

Exploring Mixed-Mode Chromatography – Column Chemistry, Properties, and Applications

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Overview

- Review mixed-mode column technology
- Demonstration of challenging applications using mixed-mode chromatography

Introduction

Although reversed-phase columns (e.g. C18) are most commonly used in broad range of applications, they often fail to retain highly hydrophilic molecules (e.g. counterions), and offer limited selectivities. 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. Mixed-mode chromatography is well-suited to retaining ionic analytes, whether hydrophobic (e.g. Naproxen) or hydrophilic (e.g. Na⁺ and Cl⁻ ions), and requires no ion-pairing agents in the method, significantly improving the MS compatibility. Most importantly, mixed-mode chromatography column chemistry can be customized to a desired selectivity during stationary phase design. This presentation will give an overview on the latest mixed-mode chromatography technology, describe unique chromatographic properties of mixed-mode columns, and discuss analytical challenges that have been addressed by mixed-mode chromatography approach. Examples include determinate on of pharmaceutical counterions, simultaneous separation of anionic, cationic, nonionic and amphoteric surfactants, high resolution and fast LC-MS analysis of paraquat and diquat, and analysis of glycans from proteins and human IgG.

Mixed-Mode Chromatography

Definition

- Hydrophobic (or hydrophilic) interaction + ion-exchange interaction

Benefits

- Adjustable selectivity for optimal separation
- Simplified mobile phase (no need for ion-pairing reagents)
- Simultaneous separation of different types of analytes

Types

- Anion-exchange/reversed-phase (AEX/RP)
- Cation-exchange/reversed-phase (CEX/RP)
- Anion-exchange/cation-exchange/reversed-phase (AEX/CEX/RP)
- AEX/HILIC
- CEX/HILIC
- AEX/CEX/HILIC

FIGURE 1. Reversed-Phase/Ion-Exchange Bimodal Phases.

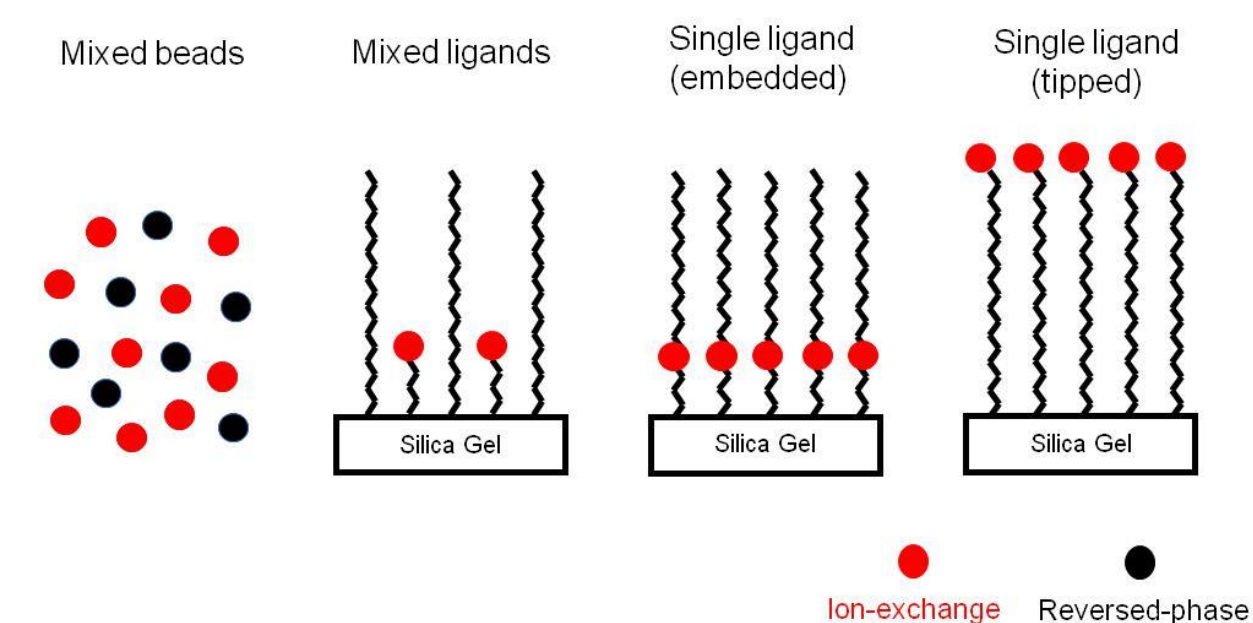
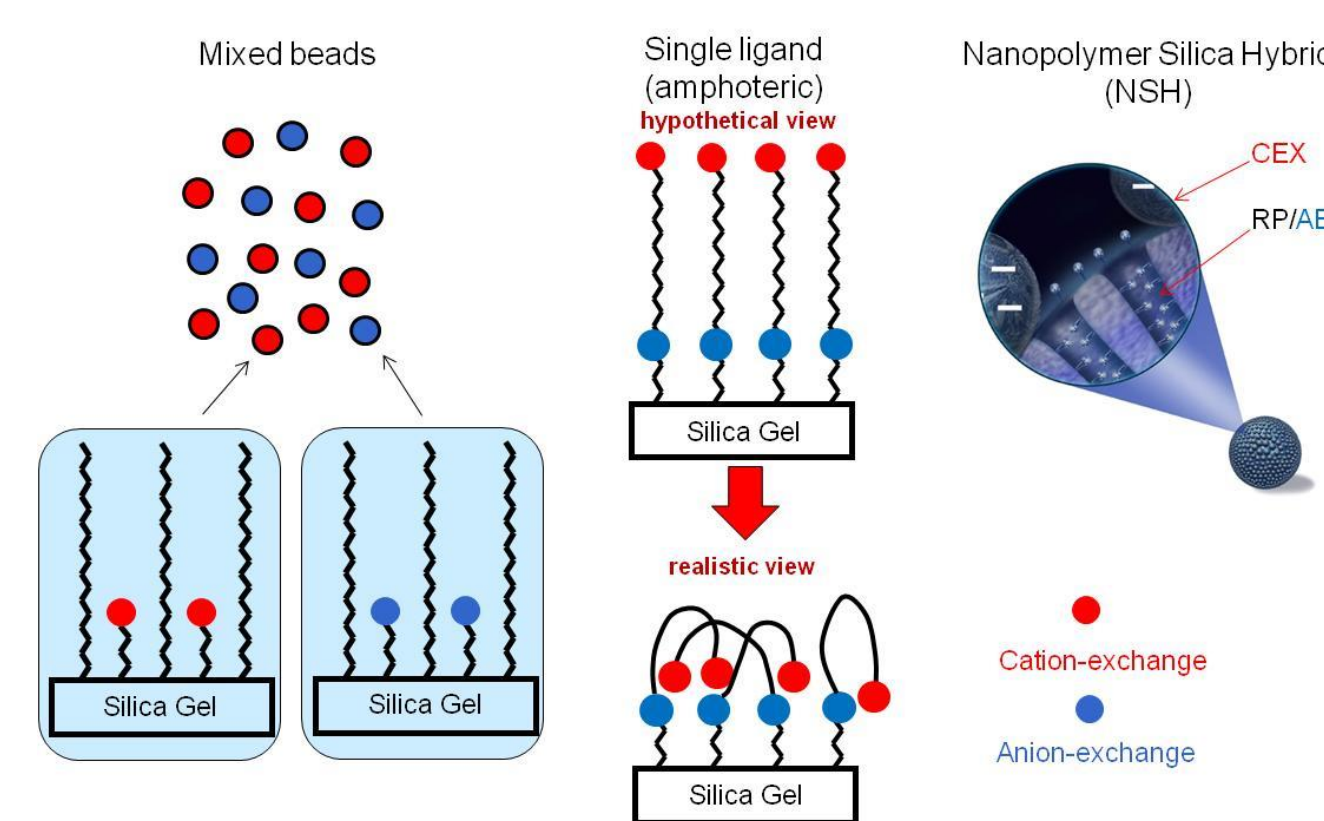


FIGURE 2. Reversed-Phase/Anion-Exchange/Cation-Exchange Trimodal Phases.



Key Applications

Pharmaceutical Counterion Analysis by Acclaim Trinity P1 & Acclaim Trinity P2

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drugs are formulated as salt forms. Assays for API and counterions are usually analyzed separately using different methods, different separation columns, and different instruments. Thermo Scientific™ Acclaim™ Trinity P1 and P2 columns are designed for the simultaneous separation of pharmaceuticals and their counterions. These columns 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. The Acclaim Trinity P1 column is designed for monovalent counterion separation and the Acclaim Trinity P2 is designed for mono- and multi-valent counterion separations.



FIGURE 3. Separation of Monovalent Counterions.

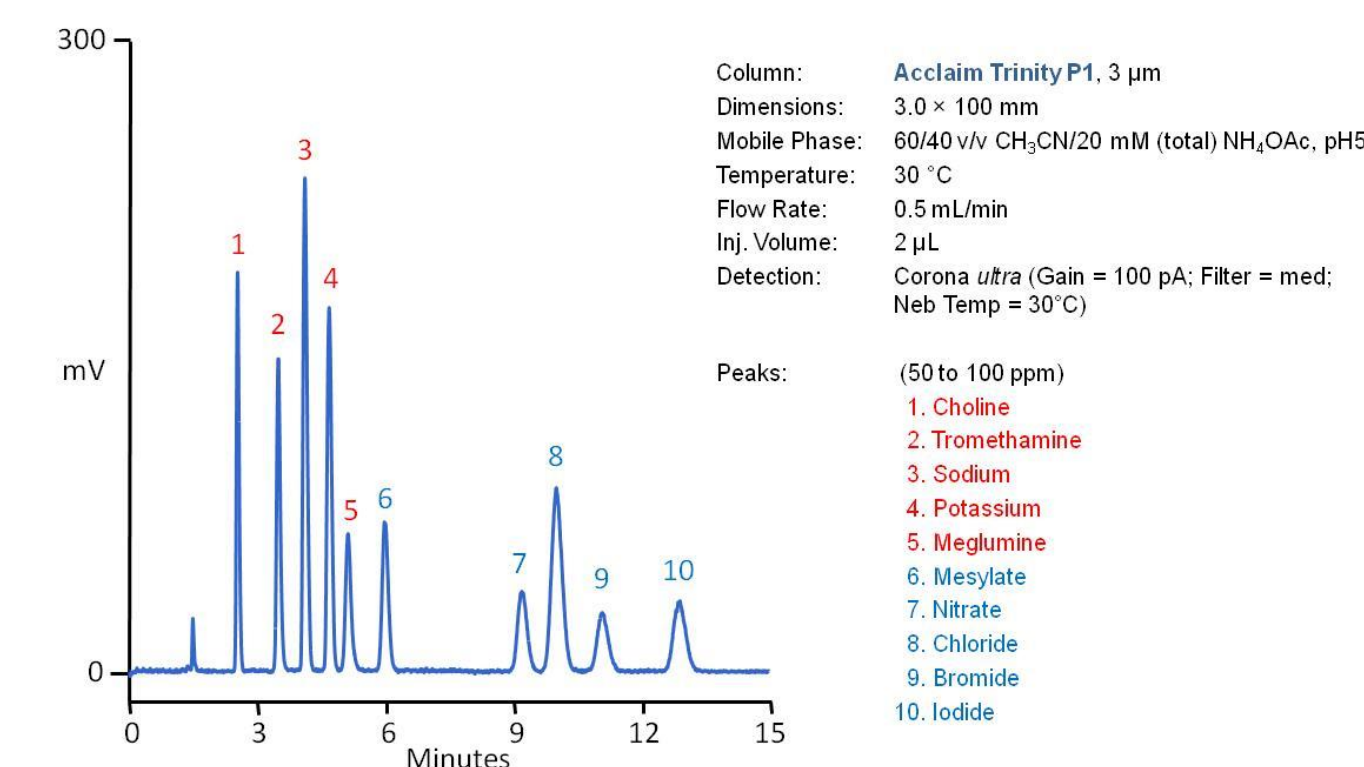
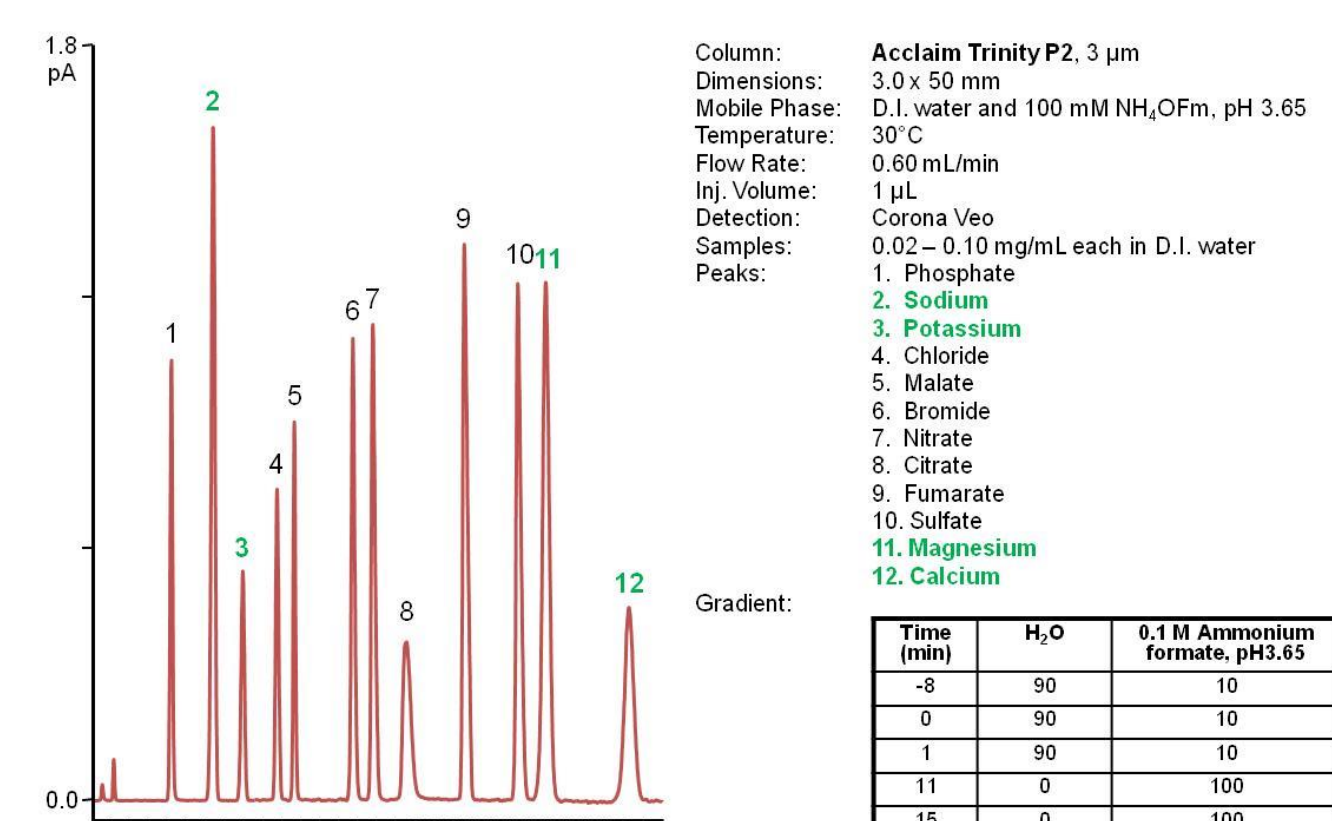


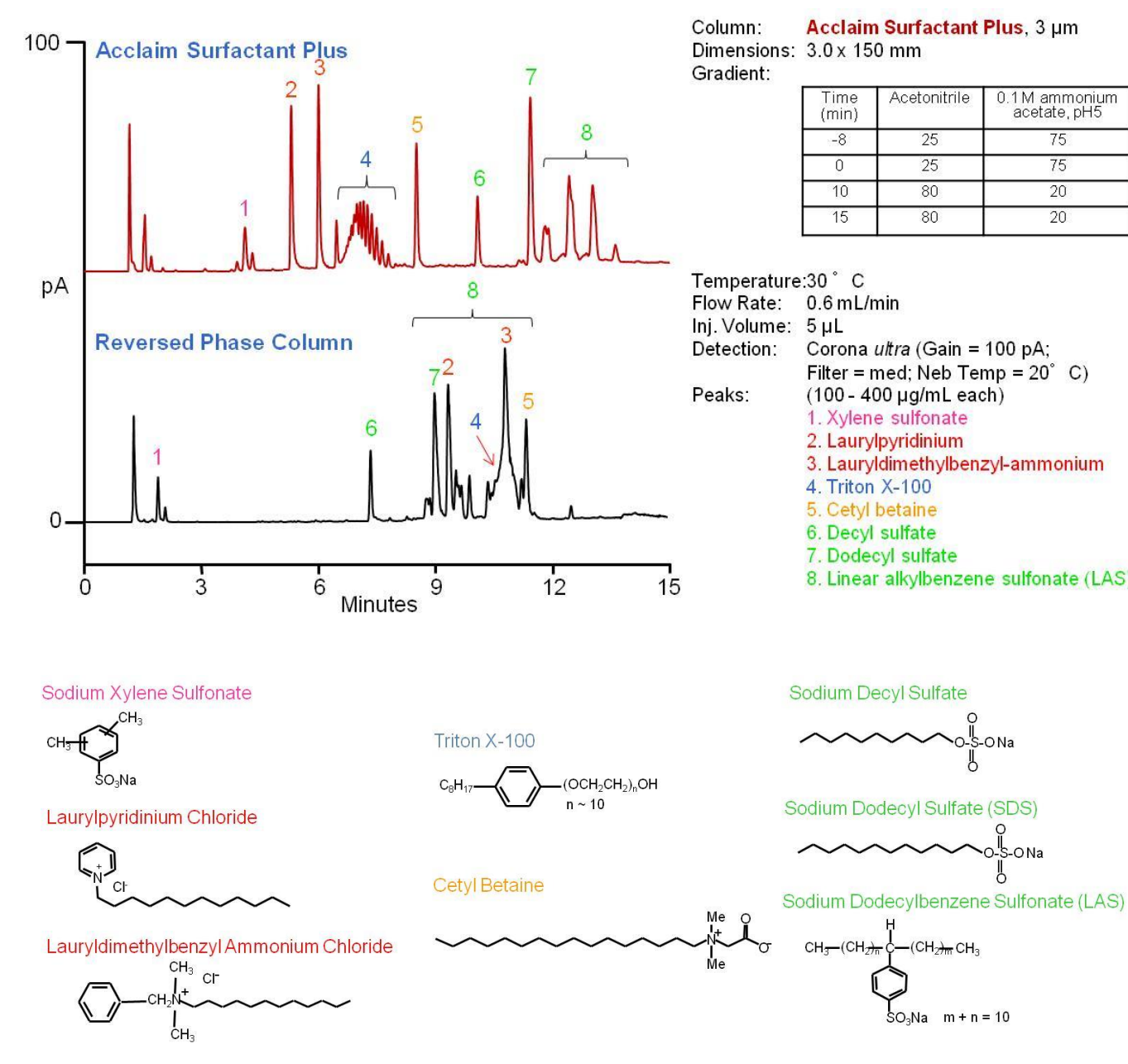
FIGURE 4. Ion (anions & cations) Screening.



Surfactant Analysis by Acclaim Surfactant Plus

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 Thermo Scientific™ Acclaim™ Surfactant Plus columns are designed for separating a wide variety of surfactants, including anionic, cationic, nonionic, and amphoteric surfactants.

FIGURE 5. Separation of Cationic, Nonionic, Amphoteric & Anionic Surfactants.



Diquat and Paraquat Analysis by Acclaim Trinity Q1

Paraquat (1,1'-dimethyl-4,4'-bipyridium dichloride, C₁₂H₁₄N₂Cl₂) and Diquat (1,1'-ethylene-2,2'-bipyridium dibromide, C₁₂H₁₂N₂Br₂) are non-selective and nonsystematic herbicides widely used in agriculture to control broadleaf and grassy weeds. However they are toxic and either compound can have serious effects as they can alter reduction-oxidation activities in biological systems. The separation of these highly charged dual quaternary amines is challenging because of their ionic nature. The Acclaim Trinity Q1 is designed for fast separation of Paraquat and Diquat with both UV and MS detections. The benefits include excellent resolution, good peak shape, high throughput, MS compatibility (no need for on-pairing reagent) and ease of use.

FIGURE 6. LC-MS-MS: Paraquat and Diquat at 10 ppb.

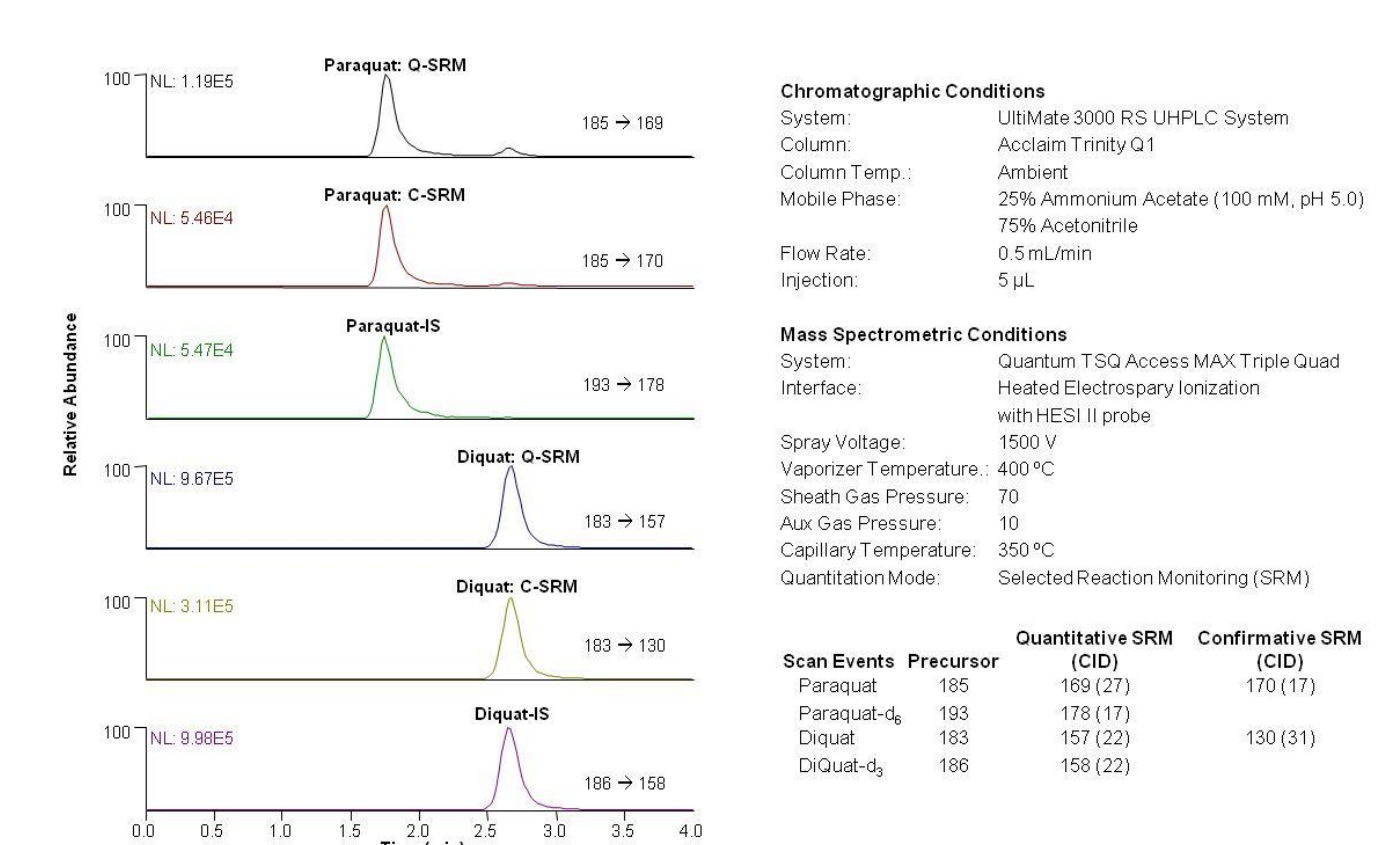
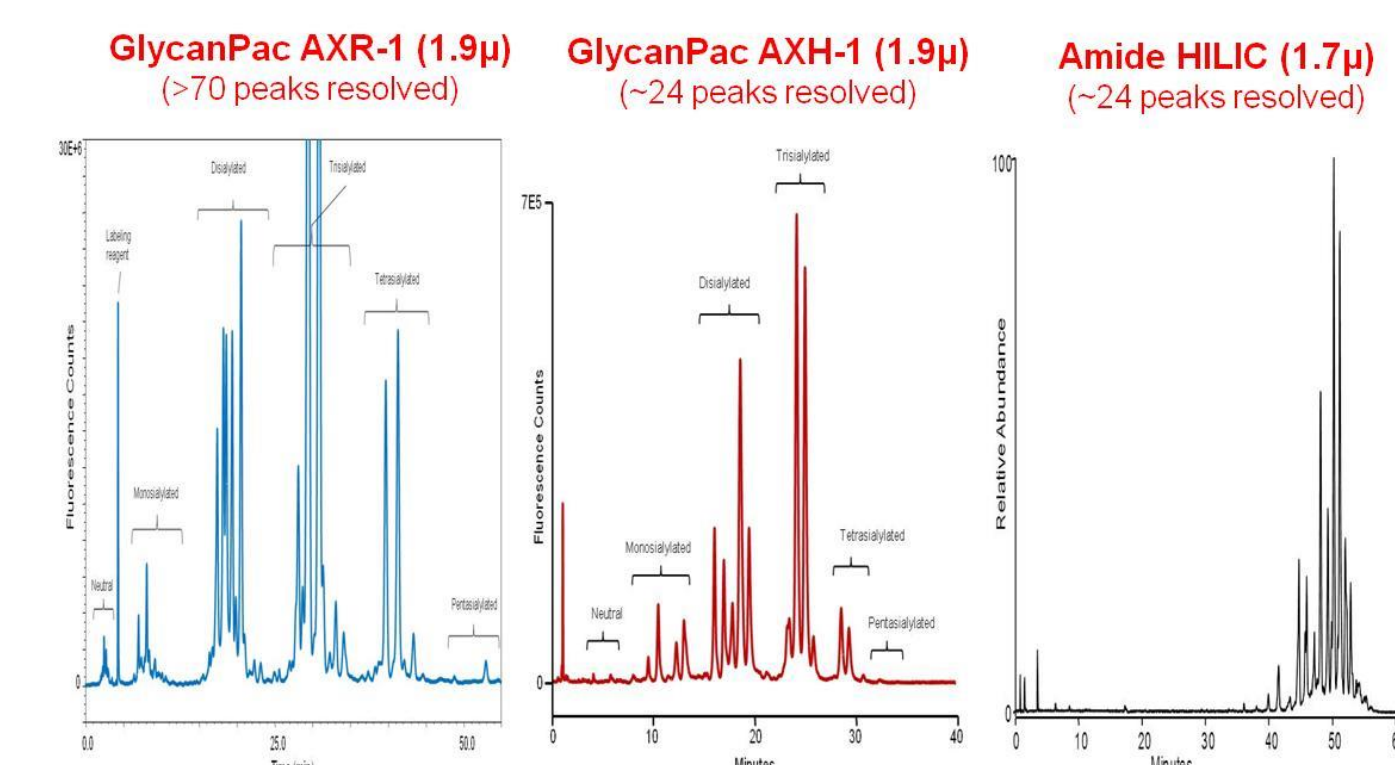


FIGURE 7. 2-AB N-Glycan from Bovine Fetuin (GlycanPac vs. Amide HILIC).



Conclusion

- Mixed-mode columns offer advantages over other separation columns through
 - Excellent performance: selectivity, resolution and retention
 - Flexibility in method development
 - Reduced cost
- Mixed-mode column technology provide versatile platform to a variety of application-specific columns
- There is a family of Thermo Scientific mixed-mode columns that facilitate instrument pull-through

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