Deconvoluted Spectral Matching Improves Target Confirmation for EPA Method 8270D

Bruce Quimby¹, Melissa Churley², Dale Walker¹ and Mike Szelewski¹

¹Agilent Technologies Inc., Wilmington, DE 19808 USA;
²Agilent Technologies Inc., Santa Clara, CA 95051 USA
Method EPA 8270D is commonly run in full scan mode (GC/MS) with target identification based on extracted ion chromatograms (EICs). The target compound’s measured ion response is compared to that of (typically) two qualifier ions within a specified, restricted retention time window for that compound, which results in a reported ratio. Identity can be further confirmed by comparing a baseline subtracted apex spectrum of the analyte to a reference spectrum. These approaches work well when there are no chromatographic interferences. Many samples, however, contain significant levels of matrix compounds that can interfere with both processes above and so analyte identification becomes more challenging. Adding deconvoluted spectral matching to the data analysis method for 8270D provides enhanced confidence in target identification.

**Introduction**

Spectral deconvolution, cont.

The second use is for identifying compounds not associated with the calibration standards; resultant deconvoluted spectra of all peaks in the chromatogram can be searched against libraries like NIST.

**Spectral deconvolution**

Spectral deconvolution is a long-used software approach to removing the ions of co-eluting compounds from the spectrum of an analyte. In deconvolution, ion chromatograms are extracted at all masses in the scan range. Ions with peaks having the same shape and RT are grouped into components. The response of ions present in multiple overlapping peaks are apportioned to each peak using a process similar to that in chromatographic integrators; these are referred to as components. Spectra are then constructed for each component found. Thus, the deconvolution process greatly reduces or eliminates interfering ions in the analyte spectra.

**Spectral deconvolution is used in two ways:**

1) **The spectrum is displayed in batch review with a reference spectrum and library match score**

The deconvoluted spectrum is presented with the reference spectrum and a library match score (LMS) in quantitative analysis software. These are displayed with the extracted ion chromatograms of the target and qualifier ions to facilitate batch review; i.e., reviewing high matrix samples in deciding if an analyte is present or absent.

2) **Identify compounds not associated with standards (known non-targets)**

The second use is for identifying compounds not associated with the calibration standards; resultant deconvoluted spectra of all peaks in the chromatogram can be searched against libraries like NIST.

**Test the workflow: Creation of a deconvoluted spectral library for method EPA 8270D**

To test this workflow, a user library was created and added to the existing 8270D quantitation method. Method calibration was performed from 0.05 to 160 ppm using 77 compounds and six ISTDs. A calibration report, based on EIC results, was generated. Batch review normally consists of determining the number of compounds that meet average relative response factor %RSD ≤ 20 and, secondarily, those that require a curve fit to meet criteria.

To create a deconvoluted spectral library, the 50 ppm standard was analyzed using a spectral deconvolution program (MassHunter Unknowns Analysis) and results were verified against entries in NIST.

A user library was created from the list of results, and names and CAS numbers were made consistent with the quantitation method. This user library, designated as Sure Target, was then specified as a reference library in the quantitation method. In the batch review screen, library match scores (LMS), retention time differences and deconvoluted spectra, head-to-tail with reference spectra, are presented.
User library added to quantitation method

Adding a SureTarget user library to the quant method in Method Edit view and Batch view

Click on Target Deconvolution Setup
Fill in library and library method information here and in Globals
Add these columns to the Batch screen for batch review

Use alternative peak feature with isomers

Chrysene and Benz[a]anthracene have nearly identical spectra and elute only 0.07 min apart. The SureTarget LMS score will sometimes be slightly better for the wrong peak, resulting in a warning in the Alternative Peak RT Diff column. In this case, the reviewer relies on RT and chooses the correct peak (choices are displayed in a separate window).

Confirmed impurity in standard mixture that co-elutes with pentachlorophenol

Apex only spectrum LMS = 88.8. There is interference with a large 169 ion.
SureTarget deconvoluted spectrum LMS = 97.2 Note that the 169 interference is gone.
Deconvolution produces clean spectra from peaks only 0.005 min apart. Pentachlorophenol co-elutes with the impurity 4-aminobiphenyl.
Challenging environmental sample analyzed: Benzo[b]fluoranthene is present using the SureTarget approach

This methodology was then applied to an environmental extract for which the target ion for benzo[b]fluoranthene had a significant response at the correct retention time, and one qualifier ion was in range, but the other qualifier was far out of range. The deconvoluted match score of 90.1 indicated a very good spectral match resulting in higher confidence for the compound to be declared present.

### Conclusions

Target identification for a regulated method based on extracted ion chromatograms from scan data is enhanced by automated spectral deconvolution. For data review in batch review mode, deconvoluted spectra with a library match score help confirm presence/absence of target analytes, especially for samples with matrix interferences.

The Alternate Peak in Window function alerts user if quant criteria has chosen wrong peak and makes it easy to correct. MassHunter Unknowns Analysis spectral deconvolution program:

- Spectral deconvolution simplifies identification of compounds, both targets and unknowns
- May be used to build libraries from calibration standards
- For users that only occasionally encounter matrix interferences, use it to inspect data file instead of in batch review

Based on the unacceptable qualifier m/z 125 and Apex spectrum LMS = 52, Benzo[b]fluoranthene is absent.

Based on the deconvoluted spectrum LMS of 90.1 and RT difference of 0.066 min, Benzo[b]fluoranthene is present.

This information is subject to change without notice.
© Agilent Technologies, Inc. 2018