# Evaluation of a UPLC hybrid zwitterionic HILIC stationary phase for global and targeted metabolomics



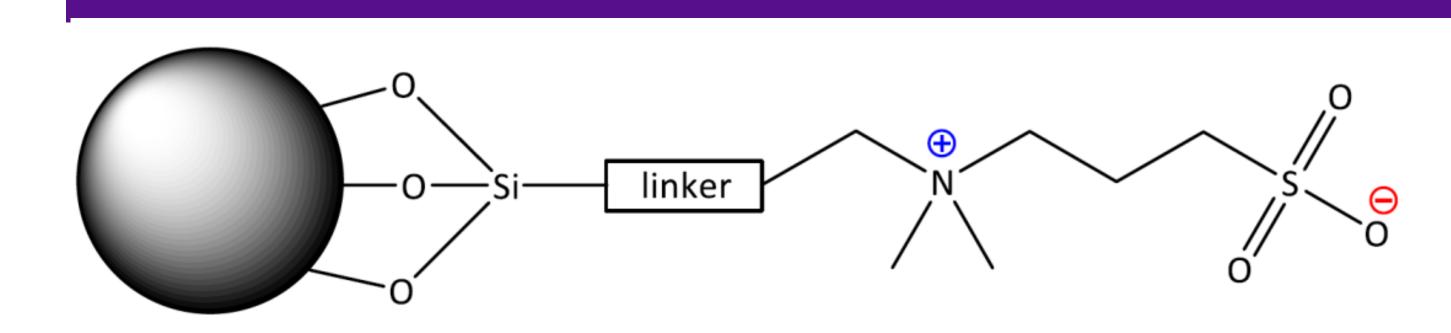
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#### Overview

AIM: Direct separation of polar metabolites has remained a challenge. Hydrophilic interaction liquid chromatography (HILIC) has made great advances in the separation of polar chromatographic performance has been lacking with only HPLC or near-UPLC particles being available. We aimed to evaluate and optimize columns containing a recently released UPLC-HILIC stationary phase "Atlantis™ Premier BEH Z-HILIC Column" produced by Waters<sup>TM</sup>.

Results: The 1.7 µm BEH Z-HILIC column efficiently separated a wide range of polar metabolites with excellent peak symmetry, and more narrow widths (FWHM) compared to other HILIC stationary phases. The column showed no "column bleed" and separated several common isoleucine/leucine) to baseline resolution. We optimized two LC methods; "Hybrid" for high throughput (MS1 only, RT Library), and a "Global" for untargeted coverage. We used the MSMLS library (~600 metabolites) to define the RT panel and optimized global coverage with human plasma (SRM1950). Future manuscripts will publish the RT library in full.

## New UPLC Particle



Structure of the sulfobetaine stationary phase for Atlantis Premier BEH Z-HILIC Columns

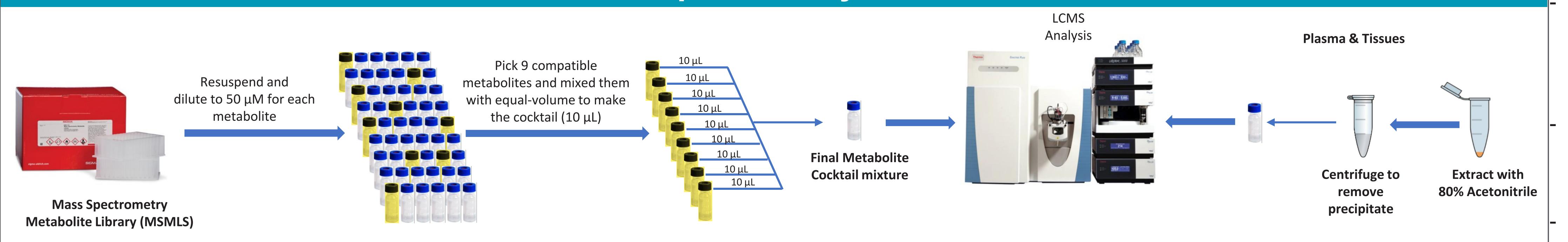
SeQuant® ZIC® -pHILIC



Particle Design – We tested two major columns of interest; the new Waters Atlantis Premier BEH Z-HILIC (top) and the Millipore SeQuant ZIC-pHILIC (bottom). Both particles utilize a sulfobetaine functional group, but with different linkers and particles. Critically, the Z-HILIC particle is available as a 1.7 μm particle while the ZIC-pHILIC is only available as a 5 μm

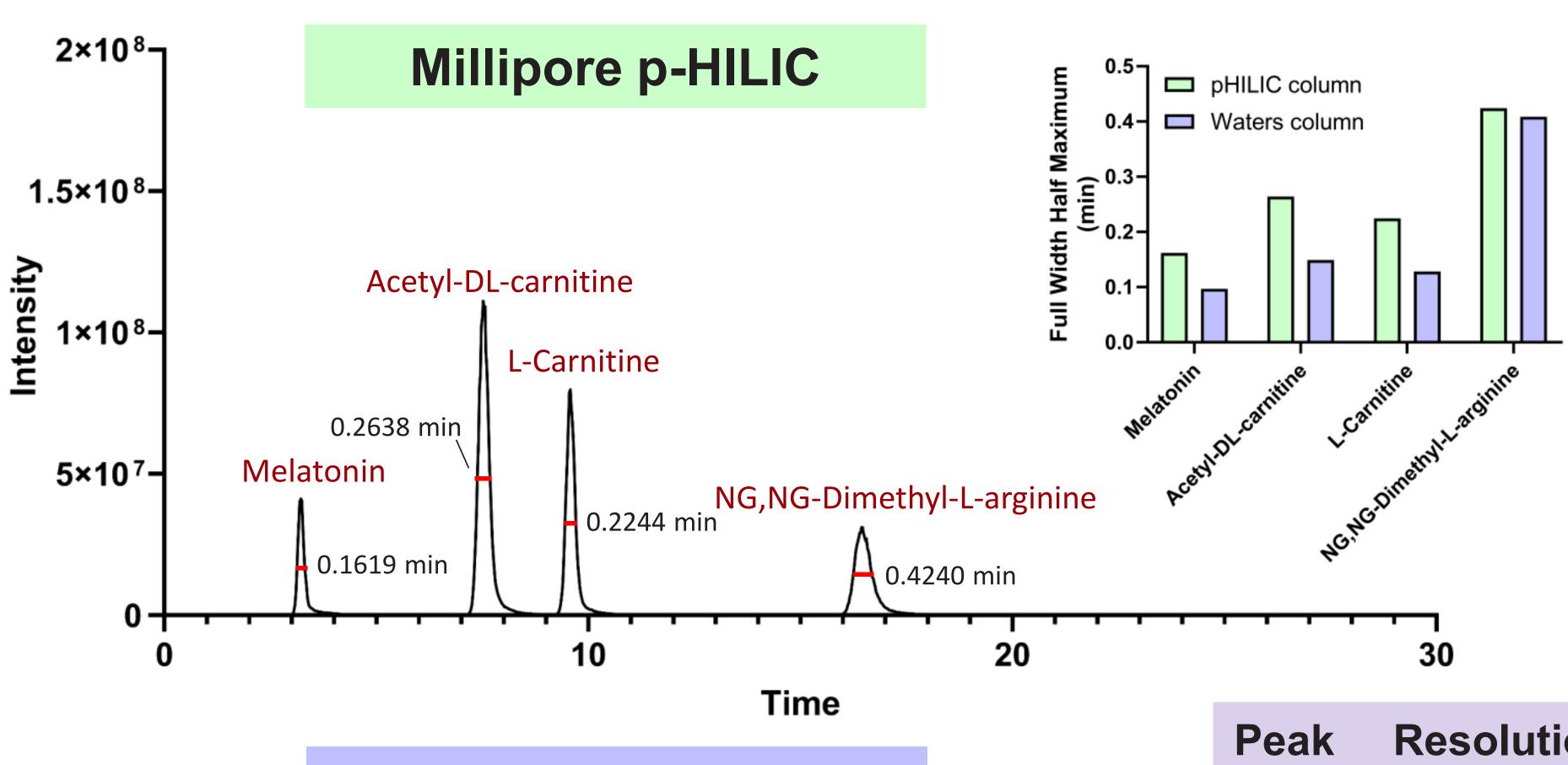
### Methods

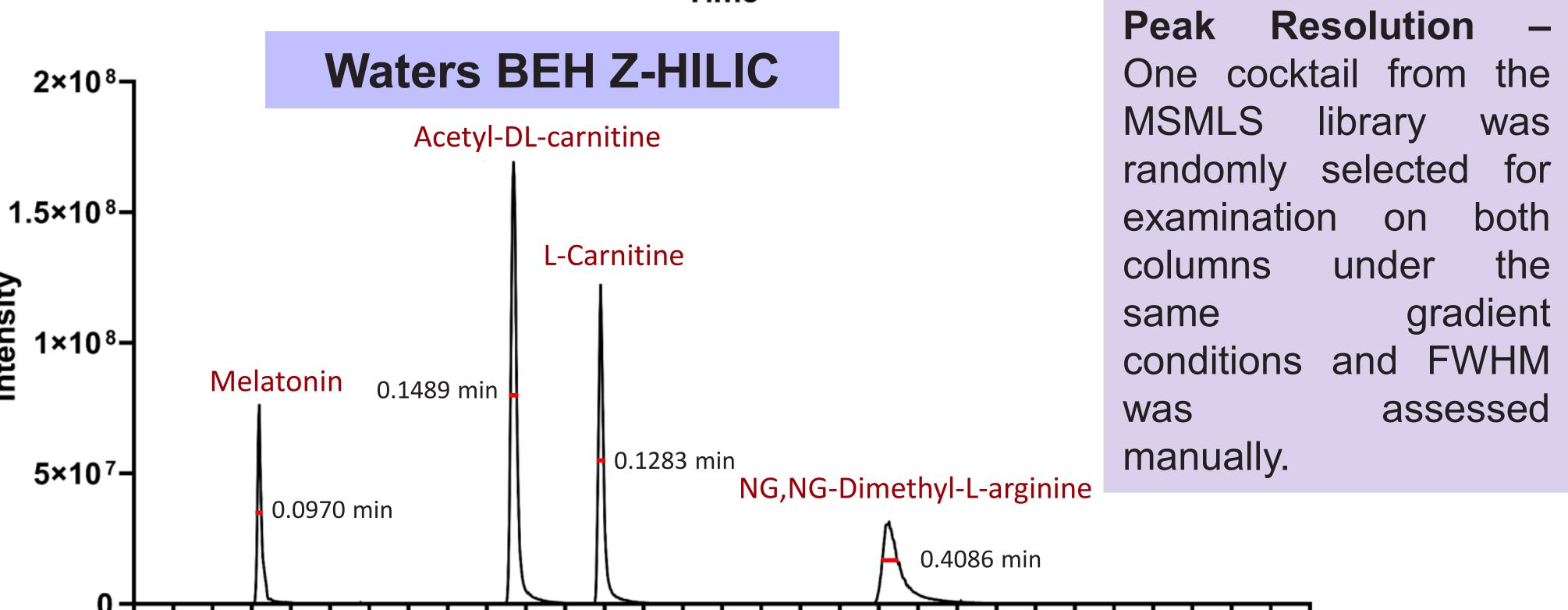
#### Overall Sample & Analysis Workflow

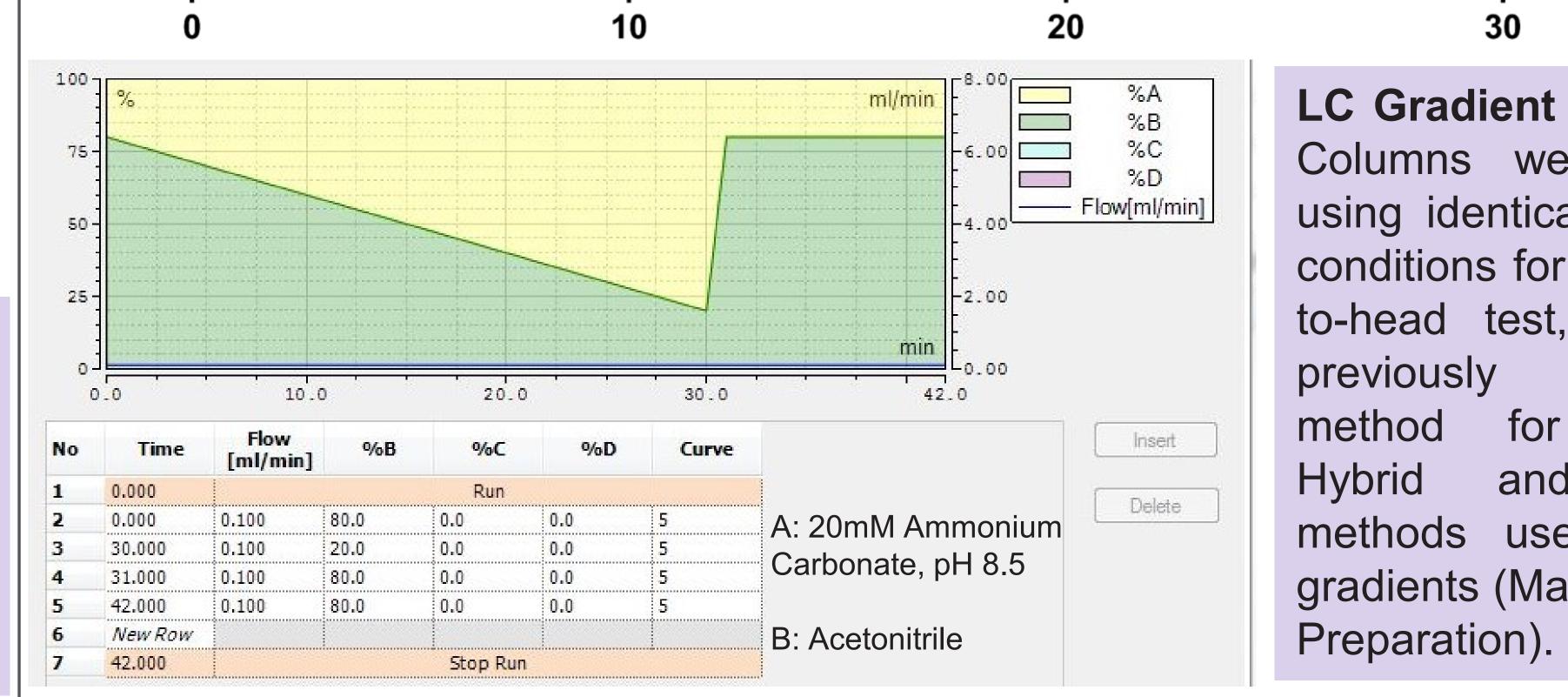


Plasma and Tissue Analysis - Samples were extracted volumetrically or by tissue weight, and extracted in a typical polar extraction solution using 80% (v/v) acetonitrile in water followed by mechanical homogenization, centrifugation, speed vacuum concentration, and reconstitution (abbreviated).

#### Retentivity & Resolution







#### LC Gradient Method -Columns were tested using identical gradient conditions for the headto-head test, using a optimized pHILIC. Global methods use different gradients (Manuscript in

for

and

# Column Bleed Assessment Millipore Column - XIC of 212.0953 m/z 212.0953 m/z Waters BEH Z-HILIC - XIC of 212.0953 m/z

"Column Bleed" Marker - The ZIC pHILIC column shows a characteristic and high intensity marker ion of column bleed at 212.0953 m/z in positive ion mode [C7H18NO4S]+ which we annotated as coming from hydrolysis of linker to the sulfobetaine zwitterion. The BEH Z-HILIC linker prevents this degradation leading to insignificant background ions coming from the

Specificity and Resolution of Millipore p-HILIC Amino Acids - The ZIC pHILIC column shows good retentivity of all amino acids (AA), but poor resolution of hydrophobic AAs (Leucine/Isoleucine). The BEH Z-HILIC column showed even higher retentivity using the same LC gradient, and was able to base-line resolve all three Waters BEH Z-HILIC hydrophobic AAs. The pHILIC (prototype 2.1µm) resolve Histidine/Threonine, while the Zcould not resolve Arginine/Lysine. compounds reflect the role of the different linker chemistries.

# Continuing Steps

MSMLS cocktails are being assessed in chemical series to probe the interaction with the HILIC stationary phase e.g., adenine, deoxyadenosine, adenosine, cAMP, AMP, ADP, ATP, cGAMP

The effect of pH and buffer strength is being assessed to optimize the coverage of complex samples such as human plasma

Manual inspection, validation, and documentation of each individual standard in the MSMLS library by +/mode at MS1 and MS2 levels, along with annotation of relative ionization efficiency, adducts, and insource fragments

Machine learning to predicting HILIC retention time using a cheminformatics approach to the MSMLS library to aid in annotation of unknown peaks

Curation of data from mouse organs at the global/untargeted level to provide reference profiles for adopters of this column/gradient/RT library, including expected contaminates

Publication of the methods and findings

#### Conclusions

- BEH Z-HILIC particle offers better retentivity of polar metabolites than similar HILIC columns
- BEH Z-HILIC particle offers better resolution and sensitivity (FWHM) than similar HILIC columns
- BEH Z-HILIC particle doesn't show "column bleed" via the 212 *m/z* sulfobetaine background ion
- The high efficiency BEH Z-HILIC UPLC particle (1.7 µm) enables fast (15 min) separations of a large library of metabolites, using MS1 scans
- The same column enables longer gradients (45 min) with less loss of sensitivity and peak broadening for global TopN dda method for maximum metabolite ID and coverage
- RT library and method will be published in full