Retention and Selectivity for Hydrophilic Interaction Liquid Chromatography (HILIC) Columns: Multivariate Modeling and Correlation with Physiochemical Properties

Tobias Jonsson[1], Ngoc Phuoc Dinh[1,2], Knut Irgum[2]

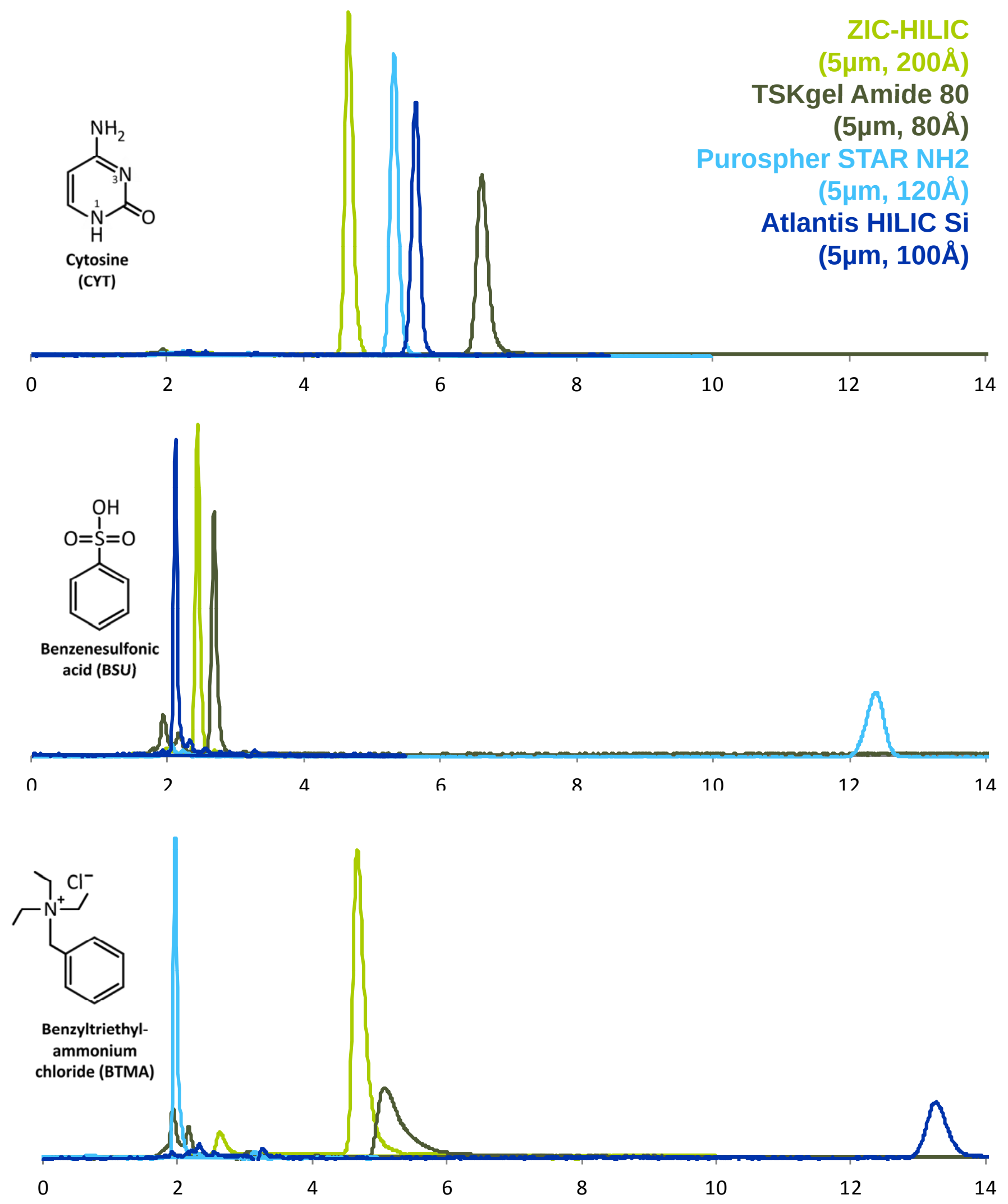
Introduction

This study have investigated retention and selectivity of HILIC type HPLC columns under LC-MS compatible conditions by injecting 20 model compounds with highly different chemicals structures and studying their retention and selectivity (retention ratios) with multivariate tools. The results were correlated to physiochemical properties of the stationary phases like functional group, surface area, and ability to adsorb water. This study enables rational selection of HILIC column based on structure of the molecules to be separated. For an introduction to HILIC theory and use we recommend Refs. 1-3.

Methods

Separations were carried out with a mobile phase containing 80:20 acetonitrile / aqueous ammonium acetate (25 mM, pH 6.8) with toluene as void volume marker. Columns were 100x4.6 mm used at 0.5 mL/min flow rate. Multivariate modeling was carried out by principal component analysis (PCA) on mean-centered, unit-variance data. Surface area were determined by BET nitrogen adsorption, and water adsorption was measured by coulometric Karl Fischer titration. Further details can be found in Refs. 4-5.

Example Chromatograms



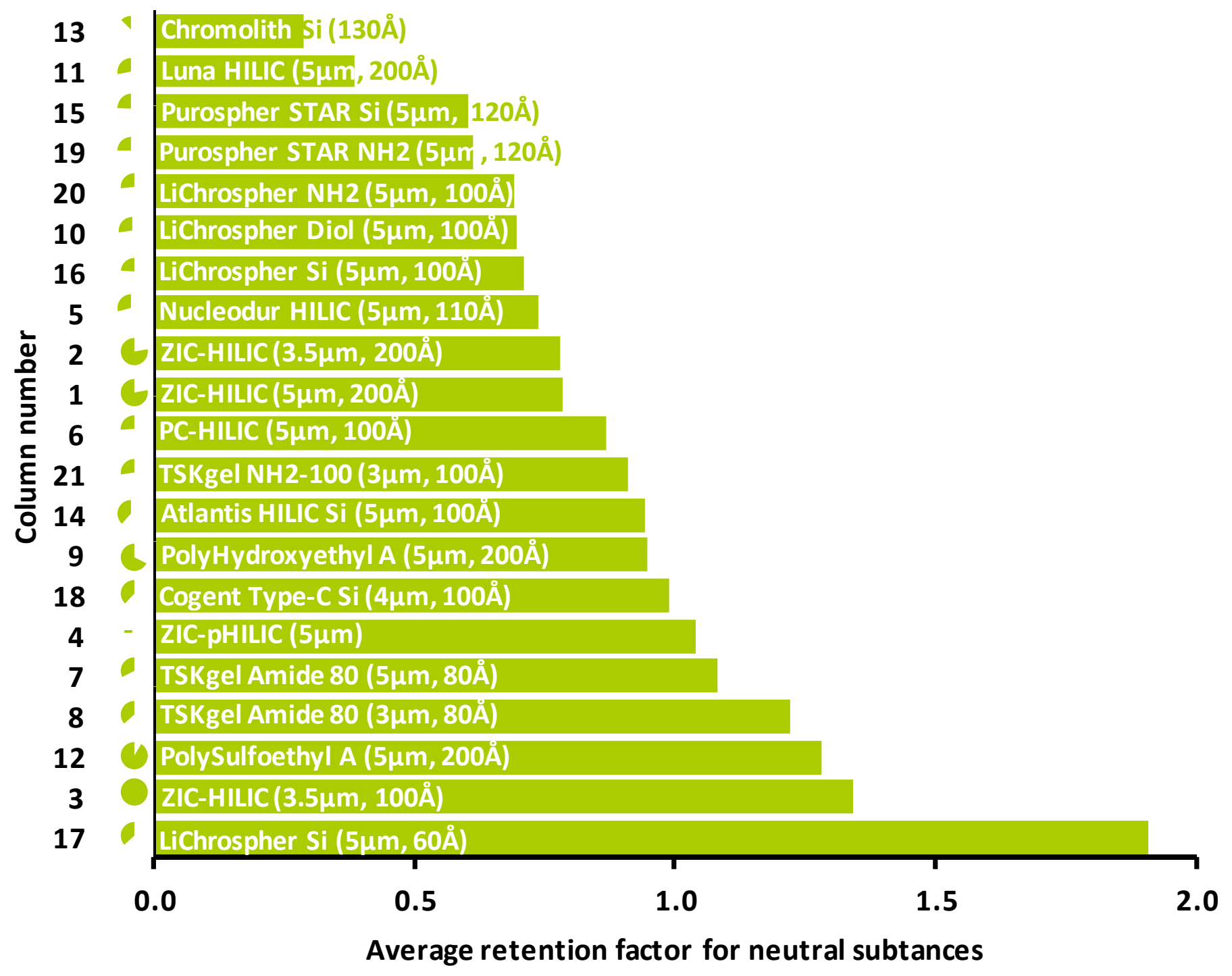
References

1. P. Hemström, K. Irgum, J. Sep. Sci. 29 (2006) 1784-1821.
2. E. Pontén, LCGC N. Amer. 30(S4) (2012) 36-42.
3. A Practical Guide to HILIC, Merck SeQuant AB (2008).
4. N.P. Dinh, T. Jonsson, K. Irgum, J. Chromatogr. A 1218 (2011) 5880-5891.
5. N.P. Dinh, F. Mihci, T. Jonsson, K. Irgum, manuscript.

Affiliates: [1]Merck SeQuant AB, Sweden; [2]Umeå University, Sweden
Corresponding Author: tobias.jonsson@merckgroup.com

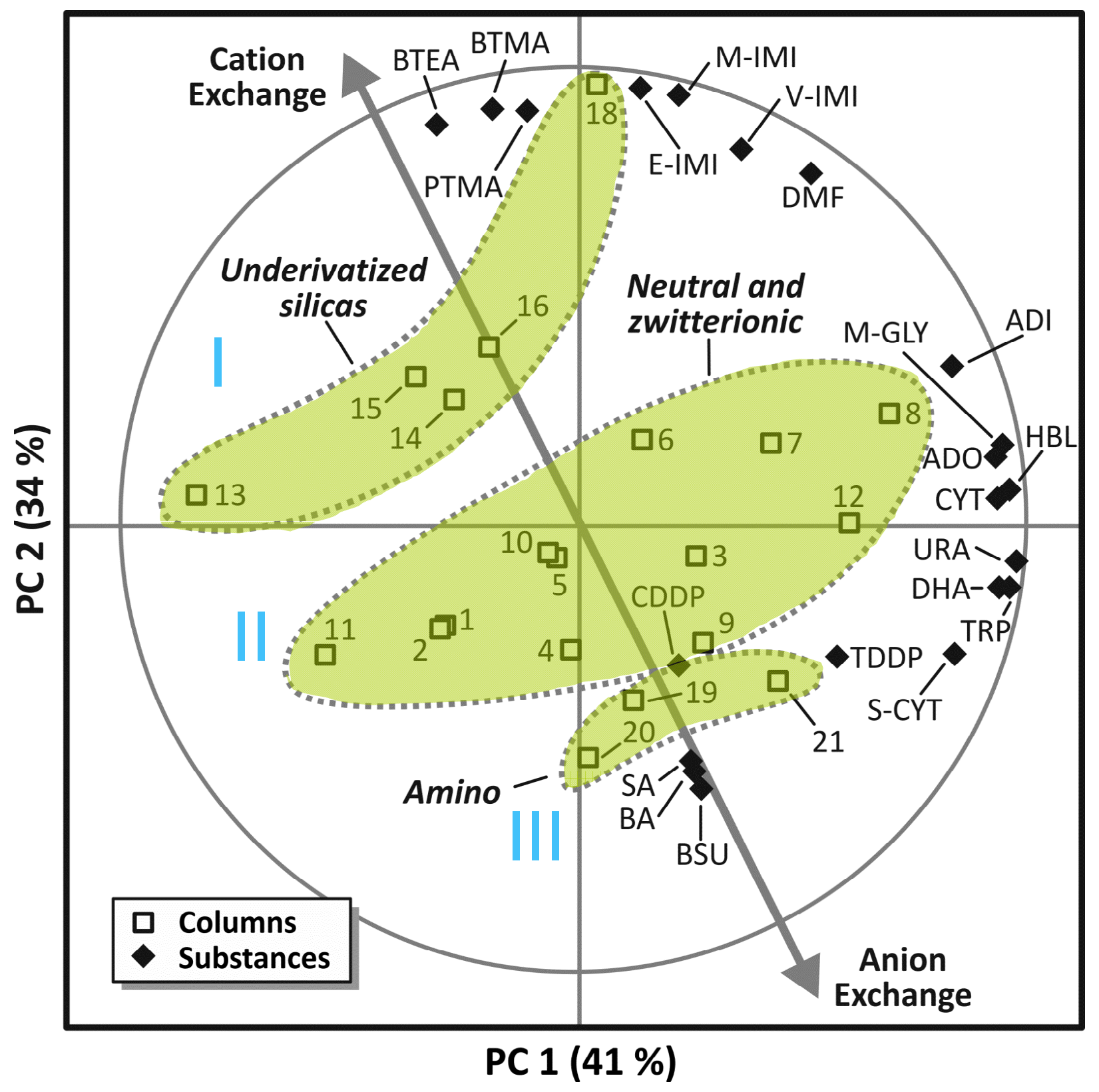
Retention on HILIC Columns

Retention of neutral substances in HILIC mode correlated well to LogD of the analytes (cf. Ref. 4) and roughly to surface area of the sorbents. Columns with poly-functionalized stationary phases showed higher retention per surface area. Multivariate classification of retention revealed three diverse clusters, making it evident that modeling on retention was not sufficient to enable rational column selection.



Average Retention of Neutral Substances
Bars indicate average retention per column and pie charts indicate relative retention per surface area. Column numbers (No. 1-21) are the same in all figures.

Cluster	Stationary Phases	Retention Characteristics
I	Bare Silica	Cation Exchange Anion Exclusion
II	Diol, Amide, Hydroxyl, All Zwitterionic	Mixed interactions; adsorbtion, partitioning and weak ion exchange
III	Amino	Anion Exchange Cation Exclusion

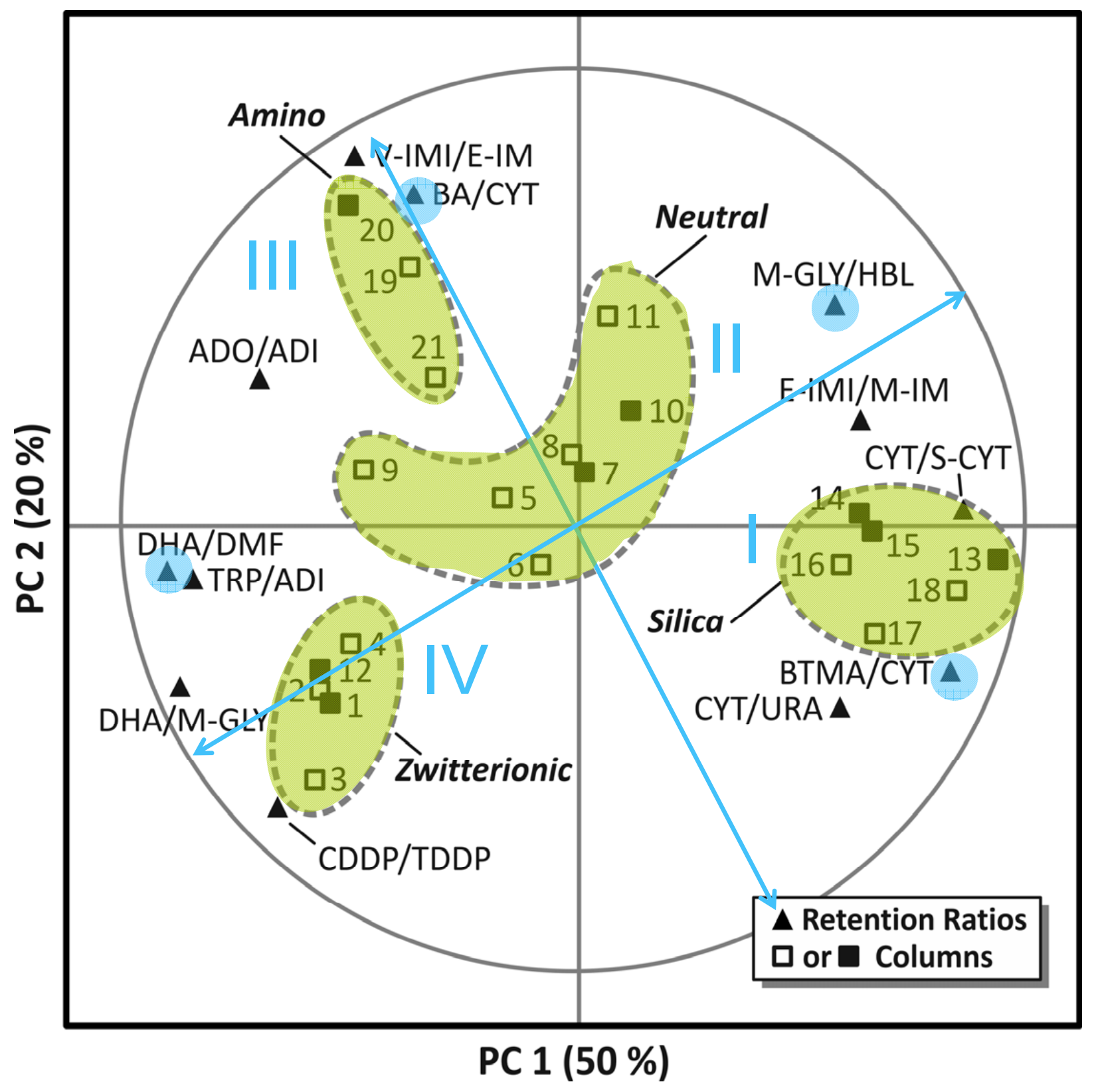


Multivariate HILIC Column Retention Comparison
PCA model of retention for commercial HILIC columns (No. 1-21). Columns with similar retention cluster more closely.

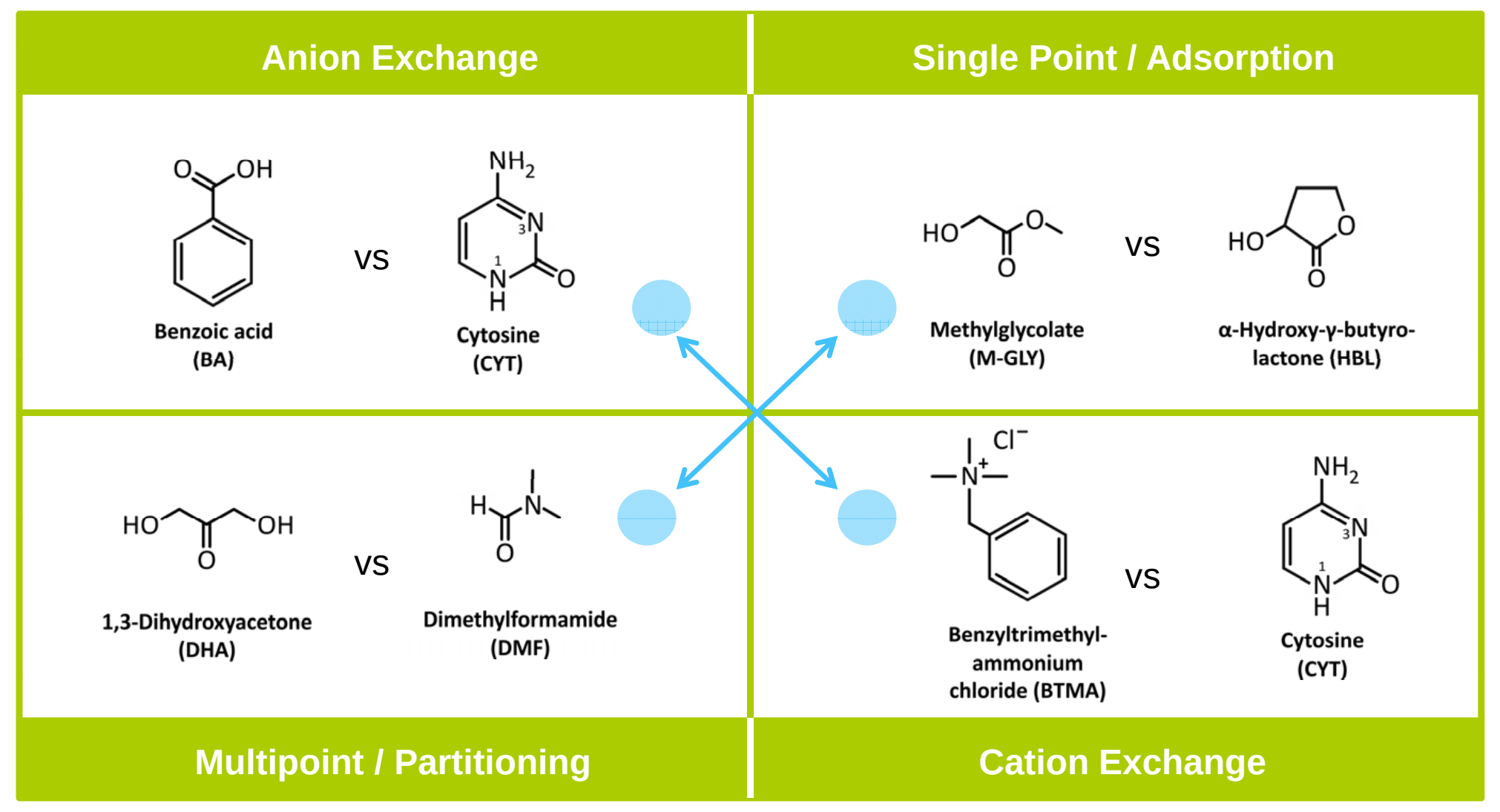
HILIC Column Selectivity Classes

A multivariate classification enabled grouping of HILIC phases into four main clusters and revealed that there are four main characteristics determining selectivity in HILIC. One dimension discriminated on cation exchange vs anion ex-change and the other on single point vs multipoint interaction (adsorption vs partitioning). Bare silica columns had similar selectivity regardless of if they were called HILIC columns or not. Some phases marketed to be zwitterionic did not group as such, but was instead clustering together with low-specific neutral columns. This is likely explained by a lower density of stationary phase on mono-functionalized materials.

Cluster	Stationary Phases	Selectivity Characteristics
I	Bare Silica Non-functionlized	Cation Exchange & Adsorption
II	Diol, Amide, Hydroxyl, Weak Zwitterionic Mono-functionalized	Low Specificity
III	Amino Mono-functionalized	Anion Exchange
IV	Zwitterionic (sulfobetaine), Sulfoethyl (w. residual Amino) Poly-functionalized	Partitioning & Weak Cation Exchange

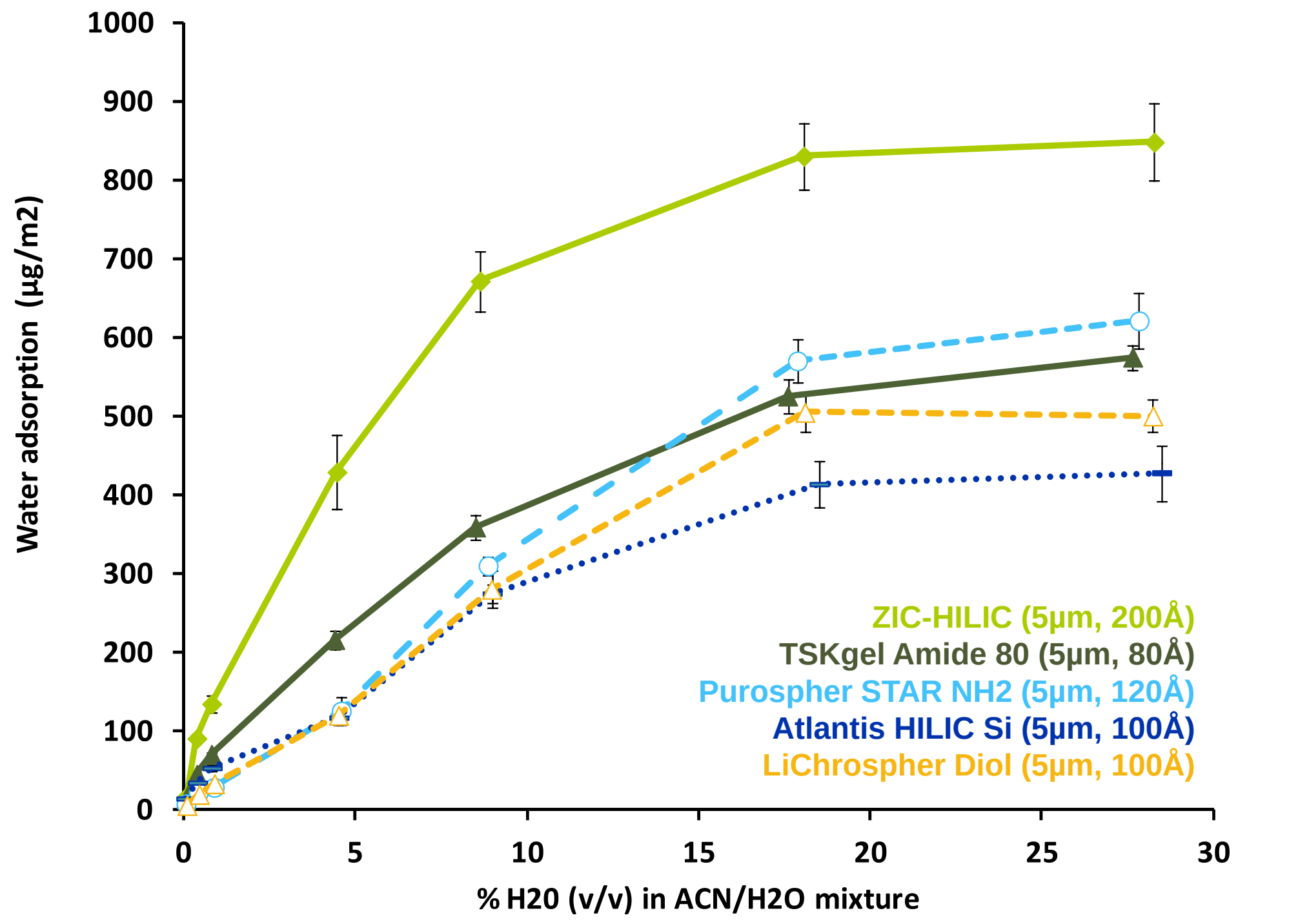


Multivariate HILIC Column Selectivity Comparison
PCA model of selectivity (retention ratio) for commercial HILIC columns (No.1-21). The more similar the selectivity, the more close the clustering. Structures below indicate typical retention ratios for each property.



Water Adsorption and Stability

The stationary phases with high retention per surface area typically adsorbed more water than bonded phases. Water adsorption isotherms for bare silica and mono-functionalized stationary phases indicated a non-homogenic multilayer formation, unlike poly-functionalized materials which showed smooth isotherms. The water layer on poly-functionalized columns was more stable and less affected by admixtures of other organic solvents.



Water Adsorption Isotherms on HILIC Materials
Accumulation of water at different acetonitrile/water ratios. Table indicates effect on water layer by replacing 5% of the acetonitrile to another solvent at 80% total organic.

Stationary Phase	THF	MeOH
ZIC-HILIC (5µm, 200Å)	88%	107%
TSKgel Amide 80 (5µm, 80Å)	77%	93%
Purospher STAR NH2 (5µm, 120Å)	48%	62%
Atlantis HILIC Si (5µm, 100Å)	48%	57%

Summary

A HILIC column classification model was presented for guided selection of stationary phase chemistry based on retention and selectivity. The model confirmed the strong impact of electrostatic interactions in HILIC, but also revealed that adsorption and partitioning are very important for selectivity. These properties varied significantly between stationary phases, sometimes even if intended to be similar, and could be correlated to physiochemical properties of the columns.

Poly-functionalized zwitterionic stationary phases like ZIC-HILIC showed selectivity depending on partitioning and weak ion exchange, and were least affected by changes in organic solvent environment. All non-functionalized bare silica columns had similar selectivity which depended on cation exchange, plus adsorption rather than partitioning.

www.sequant.com

www.merckmillipore.com/chromatography