Enhancing the chromatographic separation of polar metabolites using new generation HILIC columns

Katyeny Manuela da Silva[1], Elias Iturrospe [1], Matthias Cuykx [2], Adrian Covaci [1], Alexander van Nuijs [1]

[1] Toxicological Center, University of Antwerp, 2610 Antwerp, Belgium

[2] Laboratory of Clinical Medicine, Antwerp University Hospital, Drie Eikenstraat 655, 2650 Edegem, Belgium

Toxicological Centre
University of Antwerp

16th Annual Conference of the Metabolomics Society
METABOLOMICS2020

Introduction

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.

Objective

Development of high coverage platform for polar metabolites with significant biological function with a tree-based method development optimization

Approach

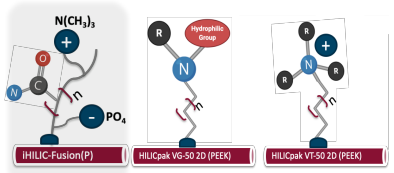
Key polar metabolic human pathways

86 panel standards

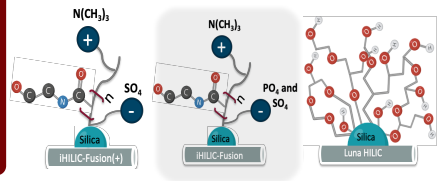
Wide range of metabolic classes including **amino acids** , **amino acid metabolites**, **peptides**, **carbohydrates**, **phosphorylated organic acids**, **energy metabolism intermediates**, **nucleic acids**, **cofactors** or **–enzymes** and **acylcarnitines**

Column Screening

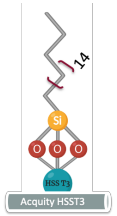
HILIC PEEK-based column



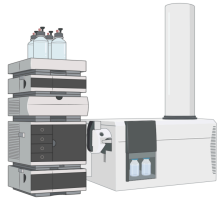
HILIC silica-based column



Reversed- phase silica-based column



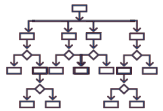
LC-QTOF analysis



Agilent LC 1290 Series
6530 QTOF-MS

ESI (-) and (+) modes

Tree-based optimization



Order of effects

Stationary phase > Mobile phase pH and modifier > Modifiers> Additional parameters (temperature, gradient, flow)

Results and Discussions

Prioritization system

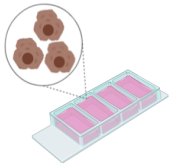
- Compounds were evaluated based on a scoring system summing the contribution of peak shape, retention time and peak intensity for each analytical standard.
- First column screening was used to prioritize the columns for future optimization. Two new zwitterionic columns were chosen: **iHILIC-Fusion(P)**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

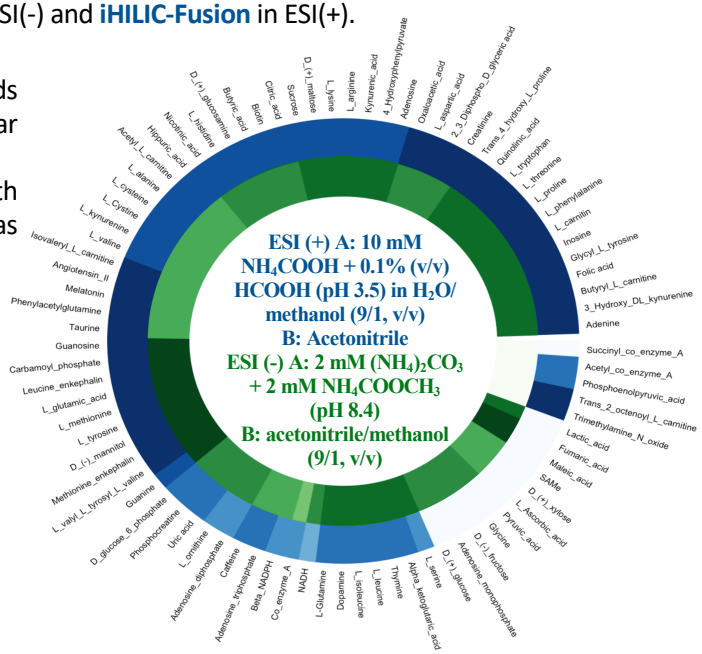
1.XCMS pre-processing
2.Blank subtraction
3.Filter features with median RSD < 30%
4. Annotated compounds as proof of concept



HepaRGs liver cells

	IC	EC	Plasma	Urine
Features ESI (+)	3652	2570	3565	3178
Features ESI (-)	1749	1622	917	577

IC=Intracellular, EC=Extracellular



Circular heatmap showing the coverage of the final optimized methods with iHILIC-Fusion(P) in ESI(-) and iHILIC-Fusion in ESI(+). Darker columns represent better scores based on peak shape, intensity and retention

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.

E. Iturrospe, K.M. Da Silva, B.T. Andújar, M. Cuykx, T. Vanhaecke, A. Covaci, A.L.N. van Nuijs, An exploratory approach for an oriented development of an untargeted multi-purpose hydrophilic interaction liquid chromatography-mass spectrometry platform for polar metabolites in biological matrices, J. Chromatogr. A. (2020). Under revision