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Application News

LCMS™-9050 High Performance Liquid Chromatograph Mass Spectrometer

Qualitative Analysis of the Components in Foods Using DART and the Quadrupole Time-of-flight Mass Spectrometer

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User Benefits

- The system can perform analysis without pretreatment, regardless of whether the sample is solid, liquid, or gas.
- Qualitative analysis can be performed with high mass accuracy, using positive/negative ion polarity switching without mass calibration by internal standard.
- Formula estimates, compound searches, and fragment attribution analysis can be performed by this workflow.

■ Introduction

In developing food and beverage products, obtaining information on the constituent components is important for maintaining and improving quality. Among them, the aroma constituents are attracting attention as an index of flavor. Normally, however, when measuring specific components in foods and beverages, extraction and other troublesome pretreatments are often necessary.

Direct Analysis in Real Time (DART®) is a method for the direct ionization of samples. It is effective for simple screening because it enables measurements to be performed quickly without pretreatment regardless of whether the sample is solid, liquid, or gas. In this article, the combination of DART-OS (IonSense, Inc.) and the LCMS-9050 quadrupole time-of-flight mass spectrometer (Q-TOF) was used to measure the components in chocolate (Fig. 1). Structural analysis of the components in the sample was performed with high mass accuracy, using the LabSolutions Insight Explore analysis software based on the MS/MS spectral information obtained.

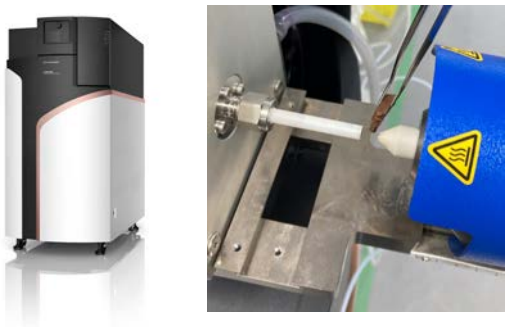


Fig. 1 Left: Exterior of LCMS™-9050, Right: Situation of Measurement

■ Direct Analysis of Chocolate

Commercially available chocolate was pinched by tweezers and then measured by suspending it over the DART ion source. The analysis conditions are shown in Table 1. Helium gas was used for ionization.

The measurement time (horizontal axis) and the peak intensity (vertical axis) are shown in Fig. 2. Peak increases were only observed while the sample was suspended over the DART ion source.

Table 1 Analysis Conditions

DART Heater Temp.:	350 °C
DL Temp.:	250 °C
BH Temp.:	400 °C
MS Scan Range:	*m/z* 100 - 1500 (Positive, Negative)
MS/MS Scan Range:	*m/z* 10 - 1500 (Positive, Negative)
Collision Energy:	35 V
Collision Energy Spread (±):	17 V

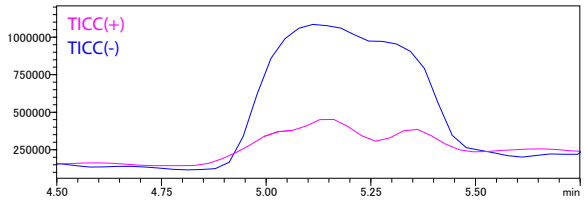


Fig. 2 Measurement Time (Horizontal Axis) and Peak Intensity (Vertical Axis)

Chocolate was measured in positive/negative ion polarity switching mode, and the mass spectra obtained are shown in Fig. 3. In the mass spectra, the peaks observed were for components A (*m/z* 181.0720) and B (*m/z* 195.0876) in positive mode, and components C (*m/z* 151.0396), D (*m/z* 255.2326), and E (*m/z* 281.2482) in negative mode.

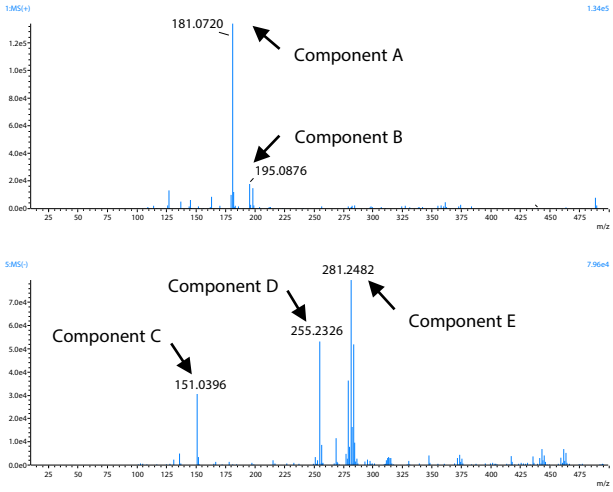
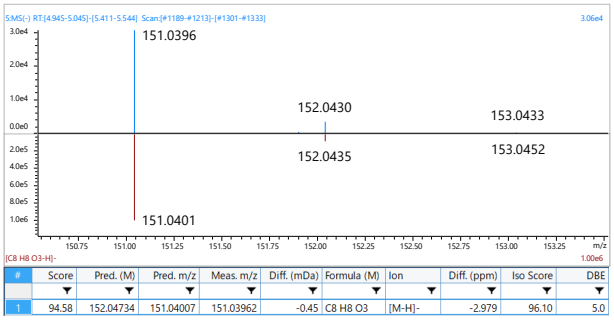


Fig. 3 Mass Spectra for Components in Chocolate
(Top: Positive Mode Measurements; Bottom: Negative Mode Measurements)

■ Formula Estimates

LabSolutions Insight Explore was used to perform formula estimates based on the mass spectral information obtained. Here, as an example, the result of a formula estimate of component C (*m/z* 151.0396) is shown in Fig. 4.

The only candidate formula suggested for component C was $C_8H_8O_3$. Similarly, $C_7H_8N_4O_2$, $C_8H_{10}N_4O_2$, $C_{16}H_{32}O_2$ and $C_{18}H_{34}O_2$ were estimated as the formulas for components A, B, D and E, respectively. For each component, the compositional formula estimates had high mass accuracy, with a mass error of 1 mDa max.



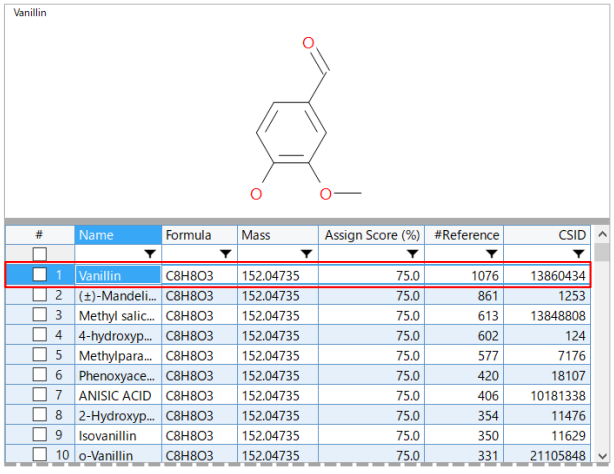
#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	94.58	152.04734	151.04007	151.03962	-0.45	C8 H8 O3	[M-H]-	-2.979	96.10	5.0

Fig. 4 Formula Estimation Results for Component C
(Top: Measured Mass Spectrum; Middle: Theoretical Spectrum;
Bottom: Compositional Formula Candidate)

■ Compound Searches and Fragment Attribution

The LabSolutions Insight Explore "Assign" function was used to perform further structural estimates for component C. Firstly, compounds matching with the formula were listed from an online search based on the ChemSpider database. Next, for the compounds obtained, the degree of congruency ("Assign score") between the product ions observed in the measured MS/MS spectrum and the product ions obtained from fragment estimation was calculated by running the "Assign" function.

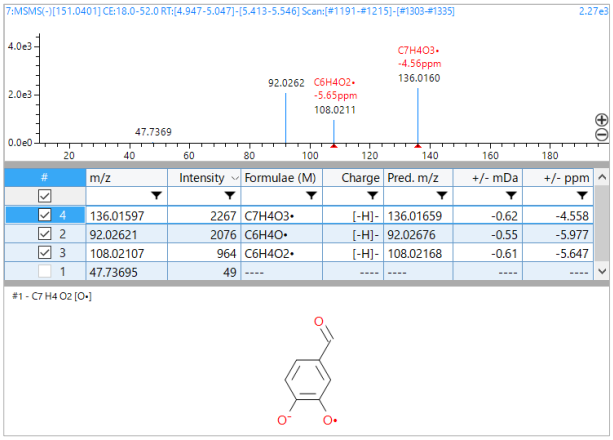
For $C_8H_8O_3$, the formula for compound C, 750 compounds were suggested in the results of running an online search. Further, for the MS/MS spectrum for component C, from the results of running the "Assign" function, vanillin (ChemSpider ID 13860434) was suggested as the compound with the highest assign score and ChemSpider reference number (#Reference). (Fig. 5) In this way, using the "Assign" function makes it possible to narrow down structural formulas and compound names from a large number of candidate compounds.



#	Name	Formula	Mass	Assign Score (%)	#Reference	CSID
1	Vanillin	C8H8O3	152.04735	75.0	1076	13860434
2	(±)-Mandeli...	C8H8O3	152.04735	75.0	861	1253
3	Methyl salic...	C8H8O3	152.04735	75.0	613	13848808
4	4-hydroxyp...	C8H8O3	152.04735	75.0	602	124
5	Methylpara...	C8H8O3	152.04735	75.0	577	7176
6	Phenoxyace...	C8H8O3	152.04735	75.0	420	18107
7	ANISIC ACID	C8H8O3	152.04735	75.0	406	10181338
8	2-Hydroxyp...	C8H8O3	152.04735	75.0	354	11476
9	Isovanillin	C8H8O3	152.04735	75.0	350	11629
10	o-Vanillin	C8H8O3	152.04735	75.0	331	21105848

Fig. 5 Compositional Formula $C_8H_8O_3$ Online Search Results

Next, an example of the automatic attribution of the fragment ions using the assign function is shown in Fig. 6.



#	m/z	Intensity	Formulae (M)	Charge	Pred. m/z	+/- mDa	+/- ppm
4	136.01597	2267	C7H4O3•	[-H]-	136.01659	-0.62	-4.558
2	92.02621	2076	C6H4O•	[-H]-	92.02676	-0.55	-5.977
3	108.02107	964	C6H4O2•	[-H]-	108.02168	-0.61	-5.647
1	47.73695	49	----	----	----	----	----

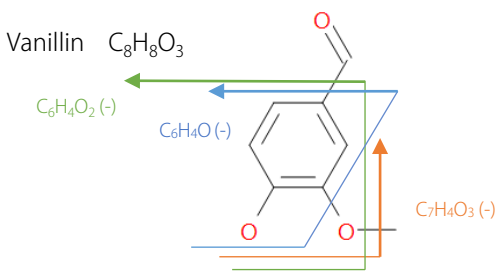


Fig. 6 Fragment Attribution Results for Component C

Finally, the analysis results for each component are shown in Table 2. Components A and B were considered to be theobromine and caffeine contained in cacao, respectively. Components D and E were considered to be palmitic acid and oleic acid, respectively, both of which are free fatty acids. All five components were measured with a mass error of less than 1 mDa compared with the theoretical mass values.

Table 2 Mass Accuracy for Components A, B, C, D, and E from Chocolate

Compo-nent	Compound Name	Formula Estimate Results	Score	Ion Type	Theoretical *m/z*	Measured *m/z*	Error (mDa)
A	Theobro-mine	$C_7H_8N_4O_2$	99.26	$[M+H]^+$	181.0720	181.0720	±0
B	Caffeine	$C_8H_{10}N_4O_2$	75.57	$[M+H]^+$	195.0877	195.0876	-0.1
C	Vanillin	$C_8H_8O_3$	94.58	$[M-H]^-$	151.0401	151.0396	-0.5
D	Palmitic acid	$C_{16}H_{32}O_2$	97.21	$[M-H]^-$	255.2330	255.2326	-0.4
E	Oleic acid	$C_{18}H_{34}O_2$	98.31	$[M-H]^-$	281.2486	281.2482	-0.4

■ Conclusion

The combination of DART and the LCMS-9050 Q-TOF mass spectrometer is capable of the direct analysis of a sample quickly and without pretreatment. Furthermore, reliable results with high mass accuracy were obtained by formula estimation and structural analysis for the measured components using the analysis software LabSolutions Insight Explore. This workflow can be applied to the analysis of components in foods and products in other fields, including chemical products and pharmaceuticals.

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