Impact of pH on Improving the Purity and Yield for Preparative Separations Using Gemini®-NX LC Columns

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Increase yield and fraction purity for basic compounds using Gemini-NX preparative columns. By neutralizing the charge on basic compounds under high pH mobile phase, resolution and retention are significantly improved. Baseline resolution can be maintained even after increasing sample concentration 10x (as seen in this technical note).

Introduction

Chemists always want to maximize throughput, but the two common problems encountered in preparative separations that limit throughput are low sample solubility and low resolution.

Gradient conditions at low pH using volatile TFA have routinely been used for preparative separations due to the pH limitations of silica-based columns. The introduction of Gemini®-NX media, which is stable in either acidic or basic conditions, provides the chemists more flexibility in choosing mobile phase conditions. Gemini-NX is available in analytical dimensions and in larger preparative 21.2, 30, and 50 mm diameter dimensions using the Axia™ technology, adding new opportunities to achieve better purifications within the pH range of 1-12.

This technical note compares the same preparative separations with low and high pH volatile buffers using Gemini-NX media, highlighting the advantages when operating at an elevated pH even if the compounds have low solubility and/or low resolution. The work also compares and demonstrates the utility of this media to improve throughput in the preparative and kilo scale laboratories.

Experimental

Gemini-NX 5 µm C18 media, packed in a 50 x 21.2 mm Axia column, was used to investigate how low sample solubility and low resolution ultimately impact the yield and purity of the preparative purification. Gradient conditions were standardized using a 5 minute gradient from 5 % to 95 % ACN (Acetonitrile) with either a pH 2 mobile phase using 0.5 % TFA or a pH 10.5 mobile phase using 0.2 % NH_4OH (Ammonium Hydroxide). UV detection at 254 nm was used for all separations.

Three different sample mixtures of basic compounds with pK_a > 7 were separated using both high and low pH gradient conditions on the Gemini-NX column and the results were compared.

1. The diphenhydramine and propranolol mixture represents a difficult separation with low resolution. The two components are separated by only 0.1 minutes at low pH.
2. The lidocaine and diphenhydramine mixture represents a typical separation where the two components are better resolved by approximately 0.7 minutes using the low pH gradient conditions.
3. The diphenhydramine, oxybutynin and terfenadine mixture represents a situation where each compound must be isolated. The compounds are separated from each other by approximately 0.5 minutes at the low pH gradient conditions.

For each of these examples the initial sample load (represented by the bottom chromatogram in **blue** in each figure) is based on previous work under the low pH conditions where the resolution between the compounds was sufficient to isolate the first compound with purity greater than 95 % and a yield greater than 85 %.

To represent the preparative separation problem of low analyte solubility, the same sample was loaded in a larger volume of DMSO in the second example (middle chromatogram in **red** in each figure). The impact of further overloading the column is shown in the third chromatogram and represents the situation where a chemist wants to increase productivity by increasing the mass loaded for each run (top chromatogram in **magenta** in each figure).

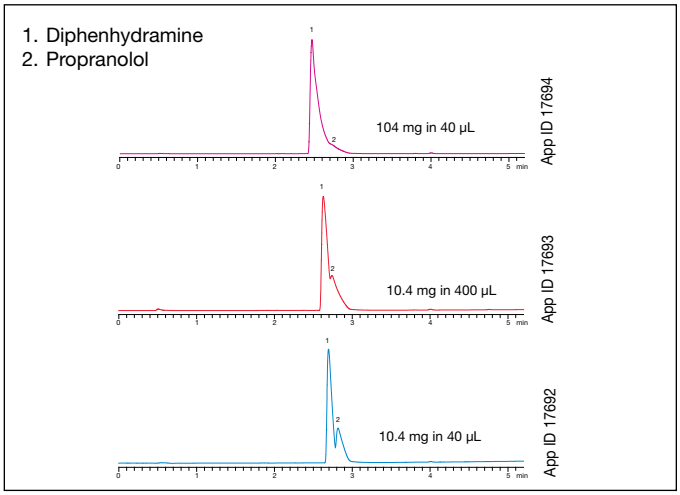
Results

The basic compounds have pK_a > 7 and when purified using 0.5 % TFA in the mobile phase the molecules are positively charged and more hydrophilic. With TFA in the mobile phase, the ionized bases have less hydrophobic interaction with the stationary phase as indicated by the shorter retention time and lower amount of acetonitrile required to elute the compounds. As seen in the chromatograms (**Figures 1**, **3**, and **5**), diphenhydramine elutes with the low pH buffer at approximately 2.8 minutes when the acetonitrile concentration is at 50 %.

When separating basic compounds at higher pH conditions, the compounds are more hydrophobic in their un-ionized state and retain longer on the reversed phase columns. A higher acetonitrile concentration is required to elute these uncharged bases from the Gemini-NX column when using the pH 10.5 NH_4OH mobile phase (**Figures 2**, **4**, and **6**). For example, diphenhydramine elutes with the high pH buffer at approximately 3.8 minutes when the mobile phase is 75 % acetonitrile, compared to 2.8 minutes with the low pH buffer when the acetonitrile concentration is 50 %.

Results at Low pH 2 with 0.5 % TFA

Figure 1
Gemini®-NX Low pH 2 with 0.5 % TFA



Diphenhydramine and propranolol are separated on the Gemini-NX 5 µm C18 column. Diphenhydramine elutes at approximately 50 % acetonitrile when using the TFA gradient conditions. If a higher loading volume is used, the resolution between the two compounds is lost, but the retention time for each of the peaks remains unchanged with this increased injection volume. If the sample mass is increased (same loading volume), the compounds elute earlier during the gradient indicating that the column is overloaded. With either the higher volume or the higher sample load there is no purification since the compounds merge together.

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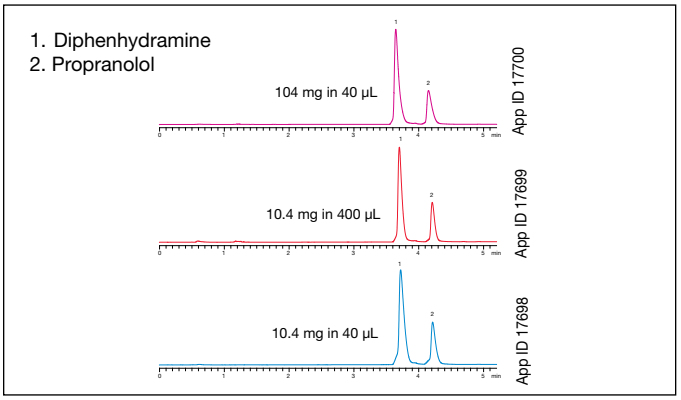
Results at High pH 10.5 with 0.2 % NH_4OH

A dramatic change is seen when separating the basic compounds at pH 10.5 for the Gemini-NX column. At pH 10.5, the basic compounds are un-ionized and are more hydrophobic. The retention for the uncharged basic compounds increases and higher concentrations of acetonitrile are required to elute the compounds.

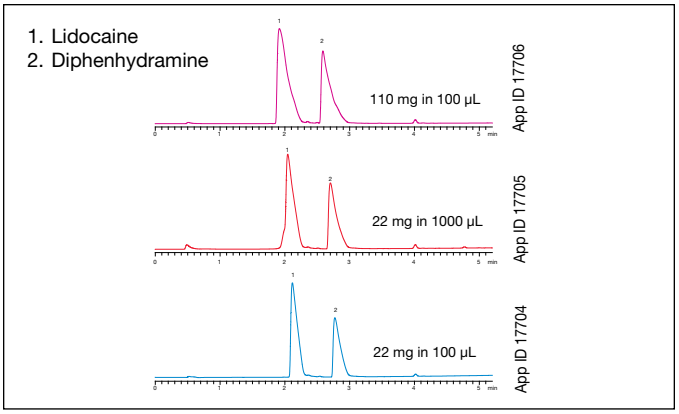
Figure 2
Gemini®-NX High pH 10.5 with 0.2 % NH_4OH



The resolution of diphenhydramine and propranolol is significantly improved at the higher pH and the two compounds are now separated by 30 seconds instead of 6 seconds. The uncharged diphenhydramine requires 75 % acetonitrile to elute it from the column. Increasing the sample volume from 40 µL to 400 µL does not affect the separation and increasing the sample load to 10X does not cause any loss of resolution.

Results at Low pH 2 with 0.5 % TFA

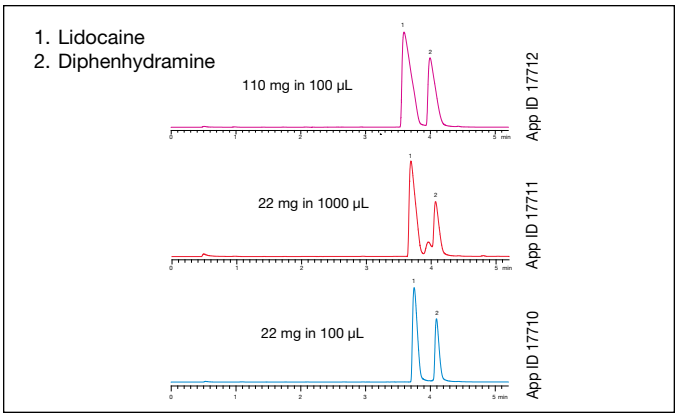
Figure 3
Gemini®-NX Low pH 2 with 0.5 % TFA



Lidocaine is better resolved from diphenhydramine compared to the propranolol example in **Figure 1** and higher sample loads (22 mg) are possible with this mixture. Lidocaine elutes from the column in approximately 40 % acetonitrile. When the loading volume is significantly increased, peaks begin to front due to the 1000 µL loading volume on the Gemini-NX 5 µm C18 column and sample breakthrough occurs as indicated by the peak at the solvent front. The larger loading volume has a major impact on the peak shape for compounds with shorter retention times since they are eluting at much lower concentrations of acetonitrile. When the mass is increased 5X, the retention time slightly decreases. With this higher load, the peak shape becomes broader for both compounds.

Results at High pH 10.5 with 0.2 % NH_4OH

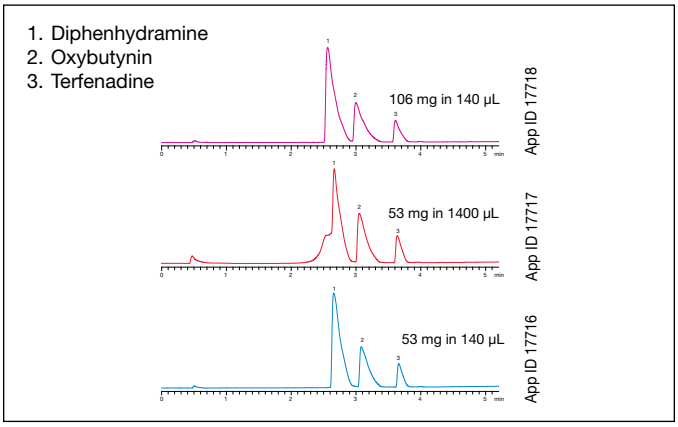
Figure 4
Gemini®-NX High pH 10.5 with 0.2 % NH_4OH



The preparative separation of diphenhydramine and lidocaine is also improved at the pH 10.5 conditions compared to the low pH 2 conditions (compare **Figure 3** and **4**). There is some peak splitting that occurs when the sample volume is increased to 1000 µL DMSO, but the higher mass loading only causes a slight broadening of the peak and shorter elution time. The resolution between the peaks is not affected by the increased mass loading.

Results at Low pH 2 with 0.5 % TFA

Figure 5
Gemini®-NX Low pH 2 with 0.5 % TFA



The three compounds are well resolved when using the Gemini-NX media and a 53 mg load with the TFA buffer. When the more dilute sample is loaded the 1400 µL injection deforms the peak shape due to the high volume of DMSO. If the sample volume is limited to 700 µL the peak shape is maintained and there is no sample breakthrough (data not shown). When the sample load is increased to 106 mg the compounds are still resolved, although they elute slightly earlier in the gradient, but there is no sample breakthrough at the solvent front.

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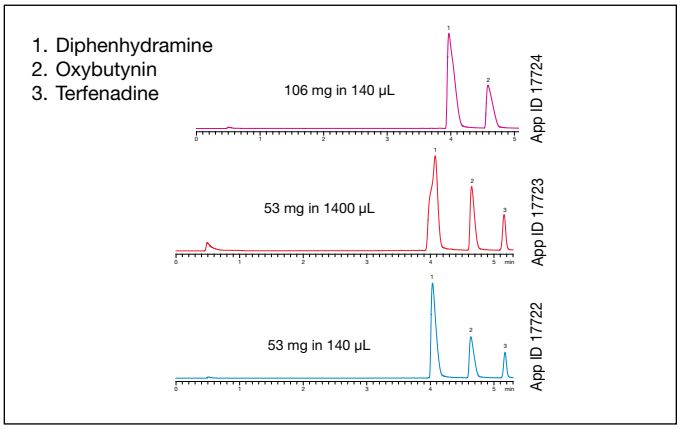
Results at High pH 10.5 with 0.2 % NH_4OH

Figure 6
Gemini®-NX High pH 10.5 with 0.2 % NH_4OH



When the mobile phase is at pH 10.5, these three compounds require a significantly higher % acetonitrile to elute from the Gemini-NX column. The resolution between the compounds is maintained as the sample volume and/or sample mass is increased. There is no loss of resolution and only a very slight shift in retention time as the sample mass increases indicating that much more sample could be loaded on the column.

Conclusions

For preparative separations of basic compounds the use of a high pH mobile phase is very advantageous:

1) At pH 10.5 the basic compounds are un-ionized and more hydrophobic, allowing increased interaction with the stationary phase.

2) Higher concentrations of acetonitrile are required to elute the compounds.

3) Separations are less affected by injection volume allowing more dilute samples to be purified.

4) Peak distortion due to column overloading is reduced and higher sample loads can be purified in a single run.

In all cases studied, the higher pH buffer allowed higher mass loading reducing the number of purification cycles required. The increased resolution at the higher pH also resulted in higher purity and yield for the desired basic compounds.

Operating at the higher pH also provides an alternative choice for the chemist to use a smaller diameter column with the higher sample load. The smaller diameter column reduces the amount of solvent consumed and reduces the volume of the collected fractions.

Gemini-NX is a new standard media for high efficiency preparative separations utilizing a patent-pending organo-silica grafting process that incorporates high stabilizing ethane cross-linking into its structure. This technology provides resistance to high pH attack at elevated temperature, which is critical for preparative separations, but also maintains the high efficiency and mechanical strength of a silica particle that allows the Axia™ process to be utilized to pack these preparative columns.

Ordering Information

Analytical Columns		Part No.	SecurityGuard™ Cartridges (mm)
			4 x 3.0*
			/10pk
Gemini®-NX 5 µm C18	50 x 4.6 mm	00B-4454-E0	AJ0-8368
	150 x 4.6 mm	00F-4454-E0	AJ0-8368
	250 x 4.6 mm	00G-4454-E0	AJ0-8368

Axia™ Packed Preparative Columns		Part No.	SecurityGuard™ Cartridges (mm)
			15 x 21**
			ea
Gemini-NX 5 µm C18	50 x 21.2 mm	00B-4454-P0-AX	AJ0-8370
	150 x 21.2 mm	00F-4454-P0-AX	AJ0-8370
	250 x 21.2 mm	00G-4454-P0-AX	AJ0-8370

guarantee

If Gemini-NX analytical columns do not provide at least an equivalent separation as compared to a competing column of similar particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

*SecurityGuard™ Analytical Cartridges require holder, Part No.: KJ0-4282
**PREP SecurityGuard™ Cartridges require holder, Part No.: AJ0-8223

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