The analysis of polar metabolites in metabolomics studies is often performed by hydrophilic interaction liquid chromatography (HILIC) coupled to mass spectrometry. The development of new generations of stationary phases, such as permanently charged zwitterionic columns, increased the applicability of HILIC-MS based methods.

**Key polar metabolic human pathways**
- 86 panel standards
  - Wide range of metabolic classes including amino acids, amino acid metabolites, peptides, carbohydrates, phosphorylated organic acids, energy metabolism intermediates, nucleic acids, cofactors or –enzymes and acylcarnitines

**Results and Discussions**

**Prioritization system**
- Compounds were evaluated based on a scoring system summing the contribution of peak shape, retention time and peak intensity for each analytical standard.
- First column screening was used to prioritize the columns for future optimization. Two new zwitterionic columns were chosen: iHILIC-Fusion(P) in ESI(-) and iHILIC-Fusion in ESI(+).

**The mechanisms of retention were explored**
- Zwitterionic amino acids with both methods had significant retention due to quadrupolar electrostatic interactions.
- Ion exchange retention was prevalent with positively charged compounds such as trimethylamine-N-oxide

**The optimized methods were applied for untargeted analysis of biological samples**
1. XCMS pre-processing
2. Blank subtraction
3. Filter features with median RSD < 30%
4. Annotated compounds as proof of concept

**Conclusions**
- Zwitterionic HILIC columns show high potential for covering polar metabolites in untargeted metabolomics.
- Tree-based LC method optimization is a fast and straightforward method to significantly improve metabolic coverage.
- Combining the final optimized HILIC-MS method in ESI (+) and ESI (-), 98.9% of polar standards could be separated and detected, covering key pathways of the polar human metabolome.