Can "Deconvolution" Improve GC/MS Detectability?

Application Note

All Industries

Authors

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Abstract

This study uses 35 pesticides spiked in spinach extracts at the 50 ppb level to find the optimal AMDIS deconvolution settings. Additional advantages of using deconvolution versus MSD ChemStation, to find more compounds in an extract are also discussed.

The detectability of compounds in a complex matrix is significantly improved with deconvolution. This can also be viewed as better or increased sensitivity through improved selectivity versus the background.

Agilent's MSD ChemStation add-on - Deconvolution Reporting Software (DRS) runs AMDIS automatically to generate an easy-to-read quantitation report.

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Introduction

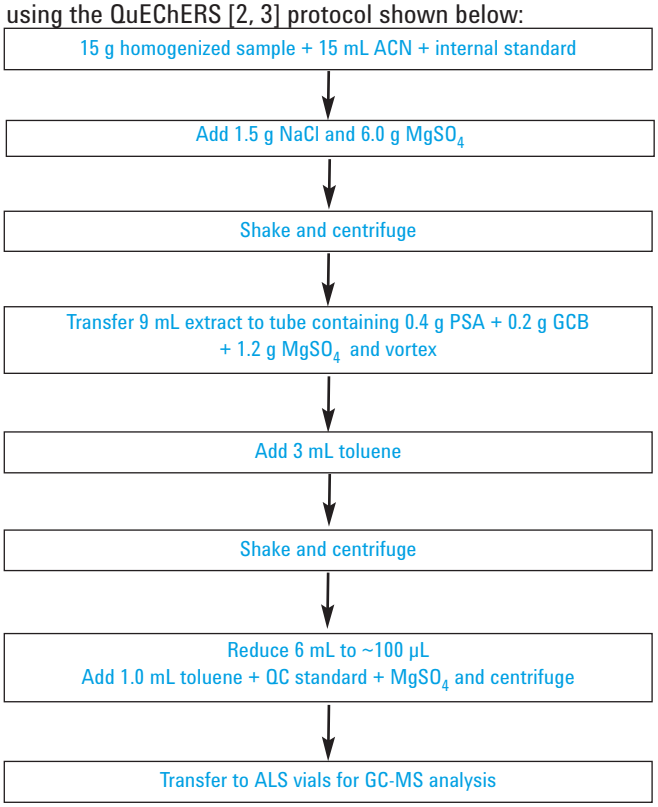
Instrument detectability is usually determined by the amount of sample injected, the responses from the detector and matrix interferences. The signal-to-noise ratio (S/N) can be used to gauge the sensitivity of an instrument in a clean sample. The presence of matrix alters this sensitivity due to a lack of selectivity between compounds of interest and background.

In a multiresidue analysis, the data reviewing process is also very important in confirming the hits found by the software and reviewing the integration and quantitation for accuracy.

Agilent Deconvolution Reporting Software (DRS) has been proven as a powerful data processing tool for finding trace compounds in complex matrices [1]. In this study, results from the Automated Mass spectral Deconvolution and Identification System (AMDIS), part of DRS is closely studied and compared to the results from ChemStation. The goal is to determine if deconvolution (DRS) can provide better results (detectability) than routine ChemStation data processing.

Experimental

Spinach extracts (see Acknowledgement) were prepared using the QuEChERS [2, 3] protocol shown below:



Thirty-five pesticides were spiked into spinach extract at 50 ppb (pg/µL).

Instrument parameters

GC: **7890A**

Autoinjector: 7693A
Retention gap: 2 m × 0.25 mm id Siltek capillary tubing
Column: HP-5MS UI (ultra inert), 15 m × 0.25 mm, 0.25 µm (from inlet to Purged Union) Agilent p/n 19091S-431 UI

Oven ramp:	Rate (°C/min)	Temp (°C)	Time (min)
Initial		100	1.6
Ramp 1	50	150	0
Ramp 2	6	200	0
Ramp 1	16	280	5

Run time: 20.933 min
Inlet: Multimode Inlet (MMI) at 17.73 psi (Retention Time Locked), constant pressure mode
RT locking: Chlorpyrifos-methyl locked to 8.297 min
Liner: Helix double taper, deactivated (Agilent p/n 5188-5398)
Injection mode: 2-µL cold splitless (fast injection)

Inlet temp. ramp:	Rate °C/min	Temp °C	Time min
Initial		50	0.01
Ramp 1	720	300	hold

Septum purge: 3 mL/min
Purged Union: 4 psi (PCM)
Split vent: 50 mL/min at 0.75 min
Gas saver: 20 mL/min after 4 min
Cryo on: Cryo use temperature 150 °C; time out at 15 min

Backflush

Postrun: 5 min
Oven: 280 °C
Purged Union: 70 psi
MMI: 2 psi
Restrictor: 0.7 m × 0.15 mm deactivated fused silica tubing (from Purged Union to MSD)

MSD: **5975C**

Solvent delay: 2.5 min
EMV mode: Gain Factor = 2
Mass Range: Full scan, 45-550
Threshold: 0
Sample number: 2 A/D Samples 4
Transfer Line: 280 °C
Source: 300 °C
Quad: 200 °C

Deconvolution

Deconvolution is a process for extracting ions from a complex total ion chromatogram (TIC), even with the target compound signal at trace levels. The software used for this technique is AMDIS developed by NIST (National Institute of Standards and Technology) [4].

As a review, let's look at the deconvolution process. AMDIS considers the peak shapes of all extracted ions and their apex retention times (RT). In this example, only some of the extracted ion chromatograms (EICs) are overlaid for clarity with the apex spectrum (Figure 1A).

Ion 160 EIC has the same RT as ions 50, 170 and 280, but has a different peak shape. Ion 185 has a different peak shape and an earlier RT. Ions 75 and 310 have similar peak shapes but they have different RTs.

Figure 1A

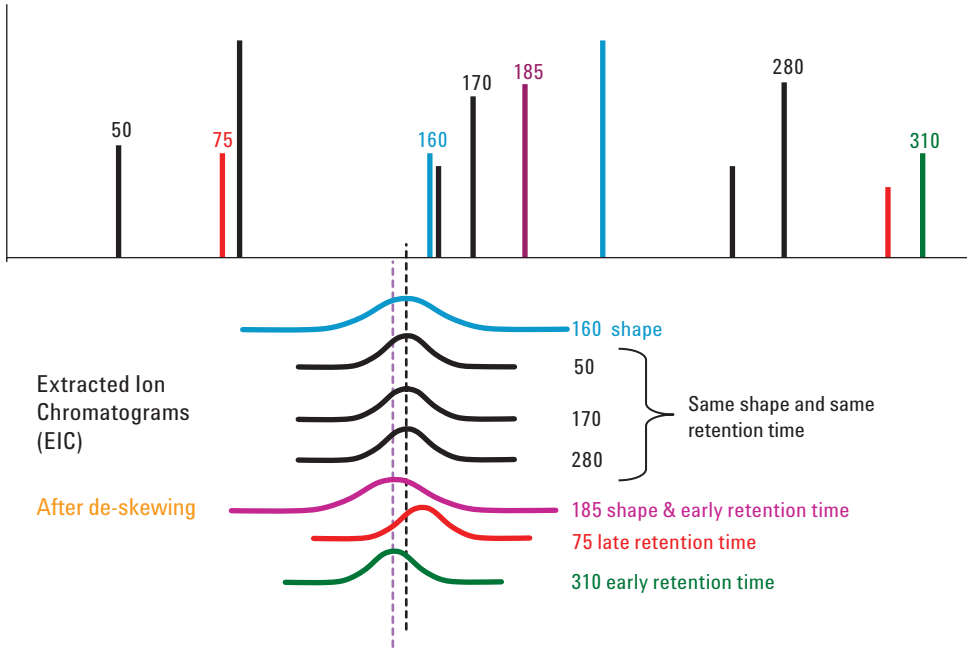


Figure 1B shows the EICs after the different peak shapes or RTs are eliminated from Figure 1A. Ions 50, 170, 280 and a few others remain.

Figure 1B

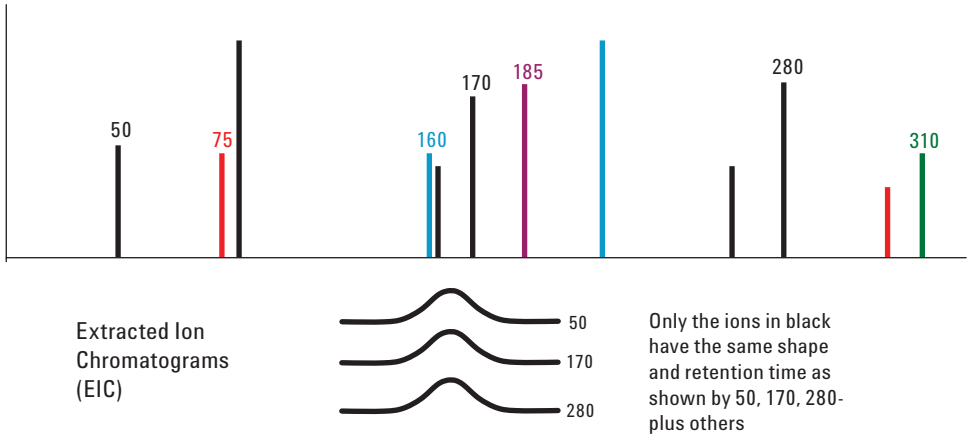


Figure 1A-1C. Simplified deconvolution process (continued).

Figure 1C shows all of the ions in black that have similar peak shapes and RTs, within the criteria set earlier by the analyst. These are grouped together and referred to as a component by AMDIS.

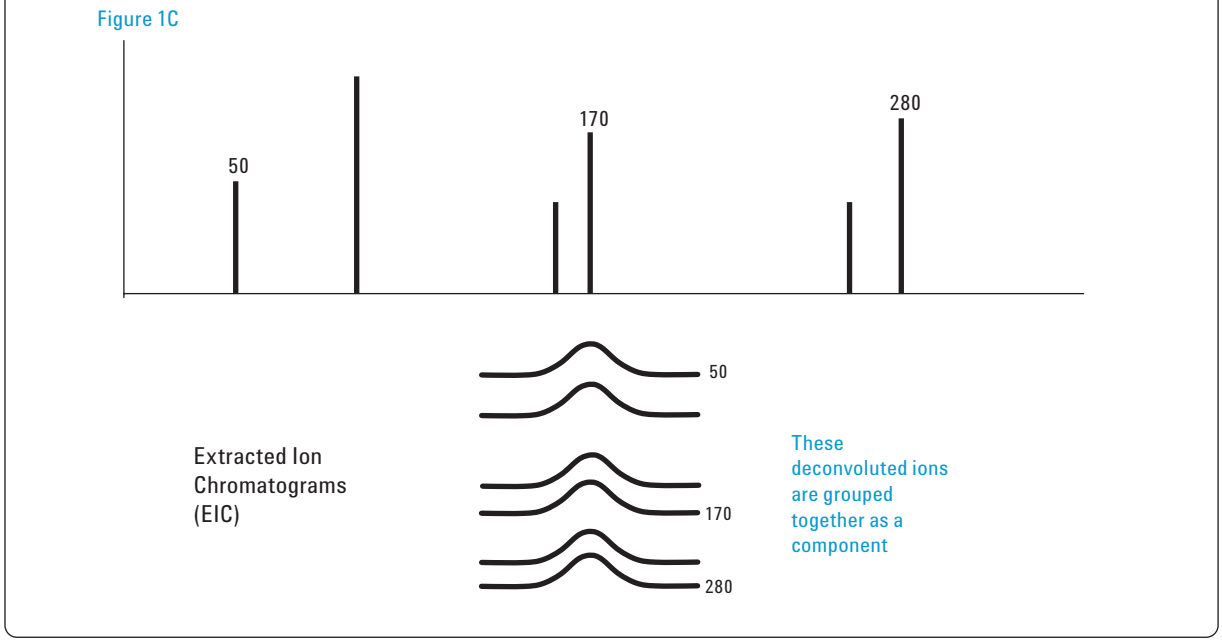


Figure 1A-1C. *Simplified deconvolution process (continued).*

Deconvolution finds the components from a complex TIC. Each component is searched against a retention time locking (RTL) library in AMDIS format. In addition to spectral matching, the locked RT can also be used as a criterion for hits. Depending on the match factor from the search, target compounds can be identified or flagged in a complex TIC. The power of deconvolution is appreciated while comparing the top two spectra in Figure 2. The raw scan or original nondeconvoluted scan is shown on top. The clean scan, that is the deconvoluted component, is shown in the middle. The bottom scan is the identified compound in the AMDIS library. Without deconvolution, the analyst would visually compare the background subtracted raw scan and library scans for confirmation. It would be very difficult, if not impossible, to say that Fenbuconazole, the target compound in this example, is present using that type of comparison.

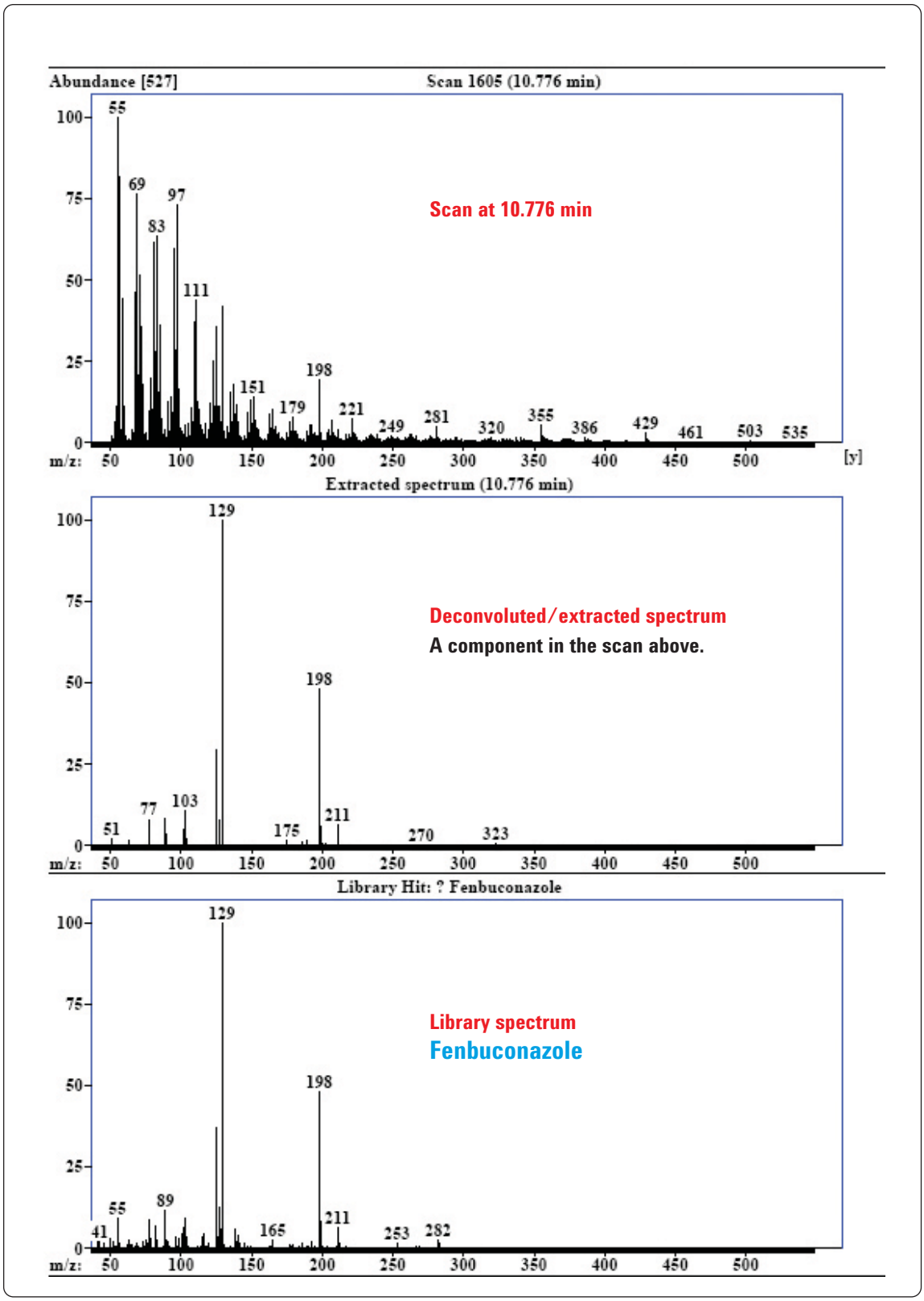


Figure 2. Comparison of raw, deconvoluted, and library spectra.

AMDIS Settings

Previous publications that discussed the power of using deconvolution to screen complex matrices, did not discuss specific AMDIS settings to define components [1, 5, 6]. In this study, several settings (that is, resolution, sensitivity, and shape requirements) are compared to find the maximum number of spiked compounds. The minimum match factor is set to 30 and the retention time window is limited to ± 30 seconds (RI window is set to 30) to qualify the hits from the retention time library search (Figure 3). The expected retention times of the compounds in the library database are obtained in acetone solvent without a retention gap. The samples in this study are in toluene solvent with a retention gap. Therefore, the retention time window is set wider than the normal 10 or 15 seconds, at ± 30 seconds.

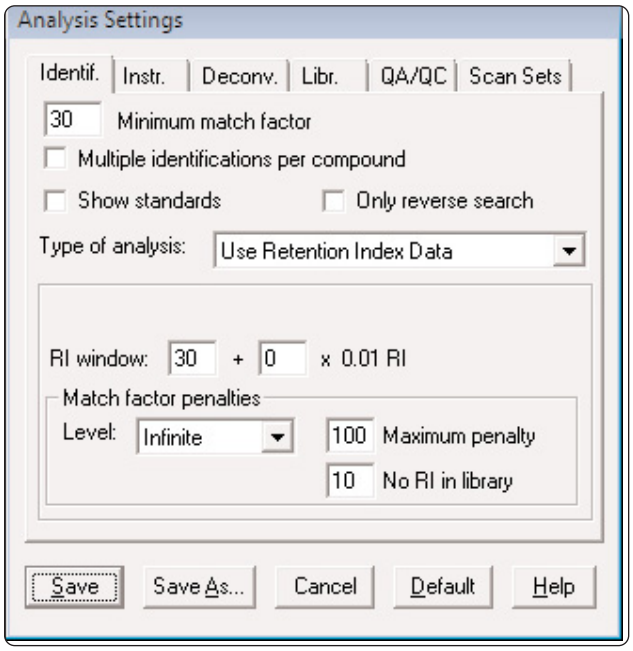


Figure 3. AMDIS identification settings.

Figures 4 and 5 describe some of the parameters in the AMDIS deconvolution tab. In this article, "1 M H M" means: adjacent peak subtraction = 1, resolution = medium, sensitivity = high, shape requirements = medium.

Settings can be optimized for chromatographic resolution, peak shape, retention time windows, acceptance criteria, and so forth. Settings can be saved to "ini" files. The chemist has control over the deconvolution and identification process by varying numerous AMDIS settings. Most of these parameter settings are not independent; so changing one parameter can affect another.

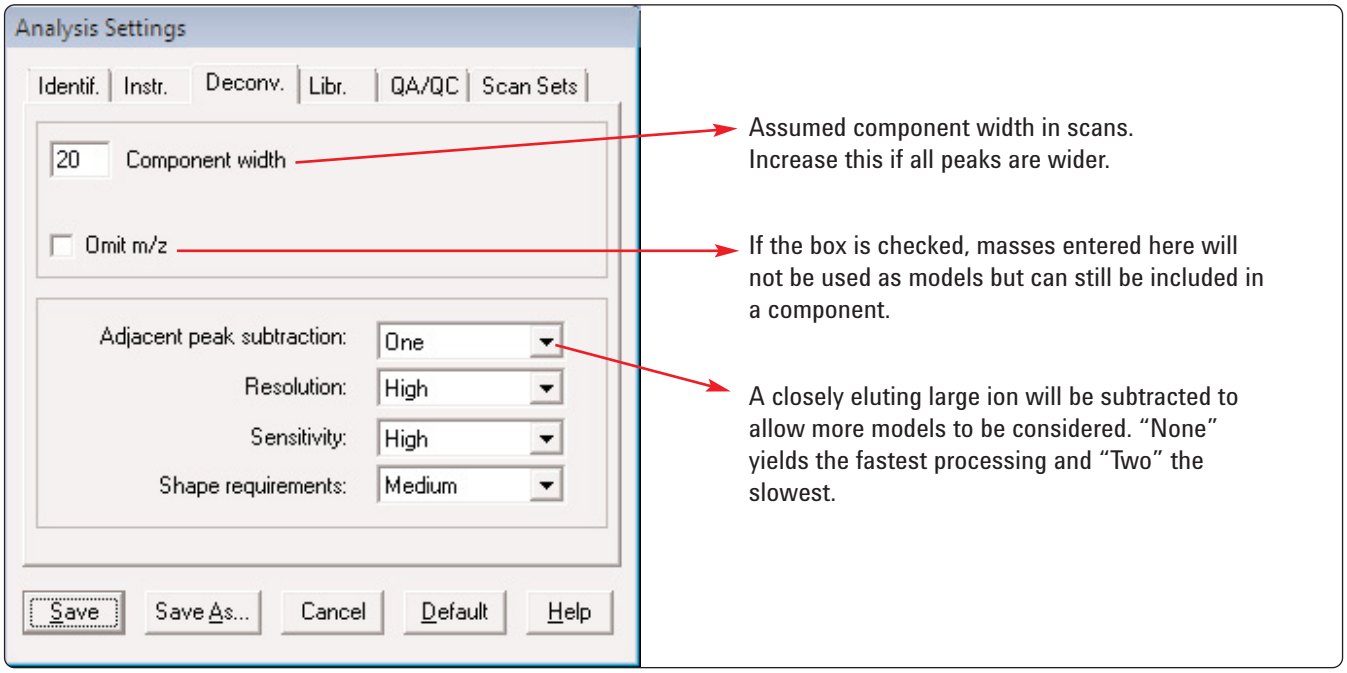


Figure 4. AMDIS deconvolution settings.

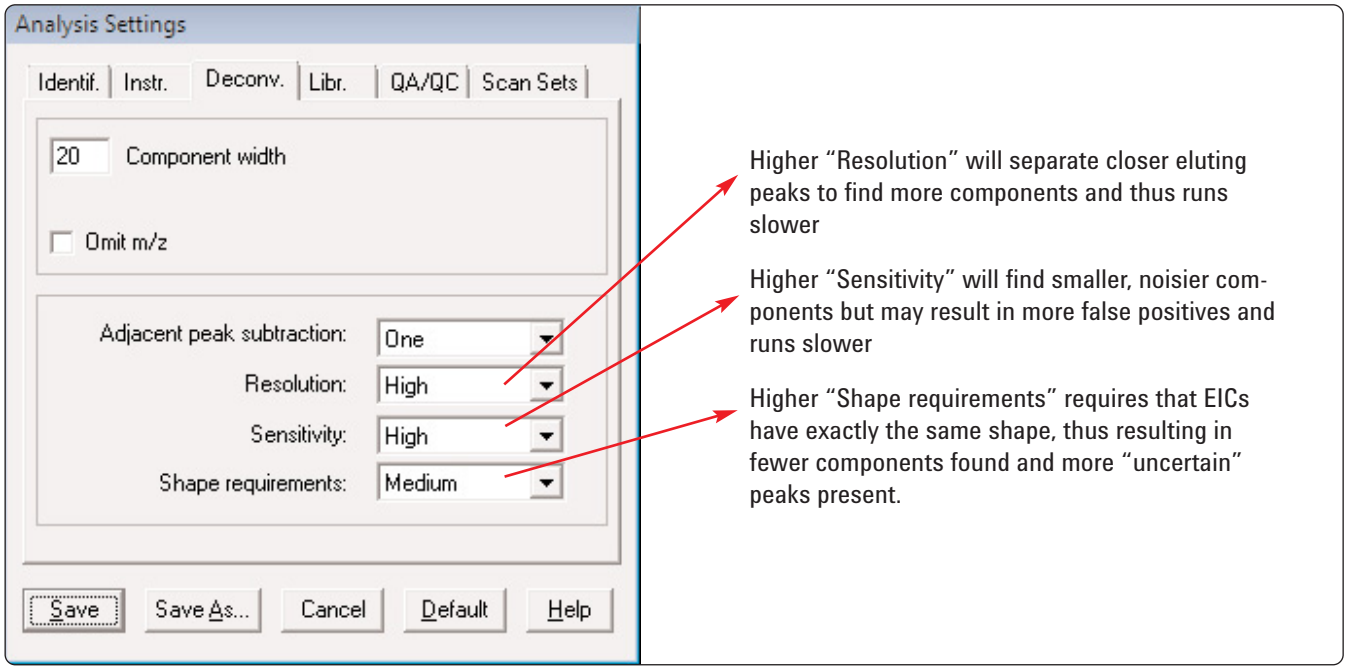


Figure 5. AMDIS deconvolution settings.

Results and Discussion

Deconvolution Settings

Figure 6 shows effects on match factors (y-axis) due to variation of adjacent peak subtraction and sensitivity across 35 pesticides (x-axis). This figure shows two things:

– The adjacent peak subtraction (1 or 2) makes little difference in match factor

– The sensitivity setting (very high and high) makes little difference in match factor

In the next few figures, the AMDIS setting is varied one at a time to observe the number of pesticides found. The reference point is the optimal setting (HHM) where the maximum number of hits were obtained.

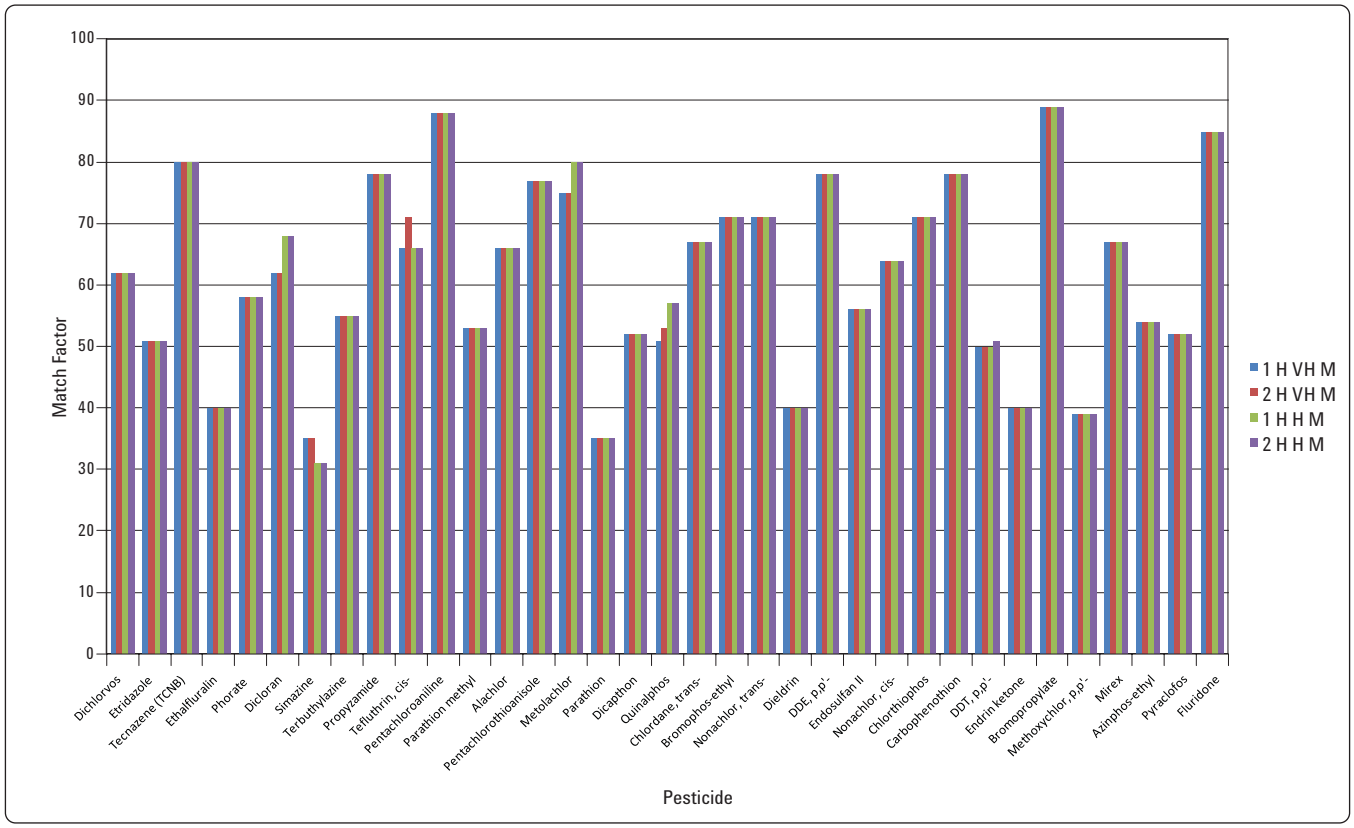


Figure 6. Comparison of match factors with four AMDIS settings.

Figure 7 shows that keeping the sensitivity and peak requirements the same, and lowering the resolution from H to M will find fewer targets. The number of targets found is in the yellow circle. A resolution setting of "low" yields even fewer targets.

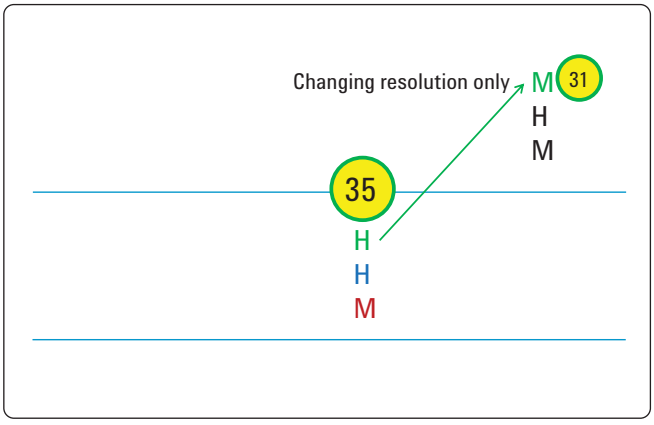


Figure 7. Number of compounds found by varying resolution.

Figure 8 shows that while keeping the resolution and peak requirement constant, lowering the sensitivity from H to M will find fewer targets. However, increasing the sensitivity from H to VH does not affect the number of targets found, similar to that in Figure 6.

Figure 9 shows that while keeping the resolution and sensitivity the same, lowering or increasing the peak shape requirement from M to L or H will find less targets.

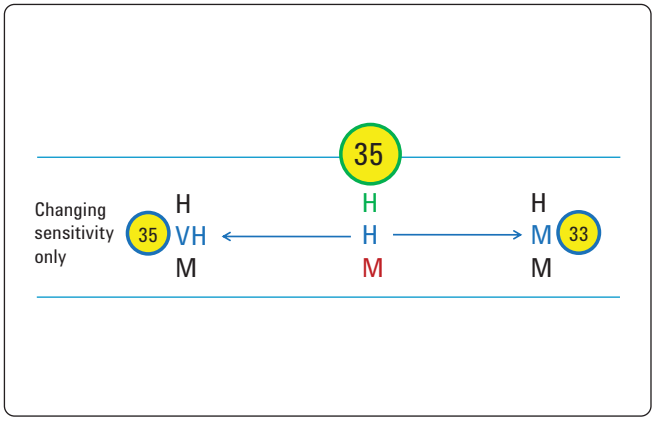


Figure 8. Number of compounds found by varying sensitivity.

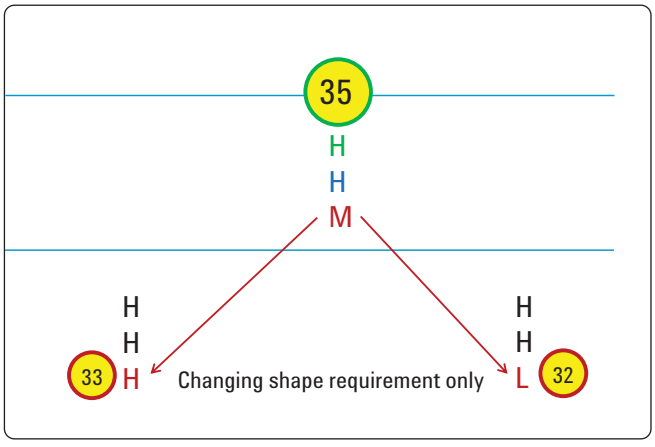


Figure 9. Number of compounds found by varying peak shape.

In addition to the number of targets found, we should look at the Average Match Factor (AMF) of all the targets found. The AMF is the number in the green triangle. Figure 10 shows that there is no significant variation in AMFs except in HHH mode (58.5) which is much lower than others (>61.6). This supports that HHM is still the optimal setting, considering processing speed and number of false positives.

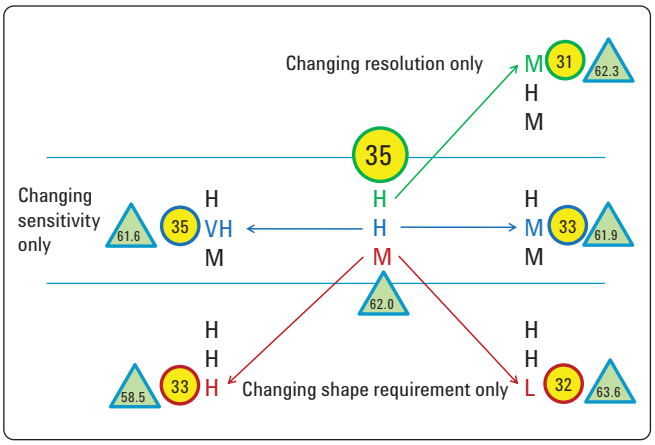


Figure 10. Comparison of average match factors with AMDIS settings.

ChemStation Quant settings

Figure 11 shows part of the "Edit Compound" screen in the MSD ChemStation. This shows the quant database for locating and confirming compounds using three ion ratios of each target analyte. The RT window is specified in the upper box and the ions and ion ratios are specified in the lower box.

As shown in Figure 11, the Extraction RT window is set to ± 0.5 min and the Qualifier Ion (Q1, Q2, and Q3), % Uncertainty is set to Absolute 50%. In ChemStation, the

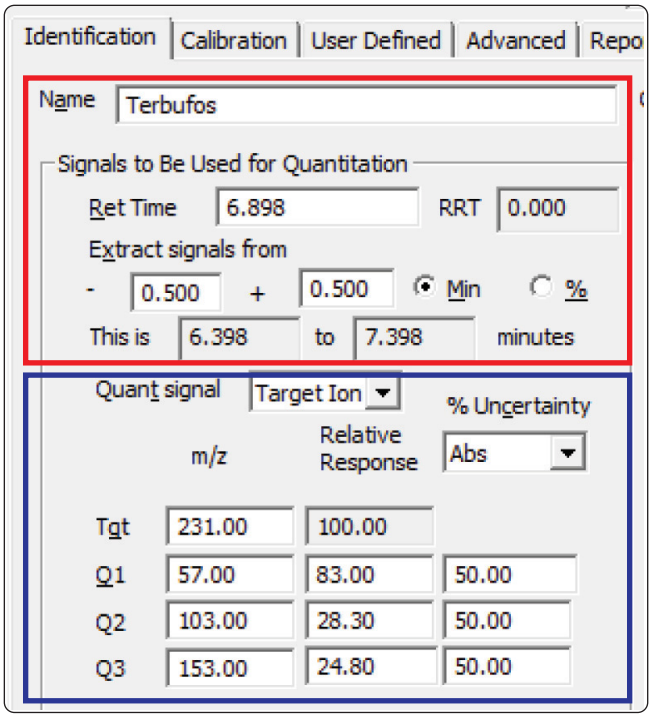


Figure 11. Target compound RT and ion setup.

target compound identification is based on four ions and three qualifier ion ratios. However, the target compound identification in AMDIS (Figure 2) was based on the full spectral library match which is more dependable.

Another key parameter in quantitation is the "Quantitation subtraction method" which is set to "Avg first and last" and not shown here.

Figure 12 is an overlay of four ions (Quant and Qualifiers) from ChemStation and the quant ion from AMDIS (in magenta).

Due to the chemical background, the four ions from ChemStation have offset and noisy baselines, which will affect the peak integration and proper quantitation results.

In comparison, the magenta trace is the deconvoluted quant ion from AMDIS. The chemical noise had been removed in the deconvolution process. It shows a flat baseline and accurate integration. There are other advantages of using deconvolution in GC/MS analysis as discussed below.

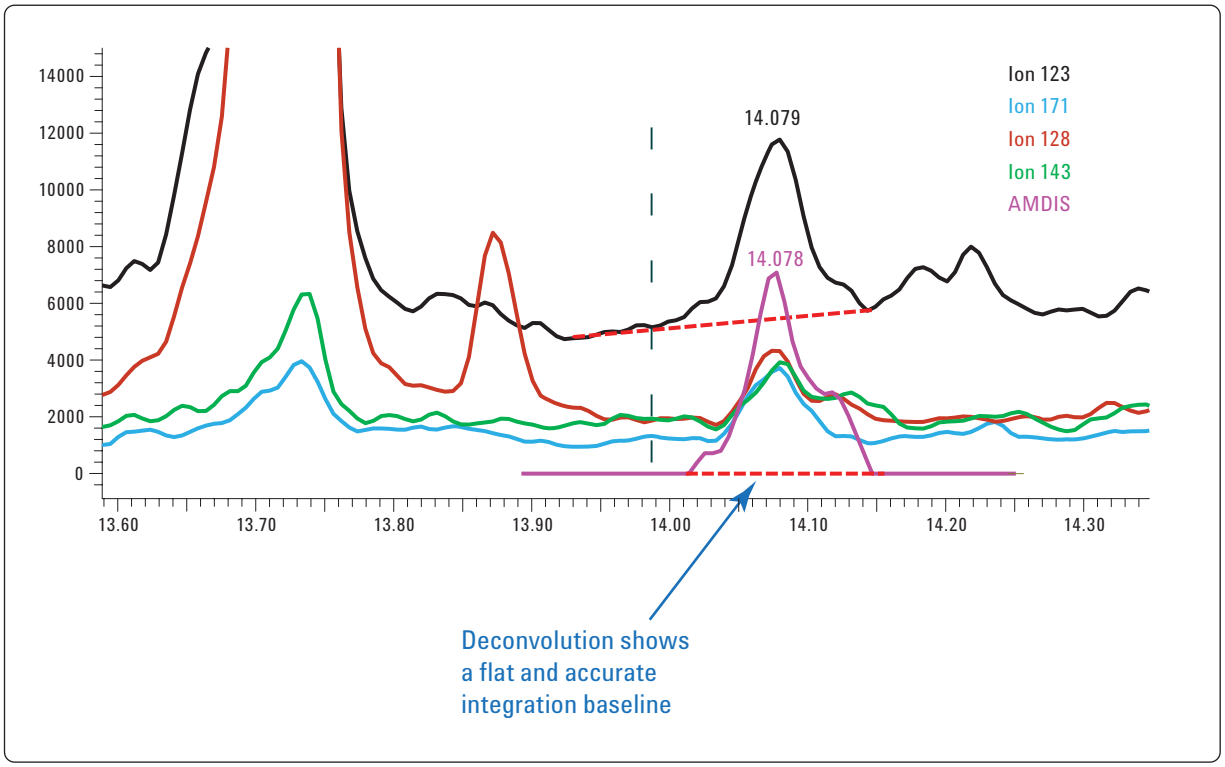


Figure 12. Target, qualifier and AMDIS deconvoluted EIC overlay.

Additional Advantages of Using Deconvolution

Finds more compounds than ChemStation does

In Figure 13, ChemStation did not integrate ion 109 (ChemStation target ion) at the expected RT, therefore, the compound was not found. AMDIS found Fonofos correctly, at 6.898 min. The qualifier ion ratios at this RT also match that required by ChemStation for identification.

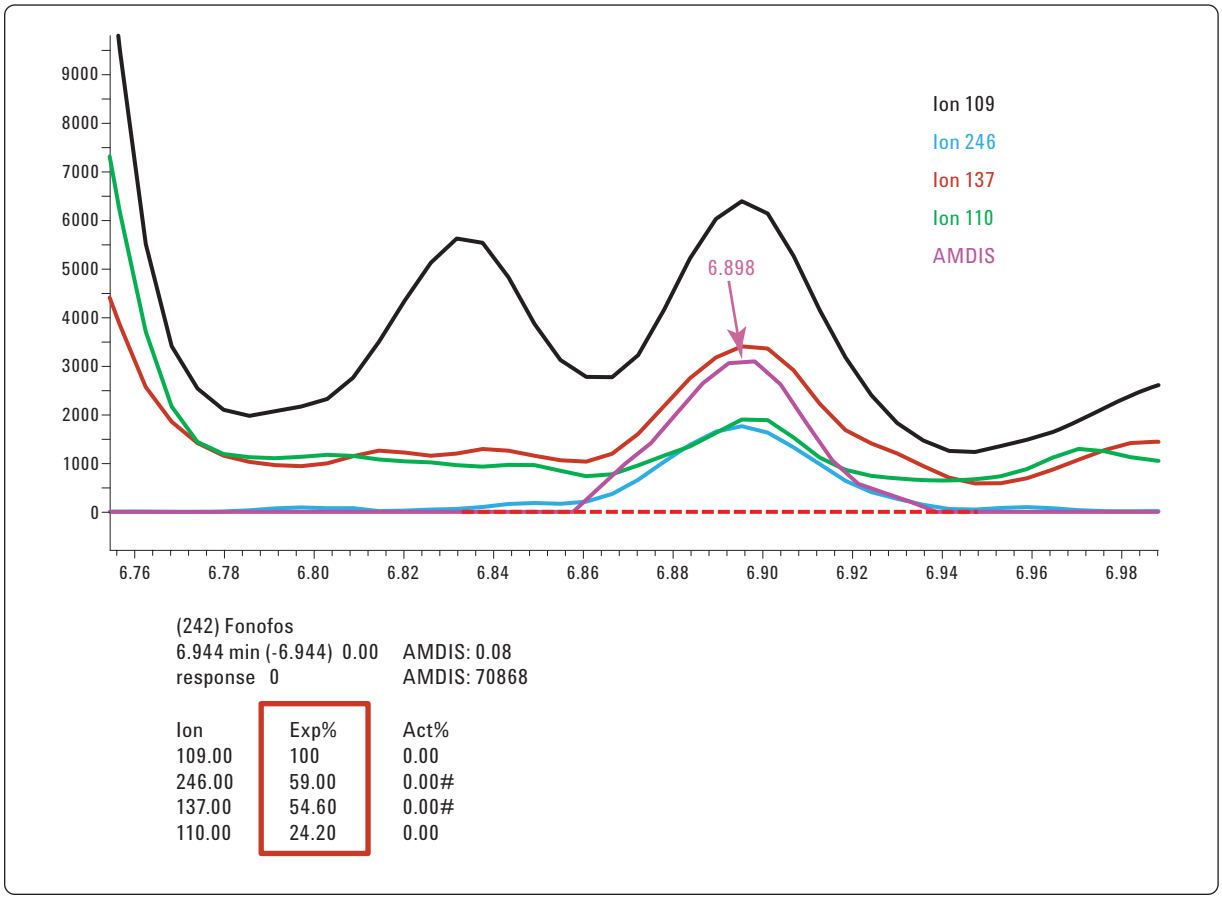


Figure 13. Target, qualifier and AMDIS deconvoluted EIC overlay.

Finds the correct peak

In Figure 14, from the size and location of the three qualifier ions, it is obvious that ChemStation picked the wrong peak (at RT = 4.067) to quantitate. However, AMDIS found a peak (at RT = 3.873) whose ion ratios are in agreement with the ChemStation qualifier ions. Again, this demonstrates that the AMDIS full-spectrum matching process is a more robust approach for identifing a compound in a complex matrix.

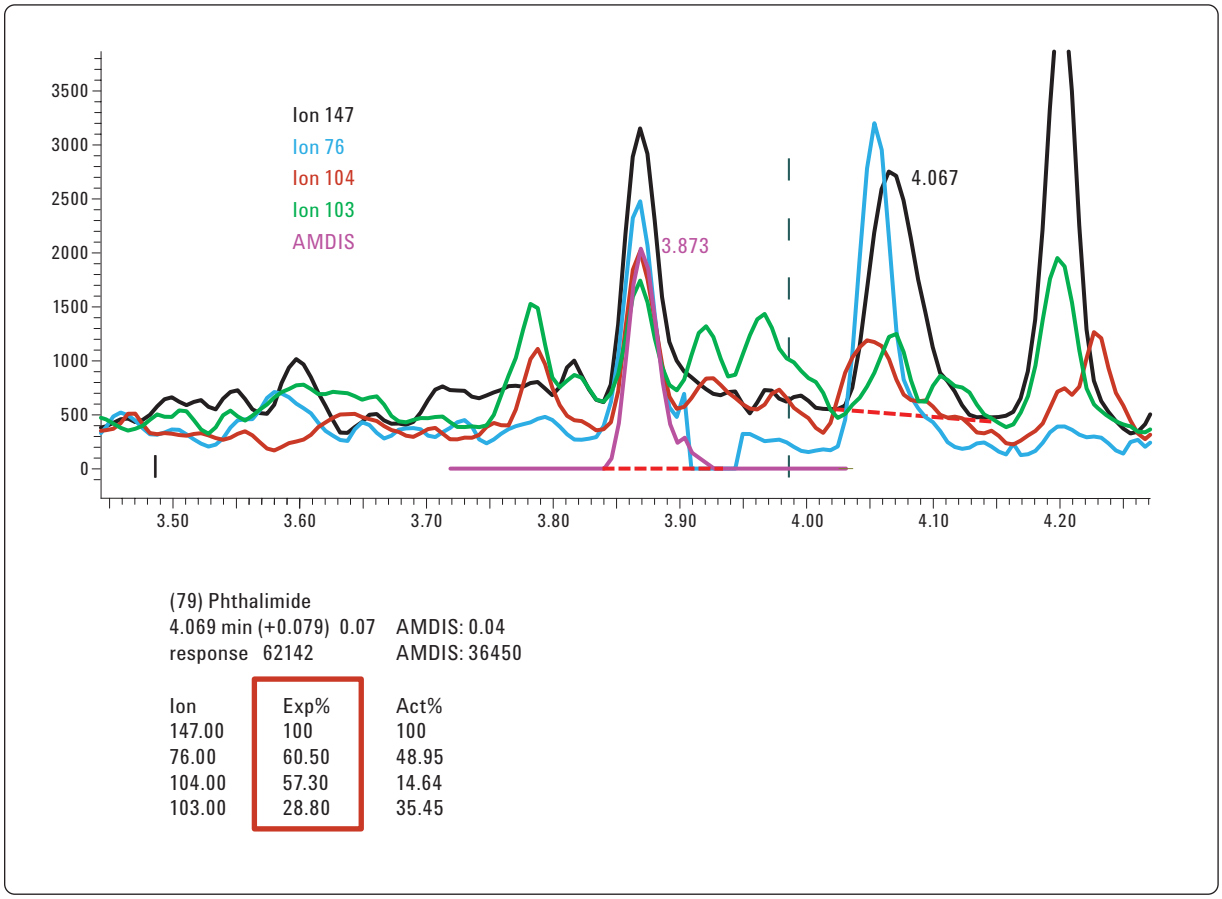


Figure 14. Target, qualifier and AMDIS deconvoluted EIC overlay.

Higher discrimination power than ChemStation

In Figure 15, the target ion (ion 235) is overwhelmed by the matrix background (shown as a large fronting peak). ChemStation was not able to differentiate the ion 235 contribution from the background or the compound; therefore it integrated the distorted peak. Due to the rising baseline, ChemStation integrated a large area of chemical background as the "target compound signal". On the other hand, AMDIS was able to deconvolute the compound signal away from the background ion and remove noise properly before the integration. This provides a more reliable quant result.

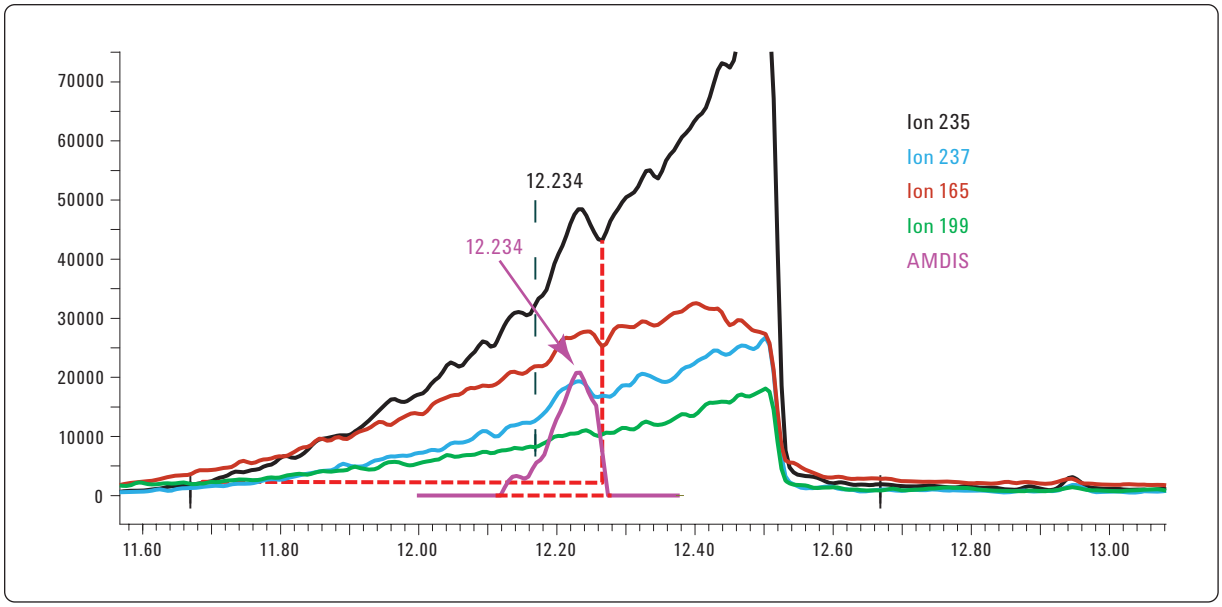


Figure 15. Target, qualifier and AMDIS deconvoluted EIC overlay.

Deconvoluted ion is noise-free, thus easier to integrate for more reliable quantitation results

In Figure 16, ChemStation and AMDIS found the same peak. Due to the noisy baseline, ChemStation drew the integration baseline (red dash line) incorrectly. Again, deconvolution removes chemical noise first, and can therefore, integrate the peak easily and reliably.

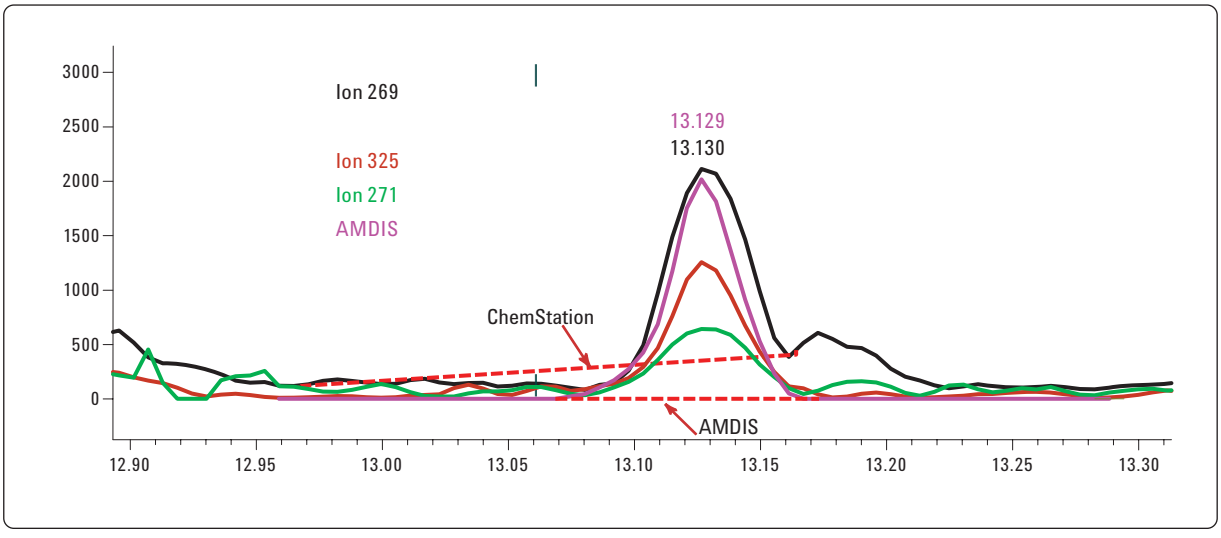


Figure 16. Target, qualifier and AMDIS deconvoluted EIC overlay.

Agilent's ChemStation add-on - Deconvolution Reporting Software (DRS) incorporates AMDIS deconvolution. Therefore, the above AMDIS advantages are automatically captured in DRS data processing which combines results from ChemStation, AMDIS, and NIST MS Search into one report.

Comparing number of compounds found between ChemStation and AMDIS

Figure 17 is a summary of the hits from ChemStation and AMDIS under four different settings, respectively. The blue bars represent the number of false positives and the red bars represent the number of actual target compounds found. On the left side of the graph, the settings of ChemStation are Ion Ratio Uncertainty. Although the absolute 30% and 50% increase the total number of compounds found, only about half of the 35 targets are found. The analyst is forced to review more hits and does not gain any additional information. The entire target list of 900+ compounds must be reviewed for false negatives. The right side of the graph shows that the four AMDIS settings gave similar results. In each case, all 35 targets were found with a reasonable number of false positives. There were no false negatives. The analyst must only review the positives, which is a significant time savings. This shows that AMDIS (DRS) is much more capable than ChemStation in finding target compounds in a complex matrix. AMDIS (DRS) provides better detectability and faster data processing.

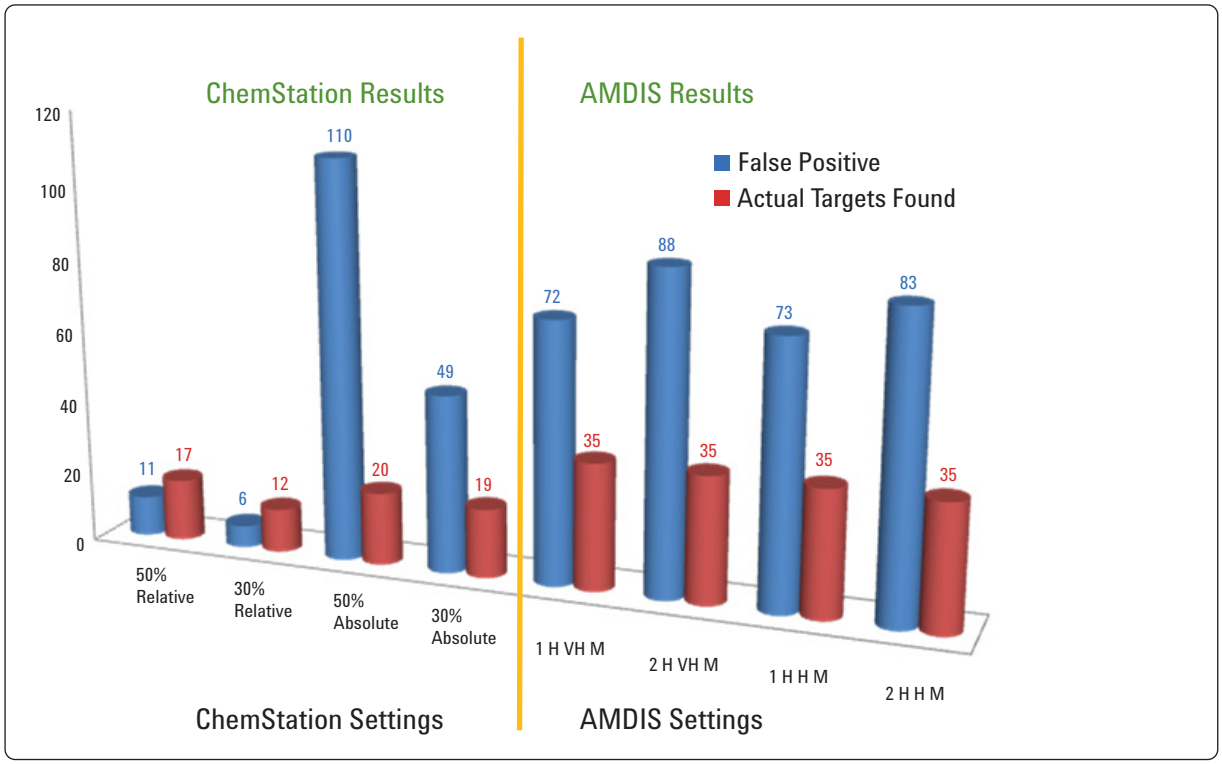


Figure 17. Overall comparison of AMDIS and MSD ChemStation compounds found.

Conclusions

- AMDIS finds more target compounds than ChemStation in a complex matrix. Deconvolution (DRS) provides a cleaned peak to integrate properly giving more reliable results.
- AMDIS did not miss any target compounds at the 50 ppb level using scan data. This minimizes the time an analyst must spend reviewing results.
- Confirmation of compounds is done in significantly less time with deconvoluted component spectra available.
- The detectability of compounds in a complex matrix is significantly improved with deconvolution. This can also be viewed as better or increased sensitivity through improved selectivity versus the background.
- Deconvolution Reporting Software (DRS) automates the deconvolution (AMDIS) process to produce an easy-to-read quantitation report.

Acknowledgement

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