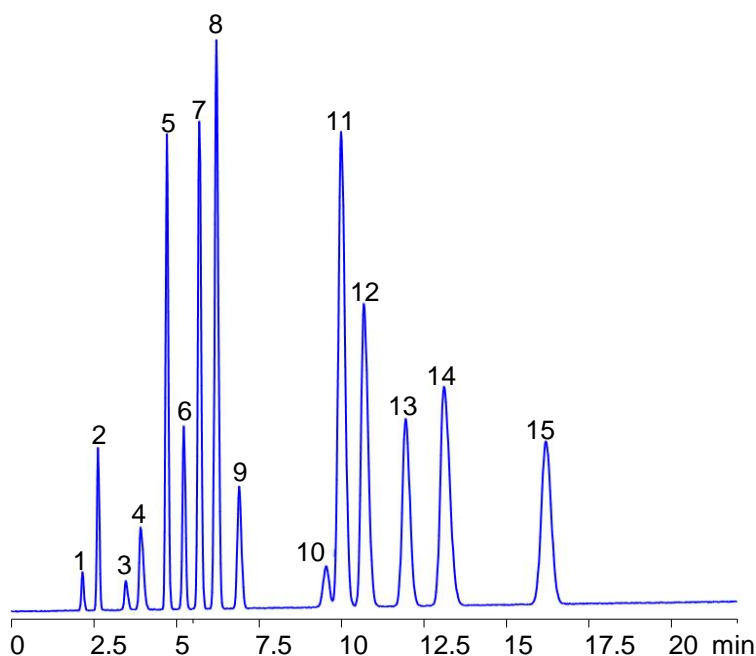


## Exploring pH Gradients for Analysis of Broad Range of Organic Acids



1. Acetic acid
2. Ascorbic acid
3. Formic acid
4. Succinic acid
5. Citric acid
6. Malonic acid
7. Fumaric acid
8. Mandelic acid
9. Oxalic acid
10. Terephthalic acid
11. Benzoic acid
12. Maleic acid
13. Phthalic acid
14. Benzenesulfonic acid
15. p-Toluenesulfonic acid

**Column:** Amaze HA  
**Dimensions:** 4.6x100 mm, 3  $\mu$ m, 100A  
**Mobile phase:** triple gradient of ACN, ammonium phosphate concentration and pH (from pH 4 to 2)  
**Flow rate:** 1 ml/min  
**Detection:** 205 nm

## Application Notes

Selectivity of separation is a key to developing robust methods. There are many more ways to adjust the selectivity of mixed-mode separations compared to single-mode chromatography whether it is RP, HILIC, or ion exchange. Mixed-mode chromatography is exploring small differences in hydrophobic/hydrophilic and ionic properties of analytes. You can control these properties of the molecules by playing with mobile phase composition.

The pH of the mobile phase can be a powerful tool to enhance or suppress the ionization of analytes as well as HELIX's stationary phases. This approach can help you to analyze acids that are very different in hydrophobicity and ionic strength. Going from a high pH (pH 4) to a low pH (pH 2) will gradually suppress the ionization of organic acids, thus reducing the ion-exchange interaction of acidic compounds with the basic nature of the Amaze HA stationary phase. Since the column is fully compatible with 100% water and 100% acetonitrile you can do single, double, and triple gradients and obtain very good and sharp peaks. Here is an application for the separation of 15 acids on the Amaze HA mixed-mode column. If you don't have a lot of compounds in your mixture you can use different conditions and a shorter column/method. Any of these acids can be analyzed individually with a simple isocratic method. See more at [www.helixchrom.com](http://www.helixchrom.com)