

HPLC Stationary Phases for Separating Highly Polar Analytes

Xiaodong Liu and Christopher Pohl
Thermo Fisher Scientific, Sunnyvale, CA, USA

Overview

Purpose: Survey column technology for hydrophilic analytes

Methods: HPLC column development and evaluation

Results: Appropriate RPLC, HILIC and mixed-mode columns are identified for separating hydrophilic analytes

Introduction

Highly polar species are omnipresent and of great importance in (bio)pharmaceutical drug development, biological processes, food and beverage, chemicals, etc. Separation of these analytes is challenging due to the diversity in charge, size and hydrophilicity, complex sample matrix, and sometimes the requirement of determination of significantly more hydrophobic compounds within a single injection. While reversed-phase C18 columns are routinely used in many HPLC applications, they often struggle to retain highly hydrophilic analytes. HILIC columns are better suited to for very polar species but challenged by less rugged methods owing to sample matrix problems or the limited solubility of analytes of interest. Mixed-mode chromatography can be a potential alternative to RPLC and HILIC by combining both hydrophobic (or HILIC) and ion-exchange retention mechanisms.

Methods

Sample Preparation

The test probes used to assess the suitability for highly polar analytes include nucleic acid bases, nucleosides, nucleotides, mono- & disaccharides, hydrophilic organic acids & organic bases, inorganic cations and anions, surfactants, etc, obtained from different sources.

Liquid Chromatography

Thermo Scientific™ Acclaim™ C30, Thermo Scientific™ Accucore™ 150-Amide-HILIC, Acclaim Surfactant Plus, Acclaim Trinity P1 and Acclaim Trinity P2 were used for this study.

Mobile phase: acetonitrile with ammonium acetate or formate

Instruments: Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC with a quaternary pump, auto-sampler, column compartment, DAD and CAD Veo.

Data Analysis

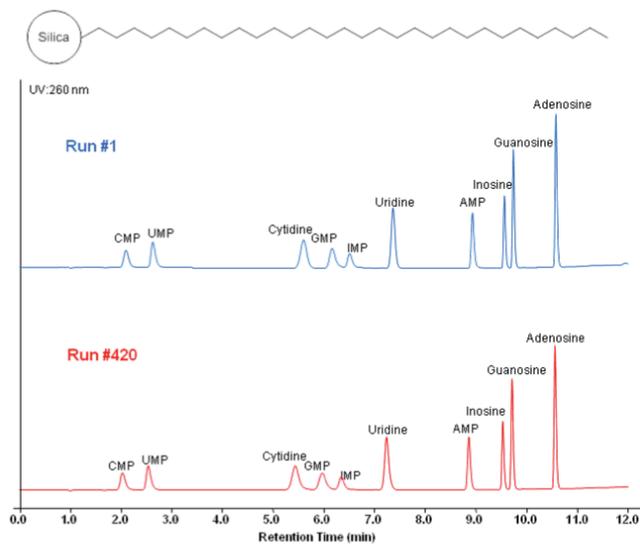
Chromatography software: Thermo Scientific™ Dionex™ Chromeleon™ 6.8

Results

Separation of Hydrophilic Analytes by RPLC

The quality of separation is affected by selectivity, retention and column efficiency. For a well-packed column, adequate retention is required and selectivity is the key. This study reveals that the Acclaim C30 column can be more versatile than a C18 column—in addition to all chromatographic characteristics of C18 column, exhibiting 100% aqueous compatibility (Figure 1) and high shape selectivity for structural closely related analytes, such as lipids.

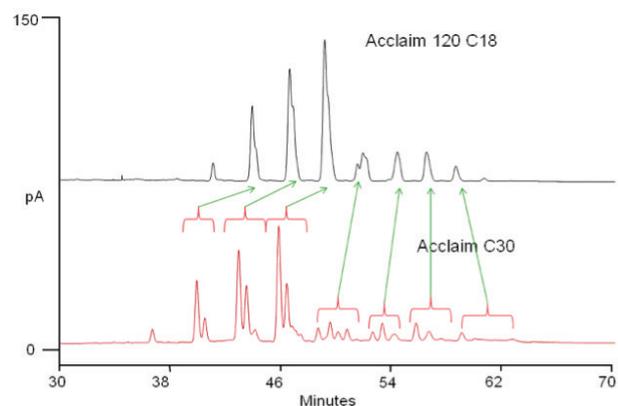
FIGURE 1. Separation of Nucleotides and Nucleosides on Acclaim C30 under Highly Aqueous Condition.



Column: **Acclaim C30**, 3 μm
 Dimension: 2.1 \times 150 mm
 Mobile Phase: A) DI water
 B) NH_4OAc (100 mM, pH5)
 C) CH_3OH
 Temperature: 15 $^\circ\text{C}$
 Flow Rate: 0.4 mL/min
 Injection: 10 μL , 1 ppm of each analyte
 Detection: UV at 260 nm

Time	%A	%B	%C
-5.0	80	20	0
0	80	20	0
3.0	80	20	0
5.9	70	20	10
8.5	40	20	40
11.9	40	20	40
12.0	80	20	0

FIGURE 2. Separation of Lipids on Acclaim C30.



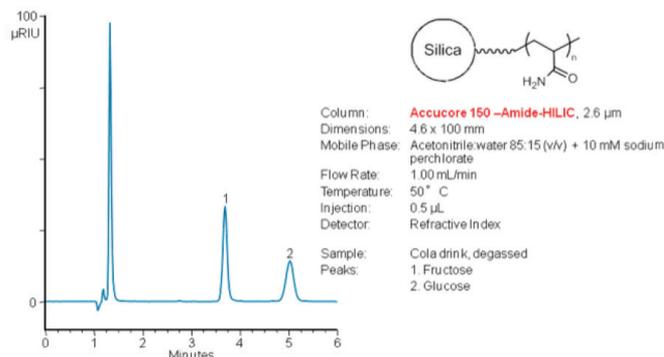
Column: **Acclaim C30** or Acclaim 120 C18, 5 μm
 Dimension: 4 \times 150 mm
 Mobile Phase: Acetonitrile (MeCN)/iso-propanol (IPA)/
 0.1 M Ammonium acetate, pH5.0 (Buffer)
 Temperature: 40 $^\circ\text{C}$
 Flow Rate: 1.0 mL/min
 Inj. Volume: 2 μL
 Detection: Corona ultra (Gain = 100 pA, Filter =
 medium, Neb. Temp = 25 $^\circ\text{C}$)
 Sample: Peanut oil (5 mg/mL in iso-propanol)

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
70	0	95	5

Separation of Hydrophilic Analytes by HILIC.

The challenge for RP columns is to generate adequate retention for hydrophilic analytes (e.g. sugars, amino acids, inorganic ions), which can be addressed by HILIC columns. However, it is challenging to generalize a method development strategy for HILIC columns. Nevertheless, amide HILIC phase has been identified as the “general-purpose” column for highly polar analytes for its simple retention mechanism and ease of use. FIGURE 3 shows the separation of sugars in a Cola drink.

FIGURE 5. Separation of Sugars in a Cola Drink.

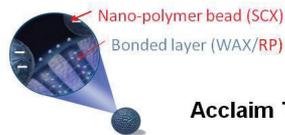


Separation of Hydrophilic Analytes by Mixed-Mode Chromatography.

Mixed-mode chromatography provides a viable solution to these challenges by combining both reversed phase and ion-exchange retention mechanisms. One major advantage of this approach is that column selectivity can easily be modified for optimal selectivity by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As a result, not only is the selectivity of a mixed-mode column complementary to that of reversed-phase columns, but it also allows for the development of multiple complementary selectivities on the same column under different appropriate conditions.

Pharmaceutical Counterion Analysis with Acclaim Trinity P1 and Acclaim Trinity P2 Columns

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Assays for API and counterions are usually analyzed separately using different methods, different separation columns, and different instruments. Acclaim Trinity P1 and P2 columns are designed for the simultaneous separation of pharmaceuticals and their counterions. They are based on nanopolymer silica hybrid (NSH) technology, a unique morphology which provides exceptional resolution of both anions and cations within the same analysis and in a reasonable amount of time. Acclaim Trinity P1 column is designed for monovalent counterion separation (FIGURE 4) and Acclaim Trinity P2 is designed for mono- and multi-valent counterion separations (FIGURE 5).



Acclaim Trinity P1



Acclaim Trinity P2

FIGURE 4. Separation of Monovalent Ions using Acclaim Trinity P1.

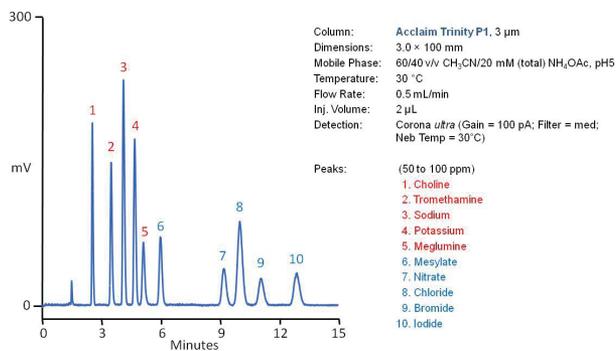
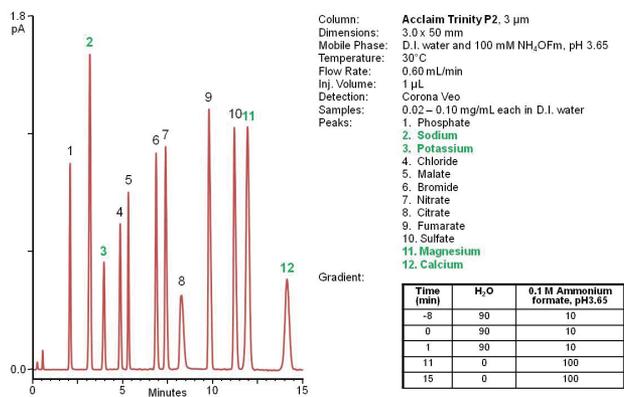
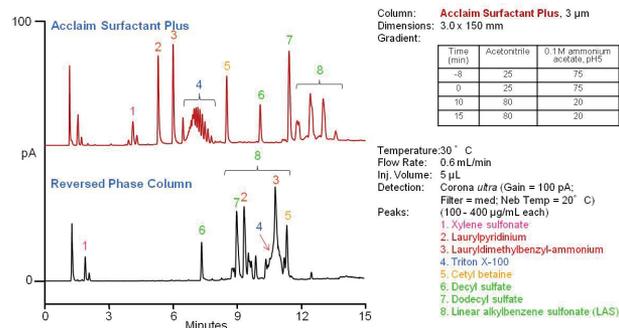


FIGURE 4. Separation of Mono- and Multi-valent Ions using Acclaim Trinity P2.



Surfactant Analysis with Acclaim Surfactant Plus Column

Surfactants are widely used in consumer products, agricultural, pharmaceutical, bio-pharmaceuticals and chemical markets, in products as diverse as pesticides, detergent powders, petroleum products, cosmetics, and pharmaceuticals. Their separation and identification can be challenging due both to the diversity of surfactants and complexity of the sample matrix. Although many HPLC columns are available and have been used for the analysis of surfactant formulations, none of these columns are capable of separating anionic, nonionic, cationic and amphoteric surfactants in a single analysis. The Acclaim Surfactant Plus columns are designed for separating a wide variety of surfactants, including anionic, cationic, nonionic, and amphoteric surfactants.



Conclusion

- Hydrophilic analytes can be separated by RPLC, HILIC and Mixed-mode chromatography
- Thermo Scientific provides comprehensive solutions for separating a broad range of hydrophilic analytes,
- Mixed-mode chromatography offers superior advantages for separating highly hydrophilic analytes through advanced column technology, excellent performance: resolution, retentivity, ruggedness, and flexibility in method development
- Mixed-mode column technology provide versatile platform to a variety of application-specific columns

For more information, visit our website at www.thermoscientific.com/chromatography

©2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

USA and Canada +1 800 332 3331
Australia 1300 735 292 (free call domestic)
China 800 810 5118 (free call domestic)
 400 650 5118
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
India +91 22 6742 9494
 +91 27 1766 2352

Japan 0120 753 670 (free call domestic)
 0120 753 671 (fax)
Korea +82 2 3420 8600
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
Singapore +65 6289 1190
All Other Enquiries +44 (0) 1928 534 050

Technical Support
 For advice and support, please visit our website:
www.thermoscientific.com/chromexpert

Thermo
SCIENTIFIC

A Thermo Fisher Scientific Brand