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NO. 181 A CHROMATOGRAPHIC COMPARISON OF A NEW FAMILY OF PACKINGS FOR REVERSE-PHASE LIQUID CHROMATOGRAPHY

Attached is documentation of a presentation given at the 1985 PITTSBURGH CONFERENCE held in New Orleans from February 25 to March 1, 1985. The published abstract is included below.

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NO. 181 A CHROMATOGRAPHIC COMPARISON OF A NEW FAMILY OF PACKINGS FOR REVERSE-PHASE LIQUID CHROMATOGRAPHY

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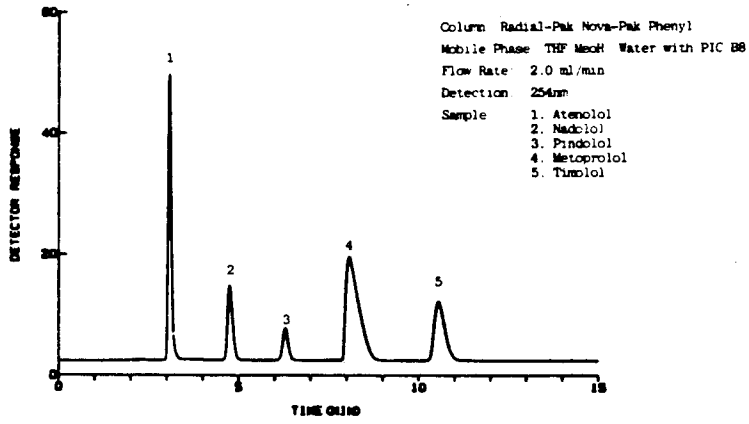
Waters' NOVA-PAK™ line of high performance packings are based on 4μ spherical silica particles, and utilize an exclusive sequential bonding process to produce a stable stationary phase with a unique surface chemistry. This line has been expanded to include two new reverse-phase packings, NOVA-PAK Phenyl and Cyano, along with NOVA-PAK C_{18}. This paper will present data from a chromatographic study of the relative retention characteristics of these packings for acidic, neutral and basic compounds. In addition separations of various classes of pharmaceutical compounds (e.g., beta blockers, see figure 1) will be optimized for each of the three NOVA-PAK packings and will be utilized to show how proper column and eluent selection can result in successful LC separations. Examples of the unique selectivity, and excellent column-to-column reproducibility of NOVA-PAK™ packings will also be shown.

Figure 1

FIVE BETA BLOCKERS



Any questions regarding the paper should be directed to the author listed at the bottom of this page. Slides are available through Marketing Communications in Milford (X2303) or directly from the photographer, Kevin Monaghan (617/528-8579).

A CHROMATOGRAPHIC COMPARISON OF A NEW FAMILY OF PACKINGS FOR REVERSE-PHASE LIQUID CHROMATOGRAPHY

Charles H. Phoebe, F. Vincent Warren, and Thomas L. Tarvin

Waters Chromatography Division
Millipore Corporation

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NOTE: Performance characteristics of NOVA-PAK Phenyl and NOVA-PAK Cyano were determined on R&D batches and mobile phase compositions may require adjustment on production batches.

NOVA–PAK™ C18

HIGH EFFICIENCY

4 micron spherical silica

BROAD SELECTIVITY

neutral, basic and acidic compounds

HIGH REPRODUCIBILITY

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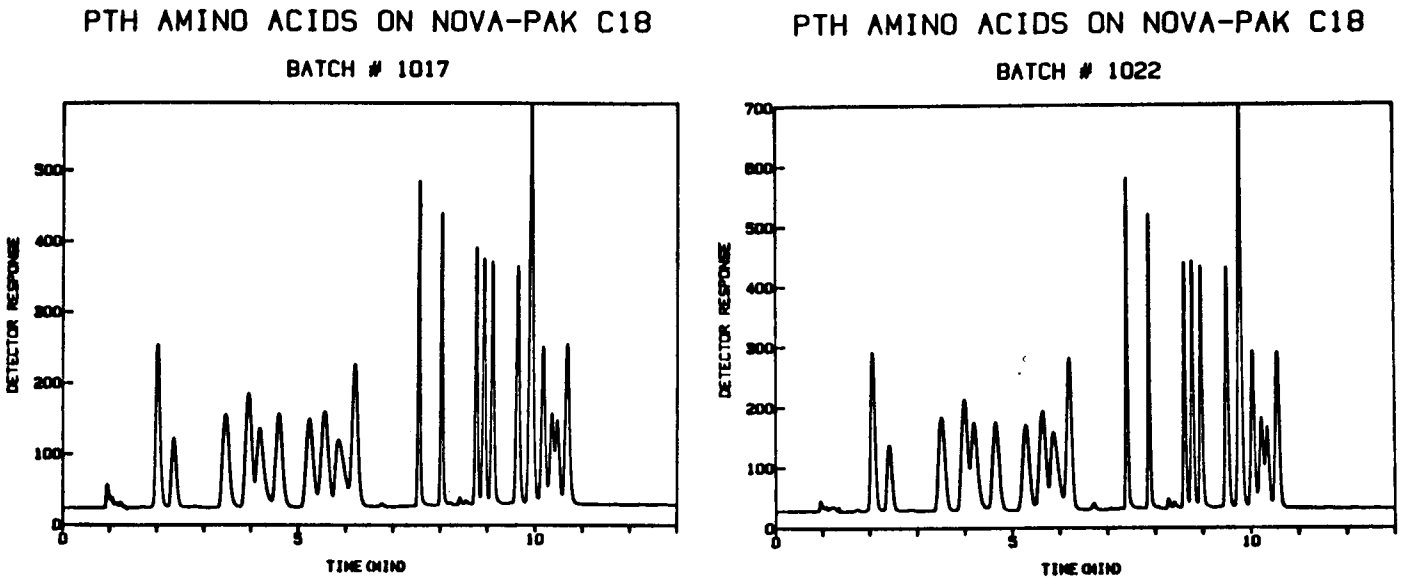
Slide 1: In 1983 Waters introduced a new C_{18} bonded-phase packing material named NOVA-PAK C_{18}. This is a high efficiency packing material based on 4 micron spherical silica. This material exhibits a broad range of selectivity being able to separate basic, acidic and neutral compounds. NOVA-PAK C_{18} is also a highly reproducible packing material since every step of the manufacturing process, from the manufacturing of the 4 micron base silica to the bonding steps, is carefully controlled and thoroughly tested.



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Slide 2: As an example of the high reproducibility of NOVA-PAK C_{18}, two batches were evaluated for their ability to perform the difficult separation of a standard mix of PTH-amino acids. The separations not only show excellent reproducibility between batches but also the broad range of selectivity for the separation of acidic, basic and neutral PTH-amino acids.

NOVA–PAK™ C18

NOVA–PAK™ PHENYL

NOVA–PAK™ CYANO

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Slide 3: In order to broaden the range of polarity and to provide the chromatographer with different selectivities, two new bonded-phase packing materials, based on the high efficiency 4 micron spherical silica (used for the manufacturing of NOVA-PAK C_{18}) have been produced. These two new bonded-phase materials, NOVA-PAK Phenyl and NOVA-PAK Cyano, combined with NOVA-PAK C_{18}, form the new family of NOVA-PAK packings.

Like NOVA-PAK C_{18}, the two new NOVA-PAK members are end-capped thereby allowing for a broad range of selectivities as the following examples of separations of neutrals, bases, and acids illustrate.

NEUTRAL COMPOUNDS

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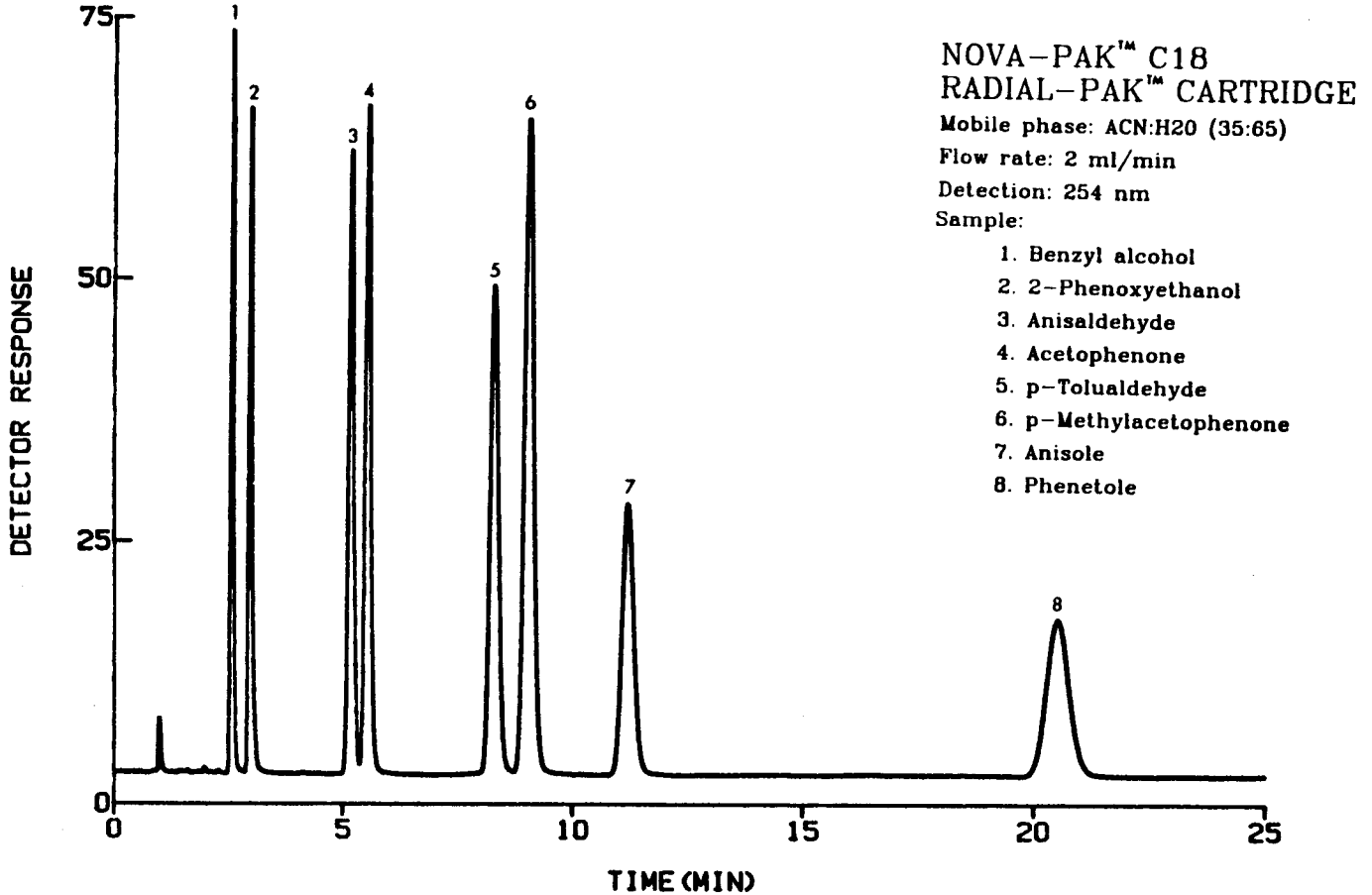
Slide 4: Neutral compounds

NOVA–PAK™ C18

AROMATIC NEUTRALS



Slide 5: A standard mix of aromatic alcohols, aldehydes, ketones, and ethers show the ability of NOVA-PAK C_{18} to separate neutral compounds of various classes.

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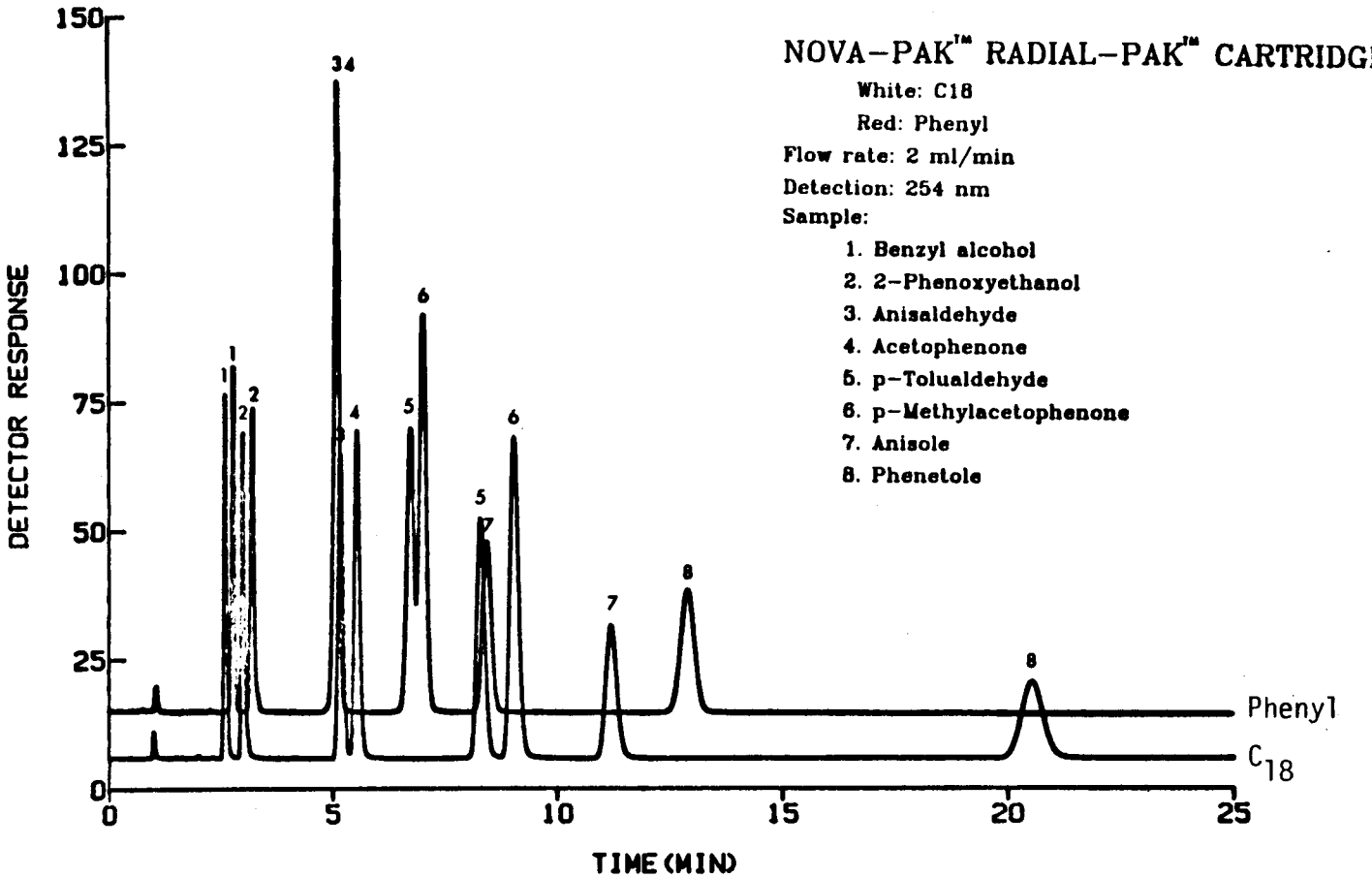
NOVA-PAK™ C18 VERSUS NOVA-PAK™ PHENYL

ACN: H20 (35: 65)



Slide 6: Using the same mobile phase (ACN:H_2O, 35:65) the separation is obtained on NOVA-PAK Phenyl. Note that the phenyl column has about 2/3 of the retention characteristics of NOVA-PAK C_{18}.

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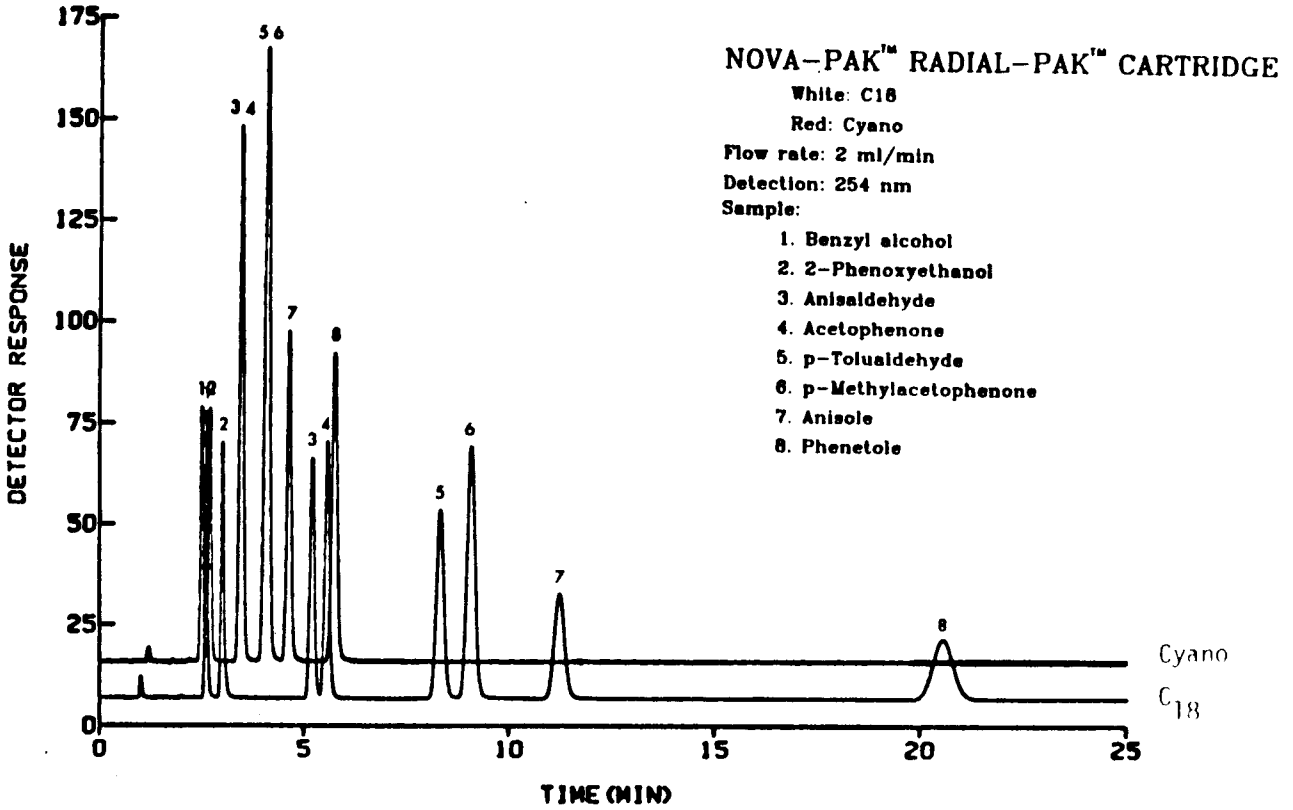
NOVA-PAK™ C18 VERSUS NOVA-PAK™ CYANO

ACN: H20 (35: 65)



Slide 7: A similar comparison with NOVA-PAK Cyano shows that the Cyano column has about 1/4 of the retention characteristics of the NOVA-PAK C_{18}.

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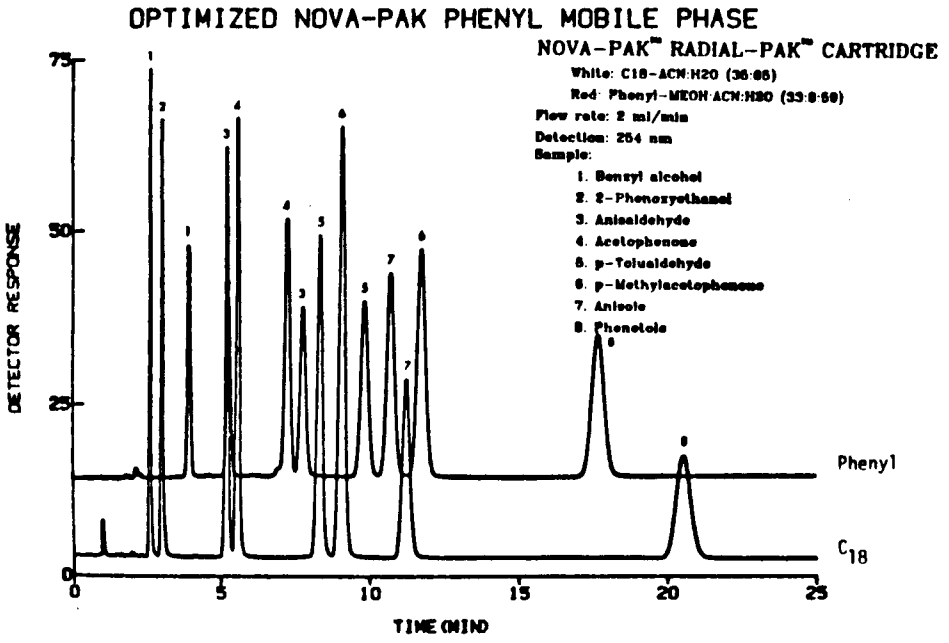
NOVA-PAK C18 VERSUS NOVA-PAK PHENYL



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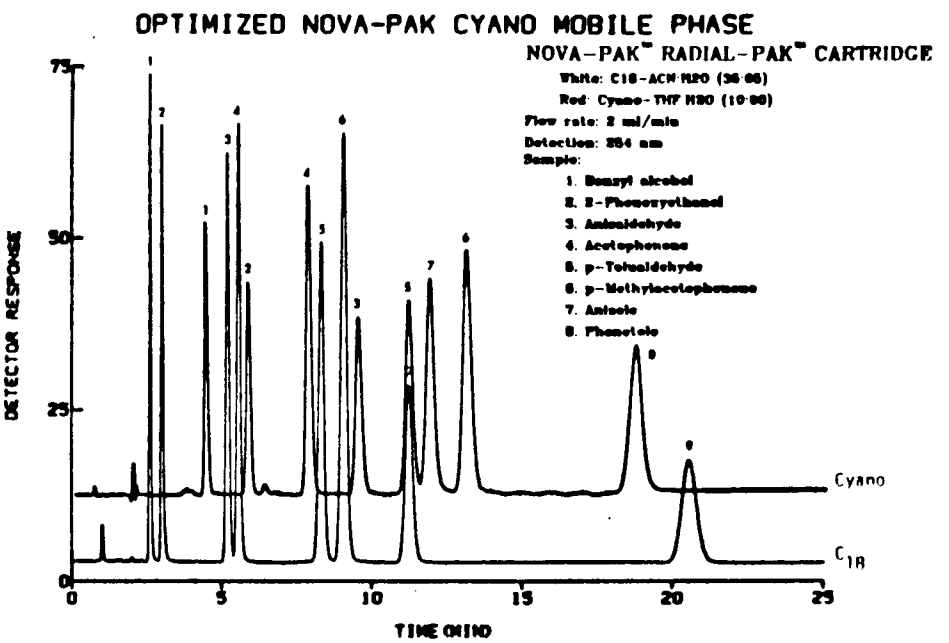
NOVA-PAK C18 VERSUS NOVA-PAK CYANO



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Slide 8 and 9: So as not to lead you to think that the NOVA-PAK Phenyl and NOVA-PAK Cyano cannot perform this separation of neutral compounds, the following two slides illustrate the separation (in comparison with NOVA-PAK C_{18}) where the mobile phase composition has been optimized for first the NOVA-PAK Phenyl and then for the NOVA-PAK Cyano. Note that all three of the packing materials are capable of performing this separation with baseline resolution of all compounds.

ACIDIC COMPOUNDS

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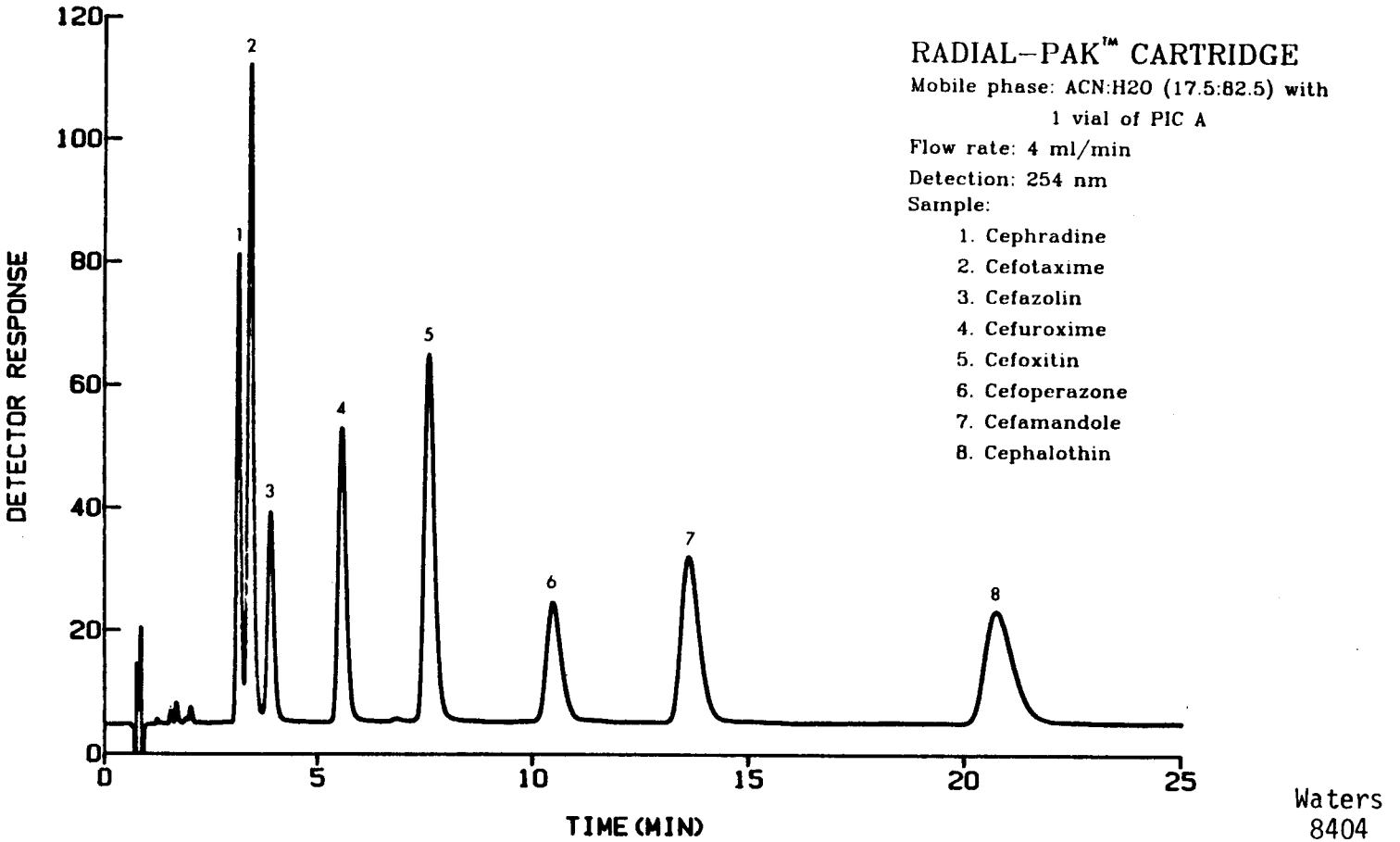
Slide 10: Acidic Compounds. The separation of eight cephalosporin antibiotics have been chosen to illustrate the ability of the NOVA-PAK family to separate acidic compounds.

NOVA–PAK™ C18

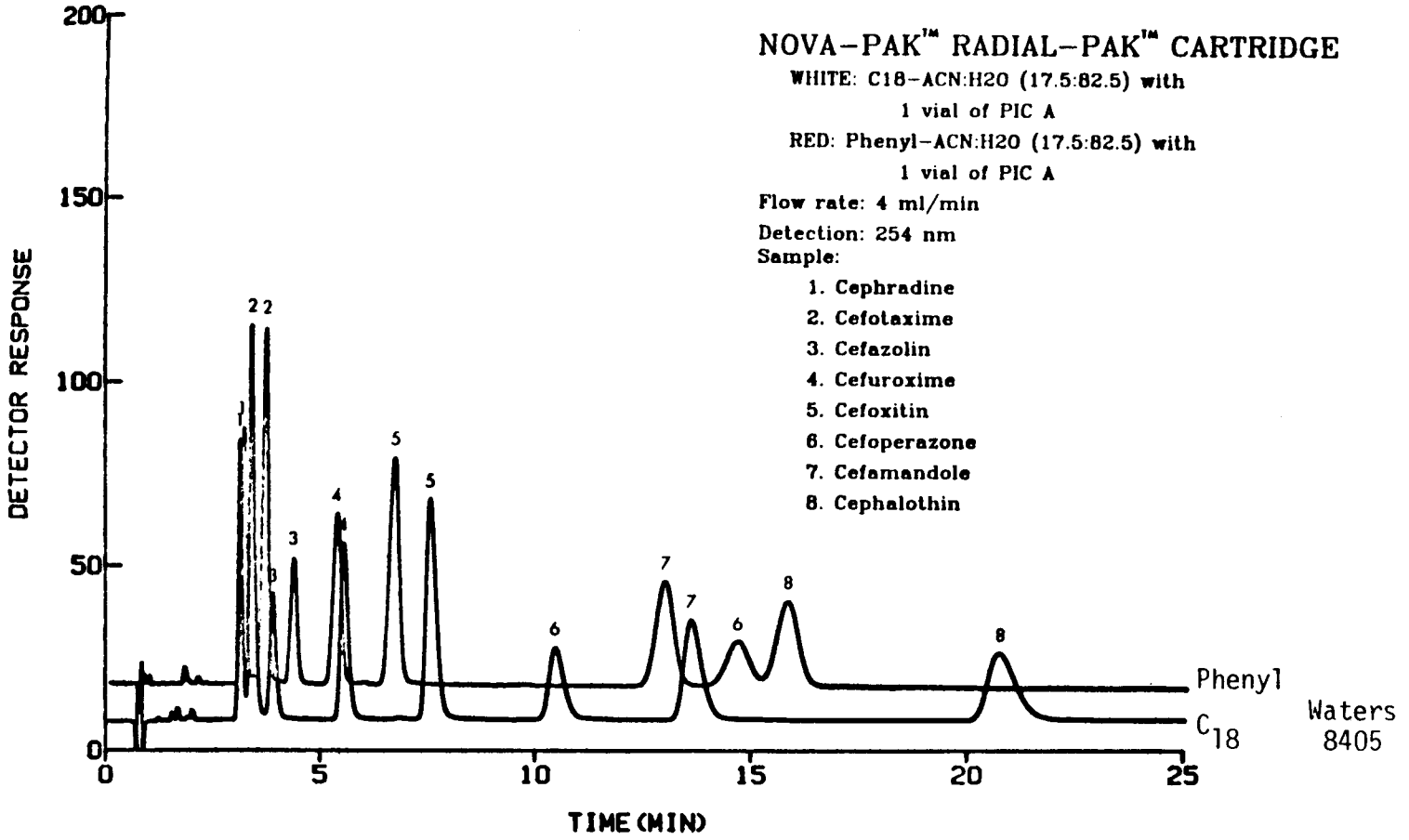
CEPHALOSPORIN ANTIBIOTICS



Slide 11: This first slide demonstrates the separation of the cephalosporin antibiotics with NOVA-PAK C_{18}.

NOVA–PAK™

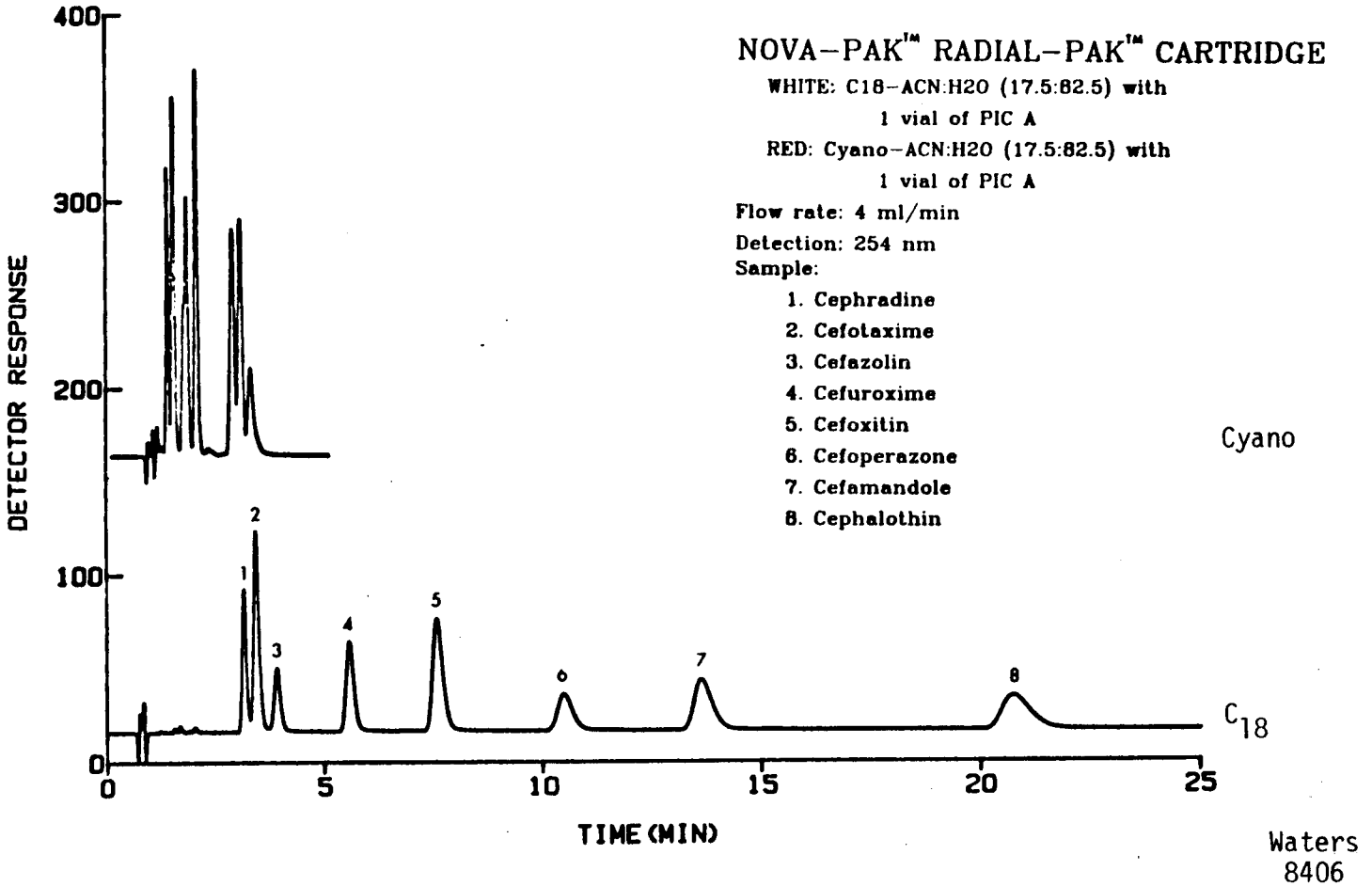
CEPHALOSPORIN ANTIBIOTICS



Slide 12: This slide shows the comparison of the separation between NOVA-PAK C_{18} and NOVA-PAK Phenyl under the same mobile phase composition. Note the baseline resolution of all compounds and the different selectivity between the C_{18} and Phenyl columns for Compounds 6, 7 and 8.

NOVA-PAK™

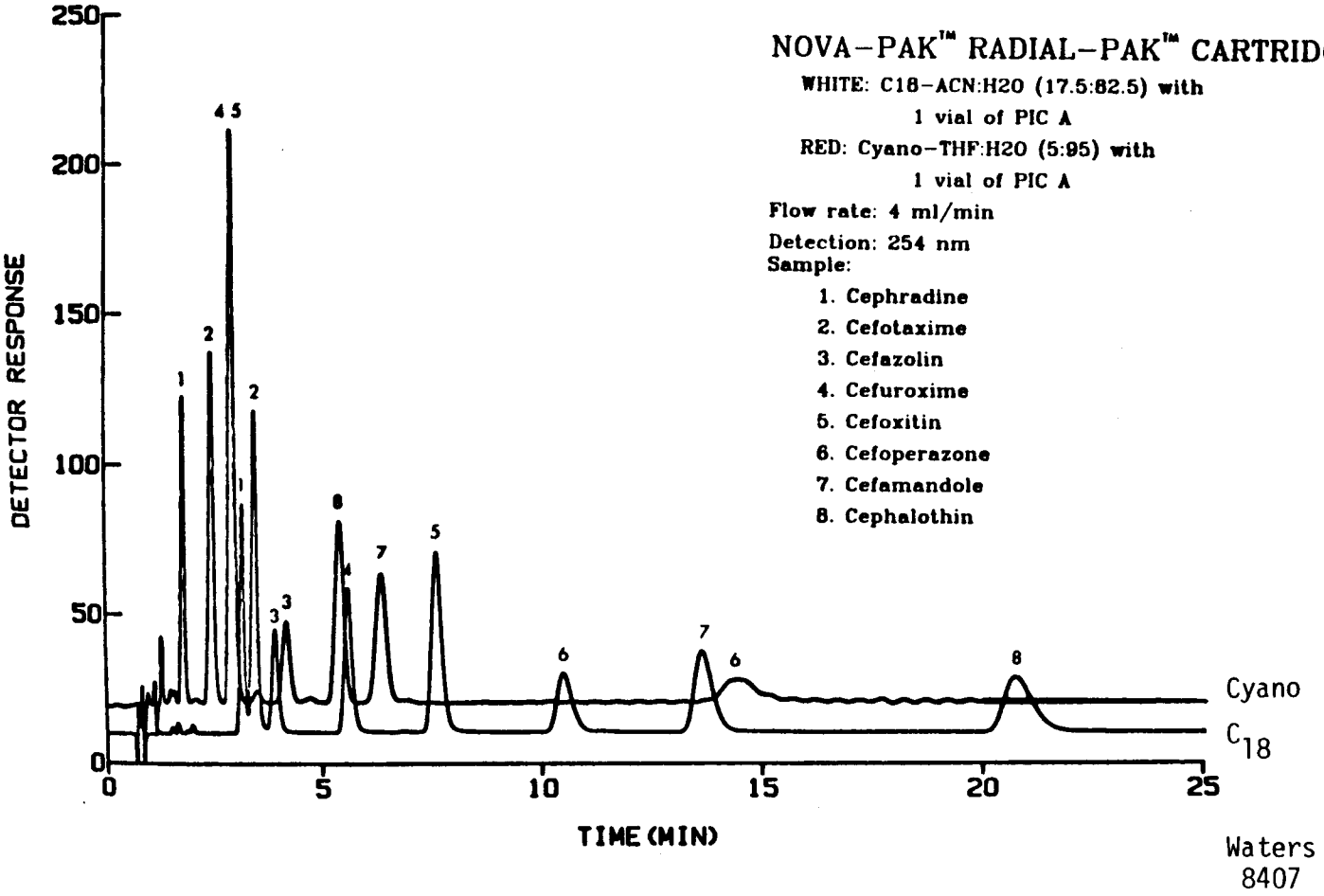
CEPHALOSPORIN ANTIBIOTICS



Slide 13: A similar comparison of NOVA-PAK C_{18} with NOVA-PAK Cyano with the same mobile phase shows a lack of retention resulting in poor resolution.

NOVA–PAK™

CEPHALOSPORIN ANTIBIOTICS



Slide 14: An optimized NOVA-PAK Cyano mobile phase now shows adequate retention, however, still results in a co-elution of compounds 4 and 5.

BASIC COMPOUNDS

Slide 15: Basic compounds. The separation of 5 Beta-adrenergic blocking agents have been chosen to illustrate the ability of the NOVA-PAK family to separate basic compounds.

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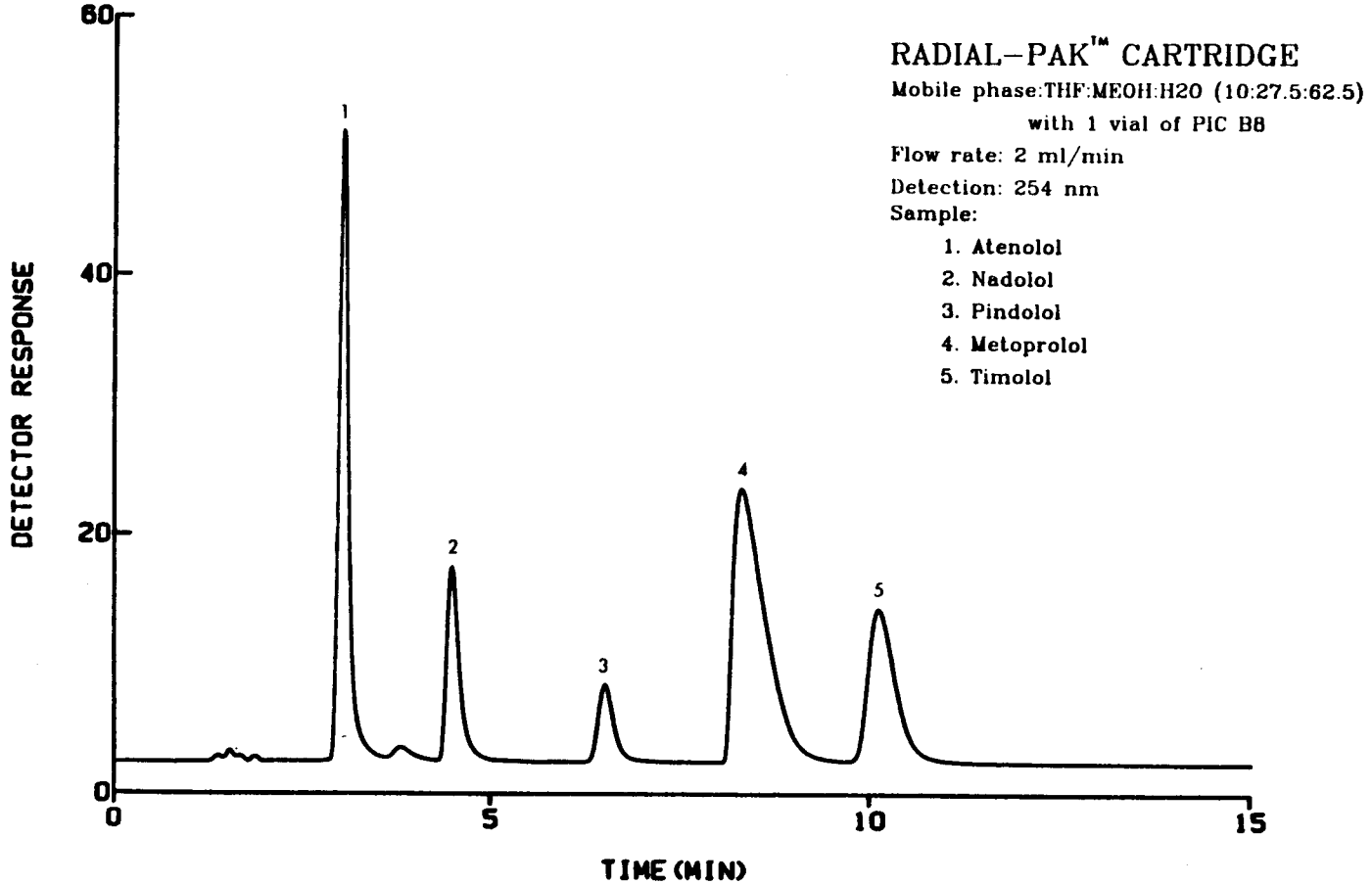
NOVA-PAK™ C18

BETA-ADRENERGIC BLOCKERS



Slide 16: The first slide illustrates the optimized mobile phase for the separation with NOVA-PAK C_{18}.

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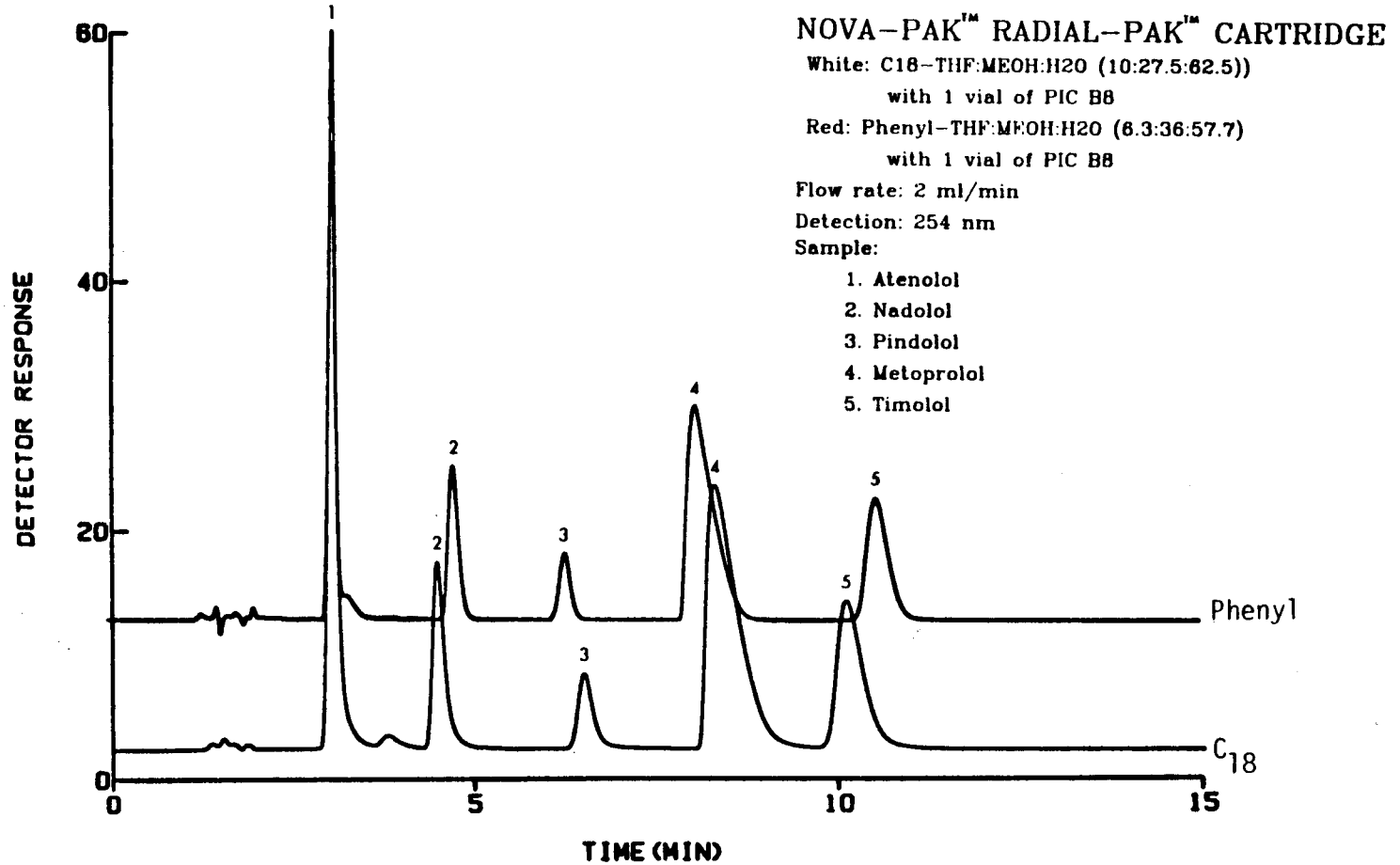
NOVA-PAK C18 VERSUS NOVA-PAK PHENYL

BETA-ADRENERGIC BLOCKERS



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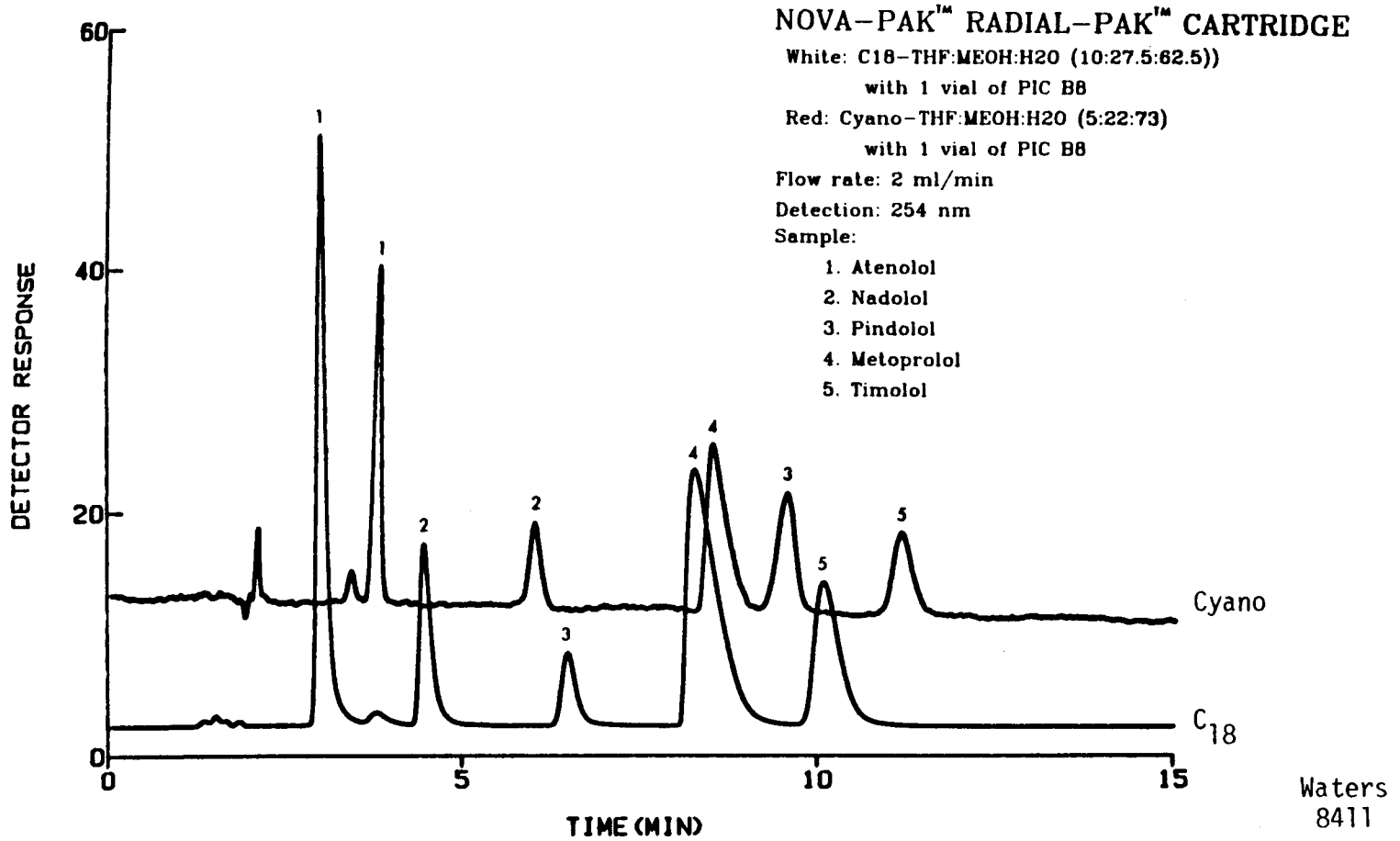
Slide 17: A comparison of the separation with the optimized mobile phases for both NOVA-PAK Phenyl and NOVA-PAK C_{18}.

NOVA-PAK C18 VERSUS NOVA-PAK CYANO

BETA-ADRENERGIC BLOCKERS



Slide 18: A comparison of the separation with optimized mobile phases for both NOVA-PAK Cyano and NOVA-PAK C_{18}.

SUMMARY

NOVA–PAK™ FAMILY

BROAD RANGE OF POLARITY

BROAD SELECTIVITY

HIGH EFFICIENCY

REPRODUCIBILITY

COST EFFECTIVE

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Slide 19: NOVA-PAK family provides the chromatographer with a broad range of polarities (note the neutral and acid separations) and broad selectivities being able to separate acidic, basic, and neutral compounds.

Since the new bonded-phase materials are based on the same 4 micron spherical silica as used for NOVA-PAK C_{18}, the whole family demonstrates high efficiency.

The new bonded-phase materials will be tested as thoroughly as the NOVA-PAK C_{18} and, therefore, will give the same high reproducibility. Finally this family demonstrates that the proper choice of a bonded-phase packing can result in equal resolution and different selectivities with decreased organic mobile phase cost and an overall decreased cost of analysis.

A CHROMATOGRAPHIC COMPARISON OF A NEW FAMILY OF PACKINGS FOR REVERSE-PHASE LIQUID CHROMATOGRAPHY

Charles H. Phoebe, F. Vincent Warren,
and Thomas L. Tarvin

Waters Chromatography Division
Millipore Corporation

[This paper describes the use of experimental water-compatible GPC columns. While these or similar columns may become products, no time has been as yet set for their introduction.]

This paper will review the various hplc methodologies for analysis of corn starch hydrolysis products, with particular attention to the use of high resolution gel permeation chromatography to analyze low dextrose equivalence corn syrups.

HPLC Characterization of Glucose Oligomer Distribution in Corn Starch Hydrolyzates

Craig A. Dorschel
W. Roy Day
Herman S. Schultz
Peter G. Alden

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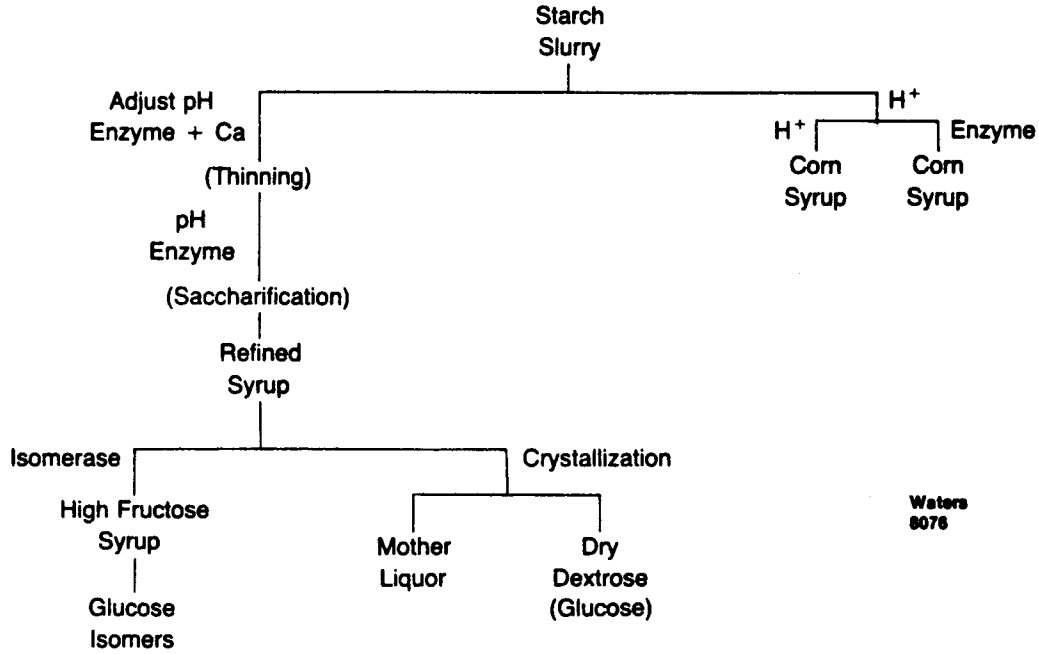
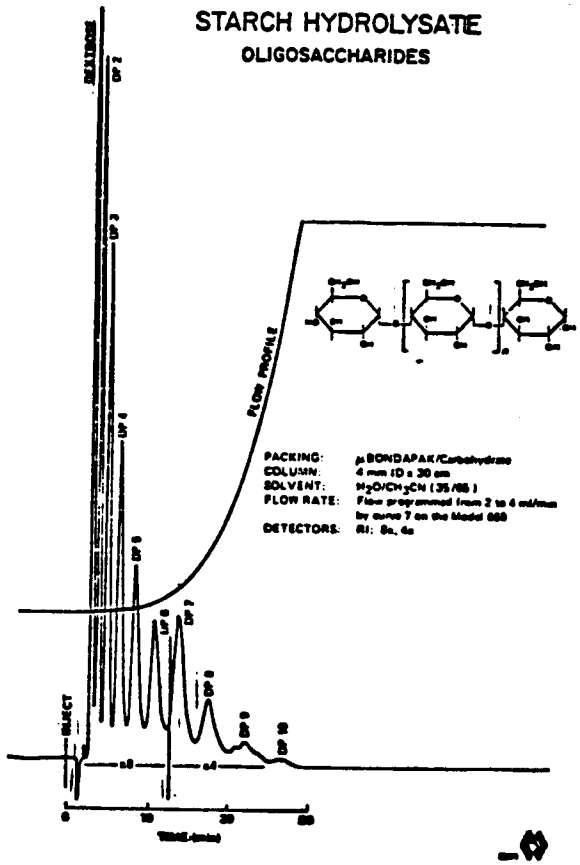
Waters Chromatography Division
Millipore Corporation

This slide gives a simplified outline of the starch hydrolysis process. Corn starch is hydrolyzed in a two stage process. The left branch of the flow chart shows an exhaustive two-stage enzyme hydrolysis leading to a refined syrup from which pure glucose (dextrose) may be crystallized or which may be further converted to high fructose corn syrup, which is widely employed as a sweetener. The right hand branch shows an initial acid hydrolysis followed by either a second acid hydrolysis or an enzyme hydrolysis. Such procedures are used to produce syrups of varying degrees of hydrolysis, expressed as dextrose equivalence (DE). A product having a low DE will be only slightly hydrolyzed and vice versa. Low DE syrups are widely employed in food processing to provide viscosity and "body" to finished products.

Starch Hydrolysis



The first hplc method for analysis of glucose oligomers used normal phase chromatography on a bonded amino phase column, such as the Waters Carbohydrate Analysis Column, and a water/acetonitrile mobile phase. Using a flow program, it is possible to see oligomers up to DP (degree of polymerization) 10.



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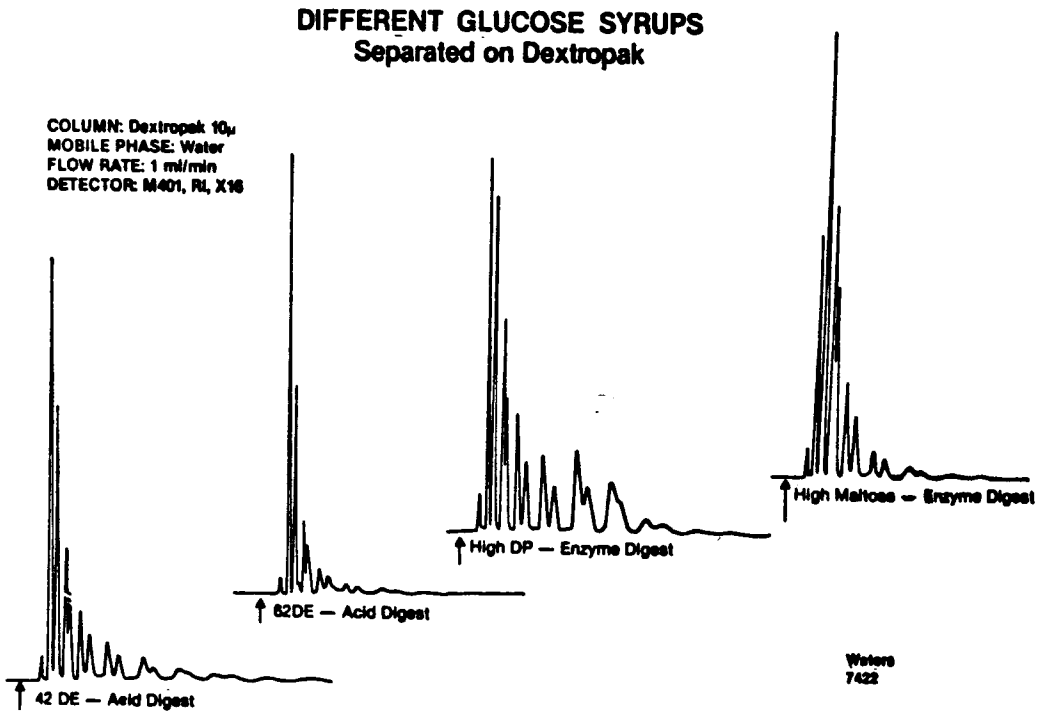
A similar-looking chromatogram may be obtained in the reversed-phase mode using the Waters Dextro-PAK Radial-PAK tm cartridge. Here the mobile phase is pure water, and no flow programming is required. An interesting aspect of chromatography on the Dextro-PAK cartridge is the partial resolution of the two anomeric forms of several of the oligomers. This column, like the Carbohydrate Analysis Column, retains oligmomers of DP > 10, which is disadvantageous in analyzing low DE syrups.



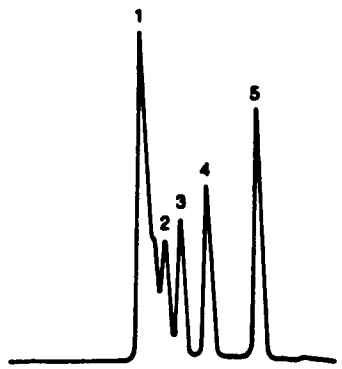
Perhaps the most widely used type of column for carbohydrate analysis today is a calcium-loaded cation exchange resin column having a small mean pore size, such as the Sugar-PAK tm I column. This type of column separates carbohydrates by a complex mechanism involving size exclusion, reversed-phase, and ion affinity effects. The resolution of oligomers is essentially all due to size exclusion effects, as is seen in this chromatogram of a 42 DE syrup. As expected, the high molecular weight material elutes first. Note that because of the small mean pore size of the packing material, anything in the sample larger than DP 5 is totally excluded from the pore volume and appears in a single large peak at the beginning of the chromatogram. All information concerning the distribution of the higher oligomers is thus hidden. Nevertheless, this type of column is quite useful in monitoring processes or end products where the aim is to compare the quantity of glucose and maltose to the remainder of the sample.

42 DE Corn Syrup

Sugar-PAK™ I Column

SAMPLE: 2% 42 DE Syrup
INJECTION VOLUME: 20 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 90°C
DETECTOR: RI 32X

1. DP5+
2. DP4
3. DP3
4. Maltose
5. Glucose



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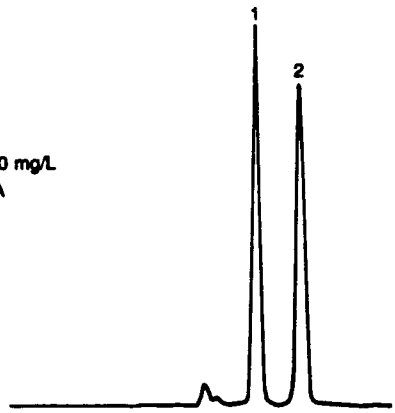
Another application where columns such as Sugar-PAK I excell is separation of monosaccharides. In this chromatogram of high fructose corn syrup we see excellent resolution of glucose and fructose, where the size exclusion effect is enhanced by interaction of the sugars with the calcium ion. The presence of a small amount of oligosaccharide may be noted.

High Fructose Corn Syrup

Sugar-PAK™ I Column

SAMPLE: 2% High Fructose Corn Syrup
INJECTION VOLUME: 20 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 90°C
DETECTOR: RI 32X

1. Glucose
2. Fructose



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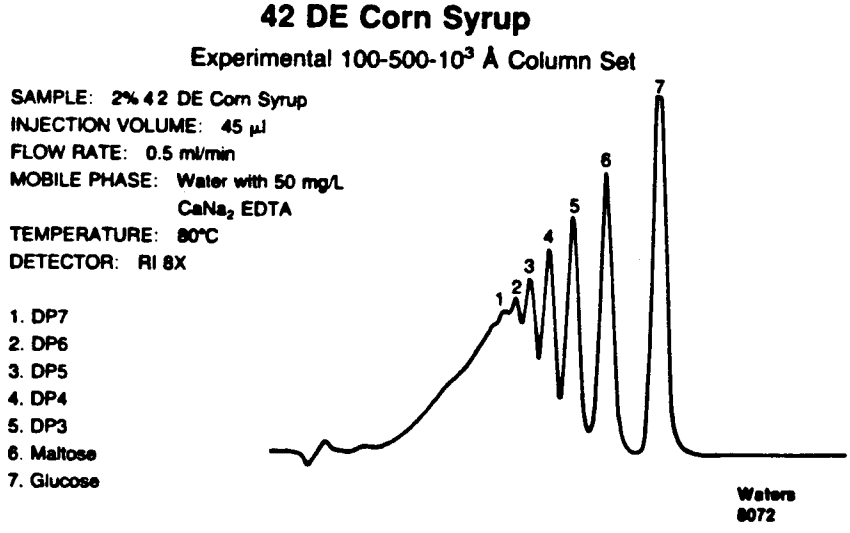
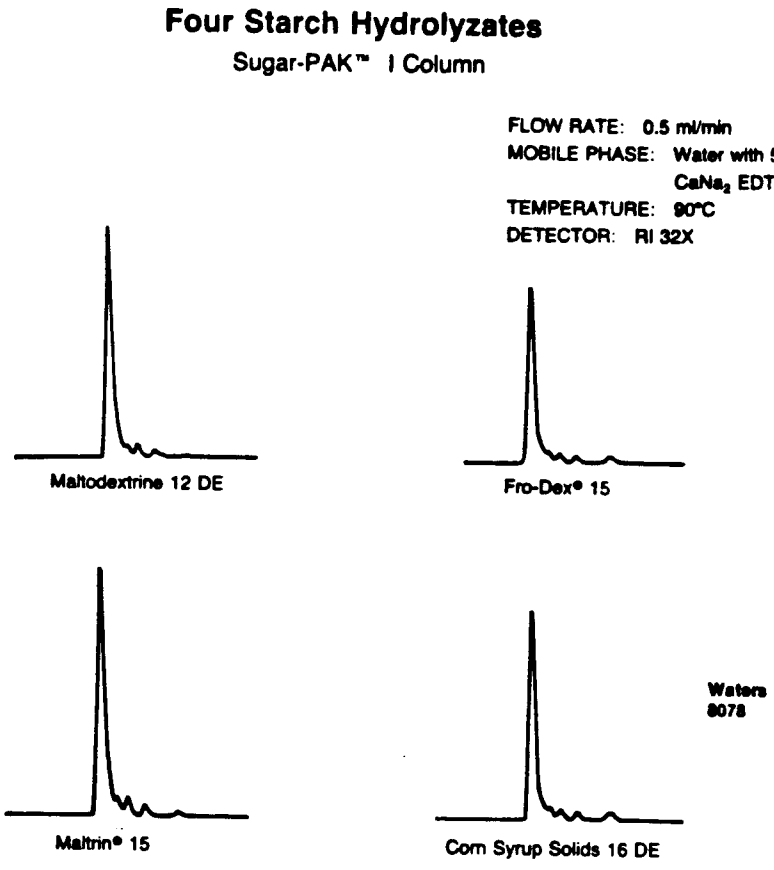
Here is another chromatogram of the same 42 DE syrup, this time run on a set of experimental high resolution gel permeation columns. The chemistry of these columns is similar to that of the Sugar-PAK I column, but the mean pore sizes of the columns - 100, 500 and 1000 angstroms (as expressed by the extended polystyrene chain convention) are all larger than that of the Sugar-PAK I. Glucose and maltose are still sufficiently resolved from the remaining material to be quantitated. In this instance, however, some degree of resolution is seen through DP 7, after which a smooth curve (representing the distribution of the higher oligomers) tails off to baseline. No peak is seen at the exclusion limit of the set, marked here by the slight baseline perturbation. The remainder of the paper illustrates how these columns show differences between four low DE samples that are not apparent using other methods.



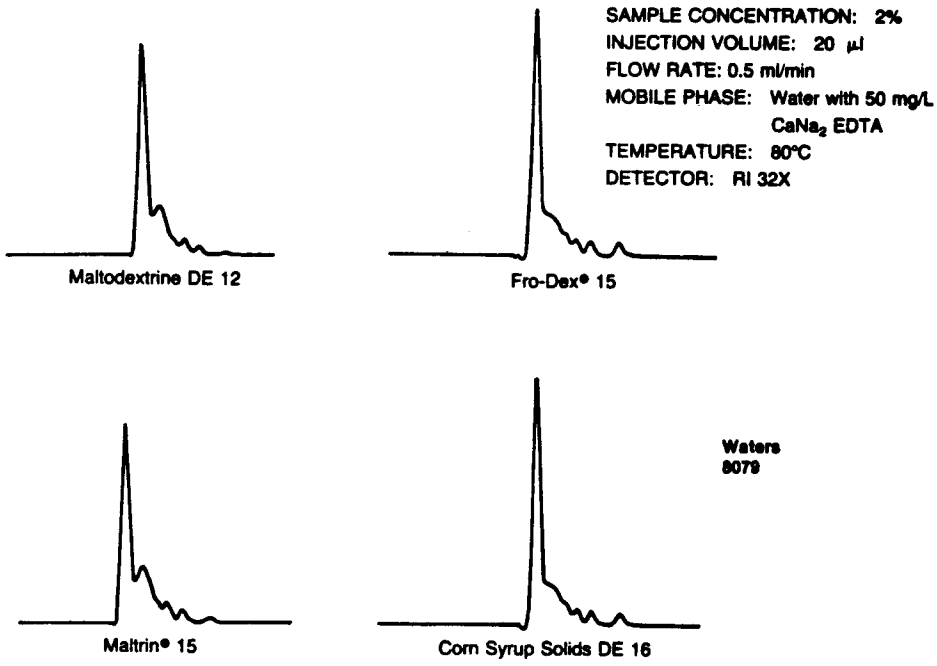
This slide shows the four samples - Maltodextrins 12 DE , Maltrin® 15, Fro-Dex® 15, and corn syrup solids 16 DE - chromatographed on a Sugar-PAK I column. Because of the low degree of hydrolysis, most of the sample elutes in the exclusion limit peak in all four cases. Other than observing the small differences in the levels of lower oligomers, no real distinction may be made between these samples.



Increasing the mean pore size by switching to an experimental 100 Å column gives the results seen in this slide. With some additional oligomers separated from the exclusion peak, the Maltodextrine and Maltrin 15 are seen to be similar, but differentiated from Fro-Dex 15 and the 16 DE solids, which again resemble each other. The members of each pair still cannot be distinguished from one another.

Four Starch Hydrolyzates

Experimental 100 Å GPC Column



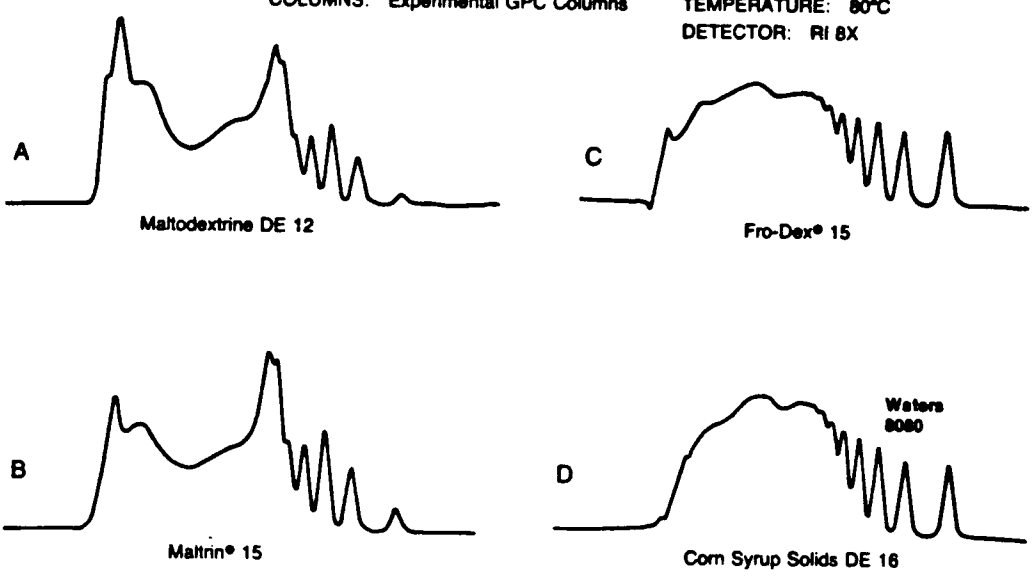
The power of high resolution GPC is evident in this slide. Maltodextrine and Maltrin 15 may be readily distinguished both from each other and from the other pair. The prominence of oligomers in the DP 5 - DP 8 range are indicative of an acid/enzyme process. The more uniform distribution of oligomers is typical of an acid/acid process in this DE range. The differences between Fro-Dex 15 and the 16 DE solids are subtle but are seen by overlaying chromatograms.

Four Starch Hydrolyzates

100-500-10^3 Å Column Set

SAMPLE CONCENTRATION: 2%
INJECTION VOLUME: A,B: 45 µl
C,D: 50 µl
COLUMNS: Experimental GPC Columns

FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 80°C
DETECTOR: RI 8X



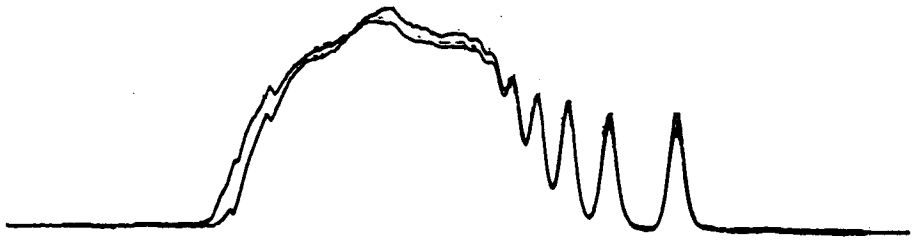
[The actual slide uses two colors for the chromatograms for clarity and has more information on operating conditions. On this copy the Fro-Dex tracing is the upper line on the left (high molecular weight) side and the lower line on the right.]

The differences between the samples may now be clearly seen. This example shows the concept of using GPC for process control and batch qualification. By comparison of chromatograms of successful and unsuccessful batches a manufacturer or user of corn syrups can develop a data base and thus be more in control of processing or confident in choice of lots and setting specifications for vendors. This is exactly what a plastics manufacturer or fabricator can do.

Fro-Dex® 15 vs. Corn Syrup Solids 16 DE

Experimental 100-500-10^3 Å GPC Column St

SAMPLE CONCENTRATION: 2%



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By substituting a second 500 Å column for the 100 Å column, the chromatograms on this slide are obtained. While sacrificing a bit of resolution in the very low DP range, additional resolution is seen in the DP 10 range.

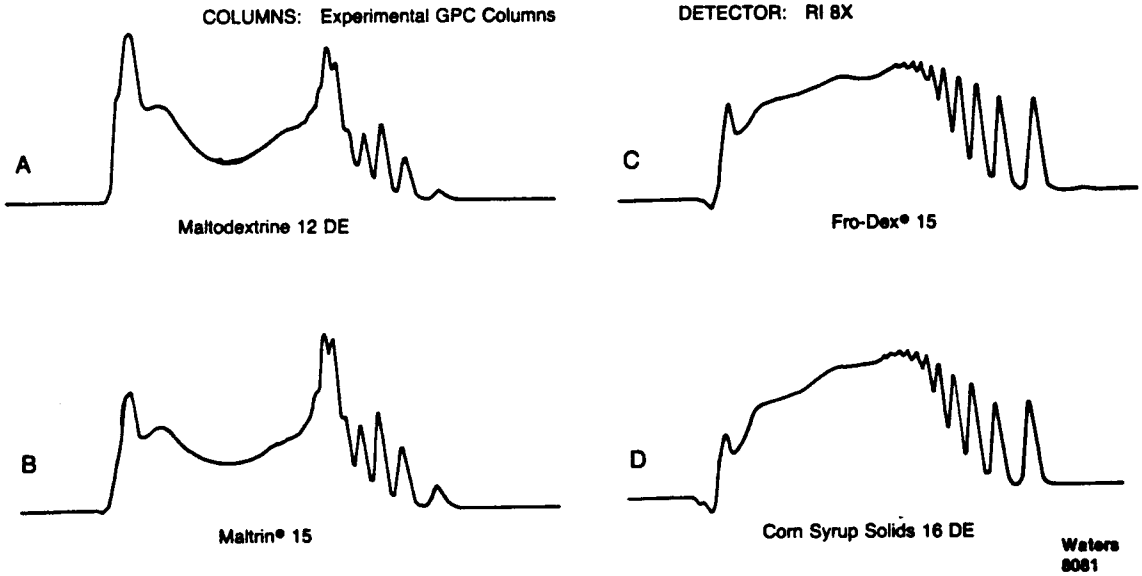
The summary point is that high resolution GPC may be seen to be the method of choice to maximize the knowledge of the nature of a sample of a corn starch hydrolyzate and shows great promise as a way to simply and reliably obtain information for batch qualification.

Four Starch Hydrolyzates

500-500-10^3 Å Column Set

SAMPLE CONCENTRATION: 2%
INJECTION VOLUME: A,B: 45µl
C,D: 60 µl
COLUMNS: Experimental GPC Columns

FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 80°C
DETECTOR: RI 8X



Dr. Jerry King provided the samples of the Maltodextrine and 16 DE solids, as well as several other samples included in the study which time did not permit inclusion in this paper.

Acknowledgement

Dr. J.W. King
CPC International
Argo, Illinois

HPLC
Characterization of
Glucose Oligomer Distribution in
Corn Starch Hydrolyzates

Craig A. Dorschel
W. Roy Day
Herman S. Schultz
Peter G. Alden

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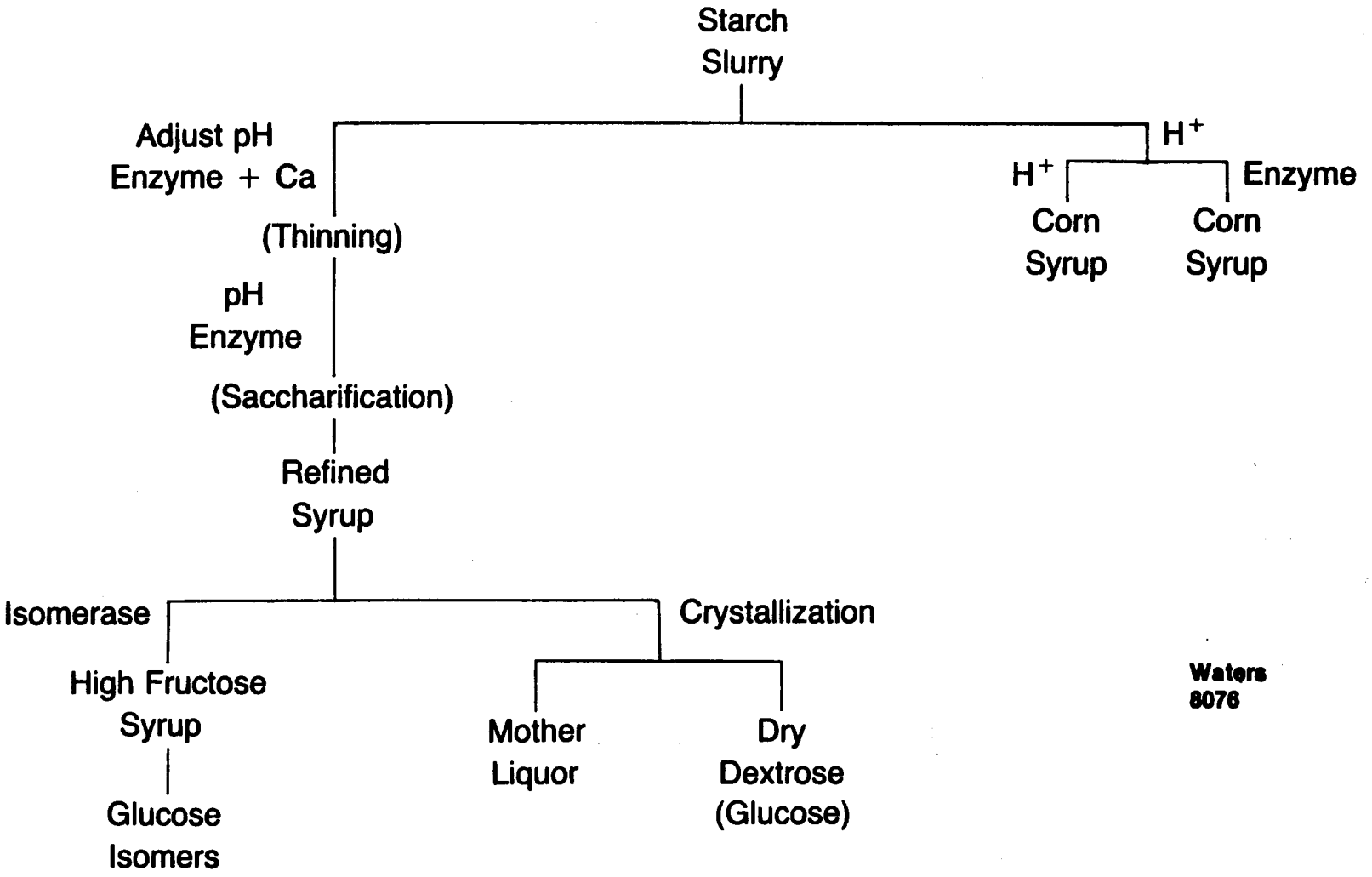
Waters Chromatography Division
Millipore Corporation

Starch Hydrolysis



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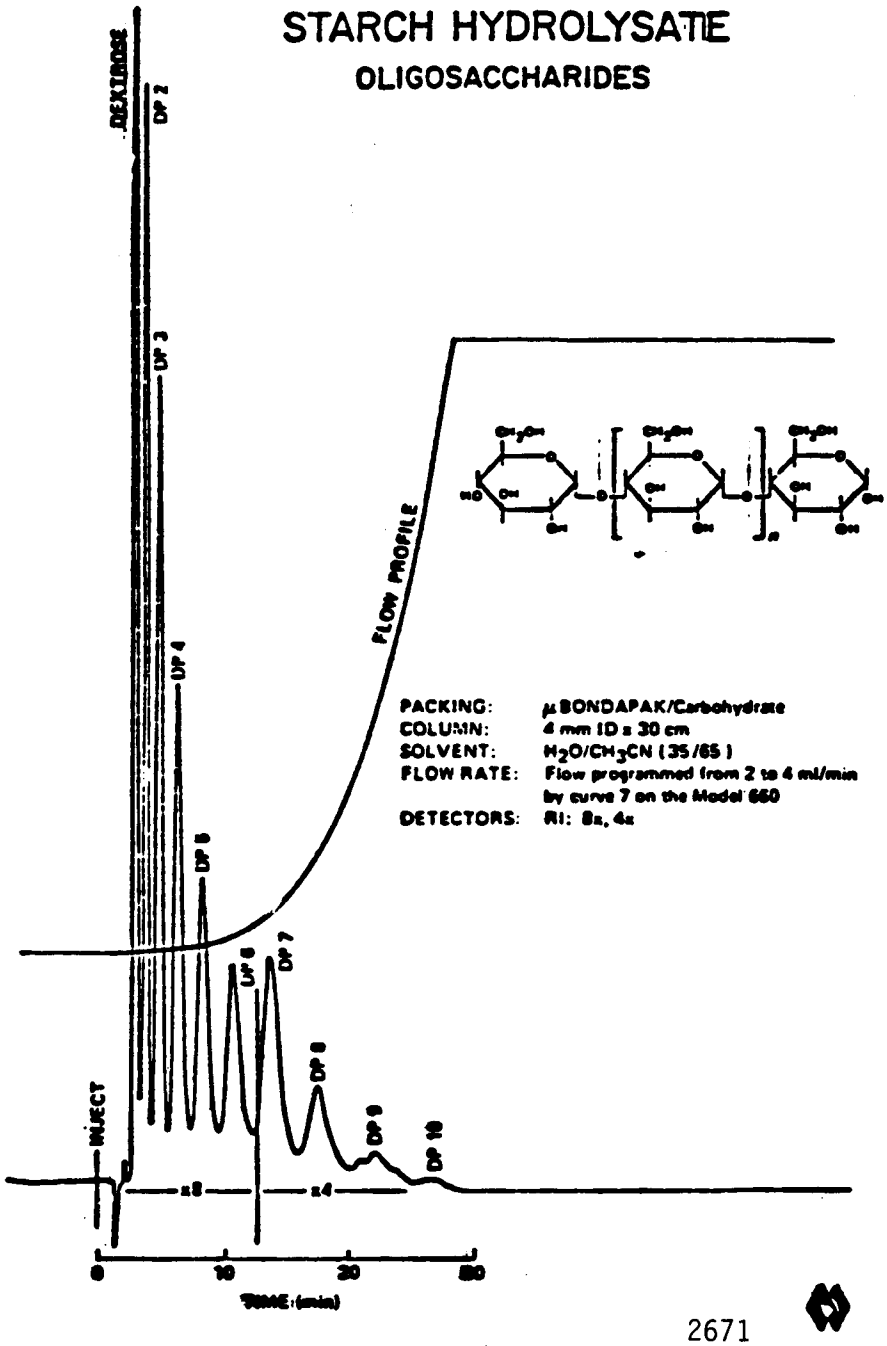
STARCH HYDROLYSATE

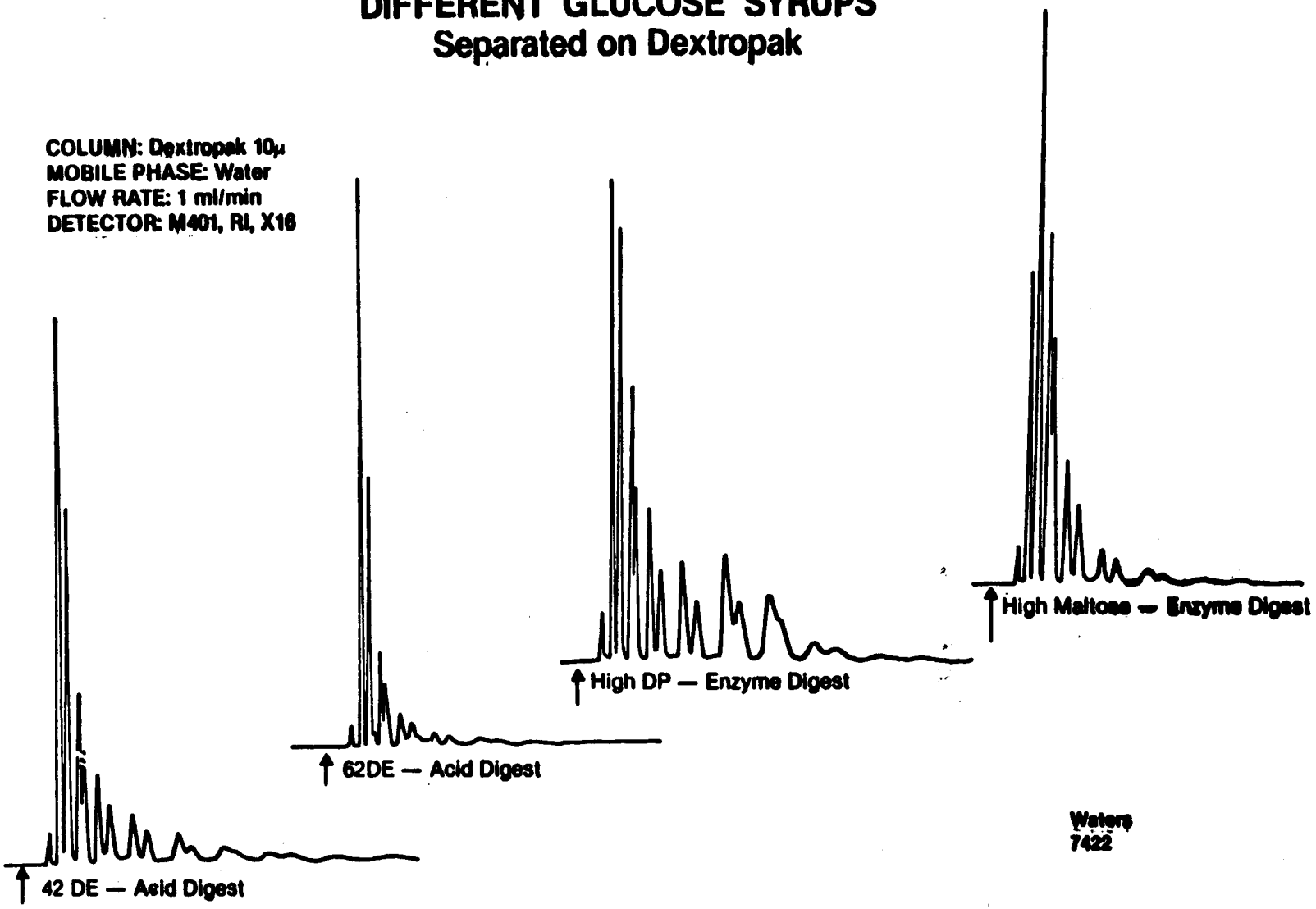
OLIGOSACCHARIDES



PACKING: μBONDAPAK/Carbohydrate
COLUMN: 4 mm ID x 30 cm
SOLVENT: H_2O/CH_3CN (35/65)
FLOW RATE: Flow programmed from 2 to 4 ml/min by curve 7 on the Model 660
DETECTORS: RI: 8x, 4x

2671

DIFFERENT GLUCOSE SYRUPS
Separated on Dextropak

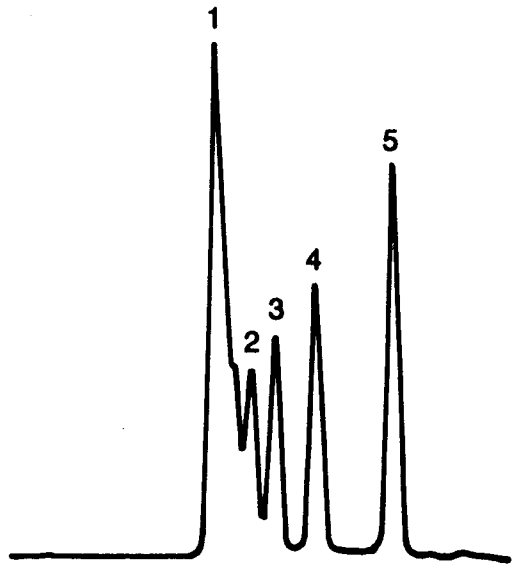


42 DE Corn Syrup

Sugar-PAK™ I Column

SAMPLE: 2% 42 DE Syrup
INJECTION VOLUME: 20 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 90°C
DETECTOR: RI 32X

1. $DP5^+$
2. DP4
3. DP3
4. Maltose
5. Glucose



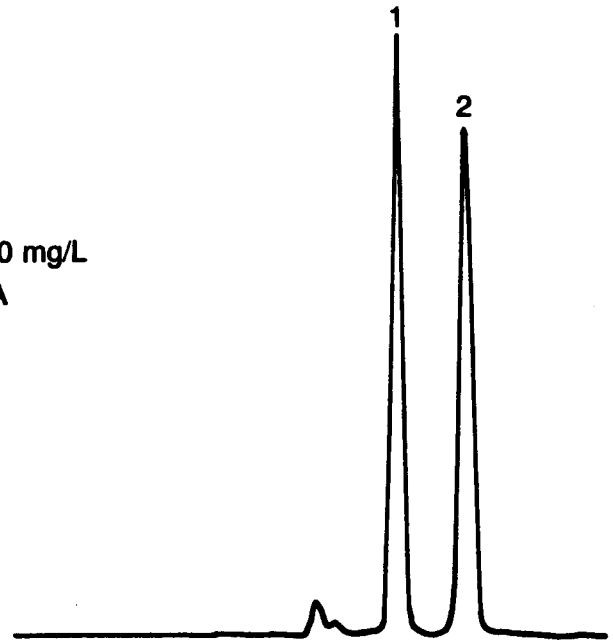
Waters
8073

High Fructose Corn Syrup

Sugar-PAK™ I Column

SAMPLE: 2% High Fructose Corn Syrup
INJECTION VOLUME: 20 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 90°C
DETECTOR: RI 32X

1. Glucose
2. Fructose



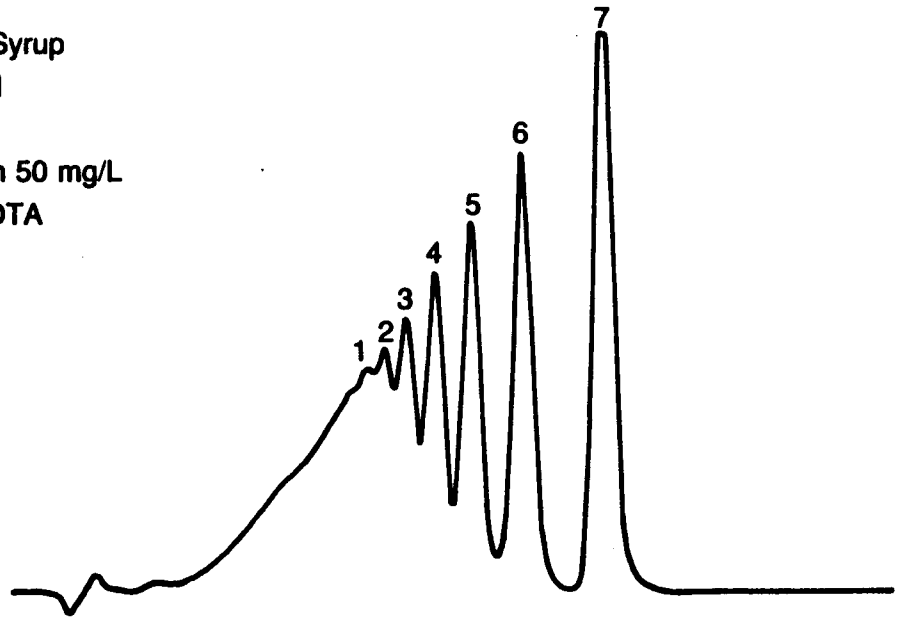
Waters
8077

42 DE Corn Syrup

Experimental 100-500-10^3 Å Column Set

SAMPLE: 2% 42 DE Corn Syrup
INJECTION VOLUME: 45 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 80°C
DETECTOR: RI 8X

1. DP7
2. DP6
3. DP5
4. DP4
5. DP3
6. Maltose
7. Glucose

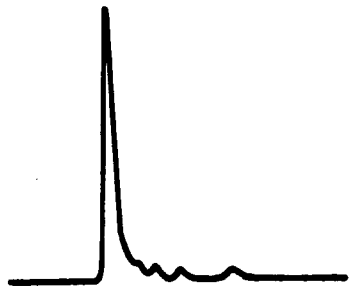


Four Starch Hydrolyzates

Sugar-PAK™ I Column

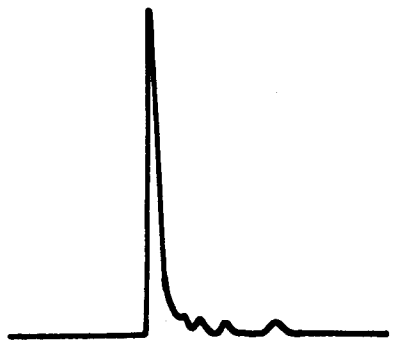
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 90°C
DETECTOR: RI 32X

Maltodextrine 12 DE



Fro-Dex® 15

Maltrin® 15

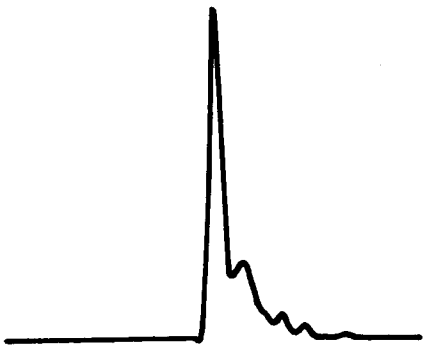


Corn Syrup Solids 16 DE

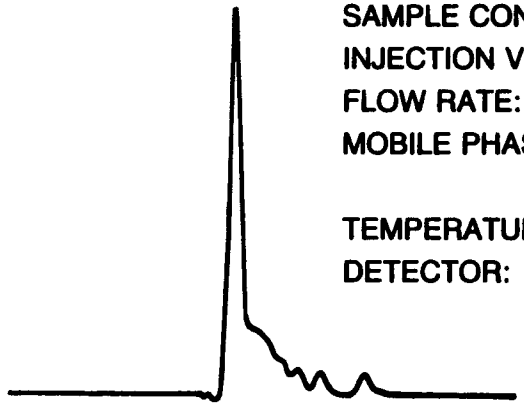
Waters
8078

Four Starch Hydrolyzates

Experimental 100 Å GPC Column

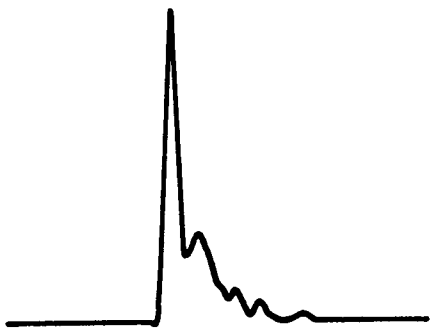


Maltodextrine DE 12

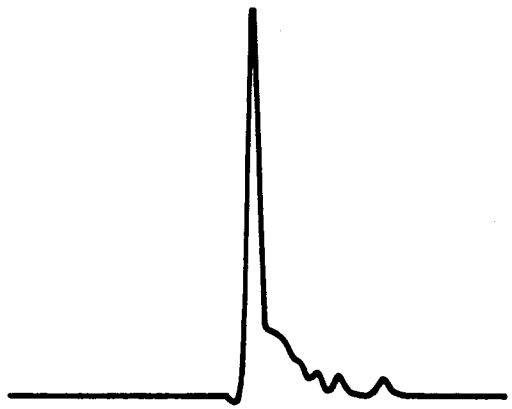


Fro-Dex® 15

SAMPLE CONCENTRATION: 2%
INJECTION VOLUME: 20 µl
FLOW RATE: 0.5 ml/min
MOBILE PHASE: Water with 50 mg/L $CaNa_2$ EDTA
TEMPERATURE: 80°C
DETECTOR: RI 32X



Maltrin® 15



Corn Syrup Solids DE 16

Waters
8079