Comparative analysis of mass spectral matching for confident compound identification using the Advanced Electron Ionization source for GC-MS

Authors
Amit Gujar,[1] Tim Anderson,[1]
Daniela Cavagnino,[2] and Amy Patel[1]

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

[2]Thermo Fisher Scientific, Milan, Italy

Keywords
Gas chromatography-mass spectrometry, GC-MS, ISQ 7000, advanced electron ionization, AEI, library match, NIST, Wiley, spectral quality, compound identification

Goal
To assess the spectral match quality of the ion spectra obtained using the Thermo Scientific™ Advanced Electron Ionization (AEI) source against the reference mass spectra of commercial libraries.

Introduction
Single quadrupole GC-MS operated in electron ionization (EI) mode has long been employed as a convenient technique for a variety of applications spanning environmental monitoring, food safety, forensic and toxicological, pharmaceutical, and industrial analysis, among others. GC-MS serves as both a qualitative and quantitative analytical tool for known compounds as well as for "unknowns" analysis. The unknowns analysis by GC-MS often relies on compound identification using spectral matches against mass spectral libraries (ex: NIST®, Wiley®, etc.) commercially available in addition to mass spectral interpretation by the user.[1] The fragmentation pattern of the EI mass spectrum is compared via spectrum matching with the reference mass spectra in the library, and a list of most probable identities is produced based on the best (forward or reverse-search) mass spectrum matching score, with 999 being a perfect match.[2]

The Thermo Scientific™ ISQ 7000™ single quadrupole mass spectrometer can be configured with a Thermo Scientific™ Advanced Electron Ionization (AEI) source. The AEI source is specifically designed to increase the ionization efficiency of the compounds eluting from the GC capillary column, which ultimately leads to increased instrument sensitivity. The AEI source utilizes a filament that is in line with the cyclotroning magnetic field and the ionized beam path and together with electrostatic mirroring of electrons leads to significant increase in the yield of ions generated within the source.[3]

Traditionally, to obtain the best sensitivity possible, the electron energy used in EI experiments is 70 eV. Thus, most commercially available libraries have spectra that have been acquired at this energy. The efficiency of ionization of most molecules increases exponentially between 10 and 20 eV, followed by plateauing of the fragment ions produced at around 35 eV.[4] It is well known that electron ionization above 40 eV generates highly reproducible spectra, providing stable molecular fingerprints.[5] With the AEI source, the optimum ionization efficiency occurs when the electron energy is set to 45 eV.

Nevertheless, according to the minimum acceptable criteria for contributing to the NIST MS database, as stated in the NIST documentation, it is required to "acquire at an electron ionization of not less than 20 eV under conditions such that isotope ratios are not grossly distorted…".[6] Therefore, it is viable to leverage electron energy other than 70 eV (but not less than 20 eV) for NIST library searching, assuming that the required matching is achieved.

An example of increased sensitivity of the AEI source is shown in Figure 1. An increase in area counts greater than a factor of 10 was observed for 10 pg on-column of terbuthylazine acquired in full-scan mode using a mass range of 35 to 550 Da when compared against the signal obtained on a traditional source, like the Thermo Scientific ExtractaBrite EI source.

The aim of this technical note is to demonstrate that the EI mass spectra obtained using the AEI source operated at its optimum electron voltage gives excellent spectral matching when compared with the commercially available reference mass spectra.
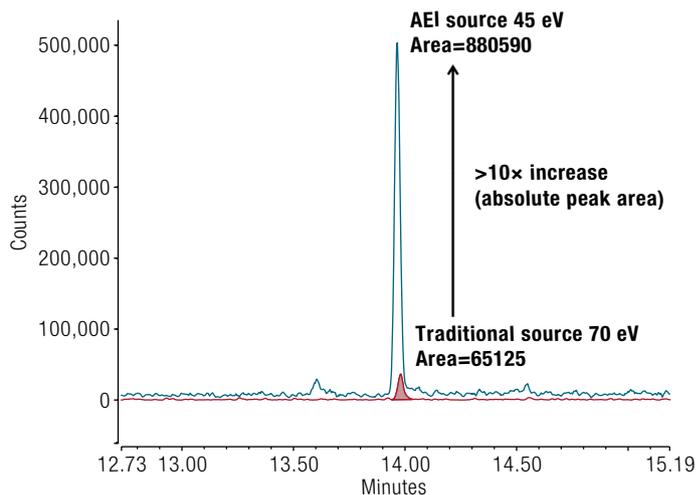

Figure 1. Example of increased sensitivity with the AEI source versus a traditional ion source for 10 pg terbuthylazine on-column, 35–550 Da full-scan

In practice, the best identification of a compound is often simply the one with the highest similarity score that represents the goodness of fit between a measured spectrum and a library reference spectrum. For this, the NIST library guidelines in Table 1 were used for interpreting the mass spectral match quality.[2]

Table 1. NIST library guidelines

Match Factor (SI) or Reverse Match Factor (RSI) Thresholds	Mass Spectral Match
900 and above	Excellent
800–900	Good
700–800	Fair
<600	Poor

Three distinct classes of compounds that are often analyzed by GC-MS were tested: semi-volatile organic compounds, pesticides, and terpenes.

Experimental

Samples

Semi-volatile organic compounds

Restek® 8270 MegaMix® standard was diluted to 10 ppm with dichloromethane before being analyzed.

Pesticides

Two multi-residue pesticide standards (organochlorine compounds #2, Restek Cat.# 32564 and organophosphorus compounds #5, Restek Cat.# 32567), were diluted to 2 ppm with toluene before analysis.

Terpenes

Individual terpenes standards were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) and diluted to 10 ppm in hexane before analysis.

The Thermo Scientific ISQ 7000 single quadrupole GC-MS equipped with the AEI source and with the ExtractaBrite EI source has been used for the analysis of the above mentioned standard mixtures. The acquired full scan spectra have been compared and matched with commercial MS libraries.

Mass spectral libraries

For the semi-volatiles and the multi-residue pesticide studies, the NIST 17 mass spectral library (mainlib) was used. For the terpenes study, the Wiley's Flavors and Fragrances of Natural and Synthetic Compounds (FFNSC), 2nd Ed. was used in conjunction with the NIST 17 library.

GC and MS conditions

Typical conditions used for these experiments are detailed in Table 2. Data was acquired in full-scan mode and processed with Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software.

Table 2. GC and MS parameters

Thermo Scientific™ TRACE™ 1310 GC system	
Column:	Thermo Scientific™ TraceGOLD™ TG-5ms GC column 30 m × 0.25 mm × 0.25 µm (P/N 26098-1420)
Liner:	Thermo Scientific™ LinerGOLD™ Splitless liner single taper with wool, 4 mm ID, 78.5 mm length (P/N 453A0924)
SSL mode:	Split (split ratio varied)
Inlet temperature:	300 °C
Septum purge flow:	5.0 mL/min
Carrier flow:	Constant He flow of 1.2 mL/min
Oven program:	35 °C (2.25 min), 18 °C/min to 100 °C (0.1 min), 23 °C/min to 280 °C (0.1 min), 10 °C/min to 320 °C (5.0 min) for SVOC and pesticides, 35 °C (2 min), 20 °C/min to 250 °C (1.0 min) for terpenes

ISQ 7000 single quadrupole GC-MS system	
MS acquisition:	Full-scan
MS transfer line temperature:	300 °C
Ion source temperature:	300 °C
Ionization mode:	EI
Electron energies:	AEI at 45eV and ExtractaBrite at 70 eV
Emission current:	50 µA
Mass range:	35–500 Da for SVOC and pesticides 35–300 Da for terpenes
Dwell time:	0.2 s

Results and discussion

Semi-volatile organic compounds (SVOCs)

SVOCs are an important class of compounds that are routinely analyzed by GC-MS especially in the field of environmental monitoring. Good spectral matching is key for confident compound identification of environmental contaminants in soil, water, and other matrices. Figure 2 shows the NIST library match factors (SI scores) for the SVOCs analyzed using both the AEI source and the ExtractaBrite ion source for the chromatographically resolved compounds. When searching NIST the match factors for both sources gave very similar results with the majority of compounds having scores of above 900 (excellent match), while all the compounds

have match factors above 870 indicating "good" to "excellent" spectral match with the NIST library spectra. No significant difference in the average match factor scores was observed for the thirty-nine compounds between spectra acquired using the AEI source (average match factor 929) and the spectra obtained using the ExtarctaBrite ion source (average match factor 921). Figure 3 shows an example of spectra for hexachlorobutadiene acquired with both the ion sources under study, in comparison to the NIST reference spectra (bottom pane). In both cases, the library match factors were excellent and very similar (936 with the ExtractaBrite ion source and 938 with the AEI source).
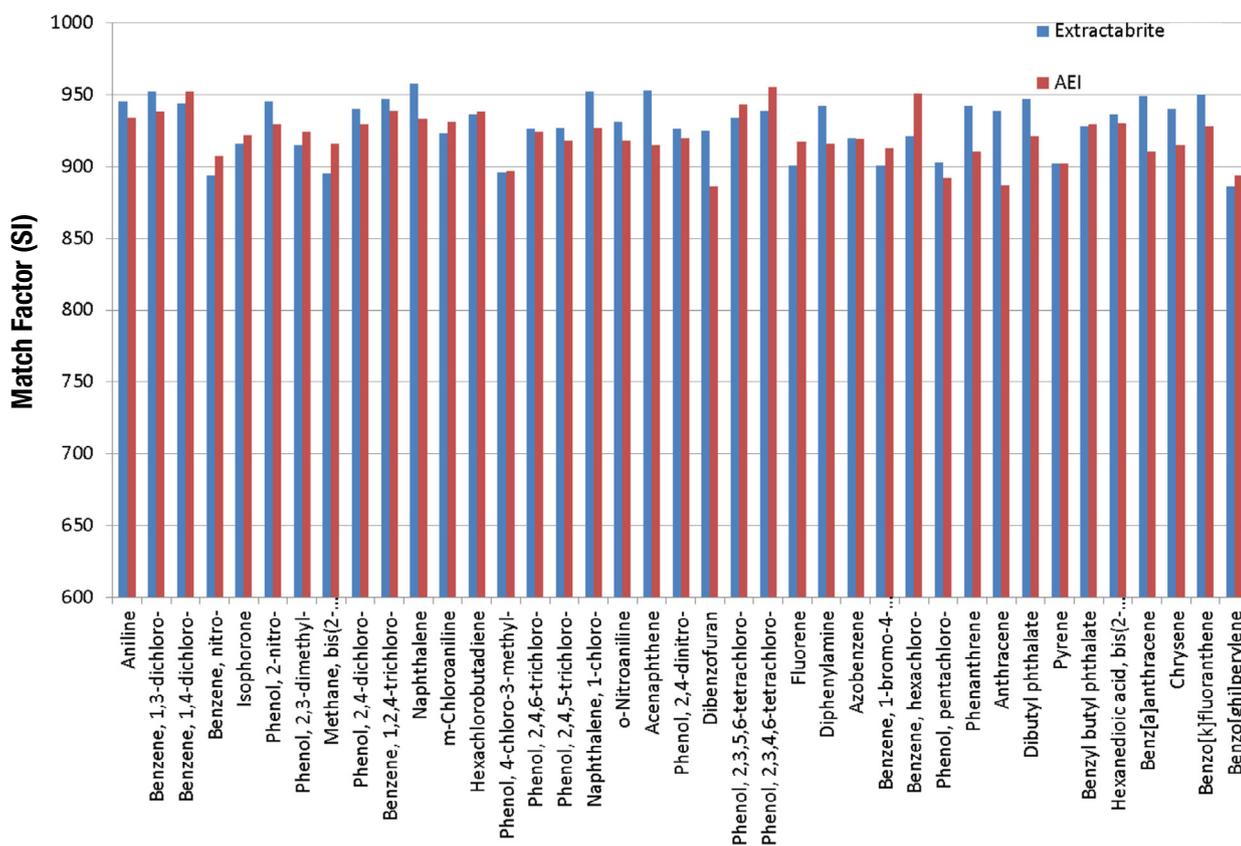

Figure 2. NIST 17 mass spectral library match factors of selected semi-volatile compounds analyzed on the conventional ExtractaBrite ion source and the AEI source
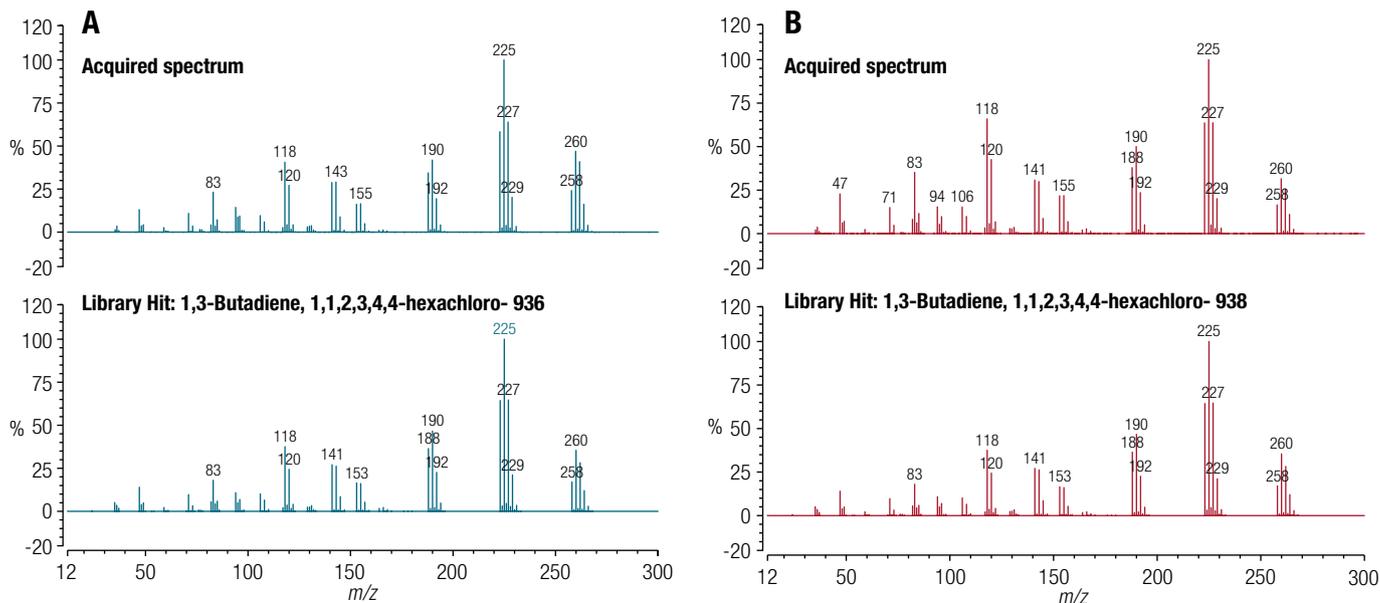
Figure 3. Mass spectra of hexachlorobutadiene obtained with (A) the ExtractaBrite ion source and (B) the AEI source

Pesticides

Pesticides are compounds that are routinely monitored in food and environmental matrices. Figure 4 shows the NIST library match factors for various organochlorine and organophosphorus pesticides. The results from the NIST search show that the mass spectra obtained from the AEI source are comparable to those from the ExtractaBrite ion source, and in both cases the match factors resulted >900 for the majority of the compounds. An example is shown in Figure 5 for the methacrifos mass spectra, which results in a match factor of >950 for the AEI source.
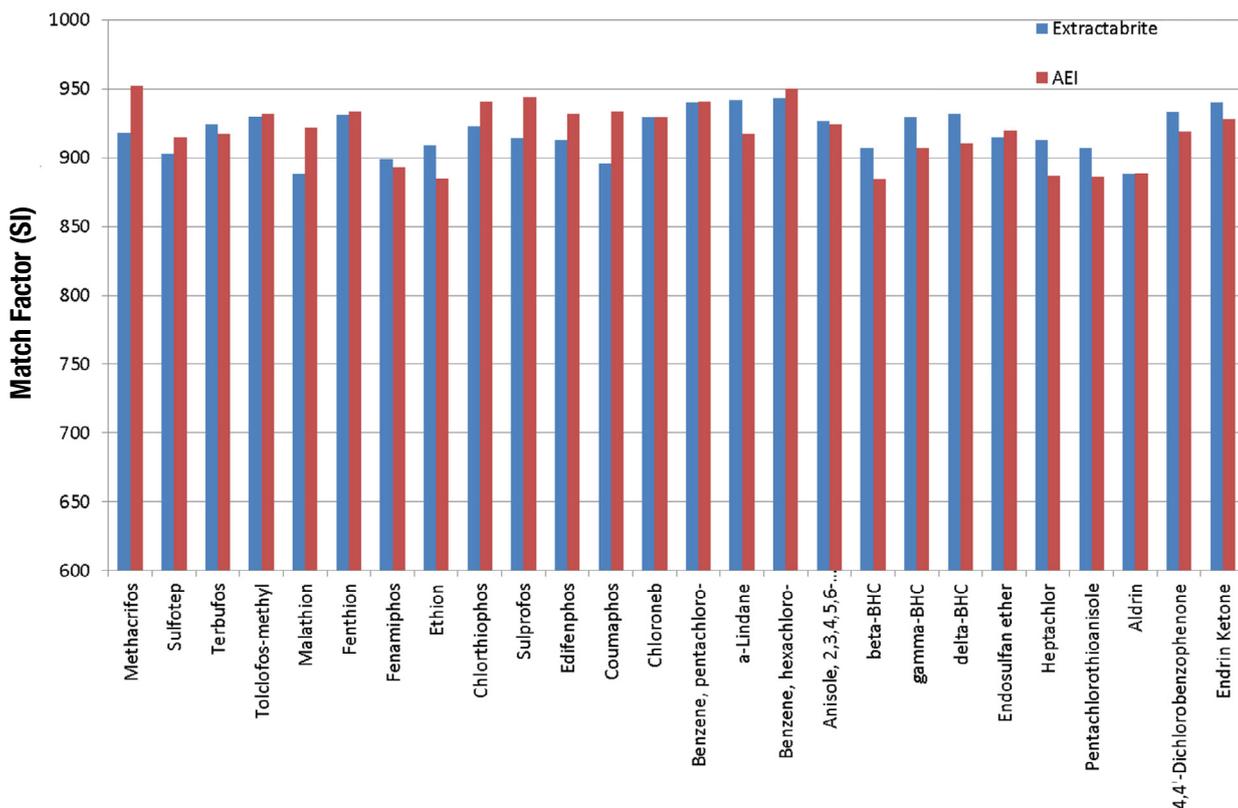

Figure 4. NIST 17 mass spectral library match factors of selected pesticides for the ExtractaBrite ion source and the AEI source
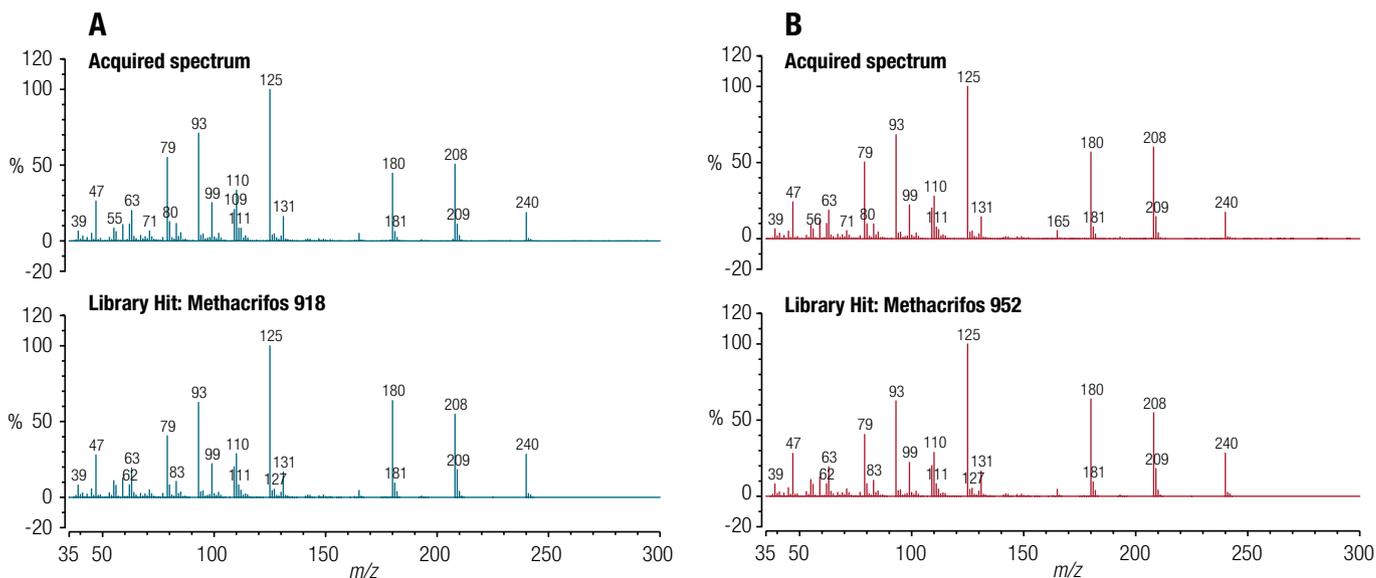
Figure 5. Mass spectra of methacrifos obtained with (A) the ExtractaBrite ion source and (B) the AEI source

Terpenes

Identification of terpenes is an important GC-MS application especially in the food and flavors industry. Many terpenes are structurally similar and therefore have very similar fragmentation patterns and common fragments; hence it is of paramount importance that GC-MS gives a high library matching score to distinguish the correct terpene and provide a reliable compound identification. Figure 6 shows the NIST match factors (SI) and reverse match factors (RSI) for various terpenes

that were tested by individual injections on the ISQ 7000 system with the AEI source. All the terpenes injected onto the system returned as the first hit following the library search with forward and reverse scores above 900. Figure 7 shows an example of AEI mass spectra for linalool compared against the Wiley's FFNSC MS spectral library. Figure 8 shows the match factor scores for 16 consecutive injections for linalool showing excellent repeatability from injection to injection with all of them showing greater than 950 for the SI scores.
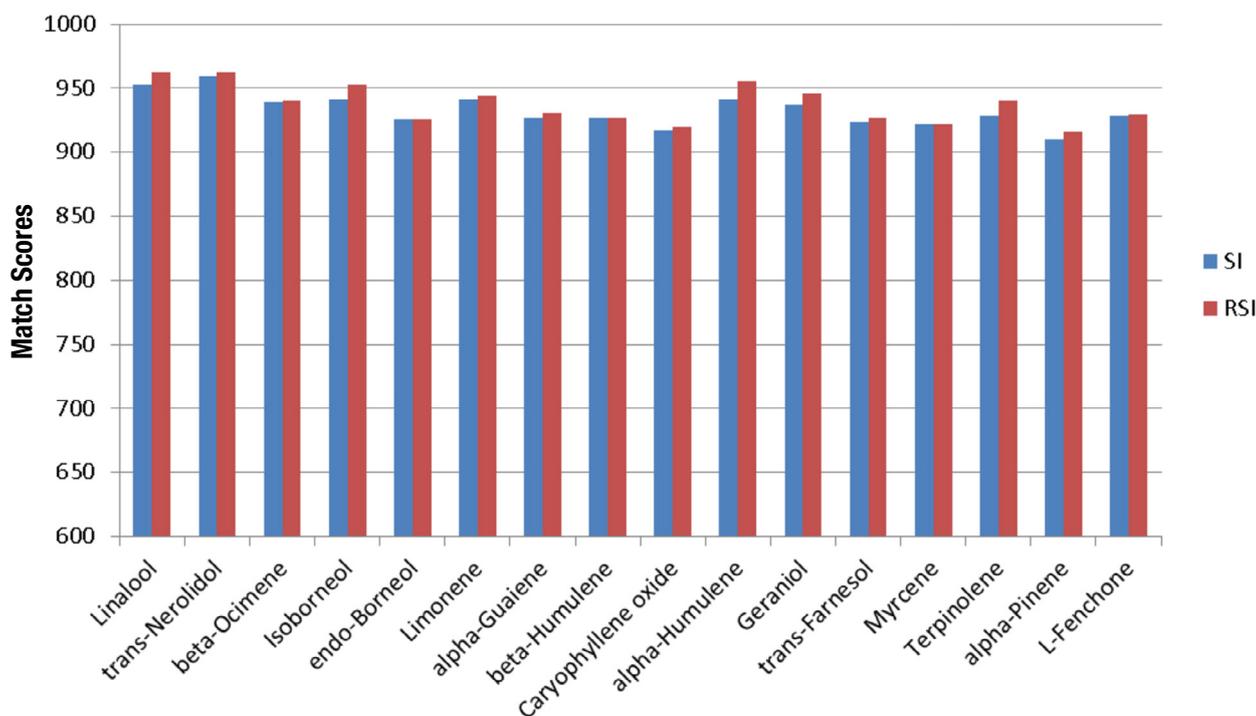

Figure 6. NIST 17 mass spectral library match factors for terpenes tested with the AEI source
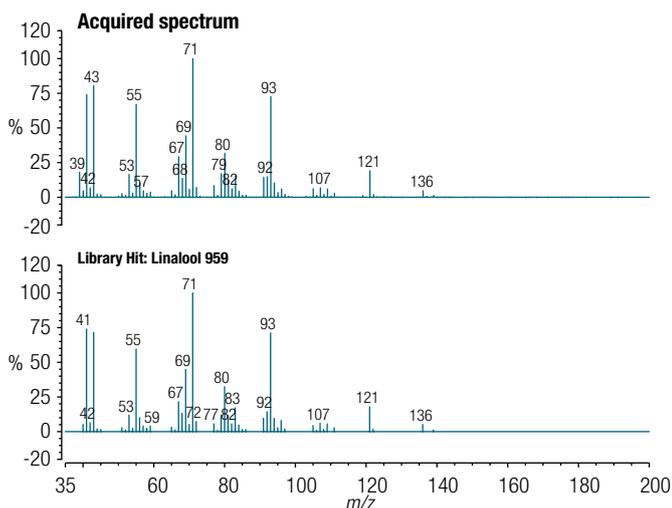
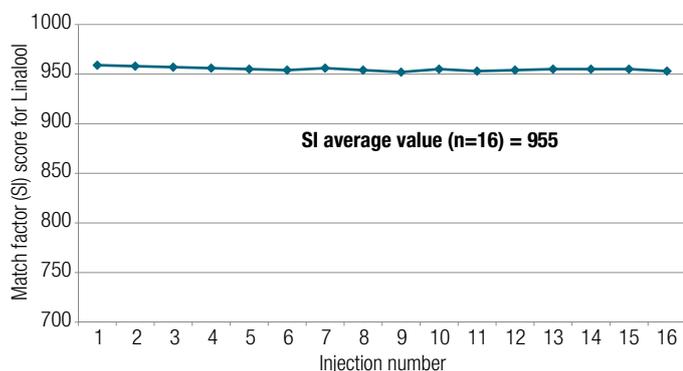Figure 7. Mass spectral fragmentation pattern for linalool and its comparison with Wiley's FFNSC, 2ed. Library

Figure 8. Match factor (SI) repeatability for linalool using the AEI ion source

Conclusions

The results of these studies demonstrate that:

- The AEI source is designed to deliver higher sensitivity compared to the conventional EI source. It shows the best performances at an optimum electron voltage setting of 45 eV without compromising on spectral results.

- Mass spectra obtained using the AEI source showed a match that was "excellent" for the majority of the compounds analyzed. The minimum value of the forward match score was >870 for all compounds analyzed.

- NIST match scores obtained with the AEI source were found to be equivalent to the ones obtained with a traditional EI source.

- The spectral match scores obtained for the 16 terpenes analyzed using the AEI source were in excellent agreement with those contained within the Wiley and NIST 17 libraries, in all instances returning the correct identification as the first "hit".

Taken together, these results indicate that the ISQ 7000 GC-MS system configured with the AEI or with the Extractabrite ion sources delivers in both cases very high quality mass spectra. In both configurations, the ISQ 7000 GC-MS can be confidently used for identification of untargeted compounds in screening experiments using the available commercial mass spectral libraries. The added advantage of the AEI source to increase ionization efficiency and extend the robustness towards difficult matrices, offers the possibility to expand the analytical capability of the laboratory. The ISQ 7000 GC-MS system configured with AEI source is therefore a valuable solution any time ultra sensitivity and robustness is required to respond to challenging analytical needs.

References

1. Sparkman, O. D.; Penton, Z. E.; Kitson, F. G. *Gas Chromatography and Mass Spectrometry – A Practical Guide;* Academic Press, 2011.

2. *Basis for Interpretation of the Library Search Results,* NIST/EPA/NIH Mass Spectral Library (NIST 17) and NIST Mass Spectral Search Program (Version 2.3) user's guide, page 41.

3. Thermo Fisher Scientific Technical Note TN 10597: Practical determination and validation of instrument detection limit for the Thermo Scientific ISQ 7000 Single Quadrupole GC-MS system with Advanced Electron Ionization source, 2018.

4. Watson, J. T.; Sparkman, O. D. Introduction to Mass Spectrometry; Wiley, 2008, page 318–319.

5. Stein, S. Mass spectral reference libraries: An ever-expanding resource for chemical identification, *Anal. Chem.* **2012,** *84*, 7274–7282.

6. *APPENDIX 7: Contributing EI Mass Spectra to the NIST/EPA/NIH Mass Spectral Library* in NIST Standard Reference Database 1A, NIST/EPA/NIH Mass Spectral Library (NIST 17) and NIST Mass Spectral Search Program (Version 2.3) user's guide.

Find out more at **thermofisher.com/ISQ7000**

Thermo Fisher
S C I E N T I F I C