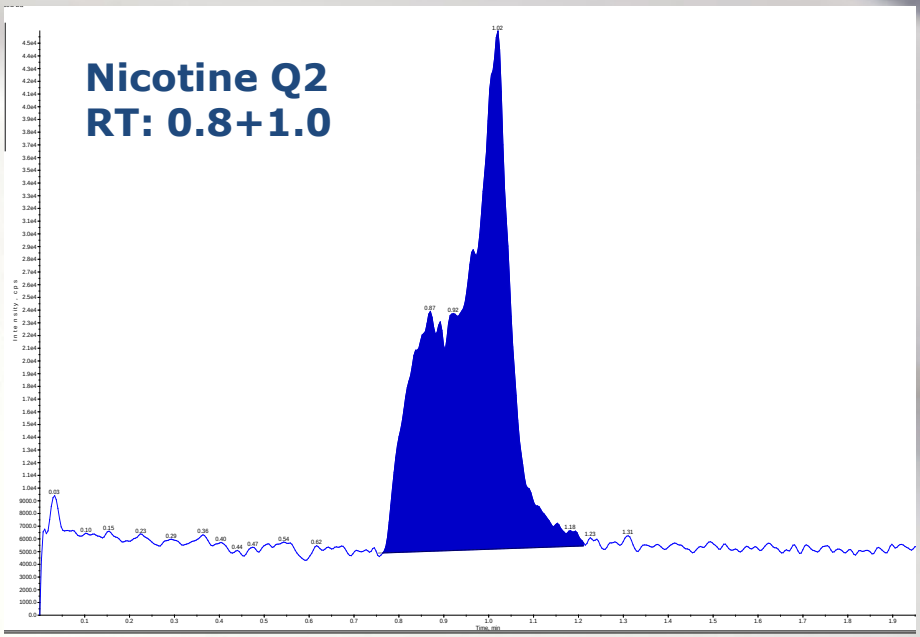
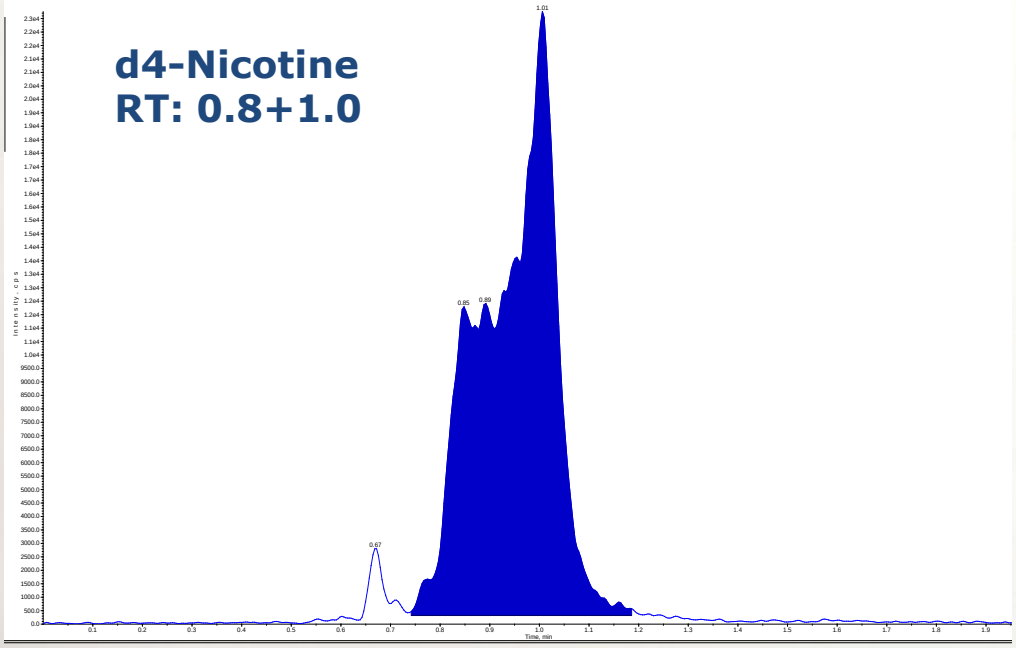
Nicotine in dried mushrooms at 0.3 mg/kg - extracted with pH 6-8







Instrument: UPLC-MSMS (Waters Aquity UPLC system - API-5000)
Column: Waters Acquity UPLC BEH C18 (100*2.1 mm , ID, 1.7 µm)
Mobile Phase A: Ammonium hydroxide 0.01 %
Mobile Phase B: Acetonitrile
Gradient: Isocratic, 40 % B for 2 min.
Flow rate: 0.45 ml/min

Future challenges

- Acid pesticides (tolufluanid, chlorthalonil..)
- Metabolites (definition of sums)
- Phenoxy acids including esters/conjugates
- Matrix effects
- Split peaks
- GC parameters
- Tea, coffee and spicies
- Mycotoxins

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Pesticides in tea and rosemary

Pesticide	Recovery Tea (%) 	Recovery Rosemary (%) 
Benfuracarb	82	91
Metconazole	91	96
Trifloxystrobine	84	92
Prothioconazole-desthio	88	108
Isoprothiolane	86	115
Ethion	72	92
Fenhexamide	96	119
Isoprothuron	86	95
Thiometon	74	106
Tridimefon	80	99
Zoxamide	80	89

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The presented method and the validation data is available at the EU RL website for fruit & vegetable AND
on the Swedish website www….
Where all presented methods will be described together with validation data etc.

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Questions?

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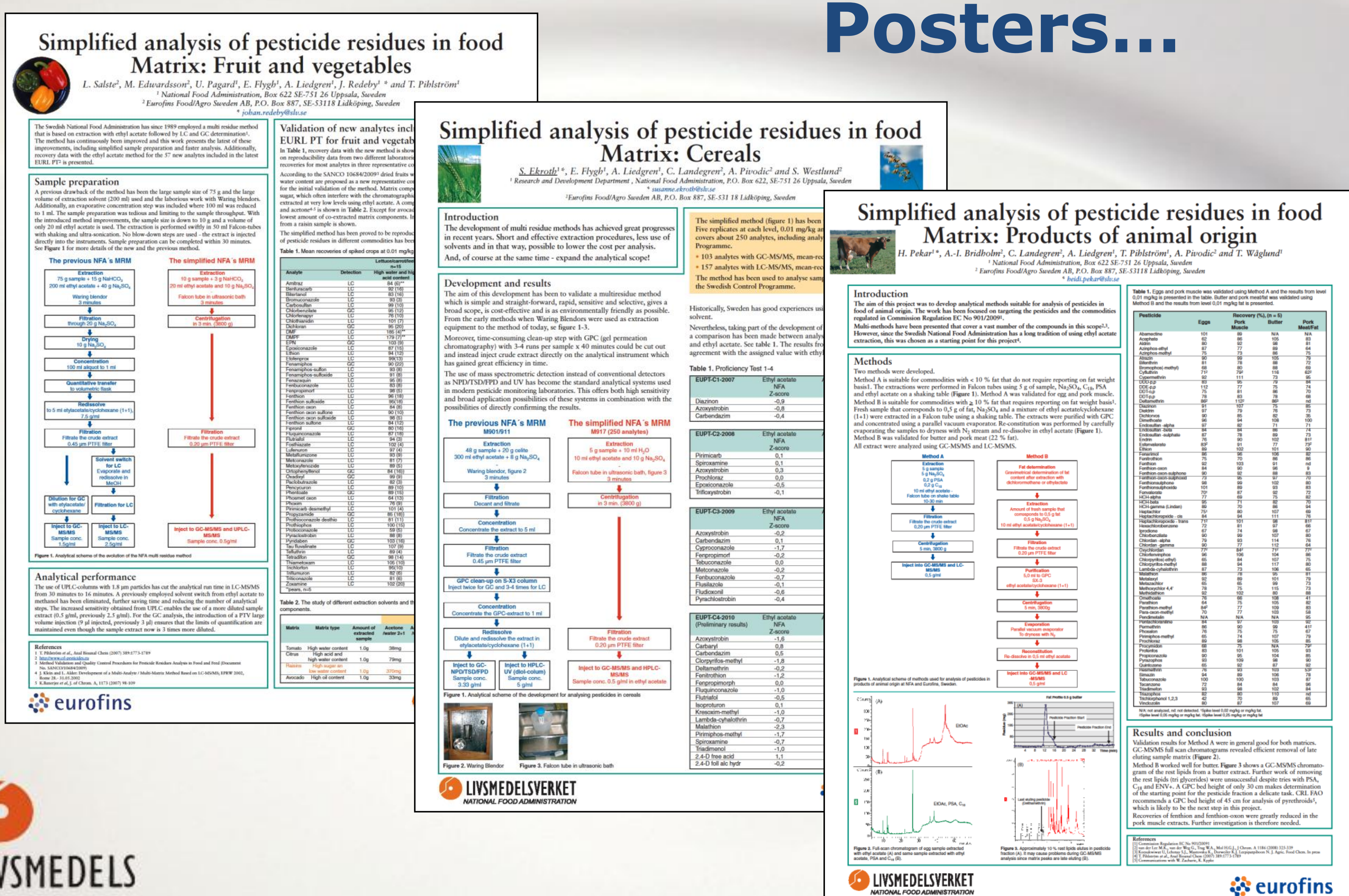
Posters...



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VERKET

Thank you for your attention!

Arbetsgrupp 3

Tuija Pihlström (arbetsledare)

Sussi Ekroth

Eva Flygh

Ulla Pagard

Heidi Pekar

Tommy Wåglund

Johan Redeby