

Intelligent Workflows (MS^M) for Metabolite Screening and Characterization on a Hybrid Dual-pressure Linear Trap and Orbitrap Mass Spectrometer

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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.

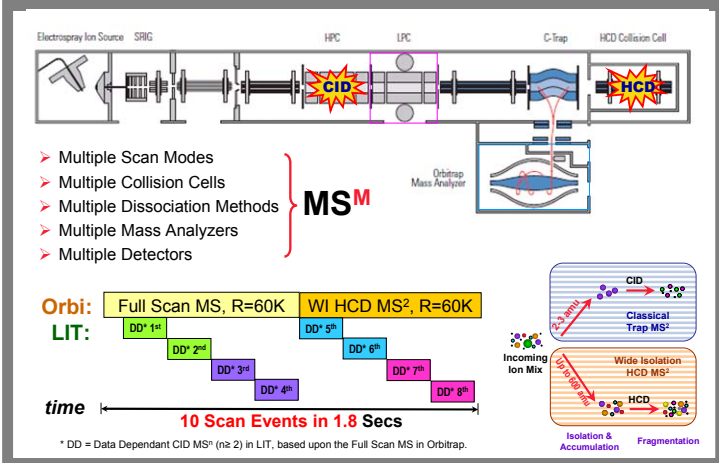
Methods: *In vitro* and *in vivo* samples of verapamil and haloperidol were analyzed using the MS^M workflow on an LTQ Orbitrap Velos™ (hybrid dual-pressure linear trap and Orbitrap). The speed and sensitivity resulting from the dual-pressure design enables detection and structural elucidation of metabolites from both *in vitro* and *in vivo* samples in a single injection.

Results: MS^M enables one-run approach for *in vitro* metabolite screening and *in vivo* metabolite profiling with great dynamic range, high sensitivity, minimal false positives and negatives under a generic method template. Comprehensive fragmentation information from both HCD and CID is available for unambiguous structural elucidation.

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 complex biological matrices. Demands are high to increase throughput, sensitivity and accuracy, while minimizing human intervention. Presented here is MS^M, an approach utilizing multiple collision cells, dissociation methods, scan modes, mass analyzers and detectors to perform intelligent metabolite identification experiments.

FIGURE 1. Schematics of the Thermo Scientific LTQ Orbitrap Velos and the MS^M experiment design. The multiple collision cells and mass analyzers are highlighted.



Methods

An LTQ Orbitrap Velos (Thermo Fisher Scientific, San Jose, CA, USA) with an HCD (Higher-energy Collisional Dissociation) collision cell was used. The schematic diagram of the instrument is shown on the top of Figure 1. An isolation mass window up to 600 amu for HCD scans was used. The experiment was designed (bottom of Figure 1) such that a high resolution full scan was acquired followed by a high resolution HCD MS/MS of all incoming ions within that 600amu window (Wide Isolation MS/MS). In parallel, the dual-pressure linear ion trap (LIT) acquired data dependent MSⁿ spectra. An Accela™ UHPLC and Hypersil Gold™ column (1x100mm, 1.9µm) with a 20-minute gradient was used. The performance of MS^M was evaluated using *in vivo* (1mg/kg by IV) samples of verapamil in rat urine, plasma and bile.

Results

The MS^M workflow has been described and demonstrated on an LTQ Orbitrap XL previously.¹ Results showed that the HCD cell can excite a large number of precursors simultaneously.

FIGURE 2. (a) Major fragmentation pathway of Verapamil and base peak chromatograms from the analysis of 10µM Verapamil rat hepatocytes incubation for (b) Orbitrap full MS and (c) the extracted ion chromatogram (EIC) of the predicted metabolites from the full MS, (d) wide isolation (WI) MS/MS, as well as the (e)-(h) EICs of the signature fragments from the WI MS/MS.

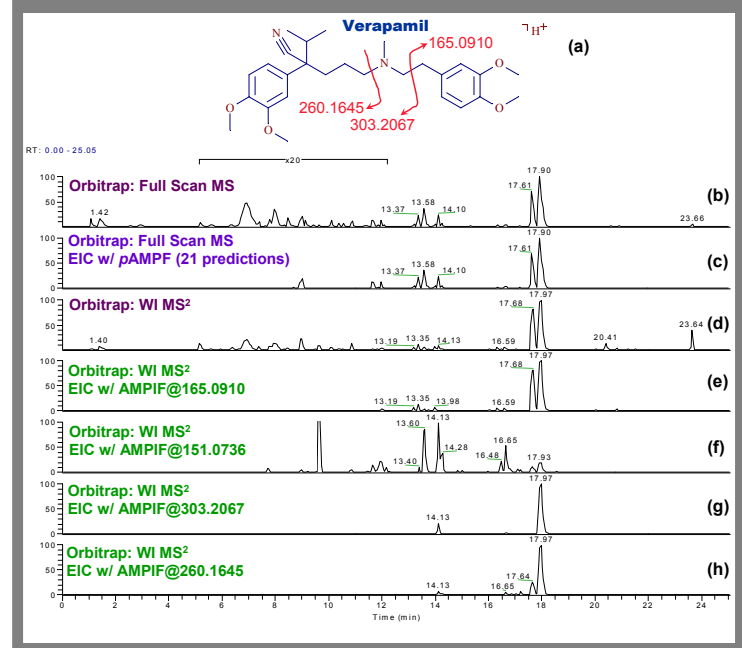
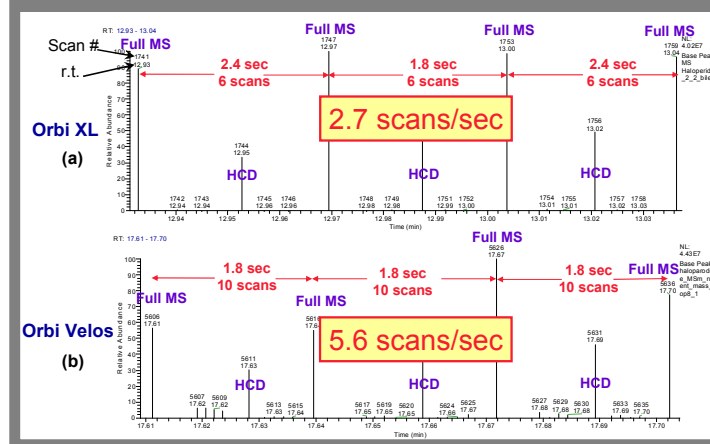


FIGURE 3. a) Scan speed for MS^M experiment in the LTQ Orbitrap XL. Each scan cycle has 2 Orbitrap scans and 4 LIT scans, and is completed in 1.8-2.4 seconds. b) Scan speed for MS^M experiment in the LTQ Orbitrap Velos. Each scan cycle has 2 Orbitrap scans and 8 LIT scans, and is completed in 1.8 seconds.



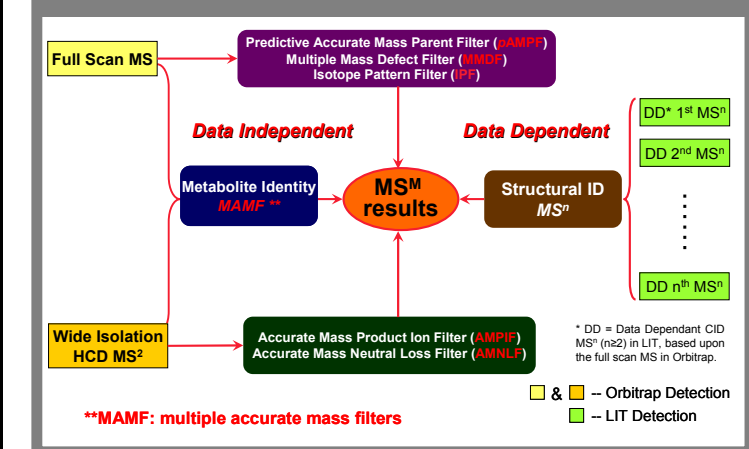
Fragmentation information of all incoming ions within the 600 amu window was recorded in the HCD spectra. By comparing the HCD MS/MS with the full MS, it is possible to mimic conventional neutral loss scanning, precursor ion scanning, and multiple reaction monitoring experiments. This was achieved by data mining from datasets with high resolution and accurate mass. Once potential metabolites were identified using such comparisons, the parallel data dependent CID MSⁿ data allowed unambiguous structure interpretation.

Using *m/z* 165.0910, a signature fragment of verapamil, accurate mass precursor ion analysis of high resolution full scan and wide isolation MS² of 10µM 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 quadrupole mass spectrometer. Similar analysis for the fragment ion at *m/z* 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 MS² or MS³ 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).

When comparing the results between the LTQ Orbitrap XL and the LTQ Orbitrap Velos, the LTQ Orbitrap Velos showed scan speeds twice as fast as the LTQ Orbitrap XL (Figure 3) due to its improved ion sources, improved ion optics, as well as the dual-pressure linear ion trap design. Within 1.8 seconds, two Orbitrap scans with resolution at 60K plus 8 MSⁿ scans in the LIT are completed in the LTQ Orbitrap Velos. This enables detection of metabolites under highly complex biological matrices.

For *in vivo* sample analysis, metabolites of Haloperidol were observed in urine / plasma / bile in a similar fashion using accurate mass precursor ion analysis as in the *in vitro* sample analysis, although matrix ions were much more prominent and complex in these samples. This demonstrates the ultra high sensitivity and excellent dynamic range of the Orbitrap mass analyzer. All metabolites were detected with less than 3ppm mass accuracy. In addition, because of its speed improvement, the LTQ Orbitrap Velos was able to trigger data dependant MSⁿ even when the intensity of the precursor ion was 2-3 orders of magnitude less than the background ions. Fragmentation information from the LIT data dependant MS² and MS³ scans were combined with Orbitrap HCD scans for structural elucidation. Figure 4 summarizes the multiple data sources in the MS^M experiment and the corresponding data analysis methods.

FIGURE 4. Multiple data sources in MS^M and the corresponding data analysis methods.



Conclusions

- MS^M is a generic workflow that does not require prior knowledge of test compounds. This enables continuous operation suitable for high-throughput data acquisition
- The LTQ Orbitrap Velos (a combination dual-pressure linear trap and Orbitrap technology) enables detection and structural elucidation of metabolites from both *in vitro* and *in vivo* samples in a single injection
- 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.
- Great dynamic range, high sensitivity, minimal false positives and negatives were demonstrated on *in vitro* rat hepatocytes at 1µM and *in vivo* rat urine/plasma/bile samples.

References

1. Y. Huang *et al.* Proceedings 57th ASMS Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, May 31 – June 4, 2009

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