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Application News

HRAM for ID / LCMS™-9030 / LabSolutions Insight Explore™

A Demonstration of HRMS with CE Spread Function for Identification of Cytotoxic Contaminant Present in Marine Bacteria Extract for Natural Product Discovery

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User Benefits

- The flexible CE spread feature on LCMS™-9030 Q-TOF enriches the fragment ions of precursors, which enables to enhance the identification reliability of compounds.
- The LabSolutions Insight Explore s/w facilitates the identification workflow by automatically formula prediction, database search and fragment annotation for easy structural elucidation.

■ Introduction

Natural products from marine bacteria are the important source of new therapeutics in drug discovery in recent years. However, raw materials such as crude microbial extracts from where these natural products come from may contain contaminants that can cause harm to human health or give a false positive result in cytotoxicity assays. Hence, detection and identification of bioactivity compounds and contaminant analysis are crucial steps towards resolving suspected contamination of raw materials. Among various analytical instruments and technologies, mass spectrometry with fragmentation has been used to provide evidences in structural elucidation and identification of unknown compounds by GC-MS and LC-HRMS for decades. In this article, an easy-to-use and reliable approach for identification of compounds by LC-Q-TOF is described.

■ Experimental

A Shimadzu LCMS-9030 Q-TOF was employed for the sample analysis. Details of the analytical conditions are compiled in Table 1.

Analysis approach

Two factors are critical for unambiguous structural elucidation and identification of compounds by LC-Q-TOF: the number of fragments obtained in MS/MS and the annotation of fragment ions. The number of fragments of a precursor ion depends on the collision energy (CE). The traditional design of collision cell with a fixed CE during MS/MS measurement generates usually lesser number of fragments, which is a main obstacle in structural analysis. An ultra fast CE spread feature is introduced on the LCMS-9030, which can generate more fragments due to a wider range of CE applied on each MS/MS measurement. For tedious fragment annotation, the LabSolutions Insight Explore software enables fully automated assigning of the mass peaks via *in-silico* fragmentation. An easy data analysis approach as illustrated in Figure 1 involves obtaining formula from accurate mass of a precursor (< 1ppm), public database search (PubChem or ChemSpider) and fragment assigning (peak annotation) [1].

Table 1 Analytical conditions

LC Conditions	
Column	Shim-pack™ GIST C18 (2.1 X 100 mm, 2
Flow Rate	µm) 0.4 mL/min
Mobile Phase	A: Water with 0.1% formic acid
	B: Methanol with 0.1% formic acid
Elution mode	Isocratic 80% B – 20% A
Oven Temp.	40°C
Injection Vol.	1 µL
Interface Conditions (LCMS-9030)	
Interface	HESI 4.5 kV
Interface Temp.	300°C
DL Temp.	250°C
Heat Block Temp.	400°C
Nebulizing Gas Flow	2 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
Data acquisition (Q-TOF)	
MS mode (TOF)	Positive, *m/z* 100-600
MS/MS (Q-TOF)	6 precursors, *m/z* 50-500, CE: -25V spread (+/-)17V
Dwell time	0.05 sec / event
Loop time	0.35 sec / data point

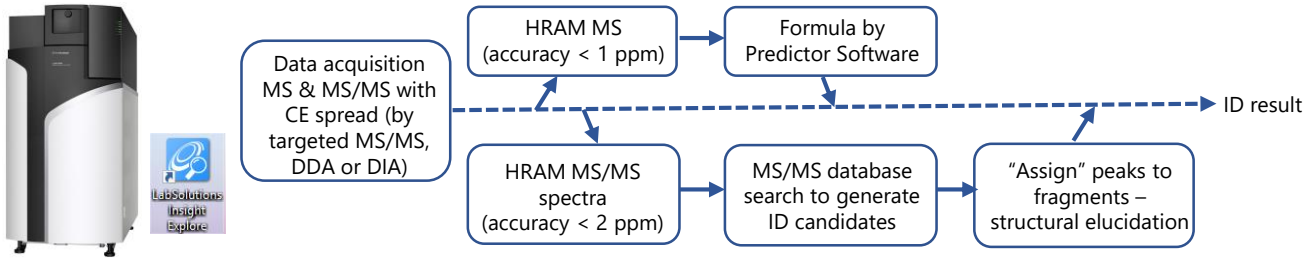

Figure 1 LCMS™-9030 with LabSolutions Insight Explore for identification & structural elucidation of unknown compounds

■ Results and Discussion

Results of MS and MS/MS of extract sample

An HPLC purified extract from marine bacteria (MBE) prepared in University of San Agustin [2] was used in this study. The sample contains a main compound and small amounts of other components as shown in MS TIC in Figure 2. The major compound P3 and the three minor components P1, P2 and P4 were detected in MS TIC firmly. The main ions of every peak were selected as the targeted precursors for MS/MS measurement. Details of the MS/MS parameters are shown in Table 2.

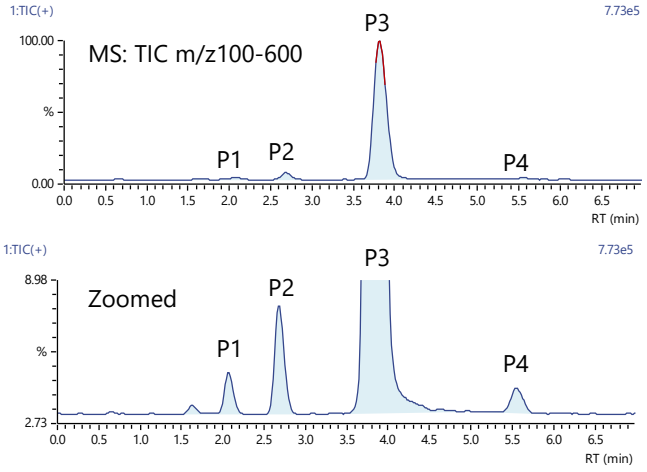

Figure 2 TIC of MBE sample on LCMS-9030

Table 2 MS & MS/MS parameters with CE spread settings

Event#	Type (+)	Precursor Ion (*m/z*)	TOF (*m/z*)	Targeted peak	CE & CE Spread
1	MS	N.A	100-600	All	N.A.
2	MS/MS	361.1861	50-500	P1	25±17
3	MS/MS	361.2223	50-500	P2	25±17
4	MS/MS	403.2338	50-500	P3	25±17
5	MS/MS	329.1598	50-500	P3	25±17
6	MS/MS	343.2117	50-500	P4	25±17
7	MS/MS	269.1382	50-500	P4	25±17

Prediction of Empirical formula

The mass accuracy of the TIC MS measurement was well maintained within 1 ppm with external standard mass calibration method [3]. The exact mass of the measured elution peaks as shown in Figure 2 were applied to the "Formula Predictor", which generates candidates of molecular formula with element settings including H, C, O, N, P and S. The formula (with highest score) that are most likely to indicate each component are listed in Table 3. As illustrated in Figure 1, these formulas are used as references to confirm the subsequent identification via MS/MS database search.

MS/MS spectra obtained with CE spread

In MS/MS measurements on Q-TOF, the fragmentation pattern of precursor ion depends on the CE value and other relevant factors such as collision gas and design of the collision cell. The most important variable factor is the CE voltage, which determines the numbers of fragments and their intensity. An ultrafast CE spread feature available on the LCMS-9030 allows to set range of CE for every MS/MS measurements. In this analysis method, CE of 25 V and CE spread of (+/-)17 V were used, which corresponds to an ultrafast ramping of CE from 8 V to 42 V for every collision process. As a result, more fragments that require different CE are obtained and combined into the same MS/MS spectrum. This CE spread collision (Table 2) has obvious advantages over the fixed CE instrument in qualitative analysis of unknown compounds because of more structural information being revealed. As can be seen in Figure 3, the MS/MS spectrum of P3 precursor ion (403.23) shows numerous fragments under the CE spread conditions (8~42 V). It is worth to note that the spectrum pattern can be optimized through settings of appropriate CE and CE spread values.

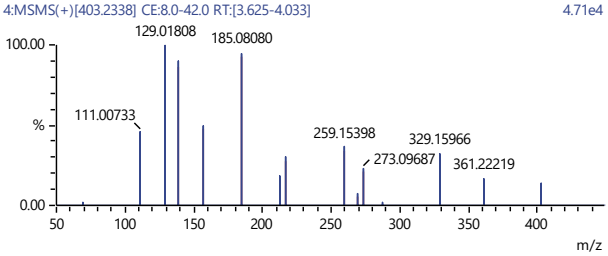

Figure 3 MS/MS spectrum of P3 main precursor (*m/z* 403.23) with CE spread setting of 8~42 V

Identification of compounds via database search and structural elucidation

The MS/MS spectrum of a targeted precursor contains structural information carried by the fragments and their accurate masses. The LabSolutions Insight Explore program has the function to send the MS/MS spectrum to database search such as ChemSpider or PubChem generating possible identification results with matching scores (Table 3, right portion). For each MS/MS database search, several structures are usually generated and listed down as candidates. In general, a higher Assign score indicates the higher probability for the best

Table 3 Summary of identification results of four components in MBE sample by HRAM on LCMS-9030 (error <1 ppm)

Peak	MS (TOF) Spectrum (error <1 ppm)						MS/MS TOF Spectra (error <2 ppm)		
	RT (min)	Area (%)	Measured [M+H]+ (*m/z*)	Formula	Diff. (ppm)	DBE*	No. of fragments	ID Result via DB search (ChemSpider)	Assign score (%)
P1	2.064	1.7	361.1858	$C_{17}H_{28}O_8$	0.415	4	11	Triisopropyl 2-acetoxy-1,2,3-propanetricarboxylate	100
P2	2.674	3.4	361.2223	$C_{18}H_{32}O_7$	0.712	3	8	Tributyl citrate	92.3
P3 (main)	3.809	94.4	403.2328	$C_{20}H_{34}O_8$	0.486	4	11	Tributyl acetyl citrate	91.7
P4	5.546	1.1	343.2116	$C_{18}H_{30}O_6$	0.162	4	5	Tributyl (E)-aconitate	87.5

** DBE: double bond equivalence*

Table 4 Annotation of fragment ions of P3 precursor using the Assign function in LabSolutions Insight Explore

#	m/z	Int. (%)	Formulae (M)	Charge	+/- ppm
0	403.2328	13.2	(precursor)		0.4
1	361.2222	15.8	C18H33O7	[+H]+	0.3
2	329.1597	34.5	C16H25O7	+	0.5
3	273.0969	21.4	C12H17O7	+	0.0
4	269.1383	6.9	C14H21O5	[+H]+	0.0
5	259.1540	34.3	C13H23O5	+	-0.1
6	217.0343	26.6	C8H9O7	+	-0.1
7	213.0757	17.2	C10H13O5	+	-0.3
8	185.0808	96.5	C9H13O4	[+H]+	-0.2
9	157.0129	43.2	C6H5O5	+	-1.6
10	139.0024	85.2	C6H3O4	+	-1.0
11	129.0181	100.0	C5H5O4	[+H]+	-1.3
12	111.0073	39.4	C5H3O3	+	-3.0

matching structure if the mass errors of the fragments are sufficiently small (e.g., < 2 ppm). The most-likely ID results of the four precursors of P1, P2, P3 and P4 are shown in Table 3 (right portion). It is worth to note that the identification result by MS/MS database search must be in accordance with the empirical formula obtained from formula predictor using the MS spectrum (Table 3, left portion).

Furthermore, the Assign function of the program annotates automatically all the fragment ions of every candidate to interpret the corresponding structure. The fragment annotations for P3 precursor (m/z 403.2328) are shown in Table 4 and few selected fragments are shown in Figure 4. This *in-silico* fragmentation provides an easy tool for the structural elucidation which is usually a very tedious and skill-required process.

The identification result for the main component (P3) by the current approach is tributyl acetyl citrate ($C_{20}H_{34}O_8$), which is supported by ^{1}H and ^{13}C NMR analysis [2]. However, the ID results for the low abundant P1, P2 and P4 are reported for reference. As shown in Figure 5, the P2 and P4 are likely the structural analogues of the main compound P3. The accurate masses of P1 and P2 are very closed (m/z 361.1858 and m/z 361.2223). However, their structures are substantially different.

■ Conclusion

An easy approach for impurity identification by HRAM on LCMS-9030 is demonstrated with an extract from marine bacteria (MBE). The flexible CE spread feature allows MS/MS producing more fragments, which is critical for ID and structural elucidation. The LabSolutions Insight Explore provides a powerful tool to facilitate the data analysis from formula prediction on MS (<1 ppm) to MS/MS (<2 ppm) database search and fragment annotations for structural elucidation.

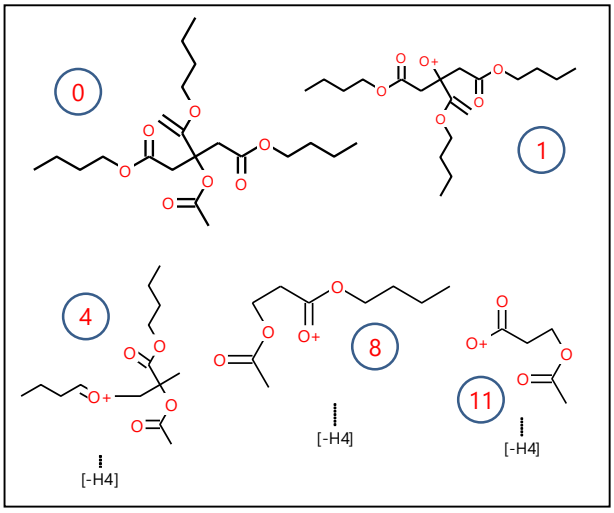

Figure 4 Representative fragment ions interpreted by the Assign function (structure # refers to Table 4)

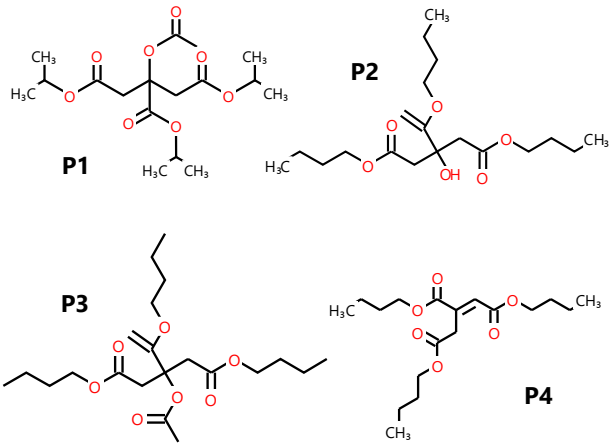

Figure 5 Identification results of MBE sample. P3 is confirmed as tributyl acetyl citrate. (Refer to Table 3 for others)

■ References

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