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Application News

CLAM™-2030 Fully Automated Sample Preparation Module for LCMS
LCMS-8060 Liquid Chromatograph Mass Spectrometer

Measuring Choline and its Metabolites in Human Plasma Using an LC/MS/MS System with Fully Automated Sample Preparation Module

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User Benefits

- Capable of quantifying choline, TMA, and TMAO levels in biological samples
- Performs time-consuming sample preparation automatically.
- Eliminates the need for manual sample preparation and reduces variability in quantitative results due to manual operations.

■ Introduction

It is known that some of the choline ingested in food is partly metabolized to trimethylamine (TMA) by intestinal bacteria and subsequently metabolized to trimethylamine N-oxide (TMAO) by enzymes in the liver. Reports have noted that TMAO is associated with cardiovascular diseases such as arteriosclerosis[1)] and that it is useful in predicting the prognosis of heart failure[2)], As a result, TMAO is now attracting interest as a new biomarker. This article describes a system for simultaneous analysis of choline and choline metabolites in human plasma using an LC/MS/MS system with fully automated sample preparation module (Fig. 1) that automatically performs deproteination of biological samples and adds internal standards.

Fig. 1 LC/MS/MS System with Fully Automated Sample Preparation Module (CLAM™-2030 + LCMS-8060)

■ Fully Automated Sample Preparation of Choline and Choline Metabolites in Plasma

The CLAM-2030 performs deproteination and other sample preparation steps automatically by simply setting the blood collection tubes directly into the system. Fig. 2 shows the sample preparation process using the CLAM-2030. Sample analysis by the LC/MS/MS system and preparation of the next sample are performed in parallel. Hence the time required per sample can be reduced substantially. The analysis cycle time from plasma sample preparation to simultaneous analysis of choline and choline metabolites by LC/MS/MS was about 5 min per sample.

■ Sample Preparation

Calibration curves were created from standard samples prepared by serial dilution of choline (1 to 1000 nmol/L), TMA (10 to 1000 nmol/L), and TMAO (10 to 10000 nmol/L) with water. Water was also used to prepare 2 µmol/L samples of choline-d13, TMA-d9, and TMAO-d9 to use as ISTDs. Choline, TMA, and TMAO levels in plasma were quantified using commercially available human plasma.

The samples were pretreated with the CLAM-2030, analyzed automatically by the LCMS-8060, then calibration curves were created, and analyte levels in the human plasma were determined.

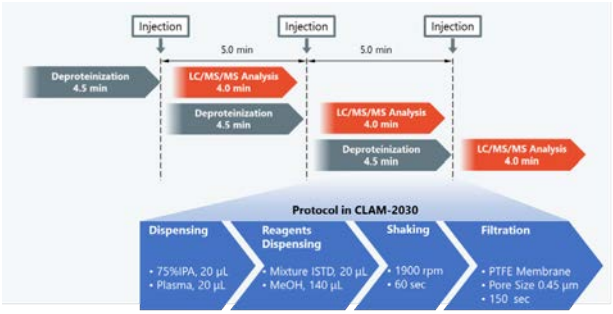

Fig. 2 Preparation of Plasma Samples Using CLAM-2030

■ Analytical Conditions

HPLC and MS analytical conditions are shown in Table 1. The MRM transitions for each compound are shown in Table 2.

Table 1 HPLC and MS Analytical Conditions

Liquid Chromatograph	
System:	Nexera™ X2
Column:	Shim-pack Velox HILIC (50 mm x 2.1 mm I.D., 2.7 µm)
Temperature	40 °C
Injection Volume:	3 µL
Mobile Phase A:	50 mM ammonium formate +0.1 % formic acid—Water
Mobile Phase B:	Acetonitrile
Flowrate:	0.4 mL/min
Gradient Program (B conc%):	70 % (0.0 min) ➡ 30 % (2.40 min) ➡ 5 % (2.41 – 3.00 min) ➡ 70 % (3.41 – 4.00 min)

Mass Spectrometer	
System:	LCMS-8060
Ionization:	ESI (Positive)
Nebulizing Gas:	3 L/min
Drying Gas:	10 L/min
Heating Gas:	10 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	OFF

Table 2 MRM Transitions for Choline and Choline Metabolites

Compound Name	Ion	Precursor ion (*m/z*)	Product ion (*m/z*)
Choline	Quantitative Ion	104.10	60.10
	Reference ion	104.10	45.10
TMA	Quantitative Ion	60.20	44.15
	Reference ion	60.20	45.05
TMAO	Quantitative Ion	76.00	58.20
	Reference ion	76.00	59.10
Choline-d13	Quantitative Ion	117.25	66.05
TMA-d9	Quantitative Ion	68.90	49.15
	Reference ion	68.90	51.15
TMAO-d9	Quantitative Ion	85.20	66.00
	Reference ion	85.20	68.25

■ Checking Calibration Curve Linearity and Reproducibility

Linearity was confirmed after calibration curve standard samples were analyzed three times. The calibration curve range, coefficient of determination (R^2), and reproducibility (concentration %RSD) and accuracy of the lowest calibration curve sample concentration are shown for each compound in Table 3. Fig. 3 also shows the calibration curves for each compound and Fig. 4 shows mass chromatograms for standard samples of each compound.

Table 3 Analysis Results from Standard Samples (n = 3)

Compound Name	Calibration Curve Range (nmol/L)	Coefficient of Determination R^2	Concentration %RSD	Accuracy (%)
Choline	1-1000	0.999	14 %	86 %
TMA	10-1000	0.997	12 %	95 %
TMAO	10-10000	0.999	2 %	96 %

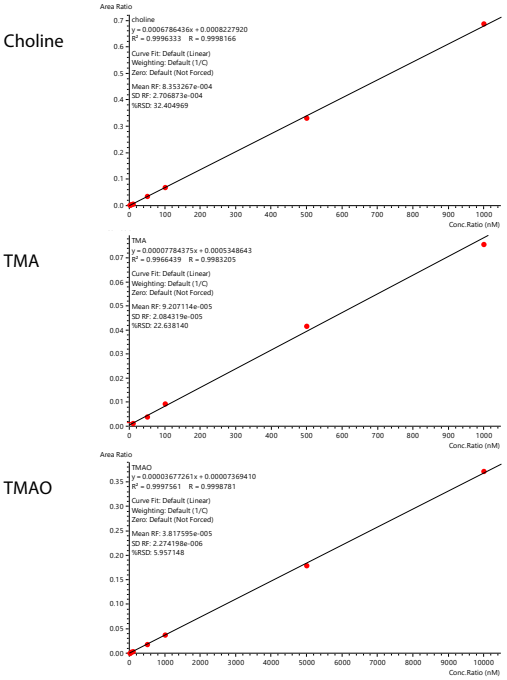

Fig. 3 Calibration Curves for Each Compound
Top: Choline 1 to 1000 nmol/L, Middle: TMA 10 to 1000 nmol/L,
Bottom: TMAO 10 to 10000 nmol/L

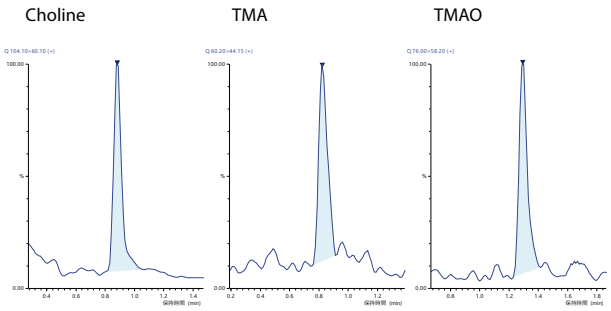

Fig. 4 Mass Chromatograms for Standard Samples of Each Compound
Left: Choline (1 nmol/L), Middle: TMA (10 nmol/L),
Right: TMAO (10 nmol/L)

■ Measuring Choline, TMA, and TMAO Levels in Human Plasma

Choline, TMA, and TMAO concentrations measured in human plasma samples (n = 3) prepared by CLAM-2030 are shown in Table 4, and the mass chromatograms of each component are shown in Fig. 5. Good reproducibility was obtained with human plasma.

Table 4 Quantitative Results from Human Plasma (n = 3)

Compound Name	Measured Concentration (nmol/L)	Concentration %RSD
Choline	938	2.8 %
TMA	685	1.1 %
TMAO	655	4.4 %

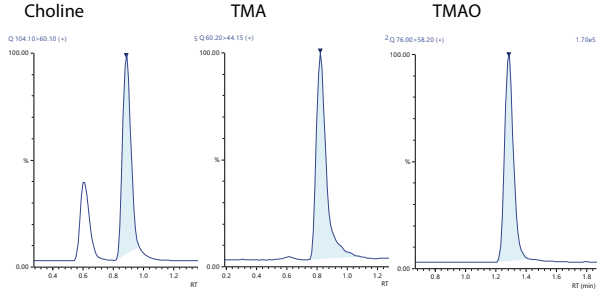

Fig. 5 Mass Chromatograms of Choline, TMA, and TMAO in Human Plasma

■ Conclusion

Sample preparation was performed using the CLAM-2030, which automatically performs deproteination of biological samples and adds internal standards. Choline, TMA, and TMAO levels in human plasma were quantified using LCMS-8060. The calibration curves for each compound showed good linearity and no reproducibility problems were encountered with human plasma samples, indicating how highly useful this analysis system is.

<References>

1) Wang Z, Stanley L Hazen et al., "Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease", *Nature* 472 (2011) 57-63.

2) Yazaki Y, Aizawa K et al., "Ethnic difference in association of outcomes with trimethylamine N-oxide in acute heart failure patients", *ESC Heart Fail* 7(5) (2020) 2373-2378.

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