thermo scientific

TECHNICAL NOTE 10556

Variable electron voltage (VeV) on Q Exactive GC and Exactive GC Orbitrap GC-MS systems

Authors
Xin Zheng,[1] Cristian Cojocariu,[2] Jason Cole,[1] Paul Silcock[2]

[1]Thermo Fisher Scientific, Austin, Texas, USA
[2]Thermo Fisher Scientific, Runcorn, UK

Keywords
Q Exactive GC, Exactive GC, variable electron voltage technology, VeV, soft EI, sensitivity, mass accuracy, fully automated tuning, Orbitrap technology, gas chromatography

Goal

To demonstrate the use of the variable electron voltages technique as a softer electron ionization that provides enhanced analytical performance.

Introduction

Electron ionization (EI)[1] refers to a hard ionization where a beam of electrons passes through a gas phase sample, resulting in positively charged fragments. Information about the molecular ion is often lacking. The transfer of energy from excited electrons to the analytes is maximized at 70 eV for most GC-amenable chemicals, so most mass spectrometers use 70 electron volts (eV) in EI mode. Chemical ionization (CI) is considered a softer ionization that often gives molecular ion information and low fragmentation through adduct patterns.[2] However, it is often low in sensitivity, the ionization can be compound specific, and it is not useful for compound identification through library searching. Thus, a softer EI technique is a promising and informative ionization mode that possesses 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.

Variable electron voltage (VeV) is a unique and effective technique that can be implemented in the high-resolution, accurate-mass (HRAM) Thermo Scientific™ Q Exactive™ GC-MS/MS and Thermo Scientific™ Exactive GC Orbitrap™ GC-MS mass spectrometers. This highly efficient

ThermoFisher SCIENTIFIC

technology enables lower eV settings 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 set-up is very simple and easy with fast, fully automated tuning.
- **Increased sensitivity:** Compounded by the full-scan sensitivity of Orbitrap technology, VeV enables enhancement of target ion signals, when comparing with standard 70 eV.
- **Increased confidence in identification:** VeV promotes molecular ion and diagnostic high mass signals, important information for compound identification and confirmation.

Experimental

The interface is very user friendly and extremely simple to operate, without extensive knowledge 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 150 to 12 eV. In addition, the mass for tuning optimization can be selected. After selections are made, autotuning is started by use of the "Tune" button. Then click the Tune button to begin tuning the system. It takes only 30 seconds to finish an auto tune on the system. The VeV tuning process is rapid and is finished within 30 seconds.

To demonstrate the utility of VeV, a routine doping screening will be used as an example of how this ionization mode is able to increase sensitivity and improve confident identification. In sports doping analysis, emerging drugs are continually added to the World Anti-Doping Agency (WADA) prohibited substances list each year. 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.

Eleven different urine blanks and four positive quality controls (positive QCs) spiked with 111 doping analytes at various concentrations ranging from 0.02 to

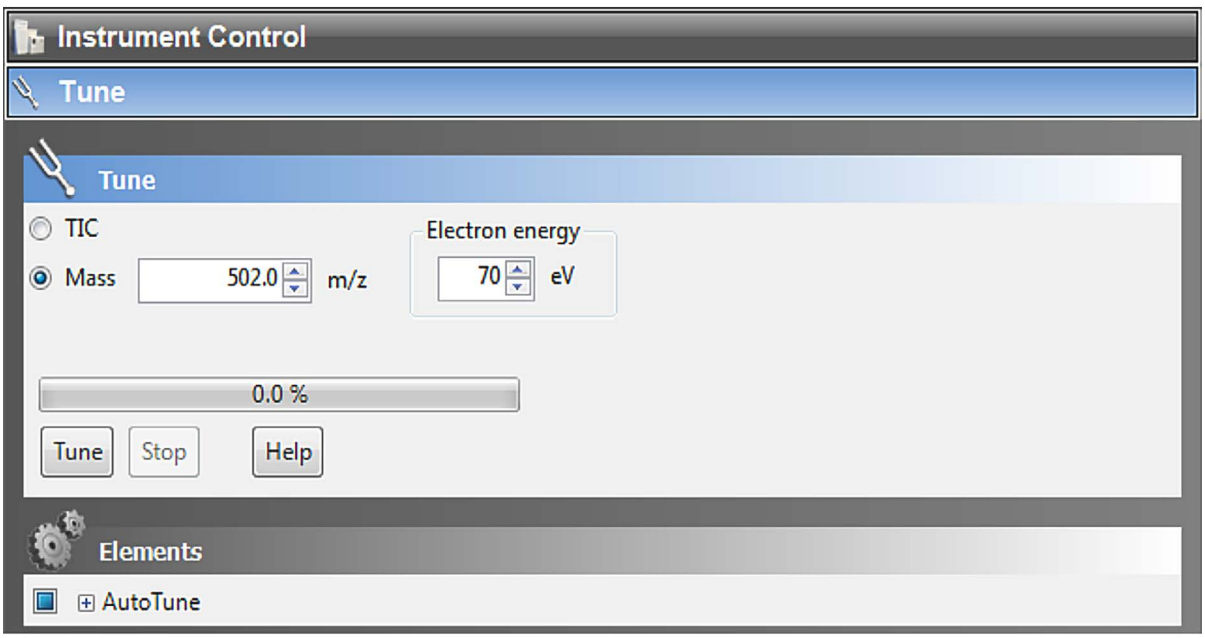

Figure 1. The VeV tuning window.

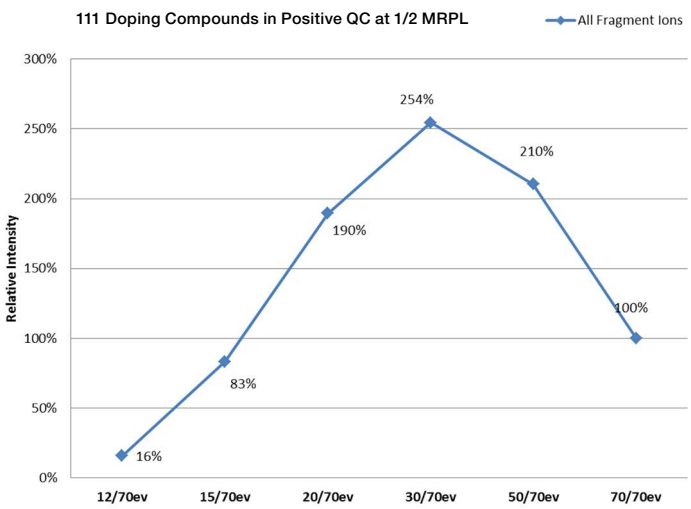

Figure 2. Comparing sensitivity at different electron energies (eV): x-axis is the lower electron energies compared to 70 eV; y-axis is the relative intensity of the sum of all target ions for 111 doping analytes.

200 ng/mL were analyzed in full-scan mode under variable electron energies with 60,000 FWHM (measured at *m/z* 200) resolution. Figure 2 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.

Results and discussion

Sensitivity

Lower electron ionization energies lead to an increase in the relative intensity of diagnostic higher *m/z* ions and/or molecular ions for doping analysis. Figure 3 exhibits a significant enhancement of the molecular ion 420.28738 *m/z* (-0.1 ppm mass accuracy) of 19-NA at 12 eV. The lower *m/z* ion intensities, for example 73.04680, 169.10428, 225.16367, and 315.21368 *m/z*, decreased almost 20%, which largely simplified the spectrum. The stronger molecular ion signal and reduced fragmentation obtained at 12 eV offers the advantage of selectivity, increased sensitivity, and thus improved spectral signal-to-noise (S/N) ratios for doping analysis. The calculated spectral S/N ratios at different electron energies based on the molecular ion of 19-NA are displayed in Table 1. The lower the electron energies, the greater S/N ratios for target ions (high *m/z* ions and/or molecular ions) and ultimately the lower the detection limits of the instrument, which is of highest importance for the detection of AAS.

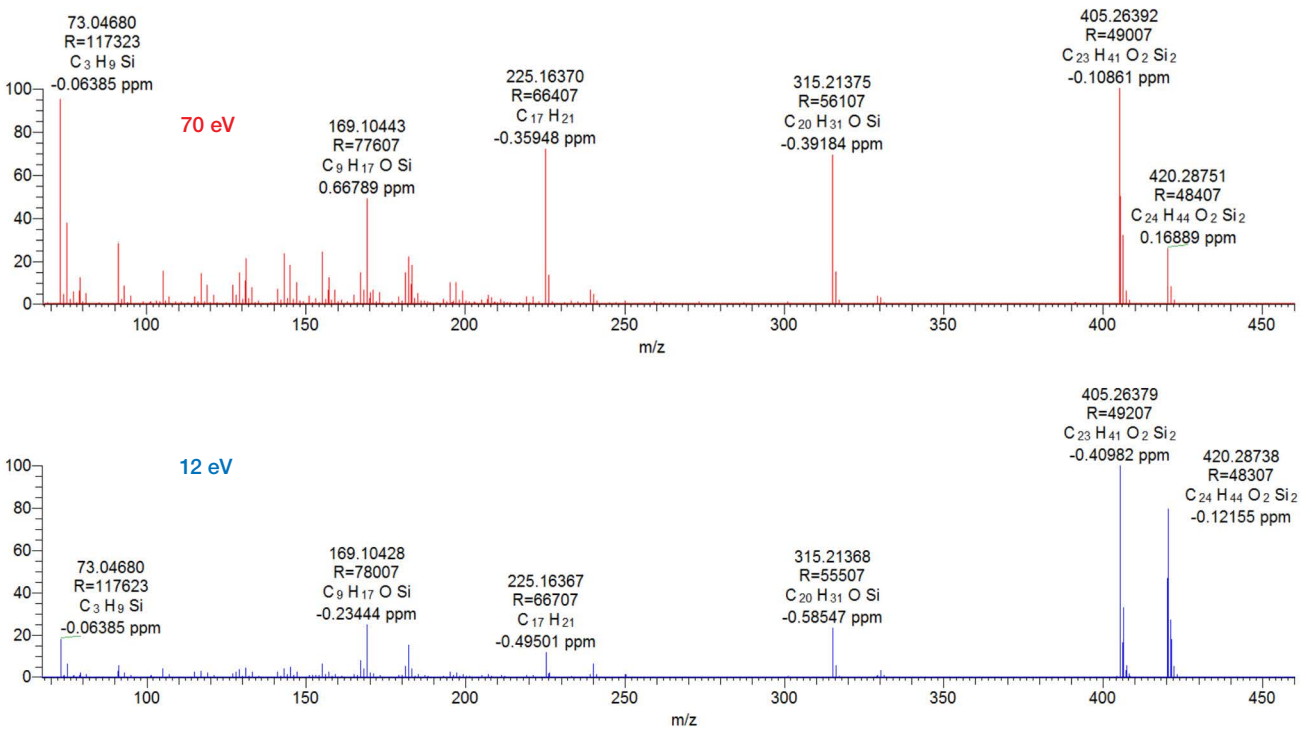

Figure 3. Comparison of 19-NA mass spectra acquired using VeV, at 70 eV and 12 eV.

Table 1. Spectral signal-to-noise ratio (S/N) of the molecular ion of 19-NA acquired at different electron energies.

Electron Energy (eV)	S/N
70	1587
50	1620
40	1627
30	1725
20	3143
15	5759
12	7790

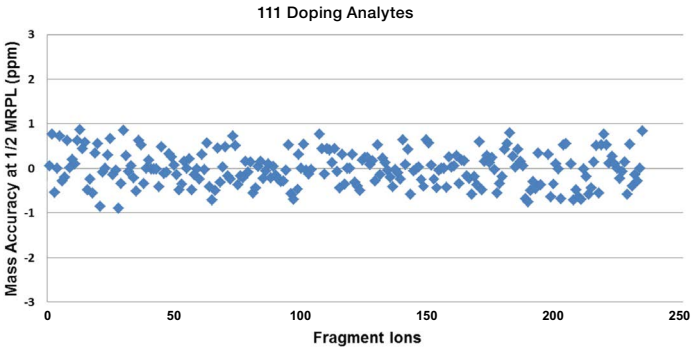

Figure 4. Mass accuracy (ppm) of 111 doping analytes at half MRPL.

Reliable accurate mass

Acquiring reliable accurate mass measurements is critical when detecting doping analytes at lower concentrations in complex urine 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. In Figure 4, 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.

Conclusion

Overall, using VeV on the Q Exactive GC and Exactive GC GC-MS systems allows for enhanced analytical performance. The automated tuning system greatly reduces complexity and improves operational efficiency in the laboratory. The VeV can significantly lower the limit of detection for confident qualitative and quantitative analysis. Mass accuracy is consistently maintained at less than 1 ppm irrespective of the ionization energy used. 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.

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.

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

ThermoFisher SCIENTIFIC