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A NEW SCIENTIFIC SOFTWARE APPROACH FOR THE ROUTINE ACCURATE MASS SCREENING

G. Bondoux , J.M. Joumier, A. Gledhill

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How is Residue Screening Classified?

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- 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?

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- 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)

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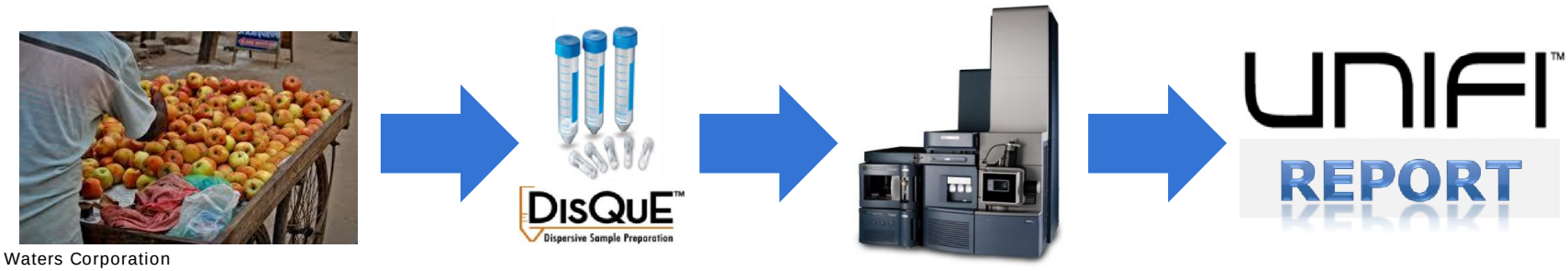
- **EU SANCO/12495/2011** *(implemented Jan 2012)*
  - Guidance criteria for method validation and analytical quality control (AQC)
  - Primarily intended for official control of pesticide residues in food and feeds
  - **Covers screening methods**
  - Industry benchmark for pesticide residue analysis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

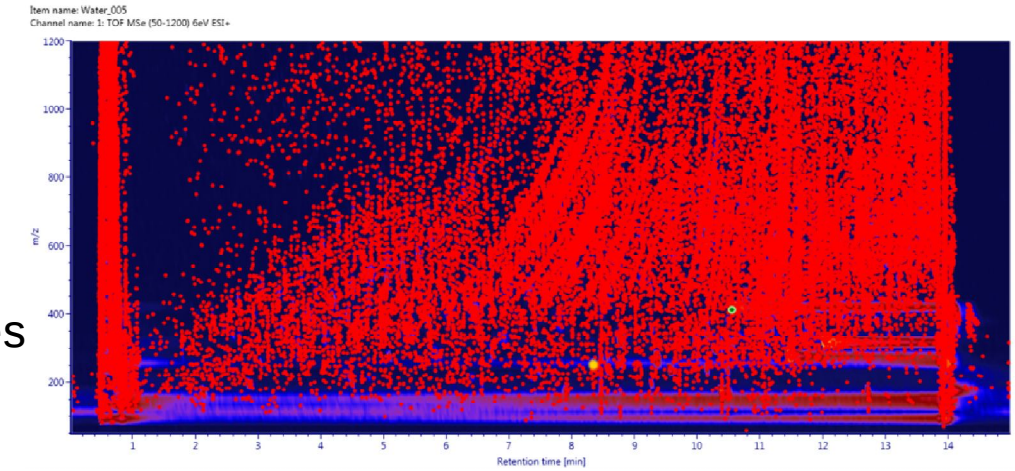
- **Applicability of the screening method is defined by the false non-compliant (positive) and false compliant (negative) rates**
  - A low false negative rate is critical for screening assays to avoid missing MRL violations
  - **Tolerance ≤ 5% false negative rate**
  - A low false positive rate is important for screening assays to reduce costly quant / confirmatory analysis
  - **Desirable ≤ 5% false positive rate**

- Mass accuracy tolerance = ≤5 ppm
- Mass resolution tolerance = ≥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!



Surface water MS data. 44829 peaks are detected

- 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

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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 Scientific Library

The ultimate reference resource

INTERPRETATION

The first comprehensive turnkey solution for routine screening

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High Resolution Chromatographic Separations: *(Pw ~3-4 s with 12 points)*

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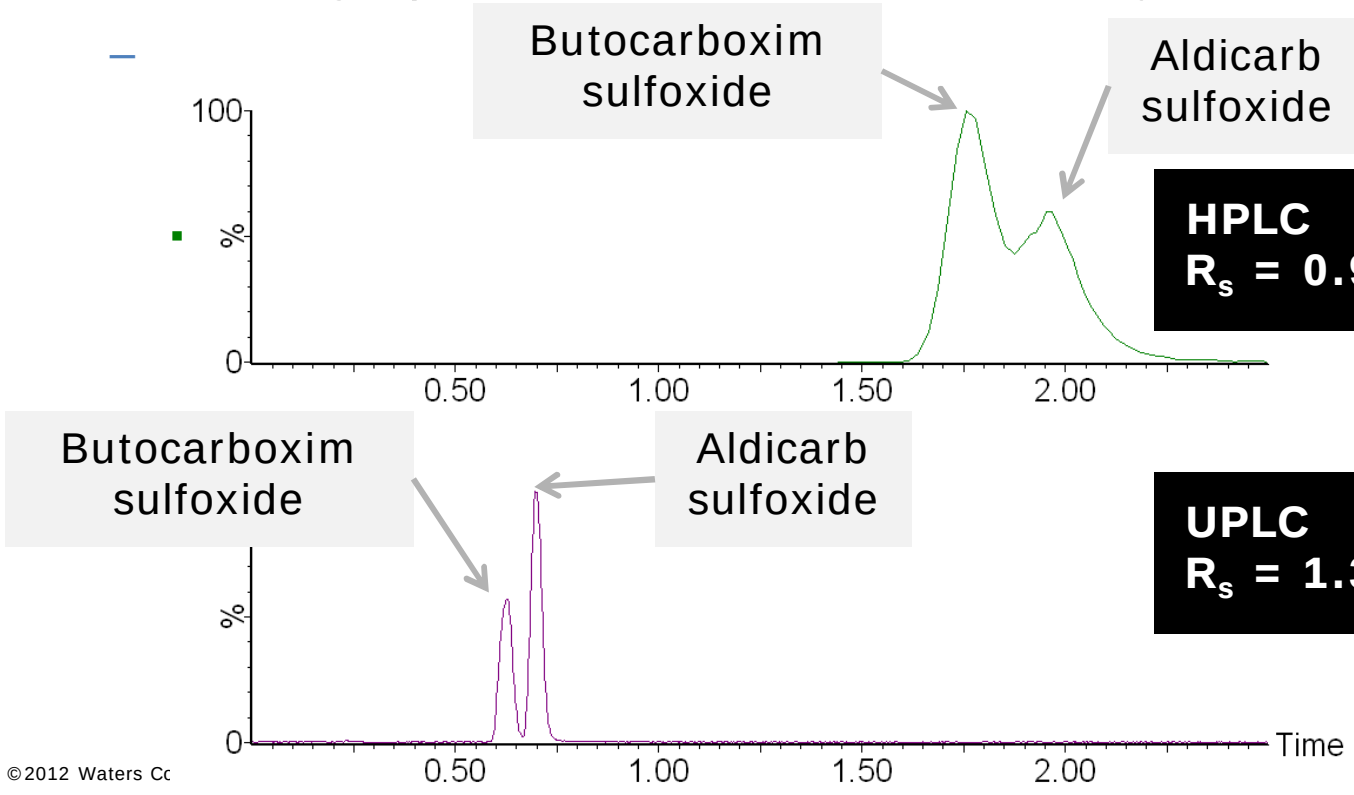
- **Complex separation**
  - **A successful screening starts with the chromatography**
- **Minimizing dispersion** to enhance chromatographic resolution and sensitivity
  -



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INTERPRETATION

Xevo G2-S QTof
Inclusion of StepWave – For Ultimate Sensitivity

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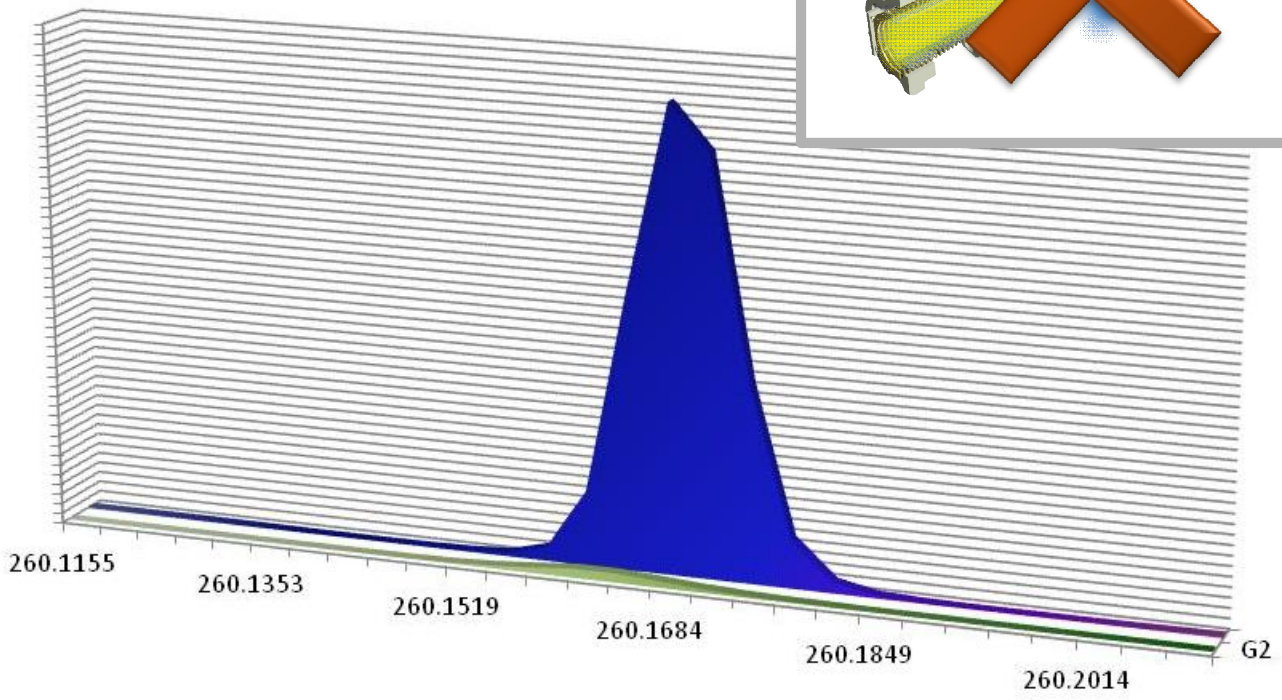
Xevo G2-S QTof

Accurate mass measurements for precursor and product ions

DETECTION



Sensitivity gain with stepwave: ~ 10 x

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INTERPRETATION

Data Treatment

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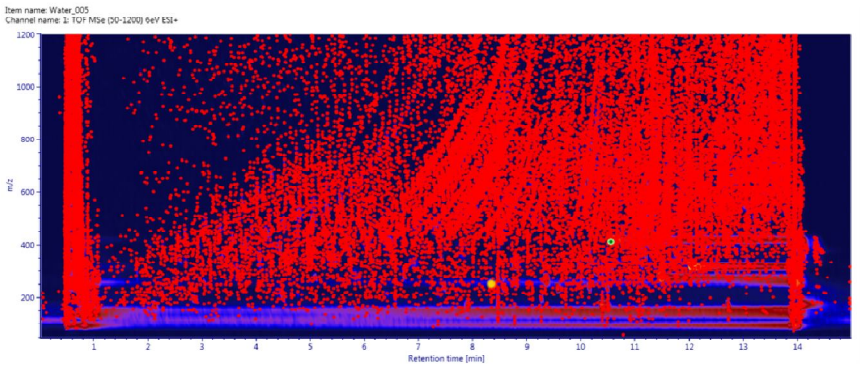
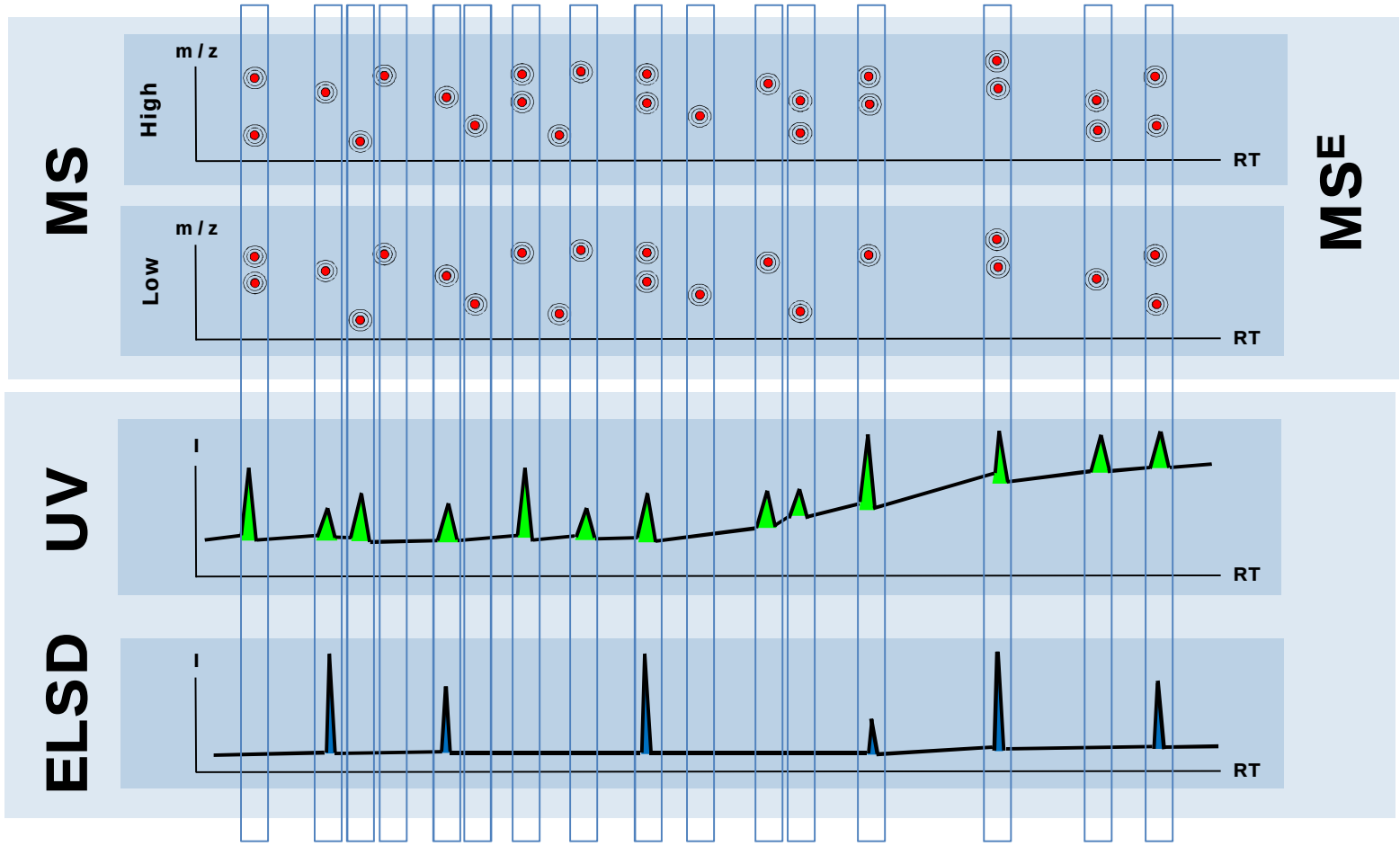
Overview



Surface water MS data. 44829 peaks are detected

The Component Approach



The software organises the data across all channels into components

The Component Approach

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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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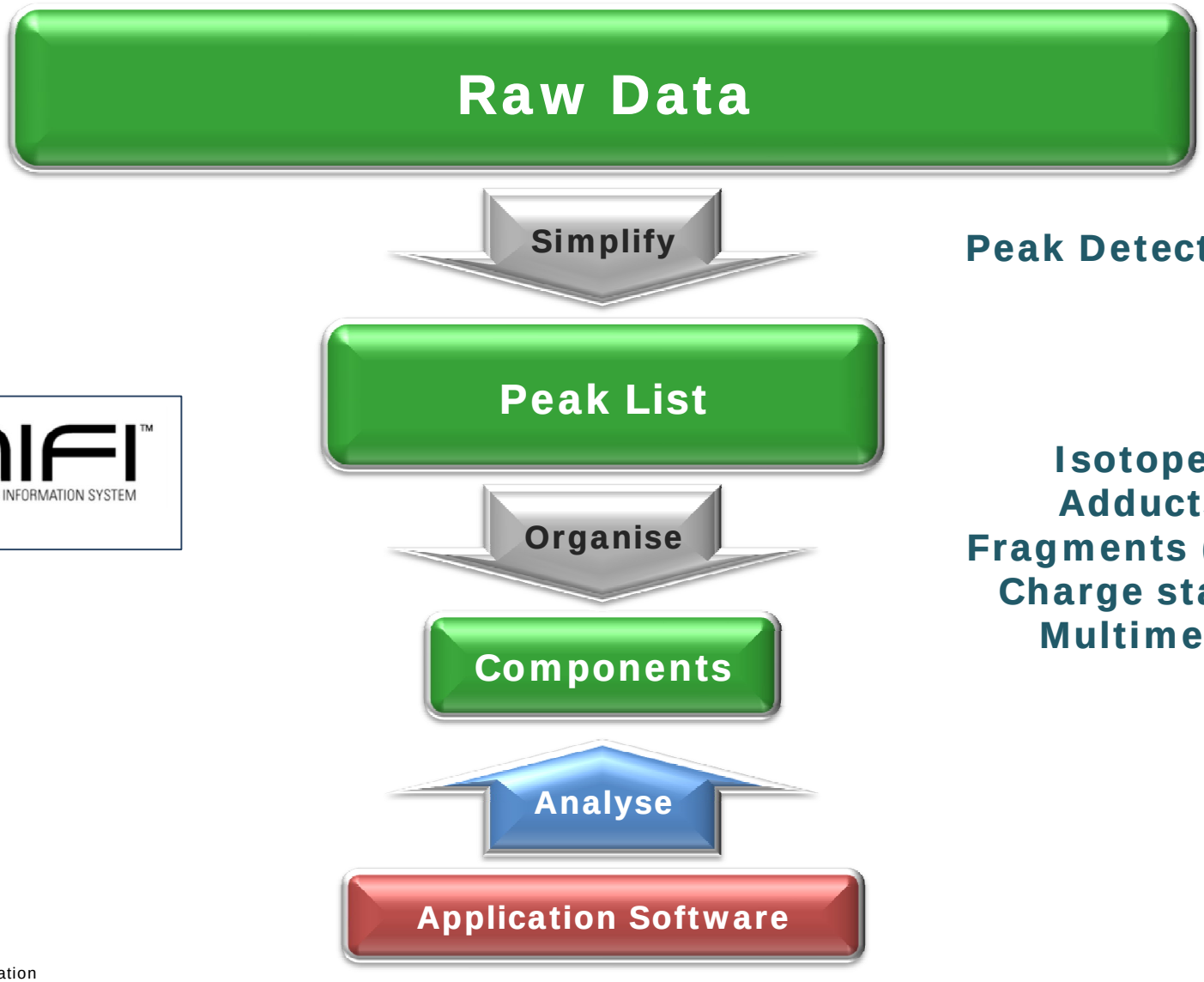

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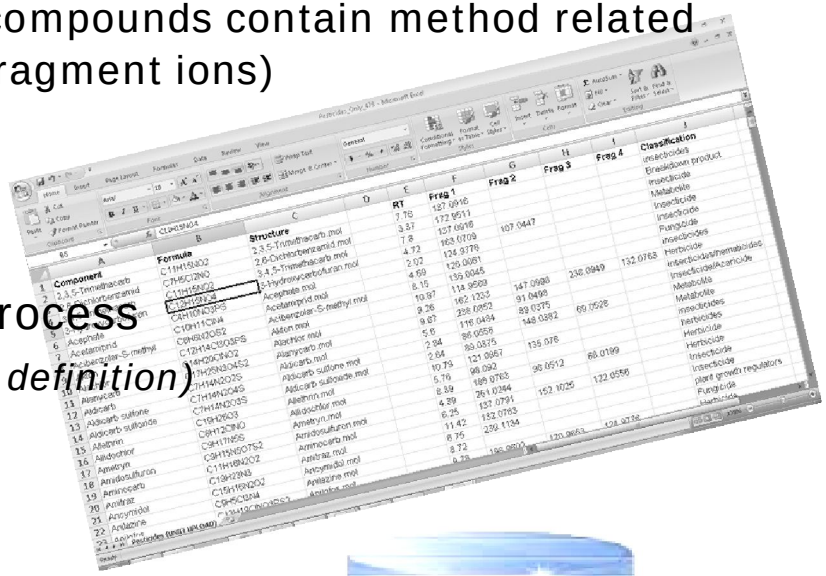
SCIENTIFIC INFORMATION SYSTEM

Scientific Library

Comprehensive 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



Data Review

Data Review
Two Key Workflows

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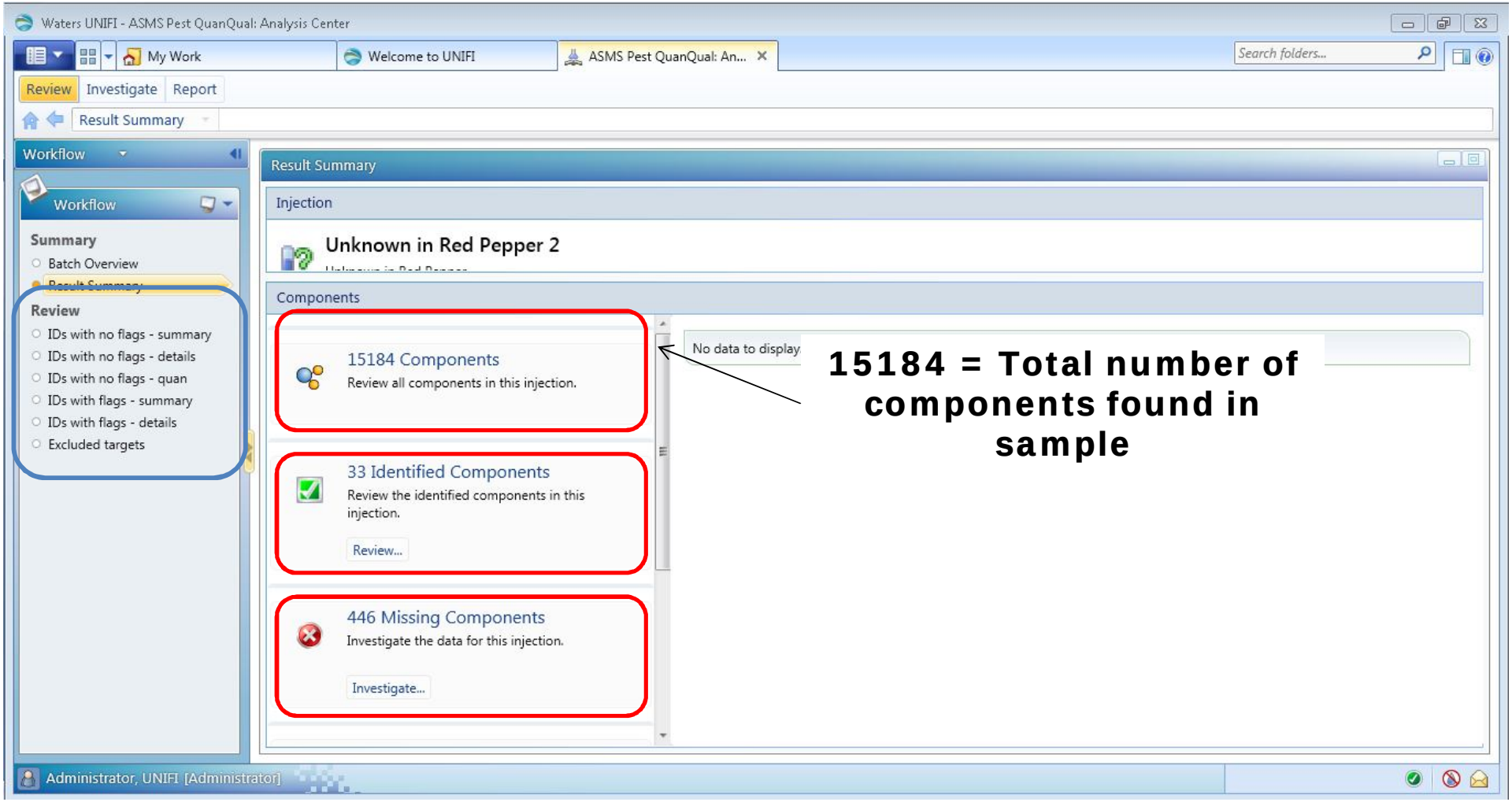
Non-targeted workflow

- 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..)

Review: Summary Page



Data review for non-targeted analysis
Fragment ion identification

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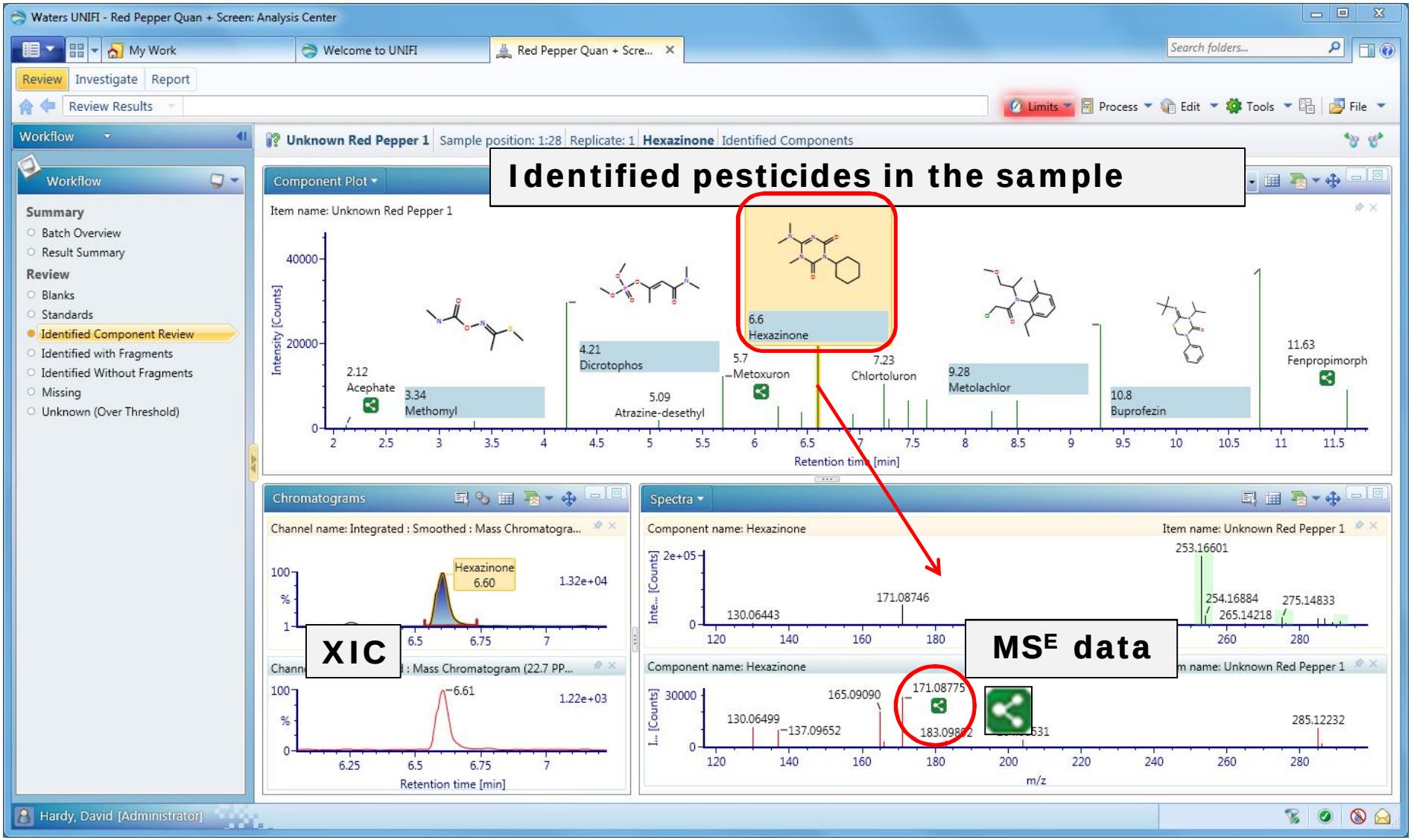

Review:
Positively Identified: Data Confirmation

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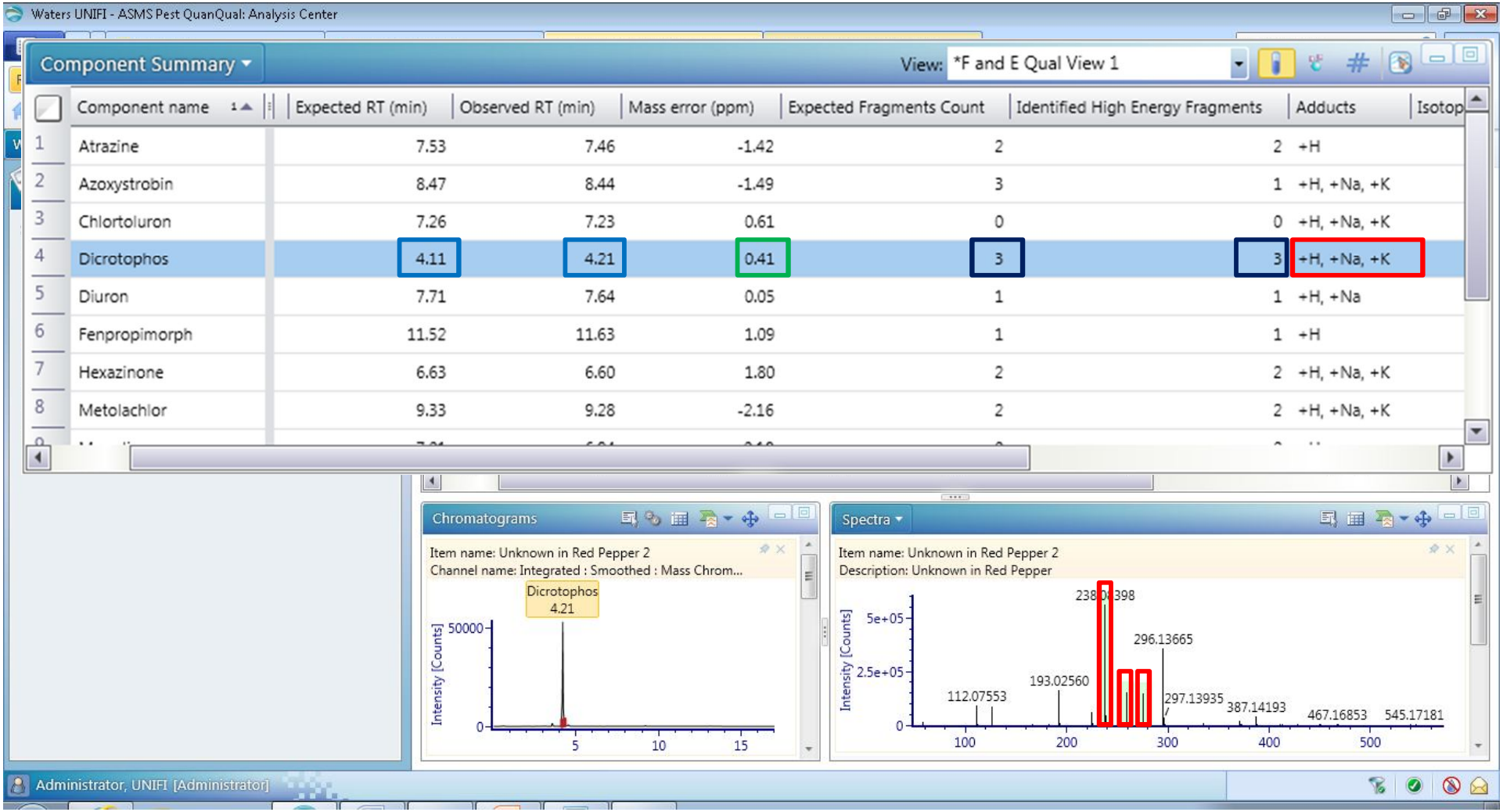

Review:
Positively Identified: Low & high energy data for all adducts

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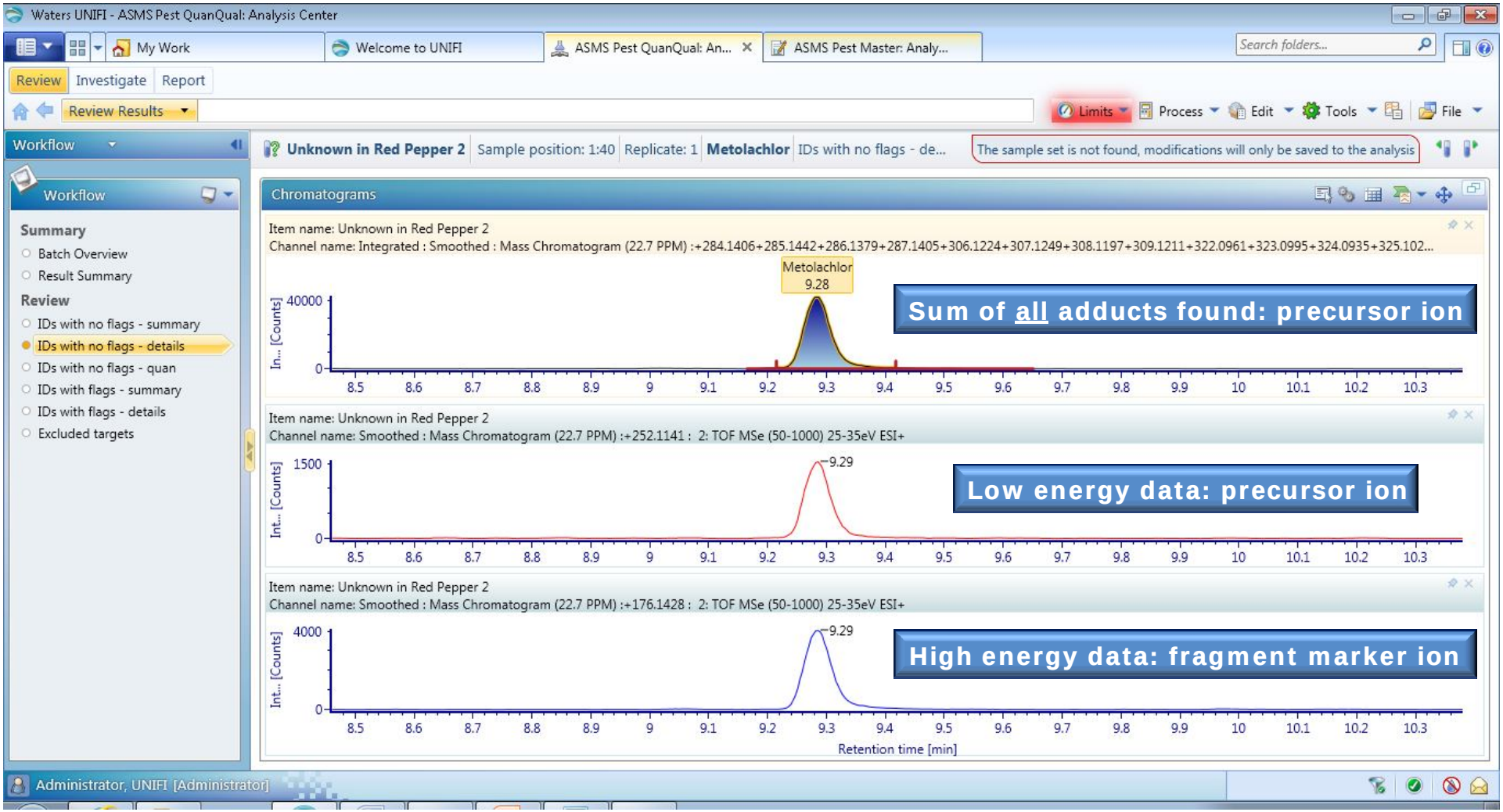

Review:
Positively Identified: Quantification Results

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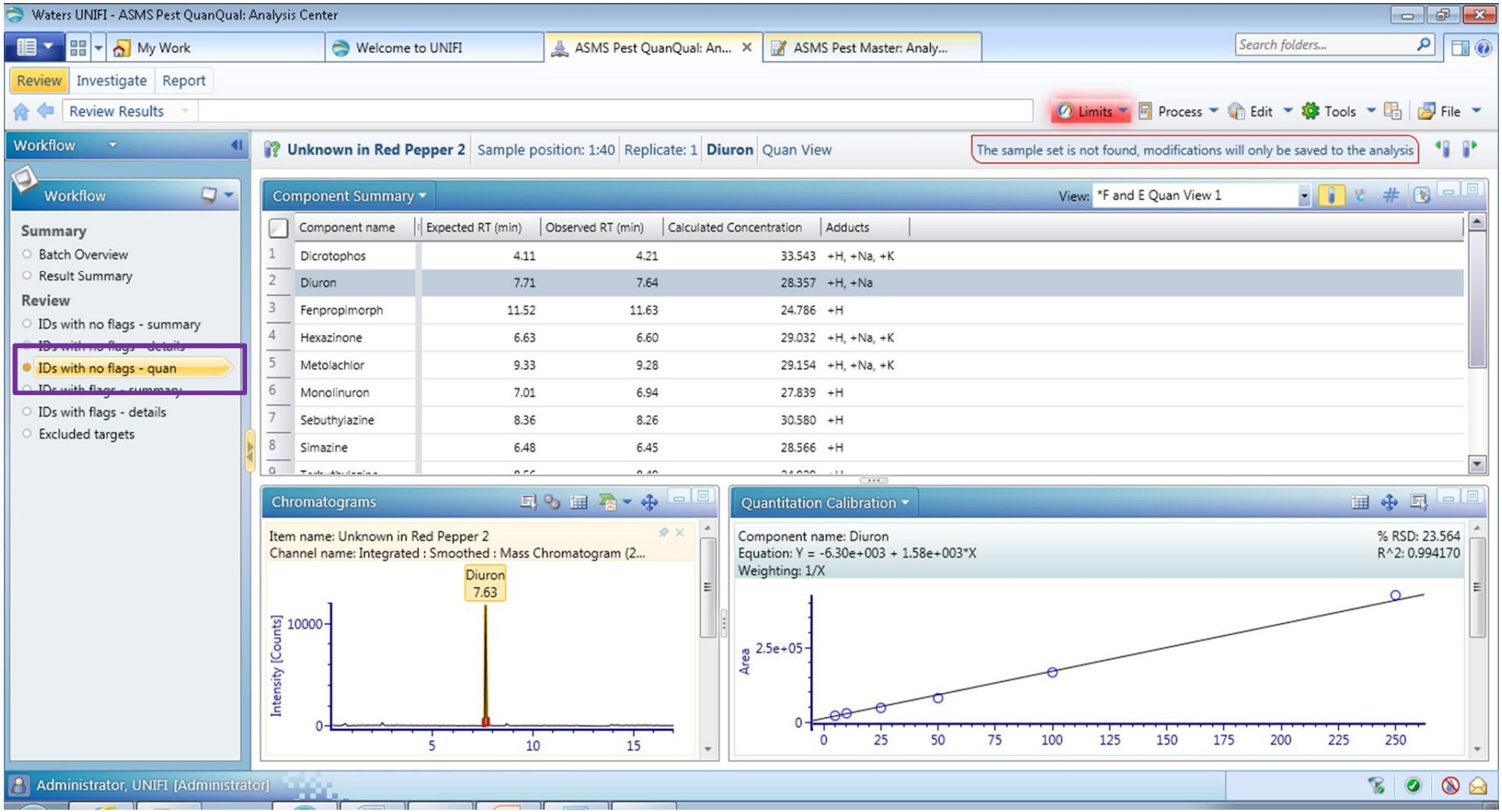

Review:
Components with Flagged Values

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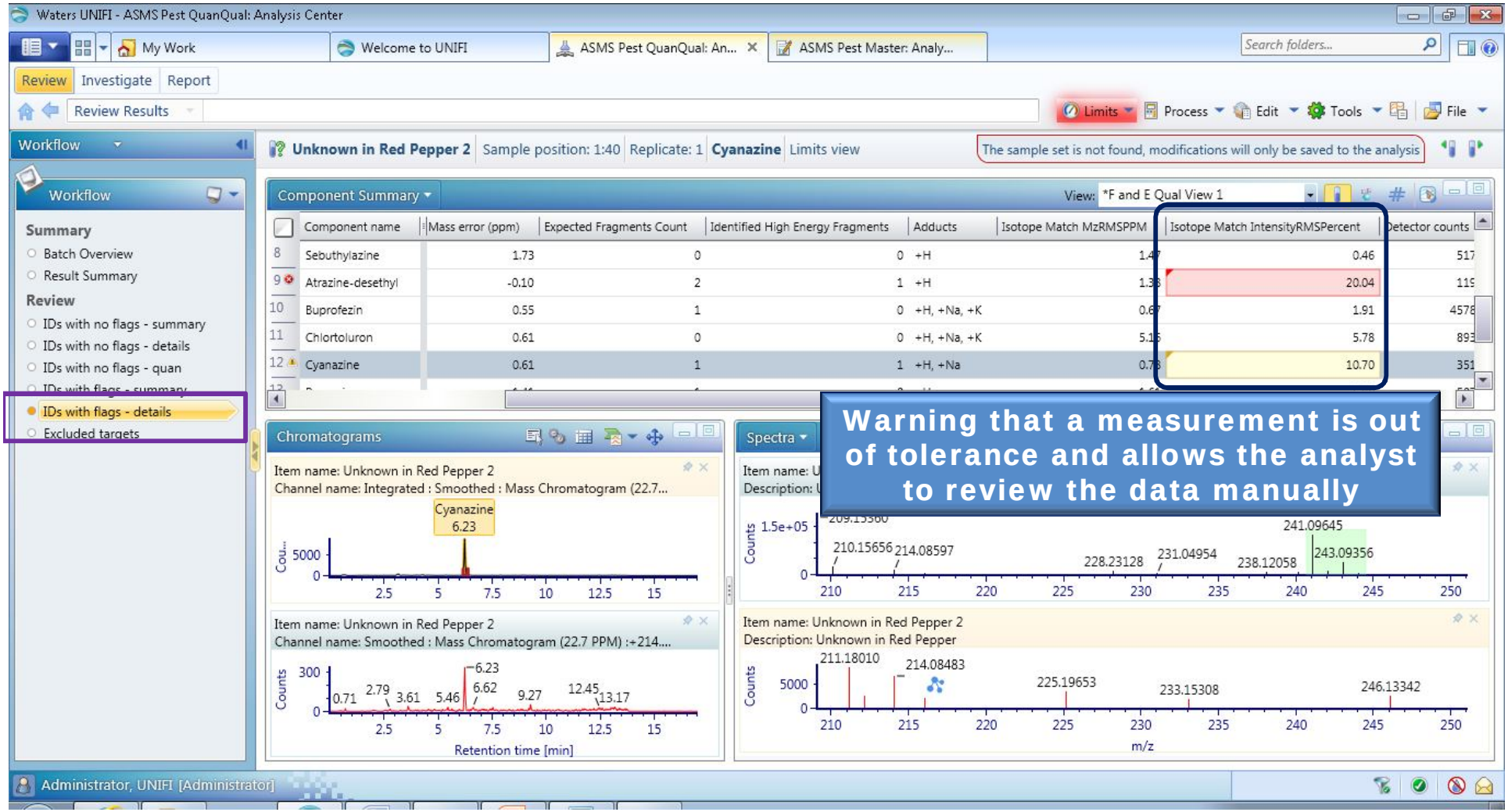

Looking for Unknown Compounds

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Supporting Software Tools

Managing the Unidentified Candidate List

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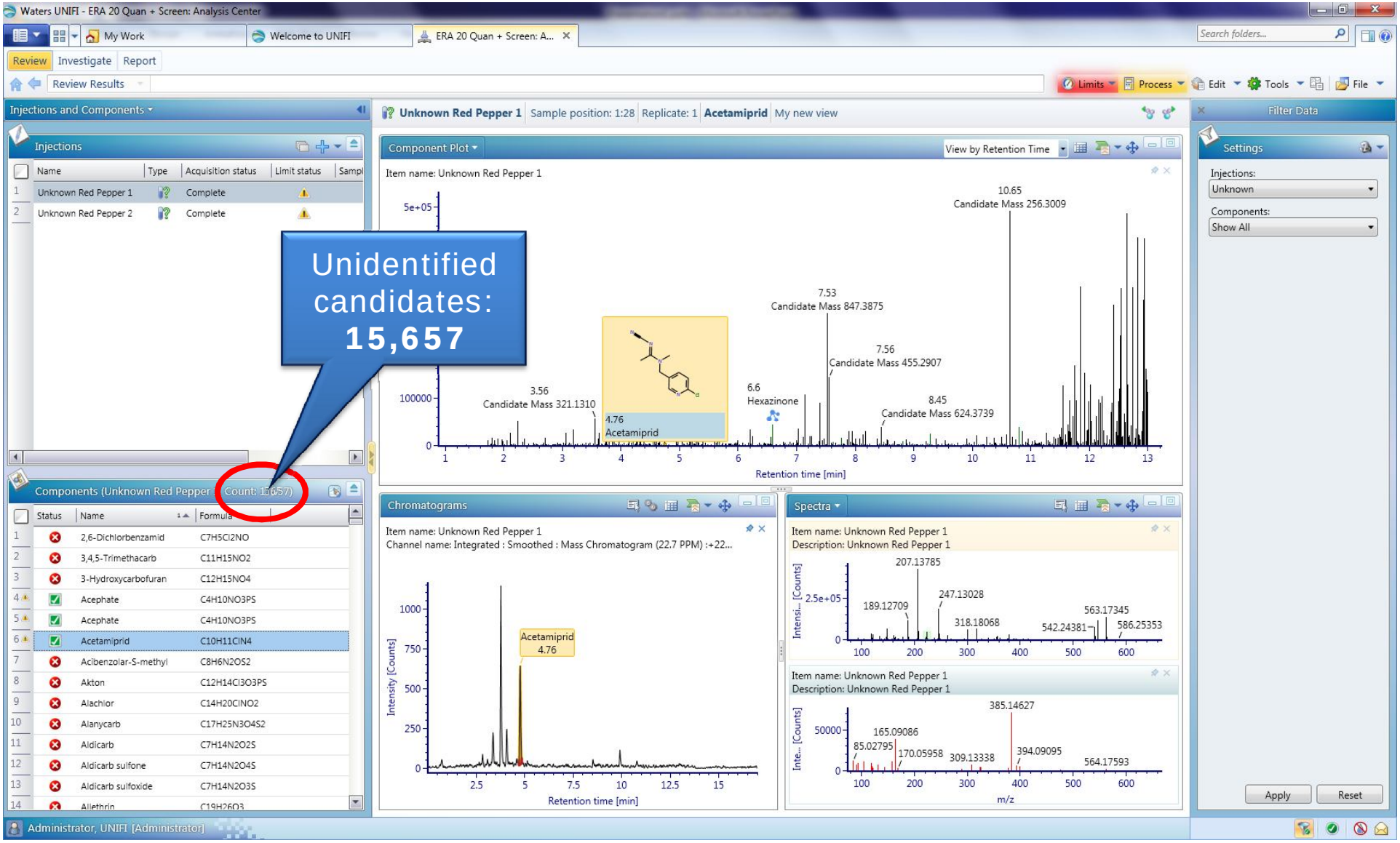

Data Review
Two Key Workflows

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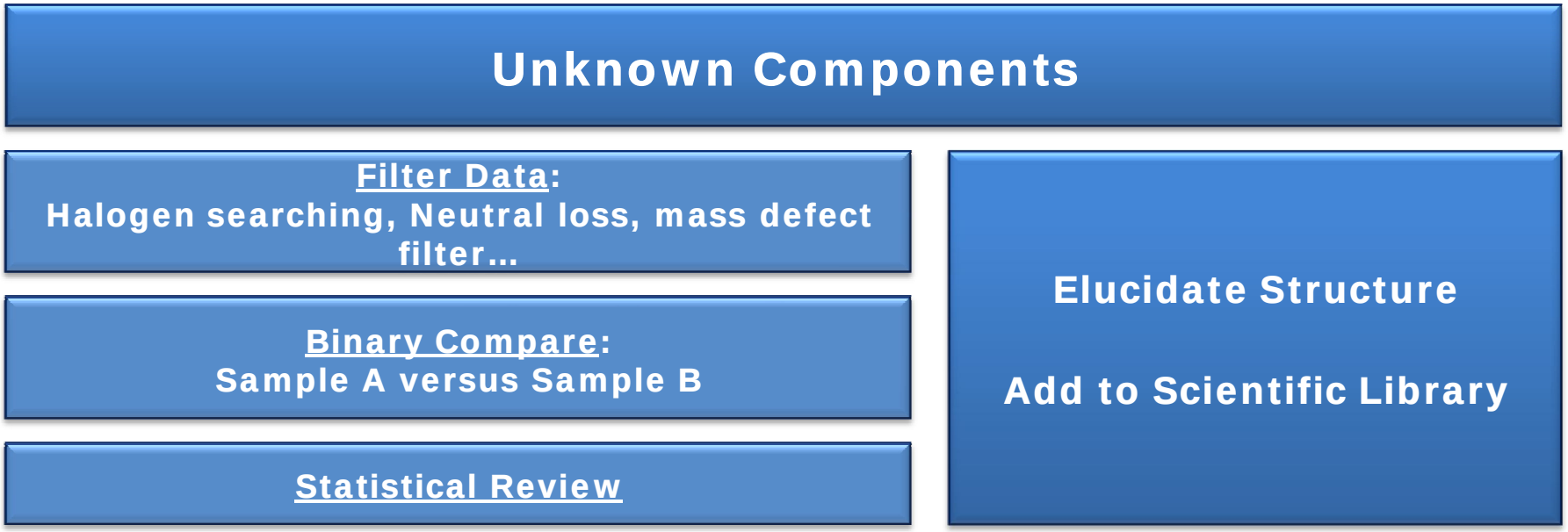

- **15,500 Unknowns!!**
  - ✔ Reduce the unknown component list - use of filters (halogen, neutral loss, mass defect filters…), sample comparison, statistical analysis
  - ✔ Elucidate structure seamlessly – Full Toolset integrated into UNIFI
  - ✔ Potential to automatically add IDed unknown to the Scientific Library

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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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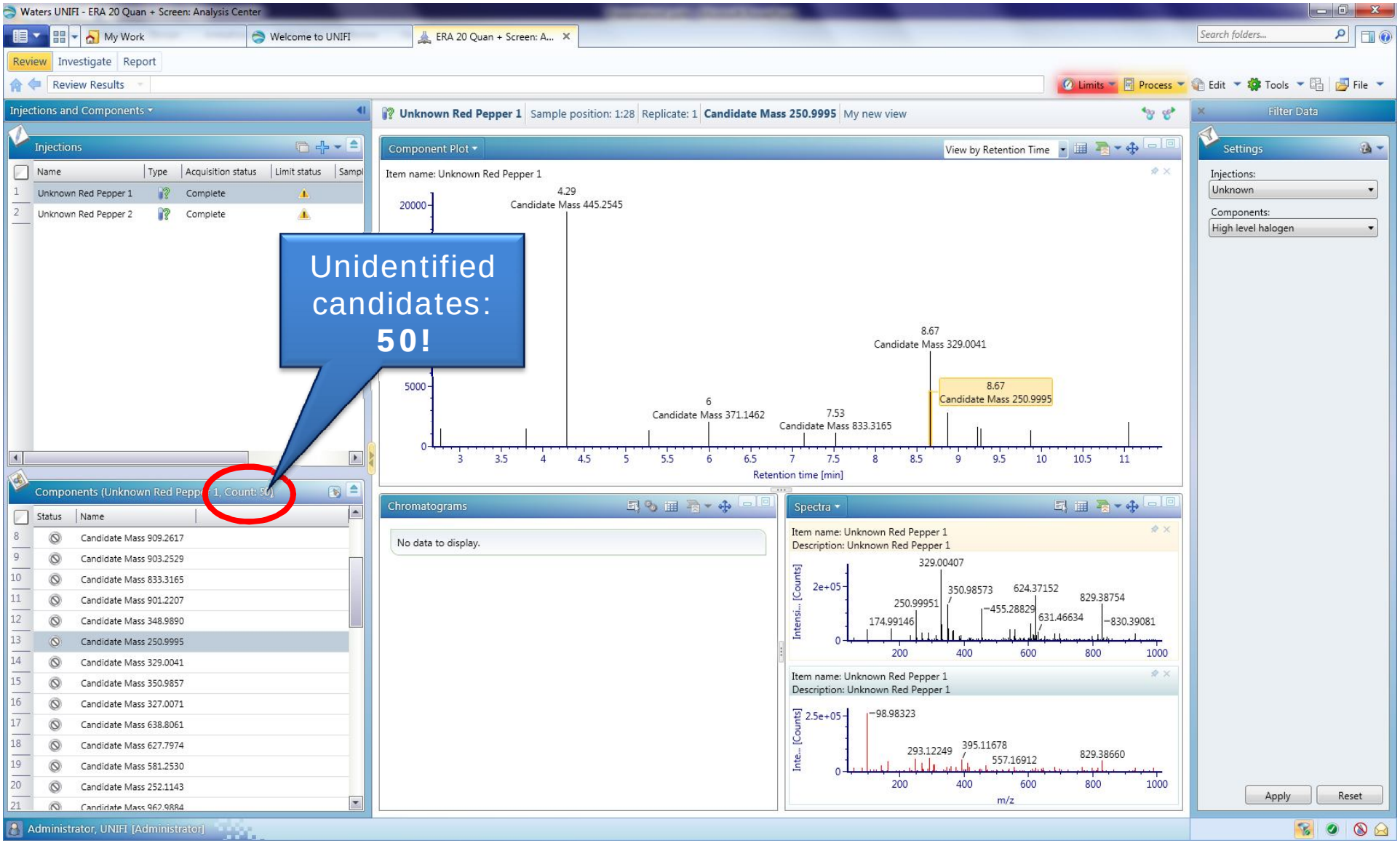

Searching external libraries in the elucidation Toolset
Online search MassBank

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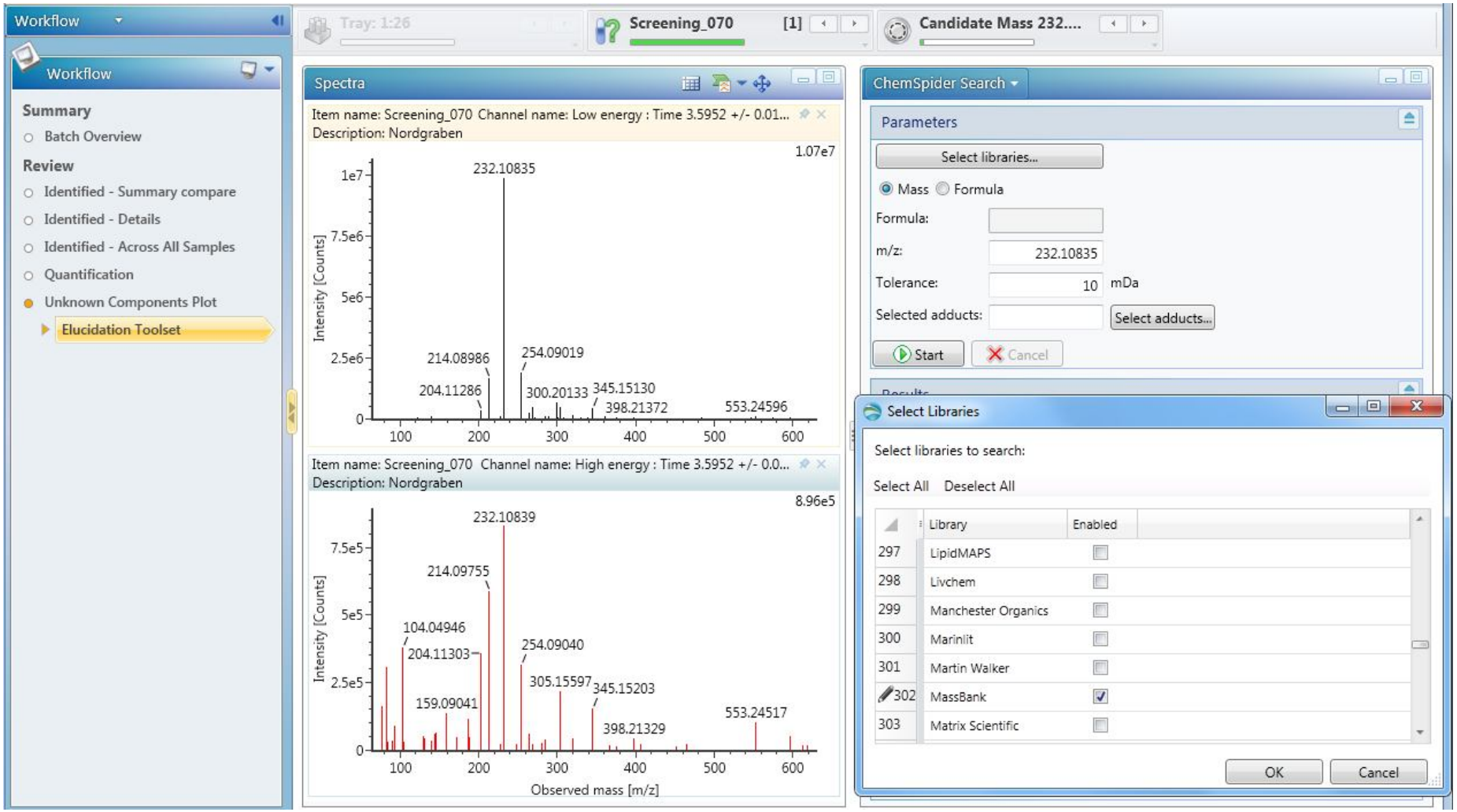

Summary

- 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**