

# Metabolite Screening and Structural Elucidation Using a New Generation of Triple Quadrupole Mass Spectrometers

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## Overview

**Purpose:** To elucidate predicted and unpredicted metabolites by using a new generation of triple quadrupole mass spectrometers.

**Methods:** SRM and SRM triggered Data Dependent product ion scans on the TSQ platform, namely the Thermo Scientific TSQ Vantage.

**Results:** Today's non-hybrid triple quadrupoles generate excellent product ion spectra along with the SRM sensitivity needed for metabolite screening and structural elucidation.

## Introduction

The triple quadrupole mass spectrometer is a commonly used instrument for LC/MS/MS analyses due to its highly sensitive quantitation of target analytes in complex matrices. Historically triple quadrupoles are not known for being sensitive instruments for collecting product ion spectra when compared to hybrid triple quad/linear ion trap systems<sup>(1,2)</sup>. The primary difference between these two instruments is the method of ion analysis. The hybrid instruments collect the product ions for amplification before detection whereas the triple quadrupole has the product ions directly strike the detector. Trapping of the ions increases the sensitivity and scan speed of a full-scan product ion spectrum. Good sensitivity in the MS/MS spectrum is necessary for structural elucidation of both drug impurities and metabolites. Thus, to perform impurity profiling and metabolite identification using triple quadrupoles, it is desirable to improve the intensity and number of ions in the MS/MS spectra.

Recently, innovations to improve the MS/MS sensitivity and ion abundance have been employed on new generation triple quadrupoles. These include a Reverse Energy Ramp (RER) and Collision Energy Gradient (CEG). The RER voltage applied to Q2 is decreased linearly from a high value to a low value as Q3 is scanned from low mass to high mass (See Figure 1). Thus, in a single scan, low mass fragments are generated by applying a higher collision energy to Q2 during a product ion scan, and high mass fragments are generated by applying a lower collision energy at the end of the Q3 scan. The result is the MS/MS spectrum rich in both low and high mass fragment ions. The CEG is a means of applying an optimized collision energy to the precursor ion depending on its mass-to-charge ratio. The collision energy varies proportionally with the mass of the precursor ion, resulting in optimized or "normalized" collision energy for each precursor and improving the MS/MS spectral quality. In addition, these triple quads have the ability to trigger an MS/MS acquisition from a SRM scan, in a data dependent manner. This enables MS/MS acquisition of multiple low level analytes from a SRM scan, improving cycle time and sensitivity compared to traditional full-scan triggered MS/MS techniques. Finally, the application of RER, CEG, and SRM-triggered MS/MS spectra greatly improve sensitivity for multiple transitions, spectral quality and number of product ions generated, thus facilitating identification of impurities and metabolites.

This study investigates the ability of the newer generation of triple quads, primarily the TSQ Vantage™, to perform metabolite screening and identification for several well characterized compounds. The presented data demonstrate the utility of these highlighted scan features and improved sensitivity for compound screening and structural elucidation, particularly for glutathione and oxidized metabolites.

## Methods

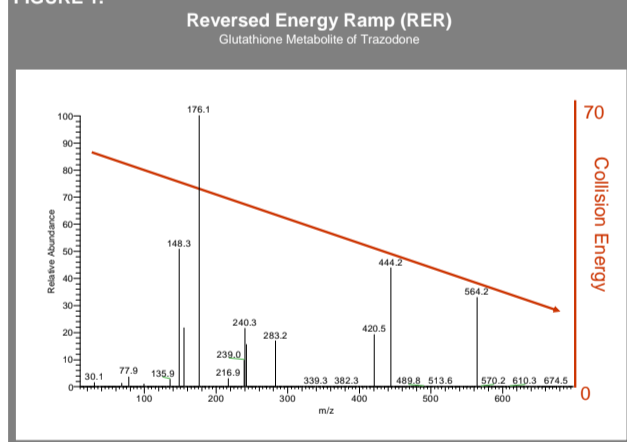
All experiments were performed using a TSQ platform, namely the TSQ Vantage (Thermo Fisher Scientific, San Jose, CA) and the AB Sciex Qtrap® 4000. Two scan events were employed in these studies. Scan event one using fixed SRM transitions to monitor for predicted metabolites. Scan event two was a Data Dependent product ion scan of the most intense ion from event one for structural elucidation of the metabolites.

For the TSQ platform, scan event two used the CEG (collision energy gradient) and RER (reverse energy ramp) techniques. CEG applies a different collision energy (CE) for each parent ion depending on mass. The following formula  $X \cdot m/z + \text{base collision energy}$  ( $0.035 \cdot 693 + 10 \text{ V} = 34 \text{ V}$ ) describes how the instrument calculates the CE for the 2nd quadrupole. This calculation therefore normalizes the collision energy according to mass and allows for optimum fragmentation of each parent ion in an analytical run. Figure 1 illustrates the function of RER on a metabolite of Trazodone, using the base energy calculated from the formula described above. Briefly, RER applies an additional collision energy to Q2 by applying more voltage at lower mass. As the triple quadrupole scans quadrupole three, quadrupole two ramps the CE voltage in a linear fashion from higher energy to low. So, all fragment ions at lower m/z will have a higher CE applied. This energy will therefore provide more fragmentation to the lower mass range, yielding a richer fragmentation pattern. The RER offset value is set by the user, and it was set to 30 V in these experiments. For the Trazodone metabolite below, the lowest fragment ion at m/z 30 will have CE = 64 V applied to Q2. The AB Sciex Q-trap 4000 was tuned and optimized for each parent compound. Scan event two employed a static collision energy in EPI mode.

A binary solvent system consisting of (A) water and (B) MeCN with 0.1% formic acid was used for all experiments using a flow rate of 300 µL/min. All chromatographic separations, employed a C18 column with a gradient.

Microsomal incubations were conducted using 20 µM of substrate for 1 hour at 37°C. The incubations were conducted in with or without the presence of glutathione (5 nM). Each incubation used 1 mg/mL of protein and 1 mM of NADPH in 0.1 mM of potassium phosphate buffer. After incubations each reaction was quenched with one volume of acetonitrile.

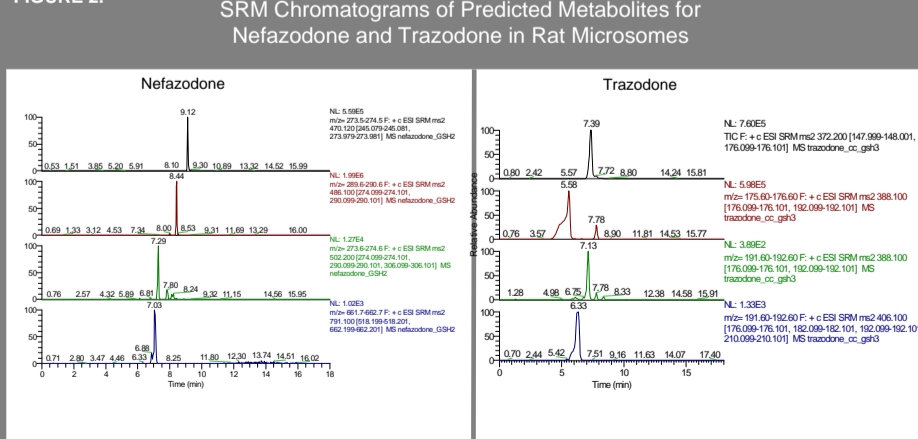
FIGURE 1.



## Results

The SRMs of a few predicted metabolites and parent compounds are illustrated in Figure 2. These SRMs are examples of the first scan event of the experiment method. The top chromatogram in both the Nefazodone and Trazodone figures corresponds to the parent compounds. For Nefazodone, the next two traces are examples of mono and dihydroxylations. The last trace on the left side is for a glutathione conjugation. On the right side of Figure 2, the second and third trace correlates to hydroxylated metabolites. A hydroxylation with addition of water is demonstrated on the final chromatogram of Trazodone. Other predicted metabolites included in this assay were GSH adducts for both compounds, dealkylation, hydroxylations for the GSH adducts.

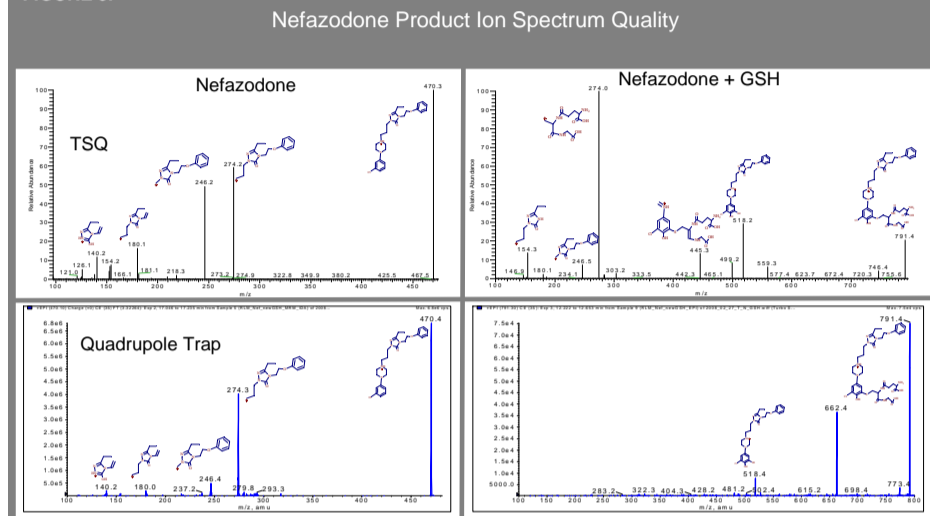
FIGURE 2.



## Results continued

The two top panels of Figure 3 were taken from the TSQ platform, whereas the bottom panels were obtained from the Q-trap platform. The left side of this figure shows spectra from the parent compound Nefazodone while the right side demonstrates the metabolite GSH adduct to Nefazodone. While looking at the top panels of the Figure 3, notice the multiple fragments, intensity, and mass range on new generation triple quadrupoles. Compare this rich fragmentation to the Q-trap in the bottom of this figure, observing that quality from the TSQ spectrum is enhanced in the lower mass range. The results also show that there are more intense ions on TSQ across the mass range when comparing the spectra. The addition of these intense ions allow for further confirmation by obtaining structural elucidation of the metabolites as well as helping to identify other transitions that may be more sensitive to identify other metabolites. In this example with the adduct of GSH we can clearly see that the m/z of 445 and 518 allows us to confidently identify that modification on the benzyl ring with the chloride. The other ions shown would further aid in identification if additional metabolites with modifications to the GSH adduct are observed.

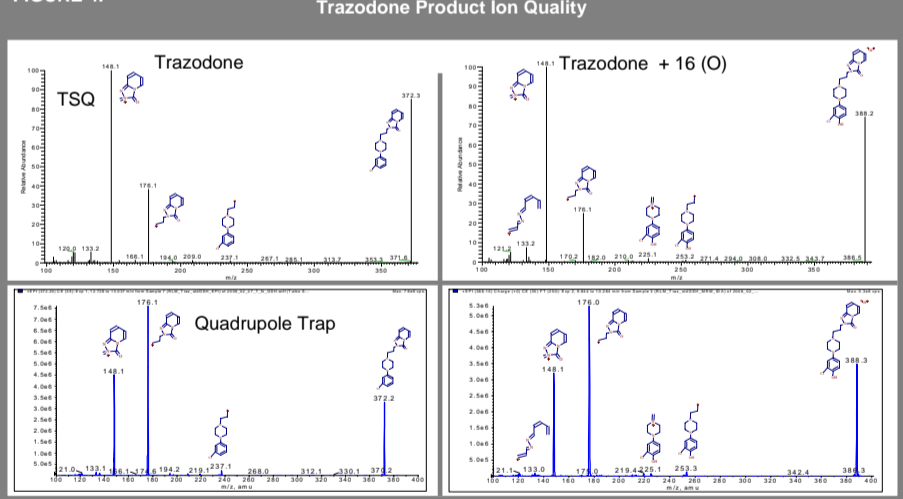
FIGURE 3.



Certain metabolites are still not polar enough to be eliminated from the body after one cycle of metabolism. Therefore, the metabolite must be metabolized again. An example of this is the case with Trazodone. This compound has a glutathione adduct as well as being hydroxylated. Figure 1 shows the spectrum related to this metabolite with the metabolite parent m/z as 693. The lower fragments (78, 148, and 176) are all mass related to the fused ring structure with no modifications. All the other mass are related to the other side of the molecule shown in Figure 4 with a hydroxylation and a GSH modification. With the two product ion spectra we are able to associate both modifications to the benzyl ring with the Chlorine.

Further examples of Trazodone and its metabolites are shown in Figure 4. The left side of the figure is demonstrating the fragmentation of Trazodone while the right side has a metabolite of a hydroxylated metabolite. Notice how the TSQ platform, top of figure, has a similar product ion spectra compared to the Q-trap spectra located in the bottom. Although both instruments yield the similar products, notice that the TSQ ions across the mass range have more intense product ions. The more intense ions allow for more reproducible spectra, which allows for higher confidence for structural elucidation.

FIGURE 4.



## Conclusions

- MS/MS spectra from newer generation of triple quadrupoles generate higher quality, richer product ion spectra.
- The improvement in the quality of the product ion spectra allows for better confidence in structure elucidation of metabolites.
- With the improvements in the quality of the ion spectra and in the overall triple quad sensitivity, using the SRM triggered product ion scan is an ideal use for screening of metabolites.

## References

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- (Hager, JW. A new linear ion trap mass spectrometer. *Rapid Communications in Mass Spectrometry*. 2002;16:512-526)

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