

The Impact of the “Reversed Energy Ramp” (RER) Scan Function on Metabolite Identification at or Below the 1µM Level on a Triple Stage Quadrupole

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Overview

Purpose: Increase the information content and sensitivity of triple stage quadrupole mass spectrometers for better structural elucidation.

Methods: The RER scan function was developed and PolyTyrosine, Reserpine, Alprazolam, Nefazodone and Paroxetine were used to evaluate the scan function.

Data Dependent™ scanning was used to trigger the RER scan for the selected compounds. The source scans used for Data Dependent scanning were: SRM, Precursor Ion Scan and full scan Q1MS.

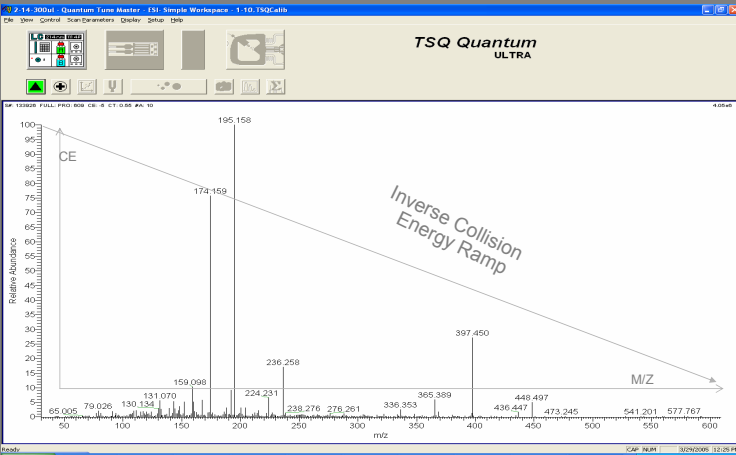
Spectra acquired with a static collision energy and with ramped collision energy are compared.

Results: The data acquired with the RER scan is more sensitive (2-3 times) and is richer in information than the data acquired with the Static Collision Energy (SCE) scan.

Introduction

A “reversed energy ramp” scan (RER) was devised for improving product ion sensitivity when using a triple stage quadrupole mass spectrometer (TSQ) and as a means for increasing the information content for better structural elucidation. Coupled to techniques such as constant neutral loss scans (CNL) and precursor ion scans (PS), a standard TSQ instrument with RER can be used as a powerful tool for metabolite identification. In collision induced dissociation (CID) experiments on a TSQ, multiple generation MS/MS spectra are seen. However, it is observed that the smaller mass fragments (second or third generation) need more collision energy at the same gas pressure to be created than the larger fragment ions (first generation). The RER scan function linearly reduces the amount of collision voltage (energy) while the product ions are scanned from low to high mass. The immediate advantage is realized during a Data-Dependent LC-MS experiment, where the speed of acquisition of the RER scan allows product ion scans to be summed, enhancing fragmentation patterns for low level metabolites.

FIGURE 1. Schematic of how the RER scan is applied. The RER scan function linearly reduces the amount of collision voltage (energy) while the product ions are scanned from low to high mass. Using this scan function the fragments are created with “normalized” collision energy. The product ions are created more efficiently, resulting in higher sensitivity and richer product ion spectra.



Methods

PolyTyrosine, Reserpine, Alprazolam, Nefazodone and Paroxetine were used to evaluate the scan function. The PolyTyrosine was used to evaluate the function in the Tune view only while the other compounds were used with a chromatographic separation method too.

HPLC Conditions: Finnigan™ Surveyor HPLC™ system was used. Samples (10µL) were injected onto a Hypersil Gold™ 2.1 x 150 mm column (3 µm particle size). A gradient LC method used mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 500 µL/min. The gradient was (time, %B): (0, 5) (0.2, 5) (10, 95) (10.1, 5) (12, 5) at 60°C.

FIGURE 2. Instrument Method. The second scan event is the Data Dependent scan. The energy ramp is set to 35 V and the collision energy to 20 V. Resulting in a ramp from 55 V at m/z 30 to 20 V at the end mass.

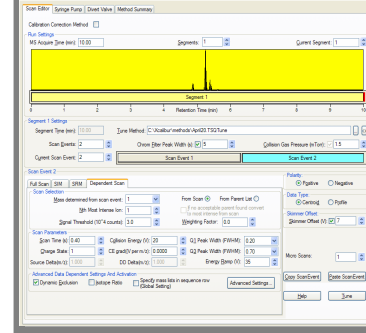
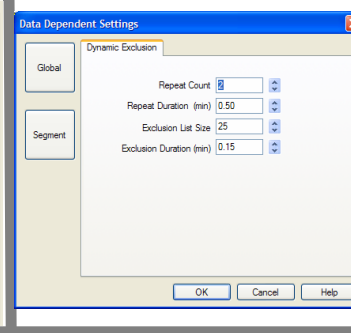


FIGURE 3. A combination of Data Dependent and Dynamic Exclusion settings were used in the experiments. Using these settings two full scan spectra per compound were obtained.



Mass Spectrometer Conditions:
Finnigan TSQ Quantum Ultra™
Ion source and polarity: H-ESI, Positive ion mode
Spray voltage: 255 V
Vaporizer temperature: 300°C
Sheath gas: 75 units nitrogen
Auxiliary gas: 30 units nitrogen
Transfer tube temperature: 350°C

Results:

FIGURE 4. RER and SCE Spectra of Nefazodone, Paroxetine, PolyTyrosine and Alprazolam. Top spectra are SCE scans and below are the corresponding RER scan spectra. RER scans ramp from 50 V at m/z 30 to 10 V at precursor m/z. Note that the RER spectra are richer in fragments/information and in intensity.

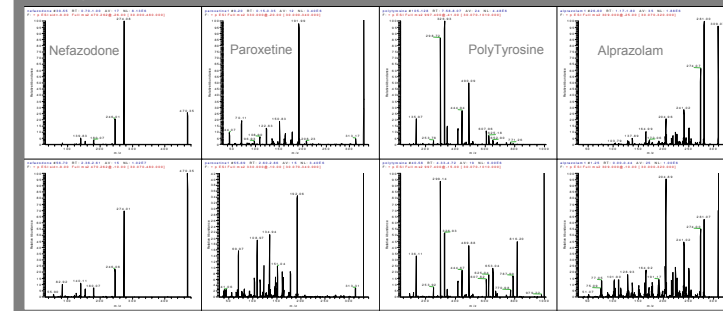


FIGURE 5. Chromatograms with the four compounds in the test mix acquired with the RER scan to illustrate the improvement in sensitivity. In comparison with the SCE scan, see Figure 6, the peaks have a factor of 2 to 3 times more intensity with the RER scan.

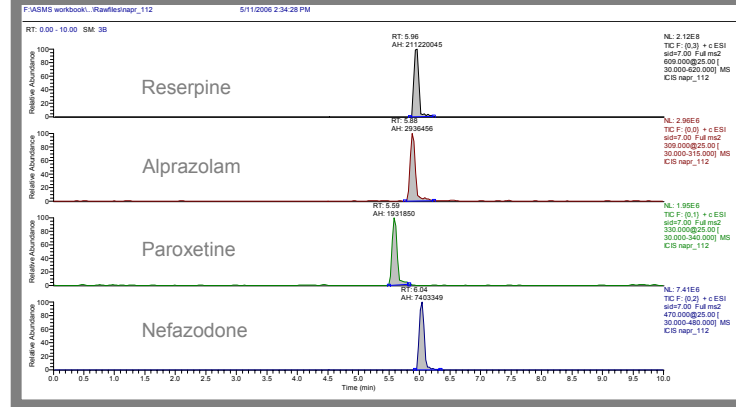
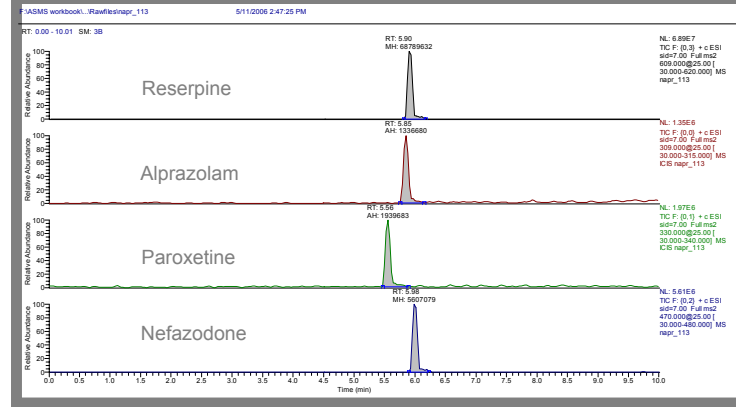


FIGURE 6. Chromatograms with the four compounds in the test mix acquired with the SCE scan to illustrate the improvement in sensitivity. In comparison with the RER scan, see Figure 5, the peaks have a factor of 2 to 3 times less intensity with the SCE scan.



In Figure 4, four compounds are shown as examples with the RER scan (top spectra) and the SCE scan (bottom spectra) for comparison. The RER scan spectra of the compounds are clearly much richer in information than the SCE scan spectra, providing more structural information.

Figures 5 and 6 illustrate the increase in sensitivity of the RER scan over the SCE scan - on average the signal increases by a factor of 2 to 3. These files are acquired on only the four product ions scan in the method. In Figure 5 the RER scan function was used and in Figure 6 the SCE scan function was used.

In Figures 7 and 8 the four compounds in the test mix are separated by the chromatographic system in to narrow peaks of less than two seconds wide at half height. Using Dynamic Exclusion, the system switches to the full scan product ion mode for two scans once the threshold is reached. The instrument is still fast enough to have more than fourteen SRM scans across the chromatographic peak for accurate quantification. Even at lower concentrations (10 pg/µL, fig. 8) the Full scan spectra are reproducible with the spectra with the higher concentration (100 pg/µL, fig. 7).

FIGURE 7. A 10 µg injection of a 100 pg/µl test mix solution. Using Quality Enhanced Data Dependent (QED) scanning, a SRM triggered Data Dependent scanning. Combined with Dynamic Exclusion this gives two full RER product ion scans for compound confirmation, leaving more than enough scans across the peak for reliable quantification.

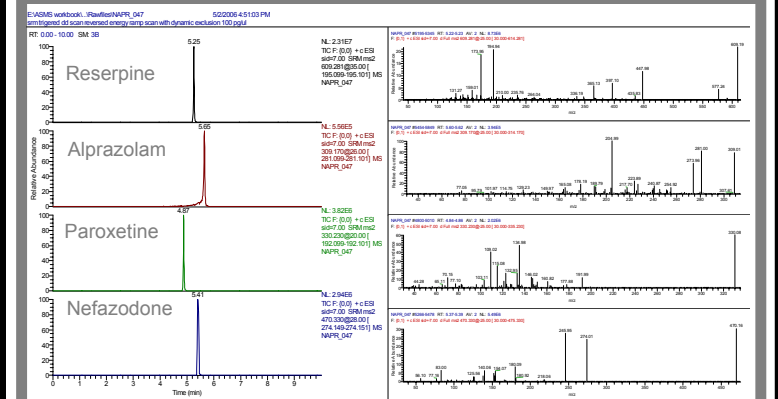
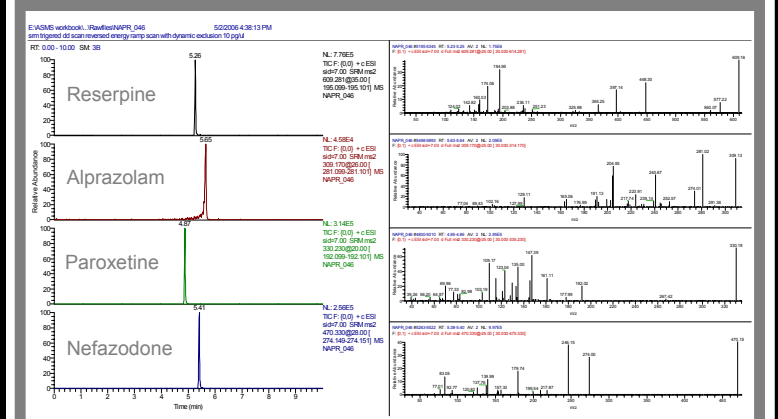


FIGURE 8. Same experiment as above only this time 10 pg/µL test mix solution. Even at low concentrations the spectra are reproducible.



Conclusions

Spectra acquired with the Reversed Energy Ramp scan function are richer in information when compared to the Static Collision Energy scan. With the SCE scan, the same amount of information can be obtained using rolling energy scans where the collision energy is increased over several scans. The disadvantage is that it takes more scans and therefore more time to obtain the data. Leaving not enough time to acquire other important information. On average, the intensities of the chromatographic peaks acquired with the RER scan are 2-3 times higher than the peaks acquired with the static collision energy scans. The RER scan is ideal to be used in a generic way and to generate product ion libraries for different applications like pesticides, food additives and residues and clinical applications.