

Sugar Analysis: An aqueous alternative to the use of amino-bonded HPLC columns with acetonitrile eluents

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Introduction

The food and beverage industry requires robust and reliable methods of analysis for simple sugars such as carbohydrates. These are extremely hydrophilic, highly polar compounds, uncharged at neutral pH, that do not have a UV chromophore. Their separation is therefore a challenge to the food technologist.

Commonly, this type of analysis requires the use of an amino-bonded HPLC column, which typically runs with high percentage acetonitrile eluents. However, global shortages in acetonitrile and increased cost of materials, have highlighted the need for a cheaper, more readily available option.

Ligand exchange HPLC is an alternative method that uses simple eluents, often only water, in an isocratic mode. However, many of the stationary phases used for ligand exchange chromatography are of a large particle size with a broad particle size distribution, and so efficiency is low. Varian's Hi-Plex columns are specifically developed for simple sugar and sugar alcohol analysis. The monodispersed particle gives improved column efficiency and resolution of simple and complex mixtures, with excellent packed bed stability and column lifetime.

The work presented here details methods for simple sugar separations, and demonstrates how ligand exchange chromatography with Hi-Plex can achieve or even exceed the capabilities of an amino column without the need for acetonitrile.

Results and Discussion

Polaris NH₂ is an aminopropylsilane-covered ultra-pure silica that can be used in either reversed or normal phased conditions. Selectivity of the amino phase is substantially different from C₁₈ columns making it ideal for difficult polar samples such as the analysis of mono- and disaccharides. An example of such a separation is shown in Figure 1, whereby a mixture of four sugar molecules is separated with a mobile phase of 80:20 acetonitrile/water:

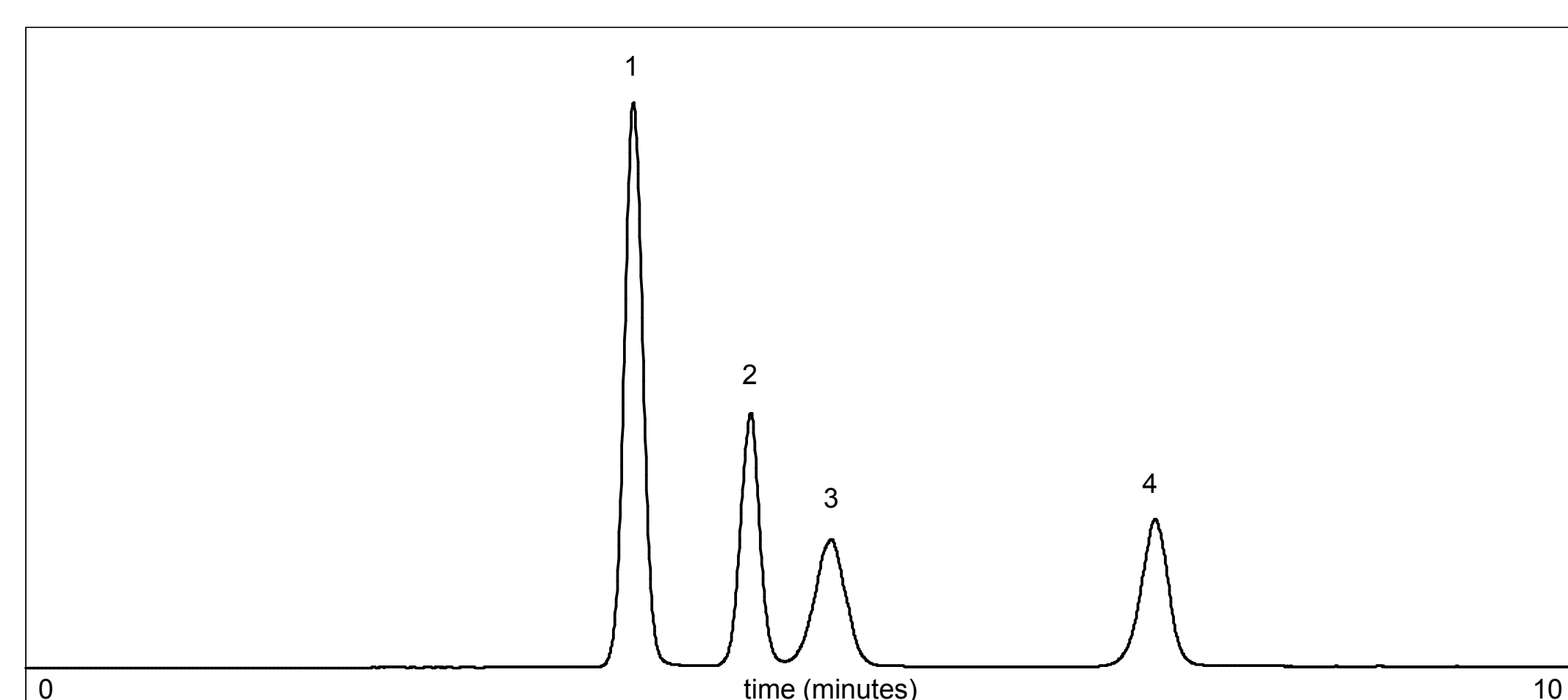


Figure 1: Separation of 4 sugars (1mg/ml each) on the Pursuit NH₂, 100Å 5µm, 150x2.1mm. Eluent: 80:20 ACN/water, 0.2ml/min. 2µl Injection, with Varian 385-LC ELS detection (Neb: 40°C, Evap: 60°C, Gas: 1.60SLM). Compounds: 1. Xylose, 2. Fructose, 3. Glucose, 4. Sucrose

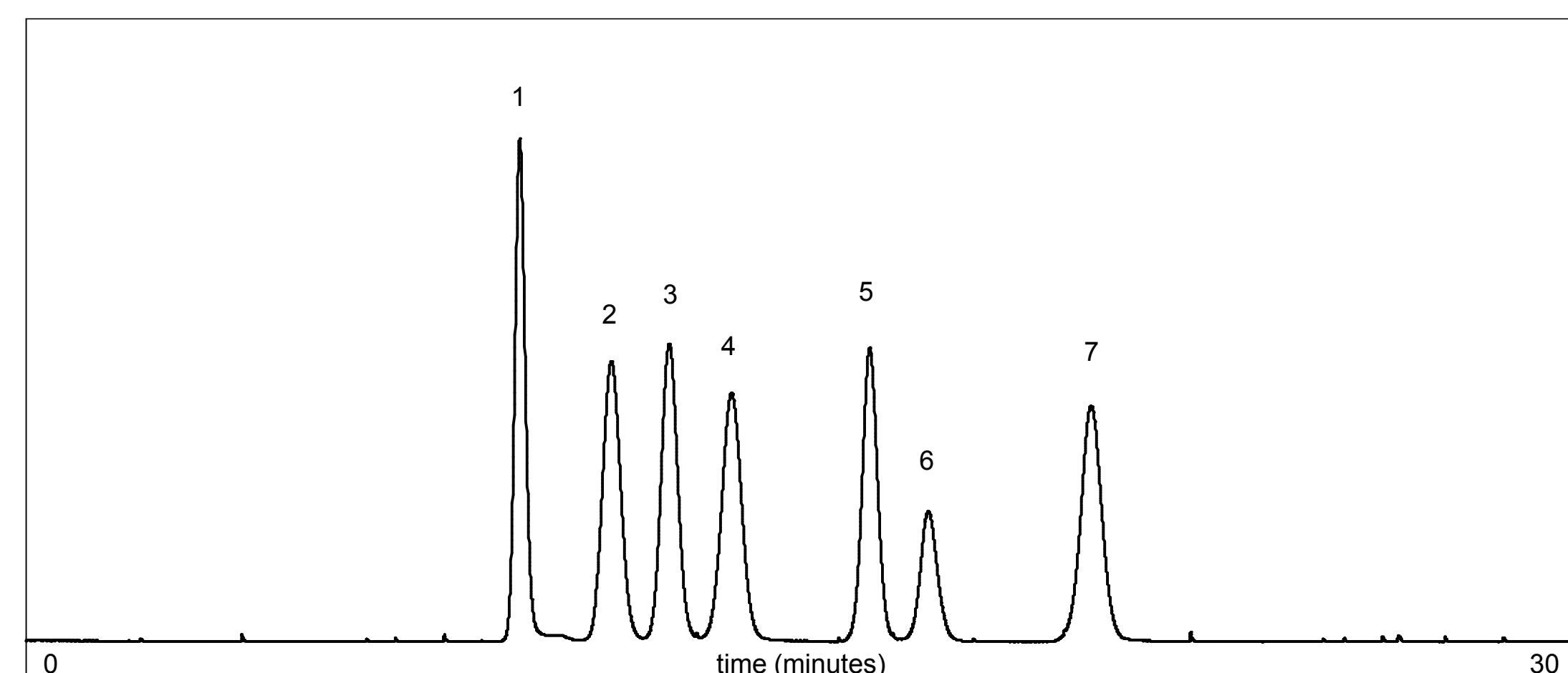
Amino bonded HPLC columns give greater retention for higher molecular weight compounds therefore, in the separation above, the disaccharide sucrose elutes later than the three monosaccharides.

Ligand Exchange Chromatography

Hi-Plex media are based on monodispersed poly(styrene/divinylbenzene) particles with a low crosslink density. The particles have sulphonic acid functional groups, which are strong cation exchange groups, and are loaded with the appropriate counter ion for the separation.

The principle behind ligand exchange is that each of the hydroxyls on a sugar molecule carries a very slight negative charge. The hydroxyl group on the anomeric carbon can be deprotonated and have a strong negative charge. It is the interaction between these negative charges on the sugar molecule and the positive charge contributed by the metal ion associated with the sulphonic acid functionality that provides the selectivity of the separation.

A common application for all Hi-Plex columns is the analysis of sugars, sweeteners and sugar alcohols in food and drink. The chromatogram in Figure 2 below shows a typical chromatographic profile for fruit wine, which is also described in Application Note number SI-02027.



Example 1: Complicated Sugar Mixtures

For complex samples, containing mixtures of mono-, di- and tri-saccharides as well as sugar alcohols, amino-bonded silica columns struggle to give resolution of all compounds present in solution. This, in combination with the global acetonitrile shortage, has caused many users of these particular columns to look towards other solutions.

This example shows how a Hi-Plex Ca column can give at least baseline resolution for a sample containing the following 8 individual compounds, using only HPLC grade water as eluent. In addition, in order to gain optimum column efficiency, and reduced column back pressure, all Hi-Plex columns need to be run at elevated temperatures.

1. Fructose	monosaccharide	MW: 180
2. Glucose	monosaccharide	MW: 180
3. Galactose	monosaccharide	MW: 180
4. Mannitol	sugar alcohol	MW: 182
5. Sorbitol	sugar alcohol	MW: 182
6. Sucrose	disaccharide	MW: 342
7. Raffinose	trisaccharide	MW: 594.5
8. Stachyose	tetrasaccharide	MW: 666

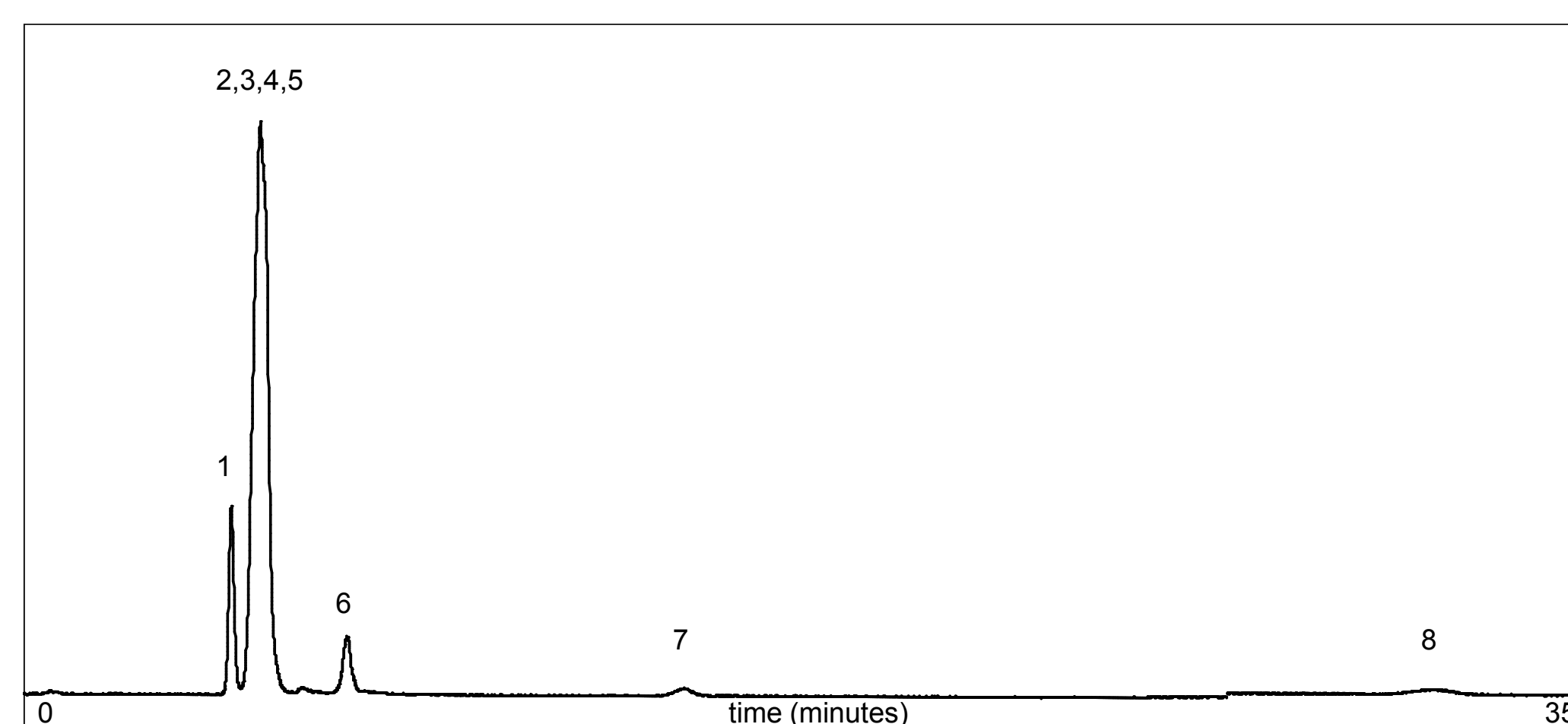
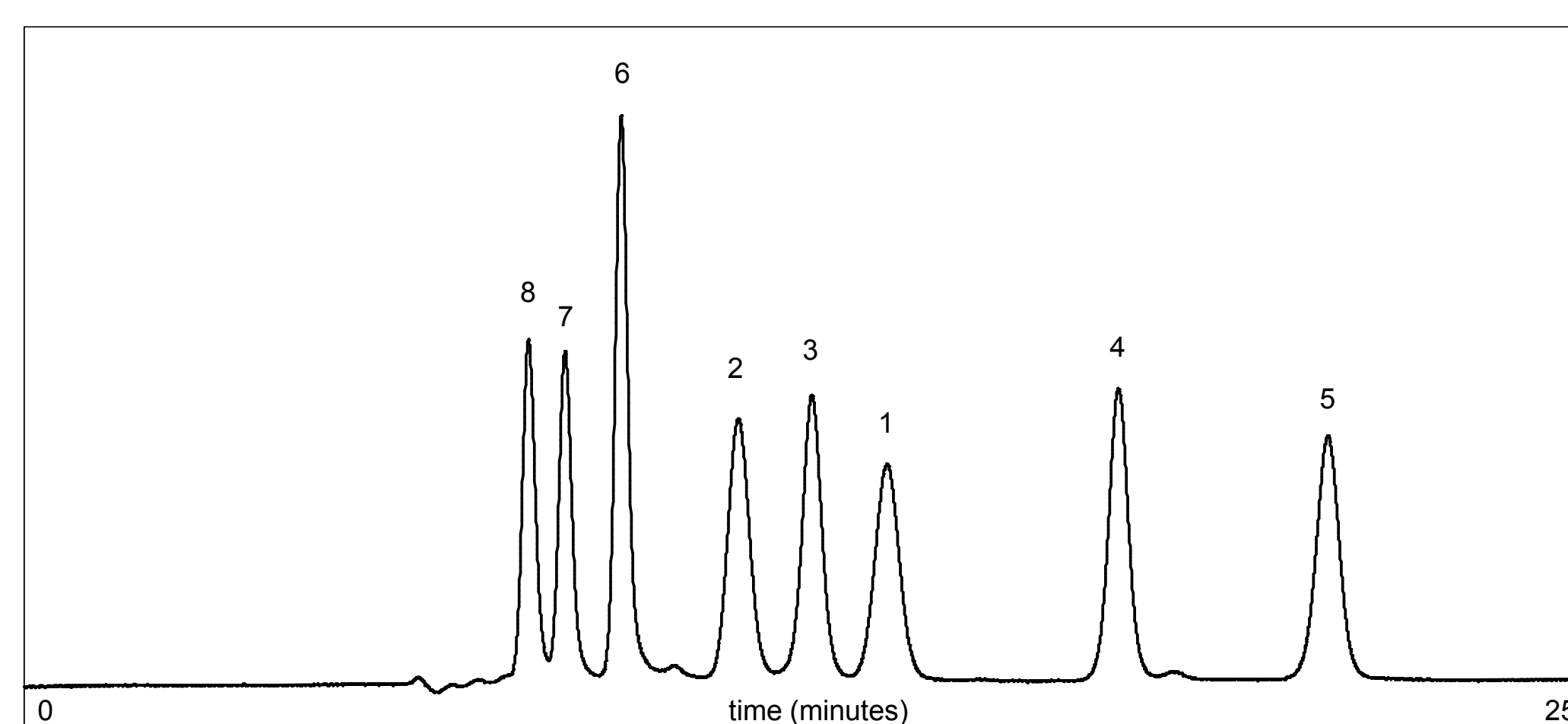


Figure 3: Separation of 8 sugars/sugar alcohols (1mg/ml each) on the Pursuit NH₂, 100Å 5µm, 150x2.1mm. Eluent: 80:20 ACN/water, 0.2ml/min. 2µl Injection, with Varian 385-LC ELS detection (Neb: 40°C, Evap: 60°C, Gas: 1.60SLM).

All of the monosaccharides and sugar alcohols elute within the first six minutes, and most are co-eluted in a single peak. The trisaccharide raffinose then takes twice as long to elute, and the tetrasaccharide stachyose finally elutes at around 32 minutes under the chosen mobile phase conditions. Therefore, a complicated mixture such as this would be very difficult to separate on this column.

The Hi-Plex Ca column elutes sugar molecules in the opposite order, as higher molecular weight compounds are excluded from the stationary phase, and elute close to the dead volume of the column. The chromatogram in Figure 4 is in indication of the type of separation that this column can achieve:



Injection, with Varian 356-LC RI detection.

Clearly, the elution order of these two columns is very different, however the Hi-Plex Ca is able to separate all the compounds in the sample to at least baseline resolution.

The tetrasaccharide stachyose now elutes first, and all compounds elute within 25 minutes.

Example 2: Different Sugar Structures

This sample contained fewer compounds than in example one, however still included one trisaccharide, one disaccharide, one sugar alcohol and three monosaccharides:

1. Ribitol (Adonitol)	sugar alcohol	MW: 152
2. Fructose	monosaccharide	MW: 180
3. Mannose	monosaccharide	MW: 180
4. Glucose	monosaccharide	MW: 180
5. Maltose	disaccharide	MW: 342
6. Melezitose	trisaccharide	MW: 504

The chromatograms shown in Figure 5 show a comparison of the separation achieved on the Polaris NH₂ and Hi-Plex Ca. As expected, the Hi-Plex Ca gives reversed elution order to the Polaris NH₂, and a much better separation of the sugars present in solution.

Compounds of the same type (i.e. monosaccharides, disaccharide etc.) tend to co-elute on the Polaris Amino, however separations of different molecular weight compounds is possible.

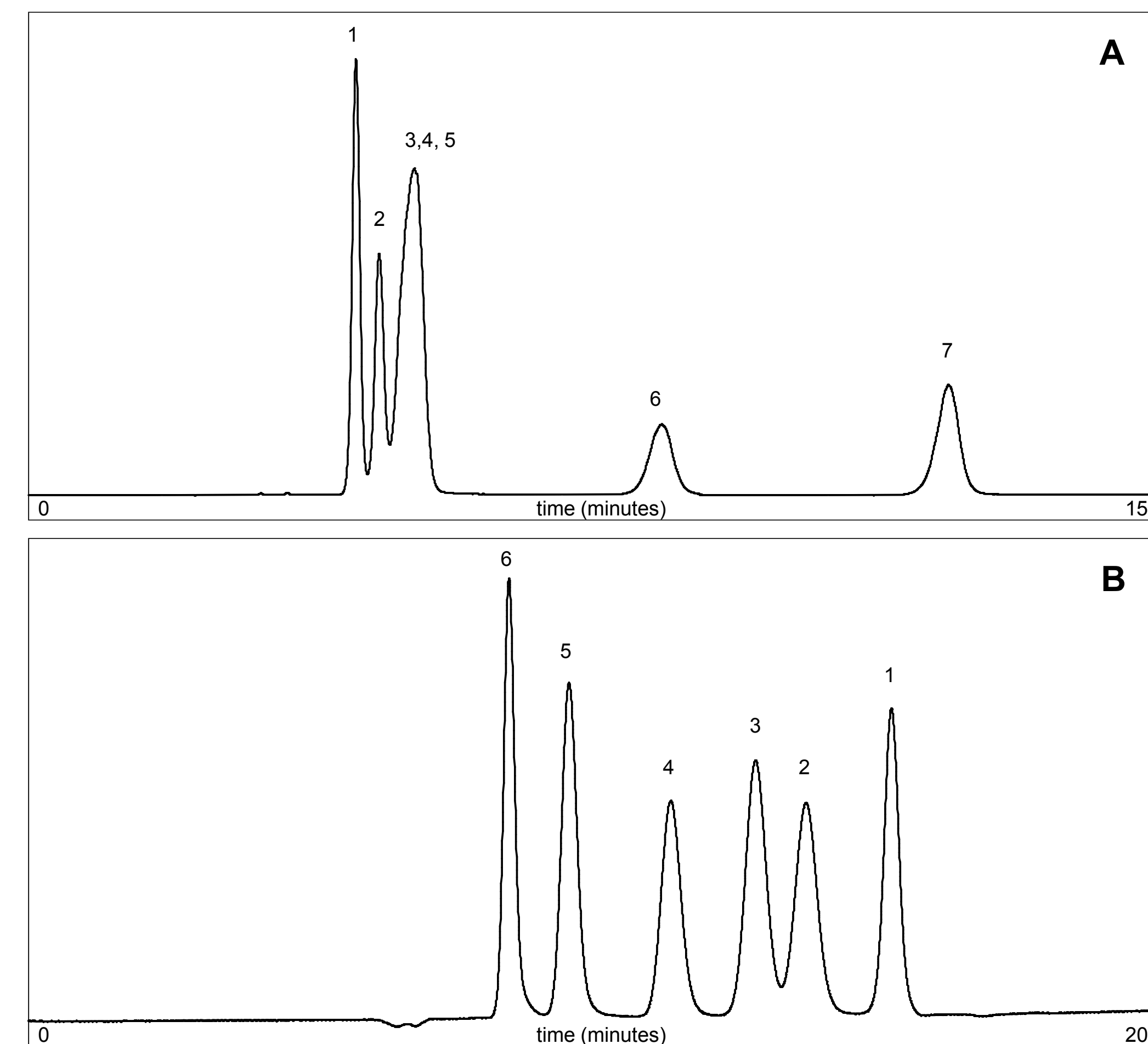


Figure 5: Separation of 8 sugars/sugar alcohols (1mg/ml each). A – Pursuit NH₂, 100Å 5µm, 150x2.1mm. Eluent: 80:20 ACN/water, 0.2ml/min. 2µl Injection, with Varian 385-LC ELS detection (Neb: 40°C, Evap: 60°C, Gas: 1.60SLM). B – Hi-Plex Ca, 8µm, 300x7.7mm. Eluent: Water, 0.6ml/min, 85°C. 20µl Injection, with Varian 356-LC RI detection.

Example 3: Sugar Alcohols

A sugar alcohol is a hydrogenated form of carbohydrate, whose carbonyl group has been reduced to a primary or secondary hydroxyl group. A mixture of seven sugar alcohols is run on both columns, that contains pairs of compounds with the same molecular weight:

1. Glycerol	MW: 92
2. Iso-erythritol	MW: 122
3. Ribitol (Adonitol)	MW: 152
4. Arabitol	MW: 152
5. Sorbitol	MW: 182
6. Dulcitol	MW: 182
7. Maltitol	MW: 344

Figure 6 show a comparison of the separation achieved on the Polaris NH₂ and Hi-Plex Ca:

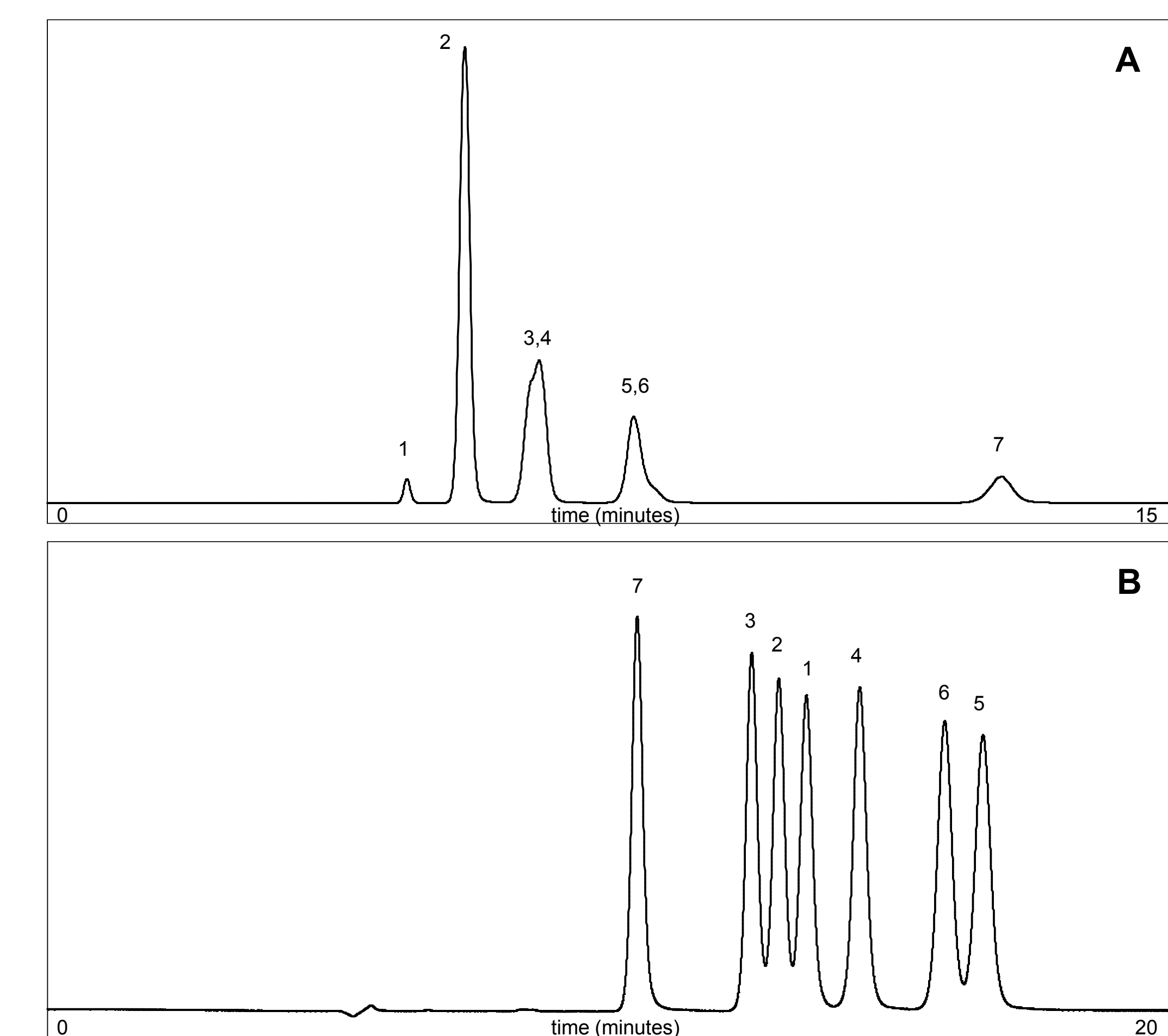


Figure 6: Separation of sugars alcohols (1mg/ml each). A – Pursuit NH₂, 100Å 5µm, 150x2.1mm. Eluent: 80:20 ACN/water, 0.2ml/min. 2µl Injection, with Varian 385-LC ELS detection (Neb: 40°C, Evap: 60°C, Gas: 1.60SLM). B – Hi-Plex Ca, 8µm, 300x7.7mm. Eluent: Water, 0.5ml/min, 90°C. 2.5µl Injection, with Varian 356-LC RI detection.

This example emphasises that amino bonded columns are clearly able to separate sugars and sugar alcohols of differing molecular weights, however gives no resolution between compounds of the same molecular weight.

The ligand exchange functionality of the Hi-Plex Ca, in combination with size exclusion effects, makes it capable of separating all of the sugar alcohols present in this sample.

Conclusions

The Hi-Plex Ca column gives improved separation of complex mixtures of sugars and sugar alcohols, and requires no acetonitrile in the mobile phase.

An amino bonded column such as the Polaris NH₂ is capable of separating more simple samples, however compounds of very similar or identical molecular weight will co-elute.

Hi-Plex columns can also be used with an ELS detector, which gives improved sensitivity at low concentrations.