thermo scientific

Automated and accurate component detection using reference mass spectra

Authors

Barbara van Cann[1] and Amit Gujar[2]

[1]Thermo Fisher Scientific, Breda, NL

[2]Thermo Fisher Scientific, Austin, TX, USA

Keywords

GC-MS, LC-MS, IC-MS, Chromatography Data System, full scan acquisition, targeted screening, reference mass spectrum, Chromeleon CDS

Goal

To demonstrate the capability of Chromeleon CDS to compare full scan MS spectra of peaks to pre-defined laboratory reference spectra, in order to increase confidence of correct component detection.

Introduction

Gas chromatography coupled with mass spectrometry (GC-MS)* is a powerful analytical technique for identifying, confirming, and quantifying compounds in complex matrices.[1] Its application ranges from analysis of pesticides in food, to environmental contaminants in air, water, and soil, to drug analysis in biological matrices.[2-4] The chromatographic separation of matrix and analytes is performed by the GC, and the mass spectrometer, typically equipped with an electron ionization (EI) source, is used for identifying the analytes based on the unique fragmentation pattern of the molecule. Extensive spectral libraries, such as NIST/EPA/NIH Mass Spectral Library with Search Program, are available to compare the generated mass spectra to ascertain the identity of the compounds.

Generally, compound identification is performed based on the retention time, though other identification methods can provide additional confidence. Using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), the targeted screening of compounds involves setting up the quantitation and confirmation ions in the processing method, along with the retention times. The identification and confirmation of the analyte is based on detection of the peak at that retention time along with matching confirming ions in specific ion ratios. This works well when having a relatively clean sample with a minimum

*This functionality is not limited to GC-MS, but also applicable to liquid chromatography (LC) or ion chromatography (IC) coupled with MS.

ThermoFisher SCIENTIFIC

number of matrix or coeluting peaks, but not in complex samples or samples contaminated with background noise. For example, when identifying pesticides in food or coeluting matrices, there is always the danger of having an incorrect identification, especially when the chromatography or spectrometry is not optimal.

Figure 1 shows an example of a tea matrix without spiked pesticides, incorrectly showing some of the analytes as positively identified. This means that the software found peaks in the extracted ion chromatogram that were within the specified retention time and within ion ratio criteria and thus determined that the component was detected and confirmed. However, when taking a closer look at the mass spectrum of the identified component (Figure 2A) and comparing this to the expected spectrum of the component from a spectral library (Figure 2B) we find a poor spectral match, indicating this is not the correct component.

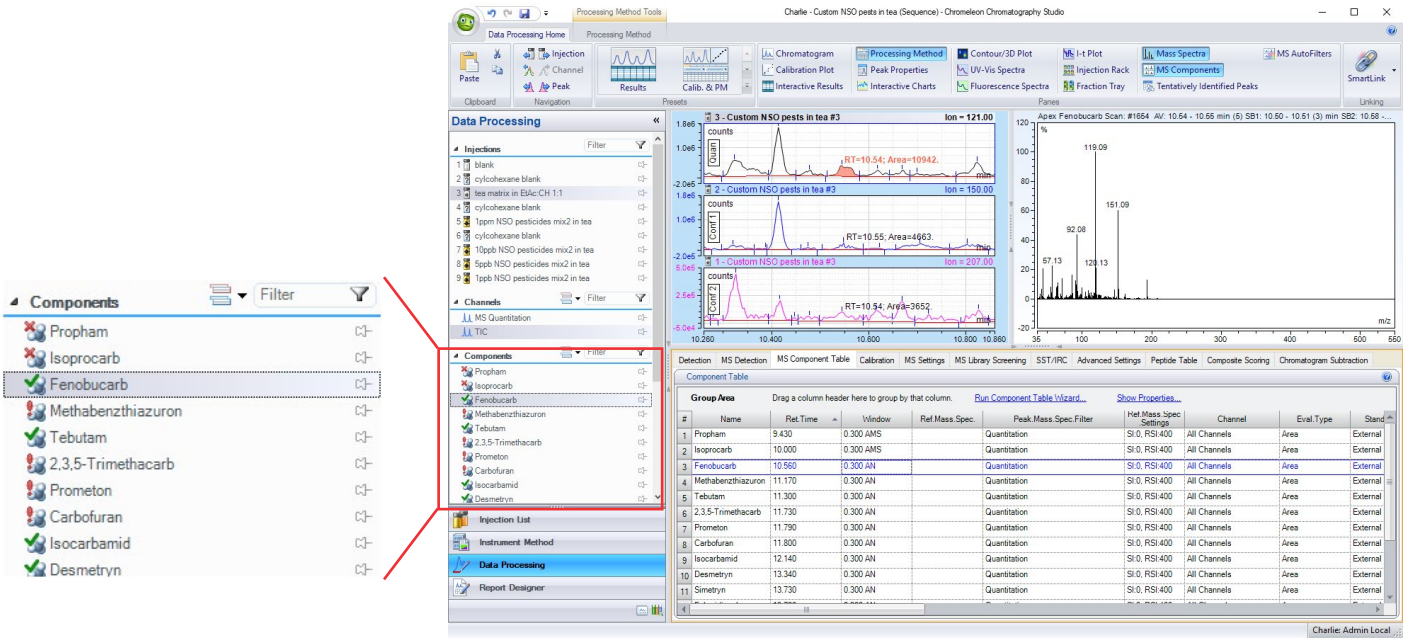


Figure 1. "False positive" identification of certain pesticides in tea matrix

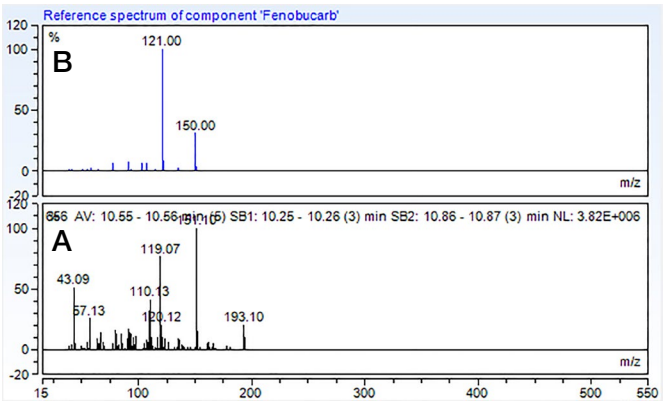


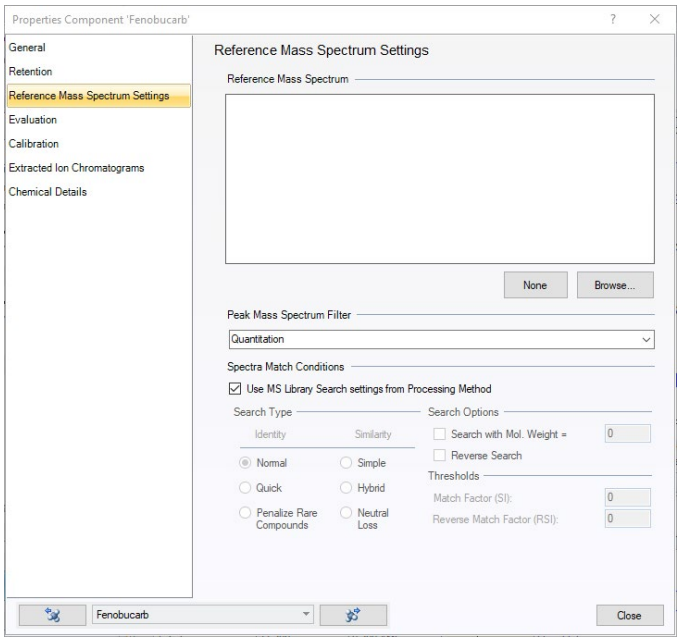
Figure 2. Comparison of components mass spectrum (A) to the fenobucarb reference mass spectrum (B)

This technical note demonstrates the use of reference mass spectra to increase the confidence in identification of the components and minimize the possibility of false positive identifications when screening for compounds based on full scan spectral library matching. In addition, this method allows for correct identification under various conditions causing retention time shifts, or even when retention times are completely unknown.

Reference mass spectrum

The ability in Chromeleon CDS to assign a reference spectrum to each component drastically increases the confidence in the identification of these components (Figure 3A). The parameters for comparing the component's spectra with the library spectra can be based on the global MS Library Screening parameters or they can be set individually for each component. The reference spectrum can be selected from any of the commercially available libraries (Figure 3B). One can also use the mass spectrum from another injection as the source of reference mass spectrum.

A



B

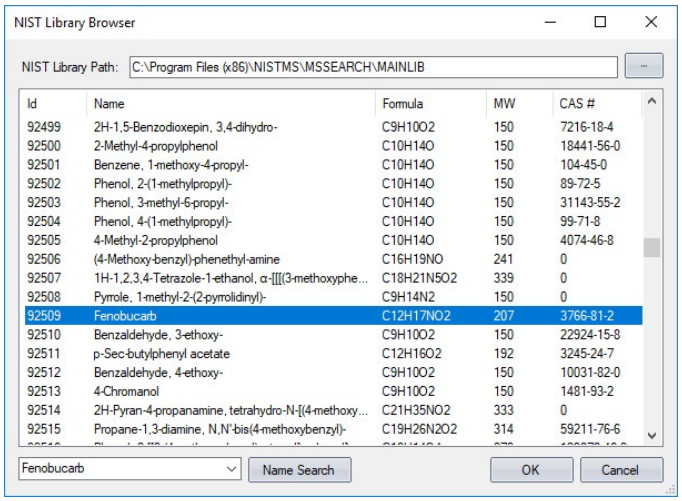


Figure 3. Addition of reference mass spectrum (A) by selecting a spectrum from a NIST library (B)

For accurate detection of components it is possible to define the component match settings (Figure 4). For chromatography, the standard match is based on detection of the component within a specified retention time window, looking for the first or greatest peak in this window, or for the one nearest to the defined retention time. A shift or a drift in retention times can cause the target peak to shift outside the time window, leading to incorrect identification of the component or components not being identified at all.

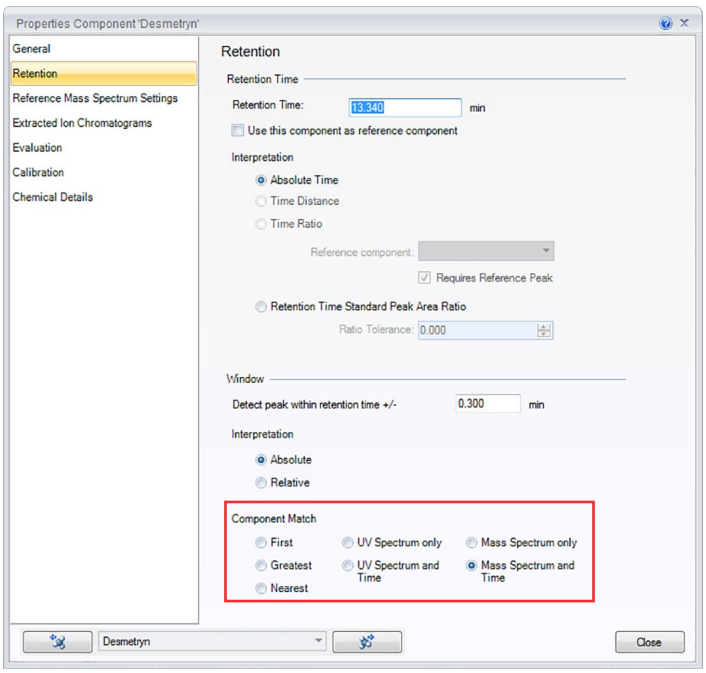


Figure 4. Component match options

Figure 4 shows the additional component match options provided by the Chromeleon software, namely “Mass Spectrum only” and “Mass Spectrum and Time”. To reliably pick the correct peak, one needs only to set an approximate retention time and window in combination with the option to match components based on comparison to a reference spectrum. Figure 5 shows the component table setup for identification based on matching the reference spectrum and time.

MS Component Table

Component Table

Group Area Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

#	Name	Ret.Time	Window	Ref.Mass.Spec.	Peak.Mass.Spec. Filter	Ref.Mass.Spec. Settings	MS Quantitation Peak	MS Confirming Peak 1	MS Confirming Peak 2
1	Propham	9.430	0.300 AMT		Quantitation	SI:0, RSI:700	93.00	43.00	179.00
2	Isoprocarb	10.000	0.300 AMT		Quantitation	SI:0, RSI:700	121.00	136.00	193.00
3	Fenobucarb	10.560	0.300 AMT		Quantitation	SI:0, RSI:700	121.00	150.00	207.00
4	Methabenzthiazuron	11.170	0.300 AMT		Quantitation	SI:0, RSI:700	164.00	136.00	135.00
5	Tebutam	11.300	0.300 AMT		Quantitation	SI:0, RSI:700	91.00	190.00	233.00
6	2,3,5-Trimethacarb	11.730	0.300 AMT		Quantitation	SI:0, RSI:700	136.00	121.00	193.00
7	Prometon	11.790	0.300 AMT		Quantitation	SI:0, RSI:700	210.00	225.00	168.00
8	Carbofuran	11.800	0.300 AMT		Quantitation	SI:0, RSI:700	164.00	149.00	221.00
9	Isocarbamid	12.140	0.300 AMT		Quantitation	SI:0, RSI:700	142.00	130.00	185.00
10	Desmetryn	13.340	0.300 AMT		Quantitation	SI:0, RSI:700	213.00	198.00	171.00
11	Simetryn	13.730	0.300 AMT		Quantitation	SI:0, RSI:700	213.10	170.00	
12	Fuberidiazole	13.730	0.300 AMT		Quantitation	SI:0, RSI:700	184.00	155.00	129.00
13	Diphenamid	15.260	0.300 AMT		Quantitation	SI:0, RSI:700	167.00	239.00	152.00

Figure 5. Component table setup for spectrum and time matching

Figure 6 shows an example for 100 fg octafluoronapthalene (OFN), where the retention time for the compound is set at 4.0 minutes in the processing method, but still the correct peak at 3.79 minutes is identified based on the closest match to the reference mass spectrum. Any other component match settings of “First”, “Greatest”, or “Nearest” would not have resulted in correct peak identification of OFN.

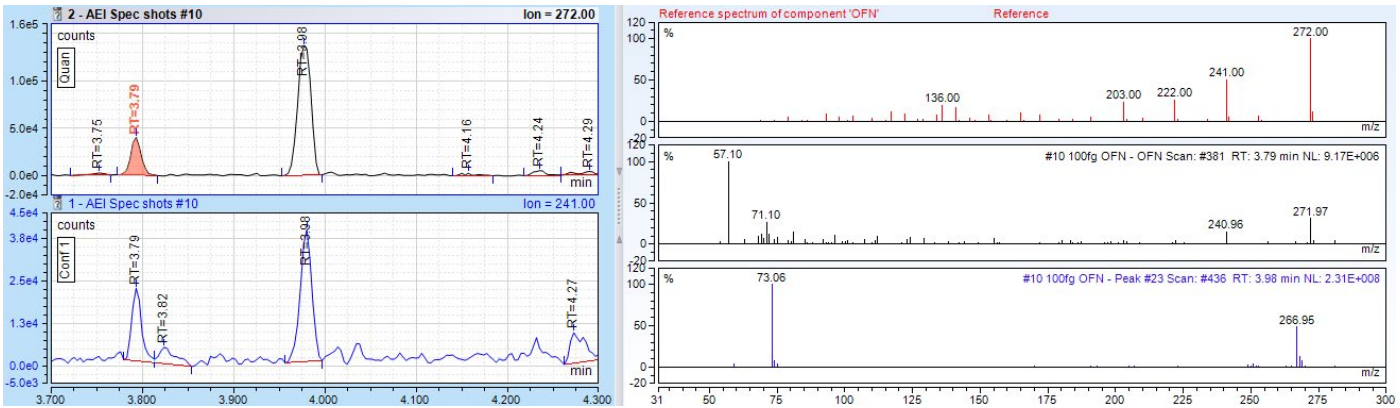
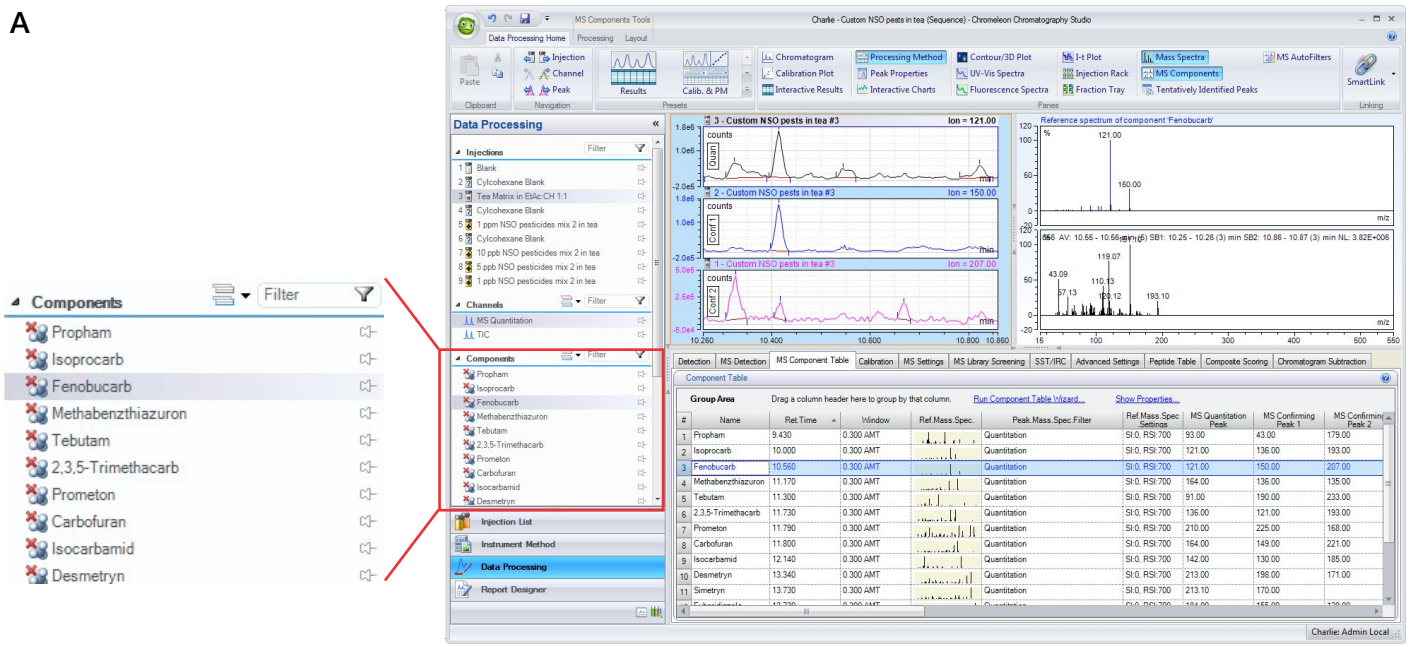


Figure 6. Example of correct assignment of OFN, based on the closest match to the reference spectrum, where the processing method has the OFN retention time assigned at 4 minutes

Figure 7 shows the same example as in Figure 1, now with the reference spectrum assigned to the components, including matching for spectrum and time.

A



B

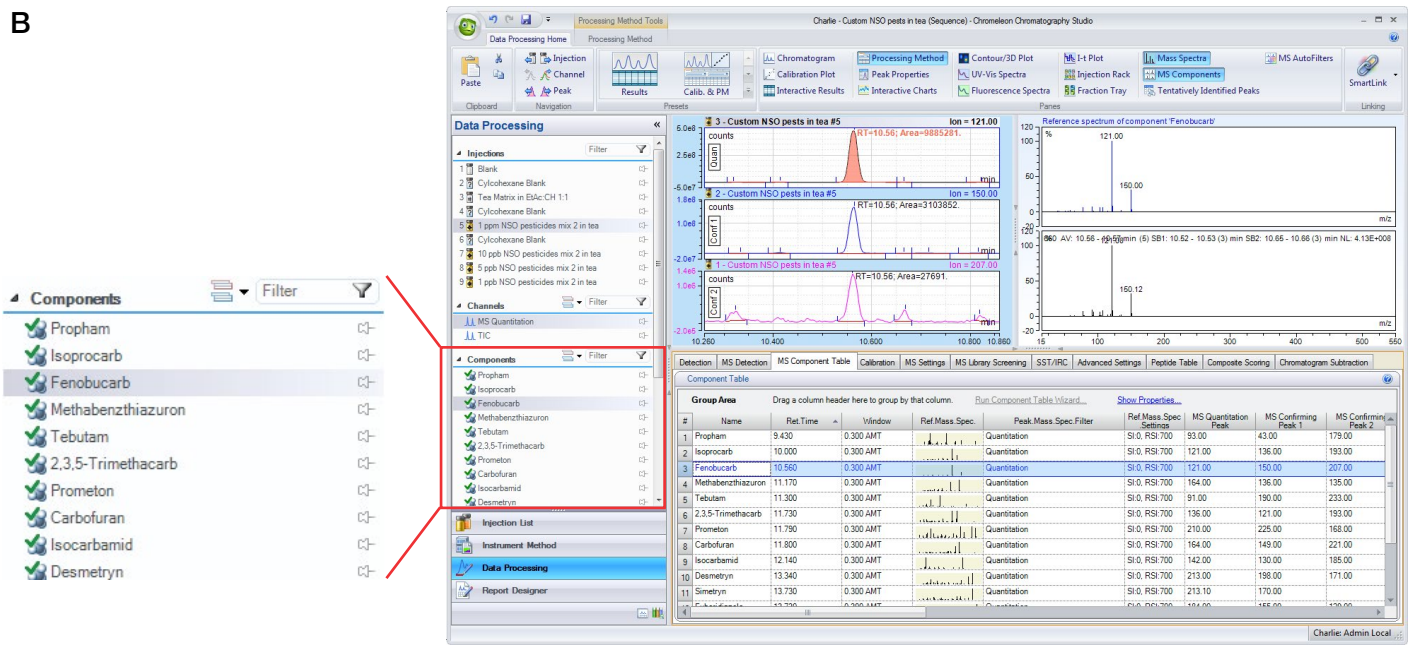


Figure 7. Correct "identification" of components in the tea matrix (A) and spiked tea matrix (B)

In a more extreme case, when the component retention times are not known at all, it still is possible to perform identification based on the spectrum. As shown in Figure 4, the component match section provides the option to match components based on the reference mass spectrum only. This targeted screening approach is useful for screening large numbers of components, for example pesticides in food or drugs of abuse in biological matrices, without the need to inject standards for retention time determination. After a component is identified by this targeted screening approach with the help of a reference mass spectrum, confirmation can be made by injecting the standard and confirming its presence by retention time. Figure 8 shows the component table setup to search for components based on comparison to the reference spectrum only. The Retention Time and Window parameters are set to cover the full chromatogram. Figure 9 shows the chromatogram with correctly identified peaks.

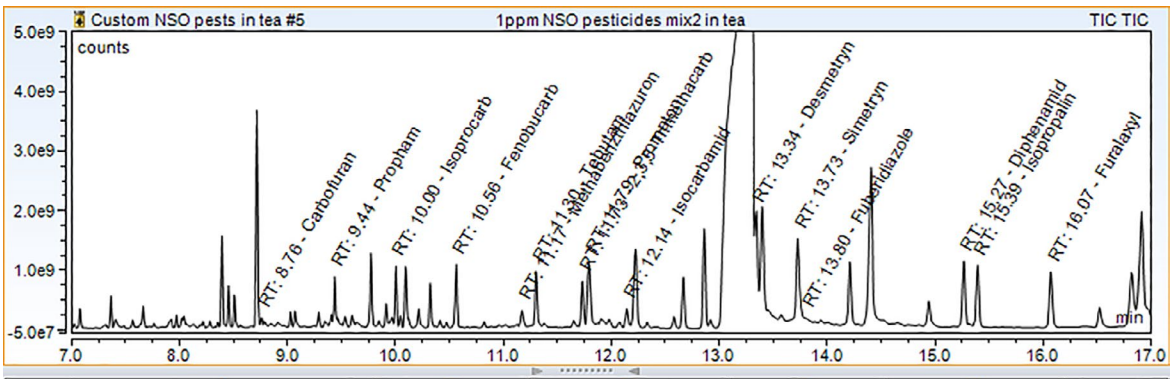
MS Component Table

Component Table

Group Area Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

#	Name	Ret.Time	Window	Ref.Mass.Spec.	Peak.Mass.Spec. Filter	Ref.Mass.Spec Settings	MS Quantitation Peak	MS Confirming Peak 1	MS Confirming Peak 2
1	Simetryn	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	213.10	170.00	
2	Fuberidiazole	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	184.00	155.00	129.00
3	Desmetryn	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	213.00	198.00	171.00
4	Diphenamid	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	167.00	239.00	152.00
5	Isopropalin	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	280.00	238.00	309.00
6	Methabenzthiazuron	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	164.00	136.00	135.00
7	Furalaxyl	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	95.00	242.00	301.00
8	Propham	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	93.00	43.00	179.00
9	Isoprocarb	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	121.00	136.00	193.00
10	Fenobucarb	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	121.00	150.00	207.00
11	Tebutam	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	91.00	190.00	233.00
12	2,3,5-Trimethacarb	18.000	18.000 AMS		Quantitation	SI:0, RSI:700	136.00	121.00	193.00

Figure 8. Component table setup for spectrum matching



	A	B	C	D	E
1	Peak Name	Ret. Time	Ref.MS.Match	Area	Rel Area
2		min		counts*min	%
7	Fenobucarb	10.56	904	24348945	0.62
8	Methabenzthiazuron	11.17	859	8075907	0.21
9	Tebutam	11.30	867	22074701	0.56
10	2,3,5-Trimethacarb	11.73	770	18836842	0.48
11	Prometon	11.79	786	39140296	1.00

Figure 9. Chromatogram with correctly identified peaks when component retention times are unknown

The reference spectra are stored in the processing method, which can be used on any other Chromeleon CDS system, even when the original source of the spectra (library or injection) is not present.

The report variable peak.refMsMatch reports the SI or RSI match factor score of the actual peak spectrum relative to the reference spectrum and thus is useful as a quick gauge of the spectral quality.

Conclusion

This technical note highlights the functionality of Chromeleon CDS to assign a reference mass spectrum to a component and use that spectrum to match against an unknown peak for ultimate confidence in results. One of the major advantages of doing this is that it significantly increases the probability of the software detecting the correct peak by eliminating false positive identification, even when there is change in the expected retention time. The second benefit, related to screening of unknowns, is that one can get tentative identification of a component, which can further be confirmed by retention times after injecting a standard. Thus, in utilizing Chromeleon CDS, the user will have increased confidence in data quality and will save time when screening for unknowns.

References

1. Hübschmann, H.-J. Handbook of GC-MS; Wiley-VCH, 2015.
2. Zweigenbaum, J. (Ed.) Mass Spectrometry in Food Safety: Methods and Protocols; Humana Press, 2011.
3. Bruner, F. Gas Chromatographic Environmental Analysis: Principles, Techniques, Instrumentation; Wiley-VCH, 1993.
4. Gerhards, P.; Bons, U.; Sawazki, J.; Szigan, J. and Wertmann, A. GC/MS in Clinical Chemistry, Wiley-VCH, 1999.
5. NIST/EPA/NIH Mass Spectral Library (NIST 17) and NIST Mass Spectral Search Program (Version 2.3), NIST Mass Spectrometry Data Center, Gaithersburg, MD.

Find out more at **thermofisher.com/Chromeleon**

ThermoFisher SCIENTIFIC