“Mass spectrum-first”- revisiting Deconvolution for low-resolution GC-MS

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ABSTRACT

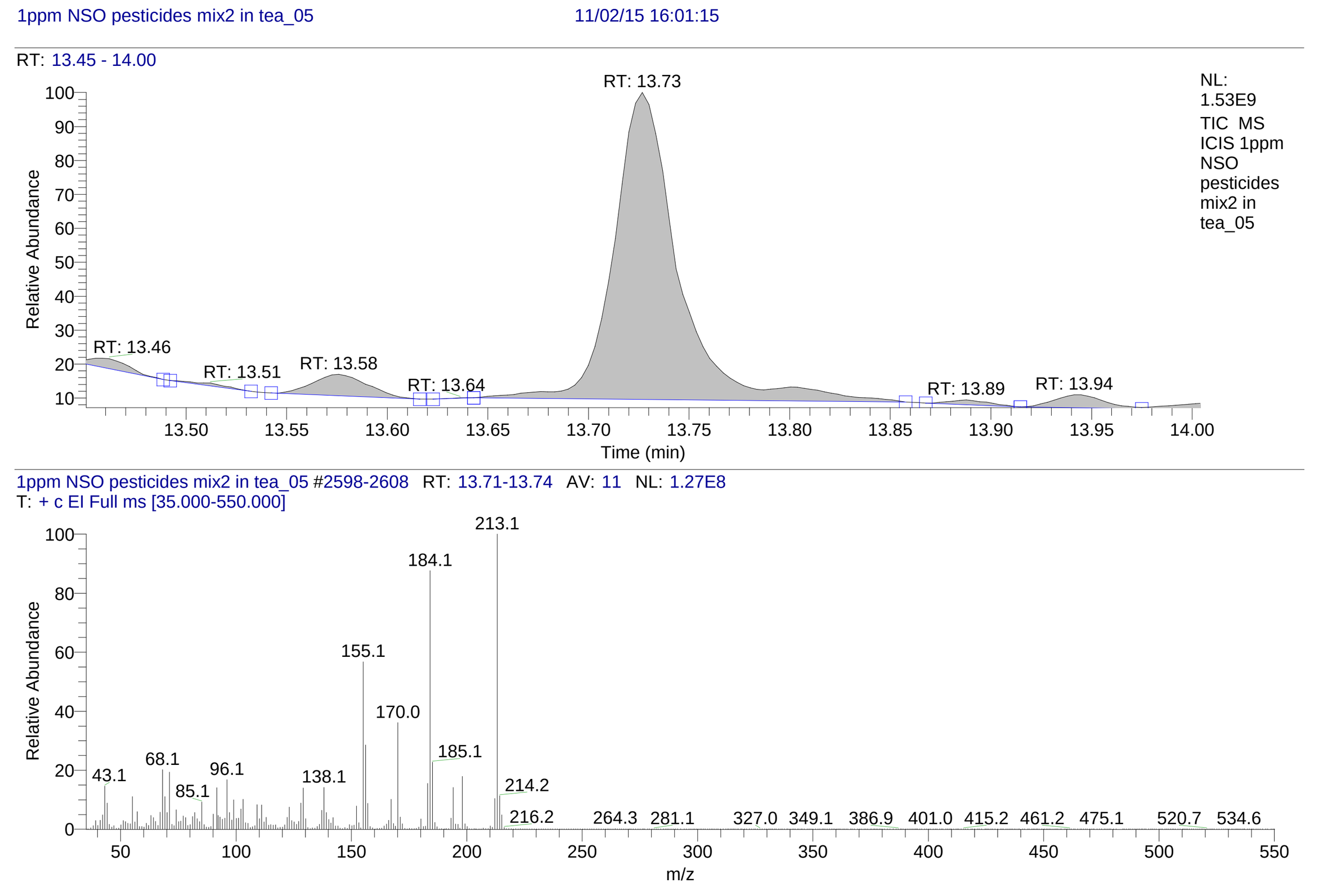
Gas chromatography coupled with a low-resolution mass spectrometer is a powerful analytical technique for identifying, confirming and quantifying organic compounds in complex matrices. Electron ionization (EI) produces a fragmentation pattern that is generally considered instrument independent. This has led to availability of a large database of EI library spectra like the NIST/EPA/NIH mass spectral library that contains hundreds of thousands of compounds and is primarily used for identification of “known” unknown compounds. Determination of unknown compounds by library matching has traditionally followed a ‘Chromatography-first’ approach. ‘Chromatography-first’ approach involves peak detection by one of the several available algorithms (like Genesis, ICIS, COBRA, etc.) and matching the detected peaks with the library to determine the closest matching compound. One of the flaws of using this approach to determine unknown compounds is that co-eluting compounds can be difficult (or almost impossible at low-levels) to identify. The deconvolution software takes the ‘Mass spectrum-first’ approach. This approach identifies compounds that would be co-eluting chromatographically but whose mass spectrum peak apexes are still separated.

In this study we revisit the concept of deconvolution and show its utility in identifying unknown compounds at both high and low level concentrations. We show how “mass spectrum-first” approach has assisted in identification of pesticides in complex matrix with the ability to deconvolve not only co-eluting pesticides at high levels, but also the ability to identify low level pesticides that are essentially “buried” in the noise of the matrix. With the availability of advanced filtering capability and retention indexing feature we hope this new approach of looking at GC-MS data will significantly decrease the burden of high-throughput labs that are screening for specific class of compounds like pesticides or drugs of abuse.

INTRODUCTION

When analyzing compounds in complex matrices, one common occurrence is co-elution of compounds of interest or co-elution of analytes with matrix compounds. Figure 1 shows an example where there are two pesticides co-eluting to form a single chromatographic peak. A NIST library search at the apex of the peak identifies the compound as Simetryn but on closer examination of the spectra one can observe that some of the mass spectral peaks (like *m/z* 184 and 129) are prominently missing from the library spectra.

Figure 1. (a) Chromatogram and mass spectrum of a peak found in tea matrix spiked with pesticides; (b) library search of the same mass spectrum showing the NIST identification.



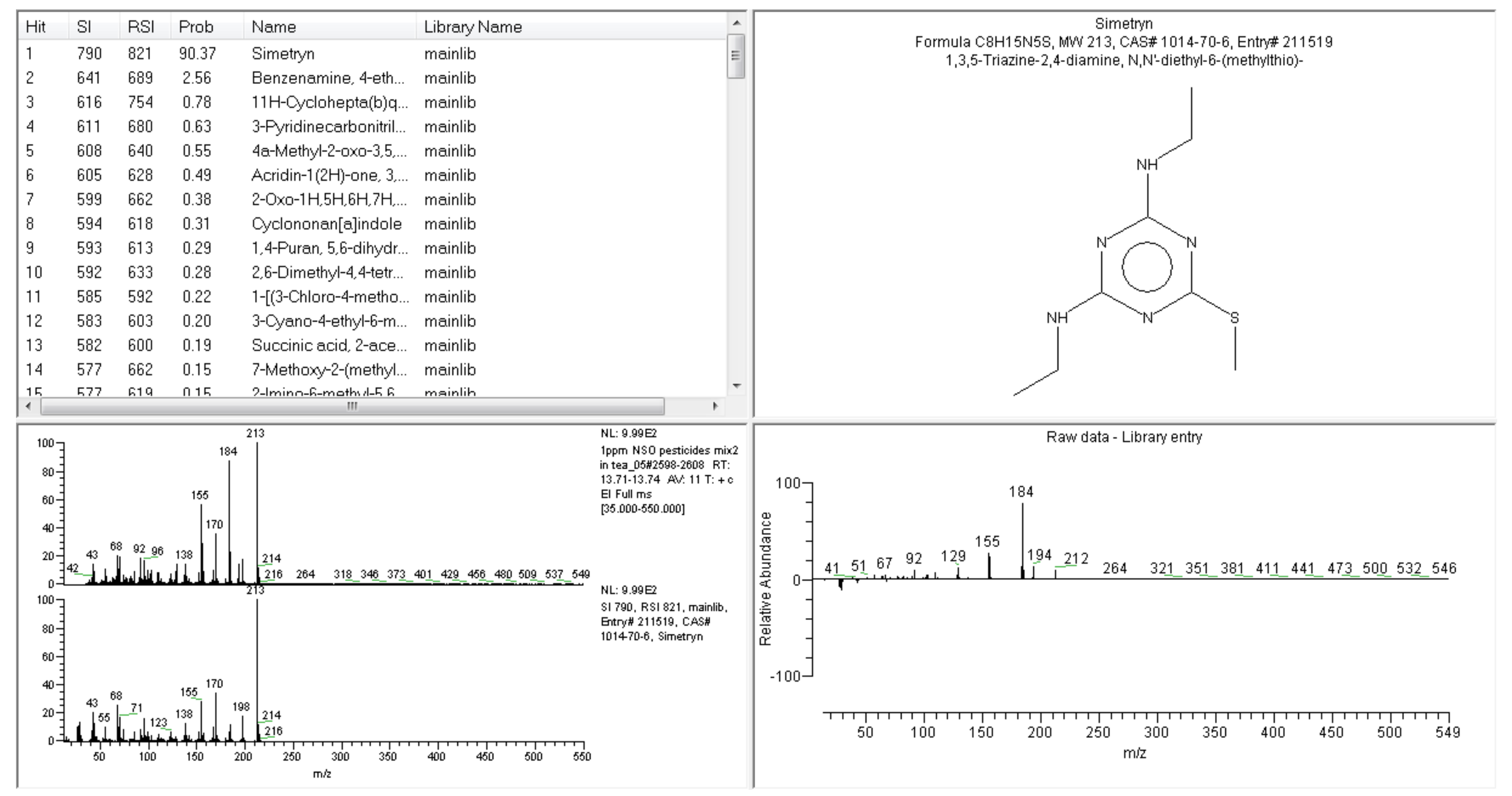
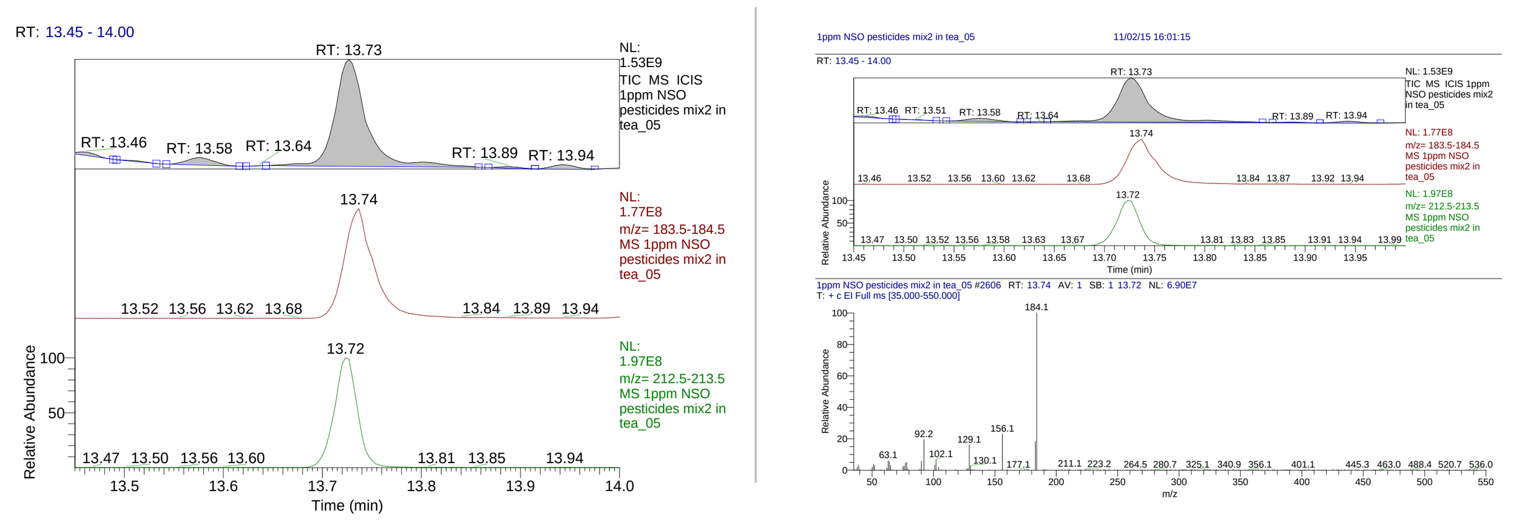


Figure 2 shows the extracted ion chromatogram (XIC) for 184 and 213 along with the total ion chromatogram (TIC). One can clearly identify that there are two peaks at apexes of 13.72 and 13.74 minutes. On performing a library search at 13.74 minutes while subtracting the mass spectra at 13.72 minutes identifies the compound to be Fuberidazole.

Figure 2. (a) Extracted ion chromatograms (XIC) of *m/z* 184 and 213 and spectra at retention time of 13.74 minutes subtracted by spectra at 13.72 minutes and (b) its library match results.



This manual process of deconvoluting a peak is tedious and requires extreme care and attention and if the concentration of the analyte is low such that it is hidden in the noise then there is no way to identify the peak by this process.
The Thermo Scientific™ Deconvolution plugin tool for Thermo Scientific™ TraceFinder™ 4.1 software is designed to automatically deconvolve chromatographic peaks into multiple components represented by aligned mass spectral peaks. In addition the plugin automatically does a library search match and has the ability to export an unknown compound to the NIST format in order to create a user-defined library of compounds. When combined with the Unknown Screening functionality of the TraceFinder software, it can be used to do cross-sample overlay of analytes that are well below the conventional detection limits of a unit resolution quadrupole instruments.

MATERIALS AND METHODS

Sample Preparation

The samples analyzed were QuEChERS extracted tea matrix which were spiked with 1 ppm, 10 ppb, 5 ppb and 1 ppb levels of a custom pesticide mix containing nitrogen, oxygen and sulfur compounds. A Thermo Scientific™TSQ™ 9000 mass spectrometer equipped with the Thermo Scientific™ ExtractaBrite ion source coupled to a Thermo Scientific™ TRACE 1310 GC system was used to analyze the samples. The mass spectrometer was operated in full-scan mode scanning 35-550 Da with dwell time of 0.2 s.

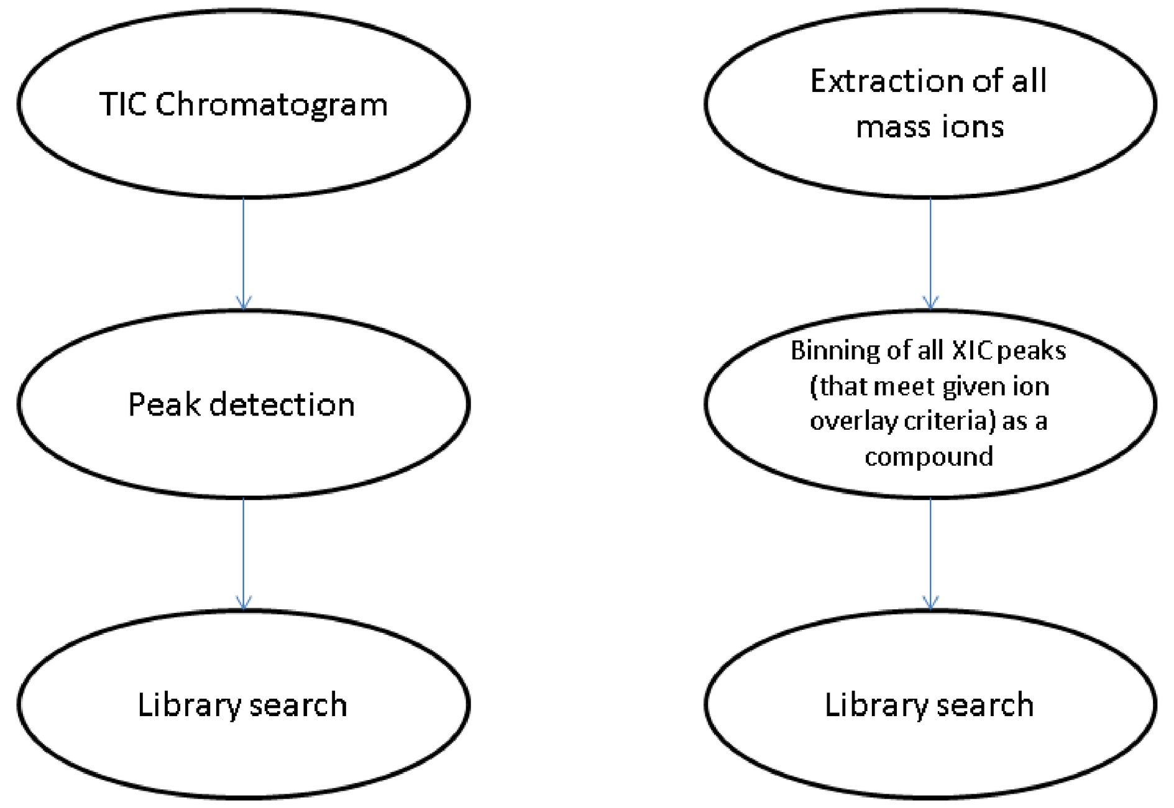
Software-

Deconvolution plugin version 1.3 was used in conjunction with TraceFinder 4.1 software.

RESULTS AND DISCUSSION

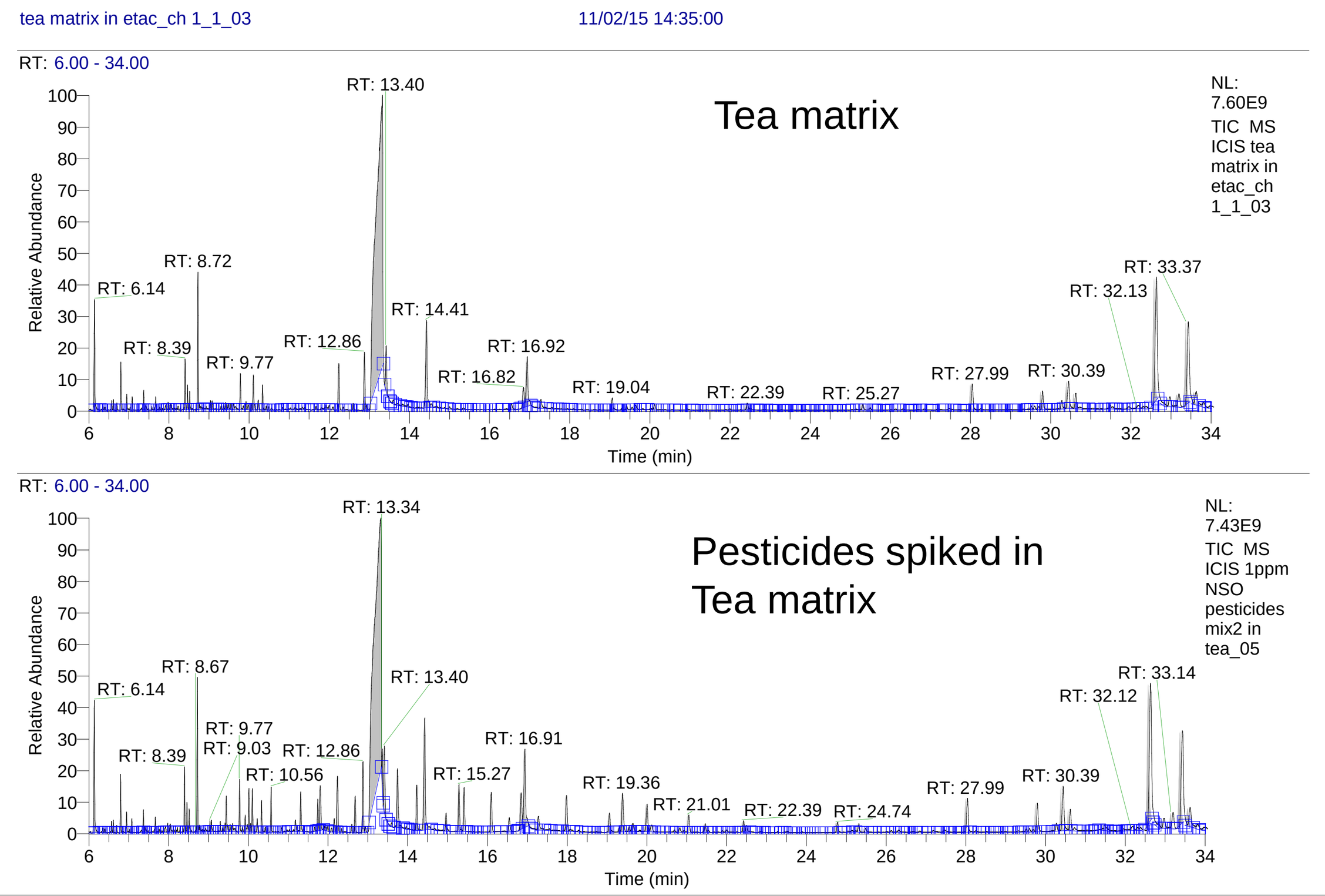
Determination of unknown compounds by library matching has traditionally followed a ‘Chromatogram-first’ approach. ‘Chromatogram-first’ approach involves peak detection by one of the several available algorithms (like Genesis, ICIS, COBRA, etc.) and matching the detected peaks with the library to determine the closest matching compound. The manual method of determination of unknown compounds also follows this approach but with greater flexibility of which scans to select and the background scans to subtract. One of the flaws of using this approach to determine unknown compounds is that co-eluting compounds can be difficult (or almost impossible at low-levels) to identify. The deconvolution plugin software takes the ‘Mass spectrum-first’ approach. It starts off with looking for masses whose peak apexes are aligned within a certain criteria (peak ion overlay %) and creates an extracted ion chromatogram (XIC) out of the mass spectrum peaks that are aligned and bins them as individual compounds. It then performs library search on these binned XICs. This approach identifies compounds that would be co-eluting chromatographically but whose mass spectrum peak apexes are still separated. The procedure is shown in Figure 3.

Figure 3. Unknowns analysis workflow - (a) Traditional ‘Chromatogram-first’ approach and (b) ‘Mass spectrum-first’ approach of the deconvolution plugin software.



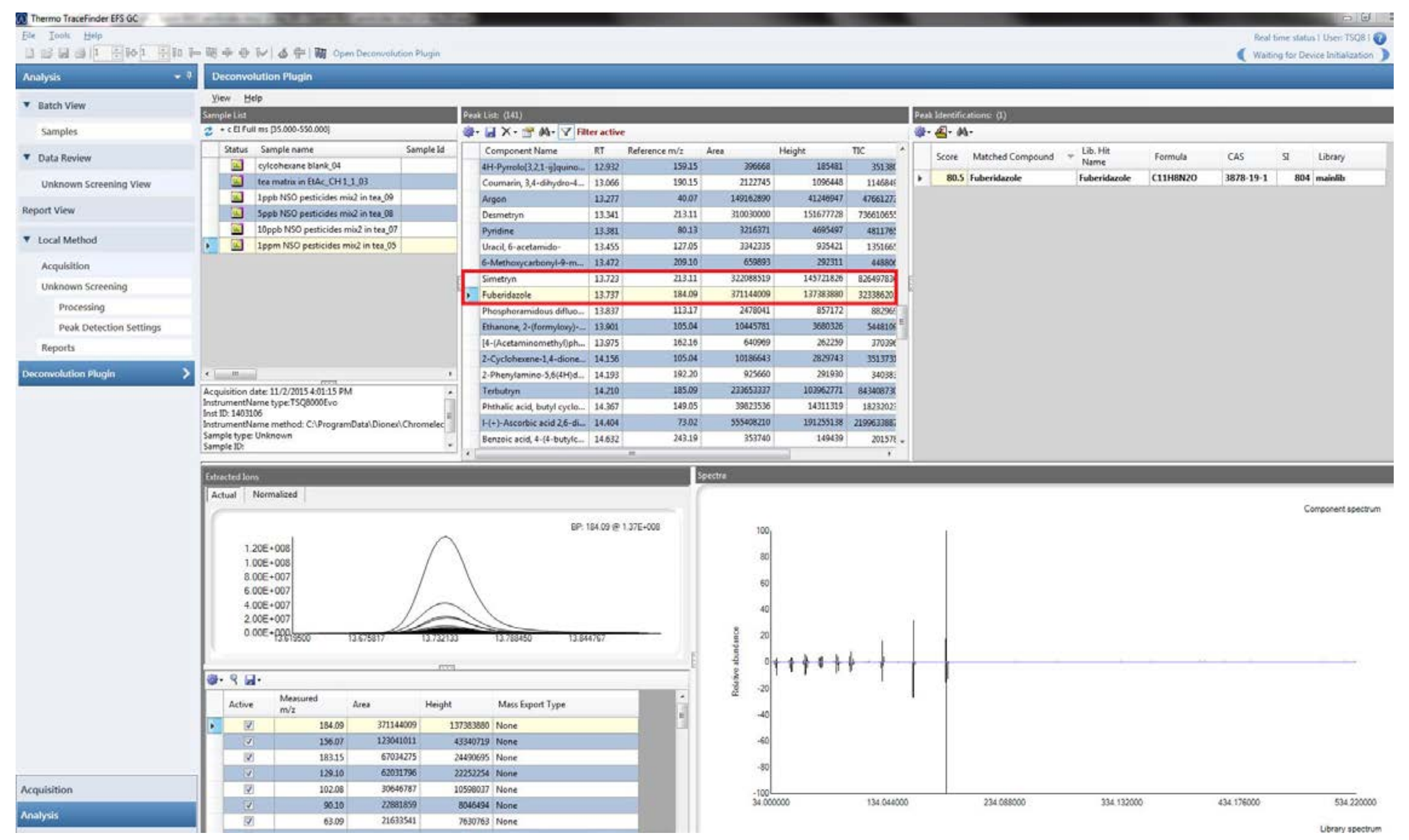
Deconvolution at high levels of analyte- Figure 4 shows the comparison of chromatograms of the QuEChERS extracted tea matrix unspiked and spiked with the custom pesticide mix at 1 ppm level. One can easily pick out pesticides from the matrix by simply subtracting the two chromatograms. Most modern chromatography data system software have the capability to do that. What is challenging are cases where you have co-eluting pesticide that show up as a single chromatographic peak. The ‘chromatogram-first’ approach mentioned above would give poor match scores for such peaks and thus fail to identify one or the other co-eluting compound.

Figure 4. Chromatogram of unspiked and pesticides spiked tea matrix.



The deconvolution plugin software computationally separates these individual compounds from the single chromatographic peak. It then proceeds to do an automatic library search and gives the results in a tabular format in form of ‘Peak List’. Figure 5 shows the snapshot of the software highlighting Peak list table. The figure also shows that it deconvoluted the single chromatographic peak of Figure 1 into its components viz. Simetryn and Fuberidazole.

Figure 5. Snapshot of the deconvolution plugin software highlighting separation of Simetryn and Fuberidazole as individual compounds.



Another example of deconvolution is shown in Figure 6 where the plugin automatically deconvolves and identifies peak at 11.79 minutes as Carbofuran and Prometon. Figure 7 shows all the aligned peaks associated with Carbofuran and Prometon as separate compounds as determined by the deconvolution plugin software.

Figure 6. Snapshot of the deconvolution plugin software highlighting separation of Carbofuran and Prometon as individual compounds.

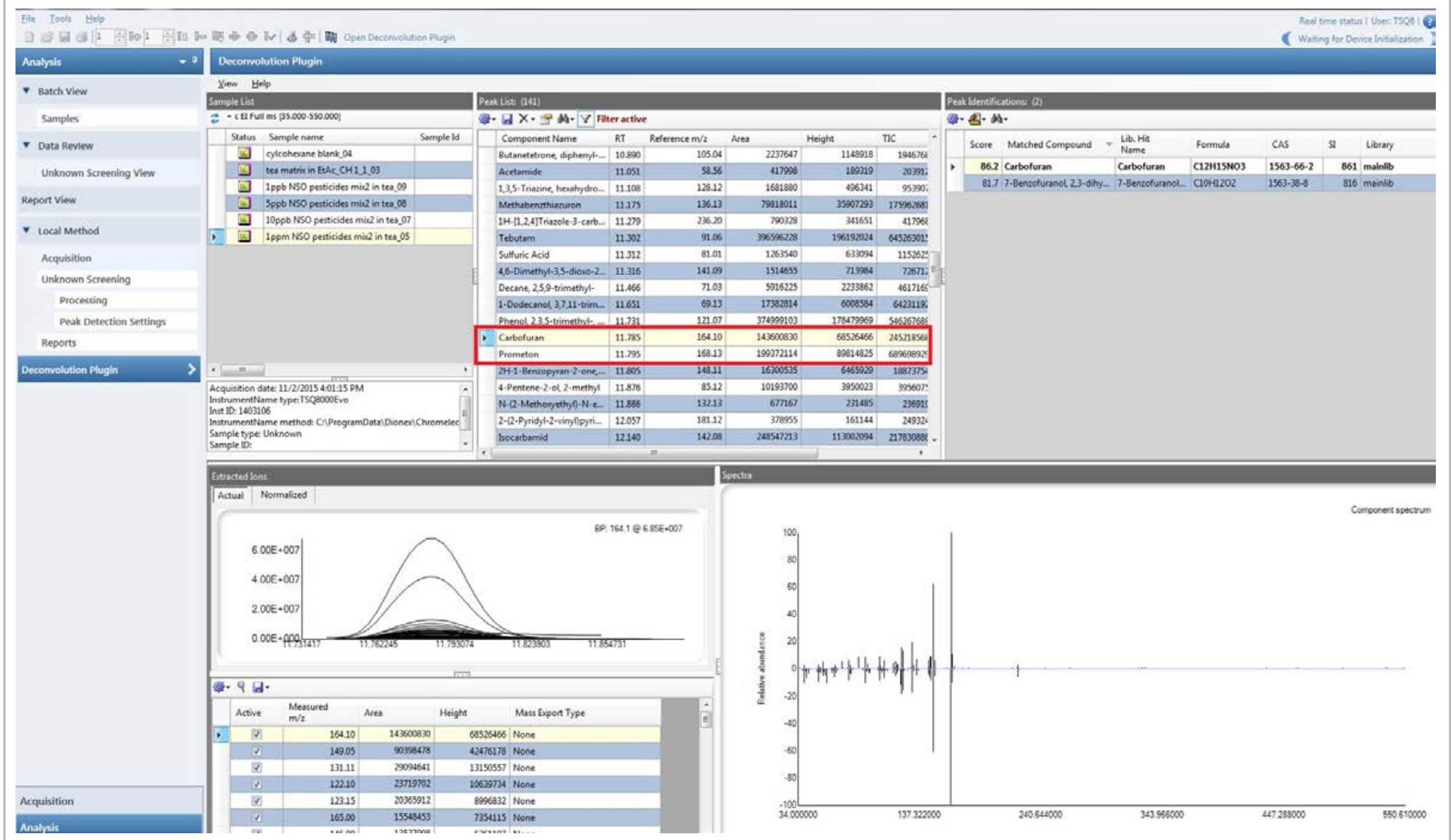
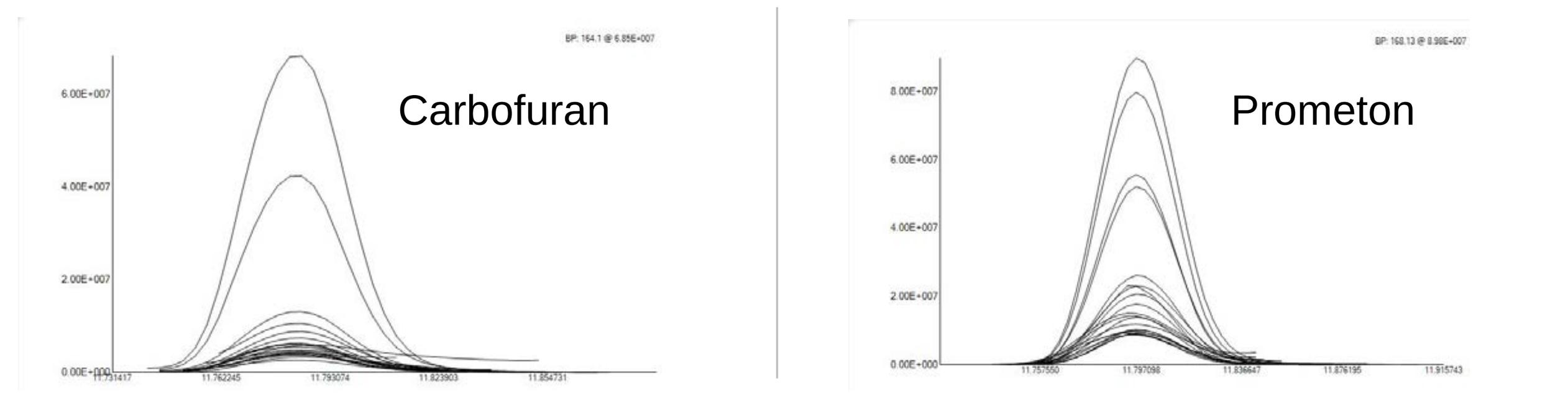
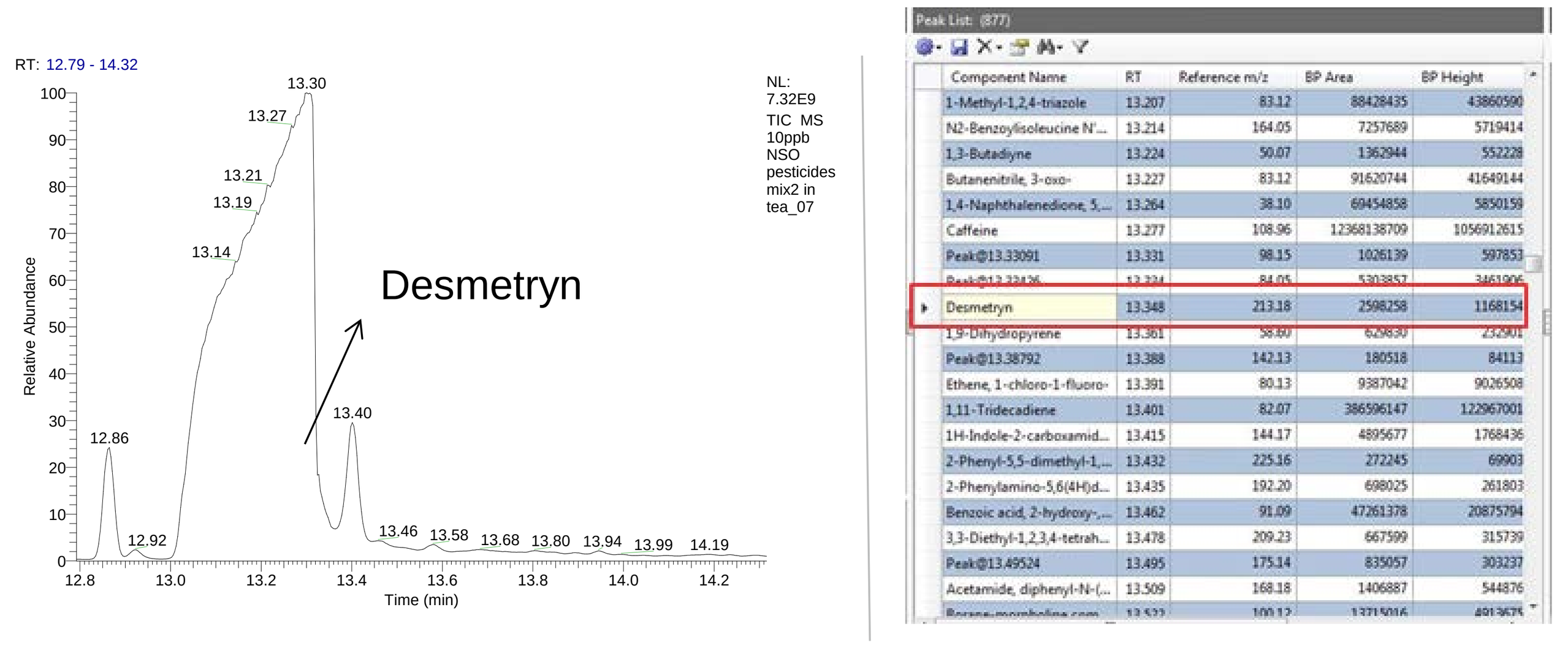


Figure 6. Deconvolution plugin result showing all the binned XICs associated with individual compounds.



Deconvolution at low levels of analyte- One of the most useful features of the deconvolution plugin software is the ability to identify compounds at low-levels even in presence of large background or heavy interference. Figure 7 shows the deconvolution software successfully identifying Desmetryn at 10 ppb levels even with a huge caffeine interference.

Figure 7. TIC showing co-elution of 10 ppb Desmetryn with the huge caffeine peak (a) and Deconvolution plugin correctly identifying Desmetryn at 10 ppb level.



CONCLUSIONS

This poster illustrates the workings of the Deconvolution Plugin software and its ability to deconvolve a single chromatographic peak composed of two or more co-eluting compounds. The software also has the ability to identify compounds at low levels whose mass traces would otherwise be a part of the noise and thus do not form well defined chromatographic peak. When combined with the unknowns screening function of TraceFinder 4.1 software one can have the ability to detect components that are well below the conventional detection limits of the unit-resolution mass spectrometer.

TRADEMARKS/LICENSING

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