The Determination of Carbohydrates, Alcohols, and Glycols in Fermentation Broths

Valoran Hanko and Jeffrey S. Rohrer
Thermo Fisher Scientific, Sunnyvale, CA, USA

Application Note 122

Introduction

Fermentation broths are used in the manufacture of biotherapeutics and many other biological materials produced using recombinant genetic technology, as well as for the production of methanol and ethanol as alternative energy sources to fossil fuels.

Recently, attention has been given to characterizing the ingredients of fermentation broths because carbon sources and metabolic by-products have been found to impact the yield of the desired products. Carbohydrates (glucose, lactose, sucrose, maltose, etc.) are carbon sources essential for cell growth and product synthesis, while alcohols (ethanol, methanol, sugar alcohols, etc.), glycols (glycerol), and organic anions (acetate, lactate, formate, etc.) are metabolic by-products that often reduce yields.

Fermentation broths are complex mixtures of nutrients, waste products, cells, cell debris, and desired products. Many of these ingredients are nonchromophoric and cannot be detected by absorbance. Carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds can be oxidized and detected by amperometry. This detection method is specific for analytes that can be oxidized at a selected potential, leaving all other compounds undetected.

Pulsed amperometric detection (PAD) is a powerful detection technique with a broad linear range and very low detection limits. High-performance anion-exchange chromatography (HPAE) is capable of separating complex mixtures of carbohydrates. For complex samples such as fermentation broths, the high resolving power of HPAE and the specificity of PAD allow the determination of carbohydrates, glycols, sugar alcohols (alditols), and other alcohols such as ethanol and methanol, with little interference from other broth ingredients.[1–3] Although biosensor and flow-injection analyzer-based methods are commonly used to evaluate fermentation broths, these techniques cannot simultaneously determine multiple compounds.[4,5] Refractive index detection has been used for the analysis of fermentation broths, but is limited by poor sensitivity and selectivity.[6,7] Postcolumn derivatization with UV/Vis detection has also been used, but is complicated by the additional reaction chemistry and poor sensitivity.[8,9] HPAE-PAD provides the analytical capability to monitor, without derivatization, a large number of different compounds simultaneously using a single instrument and chromatographic method.

This Application Note describes the use of two different anion-exchange columns with amperometric detection to analyze simple sugars, sugar alcohols, alcohols, and glycols in yeast and bacterial fermentation broths. The yeast Saccharomyces cerevisiae in Yeast Extract-Peptone-Dextrose (YPD) broth and the bacteria Escherichia coli (*E. coli*) in Luria-Bertani (LB) broth are common eukaryotic and prokaryotic fermentation systems, respectively. Both fermentation broth cultures are complex and contain undefined media ingredients, and thus are a great challenge for most separation and detection technologies. These formulations contain inorganic and organic anionic ingredients that have been analyzed using the Thermo Scientific™ Dionex™ IonPac™ AS11 and AS11-HC anion-exchange columns with suppressed conductivity detection.[10]

In the methods outlined in this Note, the selectivities of the Thermo Scientific™ Dionex™ CarboPac™ PA1 and MA1 anion-exchange columns are compared for the analysis of carbohydrate, alcohol, and glycol ingredients in fermentation broths.

The Dionex CarboPac PA1 column packing consists of a 10 µm nonporous, highly crosslinked polystyrene/divinylbenzene substrate agglomerated with 350 nm diameter latex. The Thermo Scientific Dionex MicroBead™ latex is functionalized with quaternary ammonium ions, which create a thin surface rich in anion-exchange sites. The Dionex CarboPac PA1 column has a unique MicroBead pellicular resin structure that gives it stability from pH 0–14 at all concentrations of buffer salts, and enables excellent mass transfer, resulting in rapid gradient equilibration. The Dionex CarboPac PA1 column has an anion-exchange capacity of approximately 100 µeq/column and is specifically designed as a general purpose carbohydrate column.

Thermo SCIENTIFIC

The Dionex CarboPac MA1 column resin is composed of a polystyrene/divinylbenzene polymeric core. The surface is grafted with quaternary ammonium anion-exchange functional groups. Its macroporous structure provides an extremely high anion-exchange capacity of 1450 µeq/column. The Dionex CarboPac MA1 column is designed specifically for sugar alcohol and glycol separations.

Expected detection limits, linearity, selectivity, and precision are reported for the Dionex CarboPac MA1 column using a Thermo Scientific™ Dionex™ DX-500 BioLC™ system with pulsed amperometric detection.

Equipment

- Dionex DX-500 BioLC system consisting of:
  - GP40 Gradient Pump with degas option
  - ED40 Electrochemical Detector
  - LC30 or LC25 Chromatography Oven
  - AS3500 Autosampler
- Thermo Scientific™ Dionex™ PeakNet™ Chromatography Workstation

Reagents and Standards

Reagents

- Sodium hydroxide, 50% (w/w) (Fisher Scientific and J.T. Baker)
- Deionized water, 18 MΩ-cm resistance or higher

Standards

- D-Arabinose, anhydrous (Sigma Chemical Co.)
- L-Arabitol (Aldrich Chemical Co.)
- 2,3-Butanediol (Sigma Chemical Co.)
- D-Cellobiose, anhydrous (Sigma Chemical Co.)
- 2-Deoxy-D-glucose, reference grade (Pfanstiehl Laboratories)
- Erythritol (Pfanstiehl Laboratories)
- Ethanol (EM Science)
- D-Fructose, reference grade (Pfanstiehl Laboratories)
- Fucose, reference grade (Pfanstiehl Laboratories)
- Galactitol, reference grade (Pfanstiehl Laboratories)
- D-Galactose, reference grade (Pfanstiehl Laboratories)
- Galactosamine, reference grade (Pfanstiehl Laboratories)
- D-Glucosamine, reference grade (Pfanstiehl Laboratories)
- β-D-Glucose, reference grade (Pfanstiehl Laboratories)
- Glycerol (EM Science)
- α-Lactose, monohydrate (Sigma Chemical Co.)
- Maltose, monohydrate, reference grade (Pfanstiehl Laboratories)
- Maltitol (Aldrich Chemical Co.)
- Maltotriose, hydrate (Aldrich Chemical Co.)
- Mannitol, ACS grade (J.T. Baker Inc.)
- Methanol (EM Science)
- Raffinose, pentahydrate, reference grade (Pfanstiehl Laboratories)
- L-Rhamnose, monohydrate (Pfanstiehl Laboratories)
- D-Ribose, reference grade (Pfanstiehl Laboratories)
- Ribitol, reference grade (Pfanstiehl Laboratories)
- Sorbitol (Eastman Chemical Co.)
- Sucrose (Fisher Scientific)
- α-α-Trehalose, dihydrate, reference grade (Pfanstiehl Laboratories)
- D-Xylose, anhydrous (Sigma Chemical Co.)

Culture and Media

- Bacto YPD Broth (DIFCO Laboratories, Cat. No. 0428-17-5)
- Bacto Yeast Extract (DIFCO Laboratories, Cat. No. 0127-15-1)
- Bacto Peptone (DIFCO Laboratories, Cat. No. 0118-15-2)
- LB Broth (DIFCO Laboratories, Cat. No. 0446-17-3)
- Yeast, Saccharomyces cerevisiae, Bakers Yeast type II (Sigma Chemical Co., Cat. No. 45C-2)
- Bacteria, Escherichia coli (donated by SRI International)

Conditions

Dionex CarboPac MA1 Column Method

Columns:	Dionex CarboPac MA1 Analytical, P/N 44066 Dionex CarboPac MA1 Guard, P/N 44067	
Flow Rates:	0.4 mL/min	
Eluent:	A: 480 mM Sodium hydroxide	
Program:	Time (min)	A (%)
	0.0	100
	70.0 (End)	100

Dionex CarboPac PA1 Column Method

Columns:	Dionex CarboPac PA1 Analytical, P/N 35391 Dionex CarboPac PA1 Guard, P/N 43096			
Flow Rate:	1.0 mL/min			
Eluents:	A: Water B: 100 mM Sodium hydroxide C: 250 mM Sodium hydroxide			
Program:	Time (min)	A (%)	B (%)	C (%)
	0.00	84	16	0
	60.0	84	16	0
	60.1	0	0	100
	70.0	0	0	100
	70.1	84	16	0
	90.0 (End)	84	16	0

Common to Both Methods:	
Inj. Volume:	10 µL
Temperature:	30 °C
Detection (ED40):	Pulsed amperometry, gold electrode

Waveform for the ED40:

Time (s)	Potential (V)	Integration
0.00	+0.05	
0.20	+0.05	Begin
0.40	+0.05	End
0.41	+0.75	
0.60	+0.75	
0.61	-0.15	
1.00	-0.15	

Preparation of Solutions and Reagents

Sodium Hydroxide Eluents

100 mM Sodium Hydroxide

It is essential to use high-quality water of high resistivity (18 MΩ-cm) as free of dissolved carbon dioxide as possible. Biological contamination should be absent. Additionally, borate, a water contaminant that can break through water purification cartridges (prior to any other indication of cartridge depletion), can be removed by placing the Thermo Scientific™ Dionex™ BorateTrap™ column (P/N 47078) between the pump and the injection valve. It is extremely important to minimize contamination by carbonate, a divalent anion at high pH that binds strongly to the columns, causing a loss of chromatographic resolution and efficiency. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate and is the preferred source for sodium hydroxide.

Dilute 10.4 mL of 50% (w/w) sodium hydroxide solution into 1990 mL of water to yield 100 mM sodium hydroxide. Keep the eluents blanketed under 34–55 kPa (5–8 psi) of helium at all times.

250 and 480 mM Sodium Hydroxide

When preparing these eluents, follow the same precautions described above for the 100 mM sodium hydroxide eluent.

Sodium Hydroxide (mM)	50% Sodium Hydroxide (mL)	Water (mL)
250	26	1974
480	50	1950

Keep the eluents blanketed under 34–55 kPa (5–8 psi) of helium at all times. On-line degassing is necessary because the amperometric detector is sensitive to oxygen in the eluent. Set the pump to degas for 30 seconds every 4 minutes.

Stock Standards

Keep solid standards desiccated and under vacuum prior to use. Dissolve in purified water to 10 g/L concentrations. Combine and further dilute with purified water to yield the desired stock mixture concentrations. Maintain the solutions frozen at –20 °C until needed.

For determinations of linear range and lower detection limits, combine 10 g/L solutions of 2,3-butanediol, glycerol, erythritol, rhamnose, arabitol, sorbitol, galactitol, mannitol, arabinose, glucose, galactose, lactose, ribose, sucrose, raffinose, and maltose to make a 100 mg/L standard mix solution. Add methanol to this mix at a 10 g/L concentration. Dilute serially with water to final desired concentrations. This study used the following concentrations: 90, 80, 70, 60, 50, 40, 30, 20, 10, 8, 6, 4, 2, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.08, 0.06, and 0.04 mg/L. Methanol concentrations ranged from 9 to 0.004 g/L after dilution. Prepare two standard solutions at concentrations of 1 g/L. One solution contained 2,3-butanediol, erythritol, arabitol, galactitol, arabinose, galactose, and ribose. The other solution contained glycerol, rhamnose, sorbitol, mannitol, glucose, lactose, sucrose, raffinose, and maltose. Dilute these solutions to 0.8, 0.6, 0.4, and 0.2 g/L.

Sample Preparation

Yeast Fermentation Broth Culture—Standard Media

In a sterile 500 mL Erlenmeyer flask, dissolve 10 g of Bacto YPD broth (DIFCO Laboratories, Cat. No. 0428-17-5) in 200 mL of purified sterile water. Bacto YPD broth contains 2 g of Bacto Yeast Extract, 4 g of Bacto Peptone, and 4 g of dextrose (glucose) per 10 g. Dissolve 1.0 g of yeast (Saccharomyces cerevisiae; Bakers Yeast type II; Sigma Chemical Co., Cat. No. 45C-2) in the YPD broth. Cap the flask with a vented rubber stopper. Incubate the culture in a 37 °C shaking water bath (500–600 rpm) for 24 hours. Remove aliquots at designated time points and place on ice.

For this study, samples were taken after the addition of yeast at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 24 hour intervals. The incubation began when yeast was added to the media. Aliquots were centrifuged at 14,000 × g for 10 minutes and diluted 100-fold in purified water. Diluted supernatant (10 µL) was analyzed directly.

Yeast Fermentation Broth Culture—Modified Multiple Carbohydrate Media

In a sterile 500 mL Erlenmeyer flask, dissolve 2 g of Bacto Yeast Extract (DIFCO Laboratories, Cat. No. 0127-15-1), 4 g of Bacto Peptone (DIFCO Laboratories, Cat. No. 0118-15-2), and 4 g of carbohydrates (0.4 g each of glucose, sucrose, maltose, lactose, galactose, sorbitol, ribose, arabinose, rhamnose, and raffinose) in 200 mL of purified sterile water. Dissolve 1.0 g of yeast (Saccharomyces cerevisiae; Bakers Yeast type II; and Sigma Chemical Co., Cat. No. 45C-2) in the YPD broth. Incubate and sample the culture as described for the standard media.

E. coli Fermentation Broth Culture—Standard Media

Dissolve LB broth to a concentration of 25 g/L with water, heat to a boil, and autoclave for 15 minutes at 121 psi. LB broth contains 10 g of tryptone, 5 g of yeast extract, and 10 g of sodium chloride per 25 g. Incubate and sample the culture as described for the yeast standard media.

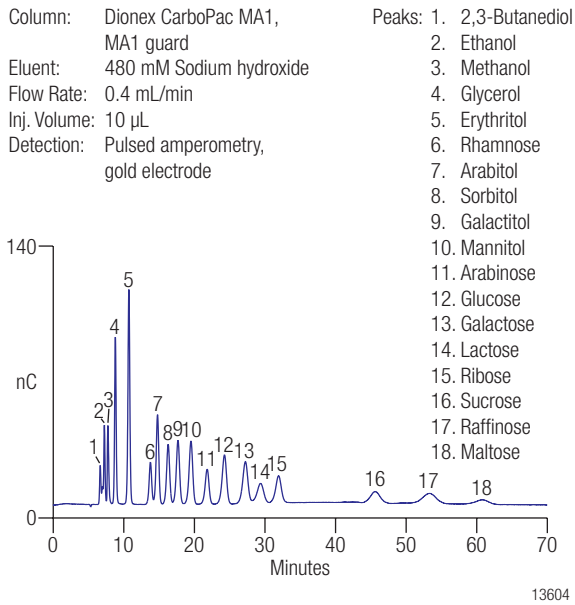

Figure 1. Common carbohydrates, alditols, alcohols, and glycols found in fermentation broths separated on the Dionex CarboPac MA1 column with pulsed amperometry.

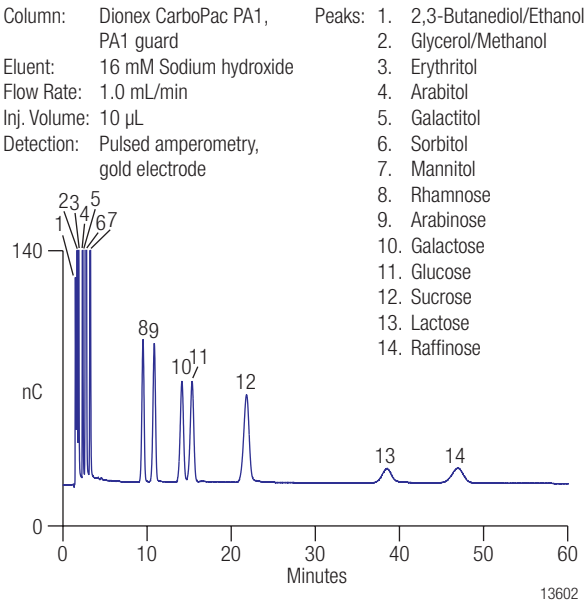

Figure 2. Common carbohydrates, alditols, alcohols, and glycols found in fermentation broths separated on the Dionex CarboPac PA1 column.

Results and Discussion

Selectivity

Dionex CarboPac MA1 Column

Figure 1 shows the separation of alcohols (2,3-butanediol, ethanol, methanol), glycols (glycerol), alditols (erythritol, arabitol, sorbitol, galactitol, mannitol), and carbohydrates (rhamnose, arabinose, glucose, galactose, lactose, sucrose, raffinose, maltose) commonly found in fermentation broths using a Dionex CarboPac MA1 column set with 480 mM sodium hydroxide eluent flowing at 0.4 mL/min. The alcohols, sugar alcohols (alditols), glycols, and carbohydrates are well resolved. Maltose elutes last at about 60 minutes.

The retention times of common fermentation broth carbohydrates under these conditions are listed in Table 1. Generally, alcohols elute first, followed by glycols, alditols, monosaccharides, disaccharides, and trisaccharides. Ethanol, methanol, and 2,3-butanediol are resolved, but the ethanol response is about 570 times less than the glucose response, and methanol is about 3600 times less responsive than glucose by mass. This can be advantageous when relatively large amounts of ethanol or methanol are produced, such as in the manufacture of alcoholic beverages and in the generation of alternative energy sources. Some large carbohydrates such as maltotriose could not be eluted under these conditions and are best analyzed using the CarboPac Dionex PA1 column.

Dionex CarboPac PA1 Column

Figure 2 shows the analysis of common fermentation broth alcohols, glycols, alditols, and carbohydrates using the Dionex CarboPac PA1 column. The elution order of the Dionex CarboPac PA1 column is similar to the Dionex CarboPac MA1 column. Alcohols elute first, followed by glycols, alditols, and mono-, di-, and trisaccharides. At elevated eluent strengths (e.g., 100–250 mM sodium hydroxide), many larger carbohydrates separate in under 20 minutes. For example, maltotriose, which elutes after 60 minutes on the Dionex CarboPac MA1 column, elutes at

Table 1. Retention times for carbohydrates and alcohols on the Dionex CarboPac MA1.

Analyte	Retention Time (min)
2,3-Butanediol	6.6
Ethanol	7.3
Methanol	7.7
Glycerol	8.7
Erythritol	10.7
Rhamnose	13.6
Fucose	13.8
Arabitol	14.7
Galactosamine	14.7
Glucosamine	15.3
Sorbitol	16.1
Trehalose	17.0
Galactitol	17.6
Ribitol	17.8
Mannitol	19.4
2-Deoxy-D-Glucose	20.2
Mannose	21.6
Arabinose	21.6
Glucose	24.0
Xylose	24.6
Galactose	27.0
Maltitol	27.7
Lactose	28.9
Fructose	29.0
Ribose	31.5
Cellobiose	43.2
Sucrose	44.9
Raffinose	51.8
Maltose	59.4
Maltotriose	>60.0

25.8 minutes with a 250 mM sodium hydroxide eluent on the PA1 column. Under these conditions, the early eluting peaks (e.g., 2,3-butanediol, methanol, and ethanol) coelute.

Table 2 summarizes the retention times for common fermentation analytes using the Dionex CarboPac PA1 column at 16, 50, 100, and 250 mM sodium hydroxide eluent conditions with 1 mL/min flow rates. These results demonstrate that adjustment of the eluent strength modifies column selectivity, sometimes changing analyte elution order. For example, at 16 mM sodium hydroxide, sucrose eluted slightly before ribose; yet at 50, 100, or 250 mM, ribose eluted significantly ahead of sucrose. Therefore, some separations can be improved by adjusting eluent strength. Adjusting eluent strengh can increase or decrease the peak area response of some analytes. Generally, response increases with increased eluent strength, but can decrease for some analytes. A decrease in response at elevated eluent strengths is probably due to hydroxide ions competing with analytes for sites on the electrode surface.

Table 2. Dionex CarboPac PA1 column with PA1 guard retention times (minutes).

	Sodium Hydroxide Concentration at 1.0 mL/min			
Analyte	**16 mM**	**50 mM**	**100 mM**	**250 mM**
2,3-Butanediol	1.5	1.5	1.5	1.5
Ethanol	1.6	1.6	1.6	1.6
Glycerol	1.7	1.7	1.7	1.7
Methanol	1.7	1.7	1.7	1.7
Erythritol	1.9	1.8	1.8	1.8
Arabitol	2.4	2.3	2.2	2.1
Galactitol	2.7	2.5	2.5	2.3
Ribitol	2.8	2.6	2.5	2.3
Sorbitol	2.8	2.6	2.5	2.2
Mannitol	3.3	3.0	2.8	2.4
Trehalose	3.7	3.3	3.1	2.6
Fucose	5.5	4.0	3.2	2.3
Maltitol	8.9	7.1	5.9	3.9
2-Deoxy-D-Glucose	9.3	6.2	4.6	3.0
Rhamnose	9.6	5.4	3.8	2.5
Galactosamine	10.7	6.2	4.4	2.7
Arabinose	10.9	6.6	4.6	2.9
Glucosamine	12.7	6.9	4.7	2.8
Galactose	14.3	8.4	5.8	3.3
Glucose	15.5	8.7	5.8	3.3
Mannose	16.9	8.6	5.5	3.1
Xylose	17.3	9.2	6.0	3.3
Fructose	20.4	10.3	6.5	3.6
Sucrose	21.7	15.4	10.9	6.0
Ribose	22.0	11.1	7.0	3.8
Lactose	38.5	18.9	10.7	5.0
Raffinose	47.1	31.0	21.1	9.3
Cellobiose	>60	31.8	17.7	6.9
Maltose	>60	55.7	27.0	9.5
Maltotriose	>60	>60	43.5	25.8

The Dionex CarboPac PA1 column is similar to the PA10 column. The Dionex CarboPac PA10 column is solvent-compatible and has better resolution between amino and neutral sugars. In some cases, the Dionex CarboPac PA10 column has a slightly different selectivity. For example, sucrose and fructose coelute on the Dionex CarboPac PA10 column at an eluent strength of 16–18 mM sodium hydroxide, but are well resolved on the Dionex CarboPac PA1 column. At low eluent strengths, sucrose and ribose coelute on the Dionex CarboPac PA1 column, but are resolved on the PA10 column, especially at lower sodium hydroxide concentrations (10 mM).

Figures 1 and 2 and Tables 1 and 2 show that the Dionex CarboPac MA1 and PA1 columns have different selectivities and therefore different strengths for determining the alcohols and carbohydrates in fermentation broths. Column choice will be dictated by the analytes, their concentrations, and the desired analysis time.

Method Detection Limits

The method detection limits (MDL) for a 10 µL injection of common fermentation broth constituents using the Dionex CarboPac MA1 column are shown in Table 3. The MDL is defined as the minimum concentration required to produce a peak height signal-to-noise ratio of 3. The MDL can be further decreased by increasing the injection volume above the 10 µL injection volume used in this Application Note, and by using smoothing algorithms available in PeakNet software.[11–13] Detection limits generally increase with longer retention times because of peak broadening. Methanol and ethanol have higher detection limits because their response factors are lower.

Table 3. Estimated detection limits* using the Dionex CarboPac MA1 column with pulsed amperometry

Analyte	ng	µg/L**
2,3-Butanediol	1	100
Ethanol	300	30000
Methanol	7000	700000
Glycerol	0.4	40
Erythritol	0.2	20
Rhamnose	1	100
Arabitol	0.5	50
Sorbitol	0.8	80
Galactitol	0.7	70
Mannitol	0.7	70
Arabinose	1	100
Glucose	0.9	90
Galactose	1	100
Lactose	2	200
Ribose	1	100
Sucrose	4	400
Raffinose	5	500
Maltose	9	900

*Lower limit of detection is based on 3× baseline noise

**10 µL injections

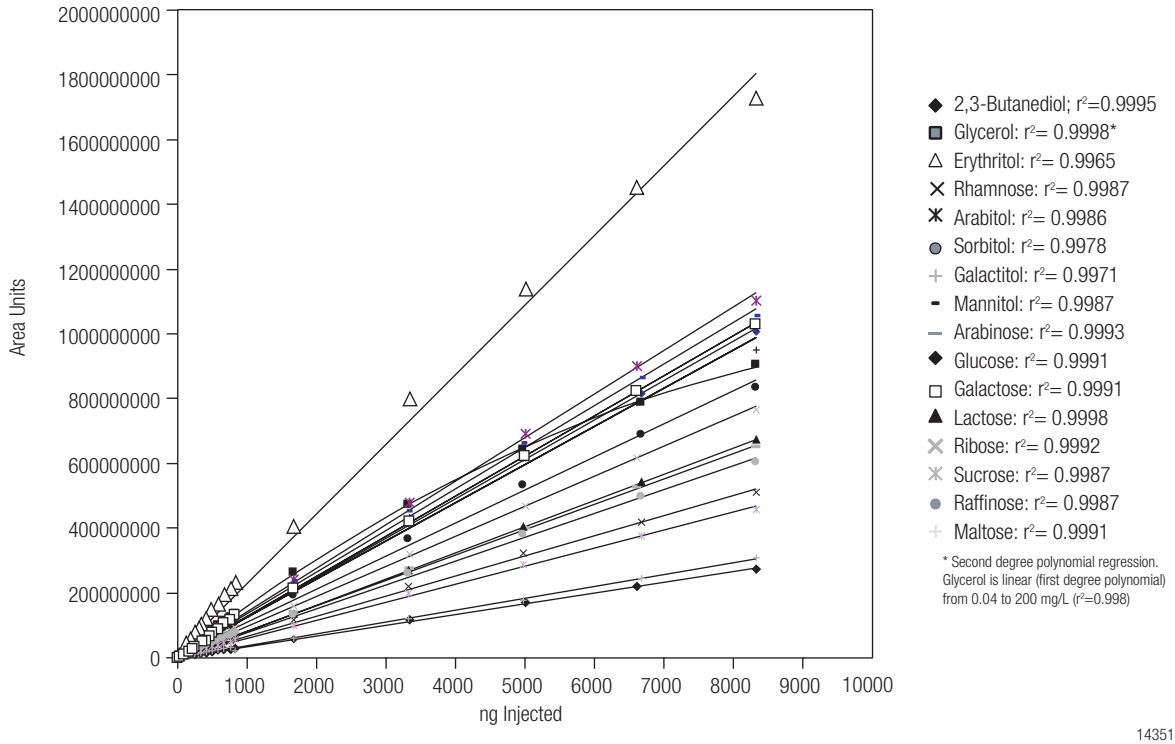

Figure 3. Method linearity using the Dionex CarboPac MA1 column with pulsed amperometric detection.

Linearity

Glycerol, 2,3-butanediol, erythritol, rhamnose, arabitol, sorbitol, galactitol, mannitol, arabinose, glucose, galactose, lactose, ribose, sucrose, raffinose, and maltose standards ranging from 0.1 to 1000 mg/L (1 to 10,000 ng) were injected (n=2 to 3 per concentration) onto a Dionex CarboPac MA1 column. Figure 3 shows that the method was linear for 2,3-butanediol, rhamnose, arabitol, sorbitol, mannitol, arabinose, glucose, galactose, lactose, ribose, sucrose, raffinose, and maltose over this range (r^2=0.998–0.999). Glycerol was linear over the range of 0.04–200 mg/L (0.04–2000 ng, r^2=0.998); erythritol over the range of 0.04–100 mg/L (0.4–1000 ng, r^2=0.999); and galactitol over the range of 0.07–100 mg/L (0.7–1000 ng, r^2=0.998). For the range of 0.1–1000 mg/L (1–10,000 ng), glycerol, erythritol, and galactitol deviated from linearity (r^2=0.987, 0.997, 0.997, respectively). Using a second order polynomial regression, the r^2 for glycerol, erythritol, and galactitol was 1.000. For all analytes, linearity was demonstrated over at least three orders of magnitude, and for most analytes, over four orders of magnitude. Broad linear ranges help reduce the need to dilute the sample and repeat the analysis when components vary greatly in concentration.

Precision and Stability

Peak area and retention time RSDs were determined for replicate injections of common carbohydrates, alditols, alcohols, and glycols spiked into yeast fermentation broth. Common fermentation broth carbohydrate and alcohol standards were added (10 mg/L) to heat-treated yeast fermentation broth culture supernatant and then analyzed over 48 hours (10 µL per injection, 42 injections) on the MA1 column. Results for precision are presented in Table 4.

Table 4. Peak area and retention time precision over 48 hours, RSD (%).

Analyte	Peak Area Units	Retention Time
2,3-Butanediol	2.4	0.2
Ethanol	2.7	0.2
Glycerol	2.0	0.2
Erythritol	3.4	0.2
Rhamnose	1.8	0.2
Arabitol	3.0	0.2
Sorbitol	2.7	0.2
Galactitol	2.7	0.3
Mannitol	2.7	0.3
Arabinose	3.1	0.3
Glucose	3.3	0.2
Galactose	3.5	0.3
Lactose	3.6	0.3
Ribose	3.1	0.3
Raffinose	4.8	0.4
Maltose	6.8	0.4

Figures 4 and 5 show the stability of peak area and retention time for fermentation broths analyzed over 48 hours. At this concentration, peak area RSDs ranged from 2 to 7%, and retention time RSDs ranged from 0.2 to 0.4%. Precision is affected by concentration (i.e., RSD values increase as the concentrations approach the MDL).

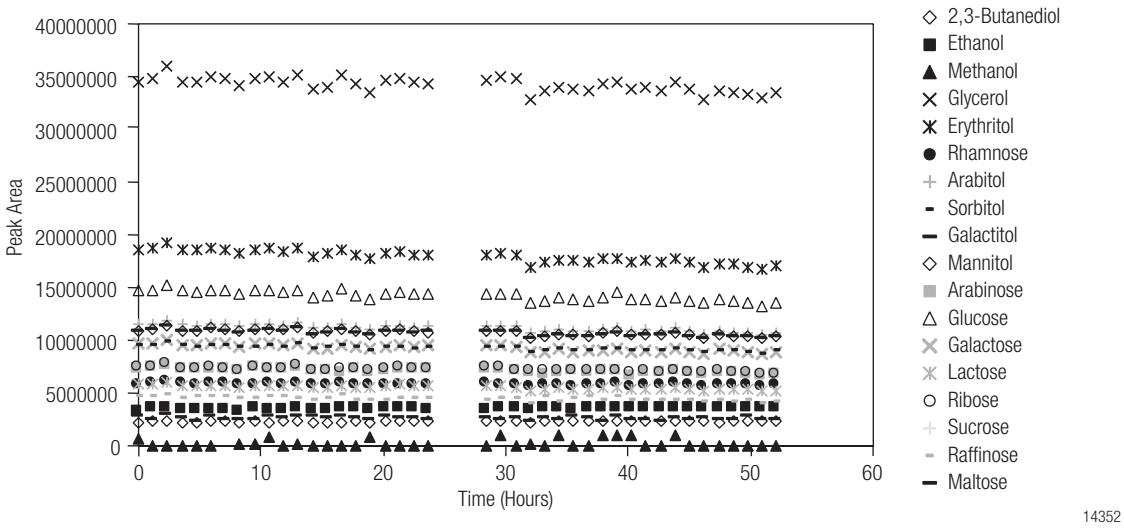

Figure 4. Stability of peak area over 48 hours for fermentation broth analysis. Injections 25 through 28 were standards in water.

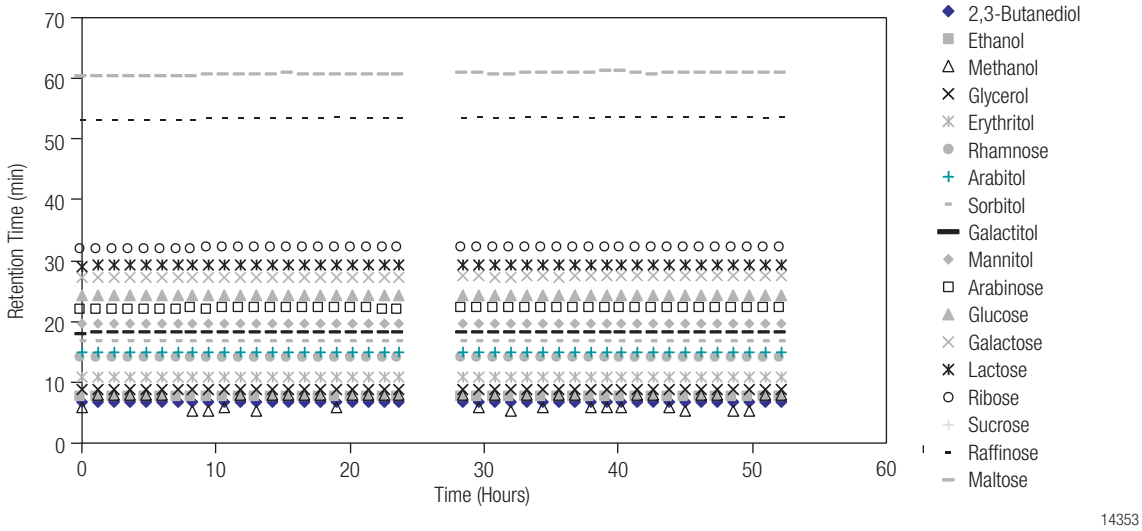

Figure 5. Stability of retention time over 48 hours for fermentation broth analysis. Injections 25 through 28 were standards in water.

Determination of Carbohydrates, Alditols, Alcohols, and Glycols in Fermentation Broth Cultures

Yeast (Saccharomyces cerevisiae) Culture

Yeast was grown in Bacto YPD broth at 37 °C for up to 24 hours. Figure 6 shows the separation of fermentation broth ingredients in a yeast culture at the beginning (Figure 6A) and after 24 hours (Figure 6B) of incubation. At the beginning of the culture, the glucose (dextrose) component was prominent. Ethanol was found at a relatively high concentration, along with trace levels of glycerol, erythritol, rhamnose, trehalose, arabinose, and cellobiose. During the first 3 hours, glucose levels decreased, and after 3 hours glucose was not detected (data not shown).

Glycerol increased over the same time period and remained constant after 3 hours. Ethanol concentration remained constant up to 7 hours. Between 7 and 24 hours, ethanol concentration decreased, presumably due to evaporative losses. Erythritol and rhamnose concentrations did not change, cellobiose concentration decreased by 50%, and trehalose and arabinose were depleted between 7 and 24 hours.

When the cell culture broth was modified to contain ten different carbohydrates and alditols, at the same total carbohydrate concentration as the standard Bacto YPD broth, it was apparent that yeast prefer to use certain carbohydrates over others, and that some carbohydrates or alditols could not be used as a carbon source during the 24-hour incubation period. Figure 7 shows the concentration of broth components over 24 hours. Glucose and raffinose were metabolized within the first hour. After one hour, the yeast began to consume maltose and galactose. Rhamnose, sorbitol, arabinose, lactose, and ribose were either unchanged or decreased slightly over 24 hours. Glycerol increased for the first hour, and then leveled off. Sucrose could not be measured, even at 0.15 hours (9 min) of incubation, which was the earliest time point possible due to the time required for full yeast dissolution. Sucrose was probably digested by the extracellular enzyme invertase, which is present in large amounts in the dried yeast. Invertase will cleave sucrose into its monosaccharides, glucose and fructose. Glucose was measured at levels higher than expected at the first time point, which supports this hypothesis.

Column: Dionex CarboPac MA1, MA1 guard
Eluent: 480 mM Sodium hydroxide
Flow Rate: 0.4 mL/min
Inj. Volume: 10 µL
Detection: Pulsed amperometry, gold electrode
Sample: 100-fold dilution, supernatant

Peaks:
1. Unknown
2. Unknown
3. Ethanol
4. Glycerol
5. Unknown
6. Erythritol
7. Unknown
8. Rhamnose
9. Arabitol
10. Trehalose
11. Unknown
12. Arabinose
13. Glucose
14. Unknown
15. Cellobiose
16. Unknown

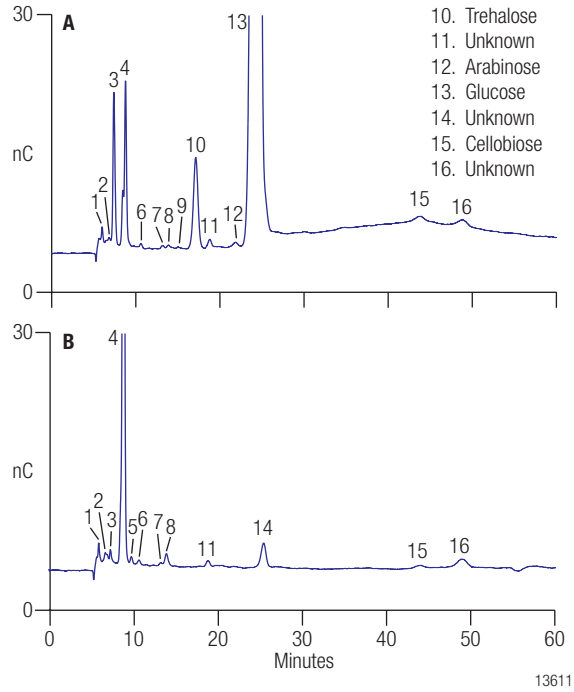

Figure 6. Saccharomyces cerevisiae fermentation broth culture using the Dionex CarboPac MA1 column, at (A) 0 hours of incubation and (B) 24 hours of incubation.

E. coli Culture

E. coli was grown on LB broth for 24 hours at 37 °C. Figure 8 shows that only trace levels of carbohydrates were found in this media. Erythritol, arabitol, arabinose, lactose, and maltose were identified by retention time in the starting media. No glucose was measured. After 24 hours, trace levels of 2,3-butanediol, erythritol, mannitol, glucose, and galactose were measured. Many unidentified peaks were consumed during this incubation period.

Conclusion

These results show that both yeast and bacterial fermentation broths can be analyzed for carbohydrate composition using high-performance anion-exchange chromatography and pulsed amperometry. Two columns (Dionex CarboPac MA1 and PA1) are available for the analysis of fermentation broth carbohydrates, alcohols, alditols, and glycols. The Dionex CarboPac MA1 column provides excellent separation of early eluting compounds such as alcohols, glycols, alditols, and monosaccharides. The run times are long for more complex carbohydrates such as trisaccharides. Separations using the Dionex CarboPac PA1 column are faster and can effectively separate di- and trisaccharides. Complex mixtures of carbohydrates and alditols can be monitored simultaneously, providing the analyst with information that is needed to optimize the fermentation.

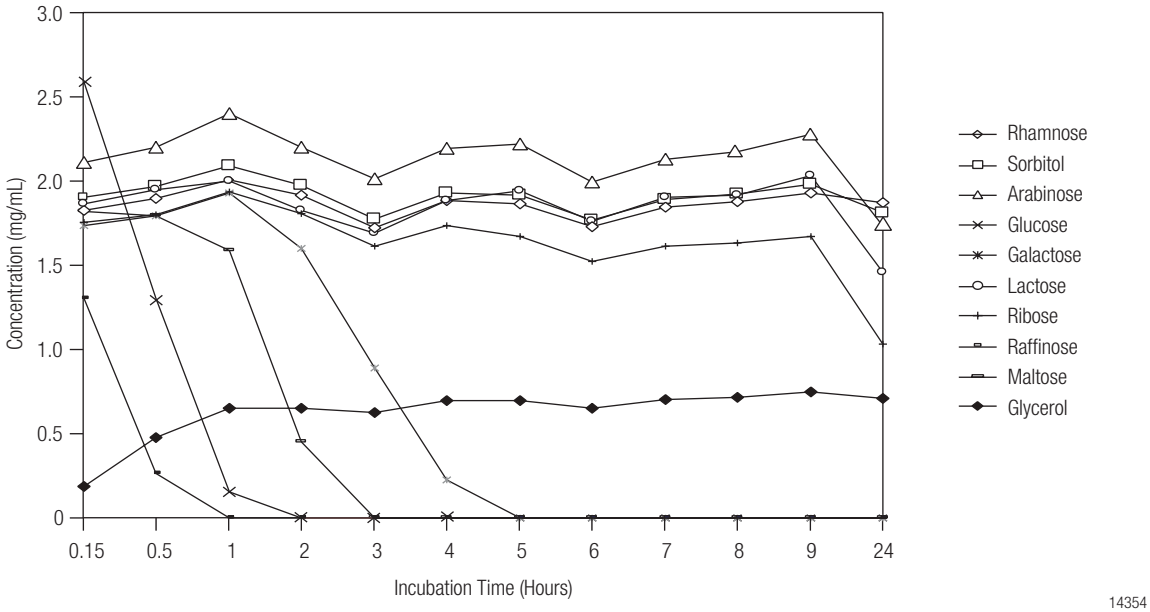

Figure 7. Saccharomyces cerevisiae culture grown on fermentation broth consisting of multiple carbohydrates and alditols, analyzed on a Dionex CarboPac MA1 column with integrated amperometry.

Column:	Dionex CarboPac MA1, MA1 guard
Eluent:	480 mM Sodium hydroxide
Flow Rate:	0.4 mL/min
Inj. Volume:	10 µL
Detection:	Pulsed amperometry, gold electrode
Sample:	10-fold dilution, supernatant
Peaks:	1. Unknown
	2. Unknown
	3. 2,3-Butanediol
	4. Ethanol
	5. Glycerol
	6. Unknown
	7. Erythritol
	8. Unknown
	9. Rhamnose
	10. Arabitol
	11. Unknown
	12. Unknown
	13. Galactitol
	14. Unknown
	15. Arabinose
	16. Unknown
	17. Lactose
	18. Unknown
	19. Unknown
	20. Unknown
	21. Unknown
	22. Maltose
	23. Unknown

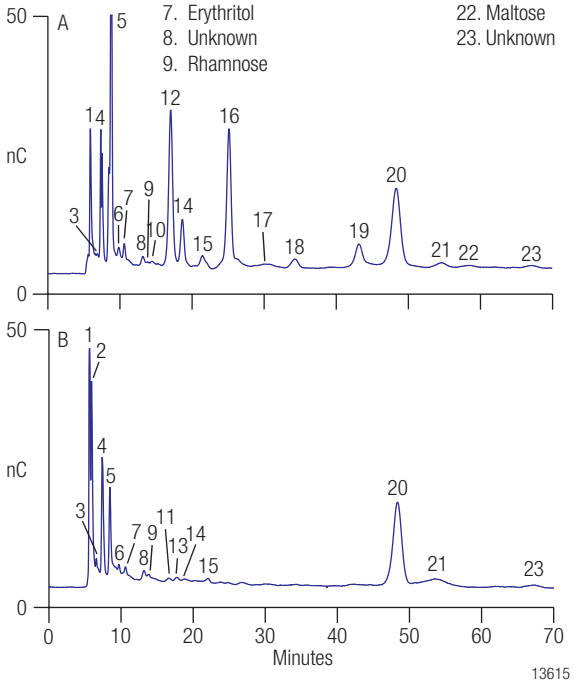

Figure 8. *E. coli* fermentation broth culture using the Dionex CarboPac MA1 column, at (A) 0 hours of incubation and (B) 24 hours of incubation.

References

1. Robinett, R.S.R.; Herber, W.K. *J. Chromatogr. A.* **1994,** *671,* 315–322.
2. Herber, W.K.; Robinett, R.S.R. *J. Chromatogr. A.* **1994,** *676,* 287–295.
3. Marko-Varga, G.; Buttler, T.; Gorton, L.; Olsson, L.; Durand, G.; Barcelo, D. *J. Chromatogr. A.* **1994,** *665,* 317–332.
4. Schugerl, K.; Brandes, L.; Wu, X.; Bode, J.; Ree, J. I.; Brandt, J.; Hitzmann, B. *Anal. Chim. Acta.* **1993,** *279,* 3–16.
5. Rank, M.; Gram, J.; Danielsson, B. *Anal. Chim. Acta.* **1993,** *281,* 521–526.
6. Buttler, T.; Gordon, L.; Marko-Varga, G. Anal. Chim. Acta. 1993, 279, 27–37.
7. van de Merbel, N.C.; Lingeman, H.; Brinkman, U.A. Th.; Kolhorn, A.; de Rijke, *L.C. Anal. Chim. Acta 1993, 279,* 39–50.
8. van de Merbel, N.C.; Kool, I.M.; Lingeman, H.; Brinkman, U.A. Th.; Kolhorn, A.; de Rijke, L.C. Chromatographia 1992, 33 (11/12), 525–532.
9. Marko-Varga, G.; Dominguez, E.; Hahn-Hagerdal, B.; Gorton, L.; Irth, H.; De Jong, G.J.; Frei, R.W.; Brinkman, U.A. *Th. J. Chromatogr.* **1990,** *523,* 173–188.
10. Dionex Corporation (now part of Thermo Scientific), Application Note 123, *Determination of organic and inorganic anions in fermentation broths.*
11. Dionex Corporation (now part of Thermo Scientific), Technical Note 40, *Glycoprotein monosaccharide analysis using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD).*
12. Schibler, J.A. *Am. Lab.* **1997,** 52–54.
13. Dionex Corporation (now part of Thermo Scientific), Technical Note 43, *Using smoothing algorithms to reduce baseline noise in chromatography.*

List of Suppliers

Aldrich Chemical Company, Inc., 1001 West Saint Paul Avenue, P.O. Box 355, Milwaukee, Wisconsin, 53233, USA. Tel.: 1-800-558-9160.

J.T. Baker Incorporated, 222 Red School Lane, Phillipsburg, New Jersey, 08865, USA. Tel.: 1-800-582-2537.

Eastman Chemical Company, 1001 Lee Road, Rochester, New York, 14652-3512, USA. Tel.: 1-800-225-5352.

EM Science, P.O. Box 70, 480 Democrat Road, Gibbstown, New Jersey, 08027, USA. Tel.: 1-800-222-0342.

Fisher Scientific, 711 Forbes Avenue, Pittsburgh, Pennsylvania, 15219-4785, USA. Tel.: 1-800-766-7000.

Pfanstiehl Laboratories, Inc., 1219 Glen Rock Avenue, Waukegan, Illinois, 60085-0439, USA. Tel.: 1-800-383-0126.

Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri, 63178, USA. Tel.: 1-800-325-3010.

www.thermoscientific.com/dionex

Thermo Scientific Dionex products are designed, developed, and manufactured under an ISO 9001 Quality System.

Australia +61 3 9757 4486
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
China +852 2428 3282
Denmark +45 70 23 62 60
France +33 1 60 92 48 00
Germany +49 6126 991 0
India +91 22 2764 2735
Italy +39 02 51 62 1267
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Netherlands +31 76 579 55 55
Singapore +65 6289 1190
Sweden +46 8 473 3380
Switzerland +41 62 205 9966
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA and Canada +847 295 7500

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

AN70494_E 01/13S