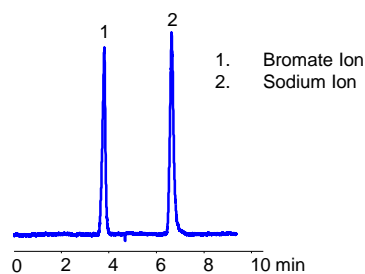


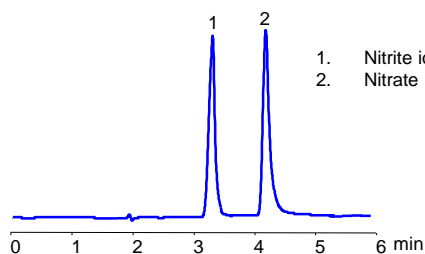
## Exploring Various Modes in HILIC Mixed-Mode Chromatography for Analysis of Various Compounds



1. Bromate Ion
2. Sodium Ion

**Column:** Amaze TH  
**Column size:** 4.6x150 mm, 5 µm, 100A  
**MP:** 50% ACN with 20 mM AmAc pH 4  
**Flow:** 1.0 mL/min  
**Detection:** ELSD, 40°C

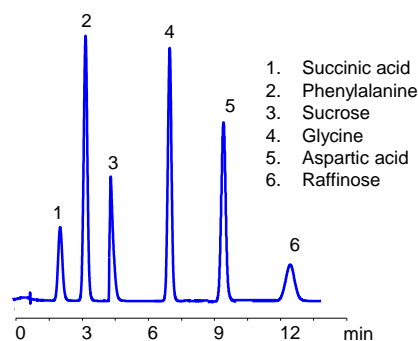
**Fig. 1 Simultaneous HPLC analysis of sodium and bromate ions in Cation- and Anion-Exchange Modes**



1. Nitrite ion
2. Nitrate ion

**Column:** Amaze TH  
**Column size:** 4.6x150 mm, 5 µm, 100A  
**MP:** 20% ACN with 20 mM AmAc pH 4  
**Flow:** 1.0 mL/min  
**Detection:** ELSD, 40°C

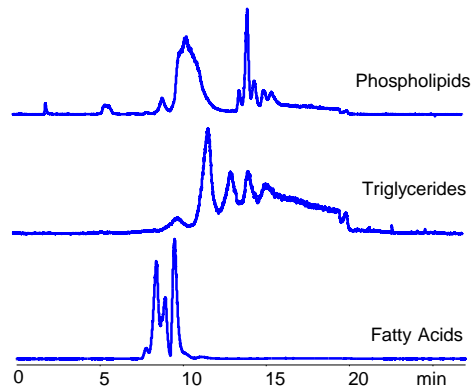
**Fig. 2 HPLC analysis of nitrite and nitrate ions in anion-exchange mode**



1. Succinic acid
2. Phenylalanine
3. Sucrose
4. Glycine
5. Aspartic acid
6. Raffinose

**Column:** Amaze TH  
**Size:** 4.6 x 100 mm  
**MP:** 80% ACN with 10 mM AmAc pH 4.8  
**Flow:** 1.0 mL/min  
**Detection:** ELSD, 40°C

**Fig. 3 HPLC analysis of sugars, amino acids and carboxylic acids in HILIC/cation- and anion-exchange modes**



**Column:** Amaze TH  
**Dimensions:** 4.6x150 mm, 5 µm, 100A  
**MP:** ACN gradient from 30% to 70%, AmFm pH 3 gradient from 20 mM to 60 mM in 12 min, 5 min hold  
**Detection:** ELSD 40°C

**Fig. 4 HPLC analysis of glycerides, triglycerides, phospholipids and fatty acids in RP and ion-exchange modes**

## Application Notes

Mixed-mode columns can be operated either in a single or multiple modes at the same time. An addition of a secondary interaction, whether it is RP, HILIC, ion-exchange or ion-exclusion, allows for alternative selectivity compared to single-mode columns. This approach helps separate compounds of different natures in one run. Retention time and selectivity of separation can be adjusted by more parameters than in pure RP or HILIC chromatography. Independent control of interaction modes guarantees better control, sharper peaks, and more robust separations. The retention time of compounds can be adjusted by a variation of the amount of organic, buffer pH, buffer concentration and buffer nature. Here is an example of Amaze TH column being used in pure cation- and anion-exchange modes (Fig. 1, 2) and an example when Amaze TH column is used in HILIC/ion-exchange mode (Fig. 3). Amaze TH also has some reversed-phase properties and can be used for the analysis of very hydrophobic molecules like phospholipids, triglycerides, and fatty acids (Fig. 4). Possibilities are endless - mixed-mode columns are a very powerful and effective alternative to single-mode columns (RP or HILIC). Check our website [www.helixchrom.com](http://www.helixchrom.com) to become an expert in mixed-mode chromatography.