SHIMADZU
Excellence in Science

TECHNOLOGY BRIEF | NO. MST-208

LC/MS – Component Annotation – Natural Product

Data-Dependent Analysis Approach in LC/HRMS: Annotation of Natural Product Components as Case Study

Written by:

Udi Jumhawan

Chua Chun Kiang

Chia Shao Hua

Loo Lai Chin

Abstract

Component annotation is considered as the most challenging step in untargeted analysis. Nowadays, liquid chromatography coupled with high resolution mass spectrometry (LC/HRMS) presents the most widely used analytical platform for untargeted analysis. Equipped with efficient data acquisition method such as data-dependent analysis (DDA), this analytical platform has been a great help for component annotation. The successful annotation requires combination of high-quality MS/MS spectrum and sophisticated data processing software. Frequently, data processing can be a bottleneck for component annotation due to data complexity. A DDA-based workflow was developed by using combination of LCMS-9030 Q-TOF and LabSolutions Insight™ Explore for component annotation. Simplified and seamless component annotation was demonstrated for natural product sample, *Aconitum carmichaeli*.

Therapeutic components in Aconitum carmichaeli (left) are analyzed and annotated for characterization

Keywords:
Component Annotation, Data Dependent Analysis, Natural Product

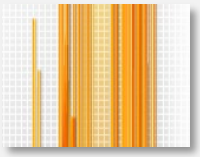
Highlights

- LCMS-9030 Q-TOF provides high quality and high purity MS/MS spectra by employing data-dependent analysis approach for component annotation of natural product
- Combination of Shimadzu's powerhouse software, LabSolutions™ LCMS and LabSolutions Insight™ Explore, demonstrates efficient data acquisition and processing workflow of complex HRMS data

Technologies Featured

LCMS-9030 Q-TOF

LabSolutions™ LCMS and Insight™ Explore



1. INTRODUCTION

LC/HRMS is currently the most prominent analytical tool for untargeted analysis. Combination of soft ionization technique and versatile mass analyzer, working in tandem or hybrid configuration, enables aid for component annotation by providing highly-resolved and accurate MS/MS spectra.

Resourceful data acquisition approaches have been developed to further facilitate a more time-efficient for data collection including data-dependent analysis (DDA) and data-independent analysis (DIA). Both approaches are commonly used and proved to be complementary. DDA approach has been recognized for providing a higher purity MS/MS spectrum. It includes survey scan followed by automated fragmentation for precursor ions above a pre-set abundance threshold.

Chemists have made tremendous efforts to annotate components in natural products especially herbal medicines. These components may exhibit therapeutic effects and thus it is important to carry out complete characterization. In this article, a simplified DDA-based LC/HRMS workflow was demonstrated for component annotation of herbal medicine, *Aconitum carmichaeli.*

2. EXPERIMENT

2.1 Sample Preparation

A 500mg of *Aconitum carmichaeli* was cut into small pieces and vortexed in 70% methanol for 30 mins. The extract was filtered using 0.22 µm PTFE filter and subsequently injected into LCMS-9030 Q-TOF.

2.2 Analytical Condition and Data Processing

Analysis was performed on LCMS-9030 Q-TOF. Analytical conditions (liquid chromatography and mass spectrometry) are described in Table 1. Data acquisition and processing were carried out by using LabSolutions™ LCMS v5.99 and LabSolutions Insight™ Explore, respectively.

Table 1. Analytical conditions for analysis of natural product by LCMS-9030 Q-TOF

Column	C18 column (100 mm x 2.1 mm x 2.7 µm)
Mobile phase	A : 0.1% formic acid in water B : acetonitrile
Gradient program	30 min gradient program
Flow rate	0.4 mL/min
Oven temperature	40˚C
Injection volume	10 µL

Interface	Heated ESI
MS mode	Full scan (MS^1) and DDA, positive
Mass range	100-1000 *m/z*
CE spread	15-55 eV
No. of dependent events	20
Heat block temperature	400˚C
DL temperature	250˚C
Interface temperature	300˚C
Nebulizing gas	N_2, 3 L/min
Drying gas	N_2, 10 L/min
Heating gas	Zero air, 10 L/min

3. RESULTS AND DISCUSSION

3.1. DDA Data Collection

DDA data acquisition was controlled by the LabSolutions™ LCMS v.5.99 software. Generic data collection was established using TOF survey scan (MS1) ranged from 100 to 1000 m/z. Base peak chromatogram (BPC) intensity threshold (BPC > 3000) was applied to execute MS/MS fragmentation using collision energy spread (15-55 eV). Twenty dependent (MS/MS) events were set to allow sufficient MS/MS data collection (Figure 1). Ion exclusion and inclusion settings are available in the LabSolutions™ LCMS software dialog to automatically exclude background ions and include ions of interest, respectively.

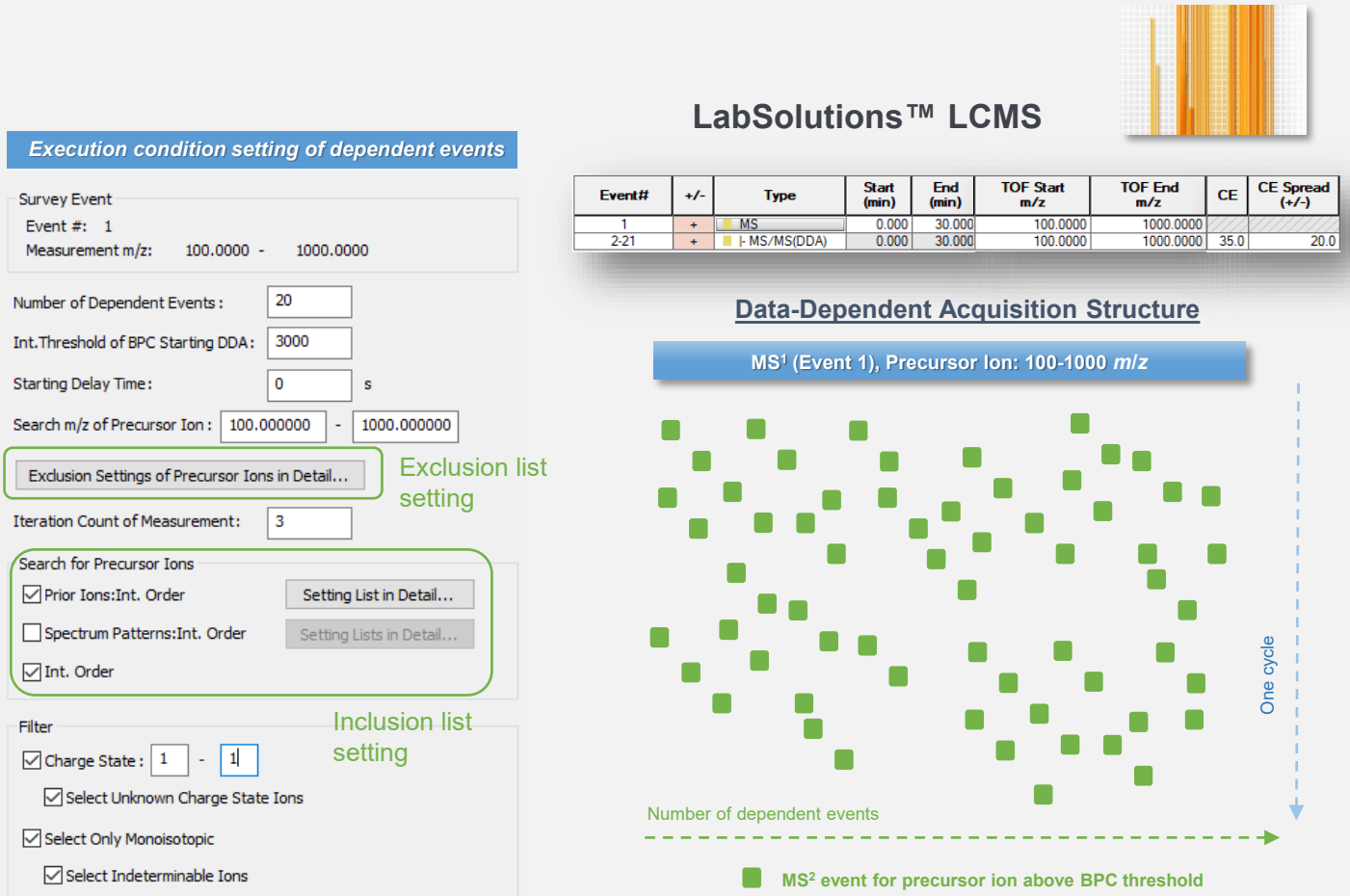


Figure 1. LabSolutions™ LCMS software dialog and the structure of DDA data collection mode for LCMS-9030

3.2. DDA Data Analysis

Analysis of DDA data is very straightforward. Since MS/MS data is automatically executed based on intensity threshold, data deconvolution is not needed. In comparison to DIA approach, DDA method should enable high quality and high purity MS/MS spectra. LabSolutions Insight™ Explore was utilized for data processing from component detection to library searching and in-silico fragmentation (Figure 2).

Component detection was carried out using the Analyze module. Components or precursor ions are detected by searching every scan for ions that behave as a chromatographic peak with a take-off, apex and landing. Ion filtering is then conducted based on peak width, peak response and signal-to-noise ratio.

Detected precursor ions can be compared against suspect screening list to expedite identification workflow. Prediction of chemical formula and overview of isotopic pattern by the Formula Predictor module will provide visual confirmation. Isotopic pattern of the acquired MS1 data highly matched to that of theoretical pattern (Iso score: 99.75). Iso score represents how well the masses of the isotopic peaks match the theoretical isotopic pattern.

LabSolutions Insight™ Explore

Analyze

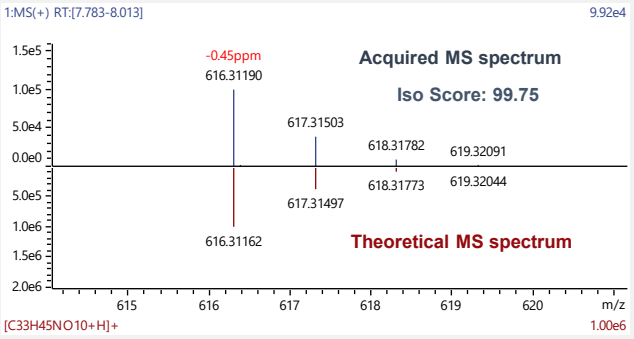
- **Component Detection**
  - The **Analyze** module automatically performs component detection (based on MS^1 or MS/MS)

Detected Components				Screening Targets		
RT	m/z	Response	Precursor Ion	Target Name	Target Formula	Target m/z
				Available		
7.965	616.31248	12126	616.31248	Hypaconitine	C33H45NO10	616.31218
12.412	177.12709	12132	177.12709	2,2',4'-trimethylpropiophenone	C12H16O	177.12795
12.387	195.13796	12196	195.13796	Cnidilide	C12H18O2	195.13851
12.387	195.13796	12196	195.13796	Neocnidilide	C12H18O2	195.13851

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

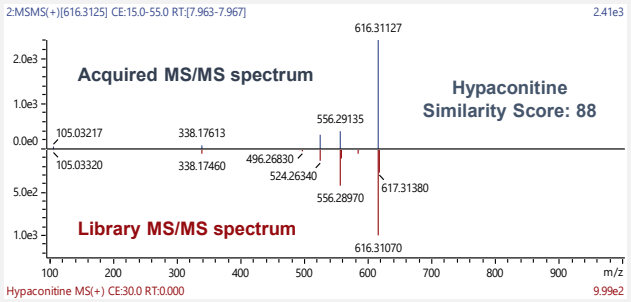
Formula Predictor

- **Isotope Pattern**
  - The **Formula Predictor** module 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** module enables matching of MS/MS spectrum to a curated local library for identification of unknown compounds



Assign

- **Public Library Search**
  - The **Assign** module 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

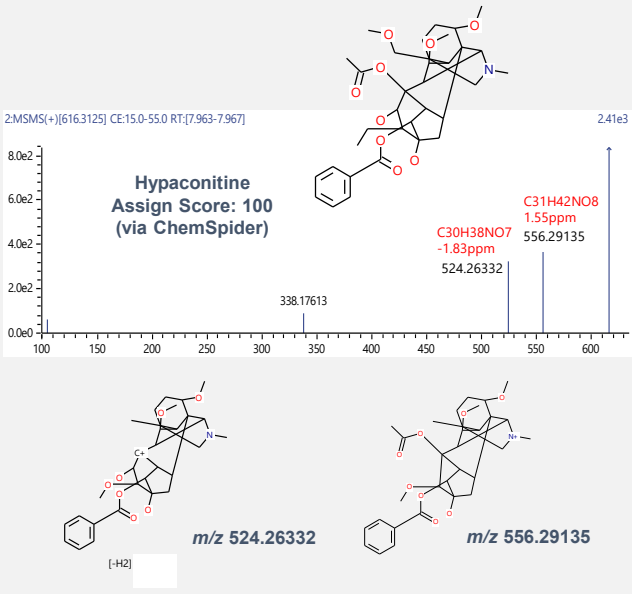


Figure 2. DDA data processing workflow with LabSolutions Insight™ Explore

Both precursor and fragment (MS/MS) ions are used for library searching in the Library Search module (under Edit function) against local library or public domains such as PubChem and ChemSpider through the Assign module. The Assign module is designed to perform in-silico fragmentation of detected component based on the acquired MS/MS data and the MOL file from public libraries. This will improve confidence level of component annotation.

To populate precursor ions and locate specific data for specific search, the Precursor module is available in the Insight™ Explore (Figure 3). It is equipped with heat map to show precursor ion's intensity distribution as well as individual pane for chromatogram and MS/MS spectrum. Especially for DDA data, selection mode of precursor ion will also be displayed in the last column (precursor ions can be selected based on intensity threshold or inclusion/preferred list). Library search and in-silico fragmentation can be performed from the Precursor module.

A total of 441 precursor ions were extracted from 7707 components found in the extract of Aconitum carmichaeli. Hypaconitine was one of the annotated components. Annotation was carried out based on screening against suspect list as well as local (similarity score: 88) and public databases (Assign score: 100). In-silico fragmentation provided substantial affirmation for Hypaconitine annotation.

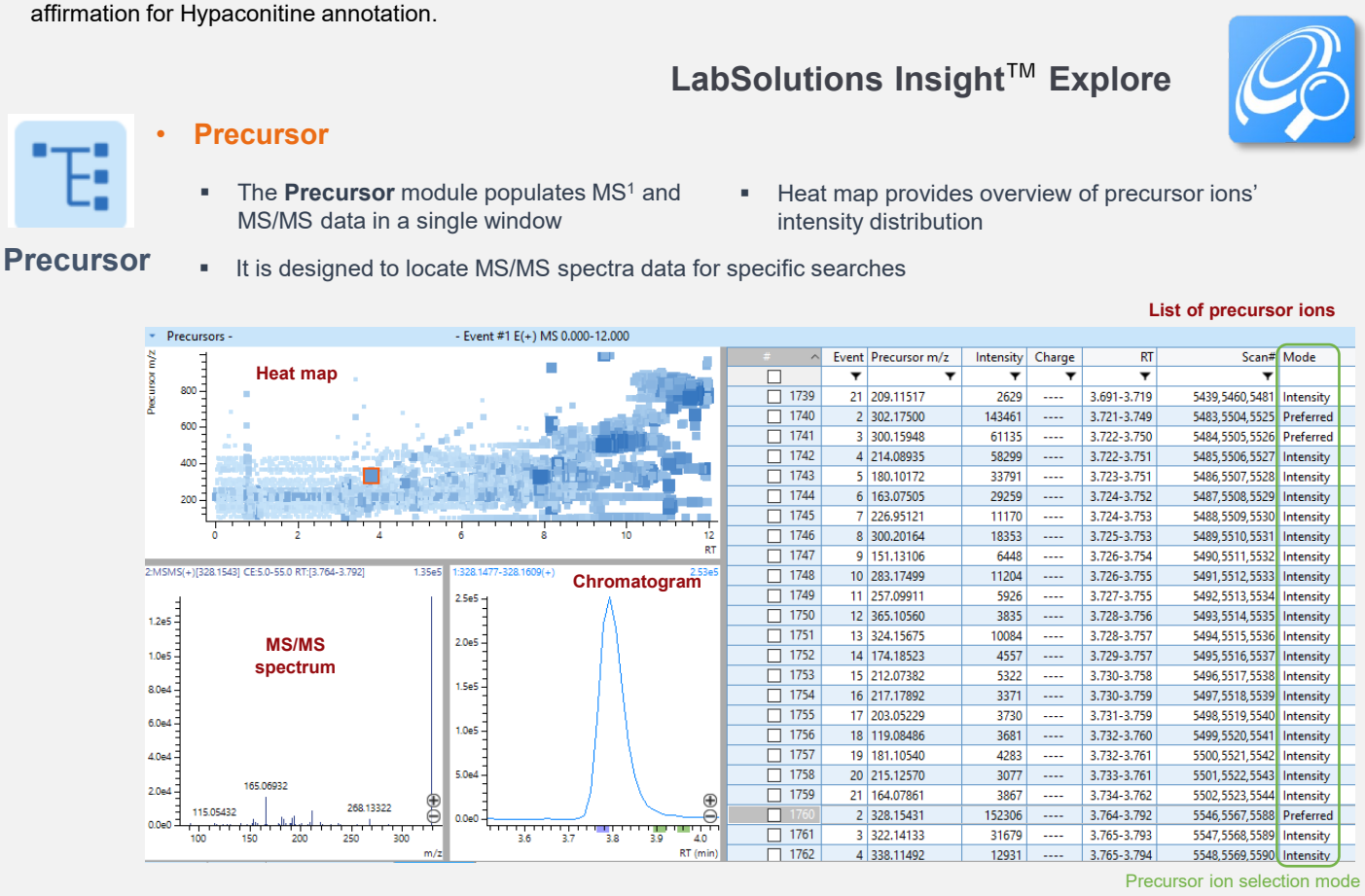


Figure 3. Precursor module in LabSolutions Insight ™ Explore

4. CONCLUSION

DDA approach provides significant aids for component annotation in natural product studies as it enables high quality and high purity MS/MS spectra. DDA approach would greatly benefit from improvements in data acquisition and data processing. With the effortless performance of LCMS-9030 Q-TOF and simplicity of Insight™ Explore, Shimadzu offers solution for component annotation and transforms it into a seamless routine work.

READ MORE

Shimadzu LCMS-9030 Q-TOF (P/N: 225-26000-58)

The LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer integrates the world's fastest and most sensitive quadrupole technology with unique TOF architecture. A product of Shimadzu's engineering DNA, the LCMS-9030 enhances the most important features of Q-TOF instrumentation - mass accuracy, sensitivity, and speed - to address qualitative and quantitative challenges with genuine confidence and ease.

Learn More

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

LabSolutions Insight Explore offers an intuitive and full-featured quantitative environment with significant qualitative enhancements. A sophisticated peak detection algorithm detects chromatographic features based on their accurate mass. Resulting peak displays enable ranked formula prediction with theoretical spectral matching. Seamless library search platform against local and public libraries. A deconvolution algorithm is also provided for analysis of polyvalent ions.

Learn More

Shimadzu (Asia Pacific) Pte. Ltd
www.shimadzu.com.sg

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
Company names, products/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation, its subsidiaries or its affliates, whether or not they are used with trademark symbol "TM" or "®".
Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu (Asia Pacific) Pte. Ltd, 2021 / First Edition: April 2021