

# Using MSM for intelligent Metabolite Screening and Structural Elucidation with an LTQ Orbitrap

Yingying Huang<sup>1</sup>, Ji Ma<sup>2</sup>, Jae Schwartz<sup>1</sup>, Robert Cho<sup>2</sup>, Yan Chen<sup>1</sup>, Tim Carlson<sup>2</sup>

<sup>1</sup>Thermo Fisher Scientific, San Jose, CA, USA

<sup>2</sup>Amgen, 1120 Veterans Boulevard, South San Francisco, CA, USA

## Overview

**Purpose:** To design a generic metabolite identification workflow suitable for high throughput data acquisition that: 1. requires no prior knowledge of test compounds; 2. accomplishes detection and structural elucidation of metabolites in a single injection; 3. detects both expected and unexpected metabolites.

**Methods:** *In vitro* and *in vivo* samples of verapamil and haloperidol were analyzed using the MS<sup>M</sup> workflow on a modified LTQ Orbitrap XL<sup>TM</sup> with HCD collision cell.

**Results:** MS<sup>M</sup> enables one-run approach for *in vitro* metabolite identification; and two-run approach for definitive *in vivo* metabolite profiling with great dynamic range, high sensitivity, minimal false positives and negatives.

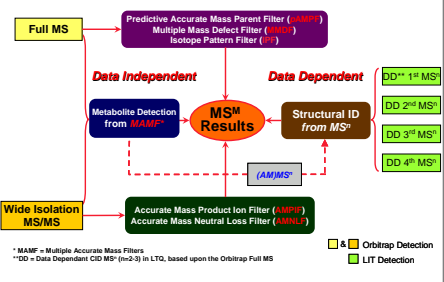
## Introduction

An integral part of drug discovery and development is the identification of drug metabolites that indicate intrinsic pharmacokinetic mechanism, pharmacological activity or specific toxicity. Depending on the stage of the drug discovery and development, two typical environments to perform metabolite identification experiments by LC-MS are discovery metabolite screening and definitive biotransformation characterization. While the focus and the data requirements for each are different, general challenges remain the detection and identification of metabolites in the presence of highly complex biological matrices. Demands are high to increase throughput, sensitivity and accuracy while minimizing human intervention. Presented here is MS<sup>M</sup>, an approach utilizing multiple collision cells, dissociation methods, scan modes, mass analyzers and detectors to perform intelligent metabolite identification experiments.

## Methods

A modified LTQ Orbitrap XL with a Higher-energy Collisional Dissociation (HCD) collision cell was used. An isolation mass window up to 600 amu for HCD scans was enabled. The experiment was designed such that a high resolution full scan was acquired followed by a high resolution HCD MS/MS of all incoming ions within that 600 amu window (Wide Isolation MS/MS). In parallel, the linear ion trap (LIT) acquired data dependent MS<sup>n</sup> spectra. Thermo Scientific Accela UPLC and Hypersil GOLD<sup>TM</sup> C18 column (1x100mm, 1.9µm) with a 20-minute gradient was used. 10µM and 1µM of haloperidol in incubation samples from verapamil and haloperidol were analyzed. The performance of MS<sup>M</sup> was also further evaluated using *in vivo* 1mg/kg (by IV) rat urine, plasma and bile samples.

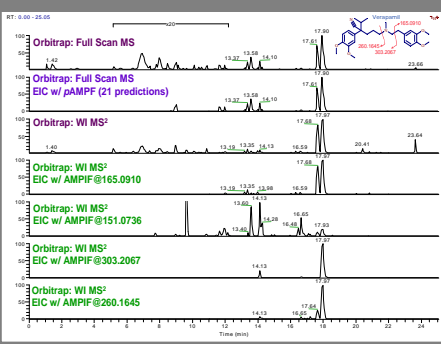
FIGURE 1. Multiple data sources in MS<sup>M</sup> and the corresponding data analysis methods.



## Results

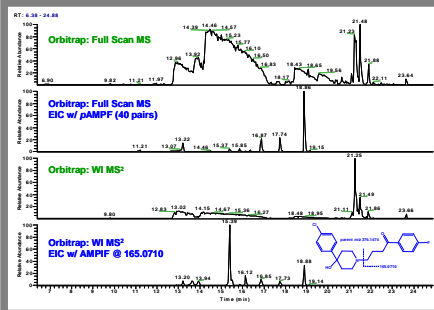
Results showed that the HCD cell can excite a large number of precursors simultaneously. Fragmentation information of all incoming ions within the 600 amu window was recorded in the HCD spectra. By comparing the data from HCD with full scan, analytical approaches comparable to neutral loss scanning, precursor ion scanning, and multiple reaction monitoring experiments were achieved through data mining using accurate mass and high resolution information. Once potential metabolites were identified using such comparisons, the parallel data dependent CID MS<sup>n</sup> data allowed unambiguous structure interpretation.

FIGURE 2. Base peak chromatograms from the analysis of 10mM verapamil rat hepatocytes incubation for Orbitrap full MS and wide isolation (WI) MS/MS, and the extracted ion chromatogram (EIC) of the predicted metabolites from the full MS, as well as the EICs of the signature fragments from the WI MS/MS.



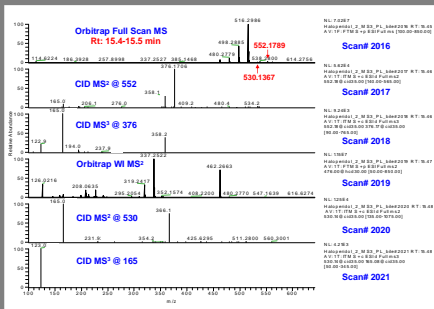
Using *m/z* 165.0910, a signature fragment of verapamil, accurate mass precursor ion analysis of high resolution full scan and wide isolation MS<sup>2</sup> of 10mM rat hepatocytes sample led to identification of more than 16 putative metabolites (Figure 2). The results are similar to those from precursor ion scanning experiments using a triple stage quadrupole mass spectrometer. Similar analysis for the fragment ion at 151.0753 resulted in the discovery of 5 additional putative metabolites. Definitive structural elucidation of these putative metabolites was achieved by analysis of the data dependent LIT CID MS<sup>n</sup> or MS<sup>n</sup> data that were collected in parallel (data not shown). Analysis of 1µM rat hepatocytes sample using the same approach also led to the identification of all Phase I and II metabolites (data not shown).

FIGURE 3. Base peak chromatograms from the analysis of Haloperidol rat bile (IV@1mg/kg) sample.



In a similar fashion, metabolites of haloperidol were observed in urine/plasma/bile samples using accurate mass precursor ion analysis (Figure 3). For *in vivo* sample analysis, an inclusion list of the accurate masses of the predicted metabolites was used to trigger data dependent MS<sup>n</sup> even when the intensity of the precursor ion was 2-3 orders of magnitude less than the background ions (Figure 4). In the case when the CID MS<sup>n</sup> scan was not triggered automatically on some unpredicted metabolites due to matrix interference, a second run using Orbitrap full scan plus LIT (or Orbitrap MS<sup>n</sup>) with an accurate mass inclusion list derived from the previous MS<sup>M</sup> experiment was performed, as indicated by the dotted line with the "(AM)MS<sup>n</sup>" label in Figure 1.

FIGURE 4. The spectra acquired in one MS<sup>M</sup> scan cycle from the analysis of haloperidol rat bile sample. MS<sup>2</sup> and MS<sup>3</sup> spectra of metabolites (552 and 530) were acquired regardless of their minimum intensity comparing to the background bile salts.



## Conclusions

- MS<sup>M</sup> is a generic workflow that does not require prior knowledge of test compounds. This enables continuous operation suitable for high throughput data acquisition.
- The comparison between the full MS and the wide isolation MS/MS enables analysis similar to triples-only experiments (precursor ion and neutral loss scanning but with high resolution and accurate mass) suitable for unexpected metabolite screening.
- The combination of LTQ and Orbitrap enables detection and structural elucidation of metabolites in a single injection.
- Great dynamic range, high sensitivity, minimal false positives and negatives were demonstrated on *in vivo* metabolite profiling.

## References

- J. Ma, Y. Huang, J. Schwartz, R. Cho, Y. Chen, T. Carlson, Thermo Scientific ASMS Users Meeting, Denver, CO, 2008

All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. The information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.