https://doi.org/10.62422/978-81-970328-7-5-028

The Development and Validation of a GC-MS Method to Quantify Short and Branched Chain Fatty Acids in Human Stool and Applied to Patients with Inflammatory Bowel Disease and Healthy Controls

Justin Gray[1], Baochuan Guo[2], Rebecca Bearden[3]
[1, 2, 3]Department of Chemistry, Cleveland State University, Cleveland, OH USA

The study of short (SCFAs) and branched chain fatty acids (BCFAs) in human stool related to gastrointestinal diseases, gut microbiota, metabolism and diet has dramatically increased. As a result, a fast, reliable method with minimal pretreatment is needed for quantification of these metabolites (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acid; µg/g stool) in stool. Therefore, a GC-MS method meeting this criterion was developed. Stool samples were homogenized, diluted with 80:20 water:methanol (v/v) and adjusted to a pH of 1.5 - 2.5. Samples were vortexed, centrifuged and directly injected into the GC-MS using pulsed splitless injection offering two-to-three-fold signal enhancement over a 10:1 split injection. DB-FATWAX Ultra Inert Polyethylene Glycol (PEG) Column showed no peak tailing, reduced responses or retention time shifts after 1476 stool injections while other columns failed before 361 injections. A case-control study was conducted using 53 remnant raw stool samples with a positive diagnosis of either ulcerative colitis (UC) or Crohn's Disease (CD) which comprised the Inflammatory Bowel Disease (IBD) group and were compared to a control group of 21 samples for SCFA and BCFA concentrations. Strong statistical differences were observed between groups whereas the IBD group contained less propionic, butyric and valeric acid ($p < 0.05$). A receiver operator curve was plotted using the sum of significantly different SCFAs normalized against acetic acid resulting in 96% AUC (95% CI: 0.89 – 0.98) demonstrating potential diagnostical application.

Biography:

My general research is focused on understanding the use of short (SCFAs) and branched chain fatty acids (BCFAs) as diagnostical biomarkers for those with IBD. The development and validation of a fast, fully validated GC-MS method has enabled reliable quantification of these analytes in stool which has lacked due to time-consuming pre-treatment methods. Understanding the role between SCFAs and BCFAs, the gut microbiota, probiotics and how indigestible fibers impact pathophysiology of IBD is of great interest.