European Commission

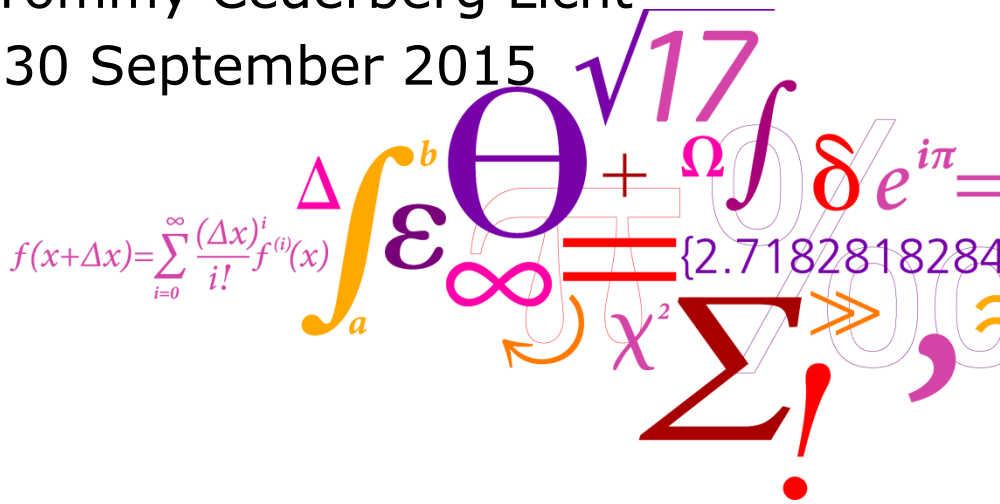
EURL-CF

DTU

Screening Method validation of Pesticide Residues in Cereals using G-QTOF and exact mass library

Mette Erecius Poulsen and Tommy Cederberg Licht

EURL workshop, Stuttgart, 30 September 2015



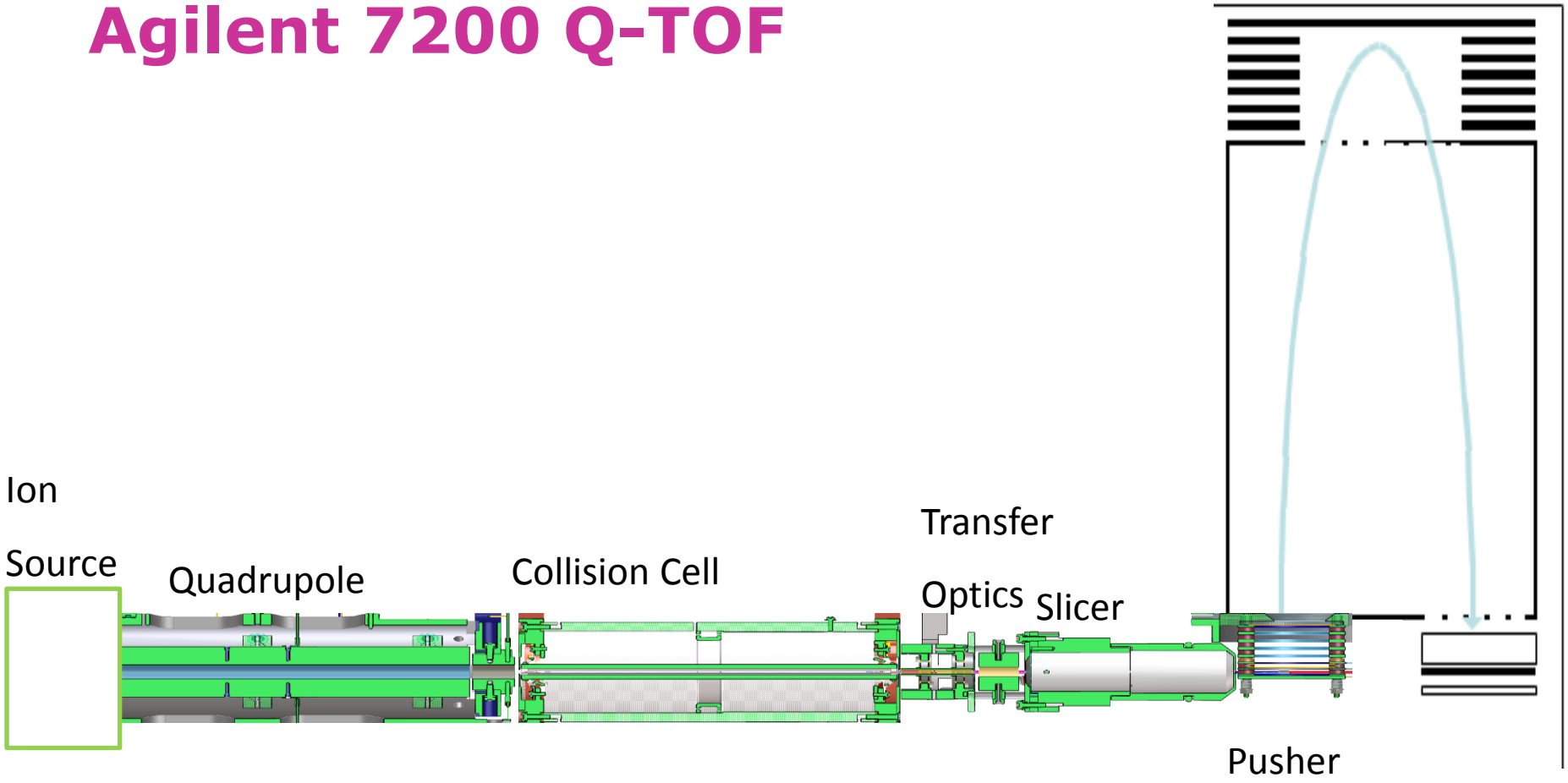
DTU Food
National Food Institute

EURL-CF and GC-QTOF

- TOF instrument: 7200 GC/Q-TOF, Agilent Technologies

- GC-system: 7890B, Agilent Technologies
  - PAL autosampler system
  - Gerstel PTV injector.
  - Back flush,
  - Two HP-5MS UI, 15 m, 0.250 mm dia, 0.25 mm film thickness

Agilent 7200 Q-TOF

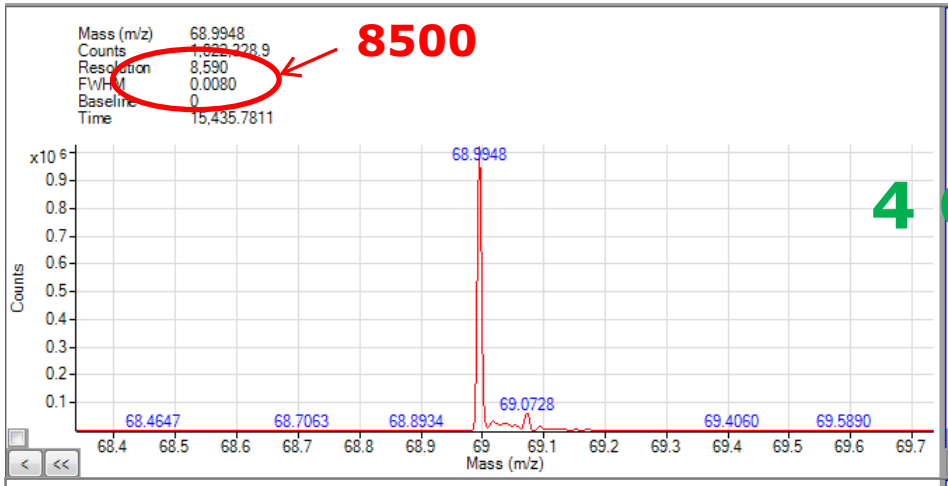


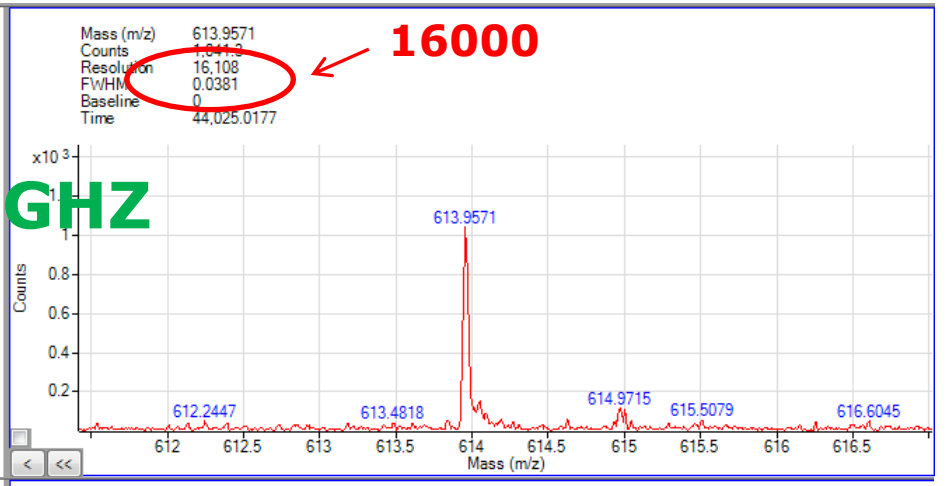
Conditions

- Ionisation mode: EI positive
- Acquisition rate:

Conditions

- Ionisation mode: EI positive
- Acquisition rate:
  - 2 GHz – higher dynamic range
  - 4 GHz – higher resolution


Mass (m/z) 68.9948
Counts 1,622,328.9
Resolution 8,590
FWHM 0.0080
Baseline 0
Time 15,435.7811
8500
68.9948
68.4647
68.7063
68.8934
69.0728
69.4060
69.5890
Counts
Mass (m/z)


Mass (m/z) 613.9571
Counts 1,641.3
Resolution 16,108
FWHM 0.0381
Baseline 0
Time 44,025.0177
16000
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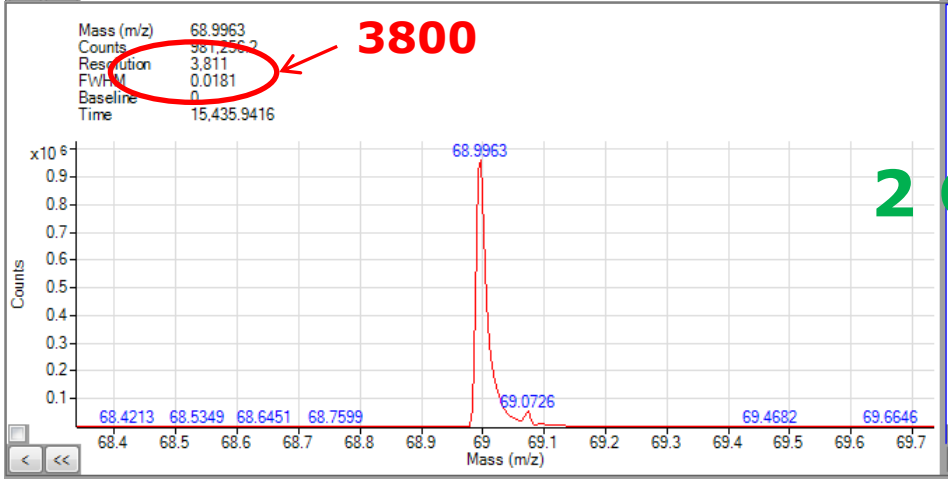
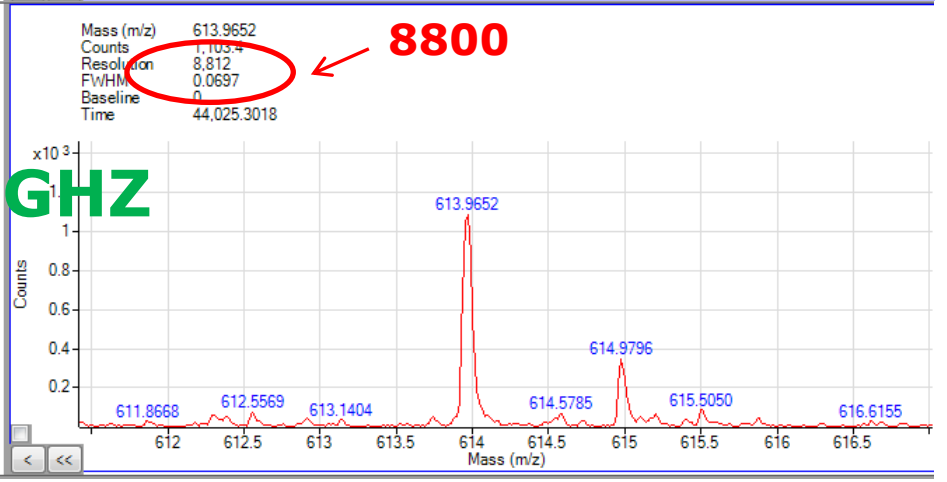
612.2447
613.4818
614.9715
615.5079
616.6045
Counts
Mass (m/z)

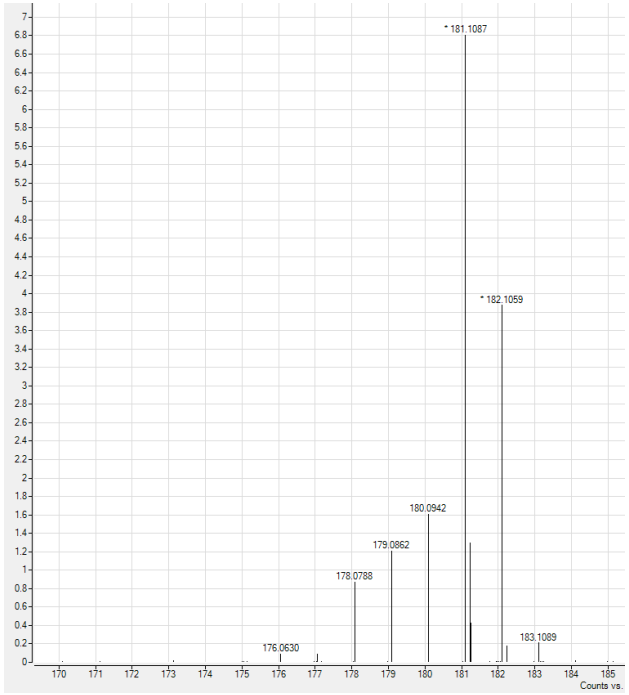
4 GHZ

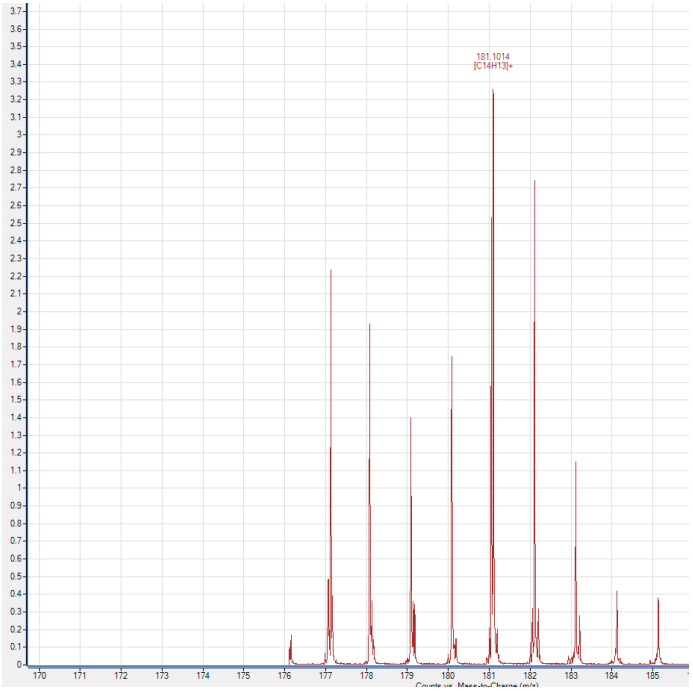

Mass (m/z) 68.9963
Counts 981,256.2
Resolution 3,811
FWHM 0.0181
Baseline 0
Time 15,435.9416
3800
68.9963
68.4213
68.5349
68.6451
68.7599
69.0726
69.4682
69.6646
Counts
Mass (m/z)


Mass (m/z) 613.9652
Counts 1,103.4
Resolution 8,812
FWHM 0.0697
Baseline 0
Time 44,025.3018
8800
613.9652
611.8668
612.5569
613.1404
614.5785
614.9796
615.5050
616.6155
Counts
Mass (m/z)

2 GHZ

- Acquisition mode: Centroid or profile – profile, big data file
- Source temperature 230 ºC
- Software: MassHunter B 06.01/07.01

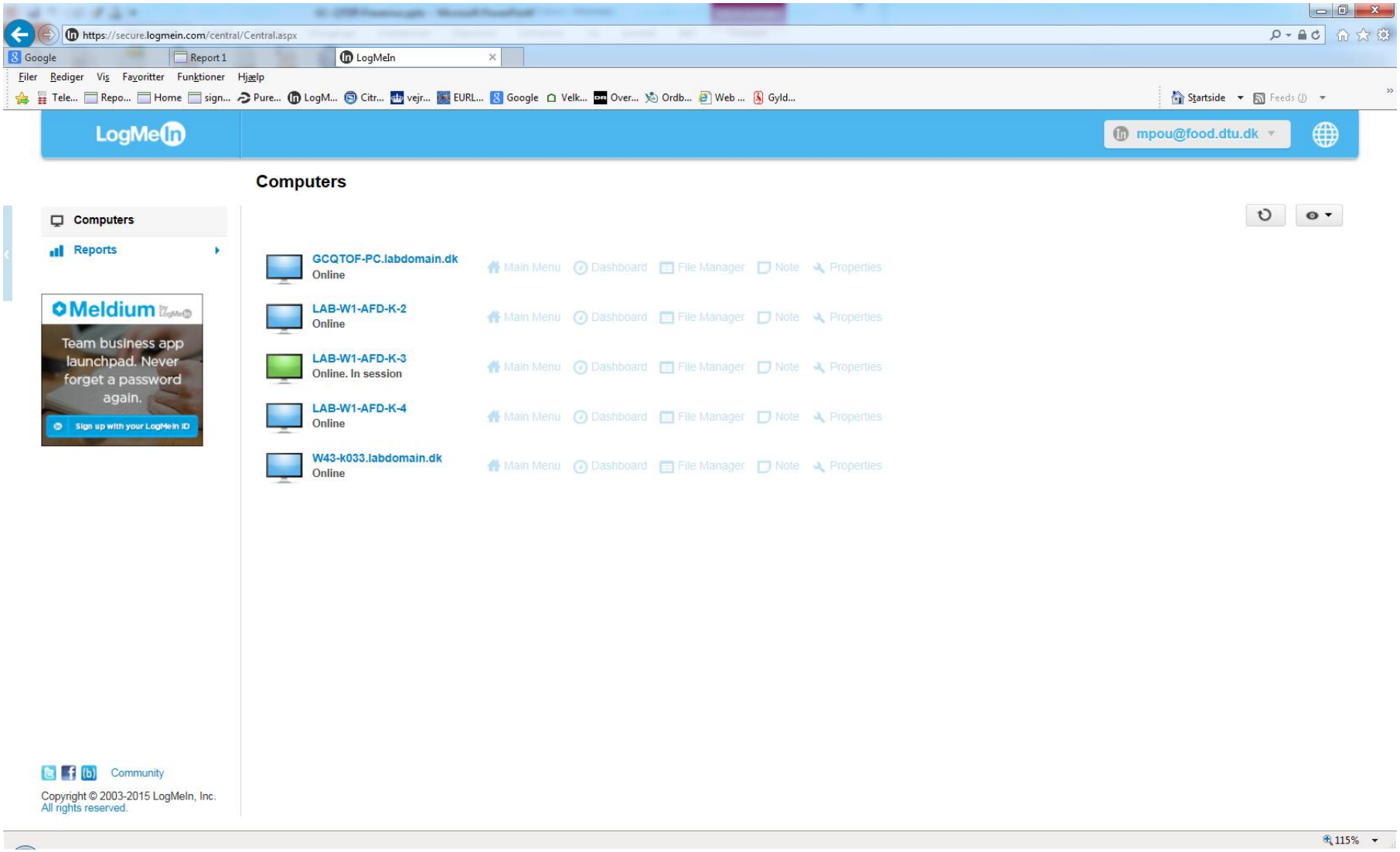




Virtual computers for data processing

- Back up of all laboratory data each day 6AM

- Virtual computers – access via Logmein
  - On the Laboratory domain
  - Windows Professional 64 bit, English version
  - All data
  - Few licenses are necessary
  - Access from all computers with internet


https://secure.logmein.com/central/Central.aspx
Google
Report 1
LogMeIn
Filer Rediger Vis Favoritter Funktioner Hjælp
LogMeIn
mpou@food.dtu.dk
Computers
Computers
Reports
Meldium
Team business app launchpad. Never forget a password again.
Sign up with your LogMeIn ID
GCQTOF-PC.labdomain.dk
Online
LAB-W1-AFD-K-2
Online
LAB-W1-AFD-K-3
Online. In session
LAB-W1-AFD-K-4
Online
W43-k033.labdomain.dk
Online
Main Menu Dashboard File Manager Note Properties
Community
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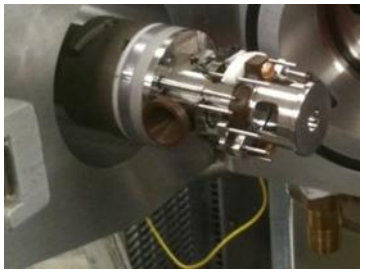
All rights reserved.
115%

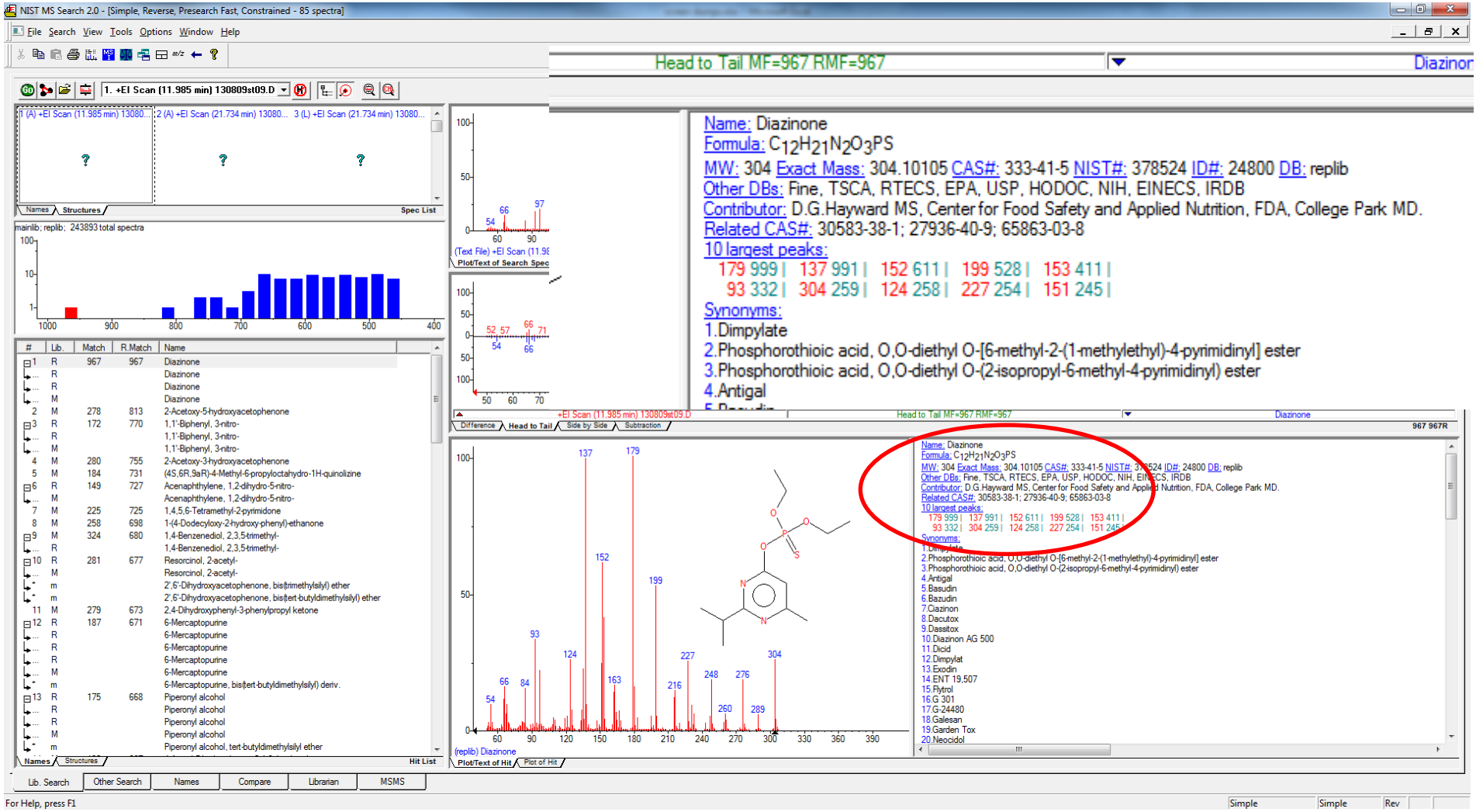
GC-QTOF - challenge



- Fragmentation in the ion source

- No library/database with exact masses for pesticide fragments and information on the elemental composition

Exact masses of fragments



Diazinon

Diazinone - MS Interpreter

File Edit View Options Help

Formula Calculator

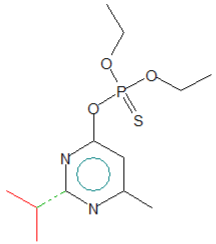
m = 179 C12H21N2O3PS

Calculate Options Parent = 304 Loss = 125

84 Ions	O+E	RDB	Mass	C	H	N	O	P
C_7O_2PS	Even	8.5-11.5	178.93566	7	0	0	2	1
C_6N_2OPS	Even	8.5-13.5	178.94690	6	0	2	1	1
C_7H_2NOPS	Odd	8-12	178.95947	7	2	1	1	1
$C_6N_2O_3P$	Even	8.5-11.5	178.96465	6	0	2	3	1
C_7HNO_3S	Odd	8-11	178.96771	7	1	1	3	0
$C_3H_4N_2O_3PS$	Even	3.5-8.5	178.96802	3	4	2	3	1
$C_{11}OP$	Even	12.5-13.5	178.96868	11	0	0	1	1
C_8H_4OPS	Even	7.5-10.5	178.97205	8	4	0	1	1
$C_7H_2NO_3P$	Odd	8-10	178.97723	7	2	1	3	1
$C_{10}N_2P$	Even	12.5-15.5	178.97991	10	0	2	0	1
$C_8H_3O_3S$	Even	7.5-9.5	178.98029	8	3	0	3	0

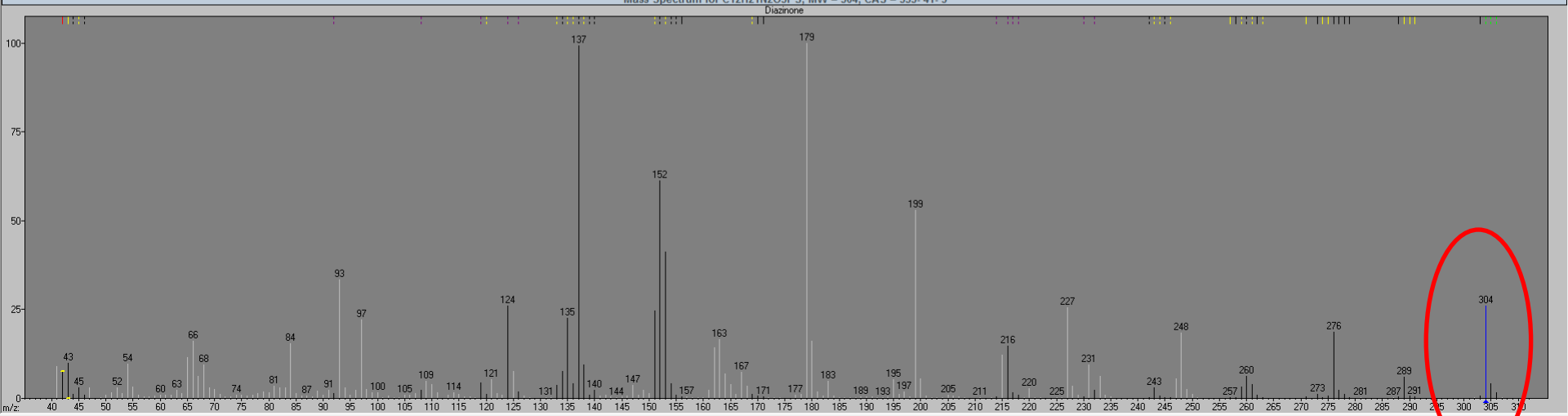
Structure, Maximum Dissociation = 93

m/z	mass	formula	loss	type	H	rate	abund
42	42.04695	C_3H_6	$C_9H_{15}N_2O_3PS$	dissociation	-1	-15*	77



Mass Spectrum for C12H21N2O3PS; MW = 304; CAS = 333-41-5

Diazinone



Exact molecular weight. Click to sort.

Diazinon

Diazinone - MS Interpreter

File Edit View Options Help

mz=216 (1/2)

Formula Calculator

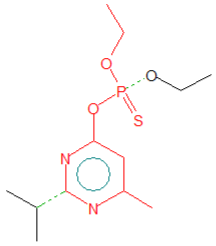
m = 216 C12H21N2O3PS

Calculate Options Parent = 304 Loss = 88

59 Ions	O+E	RDB	Mass	C	H	N	O	P
$C_{10}HO_2PS$	Odd	11-14	215.94349	10	1	0	2	1
C_9HN_2OPS	Odd	11-16	215.95472	9	1	2	1	1
$C_9N_2O_3S$	Odd	11-15	215.96296	9	0	2	3	0
$C_{10}H_3NOPS$	Even	10.5-14.5	215.96730	10	3	1	1	1
$C_9HN_2O_3P$	Odd	11-14	215.97248	9	1	2	3	1
$C_{10}H_2NO_3S$	Even	10.5-13.5	215.97554	10	2	1	3	0
$C_6H_5N_2O_3PS$	Odd	6-11	215.97585	6	5	2	3	1
$C_{11}H_5OPS$	Odd	10-13	215.97987	11	5	0	1	1
$C_{10}H_3NO_3P$	Even	10.5-12.5	215.98505	10	3	1	3	1
$C_{11}H_4O_3S$	Odd	10-12	215.98811	11	4	0	3	0
$C_7H_7NO_3PS$	Even	5.5-9.5	215.98843	7	7	1	3	1

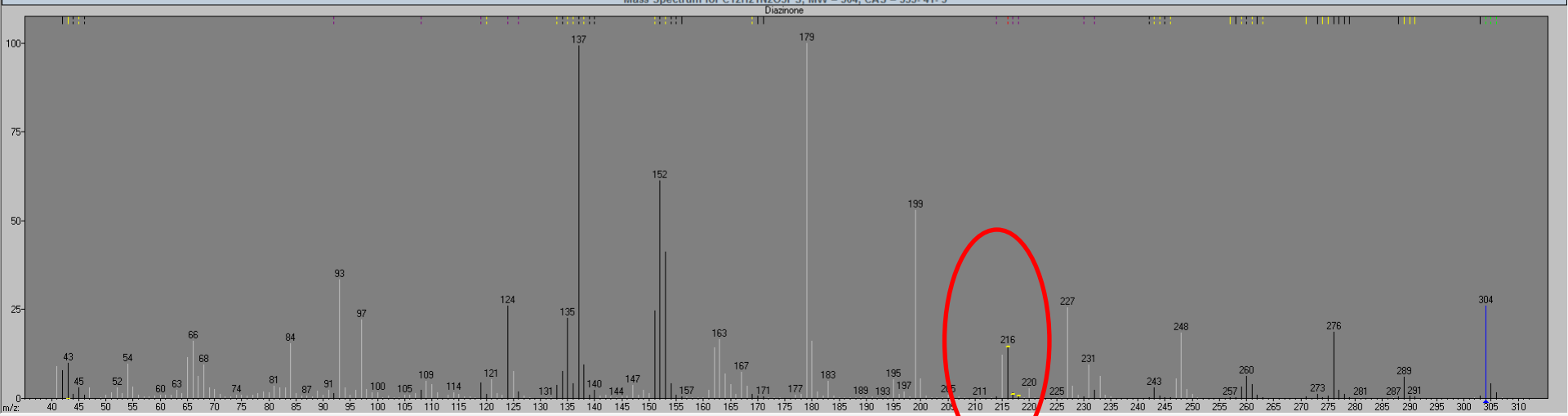
Structure, Maximum Dissociation = 93

m/z	mass	formula	loss	type	rate	abund
216 (1/2)	216.012236	$C_7HgN_2O_2PS$	$C_5H_{12}O$	unspecified	N/A	146



Mass Spectrum for C12H21N2O3PS; MW = 304; CAS = 333-41-5

Diazinone



Diazinon

Diazinone - MS Interpreter

File Edit View Options Help

Formula Calculator

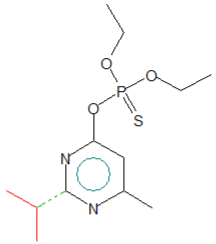
m = 179 C12H21N2O3PS

Calculate Options Parent = 304 Loss = 125

84 Ions	O+E	RDB	Mass	C	H	N	O	P
C_7O_2PS	Even	8.5-11.5	178.93566	7	0	0	2	1
C_6N_2OPS	Even	8.5-13.5	178.94690	6	0	2	1	1
C_7H_2NOPS	Odd	8-12	178.95947	7	2	1	1	1
$C_6N_2O_3P$	Even	8.5-11.5	178.96465	6	0	2	3	1
C_7HNO_3S	Odd	8-11	178.96771	7	1	1	3	0
$C_3H_4N_2O_3PS$	Even	3.5-8.5	178.96802	3	4	2	3	1
$C_{11}OP$	Even	12.5-13.5	178.96868	11	0	0	1	1
C_8H_4OPS	Even	7.5-10.5	178.97205	8	4	0	1	1
$C_7H_2NO_3P$	Odd	8-10	178.97723	7	2	1	3	1
$C_{10}N_2P$	Even	12.5-15.5	178.97991	10	0	2	0	1
$C_8H_3O_3S$	Even	7.5-9.5	178.98029	8	3	0	3	0

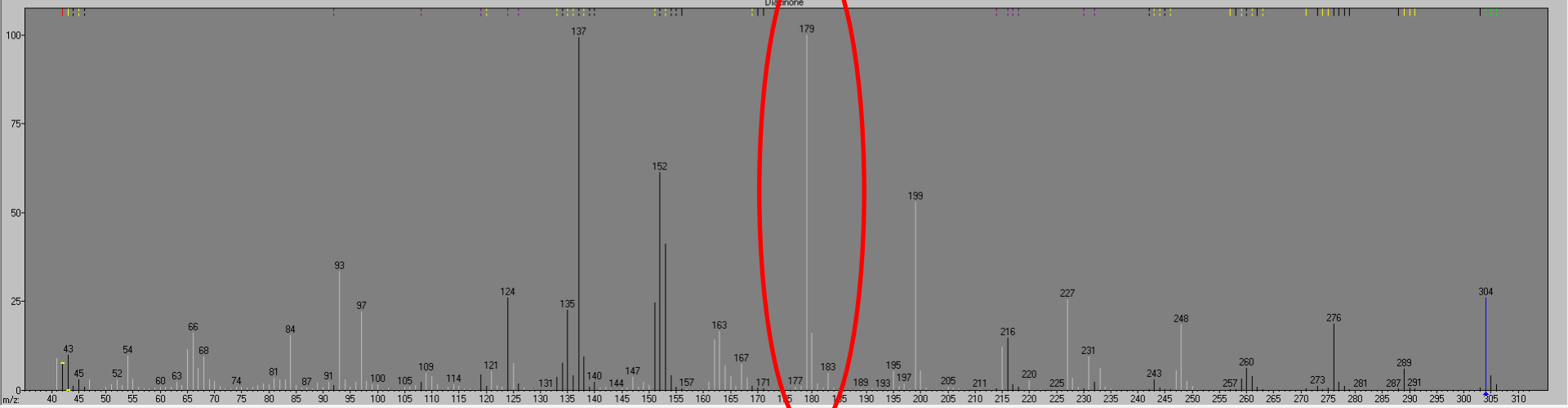
Structure, Maximum Dissociation = 93

m/z	mass	formula	loss	type	H	rate	abund
42	42.04695	C_3H_6	$C_9H_{15}N_2O_3PS$	dissociation	-1	-15*	77



Mass Spectrum for C12H21N2O3PS; MW = 304; CAS = 333-41-5

Diazinone



Exact molecular weight. Click to sort.

Butamiphos

Butamifos - MS Interpreter

File Edit View Options Help

Formula Calculator

m = 258 C13H21N2O4PS

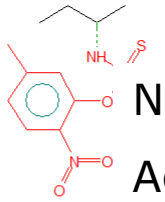
Calculate Options Parent = 332 Loss = 74

44 Ions	O+E	RDB	Mass	C	H	N	O	P	S
$C_7H_{19}N_2O_4PS$	Odd	0-5	258.08031	7	19	2	4	1	1
$C_{11}H_{16}NO_4S$	Even	4.5-7.5	258.08000	11	16	1	4	0	1
$C_{10}H_{15}N_2O_4P$	Odd	5-8	258.07694	10	15	2	4	1	0
$C_{11}H_{17}NO_2PS$	Even	4.5-8.5	258.07176	11	17	1	2	1	1
$C_{10}H_{14}N_2O_4S$	Odd	5-9	258.06743	10	14	2	4	0	1
$C_{13}H_{10}N_2O_4$	Odd	10-12	258.06406	13	10	2	4	0	0
$C_{10}H_{15}N_2O_2PS$	Odd	5-10	258.05919	10	15	2	2	1	1
$C_{13}H_{11}N_2O_2P$	Odd	10-13	258.05581	13	11	2	2	1	0

Structure, Maximum Dissociation = 147

m/z	mass	formula	loss	type	H	rate	abund
258 (1/2)	258.0228	$C_9H_{11}N_2O_3PS$	$C_4H_8 + H_2O$	g-shift/diss	+1	-62	223

- H2O



NIST: 258.022799 -C4H8 + H20

Agilent/DTU: 258.071761 -NO2 -C2H4

Mass Spectrum for C13H21N2O4PS; MW = 332; CAS = 36335-67-8

Butamifos

Log

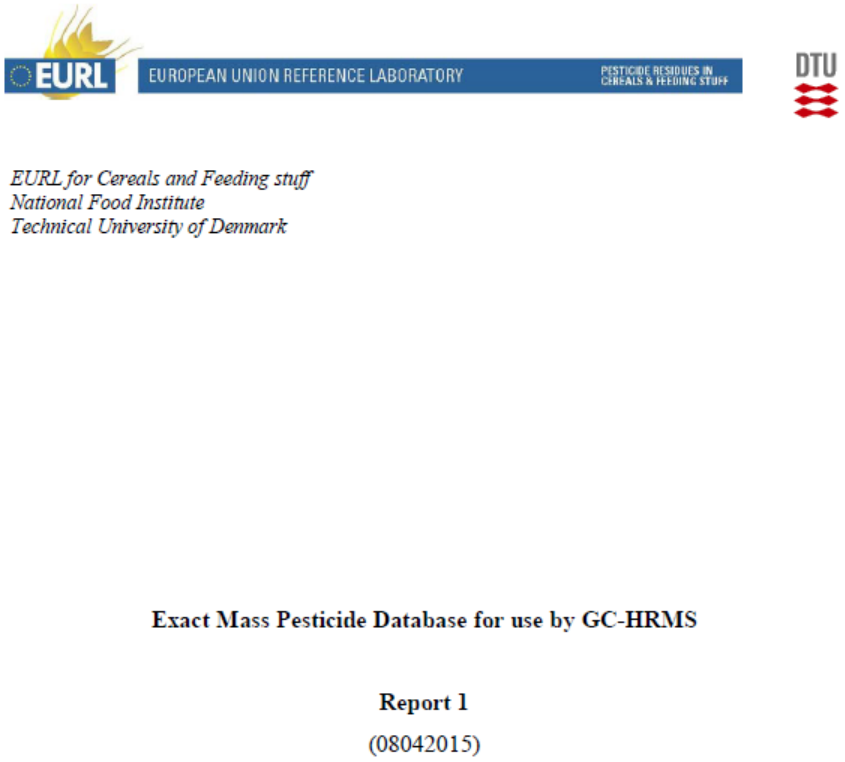
m/z:

Exact molecular weight

Exact masses

- 53 pesticides
- 2-6 fragments
  - Not included in MACP

- www.eurl-pesticides.eu
  - Cereals and feedingstuff /List of methods

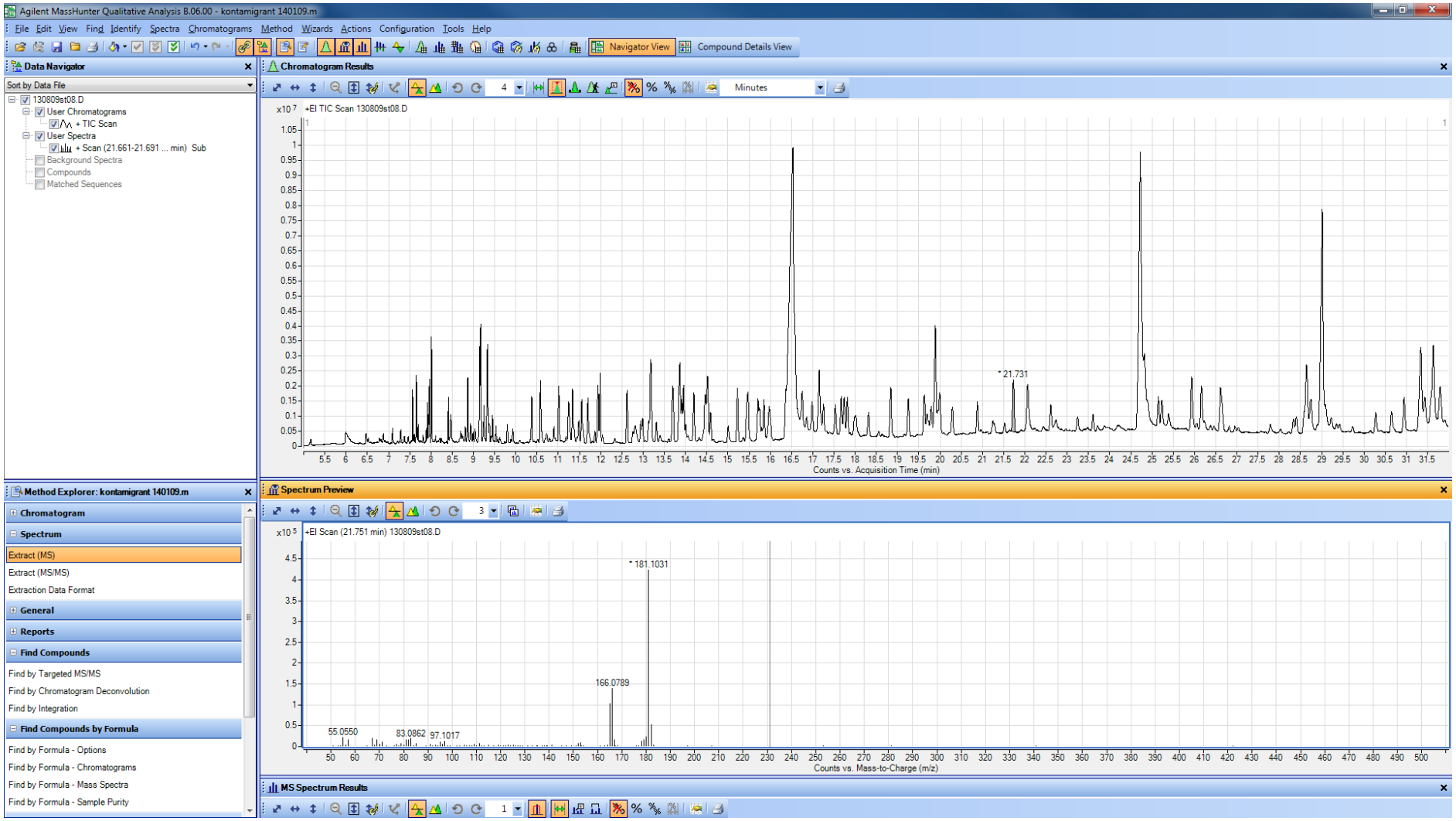


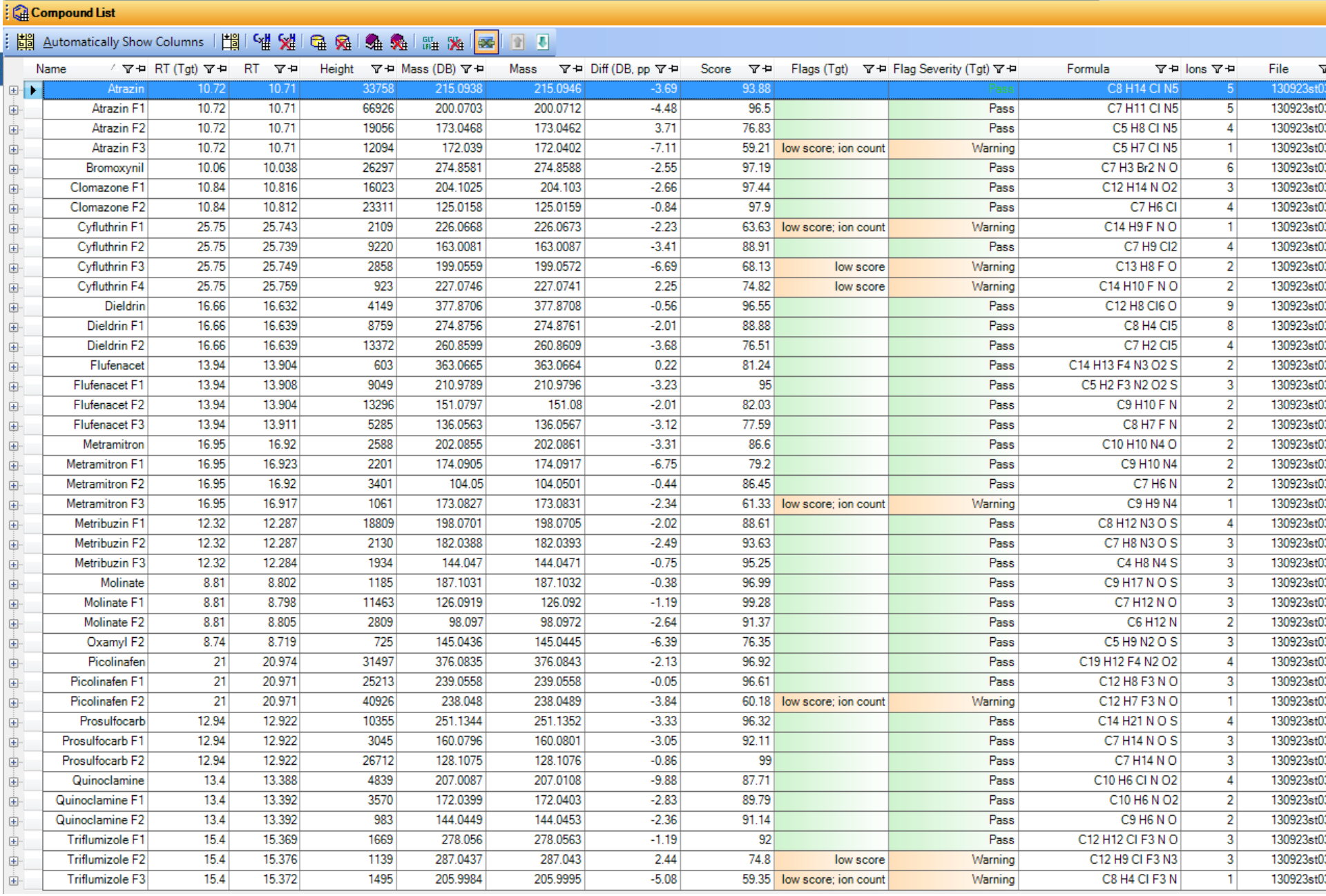
Compound	CAS	Rt	Molecular weight	Formula	Fragment m/z -e	Fragment m/z neutral
2,4-D Butyl ester	94-80-4	12.607	276.032000	C12H14O3Cl2		
2,4-D Butyl ester	94-80-4	12.607		C8H6O3Cl2	219.968851	219.969399
2,4-D Butyl ester	94-80-4	12.607		C8H6O3Cl	184.999998	185.000547
2,4-D Butyl ester	94-80-4	12.607		C7H5OCl2	174.971197	174.971745
2,4-D Butyl ester	94-80-4	12.607		C6H4OCl2	161.963372	161.963920
2,4-D Butyl ester	94-80-4	12.607		C6H3Cl2	144.960632	144.961181

- Weigth of one electron = 0.00055 unit mass

- Influence on mass accurracy at
  - 300 amu = 1.8 ppm
  - 200 amu = 2.7 ppm
  - 100 amu = 5.5 ppm

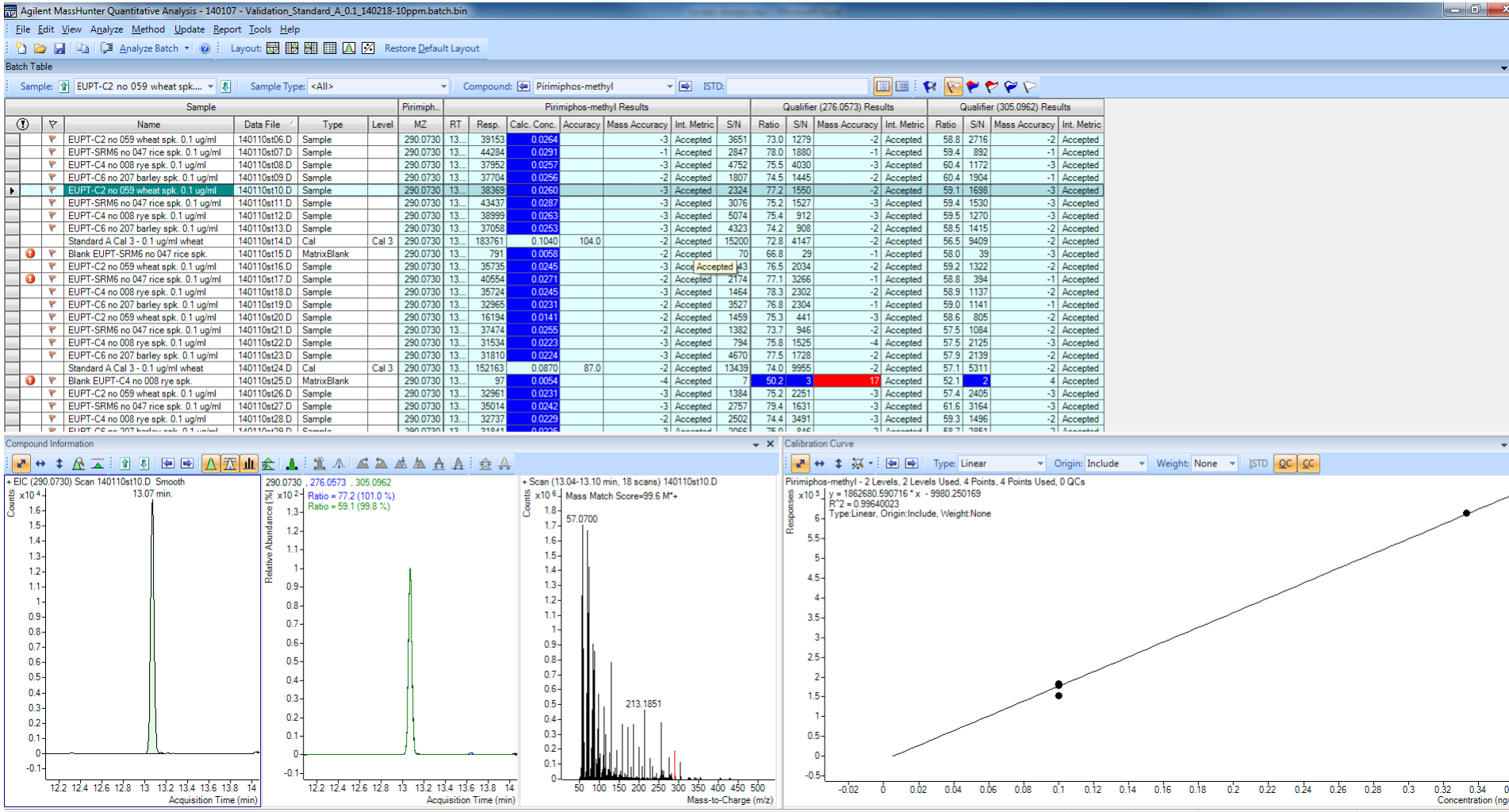
Qualitative software

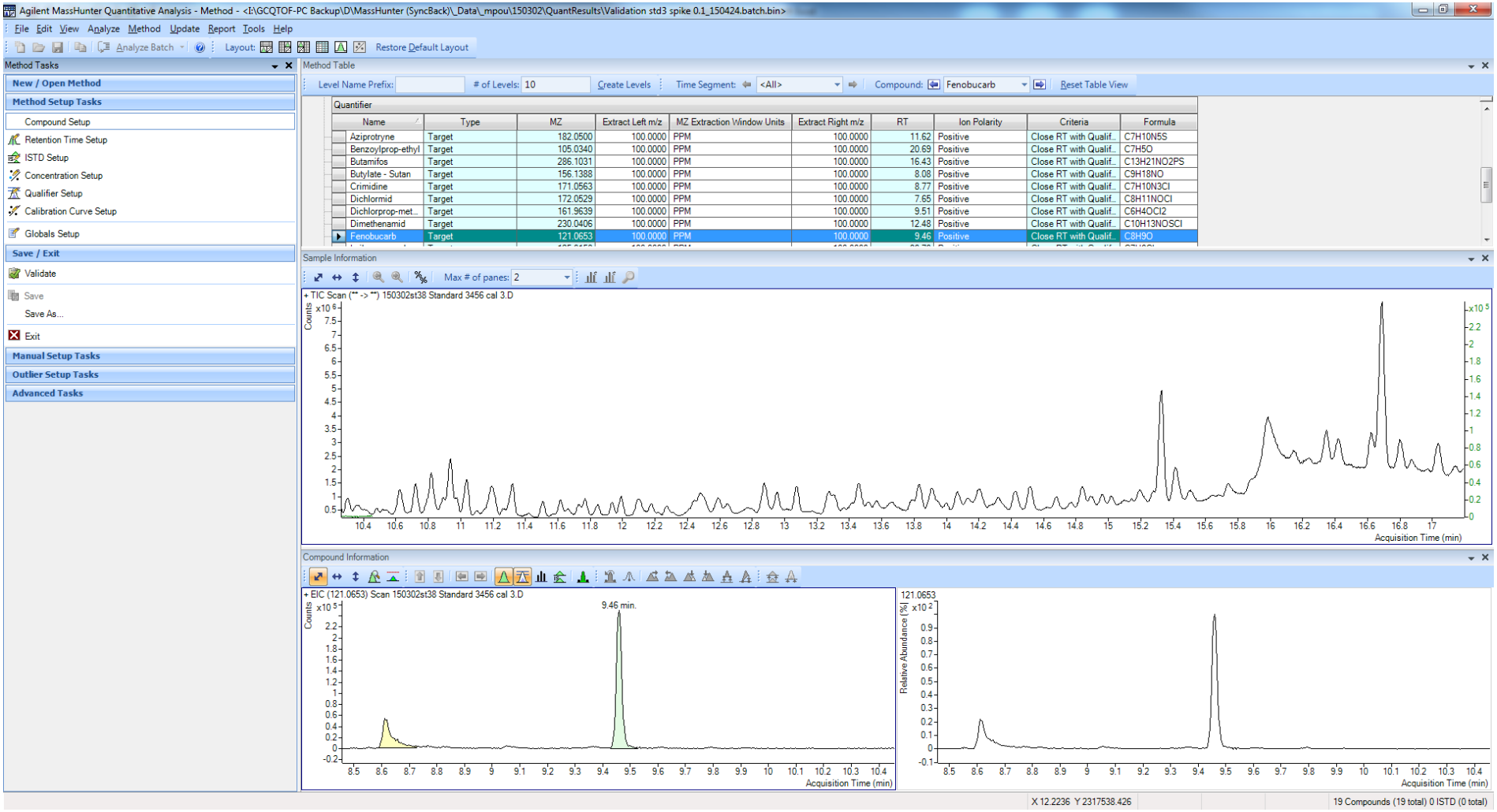




Name	RT (Tgt)	RT	Height	Mass (DB)	Mass	Diff (DB, pp	Score	Flags (Tgt)	Flag Severity (Tgt)	Formula	Ions	File
Atrazin	10.72	10.71	33758	215.0938	215.0946	-3.69	93.88		Pass	C8 H14 Cl N5	5	130923st0
Atrazin F1	10.72	10.71	66926	200.0703	200.0712	-4.48	96.5		Pass	C7 H11 Cl N5	5	130923st0
Atrazin F2	10.72	10.71	19056	173.0468	173.0462	3.71	76.83		Pass	C5 H8 Cl N5	4	130923st0
Atrazin F3	10.72	10.71	12094	172.039	172.0402	-7.11	59.21	low score; ion count	Warning	C5 H7 Cl N5	1	130923st0
Bromoxynil	10.06	10.038	26297	274.8581	274.8588	-2.55	97.19		Pass	C7 H3 Br2 N O	6	130923st0
Clomazone F1	10.84	10.816	16023	204.1025	204.103	-2.66	97.44		Pass	C12 H14 N O2	3	130923st0
Clomazone F2	10.84	10.812	23311	125.0158	125.0159	-0.84	97.9		Pass	C7 H6 Cl	4	130923st0
Cyfluthrin F1	25.75	25.743	2109	226.0668	226.0673	-2.23	63.63	low score; ion count	Warning	C14 H9 F N O	1	130923st0
Cyfluthrin F2	25.75	25.739	9220	163.0081	163.0087	-3.41	88.91		Pass	C7 H9 Cl2	4	130923st0
Cyfluthrin F3	25.75	25.749	2858	199.0559	199.0572	-6.69	68.13	low score	Warning	C13 H8 F O	2	130923st0
Cyfluthrin F4	25.75	25.759	923	227.0746	227.0741	2.25	74.82	low score	Warning	C14 H10 F N O	2	130923st0
Dieldrin	16.66	16.632	4149	377.8706	377.8708	-0.56	96.55		Pass	C12 H8 Cl6 O	9	130923st0
Dieldrin F1	16.66	16.639	8759	274.8756	274.8761	-2.01	88.88		Pass	C8 H4 Cl5	8	130923st0
Dieldrin F2	16.66	16.639	13372	260.8599	260.8609	-3.68	76.51		Pass	C7 H2 Cl5	4	130923st0
Flufenacet	13.94	13.904	603	363.0665	363.0664	0.22	81.24		Pass	C14 H13 F4 N3 O2 S	2	130923st0
Flufenacet F1	13.94	13.908	9049	210.9789	210.9796	-3.23	95		Pass	C5 H2 F3 N2 O2 S	3	130923st0
Flufenacet F2	13.94	13.904	13296	151.0797	151.08	-2.01	82.03		Pass	C9 H10 F N	2	130923st0
Flufenacet F3	13.94	13.911	5285	136.0563	136.0567	-3.12	77.59		Pass	C8 H7 F N	2	130923st0
Metramitron	16.95	16.92	2588	202.0855	202.0861	-3.31	86.6		Pass	C10 H10 N4 O	2	130923st0
Metramitron F1	16.95	16.923	2201	174.0905	174.0917	-6.75	79.2		Pass	C9 H10 N4	2	130923st0
Metramitron F2	16.95	16.92	3401	104.05	104.0501	-0.44	86.45		Pass	C7 H6 N	2	130923st0
Metramitron F3	16.95	16.917	1061	173.0827	173.0831	-2.34	61.33	low score; ion count	Warning	C9 H9 N4	1	130923st0
Metribuzin F1	12.32	12.287	18809	198.0701	198.0705	-2.02	88.61		Pass	C8 H12 N3 O S	4	130923st0
Metribuzin F2	12.32	12.287	2130	182.0388	182.0393	-2.49	93.63		Pass	C7 H8 N3 O S	3	130923st0
Metribuzin F3	12.32	12.284	1934	144.047	144.0471	-0.75	95.25		Pass	C4 H8 N4 S	3	130923st0
Molinate	8.81	8.802	1185	187.1031	187.1032	-0.38	96.99		Pass	C9 H17 N O S	3	130923st0
Molinate F1	8.81	8.798	11463	126.0919	126.092	-1.19	99.28		Pass	C7 H12 N O	3	130923st0
Molinate F2	8.81	8.805	2809	98.097	98.0972	-2.64	91.37		Pass	C6 H12 N	2	130923st0
Oxamyl F2	8.74	8.719	725	145.0436	145.0445	-6.39	76.35		Pass	C5 H9 N2 O S	3	130923st0
Picolinafen	21	20.974	31497	376.0835	376.0843	-2.13	96.92		Pass	C19 H12 F4 N2 O2	4	130923st0
Picolinafen F1	21	20.971	25213	239.0558	239.0558	-0.05	96.61		Pass	C12 H8 F3 N O	3	130923st0
Picolinafen F2	21	20.971	40926	238.048	238.0489	-3.84	60.18	low score; ion count	Warning	C12 H7 F3 N O	1	130923st0
Prosulfocarb	12.94	12.922	10355	251.1344	251.1352	-3.33	96.32		Pass	C14 H21 N O S	4	130923st0
Prosulfocarb F1	12.94	12.922	3045	160.0796	160.0801	-3.05	92.11		Pass	C7 H14 N O S	3	130923st0
Prosulfocarb F2	12.94	12.922	26712	128.1075	128.1076	-0.86	99		Pass	C7 H14 N O	3	130923st0
Quinoclamine	13.4	13.388	4839	207.0087	207.0108	-9.88	87.71		Pass	C10 H6 Cl N O2	4	130923st0
Quinoclamine F1	13.4	13.392	3570	172.0399	172.0403	-2.83	89.79		Pass	C10 H6 N O2	2	130923st0
Quinoclamine F2	13.4	13.392	983	144.0449	144.0453	-2.36	91.14		Pass	C9 H6 N O	2	130923st0
Triflumizole F1	15.4	15.369	1669	278.056	278.0563	-1.19	92		Pass	C12 H12 Cl F3 N O	3	130923st0
Triflumizole F2	15.4	15.376	1139	287.0437	287.043	2.44	74.8	low score	Warning	C12 H9 Cl F3 N3	3	130923st0
Triflumizole F3	15.4	15.372	1495	205.9984	205.9995	-5.08	59.35	low score; ion count	Warning	C8 H4 Cl F3 N	1	130923st0

Quantitativ software used for qualitative detection

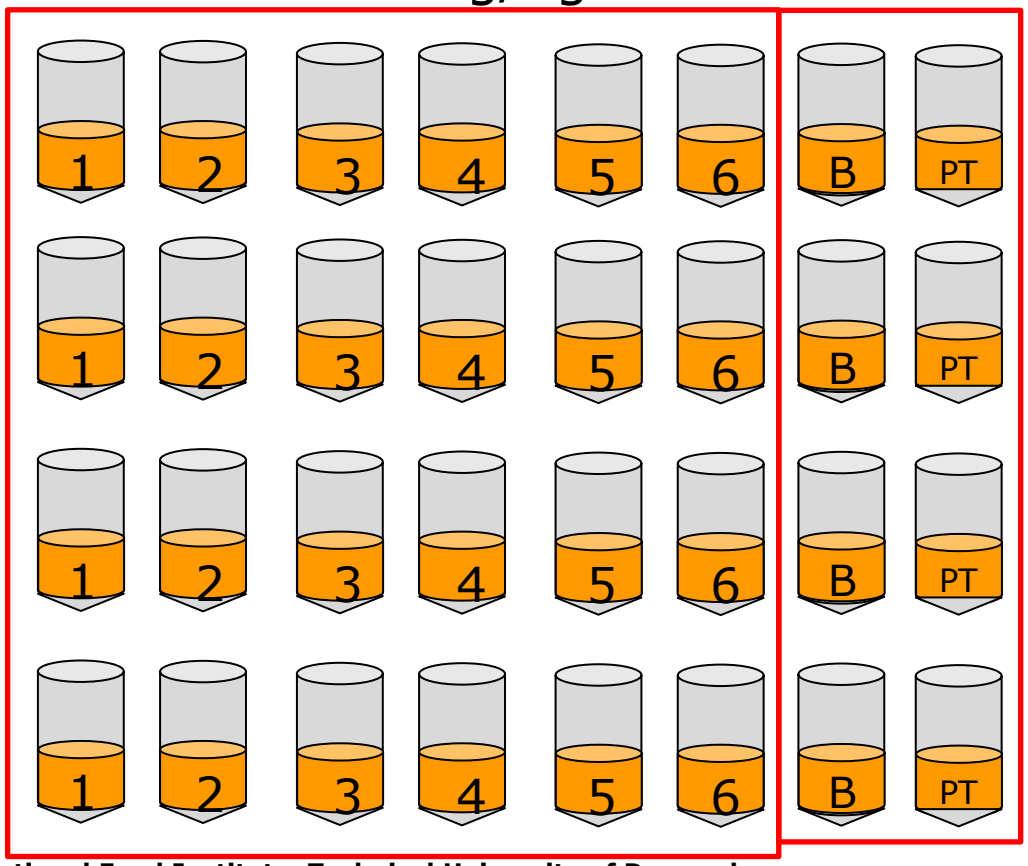



Agilent MassHunter Quantitative Analysis - Method - <I:\GCQTOF-PC Backup\D\MassHunter (SyncBack)_Data_mpou\150302\QuantResults\Validation std3 spike 0.1_150424.batch.bin>
File Edit View Analyze Method Update Report Tools Help
Analyze Batch
Layout:
Restore Default Layout
Method Tasks
New / Open Method
Method Setup Tasks
Compound Setup
Retention Time Setup
ISTD Setup
Concentration Setup
Qualifier Setup
Calibration Curve Setup
Globals Setup
Save / Exit
Validate
Save
Save As...
Exit
Manual Setup Tasks
Outlier Setup Tasks
Advanced Tasks
Method Table
Level Name Prefix:
# of Levels: 10
Create Levels
Time Segment: <All>
Compound: Fenobucarb
Reset Table View
Quantifier
Name Type MZ Extract Left m/z MZ Extraction Window Units Extract Right m/z RT Ion Polarity Criteria Formula
Aziprotryne Target 182.0500 100.0000 PPM 100.0000 11.62 Positive Close RT with Qualif... C7H10N5S
Benzoylprop-ethyl Target 105.0340 100.0000 PPM 100.0000 20.69 Positive Close RT with Qualif... C7H5O
Butamifos Target 286.1031 100.0000 PPM 100.0000 16.43 Positive Close RT with Qualif... C13H21NO2PS
Butylate - Sutan Target 156.1388 100.0000 PPM 100.0000 8.08 Positive Close RT with Qualif... C9H18NO
Crimidine Target 171.0563 100.0000 PPM 100.0000 8.77 Positive Close RT with Qualif... C7H10N3Cl
Dichlormid Target 172.0529 100.0000 PPM 100.0000 7.65 Positive Close RT with Qualif... C8H11NOCl
Dichlorprop-met... Target 161.9639 100.0000 PPM 100.0000 9.51 Positive Close RT with Qualif... C6H4OCl2
Dimethenamid Target 230.0406 100.0000 PPM 100.0000 12.48 Positive Close RT with Qualif... C10H13NOSCl
Fenobucarb Target 121.0653 100.0000 PPM 100.0000 9.46 Positive Close RT with Qualif... C8H9O
Sample Information
Max # of panes: 2
+ TIC Scan (** -> **) 150302st38 Standard 3456 cal 3.D
Counts
Acquisition Time (min)
Compound Information
+ EIC (121.0653) Scan 150302st38 Standard 3456 cal 3.D
9.46 min.
Counts
Acquisition Time (min)
121.0653
Relative Abundance [%]
Acquisition Time (min)
X 12.2236 Y 2317538.426
19 Compounds (19 total) 0 ISTD (0 total)

C12H14O3Cl2	12.607	276.0320	2,4-D Butyl ester	276.0315
C8H6O3Cl2	12.607	219.9694	2,4-D Butyl ester F1	219.9688
C8H6O3Cl	12.607	185.0005	2,4-D Butyl ester F2	185.0000
C7H5OCl2	12.607	174.9717	2,4-D Butyl ester F3	174.9712
C6H4OCl2	12.607	161.9639	2,4-D Butyl ester F4	161.9634
C6H3Cl2	12.607	144.9612	2,4-D Butyl ester F5	144.9606

Design of validation 1 MACP pesticides

- 6 samples of four different types of cereal samples, blank and EUPT blank test material – 4 matrices, two spiking levels, 0.05 and 0.01 mg/kg



Design of validation (2)

- Spiked with more than 100 pesticdes.
- As a start 38 of the compounds was evaluated
- According to SANCO/12571/2013 at least 95% of the samples should be detected (a false-negative rate of 5% is accepted).
  - This means that only 1 out of 20 spiked samples are allowed to be non-detected.

Screening detection criteria

- 1. DTU:
  - Retention time (RT): ± 0.1 min
  - Mass accuracy: 10 ppm for at least 2 fragment ions
  - Signal to noise ratio (S/N): 6

Validation results 38 MACP pesticides – spike experiments

- SDL =0.01 mg/kg (17)
  - Bifenthrin, Chlorpyrifos, Chlorpyrifos-methyl, Diazinon, Fenitrothion, Fipronil, Flutriafol, Iprodione, Krexoxim-methyl, Malathion, Methacrifos, Penconazole, Pirimicarb, Pirimiphos-methyl, Propiconazole, Trifluralin and Vinclozolin
- SDL = 0.05 mg/kg (19)
  - Azoxystrobin, Boscalid, Carboxin, Cypermethrin, Cyprodinil, Diclorvos, Difenoconazole, Epoxiconazole, Fenbuconazole, Lambda-cyhalothrin, Pendimethanil, Prochloraz, Procymidone, Pyraclostrobin, Spiroxamin, Tebuconazole, Triadimenol, Trifloxystrobin, Triticonazole
- Not validated (2)
  - Azinphos-methyl, Metconazole

Validation results – PT test material

- The test materials contained 46 residues of 27 different pesticides.
- All pesticides were detected apart from one residue of lambda-cyhalothrin.
  - "assigned value" = 0.025 mg/kg; SDL = 0.05 mg/kg
- No false positive results were seen.

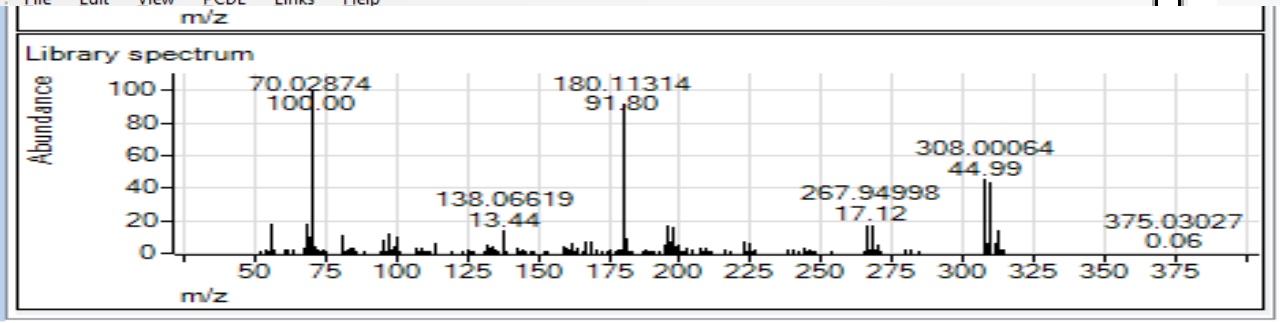
Agilent pesticide spectrum library

EURL EUROPEAN UNION REFERENCE LABORATORY PESTICIDE RESIDUES IN CEREALS & FEEDING STUFF

- Spectra of 753 pesticides/compounds

MassHunter PCDL Manager for Pesticides - D:\MassHunter\PCDL\PCDL_stdA2-changed spectrum below100.cdb

File Edit View PCDL Links Help



Library spectra

Compound Name	Ion Species	Precursor Ion	CE (V)	Polarity	Ionization	Ins
Prochloraz			0	Positive	EI	QT

Add Spectra | Delete Spectra | Update Spectra

Single Search Results: 81 hits

Compound Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSpider		
Chlorpyrifos	C9H11Cl3NO3PS	348.92628			14.520	2921-88-2	2629		
Chlorfenvinphos I	C12H14Cl3O4P	357.96953			15.750	470-90-6	4526760		
Fluquinconazole	C16H8Cl2FN5O	375.00899			25.420	136426-54-5	77933		
Prochloraz	C15H16Cl3N3O2	375.03081			25.570	67747-09-5	66316		
Ethion	C9H22O4P2S4	383.98762			18.940	563-12-2	3171	O,O,O',O'-tetraethyl S,S'-methanediyl bis(phospho...	1
Permethrin I	C21H20Cl2O3	390.07895			25.040	54774-45-7	36968	3-Phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)-2,2...	1
Azoxystrobin	C22H17N3O5	403.11682			30.900	131860-33-8	2298772	Methyl (2E)-2-(2-{[6-(2-cyanophenoxy)-4-pyrimidin...	1
Endosulfan (beta isomer)	C9H6Cl6O3S	403.81688			16.880	33213-65-9	3111	1,9,10,11,12,12-hexachloro-4,6-dioxa-5-thiatricycl...	1
Endosulfan (alpha isomer)	C9H6Cl6O3S	403.81688			18.500	959-98-8	3111	1,9,10,11,12,12-hexachloro-4,6-dioxa-5-thiatricycl...	1
Difenconazole I	C19H17Cl2N3O3	405.06470			29.420	119446-68-3	77730	1-({2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-meth...	1
Trifloxystrobin	C20H19F3N2O4	408.12969			20.100	141517-21-7	9839700	Methyl (2E)-(methoxyimino)(2-{[({(1E)-1-[3-(trifluoro...	1
Cypermethrin I	C22H19Cl2NO3	415.07420			26.770	52315-07-8	2809	Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichlorovin...	1
Fenvalerate	C25H22ClNO3	419.12882			28.520	51630-58-1	3230	(S)-Cyano(3-phenoxyphenyl)methyl (2S)-2-(4-chlor...	1

	Mass	Rel Abund
	70.02874	100.00000
	180.11314	91.79576
	308.00064	44.99226
	309.99681	43.01000
	56.04948	17.66159
	68.03690	17.54143
	267.94997	17.11979
	265.95369	17.01565
	195.92306	16.52209
	197.92117	15.38622
	311.99411	14.22519
	138.06619	13.44272
	70.06513	12.14967

Agilent MassHunter Quantitative Analysis (for TOF) - Method - [I:\GCQTOF-PC Backup\D\MassHunter (SyncBack)_Quantitative methods\150909ValidationStdA_import5Fragments.m]

File Edit View Analyze Method Update Report Tools Help

Analyze Batch | Layout: | Restore Default Layout

Method Tasks

- New / Open Method
- Method Setup Tasks
  - Compound Setup
  - Retention Time Setup
  - ISTD Setup
  - Concentration Setup
  - Qualifier Setup
  - Calibration Curve Setup
  - Globals Setup
- Save / Exit
  - Validate
  - Save
  - Save As...
  - Exit
- Manual Setup Tasks
- Outlier Setup Tasks
- Advanced Tasks

Method Table

Level Name Prefix: | # of Levels: 10 | Create Levels | Time Segment: <All> | Compound: Cyprodinil | Reset Tab

Calibration

Level	Conc.	Response
Cal 2	0.3330	369865

Quantifier

Name	TS	Scan	MZ	Uncertainty
Cyprodinil	1	Ms2Scan	224.1182	Relative

Qualifier

MZ	Rel. Resp.	Uncertainty	Area Sum
225.1261	47.8	30.0	
210.1026	9.2	30.0	
38.5190	8.6	30.0	
208.0869	7.7	30.0	
226.1278	6.1	30.0	

Calibration

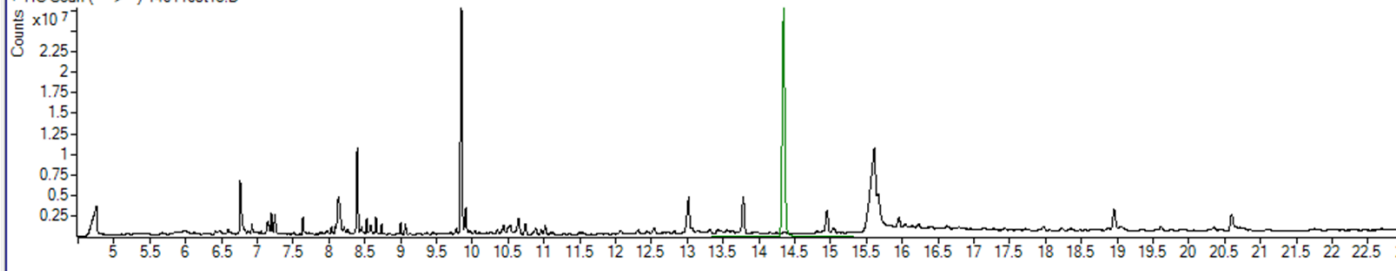
Level	Conc.	Response
Cal 3	0.1000	151327
Cal 3	0.1000	106062
Cal 3	0.1000	116406
Cal 2	0.3330	369865

Quantifier

Sample Information

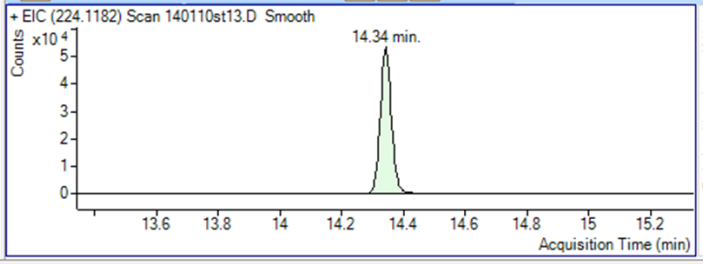
Max # of panes: 2

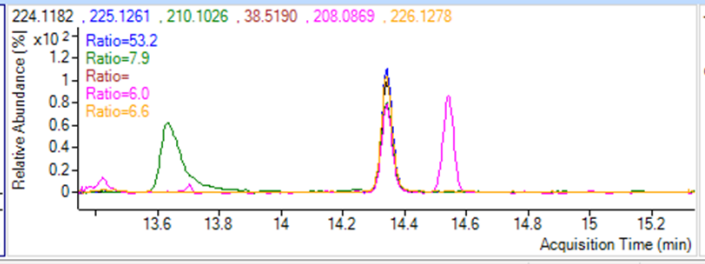
+ TIC Scan (** -> **) 140110st13.D



Compound Information

+ EIC (224.1182) Scan 140110st13.D Smooth





Screening detection/identification criteria

- 1. DTU:
  - Retention time (RT): ± 0.1 min
  - Mass accuracy: 10 ppm for at least 2 fragment ions
  - Signal to noise ratio (S/N): 6

- 2. SANCO/12571/2013 (identification):
  - Retention time (RT): ±0.2 min
  - Signal to noise ratio (S/N): 3
  - mass accuracy < 5 ppm; at least one fragment ion
- 3. Possible new SANTE document (identification):
  - Retention time (RT): ±0.1 min
  - Signal to noise ratio (S/N): 3
  - 2 ions with mass accuracy ≤ 5 ppm, < 1 mDa for m/z < 200

Validation of 72 MACP pesticides

Criteria screening	# pesticides	SDL 0.05 mg/kg	SDL 0.05mg/kg	Not validated
2 ions <10 ppm	72	21	49	2
2 ions <5 ppm	72	38 (3)	28 (27)	6
2 ions <5 ppm or < 1 mDalton for mz<200 amu	72	31	38 (23)	3

Validation of 72 MACP pesticides

Criteria screening	# pesticides	SDL 0.05 mg/kg	SDL 0.05mg/kg	Not validated
2 ions <10 ppm	72	21	49	2

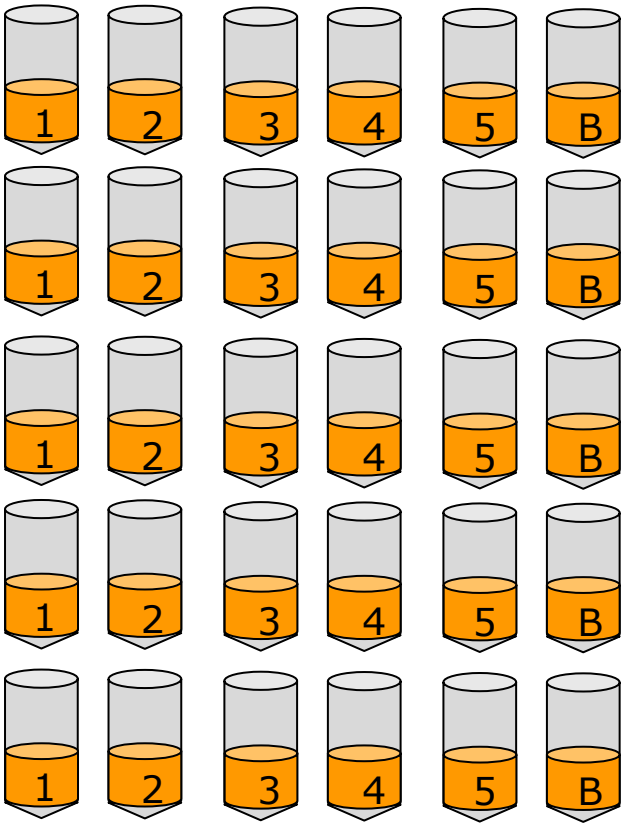
Criteria identification	# pesticides	0.05 mg/kg	0.01 mg/kg	Not identified
2 ions <5 ppm	72	38 (3)	28 (27)	6
2 ions <5 ppm or < 1 mDalton for mz<200 amu	72	31	38 (23)	3

Standard mixtures for screening

Mixtures	8 mixtures with 50-100 pesticides
Isomers	Avoiding isomers in the same mixtures
No. of compounds	432
Validation	2-4 mixtures together

Design of validation

- 5 samples of 5 different cereal types, 1 blank
  - 5 matrices, 25 samples, spike levels 0.1, 0.02 and 0.01 mg/kg



Barley

Oat

Rice

Rye

Wheat

Validation 2

- Compounds spiked with
  - 53 of the compounds where exact masses has been calculated.
- According to SANCO/12571/2013 at least 95% of the samples should be detected (a false-negative rate of 5% is accepted).
  - This means that only 1 out of 20 spiked samples are allowed to be non-detected.

Pesticides/metabolites

2.4.5-T-methylester
2.4-D Butyl ester
2.4-DB-methylester
4,4'-Dichlorobenzophenone
Acetochlor
Allethrin
Aspon (NDP)
Atrazine-desethyl
Aziprotryne
Barban
Benfluralin
Bensulide
Benzoylprop-ethyl
Butachlor
Butamifos
Butylate
Carbetamide
Chlorthiamid
Crimidine
Crotoxyphos (
Cythioate
DDM / Dichlorophen
Diazoxon
Dibutylchlorendate
Dicapthon
Dichlormid
Dichlorprop-methyl
Dimethenamid (SAN 582H)
Dithiopyr
Fenobucarb (Baycarb)
Fenoprop-methyl
Fluchloralin
Fluoroglycofen-ethyl
Fluridone
Flurprimidol
Imibenconazole
Lethane 384
Mefluidide
Methabenzthiazuron
Nitrothal-isopropyl
O.O.O-Triethylphosphorothioate
Oryzalin
Pentanochlor (Solan)
Pethoxamid
Plifenate (Penfenate)
Profluralin
Propachlor
Quinomethionate (MQD)
Resmethrin
Thenylchlor
Tiocarbazil I (Drepamon)
Triclopyr-methyl

Agilent pesticide spectrum library

MassHunter PCDL Manager for Pesticides - D:\MassHunter_PCDL\TLCE pesticid PCDL 53 fra std 3 og 4 - 20150430.cdb

File Edit View PCDL Links Help

Find Compounds

Single Search | Batch Search | Batch Summary | Edit Compounds | Spectral Search | Browse Spectra | Edit Spectra

Mass

Mass tolerance

Retention time

RT tolerance

Ion search mode

Include n

Include a

Include c

Print/Cop

Molecule:

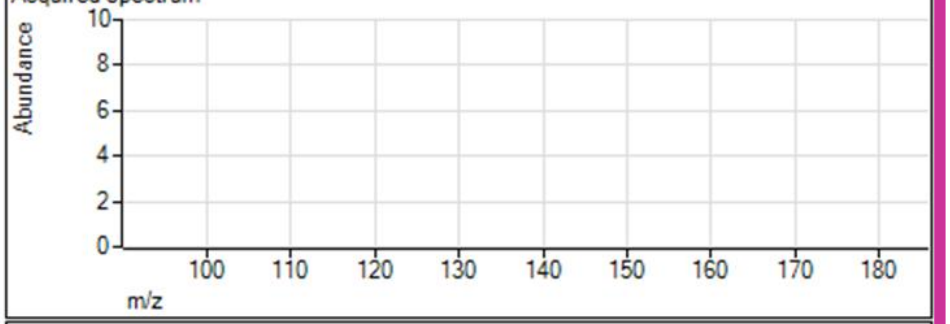
Notes:

Spider

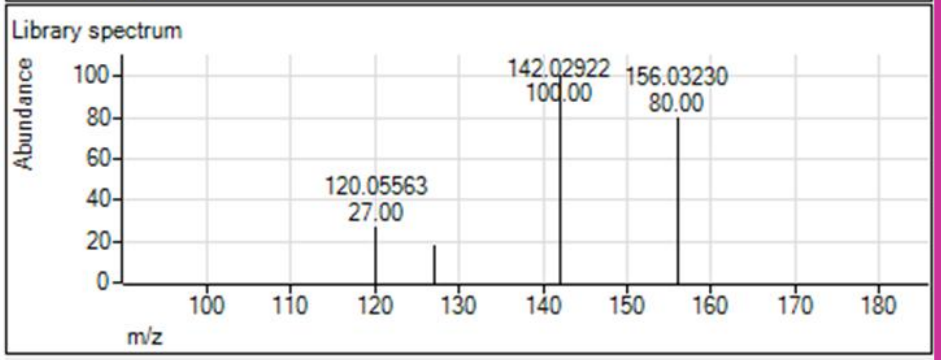
Edit Spectra

Graphics | Mass Lists

Acquired spectrum



Library spectrum



Library masses

	Mass	Rel Abund
▶	142.02923	100.00000
	156.03230	80.00000
	120.05562	27.00000
	127.00575	18.00000

Crimidine									
XMC (3,5-X									
Atrazine-des									
O.O.O-Triet									
Lethane 38									
Chlorthiamid									
Dichlormid									
Fenobucarb (Baycarb)	C12H17NO2	207.12593			9.529	3766-81-2	18452	2-(butan-2-yl)phenyl methylcarbamate	1
Propachlor	C11H14ClNO	211.07639			9.609	1918-16-7	4762	2-Chloro-N-isopropyl-N-phenylacetamide	1
Butylate	C11H23NOS	217.15004			8.137	2008-41-5	15357	S-Ethyl diisobutylcarbamothioate	1
Methabenzthiazuron	C10H11N3OS	221.06228			10.080	18691-97-9	27173	1-(1,3-Benzothiazol-2-yl)-1,3-dimethylurea	1
Aziprotryne	C7N7SH11	225.07966			11.702	4658-28-0	2297441	4-Azido-N-isopropyl-6-(methylsulfanyl)-1,3,5-triazin-...	1
Quinomethionate (MQD)	C10N2OS2H6	233.99215			15.760	2439-01-2	16193	6-Methyl[1,3]dithiolo[4,5-b]quinoxalin-2-one	1
Carbetamide	C12N2O3H16	236.11609			14.158	16118-49-3	25761	1-(Ethylamino)-1-oxo-2-propanyl phenylcarbamate	1
Pentanochlor (Solan)	C13H18ClNO	239.10769			13.560	2307-68-8	15945	N-(3-Chloro-4-methylphenyl)-2-methylpentanamide	1
Dichlorprop-methyl	C10H10Cl2O3	248.00070			9.588	57153-17-0	82169	methyl 2-(2,4-dichlorophenoxy)propanoate	1
4,4'-Dichlorobenzophenone	C13H8Cl2O	249.99522			14.183	90-98-2	6767	Bis(4-chlorophenyl)methanone	1
Barban	C11H9Cl2NO2	257.00103			16.832	101-27-9	7270	4-Chloro-2-butyn-1-yl (3-chlorophenyl)carbamate	1

Agilent MassHunter Quantitative Analysis (for TOF) - Method - [I:\GCQTOF-PC Backup\D\MassHunter (SyncBack)_Quantitative methods\150908ValidationStd3PCDLTommy.m]

File Edit View Analyze Method Update Report Tools Help

Analyze Batch | Layout: | Restore Default Layout

Method Tasks

- New / Open Method
- Method Setup Tasks
  - Compound Setup
  - Retention Time Setup
  - ISTD Setup
  - Concentration Setup
  - Qualifier Setup
  - Calibration Curve Setup
  - Globals Setup
- Save / Exit
  - Validate
  - Save
  - Save As...
  - Exit
- Manual Setup Tasks
- Outlier Setup Tasks
- Advanced Tasks

Method Table

Level Name Prefix: | # of Levels: 10 | Create Levels | Time Segment: <All> | Compound: 2.4-D Butyl ester | Reset Ta

Quantifier

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Qualifier

MZ	Rel. Resp.	Uncertainty	Area Sum
276.0315	88.2	20.0	
174.9712	72.9	20.0	
161.9634	58.8	20.0	
144.9606	35.3	20.0	
219.9689	35.3	20.0	

Quantifier

Name	TS	Scan	MZ	Uncertainty	RT
2.4-DB-methyles...	1	Ms2Scan	101.0597	Relative	12.20

Qualifier

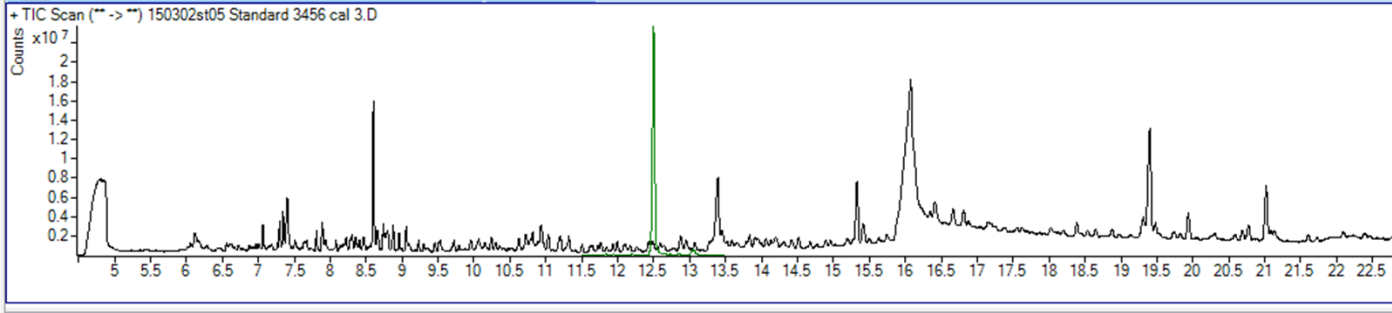
MZ	Rel. Resp.	Uncertainty	Area Sum
161.9634	22.0	20.0	
230.9974	10.0	20.0	
262.0158	2.0	20.0	

Quantifier

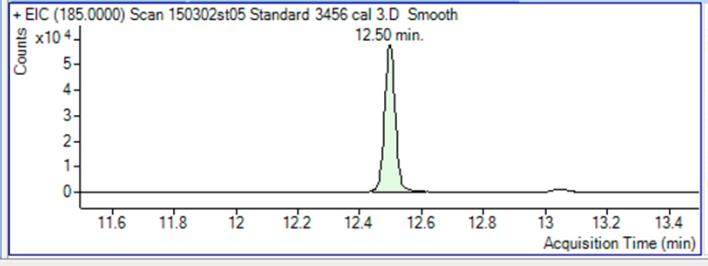
Name	TS	Scan	MZ	Uncertainty	RT

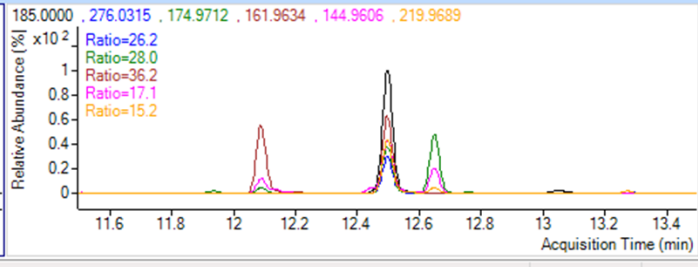
Sample Information

Max # of panes: 2



Compound Information





Screening detection/identification criteria

- 1. DTU:
  - Retention time (RT): ± 0.1 min
  - Mass accuracy:10 ppm for at least 2 fragment ions
  - Signal to noise ratio (S/N): 6
- 2. SANCO/12571/2013 (identification):
  - Retention time (RT): ±0.2 min
  - Signal to noise ratio (S/N): 3
  - mass accuracy < 5 ppm; at least one fragment ion
- 3. Possible new SANTE document (identification):
  - Retention time (RT): ±0.1 min
  - Signal to noise ratio (S/N): 3
  - 2 ions with mass accuracy ≤ 5 ppm, < 1 mDa for m/z < 200

Results

Criteria screening	# pesticides	SDL 0.10 mg/kg	SDL 0.02 mg/kg	SDL 0.01 mg/kg	Not validated
2 ions <10 ppm	52	8	6	23	15
2 ions <5 ppm	52	10 (2)	3 (3)	19 (4)	20
2 ions <5 ppm or < 1 mDalton for mz<200 amu	52	9 (1)	3	22 (2)	18

Results

Criteria screening	# pesticides	SDL 0.10 mg/kg	SDL 0.02 mg/kg	SDL 0.01 mg/kg	Not validated
2 ions <10 ppm	52	8	6	23	15

Criateria identification	# pesticides	0.10 mg/kg	0.02 mg/kg	0.01 mg/kg	Not identified
2 ions <5 ppm	52	10 (2)	3 (3)	19 (4)	20
2 ions <5 ppm or < 1 mDalton for mz<200 amu	52	9 (1)	3	22 (2)	18

Conclusions

- Exact masses of fragments are needed for GC accurate mass spectrometry due to fragmentation in the ion source
- Processing data in Mass Hunter quantitative software is easy and provide the necessary information on mass accuracy, signal/noise and ion ratios
- Prossesing methods are straightforward build by importing fragment exact masses and Rt from a library (PCDL)
- Validation of a screening method was successful for 109 compounds.
- The method was used successfully on PT test material.
- The instrument is performing well for screening purposes,
  - However, for identification the performance is not impressive.
- The Mass Hunter software can still be improved, especially to be amendable to GC.


Thank you for your attention