A validated and reproducible LC-MS/MS analytical method to quantify an emerging cardiovascular risk biomarker, trimethylamine N-Oxide (TMAO), in human plasma

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Introduction and Aim: The interplay between nutrition patterns, gut microbiota-derived metabolites and cardiovascular diseases (CVDs) has recently become a field of intensive research. Gut microbiota-derived metabolites represent an attractive source of biomarkers for CVDs, including atherosclerotic cardiovascular disease (ASCVD) and metabolic diseases. Trimethylamine (TMA), a gut-derived metabolite, undergoes oxidation in the liver, resulting in trimethylamine N-oxide (TMAO). Recent evidence from *in vivo* studies and clinical trials has correlated TMAO with increased CVD risk and occurrence due to the atherogenic potential of TMAO. TMAO has been quantified by exploiting mass spectrometry (MS) techniques, coupled either with liquid (LC) or gas chromatography (GC), and nuclear magnetic resonance (NMR) spectroscopy. However, standardization parameters and conditions still represent a critical issue when dealing with data reproducibility and robustness.
Hence, this study aimed at developing a validated and highly reproducible method for the quantification of TMAO in human-derived plasma.
Methods: A high-performance LC (HPLC) system (Shimadzu) coupled to tandem MS (MS/MS) (Sciex QTRAP 6500+) was selected, along with multiple reaction monitoring (MRM) modality and electrospray ionization (ESI) in positive polarity. Calibration curve was obtained spiking a healthy subject serum sample with different TMAO concentrations in the ng/mL range.
Results and Discussion: The HPLC-MS/MS method was optimized according to the different MS parameters and by selecting the most appropriate column for HPLC assessment. Method validation was performed evaluating the intra-/inter-assay accuracy and precision. The coefficient of variation (CV%) always resulted below 20%.
Conclusion: The HPLC-MS/MS method showed to be robust and reproducible according to standardization requirements, and useful to assess ASCVD risk in a cohort with subclinical atherosclerosis, food intake record and microbiome evaluation.[1]
Relevance to AtheroNET: TMAO data from characterized CVD patient cohorts, obtained in a reproducible and standardized manner, could be integrated by artificial intelligence/machine learning-based approach to improve CVD risk stratification toward precision medicine.

[1]Baragetti,A, et al. Gut Microbiota Functional Dysbiosis Relates to Individual Diet in Subclinical Carotid Atherosclerosis. Nutrients 2021, 13, 304. https://doi.org/ 10.3390/nu13020304.