Quantify with confidence and screen for the unexpected

New tools and workflows for your pesticide analysis

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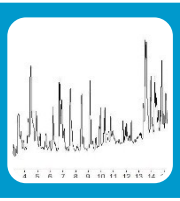
EMEA LC/MS Food Segment Scientist

Agenda

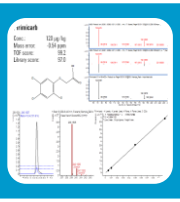


Pesticide screening

Toolbox Accurate Mass

Validation study

Real samples



Summary

Introduction

Current regulations and residue situation

- **EU regulation**
  - Maximum residue levels regulated in commision 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 ?

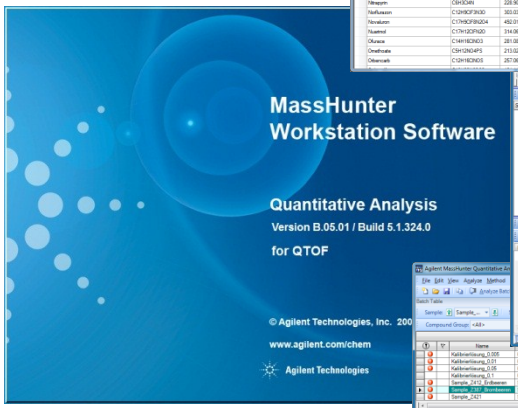
… to find what else is in the sample ?

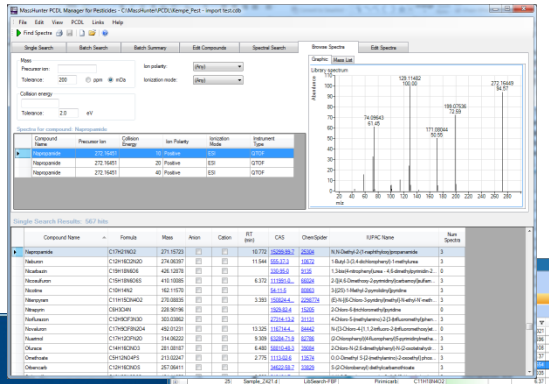
… to retrospectively look into the sample ?

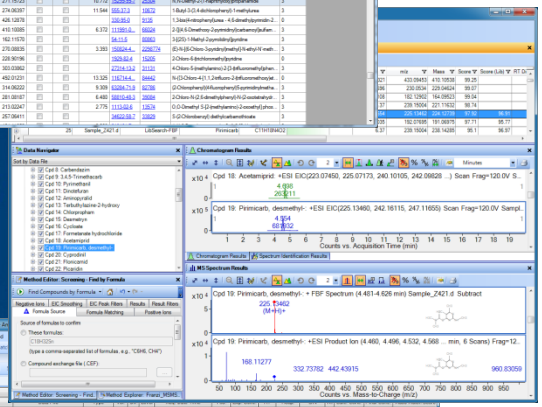
… to use profiling tools to find the unexpected ?

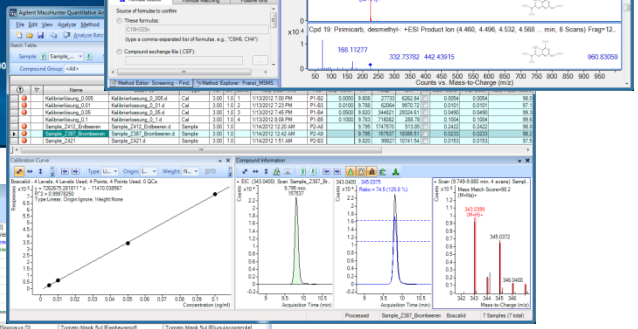
The toolbox for accurate mass screening

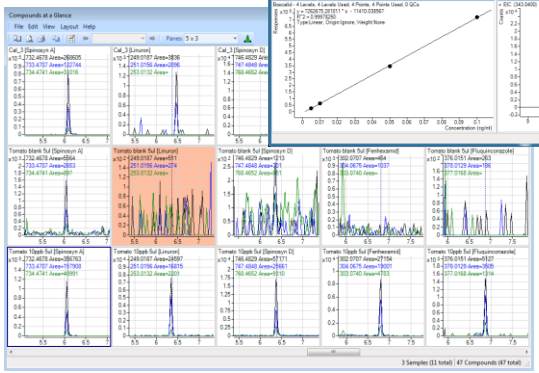
The complete solution from Agilent









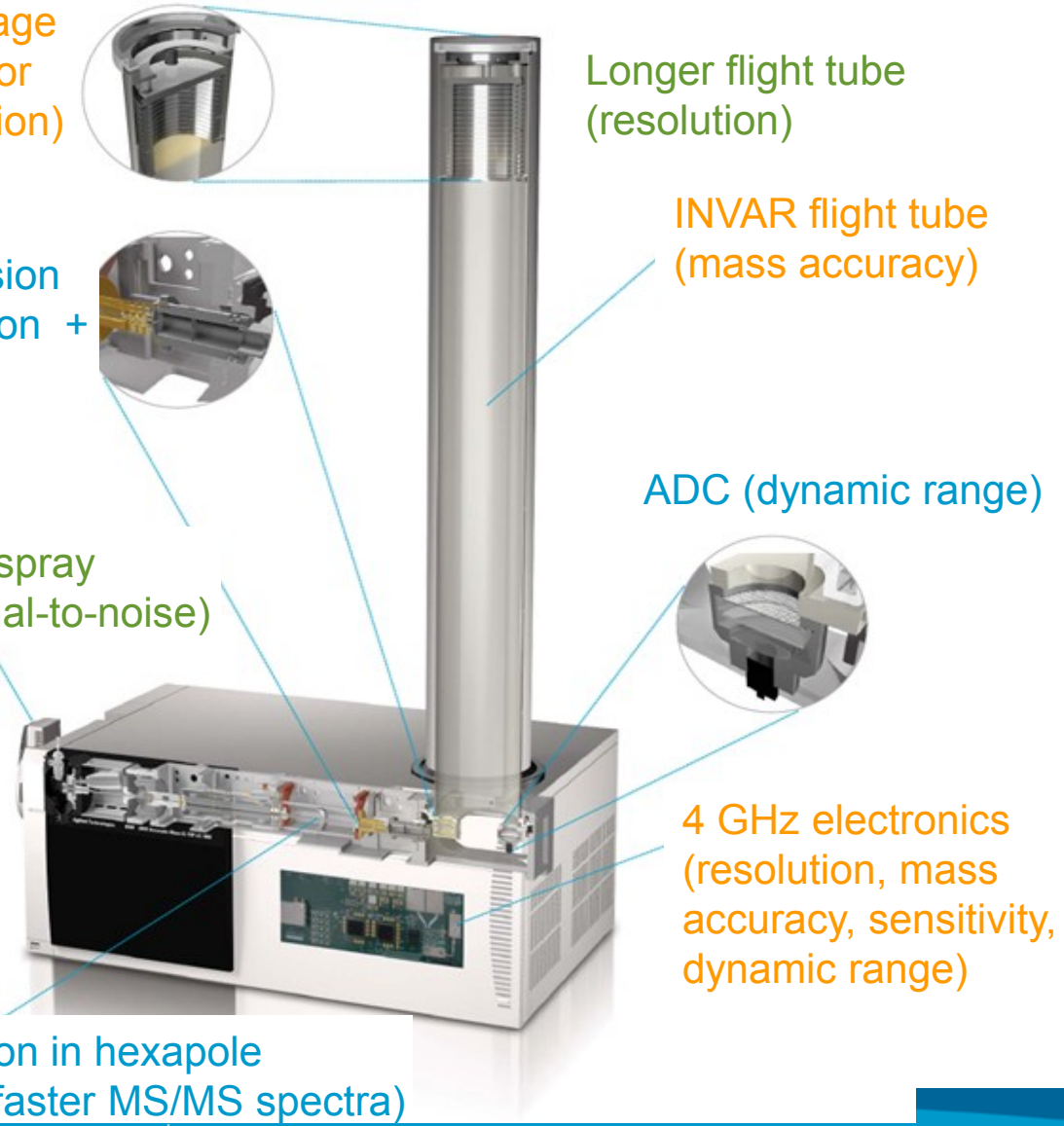


... and we also have GC-MS/MS

Screening workflow using LC-(Q)TOF-MS

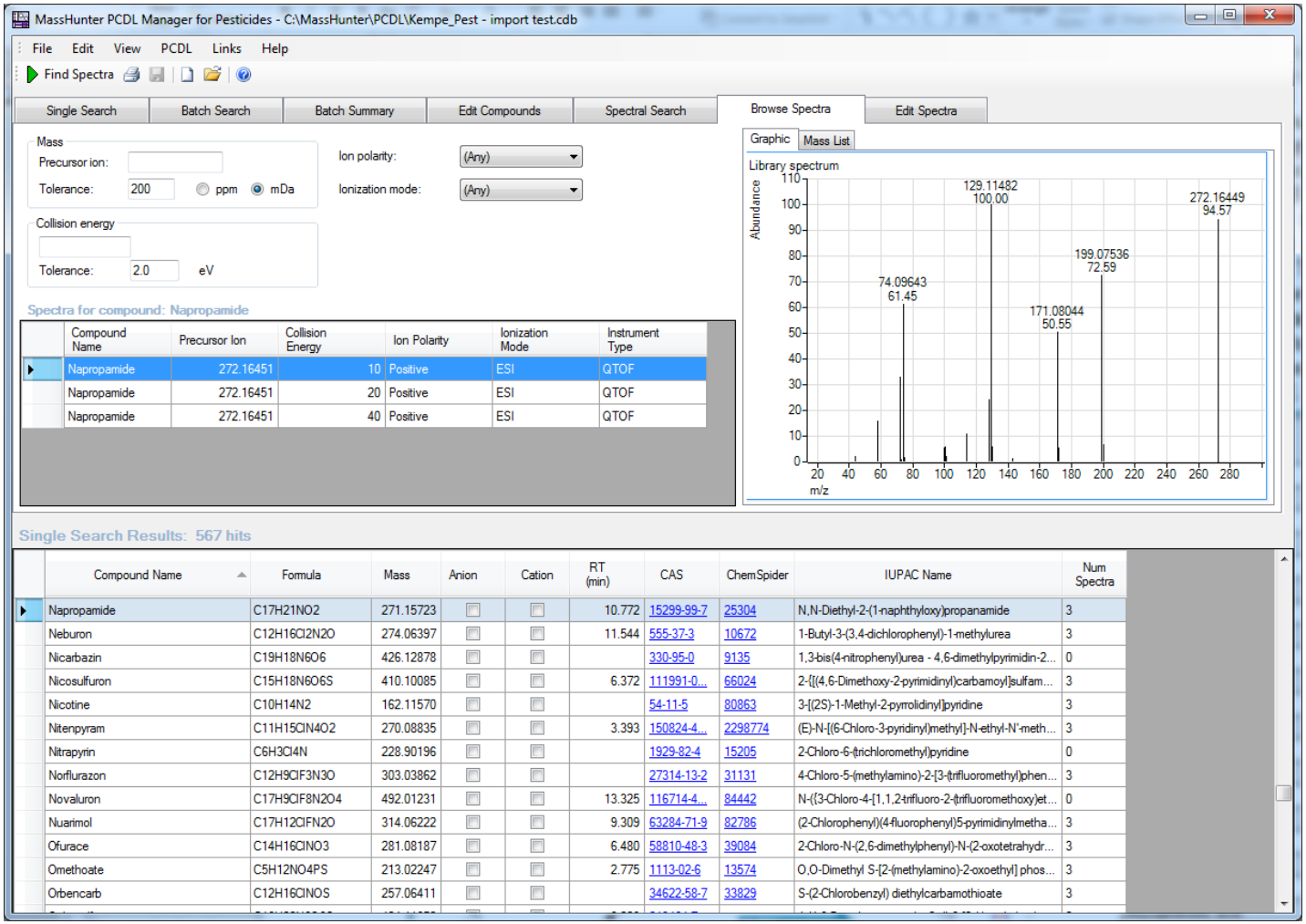
Instrumentation

Technology Innovation



Screening workflow using LC-(Q)TOF-MS

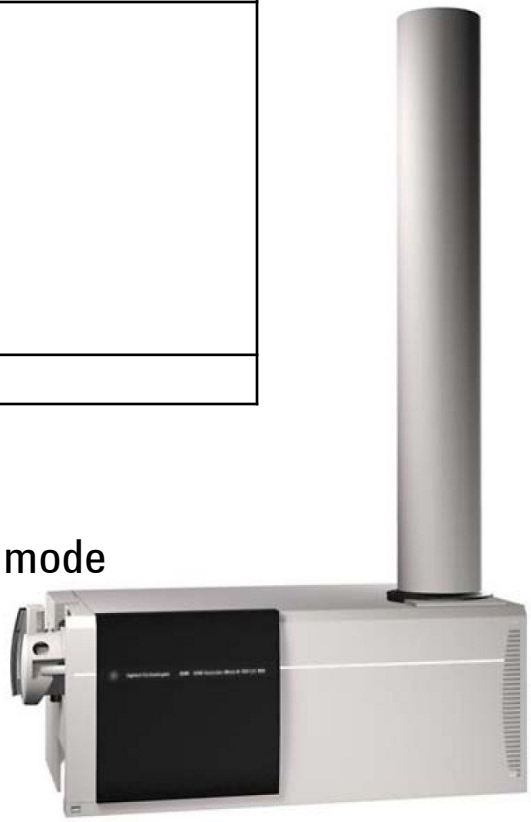
Personal compound database and library



Experimental

UHPLC and QTOF method parameters

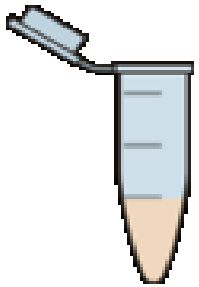
UHPLC column	Agilent ZORBAX Eclipse Plus C18 RRHD 2.1 x 150 mm, 1.8 µm @ 30°C
Mobile phase	A: 5 mM NH_4 formate + 0.1% formic acid B: 5 mM NH_4 formate + 0.1% formic acid in methanol
Gradient program	Min % B 0 10 0.5 10 3.5 50 17.0 100 20.0 100 20.1 5 Stop time 22 min
Flow rate	0.40 ml/min



- **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

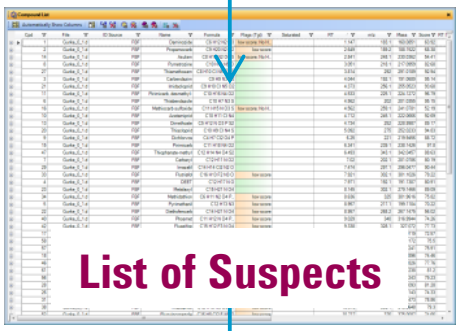
Validation study and real sample screening

Experimental setup



LC-QTOF
Target MS/MS
acquisition

Data analysis
using
„Find-by-Formula“



List of Suspects

Verified by
MS/MS library
search

Yes

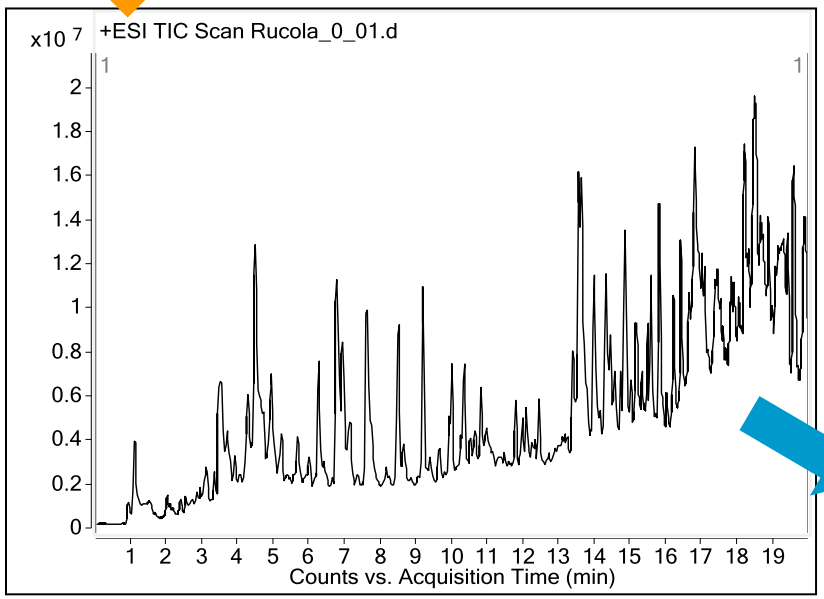
No

Quant with verification by
accurate mass metrics

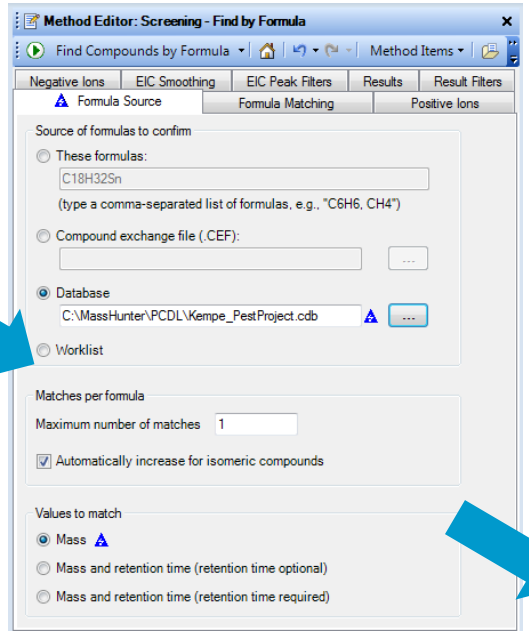
Screening workflow using LC-(Q)TOF-MS

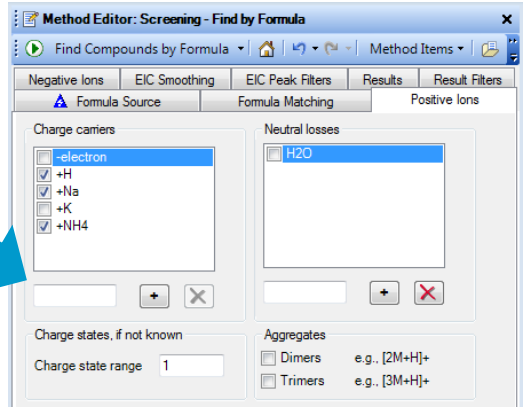
Innovative workflow – Find by Formula

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



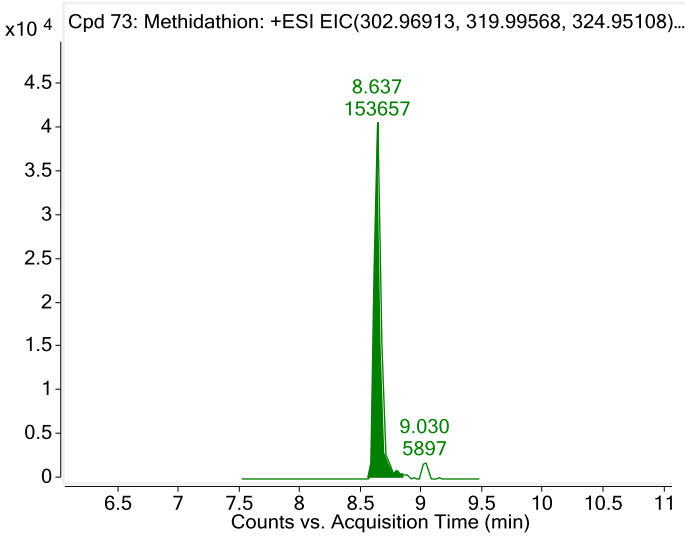
TIC chromatogram of rucola extract spiked with pesticides @ 10 µg/kg

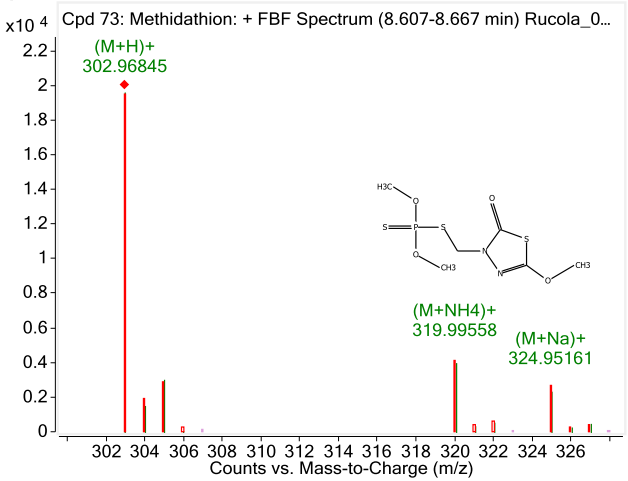


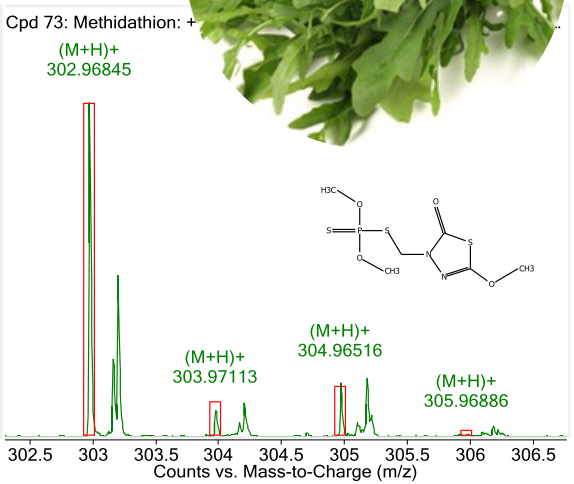


Find-by-Formula data mining algorithm

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

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

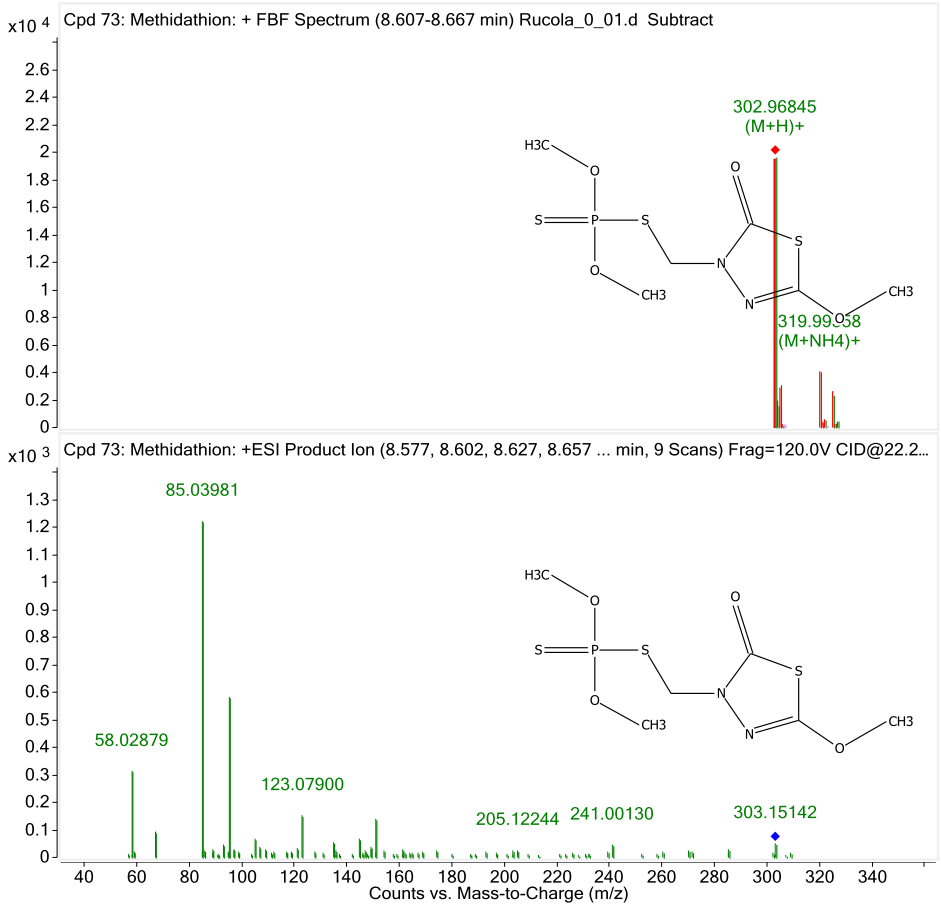
Species	Ion Formula	Score (MFG)	Score (MFG, MS/MS)	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)
(M+H)+				97	97.81	95.54	97.13

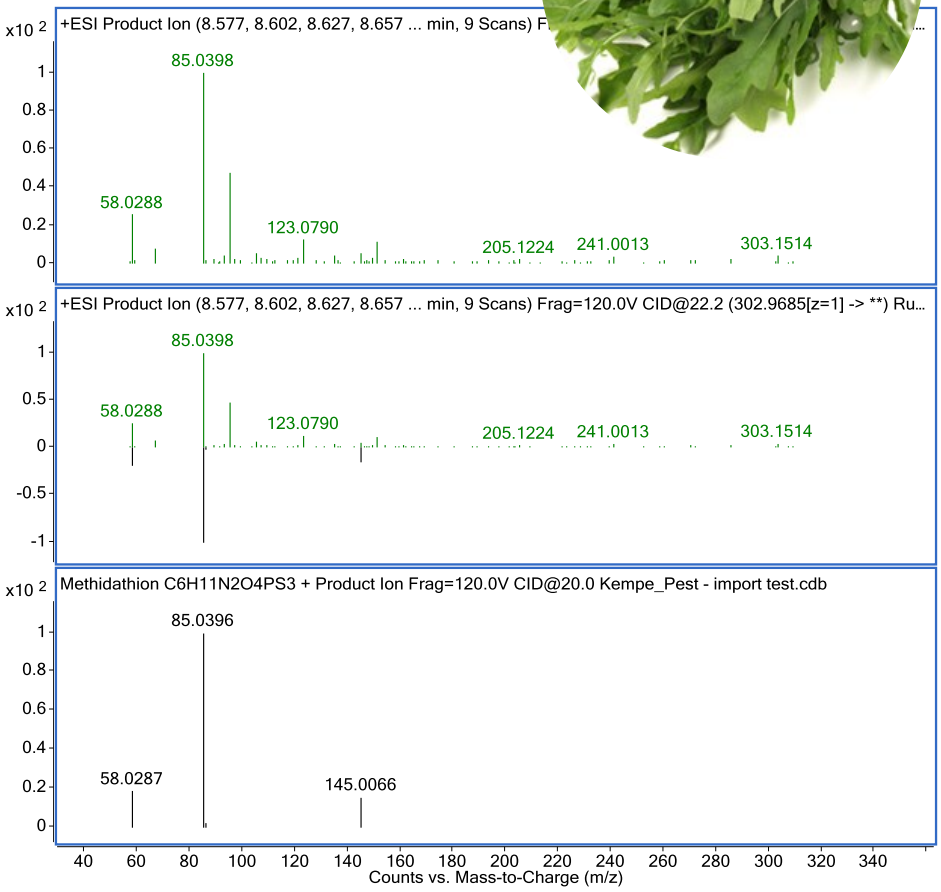
m/z	m/z (Calc)	Diff (ppm)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum% (Calc	Height Sum %
302.968...	302.96913	2.26	19496.9	19220.8				
303.971...	303.97112	-0.03	1576.9	1898.9				
304.965...	304.96565	0.18	2957.5	2822.9				
305.968...	305.96772	-3.73	163.6	252.3				

Results scoring based on RT, mass accuracy, isotope abundance and spacing

Library searching and confirmation

Rucola extract spiked with pesticides @ 10 µg/kg



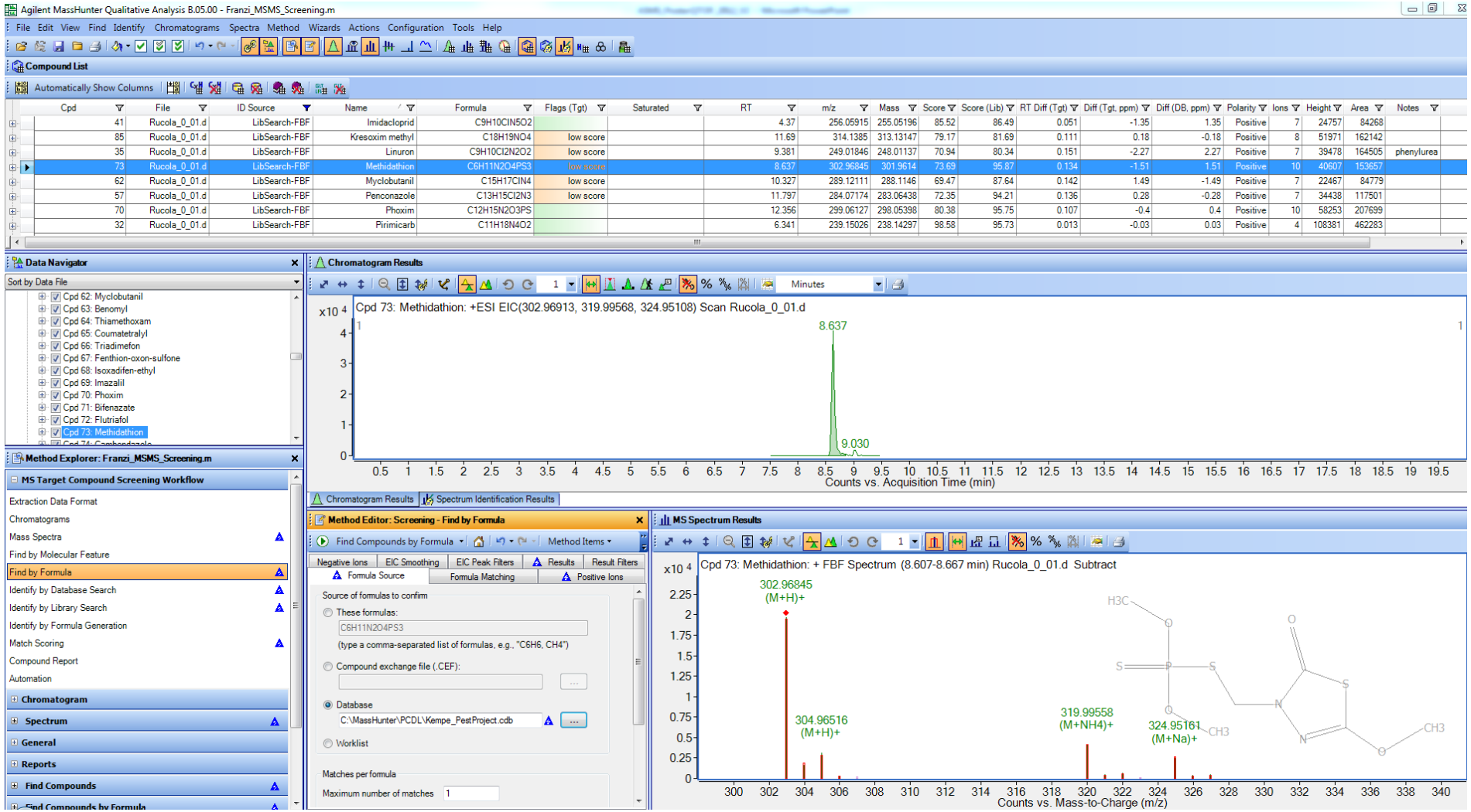


TOF spectrum and MS/MS spectrum

Comparison of acquired vs. library spectrum

Screening workflow using LC-(Q)TOF-MS

Screen shot MassHunter Qualitative analysis



Pesticides screening with accurate mass

Validation data for 3 different matrices

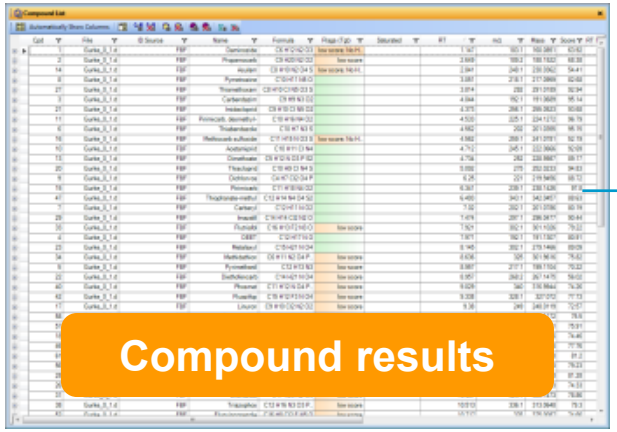
Compound	Cucumber					Lemon					Rucola				
	Blank	0.005	0.01	0.05	0.1	Blank	0.005	0.01	0.05	0.1	Blank	0.005	0.01	0.05	0.1
Acetamiprid															
Azoxystrobin															
Bifenazat															
Boscalid															
Buprofenzin															
Carbaryl															
Carbendazim															
Chlopyrifos															
Chlorpyrifos-methyl															
Cyprodinil															
DEET															
Dichlorvos															
Difenoconazole															
Diflubenzuron															
Dimethoat															
Dimethomorph															
Fenhexamid															
Fluazifop free acid															
Fludioxonil															
Fluquinconazole															
Flutriafol															
Imazalil															
Imidacloprid															
Kresoxim-methyl															
Linuron															
Mandipropamid															
Metalaxyl															
Methidathion															
Myclobutanil															
Penconazole															
Pendimethalin															
Phosmet															
Phoxim															
Pirimicarb															
Pirimicarb-desmethyl															
Propamocarb															
Propiconazole															
Prosulfocarb															
Pyraclostrobin															
Pyridaben															
Teflubenzuron															
Thiabendazole															
Thiacloprid															
Thiamethoxam															
Thiophanat-methyl															
Triadimefon															
Triazophos															
Trifloxystrobin															
Results FBF	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
Results Library search	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
False positives															
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

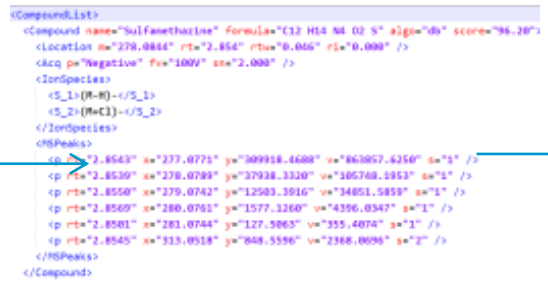
** For Cyhexatin, Famoxadone, Iprodione, Piperonyl-butoxide, and Propargite, found by the FBF algorithm in the MS domain, no MS/MS spectra was included in the accurate mass library for the observed ion species.

Validation study and real sample screening

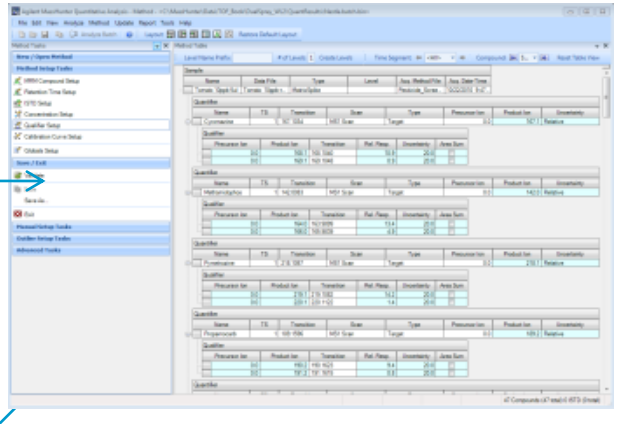
Experimental setup

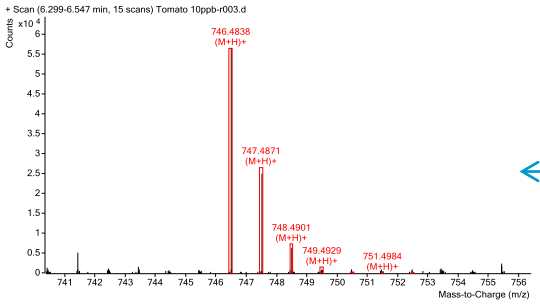


Compound results

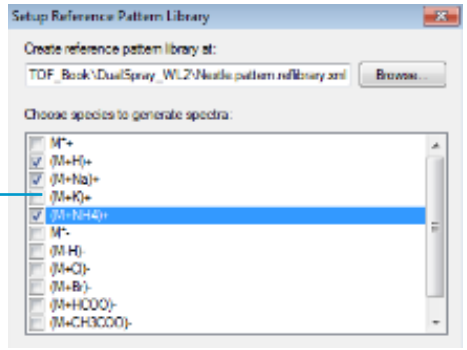


.cef file





Reference pattern library



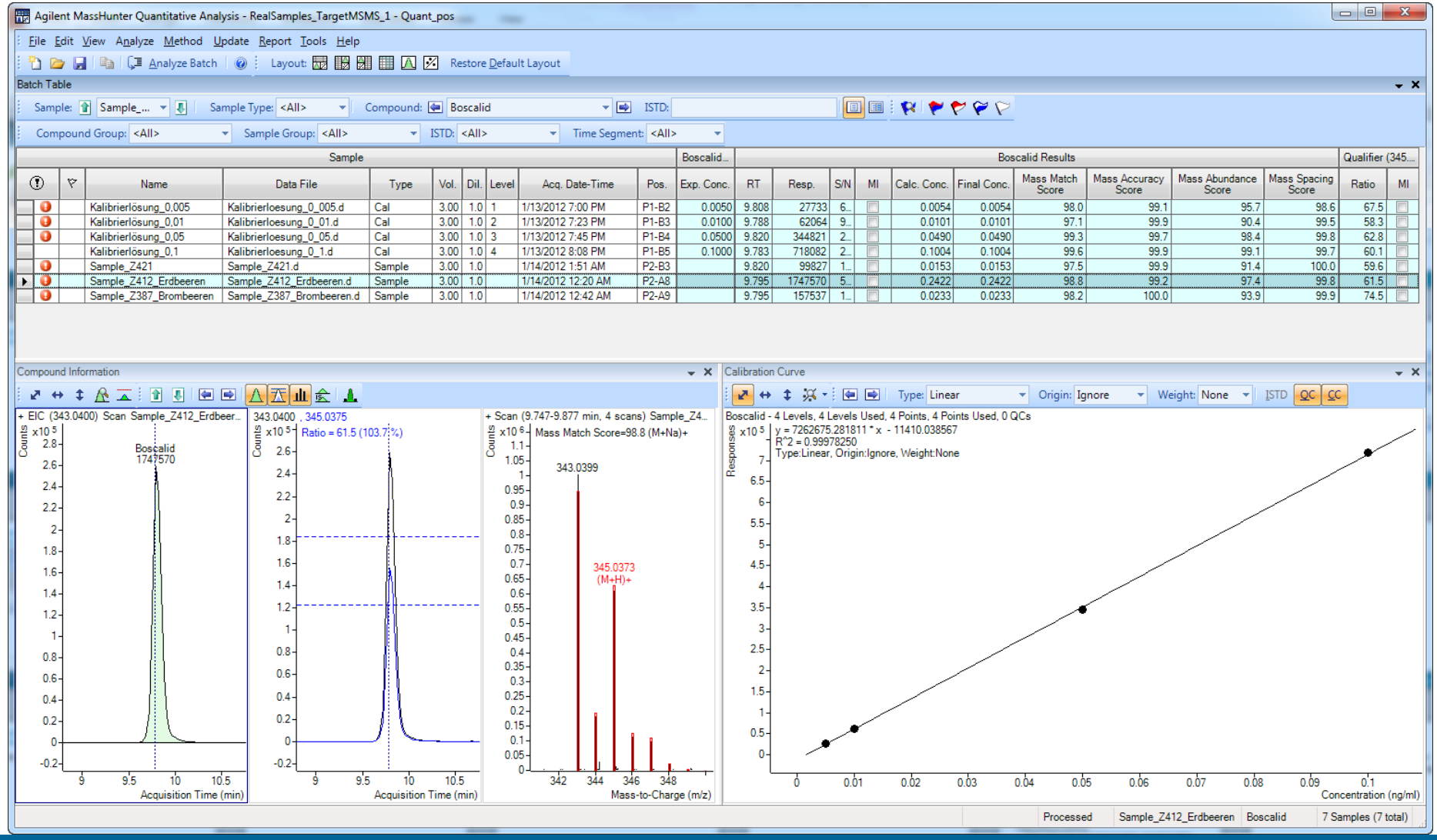
Adduct pattern

Autodynamic Quant method creation

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

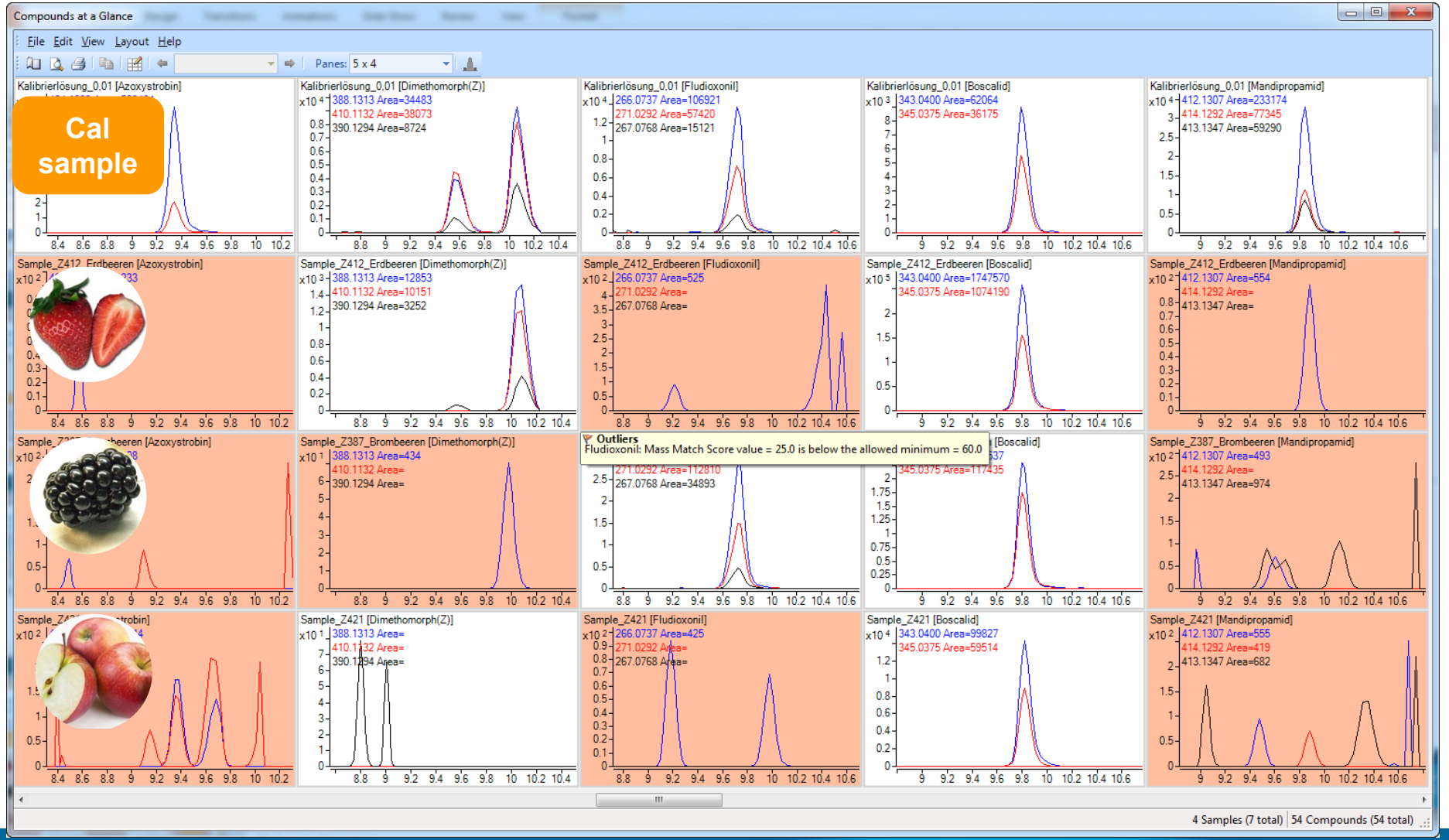
Quantitation of pesticides using accurate mass

Batch review process including accurate mass metrics



Quantitation of pesticides using accurate mass

Batch review process using Compounds at a Glance

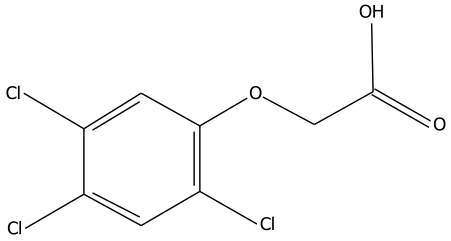


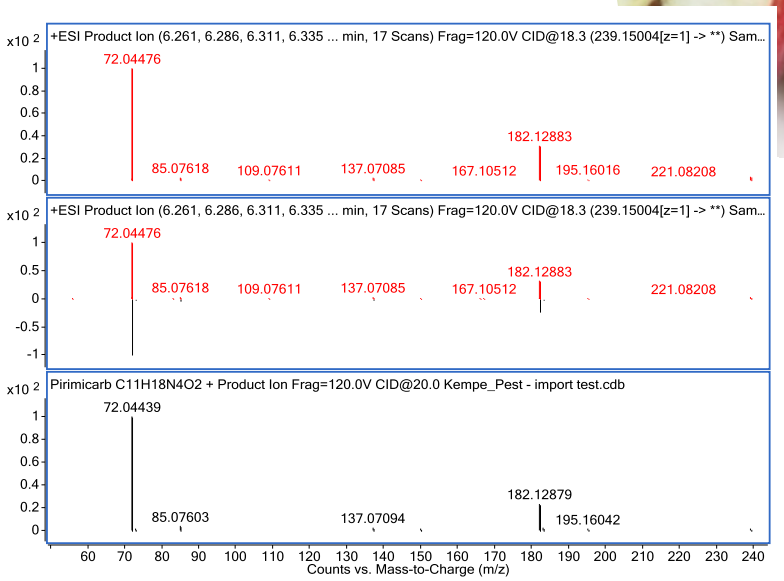
Pesticides screening and quantitation

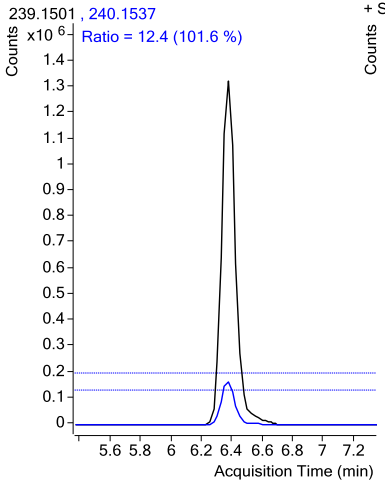
Apple sample from local market

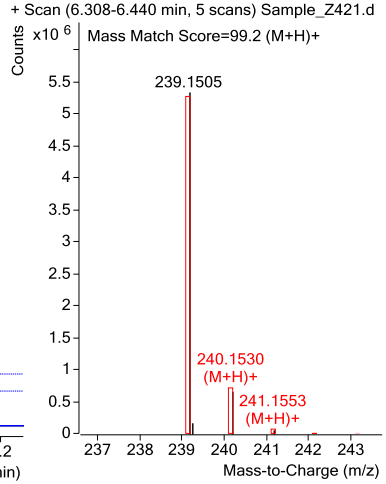
Pirimicarb

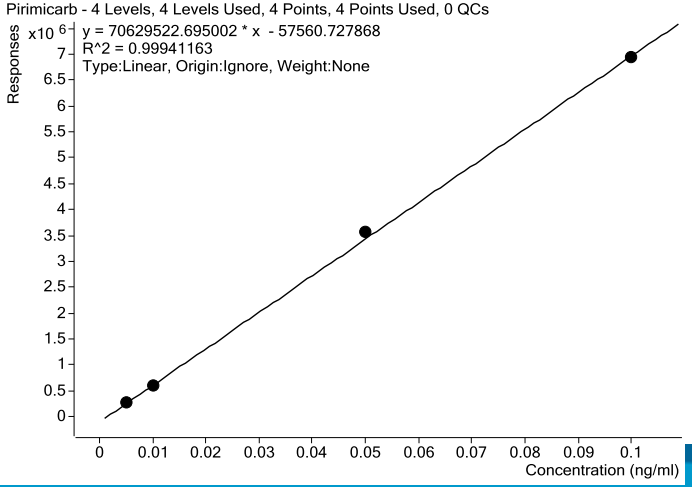
Conc.:	120 µg/kg
Mass error:	-0.54 ppm
TOF score:	99.2
Library score:	97.0









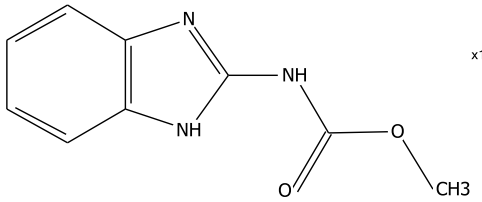


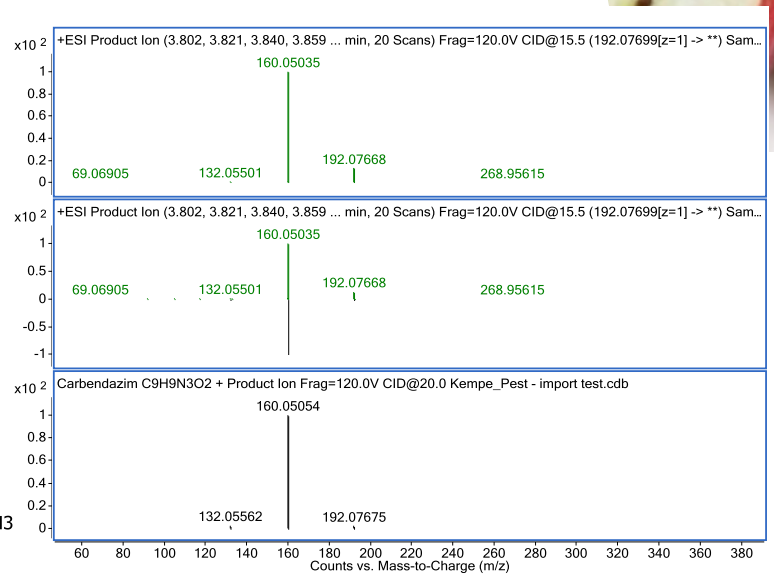
Pesticides screening and quantitation

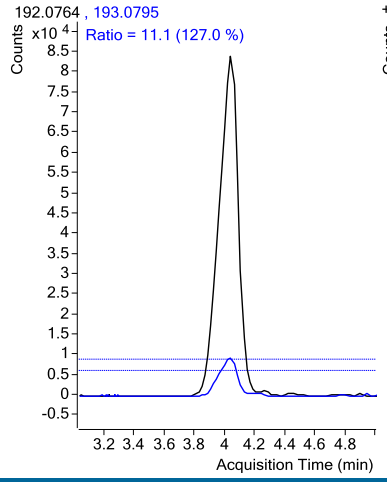
Apple sample from local market

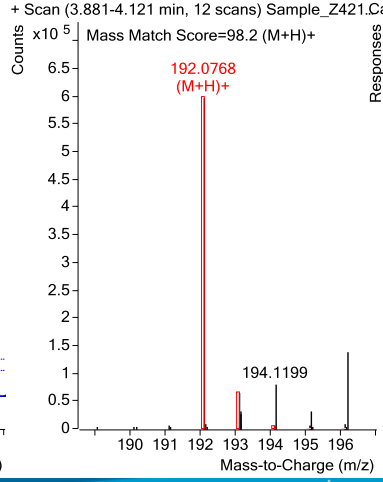
Carbendazim

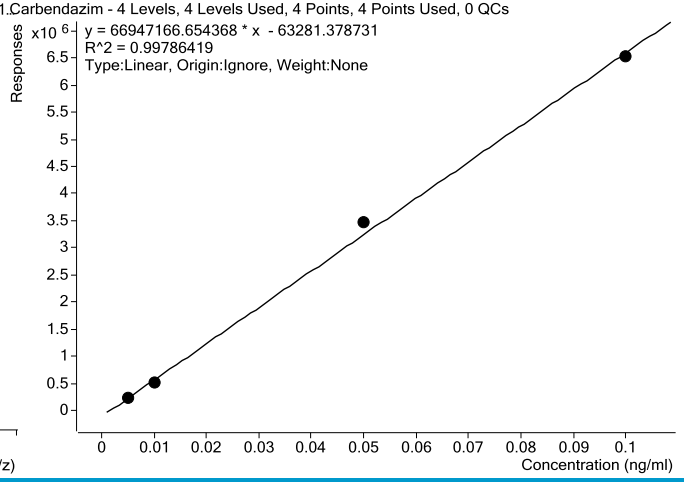
Conc.:	20 µg/kg
Mass error:	-1.57 ppm
TOF score:	98.2
Library score:	95.8









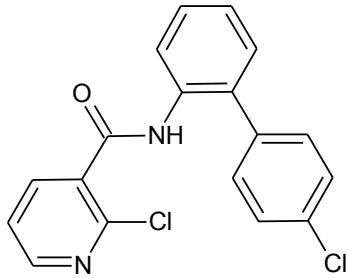


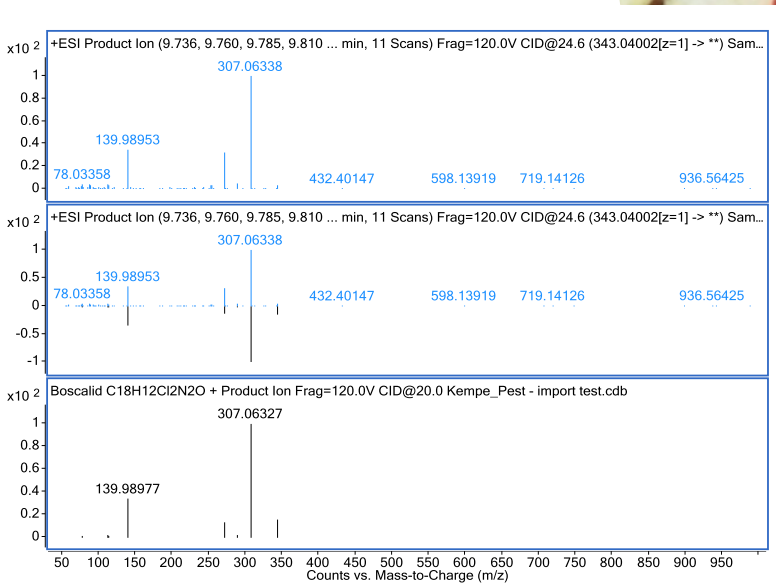
Pesticides screening and quantitation

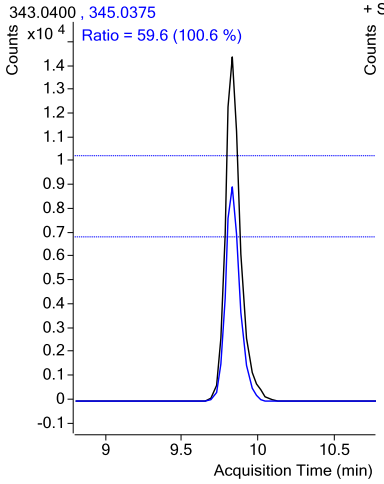
Apple sample from local market

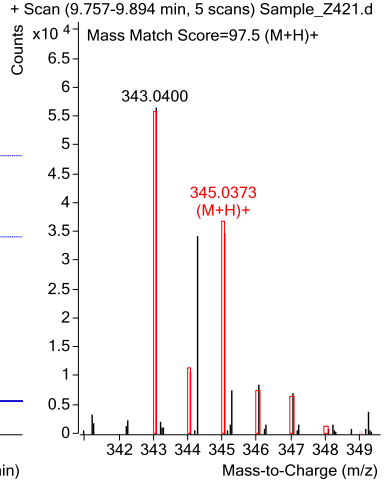
Boscalid

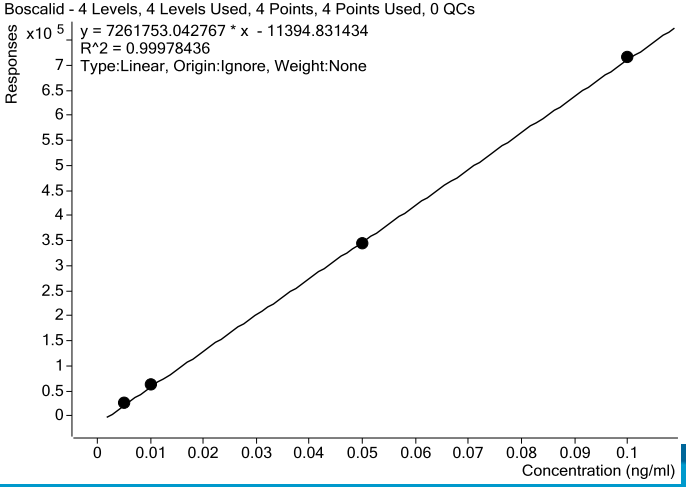
Conc.:	20 µg/kg
Mass error:	-0.15 ppm
TOF score:	97.5
Library score:	93.0









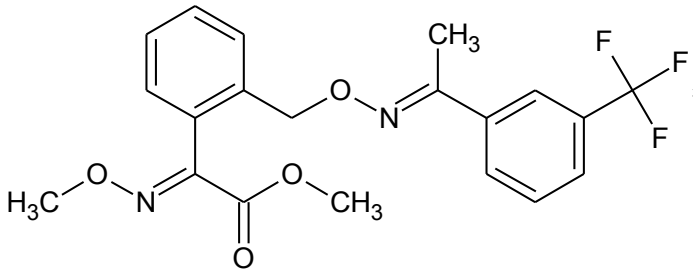


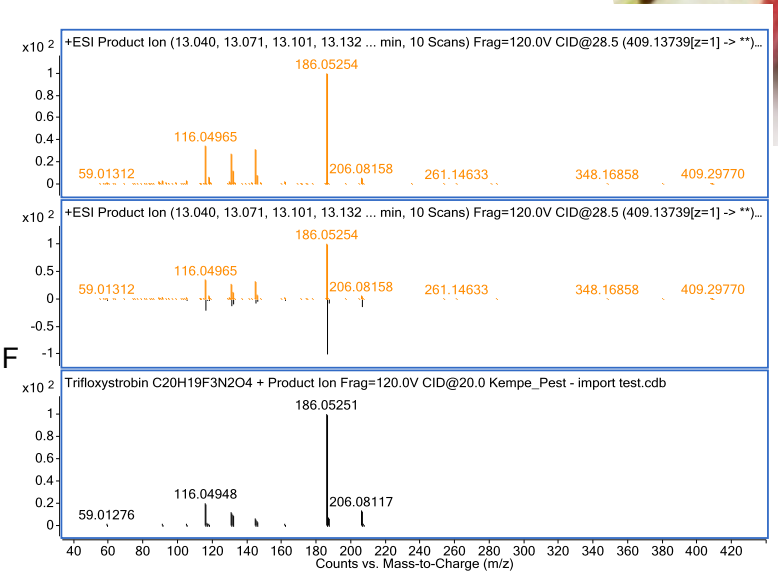
Pesticides screening and quantitation

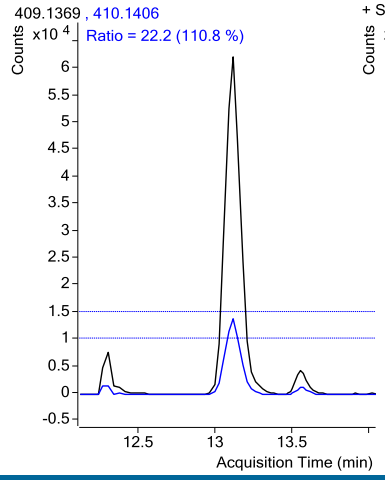
Apple sample from local market

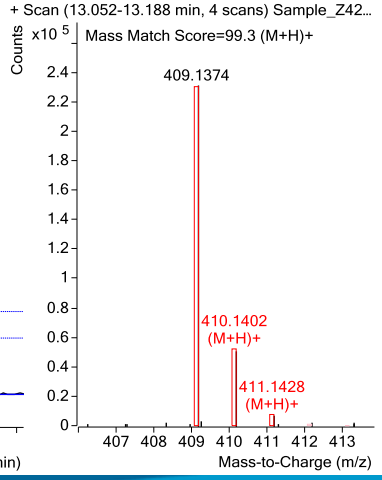
Trifloxystrobin

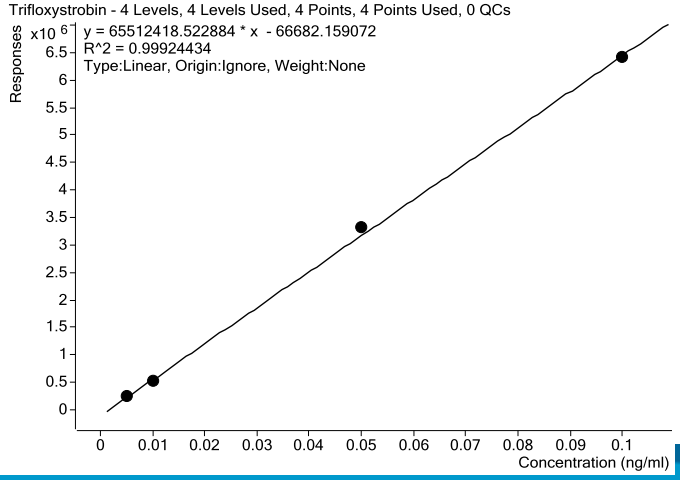
Conc.:	10 µg/kg
Mass error:	-1.00 ppm
TOF score:	99.3
Library score:	87.8











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!

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sachsen.de 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 ≥ 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