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Liquid Chromatograph Mass Spectrometer LCMS™-9030

Application News

Simplifying Natural Product Analysis with Data-Independent Acquisition Workflow

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User Benefits

◆ 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

■ 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.

■ Measurement Conditions and Samples

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.

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) H_2O 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

LabSolutions™ LCMS
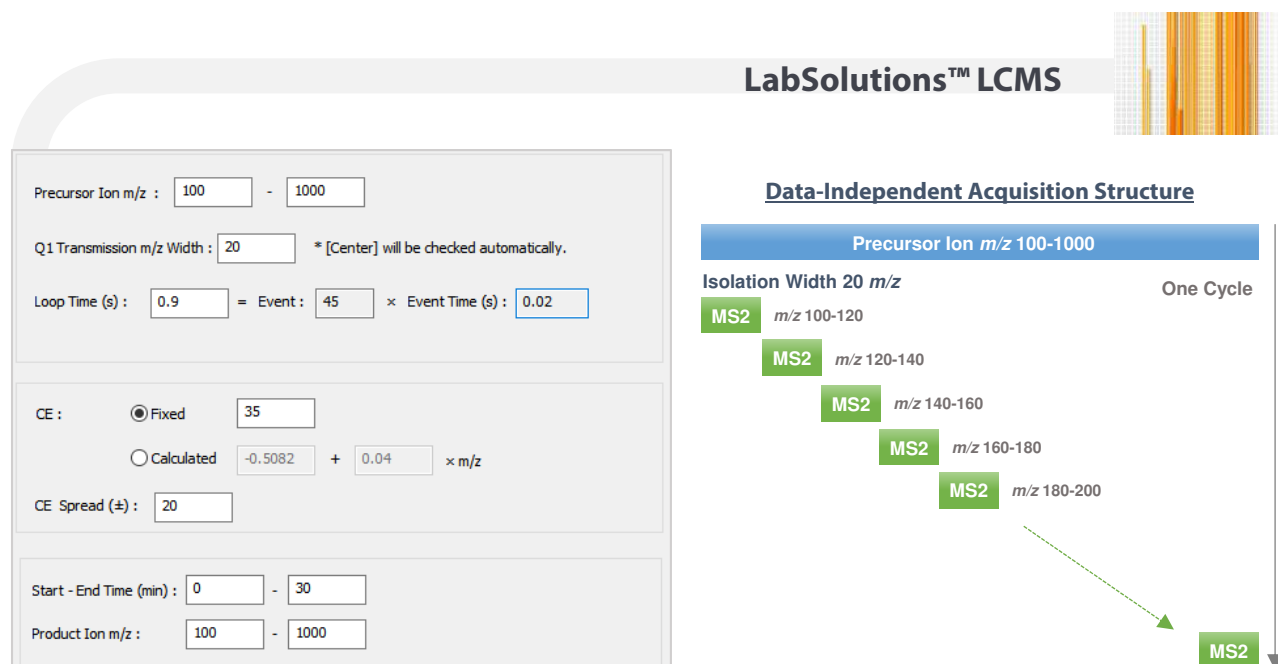
Data-Independent Acquisition Structure

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

LabSolutions Insight Explore™

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	Karacoline/aco...	C22H35NO4	378.26389
2.888	438.28464	1444335	438.28464	Bullatine B/10-h...	C24H39NO6	438.28502

MSMS(+)[450.0000] CE:15.0-55.0 RT:[2.505-2.904] Scan:[#7517-#8713]   9.94e4

Acquired MS/MS spectrum
454.27952
108.08025 167.08466 219.11622   376.21114   436.26880
108.08026 167.08466   358.20074   436.26884
Deconvoluted MS/MS spectrum
454.27952

MSMS(+)[454.2795] CE:15.0-55.0 RT:[2.505-2.904] Scan:[#7517-#8713] (Dec... 3.01e5

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

MSMS(+)[450.0000] CE:15.0-55.0 RT:[2.505-2.904] Scan:[#7517-#8713]   9.94e4

454.27952
Acquired MS/MS spectrum
454.31663 455.28292 456.28527 457.28727
455.28326 456.28588
Iso Score: 92
Theoretical MS/MS spectrum
454.27993
[C24 H39 N O7+H]+   1.00e6

Library Search

- **Local Library Search**
  - The **Library Search** tool enables matching of MS/MS spectrum local libraries for seamless identification of unknown compounds
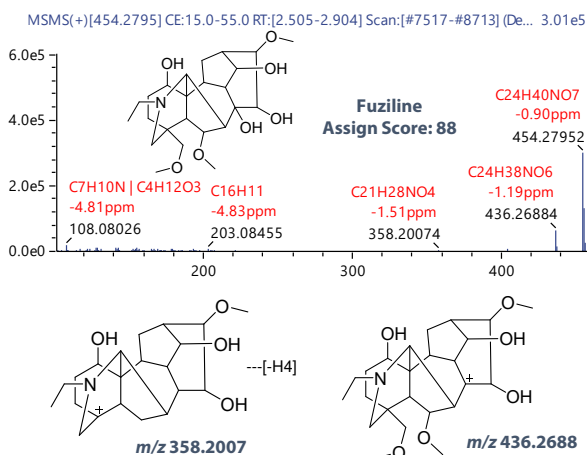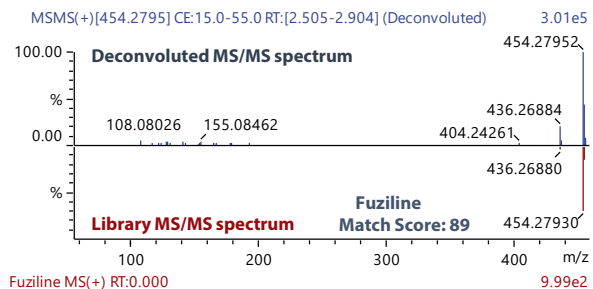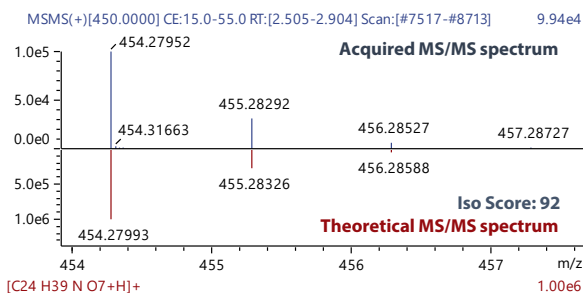
MSMS(+)[454.2795] CE:15.0-55.0 RT:[2.505-2.904] (Deconvoluted)   3.01e5

Deconvoluted MS/MS spectrum
454.27952
436.26884
108.08026   155.08462   404.24261
436.26880
Fuziline
Library MS/MS spectrum   Match Score: 89   454.27930
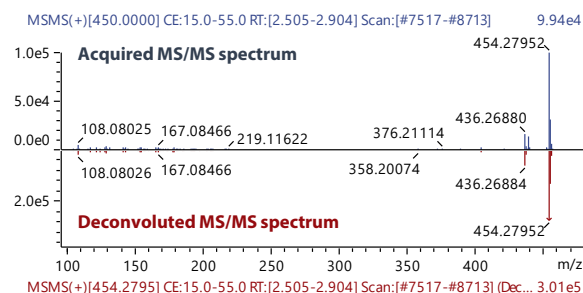Fuziline MS(+) RT:0.000   9.99e2

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

MSMS(+)[454.2795] CE:15.0-55.0 RT:[2.505-2.904] Scan:[#7517-#8713] (De... 3.01e5

C24H40NO7
-0.90ppm
454.27952
Fuziline
Assign Score: 88
C7H10N | C4H12O3   C16H11   C21H28NO4   C24H38NO6
-4.81ppm   -4.83ppm   -1.51ppm   -1.19ppm
108.08026   203.08455   358.20074   436.26884

---[-H4]

m/z 358.2007   m/z 436.2688

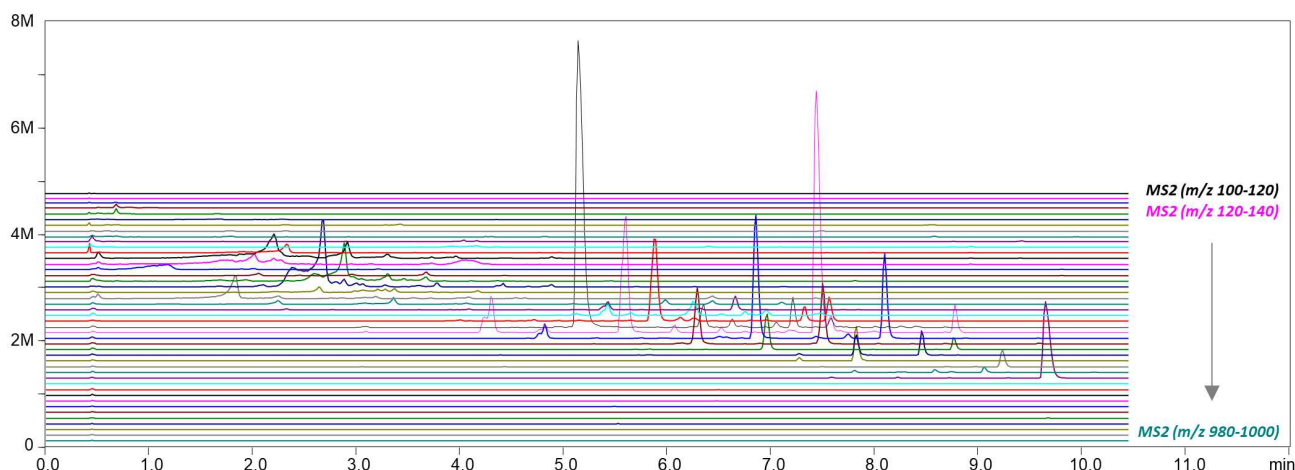Scheme 1. DIA data processing workflow with LabSolutions Insight Explore™.

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

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 (N$_2$) flow rate 10.0 L/min, nebulizing gas (N$_2$) 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.

■ Results and Discussion

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. Fig. 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. Fig. 2 shows a segment of total ion chromatograms from retention time 0-12 min for DIA-MS/MS events.

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.

■ 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.

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