

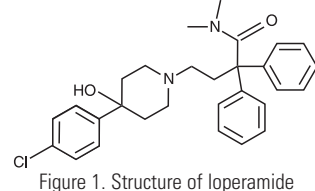
Simultaneous Metabolite Identification and Quantitation of a Parent Drug Using Reverse Energy Ramp Scanning on a Triple Stage Quadrupole Mass Spectrometer

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Introduction

Recent advances in the field of mass spectrometry have enhanced and expanded the capabilities of triple stage quadrupole (TSQ) mass spectrometers. These innovations include using linear ion traps to increase the product ion sensitivity; using high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) to increase the selectivity; and, more recently, using reverse energy ramp (RER) scanning to increase the product ion sensitivity. In addition, software developments, such as SRM-triggered data dependent scanning, have enabled the simultaneous acquisition of quantitative SRM data and qualitative product ion mass spectra on a TSQ instrument.

This application note describes the quantitation of loperamide (Figure 1), an anti-diarrheal agent, in mouse plasma and the simultaneous identification of its metabolites. Both data dependent scanning and reverse energy ramp scanning have been used to enhance the product ion sensitivity on a triple stage quadrupole mass spectrometer. Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) scanning delivers an information rich mass spectrum that can be used to confirm the existence of compounds while they are being quantified (Figure 2).



When a particular SRM transition reaches a predefined intensity threshold, the instrument automatically triggers QED-MS/MS, using the reverse energy ramp scan function. The RER scan function linearly reduces the amount of collision voltage (energy) while the product ions are scanned from low to high mass (Figure 3). By 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.

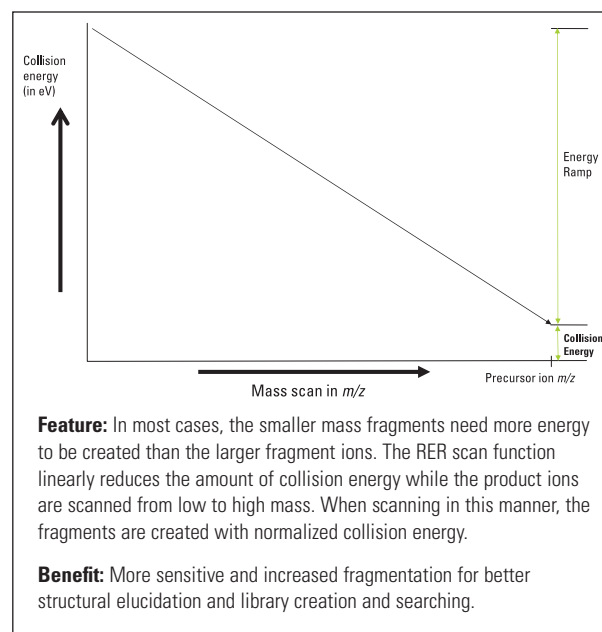


Figure 3: Schematic of RER scan

Goal

To explore the potential of simultaneous metabolite identification and quantitation of a parent drug by using a triple stage quadrupole mass spectrometer.

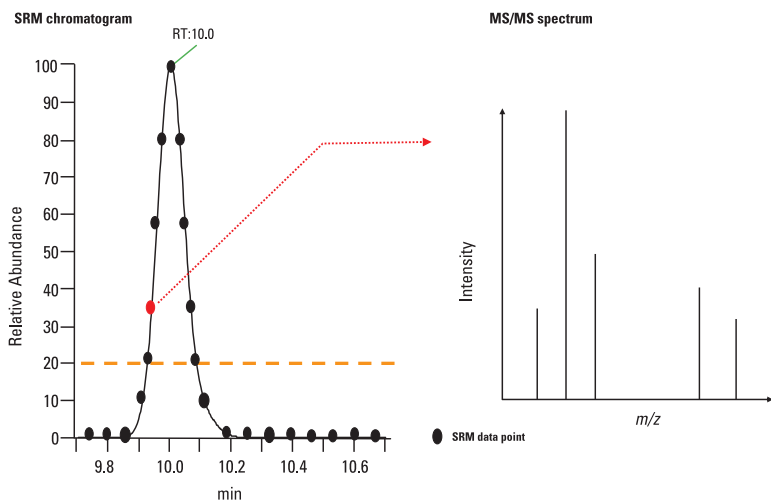


Figure 2: Schematic of QED-MS/MS

Experimental Conditions

Loperamide was administered intravenously to mice at 1 mg/kg. An SRM-triggered product ion scan with reverse energy ramp scanning was used to simultaneously identify and quantify loperamide metabolites in mouse plasma.

Sample Preparation

To prepare the sample, 25 μ L of mouse plasma was precipitated using acetonitrile and the mixture was centrifuged. Then, 10 μ L of supernatant was injected into the LC-MS/MS.

HPLC

HPLC analysis was performed using the Thermo Scientific Surveyor™ HPLC system. Each 10 μ L sample was injected onto a Thermo Scientific Hypersil GOLD™ 2.1 \times 150 mm column (3 μ m 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 μ L/min. The gradient was as follows (time, %B): (0, 5) (0.1, 5) (15, 100) (17, 100) (17.1, 5) (20, 5).

MS

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra™ triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI) probe. The MS conditions were as follows:

Ion Source Polarity:	Positive ion mode
Spray Voltage:	255 V
Vaporizer Temperature:	475 °C
Sheath Gas Pressure (N ₂):	75 units
Auxiliary Gas Pressure (N ₂):	30 units
Ion Transfer Tube Temperature:	350 °C

The SRM data dependent scan conditions were as follows:

Minimum Signal Required:	15,000.0
Dynamic Exclusion Settings:	
Repeat Count:	2
Repeat Duration:	0.70 min
List Size:	25
Exclusion Duration:	0.10 min

The SRM transitions that were monitored are summarized in Table 1.

Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)
449.15	182.00
449.15	238.00
463.15	196.00
463.15	252.00
465.15	198.00
465.15	254.00
477.15	210.00
477.15	266.19
479.15	268.00
479.15	252.00
493.15	252.00
493.15	282.00

Table 1: SRM transitions

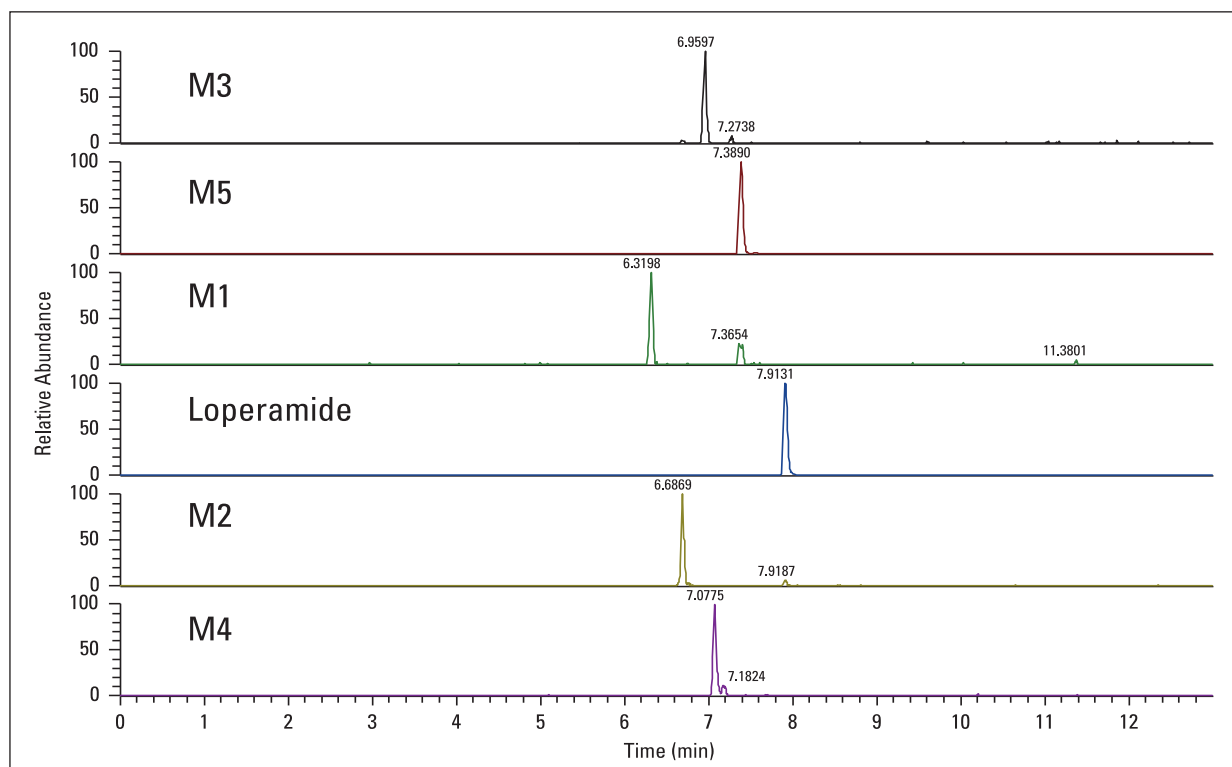


Figure 4: Chromatograms of loperamide and metabolites in mouse plasma

Results and Discussion

With the QED-MS/MS scanning and RER techniques, five metabolites of loperamide were characterized with high quality product ion spectra, and time course profiles of loperamide and its metabolites were obtained simultaneously.

Figure 4 shows representative SRM chromatograms for the analysis of loperamide and its five metabolites in

mouse plasma. Clearly identifiable and quantifiable peaks were observed. The calibration curve data is displayed in Figure 5. The weighted ($1/x$) calibration curve was quadratic over the concentration range with a correlation coefficient (R^2) of 0.9984.

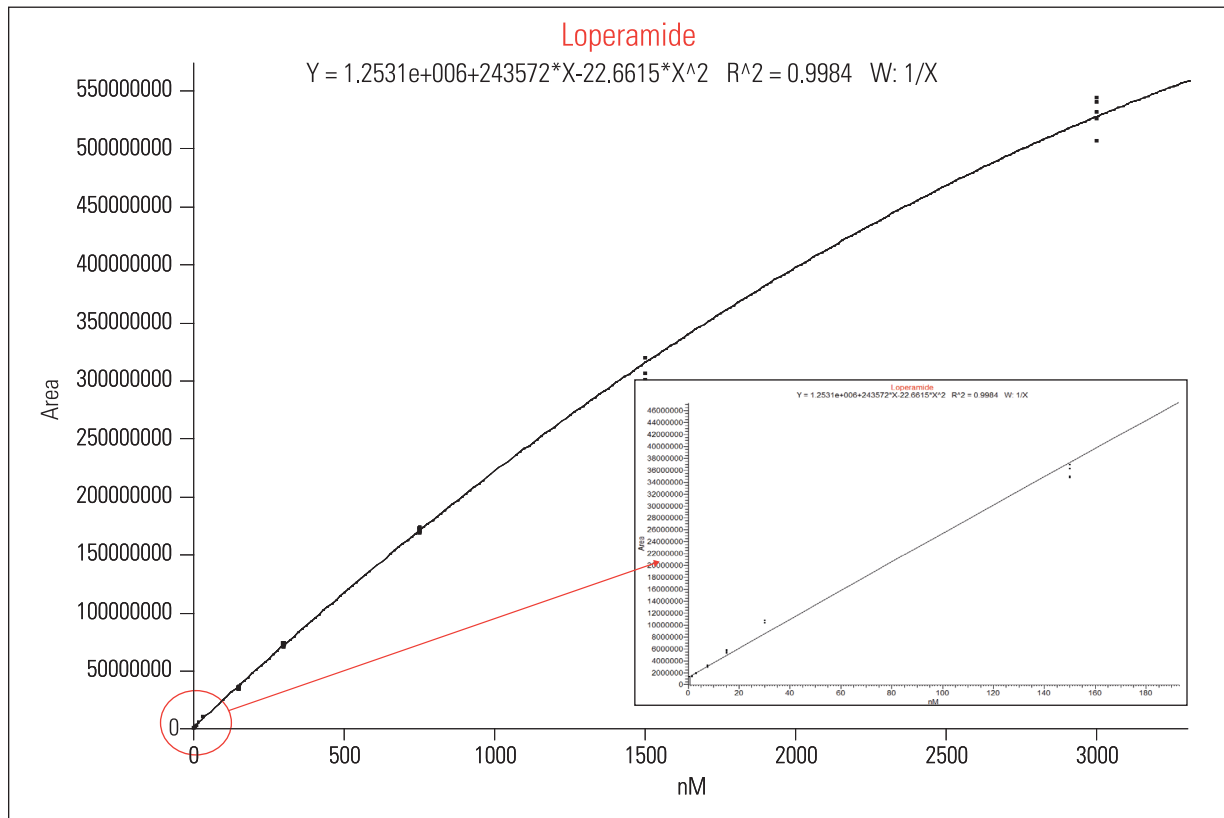


Figure 5: Calibration curve of loperamide in mouse plasma

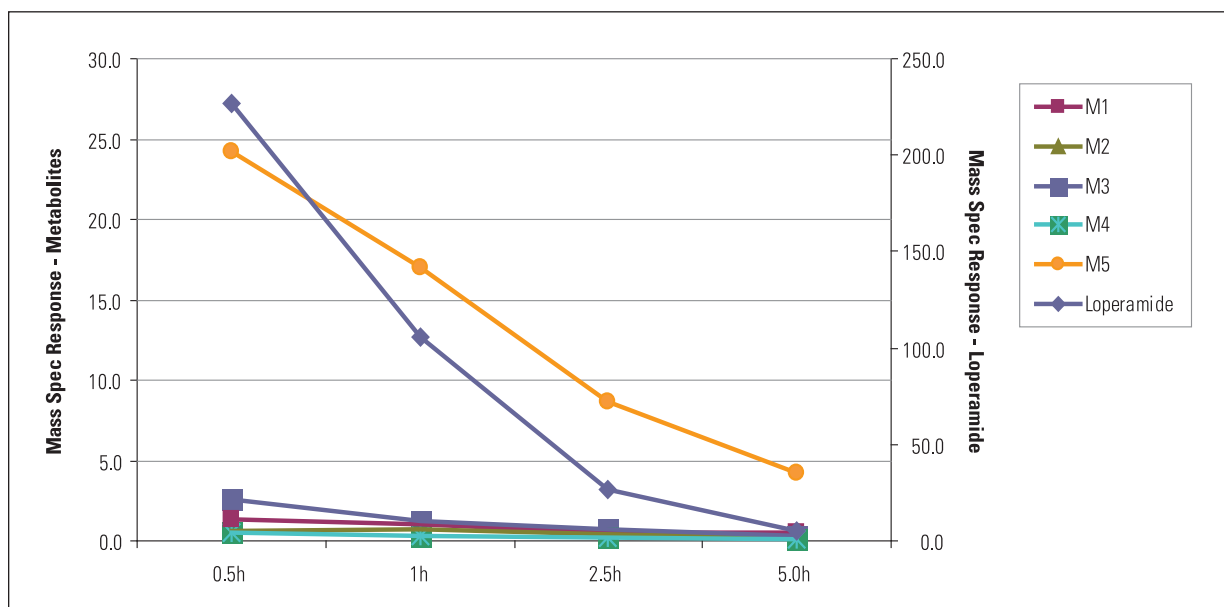


Figure 6: Concentration profiles of loperamide and metabolites in mouse plasma after intravenous administration at 1 mg/kg

The plasma concentration-time profiles for loperamide and its five metabolites were determined after intravenous administration at 1 mg/kg (Figure 6). The product ion spectra of loperamide and its five metabolites obtained by using the QED-MS/MS and RER techniques are shown in Figure 7. The spectra have rich fragment information, which facilitates the structure elucidation of the metabolites.

Conclusion

Quantitation-Enhanced Data-Dependent MS/MS scanning on a triple stage quadrupole mass spectrometer enables simultaneous metabolite identification and quantitation of a parent drug. The selectivity provided by this technique helps to increase the detection of metabolites. When the QED-MS/MS and RER techniques are coupled, the structure and concentration profiles of parent drugs and metabolites can be obtained simultaneously, thus increasing the bioanalytical throughput in drug discovery laboratories.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

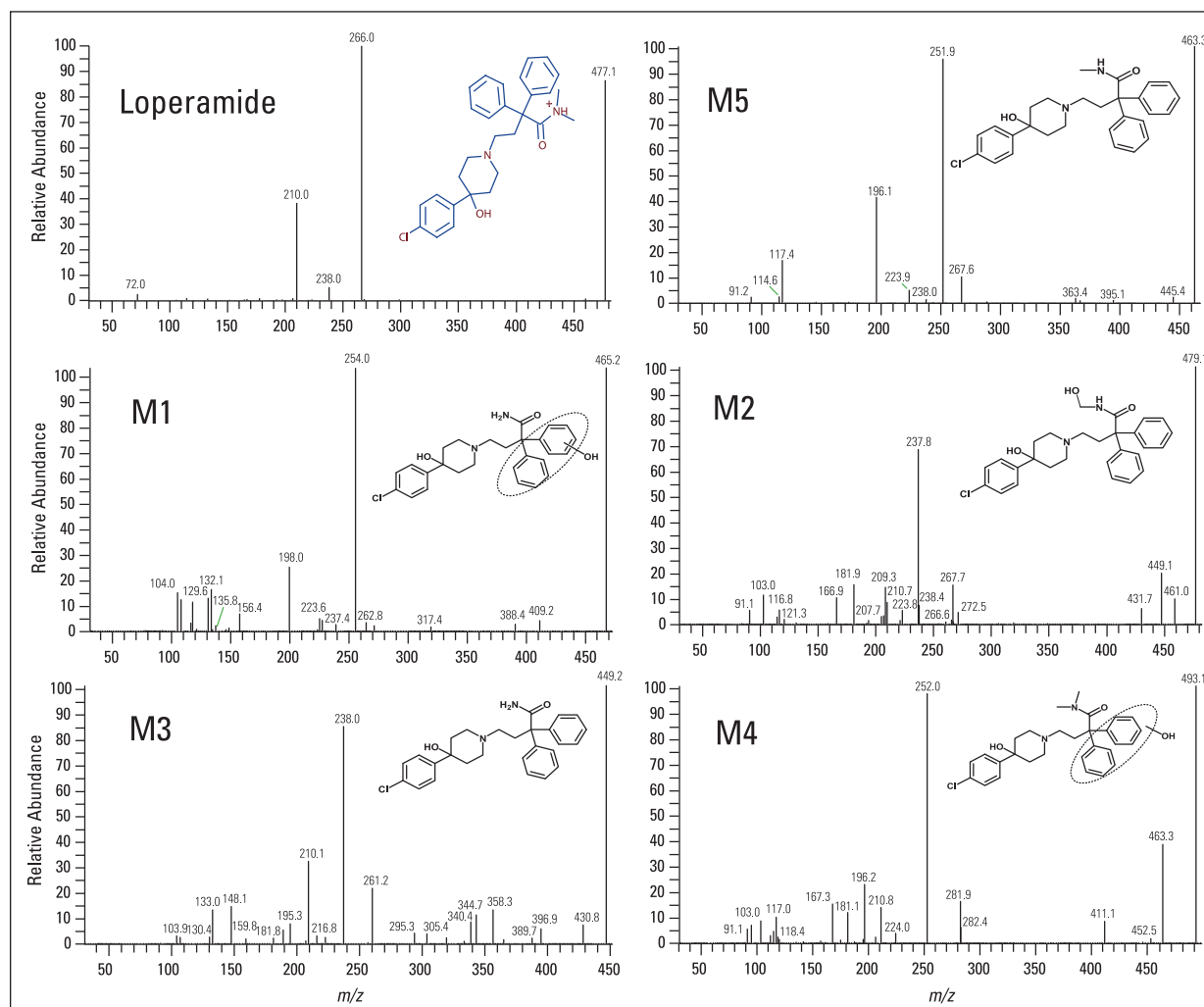


Figure 7: Product ion spectra of loperamide and metabolites using RER

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