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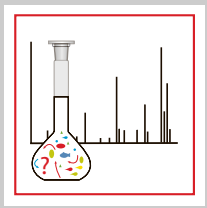

Highlights of Analytical Sciences in Switzerland

Division of Analytical Sciences

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Quantification of Quaternary Ammonium Compounds Against Surrogate Matrix by HILIC-MS/MS

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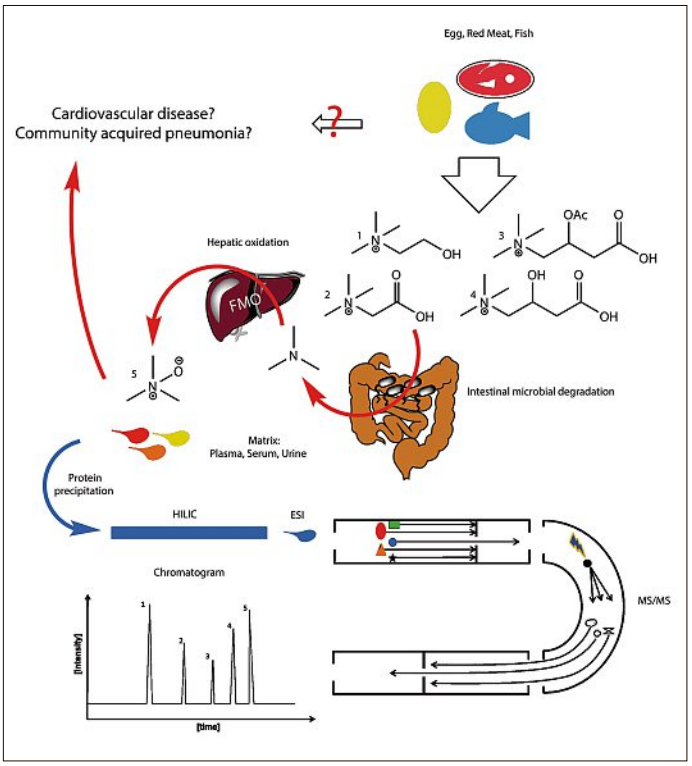
Keywords: Community acquired pneumonia (CAP) · HILIC · HPLC · MS/MS · Surrogate matrix · Trimethylamine-*N*-oxide (TMAO)

Metabolites like choline and its oxidized form betaine as well as other closely related quaternary ammonium compounds like L-carnitine and *O*-acetyl-L-carnitine, play a pivotal role in the synthesis of membrane phospholipids, energy metabolism, and as methyl group donators in a number of biosynthetic reactions. Choline, betaine, L-carnitine, and *O*-acetyl-L-carnitine are the main ingredients found in red meat, fish and many life-style products. Numerous studies have highlighted the protective effect of L-carnitine, choline and betaine on cardiac metabolism and performance. However, recent publications described the intestinal breakdown of choline, betaine and L-carnitine to trimethylamine by intestinal microorganism and subsequent oxidation to trimethylamine-*N*-oxide (TMAO) by flavin-monooxygenases (FMO) in the liver. Several researchers investigated the importance of TMAO in predicting cardiovascular events.

Due to the high polarity and low volatility of TMAO and its precursor, analysis is predominantly done by HPLC. As solid support, mostly hydrophilic interaction liquid chromatography (HILIC-), C4- or Phenyl-columns are used. In our opinion, HILIC chromatography seems to be the best method for the separation of the above-mentioned analytes. As solvent, a mixture of acetonitrile and water was used with ammonium formiate as buffer showing less background noise compared to ammonium acetate. After protein precipitation with acetonitrile and centrifugation, the supernatant was directly injected. Method validation was done according to international guidelines showing good results for selectivity, recovery and matrix effects. Calibration and quality control samples were prepared in water as surrogate matrix. Standard addition control experiments were performed to assure accuracy for non-matrix-matched calibration. The final calibration model was linear for all analytes.

The method was developed for analysis of plasma, serum and urine samples after overnight fasting. For urine samples, a 5-fold pre-dilution was necessary. In a well-defined study group, we could highlight a close association of TMAO with long-term fatal outcomes in CAP patients without coronary artery disease.



"You are what you eat": from plate to reader.

This LC-MS/MS multi-analyte approach allows the simultaneous and precise quantification of TMAO and its precursors in selected human fluids. Standard addition experiments for all matrices showed good correlation with calibrators prepared in water as surrogate matrix.

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