

A NEW SCIENTIFIC SOFTWARE APPROACH FOR THE ROUTINE ACCURATE MASS SCREENING

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Waters



How is Residue Screening Classified?

- Targeted screening
 - **Selective acquisition &/or processing modes**
 - Well defined target list of analytes

- Non-targeted screening
 - **Non selective data acquisition method.**
Acquired data can be used for
 - Checking for the presence of compounds from a (large) library
 - Finding compound not present in the library, maybe an unknown or new chemical structure. Structural elucidation required



Advantages of HR-MS screening?

- Over recent years use of high resolution mass spectrometry has gained in popularity as a screening tool in the food and environmental sector
 - ✓ **Ability to perform non-targeted analysis**
 - ✓ **Ability to perform historical (retrospective) data review**
 - ✓ **Ability to perform full spectral analysis**
 - ✓ **Ability to screen for larger number of compounds and adducts**
 - ✓ **Increased specificity in complex matrices**
 - ✓ **Elucidation of unknowns ?**

Current Guidelines for Pesticide Analysis (food safety)

- **EU SANCO/12495/2011** (*implemented Jan 2012*)
 - Guidance criteria for method validation and analytical quality control (AQC)
 - Primarily intended for official control of pesticide residues in food and feeds
 - **Covers screening methods**
 - Industry benchmark for pesticide residue analysis
 - *and in the lack of specific guidelines within other areas of residue analysis*



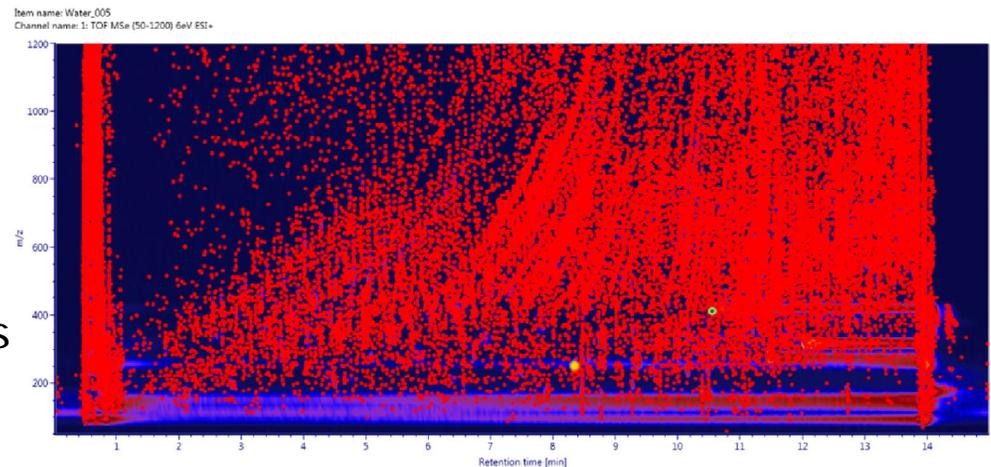
SANCO/12495/2011 – performance criteria -1

- Sensitivity in line with the relevant Regulatory limits
 - MRLs / MRPLs / RCs / ALs / ADIs etc...
- **Applicability of the screening method is defined by the false non-compliant (positive) and false compliant (negative) rates**
 - A low false negative rate is critical for screening assays to avoid missing MRL violations
 - **Tolerance \leq 5% false negative rate**
 - A low false positive rate is important for screening assays to reduce costly quant / confirmatory analysis
 - **Desirable \leq 5% false positive rate**
- Mass accuracy tolerance = \leq 5 ppm
- Mass resolution tolerance = \geq 20k (FWHM)
- Retention time mandatory for confirmation



Challenges:

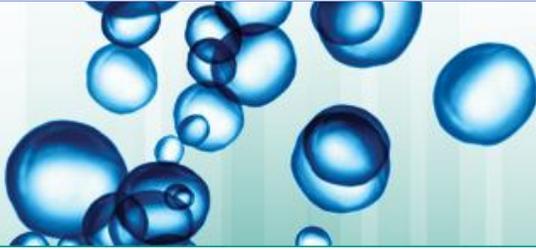
- One single chemical entity leads to multiple MS peaks, at the same retention time
 - The parent ion
 - Isotopes
 - Adducts
 - Fragments
- Multiple co-elutions in real samples
- Possible presence of isomers
- Extremely complex data sets!
- Using only the exact mass / RT to determine to presence of a contaminant may lead to too many false positives!
- Need to use isotopes, adducts, fragments... for compounds determination



Surface water MS data. 44829 peaks are detected

Introducing a new screening solution for targeted and non-targeted analysis using HR-MS





WATERS PESTICIDE SCREENING APPLICATION SOLUTION

A comprehensive solution for high-throughput, multi-residue pesticides screening.

**Pesticide-Based
Installation
Specifications**



**DisQuE™ Dispersive
Sample Preparation Kit**
Fast, simple
pesticide extractions

PREPARATION



**ACQUITY UPLC
I-Class System**
High resolution
separations of
trace analytes

SEPARATION



Xevo G2-S QTof
Accurate mass
measurements
for precursor
and product ions

DETECTION

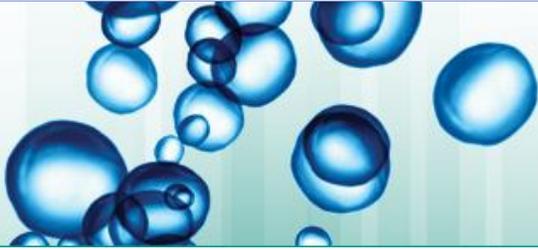


UNIFI™

UNIFI Scientific Library
The ultimate
reference resource

INTERPRETATION

The first comprehensive turnkey solution for routine screening



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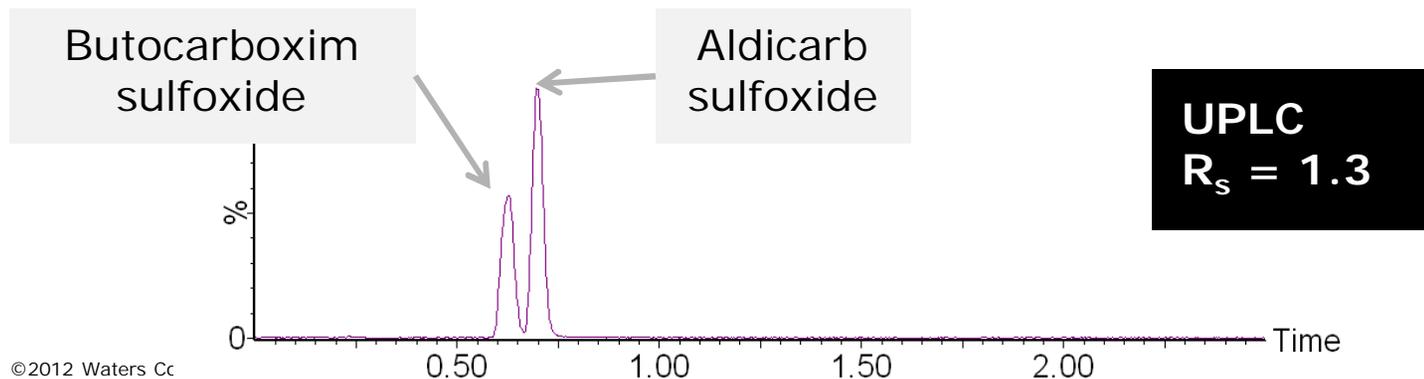
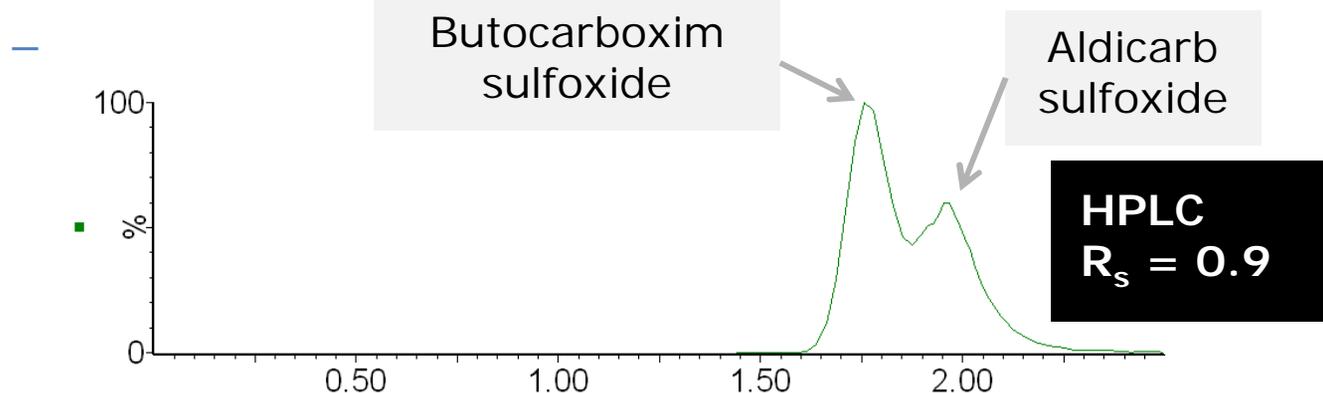
High Resolution Chromatographic Separations: ($P_w \sim 3-4$ s with 12 points)

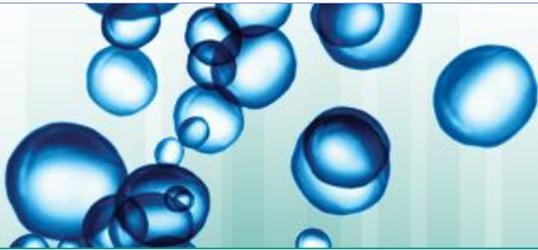
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■ Complex separation

- A successful screening starts with the chromatography

■ Minimizing dispersion to enhance chromatographic resolution and sensitivity





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Xevo G2-S QToF

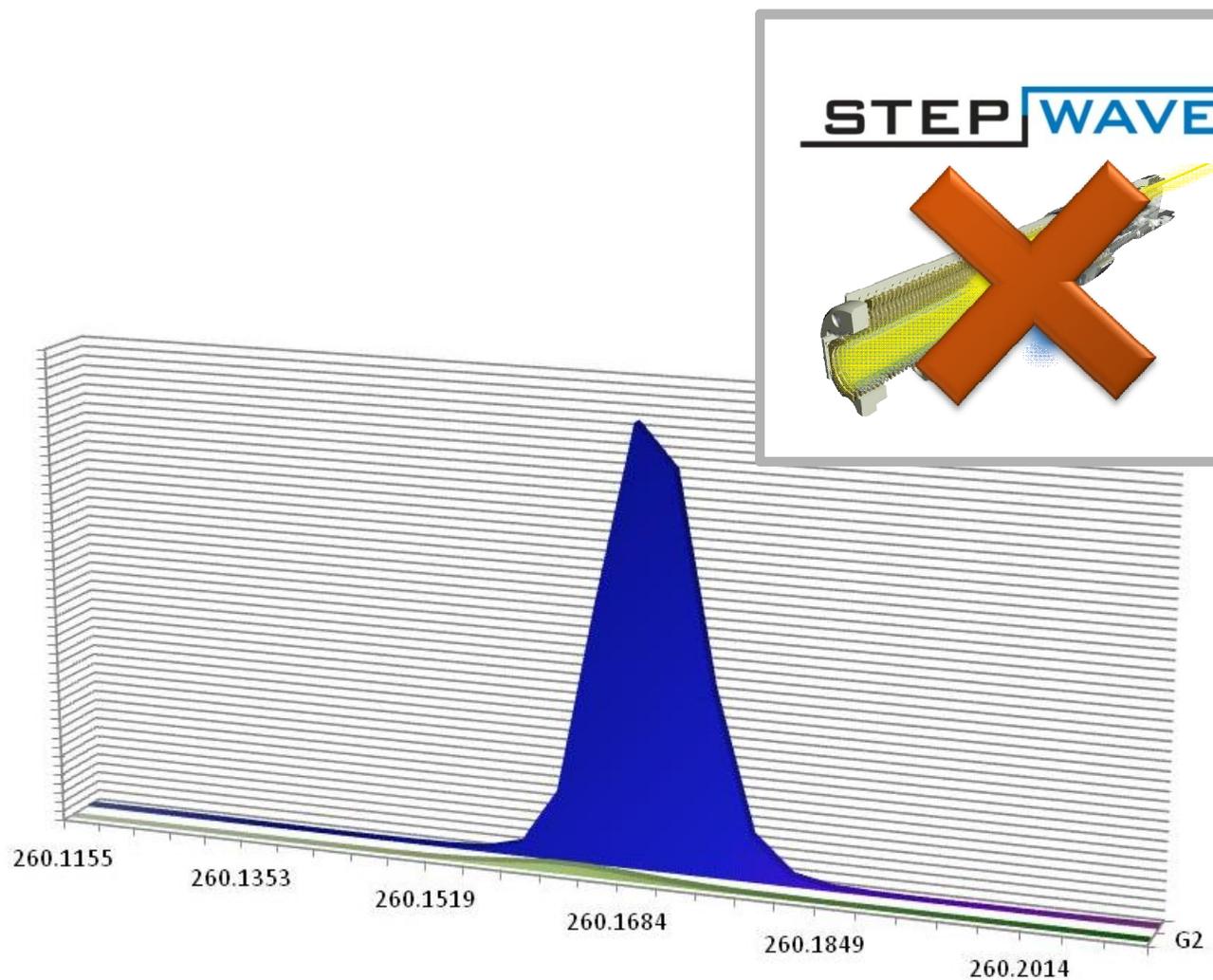
Inclusion of StepWave – For Ultimate Sensitivity

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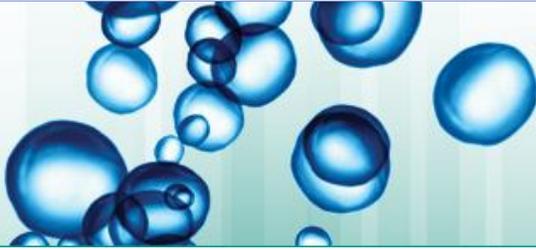


Xevo G2-S QToF
Accurate mass
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Sensitivity gain with stepwave: ~ 10 x



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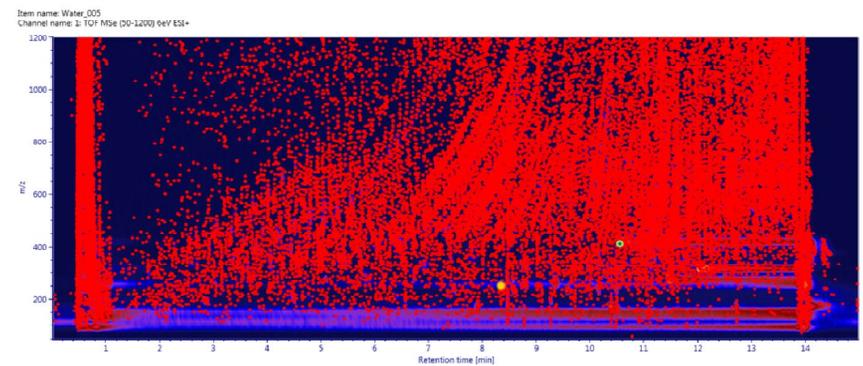
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INTERPRETATION

Data Treatment

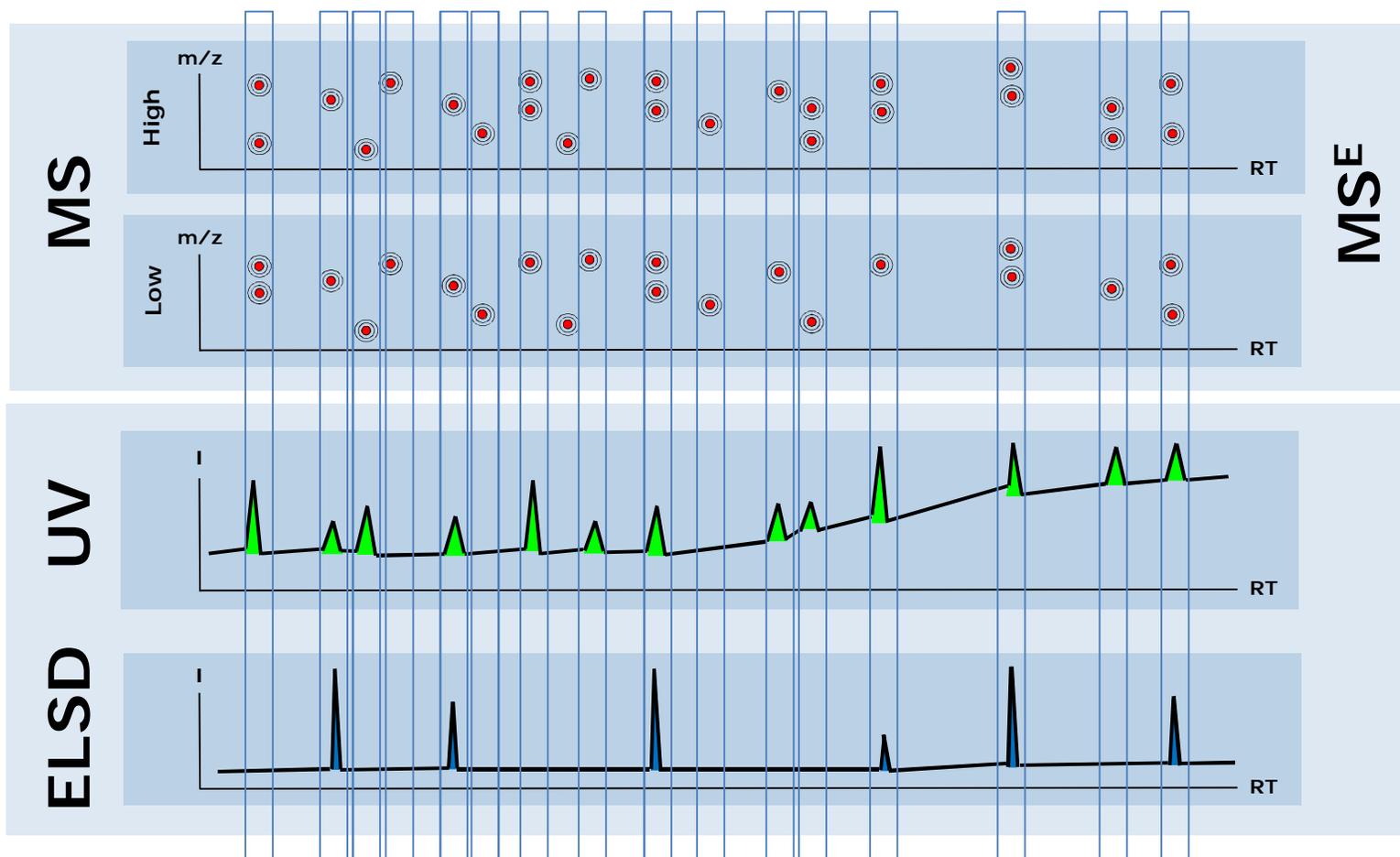
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Overview



Surface water MS data. 44829 peaks are detected

The Component Approach



The software organises the data across all channels
into components

The Component Approach

ID	Mass	RT	Area	Isotopes	Fragments	Adducts
1	206.1242	1.53	1220	2	4	H ⁺
2	545.0218	1.89	3029	3	8	H ⁺ , Na ⁺
3	376.9867	2.13	2363	3	7	H ⁺ , Na ⁺
4	252.1921	2.62	1873	2	9	H ⁺
5	259.1102	3.20	3294	3	3	H ⁺
6	462.0824	3.65	1491	2	5	H ⁺
7	328.0492	4.11	3842	3	4	Na ⁺
...

**The software organises the data across all channels
into components**

Data Processing Workflow

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Raw Data

Simplify

Peak Detection

Peak List

Organise

Isotopes
Adducts
Fragments (MS^E)
Charge states
Multimers

Components

Analyse

Application Software

Scientific Library

- Screening experiments are dependent on the **quality** of the libraries
- Libraries already available:
 - Over 2000 entries of which around 500 compounds contain method related information (RT, m/z for precursor and fragment ions)

- Easy to input your data
 - UNIFI can use Excel spreadsheets
- Critical information that is used for ID process

- ❖ **Name** (*chemical, common, marker residue definition*)
- ❖ **Chemical formula**
- ❖ **Structure**
- ❖ **Retention time**
- ❖ **Accurate mass** (*precursor and product ions*)
- ❖ **Fragment ion(s)**
- ❖ **Isotopic patterns**
- ❖ **Isotope intensity**
- ❖ **Expected ion ratios**
- ❖ **Theoretical spectra**

Component	Formula	Structure	RT	Frag 1	Frag 2	Frag 3	Classification
1	C ₇ H ₅ N ₃ O ₆	2,3,5-Trinitroanisole mol	7.78	127.0916			Insecticides
2	C ₇ H ₅ N ₃ O ₆	2,6-Dichloroanisole mol	3.37	172.0511			Fertilizers product
3	C ₇ H ₅ N ₃ O ₆	3,4,5-Trinitroanisole mol	7.8	127.0916			Metabolites
4	C ₇ H ₅ N ₃ O ₆	Hydroxycarboluran mol	4.72	134.0378			Insecticide
5	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	4.69	120.0081	107.0447		Fungicide
6	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	8.10	139.0045			Insecticides
7	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	10.87	118.9588			Insecticides
8	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	9.26	162.1332	147.0580	236.0840	Insecticides
9	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	9.67	236.0852	91.0498		Insecticides
10	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	9.67	116.0494	89.0375	69.0528	Insecticides
11	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	5.6	98.0259	148.0382		Insecticides
12	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	2.84	98.0259	136.0378		Insecticides
13	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	2.84	121.0367			Insecticides
14	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	10.79	98.0262	98.0512	68.0199	Insecticides
15	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	5.76	189.0763	152.1020	122.0596	plant growth regulators
16	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	8.39	184.0764			Fungicide
17	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	4.89	137.0791			Insecticides
18	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	6.25	152.0783			Insecticides
19	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	11.42	236.1134			Insecticides
20	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	8.76	136.0512	120.0613	124.0732	Insecticides
21	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	8.72				Insecticides
22	C ₇ H ₅ N ₃ O ₆	Acetophenone mol	6.25				Insecticides



Data Review

Data Review

Two Key Workflows

■ Non-targeted workflow

- ✓ Obtain a summary of the identified compounds that are present (and absent) and determine concentration
- ✓ Provide a list of all compounds that meet user criteria (retention time, accurate mass measurement of precursor and fragments, adducts found, isotope ratios, user-defined limits)
- ✓ Provide a list of spurious results (e.g. RT & accurate mass measurement shifted, isotope ratios questionable..)

Summary / Overview of the Results

Present & Absent.
Quantity.

Compounds that need
reviewing

Review: Summary Page

Waters UNIFI - ASMS Pest QuanQual: Analysis Center

My Work | Welcome to UNIFI | ASMS Pest QuanQual: An... | Search folders...

Review | Investigate | Report

Result Summary

Workflow

Summary

- Batch Overview
- Result Summary

Review

- IDs with no flags - summary
- IDs with no flags - details
- IDs with no flags - quan
- IDs with flags - summary
- IDs with flags - details
- Excluded targets

Result Summary

Injection

Unknown in Red Pepper 2

Components

- 15184 Components**
Review all components in this injection.
- 33 Identified Components**
Review the identified components in this injection.
Review...
- 446 Missing Components**
Investigate the data for this injection.
Investigate...

No data to display

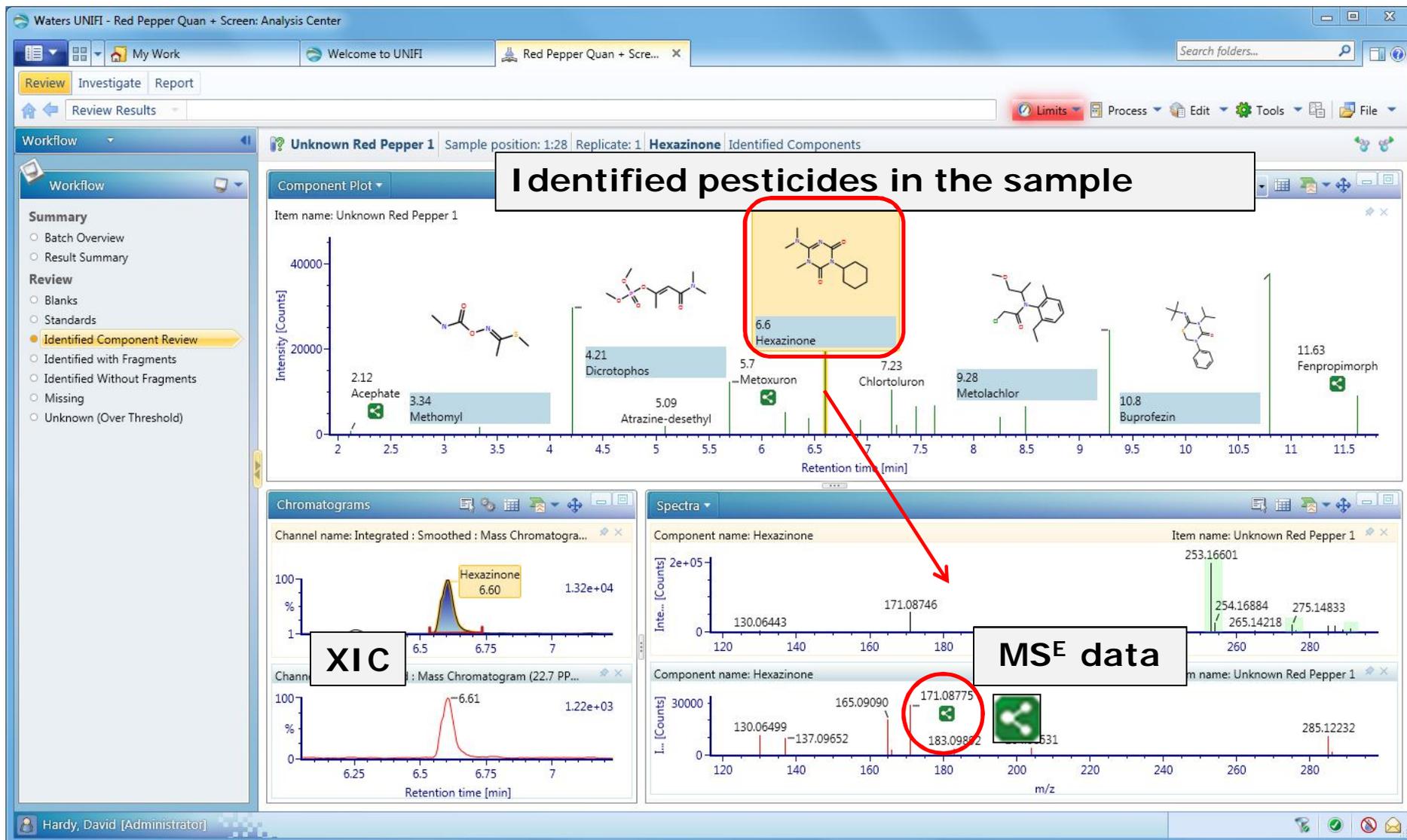
15184 = Total number of components found in sample

Administrator, UNIFI [Administrator]

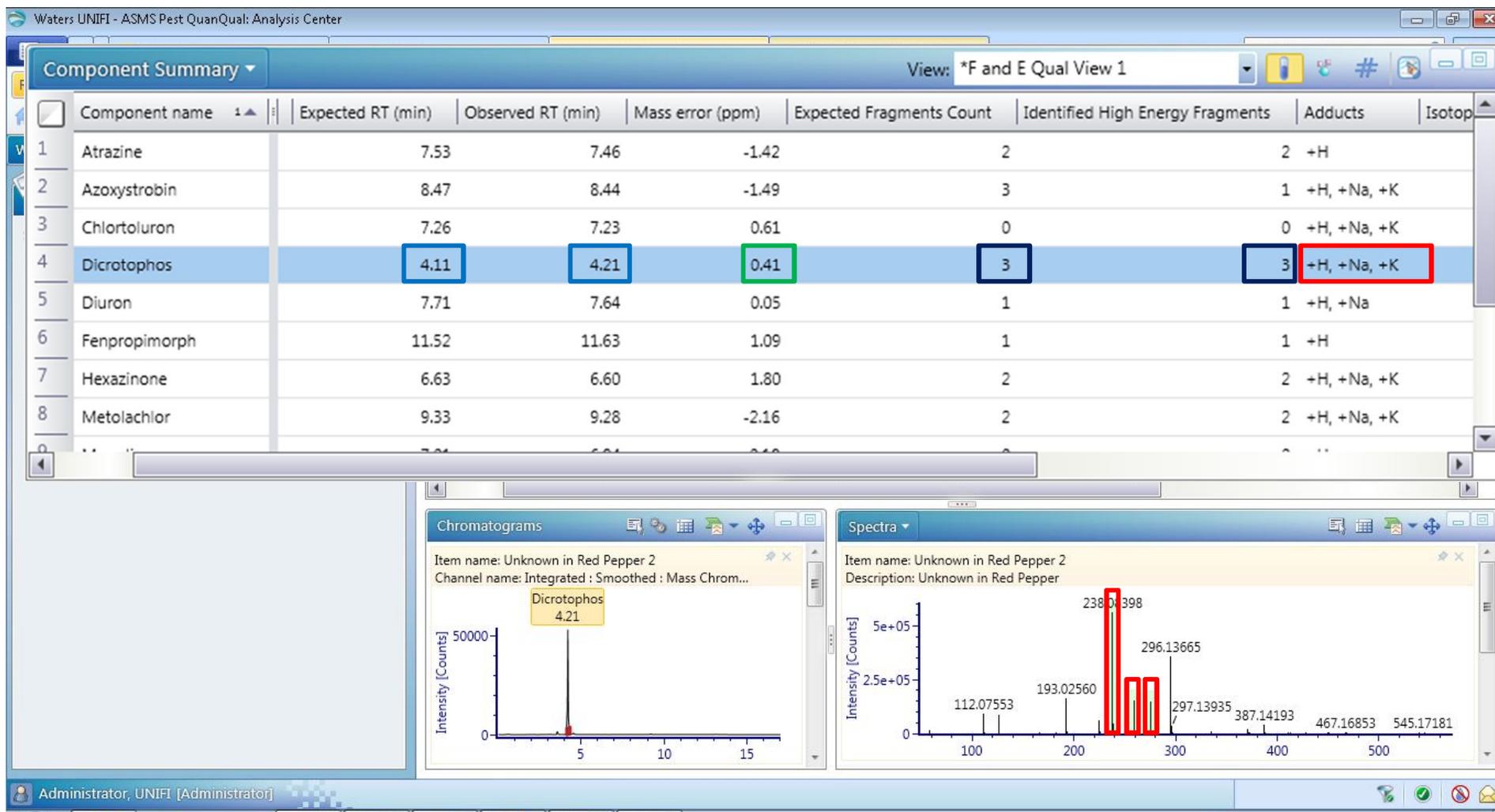
Data review for non-targeted analysis

Fragment ion identification

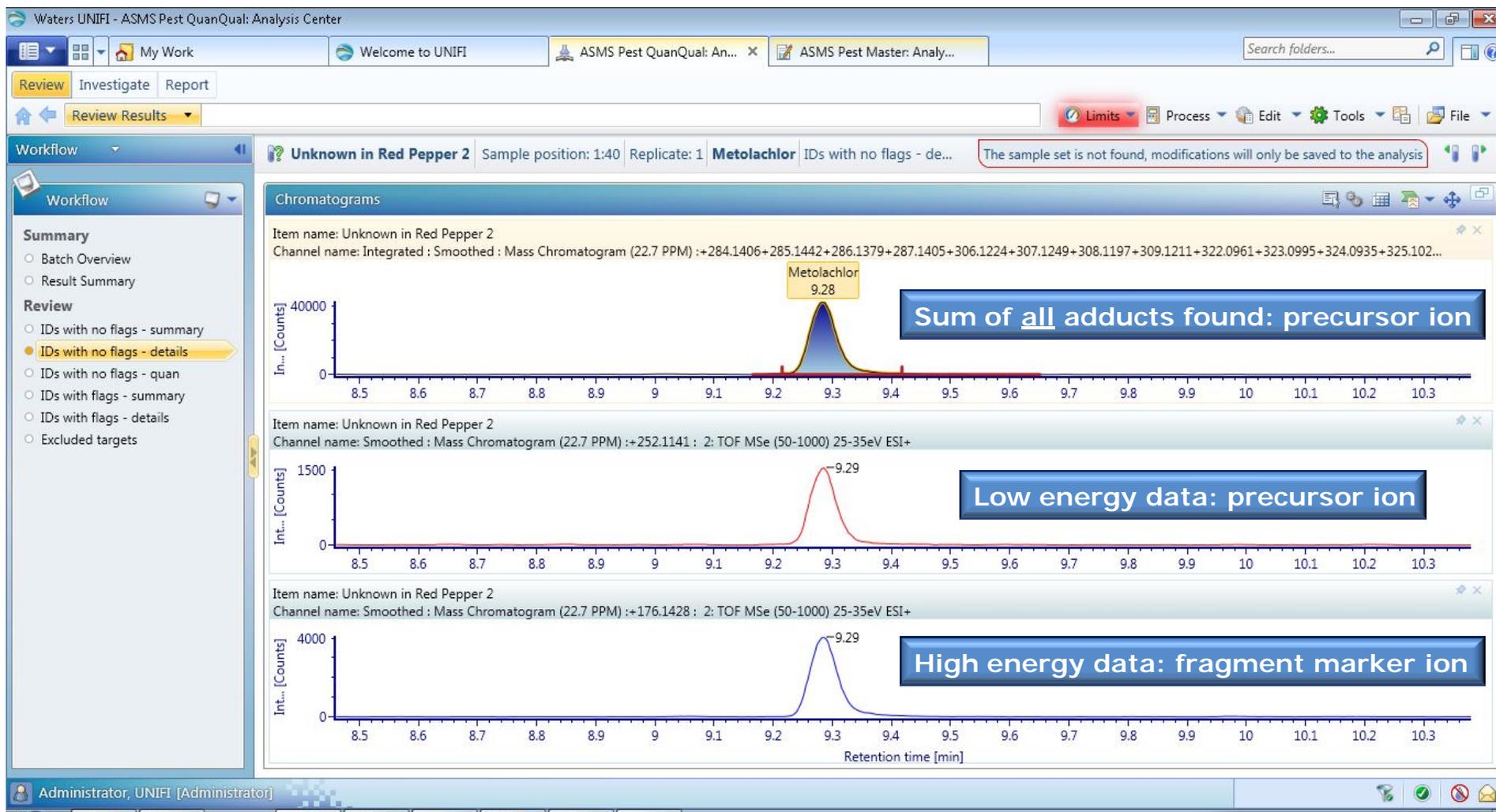
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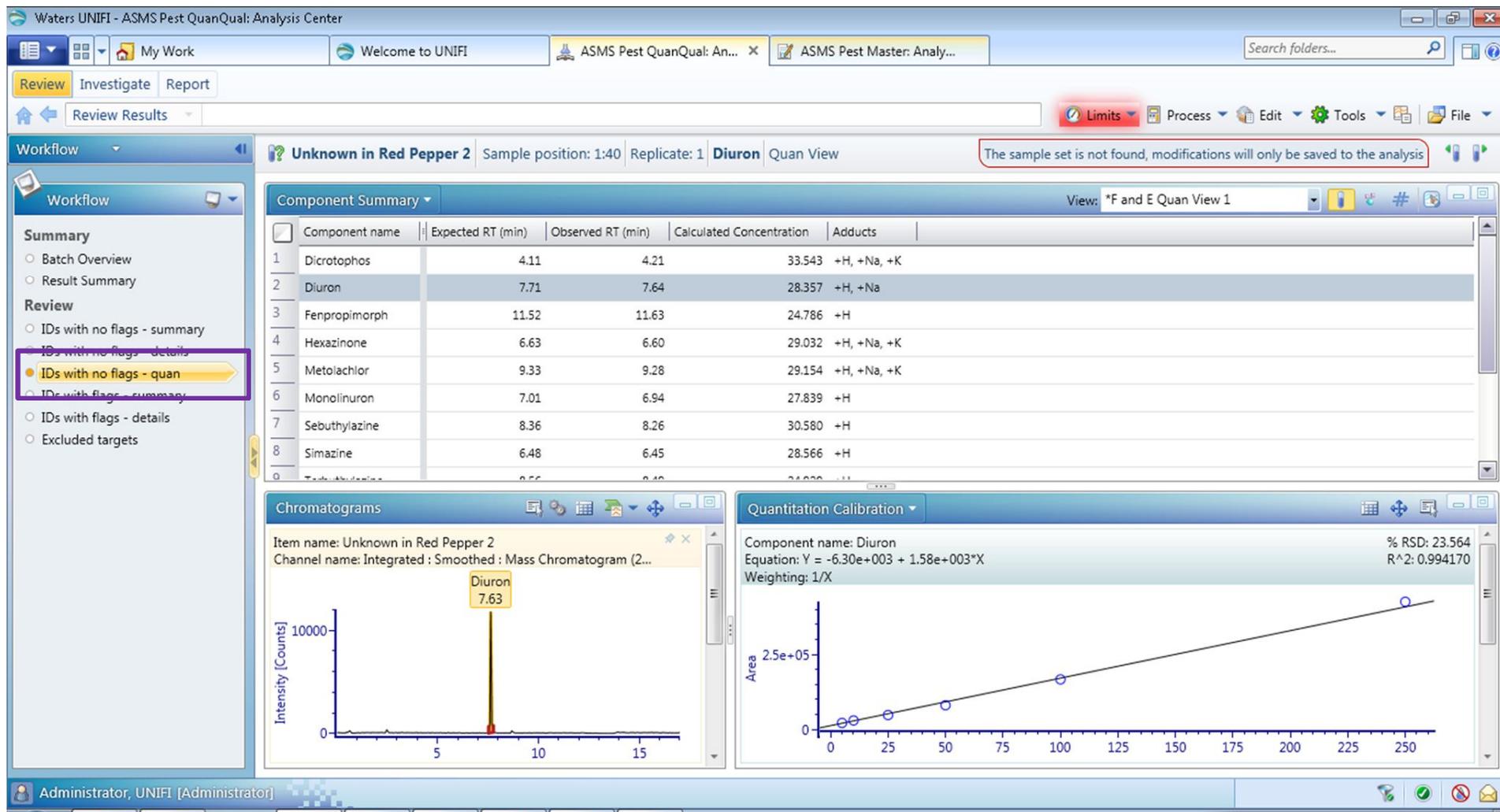
Review: Positively Identified: Data Confirmation



Review: Positively Identified: Low & high energy data for all adducts



Review: Positively Identified: Quantification Results



Review: Components with Flagged Values

Waters UNIFI - ASMS Pest QuanQual: Analysis Center

My Work Welcome to UNIFI ASMS Pest QuanQual: An... ASMS Pest Master: Analy...

Review Investigate Report

Review Results

Workflow

Unknown in Red Pepper 2 Sample position: 1:40 Replicate: 1 Cyanazine Limits view

The sample set is not found, modifications will only be saved to the analysis

Component Summary View: *F and E Qual View 1

Component name	Mass error (ppm)	Expected Fragments Count	Identified High Energy Fragments	Adducts	Isotope Match MzRMSPPM	Isotope Match IntensityRMSPercent	Detector counts
8 Sebuthylazine	1.73	0	0	+H	1.47	0.46	517
9 Atrazine-desethyl	-0.10	2	1	+H	1.38	20.04	119
10 Buprofezin	0.55	1	0	+H, +Na, +K	0.67	1.91	4578
11 Chlortoluron	0.61	0	0	+H, +Na, +K	5.15	5.78	893
12 Cyanazine	0.61	1	1	+H, +Na	0.78	10.70	351

Summary

- Batch Overview
- Result Summary

Review

- IDs with no flags - summary
- IDs with no flags - details
- IDs with no flags - quan
- IDs with flags - summary
- IDs with flags - details
- Excluded targets

Chromatograms

Item name: Unknown in Red Pepper 2
Channel name: Integrated : Smoothed : Mass Chromatogram (22.7...)

Spectra

Item name: Unknown in Red Pepper 2
Description: Unknown in Red Pepper

Item name: Unknown in Red Pepper 2
Description: Unknown in Red Pepper

Warning that a measurement is out of tolerance and allows the analyst to review the data manually

Administrator, UNIFI [Administrator]

Looking for Unknown Compounds

-

Supporting Software Tools



Managing the Unidentified Candidate List

The screenshot displays the Waters UNIFI software interface. The main window shows a 'Component Plot' for 'Unknown Red Pepper 1' with a retention time of 4.76 minutes for Acetamidrid. A blue callout box points to the 'Components (Unknown Red Pepper 1)' list, which has a count of 15,657. The 'Components' list includes various chemical compounds with their respective formulas and status indicators. The 'Chromatograms' panel shows a mass chromatogram for Acetamidrid at 4.76 minutes. The 'Spectra' panel displays two mass spectra for the sample, showing intensity versus m/z.

Unidentified candidates: 15,657

Status	Name	Formula
✗	2,6-Dichlorbenzamid	C7H5Cl2NO
✗	3,4,5-Trimethacarb	C11H15NO2
✗	3-Hydroxycarbofuran	C12H15NO4
✓	Acephate	C4H10NO3PS
✓	Acephate	C4H10NO3PS
✓	Acetamidrid	C10H11ClN4
✗	Acibenzolar-S-methyl	C8H6N2OS2
✗	Akton	C12H14Cl3O3PS
✗	Alachlor	C14H20ClNO2
✗	Alanycarb	C17H25N3O4S2
✗	Aldicarb	C7H14N2O2S
✗	Aldicarb sulfone	C7H14N2O4S
✗	Aldicarb sulfoxide	C7H14N2O3S
✗	Allethrin	C19H26O3

Data Review Two Key Workflows

Unknown Components

Filter Data:

Halogen searching, Neutral loss, mass defect filter...

Binary Compare:

Sample A versus Sample B

Statistical Review

Elucidate Structure

Add to Scientific Library

■ 15,500 Unknowns!!

- ✓ Reduce the unknown component list - use of filters (halogen, neutral loss, mass defect filters...), sample comparison, statistical analysis
- ✓ Elucidate structure seamlessly – Full Toolset integrated into UNIFI
- ✓ Potential to automatically add IDed unknown to the Scientific Library

Looking for Unknown Components...?

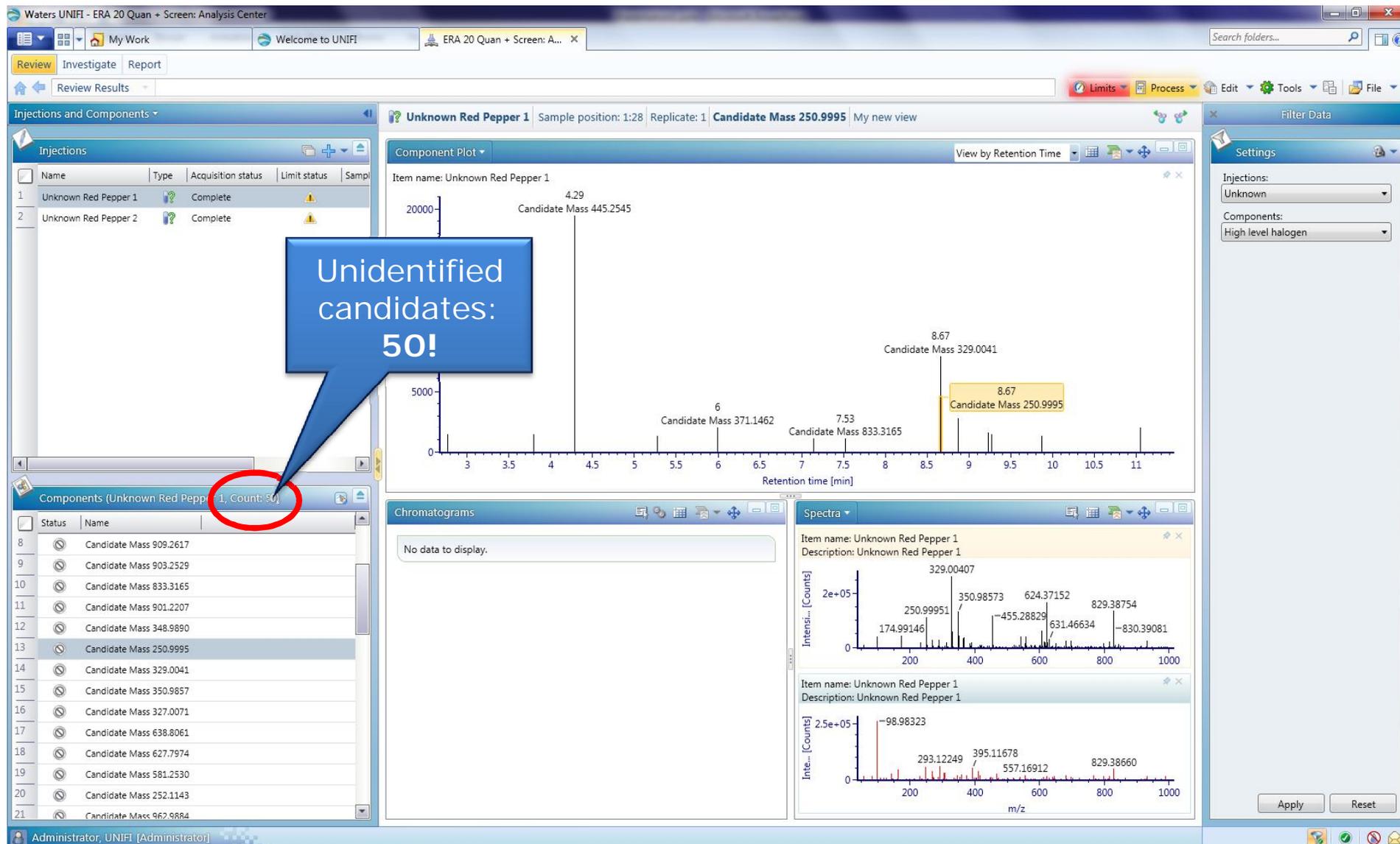
-

...Use a Filter Approach



Candidate list has been reduced from 15,000 to 50 by using halogen filtering

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Searching external libraries in the elucidation Toolset

Online search MassBank

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The screenshot displays the Waters Elucidation Toolset interface. On the left, a 'Workflow' sidebar shows 'Elucidation Toolset' selected. The main area contains two mass spectra plots for 'Screening_070' (Nordgraben). The top plot is 'Low energy' (Time 3.5952 +/- 0.01...) with a base peak at m/z 232.10835. The bottom plot is 'High energy' (Time 3.5952 +/- 0.0...) with a base peak at m/z 232.10839. To the right, the 'ChemSpider Search' window is open, showing search parameters: Mass selected, m/z 232.10835, Tolerance 10 mDa. Below it, the 'Select Libraries' dialog is open, showing a list of libraries with 'MassBank' checked.

Workflow

- Summary
 - Batch Overview
- Review
 - Identified - Summary compare
 - Identified - Details
 - Identified - Across All Samples
 - Quantification
 - Unknown Components Plot
 - Elucidation Toolset**

Spectra

Item name: Screening_070 Channel name: Low energy : Time 3.5952 +/- 0.01...
Description: Nordgraben

Intensity [Counts] vs Observed mass [m/z]

m/z	Intensity [Counts]
204.11286	~1.5e6
214.08986	~2.5e6
232.10835	1.07e7
254.09019	~3.5e6
300.20133	~2.5e6
345.15130	~2.5e6
398.21372	~1.5e6
553.24596	~1.5e6

Item name: Screening_070 Channel name: High energy : Time 3.5952 +/- 0.0...
Description: Nordgraben

Intensity [Counts] vs Observed mass [m/z]

m/z	Intensity [Counts]
104.04946	~4e5
159.09041	~2e5
204.11303	~4e5
214.09755	~6e5
232.10839	8.96e5
254.09040	~4e5
305.15597	~3e5
345.15203	~3e5
398.21329	~2e5
553.24517	~2e5

ChemSpider Search

Parameters

Select libraries...

Mass Formula

Formula:

m/z:

Tolerance: mDa

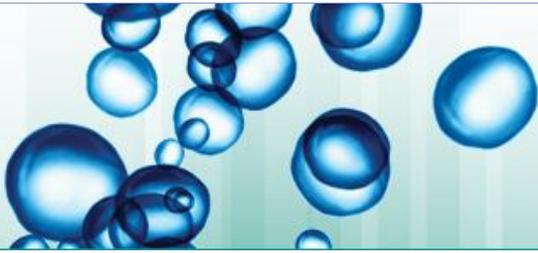
Selected adducts:

Select Libraries

Select libraries to search:

Select All Deselect All

Library	Enabled
297 LipidMAPS	<input type="checkbox"/>
298 Livchem	<input type="checkbox"/>
299 Manchester Organics	<input type="checkbox"/>
300 Marinlit	<input type="checkbox"/>
301 Martin Walker	<input type="checkbox"/>
302 MassBank	<input checked="" type="checkbox"/>
303 Matrix Scientific	<input type="checkbox"/>



WATERS PESTICIDE SCREENING APPLICATION SOLUTION

Summary

- Waters has introduced the new Pesticide Application Screening Solution
 - **Chemistries:** pesticide-specific installation specs, sample prep, columns
 - **Hardware:** ACQUITY UPLC I-Class, Xevo G2-S Qtof MS
 - **Software:** UNIFI – IntelliStart, experimental LC and MS methods , scientific library, customised screening reports
- Support routine analysis of food safety and environmental screening: pesticides,
- The pesticide solution is flexible: can be modified & adapted
- Tools to help you meet the regulatory requirements for routine accurate mass screening
 - ✓ **The most comprehensive routine application solution for food and environmental screening**