Retention modelling in hydrophilic interaction chromatography (HILIC)

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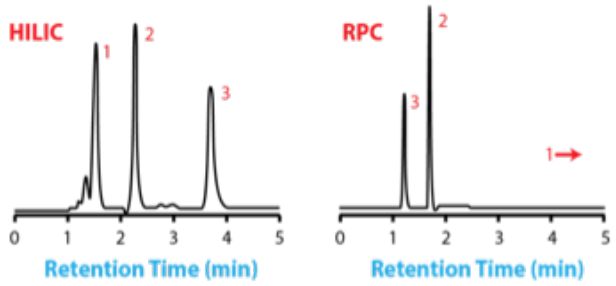
INTRODUCTION

Separation of hydrophilic and polar compounds (such as peptides) by HILIC can provide 10-1000 times increase in sensitivity compared to regular phase chromatography (RPC).



Partitioning and Adsorption are major retention mechanisms in HILIC interactions and depend on the following factors:

- Analyte
- Physicochemical properties of the stationary phase
- Type and composition of the mobile phase

CURRENT STATE

There are few reports on the accuracy of retention modelling in HILIC and none using commercially available software

GOAL OF STUDY

To evaluate the accuracy of retention modelling in HILIC and determine the importance of various factors on retention and selectivity.

METHOD

Instrument

Agilent 1290 infinity UHPLC

Software

- ChemStation
- ACD/LC Simulator
- SAS JMP
- ACD/Percepta

Compounds

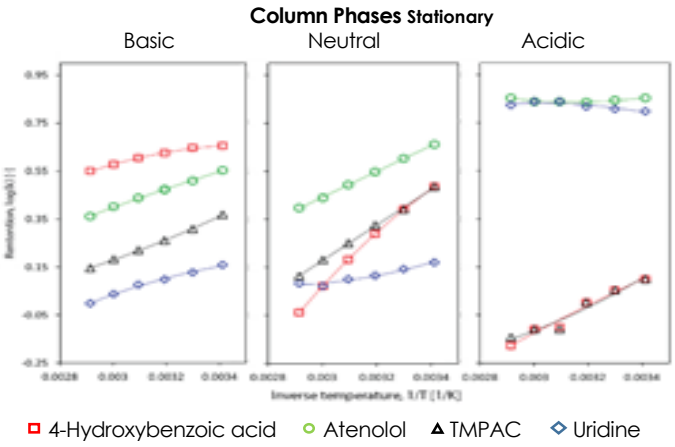
54 pharmaceutically relevant compounds covering a diverse range of pK_a and log*D* values were chromatographed with subsets used for further analyses.

RESULTS

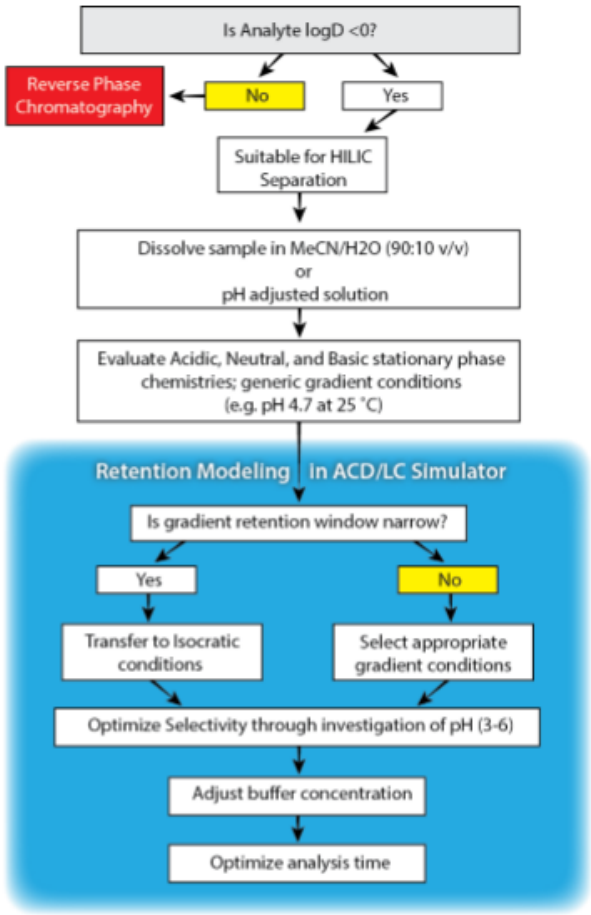
Changes in Retention Behavior

Different retention behaviors were observed for 3 HILIC columns (acidic, basic, and neutral stationary phases) under various reaction conditions.

The Van't Hoff plots illustrate retention as a function of temperature.



Method development and retention modelling strategy



IMPORTANCE OF FACTORS ON RETENTION AND SELECTIVITY

Retention: Organic Content > Stationary Phase > Temperature ≈ Mobile Phase pH ≈ Buffer Concentration

Selectivity: Stationary phase > Mobile Phase pH > Buffer Concentration > Temperature > Organic Content

CONCLUSION

This study showed that robust modelling for HILIC is possible following the proposed method development and retention modelling strategy.

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