Comprehensive Detection of Metabolites using Polarity Switching Data Collection with the New QTRAP® 5500 LC/MS/MS System

Carmai Seto
AB SCIEX, Concord, Ontario, Canada

Overview

Majority of xenobiotic metabolites tend to ionize in the same mode as the parent drug. Typically the majority of therapeutics and their down stream metabolites ionize in positive ESI with excellent sensitivity. However some Phase II conjugates such as the additions of phosphate or sulfate, completely change the ionization character of the molecule to negative ESI. Polarity switching data collection ensures a more comprehensive way of detecting metabolites in both polarities within a single LCMS run. Furthermore for GSH conjugate detection a number of pos/neg precursor/neutral loss or MRM IDA experiments provide effective coverage for all possible formations (see technical note titled "GSH Conjugate Screening with the New QTRAP® 5500 LC/MS/MS System). The new QTRAP 5500® system is especially effective at pos/neg IDA methods. The 5500 QTRAP® System's ability to perform fast Quadrupole and LIT scanning (4X Quadrupole and 5X LIT over the 4000 QTRAP), 50 ms polarity switching and enhanced sensitivity in both Quadrupole and LIT mode allow for efficient metabolic detection. These groundbreaking workflows are further optimized by the addition of LightSight® 2.1 for automated data processing and IDA method creation.

Introduction

In discovery stage pharmaceutical research, information about the extent of metabolism and the elucidation of metabolite structures is used in lead optimization. Identified metabolites can be a source of new therapeutics, toxicity assessment and drug "soft spot" optimization. LCMS is widely used for the identification of metabolites at all stages of drug development. Metabolites most often ionize with the same polarity as the compound of interest. However, there are cases where this assumption does not hold true thus leading to cases of missed metabolites. Polarity switching data collection ensures a more comprehensive way of detecting metabolites of various polarities.

There are two approaches to collecting polarity switching data; single injection polarity switching and the two injection discreet polarity analysis (one injection in positive and another in negative ion mode). The one injection approach is where the positive and negative ion survey experiments are looped in throughout the run with or with out dependent IDA. With the two injection approach, the positive and negative ion experiments are in two separate runs. This approach however takes twice as long to obtain complete data and there is a chance of LC retention time shift between samples complicating data analysis. Furthermore, greater sample amounts are required for a two injection workflow.

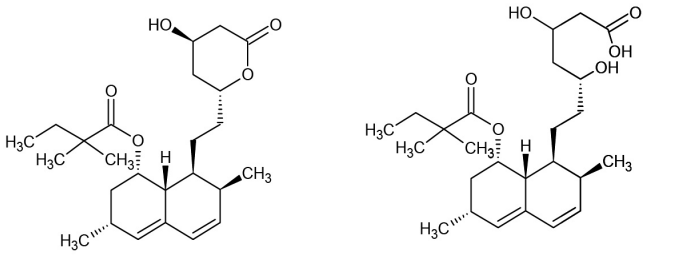

Figure 1. Structure of simvastatin and simvastatin hydroxyl acid

In this work, metabolites of Simvastatin were detected and confirmed in an in vitro incubation; using a single injection polarity switching IDA methodology

Experimental Conditions

Simvastatin was incubated in rabbit microsomes at a concentration of 10 µM under oxidative conditions using an NADPH regenerating system. The incubation was quenched with acetonitrile at time zero and 60 minutes and then centrifuged to pellet the protein. The supernatant was then diluted 3 fold with water. A QTRAP® 5500 LC/MS/MS system was used for acquisition and LightSight® software for metabolite identification, version 2.1, was used for data interpretation.

A 400 µL/min gradient (5% A – 85% A in 12.5 minutes, 85% - 95% A in 1 minute, 95% A isocratic for 1.5 minutes, where A is 0.1% formic acid in acetonitrile and B is 0.1% formic acid in

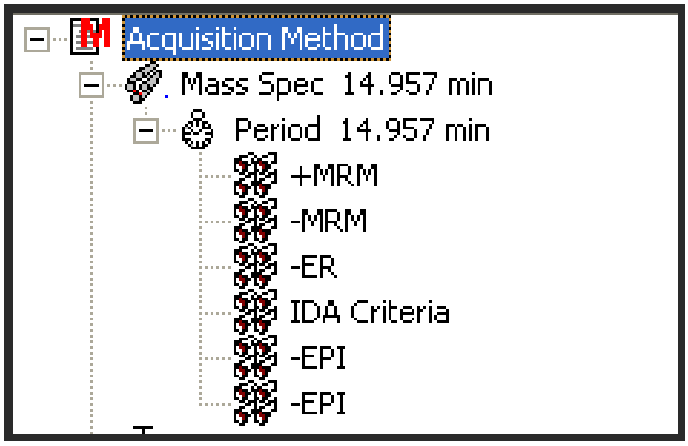

Figure 2. The polarity switching IDA method used to detect metabolites of simvastatin

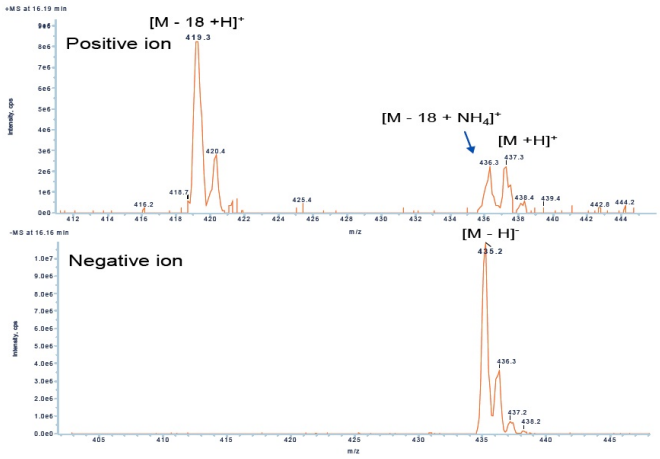

Figure 3. The detection of Simvastatin carboxylic acid in both positive and negative ion; the compound is better detected using negative ion.

water) was used with a Phenomenex Luna C18(2) 3µm, 50 x 2 mm HPLC column.

Polarity switching data collection can be achieved using various survey scans, such as EMS (Trap single MS) or Quadrupole MS/MS modes such as precursor ion, neutral loss or MRM. For this study, a pMRM method was used. With prior knowledge of the parent drug and its fragmentation pathway, theoretical MRM transitions for metabolites can be determined based on a particular biotransformation list). For this experiment, two pMRM methods (one positive and one negative ion) were combined into a single experiment. Each of the pMRM surveys contained a total of 48 MRM transitions which covered the range of phase I metabolites. MS/MS data was collected automatically in negative ion via Information Dependent Acquisition (IDA) at a scan rate of 10,000 Da/sec. The structure of the method is shown in Figure 2. The overall duty cycle time for this method was approximately 2.6 seconds.

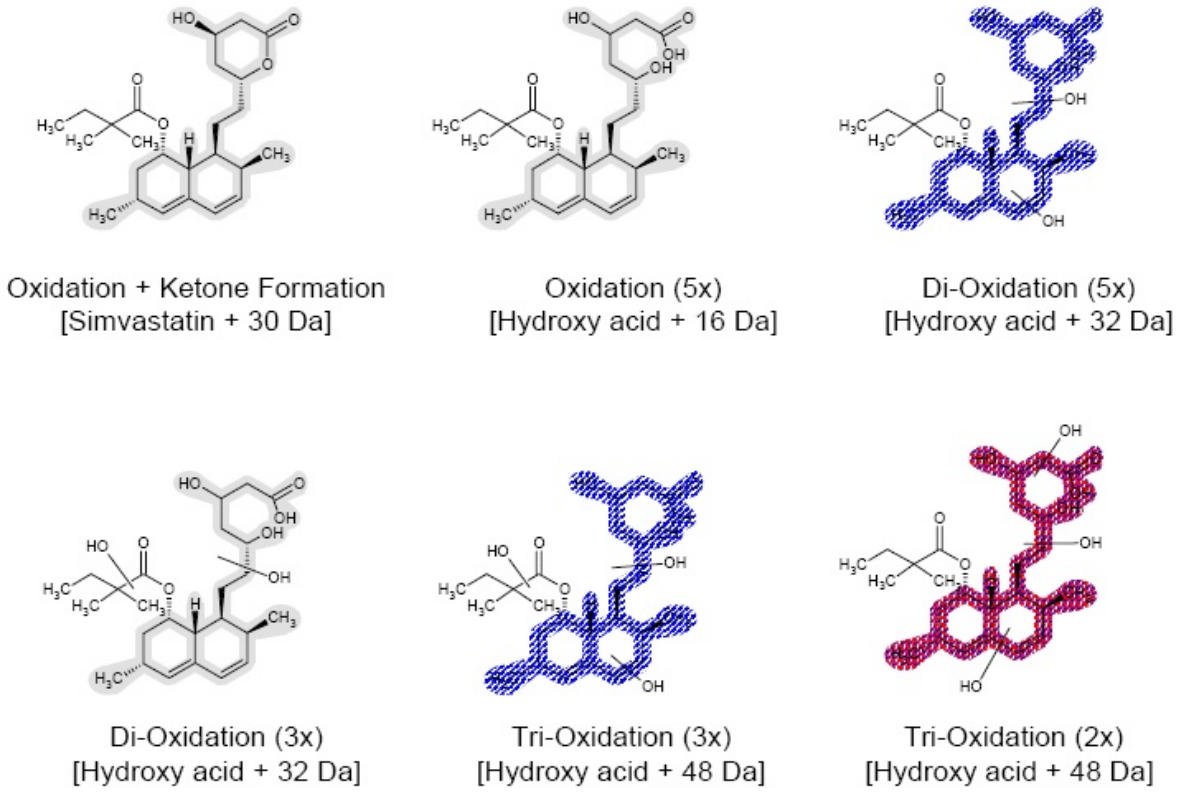

Figure 4. Metabolites of simvastatin and simvastatin hydroxy acid identified in a rabbit microsomal incubation

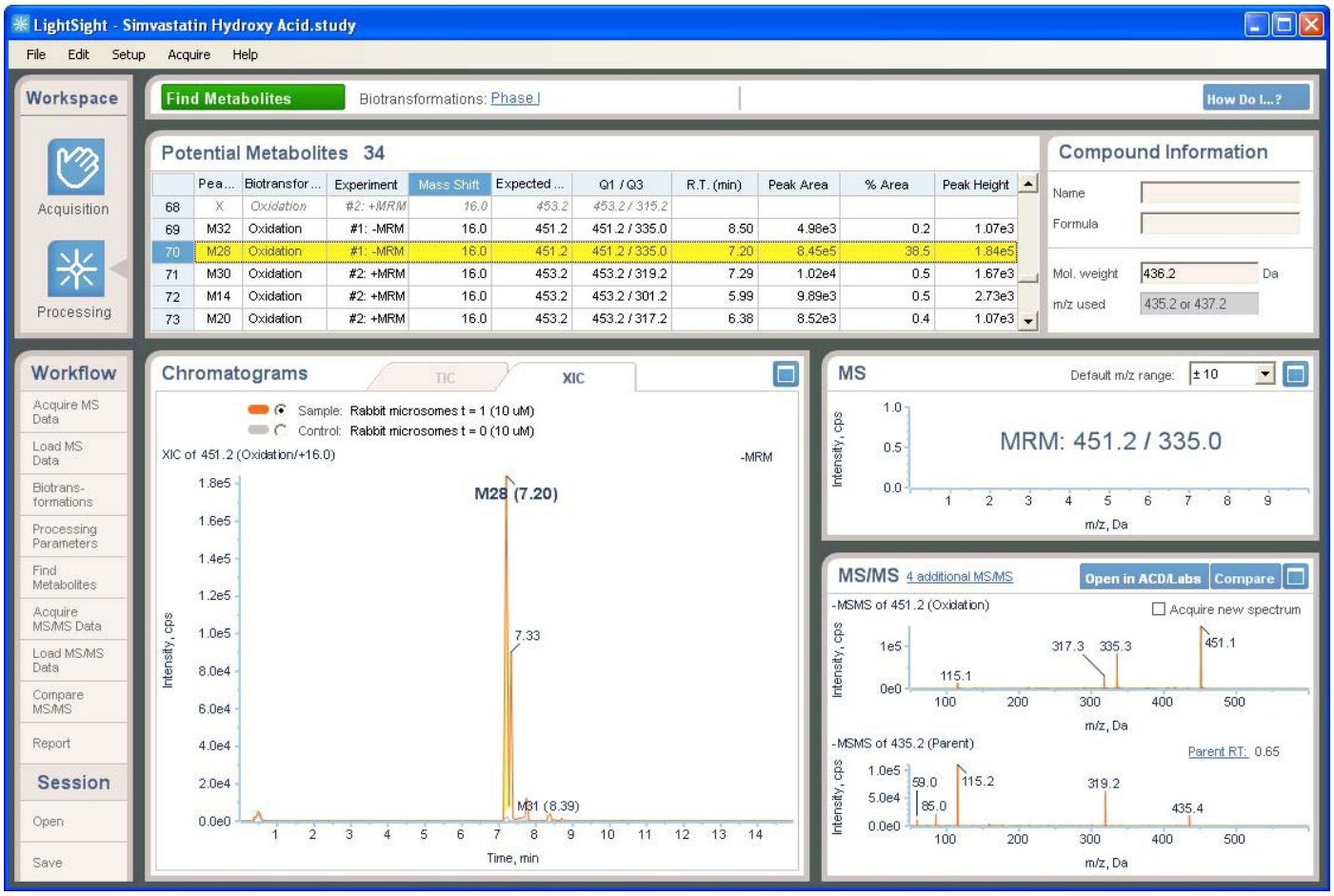

Figure 5. An oxidative metabolite of simvastatin hydroxy acid found using polarity switching data collection. The LightSight® software allows this data to be processed in the same session.

Results and Discussion

Simvastatin is a lactone prodrug and is hydrolyzed to the active compound (simvastatin hydroxy acid), (Figure 1). Both simvastatin and the hydroxyl acid ionize in positive ion. However, if the MS/MS acquisition method was only in positive ion, some metabolites could be missed because the carboxylic acid is ionizes predominately in negative mode (see Figure 3).

Since both simvastatin and the carboxylic acid can be metabolized, it is important to monitor both polarities. Previously, it was not possible to collect data in both polarities as well as confirmatory information in a single injection due to IDA cycle time limitations. However, with the improvement in polarity switching capabilities (50 ms) of the QTRAP® 5500 System, combined with the fast trap and quadrupole scanning of the new system; many single and dual survey pos/neg IDA workflows are now possible.

Proposed structures of some of the metabolites detected are given in Figure 4; some were metabolites of simvastatin, while the majority of those metabolites detected were modifications to the hydroxy acid. Using LightSight® software, a total of 34 metabolites were detected in the microsomal incubation using automated data processing. The metabolites detected in both polarities were correlated in one session (Figure 5), making reporting of results easier. MS/MS data was collected in both positive ion and negative ion; where the data in the negative polarity was collected automatically through IDA and the data in the positive polarity was collected through multi-experiment, period based MS/MS methods.

Of the 34 metabolites, 5 metabolites were only detected in positive ion, 28 metabolites were detected in negative ion and 1 metabolite (Mx) was detected in both polarities. An advantage of detecting metabolites in both polarities is that MS/MS data can be collected in both polarities which provides more information for structural elucidation. The MS/MS spectra of Mx are depicted in Figure 6. If the MS/MS spectra was only collected in negative ion, (top spectrum in Figure 6) the site of oxidation could only be localized to half of the molecule. However, with the positive ion

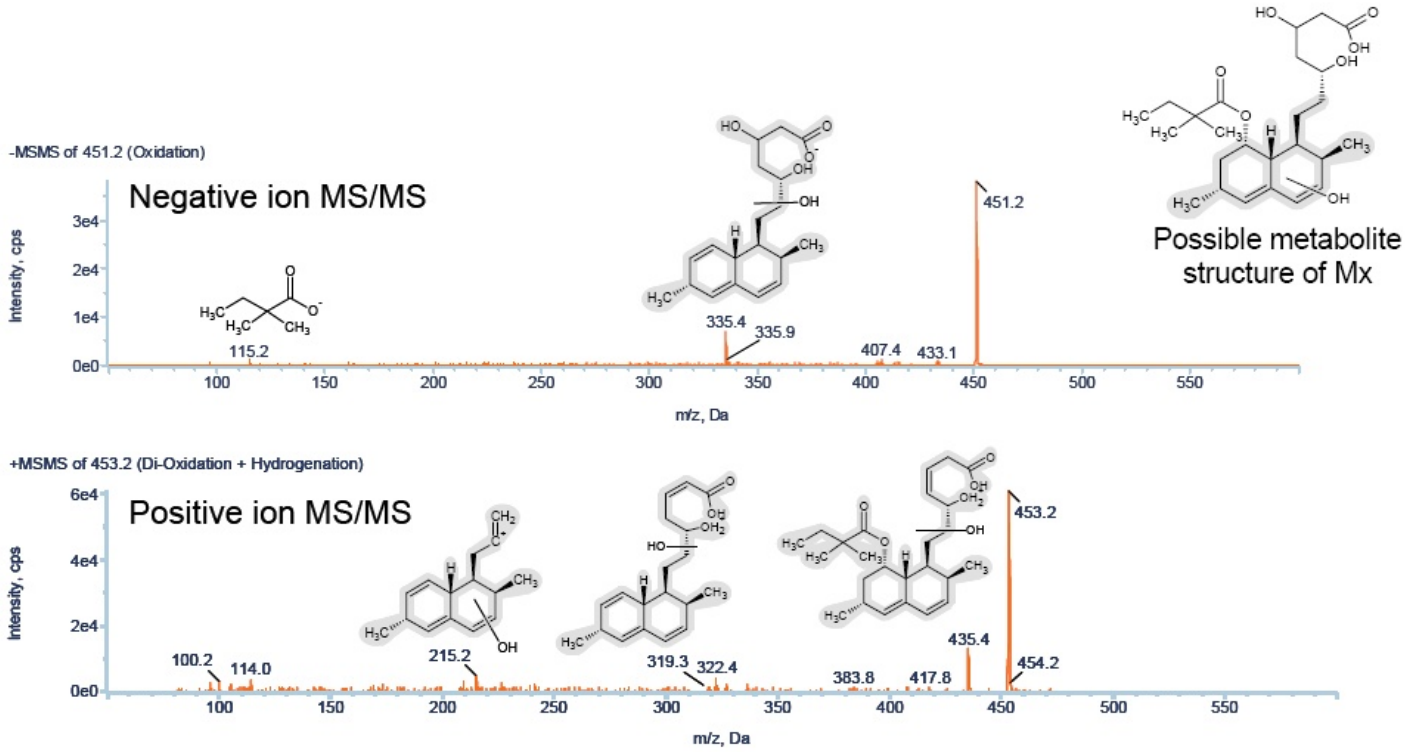

Figure 6. The MS/MS spectra of Mx (oxidation of simvastatin hydroxyl acid) in positive and negative ion. The site of oxidation could be pinpointed with better when using both spectra.

MS/MS spectrum, the site of oxidation could be further pinpointed to the core ring structure.

Summary

Single injection polarity switching IDA experiments on the QTRAP® 5500 System provide improved metabolite detection compared to two injection single polarity experiments. There are four key factors which support this conclusion. One, a single injection workflow requires less sample, thus allowing for a large injection volume and improved sensitivity for a single analysis. Two, data reduction is made easier, since only one LCMS experiment is required to be processed. Three, combined positive and negative ESI metabolite profiles, allow for complete confirmation of varied species in a single analysis. Four, the QTRAP 5500® System's fast scanning attributes in Quadrupole and LIT along with fast polarity switching capabilities allow for higher throughput analyses by incorporating faster LC strategies.

Highlights

- Improved quadrupole sensitivity (MRM response & S:N, PI & NL)
- Sensitivity improvement in TRAP modes (EPI, 10-100 fold)
- 4 times faster QqQ scanning
- Faster Polarity switching (50 ms)
- 5 times faster LIT scanning (20,000 amu/sec)
- Shorter fill time capabilities (50 µs)
- Unique workflows ideal for metabolite identification

For Research Use Only. Not for use in diagnostic procedures.

Publication number: P1034410-01

Headquarters
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

International Locations
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices