Quantitative of TMAO and Precursor in Urine and Plasma by LC-MS/MS

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Introduction

Liquid Chromatography - Mass Spectrometry is a highly reliable analytical chemistry technique that combines chromatography for separating mixtures with mass spectrometry for determining molecular weights. LC-MS/MS is regarded as one of the most accurate measurement method available. In clinical diagnostics, LC-MS/MS stands out for superior specificity compared to other techniques, such as immunoassays. We have developed a simplified LC-MS/MS method capable of simultaneously analyzing TMAO, choline, and betaine in human plasma and urine, thereby supporting biomarker research for clinical diseases.

Trimethylamine N-oxide (TMAO) is a bioactive metabolite produced by gut microbiota from dietary sources. Recent studies linked elevated plasma levels of TMAO to various diseases, including atherosclerosis, hypertension, and metabolic disorders such as diabetes and hyperlipidemia, all of which contribute to vascular dysfunction. As TMAO is recognized as a biomarker for chronic diseases, its detection in plasma is essential for early diagnosis and disease management.

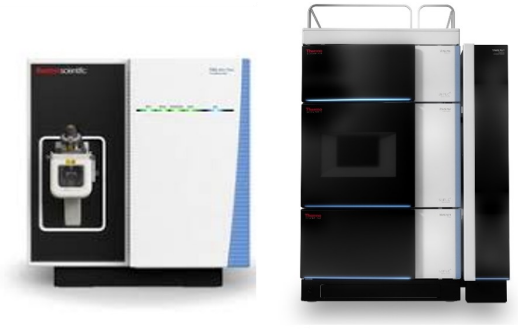


Fig. 1 Mass Spectrometry and UHPLC by Thermo Fisher Scientific

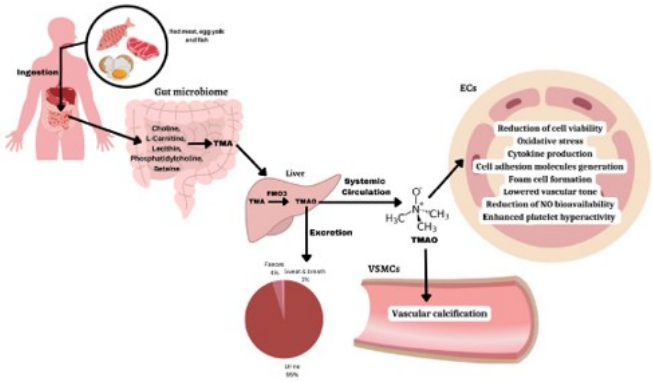


Fig.2 Biochemical Pathways of TMAO

Desorption, Clean-up and LC Conditions

Column	Acclaim™ Mixed-Mode HILIC-1 (2.1x150mm) 3µm
Column Temp.	40 °C
Mobile phase	A: 10 Mm Ammonium formate B: Methanol
Data acquisition mode	Selected ion monitoring (SRM)

MS Conditions

Ion source	H-ESI
vaporizer temperature	350 °C
Spray voltage	3500 V
Sheath gas and auxiliary	60 and 10 (arbitrary units),
Collision gas (argon) pressure	1.5 mTorr.

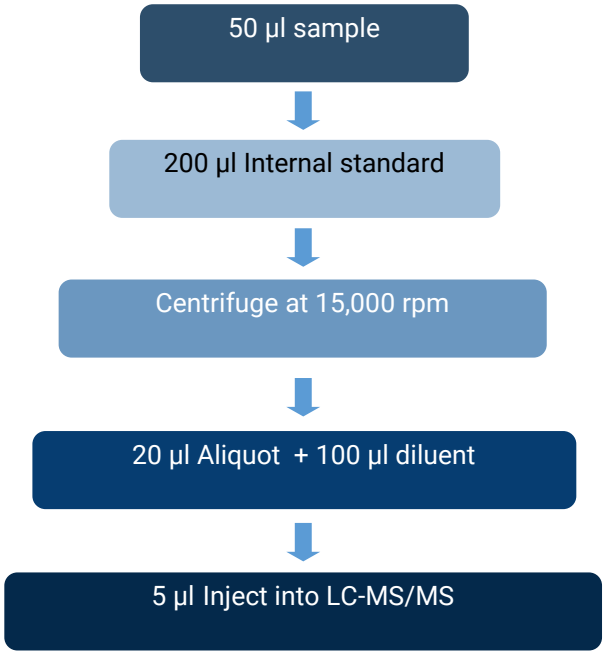
SRM transition and Collision Energy

Compound	Precursor (m/z)	Product (m/z)	CE (v)	RF (v)
TMAO	75.9	42.217	38.16	41
Betaine	117.983	42.133	55.77	61
Choline	104.05	44.967	22.19	53
TMAO-D9*	84.983	66.133	21.83	44
TMA-D9*	69.05	49.05	24.69	46

** ISTD (D-substitute)*

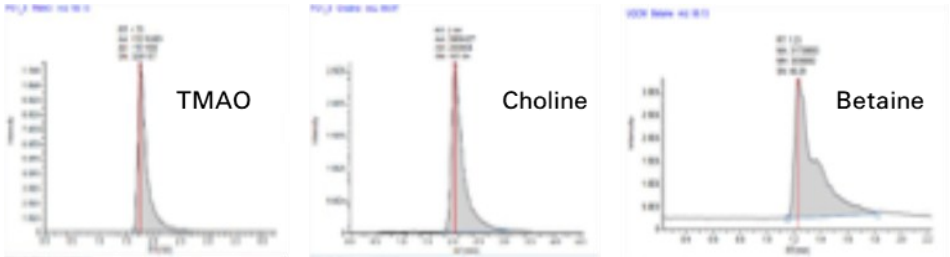
Sample Preparation

Protein precipitation was carried out, followed by the addition of 200 µL of the internal standard solution to each calibration, QC, and unknown sample (50 µL). The mixture was briefly vortex-mixed and then centrifuged at 10,000 × g for 10 minutes. After centrifugation, 20 µL of the clear supernatant was collected and diluted with 100 µL of a 75:25 acetonitrile:methanol solution, followed by a brief vortex mix. Finally, a 5 µL aliquot of the prepared sample was injected into the LC-MS/MS for analysis.



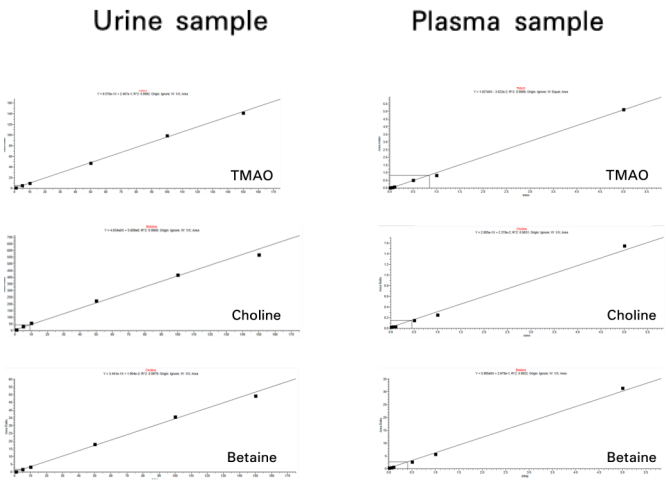
Result

LC-MS/MS Chromatogram



Calibration curve

The standard curves for each sample type were determined separately for urine and plasma samples. The linearity of both sample types is shown in the graph below, with the standard curves for both samples having an R^2 value greater than 0.99.



Signal Response

The lowest detectable concentration is 0.01 ppm. with the Signal-to-Noise (S/N) ratio for the lowest concentration presented in the table below.

Compound	Concentration (ppm)	Signal/Noise ratio (S/N)
Betaine	0.01	35
Choline	0.01	3.5
TMAO	0.01	54

Conclusion

We have developed a standardized method for analyzing TMAO, betaine, and choline in plasma and urine. This method offers a simple sample preparation process, high accuracy, and is well-suited for both research and clinical applications that require rapid and straightforward detection. Currently, this method is being used to support clinical research on disease biomarkers in medical studies.

Acknowledgment

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Reference

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- Andrew J. Ocquea, Jason R. Stubbsb, Thomas D. Nolina. Development and validation of a simple UHPLC–MS/MS method for the simultaneous determination of trimethylamine N-oxide, choline and betaine in human plasma and urine. Journal of Pharmaceutical and Biomedical Analysis 109 (2015) 128–135.