Metabolite Identification in Complex Matrices by Structurally Intelligent Acquisition and Processing

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Overview

Purpose: Demonstrate utility of a combination of structurally intelligent acquisition and data processing to detect and identify metabolites in human urine samples.

Methods: Samples of human urine were acquired after 14 days of oral dosing with omeprazole (22mg/day PO). Urine samples were collected from 0-3. 3-5, and 5-7 hours and prepared for analysis by SPE desalting / concentration. Acquisition was by HRAM LC-MS/MS on a benchtop Orbitrap mass spectrometer.

Results: A total of 27 metabolites were identified in human urine samples with intelligent acquisition of fragmentation data for all detected metabolites.

Introduction

The determination of the metabolic profile of new chemical entities in pharmaceutical development is a critical step in approval. The need to understand the major routes of elimination, potential for drug-drug interaction, presence of pharmacologically active or biologically reactive metabolites, and the need to confirm coverage of toxicology studies all drive metabolite identification. *In vitro* studies are often the first option for identification studies however the inability of such systems to completely model *in vivo* metabolism is understood. As such, *in vivo* metabolite identification on samples from pharmacokinetic, toxicological, and mass balance studies is common. The complexity of such sample matrices as plasma, urine, feces, and bile makes such studies difficult. In this study we have analyzed the metabolism of omeprazole in human urine samples using a structurally intelligent acquisition and processing technique to overcome the complexity of the matrix.

Methods

Sample Preparation

Urine samples collected from 0-3, 3-5, and 5-7 hours post dose on the last day of dosing were acquired and prepared by solid phase extraction (Hypersep C18, 50mg, Thermo Fisher Scientific, San Jose CA)

Liquid Chromatography

Samples were analyzed on a system consisting of an Open Accela™ autosampler and an Accela 1250 UHPLC pump with separation achieved on a 150 X 2.1, 1.9u Hypersil™ Gold column (Thermo Fisher Scientific, San Jose CA). Mobile phase A consisted of Water with 0.1% formic acid and mobile phase B consisted of ACN with 0.1% formic acid.

Table 1. LC Method

Time (min)	% A	% B	Flow (µL/min)
0.0	98	2	400
1.0	98	2	400
5.0	60	40	400
8.0	10	90	400
8.5	10	90	400
9.0	98	2	400
15.0	98	2	400

Mass Spectrometry

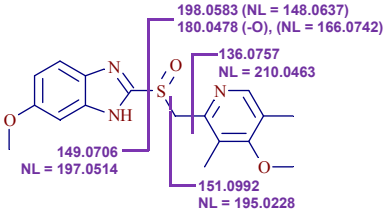
Qualitative analyses was performed on Q Exactive™ benchtop Orbitrap™ mass spectrometer connected to the LC system described above. The mass spectrometer was operated in positive ionization mode with a HESI-II probe (Sheath Gas: 40, Aux Gas: 15, 450 °C). Acquisition using structure based triggering of MS^2 fragmentation is discussed more fully in the Results section\

Results

Structure Based Acquisition – HRAM-NL

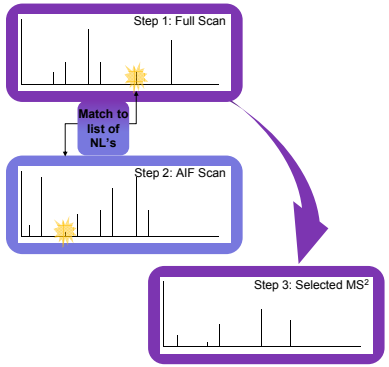
Neutral loss acquisition has been used for many years on triple quadrupoles as a sensitive, semi-selective method for detecting potentially related metabolites in samples. The limited number of neutral losses simultaneously detected and the limitations of nominal mass constrained the approach however. Using a combination of high resolution accurate mass and all ion fragmentation (AIF), the utility of neutral loss analysis becomes more powerful. All ion fragmentation scans on the instrument are performed by allowing a wide range of precursors into the HCD cell (high energy collisional dissociation cell). The full mass range of fragments generated from all precursors are then scanned. A list of observed neutral loss values from a standard injection of omeprazole (Figure 1) was created. This list was augmented using a list of known common neutral losses for phase II biotransformations (glucuronidation, sulfation, glutathione conjugation) to increase the acquisition power.

Figure 1. Fragments and Associated Neutral Loss Values used for Omeprazole.



The schematic in Figure 2 outlines the approach used. Briefly, a high resolution full scan was acquired (mz 150-900, 70,000 resolution) followed by an AIF scan (m/z 60-900, 35,000 resolution). All members of the provided neutral loss list are searched by comparing all combinations of observed full scan and AIF scan ions. Any match within 10 ppm triggers a precursor ion selected MS2 on the suspected parent ion. If no match to any provided neutral loss is observed, the next full scan AIF scan combination occurs. This pattern repeats throughout the entire LC run.

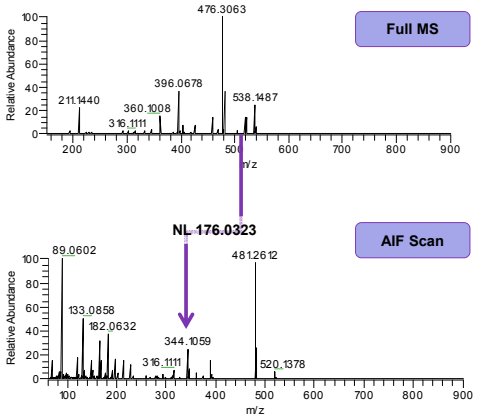
FIGURE 2. Schematic of Structurally Intelligent MS^2 Triggering.



Sensitivity of HRAM Neutral Loss Triggered MS^2 Acquisition

In the presence of the complex concentrated urine matrix minor metabolites observable in full scan were selectively triggered for MS2 analysis. A minor glucuronide of a dehydrogenation product observed at tR = 4.6 minutes was triggered due to a strong observed neutral loss of the glucuronide. Sensitive detection and fragmentation of metabolites down to 0.1% was achieved in all timecourse samples.

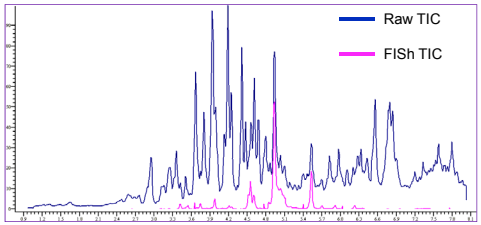
FIGURE 3. Detection of Glucuronide Metabolite



Data Processing – Fragment Based Analysis

The same fragmentation based instrument acquisition techniques were used to analyze the data. Fragment ion searching (FISh) uses the observed and theoretical fragments of omeprazole along with a list of expected biotransformations to search the data for fragments matching within 5 ppm. Fragments matched in this way create a fragment based total ion current that quickly visualized the regions with significant similar fragmentation and most likely metabolites. For this example we used a set of expected omeprazole fragments (8 total) and a small list of common phase I and phase II biotransformation (16 total) to perform the analysis. The results of the processing are shown in Figure 4 for only the raw data and the FISh trace. The trace of detected components from the FISh trace is shown in Figure 5.

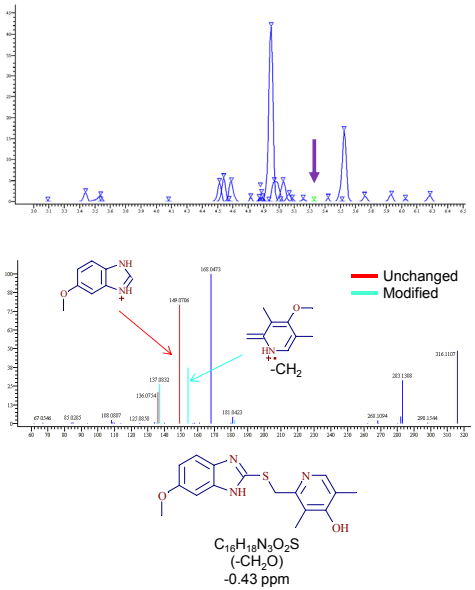
FIGURE 4. Fragment Ion Search (FISh) Results for 0-3 hour Urine



Detected Components and Structure Identification

The fragmentation data used to detect components also provided an immediate interpretation of the structure. As can be seen in Figure 5, the detected components were easily interrogated with the same fragmentation information used to detect, subsequently used to identify a demethylated sulfur reduction product. In addition, the fragment based approach does not require predictions of metabolism as is the case for mass defect based approaches where incorrectly setting a defect window can lead to false negatives.

FIGURE 5. Detected Components in Urine and Identification of Demethylation Sulfur Reduction Product



Conclusion

Chemically intelligent, structure based acquisition and processing of complex *in vivo* samples provided excellent detection of multiple omeprazole metabolites with interpretation aided by the fragmentation used to trigger acquisition.

- No need to predict metabolism *a priori* to set mass defect filters.
- Limited false positives through high resolution accurate mass and structure based detection.
- Fragment based searching of provides immediate structure interpretation information for detected metabolites.