Technical Guide

BetaMax® Columns

Introduction

BetaMax packings are designed to give maximum retention for acidic, basic and neutral analytes. Three distinct packings have been designed: BetaMax Neutral, BetaMax Acid and BetaMax Base. The 60Å pore size of BetaMax packings provides almost 50% more surface area than typical 100Å silicas, leading to greater retention. BetaMax Neutral (C18) has a significantly higher carbon load (29%) than most other C18 packings, which are typically10-20% carbon.

The three BetaMax packings provide the user with a route to rapid HPLC method development. BetaMax Neutral has been designed specifically to offer the greatest retention for a wide range of small neutral organic molecules. BetaMax Acid is designed to provide reversed phase selectivity and retention for polar compounds, particularly those that possess acidic character. BetaMax Base can be used in both reversed phase and normal phase applications, and is particularly well suited for the analysis of basic analytes. BetaMax Base columns have cyano stationary phase functionality and often provide alternative selectivity and retention when compared to BetaMax Neutral.

We have made a brief comparison of each of the BetaMax packings in order to assist with column selection. BetaBasic® 18, a general purpose C18 packing, is compared to the BetaMax family in order to highlight the differences in retention behavior towards a range of compounds.

Specifications:

Phase	Particle size	Carbon Load	Pore Size	End-capping	Silica type
BetaMax Base	5µm	9%	60Å	Yes	High purity, base deactivated
BetaMax Acid	5µm	15%	60Å	Yes	High purity, base deactivated
BetaMax Neutral	5µm	29%	60Å	Yes	High purity, base deactivated

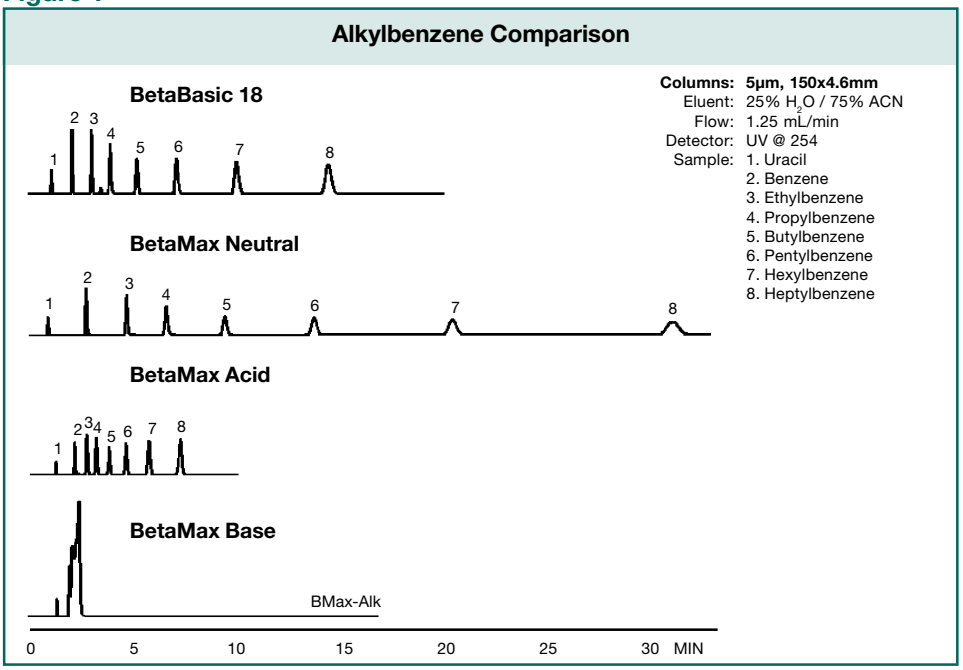
BetaMax Column Selection

Retention of Neutral Compounds - In order to compare the retention of neutral compounds, a homologous series of alkylbenzenes was analyzed, as shown in Figure 1.

For each homolog, the retention on the BetaMax Neutral column is significantly more retentive than on the BetaBasic 18 column, a highly base deactivated packing with moderate retention characteristics.

The BetaMax Neutral column provides more than twice the retention for simple neutral molecules than the BetaBasic 18 column. The BetaMax Acid column shows about one third of the retention of the BetaMax Neutral column for these compounds, while the BetaMax Base column shows very little retention or separation at all. The BetaMax Acid and BetaMax Base columns are designed to retain analytes based on additional analyte-stationary phase interactions, in addition to those purely dispersive interactions maximized by the BetaMax Neutral column. The effect of these additional interactions becomes apparent only when the analyte contains a certain degree of polar functionality (not present in the alkylbenzene homologs used in this study).

Figure 1



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Retention of Polar Basic Compounds
In order to compare the retention behavior for basic compounds, a series of procainamides was investigated and compared to the retention behavior of a BetaBasic® 18 column, as demonstrated in Figure 2.

The BetaMax® Neutral column shows the same retention behavior as the BetaBasic 18 column, but with approximately twice the retention. Excellent peak shape is shown in all cases for these very basic compounds.

The BetaMax Acid column demonstrates reduced retentionof all basic compounds relative to neutral compounds, and consequently shows very different selectivity and elution order.

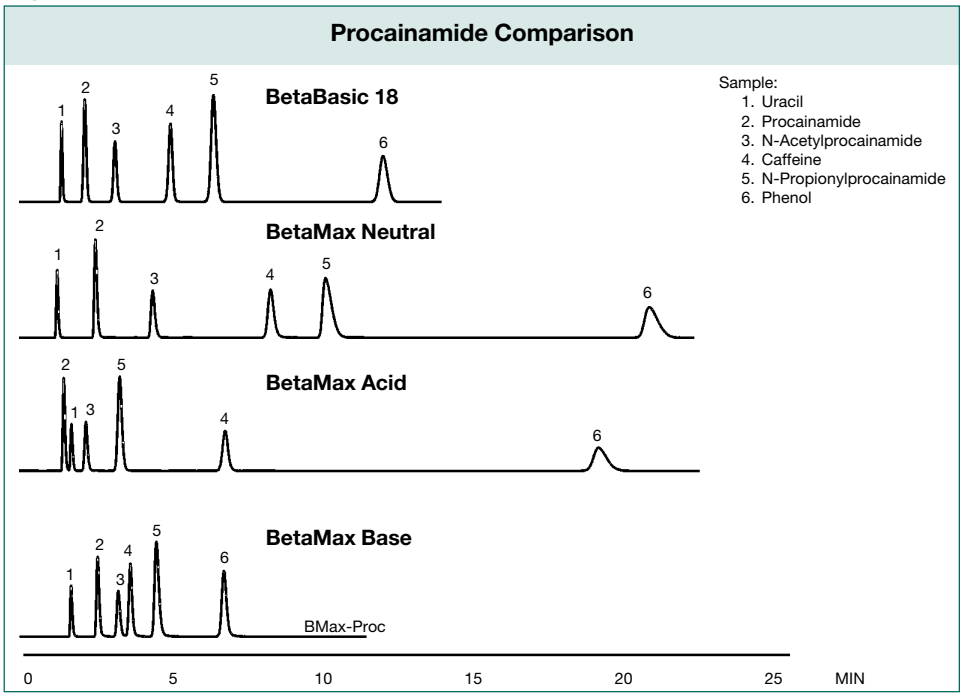
The BetaMax Base column shows the same elution order and selectivity as the C18 columns. However, it shows a significantly shorter retention than both the BetaMax Neutral and BetaBasic 18 columns due to reduced hydrophobic interactions, which are the primary mechanism of retention under these conditions.

Retention of Polar Acidic Compounds
For this study we chose a series of polyhydroxybenzenes, which range in their polarity. Problems often occur when trying to retain very polar compounds, and they typically elute near or at the unretained volume marker.

As shown in Figure 3, all of the BetaMax columns show increased retention of phloroglucinol, the most polar of the test solutes, when compared to the BetaBasic 18 column. Both the BetaMax Base column and BetaMax Acid column show more than twice the retention for this compound than the BetaMax Neutral or BetaBasic 18 columns. In this case the polar retention mechanisms of BetaMax Acid and BetaMax Base columns combine with dispersive interactions to provide additional retention.

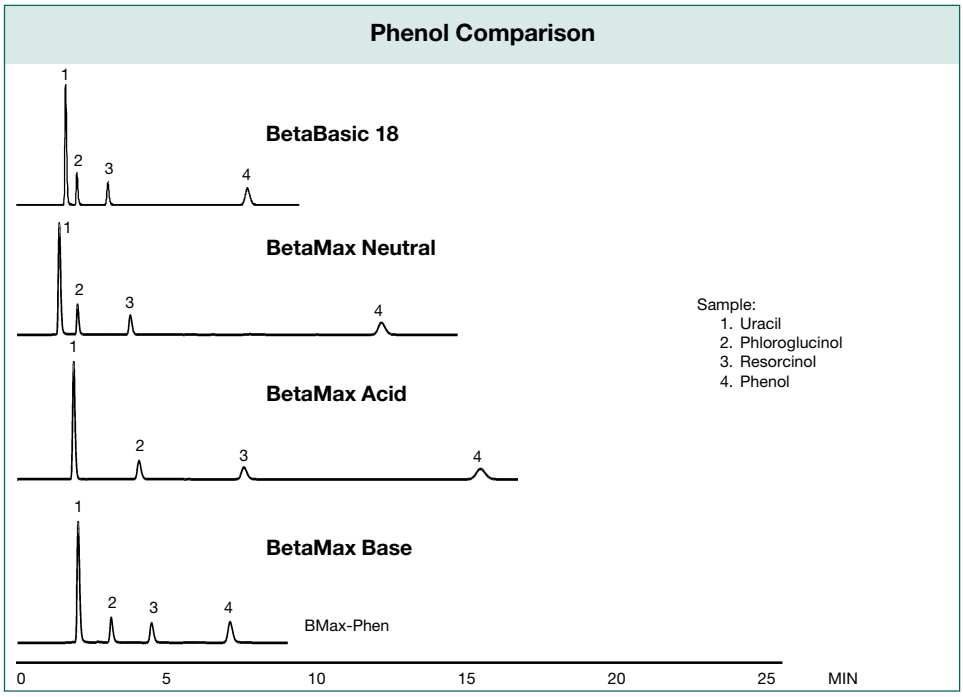
These packings offer substantial promise for the retention and quantification of polar acidic compounds. The moderately polar compound, resorcinol, was also retained to a greater extent with the BetaMax Acid and BetaMax Base columns, although to a lesser extent with the BetaMax Base column.

Figure 2



Columns: **5µm, 150x4.6mm**
Eluent: 90% 50mM KH_2PO_4, pH 3.5 / 10% ACN
Flow: 1.25 mL/min
Detector: UV @ 254

Figure 3



Columns: **5µm, 150x4.6mm**
Eluent: 80% 0.1% formic acid / 20% ACN
Flow: 1.0 mL/min
Detector: UV @ 254

BetaMax® Base

The cyano functional group has been shown to provide good retention, selectivity and peak shape for a wide variety of basic compounds. While adequate selectivity cannot always be tied to maximum retention, the achievement of a large capacity factor, k, is a vital part of the fundamental resolution equation. The cyano functional group of the BetaMax Base phase makes it a good first choice for compounds which have at least one amino group.

BetaMax Base columns are versatile because the cyano function can also retain and separate compounds under normal phase conditions. For polar molecules, this change to a new retention mechanism often occurs near 100% organic in the mobile phase, as illustrated by the graphic representation in Figure 4. Acetonitrile and THF are weaker than methanol in normal phase and can be useful as modifiers to methanol to encourage more retention and alter selectivity. BetaMax Base columns also show dramatic changes in selectivity based upon variations in pH conditions (Figure 5).

BetaMax Acid

The BetaMax Acid phase employs an embedded polar group in a long alkyl chain, similar to the PRISM® phase, for highly selective interactions with polar molecules. While primarily a reversed phase packing with special affinity for carboxylic acid groups (Figure 6), BetaMax Acid columns should be evaluated for any molecule with multiple functional groups which can engage in hydrogen bonding. Figure 6 demonstrates the unique capabilities of the BetaMax Acid packing in the retention of ascorbic acid, a polar acidic compound that requires 100% aqueous conditions for adequate retention.

Figure 4

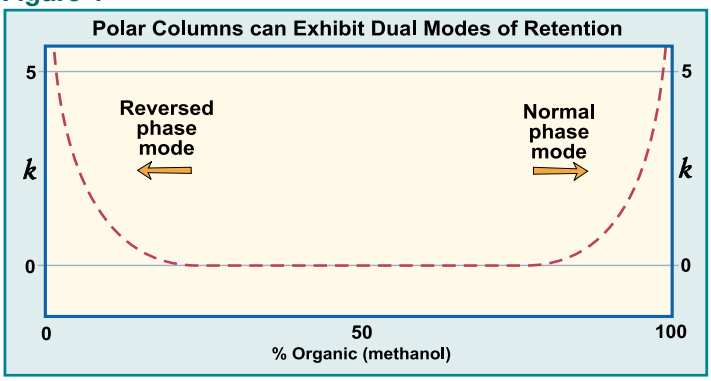
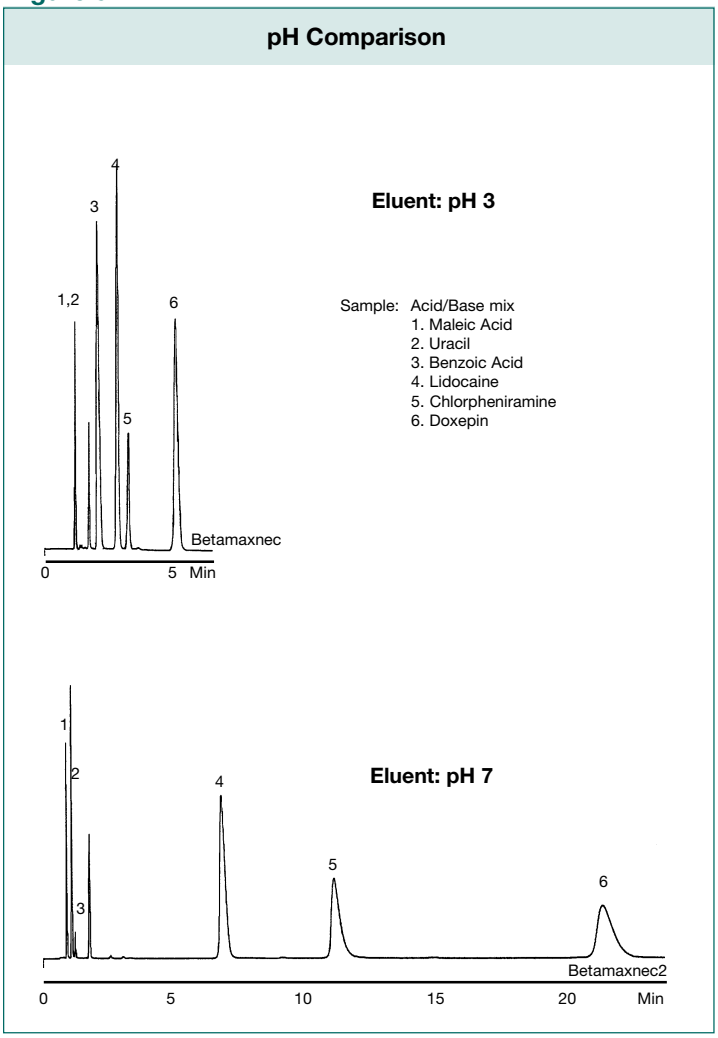
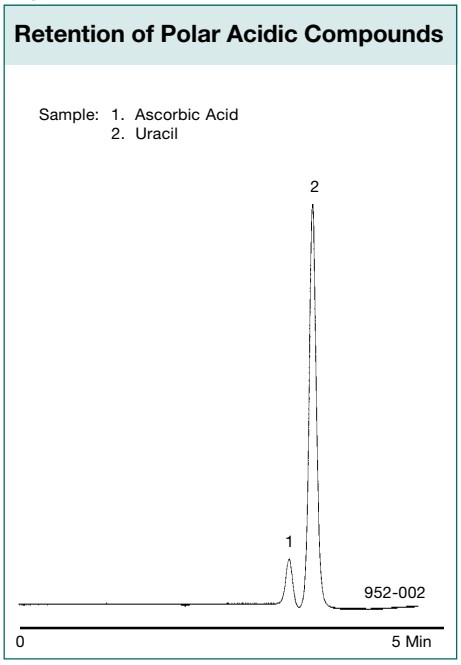


Figure 5



BetaMax Base, 5µm, 150x4.6mm
Eluent: 35% ACN / 65% 0.05M KH_2PO_4
Flow: 1.25 mL/min
Detector: UV @ 254
Temperature: Ambient

Figure 6



BetaMax Acid , 5µm, 150x4.6mm
Eluent: 0.01M KH_2PO_4, pH 2.6
Flow: 1.0 mL/min
Detector: UV @ 220

BetaMax® Neutral

While BetaMax Base and BetaMax Acid columns will often show good retention and selectivity for neutral molecules, the C18 group of the BetaMax Neutral phase will usually give greatest retention and resolution for non-ionic molecules. The extreme hydrophobicity of BetaMax Neutral, which has almost 30% carbon by weight, makes it useful primarily in mobile phases where at least 10%-20% organic can be employed in the mobile phase. Maximum retention and selectivity are achieved when larger molecules having alkyl chains are analyzed. Figures 7 and 8 demonstrate retention and selectivity for organic acids through the comparison of a BetaMax Neutral column versus two other moderately polar C18 columns. The maximum retention and separation is achieved using the BetaMax Neutral column, even in a relatively weak mobile phase. The excellent separation is based on the varying hydrophobicity of each analyte, based on the different positions of double bonds and alkyl groups.

Stability

BetaMax packings achieve low bleed and high stability through the use of uniform, high-purity silica and dense phase bonding. All BetaMax phases exhibit this superior stability as shown in Figure 9. All three BetaMax packings have been subjected to TFA hydrolysis at pH 1.8 and 50°C. This exceptional stability has benefits for LC/MS or any situation where high sensitivity and reproducibility over a large number of samples is necessary, especially under challenging mobile phase conditions.

AQUASIL™ Siliconizing Fluid for treating glass surfaces is sold by Pierce Chemical Co., Rockford, IL.

Figure 7

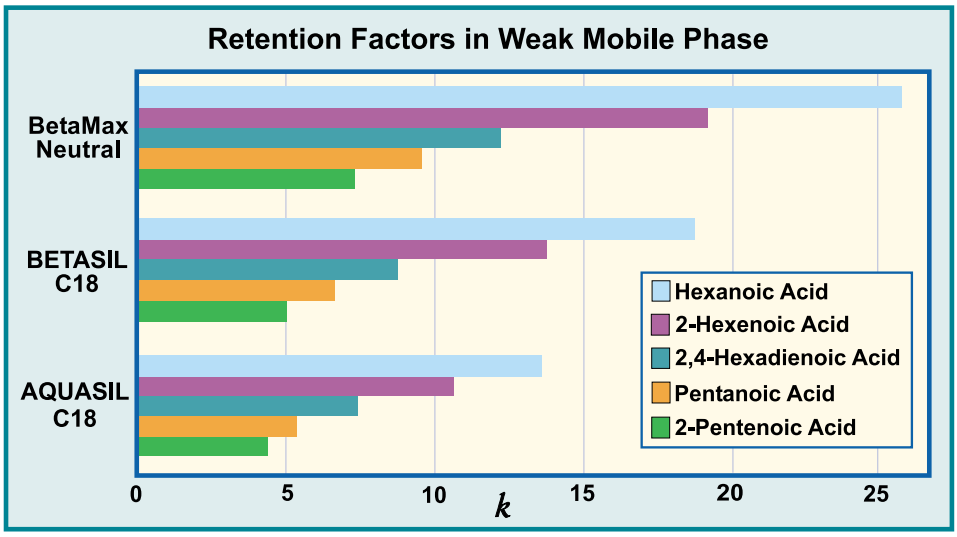


Figure 8

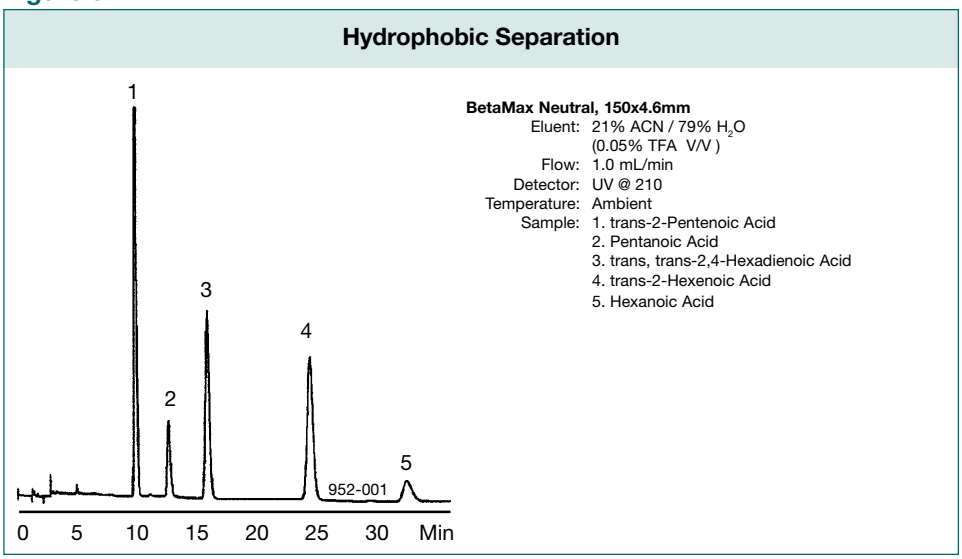


Figure 9