

LC/MS – Compound Annotation – Natural Product

Simplifying Natural Product Analysis with Data-Independent Acquisition Workflow

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Abstract

Natural product research has gained renewed attention as a diverse source of novel chemical entities with potential pharmacological properties. This renewed momentum is partly driven by the development and improvement in the technology for natural product research. High-resolution mass spectrometry (HRMS) has since become ubiquitous equipment for chemical profiling of natural products. HRMS and its various modes of analysis, such as data-independent acquisition (DIA), deliver not only accurate mass and isotopic information but in-depth analysis crucial for deciphering the structures of unknown chemical compounds. This article demonstrates the application of liquid chromatography/mass spectrometry (LC/MS) with a quadrupole time-of-flight (Q-TOF) spectrometer, the Shimadzu LCMS-9030, for the analysis of natural products. The acquisition and data processing workflow solutions for DIA mode will be demonstrated.



*Traditional Chinese herbs, such as *Aconitum carmichaeli* (right), can be analyzed for natural product discovery*

Keywords:
LCMS-9030, Q-TOF, Data-Independent Acquisition, High-Resolution Accurate Mass

Highlights

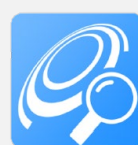
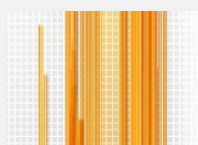
- Data-independent acquisition mode on Shimadzu LCMS-9030 Q-TOF enables comprehensive coverage of chemical compounds for natural product research
- LabSolutions™ LCMS simplifies the setup of data-independent acquisition mode
- LabSolutions Insight™ Explore delivers seamless data-processing tools to analyze data-independent acquisition data

Technologies Featured

LCMS-9030 Q-TOF



LabSolutions™ LCMS and LabSolutions Insight™ Explore



1. INTRODUCTION

Natural products are intensively studied because they are a rich source of chemical diversity capable of delivering pharmacological benefits. High-resolution mass spectrometry (HRMS) instrument such as quadrupole time-of-flight (Q-TOF) is frequently used to aid the identification of unknown natural products. In addition, natural product chemists are equally concerned with obtaining a full coverage of chemical compounds to reduce false-negative identifications.

The data-independent acquisition (DIA) method performed on the HRMS instrument is thus designed to maximize the unknown compounds coverage and their identification. DIA method acquires MS/MS scans systematically without prior knowledge of target precursor information. Due to the independence between precursors and fragment ions in DIA analysis, it is necessary to have a powerful data analysis solution that allows a rigorous and exploitable analysis of data.

In this article, we will demonstrate the acquisition and data processing workflow solutions for DIA method on Shimadzu LCMS-9030 Q-TOF. A traditional herb sample, *Aconitum carmichaeli*, will be used to illustrate the workflow.

2. EXPERIMENT

2.1 Sample Preparation

The extract of *Aconitum carmichaeli* was prepared by agitating 500 mg of *Aconitum carmichaeli* chunks in 70% methanol for 30 mins. The extract was filtered with a 0.2 µm syringe filter and directly injected into the LCMS-9030 Q-TOF.

2.2 Analytical System

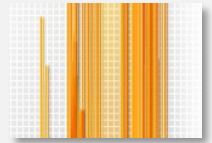
Chromatographic analysis was performed using the Shimadzu Nexera X2 system equipped with two binary pumps, degasser, autosampler, and a thermostatically controlled column unit. Chromatographic separation was carried out on a Shimadzu Velox C18 column (2.1 mm x 100 mm, 2.7 µm particle size). The column temperature was maintained at 40°C. The mobile phase were consisted of (A) H₂O with 0.1% formic acid and (B) acetonitrile. The gradient elution program of the mobile phase was 0-8 min (5-40% B), 8-10 min (40% B), 10-17.5 min (40-60% B), 17.5-21.5 min (60-95% B), 21.5-23.5 min (95% B), 23.5-24.5 min (95-5% B), 24.5-30 min (5%B). The flow rate was set at 0.4 mL/min. The sample volume injected was 10 µL.

The mass spectrometer used was LCMS-9030 Q-TOF, a quadrupole time-of-flight system. Data-independent acquisition mode was performed with a precursor ion range of m/z 100-1000, an isolation window width of 20 m/z , a collision energy spread of 15-55 eV, and a product ion range of m/z 100-1000 in positive mode. The conditions of the heated ESI source were as follows: drying gas (N₂) flow rate 10.0 L/min, nebulizing gas (N₂) flow rate 3.0 L/min, heating gas (zero air) flow rate 10.0 L/min, heat block temperature 400°C, DL temperature 250°C, and interface temperature 300°C. Data processing was performed with Shimadzu LabSolutions™ LCMS v5.99 and LabSolutions Insight™ Explore software. A suspected screening list containing compound name, accurate mass and chemical formula was used for targeted screening. NIST 2020 MS/MS database entry was used for library match.

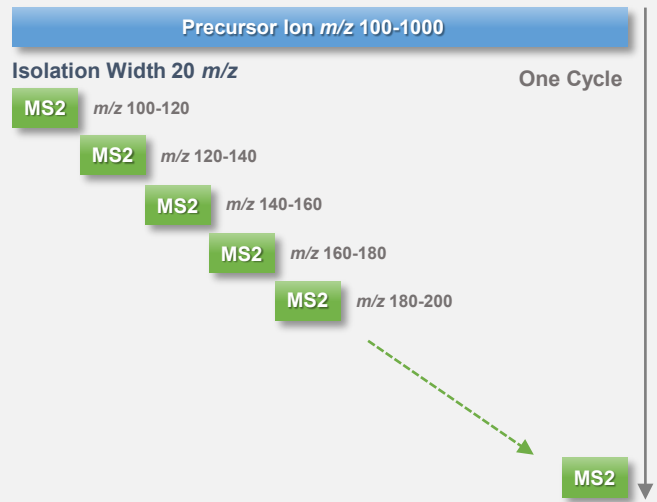
3. RESULTS AND DISCUSSION

3.1. DIA Data Collection

The collection of DIA data is greatly simplified in the LabSolutions™ LCMS v.5.99 software. With the LCMS-9030 Q-TOF, DIA data is collected by isolating and fragmenting a defined mass-to-charge (m/z) window and acquiring the MS/MS data within a cycle. Figure 1 shows the software interface for setting the DIA parameters. The interface systematically guides users to setup precursor ion m/z range, isolation window width, expected loop/cycle time, collision energy spread, acquisition time, and product ion m/z range. It is noteworthy that high-speed data acquisition up to 100 Hz and wide collision energy spread can be applied for DIA analysis. A generic DIA method was used for the analysis of the *Aconitum carmichaeli* extract. Figure 2 shows a segment of total ion chromatograms from retention time 0-12 min for DIA-MS/MS events.



Data-Independent Acquisition Structure



Precursor Ion m/z : 100 - 1000

Q1 Transmission m/z Width : 20 * [Center] will be checked automatically.

Loop Time (s) : 0.9 = Event : 45 × Event Time (s) : 0.02

CE : Fixed 35
 Calculated -0.5082 + 0.04 × m/z

CE Spread (±) : 20

Start - End Time (min) : 0 - 30

Product Ion m/z : 100 - 1000

Figure 1. LabSolutions™ LCMS software dialog for data-independent acquisition mode parameter settings and the structure of DIA data collection for LCMS-9030

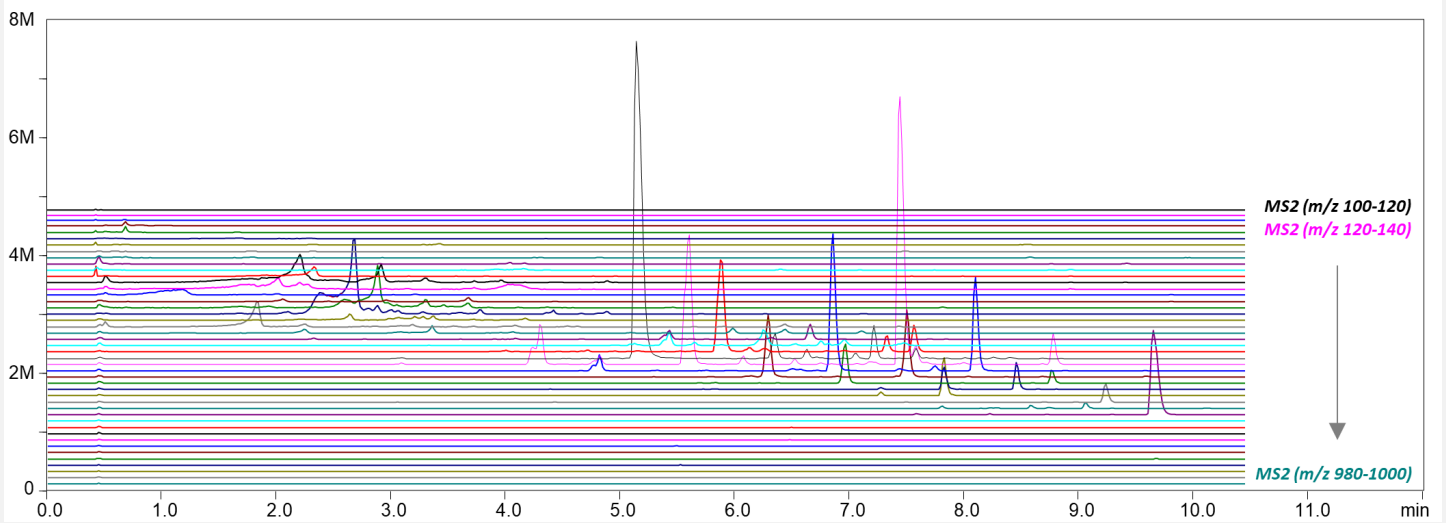


Figure 2. Total ion chromatograms of DIA-MS/MS events for the analysis of *Aconitum carmichaeli* extract

3.2. DIA Data Processing

The analysis of DIA data is usually more rigorous than conventional MS data analysis. Hence, the LabSolutions Insight™ Explore software is specifically designed to simplify the qualitative analysis of Q-TOF data. This saves time and avoid a tedious data process.

LabSolutions Insight™ Explore is consisted of a series of tools like Analyze, Formula Predictor, Library Search, and Assign, which support the qualitative data analysis. The DIA data analysis workflow is shown in Scheme 1.

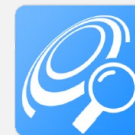
The Analyze tool incorporates enhanced component detection and deconvolution algorithms for DIA data. The deconvolution process hence re-establishes the link between precursors and fragment ions. Precursor ions of deconvoluted components can then be compared with target list to locate suspected targets. The Formula Predictor tool can be subsequently used to predict chemical formulas for results generated by the Analyze tool. Isotopic scores are calculated for each predicted formula to increase screening confidence. Furthermore, the isotopic pattern of acquired and predicted mass peaks can visually compared.

The deconvoluted MS/MS spectra can be matched against a local MS/MS database using the Library Search tool to aid the identification of unknown compounds. In the event that the local MS/MS database has limited entries, the Assign tool can be used to search against public libraries such as PubChem and ChemSpider using accurate mass or chemical formulas. An in-silico fragmentation algorithm built within the Assign tool annotates MS/MS fragment ions based on the chemical structure of the suspected compound. It further helps to identify unknown compounds by providing scores on the goodness of fragment ions annotation.

For the analysis of this *Aconitum carmichaeli* extract, a total of 5150 components were detected within the retention time of 0-12 min. 260 unique precursor ions were found. Amongst them, a deconvoluted component with precursor ion of m/z 454.2797 was annotated as fuziline, a steroid alkaloid (Scheme 1). It was successfully matched against the NIST 2020 MS/MS database entry with a match score of 89. Furthermore, the Assign tool shortlisted fuziline as possible target with an Assign score of 88. Based on in-silico fragmentation calculation, the fragment ions from the deconvoluted MS/MS spectrum could be structurally annotated to support the identification of the unknown component.

4. CONCLUSION

Natural product research greatly benefits from the availability of DIA mode. This analysis mode increases the coverage of chemical compounds discovered and simplifies the discovery efforts. The LCMS-9030 Q-TOF system enables the analysis of even the most complex natural products. Supported by LabSolutions Insight™ Explore as a dedicated software solution to deliver data processing solutions for DIA data, the workflow of natural product discovery can be greatly simplified.



Analyze

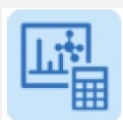
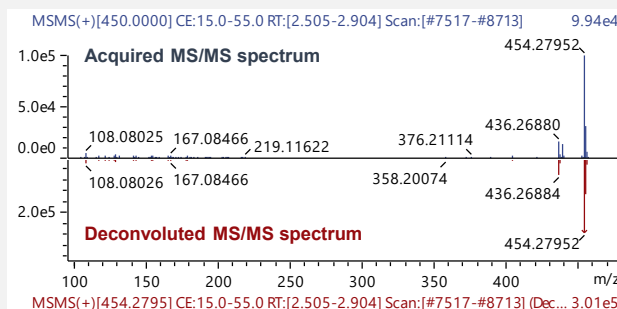
• Deconvolution

- The **Analyze** tool automatically performs component detection and MS/MS deconvolution
- Fragment ions are correlated to their respective precursor ions

• Suspect Screening

- Screening against a target list enables quick detection of suspected targets

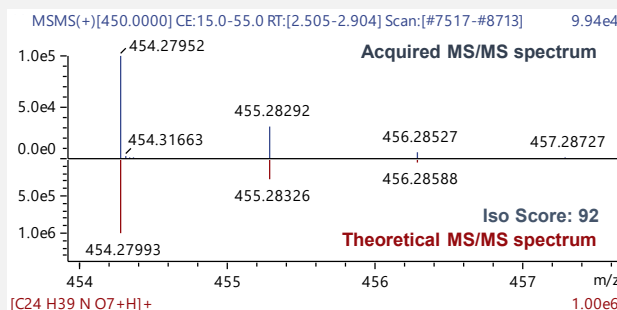
Deconvoluted Components				Screening Targets		
RT	m/z	Response	Precursor Ion	Target Name	Target Formula	Target m/z
2.689	454.27952	2684546	454.27952	Fuziline	C24H39NO7	454.27993
2.780	378.26359	14172	378.26359	Karacolone/aco...	C22H35NO4	378.26389
2.888	438.28464	1444335	438.28464	Bullatine B/10-h...	C24H39NO6	438.28502



Formula Predictor

• Isotope Pattern

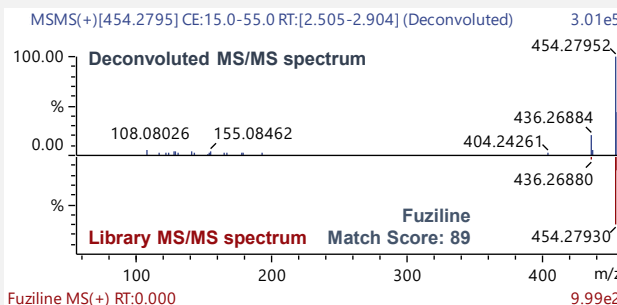
- The **Formula Predictor** tool provides scores as a metric to shortlist the correct chemical formula for unknown compounds
- Mirror plot displaying acquired and theoretical isotopic patterns provides fast visual confirmation



Library Search

• Local Library Search

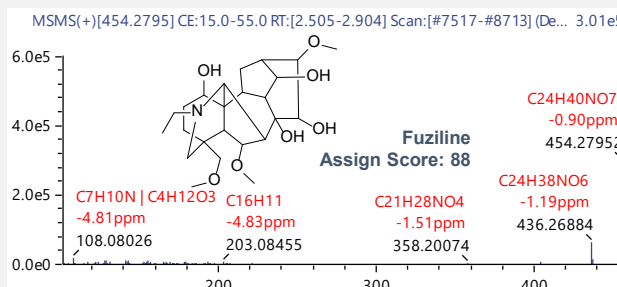
- The **Library Search** tool enables matching of MS/MS spectrum local libraries for seamless identification of unknown compounds



Assign

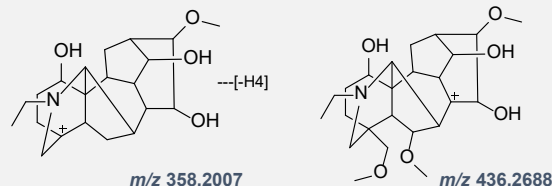
• Public Library Search

- The **Assign** tool enables searching and scoring against public libraries such as PubChem and ChemSpider



• In-silico Fragmentation

- Assign scores are provided based on *in-silico* fragmentation analysis on the MOL files automatically retrieved from public libraries to help analysts annotate unknown compounds



Scheme 1. DIA data processing workflow with LabSolutions Insight™ Explore

READ MORE

LCMS-9030 Q-TOF



The LCMS-9030 Q-TOF uses newly patented technologies to deliver both high resolution and accurate mass, attributes essential for confident chemical formula assignment and unknown identification. High-efficiency ion guides, quadrupole, and collision cell enable high sensitivity for the detection of trace-level compounds.

Unique UFgrating™ and *iReTOF*™ technologies ensure ultrafast acceleration of ions into the flight tube (UF-FlightTube™) and ideal reflection of those ions back to the detector. The result is high-speed data acquisition compatible with the high-throughput laboratory.

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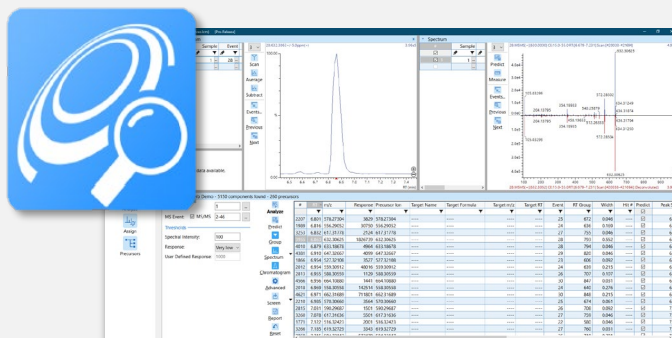
LabSolutions Insight™ Explore (P/N: 225-39181-91)

LabSolutions Insight™ Explore offers an intuitive and full-featured quantitative environment with significant qualitative enhancements.

Sophisticated peak detection and deconvolution algorithms detect chromatographic features based on their accurate mass and simplify the data processing workflow of data-independent acquisition.

Formula prediction and *in-silico* fragmentation algorithms improve the confidence of annotation for unknown compounds.

[Learn More](#)



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