



## Using Mass-MetaSite and WebMetabase to Process HDMS<sup>E</sup> Data Acquired on the Vion IMS QToF Mass Spectrometer

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This Application brief demonstrates a new Application Programming Interface (API) released in UNIFI 1.9.2 enables third party applications to read and process data acquired on instruments using the UNIFI platform. We describe the integration of Mass-MetaSite and WebMetabase with UNIFI in order to identify drug metabolites in hepatocyte incubations.

### Benefits

Third party applications have the potential to add significant value to the native capabilities of the UNIFI Scientific Information System. Here, we describe the integration of Mass-MetaSite and WebMetabase to process drug metabolism data in a drug discovery environment for discovery applications.

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## Introduction

Mass-MetaSite and WebMetabase (Lead Molecular Design, Barcelona, Spain) represent a suite of applications targeted at addressing a fundamental limiting issue in drug discovery – designing out metabolic liabilities in order to bring forward compounds into development that are likely to have acceptable pharmacokinetics in humans. High resolution, ion mobility enhanced mass spectrometry, combined with UPLC and UNIFI, excels at generating datasets, which can be used to identify drug metabolites. Mass-MetaSite and WebMetabase complement this by providing additional functionality including:

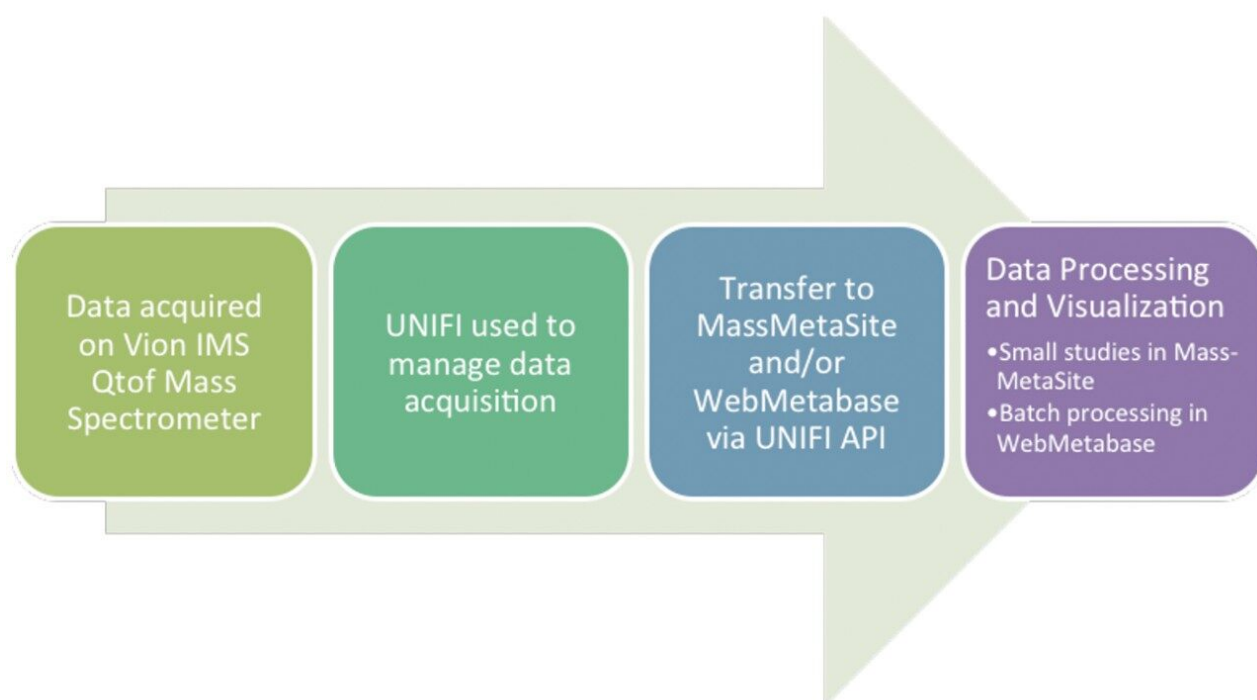
- Batch processing of datasets for a larger number of compounds
- Calculating formal metabolic clearance outcomes
- Modeling the most likely site of oxidation based on CYP450 homology and identifying the structural determinants of interactions with key CYP450 isoforms
- Suggesting isosteric replacements for metabolically labile moieties

Waters and Lead Molecular Design developed a seamless workflow in which UPLC/HDMS<sup>E</sup> data are acquired in UNIFI, then transferred, processed, and visualized within Mass-MetaSite and/or WebMetabase. The combination of Vion IMS QToF Mass Spectrometer, UNIFI, and High Definition MS<sup>E</sup> acquisition schema affords a user-friendly, robust, generic, and selective approach to the characterization of complex samples.

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## Results and Discussion

Verapamil was incubated in a rat hepatocyte suspension at a 10  $\mu$ M substrate concentration and aliquots taken at defined time points, quenched with acetonitrile (2 volumes acetonitrile:1 volume of incubation), centrifuged, and the supernatant transferred to a 96 well plate for subsequent analysis. Samples were further diluted 1 in 10 with urine. HDMS<sup>E</sup> datasets were acquired on a Vion IMS QToF Mass Spectrometer equipped with an ACQUITY UPLC I-Class System, running under UNIFI control. The general workflow for the solution is shown in Figure 1. Acquired datasets were transferred onto a processing workstation running UNIFI 1.9.4 and UNIFI API 1.0, though it is also possible for data to be processed directly from the acquisition computer. Secure access by Mass-MetaSite and WebMetabase to the UNIFI system was achieved by password-based authentication. Subsequent selection of samples for downstream processing within Mass-MetaSite was achieved by navigation within the UNIFI folder structure (Figure 2).



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*Figure 1. Workflow used for data acquisition and processing.*

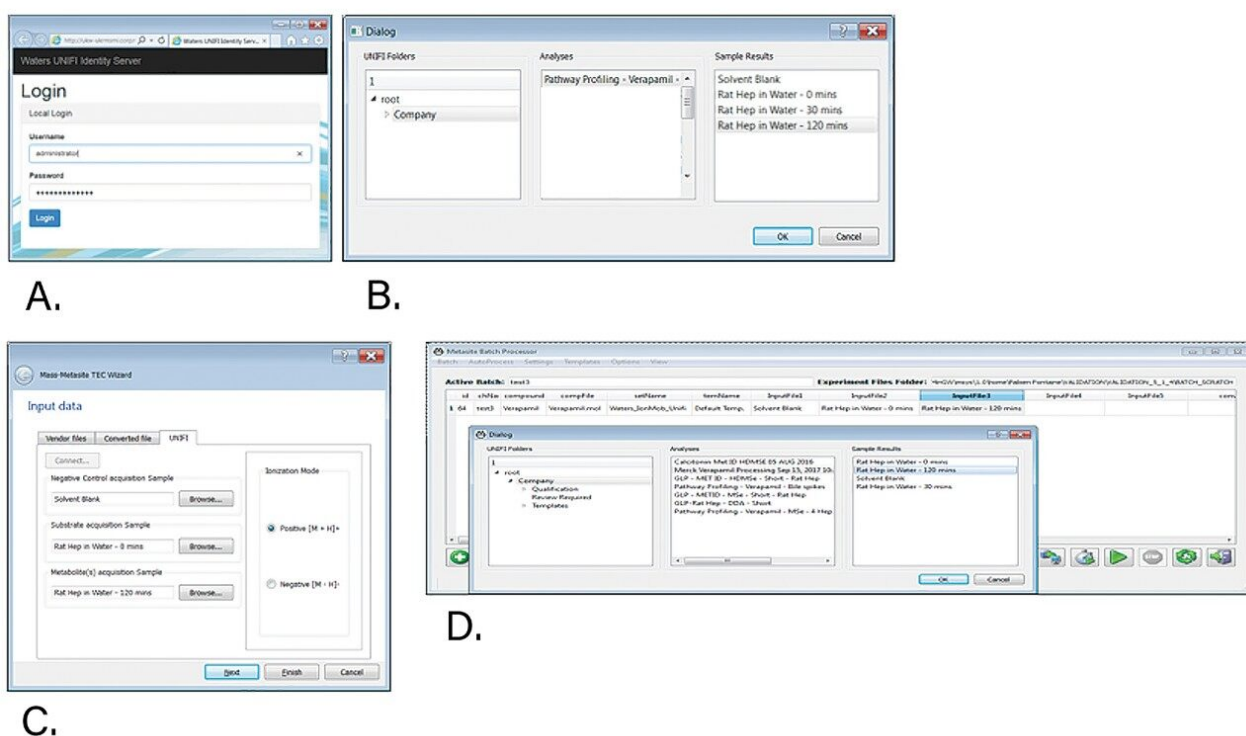


Figure 2. A. Authentication of access to UNIFI. B. navigation of the UNIFI folder structure within Mass-MetaSite allows C. selection of datasets for processing in Mass-MetaSite and/or D. population of batch processing in WebMetabase.

Two modes of data processing are possible, either single sample processing with comparison to a defined control within Mass-MetaSite or batch processing of multiple compounds with multiple conditions (species, time, matrix) within WebMetabase (Figure 2). Data review can be achieved in a standalone mode within the application, or as a cloud-enabled solution within a standard web browser.

Representative outcomes within Mass-MetaSite for the 30 minute time point of the verapamil incubation are shown in Figure 3. Within UNIFI, metabolites are characterized not only on the basis of  $m/z$  and retention time, but with their collision cross section (CCS), allowing isobaric metabolites to be distinguished across experiments. Like UNIFI, Mass-MetaSite generates product ion spectra for identified metabolites by utilizing drift time correlation, resulting in clean spectra comparable in quality to those obtained with targeted MS/MS. A formal validation of the outcomes from data processed within both Mass-MetaSite and UNIFI confirmed that  $m/z$  and CCS values were consistent.

For CCS values, there was an average difference between UNIFI and Mass-MetaSite of 0.04%. For  $m/z$  values, there was an average difference between UNIFI and Mass-MetaSite of 0.05 ppm.

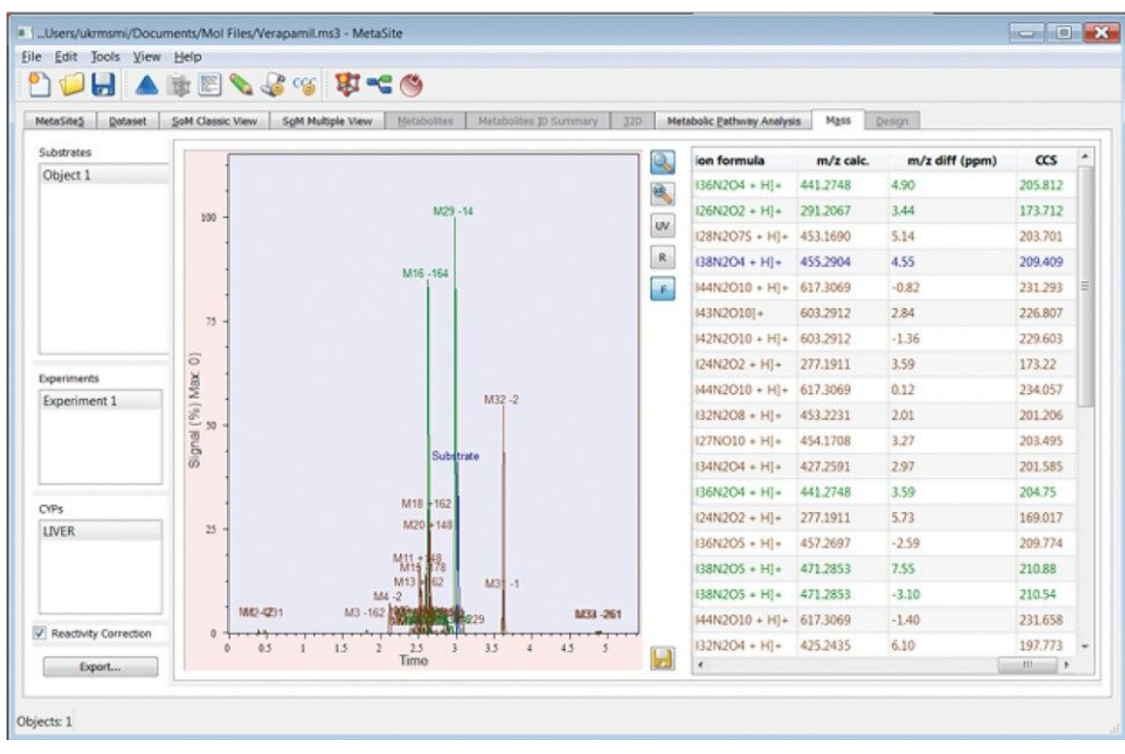
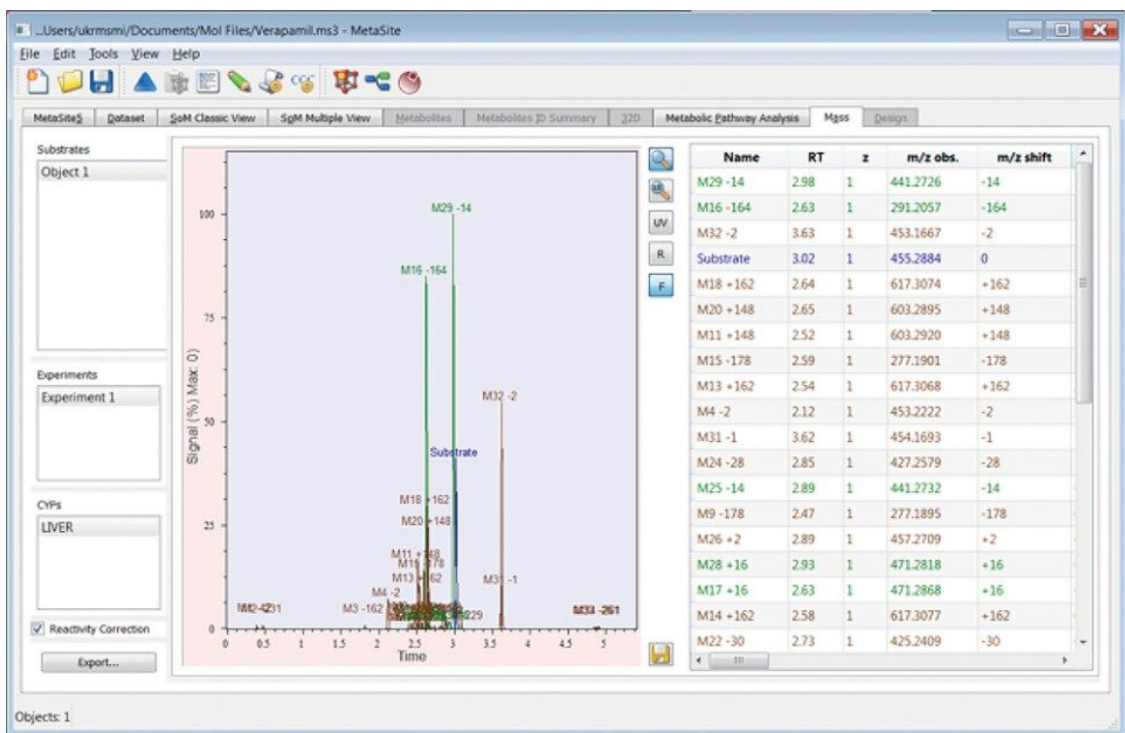


Figure 3. Metabolic profile for the 30 min incubation of verapamil in rat hepatocytes showing two different views of the data table with columns for RT, m/z, mass change, mass accuracy, and CCS data, along with other relevant outcomes.

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## Conclusion

The UNIFI API provides a platform by which third party applications can take advantage of the exceptional data quality afforded by UPLC-HR-IMS-MS, enabling a rich ecosystem of software to thrive. The capabilities of Mass- MetaSite and WebMetabase are complementary to those offered by UNIFI, and afford access to a comprehensive toolset which can be used to understand the metabolism and optimize the pharmacokinetics of lead compounds within a drug discovery environment.

### Glossary:

CCS (Collision Cross Section): Value describing an ion's structure, charge, and conformation, determined by the interaction of an individual ion with a neutral gas in an ion mobility drift cell. CCS is directly related to drift time.

IMS (Ion Mobility Spectrometry): A separation technique in which ions are separated based on their size, shape, and/or charge as they pass through a neutral gas. IMS is frequently coupled with mass spectrometry as IMS-MS.

HDMS<sup>E</sup> (High Definition MS<sup>E</sup>): Data-independent acquisition method combining ion mobility separation and MS<sup>E</sup> data acquisition. Low collision energy IMS-MS scans and high collision energy IMS-MS scans are alternated, allowing both precursor and product ion accurate mass measurements to be obtained.

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ACQUITY UPLC I-Class PLUS System <<https://www.waters.com/134613317>>

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