Detection and Identification of Labeled Compounds From High Resolution Tandem Mass Spectrometry

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ABSTRACT

Purpose: Confident detection and identification of 14C containing metabolites with effective matrix background removal utilizing high resolution tandem mass spectrometry and Thermo Scientific™ Compound Discoverer™ software in one single workflow.

Methods: The parent compound in study was fully labeled with one 14C. Rat urine samples from 0 to 24hr collected post dose were pooled using the AUC pooling method. HRMS full scan followed by data dependent ms2 and ms3 data were collected on the Thermo Scientific™ LTQ Orbitrap Velos™ mass spectrometer. Data analysis was performed by Compound Discoverer software using one single processing workflow. The workflow employed nodes for expected compounds and unknown compounds detection and Pattern Scoring node to mark the compounds matching the specified [14]C pattern. Identification of these compounds were achieved by the FISh Scoring node.

Results: The Pattern Scoring node along with unknown compound detection in Compound Discoverer software effectively removed matrix background and extracted out the [14]C containing compounds. Comparison of the 14C containing compounds trace and radio trace for the same sample confirmed the detections. The FISh Scoring node in Compound Discoverer software provided structural information of the fragments for these compounds and helped elucidate the structures for these compounds.

INTRODUCTION

Radio-labeled compounds have been used extensively to trace the path of biochemical reactions in metabolism or biomarker studies. Although LC/HRMS techniques are commonly employed for these studies, labeled compound profiling in complex biological samples remains a challenge due to factors such as complex matrixes and insufficient resolution. This study demonstrates a simple yet powerful workflow for radio-labeled compound profiling using high resolution Orbitrap™-based mass spectrometer and Compound Discoverer software.

MATERIALS AND METHODS

Sample Preparation

Urine: For each individual subject, urine samples from 0 to 24hr collected post dose were pooled using the AUC pooling method. Pooled urine samples were added to one volume of acetonitrile containing 3% formic acid and centrifuged for 10 min at 1,460 x g with an Eppendorf benchtop centrifuge to remove particulates. Supernatants were evaporated under nitrogen and reconstituted into 3:1 water : methanol without further purification. Approximately 99% of total radioactivity in urine was recovered in extracts.

Analytical Method for LC-radiometric-MS analysis

Mass spectrometer: LTQ Orbitrap Velos MS

LC: Agilent 1200 RPLC binary pumps and degasser
CTC HTS PAL autosampler

Injection Volume: 10ul

Column: Shiseido Capcell PAK UG120, 2 X 100 mm , 3 µm particle size

Mobile Phase A: 0.1% formic acid in water

Mobile Phase B: 0.1% formic acid in acetonitrile

Gradient (linear):

Time (min)	% Mobile Phase B
0	5
4	5
38	20
38.1	100
42	100
42.1	5
50	5

Parent Compound

Reference to "Discovery of AMG 925, a FLT3 and CDK4 Dual Kinase Inhibitor with Preferential Affinity for the Activated State of FLT3", Journal of Medicinal Chemistry, 2014, 57, 3430−3449

Data Analysis

The HRAM data was processed by Compound Discoverer software using a single processing workflow (Figure 1). The workflow employed expected compound search based on parent and transformations, unknown compound detection and pattern scoring node. FISh Scoring node was included to explain the fragments of the detected compounds for structure ID purpose.

Experimental pattern from parent compound in the urine sample is displayed in Figure 2. The pattern was imported into the Compound Discoverer Pattern Scoring node for pattern matching (Figure 3).

Figure 1. Workflow tree in Compound Discoverer software

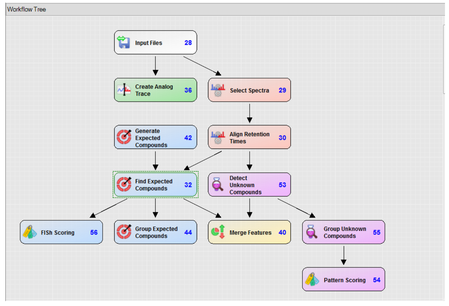
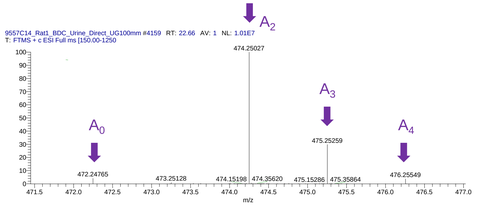


Figure 2. Raw full ms spectrum showing[14]C labeled and unlabeled [12]C pattern

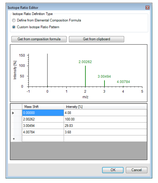


RESULTS

Setting Up the Pattern

Experimental pattern was extracted from raw standard data. The Pattern Scoring node takes either natural or custom pattern. By using the experimental pattern instead of theoretical pattern helps to achieve better pattern matching results. From previous experience, the custom pattern should consist of at least four isotopic masses or packets in order to effectively reduce or suppress matrix interferences from complex biological samples. (Figure 3)

Figure 3. Isotope Ratio Editor



Matched Compounds

Compound Discoverer software marks the unknown compounds that match the specified custom pattern in the Compounds table (Figure 4). For each compound that matches the pattern, the matched pattern is displayed in the spectrum window (Figure 5)

Figure 4. Compounds table with Pattern Matching column

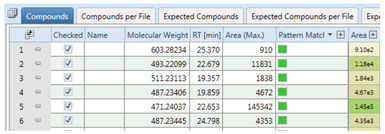
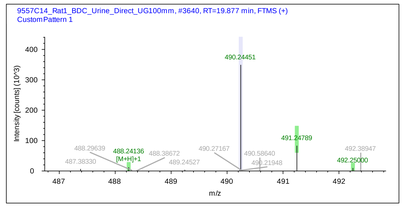
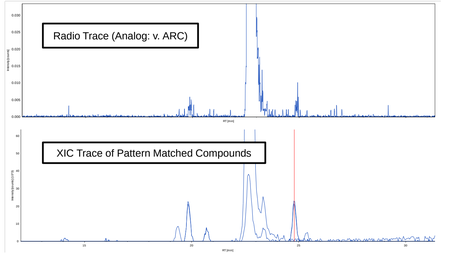


Figure 5. Visualization of matched pattern in spectrum view



To validate the workflow using unknown compound detection combined with pattern scoring in Compound Discoverer software, we compared the radio trace and the XIC traces from matched compounds for the same sample. Figure 6 shows the stacked view of the radio trace and the matched compounds trace in Compound Discoverer software. Low level metabolites were confidently detected by this approach.

Figure 6. Stacked radio metric trace ([14]C) and XIC trace of pattern matched compounds

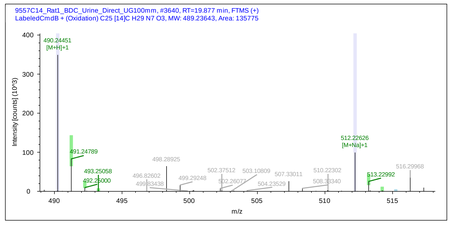


Compound Identification

The next step is to identify these [14]C containing compounds. Identification was achieved within the same workflow by including expected compound search based on the parent and transformations.

Figure 7 shows automatic adduct detection and visualization of isotopic pattern fit in Compound Discoverer software. Isotope pattern fit confirmed the formula assignment for these compounds.

Figure 7. Expected compound detection with automatic adduct assignment



Structure elucidation of these compounds were achieved by the FISh Scoring node in the same workflow. FISh stands for fragment ion searching. FISh Scoring works on both MS[2] and MS[3] spectra. See Figure 8 and 9. FISh Scoring uses Mass Frontier™ Fragmentation Libraries to predict in silico fragments based on the structure of the parent compound or dealkylation / dearylation products. It automatically annotated the MS[2] and MS[3] spectra with fragment structures and color coded the direct match fragments in green and transformation shifted fragments in blue.

Figure 8. Structure annotations from FISh Scoring node on MS[2] spectrum of one of the oxidation metabolites.

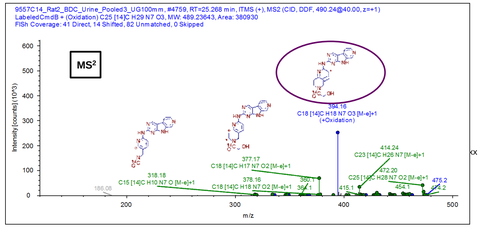
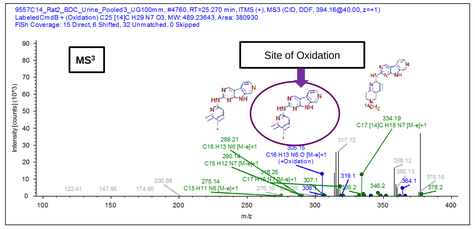


Figure 9. Structure annotations from FISh Scoring node on MS[3] spectrum of one of the oxidation metabolites.



CONCLUSIONS

- Compound labeling combined with high resolution LC/HRAM mass spectrometry and MS[n] is an effective way for confident compound detection and identification from complex biological samples.
- Compound Discoverer software provides a suite of advanced algorithms (nodes) which enable flexible yet powerful data processing that was previously not possible.
- Correlation between the radiometric trace and [14]C pattern matched compounds trace proves the workflow is effective and powerful.
- The FISh Scoring node in Compound Discoverer helps to elucidate the structure of detected metabolites based on MS[2] and MS[3] spectra.
- The approach described here can be applied to any small molecule identifications utilizing labeling technique.
- Future considerations include further improvement to the pattern search algorithm and developing a mechanism to detect only compounds that match specified pattern(s).

TRADEMARKS/LICENSING