

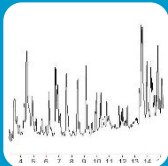


# Quantify with confidence and screen for the unexpected

New tools and workflows for your pesticide analysis

Dr. Thomas Glauner  
EMEA LC/MS Food Segment Scientist

# Agenda



Pesticide screening



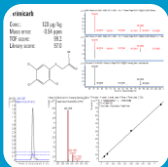
Toolbox Accurate Mass



Validation study



Real samples



Summary

# Introduction

## Current regulations and residue situation

- **EU regulation**

- Maximum residue levels regulated in commission regulation (EC) 396/2005
  - appendix II – IV regulates >170 000 MRLs (matrix-pesticide combinations)
- European and national monitoring programs, regulated by (EC) 396/2005
- 173 alert notifications related to pesticide residues entered in the Rapid Alert System for Food and Feed (RASFF) in 2009

- **US regulation**

- 40 CFR Part 180:
  - Regulates tolerances and exemptions for pesticides in food
  - Specifies allowed methodologies for analysis
- Special regulations are in place for:
  - Organic food
  - Baby food



# “Check your scope” ranking for pesticides

Published by EURL for pesticides



- Includes more than 1100 compounds
  - Pesticides currently used or used in the past
  - Pesticide metabolites of importance
- Ranking of pesticides based on:
  - Toxicological data (toxicological endpoints and endocrine disruptive activity)
  - Residue situation in crops (reporting from labs in Europe and RASFF notifications)
  - Agricultural usage (including potential for misuse and persistent pesticides)
- Current situation
  - Around 600 compounds included in routine monitoring programs
  - Only about 150 pesticides often found in food commodities

# Accurate Mass Screening and Quantitation

Wouldn't it be nice ...

- ... to measure everything ?
- ... to only calibrate and quantify what is likely ?
- ... to find what else is in the sample ?
- ... to retrospectively look into the sample ?
- ... to use profiling tools to find the unexpected ?



# The toolbox for accurate mass screening

## The complete solution from Agilent



**MassHunter Workstation Software**

**Quantitative Analysis**  
Version B.05.01 / Build 5.1.324.0  
for QTOF

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[www.agilent.com/chem](http://www.agilent.com/chem)  
Agilent Technologies

The screenshot displays the MassHunter software interface with several key components:

- Top Panel:** Search and analysis parameters, including Mass, Precision, and Collision energy.
- Table:** A list of compounds with columns for Compound Name, Formula, Mass, Aton, Cation, RT (min), CID, OverScan, N3, and EUPIC Type.
- Chromatogram:** A plot showing detector response versus time (min) with peaks labeled at 12.2963, 12.5102, 11.0904, and 21.7608.
- Method Editor:** A window showing the configuration of the acquisition method, including the instrument type (QTOF) and various parameters.
- Quantitation Results:** A table showing the results of the quantitative analysis, including peak names, retention times, and areas.
- Mass Spectrum:** A plot showing relative intensity versus m/z for a specific peak, with a base peak at m/z 225.0422.
- Reference Spectrum:** A plot showing the reference mass spectrum for a specific compound, with a base peak at m/z 332.7302.
- Reference Library:** A table showing the reference library entries, including compound names, formulas, and retention times.
- Reference Spectrum:** A plot showing the reference mass spectrum for a specific compound, with a base peak at m/z 348.0372.

... and we also have GC-MS/MS

# Screening workflow using LC-(Q)TOF-MS

## Instrumentation

### Technology Innovation



Ion Beam Compression Technology (resolution + mass accuracy)

Dual-stage ion mirror (resolution)



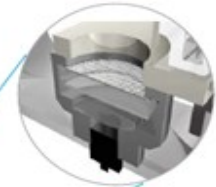
Longer flight tube (resolution)

INVAR flight tube (mass accuracy)



Orthogonal spray source (signal-to-noise)

ADC (dynamic range)



4 GHz electronics (resolution, mass accuracy, sensitivity, dynamic range)

Ion acceleration in hexapole collision cell (faster MS/MS spectra)

# Screening workflow using LC-(Q)TOF-MS

## Personal compound database and library

MassHunter PCDL Manager for Pesticides - C:\MassHunter\PCDL\Kempe\_Pest - import test.cdb

File Edit View PCDL Links Help

Find Spectra

Single Search Batch Search Batch Summary Edit Compounds Spectral Search

Browse Spectra Edit Spectra

Mass  
Precursor ion:   
Tolerance: 200  ppm  mDa  
Collision energy  
Tolerance: 2.0 eV

Ion polarity: (Any)  
Ionization mode: (Any)

Spectra for compound: Napropamide

Compound Name	Precursor Ion	Collision Energy	Ion Polarity	Ionization Mode	Instrument Type
Napropamide	272.16451	10	Positive	ESI	QTOF
Napropamide	272.16451	20	Positive	ESI	QTOF
Napropamide	272.16451	40	Positive	ESI	QTOF

Graphic Mass List

Library spectrum

Abundance

m/z

Single Search Results: 567 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider	IUPAC Name	Num Spectra
Napropamide	C17H21NO2	271.15723	<input type="checkbox"/>	<input type="checkbox"/>	10.772	15299-99-7	25304	N,N-Diethyl-2-(1-naphthoxy)propanamide	3
Neburon	C12H16Cl2N2O	274.06397	<input type="checkbox"/>	<input type="checkbox"/>	11.544	555-37-3	10672	1-Butyl-3-(3,4-dichlorophenyl)-1-methylurea	3
Nicarbazin	C19H18N6O6	426.12878	<input type="checkbox"/>	<input type="checkbox"/>		330-95-0	9135	1,3-bis(4-nitrophenyl)urea - 4,6-dimethylpyrimidin-2...	0
Nicosulfuron	C15H18N6O6S	410.10085	<input type="checkbox"/>	<input type="checkbox"/>	6.372	111991-0...	66024	2-[[[4,6-Dimethoxy-2-pyrimidinyl]carbonyl]sulfam...	3
Nicotine	C10H14N2	162.11570	<input type="checkbox"/>	<input type="checkbox"/>		54-11-5	80863	3-[(2S)-1-Methyl-2-pyrrolidinyl]pyridine	3
Nitenpyram	C11H15ClN4O2	270.08835	<input type="checkbox"/>	<input type="checkbox"/>	3.393	150824-4...	2298774	(E)-N-[[6-Chloro-3-pyridinyl]methyl]-N-ethyl-N'-meth...	3
Nitrapyrin	C6H3Cl4N	228.90196	<input type="checkbox"/>	<input type="checkbox"/>		1929-82-4	15205	2-Chloro-6-(trichloromethyl)pyridine	0
Norflurazon	C12H9ClF3N3O	303.03862	<input type="checkbox"/>	<input type="checkbox"/>		27314-13-2	31131	4-Chloro-5-(methylamino)-2-[3-(trifluoromethyl)phen...	3
Novaluron	C17H9ClF8N2O4	492.01231	<input type="checkbox"/>	<input type="checkbox"/>	13.325	116714-4...	84442	N-[(3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)et...	0
Nuarimol	C17H12ClFN2O	314.06222	<input type="checkbox"/>	<input type="checkbox"/>	9.309	63284-71-9	82786	(2-Chlorophenyl)(4-fluorophenyl)5-pyrimidinylmetha...	3
Ofurace	C14H16ClNO3	281.08187	<input type="checkbox"/>	<input type="checkbox"/>	6.480	58810-48-3	39084	2-Chloro-N-(2,6-dimethylphenyl)-N-(2-oxotetrahydr...	3
Omethoate	C5H12N2O4PS	213.02247	<input type="checkbox"/>	<input type="checkbox"/>	2.775	1113-02-6	13574	O,O-Dimethyl S-[2-(methylamino)-2-oxoethyl] phos...	3
Orbencarb	C12H16ClNOS	257.06411	<input type="checkbox"/>	<input type="checkbox"/>		34622-58-7	33829	S-(2-Chlorobenzyl) diethylcarbamothioate	3



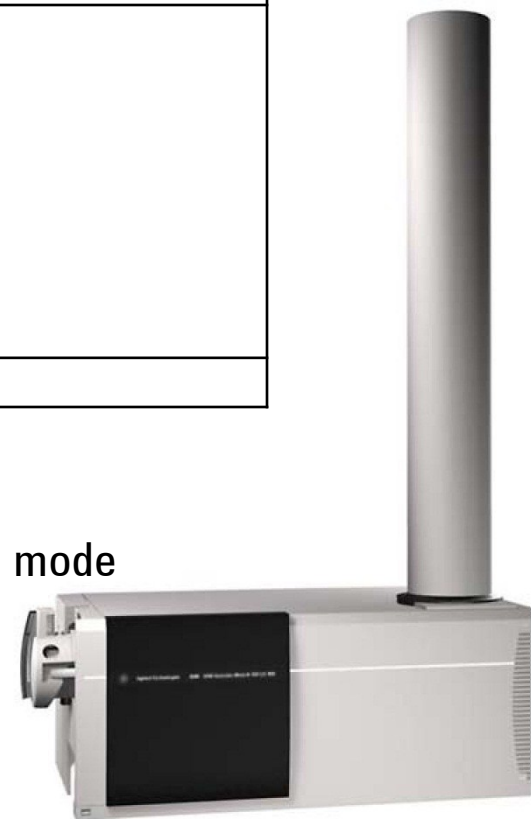
# Experimental

## UHPLC and QTOF method parameters



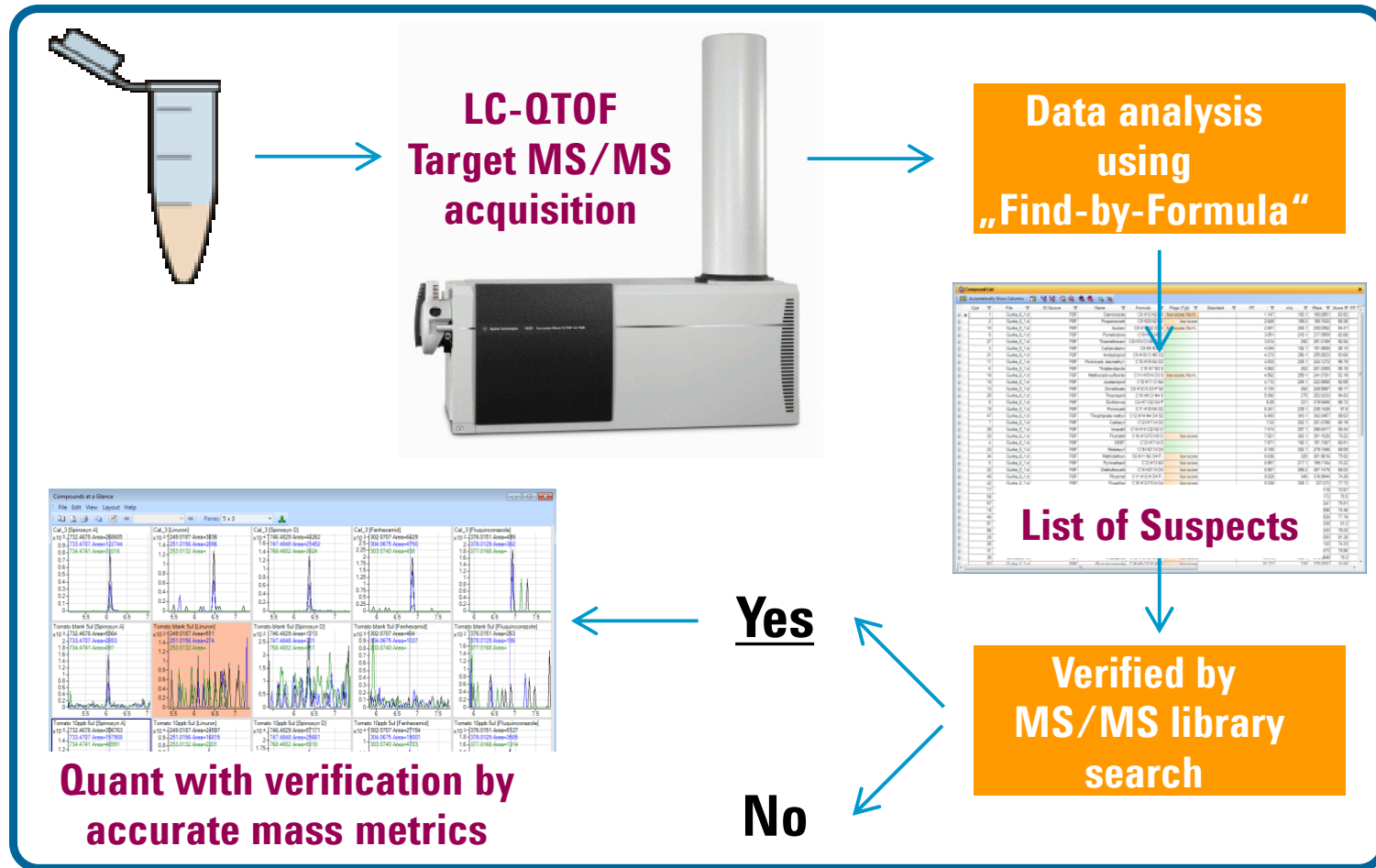
<b>UHPLC column</b>	Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 x 150 mm, 1.8 $\mu$ m @ 30°C	
<b>Mobile phase</b>	A: 5 mM NH <sub>4</sub> formate + 0.1% formic acid B: 5 mM NH <sub>4</sub> formate + 0.1% formic acid in methanol	
<b>Gradient program</b>	Min	% B
	0	10
	0.5	10
	3.5	50
	17.0	100
	20.0	100
	20.1	5
	Stop time	22 min
<b>Flow rate</b>	0.40 ml/min	

- **G6540 UHD QTOF** operated in 2GHz High extended dynamic range mode
- Acquisition rate **5 scans/sec in MS** and **3 scans/sec in MS/MS**
- Dynamic **target MS/MS acquisition** with > 200 target masses



# Validation study and real sample screening

## Experimental setup

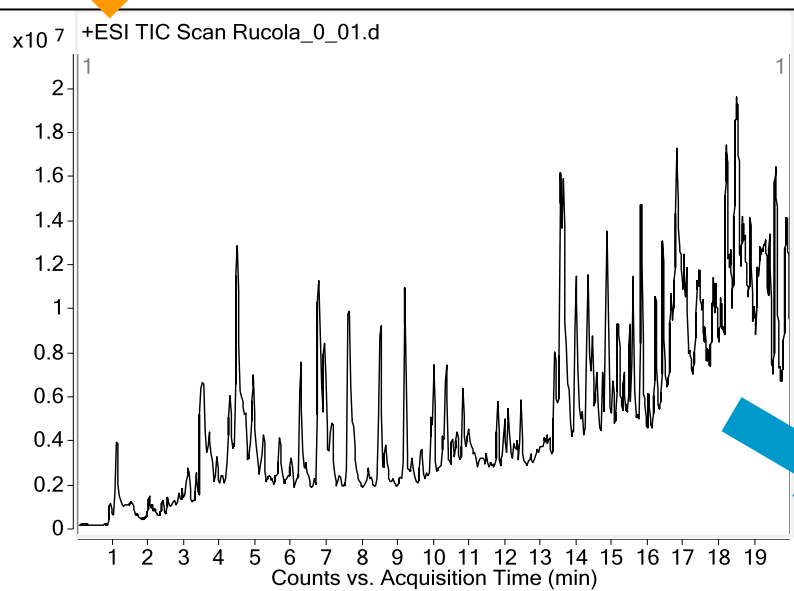


# Screening workflow using LC-(Q)TOF-MS

## Innovative workflow – Find by Formula



- Fully automatic compound identification based on formula information included in database and definition of ion species



TIC chromatogram of rucola extract spiked with pesticides @ 10 µg/kg

Method Editor: Screening - Find by Formula

Find Compounds by Formula

Negative Ions | EIC Smoothing | EIC Peak Filters | Results | Result Filters

Formula Source | Formula Matching | Positive Ions

Source of formulas to confirm

These formulas:  
C18H32Sn  
(type a comma-separated list of formulas, e.g., "C6H6, CH4")

Compound exchange file (.CEF):

Database  
C:\MassHunter\PCDL\Kemp\_PestProject.cdb

Worklist

Matches per formula

Maximum number of matches 1

Automatically increase for isomeric compounds

Values to match

Mass

Mass and retention time (retention time optional)

Mass and retention time (retention time required)

Method Editor: Screening - Find by Formula

Find Compounds by Formula

Negative Ions | EIC Smoothing | EIC Peak Filters | Results | Result Filters

Formula Source | Formula Matching | Positive Ions

Charge carriers

-electron

+H

+Na

+K

+NH4

Neutral losses

H2O

Charge states, if not known

Charge state range 1

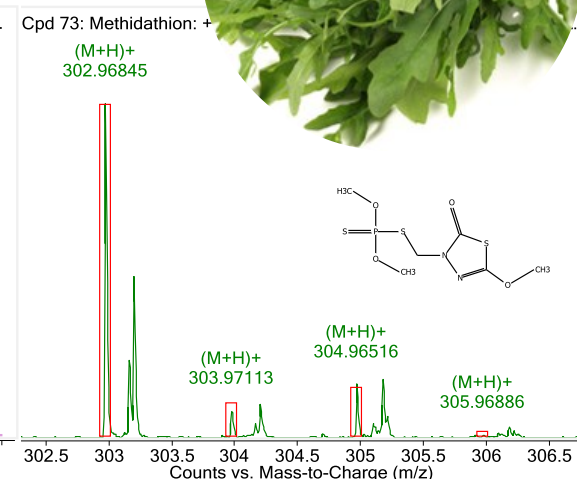
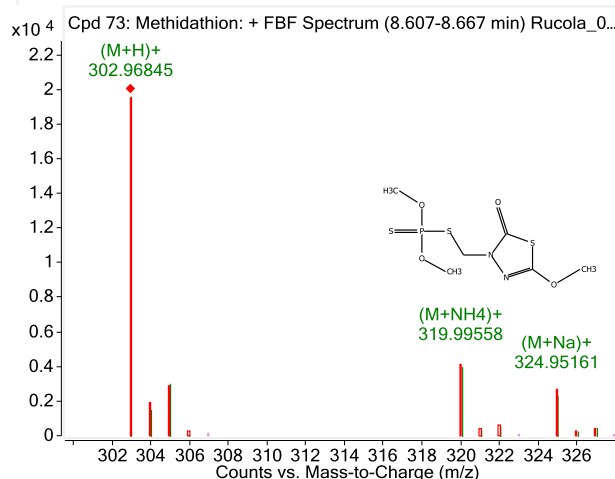
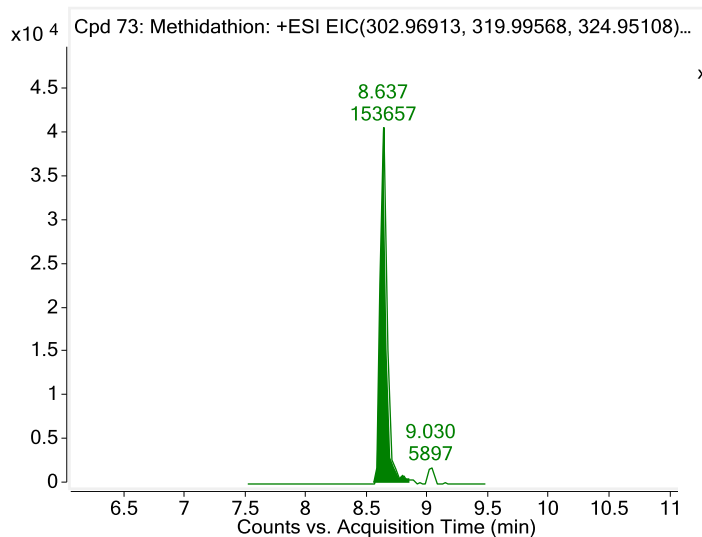
Aggregates

Dimers e.g., [2M+H]<sup>+</sup>

Trimers e.g., [3M+H]<sup>+</sup>

# Find-by-Formula data mining algorithm

## Rucola extract spiked with pesticides @ 10 µg/kg



Automatic EIC extraction for all adducts and isotopes

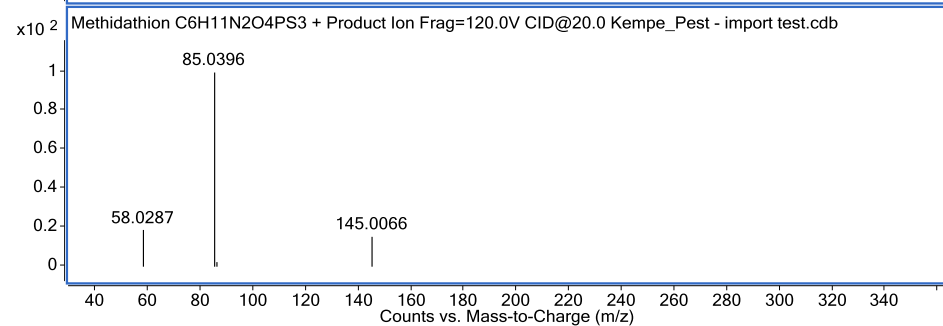
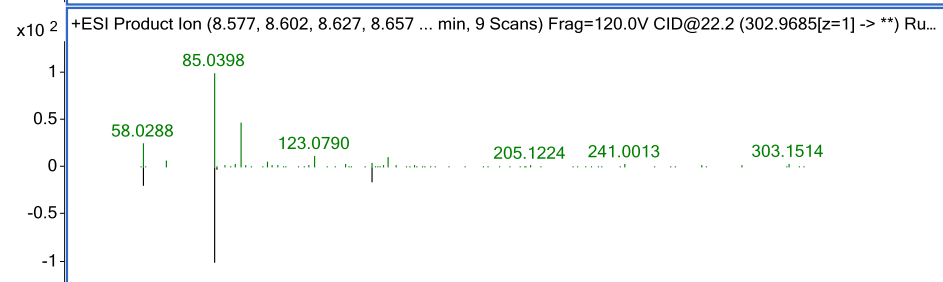
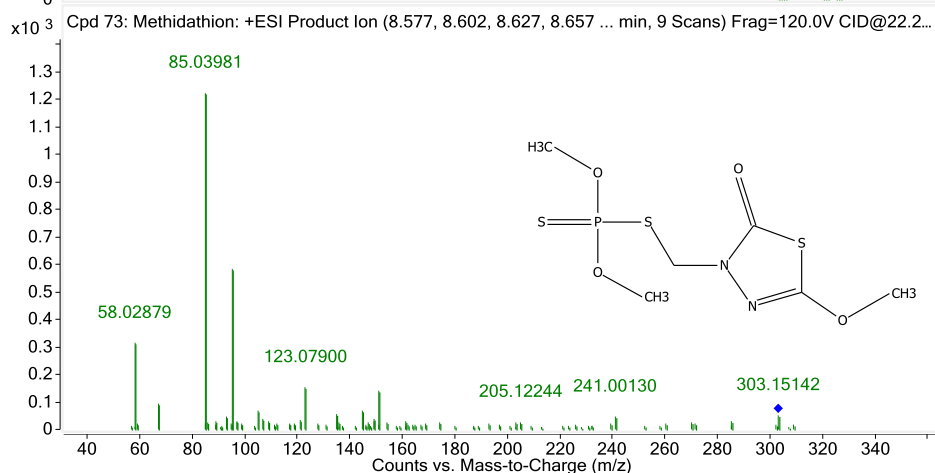
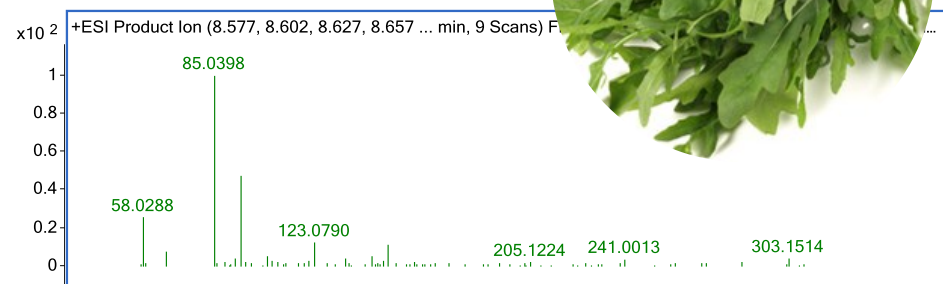
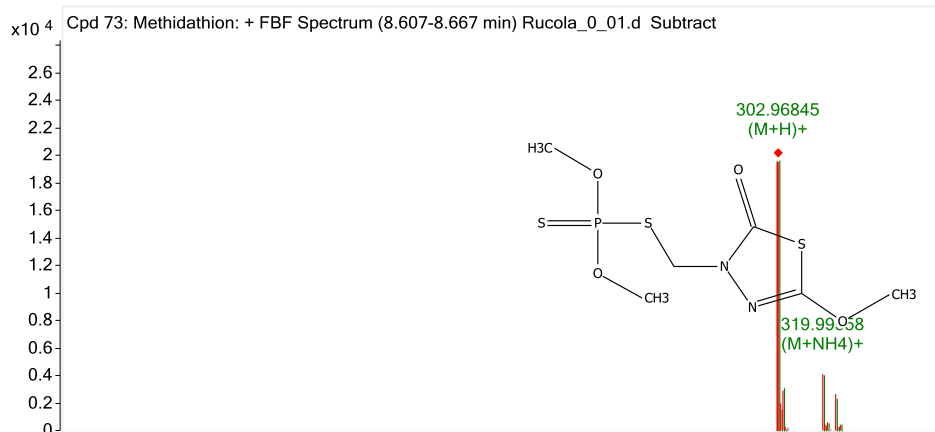
Extraction of MS and MS/MS spectra and visual comparison of isotope patterns

Cpd	File	ID Source	Name	Formula	Flags (Tgt)	Saturated	RT	m/z	Mass	Score																																													
173	Rucola_0_01.d	FBF	Methidathion	C6 H11 N2 O4 P S3			8.637	302.96845	301.96141	88.1																																													
<table border="1"> <thead> <tr> <th>Best</th> <th>ID Source</th> <th>Name</th> <th>Formula</th> <th>Score</th> <th>Score (RT)</th> <th>RT Diff</th> <th>Notes</th> <th>RT (Tgt)</th> </tr> </thead> <tbody> <tr> <td></td> <td>FBF</td> <td>Methidathion</td> <td>C6 H11 N2 O4 P S3</td> <td>88.1</td> <td>25.78</td> <td>0.134</td> <td></td> <td>8.503</td> </tr> </tbody> </table>											Best	ID Source	Name	Formula	Score	Score (RT)	RT Diff	Notes	RT (Tgt)		FBF	Methidathion	C6 H11 N2 O4 P S3	88.1	25.78	0.134		8.503																											
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<table border="1"> <thead> <tr> <th>Species</th> <th>Ion Formula</th> <th>Score (MFG)</th> <th>Score (MFG, MS/MS)</th> <th>Score (MS)</th> <th>Score (mass)</th> <th>Score (iso. abund)</th> <th>Score (iso. spacing)</th> </tr> </thead> <tbody> <tr> <td>(M+H)+</td> <td></td> <td></td> <td></td> <td>97</td> <td>97.81</td> <td>95.54</td> <td>97.13</td> </tr> </tbody> </table>											Species	Ion Formula	Score (MFG)	Score (MFG, MS/MS)	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)	(M+H)+				97	97.81	95.54	97.13																													
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<table border="1"> <thead> <tr> <th>m/z</th> <th>m/z (Calc)</th> <th>Diff (ppm)</th> <th>Height</th> <th>Height (Calc)</th> <th>Height %</th> <th>Height % (Calc)</th> <th>Height Sum% (Calc)</th> <th>Height Sum %</th> </tr> </thead> <tbody> <tr> <td>302.968...</td> <td>302.96913</td> <td>2.26</td> <td>19496.9</td> <td>19220.8</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>303.971...</td> <td>303.97112</td> <td>-0.03</td> <td>1576.9</td> <td>1898.9</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>304.965...</td> <td>304.96565</td> <td>0.18</td> <td>2957.5</td> <td>2822.9</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>305.968...</td> <td>305.96772</td> <td>-3.73</td> <td>163.6</td> <td>252.3</td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>											m/z	m/z (Calc)	Diff (ppm)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum% (Calc)	Height Sum %	302.968...	302.96913	2.26	19496.9	19220.8					303.971...	303.97112	-0.03	1576.9	1898.9					304.965...	304.96565	0.18	2957.5	2822.9					305.968...	305.96772	-3.73	163.6	252.3				
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Results scoring based on RT, mass accuracy, isotope abundance and spacing

# Library searching and confirmation

## Rucola extract spiked with pesticides @ 10 µg/kg



TOF spectrum and MS/MS spectrum

Comparison of acquired vs. library spectrum

# Screening workflow using LC-(Q)TOF-MS

## Screen shot MassHunter Qualitative analysis

Agilent MassHunter Qualitative Analysis 8.05.00 - Franzi\_MSMS\_Screening.m

File Edit View Find Identify Chromatograms Spectra Method Wizards Actions Configuration Tools Help

Compound List

Automatically Show Columns

Cpd	File	ID Source	Name	Formula	Flags (Tgt)	Saturated	RT	m/z	Mass	Score	Score (Lib)	RT Diff (Tgt)	Diff (Tgt, ppm)	Diff (DB, ppm)	Polarity	Ions	Height	Area	Notes
41	Rucola_0_01.d	LibSearch-FBF	Imidacloprid	C9H10ClN5O2			4.37	256.05915	255.05196	85.52	86.49	0.051	-1.35	1.35	Positive	7	24757	84268	
85	Rucola_0_01.d	LibSearch-FBF	Kresoxim methyl	C18H19NO4	low score		11.69	314.1385	313.13147	79.17	81.69	0.111	0.18	-0.18	Positive	8	51971	162142	
35	Rucola_0_01.d	LibSearch-FBF	Linuron	C9H10ClN2O2	low score		9.381	249.01846	248.01137	70.94	80.34	0.151	-2.27	2.27	Positive	7	39478	164505	phenylurea
73	Rucola_0_01.d	LibSearch-FBF	Methidathion	C6H11N2O4PS3	low score		8.637	302.96845	301.9614	73.69	95.87	0.134	-1.51	1.51	Positive	10	40607	153657	
62	Rucola_0_01.d	LibSearch-FBF	Myclobutanil	C15H17ClN4	low score		10.327	289.12111	288.1146	69.47	87.64	0.142	1.49	-1.49	Positive	7	22467	84779	
57	Rucola_0_01.d	LibSearch-FBF	Penconazole	C13H15ClN2	low score		11.797	284.07174	283.06438	72.35	94.21	0.136	0.28	-0.28	Positive	7	34438	117501	
70	Rucola_0_01.d	LibSearch-FBF	Phoxim	C12H15N2O3PS			12.356	299.06127	298.05398	80.38	95.75	0.107	-0.4	0.4	Positive	10	58253	207699	
32	Rucola_0_01.d	LibSearch-FBF	Pririmcarb	C11H18N4O2			6.341	239.15026	238.14297	98.58	95.73	0.013	-0.03	0.03	Positive	4	108381	462283	

Data Navigator

Sort by Data File

- Cpd 62: Myclobutanil
- Cpd 63: Benomyl
- Cpd 64: Thiamethoxam
- Cpd 65: Coumatralyl
- Cpd 66: Triadimefon
- Cpd 67: Fenitrothion-sulfone
- Cpd 68: Isoxadifen-ethyl
- Cpd 69: Imazalil
- Cpd 70: Phoxim
- Cpd 71: Bifenazate
- Cpd 72: Flutriafol
- Cpd 73: Methidathion
- Cpd 74: Carbendazim

Method Explorer: Franzi\_MSMS\_Screening.m

MS Target Compound Screening Workflow

Extraction Data Format

Chromatograms

Mass Spectra

Find by Molecular Feature

Find by Formula

Identify by Database Search

Identify by Library Search

Identify by Formula Generation

Match Scoring

Compound Report

Automation

Chromatogram

Spectrum

General

Reports

Find Compounds

Find Compounds by Formula

Chromatogram Results

Sort by Data File

Minutes

Cpd 73: Methidathion: +ESI EIC(302.96913, 319.99568, 324.95108) Scan Rucola\_0\_01.d

Chromatogram Results

Spectrum Identification Results

Method Editor: Screening - Find by Formula

Find Compounds by Formula

Method Items

Negative Ions

EIC Smoothing

EIC Peak Filters

Results

Result Filters

Formula Source

Formula Matching

Positive Ions

Source of formulas to confirm

These formulas:

C6H11N2O4PS3

(type a comma-separated list of formulas, e.g., "C6H6, CH4")

Compound exchange file (.CEF):

Database

C:\MassHunter\PCDL\Kemp\_PestProject.cdb

Worklist

Matches per formula

Maximum number of matches 1

MS Spectrum Results

Cpd 73: Methidathion: + FBF Spectrum (8.607-8.667 min) Rucola\_0\_01.d Subtract

302.96845 (M+H)+

304.96516 (M+H)+

319.99558 (M+N4)+

324.95161 (M+Na)+

H3C

S

N

CH3

Counts vs. Mass-to-Charge (m/z)

# Pesticides screening with accurate mass

## Validation data for 3 different matrices



Compound	Cucumber					Lemon					Rucola				
	Blank	0.005	0.01	0.05	0.1	Blank	0.005	0.01	0.05	0.1	Blank	0.005	0.01	0.05	0.1
Acetamiprid															
Azoxystrobin															
Bifenazat															
Boscalid															
Buprofenzin															
Carbaryl															
Carbendazim															
Chlorpyrifos															
Chlorpyrifos-methyl															
Cyprodinil															
DEET															
Dichlorvos															
Difenoconazole															
Diflubenzuron															
Dimethoat															
Dimethomorph															
Fenhexamid															
Fluazfop free acid															
Fludioxonil															
Fluquinconazole															
Flutriafol															
Imazalil															
Imidacloprid															
Kresoxim-methyl															
Linuron															
Mandipropamid															
Metaxyl															
Methidathion															
Myclobutanil															
Penconazole															
Pendimethalin															
Phosmet															
Phoxim															
Pirimicarb															
Pirimicarb-desmethyl															
Propamocarb															
Propiconazole															
Prosulfocarb															
Pyraclostrobin															
Pyridaben															
Teflubenzuron															
Thiabendazole															
Thiacloprid															
Thiamethoxam															
Thiophanat-methyl															
Triadimefon															
Triazophos															
Trifloxystrobin															
<b>Results FBF</b>	6/53	49/53	51/53	53/53	53/53	6/53	42/53	45/53	52/53	52/53	2/53	41/53	48/53	52/53	53/53
<b>Results Library search</b>	4/53	42/53	44/53	48/53	48/53	1/53	37/53	41/53	45/53	47/53	1/53	37/53	40/53	46/53	47/53
<b>False positives</b>															
RT required	7	3	4	8	6	9	9	8	15	22	19	59	65	66	65
RT required + Library search	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RT optional	73	71	72	72	76	114	113	119	121	127	126	171	168	185	180
RT optional + Library search	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

- Most compounds found in lowest spiking level in all matrices
- Even at lowest spiking level reliable MS/MS spectra
- No false positives for Find-by-Formula data mining and library searching even without using RT information

\*\* For Cyhexatin, Famoxadone, Iprodione, Piperonyl-butoxide, and Propargite, found by the FBF algorithm in the MS domain, no MS/MS spectra was included in the accurate mass library for the observed ion species.

# Validation study and real sample screening

## Experimental setup

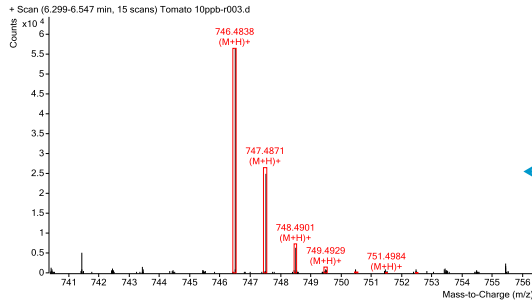
ID	Name	Formula	MW	Score
1	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
2	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
3	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
4	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
5	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
6	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
7	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
8	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
9	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
10	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
11	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
12	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
13	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
14	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
15	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
16	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
17	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
18	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20
19	Sulfamethoxazole	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	253.27	96.20
20	Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O	258.30	96.20

Compound results

```
<CompoundList>
<Compound name="Sulfamethoxazole" formula="C10 H11 N3 O3 S" alg="ds" score="96.20">
<Location m="379.8844" r="3.854" rtu="0.046" ri="0.008" />
<ccq p="Negative" f="100" s="2.008" />
</Compound>
<IonSpecies>
<S_1>[M-H]^-/5.1>
<S_2>[M-C1]^-/5.2>
</IonSpecies>
</Compound>
<MSPeaks>
<cp m="3.854" s="377.0771" y="309918.4688" v="863857.6358" s="1" />
<cp m="3.853" s="378.0789" y="37938.3320" v="105748.2553" s="1" />
<cp m="3.855" s="379.0742" y="12583.3910" v="34851.5859" s="1" />
<cp m="3.856" s="380.0761" y="1577.3260" v="4396.8347" s="1" />
<cp m="3.851" s="381.0744" y="127.5063" v="355.4874" s="1" />
<cp m="3.845" s="313.0518" y="848.5596" v="2368.0696" s="2" />
</MSPeaks>
</Compound>
```

.cef file

Name	Date File	Type	Level	Score
Sulfamethoxazole	11/11/11	MS/MS	1	96.20
Trimethoprim	11/11/11	MS/MS	1	96.20



Reference pattern library

Setup Reference Pattern Library

Create reference pattern library at:

TDF\_BookDualSpray\_WL2\Neatle pattern library.xml

Choose species to generate spectra:

- [M-H]<sup>-</sup>
- [M+H]<sup>+</sup>
- [M+K]<sup>+</sup>
- [M+NH<sub>4</sub>]<sup>+</sup>
- M<sup>-</sup>
- [M-H]
- [M-C]
- [M-B]
- [M+COO]<sup>-</sup>
- [M+CH<sub>3</sub>COO]<sup>-</sup>

Adduct pattern

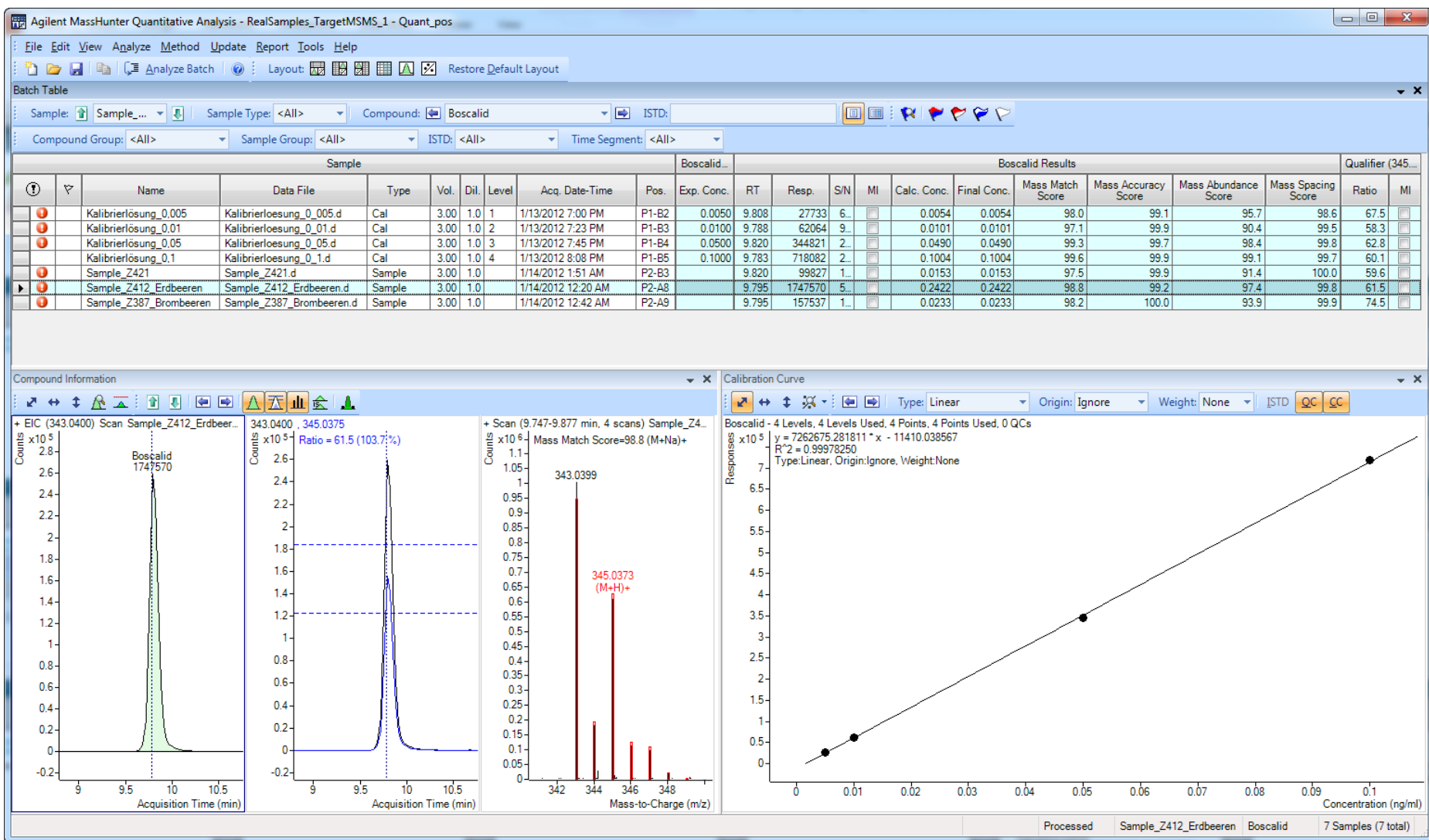
**Autodynamic Quant method creation**

- Accurate mass EICs for quantifier and qualifier
- Relative responses
- Retention times



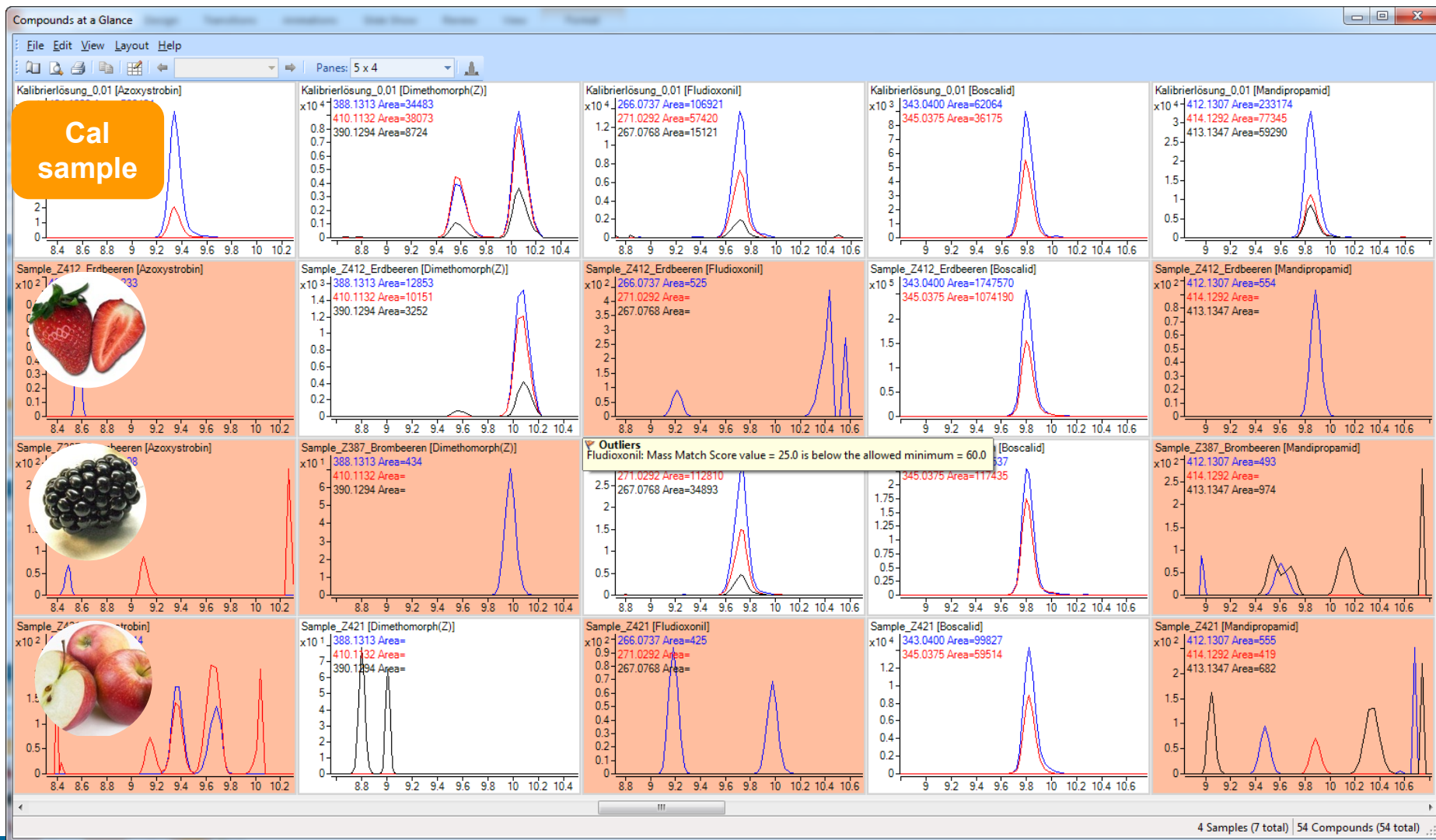
# Quantitation of pesticides using accurate mass

## Batch review process including accurate mass metrics



# Quantitation of pesticides using accurate mass

## Batch review process using Compounds at a Glance



# Pesticides screening and quantitation

## Apple sample from local market



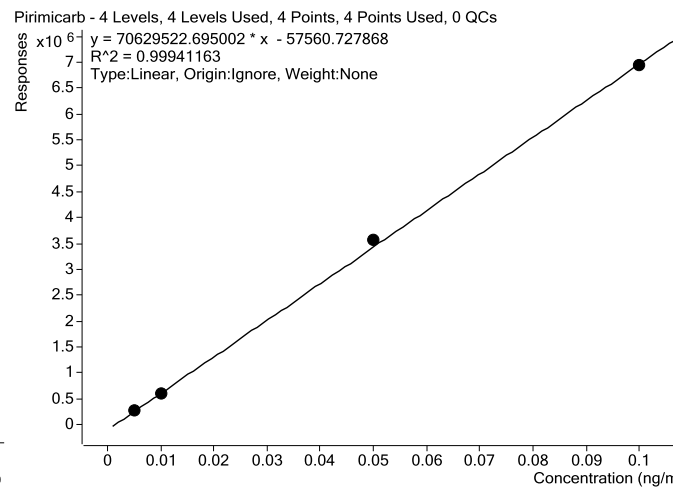
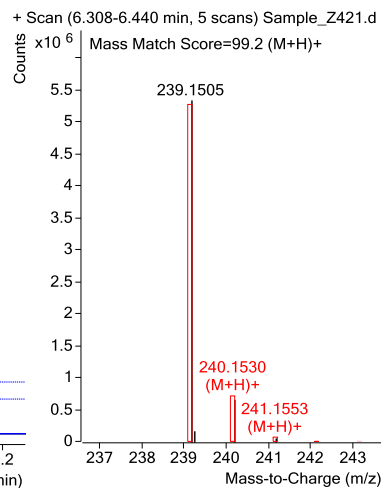
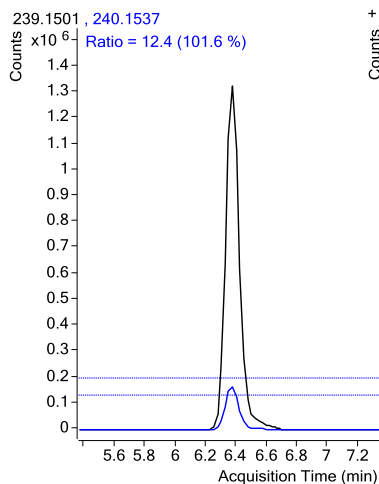
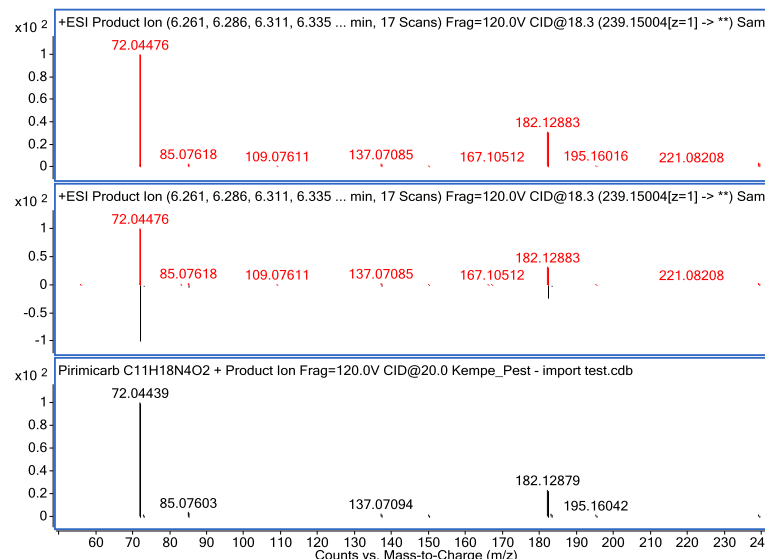
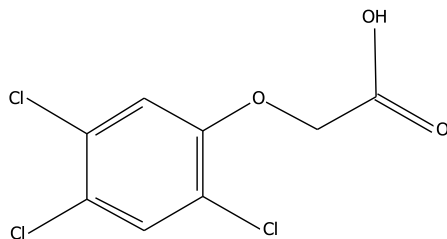
### Pirimicarb

Conc.: 120 µg/kg

Mass error: -0.54 ppm

TOF score: 99.2

Library score: 97.0



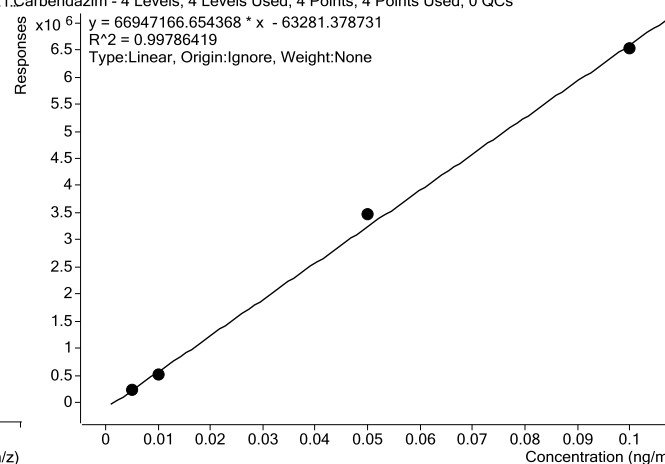
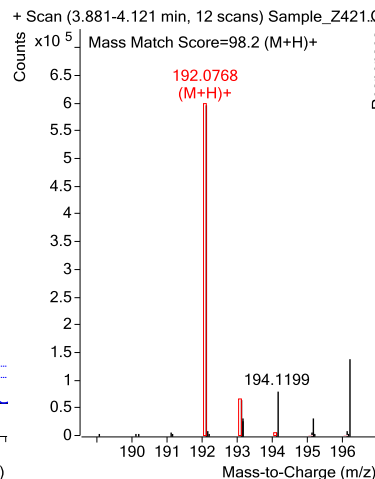
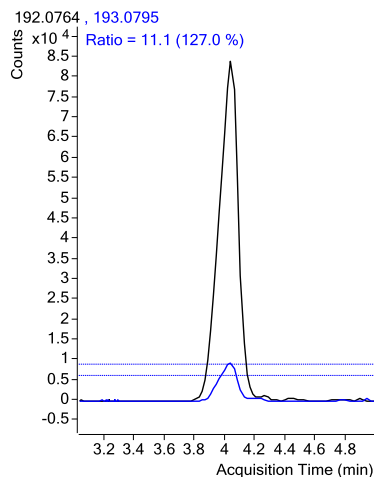
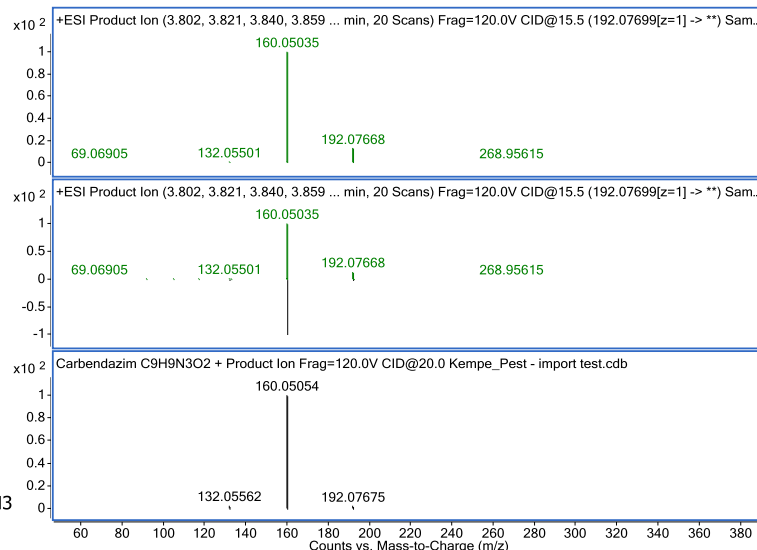
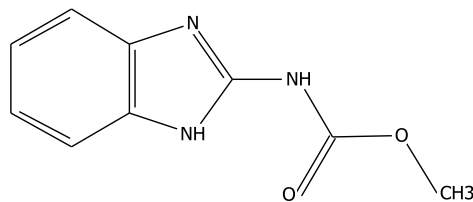
# Pesticides screening and quantitation

## Apple sample from local market



### Carbendazim

Conc.: 20 µg/kg  
Mass error: -1.57 ppm  
TOF score: 98.2  
Library score: 95.8



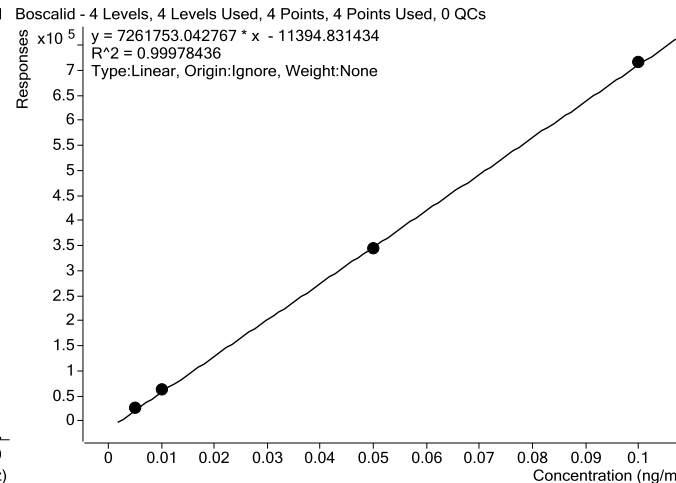
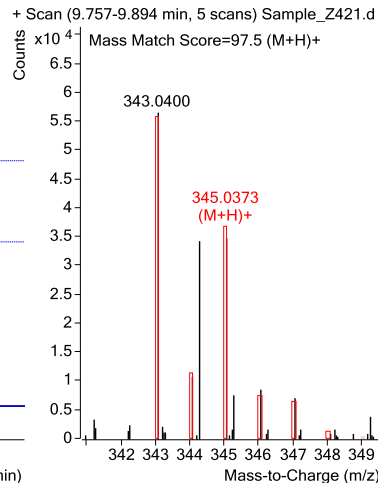
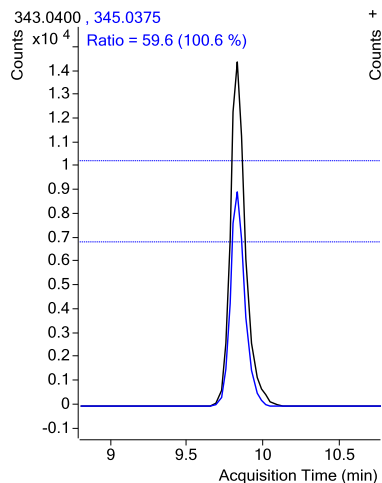
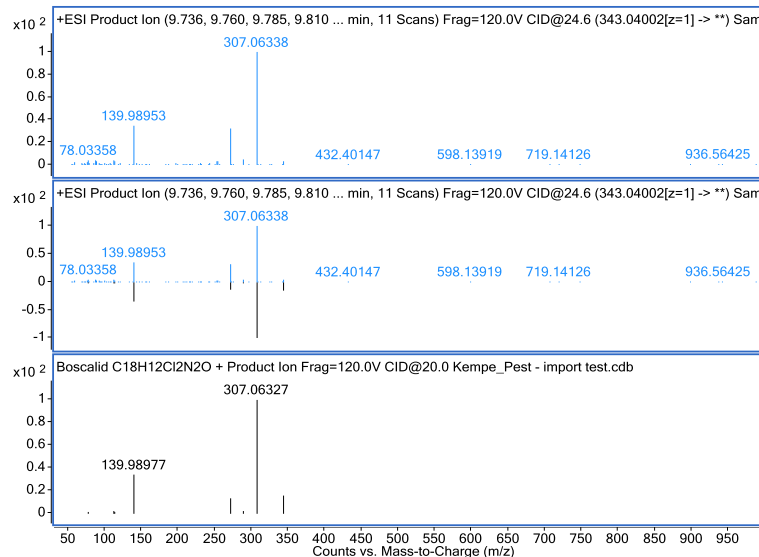
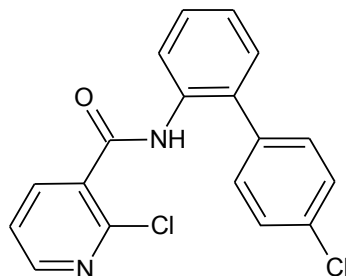
# Pesticides screening and quantitation

## Apple sample from local market



### Boscalid

Conc.: 20 µg/kg  
Mass error: -0.15 ppm  
TOF score: 97.5  
Library score: 93.0



# Pesticides screening and quantitation

## Apple sample from local market



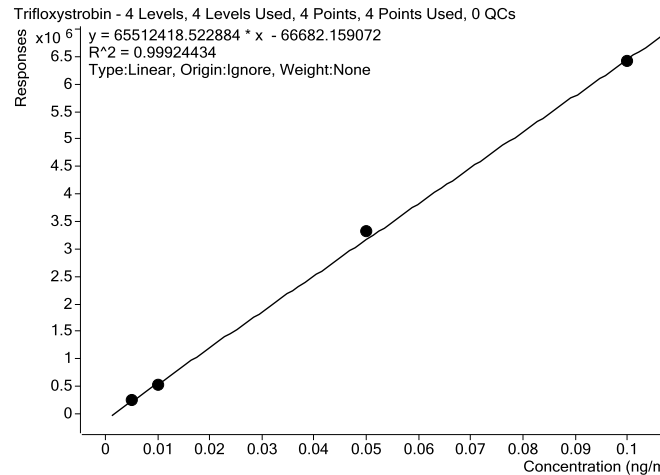
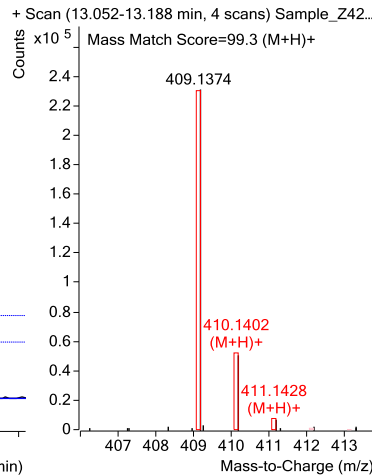
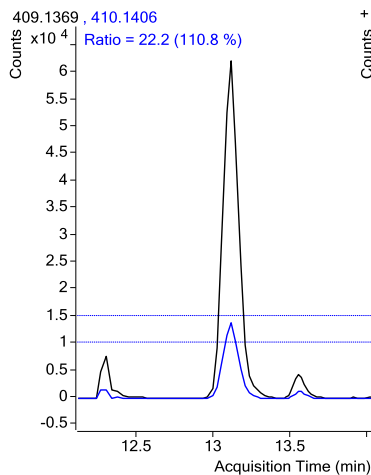
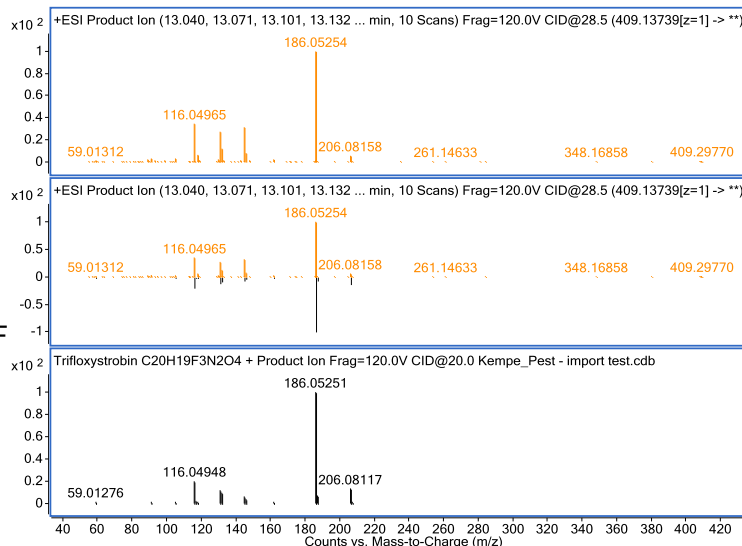
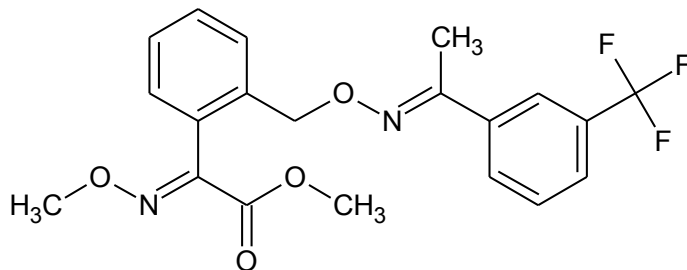
### Trifloxystrobin

Conc.: 10 µg/kg

Mass error: -1.00 ppm

TOF score: 99.3

Library score: 87.8



# Summary

- Trend towards comprehensive screening methods using accurate mass instruments
- UHPLC for improved chromatographic resolution, short runtimes and highest sensitivity
- Personal compound database and library containing > 600 pesticides with molecular formulas and accurate mass MS/MS spectra
- Innovative data mining tools for identification of trace contaminants in complex matrices
- Unambiguous confirmation of pesticide residues in complex matrices by accurate mass library searching
- Seamless integration of compound identification and quantitation for easy and quick batch review
- Accurate quantitation of pesticides in real samples using UHPLC-(Q)TOF technology

# Thanks!

thomas\_glauner@agilent.com

## Acknowledgements

- Franziska Spitzbarth, Hochschule Fresenius, Zwickau
- Dr. Günther Kempe

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Landesuntersuchungsanstalt Sachsen



# SANCO guidelines for pesticides screening

## Implementations for qualitative and quantitative methods

- **Qualitative multi-residue methods**

- To extend the analytical scope in a cost-effective way to analytes which have low probability to be present in samples
- Screening Detection Level (SDL) for individual compounds must be established and confidence of detection must be validated for different commodities
- Method performance must be shown for every batch with  $\geq 10$  indicator analytes at the SDL

- **Quantitative multi-residue methods**

- Initial method validation (intra-laboratory)
- Recovery determination for 10% of analytes in each batch in a rolling program
- Strict compliance with identification criteria (e.g. accurate mass for  $> 2$  ions and RTs)
- Usually calibration with all targeted analytes for each batch, but at least for a number of representative analytes (minimum 25 + 10% of total number of analytes)
- Re-calibration for positive compounds possible, re-analyze in case of MRL violation