Enhanced Target Identity Confirmation In 8270D Using Deconvoluted Spectral Matching

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Data Review In Routine Quantitative Analysis Like Semi-VOAS

- After method is developed and calibration is sucessful, running real samples begins.
- Identification of targets is based on:
  - Response at the target ion within the RT window for that compound
  - Ratio of qualifier ion responses to target response
- With Semi-VOA extracts, there are often significant matrix interferences that can make the qualifier ratios fall out of the calibrated range. The data reviewer must now decide between:
  - Do not report the compound as present because the qualifier ratios were not met
  - Report the compound as present because there was a response at the target at the correct RT and (for example) one of the two qualifier ratios was met but not the other one.
- A means of getting more information to decide if a compound is present would be very helpful
- As part of our 8270 method project, we wanted to use spectral matching to help in the decision making.

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Use of Spectra In Confirming Presence Of Target Analytes

MSD ChemStation

- **QEdit**
  - users can display spectrum at apex of target ion response with baseline spectrum (lowest of either before or after) automatically subtracted.
  - Library reference spectrum can also displayed for comparison.
  - No library match score is provided.
- **DRS** (Deconvolution Reporting Software)
  - Deconvoluted spectrum from AMDIS is imported and displayed with library match score (LMS), reference spectrum and baseline subtracted spectrum.
  - Spectra imported only for those analytes where LMS > user set minimum.
  - Deconvolution component is independent of target found by quant criteria.

Use of Spectra In Confirming Presence Of Target Analytes

MassHunter Quant

- In MHQ, analysts can display AVERAGE (default) or APEX spectrum in data review (baseline spectrum is not subtracted.)
- Reference spectrum and Library Match Score (LMS) can be displayed.
- Works for clean, well separated peaks that are not setting on high column bleed or background ions

MassHunter Quant with SureTarget Deconvolution

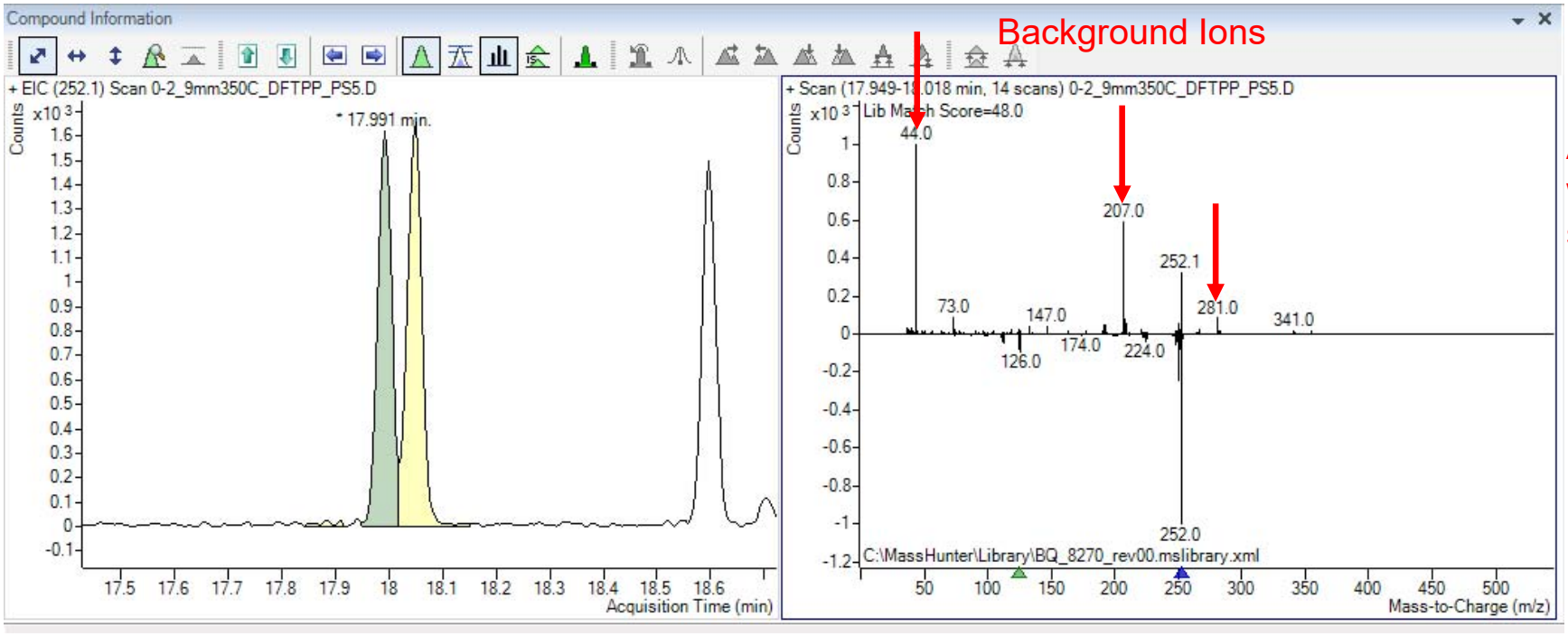
- Deconvolution greatly improves library searching of spectra by removing interfering ions from co-eluting peaks and background
  - Used to confirm identity of target identified by traditional target/qualifiers ratio method
  - **Alternate Peak In Window** feature helps detect if wrong the target peak is chosen by Quant criteria and displays better choice

SureTarget in Unknowns Analysis (UA)

- Unknowns Analysis uses same deconvolution engine as MassHunter Quant
- UA is a convenient way to build libraries
  - Use library to create Quant method
- Use like AMDIS for identifying unknowns
  - Search large RT locked libraries
  - Search NIST and industry group libraries (like AAFS)

MassHunter Quant Using AVERAGE Spectrum

Same peak as in previous MSD ChemStation slides



AVERAGE spectrum with no baseline subtracted

LMS = 48

Library reference spectrum

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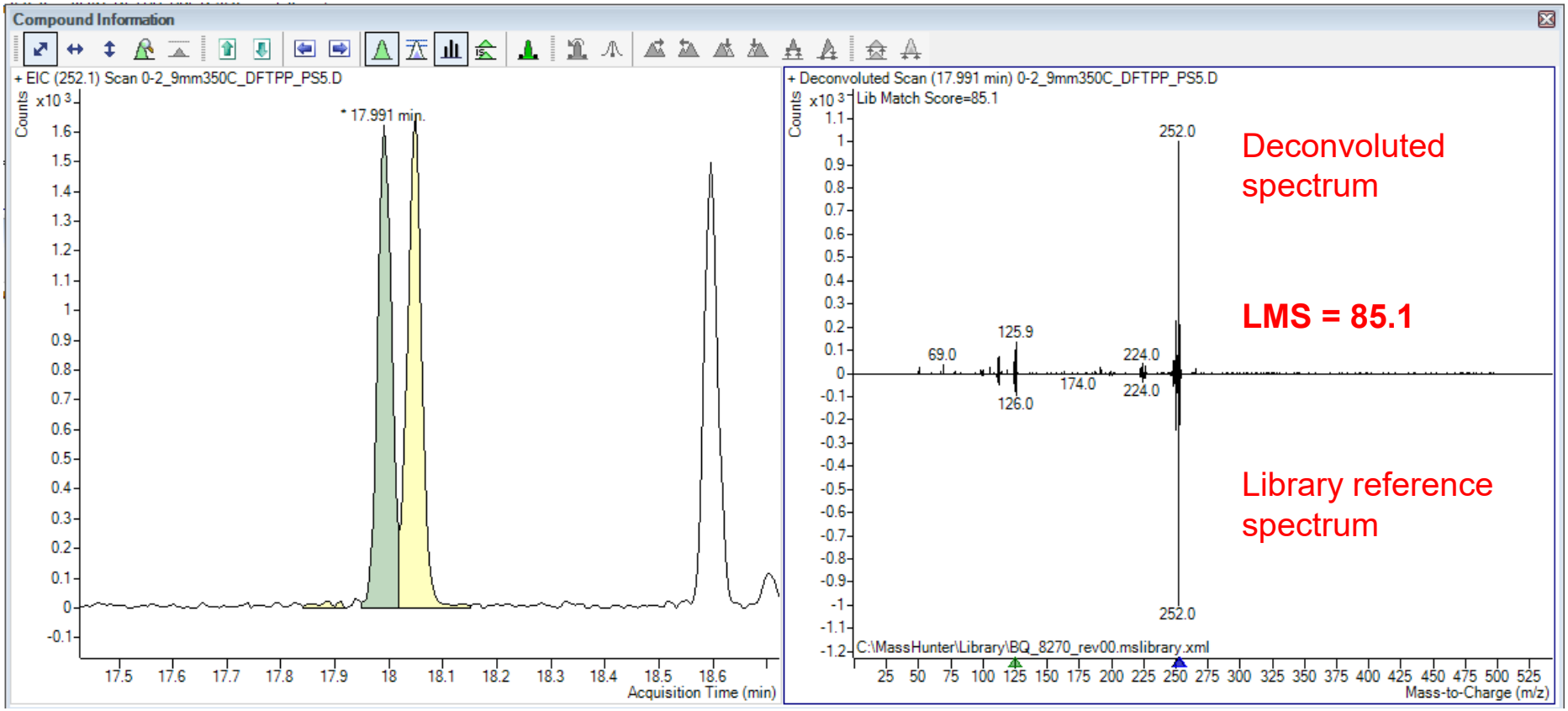
MassHunter Quant Using SureTarget Deconvolution

SureTarget deconvolution removes bleed and background ions, giving much better LMS

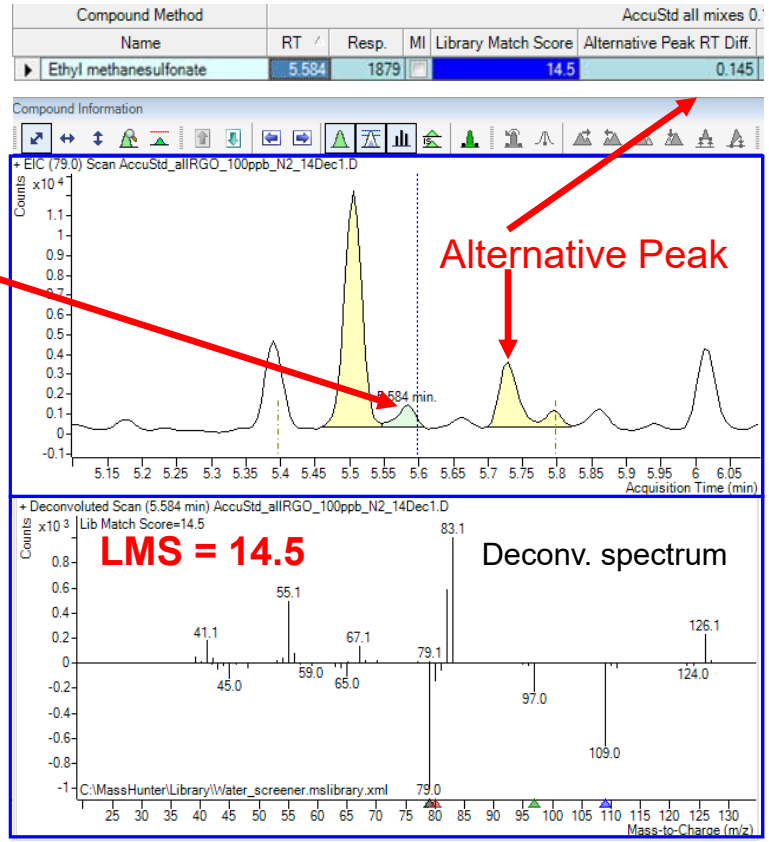


Alternative Peak in Window
Alternative Peak RT diff

Peak Found By Criteria

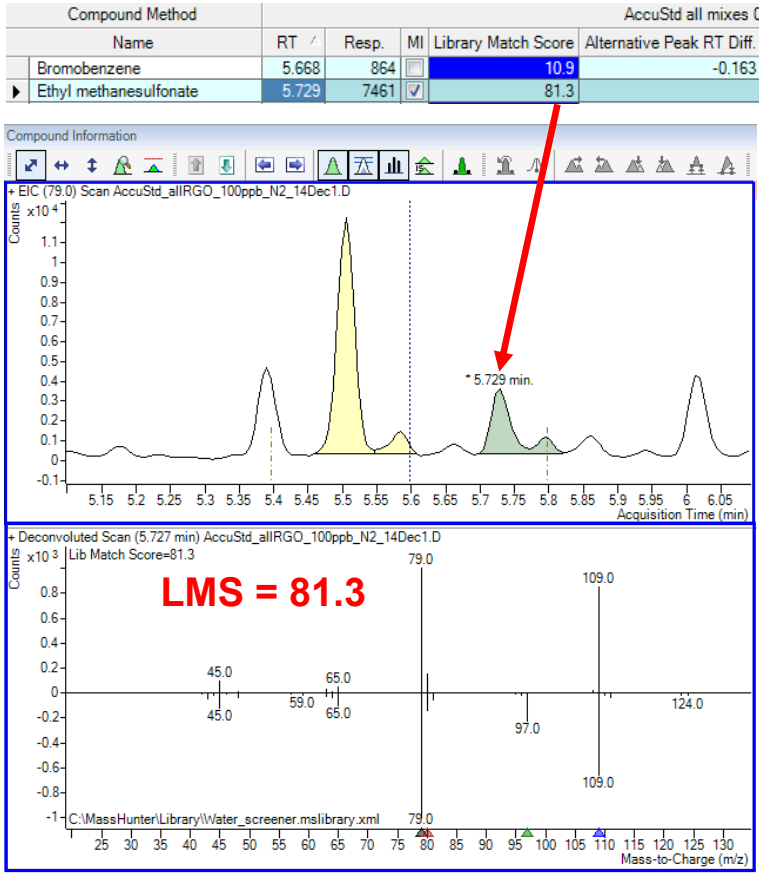
Spectrum of peak found by criteria

Library reference spectrum



- Quant method criteria chooses the primary peak based on target qualifier ratio and RT
- SureTarget looks for alternative peaks in RT window based on deconvoluted LMS
- Indicates alternative peak with highest LMS as entry in Alternative Peak RT Diff column.
- Warns reviewer of possible better choice based on deconvoluted LMS

Alternative Peak in Window
Alternative Peak RT diff



Spectrum of alternative peak

Library reference spectrum

- Use Manual Integration button to select alt peak
- Corresponding info of selected peak is updated in the batch table (RT, LMS, response, etc.)

Adding Deconvolution To MassHunter 8270 Semi-VOA Quant Method

Build library of spectra from 10 ppm calibration standard containing all 84 analytes and ISTDs

- Use **Unknowns Analysis** to deconvolute spectra for library
- Search spectra against NIST
- Delete all identified impurities components that are not part of the method
- Use **Show Alternate Hits** feature to correct misidentified spectra (isomers, etc)
- Export desired spectra to MassHunter Library Builder program
- Edit names and CAS numbers to match those entered in Quant table

In Quant Edit Method screen

- Select **Workflow/Target Deconvolution Setup**
- In Setup screen, fill in **Reference Library**, **Library Method**, and **Outlier Setup Items**

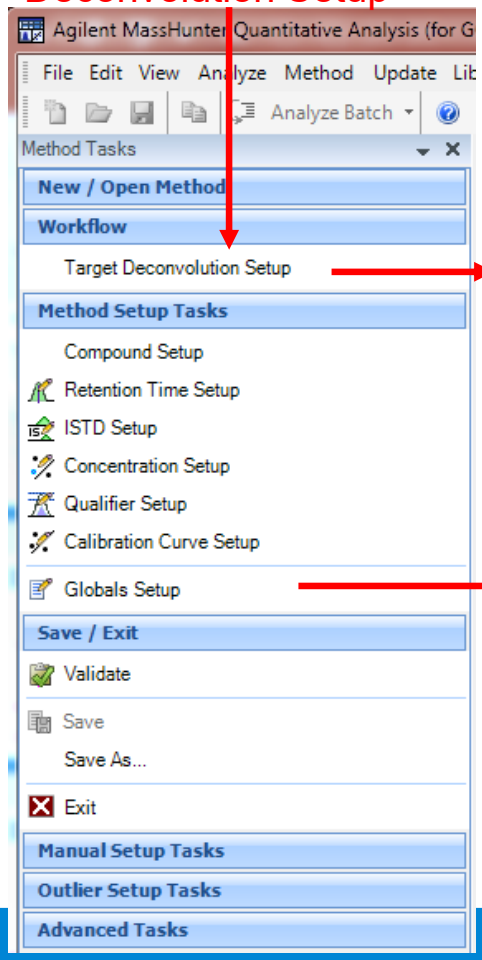
In Quant Batch screen

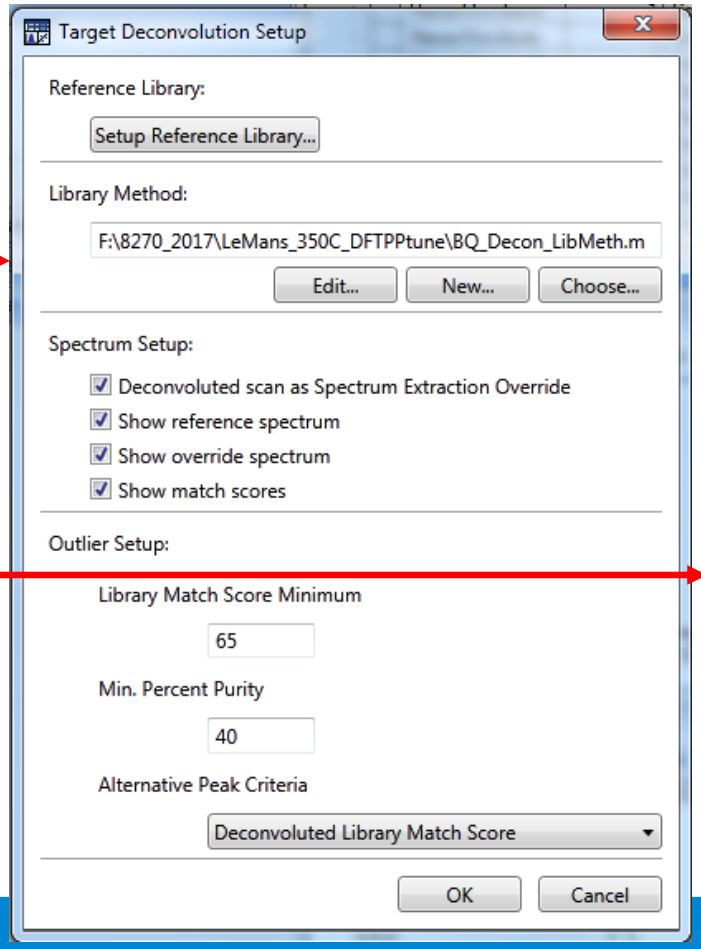
- Add columns for **RT Diff**, **Library Match Score**, and **Alternative Peak RT Diff**

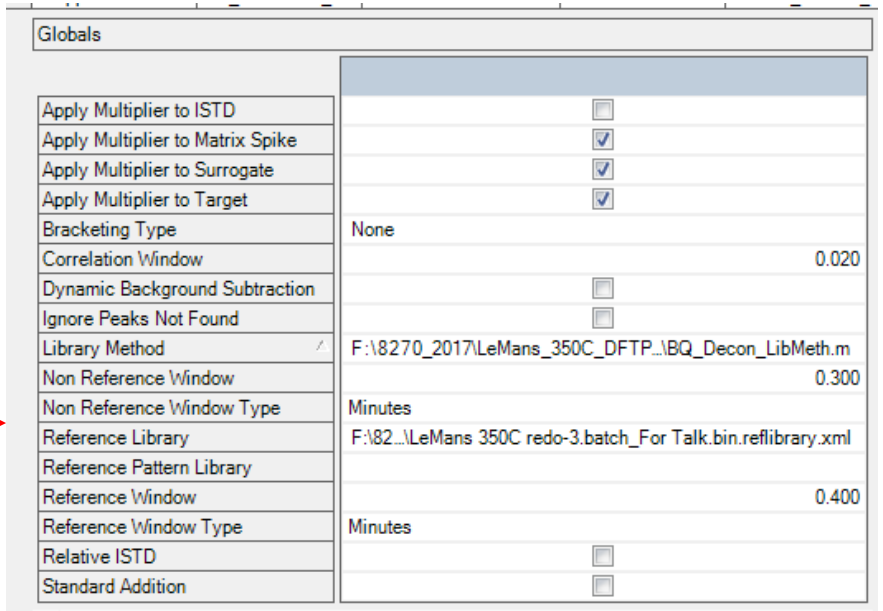
Adding Deconvolution To Quant Method: In Method Edit View

Click On Target Deconvolution Setup

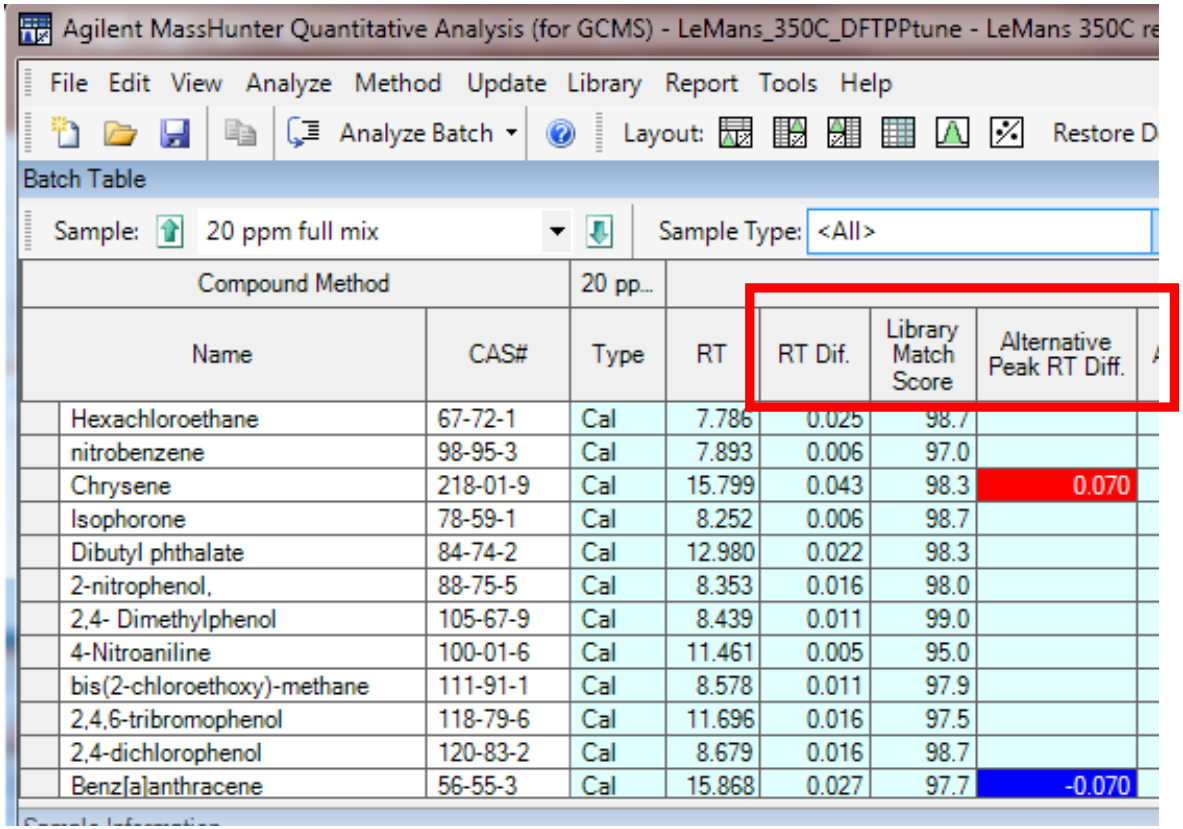
Fill In Library and Library Method Info Here AND Globals







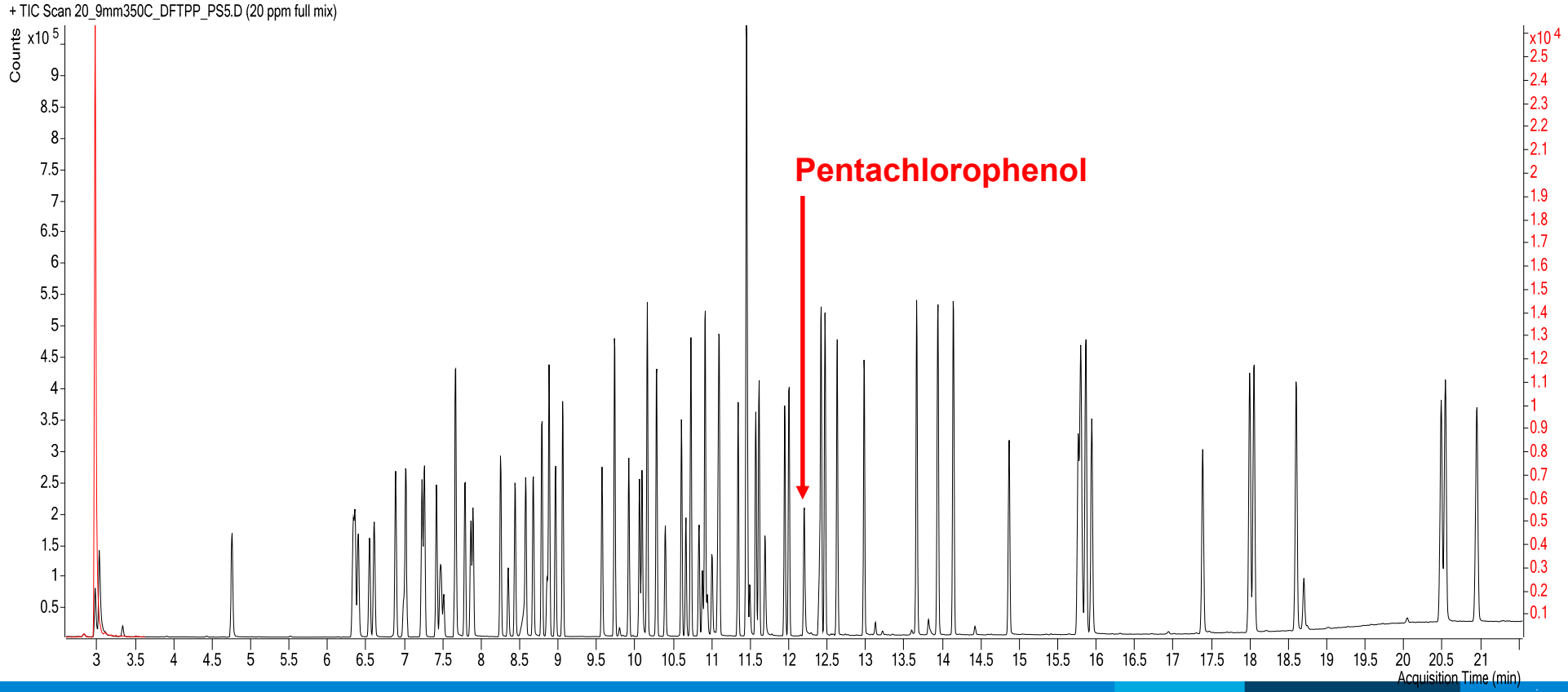
Add These Columns To the Batch Screen



Compound Method		20 pp...					
Name	CAS#	Type	RT	RT Dif.	Library Match Score	Alternative Peak RT Diff.	
Hexachloroethane	67-72-1	Cal	7.786	0.025	98.7		
nitrobenzene	98-95-3	Cal	7.893	0.006	97.0		
Chrysene	218-01-9	Cal	15.799	0.043	98.3	0.070	
Isophorone	78-59-1	Cal	8.252	0.006	98.7		
Dibutyl phthalate	84-74-2	Cal	12.980	0.022	98.3		
2-nitrophenol,	88-75-5	Cal	8.353	0.016	98.0		
2,4- Dimethylphenol	105-67-9	Cal	8.439	0.011	99.0		
4-Nitroaniline	100-01-6	Cal	11.461	0.005	95.0		
bis(2-chloroethoxy)-methane	111-91-1	Cal	8.578	0.011	97.9		
2,4,6-tribromophenol	118-79-6	Cal	11.696	0.016	97.5		
2,4-dichlorophenol	120-83-2	Cal	8.679	0.016	98.7		
Benz[a]anthracene	56-55-3	Cal	15.868	0.027	97.7	-0.070	

20 ppm 8270 Semi-VOA Calibration Standard, 84 Compounds Including ISTDs

With so many compounds, it is almost impossible to avoid co-elutions, which cause identification problems. Deconvolution helps resolve these



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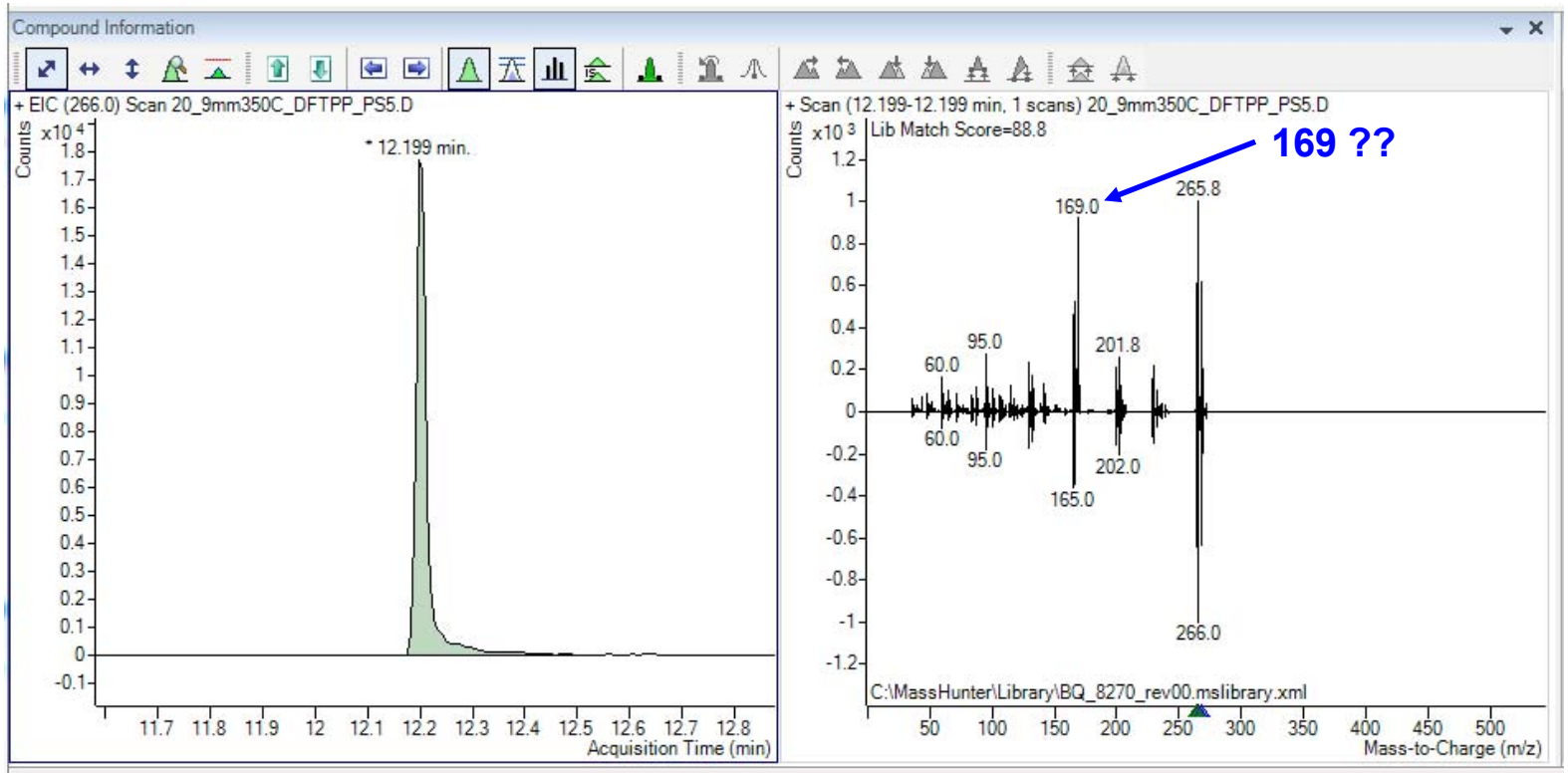
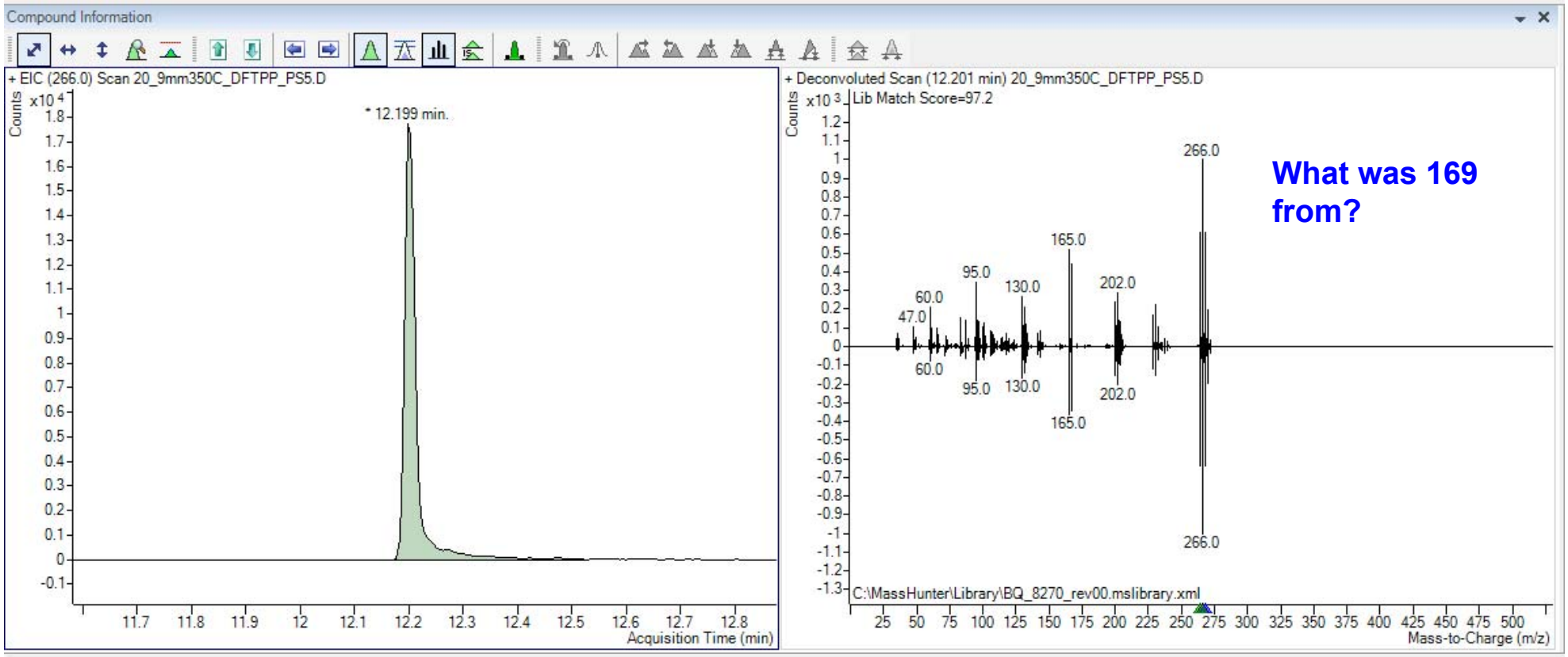
Pentachlorophenol With Apex Spectrum

APEX spectrum has library match score (LMS) of 88.8. There is still some interference with a large 169 ion



Pentachlorophenol With Deconvolution In Quant

Deconvoluted spectrum has improved LMS = 97.2 Note that 169 interference is gone.



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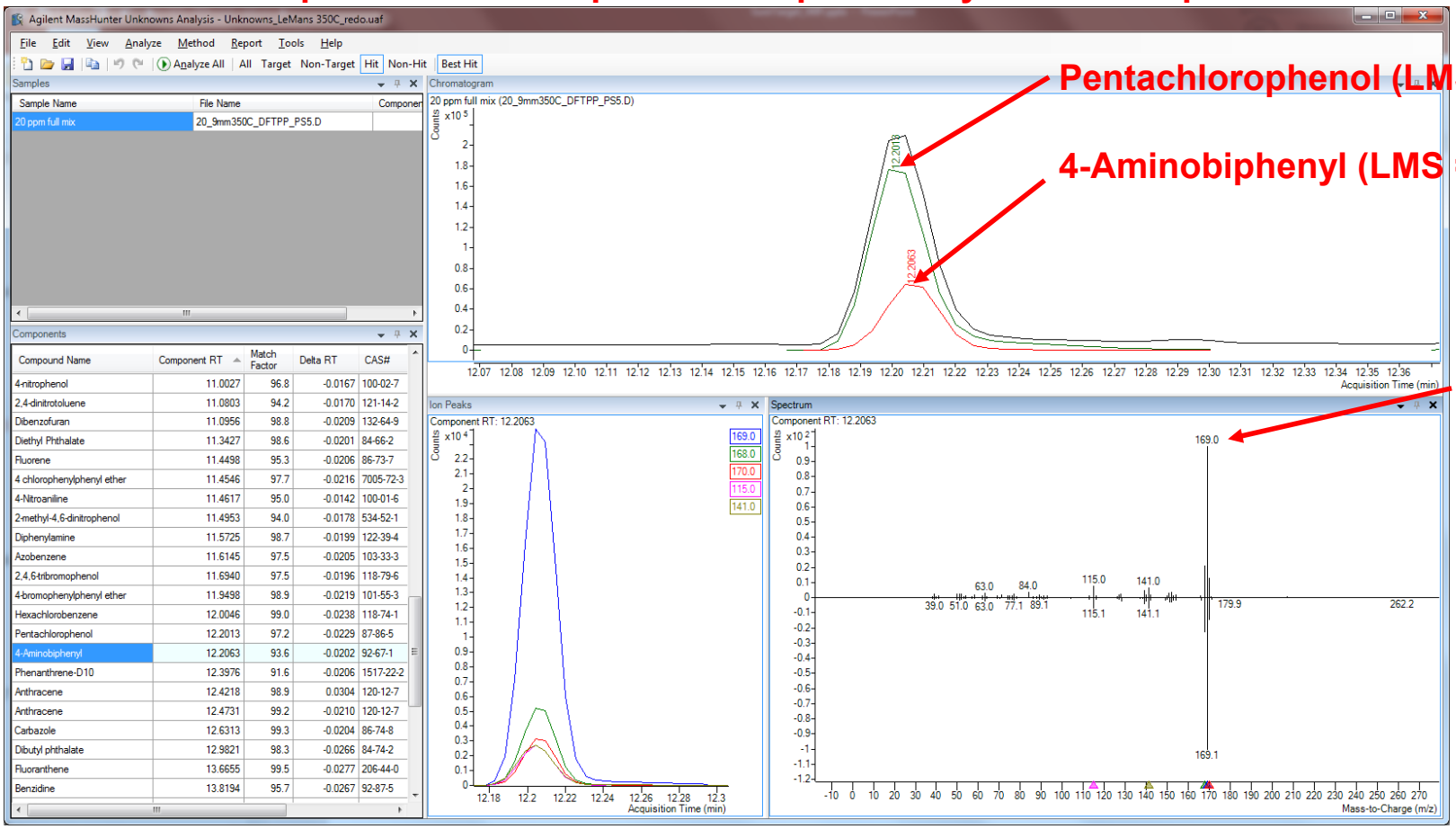
Unknowns Analysis Deconvolution Finds Co-eluting Impurity

Deconvolution produces clean spectra from peaks only 0.005 min apart



Pentachlorophenol (LMS = 97.2)

4-Aminobiphenyl (LMS = 93.6)

169 base peak of 4-Aminobiphenyl interferes in Pentachlorophenol apex spectrum

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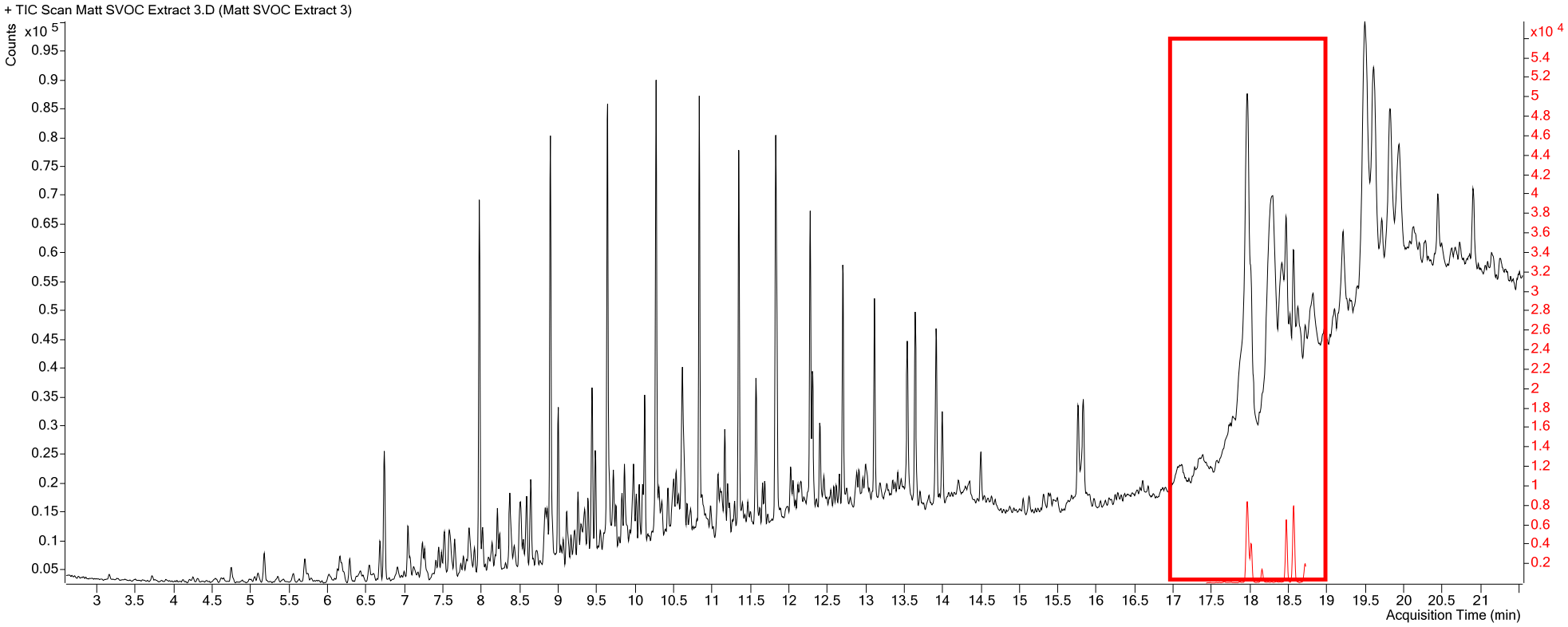
Analysis Of Semi-VOA Extract Provided Environmental Lab

Semi-VOA extracts often look like diesel fuel, which means lots of potential interferences

Is Benzo[b]fluoranthene in here?



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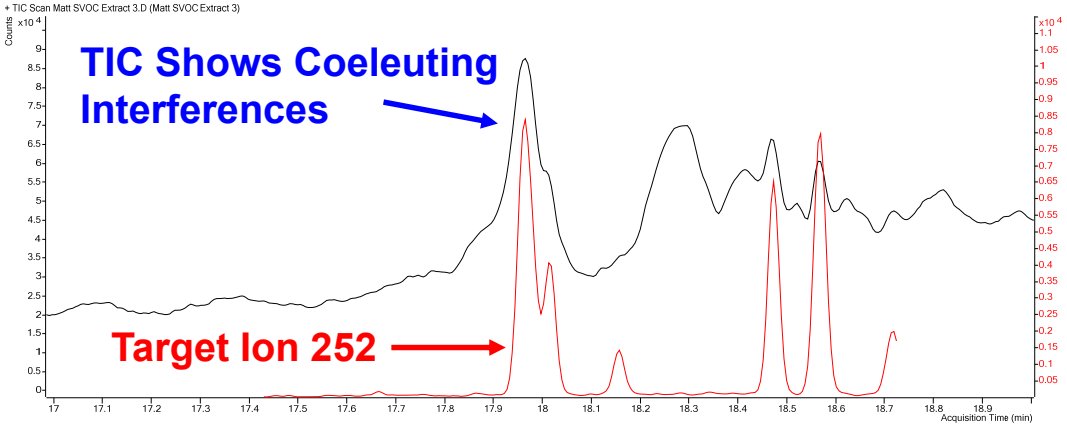
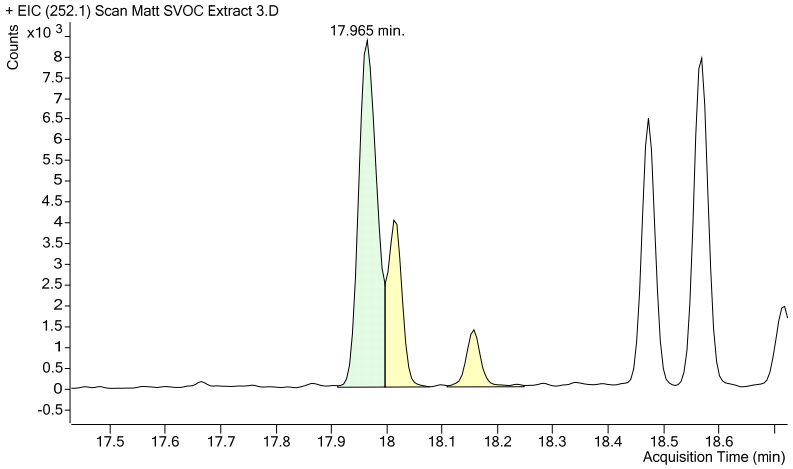
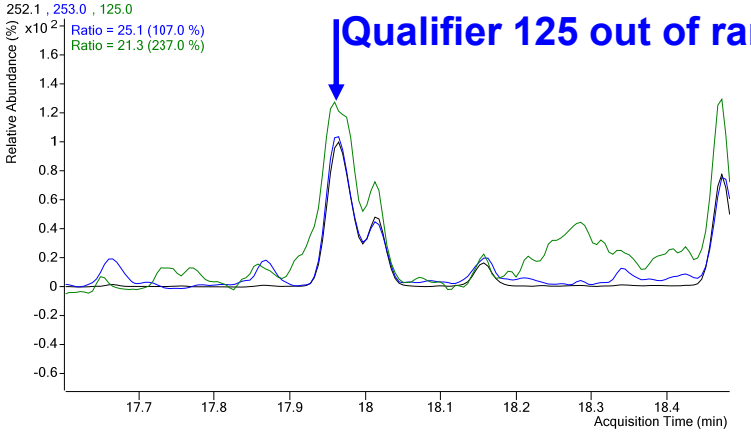
Analysis Semi-VOA Extract Provided By Environmental Lab



- **Target ion 252 has response at 0.066 min from quant table RT.**
- **Qualifier 253 is good (107%)**
- **Qualifier 125 is out (237%)**
- **TIC shows interference(s)**
- **Is it there or not?**



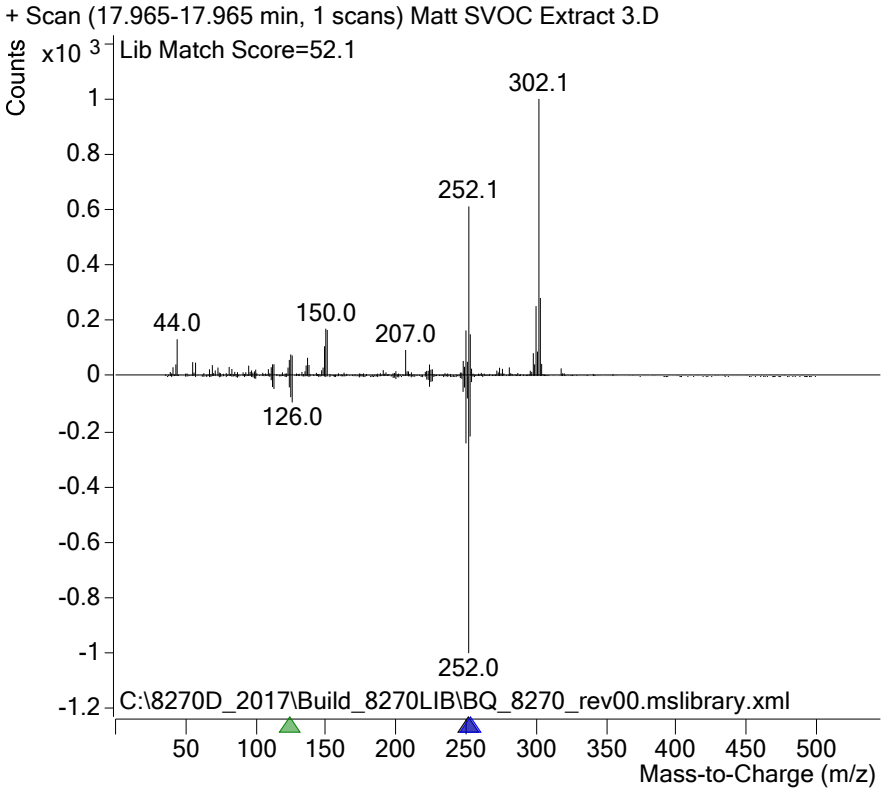


Analyze Semi-VOA Extract Provided By Environmental Lab

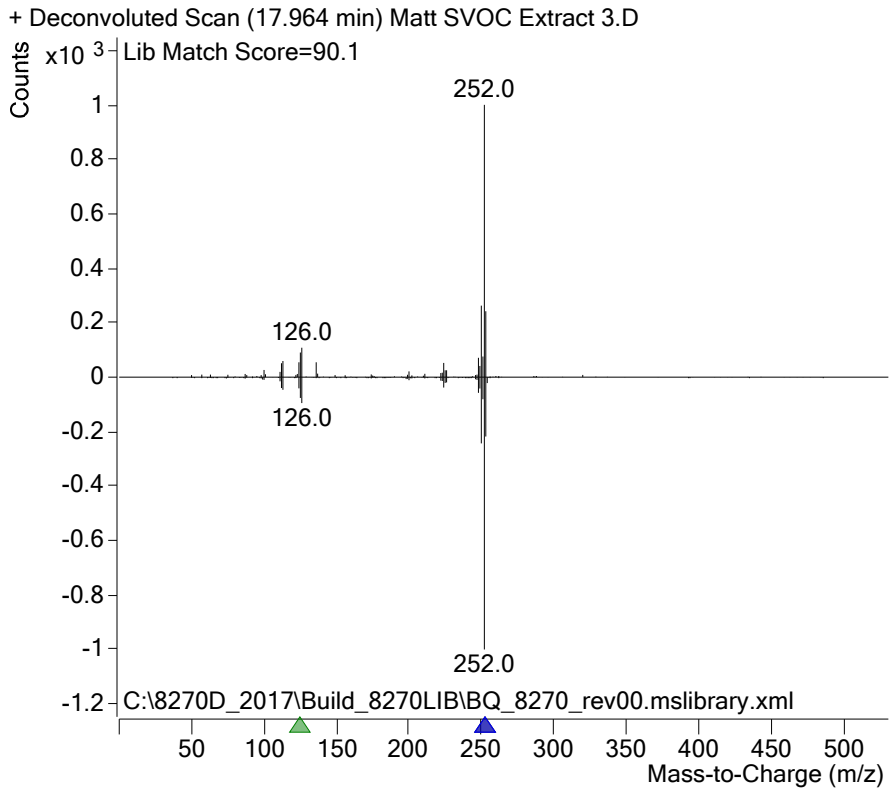
Based on qualifier way out and APEX spectrum LMS = 52, Benzo[b]fluoranthene is absent

Based on deconvoluted LMS of 90.1 AND RT diff of only 0.066 min, Benzo[b]fluoranthene is present

APEX Spectrum LMS = 52.1



Deconvoluted Spectrum LMS = 90.1



Summary

SureTarget Deconvolution features added to MassHunter Quant programs offer useful new tools for routine analyses:

In MH Quant

- For data review in Quant Batch table, deconvoluted spectra with library match score helps confirm presence/absence of target analytes, especially for samples with matrix interferences
- Alternate Peak in Window function alerts user if Quant criteria has chosen wrong peak and makes it easy to correct.

Unknowns Analysis

- Deconvolution simplifies identification of compounds, both targets and unknowns
- Use it to build libraries from calibration standards
- For users that only occasionally encounter matrix interferences, use Unknowns to inspect data file instead of in Quant review.

Thank you for your attention!