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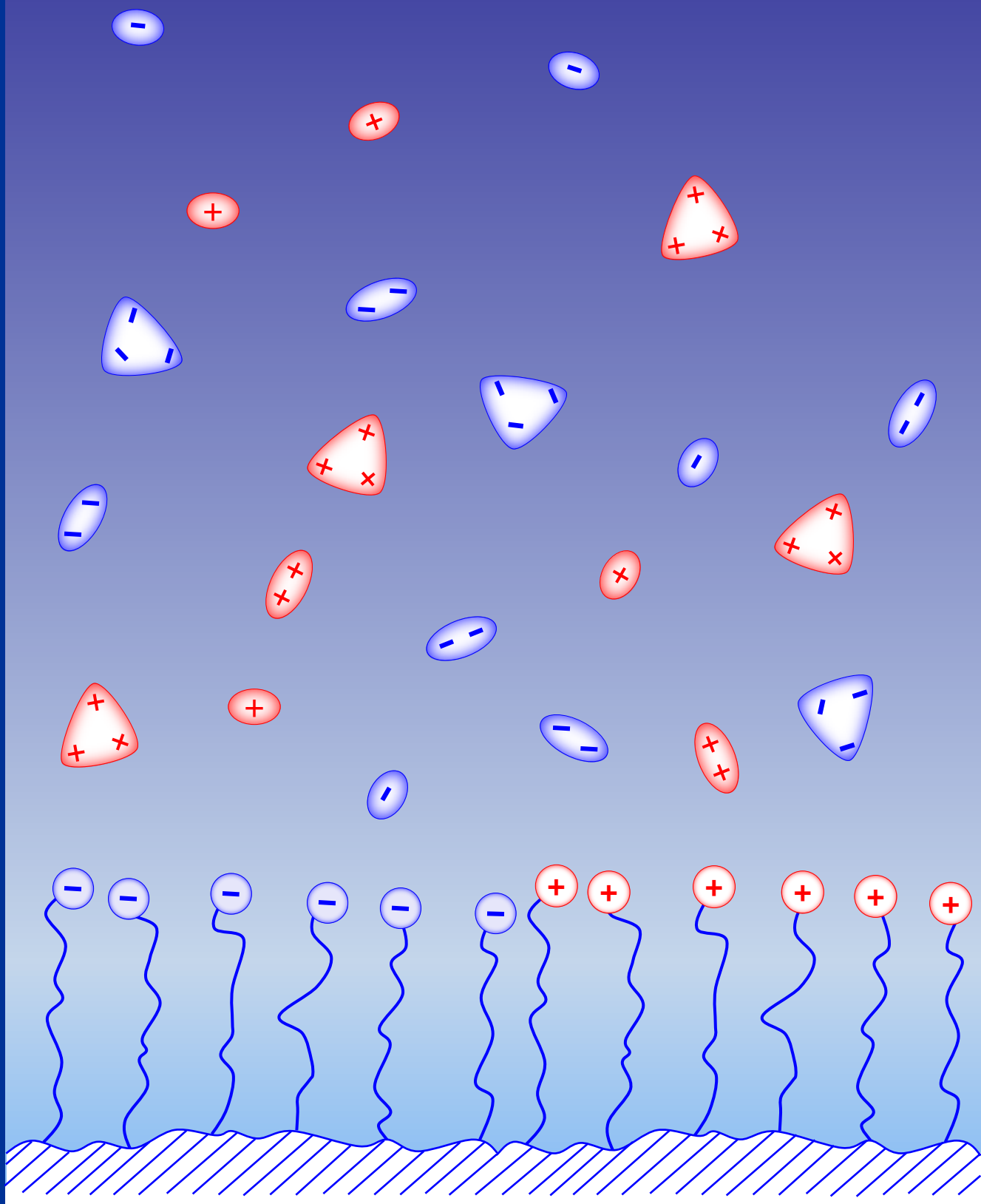
SIELC



Newcrom

A new type of LC separation media

Introduction

SIELC Technologies has developed a line of Newcrom columns, a mixed-mode column based on a new type of bonded stationary phase. This set includes four mixed-mode columns with anion and cation exchange properties and differing ionic capacities. All four columns are both reverse-phase (RP) type and ion-exchange (IE) type.

What makes Newcrom columns special? It is the position of the ion-exchange groups in the ligand structure bonded to the silica surface (Fig. 1).

Column	Ion-exchange	Separation mode	Ion capacity
Newcrom A	Cation exchange	RP + IE	Low
Newcrom AH	Cation exchange	RP + IE	High
Newcrom B	Anion exchange	RP + IE	Low
Newcrom BH	Anion exchange	RP + IE	High

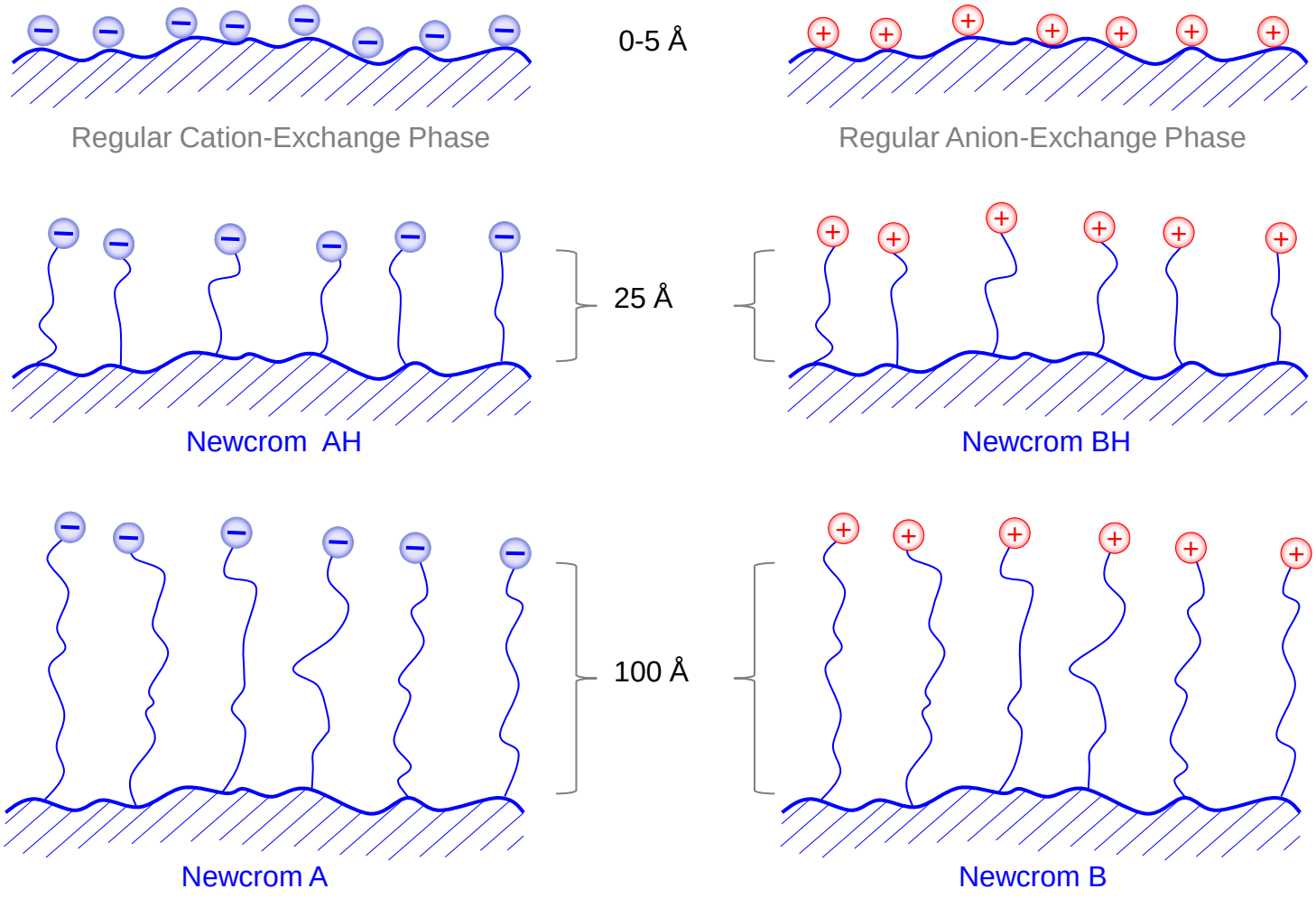


Figure 1. Newcrom mixed-mode stationary phase ligand structure representation. All phases comprise of a long hydrophobic chain bonded to the silica gel surface and a terminal ion-exchange group.

Introduction

Most ion-exchange columns have the ion-exchange groups placed on or near the surface of the solid support (Fig. 2). This creates a problem with getting proper uniform surface charge density. There are regions with relatively high charge density and other regions with relatively low charge density, which can be attributed to normal statistical distributions of the ions.

Explained another way, it is due to the differing accessibility of the pores to the chemical reagents. As a result, the interaction of the charged molecules, especially multi-charged molecules, will be non-uniform. Depending on the surface charge density, the multi-charged molecules can be retained better or worse on such a surface (Fig. 2).

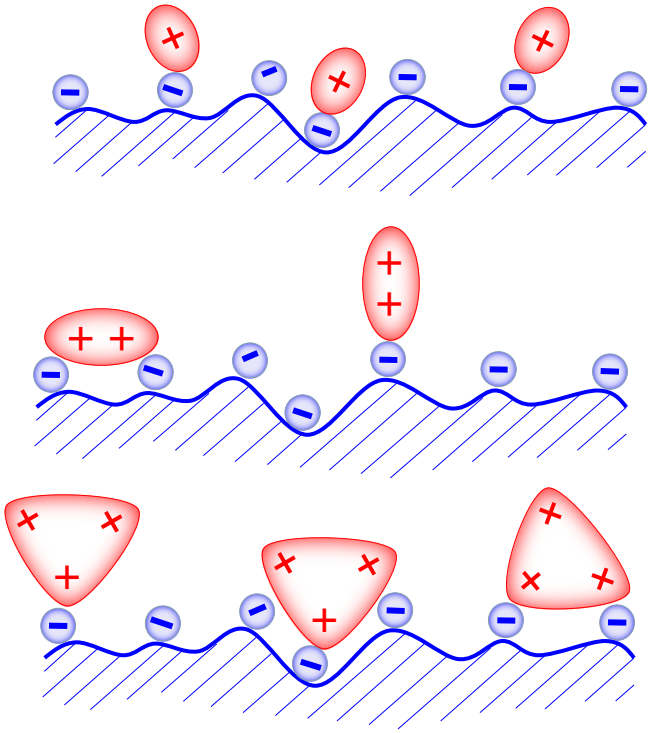


Figure 2. Traditional Ion-exchange column interaction with charged analytes.

When a charged group of the stationary phase is placed at the end of long flexible linkages far away from the surface, such as in the Newcrom columns, the interaction between the stationary phase and the charged analytes mimics the interaction in solution with ions distributed uniformly and the interaction rate is the same for all the ions of similar charge (Fig. 3).

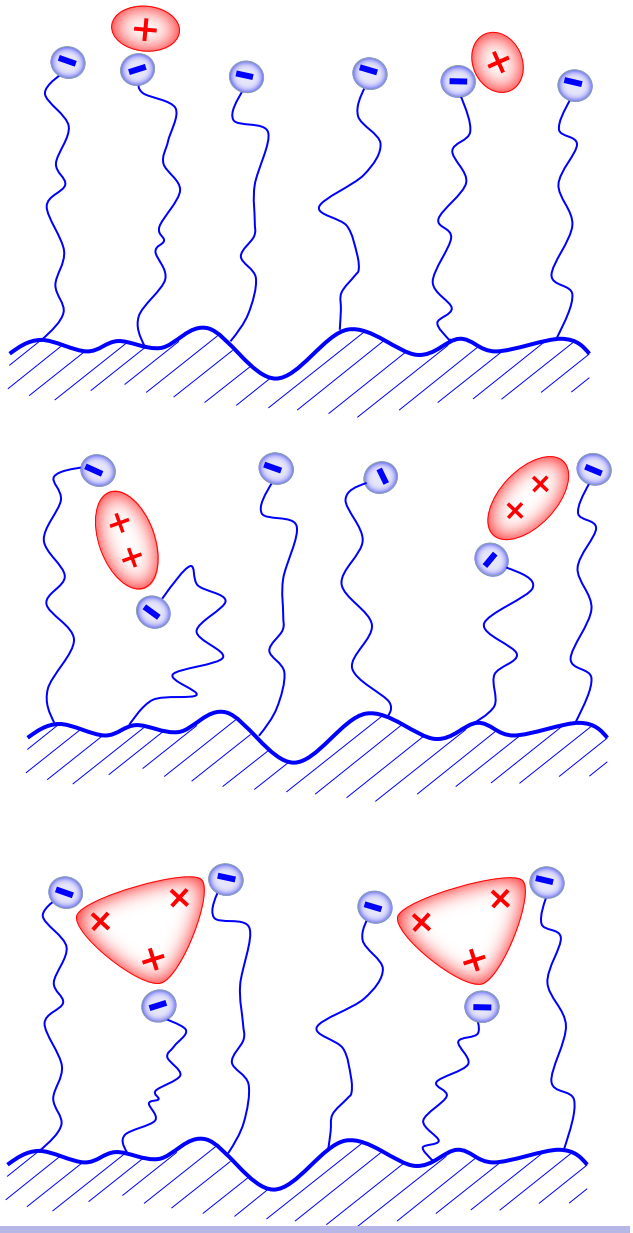


Figure 3. Newcrom Ion-exchange column interaction with charged analytes.

Mixed-mode column advantages

- Retains polar charged compounds.
- Retains hydrophobic neutral compounds.
- No secondary silanol interaction effect.
- Operation possible in either pure water or pure organic mobile phase (MP).
- Two dimensional retention characteristics. Both the buffer concentration and the organic modifier concentration in the MP affect the degree of retention and selectivity.
- Earlier mixed-mode columns were not able to work in MPs containing MeOH, whereas the Newcrom stationary phase can operate in MPs containing either MeOH and MeCN.
- Low buffer concentration needed to generate efficient ion-exchange.
- Short equilibration times when switching between buffers.

Mechanism

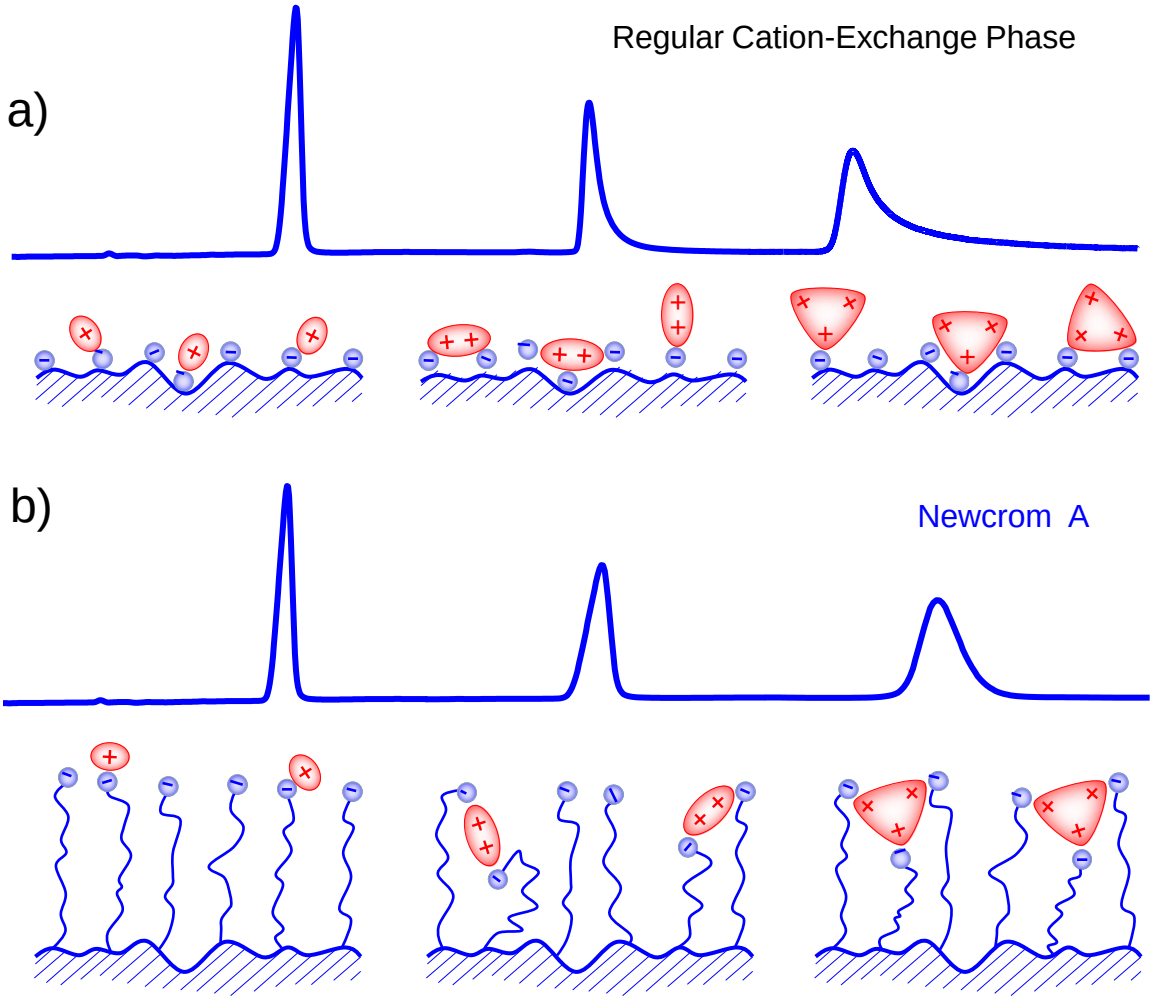


Figure 4. Chromatogram examples of a regular ion-exchange stationary phase with charged analytes (a) and Newcrom type stationary phase with charged analytes (b). Single, double, and triple charged analytes examples are shown.

Fig.4 above depicts how the chromatogram is affected by where the charged functional group is placed relative to the surface. For single-charged species (the 1st peak), there is little, if any, change in the peak symmetry when comparing a regular CE column (Fig. 4a) and a Newcrom A column (Fig. 4b). For multi-charged species, the tail of the peak drifts on a regular CE column (the 2nd and 3rd peaks). As depicted below the chromatogram in Fig. 4a, this is due to the fact that multi-charged species are retained unevenly on the surface due to normal statistical variance in how each multi-charged analyte approaches the surface. With Newcrom A, the charged analytes interact with a terminal charge on an extended ligand, which gives the stationary phase the ability to uniformly retain multi-charged species, as depicted in Fig. 4b. This produces significantly more symmetric peak characteristics.

Polar Compounds

Reverse-phase (RP) columns are the workhorses of HPLC analysis. The four main problems of typical RP columns familiar to anybody who has developed a reverse phase method are:

- Poor retention of polar compounds.
- Residual unwanted silanol interaction with basic compounds.
- Dewetting at lower organic concentration in the mobile phase.
- Zero or limited control of elution order and resolution of analytes.

To address each problem, different modifications on standard C18 chemistry were developed and used instead of the standard C18. Over 500 modifications of C18 columns are available on the market as a result.

We are introducing Newcrom A and Newcrom B columns, which address all four problems stated above, using brand new mixed-mode chemistry. Now with just these two columns, you can do almost any type of separation by modifying just the mobile phase.

Notice in Fig. 5, for example, how the retention times and order of the various compounds change from Fig. 5a to Fig. 5b. These separations were done on the same Newcrom A column, and compound retention was altered significantly just by inverting the ratio of Acetonitrile (MeCN) to H_2O in the mobile phase. Fig. 5c shows another significant change in retention times and order just by replacing MeCN with Methanol (MeOH) as the organic component in the MP.

A significant number of polar compounds have at least one charge. This charge is not efficiently utilized for separation in traditional RP chromatography. For separation using Newcrom columns, the stationary phase incorporates ion-exchange functional groups, where the strong ionic interaction plays an important role in the retention of polar charged molecules.

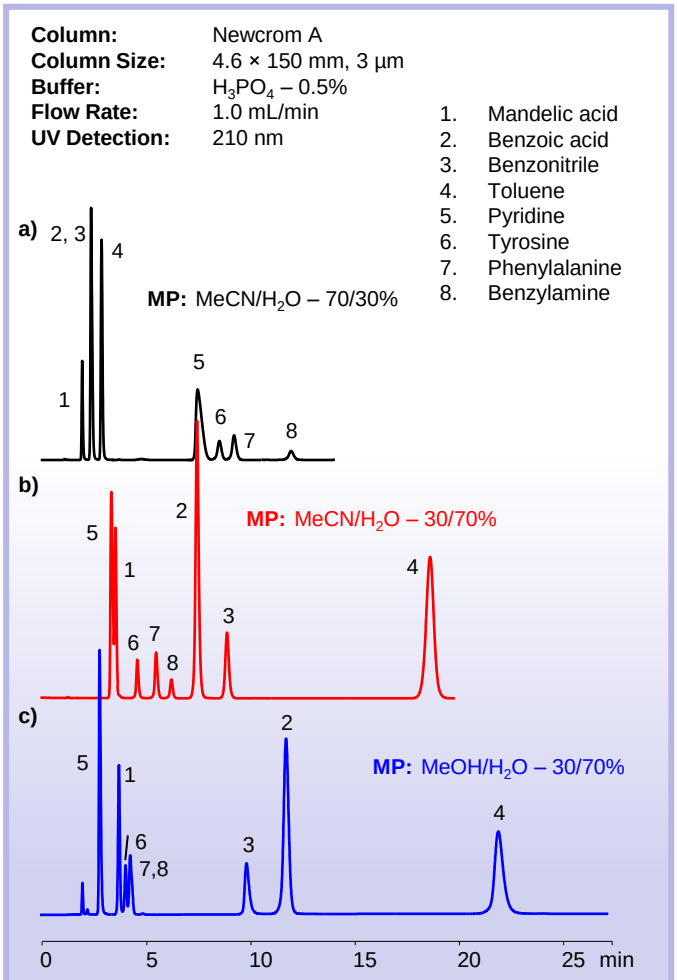


Figure 5 – Change in Retention Order and Times Based on the Concentration & Identity of the Organic Component of the MP.

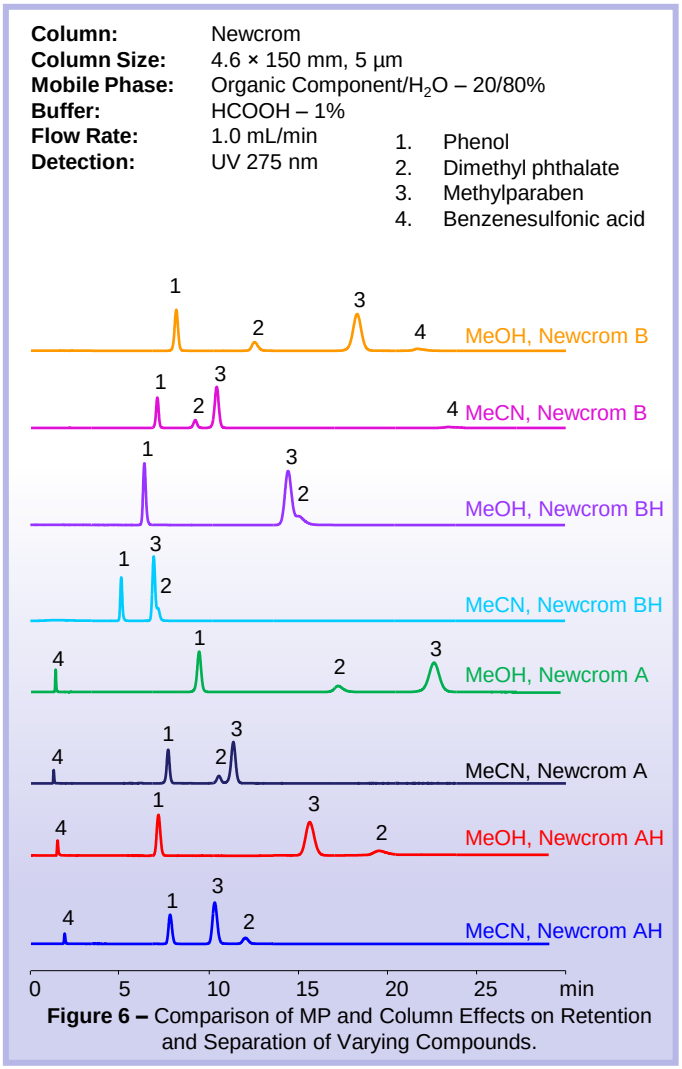


Figure 6 – Comparison of MP and Column Effects on Retention and Separation of Varying Compounds.

Polar and Neutral Compounds

Mixed-mode columns retain polar charged molecules and hydrophobic molecules equally well. The retention control can be significantly independent for charged and non-charged analytes. The buffer concentration is the main factor in retaining charged molecules, while the organic modifier concentration is the main factor in retaining hydrophobic molecules.

The type of organic modifier will also affect the retention profile of these compounds, such as when MeCN is replaced by MeOH. The nature of the organic modifier effects not only retention times, but also the relative retention of different molecules. This can provide varying selectivity in complex mixtures. The Newcrom mixed-mode columns provide a different combination of charge type and level of ionic capacity.

Fig. 6 shows how the retention of different neutral and acidic compounds changes based on which of the 4 different Newcrom columns are in use and based on the organic component of the MP.

Column: Newcrom B
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 10/90%
Buffer: H_2SO_4 – 0.1%
Flow Rate: 1.0 mL/min
Detection: UV 260 nm

1. Adenosine 5'- Monophosphate
2. Adenosine 5'- Diphosphate
3. Adenosine 5'- Triphosphate

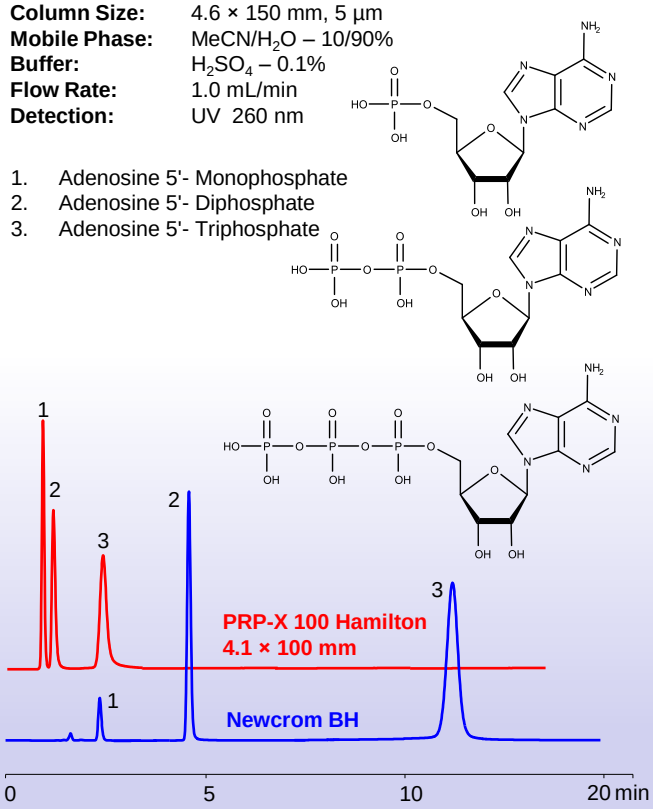


Figure 7 – Retention and Separation Comparison of AMP, ADP, and ATP on Ion-Exchange and Mixed-Mode Columns.

Column: Newcrom
Column size: 4.6 × 150 mm, 5 µm
Mobile phase: MeCN/H_2O – 70/30%
Buffer: AmFm pH 3.0 – 60 mM
Detection: CAD
Flow rate: 1.0 ml/min

1. Sodium
2. Dodecyl Sulfate

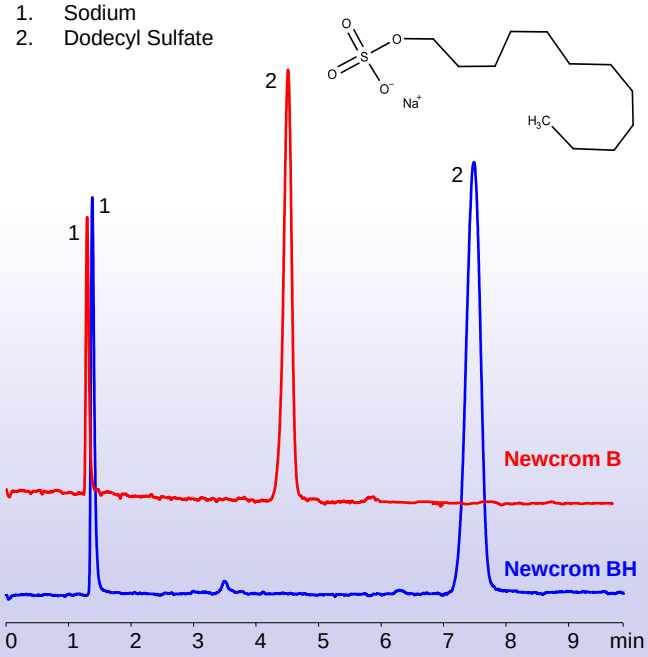


Figure 8 – Retention and Separation of Sodium Dodecyl Sulfate on Newcrom B and Newcrom BH Columns.

No Dewetting

Column: Newcrom A
Column Size: 4.6 × 50 mm, 5 µm
Mobile Phase: MeCN/H_2O
Flow Rate: 1 mL/min
Detection: UV 270 nm

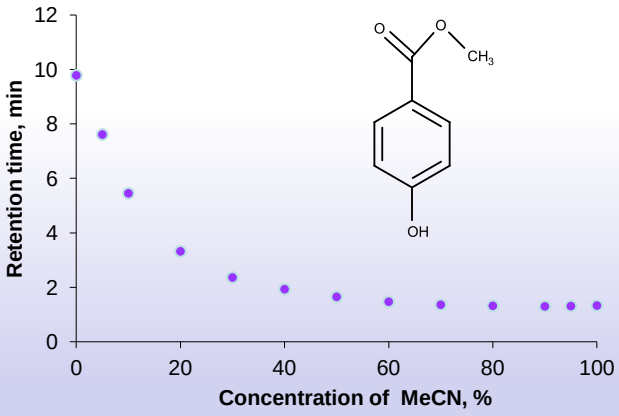


Figure 9 – Dewetting Study of Methylparaben in Varying Aqueous Concentrations on Newcrom A.

Typically, C18 reverse-phase columns require some degree of organic modifier in the mobile phase to provide sufficient wetting (contact) of the hydrophobic stationary phase surface with the water-based mobile phase. If the organic modifier is completely removed, in order to get more retention of polar compounds, a decrease in retention is commonly observed instead of the expected increase. This phenomenon is called dewetting and it often complicates method development for polar compounds. Newcrom is completely free from this complication due to the presence of terminal ionic groups on every bonded ligand molecule. As a result, any mobile phase can be used with 100% water or 100% organic modifier without any negative effect.

You can see this explicitly in the experiment shown in Fig. 9. The data shows that as the concentration of the organic component (MeCN) of the MP decreases, the retention time of Methylparaben increases, and no dewetting occurs.

Mixed-Mode (2-D Chromatography)

Mixed-mode Newcrom columns allow for multiple interactions on a single column and offer 2D-type chromatography (Fig. 10) as a tool to separate complex mixtures with a variety of compounds possessing opposing properties: hydrophobic and hydrophilic, neutral and ionic, positively charged and negatively charged, etc. Newcrom columns can be designed with either Cation-Exchange (CE) or Anion Exchange (AE) technology. With a mixed-mode column, the two unique exchange separation modes (ion-exchange and reverse-phase) have different hydrophobicity, and vice versa. Mixed mode columns can use these two-dimensional differences to separate compounds previously inseparable in a one-dimensional column.

When a single mode of interaction is employed on a reverse-phase column, compounds elute according to their hydrophobicity (designated as Log P). Analytes with similar hydrophobicity may co-elute or show poor resolution, as shown in Figs. 10a and 10d. Notice how the retention order in Fig. 10d corresponds to the ascending order of each analyte on the y-axis in Fig. 10a. An opposite approach is to use ion-exchange chromatography, where compounds are retained based on their ionic properties (designated as Log D). Similarly, using ion-exchange chromatography, some compounds may closely elute because they are identical in terms of the strength of ionic interaction between the stationary phase and analyte; this can bee seen in Figs. 10c and 10f. Notice here that the retention order in Fig. 10f corresponds to the ascending order of each analyte on the x-axis in Fig. 10c. In mixed-mode chromatography, both reverse-phase and ion-exchange modes are used, so there are two possible types of interactions – hydrophobic and electrostatic – and analytes elute based on the combined strength of both interactions relative to the mobile phase composition.

Mixed-mode chromatography is characterized by the diagonal in Fig. 10b, where the slope, α, is a function of the ratio of ion-exchange vs. reversed-phase governed by the mobile phase composition. The selectivity of the mixed-mode column is governed by the composition of the mobile phase – specifically, by the buffer and organic component concentrations. Low concentrations of organic component or high buffer concentrations will correspond with a high value of α (~90°), and thus RP-like retentions, whereas high concentrations of the organic component or hydrophilic analytes will correspond with a low value of α (~0°), and thus more IE-like retentions.

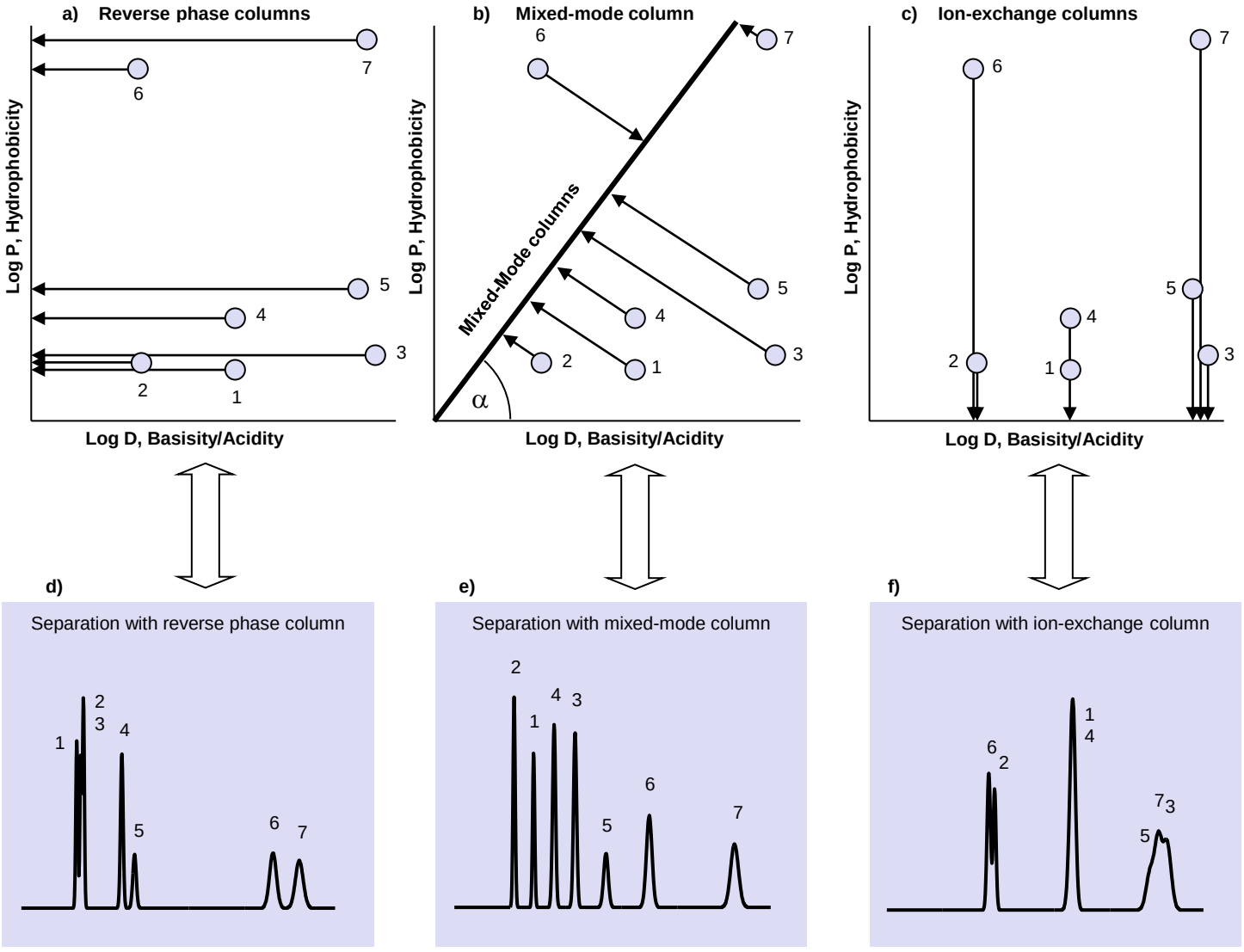


Figure 10. a-c) Plots of analytes' hydrophobicity and ionic strength on reverse-phase, mixed-mode, and ion-exchange columns, respectively. d-f) Diagrams of the retention order for reverse-phase, mixed-mode, and ion-exchange columns.

This property of mixed-mode columns – that they can be tuned to utilize both the RP and IE retention modes - is illustrated clearly by the difference in the retention profiles shown in Fig. 5 (on Pg. 5), and Figs. 11 and 12 below. Remember, in Fig. 5, the elution order of the various charged and hydrophobic compounds changed depending upon whether the aqueous or organic component of the mobile phase was higher. Similarly, in Fig. 11, the charged compounds, like Iron (III) and Iron (III)-EDTA Complex, elute first in the high-aqueous MP found early in the gradient. Only later in the gradient, as the organic component increases in concentration, do the hydrophobic, non-charged compounds elute. Similarly in Fig. 12, Fenofibric acid prefers the aqueous-majority MP and elutes first, while Fenofibrate takes more than twice as long to elute.

Newcrom's mixed-mode stationary phase has at least two strong interactions with the analytes: an ionic interaction based on charge and a hydrophobic interaction based on polarity. Each interaction is regulated independently by its respective component in the mobile phase. The ionic interaction is governed by the pH and the ion-strength of the buffer in the mobile phase. The hydrophobic interaction is governed by the concentration of organic modifier in the mobile phase. By changing these three parameters, the degree of retention and the elution order of analytes can be tuned to your specific needs.

Column: Newcrom BH
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 2/98% to 70/30%
Gradient: 10 min
Buffer: H_2SO_4 – 0.1%
Flow rate: 1 ml/min
Detection: UV 260 nm

1. Fe(III)
2. Fe(III)-EDTA complex
3. Phenoxyethanol
4. Methylparaben

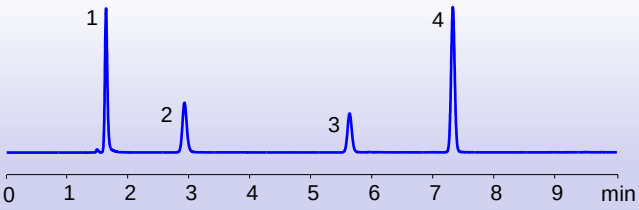


Figure 11 – Retention of Active Ingredients in Facial Cleanser.

Column: Newcrom AH
Column size: 3.2 × 100 mm, 3 µm
Mobile phase: MeCN/H_2O – 45/55%
Buffer: Formic Acid - 0.05%
Flow rate: 0.5 mL/min
UV Detection: UV 286 nm

1. Fenofibric acid
2. Fenofibrate

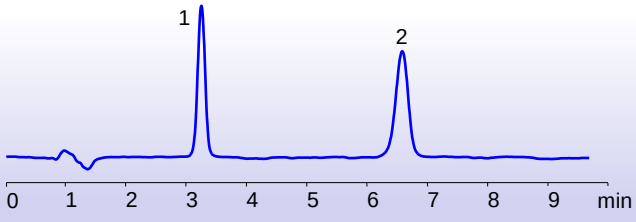


Figure 12 – Retention Time of Fenofibric acid and Fenofibrate.

Charged and Polar Compounds

Charged, polar compounds can take full advantage of the mixed-mode stationary phase on Newcrom columns. They will be affected by both ion-exchange and reverse-phase mechanisms.

A Newcrom B column can clearly separate and retain Phosphoric acid and Phosphorous acid, as shown in Fig. 13. This same column can also separate and retain various compounds with Phosphate functional groups. Fig. 14 shows a separation of various phosphate-containing compounds on a Newcrom B column. Less polar compounds are retained longer, such as Glyphosine, and more polar compounds elute faster, such as Ethylphosphonic acid.

Column: Newcrom B
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 20/80%
Buffer: HCOOH – 2%
Flow Rate: 1.0 mL/min
Detection: CAD

1. Phosphoric acid
2. Phosphorous acid

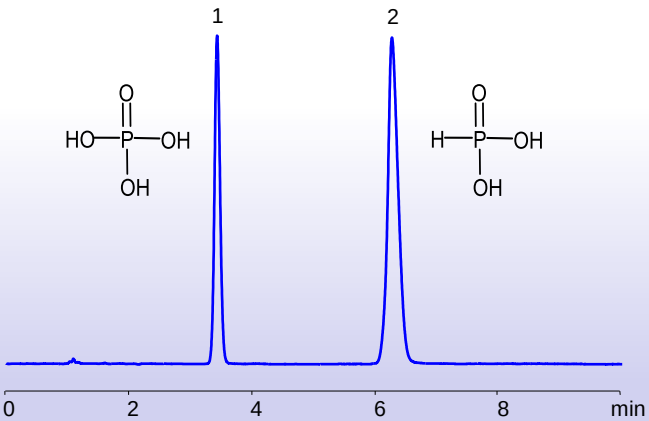


Figure 13 – Separation of Phosphoric and Phosphorous acid on Newcrom B.

Column: Newcrom B
Column Size: 4.6 × 150 mm, 5 µm
Mobile Phase: MeCN/H_2O – 40/60% to 10/90%
Buffer: HCOOH – 1.0% to 5.0%
Gradient: 15 min
Flow Rate: 1.0 mL/min
Detection: CAD

1. Ethylphosphonic acid
2. Glyphosate
3. Phosphoric acid
4. Methylphosphonic acid
5. Methylenediphosphonic acid
6. Glyphosine

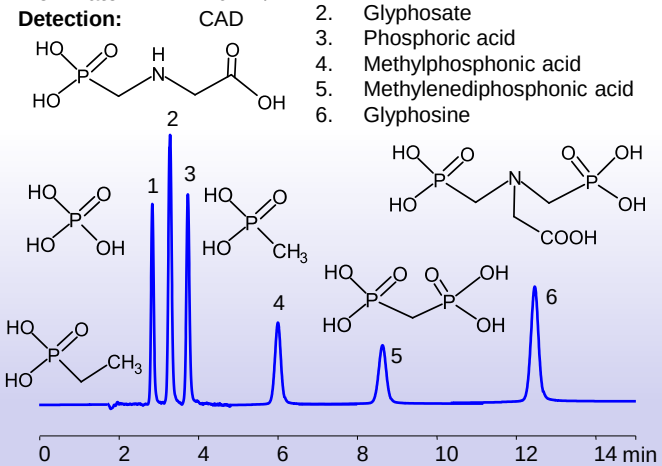


Figure 14 – Separation of Various Phosphorylated Compounds.

Since Newcrom B and Newcrom BH have different ligand lengths, their ion capacities are different enough to affect the retention of charged compounds. Longer ligand chains reduce the amount of ionic groups over any given surface area. This means a column with shorter ligands, such as Newcrom BH (or Newcrom AH) will have stronger ionic-exchange retention relative to Newcrom B (or Newcrom A, respectively). This can be seen explicitly in Fig. 15, where Maleic acid has a retention time nearly three-times as long on a Newcrom BH column compared to that on a Newcrom B column.

Newcrom columns can also separate and retain inorganic cations. Fig. 16 shows how several different inorganic cations were separated simultaneously on a Newcrom BH and on a Newcrom B column, respectively. The change in retention times based on the ligand length of the stationary phase (and therefore the stationary phase's ion capacity) is also apparent.

Simple mass spec (MS) compatible conditions can be used for the separation of many otherwise difficult compounds. A simple mobile phase buffered by Formic acid (HCOOH), Ammonium formate (AmFm), or Ammonium acetate (AmAc) is very convenient for such applications. The same method can be converted to a low UV condition with simple replacement of the Formic acid buffer with ⅓ the amount of Phosphoric acid buffer. The separation and retention of several organic acids in an MS-compatible mobile phase on a Newcrom B column can be seen in Fig. 17.

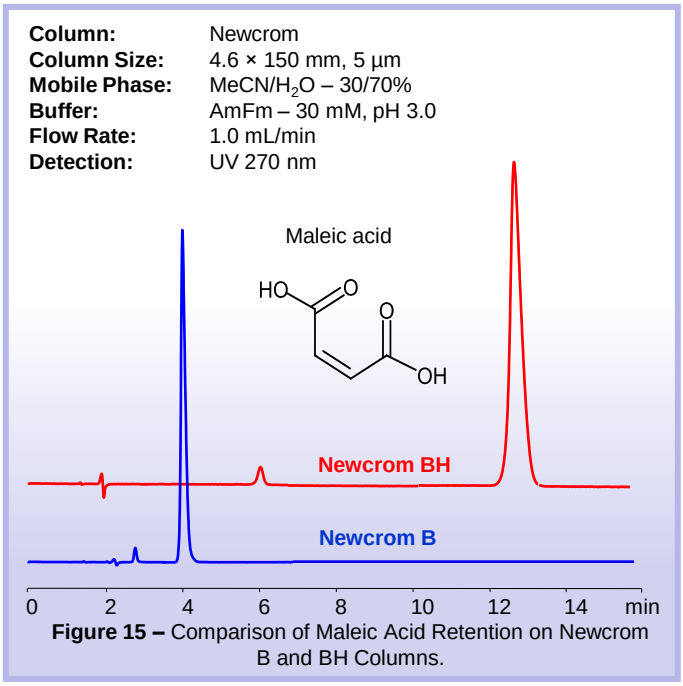


Figure 15 – Comparison of Maleic Acid Retention on Newcrom B and BH Columns.

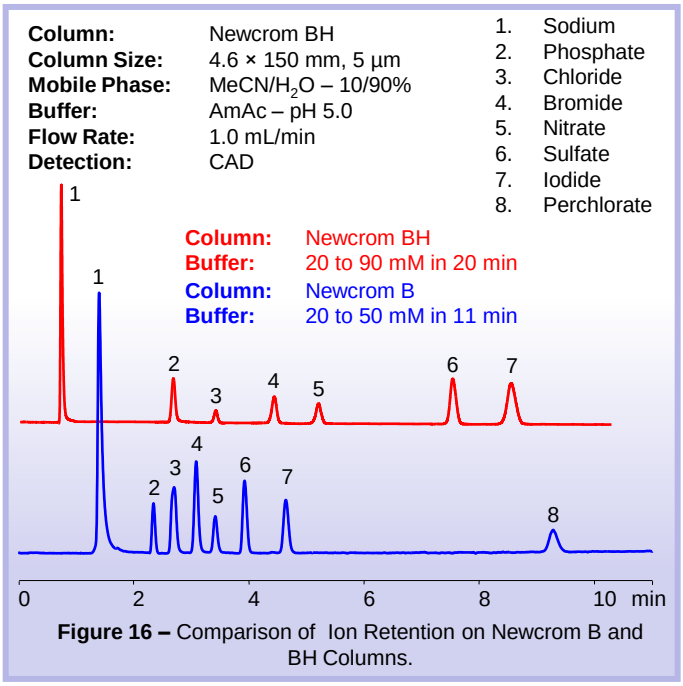


Figure 16 – Comparison of Ion Retention on Newcrom B and BH Columns.

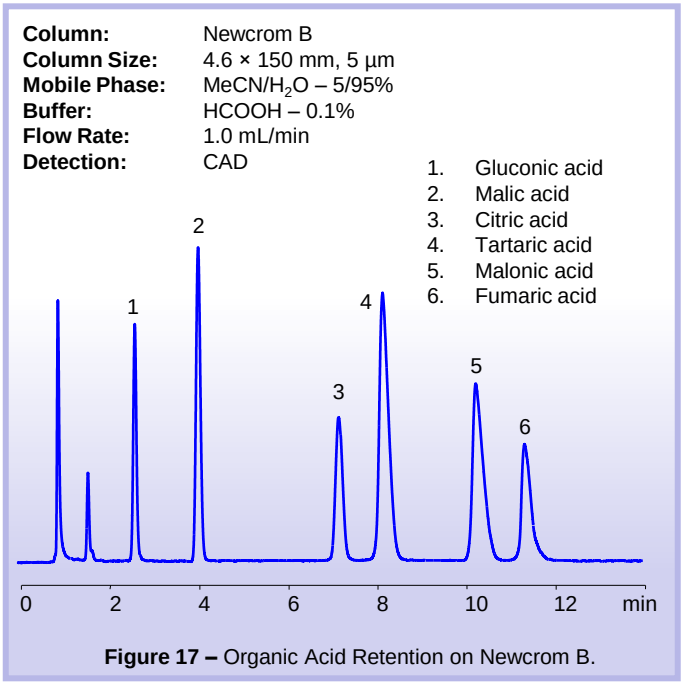


Figure 17 – Organic Acid Retention on Newcrom B.

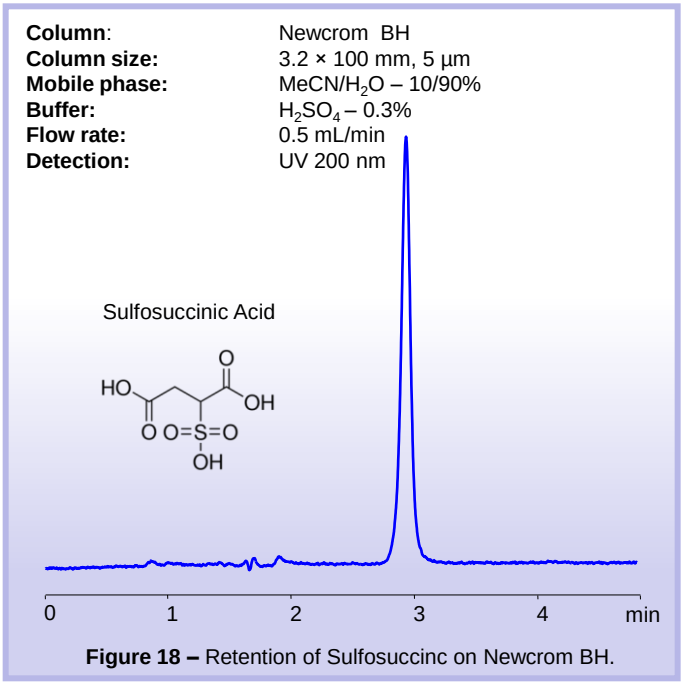


Figure 18 – Retention of Sulfosuccinc on Newcrom BH.

Changing the buffer strength can vastly affect the retention times of charged analytes (but have little-to-no effect on non-charged analytes). This is shown explicitly in Figs. 19a and 19b, where a mixture of Benzenesulfonic acid and Benzonitrile were injected onto a Newcrom B column with various buffer strengths. As the buffer strength increases, Benzenesulfonic acid elutes faster, while Benzonitrile maintains relatively consistent retention times.

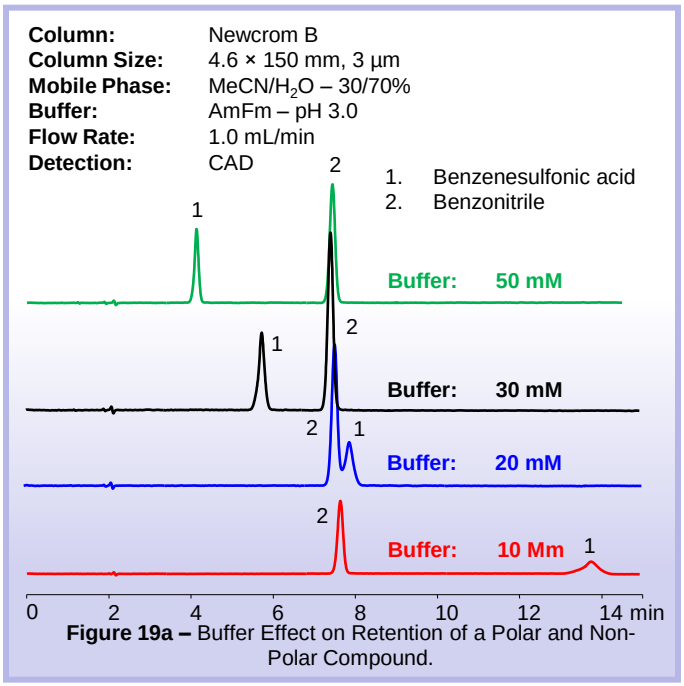


Figure 19a – Buffer Effect on Retention of a Polar and Non-Polar Compound.

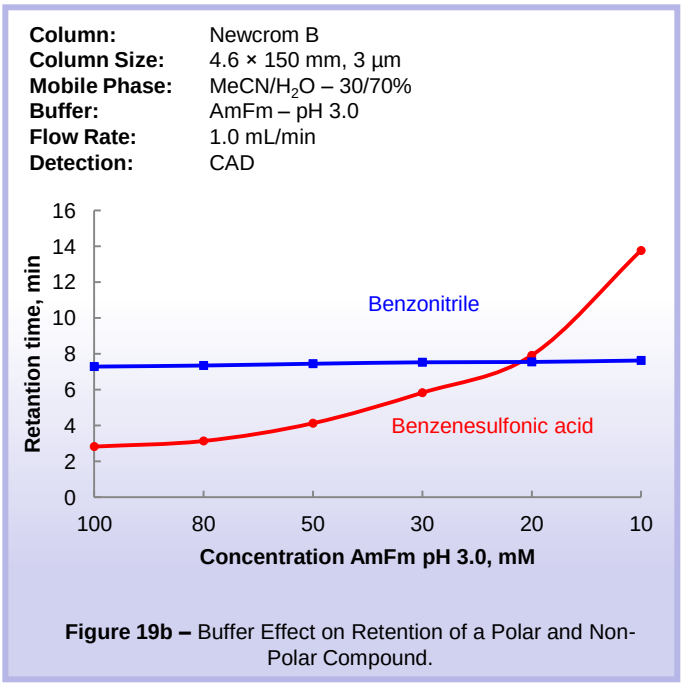


Figure 19b – Buffer Effect on Retention of a Polar and Non-Polar Compound.

This idea, that the buffer strength (and composition) can significantly impact retention times, is further expressed in Fig. 20. The retention times are plotted against the buffer concentration for two different buffers, Formic acid and Phosphoric acid. For any given concentration, Phosphoric acid delivers faster retention times than Formic acid. This is most likely due to the extra protons Phosphoric acid releases in solution compared to Formic acid.

As mentioned previously, Newcrom columns support MS-compatible mobile phases. Fig. 21 shows retention and separation of two different phosphorylated glucose sugars on a Newcrom B column using an MS-compatible MP.

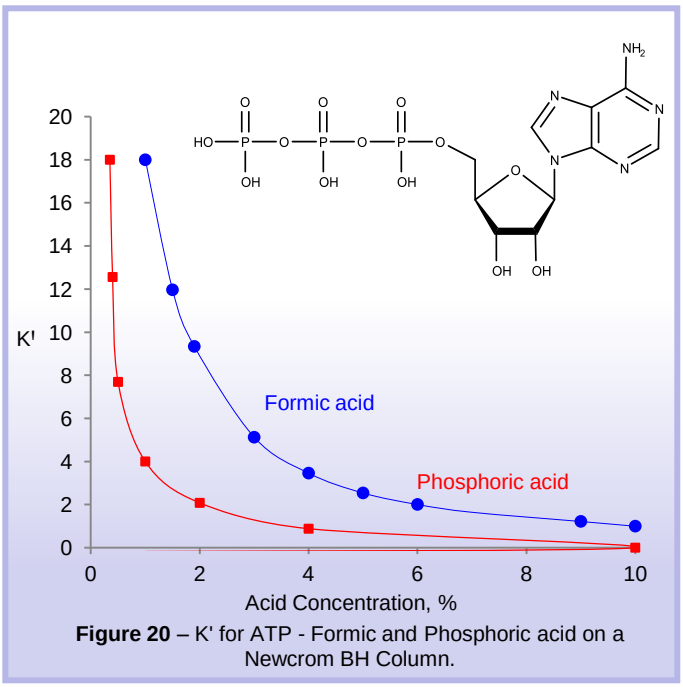


Figure 20 – K' for ATP - Formic and Phosphoric acid on a Newcrom BH Column.

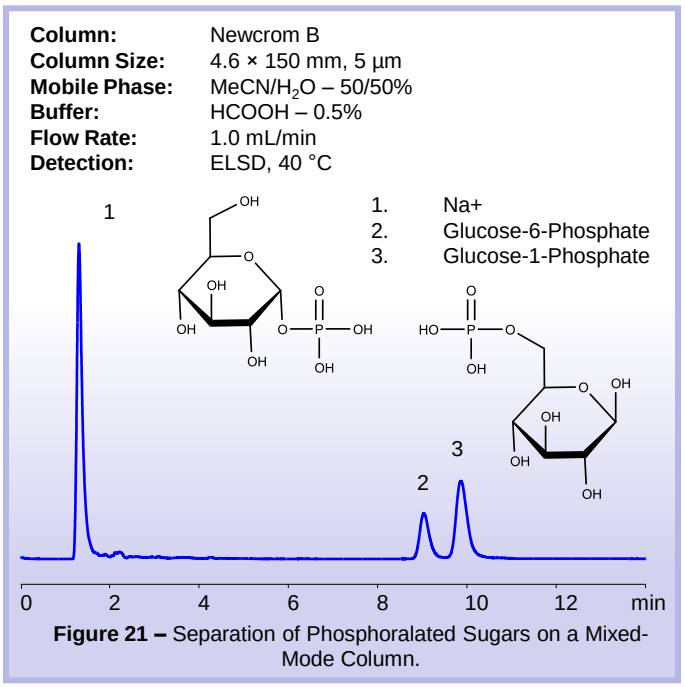


Figure 21 – Separation of Phosphoralated Sugars on a Mixed-Mode Column.

This effect of altering the MP composition, either through changing the organic concentration or the buffer strength, can be used to control the retention of amino acids. This can be shown clearly in Fig. 22, where the retention times of different amino acids increase with a buffer (compared to without a buffer) and with increased MeCN concentration.

Whereas increasing the concentration of the organic component led to increases in the retention times of charged analytes, the inverse effect occurs for uncharged, hydrophobic compounds. This can be seen in Fig. 23, where decreasing concentrations of MeCN resulted in longer retention times of the analytes, with the effect of getting stronger for compounds with increasingly larger hydrophobic carbon chains.

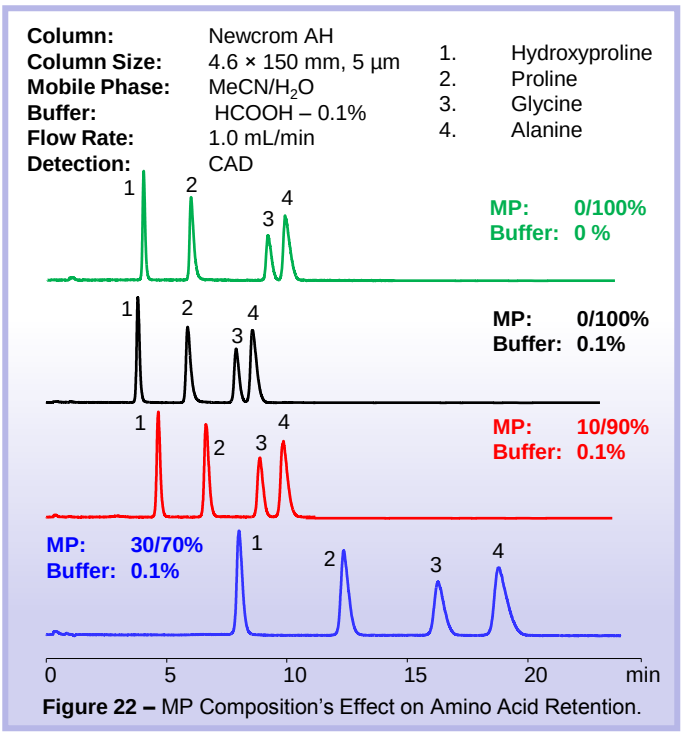


Figure 22 – MP Composition's Effect on Amino Acid Retention.

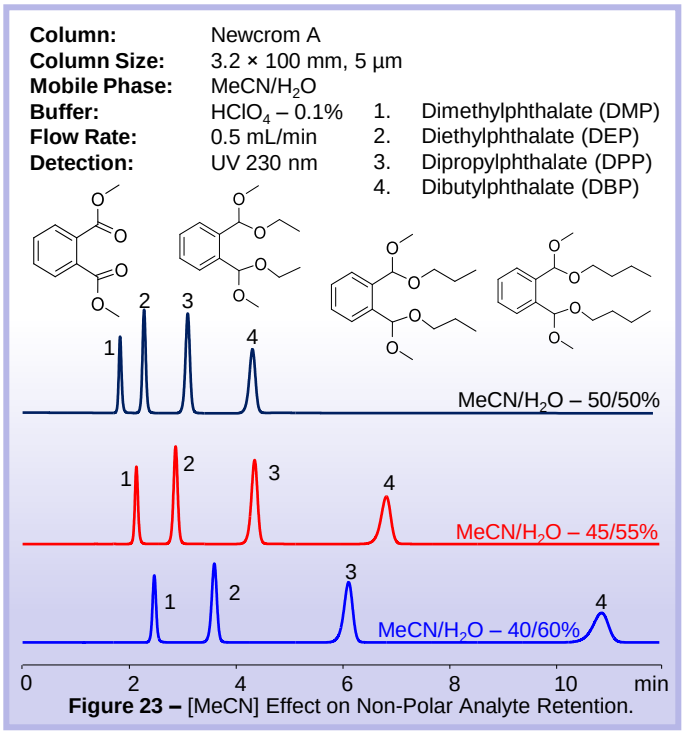


Figure 23 – [MeCN] Effect on Non-Polar Analyte Retention.

As discussed before, the structure of the column's stationary phase can also have a pronounced effect on analytes. Fig. 24 shows how the retention times for parabens are affected by the different surface chemistry on each of the 4 different Newcrom columns. It is of note that the columns with shorter ligands (Newcrom AH and BH) have shorter retention times than those with longer ligands (Newcrom A and B).

The type of the organic component of the MP can also have an effect on the retention times of analytes. Fig. 25 shows selectivity changes for nucleic acids when the MeCN switched to MeOH.

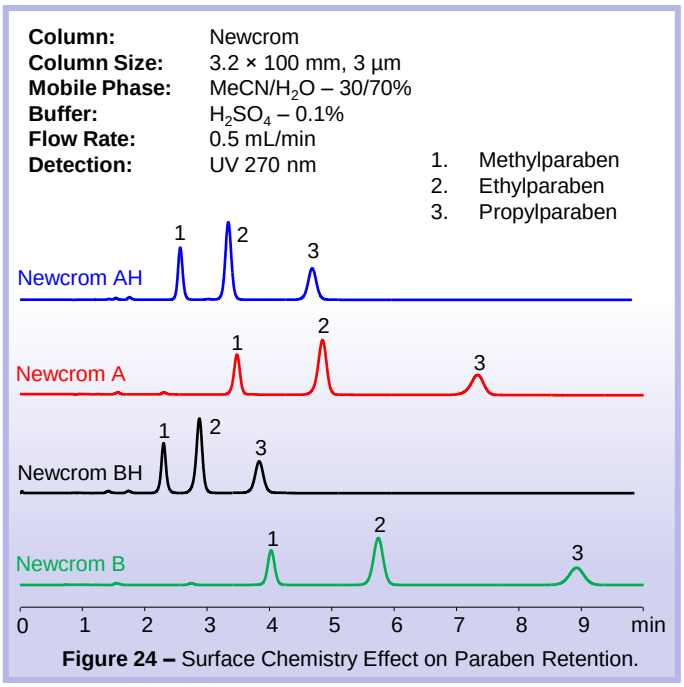


Figure 24 – Surface Chemistry Effect on Paraben Retention.

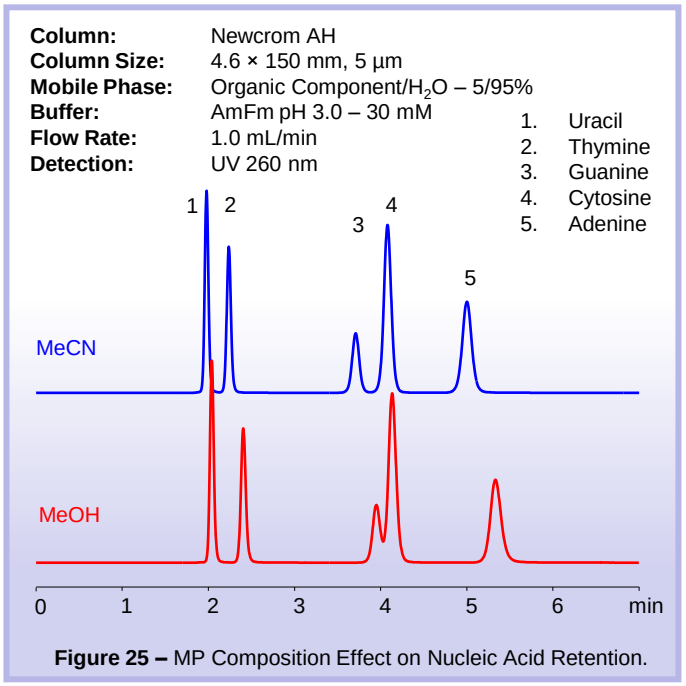


Figure 25 – MP Composition Effect on Nucleic Acid Retention.

Cordycepin has been shown to inhibit the COVID-19 coronavirus. It has a very similar structure to adenosine. Due to their similar structures, their separation can be challenging. Using a mixed-mode Newcrom AH column, however, this separation, shown in Fig. 26, can be done quickly and easily.

Fig. 27 shows the ability of a Newcrom AH column to separate the two similar forms of Vitamin B_3 with a small fraction of phosphoric acid as a buffer, while Fig. 28 displays the ability of a Newcrom AH column to separate 3 common neurotransmitters.

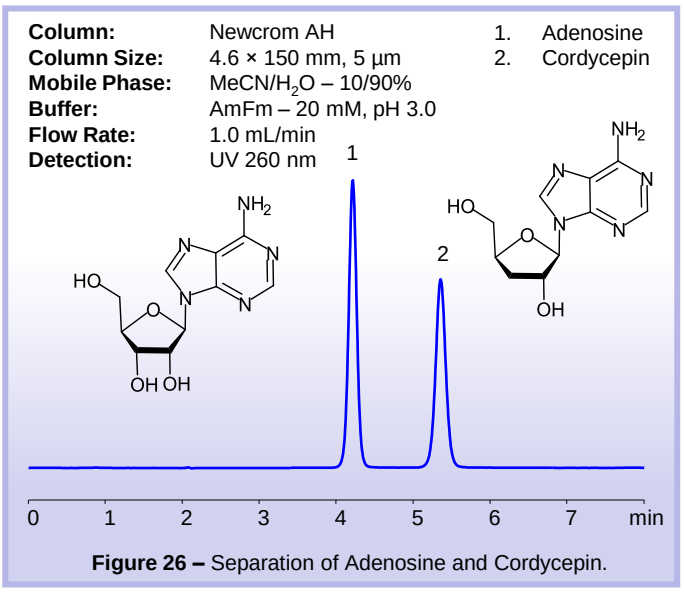


Figure 26 – Separation of Adenosine and Cordycepin.

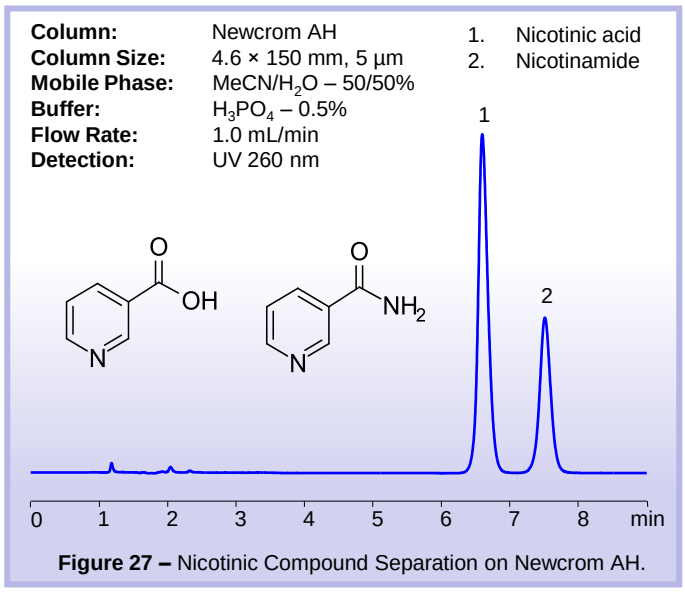


Figure 27 – Nicotinic Compound Separation on Newcrom AH.

Typical mobile phases used with Newcrom columns are based on acetonitrile or methanol, water, and the mass-spec compatible buffers ammonium formate (pH 3) and ammonium acetate (pH 5). If it is necessary to detect in low UV (<220 nm) then phosphate buffer is recommended.

Newcrom columns are available in lengths of 25, 50, 100, 150, and 250 mm and inner diameters of 1.0, 2.1, 3.2, 4.6, and 10 mm. Newcrom phases are based on spherical silica particles with 100Å pores and particle sizes of 3.0 and 5.0 um. Other column dimensions are available upon request. Guard columns are integrated with a male connector and do not require holders. Special hardware coating is available to minimize analyte contact with metal column surface.

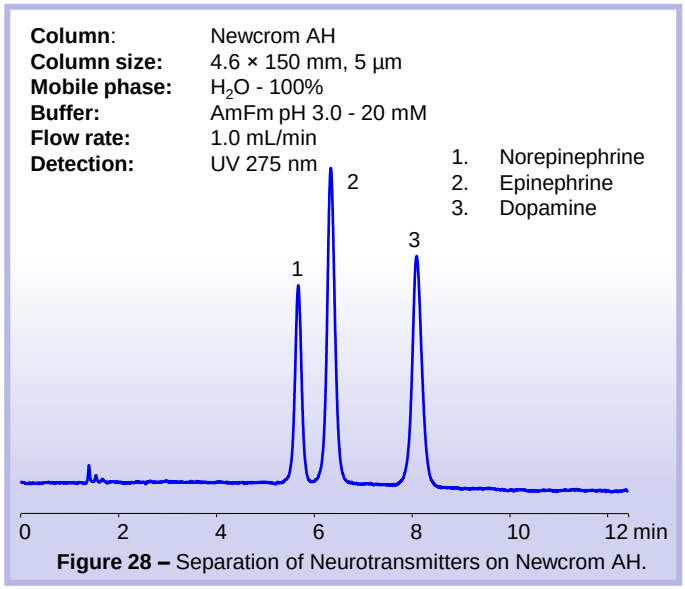


Figure 28 – Separation of Neurotransmitters on Newcrom AH.

All data were obtained in SIELC Technologies labs

Newcrom Column Part Number Generator

NA – 32 . 150 . 0510

Type of packing

Newcrom A	**NA**
Newcrom AH	AH
Newcrom B	NB
Newcrom BH	BH

Column id

4.6 mm	46
3.2 mm	**32**
2.1 mm	21
1.0 mm	10

Column length

250 mm	250
150 mm	**150**
100 mm	100
50 mm	50
25 mm	25
Guard	G

Packing size

5 µm	**05**
3 µm	03

Pores size

100 Å	**10**

To order a column or ask a question send your message to **sales@sielc.com** or call us at **+1 (847) 229-2629**