Radio-Labeled Compound Detection Using Isotopic Structures From Very High Resolution Mass Spectrometry

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Overview

Purpose: Confident detection and profiling of metabolites with effective matrix background removal by employing ^{14}C labeling and utilizing very high resolution mass spectrometry in one single workflow.

Methods: The parent compound in study is fully labeled with one ^{14}C. Samples were prepared by incubating with RLM and NAPDH and collected at T0hr and T1hr time points. HRMS full scan followed by data dependent data were collected on the Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer with 240k and 120k resolution respectively. Data analysis was done within Thermo Scientific™ Compound Discoverer™ 1.0 software using one single processing workflow. The workflow employed the Pattern Tracer node to extract out chromatographic traces from both time points representing ^{14}C containing compounds, the Expected Finder node for targeted compound detection, and the Unknown Detector node for untargeted compound detection.

Results: The Pattern Trace node in Compound Discoverer software effectively removed matrix background and revealed ^{14}C containing compounds. Comparison of pattern traces between the two time points helped eliminate impurity compounds. Linking compound detection with the pattern traces to get m/z, isotope pattern and spectrum were nicely done by manual peak integration on the pattern trace. The manually integrated pattern trace peaks were automatically linked to detected compounds from targeted and untargeted mechanisms.

Introduction

^{14}C labeling is 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 labeled compound detection and profiling workflow using the very high resolution Orbitrap Fusion mass spectrometer and Compound Discoverer software.

Methods

Parent Compound:

The compound is Amgen proprietary.

Formula: $C_{26}H_{29}N_7O_2$ with one carbon replaced with $^{14}C \rightarrow {}^{14}CC_{25}H_{29}N_7O_2$

Monoisotopic mass: 473.24152

Sample Preparation

The sample was dosed and incubated in RLM with NADPH at a concentration of 1uM. The sample was quenched with 3 volumes of methanol containing 3% formic acid and collected at T0hr and T1hr. After centrifugation, the supernatant was subjected to LC-MS analysis.

Liquid Chromatography

Samples were chromatographically separated by a gradient using an Agilent 1290 UPLC and a CAPCELL PAK IF column (2X100mm, 2um).

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Mass Spectrometry

The HRAM analysis was conducted on an Orbitrap Fusion mass spectrometer equipped with a HESI NG ion source. Full scan MS data were collected at resolving powers of 240K and data dependent at 120K.

Data Analysis

The HRAM full scan data was processed by Compound Discoverer software using a single processing workflow (Figure 1).

Experimental patterns from parent compound (Figure 2) were used instead of theoretical enrichment ratios to achieve better results. Comparison of three different patterns used by the Pattern Trace node were evaluated (Figure 3) in order to select the best pattern that most effectively reduces background, in the mean time, retains relevant peak information.

FIGURE 1. Workflow tree in Compound Discoverer software which includes Pattern Tracer node to create a trace for ^{14}C compounds, Expected Finder node to detect targeted transformation compounds and Unknown Detector node to detect untargeted compounds.

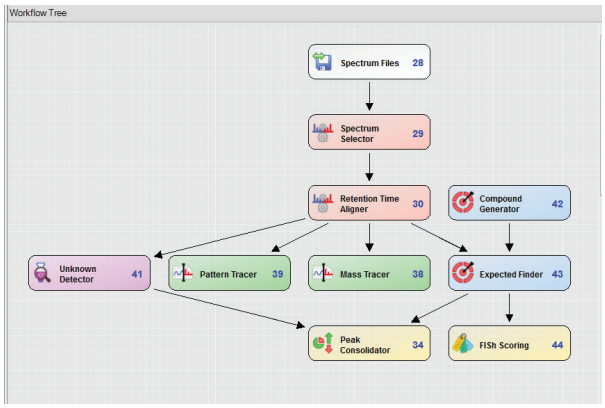

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FIGURE 2. Raw full ms spectrum showing full ms pattern of parent compound from ^{14}C labeling.

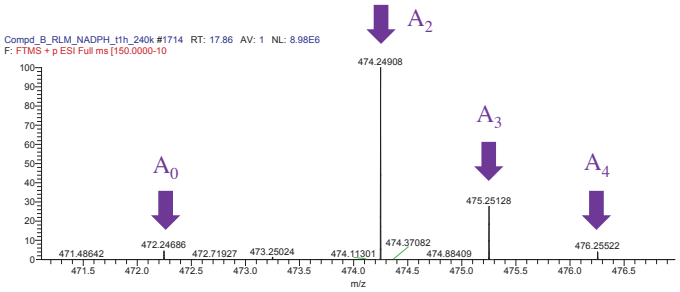
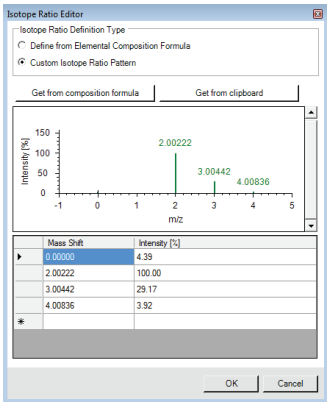


FIGURE 3. Isotope Ratio Editor in Pattern Trace node showing input of experimental custom pattern.



Three different patterns were evaluated

1. Pattern consisting A_0 and A_2 only

Mass Shift	Intensity [%]
0.00000	4.39
2.00222	100.00

2. Pattern consisting A_0, A_2 and A_3

Mass Shift	Intensity [%]
0.00000	4.39
2.00222	100.00
3.00442	29.17

3. Pattern consisting A_0, A_2, A_3 and A_4

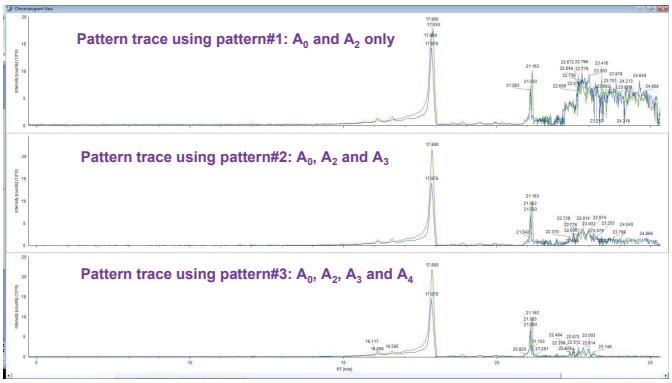
Mass Shift	Intensity [%]
0.00000	4.39
2.00222	100.00
3.00442	29.17
4.00836	3.92

Results

Pattern Selection

Three different patterns as shown in Figure 3 were used to extract out pattern traces. The results from the Compound Discoverer Pattern Tracer node indicates the more specific the pattern is, the better it removes matrix background. (Figure 4)

FIGURE 4. Pattern traces from different custom patterns



Where are my ^{14}C containing metabolites?

The Pattern Tracer node using pattern #3 (consisting of A_0, A_2, A_3 and A_4) effectively removed matrix background and other interferences. Metabolites containing ^{14}C are revealed in the pattern traces when overlaying traces from T_0 hr and T_1 hr time points. These metabolites are not visible in the overlaid base peak chromatograms. (Figure 5)

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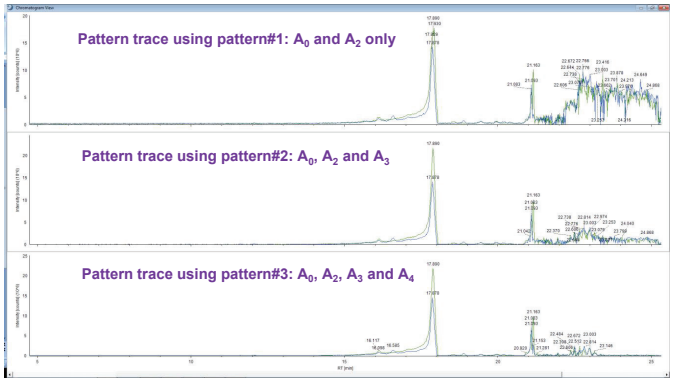

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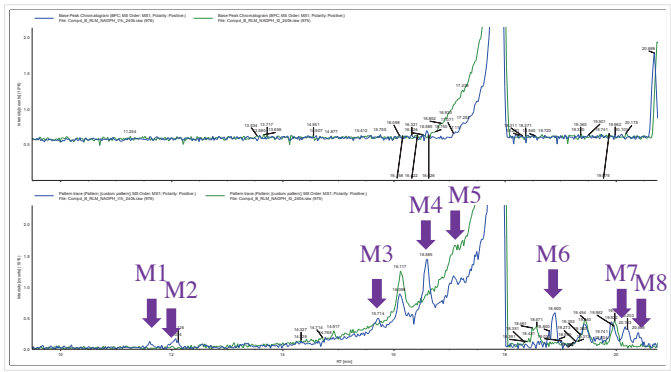
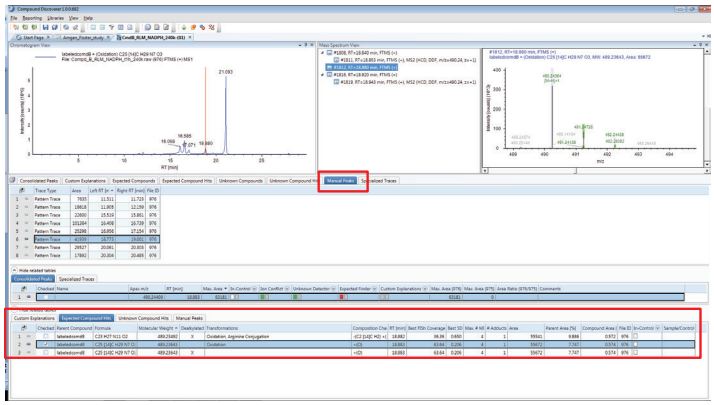
FIGURE 5. The top plot shows overlaid base peak chromatograms from T0hr and T1hr; the bottom plot shows overlaid pattern traces from T0hr and T1hr.



What are they?

Finding the identities of these metabolites from the pattern trace was achieved easily within Compound Discoverer software. The workflow used to process the data included Expected Finder node which looks for modification compounds and Unknown Detector node which detects compounds based on untargeted component detection. By manually integrating the selected peaks on the pattern trace, Compound Discoverer software links peaks detected by Expected Finder and Unknown Detector to the manually integrated pattern trace peaks (Figure 6). m/z, compound explanations, isotope pattern fit score, fragmentation ion match score, and spectral tree information became readily available to help make the correct assignment of these compounds.

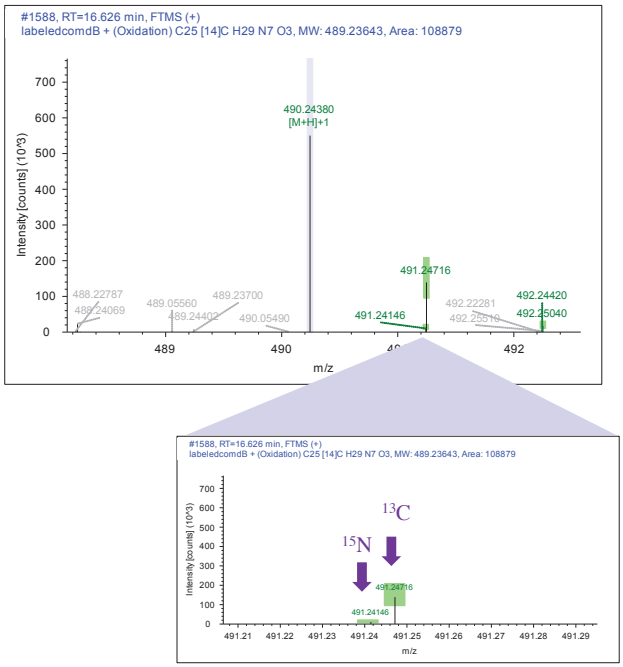
FIGURE 6. Manual peaks from pattern trace are linked with peaks detected by Expected Finder and Unknown Detector nodes.



Fine isotopic structure confirmation for very high resolution data

The elemental composition proposals from Expected Finder for these putative ^{14}C containing compounds were confirmed by isotopic pattern fit calculation which is part of the Expected Finder node. Since the parent compound contains 7 nitrogen atoms, and these metabolites also contain the same number of nitrogen, the fine isotopic peaks from ^{15}N and ^{13}C isotopes gave greater confidence in the final metabolite assignment (Figure 7).

FIGURE 7. At 240K resolution, the ^{15}N and ^{13}C isotopes are resolved and visualized within Compound Discoverer software.



^{14}C containing metabolites identified by Compound Discoverer software

The following ^{14}C containing metabolites were detected by Compound Discoverer software using a single processing workflow employing Pattern Tracer, Expected Finder and Unknown Detector nodes (Table 1). Trace level metabolites were identified by this approach. The smallest metabolite identified had a relative to parent area percent of 0.33%.

In this study, all the ^{14}C containing peaks from pattern trace were explained by the Expected Finder node. The explanations from Expected Finder provided elemental composition, transformation, formula change, mass accuracy, retention time, isotopic pattern score and FISh coverage score. These information helped quicker and more confident metabolite identification.

TABLE 1. ^{14}C containing metabolites including trace level metabolites identified by Compound Discoverer software

	Formula	Monoisotopic mass	Transformation	Composition Change	RT(min)	Mass Accuracy (ppm)	Area
P	$C_{25}{}^{14}CH_{29}N_7O_2$	473.24152	--	--	17.88	-0.10	1988333
M1	$C_{25}{}^{14}CH_{29}N_7O_3$	489.23643	Oxidation	+(O)	11.64	-0.26	6684
M2	$C_{25}{}^{14}CH_{29}N_7O_3$	489.23643	Oxidation	+(O)	12.06	-0.20	15769
M3	$C_{25}{}^{14}CH_{31}N_7O_3$	491.25208	Hydration	+(H2O)	15.70	-0.21	27945
M4	$C_{25}{}^{14}CH_{29}N_7O_3$	489.23643	Oxidation	+(O)	16.59	-0.14	108879
M5	$C_{25}{}^{14}CH_{29}N_7O_3$	489.23643	Oxidation	+(O)	17.07	-0.33	39559
M6	$C_{25}{}^{14}CH_{29}N_7O_3$	489.23643	Oxidation	+(O)	18.88	-0.26	55672
M7	$C_{25}{}^{14}CH_{29}N_7O_4$	505.23134	Oxidation + Oxidation	+(O2)	20.17	-0.41	42093
M8	$C_{26}{}^{14}CH_{29}N_7O_3$	501.23643	Desaturation, Oxidation + Methylation	+(CO)	20.40	-0.26	26039

Conclusion

- Compound labeling combined with very high resolution LC/HRAM mass spectrometry is an effective way for confident compound detection and profiling 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.
- The Pattern Tracer node is able to effectively reduce background and extract out labeled compounds based on experimental custom pattern. When it is combined in a single workflow with peak detection mechanisms, compound identification and profiling can be achieved without use of a radio detector.
- The pattern recognition algorithm in Compound Discoverer software is capable of utilizing very high resolution data and fine isotopic structures, which gives user greater confidence in results and helps get the answers quicker.
- The approach described here can be applied to any labeling studies.
- Future considerations include further improvement to the pattern search algorithm and developing a mechanism to detect compounds based on custom pattern.

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