Technical note | 000769

Gas chromotography

Enhance sensitivity using variable electron voltage (VeV) on Orbitrap Exploris GC Mass Spectrometers

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Keywords

Orbitrap Exploris GC 240, variable electron voltage technology, VeV, soft EI, chemical ionization, sensitivity, mass accuracy, Orbitrap technology, gas chromatography, steroids, pesticides

Goal

To demonstrate the application of variable electron voltages as a softer electron ionization to provide enhanced instrumental sensitivity and options for compound identification

Introduction

The classic mode of ionization in gas chromatography-mass spectrometry (GC-MS) is electron ionization (EI),[1] which refers to a hard, but efficient, ionization where a beam of electrons passes through a gas phase sample, resulting in positively charged fragments. It has the advantage of being sensitive, robust, and consistent, but in some cases information about the molecular ion is lacking due to compound fragmentation. The transfer of energy from excited electrons to the analytes is typically maximized at 70 electron volts (eV) for most GC-amenable chemicals, so most mass spectrometers use this setting in EI mode. This feature enables extensive commercially available spectral libraries to be used to match compounds of interest or propose identity to unknown peaks.

In GC-MS, an alternative and complimentary form of ionization is chemical ionization (CI), which is considered a softer ionization that often gives molecular ion information through mass adduct patterns and lower fragmentation.[2] It is often seen as an important option when an unknown compound is suspected. The mass adduct ions in the spectrum enable the swift identification of the molecular ion, from which an elemental composition can be proposed. In EI, this adduct pattern is not evident and so the user could be faced

with a molecular ion or a high mass fragment in the spectrum. However, CI is often lower in sensitivity than EI, the ionization can be compound specific, and it is generally less useful for compound identification through library searching. Thus, a softer EI technique is a promising and informative ionization mode that possesses some of the merits of both EI and CI, reducing or eliminating low mass ions that do not contain useful structural information, while simultaneously boosting higher mass ions and/or molecular ions that can be very helpful for structural elucidation or improving compound selectivity/sensitivity. For targeted analysis the eV is a parameter that can be tuned to give optimal compound response, and this aspect is evaluated in this work.

Variable electron voltage (VeV) is an effective technique that can be implemented in the high-resolution, accurate-mass (HRAM) Thermo Scientific™ Orbitrap Exploris™ GC Mass Spectrometer Series. This highly efficient technology enables lower eV settings, essentially soft EI as previously described, for electron ionization and routinely delivers very robust tuning results. It is a softer EI technique that promotes higher mass signals and increases sensitivity for compounds prone to extensive fragmentation. The key benefits of VeV are:

- Fully automated for optimum performance: Following the simplicity of Orbitrap™ GC-MS operation, VeV setup is very simple and easy with fast, fully automated tuning.
- Increased sensitivity: Enabled by the full scan sensitivity of Thermo Scientific™ Orbitrap™ technology, VeV provides enhancement of target ion signals, when comparing with standard 70 eV to deliver improved sensitivity.
- Increased confidence in identification: VeV promotes molecular ion and diagnostic high mass signals, important information for compound identification and confirmation.

Experimental

The system tune interface is user friendly and extremely simple to operate, without extensive experience or training. The VeV tuning window, shown in Figure 1, contains the electron energy spin box in which the variable electron energy can be set to values ranging from 8 to 150 eV. In addition, the mass for tuning optimization can be selected. After selections are made, autotuning is started by use of the “Optimize” button. The VeV tuning process is rapid and is finished within 30 seconds. To demonstrate how this ionization mode is able to increase sensitivity and improve compound identification, a routine doping screening standard and pesticide standard at 50 ng/mL were analyzed and the results compared to each other.

In sports doping analysis, emerging drugs are continually added to the World Anti-Doping Agency (WADA) prohibited substances list. A comprehensive screening method with exceptional sensitivity and an even lower limit of detection is needed, especially for the low ng/mL detection of anabolic androgenic steroids (AAS). In most cases, it is impossible to obtain more intense higher *m/z* ions and/or the molecular ions using the conventional 70 eV ionization energy. Particularly, the lower mass fragments that are commonly formed from endogenous steroids in the urine matrix closely co-elute with target compounds that are barely distinguishable from each other in complex matrices in conventional EI analysis. Similar challenges of sensitivity exist for targeted pesticides analysis where additional sensitivity can be utilized to reduce sample loading, lower detection limits, or enable software peak integration algorithms to work more efficiently.

Eleven different urine blanks and four positive quality controls (positive QCs) spiked with 111 doping analytes at various concentrations ranging from 0.02 to 200 ng/mL were analyzed in full-scan mode under variable electron energies ranging from 12 to 70 eV with 60,000 FWHM (measured at *m/z* 200) resolution. A pesticide standard was analyzed using EI at 12 and 70 eV. Chemical ionization analysis was also performed using methane as reagent gas at a flow rate of 1.3 mL/min.

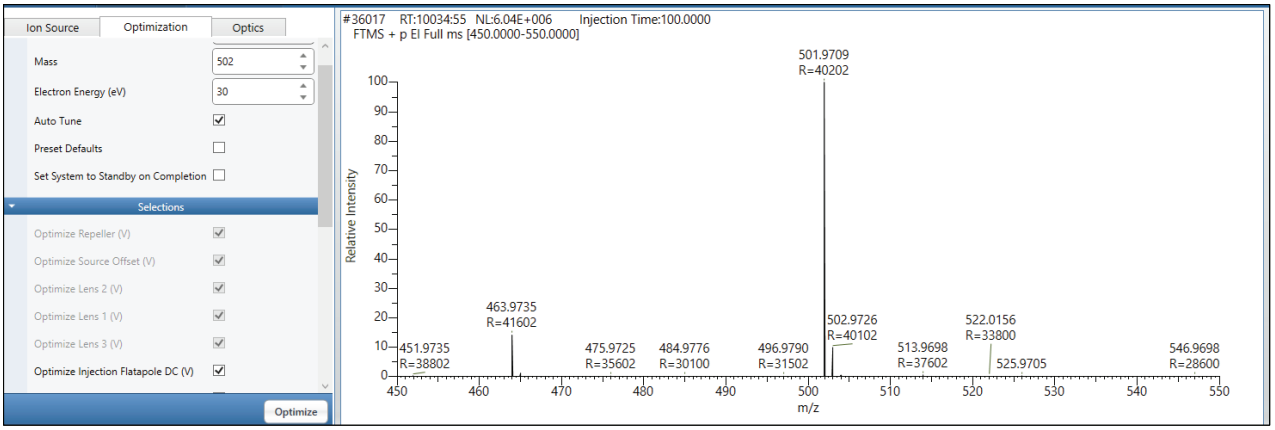

Figure 1. Orbitrap Exploris GC tune VeV window. The eV and tune mass can be selected by the user prior to tuning that takes < 1 minute.

Results and discussion

Sensitivity gains

Lower electron ionization energies can also lead to an increase in the relative intensity of diagnostic higher *m/z* ions and/or molecular ions for doping analysis or other targeted compounds. Figure 2 exhibits a significant enhancement of the molecular ion *m/z* 420.28738 (-0.1 ppm mass accuracy) of 19-NA at 12 eV. The lower *m/z* ion intensities, for example *m/z* 73.04680, 169.10428, 225.16367, and 315.21368, decreased almost 20%, which largely simplified the spectrum. The stronger molecular ion signal and reduced fragmentation obtained at 12 eV offer the advantage of selectivity, increased sensitivity, and thus improved spectral signal-to-noise (S/N) ratios for doping analysis.

To demonstrate the optimization of response, Figure 3 shows the positive QC sample that was analyzed at half the minimum required performance limit (MRPL) level in EI full-scan mode at variable electron energies (12 eV, 15 eV, 20 eV, 30 eV, 50 eV, and 70 eV). One quantitation ion and one confirming ion were selected for each compound. The y-axis of this chart shows the relative intensity of the sum of all target ions increased when compared to their 70 eV intensities. An energy of 30 eV was then chosen as the optimum energy that provides the highest sensitivity on average for the target ions of all analytes (254%) as compared to 70 eV.

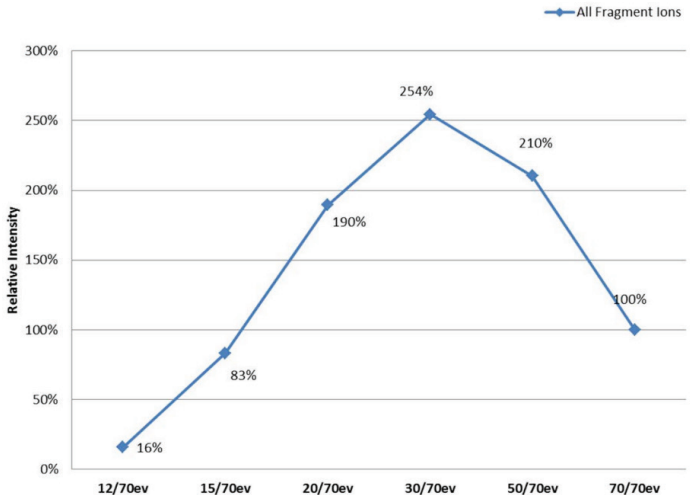

Figure 3. Comparing sensitivity at different electron energies (eV): x-axis is the lower electron energies compared to 70 eV; y-axis is the relative intensity of the sum of all target ions for 111 doping analytes in positive QC at 1/2 MRPL.

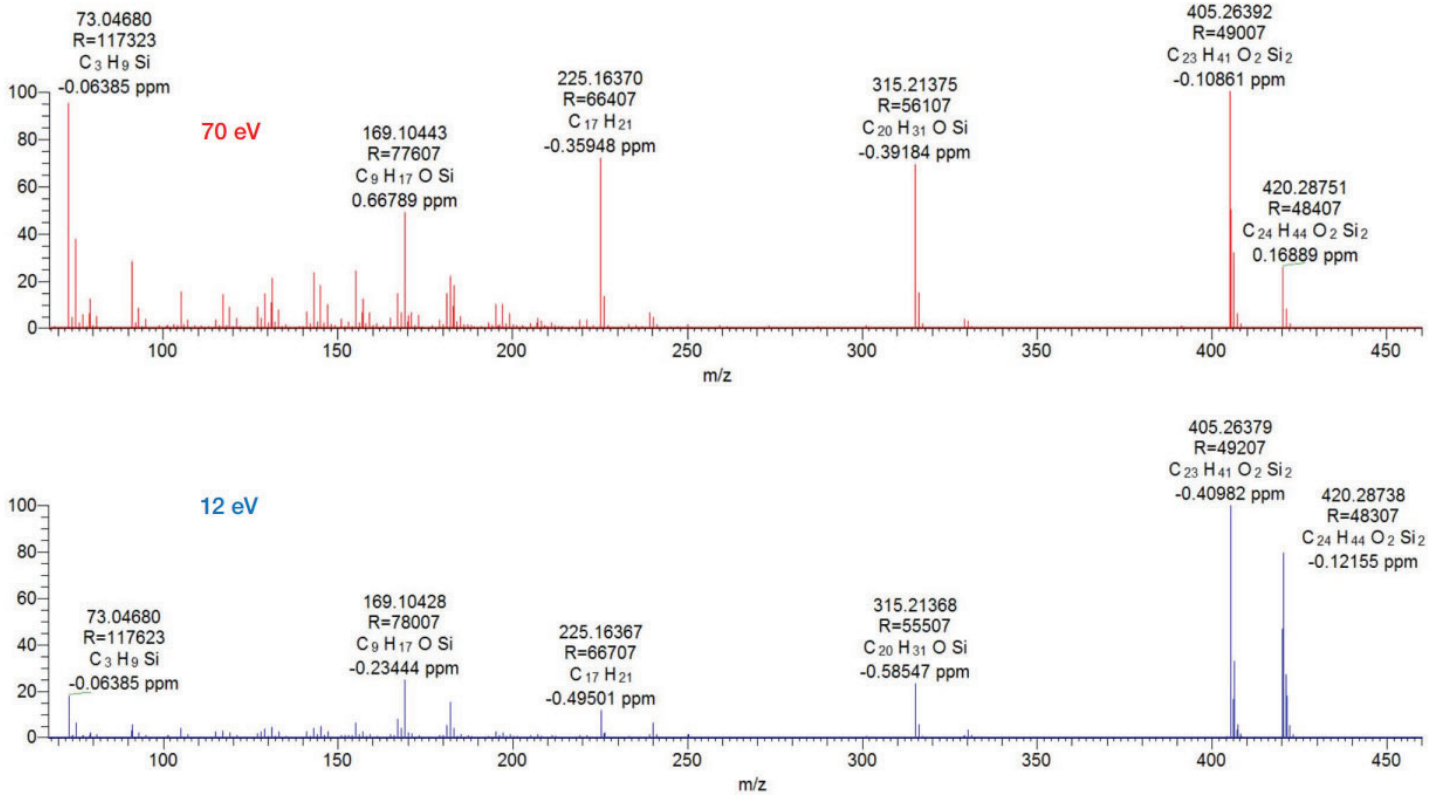

Figure 2. Comparison of 19-NA mass spectra acquired using VeV, at 70 eV and 12 eV. There is reduction in fragmentation at the lower end and enhancement of the molecular ion when using 12 eV.

Pesticide residue analysis is an additional application where optimization of the compound response through VeV would be a potential advantage. Analysis of a 50 ng/mL standard at different energies—20, 25, 30, and 70 eV—was performed. Comparing to the peak area responses at 70 eV, it was observed that a 25 eV value provided optimal benefits in response to the main quantifier ion. This was compound dependent as the histogram in Figure 4 indicates, with response gains ranging from 16% to 295% of the 70 eV peak area. The average is approximately a 2–2.5 times gain in response, which is similar to those shown for steroids.

Consistent mass accuracy

Acquiring reliable accurate mass measurements is critical when detecting analytes at lower concentrations in complex sample matrices. This is important as any compromise in accuracy of mass measurements can result in false identification, erroneous quantification, and interferences from matrix ions. Low mass accuracies ensure that compound selectivity is high and detection is robust. Also, the low mass accuracy allows for tighter tolerances to be applied for extracted ion chromatograms, which significantly reduces the possibility for false positive detects, thus increasing efficiency by eliminating the need for manual review. Typical accurate mass extraction windows on Orbitrap Exploris GC are ±5 ppm. On alternative GC-HRAM technology where lower mass accuracy is encountered, this window is typically increased to ±20 ppm, raising the likelihood of interference. In Figure 5, outstanding mass accuracy (<1 ppm) was maintained across all quantitation ions at the half MRPL level at 30 eV for all 111 analytes of interest.

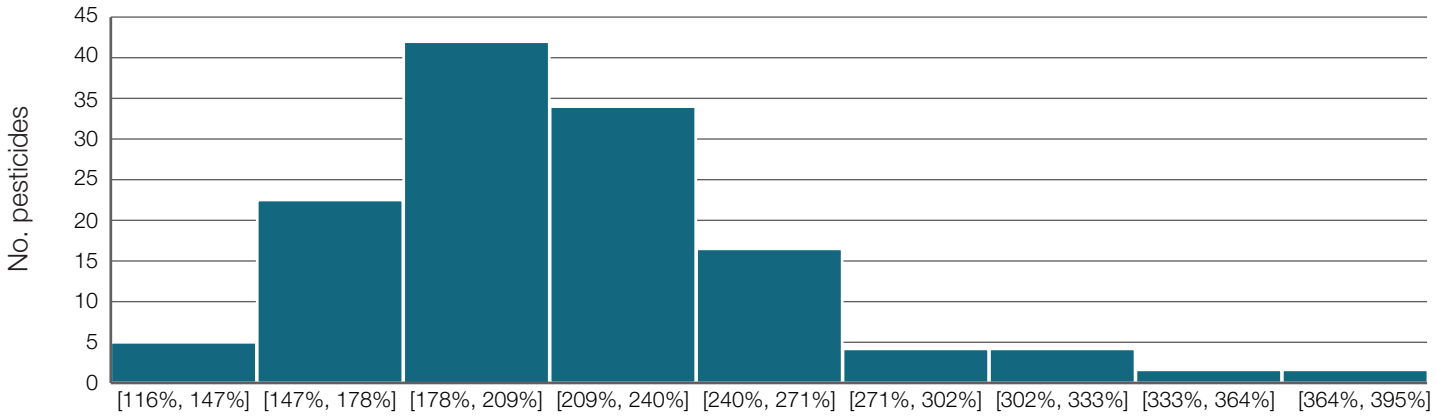

Figure 4. Histogram showing the relative peak area in peak area response of 130 pesticides between 70 eV and 25 eV

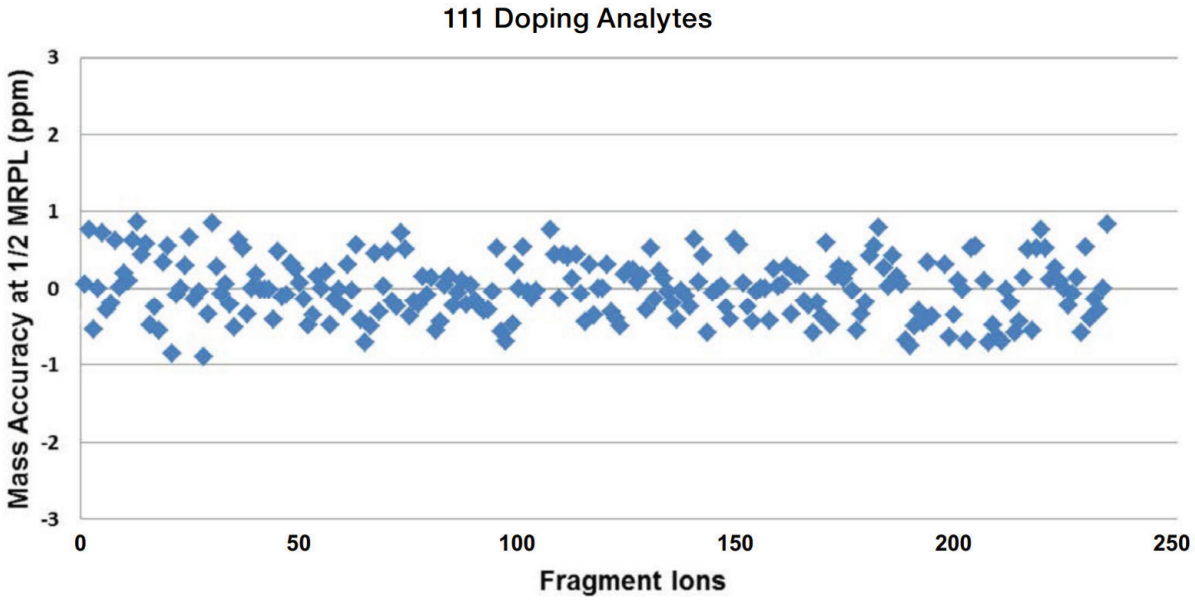

Figure 5. Mass accuracy at the half MRPL level at 30 eV for all 111 analytes of interest

Compound identification – the importance of chemical ionization

The spectral information obtained from either EI or CI is often complimentary to each other. In Figure 6, an example compound, ethoprophos ($C_8H_{19}O_2PS_2$), acquired with EI at 70 eV, EI at 12 eV, and chemical ionization with methane as the reagent gas is shown. If this compound was unknown, it would be vital to identify the molecular ion so that an elemental composition could be proposed using the accurate mass information from the Orbitrap Exploris GC. Using only EI with classic 70 eV or the lower 12 eV, the molecular ion (*m/z* 242.05586) is not visible in the spectrum. In the 12 eV spectrum, the degree of compound fragmentation is reduced, but still does not reveal the molecular ion. However, in the CI spectrum, the molecular ion can be identified through the expected pattern of adducts to the molecular ion, [M+H] [M+C_2H_5] [M+C_3H_5]. Without the adducts, it would be unclear if the ion was indeed the molecular ion or a higher mass fragment in the spectrum. On the Orbitrap Exploris GC systems, it is possible to switch from EI to CI in minutes without breaking system vacuum, enabling this critical information to be quickly obtained. Having identified the correct molecular ion of the compound, the next step would be to propose an elemental composition. With good mass accuracy of <1 ppm, the number of possibilities is reduced, providing a fast and efficient route to identification of the correct formula. Without this level of mass accuracy, the number of possible formulae could be higher, meaning the time taken to get to the correct result would be longer and the certainty in the result lower.

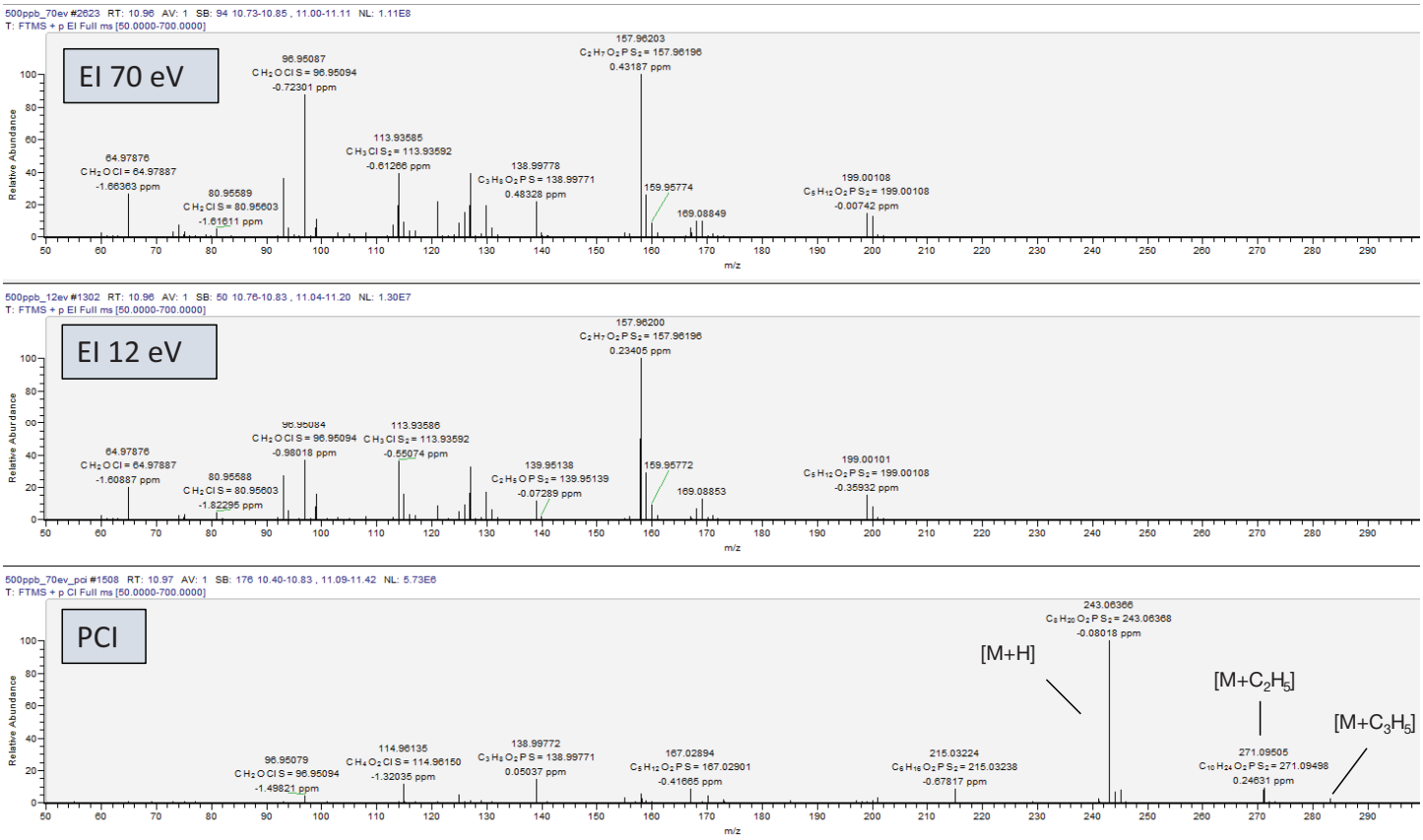

Figure 6. Spectra obtained for the compound ethoprophos at EI 70 eV (upper), 12 eV (middle), and chemical ionization (lower)

Conclusion

Overall, using VeV on the Orbitrap Exploris GC MS systems allows for enhanced analytical performance and flexibility.

- The automated tuning system greatly reduces complexity and improves operational efficiency in the laboratory.
- VeV can significantly increase compound sensitivity for confident qualitative and quantitative analysis, which is particularly advantageous in trace analysis in complex matrices.
- The enhanced signal obtained for high mass fragments, including molecular ions, in addition to outstanding mass accuracy is an effective way to identify specific compounds and help to yield useful structural information.
- Where unknown compounds are encountered, chemical ionization is highly advantageous to identify the molecular ion. The mass accuracy is consistently at less than 1 ppm, irrespective of the ionization energy used.

References

1. Märk, T.D.; Dunn, G.H. (29 June 2013). Electron Impact Ionization. Springer Science & Business Media. ISBN 978-3-7091-4028-4.
2. Munson, M.S.B.; Field, F.H. Chemical Ionization Mass Spectrometry. I. General Introduction. J. Am. Chem. Soc. 1966, 88, 2621–2630.

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