

METABOLITE STRUCTURAL ELUCIDATION USING A REVERSE ENERGY RAMP AND COLLISION ENERGY GRADIENT ON A TRIPLE QUADRUPOLE MASS SPECTROMETER

Mark A. Szewc, Kevin J. McHale

Thermo Electron Corporation, 265 Davidson Ave., Somerset, NJ 08873, USA.

The triple quadrupole mass spectrometer is the most widely used instrument for LC/MS/MS worldwide. Although triple quadrupoles are known mainly for their highly sensitive quantitative capabilities in complex matrices, the qualitative information these instruments provide can be extremely valuable. The problems that are historically associated with the qualitative data from triple quadrupole mass spectrometers have been a deficient amount of intense low molecular ions in a product ion spectrum, and the lack of dynamic collision energy normalizing for mass. The addition of intense low molecular ions could help in structure elucidation, in particular for impurities and metabolite identification. Adding a “normalized” collision energy would allow for most compounds to have an optimal fragmentation spectrum in one analytical LC/MS acquisition. Recently triple quadrupole mass spectrometers have added a Reverse Energy Ramp (RER) and Collision Energy Gradient (CEG) to solve the aforementioned issues. Briefly, RER is a collision energy ramp during a product ion scan in which the voltage applied to the 2nd quadrupole (i.e., collision cell) is varied linearly from a high value to a low value during the forward Q3 scan from low m/z to high m/z. Hence, RER aids in creating a richer product ion spectrum by applying the highest collision energy to the parent mass (selected by Q1) while Q3 is passing the low m/z ions, while the high mass fragment ions are generated at a lower collision energy. The CEG is a means of applying different collision energy to the parent ion depending on its m/z. Therefore, CEG yields a “normalized” collision energy for all potential parent masses during an LC/MS/MS acquisition, allowing a more optimized collision energy for each parent ion to yield quality MS² spectra. Here we investigate the advantages of RER and CEG compared to fixed collision energy CID methods on the triple quadrupole instrument using the TSQ Quantum Ultra for metabolite identification and structural elucidation of several well characterized compounds. Each compound studied was incubated at 20 uM for 60 minutes in rat liver microsomes. After termination of the reaction with the addition of two volumes of acetonitrile, the supernatant was analyzed by high pressure liquid chromatography (HPLC) in combination with tandem mass spectrometry. The presented data will demonstrate the utility of both RER and CEG in obtaining a richer fragmentation pattern that will aid with metabolite identification and structure elucidation.