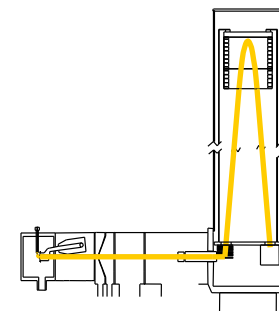


# Agilent TOF Screening & Impurity Profiling Julie Cichelli, PhD LC/MS Small Molecule Workshop Dec 6, 2012



# Review: Technology for Accurate Mass Analysis: TOF – LC/MS

- Mass measurements accurate to several decimal places (rather than "nominal" or "unit" mass measurements of  $\pm 0.1$  Da), due to *high resolution* mass analyzer.
- Most Common Uses:
  - Rapid selective and specific screening for target compounds
  - Confirmation of molecular formula for proposed compounds
  - Calculation of possible molecular formulae for unknowns

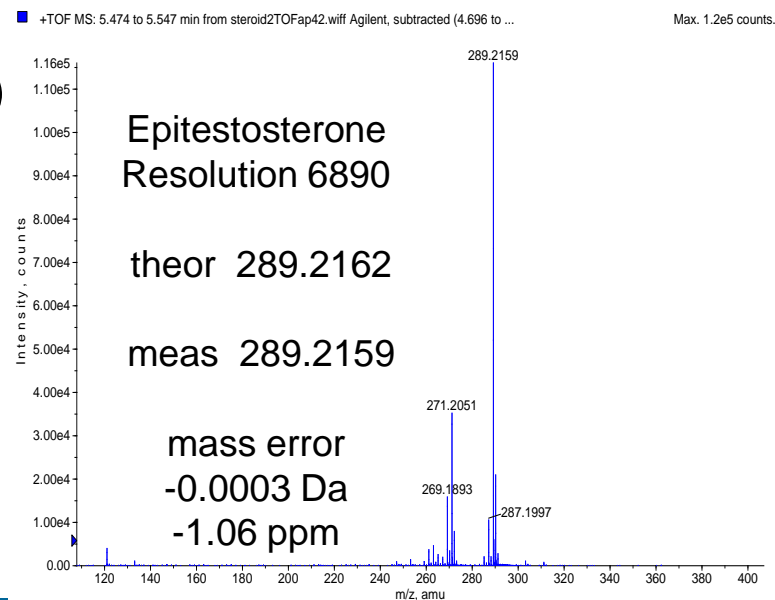


## Definition of Mass accuracy (mass error)

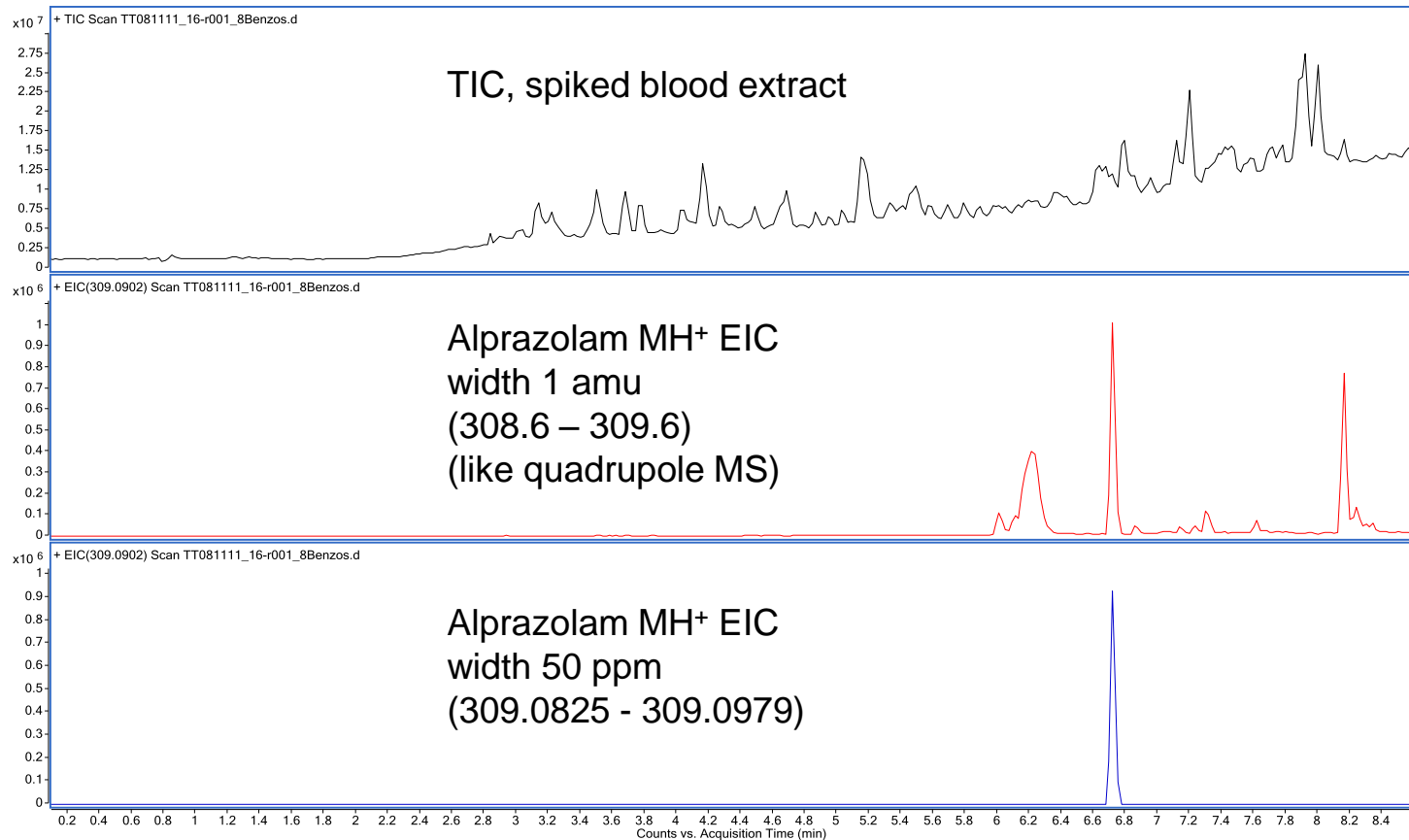
$$\text{Mass accuracy} = \left( \frac{\text{measured} - \text{theory}}{\text{theory}} \right) \times 10^6$$

$$(1000.001 - 1000.000) / 1000 \times 10^6 = 1 \text{ ppm}$$

Therefore: 0.001 Da (1 mDa) error  
@ MW 300 Da is approximately 3 ppm



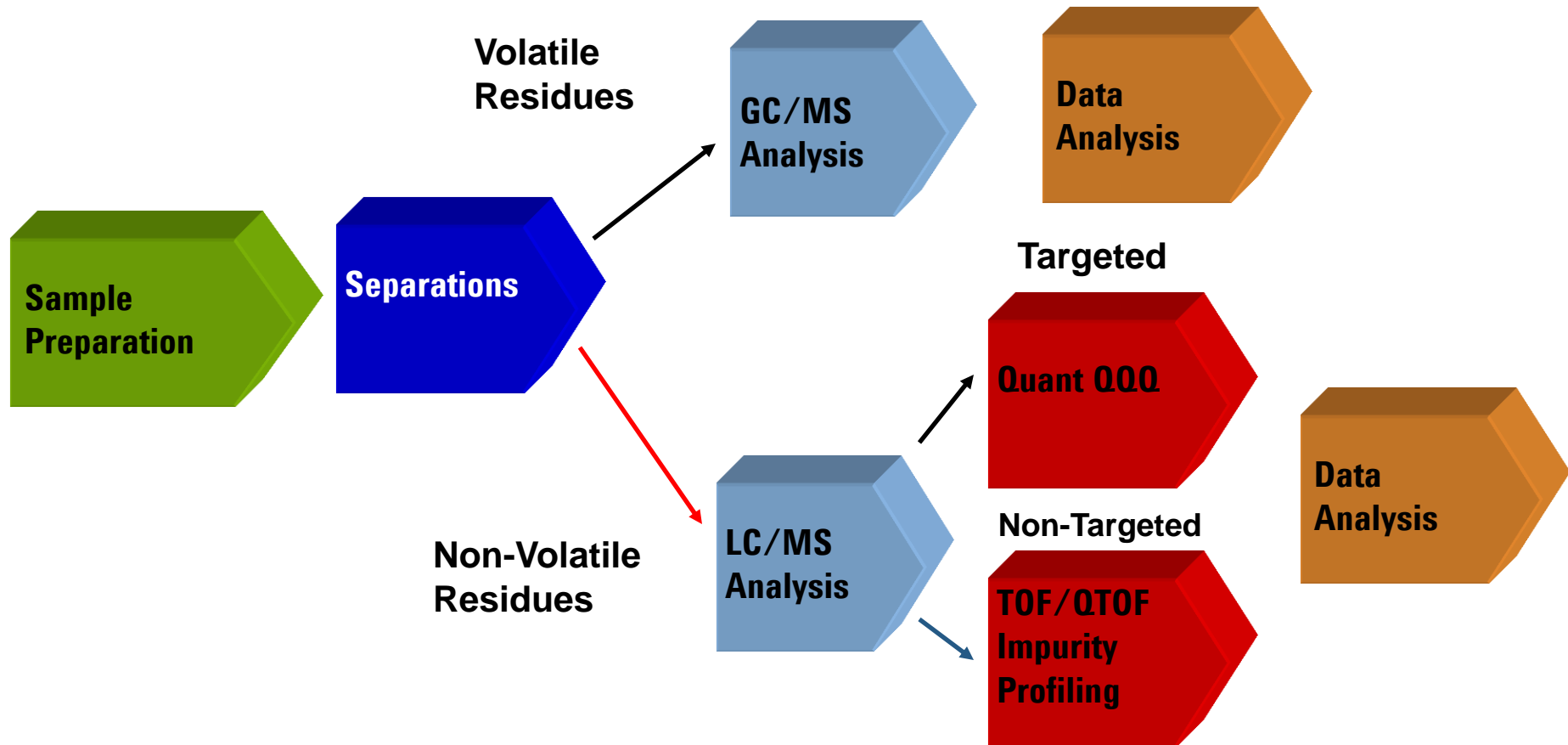
# Specificity of narrow-mass EICs: locating compounds



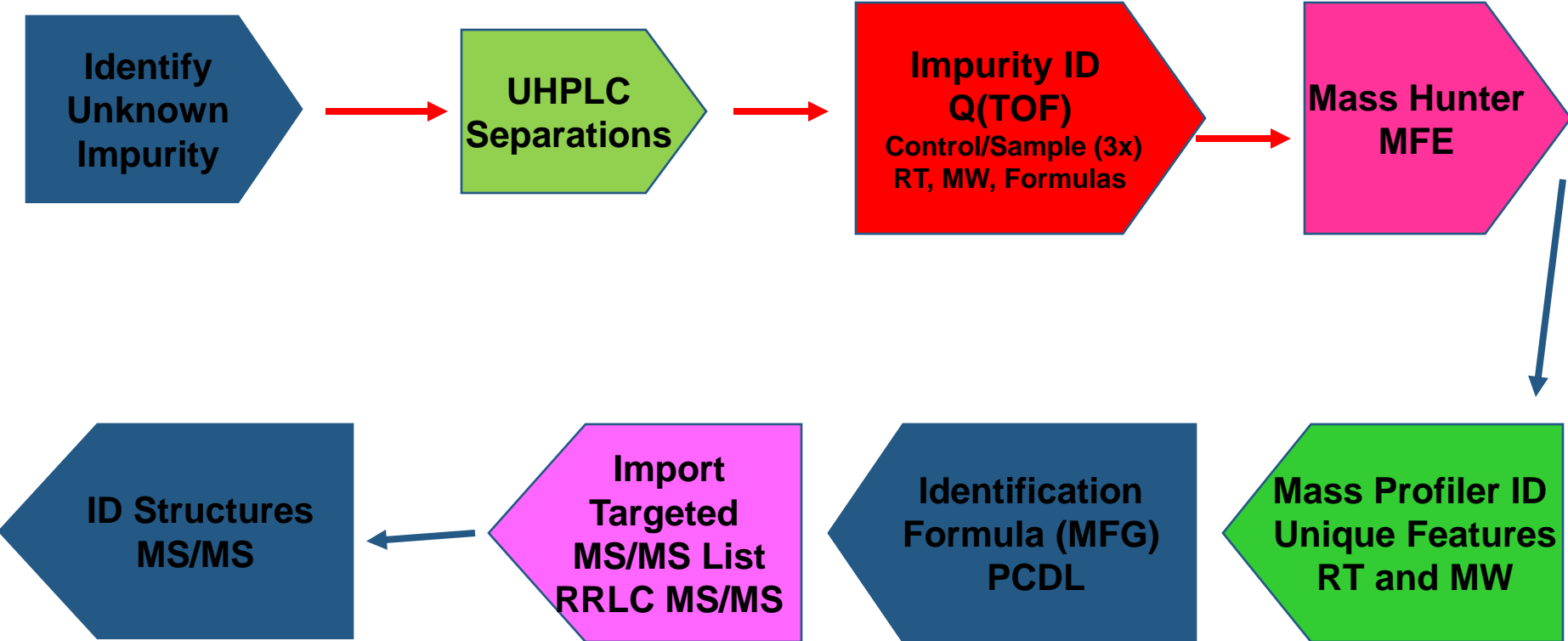
TOF's unique advantage over MS/MS: all-scan data allows retrospectively finding compounds **not originally sought**

# Agilent's Solution for Impurity Profiling

**Objective:** Detect low-level Organic Impurities in Drugs: Tablets, Capsules, Suspension, Transdermal, Meter Dose Inhalers (MDI)



# The LCMS Impurity Profiling Unknown Workflow





# What, How Many, How Much, In What Information Needed to Optimize Analyses

- **What** are you trying to detect
  - Compound Class, Polarity, Solubility, Ionization Methods
- **How Many** Compounds
  - LC Separations (C18, ANP), Mobile Phases-Buffers, Ion Suppression, Matching LC and MS Peak Widths
- **How Much** and Over What Range
  - Trace level detection (LOD/LOQ) vs. Impurities
  - Wide Dynamic Range – Extended Dynamic Range Model
  - Co-eluting low molecular weight analytes – High Resolution Mode
- **In What** Matrix
  - Complexity of Matrix, Major Components, Ion Suppression, Salts
  - Co-eluting components – what instrument state to use

# The Match Game....

Choosing The Mass Spectrometer for the Task  
**Sensitivity, Selectivity, Identification..**

**Unknown Identification**  
**Accurate Mass MS, MSMS**  
**MS/MS Sensitivity**



**6490 QQQ**



**Unknown Identification**  
**Accurate Mass MS**

**Target Analysis**  
**Enhanced Sensitivity by MS/MS**

# Find Compounds Functions in MassHunter Qual

## Find by Molecular Feature (MFE)

The screenshot shows the 'Method Editor: Find Compounds by Molecular Feature' window. It features a toolbar with navigation icons and a 'Method Items' dropdown. Below the toolbar are several tabs: 'Extraction', 'Ion Species', 'Charge State', 'Compound Filters', 'Mass Filters', 'Mass Defect', 'Results', and 'Advanced'. The 'Extraction' tab is active, showing three main sections: 'Extraction algorithm' with a 'Target data type' dropdown set to 'Small molecules (chromatographic)'; 'Input data range' with checkboxes for 'Restrict retention time to' (minutes) and 'Restrict m/z to' (m/z); and 'Peak filters' with two radio button options: 'Use peaks with signal-to-noise (Profile spectra only)' with a value of 5.0, and 'Use peaks with height (Profile and centroid spectra)' with a value of 100 counts.

“Naïve”

## Find by Formula/ Ion FBF & FBI

The screenshot shows the 'Method Editor: Find Compounds by Formula - Options' window. It has a toolbar and tabs for 'Formula Source', 'Formula Matching', 'Positive Ions', 'Negative Ions', 'Scoring', and 'Results'. The 'Formula Source' tab is active, displaying options for 'Source of formulas to confirm': 'These formulas:' (with a text input field containing 'C42H48N18'), 'Compound exchange file (.CEF):', 'Database' (with a path 'D:\MassHunter\databases\Metlin.mtl'), and 'Worklist'. Below this is a section for 'Database values to match' with radio button options: 'Mass', 'Mass and retention time (retention time optional)', and 'Mass and retention time (retention time required)'.

“Targeted”

## 2 Data Processing Approaches using Peak Picking Algorithms

- **Find by Formula**
- **Find by Molecular Feature**



# Data Processing Approaches

## Approach 1:

1. Run ***Find by Formula***
2. Advantage: no threshold setting allows data to be extracted from the “grass”
3. Disadvantage: will only show results for compounds in a compound database or specified

The screenshot shows the 'Method Editor: Find Compounds by Formula - Options' dialog box with the 'Formula Source' tab selected. The 'Source of formulas to confirm' section has three radio buttons: 'These formulas:' (unselected), 'Compound exchange file (.CEF):' (unselected), and 'Database' (selected). The 'Database' option is set to 'C:\MassHunter\PCDL\UUtah.cdb'. Below this, the 'Matches per formula' section has a 'Maximum number of matches' set to 1 and a checked box for 'Automatically increase for isomeric compounds'. The 'Values to match' section has three radio buttons: 'Mass' (selected), 'Mass and retention time (retention time optional)' (unselected), and 'Mass and retention time (retention time required)' (unselected).

The screenshot shows the 'Method Editor: Find Compounds by Formula - Options' dialog box with the 'Formula Matching' tab selected. The 'Match tolerance' section has 'Masses:' set to '+/- 10.00 ppm' and 'Retention times:' set to '+/- 0.350 minutes'. The 'Expansion of values for chromatogram extraction' section has 'Possible m/z:' set to 'Symmetric (ppm)' and '+/- 35.0'. The 'Limit EIC extraction range' checkbox is checked, and 'Expected retention time:' is set to '+/- 1.00 minutes'.

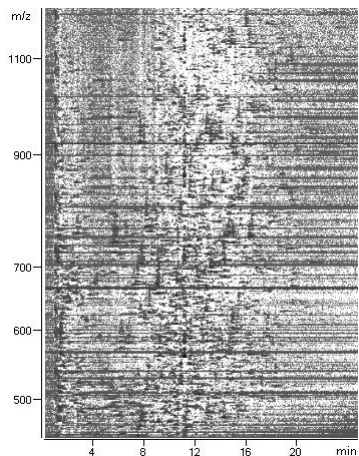
# Data Processing Approaches

## Approach 2:

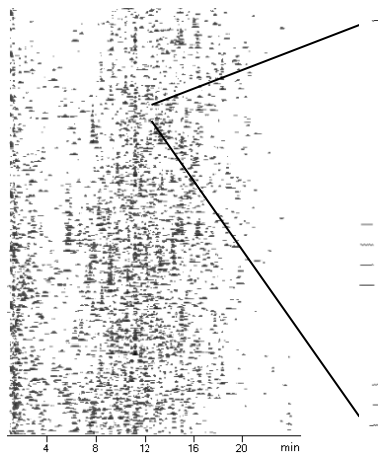
1. Run Find Compounds by *Molecular Feature Extractor*
2. Search PCD for matching compounds
3. Run Generate Formulas from Compounds for unknowns
4. Advantage: will show information for unknowns

# Molecular Feature Extractor (MFE)

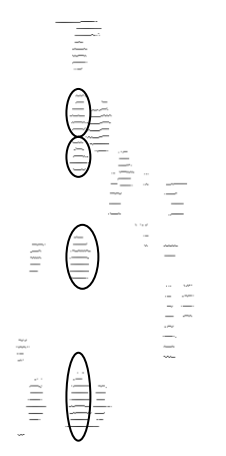
## Transforming Data to Chemical Information



Raw data



Background noise removed



Individual m/z peaks grouped into isotope clusters

| species   | RT     | m/z      | mass      | abund. |
|-----------|--------|----------|-----------|--------|
| M+3H      | 13.248 | 921.5025 | 2761.4896 | 356764 |
| M+3H+1    | 13.247 | 921.8373 | 2761.4874 | 548320 |
| M+3H+2    | 13.248 | 922.1722 | 2761.4897 | 476780 |
| M+3H+3    | 13.249 | 922.5072 | 2761.4924 | 256680 |
| M+3H+4    | 13.249 | 922.8416 | 2761.4934 | 117149 |
| M+3H+5    | 13.251 | 923.1744 | 2761.4892 | 45705  |
| M+3H+6    | 13.252 | 923.5058 | 2761.4810 | 15721  |
| M+3H+7    | 13.246 | 923.8387 | 2761.4775 | 4492   |
| M+3H+8    | 13.263 | 924.1649 | 2761.4535 | 677    |
| M+2H+Na   | 13.248 | 928.8293 | 2761.4842 | 29521  |
| M+2H+Na+1 | 13.249 | 929.1643 | 2761.4863 | 46140  |
| M+2H+Na+2 | 13.247 | 929.4957 | 2761.4781 | 43276  |
| M+2H+Na+3 | 13.247 | 929.8283 | 2761.4737 | 33217  |
| M+2H+Na+4 | 13.258 | 930.1614 | 2761.4706 | 23203  |
| M+2H+Na+5 | 13.295 | 930.5000 | 2761.4842 | 11741  |
| M+2H+Na+6 | 13.277 | 930.8343 | 2761.4846 | 3970   |
| M+2H+K    | 13.247 | 934.1483 | 2761.4673 | 8064   |
| M+2H+K+1  | 13.241 | 934.4899 | 2761.4863 | 13793  |
| M+2H+K+2  | 13.244 | 934.8196 | 2761.4759 | 11070  |
| M+2H+K+3  | 13.250 | 935.1566 | 2761.4847 | 5695   |
| M+2H+K+4  | 13.247 | 935.4933 | 2761.4926 | 3721   |
| M+2H+K+5  | 13.280 | 935.8045 | 2761.4237 | 1182   |
| M+H+2Na   | 13.238 | 936.1547 | 2761.4783 | 6951   |
| M+H+2Na+1 | 13.241 | 936.4933 | 2761.4913 | 9925   |
| M+H+2Na+2 | 13.244 | 936.8254 | 2761.4853 | 8407   |
| M+H+2Na+3 | 13.251 | 937.1567 | 2761.4769 | 7074   |
| M+H+2Na+4 | 13.256 | 937.4817 | 2761.4497 | 3386   |
| M+H+2Na+5 | 13.242 | 937.8123 | 2761.4392 | 2586   |

Export features:4664 / groups:1167

| RT  | mass   | abund     | height  | #ions |    |
|-----|--------|-----------|---------|-------|----|
| 463 | 7.715  | 1271.5948 | 11146   | 1329  | 3  |
| 464 | 7.716  | 1579.7000 | 1223    | 188   | 2  |
| 465 | 7.716  | 748.3860  | 18753   | 1216  | 4  |
| 466 | 7.718  | 1970.7935 | 2798    | 281   | 3  |
| 467 | 13.242 | 2826.4049 | 11853   | 662   | 4  |
| 468 | 13.244 | 2814.4034 | 60168   | 1698  | 8  |
| 469 | 13.245 | 2816.4119 | 8707    | 680   | 3  |
| 470 | 13.246 | 2331.1504 | 3191    | 291   | 3  |
| 471 | 13.247 | 1407.6968 | 4377    | 385   | 2  |
| 472 | 13.249 | 2761.4877 | 4778072 | 79795 | 78 |
| 473 | 13.250 | 2433.2736 | 3736    | 399   | 3  |
| 474 | 13.250 | 2806.4575 | 16286   | 650   | 7  |
| 475 | 13.252 | 1123.6411 | 4246    | 416   | 2  |
| 476 | 13.253 | 2068.0019 | 14029   | 665   | 7  |
| 477 | 13.253 | 2783.4528 | 27728   | 1212  | 8  |
| 478 | 9.159  | 677.2975  | 8801    | 714   | 2  |
| 479 | 9.159  | 2173.8394 | 3492    | 427   | 3  |
| 480 | 9.161  | 1310.6477 | 1838172 | 70500 | 23 |
| 481 | 9.161  |           | 12218   | 1585  | 1  |
| 482 | 9.162  | 1334.6137 | 75374   | 2869  | 10 |
| 483 | 10.385 | 1540.6853 | 2023    | 344   | 2  |
| 484 | 10.387 | 1026.8731 | 27677   | 4107  | 4  |

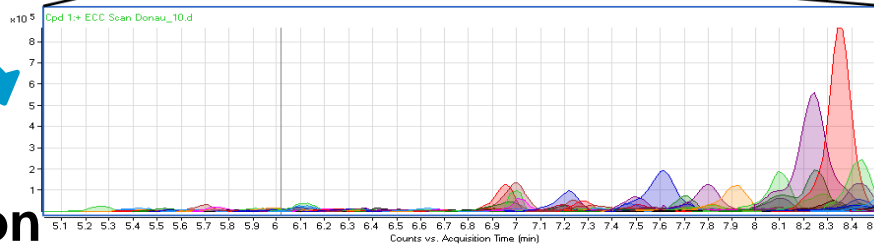
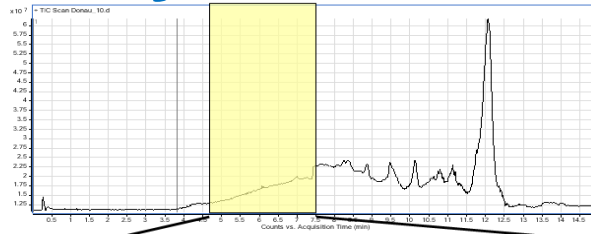
Isotope clusters grouped into molecular features

Identification, Quantification, Differential Analysis are performed on *chemically qualified compound data*

- Looks at Mass spectral data first → Groups co-eluting isotopes & converts to neutral mass
- Checks that there is a chromatographic response
- Groups all charge states, adducts, etc. associated with a given feature into a “peak volume”
- Feature consists of an accurate neutral mass, a retention time, and a peak volume

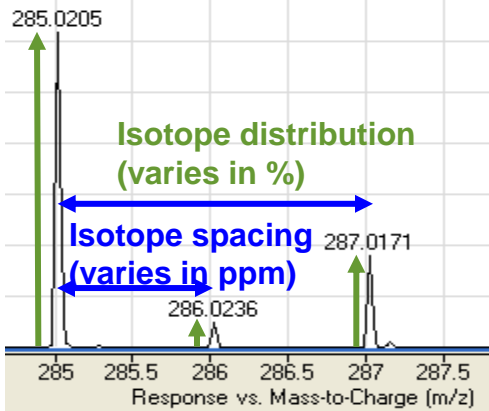


# Molecular Feature Extraction (MFE): Proprietary Peak Finding Algorithm



Scoring based on

↓ Monoisotopic mass  
(varies in ppm)



| species | RT    | m/z      | mass     | abund. |
|---------|-------|----------|----------|--------|
| M       | 8.162 |          | 342.1467 | 130643 |
| M+H     | 8.165 | 343.1547 | 342.1474 | 11889  |
| M+H+1   | 8.162 | 344.1581 |          | 2290   |
| M+H+2   | 8.157 | 345.1748 |          | 369    |
| M+H4N   | 8.164 | 360.1807 | 342.1469 | 8420   |
| M+H4N+  | 8.156 | 361.1893 |          | 1227   |
| M+Na    | 8.162 | 365.1359 | 342.1466 | 75678  |
| M+Na+1  | 8.163 | 366.1394 |          | 15324  |
| M+Na+2  | 8.162 | 367.1429 |          | 1901   |
| 2M+Na   | 8.164 | 707.2810 | 342.1459 | 4629   |
| 2M+Na+1 | 8.162 | 708.2860 |          | 1808   |
| 2M+Na+2 | 8.173 | 709.2895 |          | 336    |

*Approach 1:  
Find by Formula  
(FbF)*

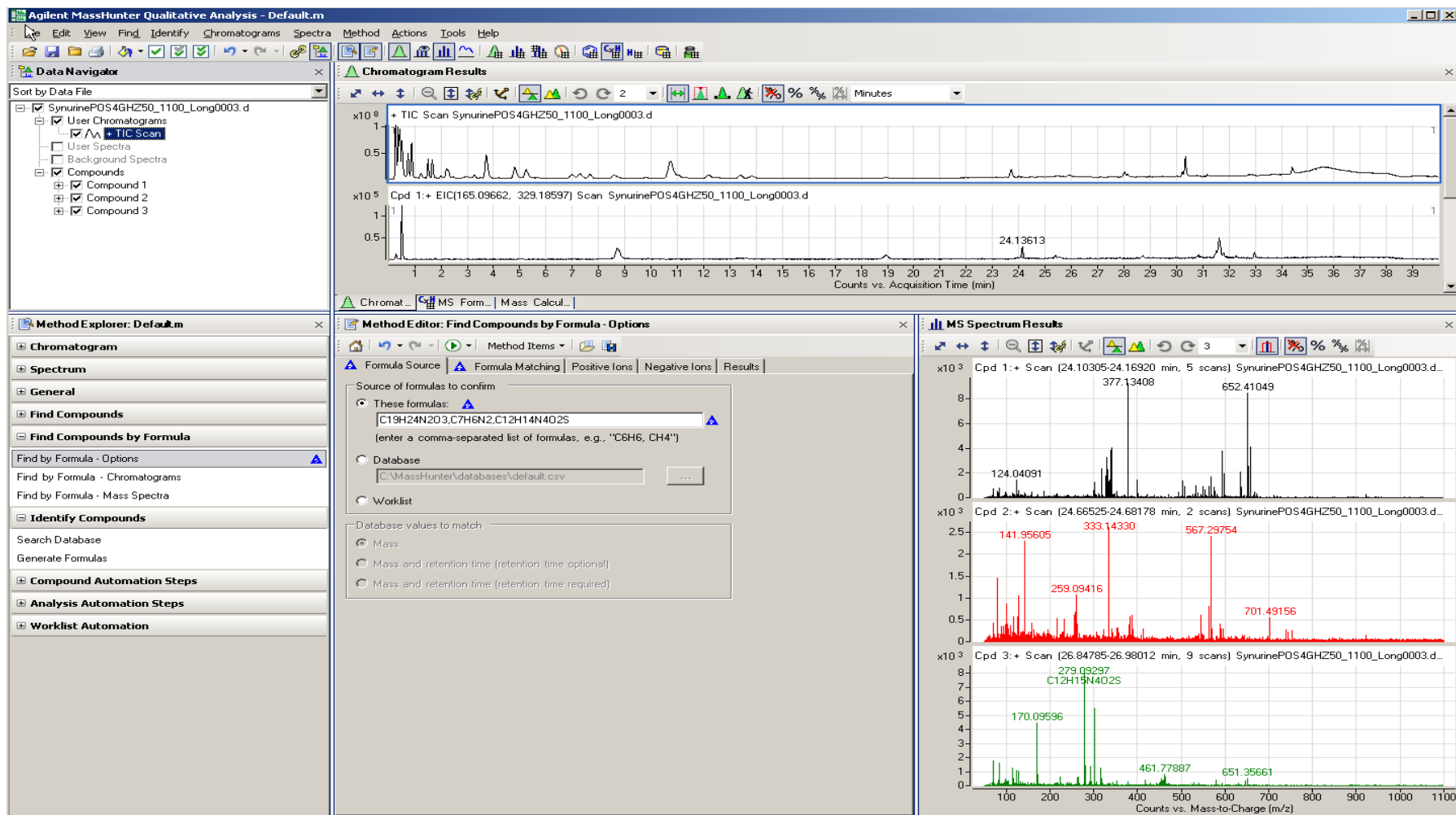
# Targeted Screening Criteria for Method

Compound is "found" if:

- Retention time is within  $\pm 0.15$  minutes of standard (typical match within  $\leq 0.05$  min)
- Mass error for adduct is within  $\pm 15$  ppm of theoretical (most are  $< 5$  ppm)
- Target compound Score is  $> 50/100$ 
  - Combination of retention time match, mass matches and isotope abundance match
  - Weighting for isotope match and abundance can be adjusted to compensate for chemical interferences with low-abundance isotopes if overly affecting Score

# Find Compounds by Formula (FBF)

## Target Compounds Adducts, Dimers, Trimers



# Find Compounds by Formula Parameters

1. **Formulas Separated by Commas**

2. **CEF File Format**

3. **Databases (Custom CSV/Metlin)**

4. **Import from Worklist**

In Acquisition Worklist

Add Neutral Formulas Imported

Negative Ions      Scoring      Results  
▲ Formula Source      Formula Matching      Positive Ions

Source of formulas to confirm

These formulas:  
C10H7N3S, C9H7N3S, C12H11NO2, C9H16N5Cl  
(type a comma-separated list of formulas, e.g., "C6H6, CH4")

Compound exchange file (.CEF):  
.....

Database ▲  
D:\MassHunter\Databases\default.csv      ...

Worklist

Database values to match

Mass  
 Mass and retention time (retention time optional)  
 Mass and retention time (retention time required)

| Method    | Azoxystrobin | Carbaryl  | Linuron      | propazine | Tetraconazole  | Thiabendazole | Triclazole |
|-----------|--------------|-----------|--------------|-----------|----------------|---------------|------------|
| Default.m | C22H17N3O5   | C12H11NO2 | C9H10Cl2N2O2 | C9H16ClN5 | C13H11Cl2F4N3O | C10H7N3S      | C9H7N3S    |

# Find Compounds by Formula Parameters

## Mass and RT Matching Criteria

Method Items

Negative Ions   Scoring   Results

Formula Source   Formula Matching   Positive Ions

Match tolerance

Masses: +/- 5.00 ppm

Retention times: +/- 0.500 minutes

Expansion of values for chromatogram extraction

Possible m/z: Symmetric (ppm) +/- 100.0

Limit EIC extraction range

Expected retention time: +/- 1.50 minutes

Negative Ions   Scoring   Results

Previous results

Delete previous compounds

New results

Highlight first compound

Highlight all compounds

Matched results

Only generate compounds for matched formulas

Chromatograms and spectra

Extract EIC

Extract raw spectrum

Extract cleaned spectrum

## Pre-Specify Adducts

Negative Ions   Scoring   Results

Formula Source   Formula Matching   Positive Ions

Charge carriers

- electron
- +H
- +Na
- +K
- +NH4

Neutral losses

- H2O

Charge states, if not known

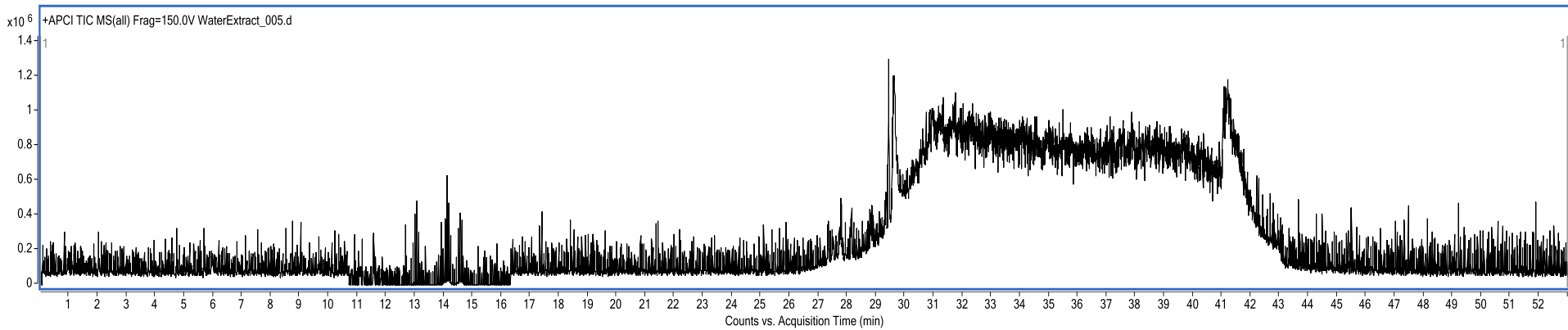
Charge state range 1-2

Aggregates

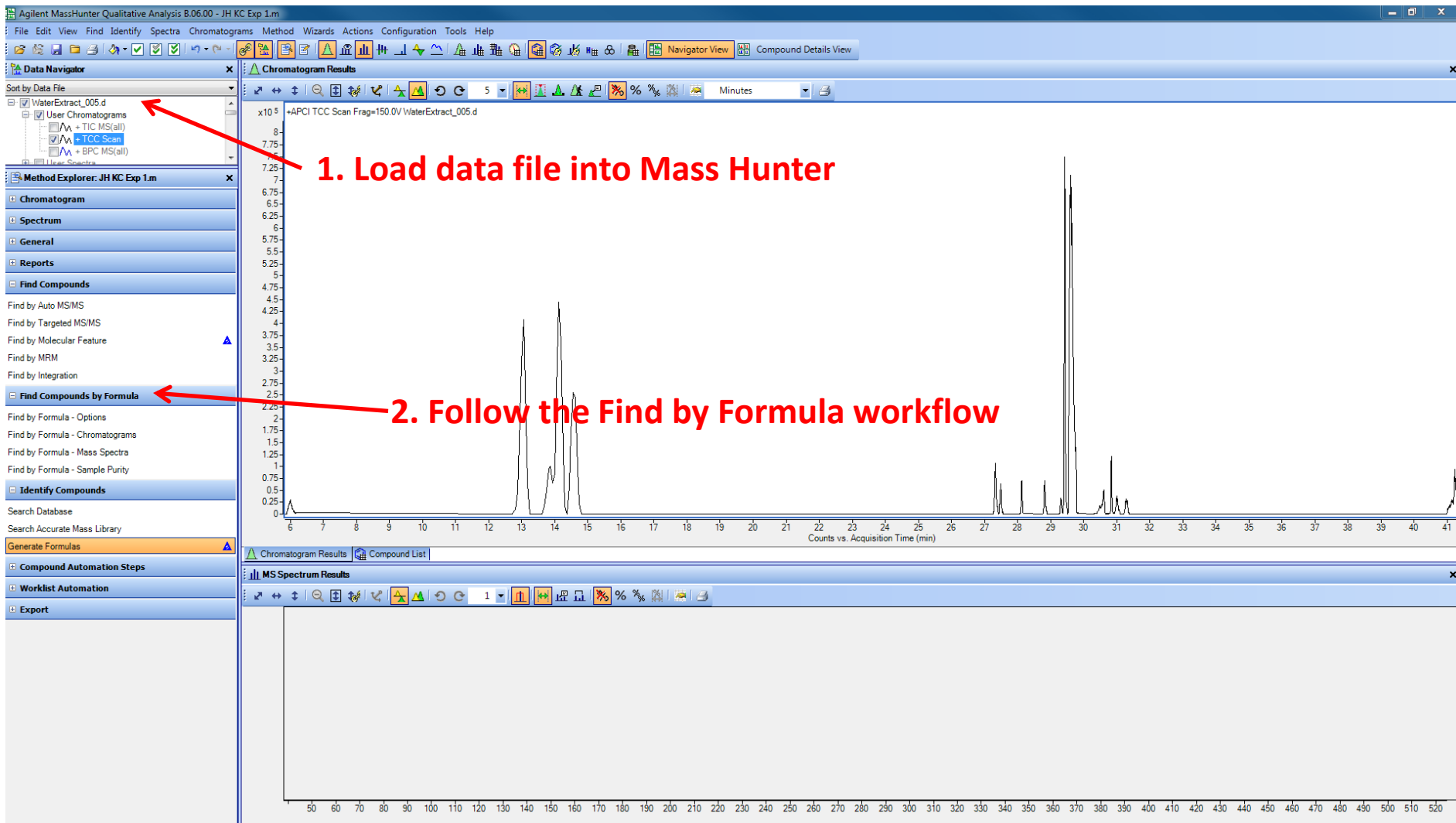
- Dimers e.g., [2M+H]<sup>+</sup>
- Trimers e.g., [3M+H]<sup>+</sup>

Check "Electron" to find Quaternary Amines

# Qualitative Analysis Water Extracts: TIC +



# Qualitative Analysis Water Extracts: FbF



# Qualitative Analysis: FBF

Method Editor: Find Compounds by Formula - Options

Find Compounds by Formula | Formula Matching | Positive Ions | Negative Ions | Results | Result Filters | Fragment Confirmation

Source of formulas to confirm

- These formulas:  
C12H16O,C11H12O,C13H18O,C12H14,C11H14O2,C13H18O  
(type a comma-separated list of formulas, e.g., "C6H6, CH4")
- Compound exchange file (.CEF):
- Database / Library  
D:\MassHunter\PCDL\default.csv
- 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: Find Compounds by Formula - Options

Find Compounds by Formula | Formula Matching | Positive Ions | Negative Ions | Results | Result Filters | Fragment Confirmation

Match tolerance

Masses: +/- 5.00

Retention times: +/- 0.500

Expansion of values for chromatogram extraction

Possible m/z: Symmetric (ppm)

Limit EIC extraction range

Expected retention time: +/- 1.50

Method Editor: Find Compounds by Formula - Options

Find Compounds by Formula | Formula Matching | Positive Ions | Negative Ions | Results | Result Filters | Fragment Confirmation

Charge carriers

- electron
- +H
- +Na
- +K
- +NH4

Neutral losses

- H2O

Charge states, if not known

Charge state range 1-2

Aggregates

- Dimers e.g., [2M+H]<sup>+</sup>
- Trimers e.g., [3M+H]<sup>+</sup>

# Qualitative Analysis: FBF

**Method Editor: Find Compounds by Formula - Options**

Find Compounds by Formula | Method Items

Formula Source | Formula Matching | Positive Ions | Negative Ions | **Results** | Result Filters | Fragment Confirmation

Previous results

- Delete previous compounds

New results

- Highlight first compound
- Highlight all compounds

Chromatograms and spectra

- Extract EIC
- Extract cleaned spectrum  Include structure
- Extract raw spectrum
  - Prefer profile for raw spectra, if available
  - Clip extracted raw spectra
- Extract MS/MS spectrum
  - Separate MS/MS spectrum per CE
  - Average MS/MS spectrum for all CEs
- Precursor tolerance: +/- 20.00 ppm
- Deisotope MS/MS spectrum

**Method Editor: Find Compounds by Formula - Options**

Find Compounds by Formula | Method Items

Formula Source | Formula Matching | Positive Ions | Negative Ions | **Results** | Result Filters

Unmatched formulas

- Only generate compounds for matched formulas

Matching criteria

Low score matches

Matches for which the overall score is low

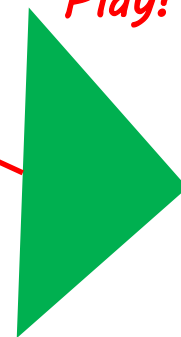
- Warn if score is < 75.00
- Do not match if score is < 70.00

Single ion matches

Matches for which only a single evidence ion is observed, but a second evidence ion of significant abundance is predicted from the formula

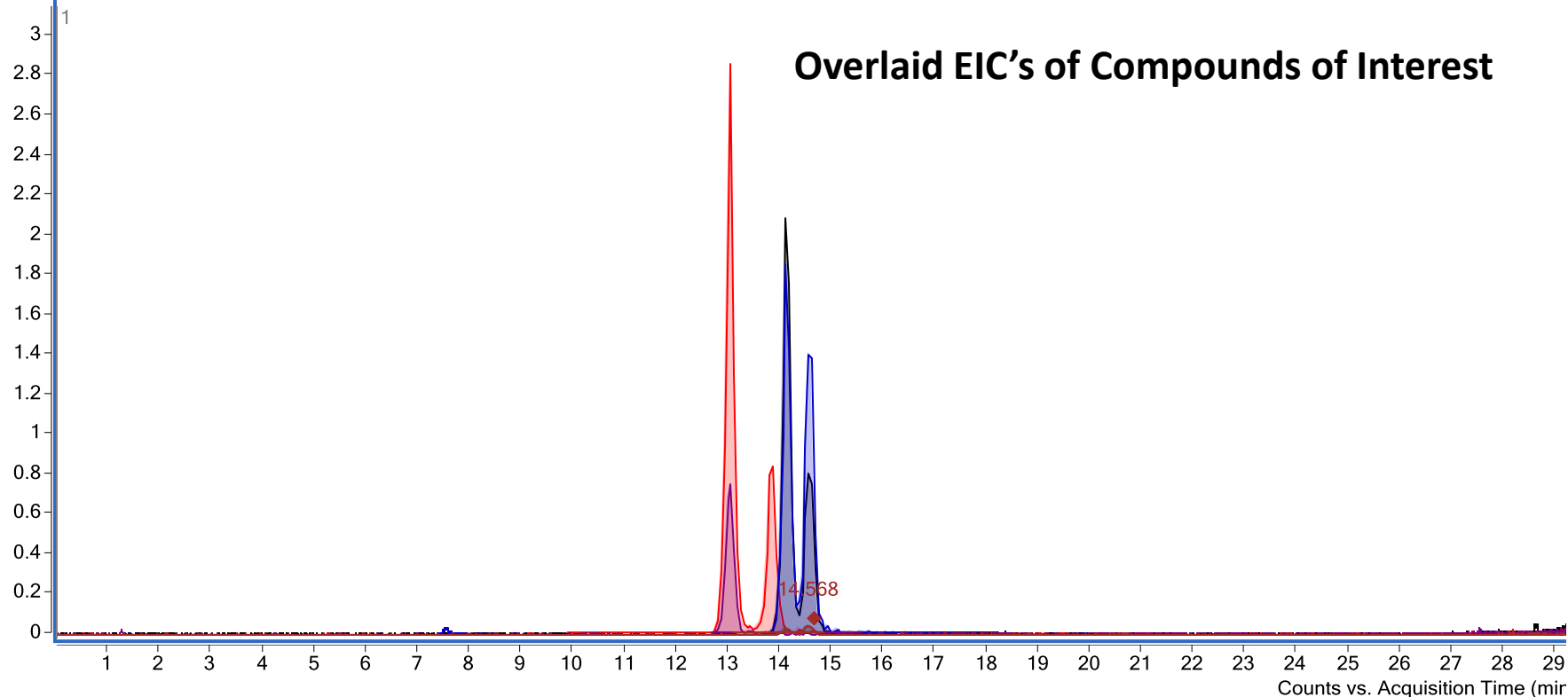
- Warn if the (unobserved) second ion's abundance is expected to be > 50.00
- Do not match if the (unobserved) second ion's abundance is expected to be > 200.00

*Press Play!*



# Qualitative Analysis FBF Results: Water Extracts

x10<sup>5</sup> Cpd 6: C13 H18 O: +APCI EIC(96.0752, 118.0571, 191.1430, 213.1250) Scan Frag=150.0V WaterExtract\_005.d

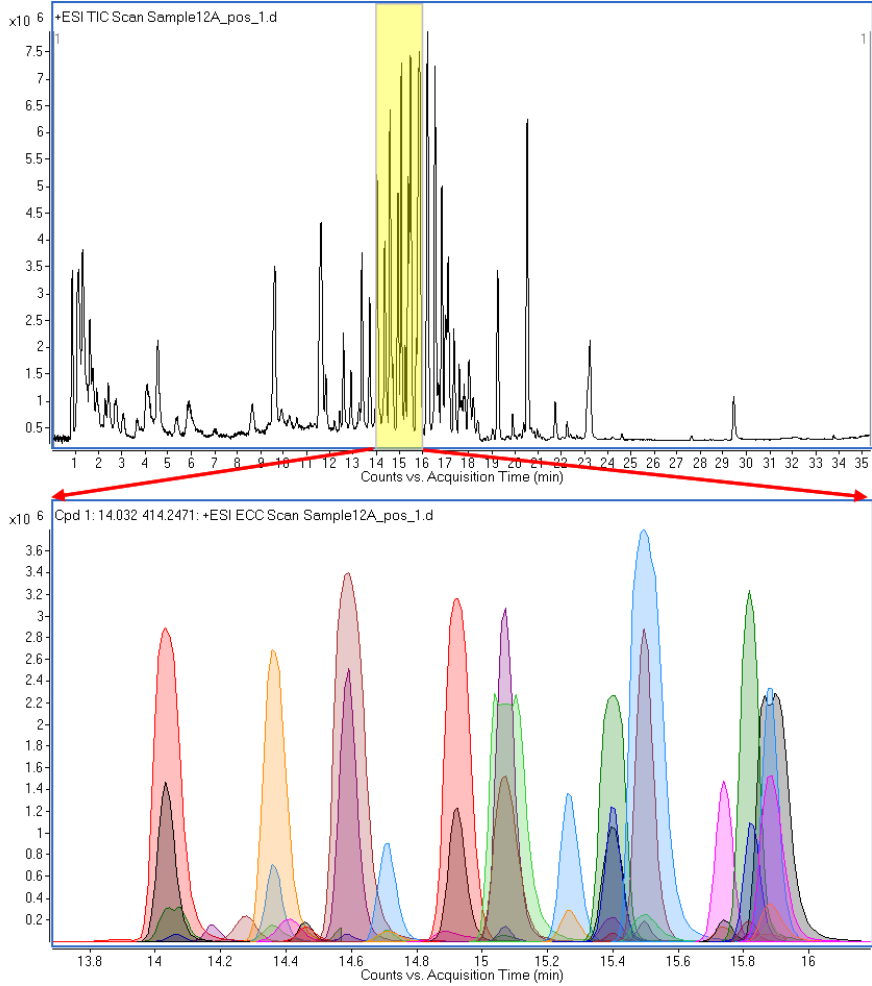


| Compound List                       |     |                    |            |        |          |          |       |          |          |        |         |                 |           |      |           |  |
|-------------------------------------|-----|--------------------|------------|--------|----------|----------|-------|----------|----------|--------|---------|-----------------|-----------|------|-----------|--|
| Show/Hide                           | Cpd | File               | Formula    | RT     | m/z      | Mass     | Score | Diff (T) | Polarity | Height | Area    | Algorithm       | Flag Seve | Ions | ID Source |  |
| <input checked="" type="checkbox"/> | 1   | WaterExtract_005.d | C12 H14    | 14.12  | 159.1168 | 158.1095 | 99.96 | -0.16    | Positive | 185405 | 2016646 | Find By Formula | Pass      | 3    | FBF       |  |
| <input checked="" type="checkbox"/> | 2   | WaterExtract_005.d | C11 H12 O  | 13.057 | 161.0961 | 160.0889 | 99.32 | 0.7      | Positive | 285440 | 3080654 | Find By Formula | Pass      | 3    | FBF       |  |
| <input checked="" type="checkbox"/> | 3   | WaterExtract_005.d | C12 H16 O  | 14.558 | 177.1275 | 176.1202 | 99.73 | 0.55     | Positive | 81152  | 1097967 | Find By Formula | Pass      | 3    | FBF       |  |
| <input checked="" type="checkbox"/> | 4   | WaterExtract_005.d | C11 H14 O2 | 13.057 | 179.1067 | 178.0993 | 99.62 | -0.19    | Positive | 75401  | 956647  | Find By Formula | Pass      | 3    | FBF       |  |
| <input checked="" type="checkbox"/> | 5   | WaterExtract_005.d | C13 H18 O  | 14.558 | 191.1427 | 190.1354 | 98.18 | -1.79    | Positive | 3962   | 47566   | Find By Formula | Pass      | 3    | FBF       |  |

*Approach 2:  
Molecular Feature  
Extractor  
(MFE)*



# The Workflow: Automated Data Mining Tools

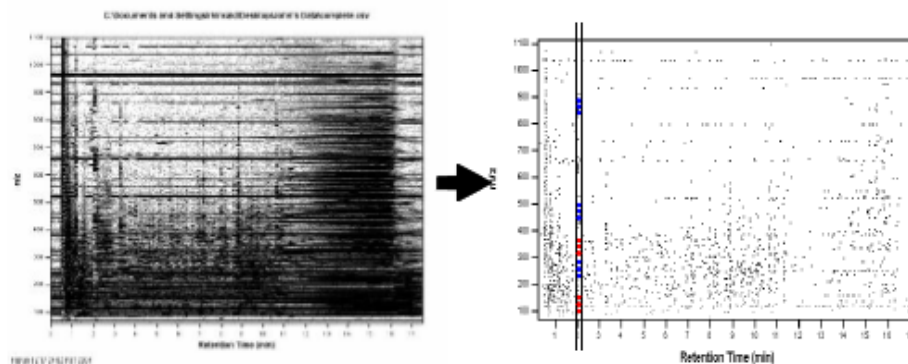
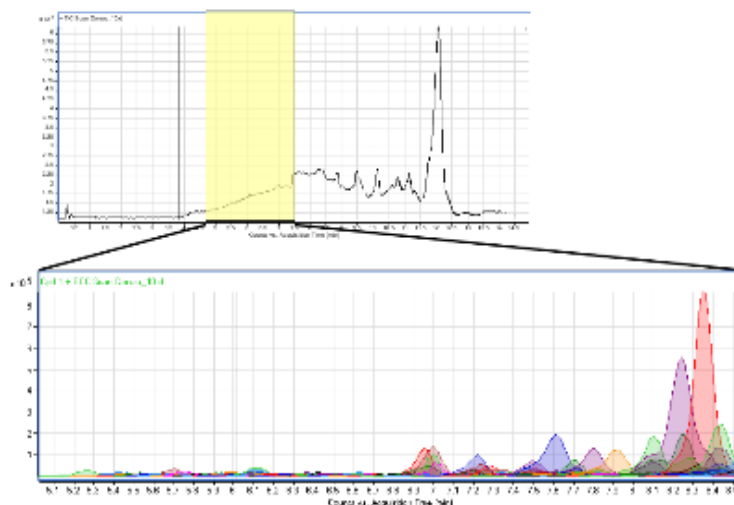


## MassHunter Molecular Feature Extraction

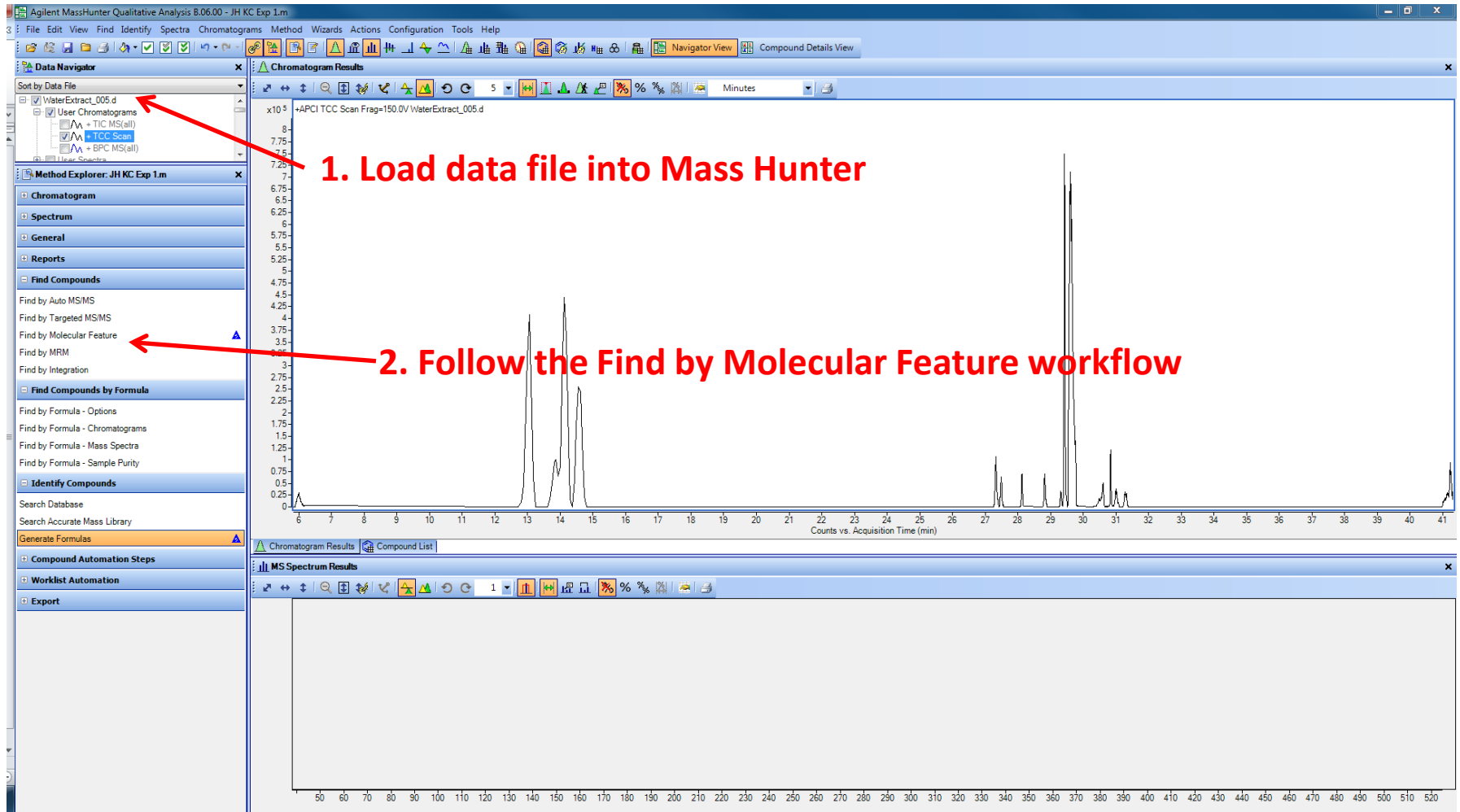
# Molecular Feature Extraction: Peak Finding Algorithm

Data is processed by proprietary feature finding algorithm

- Find chromatographic peaks
  - Find all ions that are related
  - Include any adducts, such as  $\text{Na}^+$  or  $\text{K}^+$
  - Include isotopes ( $[\text{M}+\text{H}]^+$ ,  $[\text{M}+\text{H}+1]^+$ , ...)
  - Check for dimers
  - Create a compound chromatograms (ECC) and spectra
- Sum all ion signals into one value (Feature)
- Fully automated processing
- Create data file for export



# Qualitative Analysis Water Extracts: MFE



# Qualitative Analysis Water Extracts: MFE

Method Editor: Find Compounds by Molecular Feature

Find Compounds by Molecular Feature

Extraction Ion Species Charge State Compound Filters Mass Filters Mass Defect Peak Filters (MS/MS) Results

Extraction algorithm

Target data type **Small molecules**

Input data range

Restrict retention time to

Restrict m/z to

Peak filters

Use peaks with signal-to-noise (Profile spectra only)

Use peaks with height (Profile and centroid spectra)

Allowed ion species

Positive ions

- +H
- +Na
- +K
- +NH4

Negative ions

- H
- Cl
- Br
- HCOO
- CH3COO
- CF3COO

Neutral losses

- H2O
- H3PO4

Isotope grouping

Peak spacing tolerance: 0.0025

Isotope model: Common organic

Charge state

Limit assigned charge states to a maximum

Treat ions with unassigned charge as singly

Height

- Relative height  $\geq$  2.500 %
- Absolute height  $\geq$  500 counts
- Limit to the largest 100 compounds

Compound quality

Quality score  $\geq$  50.00

Compound location

Restrict retention times to minutes

Charge states

Restrict charge states to Z

Ability to add r

# Qualitative Analysis Water Extracts: MFE

Method Editor: Find Compounds by Molecular Feature

Find Compounds by Molecular Feature | Method Items

Extraction | Ion Species | Charge State | Compound Filters | Mass Filters | Mass Defect | Peak Filters (MS/MS) | Results

Mass filters

Filter mass list  ppm

Include only these mass(es)

Source of masses

These masses:

(type a comma-separated list of masses like \*142.1012, 253.4003)

Database

D:\MassHunter\PCDL\default.csv

Method Editor: Find Compounds by Molecular Feature

Find Compounds by Molecular Feature | Method Items

Extraction | Ion Species | Charge State | Compound Filters | Mass Filters | Mass Defect | Peak Filters (MS/MS) | Results

Previous results

Delete previous compounds

New results

Highlight first compound

Highlight all compounds

Chromatograms and spectra

Extract MFE spectrum

Extract ECC

Extract raw spectrum

Extract EIC

Prefer profile for raw spectrum, if available

Clip extracted raw spectrum

Asymmetric (m/z) -  +

Extract MS/MS Spectrum

Extract separate MS/MS spectrum per collision energy

Extract average MS/MS spectrum for all collision energies

Method Editor: Find Compounds by Molecular Feature

Find Compounds by Molecular Feature | Method Items

Extraction | Ion Species | Charge State | Compound Filters | Mass Filters | Mass Defect | Peak Filters (MS/MS) | Results

Height filters

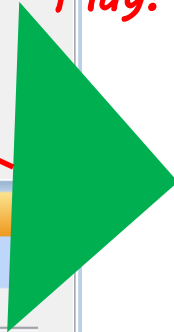
Absolute height  $\geq$   counts

Relative height  $\geq$   % of largest peak

Maximum number of peaks

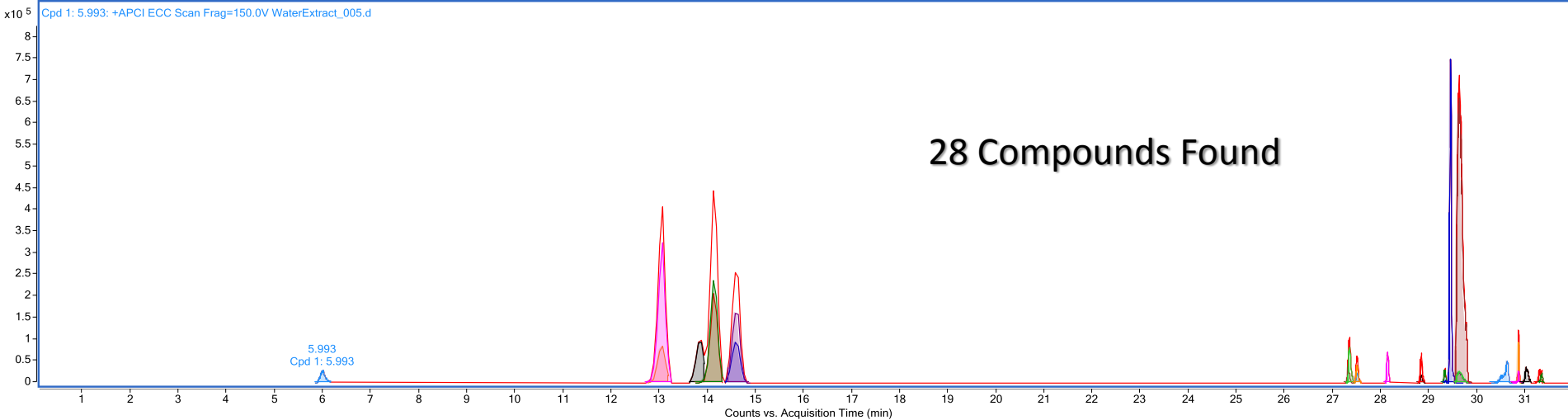
Limit (by height) to the largest

*Press Play!*



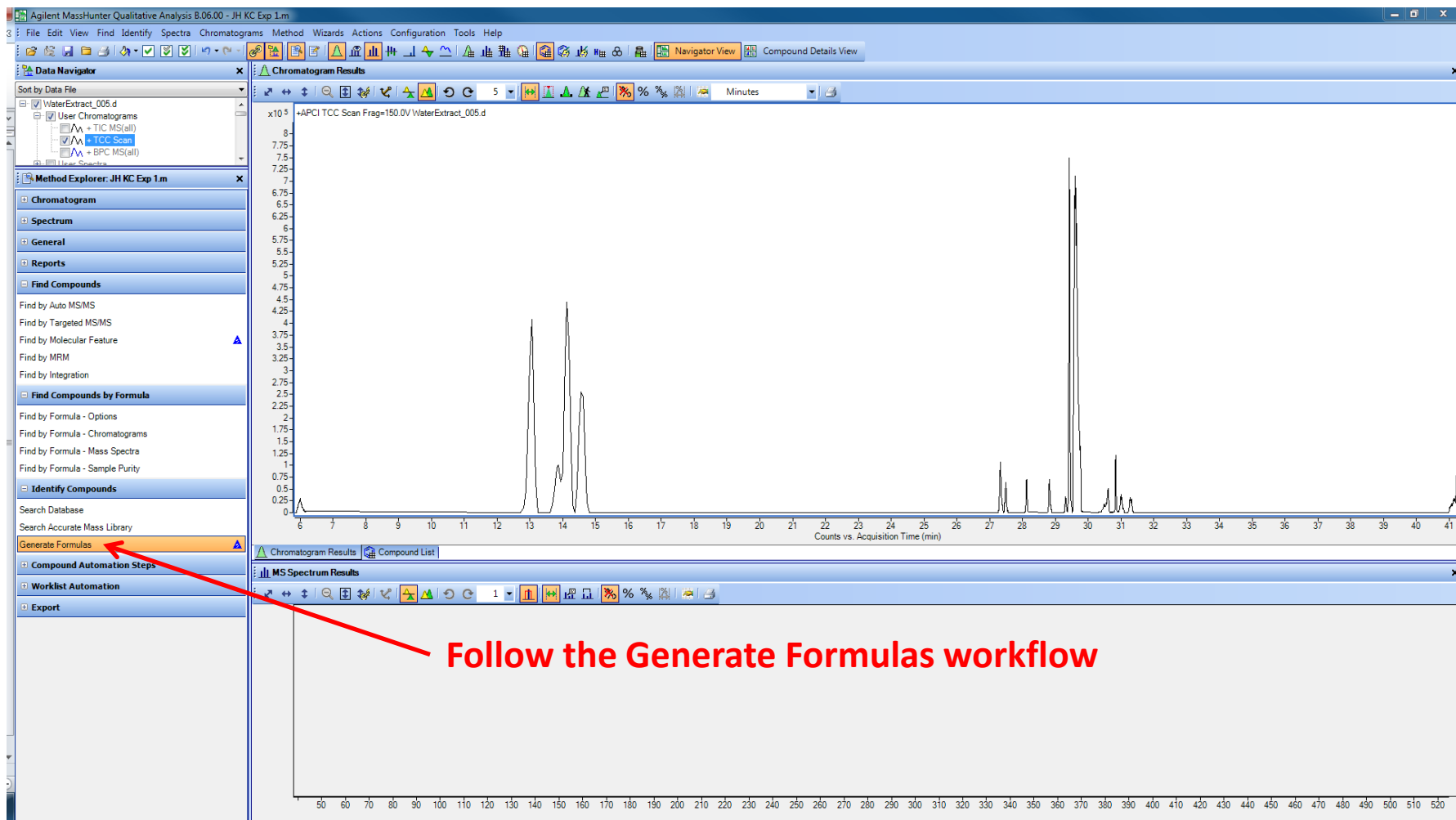
# Qualitative Analysis MFE Results: Water Extracts

Overlaid Extracted Compound Chromatograms of Compounds resulting from the parameters set in MFE peak picking algorithm



Next step: Generate formulae from compounds

# Qualitative Analysis: Generate Formulae from MFE Results



# Qualitative Analysis: Generate Formulae from MFE

## Results

Method Editor: Generate Formulas

Generate Formulas from Compound

Allowed Species Limits Charge State Fragment Formulas Scoring

Charge carrier to be assumed if not known

Positive ions:

- electron
- +H
- +Na
- +K
- +NH4
- +C2H5
- +C3H5

Negative ions:

- +electron
- H
- +Cl
- +Br
- +COOH
- +CF3COO
- +CH3COOH

MS ion electron state: even electron

Group hits with same formula (but different charge carriers)

Elements and limits

| Element | Minimum | Maximum |
|---------|---------|---------|
| C       | 3       | 60      |
| H       | 0       | 120     |
| O       | 0       | 30      |
| N       | 0       | 30      |
| S       | 0       | 5       |
| Cl      | 0       | 3       |

Method Editor: Generate Formulas

Generate Formulas from Compound

Allowed Species Limits Charge State Fragment Formulas Scoring

Limits on input masses

Maximum neutral mass for which formulas should be calculated: 750.0000

Limits on results

Minimum overall charge carrier

Maximum MS mass

Require DBE from

Maximum number charge carrier

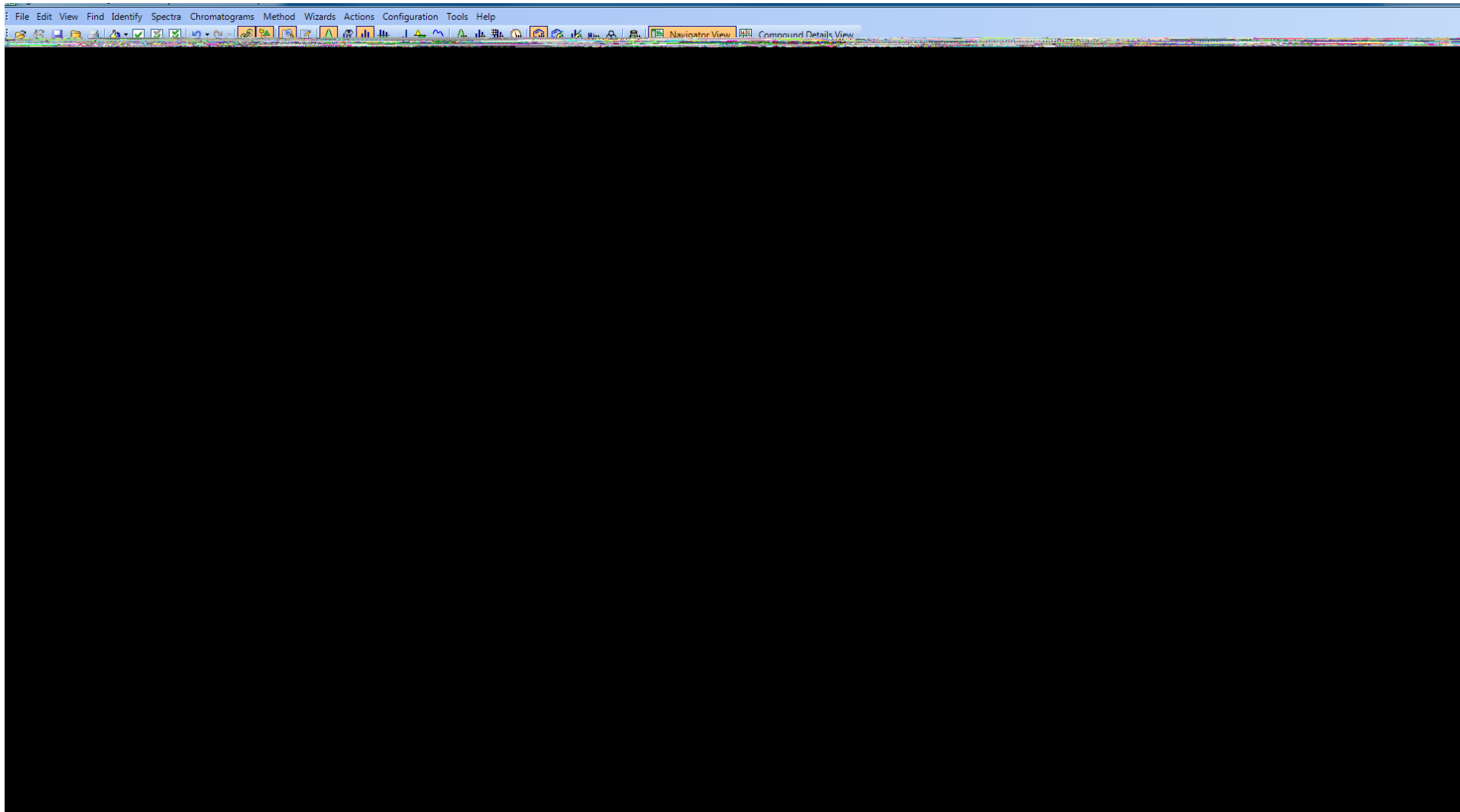
Contribution to overall score

|                         |        |
|-------------------------|--------|
| Mass score              | 100.00 |
| Isotope abundance score | 60.00  |
| Isotope spacing score   | 50.00  |
| Retention time score    | 100.00 |

Expected data variation

|                       |     |     |   |       |     |
|-----------------------|-----|-----|---|-------|-----|
| MS mass:              | 2.0 | mDa | + | 5.6   | ppm |
| MS isotope abundance: |     |     |   | 7.5   | %   |
| MS/MS mass:           | 5.0 | mDa | + | 7.5   | ppm |
| Retention time:       |     |     |   | 0.115 | min |

# Qualitative Analysis: Formula Generation Results

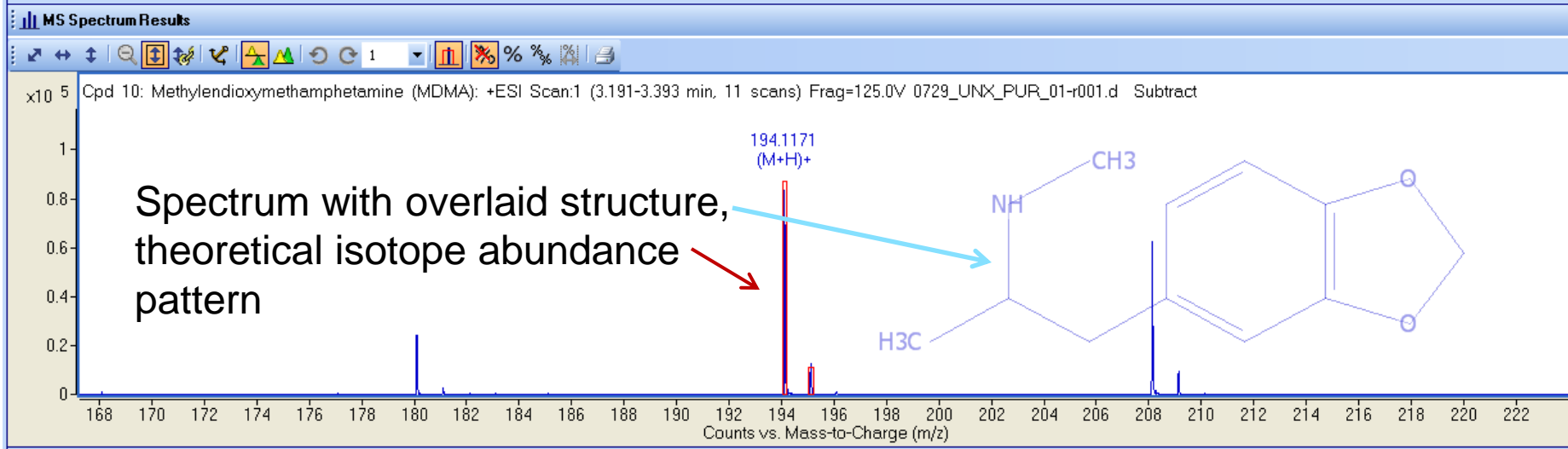
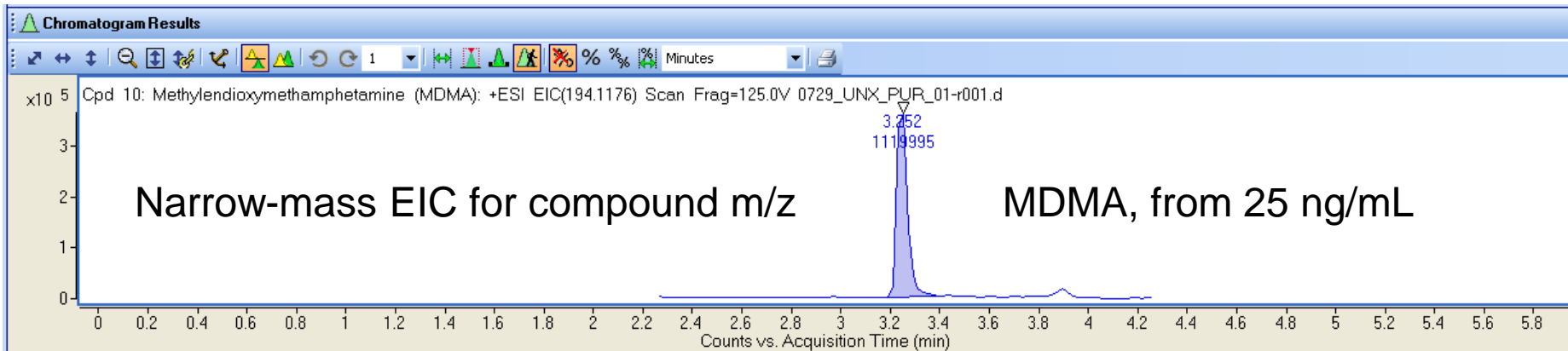


# Qualitative Analysis: Formula Generation Results

| Show/Hide                           | Cpd    | File               | ID Source | Formula      | RT     | m/z      | Mass     | Score | Di    | Ions | Height | Score (Lib) | Score (DB) | S |
|-------------------------------------|--------|--------------------|-----------|--------------|--------|----------|----------|-------|-------|------|--------|-------------|------------|---|
| <input checked="" type="checkbox"/> | 1      | WaterExtract_005.d | MFG       | C8 H8 O2     | 5.993  | 137.0598 | 136.0525 | 99.66 | -0.78 | 3    | 27242  | 99.66       |            |   |
| <input checked="" type="checkbox"/> | 23     | WaterExtract_005.d | MFG       | C8 H4 O3     | 30.842 | 149.0216 | 148.0143 | 71.3  | 11.53 | 2    | 24725  | 71.3        |            |   |
| <input checked="" type="checkbox"/> | 15     | WaterExtract_005.d | MFG       | C8 H4 O3     | 28.824 | 149.0227 | 148.0154 | 97.48 | 4.05  | 3    | 46558  | 97.48       |            |   |
| Best                                |        |                    |           |              |        |          |          |       |       |      |        |             |            |   |
| <input checked="" type="checkbox"/> | MFG    |                    |           | C8 H4 O3     | 97.48  |          |          | 4.05  |       |      |        | 97.48       |            |   |
| m/z                                 |        |                    |           |              |        |          |          |       |       |      |        |             |            |   |
| <input checked="" type="checkbox"/> | 149.02 | (M+H)+             |           | C8 H5 O3     | 97.48  |          |          | 97.48 | 95.62 |      | 98.92  | 99.48       | 46558.5    |   |
| Show/Hide                           | Cpd    | File               | ID Source | Formula      | RT     | m/z      | Mass     | Score | Di    | Ions | Height | Score (Lib) | Score (DB) | S |
| <input checked="" type="checkbox"/> | 17     | WaterExtract_005.d | MFG       | C8 H4 O3     | 29.431 | 149.0233 | 148.0161 | 99.85 | -0.15 | 3    | 690141 | 99.85       |            |   |
| <input checked="" type="checkbox"/> | 5      | WaterExtract_005.d | MFG       | C12 H14      | 14.128 | 159.1168 | 158.1095 | 90.05 | 0.27  | 3    | 185699 | 90.05       |            |   |
| <input checked="" type="checkbox"/> | 8      | WaterExtract_005.d | MFG       | C12 H14      | 14.581 | 159.117  | 158.1097 | 90.04 | -0.92 | 5    | 140592 | 90.04       |            |   |
| <input checked="" type="checkbox"/> | 3      | WaterExtract_005.d | MFG       | C11 H12 O    | 13.039 | 161.0962 | 160.089  | 95.74 | -1.06 | 4    | 285557 | 95.74       |            |   |
| <input checked="" type="checkbox"/> | 4      | WaterExtract_005.d | MFG       | C11 H12 O    | 13.837 | 161.0962 | 160.089  | 88.87 | -1.25 | 3    | 84073  | 88.87       |            |   |
| <input checked="" type="checkbox"/> | 14     | WaterExtract_005.d | MFG       | C10 H8 O3    | 28.824 | 177.0541 | 176.0468 | 97.98 | 3.09  | 3    | 17472  | 97.98       |            |   |
| <input checked="" type="checkbox"/> | 6      | WaterExtract_005.d | MFG       | C12 H16 O    | 14.133 | 177.1276 | 176.1203 | 99.59 | -1.01 | 4    | 207372 | 99.59       |            |   |
| <input checked="" type="checkbox"/> | 7      | WaterExtract_005.d | MFG       | C12 H16 O    | 14.576 | 177.1276 | 176.1203 | 99.3  | -0.92 | 3    | 81097  | 99.3        |            |   |
| <input checked="" type="checkbox"/> | 2      | WaterExtract_005.d | MFG       | C11 H14 O2   | 13.032 | 179.1067 | 178.0993 | 97.12 | 0.25  | 3    | 75380  | 97.12       |            |   |
| <input checked="" type="checkbox"/> | 16     | WaterExtract_005.d | MFG       | C13 H22 O    | 29.313 | 195.1738 | 194.1665 | 96.79 | 3.02  | 3    | 25836  | 96.79       |            |   |
| <input checked="" type="checkbox"/> | 9      | WaterExtract_005.d | MFG       | C12 H21 N... | 27.31  | 196.1695 | 195.1623 | 86.6  | 0.3   | 2    | 32882  | 86.6        |            |   |
| <input checked="" type="checkbox"/> | 10     | WaterExtract_005.d | MFG       | C12 H23 N... | 27.335 | 198.1851 | 197.1778 | 99.8  | 0.88  | 3    | 73554  | 99.8        |            |   |
| <input checked="" type="checkbox"/> | 12     | WaterExtract_005.d | MFG       | C13 H11 N... | 27.49  | 214.0894 | 213.0818 | 63.05 | -13.2 | 3    | 34742  | 63.05       |            |   |
| <input checked="" type="checkbox"/> | 13     | WaterExtract_005.d | MFG       | C18 H30 O3   | 28.125 | 295.2266 | 294.2193 | 99.83 | 0.59  | 4    | 57750  | 99.83       |            |   |
| <input checked="" type="checkbox"/> | 25     | WaterExtract_005.d | MFG       | C22 H44 N... | 31.281 | 369.3484 | 368.3411 | 92.81 | -2.24 | 3    | 21926  | 92.81       |            |   |
| <input checked="" type="checkbox"/> | 22     | WaterExtract_005.d | MFG       | C24 H38 O4   | 30.842 | 391.2829 | 390.2756 | 94.5  | 3.65  | 4    | 70912  | 94.5        |            |   |
| <input checked="" type="checkbox"/> | 24     | WaterExtract_005.d | MFG       | C22 H38 N... | 31.01  | 419.3131 | 418.306  | 82.05 | -0.89 | 2    | 29684  | 82.05       |            |   |
| <input checked="" type="checkbox"/> | 26     | WaterExtract_005.d | MFG       | C28 H46 O4   | 31.326 | 447.3457 | 446.3385 | 77.55 | 2.47  | 2    | 17710  | 77.55       |            |   |
| <input checked="" type="checkbox"/> | 19     | WaterExtract_005.d | MFG       | C20 H38 N... | 29.617 | 487.2974 | 486.2903 | 93.37 | 2.42  | 8    | 487688 | 93.37       |            |   |

Identifies isomers as separate entities due to different retention times

# Typical Target Compound Screening Result

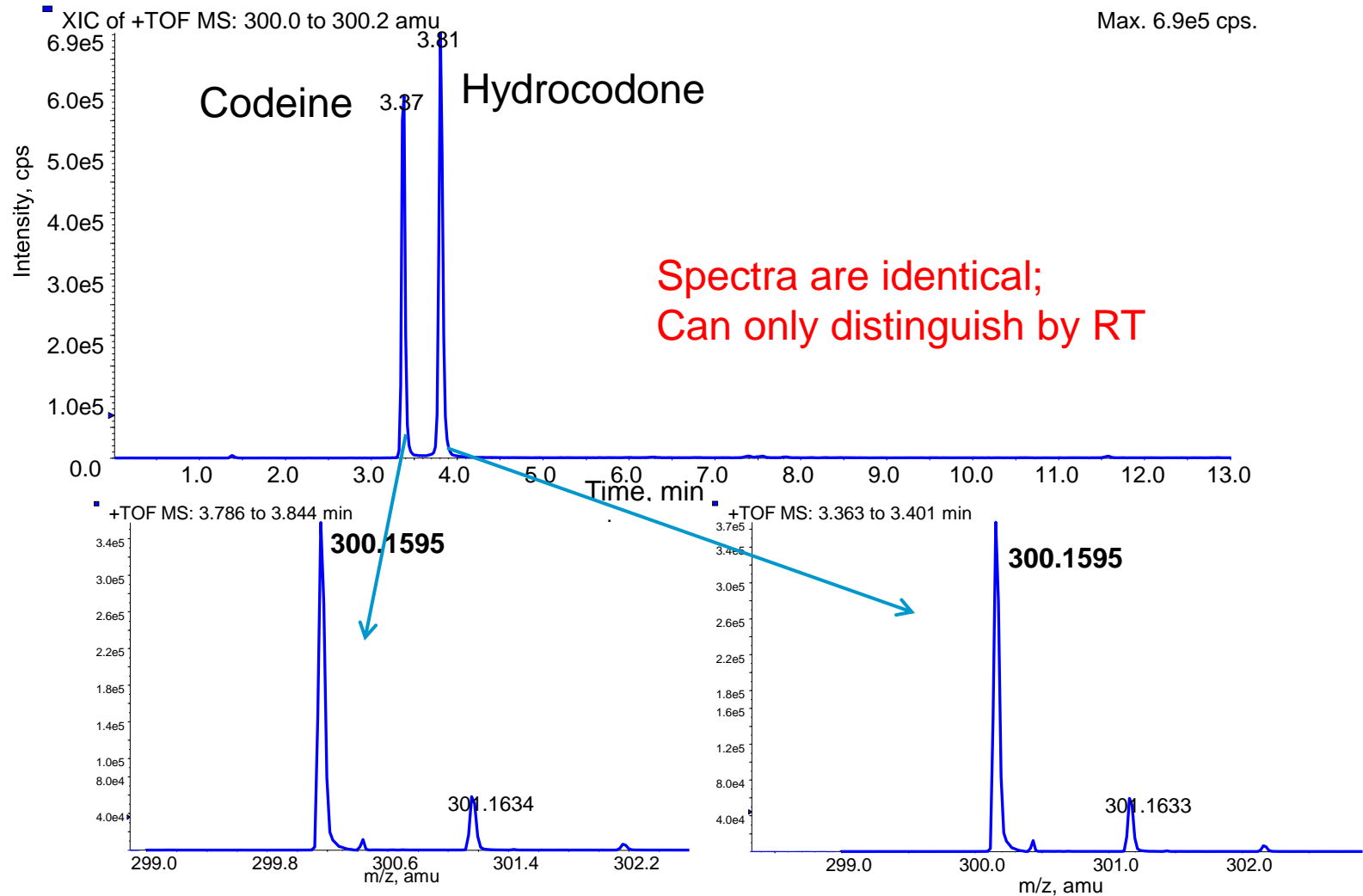


## Text Result and Figures of Merit

**DB Search Results: Cpd 10: Methylenedioxyamphetamine (MDMA)**

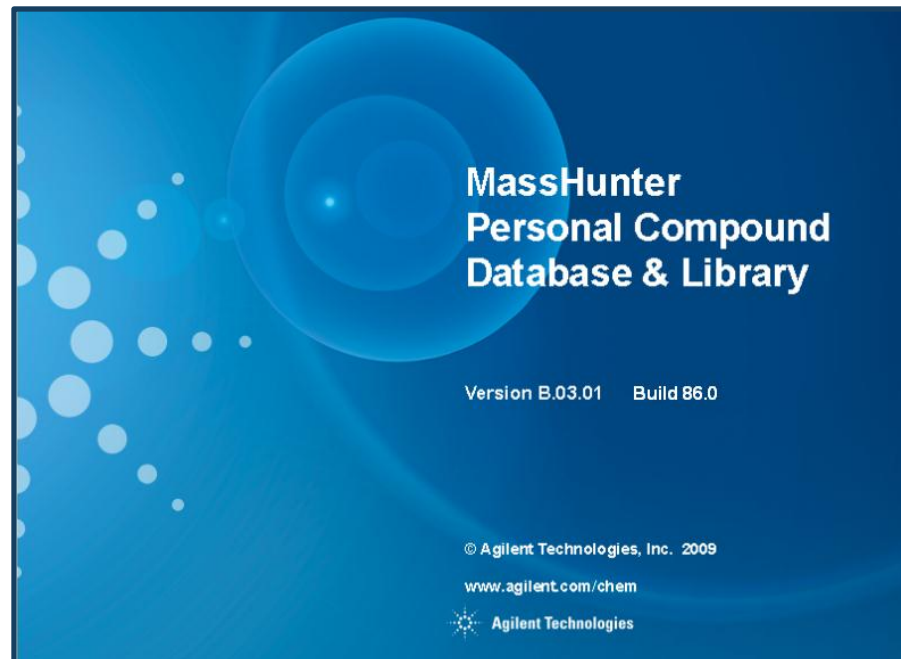
| Best                                | Name                             | Formula      | Mass     | Mass (DB) | Diff (ppm) | RT    | RT Diff | RT Match | Mass Match | Spacing Match | Abund Match | Score |
|-------------------------------------|----------------------------------|--------------|----------|-----------|------------|-------|---------|----------|------------|---------------|-------------|-------|
| <input checked="" type="checkbox"/> | Methylenedioxyamphetamine (MDMA) | C11 H15 N O2 | 193.1099 | 193.1103  | 2.16       | 3.252 | 0.006   | 99.61    | 98.19      | 10            | 91.82       | 83.19 |

# Chromatography still matters: Identification of isobaric compounds $C_{18}H_{21}NO_3$



# Personal Compound Database (PCDL)

- Fully integrated Agilent solution
- METLIN Metabolite-specific database content
- Contains over 25,000 compounds
- Includes 8,000 lipids
- Manual and batch searches
- Query based on monoisotopic mass, formula, name or retention time
- Build customized database using your own compounds and assign retention time



Optional accurate mass & retention time (AMRT) database based on the analysis of hundreds of standards

# PCDL Manager B.04

- Newest version automatically converts previous PCDL's to B.04 format, saves original as [filename].cdb.bk backup
- Includes ability to use Agilent QTOF MS/MS PCDL database/libraries, or to add user spectra to custom cdb.
- Easier to make custom databases with "Create subset PCDL"
  1. Search and select entries
  2. R-click in listing, select Create (to make new cdb) or Append to (existing) PCDL.

| Notes                               | Compound Name               | Formula  | Mass      | Anion | Cation | RT (min) | CAS        | ChemSpider |
|-------------------------------------|-----------------------------|----------|-----------|-------|--------|----------|------------|------------|
| Alkaloid                            | Nicotine                    | C10H14N2 | 162.11570 |       |        | 1.386    | 54-11-5    | 80863      |
| Alkaloid, used as insecticide       | Anabasine                   | C10H14N2 | 162.11570 |       |        | 1.885    | 494-52-9   | 178119     |
| Amiodarone metabolite, RT estimated | Desethylamiodarone          | C23H29O2 | 616.99238 |       |        | 5.226    | 83409-32-8 |            |
| Analgesic                           | Acetaminophen (paracetamol) | C8H9NO2  | 151.06333 |       |        | 2.312    | 103-90-2   | 1906       |
| Analgesic                           | Ibuprofen                   | C13H18O2 | 206.13068 |       |        | 5.309    | 15687-27-1 | 3644       |
| Analgesic                           | Naproxen                    | C14H14O3 | 230.09429 |       |        | 4.821    | 22204-53-1 | 137720     |
| Analgesic                           | Tramadol                    | C16H25N  | 263.18853 |       |        | 3.325    | 27203-92-5 | 31105      |
| Analgesic                           | Pentazocine                 | C19H27NO | 285.20926 |       |        | 3.612    | 399-83-1   | 390041     |
| Analgesic, at morphine              | Nomependine                 | C14H19N  | 233.14158 |       |        | 3.553    | 77-17-8    | 30039      |
| Analgesic, non-narcotic             | Amantadine                  | C10H17N  | 151.13610 |       |        | 3.051    | 788-84-5   |            |
| Analgesic/Dermatic                  | Salicylic acid              | C7H6O3   | 138.03169 |       |        | 3.313    | 69-72-7    | 331        |
| Anesthetic                          | Hexobarbital                | C12H16N2 | 236.11609 |       |        | 3.522    | 56-29-1    | 3482       |

|                |                       |
|----------------|-----------------------|
| Anticonvulsant | Clonazepam            |
| Anticonvulsant | Eterobarb             |
| Anticonvulsant | Progabide             |
| Anticonvulsant | Benzobarbital         |
| Anticonvulsant | Decimemide            |
| Anticonvulsant | Topiramate            |
| Anticonvulsant | Pinqalone             |
| Anticonvulsant | Suclofenide           |
| Anticonvulsant | Phenytoin-3-norvaline |
| Anticonvulsant | Ropizine              |
| Anticonvulsant | Nabazenil             |
| Anticonvulsant | Ramoplanin            |

# MassHunter PCDL: AMRT Database for Forensics

The screenshot displays the MassHunter PCDL software interface. The top menu includes File, Edit, View, Database/Library, Links, and Help. Below the menu are tabs for Single Search, Batch Search, Batch Summary, Edit Compounds, Spectral Search, Browse Spectra, and Edit Spectra. The main search area contains several input fields: Mass (with radio buttons for [M+H]<sup>+</sup>, Neutral, and [M-H]<sup>-</sup>), Mass tolerance (10.0 ppm), Retention time (with a 'Require' checkbox and a 0.1 min tolerance), and Ion search mode (with checkboxes for Include neutrals, Include anions, and Include cations). On the right, there are fields for Formula, Name (THC), Notes, IUPAC, CAS, and ChemSpider. A 'Molecule' section shows a chemical structure of THC and a 'Notes' field containing 'Cannabinoid metabolite'. Red arrows point from the search criteria to the results table below. The results table has columns for Compound Name, Formula, Mass, RT (min), CAS, and Notes. The first result is 11-Hydroxy-THC with a note of 'Cannabinoid metabolite'. A large text box on the right states: 'Approximately 7600 drugs, metabolites, potentially toxic chemicals'.

**Searchable fields**

**Molecular formula Ret time**

**Structure**

**Compound class**

**Single Search Results: 60 hits**

| Compound Name                            | Formula    | Mass      | RT (min) | CAS                          | Notes                        |
|--|------------|-----------|----------|------------------------------|------------------------------|
| 11-Hydroxy-THC                           | C21H30O3   | 330.21949 | 5.707    | <a href="#">36557-05-9</a>   | Cannabinoid metabolite       |
| 11-nor-9-Carboxy-tetrahydrocannabinol    | C21H28O4   | 344.19876 | 5.766    | <a href="#">64280-14-4</a>   | Cannabinoid metabolite       |
| 3,4-Methylenedioxyamphetamine (MDA)      | C10H13NO2  | 179.09463 | 2.830    | <a href="#">4764-17-4</a>    | PsychedelicDesignerDrug      |
| 3,4-Methylenedioxyamphetamine (MDEA)     | C12H17NO2  | 207.12593 | 3.037    | <a href="#">82801-81-8</a>   | PsychedelicDesignerDrug      |
| 3,4-Methylenedioxymethamphetamine (MDMA) | C11H15NO2  | 193.11028 | 2.868    | <a href="#">42542-10-9</a>   |                              |
| 6-Monoacetylmorphine                     | C19H21NO4  | 327.14706 | 2.736    | <a href="#">2784-73-8</a>    |                              |
| 7-Aminoclonazepam                        | C15H12ClN2 | 285.06689 | 3.427    | <a href="#">4959-17-5</a>    | Benzodiazepine metabolite    |
| 7-Amino-Flunitrazepam                    | C16H14FN3  | 283.11209 | 3.694    | <a href="#">34084-50-9</a>   | Benzodiazepine metabolite    |
| alpha-Hydroxyalprazolam                  | C17H13ClN4 | 324.07779 | 4.658    | <a href="#">37115-43-8</a>   | Benzodiazepine metabolite    |
| alpha-Hydroxyethylflurazepam             | C17H14ClN3 | 332.07278 | 4.751    | <a href="#">23071-53-3</a>   |                              |
| alpha-Hydroxytriazolam                   | C17H12ClN4 | 358.03882 | 4.586    | <a href="#">37115-45-0</a>   | 4-hydroxytriazolam           |
| Alprazolam                               | C17H13ClN4 | 308.08287 | 4.785    | <a href="#">28981-97-7</a>   | Benzodiazepine metabolite    |
| Amobarbital                              | C11H18N2O3 | 226.13174 | 4.552    | <a href="#">57-43-2</a>      | Hypnotic                     |
| Amphetamine                              | C9H13N     | 135.10480 | 2.781    | <a href="#">300-52-9</a>     | Stimulant                    |
| Benzoylcegonine                          | C16H19NO4  | 289.13141 | 3.275    | <a href="#">519-09-5</a>     | LocalAnestheticAddictiveDrug |
| Buprenorphine                            | C29H41NO4  | 467.30356 | 4.312    | <a href="#">52485-79-7</a>   | Opioid                       |
| Buprenorphine 3-glucuronide              | C35H49NO10 | 643.33565 | 3.829    | <a href="#">101224-2-...</a> |                              |
| Butalbital                               | C11H16N2O3 | 224.11609 | 4.291    | <a href="#">77-26-9</a>      | Hypnotic                     |

Approximately 7600 drugs, metabolites, potentially toxic chemicals

# Create Custom PCDL

MassHunter PCDL Manager - C:\MassHunter\PCDL\Scripts.cdb

File Edit View PCDL Links Help

Find Compounds

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

Masses: File ... Clear

| Mass | RT | Hits |
|------|----|------|
|------|----|------|

Masses:  [M+H]<sup>+</sup>  Neutral  [M-H]<sup>-</sup>

Mass tolerance: 10.0  ppm  mDa

Molecule: Structure MOL Text

Ion search mode

- Include neutrals
- Include anions
- Include cations

Notes:

1 Create new PCDL from empty Or create from a master

2 Name the custom PCDL

PCDL path: C:\MassHunter\PCDL

Select an existing PCDL:

- Empty
- ForensicsTox\_AM\_PCD
- ForensicsTox\_AM\_PCD\_JC
- HMDB\_v2.5
- Lipids03
- Metabolomics Test
- Metlin\_AMRT\_PCD
- Metlin\_AM\_PCD
- NPL5
- Pesticides\_AM\_PCD
- PlantCycPCD

General

Enter the new PCDL name: Empty

Enter a description of this PCDL:

Create Cancel

Search Results

| Best | RT (min) | IUPAC Name |
|------|----------|------------|
|------|----------|------------|

# Export Compound List to Excel

After data analysis using Find by Formula with Database search or MFE / MFG

The screenshot displays a software interface with a table of compound data, a dialog box for exporting to CSV, and a chromatogram plot.

**Table Data:**

| Show/Hide                           | Cpd | File            | Formula    | m/z      | Mass     | RT     | Score | Diff (MFG, ppm) | ID Techniq | Ions | Height | Polarity | End    | Mass (MFG) | Score (MFG) |
|-------------------------------------|-----|-----------------|------------|----------|----------|--------|-------|-----------------|------------|------|--------|----------|--------|------------|-------------|
| <input checked="" type="checkbox"/> | 38  | NPL31000_001.d  | C27 H49 N  | 388.3941 | 387.3867 | 23.053 | 99.69 | -0.57           | MFG        | 3    | 45225  | Positive | 23.167 | 387.3865   | 99.69       |
| <input checked="" type="checkbox"/> | 40  | NPL41000_001.d  | C27 H49 N  | 388.3943 | 387.387  | 23.051 | 99.24 | -1.23           | MFG        | 3    | 43628  | Positive | 23.181 | 387.3865   | 99.24       |
| <input type="checkbox"/>            | 34  | NPL51000_001.d  | C27 H49 N  | 388.3945 | 387.3872 | 23.041 | 98.62 | -1.7            | MFG        | 3    | 42845  | Positive | 23.197 | 387.3865   | 98.62       |
| <input checked="" type="checkbox"/> | 45  | NPL1_1000_001.d | C26 H47 N5 | 430.3902 | 429.3835 | 22.956 | 98.15 | -0.84           | MFG        | 3    | 47761  | Positive | 23.057 | 429.3831   | 98.15       |
| <input checked="" type="checkbox"/> | 53  | NPL21000_001.d  | C26 H47 N5 | 430.3892 | 429.383  | 22.972 | 93.62 | 0.33            | MFG        | 3    | 37993  | Positive | 23.101 | 429.3831   | 93.62       |
| <input checked="" type="checkbox"/> | 37  | NPL31000_001.d  | C26 H47 N5 | 430.3898 | 429.3829 | 22.981 | 96.98 | 0.64            | MFG        | 3    | 39852  | Positive | 23.076 | 429.3831   | 96.98       |
| <input checked="" type="checkbox"/> | 39  | NPL41000_001.d  |            |          |          |        |       |                 | FG         | 3    | 42428  | Positive | 23.065 | 429.3831   | 97.78       |
| <input type="checkbox"/>            | 33  | NPL51000_001.d  |            |          |          |        |       |                 | FG         | 3    | 41330  | Positive | 23.089 | 429.3831   | 96.69       |
| <input type="checkbox"/>            | 54  | NPL21000_001.d  |            |          |          |        |       |                 | FG         | 2    | 24862  | Positive | 23.159 | 399.3712   | 82.31       |
| <input checked="" type="checkbox"/> | 25  | NPL1_1000_001.d |            |          |          |        |       |                 | FG         | 5    | 61772  | Positive | 12.154 | 452.3363   | 99.52       |
| <input checked="" type="checkbox"/> | 32  | NPL21000_001.d  |            |          |          |        |       |                 | FG         | 6    | 36206  | Positive | 12.579 | 452.3363   | 97.48       |
| <input checked="" type="checkbox"/> | 17  | NPL31000_001.d  |            |          |          |        |       |                 | FG         | 5    | 65854  | Positive | 12.496 | 452.3363   | 98.95       |
| <input checked="" type="checkbox"/> | 19  | NPL41000_001.d  |            |          |          |        |       |                 | FG         | 5    | 69201  | Positive | 12.517 | 452.3363   | 99.26       |
| <input checked="" type="checkbox"/> | 13  | NPL51000_001.d  |            |          |          |        |       |                 | FG         | 6    | 69362  | Positive | 12.476 | 452.3363   | 99.53       |
| <input checked="" type="checkbox"/> | 44  | NPL1_1000_001.d |            |          |          |        |       |                 | FG         | 3    | 54314  | Positive | 23.14  | 368.3767   | 98.34       |

**Export Compound Summary CSV Options Dialog:**

- List of opened data files:
  - NPL1\_1000\_001.d
  - NPL21000\_001.d
  - NPL31000\_001.d
  - NPL41000\_001.d
  - NPL51000\_001.d
- Export contents:
  - Only highlighted results
  - All results
- Export destination:
  - Worklist
  - Qualitative method
    - One export file per data file
      - At the location of the data file
      - At specified directory (C:\MassHunter\Data)
    - Combine into one export file (c:\temp\exportFile)

- If export file already exists:
- Overwrite existing export file
- Auto-generate new export file name

**Chromatogram Results:**

Cpd 13: C24 H44 N4 O4: +ESI ECC Scan

12.303  
Cpd 13: C24 H44 N4 O4

# Template for Import into PCDL

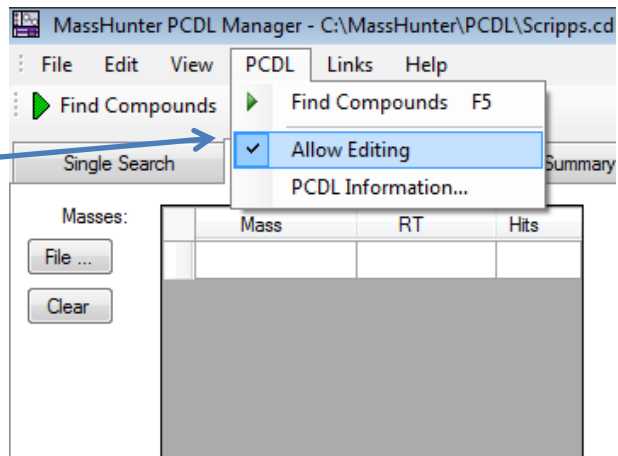
Insert the exported data into  
a **template formatted for  
importing into PCDL**  
**Not intuitive...**

**Note:** PCDL contains  
mass not m/z!

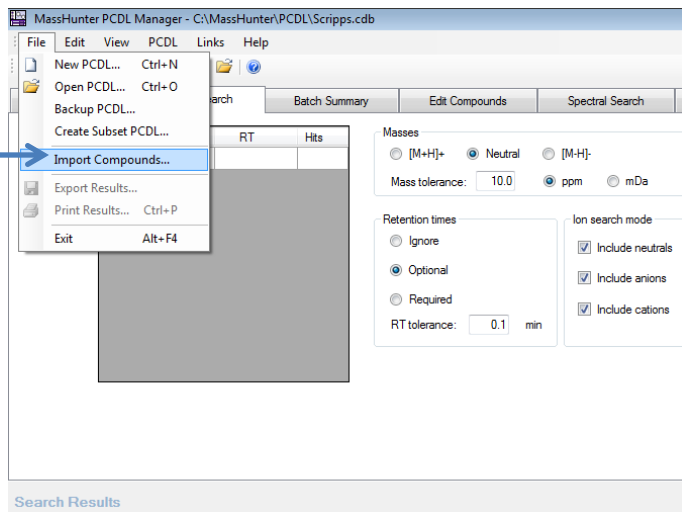
| Compounds |           |          |          |     |       |
|-----------|-----------|----------|----------|-----|-------|
| Formula   | Retention | Mass     | Compound | CAS | Notes |
| C20 H42 O | 10.303    | 458.2728 | 12       |     |       |
| C24 H44 N | 12.303    | 452.3363 | 13       |     |       |
| C26 H47 N | 22.967    | 429.384  | 33       |     |       |
| C27 H49 N | 23.041    | 387.3872 | 34       |     |       |
| C11 H26 N | 21.828    | 378.1481 | 27       |     |       |
| C16 H34 O | 9.116     | 370.2207 | 10       |     |       |
| C23 H48 N | 22.909    | 368.3776 | 32       |     |       |
| C14 H26 O | 8.68      | 354.1528 | 9        |     |       |
| C14 H30 O | 8.383     | 326.1942 | 8        |     |       |
| C20 H10 N | 20.354    | 326.0712 | 22       |     |       |
| C18 H29 N | 21.829    | 323.2101 | 29       |     |       |
| C18 H18 O | 19.193    | 314.1157 | 20       |     |       |
| C19 H20 O | 21.64     | 312.1367 | 25       |     |       |
| C12 H22 O | 7.845     | 310.1268 | 7        |     |       |
| C12 H24 O | 7.68      | 296.1475 | 6        |     |       |
| C19 H41 N | 21.593    | 283.3243 | 24       |     |       |
| C12 H26 O | 7.524     | 282.1683 | 5        |     |       |
| C16 H22 O | 21.83     | 278.1526 | 30       |     |       |
| C16 H22 O | 21.585    | 278.1523 | 23       |     |       |
| C12 H12 N | 17.43     | 272.103  | 18       |     |       |
| C10 H18 O | 6.805     | 266.1004 | 4        |     |       |
| C13 H16 N | 22.577    | 256.1449 | 31       |     |       |
| C10 H20 O | 6.634     | 252.1211 | 3        |     |       |
| C14 H18 O | 19.46     | 250.1208 | 21       |     |       |
| C11 H12 N | 17.364    | 244.1073 | 17       |     |       |
| C10 H8 N6 | 16.799    | 244.072  | 16       |     |       |
| C10 H22 O | 6.484     | 238.1418 | 2        |     |       |
| C14 H31 N | 18.764    | 229.2408 | 19       |     |       |
| C12 H12 O | 21.828    | 204.0791 | 26       |     |       |
| C9 H18 O4 | 14.647    | 190.1207 | 15       |     |       |
| C8 H18 O3 | 12.931    | 162.1257 | 14       |     |       |
| C8 H4 O3  | 21.828    | 148.0163 | 28       |     |       |
|           |           |          |          |     |       |

# Import Compounds into PCDL

3



4



PCDL Information

File name: C:\MassHunter\PCDL\Scripps.cdb

# Compounds: 59

Master or User: User Type: General

Edit allowed: No Last modified: 4/4/2012 4:23:50 PM

Version: 4.0

Description:

Single Search Results: 59 hits

|   | Compound Name | Formula    | Mass      | Anion                    | Cation                   | RT (min) |
|---|---------------|------------|-----------|--------------------------|--------------------------|----------|
| ▶ | 17            | C6H13N     | 99.10480  | <input type="checkbox"/> | <input type="checkbox"/> | 4.000    |
|   | 47            | C8H4O3     | 148.01604 | <input type="checkbox"/> | <input type="checkbox"/> | 21.832   |
|   | 28            | C10H17N    | 151.13610 | <input type="checkbox"/> | <input type="checkbox"/> | 9.005    |
|   | 14            | C8H18O3    | 162.12570 | <input type="checkbox"/> | <input type="checkbox"/> | 12.931   |
|   | 32            | C11H14O2   | 178.09950 | <input type="checkbox"/> | <input type="checkbox"/> | 17.807   |
|   | 19            | C9H18O4    | 190.12040 | <input type="checkbox"/> | <input type="checkbox"/> | 14.648   |
|   | 29            | C11H14O3   | 194.09429 | <input type="checkbox"/> | <input type="checkbox"/> | 17.452   |
|   | 18            | C8H18O5    | 194.11530 | <input type="checkbox"/> | <input type="checkbox"/> | 5.339    |
|   | 35            | C10H13N... | 195.08954 | <input type="checkbox"/> | <input type="checkbox"/> | 19.399   |
|   | 26            | C12H12O3   | 204.07910 | <input type="checkbox"/> | <input type="checkbox"/> | 21.828   |
|   | 19            | C14H22O    | 206.16707 | <input type="checkbox"/> | <input type="checkbox"/> | 22.340   |
|   | 19            | C8H16O6    | 208.09490 | <input type="checkbox"/> | <input type="checkbox"/> | 5.484    |
|   | New Compound  | C12H14O4   | 222.08921 | <input type="checkbox"/> | <input type="checkbox"/> | 16.773   |
|   | 5             | C13H18O3   | 222.12559 | <input type="checkbox"/> | <input type="checkbox"/> | 17.367   |
|   | 19            | C14H31NO   | 229.24080 | <input type="checkbox"/> | <input type="checkbox"/> | 18.764   |
|   | 39            | C15H22O2   | 234.16198 | <input type="checkbox"/> | <input type="checkbox"/> | 20.944   |
|   | 2             | C10H22O6   | 238.14180 | <input type="checkbox"/> | <input type="checkbox"/> | 6.484    |
|   | 20            | C10H22O6   | 238.14180 | <input type="checkbox"/> | <input type="checkbox"/> | 6.540    |
|   | 20            | C10H8N6... | 244.07160 | <input type="checkbox"/> | <input type="checkbox"/> | 16.802   |
|   | 23            | C11H12N... | 244.10750 | <input type="checkbox"/> | <input type="checkbox"/> | 17.366   |
|   | New Compound  | C9H16N2... | 248.10084 | <input type="checkbox"/> | <input type="checkbox"/> | 1.960    |
|   | 27            | C14H18O4   | 250.12120 | <input type="checkbox"/> | <input type="checkbox"/> | 19.461   |
|   | 6             | C10H20O7   | 252.12030 | <input type="checkbox"/> | <input type="checkbox"/> | 6.637    |

# View and Edit Compounds in PCDL

MassHunter PCDL Manager for Forensics and Toxicology - C:\MassHunter\PCDL\ForensicsTox\_AM\_PCD.cdb

File Edit View PCDL Links Help

Find Compounds

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

Name: Guanidine

IUPAC: Guanidine

Mass: 59.04835 CAS: 113-00-8

RT: ChemSpider: 3400

Formula: CH5N3

Ion type

- Neutral
- Anion
- Cation

Edit actions

- Add New
- Save As New
- Update Selected
- Delete Selected

Molecule: Structure MOL Text

Chemical structure of Guanidine: NC(=[NH2+])N

Notes: Biomolecule

Single Search Results: 7360 hits

|   | Compound Name    | Formula      | Mass            | Anion                    | Cation                   | RT (min) | CAS                      | ChemSpider               | IUPAC Name       | N. Spe |
|---|------------------|--------------|-----------------|--------------------------|--------------------------|----------|--------------------------|--------------------------|------------------|--------|
|   | Prussic acid     | CHN          | 27.01090        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">74-90-8</a>  | <a href="#">748</a>      | Hydrocyanic acid | 0      |
|   | Glyoxal          | C2H2O2       | 58.00548        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">107-22-2</a> | <a href="#">7572</a>     | Oxalaldehyde     | 0      |
|   | Allyl alcohol    | C3H6O        | 58.04186        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">107-18-6</a> | <a href="#">13872989</a> | 2-Propen-1-ol    | 0      |
| ▶ | <b>Guanidine</b> | <b>CH5N3</b> | <b>59.04835</b> | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">113-00-8</a> | <a href="#">3400</a>     | <b>Guanidine</b> | 0      |
|   | Urea             | CH4N2O       | 60.03236        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">57-13-6</a>  | <a href="#">1143</a>     | Urea             | 0      |
|   | Ethylchloride    | C2H5Cl       | 64.00798        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">75-00-3</a>  | <a href="#">6097</a>     | Chloroethane     | 0      |
|   | Acrylamide       | C3H5NO       | 71.03711        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">79-06-1</a>  | <a href="#">6331</a>     | prop-2-enamide   | 0      |
|   | Propiolactone    | C3H4O2       | 72.02113        | <input type="checkbox"/> | <input type="checkbox"/> |          | <a href="#">57-57-8</a>  | <a href="#">2275</a>     | 2-Oxetanone      | 0      |

!!! If quaternary salt it must be noted as such !!!

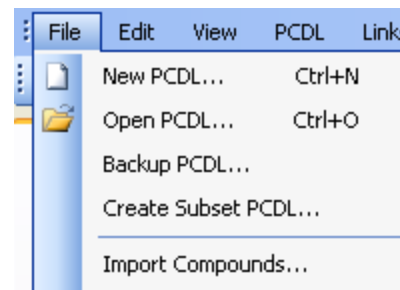
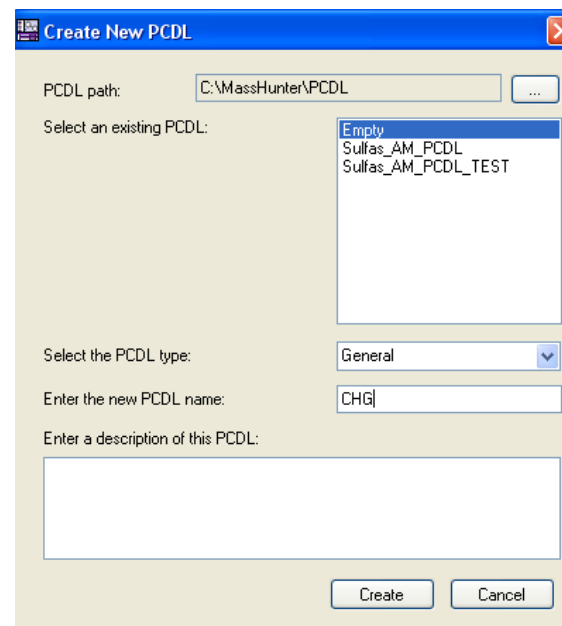


Click cation radio button to make this identification

# Personal Databases Cut/Paste to Template

Template\_for\_Import\_PCDL.csv - Microsoft Excel

|    | A   | B  | C         | D             | E           |
|----|---|----|-----------|---------------|-------------|
| 1  | # Agilent TOF Formula data store          |    |           |               |             |
| 2  | # Version: 1.0                            |    |           |               |             |
| 3  | # Copyright (c) 2004 Agilent Technologies |    |           |               |             |
| 4  | # Drugs of abuse database                 |    |           |               |             |
| 5  | Formula                                   | RT | Mass      | Compound Name | Description |
| 6  | C35H36ClNO3S                              |    | 585.21044 | Parent        |             |
| 7  |   |    |           |               |             |
| 8  |   |    |           |               |             |
| 9  |   |    |           |               |             |
| 10 |   |    |           |               |             |
| 11 |   |    |           |               |             |
| 12 |   |    |           |               |             |
| 13 |   |    |           |               |             |
| 14 |   |    |           |               |             |
| 15 |   |    |           |               |             |
| 16 |   |    |           |               |             |
| 17 |   |    |           |               |             |
| 18 |   |    |           |               |             |



## Import Compounds into New PCDL Database

# Frequently Asked Questions

1. *What criteria do labs typically use to call a positive using this methodology?*  
Typical criteria are: Score  $\geq 70$ , mass error  $\leq 12$  ppm, RT difference  $\leq 0.12$  min

Crashed serum has good hits that can have Scores in the 50-70 range due to chemical interferences with the isotopes. You can lower the effect of isotopes using Match Scoring section of the Qual method, but you are just artificially adjusting your Scores upwards by doing that. We have seen mass errors  $> 15$  ppm for a few true positive compounds in particular samples.

Labs with better sample cleanup (SPE, LLE, TOXI-TUBES) typically have Scores in the 80-90 range and mass errors  $< 10$  ppm.

Typical RT differences are 0.02-0.05 minutes. Values larger than those lower the Score, call the identification into question, or may indicate an LC or mobile phase problem. The RT Match Tolerance in the method must be sufficiently small to differentiate any close-eluting isobars such as codeine/hydrocodone (UT State Health Dept uses 0.1min rather than 0.12)

Part of your method validation must include:

- establishing what the positive identification criteria will be for your SOP.
- establishing the variation in Score, mass error and RT difference for replicate preps on multiple days at the cutoff and 50% of the cutoff for each drug. Running fortified samples at 50% of the desired cutoff will help establish your identification criteria and something like an LOD for this screen.

# Frequently Asked Questions

*2. Can you somehow import Qual results into MassHunter Quant to facilitate accurate-mass Quant method setup?*

The most important feature is importing FbF compound information (name, EIC m/z, RT) from results exported as a .cef file (rather than .csv). This takes the place of typing in information into Quant for each compound.

This release had not been sufficiently tested or documented to cover its use during this meeting. The export/import process and new Quant features will be covered during the next TOF Drugscreen User meeting in (approximately) February 2013.