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Using Variable Electron Voltage (VeV) on GC Orbitrap

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ABSTRACT

Variable Electron Voltage (VeV) tuning was evaluated on the Thermo Scientific™ Q Exactive™ GC and Exactive™ GC Orbitrap high resolution accurate mass (HRAM) mass spectrometers. This technology enables optimization of lower filament electron energy settings for electron ionization (EI), and was shown to routinely deliver robust tuning results. Optimizing the EI source at lower electron energies through this technique promotes higher mass signals and increases sensitivity for compounds prone to extensive fragmentation. In this work, a routine sports-doping screening method in complex human urine samples was used as an example of how this new VeV tuning capability is able to increase sensitivity and improve confidence of compound identification. Performance examples of VeV from other applications are also briefly discussed.

INTRODUCTION

Electron ionization (EI)[1] refers to a hard ionization where a beam of electrons passes through the gas phase sample, resulting in positively charged fragments. EI sources are most often operated with an electron energy of 70 eV because this shows good sensitivity to most GC-amenable compounds. However, EI sources operated at 70 eV produce only low mass fragments with no detectable molecular ion for many compounds. Chemical ionization (CI)[2] is considered a softer ionization that often gives molecular ion information and reduced fragmentation relative to EI, but is also lower in sensitivity, ionization can be compound specific, and 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. The key benefits of VeV are listed below:

– Fully automated for optimum performance - VeV set-up is very simple and easy with fast, fully automated tuning

– Increased sensitivity - VeV enables enhancement of higher mass ions which have higher specificity than low mass fragments

–Increased confidence in identification - VeV promotes molecular ion and other diagnostic high mass signals

MATERIALS AND METHODS

Sample Preparation

A simple sample preparation was processed in four steps: an enzymatic hydrolysis, liquid-liquid extraction, evaporation and trimethylsilylation. Twelve different blank urine samples (negative QCs) and positive QCs spiked with 111 doping analytes at various concentrations from 0.02 to 200 ng/mL were analyzed in full-scan mode at various electron energies with 60,000 FWHM (measured at *m/z* 200) resolution. SIM was also performed simultaneously with full-scan for select challenging steroids. Details of the analytical conditions are given in Table 1.

Data Analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software which allowed for both quantitative and qualitative sample analysis. This includes peak integration and calculation of compound concentration and recoveries, as well as data review and reporting. In addition, for qualitative analysis, TraceFinder software automatically generates clean mass spectra through automated peak deconvolution and compound identification by library searching either a custom made or commercially available spectral library.

Table 1. Gas chromatograph/mass spectrometer analytical parameters.

TRACE 1310 GC Parameters	
Injection Volume (µL):	2.0
Liner	Thermo single taper with wool
Inlet (°C):	280
Inlet Module and Mode:	SSL, split (5:1)
Carrier Gas	He, 0.83 ml/min
Oven Temperature Program:	
Temperature 1 (°C):	140
Hold Time (min):	0
Temperature 2 (°C):	180
Rate (°C/min)	40
Hold Time (min):	0
Temperature 3 (°C):	230
Rate (°C/min)	3
Hold Time (min):	0
Temperature 4 (°C):	300
Rate (°C/min)	40
Hold Time (min):	2
Total (min)	21

Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	12-70
Acquisition Mode:	Simultaneous Full scan & SIM
Microscans:	2
Mass range (*m/z*):	100-700
Mass resolution (FWHM at *m/z* 200):	60,000
Lock masses (*m/z*):	73.04683; 133.01356; 207.03235; 281.05114; 355.06994

Tuning Software

The VeV tuning window is shown in Figure 1. Electron energies ranging from 10 – 150 eV can be set in the spin box indicating the energy to be tuned at. The user also can set the mass to be tuned, or can select "TIC" to tune on all calibration gas masses. By clicking the "Tune" button, the system will start tuning at the desired electron energy. It takes approximately 30 seconds to finish an auto tune on the system. During the sample acquisition described in this poster, the tuning algorithm showed stable calibration gas intensities when tuning at each electron energy over successive days, which is important for reliable operation.

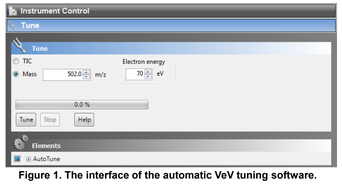

Figure 1. The interface of the automatic VeV tuning software.

RESULTS

Sensitivity

A positive steroid QC sample at half the minimum required performance limit (MRPL) was analyzed in EI full scan at various electron energies (12 eV, 15 eV, 20 eV, 30 eV, 50 eV, and 70 eV) (Figure 2). One quantitation ion and one confirming ion were selected for each compound. For most steroids, the target ions selected were higher mass ions since many lower mass fragments are also common to endogenous steroids in the urine matrix, and thus show problematic interference. The y-axis of this chart shows that the relative intensities of the sum of all target ions increased at 20 eV, 30 eV and 50 eV 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 as compared to 70 eV (254% increase). Particularly, molecular ions were observed to be increased almost four times at 30 eV compared to 70 eV for the challenging compounds listed in Figure 3. Because of this, the limit of detection at 30 eV can be lowered from that at 70 eV (Figure 4).

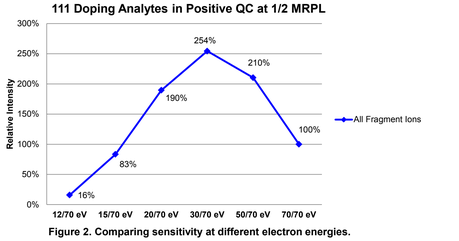

Figure 2. Comparing sensitivity at different electron energies.

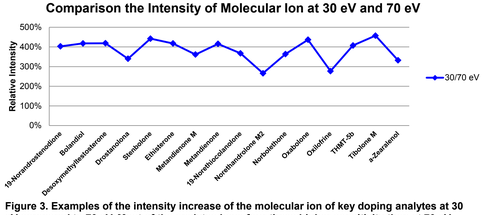

Figure 3. Examples of the intensity increase of the molecular ion of key doping analytes at 30 eV compared to 70 eV. Most of the analytes have four times higher sensitivity than at 70 eV.

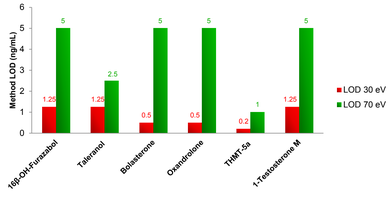

Figure 4. Examples of key doping analyte LODs at 30 eV and 70 eV.

Mass Accuracy

Acquiring reliable accurate mass measurements is critical when detecting doping analytes at lower concentrations in complex urine sample matrices using HRAM GC/MS. This is essential as any compromise in the accuracy of mass measurements can result in false identification, erroneous quantitation, and interferences from matrix ions in the extracted ion chromatogram. Low mass errors 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 result in fewer false positive detects, thus increasing efficiency by reducing the need for manual review. In Figure 5, outstanding mass accuracy (<1 ppm) was maintained across all quantitation ions at the half MRPL level at 30 eV.

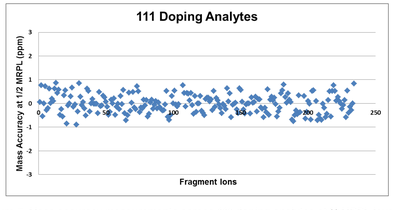

Figure 5. Mass accuracy measurements across 111 doping analytes at ½ MRPL level.

Linearity of response

Excellent linearity was obtained at 30 eV for most of doping analytes. Figure 6 shows the linearity of clenbuterol (left) in the range of concentration from 0.02-0.2 ng/mL in SIM mode, which is one of the key compounds in the World Anti-Doping Agency (WADA) list that has the lowest MRPL. The right side shows the linearity of stanozolol 3'OH in the range of concentration from 0.2-2 ng/mL in SIM mode.

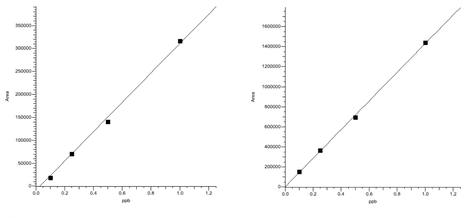

Figure 6. Linearity of clenbuterol (left) and Stanozolol 3'OH (right) over a 4-point calibration curve.

Comparison of spectra at 70 eV and 12 eV

At electron ionization energies even lower than 30 eV, a further increase in relative intensity of diagnostic higher m/z ions and/or molecular ions can be seen for doping analysis. Figure 7 exhibits a significant enhancement of the molecular ion 420.28738 Da (-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 to almost 20%, which largely simplified the spectrum. The stronger molecular ion signal and reduced fragmentation obtained at 12 eV offers the advantage of increased selectivity and thus improved spectral signal-to-noise (S/N) ratios for doping analysis.

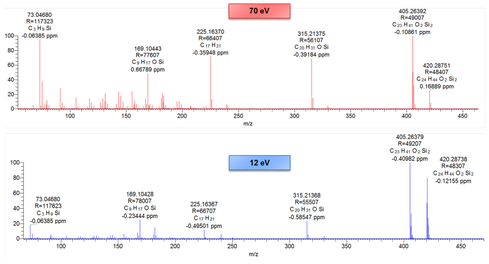

Figure 7. Comparison of 19-NA mass spectra acquired using VeV at 70 eV and 12 eV; Note that the mass accuracies of all intense ions are within 1 ppm.

Repeatability

To evaluate the repeatability of VeV, a positive QC sample at half MRPL level was chosen and analyzed in full-scan mode at 30 eV. A total of 18 replicate injections were made from a single vial. Table 2 lists the %RSDs of some key doping compounds, all with %RSDs less than 7%. .

Table 2. Repeatability (%RSD) for a positive QC sample at half MRPL level (n=18)

Compounds	RSD%	Compounds	RSD%
11-nor-9-carboxy-THC	4.95	Etamivan	5.13
19-NA (19-norandrosterone)	6.56	EMD (epimetendiol)	5.74
19-NE (19-noretiocholanolone)	5.48	Fluoxymesterone M2	4.89
(Z)-4OH-Tamoxifen	6.40	Letrozole M	5.96
Androstenodione 1	5.14	Methylnortestosterone	5.20
Boldenone	5.24	Mestanolone	5.26
Boldione	5.81	Norbolethone	6.05
Bromantane 6OH	4.76	Norbolethone M1	4.85
Calusterone	6.38	Norbolethone M2	5.26
Calusterone M	4.52	Nandrolone	5.87
Clostebol M	6.80	Oxabolone	5.93
Codeine	4.98	Tibolone M	6.08
Drostanolone M	5.13	Taleranol	6.09
Desoxymethyltestosterone	4.71	THMT-5β	6.40

Other examples

For further evaluating the utility of the VeV ionization mode, different types of compounds have been tested, such as fatty acid methyl esters (FAMEs), straight chained hydrocarbons (C8-C40), and acids and bases of environmental interest (Figure 8). All of these analytes have higher molecular ion sensitivity at 30 eV compared to 70 eV. For FAMEs, over four times increase of their molecular ions was observed. Acids and bases were almost double the intensities of their molecular ions. Hence, this data suggests that VeV is a valuable tool for a wide number of compounds to boost sensitivity, especially for molecular ions.

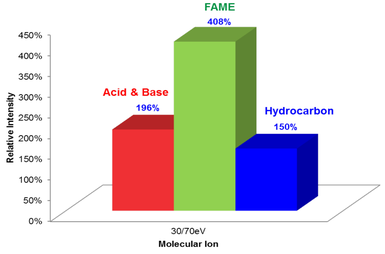

Figure 8. Comparison of the intensities of molecular ions at 30 eV compared to 70 eV.

CONCLUSIONS

- Using VeV on the Q Exactive GC and Exactive GC GC-MS systems can allow for enhanced sensitivity for more specific high mass ions
- The automated tuning system greatly reduces complexity and improves operational efficiency for optimizing sensitivity at electron energies ranging from 10 and 150 eV
- VeV can significantly lower the limit of detection of compounds in matrix for confident qualitative and quantitative analysis
- Mass accuracy is consistently maintained at less than 1 ppm irrespective of the electron energy used. The enhanced signal obtained at 30 eV for high mass fragments, including molecular ions, in addition to this outstanding mass accuracy, makes for an effective way to yield useful structural information to aid in the identification of unknown compounds.

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1. T.D. Märk; G.H. Dunn (29 June 2013). *Electron Impact Ionization*. Springer Science & Business Media. ISBN 978-3-7091-4028-4.
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TRADEMARKS/LICENSING