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Liquid Chromatograph Mass Spectrometer LCMS™-9030

Application News

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

Udi Jumhawan[1], Chua Chun Kiang[1], Chia Shao Hua[1], Iida Tetsuo[2]
[1]Shimadzu (Asia Pacific) Pte Ltd, [2]Shimadzu Corporation Japan

User Benefits

- 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

■ Introduction

LC/HRMS is currently the most prominent analytical tool for untargeted analysis. Combination of soft ionization technique and versatile mass analyser, 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*.

Fig. 1 LCMS™-9030 Q-TOF

■ Measurement Conditions and Samples

A 500 mg 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 (Fig. 1).

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 and LabSolutions Insight Explore, respectively.

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

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

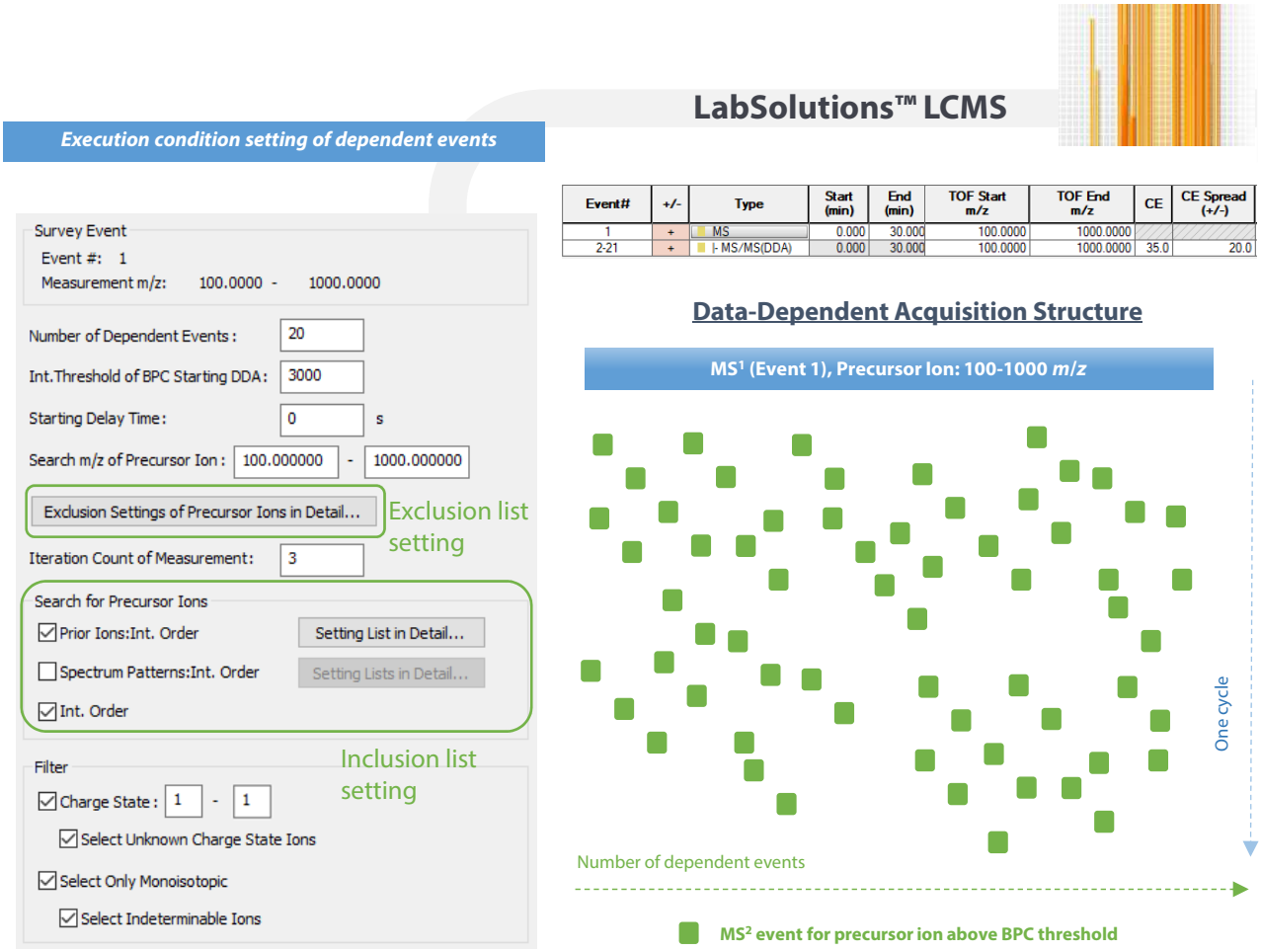


Fig. 2 LabSolutions™ LCMS software dialog and the structure of DDA data collection mode for LCMS-9030 Q-TOF

■ Results and Discussion

DDA Data Collection

DDA data acquisition was controlled by the LabSolutions LCMS software. Generic data collection was established using TOF survey scan (MS^1) 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 (Fig.2). Ion exclusion and inclusion settings are available in the LabSolutions LCMS software dialog to automatically exclude background ions and include ions of interest, respectively.

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 (Fig.3).

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 MS^1 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.

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.

LabSolutions Insight Explore™

Analyze

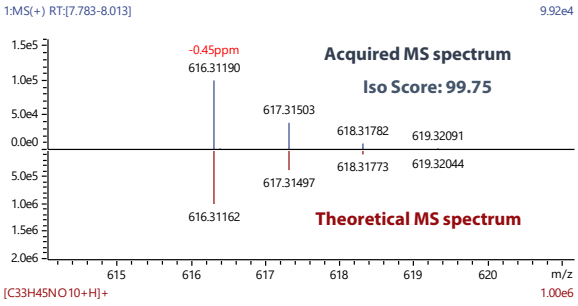
- **Component Detection**
  - The **Analyze** module automatically performs component detection (based on MS^1 or MS/MS)

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

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

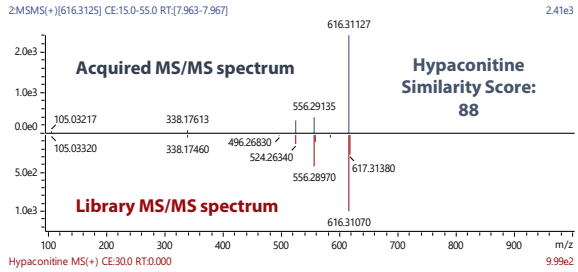
Formula Predictor

- **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



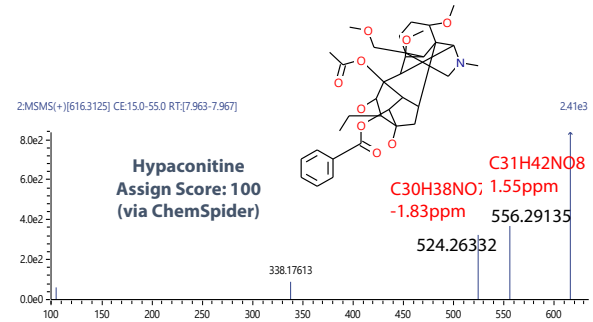
Library Search

- **Local Library Search**
  - The **Library Search** module enables matching of MS/MS spectrum to a curated local library for identification of unknown compounds

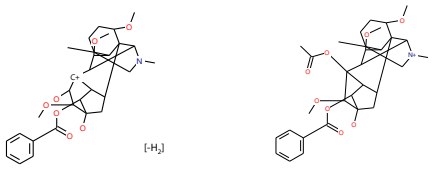


Assign

- **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



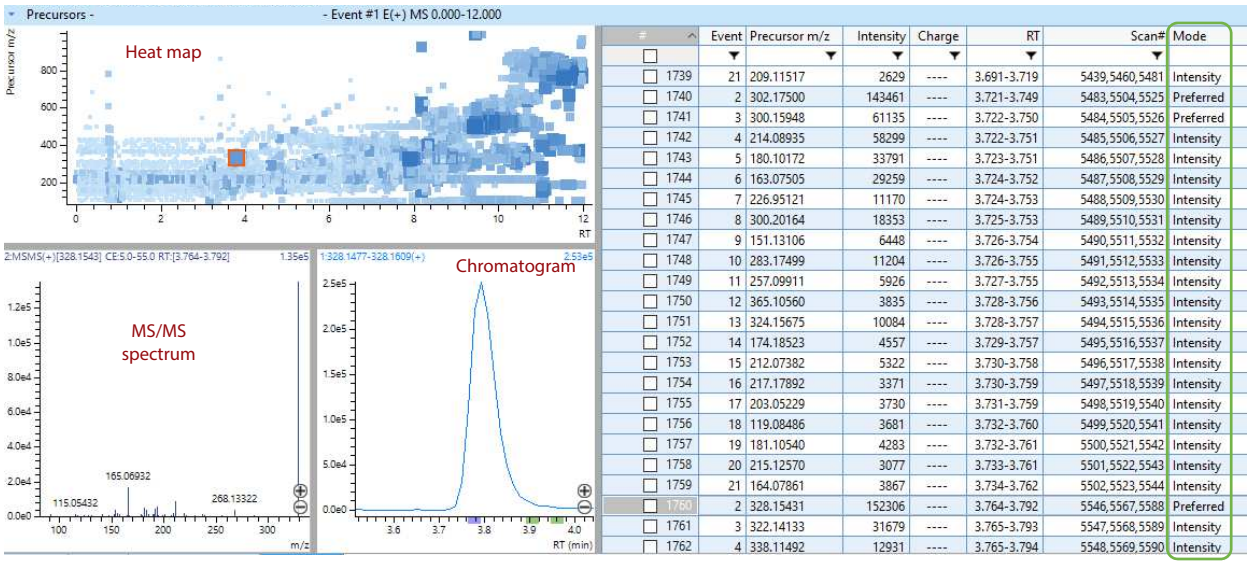
***m/z* 524.26332** ***m/z* 556.29135**

Fig. 3 DDA data processing workflow with LabSolutions Insight Explore™

LabSolutions Insight Explore™

Precursor

- **Precursor**
  - The **Precursor** module populates MS[1] and MS/MS data in a single window
  - Heat map provides overview of precursor ions' intensity distribution
  - It is designed to locate MS/MS spectra data for specific searches



#	Event	Precursor m/z	Intensity	Charge	RT	Scan#	Mode
1739	21	209.11517	2629	----	3.691-3.719	5439,5460,5481	Intensity
1740	2	302.17500	143461	----	3.721-3.749	5483,5504,5525	Preferred
1741	3	300.15948	61135	----	3.722-3.750	5484,5505,5526	Preferred
1742	4	214.08935	58299	----	3.722-3.751	5485,5506,5527	Intensity
1743	5	180.10172	33791	----	3.723-3.751	5486,5507,5528	Intensity
1744	6	163.07505	29259	----	3.724-3.752	5487,5508,5529	Intensity
1745	7	226.95121	11170	----	3.724-3.753	5488,5509,5530	Intensity
1746	8	300.20164	18353	----	3.725-3.753	5489,5510,5531	Intensity
1747	9	151.13106	6448	----	3.726-3.754	5490,5511,5532	Intensity
1748	10	283.17499	11204	----	3.726-3.755	5491,5512,5533	Intensity
1749	11	257.09911	5926	----	3.727-3.755	5492,5513,5534	Intensity
1750	12	365.10560	3835	----	3.728-3.756	5493,5514,5535	Intensity
1751	13	324.15675	10084	----	3.728-3.757	5494,5515,5536	Intensity
1752	14	174.18523	4557	----	3.729-3.757	5495,5516,5537	Intensity
1753	15	212.07382	5322	----	3.730-3.758	5496,5517,5538	Intensity
1754	16	217.17892	3371	----	3.730-3.759	5497,5518,5539	Intensity
1755	17	203.05229	3730	----	3.731-3.759	5498,5519,5540	Intensity
1756	18	119.08486	3681	----	3.732-3.760	5499,5520,5541	Intensity
1757	19	181.10540	4283	----	3.732-3.761	5500,5521,5542	Intensity
1758	20	215.12570	3077	----	3.733-3.761	5501,5522,5543	Intensity
1759	21	164.07861	3867	----	3.734-3.762	5502,5523,5544	Intensity
1760	2	328.15431	152306	----	3.764-3.792	5546,5567,5588	Preferred
1761	3	322.14133	31679	----	3.765-3.793	5547,5568,5589	Intensity
1762	4	338.11492	12931	----	3.765-3.794	5548,5569,5590	Intensity

Fig. 4 Precursor module in LabSolutions Insight Explore™

To populate precursor ions and locate specific data for specific search, the Precursor module is available in the Insight™ Explore (Fig.4). 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.

■ 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.

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www.shimadzu.com/an/

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