DEVELOPMENT OF A GC-MS BASED METABOLOMICS METHOD FOR THE ANALYSIS OF BLOOD SAMPLES FROM CHILDREN WITH VENTILATOR ASSOCIATED PNEUMONIA

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ABSTRACT

Metabolomics, the popular modern approach to screening large numbers of low molecular mass compounds in biological samples, has been successfully applied in different fields of research such as biomarker discovery and personalized medicine studies. Metabolomics has evolved greatly over the last years, although limitations on method standardization including reproducibility and validation need to be addressed. Improvements on GC-MS methods and instrumentation, and existence of commercial and publicly available spectral libraries render GC-MS an important tool for metabolomics applications. This study covers the development and optimisation of an untargeted metabolomics method for the analysis of whole blood samples by GC-MS after the application of a two-step derivatization procedure. The first step includes methoximation using methoxyamine hydrochloride and the second one involves silylation using N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The stability of the derivatives was examined for a 24-hour stay in the autosampler and for storage in the freezer (-22^{o}C) for up to 48 hours. Additionally, the storage of dried extracts for up to 48 hours, before derivatization was investigated. Acquired data from the analysis of 121 standard compounds were used to ensure Level 1, MSI identification, of the metabolites. The untargeted method was employed for the analysis of blood samples from children with ventilator-associated pneumonia (VAP), resulting in the identification of 43 metabolites. Multivariate statistical analysis revealed statistically significant metabolites such as aspartic acid, alanine and pyroglutamic acid that correlated with the suspicion of the disease and could possibly contribute to early diagnosis of VAP. The method can find wide applicability in the context of metabolomics for clinical studies.

KEYWORDS: GC-MS, Metabolomics, Blood, Ventilator associated pneumonia

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