



Overview and Strategies for Bio-Organic Molecule Purification

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Section 1

Introduction

The type of HPLC column used for small molecule analysis will be dictated at least in part by the chemical nature of the compounds of interest. Recent literature suggests that the Aminex polystyrene-divinylbenzene ion exchange resins are applicable to a wide variety of water soluble and partially water soluble small organic compounds. As illustrated in Figure 1.1, Aminex columns are complementary to reversed phase columns. Certain classes of water insoluble or sparingly water soluble compounds are best separated on reversed phase columns, while other water soluble compounds such as sugars, alcohols, and short chain organic acids are better separated on the ion exchange resins. However, there is a large group of compounds in the middle range of solubility that are amenable to analysis on both the Aminex and reversed phase columns.

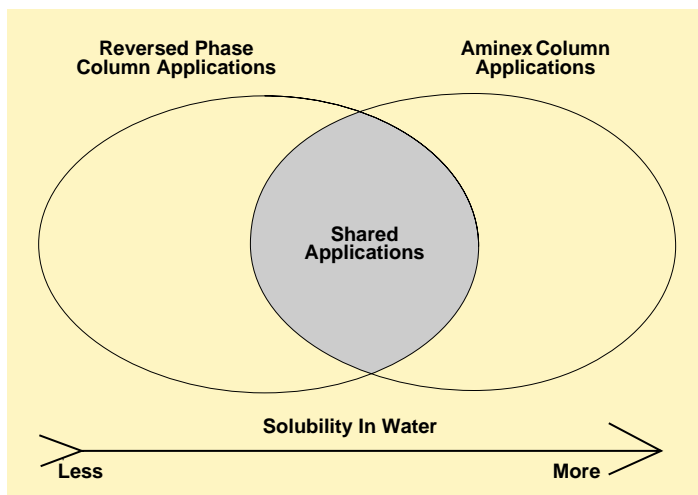


Fig. 1.1. Complementary nature of Aminex and reversed phase columns.

The power of the Aminex columns becomes apparent when examining the array of small molecular weight bio-organic molecules involved in cell metabolism (see Metabolic Pathways inside front cover) which have been analyzed on Aminex columns.

Every chromatographic separation is unique, and requires a unique combination of columns and conditions. Bio-Rad offers a wide selection of Aminex columns for the analysis of carbohydrates, organic acids, and numerous other small molecules. These columns are based on polystyrene-divinylbenzene cation exchange

resins that have a high porosity and high concentration of functional groups within their chemical structure. In addition, the remarkable chemical stability of these resins makes them useful under a wide variety of conditions.

Since their introduction in the late 1970s, Aminex columns have become the standard in a number of different industries. This is evidenced by the several hundred scientific publications using Aminex columns. A number of these publications represent pioneering reports using Aminex technology to develop methods for the analysis of diverse small molecular weight polar compounds. A list of these publications is given in bulletin 1895, and on page 59 of this guide.

These references contain useful information on the elution behavior of a variety of sugars, alcohols, organic acids, ketones, and other molecules. To make this information more accessible, the data from these references has been compiled into an Aminex HPX-87H small molecule index containing over 200 organic molecules. In addition, relative retention time data at two different temperatures is presented on 100 of these compounds that have been analyzed using dual UV and refractive index detection. For these compounds, the ratio of the UV and RI detector responses is presented. This information can be a powerful tool in the identification and quantification of analytes (see bulletin 1847).

1.1 Guaranteed Quality

To insure the highest quality products, Aminex resins and columns are custom manufactured at Bio-Rad. Each Aminex column carries an individual performance guarantee, which assures you that the column meets or exceeds the stated specifications. A test chromatogram and a computer generated report giving performance data and test conditions accompany each column. This high quality is reflected in reproducibility, which is very important when establishing routine methods to be used over extended periods and when setting up methods between laboratories. Aminex columns exhibit long lifetimes, maintaining reproducibility over hundreds of injections. Figure 1.2 compares the first and two hundredth injection of an organic acid standard on an Aminex HPX-87H column. Over the range of 200 injections the retention times for the components varied from 0–0.3%, while the efficiencies decreased by 1.9–6.3%.

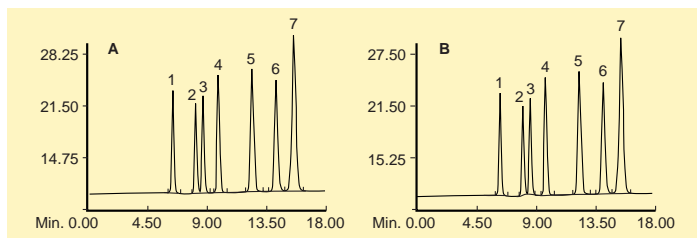


Fig. 1.2. Column lifetime studies performed on the Aminex HPX-87H column. After one injection (A) and 200 injections (B). Peak number 1) oxalic acid, 2) citric acid, 3) tartaric acid, 4) malic acid, 5) succinic acid, 6) formic acid, 7) acetic acid.

In addition, excellent column-to-column as well as lot-to-lot reproducibility has been shown for Aminex columns. Figure 1.3 examines the variations of 203 representative Aminex HPX-87H columns from six different lots using an organic acid standard. Relative standard deviations of 4.1% and 7.6% were observed between the 203 columns for the retention time and efficiency of succinic acid. No significant lot-to-lot variations were observed for the measured parameters. The average values of the individual batches were within one standard deviation of the overall averages for the 203 columns.

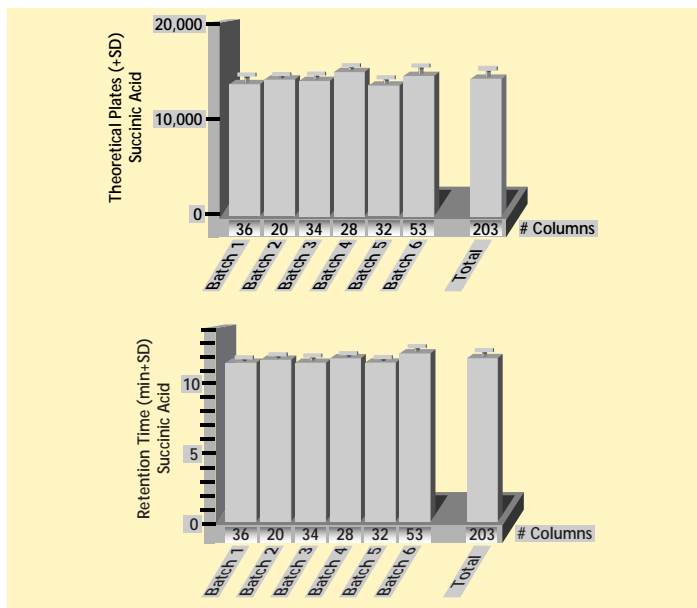


Fig. 1.3. Column-to-column and lot-to-lot reproducibility of Aminex HPX-87H columns.

1.2 Dedicated Service

To complement the HPLC and sample preparation products, Bio-Rad employs a staff of experts in HPLC separations, who can help you design the separation scheme that will be most effective in your application, taking advantage of both the state-of-the-art HPLC isocratic analyzers and the widest available selection of column chemistries. Technical support, including protocols, technical information, and a staff of HPLC specialists ready to answer any questions you might have, is available. To reach a technical specialist, contact your Bio-Rad office. In the USA, call 1-800-4BIORAD.

Section 2 Separations Mechanisms on Aminex Resins

Aminex HPLC columns, packed with a polymer-based matrix, offer many advantages for the analysis of carbohydrates, alcohols, and organic acids, in food and beverage, biochemical, biomedical, and biotechnology applications. These columns allow the use of simple isocratic methods, eluting with water or dilute acid. There is minimal sample preparation, usually just filtering through a 0.45 μm filter with no derivatization necessary. These resins exhibit high pressure stability as well as pH stability over a wide range. The Aminex resin packings allow a variety of partition type separations without the disadvantages inherent in bonded phase silica materials. The fundamental partition process responsible for separation is moderated by the ionic group bound to the resin, and by the choice of counterion. HPLC separations on Aminex resins use the mechanisms of ion exclusion, ion exchange, ligand exchange, size exclusion, reversed phase, and normal phase partitioning. These multiple modes of interaction, which have been termed ion-moderated partitioning [Jupille *et al.*, *Amer. Lab.*, **13**, 80 (1981)], offer a unique ability to separate compounds.

In ion exclusion, mobile ions with like charge cannot penetrate the resin bead, which carries a fixed charge. Thus, in pure form, ion exclusion would require that all components elute within the total column internal volume. Highly charged species are excluded from the intraparticle volume and elute sooner. If elution requires more than one column volume, ion exclusion must be accompanied by other retention mechanisms. In many separations, conditions can be chosen to make partition the accompanying mode. In normal phase partition, the sample is distributed between intra-

particle (bound) water and a less polar mobile phase. By choosing an appropriate buffer, column selectivity can be fine-tuned for a particular compound. Nonpolar compounds are retained more strongly than polar compounds, which shows that reversed phase partition is involved in the separation. In reversed phase partition, the sample molecules are distributed between a polar, usually aqueous, mobile phase and a nonpolar (aromatic) resin backbone. Proof of its involvement in the separation is the linear relationship between the logarithm of capacity factor and carbon number for a homologous series (see Figure 2.1). That is, the more hydrophobic molecules (increasing n) elute later, as manifested by the increasing k' , than the less hydrophobic ones. The reversed phase partition mechanism provides a useful basis for selecting an organic modifier to control retention. Acetonitrile, for example, is particularly effective when phenolic acids (Figure 2.2) or similar compounds are being analyzed. Ligand exchange and size exclusion mechanisms are also exploited in HPLC on Aminex resins. In size exclusion, molecules too large to penetrate the effective pore structure of the resin are physically excluded from the intraparticle volume. Figure 2.3 illustrates the way ligand exchange imparts a secondary selectivity to what is primarily a partition separation. The Aminex HPX-87C column resolves the alpha and beta anomers of glucose, but the Aminex HPX-87H column, while it provides similar retention, does not resolve the anomers. Thus, appropriate selection of counterion can moderate the primary partition mechanism.

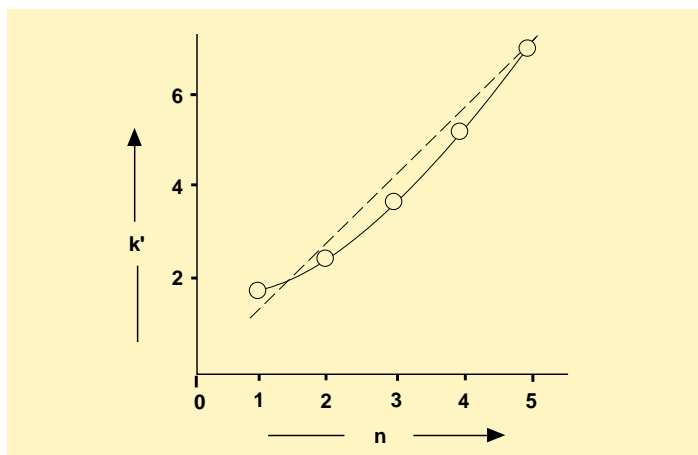


Fig. 2.1. Relation between retention (k') and carbon number (n) for straight chain aliphatic alcohols on the Aminex HPX-87H column.

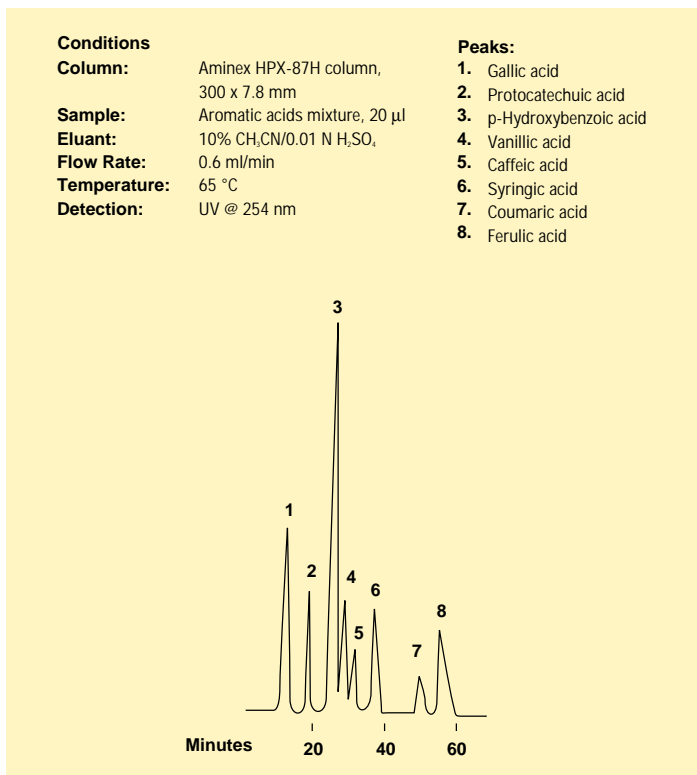


Fig. 2.2. Phenolic acid separation. Acetonitrile is used as an organic modifier to control retention in what is essentially a reversed phase separation.

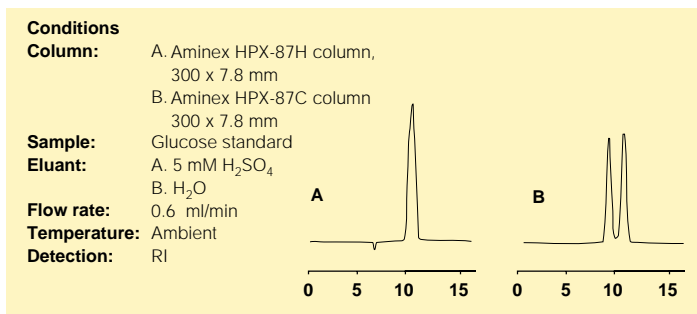


Fig. 2.3. Influence of ligand exchange on the retention of glucose. Alpha and beta anomers are separated on the calcium form resin.

Reversed phase and ion-pairing techniques on silica require complex eluants for effective separations. These mechanisms work on the principle of modifying the compound to be analyzed until it

is compatible with the column. Aminex resins separate compounds another way. Instead of modifying the compound to be analyzed, the column packing is modified, and chromatographic conditions are optimized to be compatible with the compound. Therefore, Aminex resins allow the use of isocratic elution, and easy sample preparation without sample derivatization. Aminex HPLC columns can reduce total analysis time measured from the start of sample preparation. For example, a chromatographic analysis time of 2 or 3 minutes is insignificant if the sample preparation takes 15 minutes. The separation mechanisms built into the Aminex columns simplify sample preparation. Filtration is usually the only sample preparation needed in most separations. The durable nature of the Aminex resins combines with selectivity that focuses in on a compound or class of compounds to make Aminex columns the method of choice for rapid, high resolution analysis.

Section 3

Selecting the Proper Column

In effect, selectivity for a particular separation is built into each Aminex HPLC column. Particle size, ionic form, crosslinkage, and column configuration have been optimized to provide the fastest possible analysis of a particular sample, with the highest possible resolution.

3.1 Resin Crosslinkage

Resin crosslinkage is an important parameter in any chromatographic separation. Styrene divinylbenzene resin is a relatively rigid gel-type media. The lower the crosslinkage, the more open the structure, and the more permeable it is to higher molecular weight substances. A 4% crosslinked resin is used in the Aminex HPX-42A and HPX-42C columns, which can resolve higher oligosaccharides. For smaller molecular weight compounds an 8% crosslinked resin is used, as in the Aminex HPX-87C and HPX-87H columns.

3.2 Resin Ionic Form

Selecting the proper Aminex column depends to a large extent on the ionic form of the packing material. Selecting a resin in the ionic form tailored to the compound being analyzed is the most effective way to optimize resolution. Aminex columns have been called fixed-ion resin columns, since the resin is converted to a specific ionic form before packing and is maintained in that ionic form for the life of the column. In-column conversion from one form to

another is not recommended, since resins can shrink and swell with changes in ionic form. The ionic form of the resin contributes to selectivity by charge, or by steric hindrance with the pores of the resin, or by a combination of these and other factors. Particularly in carbohydrate analysis, the nature of the cation is an important factor, due to differences in coordinating ability of the adjacent hydroxyl groups of the sugars with the fixed-cation of the resin. For example, stronger binding will occur in sugars which can favorably complex three adjacent hydroxyls to the fixed-cation than those sugars binding with only two hydroxyls.

Figure 3.1 shows the effect of resin ionic form. Carbohydrates derived from cellulose hydrolysis, specifically glucose, xylose, galactose, arabinose, and mannose, were separated on the Aminex HPX-87C column and the Aminex HPX-87P column. While the Aminex HPX-87C column is ideal for most monosaccharide separations, some of the cellulose derived carbohydrates co-elute on this column. Taking advantage of the mechanism of ligand exchange, the Aminex HPX-87P column separates the compounds with better resolution.

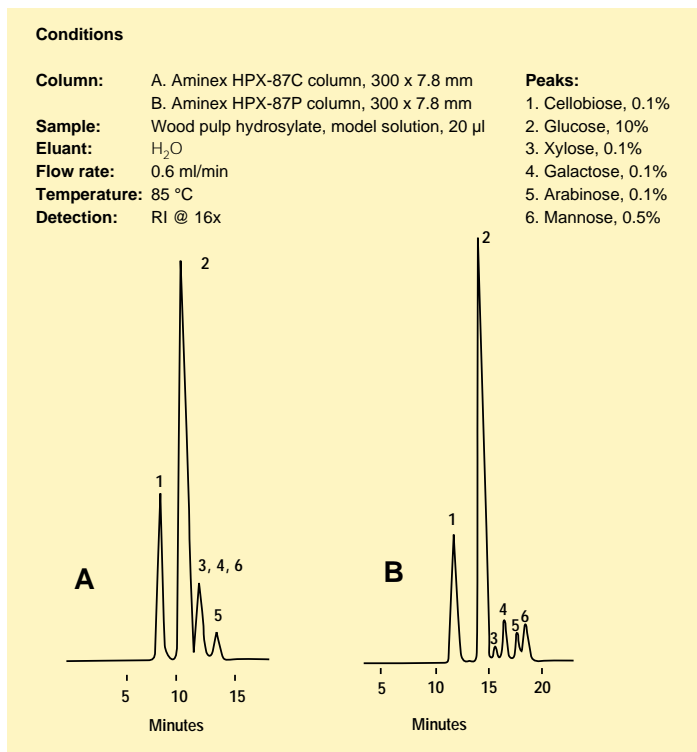


Fig. 3.1. Effect of resin ionic form on sugar separations.

3.3 Column Configuration

Column length and diameter also affect resolution and analysis time. The goal is to use only as much resin as necessary to achieve the desired separation. If the compound is strongly retained by the resin and analysis time is long on a 300 x 7.8 mm column, a shorter 100 mm column can significantly decrease the analysis time. An example of column optimization is shown in Figure 3.2, which illustrates the complete profile of a wine sample on an Aminex HPX-87H column (300 mm x 7.8 mm). If ethanol is the only compound of interest, the analysis can be completed in 5 minutes on a Fast Acid column (100 mm x 7.8 mm) instead of the 22 minutes on the longer column.

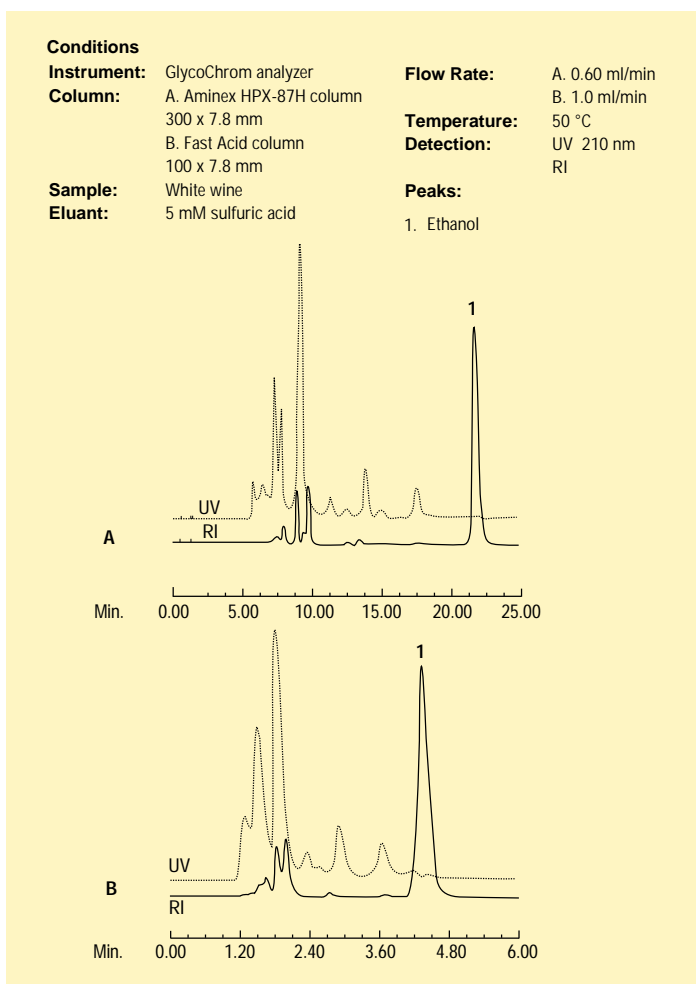


Fig. 3.2. Effect of column length on analysis time of ethanol.

Section 4

Optimizing the Analysis

With Aminex columns, several chromatographic variables have an impact on resolution and retention times, and help to control the speed of analysis. These variables include eluant ionic strength, eluant pH, column temperature, flow rate, and organic modifier concentration. After the proper column has been selected, each of these variables should be optimized to achieve the best possible separation. Variables can be manipulated, either singly or in combination, to improve resolution and to enhance selectivity.

4.1 Solvent Selection

Simplified solvent selection is a major advantage of Aminex columns, especially by comparison with polar bonded phase silica columns. Because selectivity for sugars is not strongly affected by the solvent, most carbohydrate separations, aside from sugar alcohols, can be carried out with deionized water as the mobile phase. The addition of acetonitrile can improve the resolution of sugar alcohols (Figure 4.1). A further advantage of Aminex carbohydrate columns is that large molecules like polysaccharides elute early in the separation, rather than binding irreversibly to the matrix. Table 4.1 compares the retention times of some carbohydrates and sugar alcohols on similar-sized columns packed with resins in the Aminex HPX-87 series and Aminex disaccharide column.

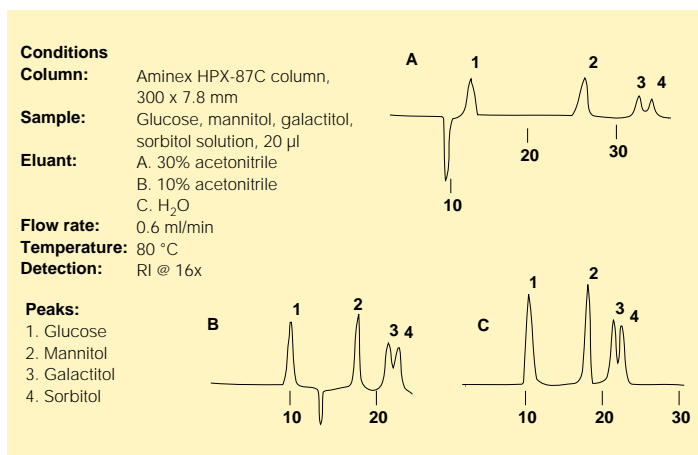


Fig. 4.1. Effect of the organic modifier concentration on retention and resolution.

Table 4.1 Retention times of sugars and alditols on Aminex carbohydrate analysis columns.

Column	HPX-87C	HPX-87P	HPX-87H	Aminex disaccharide
Mobile Phase	H ₂ O	H ₂ O	0.005 M H ₂ SO ₄	70% CH ₃ CN
Flow Rate (ml/min)	0.6	0.6	0.6	1.0
Temperature °C	85°	85°	65°	Ambient
Carbohydrate				
Stachyose	7.75	9.54	6.94	24.15
Melezitose	8.12	9.60	7.79	11.52
Raffinose	8.25	9.84	7.65	13.20
Maltotrios	8.25	10.26	7.18	13.20
Cellobiose	8.78	10.74	7.80	8.76
Sucrose	8.95	10.62	Inverts	7.62
Trehalose	9.00	10.80	8.03	8.94
Maltose	9.03	11.16	7.89	8.82
Melibiose	9.28	11.70	7.89	10.92
Lactose	9.37	11.52	8.13	9.72
Glucose	10.87	12.72	10.16	6.00
Xylose	12.00	13.86	10.39	4.56
Sorbose	12.20	14.52	9.92	5.34
Galactose	12.25	14.70	10.49	6.42
Mannose	12.47	16.44	10.02	5.85
Rhamnose	12.58	14.88	11.20	4.08
Fructose	13.58	17.04	10.39	5.52
Arabinose	13.75	15.48	11.34	5.04
Fucose	13.78	15.84	12.05	4.62
Digitoxose	13.92	14.76	13.23	3.00
Glucoheptose	15.17	19.14	9.59	6.60
Ribose	21.42	30.54	9.21	4.20

For separations on the Aminex HPX-87H column, eluant composition can be manipulated to adjust the relative retention times of the compounds to be resolved, or to exclude compounds that are not of interest. Either the acid strength of the eluant can be adjusted, or organic modifiers can be added. The retention of organic acids with pK_a less than 4 is particularly influenced by the pH of the solvent. Figure 4.2 illustrates the change in retention times of 14 organic acids with increasing eluant acid strength. For a few of the acids, such as maleic, pyruvic, and fumaric, relative retention increases with increased acidity, and this can be used to increase resolution. Figure 4.3 examines more closely the effects of sulfuric acid mobile phase changes on the retention times of different classes of compounds on the Aminex HPX-87H column. The neutral sugars, sorbitol and N-acetylglucosamine, exhibited very little change in retention times in going from 2.0 mM to 12 mM acid. The monocarboxylic acids showed modest increases in retention times. For the dicarboxylic acids, the most dramatic increase in retention time was seen

with oxalic acid, with the change decreasing as the chain length of the acids increased. The aromatic acids all exhibited significant increases in retention time with increased acidity. This retention time information can be a powerful tool in optimizing a given separation, as well as assisting in the identification of unknown substances.

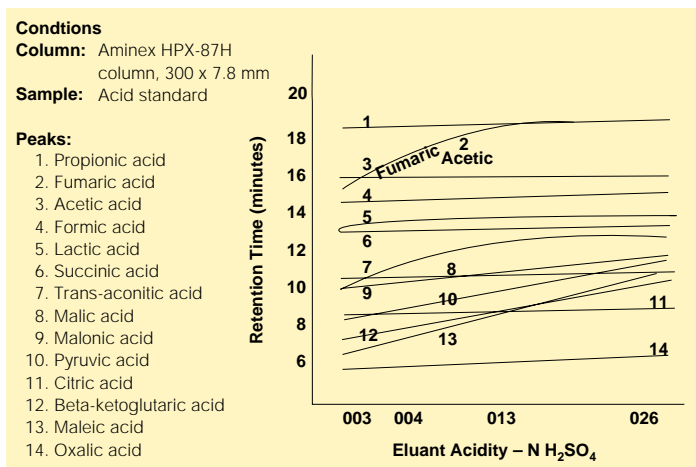


Fig. 4.2. Effect of eluant acid strength on retention times of organic acids on the Aminex HPX-87H column.

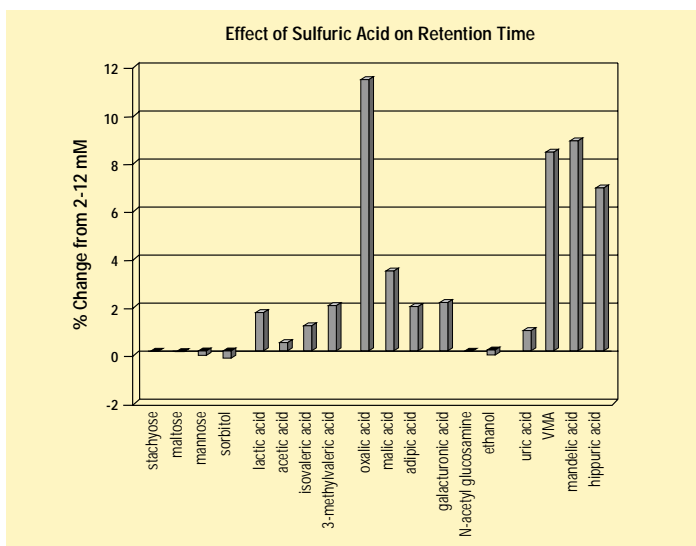


Fig. 4.3. Effect of acid strength of the elution of different classes of compounds on the Aminex HPX-87H column. The results are expressed as the percent retention time change in going from 2 mM to 12 mM sulfuric acid.

4.2 Column Temperature

Temperature control is critical with Aminex columns, since retention times and peak heights vary with changes in temperature. Consistent, reproducible temperatures are absolutely necessary for accurate, quantitative and qualitative analysis. The effect of temperature on a given analysis depends on the individual chemistry, as well as on the type of column packing and the mobile phase. Usually, increasing the column temperature decreases retention time, and increases column efficiency. In many separations, operating at high temperatures can optimize efficiency by minimizing the band spreading from slow mass transfer in the stationary phase. Column back pressure also decreases at higher temperatures. For carbohydrate analysis, the columns are heated to 85 °C to increase the theoretical plates and decrease the viscosity of the eluant and sample. This allows deeper penetration of sugars into the interior of the resin, thus allowing higher resolution. As an example, the Aminex HPX-87C column was run at ambient and 85 °C (Figure 4.4). The increased efficiency and minimized band spreading with an increase in column temperature is dramatically shown by the shape of the glucose peak. The silica based Aminex disaccharide column is not heated, though the temperature must be kept stable. On this column, retention times can change with even a 1 °C change in temperature.

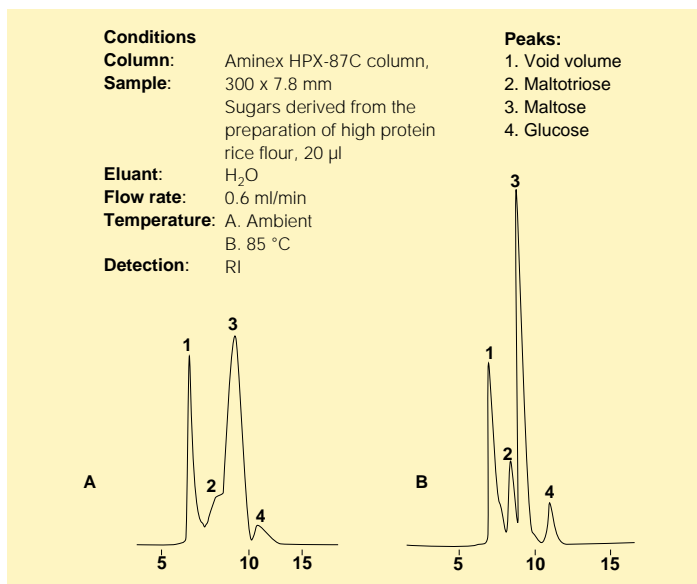


Fig. 4.4. Effect of temperature on the separation of sugars on the Aminex HPX-87C column.

Heating the Aminex HPX-87H column can increase column efficiency. As shown in Figure 4.5, theoretical plates increase by 54% when column temperature is increased from 20 ° to 60 °C. Although most organic acid separations do not require heated conditions, elevated temperatures can be used to resolve compounds that elute close together. Increasing column temperature also decreases the retention time of most organic acids. This effect varies from compound to compound due to differences in interaction with the resin. This is demonstrated in Figure 4.6 which shows the retention time changes for different classes of compounds in going from 50 °C to 22 °C. The most significant increases in retention time were observed for the aromatic acids (hippuric 56%, VMA 52%, and mandelic 35%). For the dicarboxylic acids, the retention time increases correlate with the chain length of the acid, for example oxalic 2.3%, malic 6.4%, adipic 27.7%. A similar pattern was observed for the monocarboxylic acids, for example acetic 4.6%, lactic 1.8%, isovaleric 10.6%, 3-methylvaleric 20.6%. The neutral sugars, N-acetylglucosamine and galacturonic acid, exhibited little change with temperature. The most significant decrease in retention time was seen for ethanol (-7.0%). This type of information can be very useful in optimizing a given separation, as well as assisting in the identification of unknown substances.

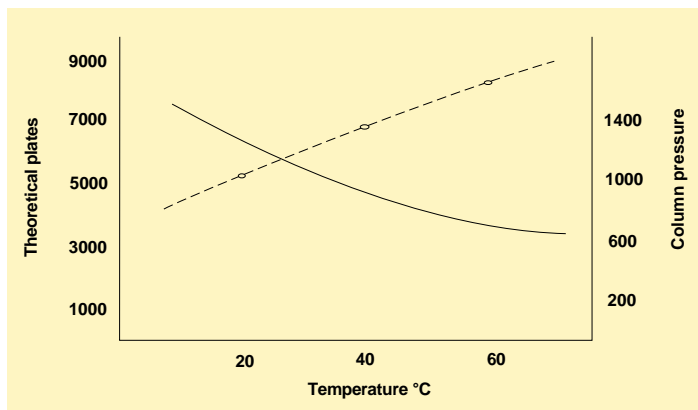


Fig. 4.5. Relationship of theoretical plates (---o---) and column back-pressure (——) to column temperature for fumaric acid on the Aminex HPX-87H column.

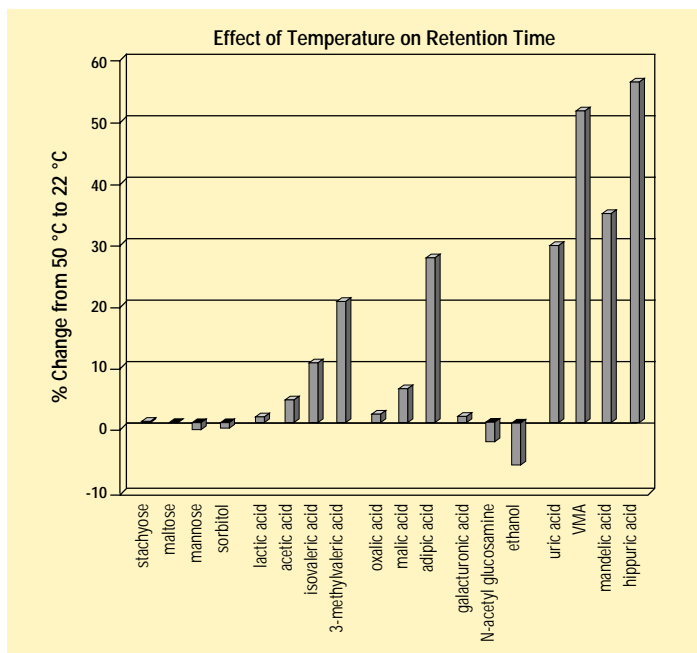


Fig. 4.6. Effect of temperature on the elution of different classes of compounds on the Aminex HPX-87H column. The results are expressed as the percent retention time change in going from 50 °C to 22 °C.

4.3 Flow Rate

Aminex columns typically require low flow rates for efficient operation, because slow mass transfer in the stationary phase contributes to band broadening. Flow rates below 1 ml/min should be used with 300 x 7.8 mm columns. Analysis times are typically short, however, because compounds of interest usually elute at low k' . For routine analysis, flow rates of 0.4–1.0 ml/min are recommended. Flow rates above 1.2 ml/min can produce excessive back pressure and cause a breakdown in peak symmetry, while flow rates lower than 0.4 ml/min increase the analysis time. However, with Aminex columns, lower flow rates also increase efficiency. For research purposes, a low flow rate combined with two or three columns in series offers the ability to isolate and examine compounds within a complex sample matrix.

4.4 Organic Modifier Concentration

Organic modifier concentration is another critical variable in optimizing an analysis. Organic modifiers, up to 30% acetonitrile or

less than 5% tert-butanol or isopropanol, can be added to the eluant to decrease adsorption of organic compounds to the column matrix. This is particularly useful in the separation of aromatic acids on the Aminex HPX-87H column. Organic modifiers can be used to reduce analysis time (Figure 4.7).

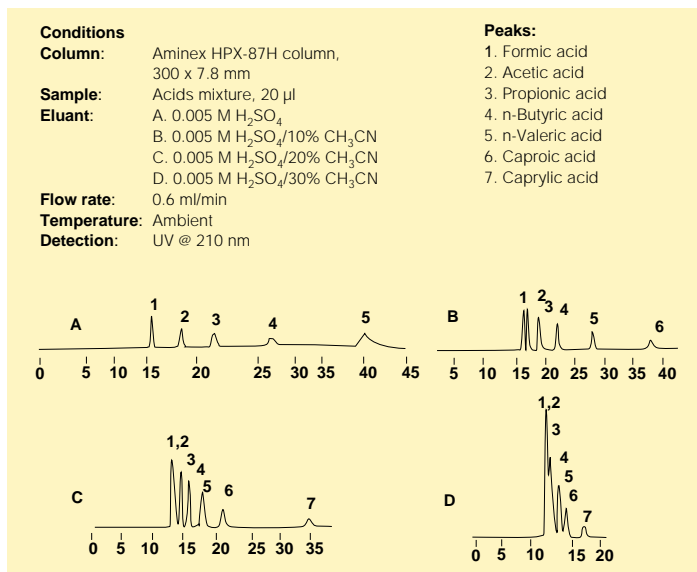


Fig. 4.7. Effect of organic modifier concentration on retention times.

The possibility of resin volume change must be considered when deciding the identity and the concentration of an organic modifier. Ideally, the organic modifier penetrates and swells the organic backbone of the resin to the same extent that decreased osmotic pressure decreases the intra-particle water volume. Acetonitrile concentrations between 5 and 30% approximate this ideal well enough to minimize void volume formation (bed shrinkage) and excessive back pressure (bed swelling). It is best to start with 5% acetonitrile at a low flow rate (0.1–0.2 ml/min) so as not to drastically increase back pressure. If acetonitrile concentrations greater than 40% must be used, the column should be packed in an acetonitrile/aqueous buffer. Ethanol and isopropanol are similar to acetonitrile. Methanol is not recommended (bed shrinkage), and neither are tetrahydrofuran, dimethylformamide, and other relatively non-polar solvents (bed swelling). With the Amminex disaccharide column, increasing the acetonitrile content of the buffer allows better resolution of disaccharides, while lowering the acetonitrile content allows better resolution of oligosaccharides.

4.5 Optimizing Compound Detection

The Aminex chemistry, using isocratic elution with water or dilute aqueous buffers, offers the important advantage of allowing dual refractive index and UV detection to produce a complete profile of mixtures which contain two or more classes of compounds which cannot be seen with one detection system. A refractive index monitor detects carbohydrates and alcohols while a UV monitor simultaneously detects organic acids, aromatic compounds, and various other compounds. The Aminex HPX-87H column is especially useful for profiling monosaccharides and organic acids simultaneously. Figure 4.8 shows a fermentation standard separated on the Aminex HPX-87H column and detected with a UV monitor and with a refractometer. Dual detection offers the possibility of using detector response ratios to aid in the quantitation, and, more importantly, in the identification, of compounds. Advantage can be taken of a great difference in the respective response factors in the UV and RI detectors to better quantitate compounds which are only partially resolved. The purity and homogeneity of peaks can be better assessed using this double detection method.

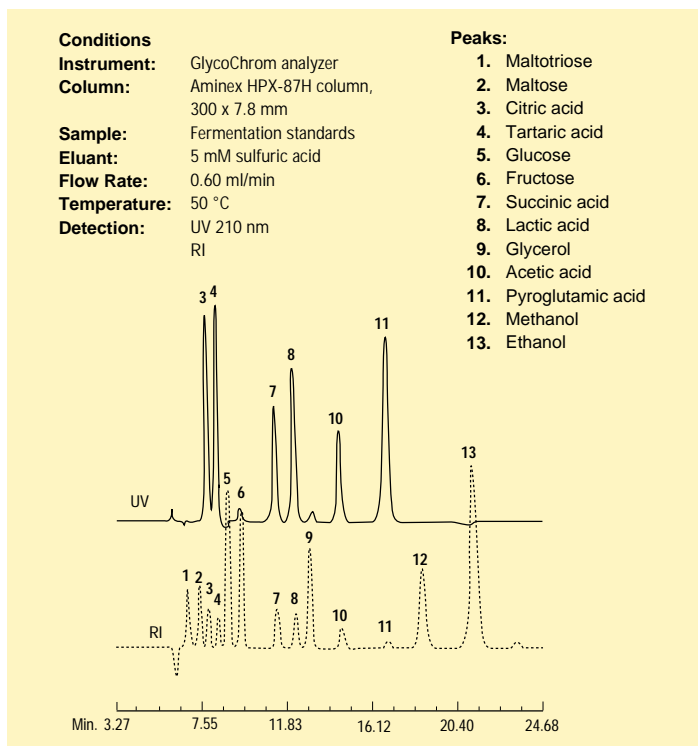


Fig. 4.8. Dual UV and refractive index detection of a series of fermentation standards on the Aminex HPX-87H column.

Section 5

Carbohydrate Analysis Columns

Sensitive and specific analyses of carbohydrates is important throughout the food industry. The presence and the quantity of carbohydrates in samples can indicate the quality and the properties of a product, and can reflect contamination or adulteration. Carbohydrate analysis is likewise important in biotechnology and pharmaceutical applications, as well as in biomedical research. In biomedical specimens, the presence of carbohydrates or the elevation of carbohydrate levels can often be correlated with changes in metabolism. Separating complex carbohydrate mixtures by HPLC is truly a challenge, in part, because of the wide variety of carbohydrates and the intricacy of carbohydrate mixtures existing in nature. Aminex carbohydrate columns provide a simple, non-destructive method for separating carbohydrates using a simple deionized water mobile phase.

Bio-Rad offers a complete line of carbohydrate analysis columns optimized to provide high selectivity for a specific carbohydrate or carbohydrate class. Research length (30 cm) columns provide high resolution separations of complex carbohydrate mixtures in approximately 20 minutes, while the smaller columns provide efficient separations of specific carbohydrates in 3–5 minutes. For complete profiles of complex mixtures, two or more columns may be used together, and dual UV/RI detection may be employed.

Aminex carbohydrate columns separate compounds using a combination of size exclusion and ligand exchange mechanisms. In oligosaccharide separations, size exclusion is the primary mechanism. Low crosslinked resins allow carbohydrates to penetrate, and oligosaccharides separate by size. For monosaccharide separations, ligand exchange is the primary mechanism which involves the binding of hydroxyl groups of the sugars with the fixed-counterion of the resin. Ligand exchange is affected by the nature of the counterion (Pb^{++} , Ca^{++} , etc.) and by the spatial orientation of the carbohydrate's hydroxyl groups.

The **Aminex HPX-87C column** is the column of choice for most general sweetener analyses. This 300 x 7.8 mm ID calcium-form column is optimized for analyzing monosaccharides, and also provides class separation of di-, tri-, and tetrasaccharides. It is used primarily for the quantitation of glucose and fructose in high fructose corn syrup, and for general monosaccharide analysis. The 250 x 4.0 mm ID column is appropriate for sugar alcohol separations.

The **Aminex HPX-87P column** is tailored for the separation of cellulose-derived monosaccharides. This lead-form column is well suited for analysis of pentoses and hexoses in wood products, especially cellobiose, glucose, xylose, galactose, arabinose, and mannose, and also provides excellent resolution of sucrose, lactose, and fructose in dairy products.

The **Fast Carbohydrate Analysis Column** is tailored for extremely fast separations of specific carbohydrates in samples where only certain components are of interest. The 100 x 7.8 mm ID lead-form column is optimized for 5 minute analysis of sucrose, glucose, galactose, and fructose.

The **Aminex HPX-87H column** is used for analysis of carbohydrates in solution with carboxylic acids, volatile fatty acids, short chain fatty acids, alcohols, ketones, and many neutral metabolic by-products. Most often used for organic acids analysis, this hydrogen-form column is also useful for fermentation monitoring, biological fluid analysis, and acetylated amino sugar separations.

The **Aminex HPX-87N column** is optimized for the analysis of sugars in samples with high salt concentrations, such as molasses. This sodium-form column is compatible with salts, so there is no need to desalt samples before analysis.

The **Aminex HPX-87K column** is optimized for the analysis of mono-, di-, and trisaccharides in samples such as corn syrup and brewing wort. This potassium-form column exhibits good separations of glucose, maltose, and maltotriose.

The **Aminex HPX-42A column** provides fast, high resolution oligosaccharide analysis. This silver-form column separates oligosaccharides through Dp 11 in approximately 25 minutes.

The **Aminex HPX-42C column** is optimized for analysis of mono- and disaccharides in starch hydrolysates. This calcium-form column also provides excellent resolution of oligosaccharides as large as Dp 10.

The **Aminex d.isaccharide column** is an amino-derivatized column tailored for quantitation of individual disaccharides but may also be used for some monosaccharide analyses. This silica-based column resolves glucose, fructose, sucrose, maltose, and lactose in 10 minutes, making it especially useful in situations requiring the analysis of many samples quickly.

The **In-Line Carbohydrate Deashing System** protects the Aminex carbohydrate columns from contamination by removing

all inorganic salts from the sample allowing only neutral carbohydrates to pass to the analytical column. This in-line deashing system consists of two Micro-Guard® cartridges, one packed with anion and one with cation exchange resin. Installed in series in front of Bio-Rad's analytical columns, this system offers a convenient and highly efficient in-line purification of sugar samples (Figure 5.1).

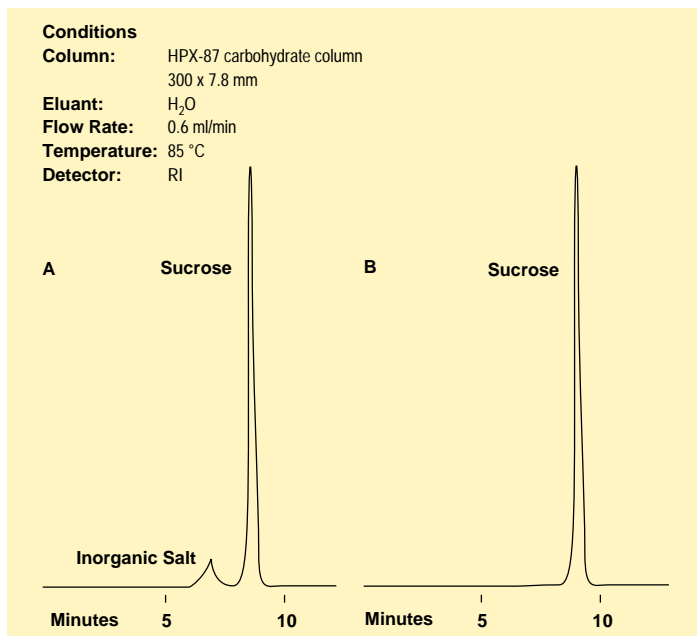


Fig. 5.1. Comparison of carbohydrate analysis without (A) and with (B) an in-line deashing system.

Section 6

Biotechnology Applications of Carbohydrate Analysis Columns

6.1 Carbohydrates in Biomedical and Pharmaceutical Samples

Increasingly, the investigation of protein structure and function has come to include, in addition to the protein itself, the carbohydrate moieties and other components bound to it. Investigation of the significance of protein bound carbohydrates begins with identifying and measuring these components. The Aminex HPX-87H column

can be used for the rapid analysis of two types of carbohydrate compounds typically obtained from protein hydrolysates: acetylated amino sugars and certain monosaccharides that have been shown to be involved in glycoprotein structure (Figure 6.1).

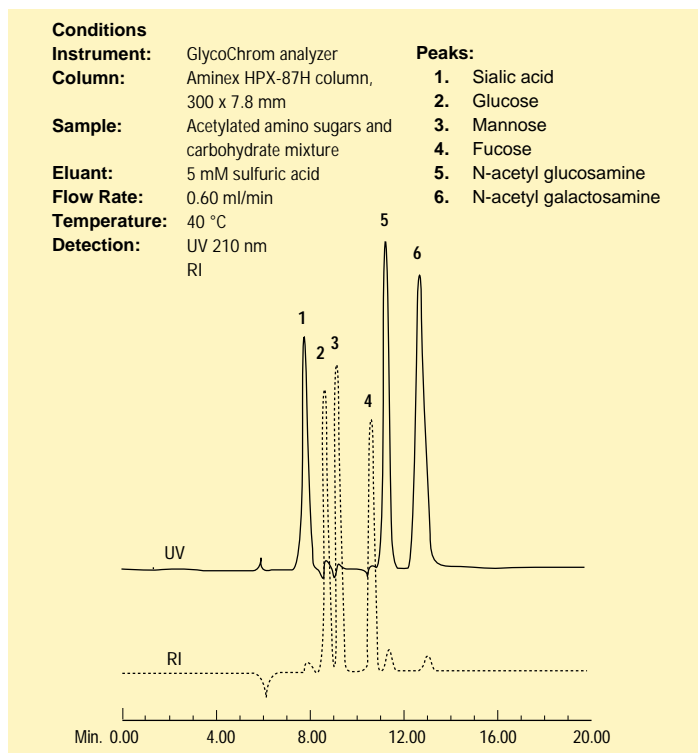


Fig. 6.1. Analysis of sugars found in glycoproteins on the Aminex HPX-87H column using dual RI and UV detection.

H. A. B. Linke and S. J. Moss [*Z. Ernaehrungswiss*, **31**, 147 (1992)] developed a sensitive HPLC method for the separation and quantitation of both carbohydrate sweeteners and organic acids in oral fluids (plaque, saliva) using an Aminex HPX-87H column. This method is useful in caries research, detecting minute amounts of sugars and organic acids in oral fluid during clearance studies of various foods in the mouth.

Ruo, *et al.* [*Clin. Chim. Acta*, **204**, 217 (1991)] developed an assay for inulin in plasma and urine on an Aminex HPX-87C column using post-column pulsed amperometric detection. This assay aids in clinical studies using inulin clearance from the kidney as a measure of glomerular filtration rates and extracellular fluid space.

Warner *et al.* [*Clin. Chim. Acta*, **127**, 313 (1983)] used the Bio-Sil® amino 5S column to develop a rapid and sensitive HPLC method to diagnose GM₁ gangliosidosis, an inherited storage disorder caused by a deficiency of the lysosomal enzyme beta-galactosidase. Because beta-galactosidase activity is nearly absent in this disease, increased amounts of GM₁ ganglioside and galactosyloligosaccharides accumulate in the viscera and are excreted in the urine. Using the Bio-Sil column with a linear gradient of acetonitrile-water, Warner was able to quantitate urinary galactosyloligosaccharides.

Denutte *et al.* [*Int. J. Appl. Radiat. Isot.*, **36**, 82 (1985)] used the Aminex HPX-87C column to develop a biosynthetic method for the preparation of pure, carrier-free [¹¹C] glucose. Green algae were fed ¹¹CO₂, and the labeled glucose was isolated by HPLC.

The capacity of lung explant cultures to synthesize collagen can be estimated by determining the content of [³H] hydroxyproline in protein following incubation with [³H] proline. After acid hydrolysis the hydroxyproline is completely separated from proline using the Aminex HPX-87C column in 10 mM calcium acetate, pH 5.5, at 85 °C [Stimler, N. P., *Anal. Biochem.*, **142**, 103 (1984)].

The Aminex HPX-87C column is also useful for analyzing pharmaceutical samples which contain carbohydrates in combination with other compounds. Figure 6.2 shows the analysis of a pharmaceutical solution containing dextrose, performed using the Aminex HPX-87C column.

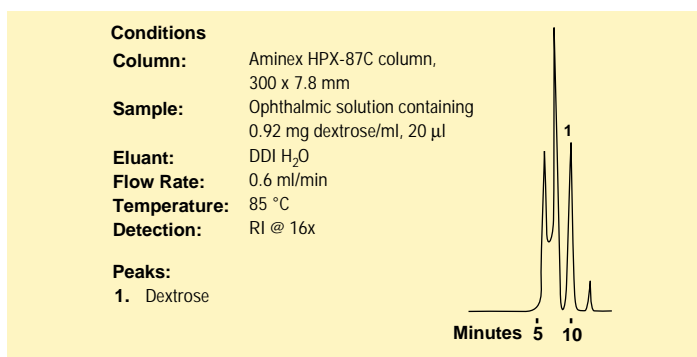


Fig. 6.2. Analysis of an ophthalmic solution on the Aminex HPX-87C column.

Often with complex samples such as cell cultures, mixtures of compounds must be resolved. Figure 6.3 shows the ability of the Aminex HPX-87H column to separate glucose and lactic acid used

to monitor cell metabolism in a cell culture medium [Tedesco, J. L., *BioTechniques*, **5**, 46 (1987)].

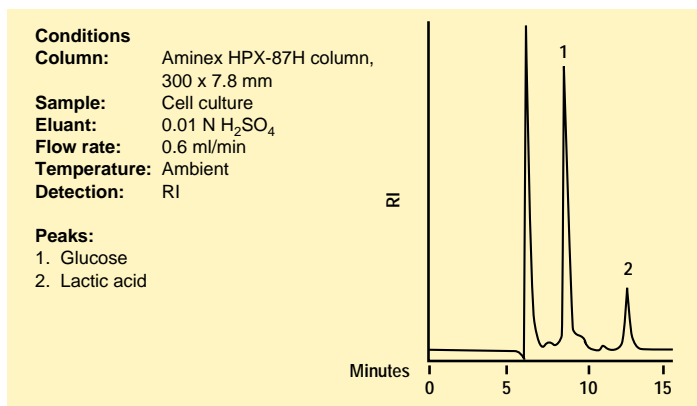


Fig. 6.3. Separation of glucose and lactic acid in conditioned DMEM containing 10% FBS on the Aminex HPX-87H column. Courtesy of John L. Tedesco, Invitron Corporation.

6.2 Carbohydrates in Natural Biological Materials

The analysis of carbohydrates in natural biological materials, particularly plants and plant products, is of interest in the study of plant physiology, and provides useful information for industrial research, biomedical research, and food technology. Analysis of these carbohydrates has become increasingly important with the attempt to use forest products and by-products more completely for energy production, chemical feedstocks, and similar applications. HPLC on Aminex columns provides one of the simplest and most rapid ways to study carbohydrates in these natural products.

Natural biological materials often contain disproportionate amounts of carbohydrates. The large quantity of one carbohydrate can mask the detection of a smaller quantity of another. An example is the separation of glucose, galactose, xylose, arabinose, and mannose found in wood pulp hydrolysates. The Aminex HPX-87P column is highly selective for the monosaccharides typically derived from cellulose, and provides excellent resolution of these compounds, even when disproportionate quantities of some carbohydrates are present (Figure 6.4). The Aminex HPX-87P column has been used to develop HPLC methods for analyzing wood sugars of interest to the pulp and paper industry.

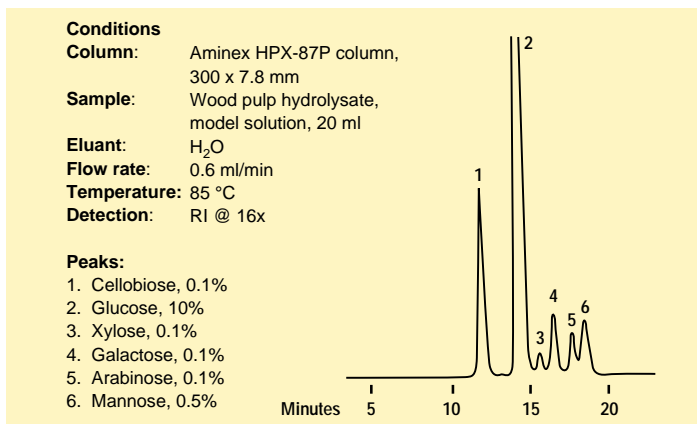


Fig. 6.4. Analysis of a wood pulp hydrolysate on the Aminex HPX-87P column.

Landolt *et al.* [*Trees*, **3**, 85 (1989)] have studied alterations in plant metabolism to better understand possible damaging effects of air pollution. They analyzed the effects of ozone and season on the pool size of cyclitols (myo-inositol and pinitol) in Scots pine using an Aminex HPX-87P column and found that ozone decreased levels of myo-inositol while increasing pinitol levels.

Hydrolyzed wood samples often contain complex mixtures of sugars, phenolics, organic acids, furfurals, and other compound classes. Analysis of the sugar fractions may be complicated by the complex array of additional compounds present. Patrick, D. W. and Kracht, W. R. [*J. Chrom.*, **318**, 269 (1985)] developed a novel column switching system, using a single pump and a 10 port valve, to simplify and speed the analysis of both water soluble and lipophilic organics in reaction products of wood hydrolysis. They were able to determine furfural and hydroxymethyl furfural, in addition to water soluble sugars and aliphatic acids, using an Aminex HPX-87H column.

Plant gums and mucilages are an important group of plant constituents with pharmaceutical and technical uses. The Aminex HPX-87P column has been used in a method which provides complete separation and quantitation of neutral sugars from plant cell walls and mucilages [Blaschek, W., *J. Chrom.*, **256**, 157 (1983)]. A mixture containing L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose, and D-glucose was baseline separated using a two-step procedure combining HPLC on an amino bonded phase column with analysis on the Aminex HPX-87P column. Lenherr, A. *et al.* [*J. Chrom.*, **388**, 455 (1987)] used an Aminex HPX-87P column to

detect glucose, mannose, allose, and galactose in plant glycosides. In samples of reference sugars, the minimum injection amount was 1 µg each using refractive index detection.

Schwald, W. *et al.* [*Chromatographia*, **20**, 35 (1985)] used a two column procedure combining the Aminex HPX-87P column and the Aminex HPX-42A column to analyze the oligomeric and monomeric carbohydrates from hydrothermal degradation of cotton-waste materials. The Aminex HPX-87P column separated the monomeric sugars and degradation products, while the Aminex HPX-42A column separated the oligomeric sugars.

Increased interest in the use of crop biomass for energy has created a demand for a rapid method to analyze the common sugars in crop plants, especially sucrose, glucose, and fructose. McBee, G. G. and Maness, N. O. [*J. Chrom.*, **264**, 474 (1982)] developed a rapid and reproducible method for the detection of these sugars in sorghum cultivar culms by HPLC following oven drying of plant tissues and refluxing with boiling 95% EtOH. HPLC was performed on an Aminex HPX-87C column and a Bio-Sil® amino 5S column was used to ascertain that the sucrose peak was pure and did not contain maltose. Kubadinow, N. [*Zuckerindustrie*, **111**, 37 (1986)] developed a quantitative method to detect carbohydrates and aconitic acid in sorghum juices by HPLC, as a control method for sorghum processing. The method uses an Aminex HPX-87H column which permits quantitation of sucrose, ketose, glucose, fructose, and cis- and trans-acetic acid within 14 minutes.

Analytically useful separations of the alpha- and beta-anomers of five economically important monosaccharides, glucose, xylose, galactose, mannose, and arabinose, can be obtained by aqueous chromatography on the Aminex HPX-87C column [Baker, J. O. and Himmel, M. E., *J. Chrom.*, **357**, 161 (1986)]. Such analyses are important in determining the mechanisms of action of different enzymes converting the polysaccharides found in woody biomass to monomeric units fermentable to fuel alcohols. Chromatography on the lead-form Aminex HPX-87P column separated the anomers of glucose, but failed to separate the anomers of the other four sugars due to the substantially higher rate of mutarotation of these sugars in the presence of the lead form packing material.

6.3 Alcohol Analysis

The measurement of alcohol is an important and frequently performed determination for the analysis of alcoholic beverages and foods. However traditional methods make this a relatively difficult pro-

cedure. Distillation, GC, enzymatic analysis, and gravimetric/refractive index can be used for accurate analysis, but they are, in varying degrees, time consuming, complex, highly technique dependent, and destructive of the sample. Aminex columns can provide rapid and non-destructive analysis of alcohols. A profile of different alcohols performed using an Aminex HPX-87H column is shown in Figure 6.5. Sugar alcohols are routinely separated on the 250 x 4.0 mm Aminex HPX-87C column according to one USP procedure (Figure 6.6).

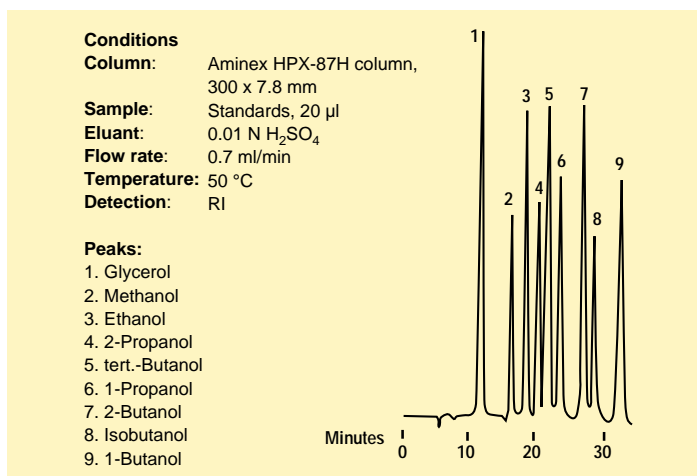


Fig. 6.5. Separation of alcohols on the Aminex HPX-87H column. Reproduced with the permission of Pecina, R. and Bonn, G., *J. Chrom.*, **287**, 245 (1984).

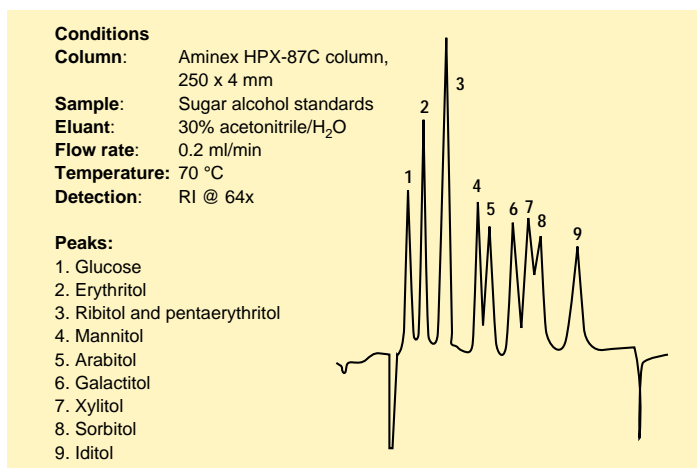


Fig. 6.6. Sugar alcohol separation.

Inositol is a cyclic polyol which is a ubiquitous component of living cells and body fluids. Inositol and its derivatives have been recognized as intracellular second messengers. These compounds appear to serve in a variety of important biochemical roles. A number of inositol isomers have been separated on an Aminex HPX-87C column by Sasake, M. *et al.* [*Carbohydr. Res.*, **183**, 1 (1988)] using refractive index detection and by Wang, W. T. *et al.* [*Anal. Biochem.*, **188**, 432 (1990)] using postcolumn pulsed amperometric detection.

6.4 Fermentation Analysis

Aminex columns are used with a combination of UV and RI detection to provide a complete picture of fermentation kinetics. The RI monitor detects fermenting carbohydrates, glycerol, and alcohol, while the UV monitor detects carboxylic acids, volatile fatty acids, and other fermentation by-products which can cause feedback inhibition. The Aminex HPX-87H column provides superior separations of both carbohydrates and organic acids by combining the mechanisms of ion exclusion and partition. A fermentation broth consisting of a complex carbohydrate mixture was monitored at 0, 24, and 96 hours. Figure 6.7-1 shows the profile of the broth at 0 hours. The carbohydrate content of the broth was profiled by RI monitoring, while the acid content was measured by UV monitoring at 210 nm. Because molasses was one of the broth components, the UV trace shows that some carboxylic acids existed in the broth prior to fermentation. Figure 6.7-2 shows the production of glycerol and alcohol, with a reduction in carbohydrates, after 24 hours. Figure 6.7-3 shows that after 96 hours most of the fermentable carbohydrates have been consumed. By providing an easy and rapid method for analyzing the major parameters of fermentation, the Aminex HPX-87H column allows researchers to accurately measure fermentation kinetics. This method is particularly useful for evaluating strains of yeast and bacteria for efficient alcohol production and for production of important by-products. Because the method is so simple, it is particularly useful for routine industrial quality control analyses.

The total utilization of biomass is very important from environmental, industrial, and agricultural points of view. Plant biomass such as lignocellulose materials of waste waters, agricultural residues, and wood are important renewable sources for fermentation to produce needed fuels. Aminex columns have been used extensively in the analysis of sugars and other organic compounds in sulphite liquors used widely as fermentation substrates. Gey, M. *et al.* [*Acta. Biotechnol.*, **11**, 511 (1991)] separated pentoses, hexoses, furfural, and hydroxymethyl furfural (HMF) from sulphite liquor on an

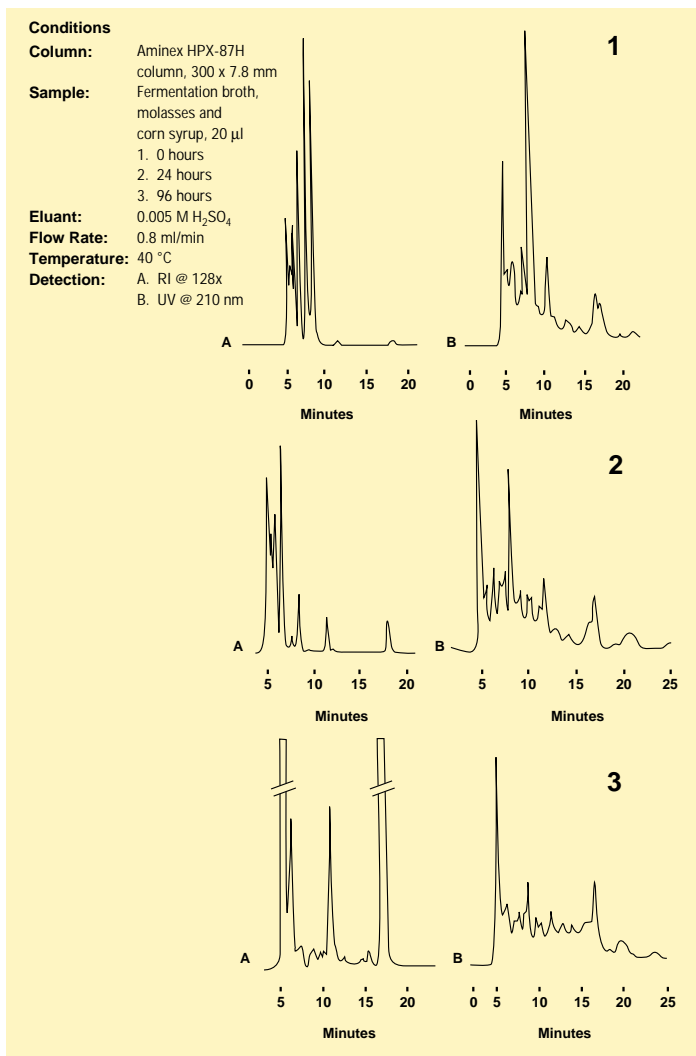


Fig. 6.7 Analysis of fermentation broths on the Aminex HPX-87H column using dual RI and UV detection.

Aminex HPX-87H column using dual refractive index and UV detection at 283 nm. Marko-Vargo, G. *et al.* have studied a number of enzyme-based detection systems for HPLC. These detection systems have been coupled with Aminex HPX-87C, HPX-87H, and HPX-87P columns to analyze sulphite liquor [*Chromatographia*, **36**, 381 (1993)] as well as penicillin fermentation [*J. Chrom.*, **408**, 157 (1987)].

The Aminex HPX-87H column can also be used in combination with other columns for a more complete profile of carbohydrates in fermentation solutions. For example, Bonn, G. and Bobleter, O. [*Chromatographia*, **18**, 445 (1984)] used the combination of an Aminex HPX-87H column and an Aminex HPX-42A column for HPLC analysis of plant biomass hydrolysis and fermentation solutions. The Aminex HPX-42A column is especially useful for the analysis of the gluco-oligomers in the solution, while the Aminex HPX-87H column separates the monomeric sugars and their degradation and fermentation products such as furfurals and alcohols very well. Dadic, M. and Belleau, G. [*J. Amer. Soc. Brewing Chem.*, **40**, 141 (1982)] used the same combination of columns to quantitate individual oligosaccharides along with the fermentable sugars maltotriose, maltose, glucose, and fructose.

Section 7

Food Applications with Carbohydrate Analysis Columns

Most industries operate within narrow profit margins, requiring large volume production and rapid product turnover for survival. As a result, accurate knowledge and control of ingredients and reliable monitoring of production processes are important cost control factors. HPLC on Aminex columns is a useful, versatile tool which can help achieve these goals. The wide range of both analytical and quality control analyses which can be performed on the Aminex columns can provide industries with information concerning raw materials, processes, and products.

7.1 Carbohydrates in Raw Materials

Since the price of corn products is often determined by the amount of fermentable sugars in them, it is necessary to determine the amount of maltotriose, maltose, and glucose, accurately. Even though differences in quantitation may seem small, when a small error is applied to thousands of pounds of corn product, the price differential can be large. Aminex HPX-87C columns are optimized for rapid analysis of high fructose corn syrup. Examples of the speed and resolution possible with these columns are shown in Figures 7.1 and 7.2. Figure 7.1 shows the separation of maltose, dextrose, fructose, and ribose. The peaks are well separated, and the analysis took only 10 minutes. Figure 7.2 shows the separation of a starch hydrolysate reported to contain 50% each of dextrose

and fructose. Again, the peaks were narrow and well separated, and the run took less than 10 minutes. If faster analysis is required and fructose is the only component of interest, the Fast Carbohydrate Analysis Column is appropriate. The column separates glucose and fructose in approximately 4 minutes, although other components of the syrup are not separated.

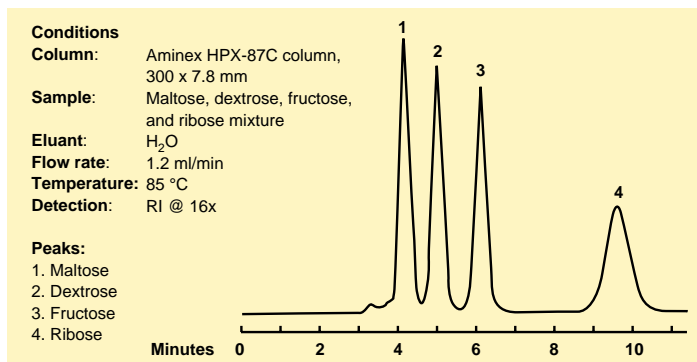


Fig. 7.1. Rapid sugar separation on the Aminex HPX-87C column.

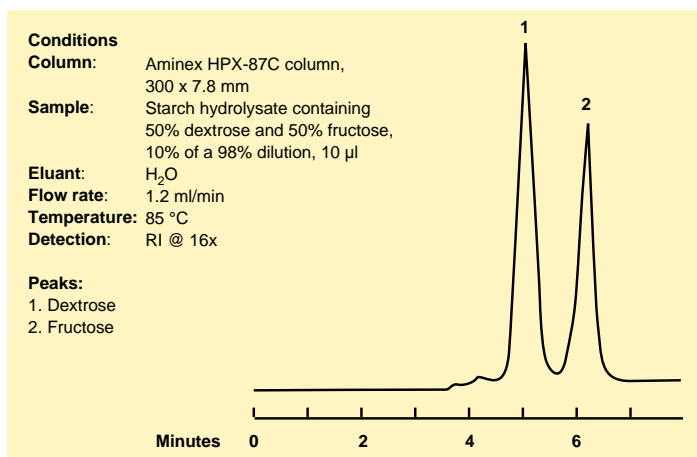


Fig. 7.2. Analysis of a starch hydrolysate on the Aminex HPX-87C column.

Sorghum is often used in commercial food products. The carbohydrate composition of sorghum stalks can be an indication of fungal stalk rot, a disease which makes the sorghum unfit for use. HPLC analysis, using either the Aminex HPX-87C column or the Fast Carbohydrate Analysis column, can provide a quick analysis. The sugars are extracted with hot 80% ethanol, evaporated, and redissolved

in water before analysis. With this method, a sorghum sample can be analyzed in 3.5 minutes on the Fast Carbohydrate Analysis column, or in 15 minutes on the Aminex HPX-87C column (Figure 7.3).

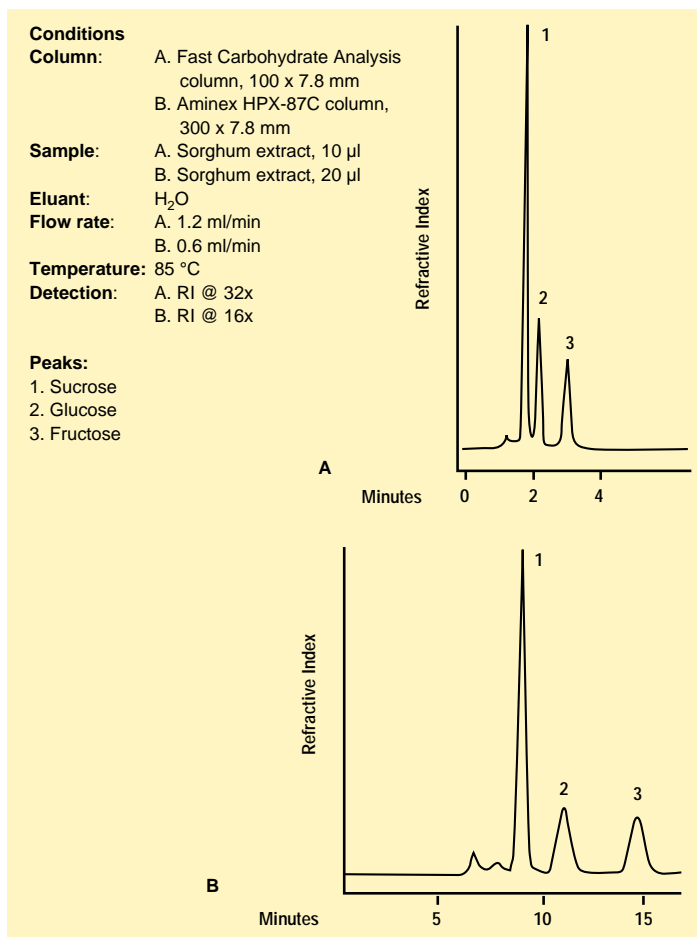


Fig. 7.3. Sorghum sugar analysis on the (A) Fast Carbohydrate and (B) Aminex HPX-87C columns.

Charles, D. F. [*Int. Sugar J.*, **83**, 169 (1981)] used an Aminex HPX-87C column to analyze samples of raw sugar for carbohydrates. He found that the presence of glycerol in raw sugars can serve as an indication of microbiological degradation. He then used the column to detect microbiological degradation in a refined product made from the sugars. The column quantitatively detected glucose, mannose, levulose, and glycerol, providing a fast, simple method to detect spoilage in raw materials and finished goods.

Water soluble carbohydrate polymers are becoming increasingly important to industry, as their use as thickening agents for foods becomes more widespread. The Aminex HPX-42C column provides excellent resolution of these oligosaccharides (Figure 7.4). Analysis times for monosaccharides, however, are relatively long on this column. The Aminex HPX-42A column can resolve glucose polymers as large as Dp 11 within 30 minutes (Figure 7.5). For better resolution of oligosaccharides greater than Dp 11, two Aminex HPX-42A columns can be used in series to separate oligosaccharides to Dp 14. This dual column method is especially useful in the carbohydrate industry, where it can be employed to determine enzymatic or acid hydrolysis in the conversion of corn syrup to fermentable carbohydrates.

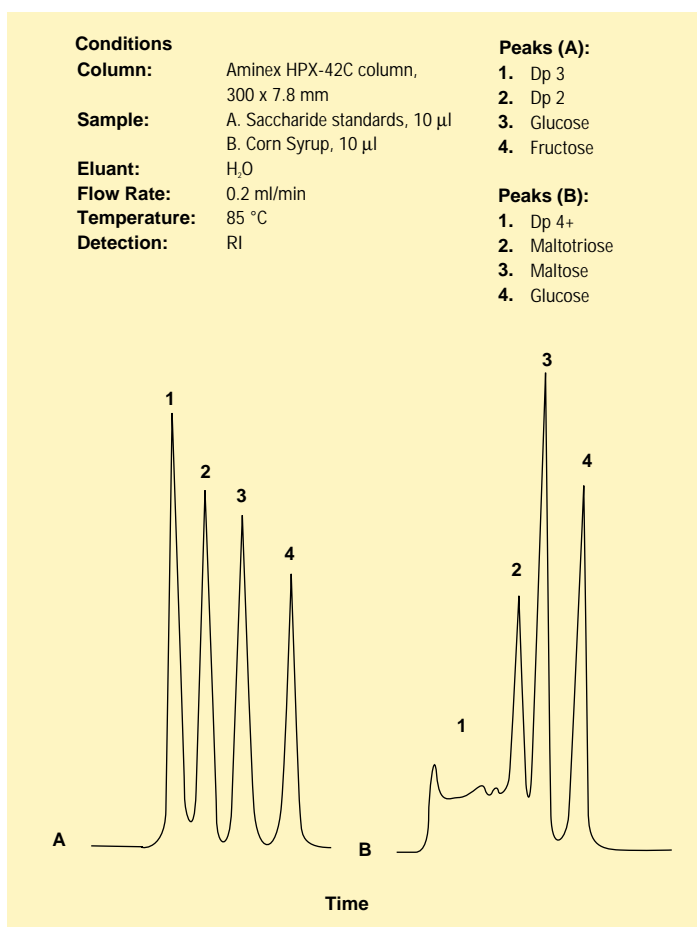


Fig. 7.4. Sugar and corn syrup analysis on the Aminex HPX-42C column.

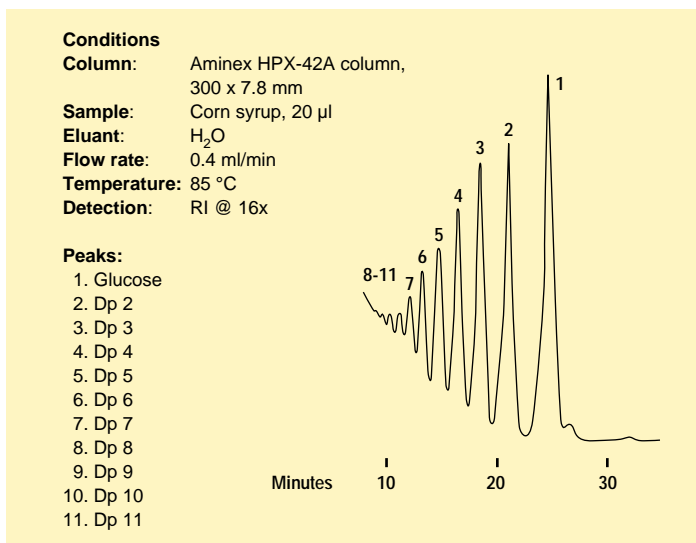


Fig. 7.5 Corn syrup analysis on the Aminex HPX-42A column.

7.2 Carbohydrates in Dairy Products

Analysis of the carbohydrate content of dairy products is important in flavor studies and nutritional assessments. Different types of sweeteners are added to dairy products to create or enhance flavors. HPLC with Aminex columns provides a rapid and simple method for assessing the type and quantity of sweetener present. The Aminex HPX-87P column is useful for analyzing samples which contain different types of sweeteners. Figure 7.6 shows the carbohydrate profile of plain yogurt which contains natural lactose and galactose, strawberry yogurt to which corn sweetener has been added for flavor enhancement, and blueberry yogurt which contains added corn syrup.

Ice cream is made up of combinations of monosaccharides and disaccharides. The type and quantity of sugars used influence both the nutritional quality and the flavor. Analysis of the carbohydrate matrix of ice cream can show the quality and sweetness of the final product. The disaccharide column is ideal for the analysis of sugars in ice cream and other dairy products (Figure 7.7). An ethanol extract of the dairy product eliminates proteins, which would rapidly deplete the guard column. The speed of analysis possible with the disaccharide column allows high sample throughput.

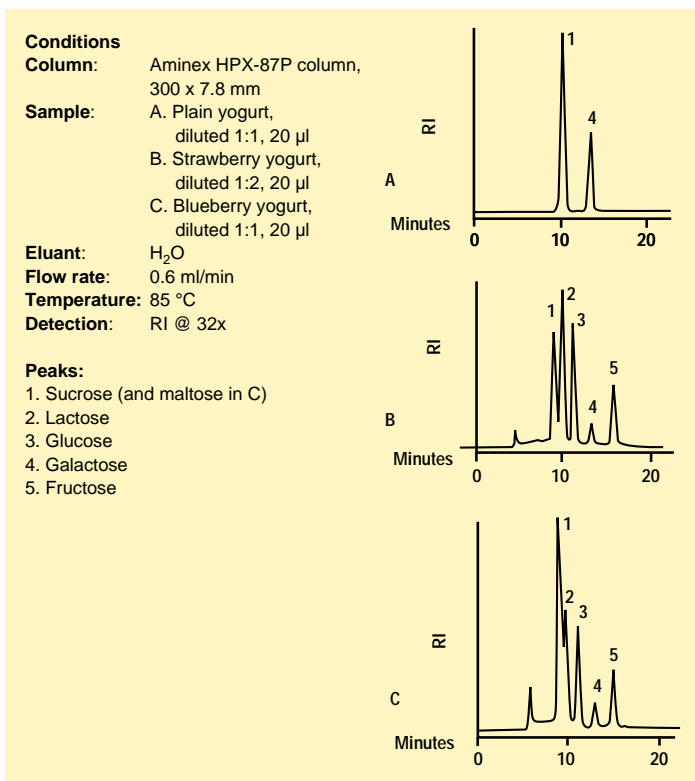


Fig. 7.6. Carbohydrate profiles of plain and flavored yogurts on the Aminex HPX-87P column.

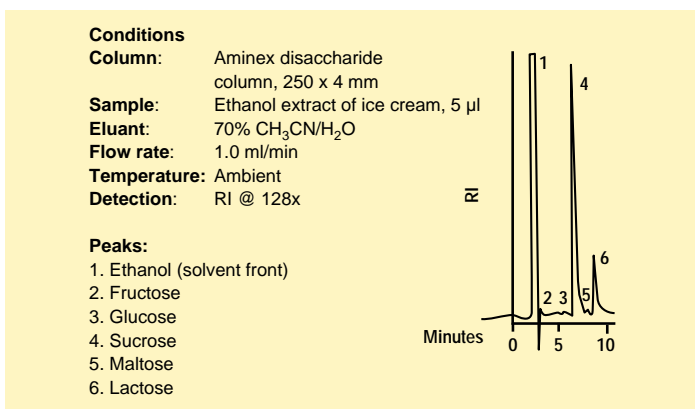


Fig. 7.7. Ice cream analysis on the Aminex disaccharide column. Courtesy of Dean Foods.

Richmond, M. L. *et al.* [*J. Dairy Sci.*, **65**, 1394 (1982)] used an Aminex HPX-87C column to develop a method for determining sucrose, lactose, glucose, galactose, and fructose in strawberry and plain yogurt, milk, buttermilk, and wheys. Operated at a flow rate of 1.0 ml/min, the column separated lactose, glucose, and galactose with near-baseline resolution in 8 minutes.

Pirsino, J. F. [*J. Food Sci.*, **48**, 742 (1983)] used the Aminex HPX-87C column to develop a simple, rapid method for simultaneous detection of lactose, glucose, and galactose in lactose-reduced milk. The milk samples were rapidly prepared for injection by precipitating protein and fat with MeCN. For spiked milk samples, recoveries from 94 to 103% were obtained.

7.3 Carbohydrates in Fruit Juice

Many compounds affect the appearance, taste, odor, and nutritional quality of fruit juice. Carbohydrates and organic acids are both present in juices at natural or supplemental levels. Both are important flavor components, and profiles of the amounts of each in a sample can be useful indicators of flavor and quality. In addition, combinations of carbohydrates and organic acids are frequently added to juices to enhance or to create flavors. Carbohydrate analysis can also be used to detect adulterations or substitutions in fruit juice components. HPLC on Aminex columns is the method of choice for the analysis of carbohydrates in fruit juice. Figure 7.8 shows the analysis of apple juice on a Fast Carbohydrate Analysis column. The analysis took 6.5 minutes and provided baseline resolution between glucose and fructose.

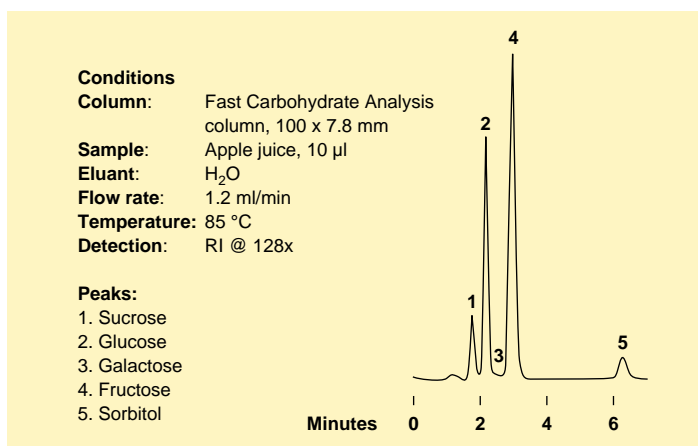


Fig. 7.8. Apple juice analysis on the Fast Carbohydrate column.

Sometimes in the beverage industry a complete profile of the carbohydrate and acid content of a formulation is required. Because of the complexity of carbohydrates as a class, no single HPLC column can be optimized to give the complete profile, so several different Aminex columns, each optimized to provide high selectivity for a different class of compounds, should be used. The Aminex HPX-87C column provides a good beginning for most sweetener analyses. This column separates glucose, fructose, and sucrose, and allows detection and quantitation of sorbitol, which co-elutes on other columns. Figure 7.9 shows the analysis of orange juice on this column. While the Aminex HPX-87C column is excellent for analyzing simple sugars, disaccharides will co-elute on the column. The Aminex disaccharide column is optimized for disaccharide analysis.

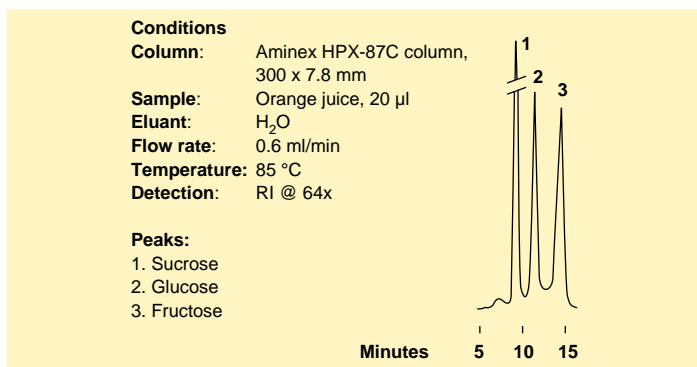


Fig. 7.9. Orange juice analysis on the Aminex HPX-87C column.

Lee, H. S. and Wrolstad, R. E. [*J. Assoc. Off. Anal. Chem.*, **71**, 789 (1988)] used the Aminex HPX-87C column to analyze the sugars in apple juice. Gancedo, M. C. and Luh, B. S. [*J. Food Sci.*, **51**, 571 (1986)] used the Aminex HPX-87H column to profile the sugars and organic acids in tomato juice.

7.4 Fermentation Analysis

The Aminex HPX-87H column provides simultaneous separations of carbohydrates and organic acids, by combining the mechanisms of ion exclusion and partition. The RI monitor detects fermenting carbohydrates, glycerol, and alcohol, while the UV monitor detects carboxylic acids, volatile fatty acids, and fermentation by-products. The ethanol and glycerol content of wine is not only an excellent indicator of the progress of fermentation, it can also be used to predict the quality of the final product, since the amount of glycerol correlates with the smoothness of the wine. Figure 7.10 shows a typical sugar profile of a Cabernet Sauvignon wine on the Aminex HPX-87C column.

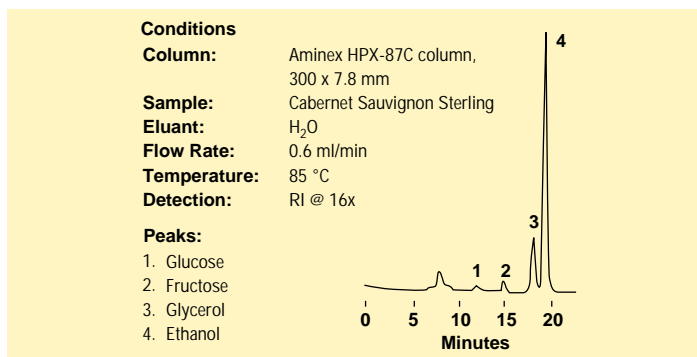


Fig. 7.10. Sugar and alcohol analysis in wine on the Aminex HPX-87C column.

7.5 Carbohydrates in Food Products

Aminex HPLC columns provide a fast and convenient method for analyzing the components of food products, making them especially useful in such applications as quality control. Not only can total sugar measurements be made quickly, but individual sugars can be quantitated with the columns. For example, Figure 7.11 shows the ability of the Aminex HPX-87C column to analyze the sweetener composition of a cookie. The Aminex HPX-87C column has been used in conjunction with the Bio-Sil amino 5S column (Aminex disaccharide) for the quantitation of sugars in raw and baked sweet potato products [Picha, D. H., *J. Food Sci.*, **50**, 1189 (1985)]. Quantitation of maltose and sucrose in baked potatoes is performed on the Aminex disaccharide column, since these compounds co-elute on the Aminex HPX-87C column.

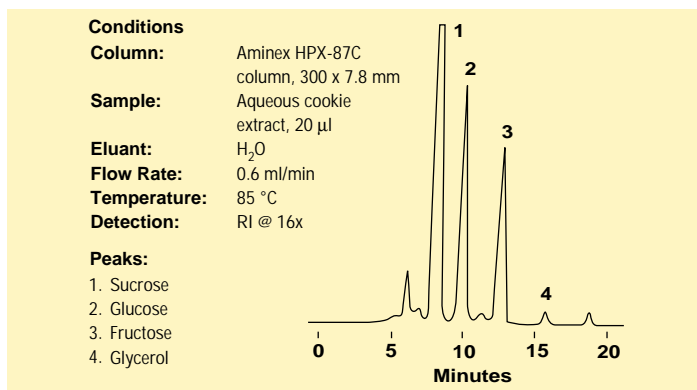


Fig. 7.11. Sugar analysis of cookie extracts on the Aminex HPX-87C column. Courtesy of Mike Gross, Keebler Company.

Polydextrose is a synthetic water soluble polymer not exceeding 22,000 daltons molecular weight. Due to the low metabolism of polydextrose (1 calorie/g) it is used as a bulking agent in the manufacture of low-calorie diet foods. With polydextrose, a variety of reduced-calorie foods has been created that simulate the texture and mouth-feel of higher calorie substances. Noffsinger, J. B. *et al.* [*J. Assoc. Off. Anal. Chem.*, **73**, 51 (1990)] have developed a simple method to assay polydextrose in foods using the Aminex HPX-87C column eluted with 5 mM calcium sulfate (Figure 7.12).

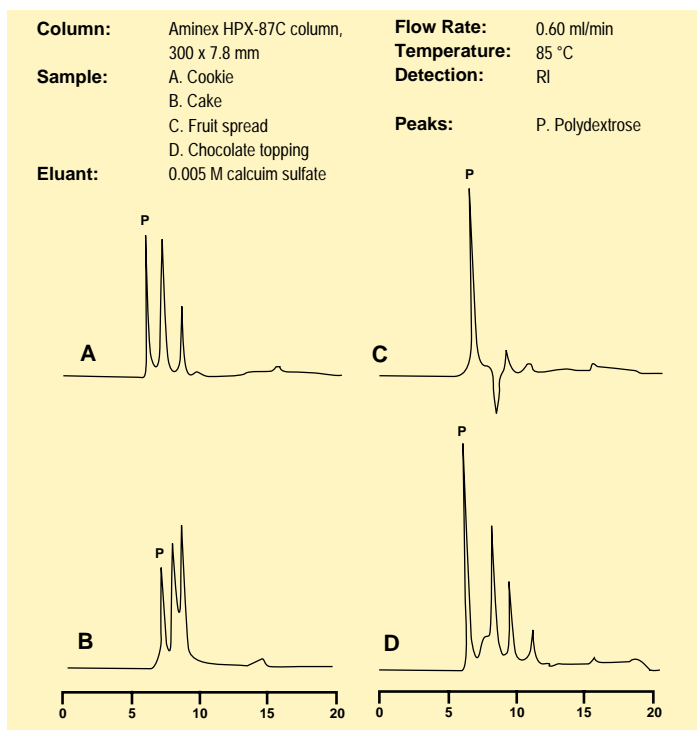


Fig. 7.12. Analysis of polydextrose in different foods on the Aminex HPX-87C column. Reproduced with permission, Noffsinger J. B. *et al.*, *J. Assoc. Off. Anal. Chem.*, **73**, 51 (1990).

Section 8

Organic Acid Analysis Columns

Sensitive analysis of small acidic and polar compounds is important in products throughout the food, beverage, and brewing industries. These compounds are not only naturally present in many foods, they are also used as food additives. The presence and quantity of organ-

ic acids in samples can indicate the quality and properties of a product, and can reflect contamination or adulteration. The current interest in human nutrition has increased the demand for simple yet specific methods of food and beverage analysis which can detect and quantitate these compounds. Analysis of organic acids, aldehydes, ketones, alcohols, and carbohydrates is important in biotechnology, as well as in biomedical applications. Aminex HPLC methods not only increase the ease with which sample components can be determined, but they also allow determination of compounds that were previously undetectable. The column decreases operator involvement and, because sample handling is kept to a minimum, sample loss and method error decrease substantially. One of the major advantages of the Aminex HPX-87H columns for biotechnology applications is the durability of the resin packing, which makes it easier to obtain consistent, reproducible results. In addition, if the columns do become contaminated they are easy to regenerate to their original high quality.

Several Aminex columns are available for small acidic and polar molecule analysis. When many compounds in a formulation must be analyzed, or when high resolution separations are required, the 300 x 7.8 mm ID Aminex HPX-87H column is the choice, although the Aminex HPX-87C column is also suited for analyzing organic acids in combination with carbohydrates. The smaller fast acid and fermentation monitoring columns provide fast separations of specific organic acids in samples where only certain components are of interest. With these columns, analyses can be completed in 3–5 minutes. As with the Aminex HPX-87H column, dilute sulfuric acid is the only eluant used in the analyses, and filtering is generally the only sample preparation required.

The Aminex HPX-87H columns separate organic acids using primarily ion exclusion and reversed phase mechanisms. When dilute sulfuric acid is used as the eluant, organic acids elute from the column in order of increasing pK_a . Anions are eluted near the void volume, and acids which have been ionized in the acidic eluant elute according to the fraction of the acid ionized. Column selectivity is controlled by changing the column temperature, the pH of the eluant, or by adding organic modifiers such as acetonitrile to the eluant to reduce resin/compound interactions and to cause strongly bound compounds such as aromatic acids to elute more rapidly. The column separates neutral species, such as carbohydrates and alcohols, by reversed phase partitioning. The eluant is polar while the resin matrix is nonpolar, so the aliphatic nonpolar alcohols are adsorbed by the resin and are eluted after charged molecules. This ability to separate a variety of compounds makes the columns ideal

for fermentation and by-product analysis, or for separating mixtures which contain several classes of components.

The **Aminex HPX-87H column** performs most analyses in about 20 minutes, with sensitivity to the ng level. A simple isocratic elution with slightly acidified water is all it takes to analyze organic acids in most samples with the 300 x 7.8 mm column, and filtration is the only sample preparation required before injection. Separations can be performed from ambient temperature to 60 °C at flow rates between 0.4 and 1.0 ml/min.

The **Fast Acid Analysis Column** is optimized for the analysis of alcohols, glycols, and hydrophobic organic acids. Typically, analyses can be shortened four-fold over those obtained with research length columns. Because the fast acid column is shorter, components elute as taller, narrower peaks. Detection limits are improved, and smaller sample loads can be used.

The **Fermentation Monitoring Column** is optimized to resolve maltotriose, maltose, glucose, and fructose while also resolving acids and alcohols. When analysis of sugars in a fermentation broth is required, this is the column of choice.

The **Aminex HPX-87C column** can be fine-tuned for organic acids analysis by using an eluant which contains a mixture of water, calcium sulfate, sulfuric acid, and acetonitrile.

Section 9

Biotechnology Applications with Organic Acid Analysis Columns

9.1 Biomedical Applications

The identification and quantitation of plasma and urinary carboxylic acids can assist in diagnosing inborn metabolic disorders. Most methods of carboxylic acid analysis require derivatization, followed by GC, GC/MS, or HPLC analysis. However, derivatization is time consuming and in addition may not be quantitative. Buchanan, D. N. and Thoene, J. G. [*Anal. Biochem.*, **124**, 108 (1982)] developed a rapid HPLC screening procedure for organic acids, which eliminates both sample extraction and derivatization, using an Aminex HPX-87H column with tandem UV and amperometric detection (Figure 9.1). Since then, several rapid HPLC screening procedures for these acids have been developed which eliminate both sample extraction and derivatization using the Aminex HPX-

87H column [Bennett, M. J. and Bradey, C. E., *Clin. Chem.*, **30**, 542, (1984); Buchanan, D. N. and Thoene, J. G., *Clin. Chem.*, **32**, 169 (1986); Chong, W. K. *et al.*, *J. Chrom.*, **487**, 147 (1989)].

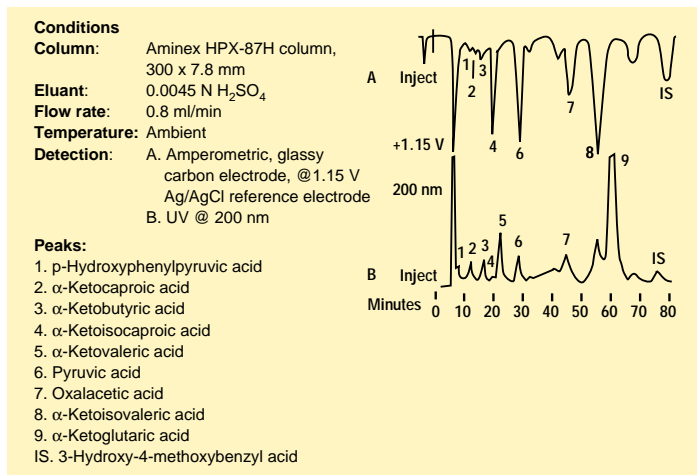


Fig. 9.1. Urinary acid analysis on the Aminex HPX-87H column using amperometric (A) and UV (B) detection.

An increase in serum lactate and pyruvate levels is associated with several medical disorders. High serum lactate levels can indicate lactic acidosis in diabetes, and can be a useful prognostic indication in myocardial infarction complicated by shock. A rise in serum pyruvate levels can indicate heavy metal poisoning or thiamine deficiency. The Aminex HPX-87H column can solve many of the analytical problems associated with the determination of serum lactate and pyruvate.

Analysis of plasma can be extremely useful as a diagnostic tool. Figure 9.2 shows an acid profile from normal plasma and six additional acid profiles indicative of various diseases. Although some sample clean-up is necessary to preserve the life of the column, the Aminex HPX-87H column provides the most convenient method of analysis for acids in biological samples.

Elevated serum sialic acid levels, as reflected by N-acetylneuraminic acid (NANA) concentration, are associated with tumor growth in neoplasms. Silver, H. K. B. *et al.* [*J. Chrom.*, **224**, 381 (1981)] analyzed serum hydrolysates for sialic acid with an Aminex HPX-87H column. They found the HPLC method easy and a valuable tool for quantitating serum sialic acid. Sialuria is a disease characterized by increased levels of free N-acetylneuraminic acid in

Conditions

Column: Aminex HPX-87H column, 300 x 7.8 mm
Sample: Plasma treated with anion exchange resin
Eluant: 0.005 M H₂SO₄
Flow rate: 0.6 ml/min
Temperature: Ambient
Detection: UV

Peaks:

Pyruvate (P) 1. Methylmalonate
 Lactate (L) 2. Propionate
 Urate (U) 3. 2-Oxoisovalerate
 4. 2-Oxo-3-methyl-*n*-valerate
 5. 2-Oxoisocaproate
 6. 3-Hydroxybutyrate
 7. Acetoacetate
 8. Isovalerate
 9. 3-Methylglutaconate

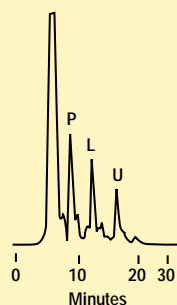
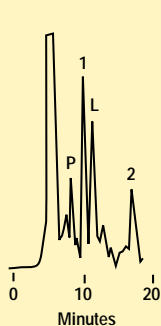
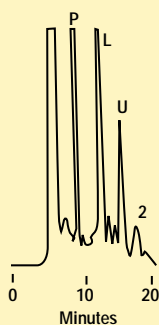
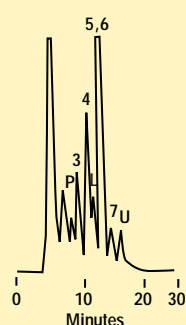
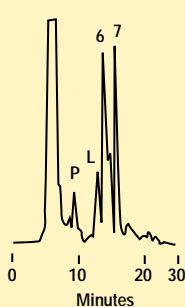
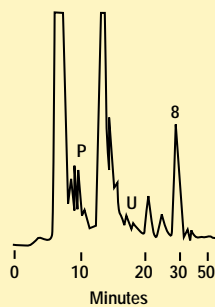
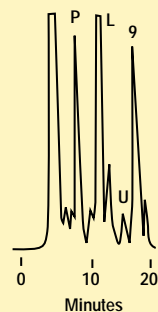
**A. Normal****B. Methylmalonic acidemia****C. Propionic acidemia****D. Maple syrup urine disease****E. Severe ketosis****F. Isovaleric acidemia****G. 3-Hyd CoA Lyase deficiency**

Fig. 9.2. Plasma profiles: (a) normal, 0.7 ml plasma; (b) methylmalonic acidemia, 0.5 ml plasma; (c) propionic acidemia 0.7 ml plasma; (d) maple syrup urine disease, 0.4 ml plasma; (e) severe ketosis, 0.1 ml plasma; (f) isovaleric acidemia, 0.5 ml plasma; (g) 3-hydroxy-3-methylglutaryl CoA lyase deficiency, 0.4 ml plasma. Reproduced with permission, Daish, P., *et al.*, *Clinica Chimica Acta*, **146**, 87 (1985).

urine. Mononen, I. [*J. Chrom.*, **381**, 219 (1986)] has described a simple method for the detection of sialuria using an Aminex HPX-87H column (Figure 9.3).

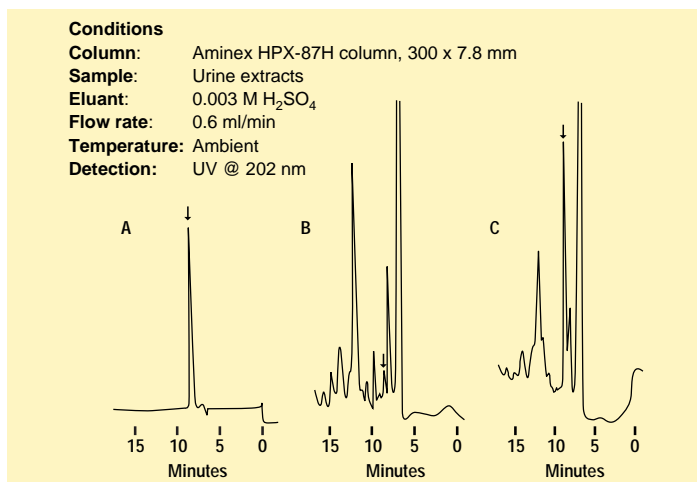


Fig. 9.3. Analysis of N-acetylneuramic acid in urine samples on the Aminex HPX-87H column. **A.** Standard N-acetylneuramic acid; **B.** Urine sample from control subject; **C.** Urine sample from a patient with sialuria. Reproduced with permission, Mononen, I., *J. Chrom.*, **381**, 219 (1986).

Chromatographic profiles of other N-acetylated amino sugars have been shown to be useful in quantitative analysis. Detection of N-acetyl galactosamine on an Aminex HPX-87H column is used for indirect assay of beta-hexosaminidase, an enzyme which catalyzes the cleavage of particular oligosaccharides to N-acetyl galactosamine. Figure 9.4 shows the profile achieved by direct injection of digest over the Aminex HPX-87H column.

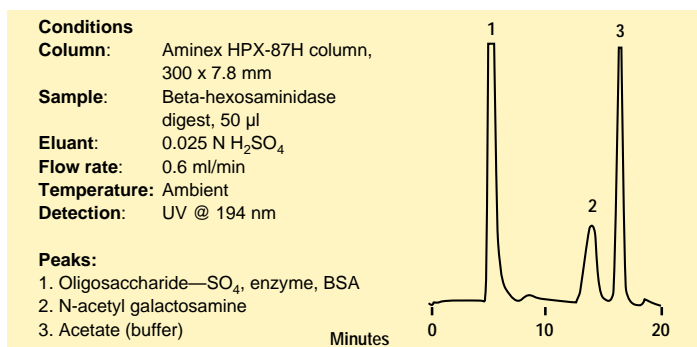


Fig. 9.4 Analysis of N-acetyl galactosamine on the Aminex HPX-87H column.

The identification and quantitation of short chain fatty acids plays an important role in several research fields. In microbiology, the measurement of volatile fatty acids is used as a presumptive test for anaerobic bacterial infection. In nutritional science, fatty acid analysis can provide information on animal metabolism. Guerrant, G. O. *et al.* [*J. Clin. Microbiology*, **16**, 355 (1982)] described a simple HPLC method for the determination of short chain fatty acids in bacterial cultures. They separated 25 standard fatty acids on an Aminex HPX-87H column, using 10.8% acetonitrile in dilute sulfuric acid. They used the HPLC method to analyze a bacterial culture of *Peptostreptococcus anaerobius*. Thirteen acids from the culture were identified by comparing their retention times to those of known standards (Figure 9.5). Krausse, R. and Ullmann, U. [*Zentralbl. Bakteri.*, **276**, 1 (1991)] have developed a rapid method for analyzing volatile and nonvolatile short chain fatty acids using the Aminex HPX-87H column.

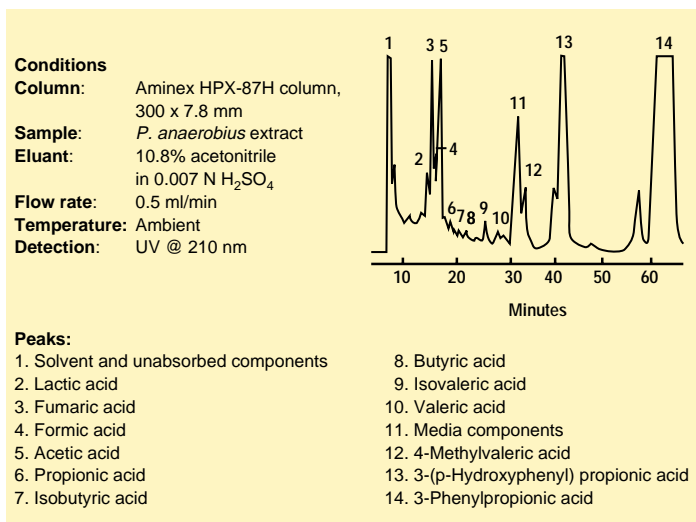


Fig. 9.5. Organic acid analysis of an extract of *P. anaerobius* on the Aminex HPX-87H column. Reproduced with permission. Guerrant, G. O., *et al.*, *J. Clin. Microbiol.*, **16**, 355 (1982).

Martillotti, F. and Puppo, S. [*Ann. Ist. Sper. Zootec.*, **18**, 1 (1985)] described a procedure for the detection of lactic acid and volatile fatty acids in silages and rumen fluids. Rumen samples were diluted 1:1 with 1 N H₂SO₄ while silage was homogenized 1:2 with 1 N H₂SO₄. After filtration and centrifugation, the supernatant was analyzed on an Aminex HPX-87H column.

Chiu, G. [*JHRC and CC*, **9**, 57 (1986)] separated and detected cyanovaleric acid in a mixture of carboxylic acids in process streams by using an Aminex HPX-87H column. The detection limits for C₄₋₆ dicarboxylic acids and cyanovaleric acid were 2 and 3 ppm, respectively. For 50 µl samples, these limits correspond to 0.1 and 0.15 µg, respectively. A complete analysis by this method took less than 25 minutes.

9.2 Pharmaceutical Solution Analysis

Pharmaceutical solutions must be strictly monitored to insure product integrity. The Aminex HPX-87H column permits the resolution and quantitation of the three different classes of compounds often found in IV solutions, carbohydrates, acids, and inorganic anions. Substances with a strong positive charge, such as sodium, potassium, and amino acids, will bind tightly to the cation exchange resin in the guard column. Substances with a strong negative charge, such as chloride ions, are excluded from the packing material and elute in the void volume. For a complete quantitative profile of an IV solution, a dual detection system should be employed. Carbohydrates such as glucose and fructose are detected with a refractometer, while acids such as lactic, gluconic, and acetic are best detected with a UV monitor at 210 nm. Figure 9.6 shows the separation of a typical IV solution in less than 25 minutes. Figure 9.7 shows the separation of acids from a urological sequestering agent.

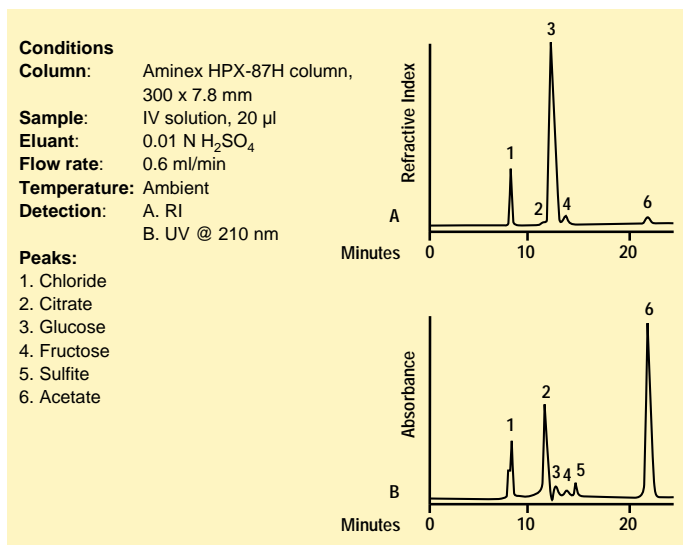


Fig. 9.6. Analysis of an IV solution on the Aminex HPX-87H column.

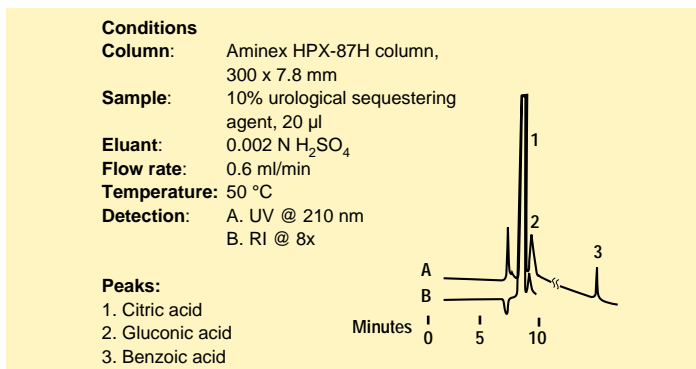


Fig. 9.7. Analysis of the acids in a urological sequestering agent on the Aminex HPX-87H column.

The measurement of alcohol is an important and frequently performed determination for the analysis of alcoholic beverages, pharmaceutical preparations, and biochemicals. The ability of the column to measure alcohol in pharmaceutical preparations is demonstrated in Figure 9.8, a chromatogram of diluted cough syrup.

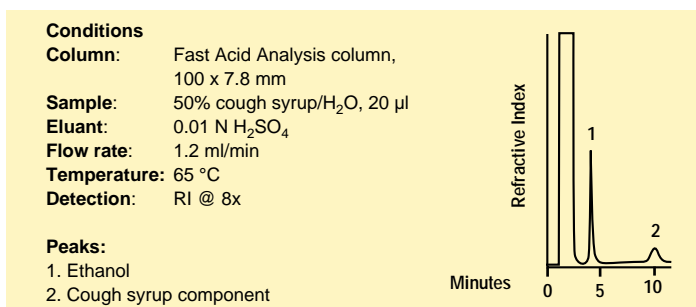


Fig. 9.8. Analysis of the ethanol in cough syrup on the Fast Acid column.

9.3 Fermentation Monitoring

The Aminex HPX-87H columns are well suited for monitoring fermentable carbohydrates and the major by-products of fermentation. The columns allow researchers to measure the kinetics of fermentation accurately by providing an easy and fast method for detecting fermentations' major parameters. Additionally, contamination is monitored through the production of acidic compounds. Acetic and lactic acids are formed by the action of bacteria and wild yeast. Fermentation cultures must be carefully monitored through all stages of the fermentation. The Aminex HPX-87H column is especially useful for this

type of fermentation monitoring. Figure 9.9 shows the analysis of a fermentation process taking place in a culture broth of *A. niger*.

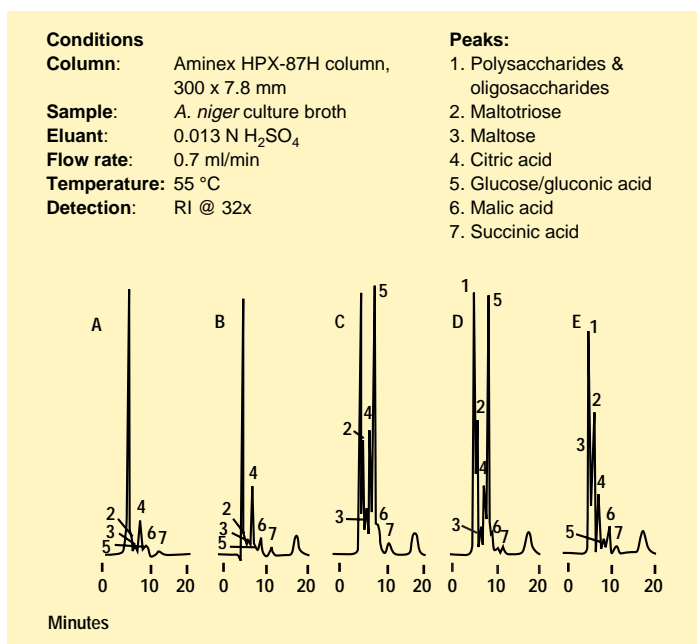


Fig. 9.9. Analysis of sugars and organic acids from the culture broths of *A. niger* on the Aminex HPX-87H column. (A) 0-hr broth (control medium); (B) 20-hr broth; (C) 24-hr broth; (D) 28-hr broth, and (E) 42-hr broth. Reproduced with permission, Tan, K.H. *et al.*, *J. App. Biochem.*, **6, 80 (1984).**

9.4 Cell Metabolism

Masson, S. *et al.* [*J. Chrom.*, **563**, 213 (1991)] have analyzed the effluents from perfused rat liver on an Aminex HPX-87H column using dual UV and refractive index detection. They were able to quantitate in a single run the metabolites derived from the tri-carboxylic cycle, glycolysis, ketogenesis and ethanol oxidation (Figure 9.10).

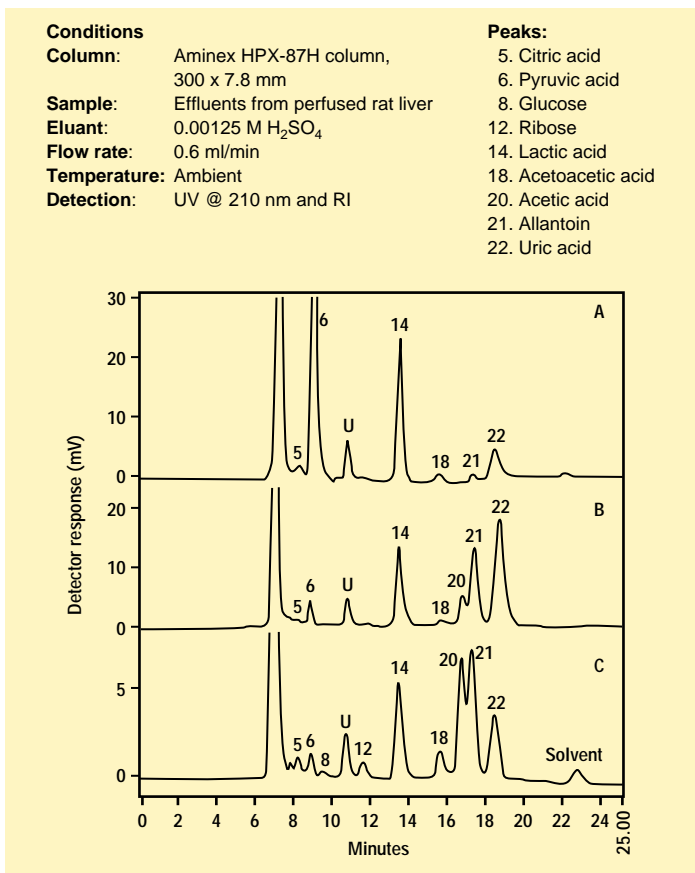


Fig. 9.10. Analysis of the effluents of perfused rat liver on the Aminex HPX-87H column using dual UV and RI detection. A. Perfusion with a Krebs-Henseleit medium; **B.** Perfusion with a Krebs-Henseleit medium supplemented with 2 mM ethanol; **C.** Perfusion with a Krebs-Henseleit medium supplemented with 2 mM ethanol and 1 mM ribose. Reproduced with permission, Masson, S. *et al.*, *J. Chrom.*, **563**, 213 (1991).

9.5 Acrylamide and Methacrylate Monomers

Acrylamide is a commercially important monomer with a wide variety of industrial applications. It is an important constituent of chromatography and electrophoresis gels used in laboratories for the analysis of proteins and nucleic acids. There is an interest in an accurate assay for these monomers from an environmental aspect as well as to assess the monomer purity. As an example, reliable electrophoresis results depend upon the purity of the starting reagents. Figure 9.11 shows that several different monomers used in the poly-

merization and/or crosslinking process can be checked for purity in one analysis using the Fast Acid Column.

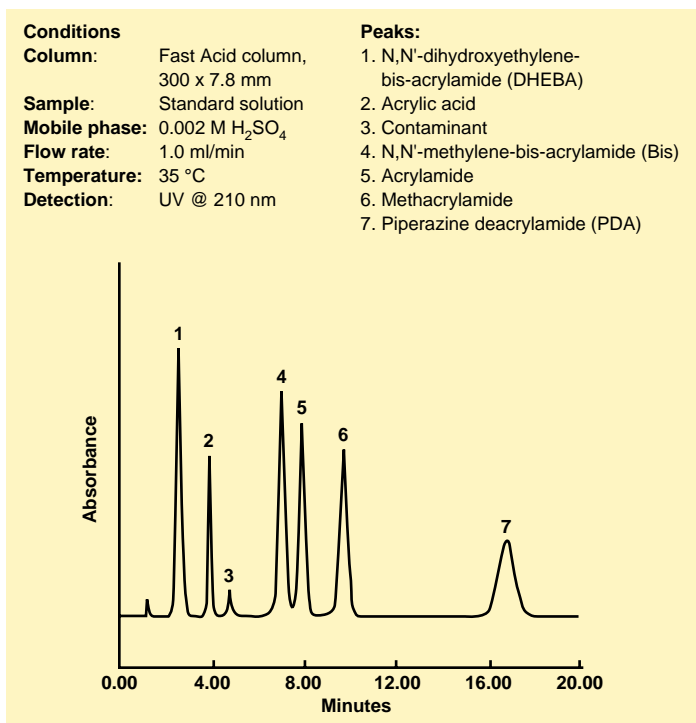


Fig. 9.11. Analysis of acrylamide monomers on the Fast Acid column.

Section 10

Food Applications of Organic Acid Analysis Columns

10.1 Process Control and Spoilage Detection

Quantitative determinations of organic acids in food products can provide important information in flavor studies, nutritional assessments, and spoilage detection. Analysis of organic acids in dairy products, for example, gives information on process control parameters and flavor indicators.

The Aminex HPX-87H column provides a rapid method for quantitation of pyruvic acid in milk (Figure 10.1A). The production of pyruvic acid by metabolizing bacteria is a measure of the con-

centration of psychrotrophic bacteria, which, while not pathogenic, can generate off flavors in milk, and will continue to grow at commercial refrigeration temperatures of 2–7 °C. At the same time, the method can be used to measure lactose, a sweetness indicator (Figure 10.1B), and to obtain a total organic acid profile of the milk. The Aminex HPX-87H column is much faster than bacterial plating techniques, which may require several days.

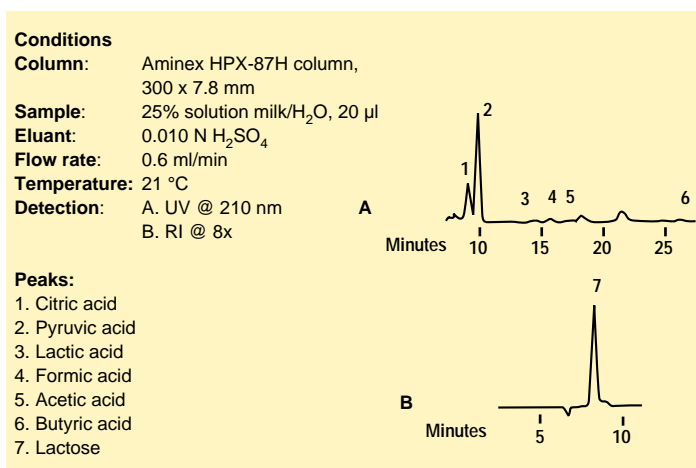


Fig. 10.1. Analysis of organic acids and lactose in milk on the Aminex HPX-87H column.

Marsili, R. T. *et al.* [*J. Food Sci.*, **46**, 52 (1981)] used the Aminex HPX-87H column to detect organic acids in whole milk, skim milk, cultured buttermilk, sour cream, cottage cheese, yogurt, cheddar cheese, and blue cheese. They were able to quantitate orotic, citric, pyruvic, lactic, uric, formic, acetic, propionic, butyric, and hippuric acids in these products. Recoveries of more than 90% of the organic acids added to the dairy products were observed for every acid but butyric.

The Aminex HPX-87H column also provides a method for better control over the production of cultured dairy products such as sour cream, yogurt, and cheese. Each microbial culture produces a distinct organic acid profile (Figure 10.2). Organic acid analysis can therefore be used both to monitor the microbiological process and to define the final product. For example, distinct and different profiles were obtained for plain and cherry yogurt. Such characteristic organic acid fingerprints give food processors the capability of correlating the levels of individual organic acids with the specific taste desired in a product. Bouzas, J. *et al.* [*J. Food Sci.*, **56**, 276 (1991)]

have developed a method for the analysis of sugars and organic acids (Figure 10.3) in cheddar cheese on an Aminex HPX-87H column using dual UV and refractive index detectors in series.

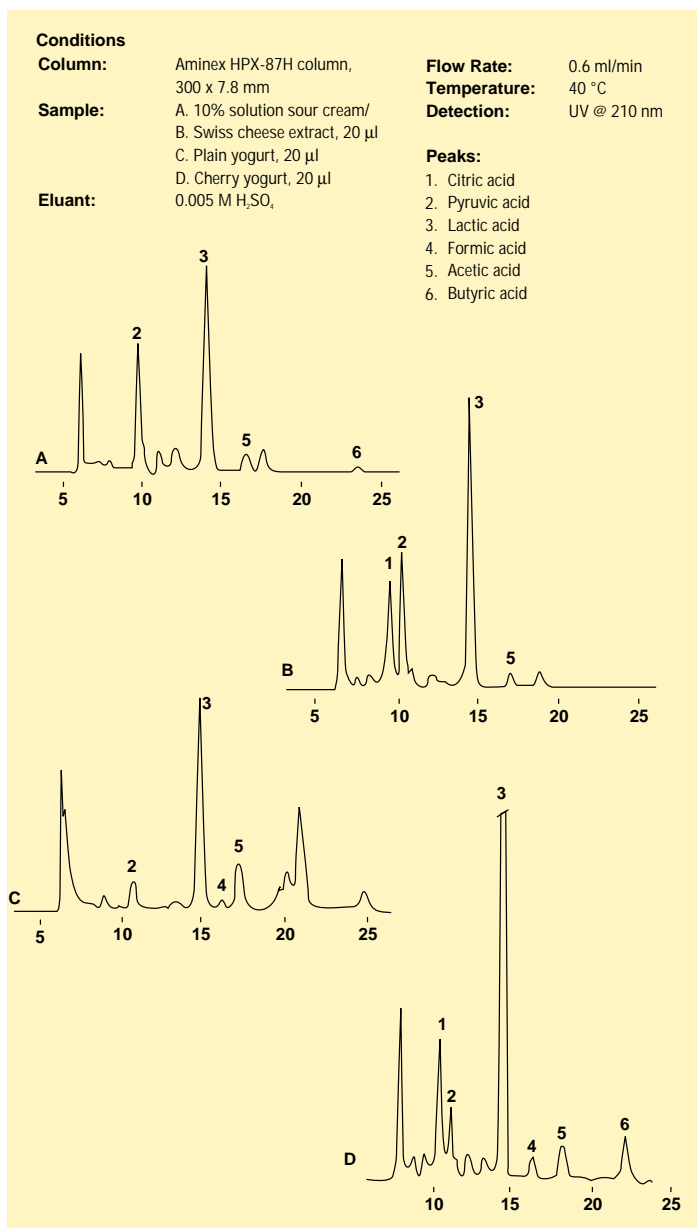


Fig. 10.2. Analysis of organic acids in dairy products on the Aminex HPX-87H column.

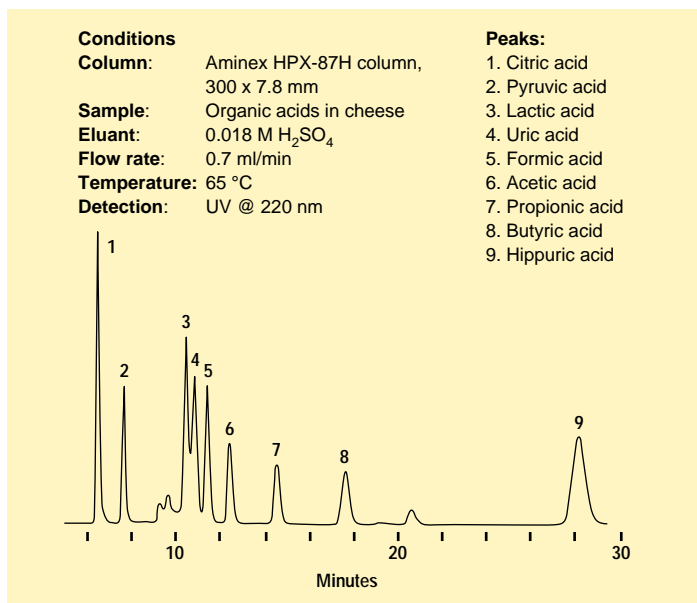


Fig. 10.3. Analysis of organic acids in cheddar cheese on the Aminex HPX-87H column. Reproduced with permission, Bouzas, J., *et al.*, *J. Food Sci.*, **56**, 276 (1991).

The Aminex HPX-87H column is also useful for detecting microbial spoilage in meats. Nassos, P. S. *et al.* [*J. Food Sci.*, **49**, 671 (1984)] used the column to develop a rapid method for measuring lactic acid, a spoilage indicator, in refrigerated ground beef.

10.2 Detecting Food Additives

Organic acids are often added to food products to enhance or modify the flavor, or to make the product more palatable and more acceptable to consumers. Citric acid, for example, is often added to fruit drinks to produce a tart flavor, and thus achieve a more natural taste. The organic acid analysis columns can provide a fast profile of the citric acid content of fruit juices, which can be used to detect product adulteration (Figure 10.4). Ashoor, S. H. and Knox, M. J. [*J. Chrom.*, **299**, 288 (1984)] used the Aminex HPX-87H column to detect citric acid in a variety of food products. The detection limit with this HPLC method was $\geq 2 \mu\text{g}$ citric acid, with a coefficient of variation of 0.2–4.4%. Recoveries of added citric acid were between 98.0 and 101.9%.

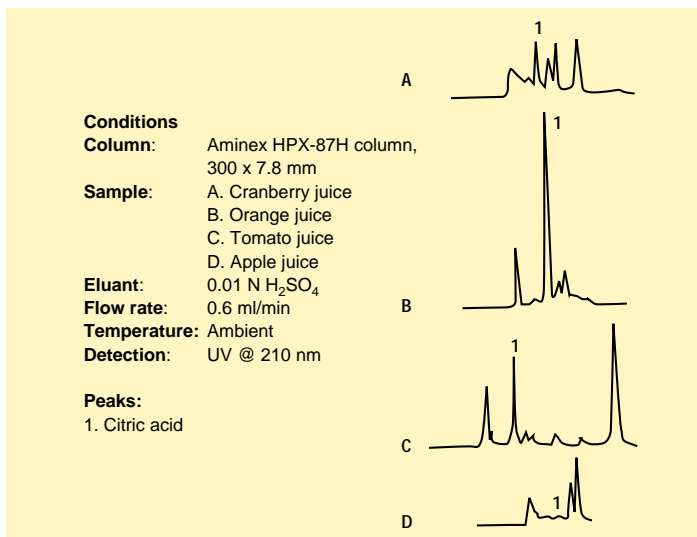


Fig. 10.4. Analysis of citric acid in fruit juices on the Aminex HPX-87H column.

Fumaric acid is another organic acid frequently added to food products to enhance flavor. Figure 10.5A shows the chromatographic profile of natural grape juice, while Figure 10.5B shows the profile of a grape ade, to which fumaric acid has been added for flavor.

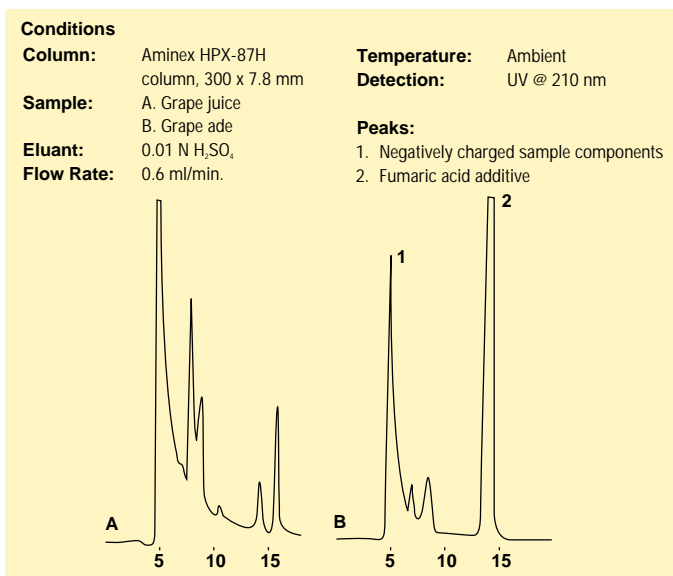


Fig. 10.5. Analysis of fumaric acid on the Aminex HPX-87H column.

Apple juice is sometimes diluted with water to extend the pure juice. When juice is diluted, synthetic malic acid and sugar are added to restore the sugar-acid balance and satisfy consumer taste. Since malic acid is the predominant acid in apple juice, the addition of synthetic malic acid allows the natural acidity of the juice to be imitated. It is sometimes difficult to detect adulteration, since the level of natural malic acid in apple juice can vary so much that monitoring malic acid alone is not a reliable indicator of adulteration. But because fumaric acid is an inherent contaminant of all commercially available malic acid, the level of fumaric acid in a sample of apple juice is an excellent indication that synthetic malic acid has been added to the juice. Since fumaric acid is present at levels less than 1 ppm in pure apple juice, juices with fumaric acid levels greater than 3 ppm can be suspected of containing synthetic malic acid. The Aminex HPX-87C column provides a fast, accurate analysis of fumaric acid levels in a juice sample (Figure 10.6). Alternatively, Evans, R. H. *et al.* [*JAOAC*, **66**, 1517 (1983)] used the Aminex HPX-87H column to evaluate the authenticity of apple juice.

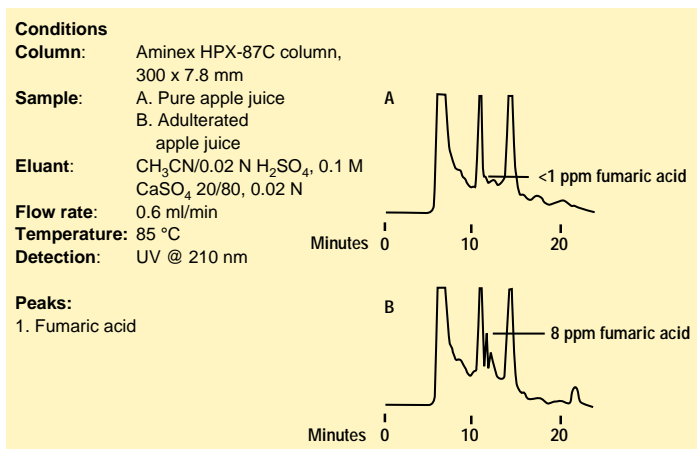


Fig. 10.6. Analysis of fumaric acid in apple juice on the Aminex HPX-87C column.

Benzoic and sorbic acids are used as preservatives in many foods and ingredients. The concentration of these acids must be monitored to achieve a level that is both effective and within legal limits. The Fast Acid Analysis Column can perform this analysis in less than 8 minutes. Phenolic acids are strongly retained on the Aminex HPX-87H column, and while acetonitrile can be used to reduce hydrophobic interactions, analyses can still take up to 1 hour. Figure 10.7 shows the results achieved when the Fast Acid Analysis

Column is used to detect sorbic and benzoic acid in maple flavored syrup, with 0.01 N sulfuric acid as the eluant.

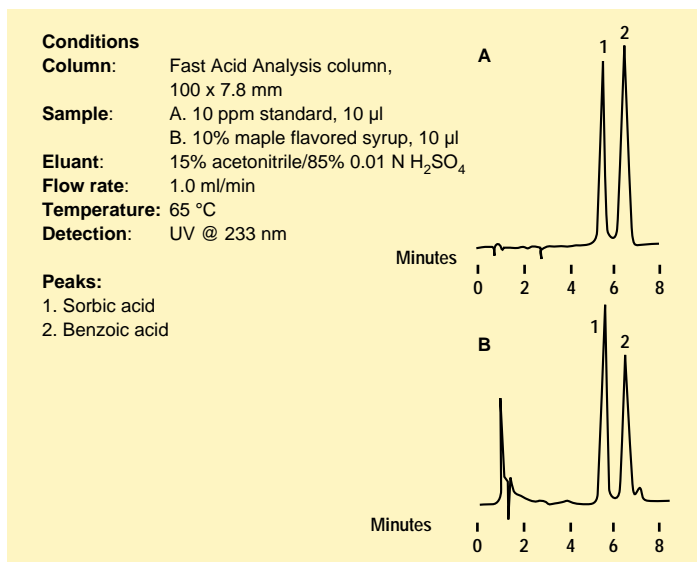


Fig. 10.7. Analysis of preservatives in maple-flavored syrup on the Fast Acid column. Courtesy of Dr. D. G. Rowsell, Footscray Institute of Technology.

Lactic acid is used as a food acidulant, and is a major component of foods such as fermented meat sausage and fermented dairy products. Because the lactic acid content of such products influences their flavor, stability, and quality after storage, quantitative determination of lactic acid in food products is important for quality control, for meeting legal regulations, and for labeling. Ashoor, S. H. and Welty, J. [*J. Chrom.*, **287**, 452 (1984)] used the Aminex HPX-87H column to develop a simple, fast HPLC method for quantitating lactic acid in food products. The detection limit was 2 μ g lactic acid, with a variation coefficient of 2.0–4.9% and recoveries of 93.4–97.8%.

Diethylene Glycol (DEG), a colorless, odorless, sweetish tasting compound that is primarily used as an antifreeze, has sometimes been added to low quality wines to enhance the body and sweetness, making the final product appear to have the quality of a better wine. Methanol, which may be naturally present in wine at low levels, has also been added to low quality wines, to increase the alcohol content. Because DEG and methanol are toxic when ingested, detecting their presence in food and beverage products is crucial for consumer safety. The Aminex HPX-87H columns provide a

reliable method to rapidly screen samples for DEG and methanol. Figure 10.8 shows the analysis of a wine sample intentionally adulterated with 20 ppm DEG. The Aminex HPX-87H column can also detect methanol in wine samples in approximately 20 minutes.

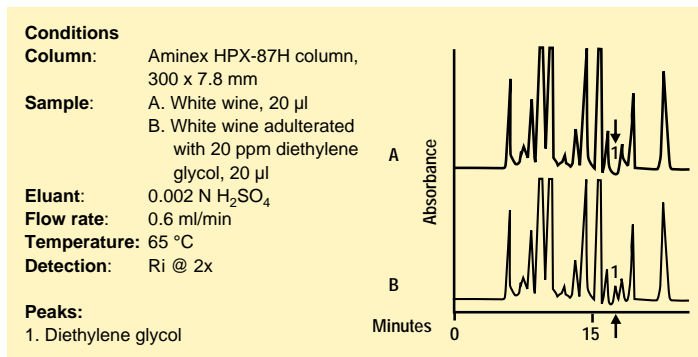


Fig. 10.8. Analysis of diethylene glycol in wine on the Aminex HPX-87H column.

Sulfite is a common additive to food and beverages. If the levels of sulfite exceed 10 ppm the FDA requires food labeling. Kim, H. J. [*J. Assoc. Off. Anal. Chem.*, **73**, 216 (1990)] describes the determination of sulfite in food and beverages using ion exclusion chromatography on the Aminex HPX-87H column with electrochemical detection.

10.3 Flavor Indicators and Product Stability

The flavor of a product can often be predicted based on the type and proportion of organic acids present. For example, the acetic acid profile of fermented products is a useful determinant of flavor. In some cases, like production control of vinegar, it is important to quantitate acetic acid in the solution to control the quality and the taste of the final product. In wine, large amounts of acetic acid indicate poor quality, and impart a vinegary taste to the final product. The Aminex HPX-87H column allows the complete analysis of the major components of a wine sample (Figure 10.9).

5-(hydroxymethyl)-2-furaldehyde (HMF) and furfural are produced as the result of dehydration of saccharides when sugars are subjected to heat processing. For example, when alcoholic beverages like brandy are distilled, the heat treatment acts as a catalyst, producing HMF and furfural. Elevated levels of HMF and furfural can give the product an off-flavor, and indicate overheating. Monitoring the levels of HMF and furfural can provide information about the

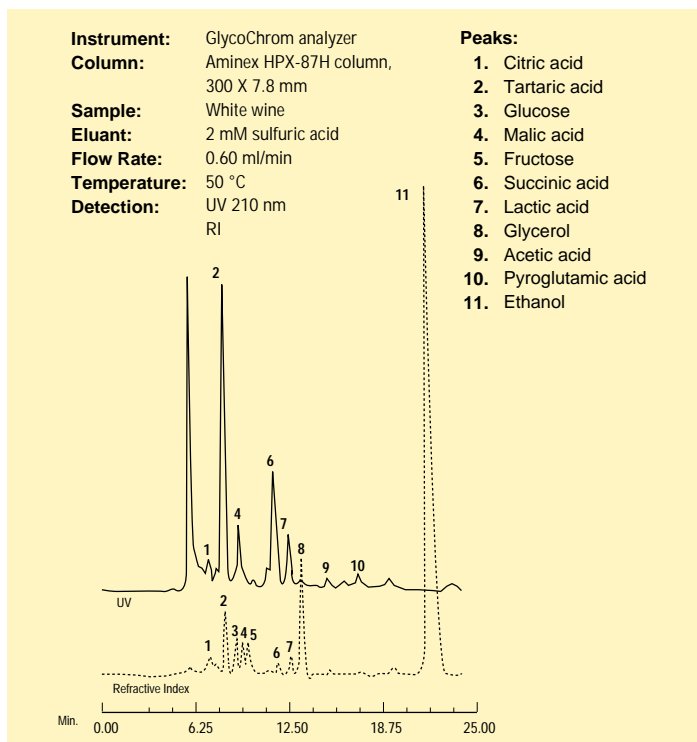


Fig. 10.9. Analysis of white wine on the Aminex HPX-87H column using dual RI and UV detection.

processing and the quality of the final product. Users of corn syrup, such as beverage industries, have put limits on the levels of HMF which are acceptable. Figure 10.10 shows a fast analysis of HMF and furfural in brandy.

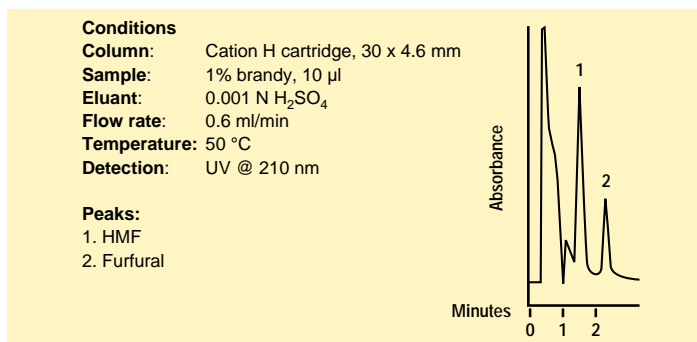


Fig. 10.10. Analysis of HMF and furfural in brandy on the Cation H cartridge.

The organic acids profile of canned foods is a useful indication of product quality and stability, as well as flavor. The organic acid analysis columns have been used to profile the organic acid content of such canned foods as sauerkraut to determine the optimal acid content for longest shelf life (Figure 10.11).

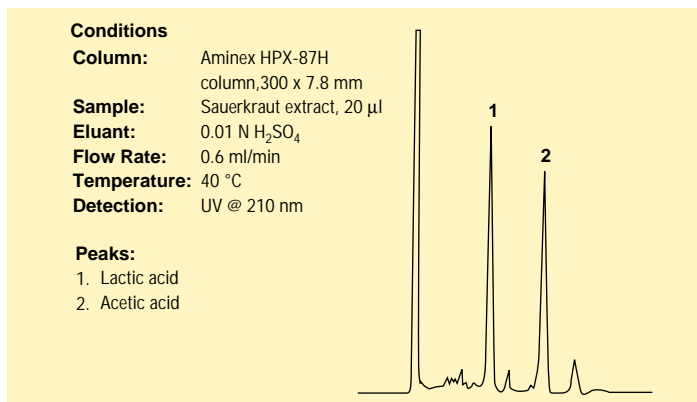


Fig. 10.11 Analysis of lactic and acetic acids in sauerkraut on the Aminex HPX-87H column.

10.4 Vitamin Content and Nutritional Quality

Vitamin C (ascorbic acid) is naturally present in many food products, and is supplementally present in fortified foods and drinks. Because vitamin C is the nutrient most affected by processing foods, the depletion of vitamin C in the final product can indicate the depletion of other nutrients. For these reasons, it is important to monitor vitamin C throughout the food processing. The Aminex HPX-87H columns provide a rapid, precise method for monitoring vitamin C in foods and beverages. Ascorbic acid can be analyzed in less than 3 minutes with the Fast Acid Analysis Column and electrochemical detection. Selective detection can permit rapid analysis of vitamin C even in complex samples. Figure 10.12 compares the results of an analysis of bean leaf on the fast acid column to that achieved on the Aminex HPX-87H column. The Aminex HPX-87H column has been used to determine ascorbic acid quantitatively in fresh and frozen fruits and vegetables, fresh and canned juices, and powdered drinks [Ashoor, S. H. and Welty, J. J., *J. Assoc. Off. Anal. Chem.*, **67**, 885 (1984)]. Graham W. D. and Annette, D. [*J. Chrom.*, **594**, 187 (1992)] have analyzed both ascorbic and dehydroascorbic acids in potatoes and strawberries using and Aminex HPX-87H column with detection at 245 nm.

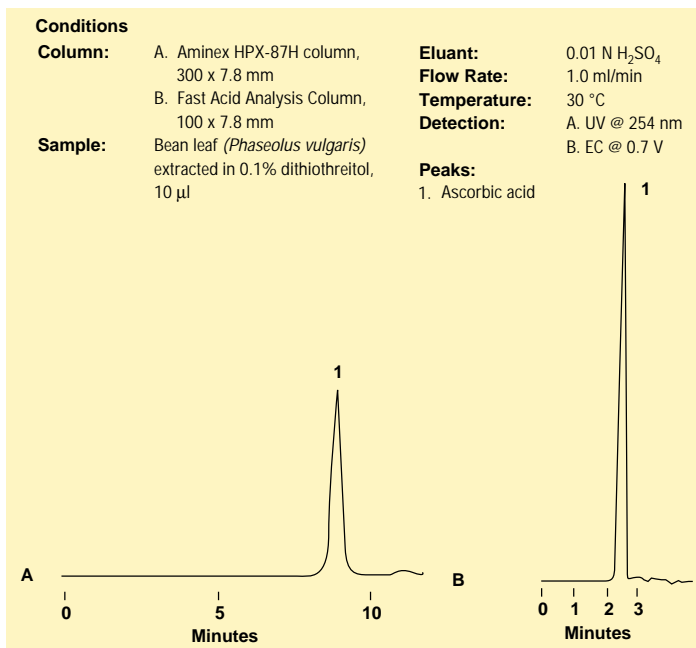


Fig. 10.12 Analysis of vitamin C in bean leaves. Courtesy of Cecelia Leung, Washington State University.

Section 11

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