

GC-MS Orbitrap and GC×GC-(HR)TOFMS in Colorectal Cancer Metabolomics

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Colorectal cancer globally affects more than one million new persons each year, and kills more than 700.000. Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one of the approaches that can be used to solve these issues.

To do so, optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS and newly developed GC-MS Orbitrap were applied, along with an in-house QC system based on all study samples aliquots and control charts to guarantee high-quality data.

Practically, replicates of 48 serum samples were analyzed on both platforms: samples from patients affected by colorectal cancer (CRC, n = 12), by colorectal cancer in remission (R-CRC, n = 12), and samples from healthy patients matched for as many biases as possible (HC and R-HC, both n = 12). The aim was to highlight candidate biomarkers able to discriminate between matched HC and CRC or R-CRC.

Each technique was investigated in terms of peak capacity, stability, with a consideration for the effect of the QC system, and identification power. The results were analyzed through their respective specificities, especially the trade between mass resolution power and chromatographic separation capacity.

The discrimination potential of the candidate biomarkers was assessed using supervised and unsupervised models (PLS & OPLS / PCA and HCA), discriminant analysis and ROC curves, with overfitting of the experimental data avoided by re-sampling and test validation testing.

They were then identified using full mass spectrum, linear retention indices and exact mass, which role was particularly scrutinized. Finally, confident identifications were used to study the main metabolic pathways altered in the disease, whether in active or in remission state.