Determination of Glycols and Alcohols in Fermentation Broths Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection

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Application Note 188

Key Words
Dionex ICS-5000, Dionex IonPac, Alcohols, Broths, Fermentation, Glycols, Ion Chromatography, Ion-Exclusion Chromatography, PAD

Fermentation with yeast, bacteria, or other microorganisms has been used for centuries to produce alcoholic beverages, bread, cheese, yogurt, and feed stock for animals. Fermentation with other microorganisms has more recently been used to produce antibiotics such as penicillin and pharmaceutical compounds, enzymes, amino acids, and organic acids,[1-4] as well as ethanol for fuel or fuel additives.[2,3]

Fermentation broths are complex mixtures of microorganisms and both organic and inorganic compounds. Ionic compounds, carbohydrates, and amino acids are essential for cellular growth and structure. Organic acids, alditols (sugar alcohols), glycols, alcohols, and other compounds are metabolic byproducts.[2,3]

To optimize growth and yields, it is crucial to monitor fermentation broths for both cellular fuel sources as well as metabolic byproducts. Many of these carbohydrates, amino acids, anions, and organic acids have been successfully monitored in fermentation media and broths using ion chromatography (IC) (Thermo Scientific Application Notes 122,[5] 123,[6] and 150[7]).

For beer and wine, the absence or presence of aldehydes and glycols affects their quality and flavor. Alcohols and glycols have been determined using ion-exclusion chromatography with an Thermo Scientific™ Dionex™ IonPac ICE-AS1, a perchloric acid eluent, and pulsed amperometric detection (PAD) with a platinum working electrode and a three-potential waveform.[8] However, perchloric acid is not an optimal eluent, as perchlorate poses a health risk to women of childbearing age and children by disrupting the uptake of iodide by the thyroid gland and causing hypothyroidism and birth defects.[9]

Perchlorate is highly regulated in the U.S. because of these health risks. To avoid the use of perchlorate, an ion-exclusion method was developed that uses a more stable and environmentally benign acid, methanesulfonic acid.

Disposable electrodes and an optimized waveform[10] have also been developed to further improve amperometric detection of a variety of analytes. The disposable platinum electrode used in this application is easy to install, does not require reconditioning or polishing, allows faster equilibration, and provides lower detection limits than a conventional platinum electrode. The waveform and electrode used here allow fast, accurate determinations of alcohols and glycols in fermentation media and alcoholic beverage samples through ion-exclusion chromatography with PAD.

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Equipment

- Thermo Scientific™ Dionex ICS-3000 Reagent-Free™ IC (RFIC™) system consisting of:
  - SP Single Pump module, gradient pump with degas option
  - DC Detector/Chromatography module, single or dual zone
  - ED Electrochemical Detector (P/N 061718)
  - Thermo Scientific™ Dionex™ AS Autosampler with sample tray temperature controlling option and 1.5 mL sample tray
- An electrochemical cell containing a combination pH-Ag/AgCl reference electrode (cell and reference electrode, P/N 061756, reference electrode P/N 061879) and a disposable platinum (Pt) working electrode (package of 6 electrodes, P/N 064440)
- Knitted reaction coil, 375 µL, (P/N 043700) with two PEEK unions (¼-28 thread female to 10-32 thread female, P/N 042806)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System, version 6.8
- 1.5 mL glass sample vials, with caps and slit septa (vial kit, P/N 055427)
- PEEK tubing:
  - Red (0.127 mm or 0.005 in i.d.) tubing, used for eluent connections from Inj. Valve 1 to column and cell.
  - Black (0.25 mm or 0.010 in i.d.) tubing used for eluent connections from Pump 1 to Inj. Valve 1 and the waste line from the cell to waste container (5 ft, P/N 052306)
  - Green (0.76 mm or 0.030 in i.d.) tubing, waste line to Dionex AS Autosampler (5 ft, P/N 052305)
- Heated water bath (VWR Scientific 1200 series)
- Shaker table (Lab Line)
- Centrifuge (Eppendorf™ 5400 series)
- Sterile assembled micro-centrifuge tubes with screw cap, 1.5 mL (Sarstedt 72.692.005)

Reagents and Standards

Reagents

Use only ACS reagent grade chemicals for all reagents and standards.

Deionized water, Type 1 reagent-grade, 18 MΩ cm resistivity or better, freshly degassed by vacuum filtration

L-Arabitol (Aldrich, P/N A3506)

Meso-Erythritol, HPLC grade, (1,2,3,4-butanetetrol; Fluka BioChemika, P/N 45670)

Ethanol, denatured, (VWR, P/N JT9401-1, 90% pure)

Ethylene glycol (1,2-ethanediol; VWR, P/N JT9300-33)

Galactitol (dulcitol; Aldrich P/N D0256)

Myo-Inositol (Aldrich, P/N I5125)

D-Mannitol (Aldrich, P/N 240184)

Methanesulfonic acid (Aldrich, P/N 64280; Thermo Scientific, P/N 033478)

Methanol (VWR, P/N JT9070-33)

1,2,3-Propanetriol (glycerol, glycerin; VWR, P/N JT2142-1)

2-Propanol (isopropyl alcohol, VWR, P/N BDH1133-4LG)

1,2-Propanediol (propylene glycol, Aldrich, P/N 241229)

pH 7 and pH 4 (yellow, blue) buffer solutions, NIST traceable (VWR International, P/N 34170-130, 34170-127)

Ribitol (adonitol; Aldrich, P/N A5502)

D-Sorbitol, HPLC (D-glucitol; Fluka BioChemika Ultra, P/N 85529)

Fermentation Medium

BD Bacto™ Yeast Extract-Peptone-Dextrose (YPD) Broth (BD Diagnostic, P/N 242820; VWR, P/N 90003-284)

Samples

Wyeast™ "German Ale," "Bohemian Lager," and "American Wheat" Saccharomyces cerevisiae samples purchased from Hop Tech Home Brewing (Dublin, CA, USA, P/N 1007XL, 2124XL, and 1010XL, respectively)

S. cerevisiae samples incubated from a Bacto YPD fermentation broth

American, German, and British beer and American wine beverages

CONDITIONS	
Column:	Dionex IonPac ICE-AS1 Analytical, 4 × 250 mm (P/N 064198)
Flow Rate:	0.2 mL/min
Eluent:	100 mM Methanesulfonic acid
Column Temperature:	30 °C
Oven Temperature:	30 °C
Tray Temperature:	10 °C
Inj. Volume:	10 µL (PEEK sample loop, P/N 042949), full loop injection
Detection:	Pulsed amperometric detection (PAD)
Waveform:	See Table 1.
Reference Electrode:	pH-Ag/AgCl electrode (P/N 061879) in AgCl mode
Working Electrode:	Disposable platinum working electrode (P/N 064440, package of six)
Typical Background:	60–90 nC versus Ag/AgCl
Typical System Backpressure:	~800 psi
Noise:	<10 pC
Typical pH:	1.0
Run Time:	30 min
Syringe Speed:	4
Flush Volume:	250 µL

Table 1. Analyte identity: UV1–UV6 are analytes that have strong UV but weak EC response.

Time (sec)	Potential vs Ag/AgCl (V)	Gain Region	Integration	Ramp
0.00	+ 0.30	Off	Off	Ramp
0.31	+ 0.30	On	Off	Ramp
0.32	+ 1.15	On	Off	Ramp
0.64	+ 1.15	On	On (Start)	Ramp
0.66	+ 1.15	On	Off (End)	Ramp
0.67	- 0.30	On	Off	Ramp
1.06	- 0.30	Off	Off	Ramp
1.07	+ 0.30	Off	Off	Ramp

Preparation of Solutions and Standards

Eluent (100 mM Methanesulfonic Acid)

An eluent generator cannot be used for this application because the pressure limitations of the ICE-AS1 (see Precautions at the end of this note). When manually preparing eluents, it is essential to use high quality, Type 1 water, 18 MΩ cm resistivity or better, that contains as little dissolved gas as possible. Dissolved gases can increase noise levels. Degas the deionized water before eluent preparation. Prepare freshly degassed deionized water weekly for the Dionex AS Autosampler flush solution.

Mix 994 g of degassed Type 1 deionized water with 9.6 g of methanesulfonic acid (MSA) in a 1 L glass eluent bottle. Swirl gently to mix. Connect the prepared eluent to Eluent A line from Pump 1 and place the eluent bottle under ~4–5 psi of helium or other inert gas. Swirl the eluent bottle to thoroughly mix the eluent. Prime the pump with the new eluent.

Standard Preparation

To prepare separate 100 mM stock solutions of *meso*-erythritol, glycerol, propylene glycol, ethanol, *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, ethylene glycol, methanol, and isopropyl alcohol, weigh the amount of reagent grade compound stated in Table 2 into individual 20 mL glass scintillation bottles. Add degassed deionized water to a total weight of 20.00 g. The stock standards are stable for more than a month when refrigerated.

Working Standards

To prepare 25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µM working standards of *meso*-erythritol, glycerol, propylene glycol, and ethanol from the 100 mM stock standards, pipette 5, 10, 20, 40, 80, 160, 320, 640, and 1280 µL, respectively, of each stock standard into 20 mL glass scintillation bottles. Dilute these working standards to 20.00 g total weight with degassed deionized water. The working standards should be prepared weekly.

Standards for Retention Time Determination and Alditol and Glycol Separation Experiments

Dilute the 100 mM stock standards of *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, ethylene glycol, methanol, isopropyl alcohol, *meso*-erythritol, glycerol, propylene glycol, and ethanol to 50 µM (10 µL of stock in 20.00 g total with deionized water) for retention time determination experiments. Prepare combined 50 µM standards of *myo*-inositol, D-mannitol, D-sorbitol, galactitol, ribitol, L-arabitol, and *meso*-erythritol in a similar way. Prepare combined 25, 50, and 100 µM ethylene glycol and propylene glycol standards in a similar way, by diluting 5, 10, and 20 µL of stock solutions to 20.00 g total weight with deionized water.

Table 2. Amount of Compound Used to Prepare 20.00 g of Individual 100 mM Stock Standard Solutions.

Compound	Formula Weight (g/mole)	Mass (g)
myo-Inositol ($C_6H_{12}O_6$)	180.16	0.360
D-Mannitol [$CH_2OH(HO\text{-}CH)_2(CHOH)_2CH_2OH$]	182.17	0.364
D-Sorbitol [D-glucitol, $CH_2OH(CHOH)(HO\text{-}CH)(CHOH)_2CH_2OH$]	182.17	0.364
Galactitol [dulcitol, $CH_2OH(HO\text{-}CH)(CHOH)_2(HO\text{-}CH)CH_2OH$]	152.17	0.364
D-Ribitol (adonitol, 1,2,3 4 5-pentanol) [$CH_2OH(HO\text{-}CH)(CHOH)(HO\text{-}CH)CH_2OH$]	152.15	0.304
L-Arabitol [$CH_2OH(CHOH)(HO\text{-}CH)_2CH_2OH$]	152.15	0.304
meso-Erythritol [1,2,3,4-butanetetrol, $HO\text{-}CH_2(CHOH)_2CH_2OH$]	122.12	0.244
Glycerol (1,2,3-propanetriol, $HO\text{-}CH_2CHOHCH_2OH$)	92.09	0.184
Ethylene glycol (1,2-ethanediol, $HO\text{-}CH_2CH_2OH$)	62.07	0.124
Propylene glycol (1,2-propanediol, $CH_3CHOHCH_2OH$)	76.09	0.152
Methanol (CH_3OH)	32.04	0.064
Denatured ethanol, anhydrous 90% (CH_3CH_2OH)	62.07	0.138
Isopropyl alcohol (2-propanol, $CH_3CHOHCH_3$)	60.09	0.120

Sample Preparation

Bacto YPD Broth Medium Preparation

To prepare the Bacto Yeast Extract-Peptone-Dextrose (YPD) broth, dissolve 10.0 g in 200 mL of aseptically prepared deionized water (0.2 µm, nylon). The Bacto YPD broth contains a 1:2:2 ratio of Bacto Yeast Extract, Bacto Peptone, and dextrose. The growth medium was used for fermentation experiments and as a matrix blank.

Fermentation Samples

Fermentation Samples for Dilution Experiments

To prepare fermentation broth samples for the dilution experiments, first prepare the matrix control sample according to the instructions in AN 122.[5] Add 20–25 g of American Wheat *S. cerevisiae* mixture to 200 g of Bacto YPD broth. Immediately heat-quench 1 mL aliquots of this time = zero fermentation broth in boiling water for 10 min and then centrifuge them at 14,000 x g for 10 min. Transfer the supernatant to another vial and dilute with purified (0.2 µm nylon filter) deionized water according to dilution experiments.

Fermentation Samples for Robustness Experiments

To prepare samples (50 µM glycerol, propylene glycol, and ethanol in the control fermentation broth) for robustness experiments, first prepare the fermentation broth as prepared for the dilution experiments. Pipette 66.7 µL of diluted (300-fold) supernatant from the centrifuged, heat-quenched fermentation broth into a 20 mL scintillation bottle and dilute to 20.00 g with purified deionized water. Pipette 5.0 µL of 100 mM glycerol, propylene glycol, and ethanol stock standards into a 20 mL glass scintillation bottle and dilute to 10.00 g total weight with 300-fold dilution fermentation broth.

Fermentation Samples for Growth Experiments

To prepare fermentation samples for growth experiments, add 20–25 mL of wine or beer *S. cerevisiae* mixture to 200 mL of Bacto YPD medium (see Medium Preparation, above) in a sterile 500 mL Erlenmeyer flask. Cap the flask with a rubber stopper prepared with an air line pointed downward. Incubate for 26–28 h at 37 °C in a shaking (500–600 rpm) water bath. Remove 1 mL aliquots at selected time points (0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, and 28 h), heat-quench, centrifuge, and dilute the supernatant 300-fold with purified deionized water in the same way as previously discussed. Spike recovery of select *S. cerevisiae* samples incubated in the Bacto YPD medium are prepared in a similar way as the spike recovery samples for the control fermentation broth.

Beverage Samples

To prepare the wine samples, centrifuge a 1 mL aliquot at 14,000 x g for 10 min. Transfer the supernatant to another vial and dilute with purified deionized water according to the dilution experiments described in the Results and Discussion section under Beverage Samples. The beer samples are first degassed with ultrasonic agitation and applied vacuum, and treated in the same manner as the wine beverage samples.

To prepare the 40 µM glycerol and 400 µM ethanol spike recovery samples for wine, pipette 4 and 40 µL, respectively, of 100 mM stock standards into a 20 mL glass scintillation bottle, and dilute to 10.00 g total weight with a 400-fold dilution of the supernatant from the centrifuged sample. Prepare the spike recovery samples for beer in the same way with a 400-fold dilution of the supernatant from the centrifuged, degassed sample.

System Preparation and Setup

The setups for the individual modules, components, and system are thoroughly described in the *Dionex ICS-3000 Operator's Manual,*[12] *Dionex ICS-3000 Installation Manual,*[13] *Dionex AS Autosampler Operator's Manual,*[14] and the Chromeleon Help menus.

Plumbing the Chromatography System

Connect black PEEK (0.254 mm or 0.010 in i.d.) tubing from Pump 1 to position P on Inj. Valve 1 inside the DC module. Connect red PEEK (0.127 mm or 0.005 in i.d.) tubing from Inj. Valve 1, position C to the heat exchanger. Install the Dionex IonPac ICE-AS1 column, according to the *Dionex IonPac ICE-AS1 Product Manual,*[15] by connecting the red PEEK tubing exiting the System 1 heat exchanger to the column and another 5 cm of red PEEK tubing to the free end the column.

Install a 375 µL knitted reaction coil between the end of the column and the cell to minimize dissolved oxygen from the sample and, therefore, the oxygen dip in the chromatogram. First connect both ends of the reaction coil to the large end (¼-28 thread female) of two unions (¼-28 thread female, 10-32 thread female).

Caution: tighten to finger tight only. Do not over tighten. Over tightening this connection can break the thermoformed end of the knitted reaction coil.

Connect the free end of the 5 cm of red PEEK tubing from the column to a free end of one of the unions. The free end of the remaining union will be connected to the cell (with another 5 cm of red PEEK tubing) after the combination pH/Ag/AgCl electrode is calibrated. The waste line from the cell will also be installed after the cell is assembled and installed. Install a 10 µL loop in DC Inj. Valve 1, in both L positions. Connect the Dionex AS Autosampler Injection Port tubing and the green PEEK (0.76 mm or 0.030 in i.d.) tubing waste line to DC Inj. Valve 1 positions S and W, respectively.

Configuring the Dionex AS Autosampler

Configure the Dionex AS Autosampler and connect the Sample Prep and Sample syringes according to the *Dionex AS Autosampler Operator's Manual.*[14] Enter the loop size (10 µL) in Loop Size V1, on the Autosampler's front panel, under Menu and Plumbing Configuration. Select the syringe sizes of the sample prep and the sample syringe from the pull down menus, under Menu and System Parameters. Also select Normal sample mode and enable Wait function, under Menu and System Parameters.

Configuring the System

Install the ED module in the middle DC chamber, above Inj. Valve 1 before turning on the DC and configuring the system. Do not remove or install the ED module while the DC is turned on. Turn on the Dionex AS Autosampler and DC and SP modules and wait until the autosampler finishes its startup process. To configure the system, open and start the Chromeleon Server Monitor program, and then open and start the Chromeleon Server Configuration program. Create a timebase (named "Fermentation" for this discussion) if one is not already present for this system, and then add the devices: Dionex ICS-3000 SP pump module, DC module, and AS Autosampler. Assign Pump 1 to the timebase (right click on SP module, select Properties, select Devices tab, and select the timebase on the pull-down menu for the pump). Verify that the Dionex AS device has the same options (e.g., SamplePreparation, Temperature Tray Control, etc.) listed on the Dionex AS Autosampler module. Save and check the configuration before leaving the program.

Configuring a Virtual Channel to Monitor pH

It is useful to monitor and record the pH during sample analyses. To record periodic pH measurements, manually enter log commands into the program (see Program section).

To continuously record the pH during sample determinations, create a virtual channel in Server Configuration. Open the Server Configuration program, right click on the timebase and select Add Device, Generic, and Virtual Channel Driver. Right click on the newly created Virtual Channel Driver device, and select Properties and the General tab. The Device Name should be automatically entered as VirtualChannel_01. Select the Signal tab, and select and double click on VirtualChannel_01 to open the Signal Configuration window. Enter pH for Unit, 1.0 for Factor, click on Analog for Type, and enter pH.value for Formula. Save and check the configuration before leaving the program. The pH virtual channel becomes one of the available signal channels. More information can be found in the Chromeleon Help program.

Amperometry Cell

Calibration, handling, and installation tips for the reference electrode and Certified Disposable Platinum working electrodes are thoroughly described in the System Preparation and Setup section of this application note, the *Dionex ICS-3000 Operator's Manual,*[12] and the product[16] and installation[17] manuals for disposable electrodes. To calibrate the combination pH-Ag/AgCl reference electrode, remove the storage cap from the reference electrode but leave the storage cap o-ring in place on top of the reference electrode. The storage cap o-ring will be used again when the reference electrode is removed and sealed into the storage cap. It does not interfere with the installation of the reference electrode. Rinse the KCl storage solution off the reference electrode, pat dry, and place the reference electrode in pH 7 buffer.

Open Chromeleon CDS and connect to the Fermentation timebase. Click on the Chromeleon Panel icon, expand the timebase panel, and select the EC Detector tab. Connect the blue lead of the reference electrode to the ED black port. Check the cell on/off button to ensure that the cell is turned off. (The pH electrode remains active regardless of the cell power.) Click on the Calibration button which opens the ED Wellness Panel. Follow the calibration instructions in the Instructions button or in the *Dionex ICS-3000 Operator's Manual*. Wait for the pH reading to stabilize, then press the pH Offset Cal button and wait while it calculates the pH offset. After it is finished, remove the reference electrode, rinse, and pat it dry. Place the reference electrode in pH 4 buffer and wait until the reading is stable. Enter 4.00 in the pH Slope Buffer value, press the pH Slope Cal. button, and wait while it calculates the slope and intercept. When it is finished, save, upload the new calibration values, and close the ED Wellness Panel.

Assembling the Electrochemical Cell

Check that the reference electrode o-ring on the bottom of the reference electrode is in place and install one if it is missing or damaged. Gently screw the reference electrode into the electrochemical cell body. Tighten finger tight to a snug fit. (Do not use tools.) Install the disposable platinum electrode in the electrochemical cell, according to the *Disposable Platinum Electrode Installation Guide for ED* shipped with the electrodes. Install the electrochemical cell into the ED. Connect the yellow and blue leads on the cell to the yellow and black ports on the ED (Figure 1).[12] Connect the free end of union-knitted reaction coil to another 5 cm length of red PEEK tubing. Connect the remaining end to the cell inlet and direct the black cell outlet tubing to waste. Loosen the waste line until after the pump has started to prevent trapping bubbles in the cell (a source of high noise). Retighten the fitting when eluent is observed in the outlet.

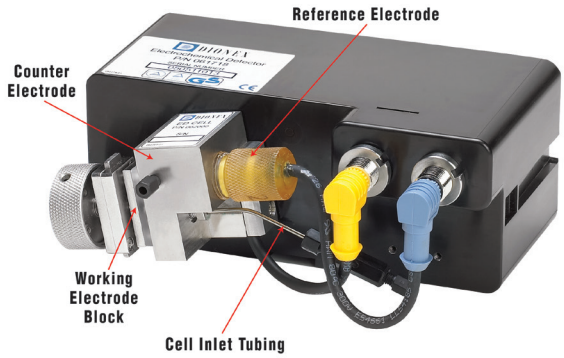

Figure 1. Amperometry cell.

In this application, the working electrode is a disposable platinum working electrode. When used with a recommended waveform and integration, the disposable platinum working electrodes have a background specification of 0 to 200 nC against the reference electrode in AgCl mode. Typically, the background will stabilize within 10 min. However, the pump may cause minute fluctuations in the background for up to an hour after installation. For trace analysis it is advisable to allow for an hour of equilibration before running samples.

Program

To make a new program, use the Program Wizard to enter the parameters from Table 1 and the Conditions section. In the EDet 1 Mode Options tab, select Integrated Amperometry. If a Virtual Channel was created in Configuration, the pH channel will be present in the Acquisition Options (see the section Configuring Virtual Channel to Monitor pH.) Select the pH, Pressure, and EDet1 detector channels. If a Virtual Channel was not created, select the Pressure and EDet1 detector channels. In the Pump_1_Pressure Options, select Auto for Step and check Average. In the EDet1 Options, select On for the amperometry cell, select all channels, enter 1.00 (Hz) for Data Collection Rate, 0 and 3 for pH Lower and Upper Limit, and enter the waveform (Table 1) in the Waveform selector. After the EDet1 Options tab, a Virtual Channel Options tab should appear with the same parameters as those entered when the Virtual Channel was configured: pH.value in Formula, Analog for Type, and Select Auto and Average for Step. Enter the title of the program and select Review Program. The new program will open in command mode in a new window.

To improve the signal to noise response for the detector signals, add signal averaging commands into the program. Find the following two commands in the program, and enter four empty lines. Using Control, Command, EDet1, ED_1, enter the commands Average, On and Step, Auto. Enter the same commands for ED_1_total. Check the program, using the Control, Check commands, and Save and Close the program.

EDet1.Mode =	IntAmp
EDet1.CellControl =	On
ED_1.Average =	On
ED_1.Step =	Auto
ED_1_total.Average =	On
ED_1_total.Step =	Auto

To record periodic pH measurements (optional), manually enter log commands into the program using Control, Command. Select System, Log, EDet1, pH, and Value, enter the retention time, and press Execute. Repeat the process for each pH reading. Save and close the program.

Results and Discussion

Separation

Ion-exclusion chromatography uses a fully sulfonated resin with a strong acid eluent to exclude strongly ionic compounds by Donnan exclusion and large compounds by steric exclusion. Small neutral compounds are separated by adsorption partition.[18] Alcohols and glycols are neutral compounds and therefore not subject to Donnan exclusion. Organic acids are protonated by the strong acid eluent to neutral compounds. The stronger acids elute earlier than the weaker acids, therefore the compounds elute in the order of their pKa. Carbohydrates are typically neutral aliphatic or cyclic poly-hydroxyl compounds. Most of the carbohydrates have little adsorption to the column and are found in the exclusion volume. Smaller linear carbohydrates are slightly adsorbed and elute early. Thus, ion-exclusion chromatography excludes strongly ionic compounds, dissacharides, polysaccharides, and some hexoses and pentoses that are typically present in high concentrations in the sample matrix. This allows better resolution of alcohols and glycols in the sample.

Figure 2 shows the separation of a *meso*-erythritol [$C_4H_6(OH)_4$], glycerol [$C_3H_5(OH)_3$], propylene glycol [$C_3H_6(OH)_2$], methanol (CH_3OH), ethanol (C_2H_5OH), and isopropanol (C_3H_7OH) standard in deionized water using 100 mM MSA. Generally, the steric interferences of the hydroxyl group(s) have a stronger influence than compound size on the elution order. That is, alditols with six hydroxyl groups elute first, followed by alditols with five and then four hydroxyl groups, glycols with three and then two hydroxyl groups, and alcohols with one hydroxyl group. Within the group, the compounds elute from most polar to least polar. For example, the alcohols elute in the following order: methanol, ethanol, and propanol.

		Peaks:		
Column:	Dionex IonPac ICE-AS1 (4 mm)			
Eluent:	100 mM methanesulfonic acid		1. Exclusion volume	— µM
Temperature:	30 °C		2. *meso*-Erythritol	200
Flow Rate:	0.2 mL/min		3. Glycerol	200
Inj. Volume:	10 µL		4. Propylene glycol	200
Detection:	PAD, Pt (disposable)		5. Methanol	200
			6. Ethanol	200
			7. Isopropanol	200

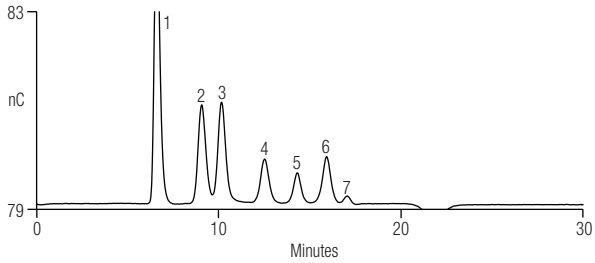

Figure 2. Separation of *meso*-erythritol, glycerol, propylene glycol, ethanol, methanol, and isopropanol.

The separation of a seven alditol standard (D-ribitol, D-sorbitol, L-arabitol, D-mannitol, galactitol, *myo*-inositol, *meso*-erythritol, 50 µM each) was evaluated to determine method suitability for alditol determinations. As expected, all of the alditols eluted early and were poorly resolved (not shown) from the other enantiomers with the exception of *meso*-erythritol. *Myo*-Inositol (8.0 min) was barely resolved from the exclusion volume peak (7.0 min), followed by D-mannitol (8.3 min), D-sorbitol and galactitol (8.4 min), D-ribitol (8.6 min), L-arabitol (8.8 min), and *meso*-erythritol (9.2 min). This method can detect alditols but cannot resolve them with this separation. An HPAE-PAD carbohydrate method with a gold working electrode has already established acceptable resolution and quantification for alditols and should be used for their determination.[5]

The separation of ethylene glycol and propylene glycol in 25, 50 and 100 µM standards was also evaluated. Ethylene glycol and propylene glycol have a 0.4 min difference in retention time, 12.1 and 12.5 min, respectively (not shown), and they co-elute as a bimodal peak. When both are present, the bimodal peak can be forced into two separate peaks, allowing the quantification of each peak. This practice does increase the uncertainty associated with the two determined values. Ethylene glycol, propylene glycol, and diethylene glycol can be fully resolved by combining an ion-exclusion guard column (Dionex IonPac ICE-AS1, 2 × 50 mm as the guard column) with cation-exchange separation (Dionex IonPac CS14, 2 × 250 mm), using the same eluent conditions, waveform, and Pt working electrode conditions, as described in this application.[10]

Waveform

Alcohols and glycols are detected with a three-potential waveform, using E_1, E_2, and E_3. These voltages are applied at the designated times during a 1.07-min waveform. E_1, the initial potential, is + 0.30 V vs Ag/AgCl and maintained from 0.00 to 0.31 min. E_2 is the oxidation cleaning potential, +1.15 V vs Ag/AgCl from 0.32 to 0.64 min with detection (integration of current) occurring from 0.64 to 0.66 min. E_3 is the reductive cleaning potential, -0.30 V vs Ag/AgCl from 0.67 to 1.06 min. At 1.06 to 1.07 min, the potential reverts to the E_1 potential. Most of the period at E_2 and all of E_3 clean and restore the working electrode. The detection and integration occurs at the end of E_2.

Method Qualification

Prior to determining *meso*-erythritol, glycerol, propylene glycol, and ethanol concentrations in a fermentation broth, the alcohol and glycol method was qualified by determining linearity over a 250-fold concentration range, typical noise, estimated limits of detection, reproducibility, and robustness. The linearity of peak response was determined by measuring *meso*-erythritol, glycerol, propylene glycol, and ethanol in five replicates each of nine standards (25, 50, 100, 200, 400, 800, 1600, 3200, and 6400 µM). The calibration results showed a quadratic relationship for *meso*-erythritol, glycerol, and propylene glycol and a linear relationship for ethanol over this concentration range, $r^2 > 0.999$ (not shown). *Meso*-erythritol, glycerol, and propylene glycol exhibit linear behavior from 25 to 2000 µM.

The noise over two 60 min runs, when no sample was injected, was determined for each of three disposable electrodes by measuring the noise in 1-min intervals from 5 to 60 min. The noise value determined by this experiment was 8.0 ± 2.7 pC (n=10). The noise was similar for all three disposable platinum working electrodes. The method detection limit (MDL) was defined as the standard with the peak height three times the noise level. For this application with a 10 µL injection, the estimated limits of detection for *meso*-erythritol, glycerol, propylene glycol, and ethanol were 1.9, 1.6, 4.2, and 4.5 µM, or 2, 2, 4, and 2 ng, respectively. The signal-to-noise ratios for *meso*-erythritol, glycerol, propylene glycol, and ethanol in the 25 µM combined standard were 11.6 ± 0.1, 13.4 ± 0.2, 5.5 ± 0.1, and 4.4 ± 0.1 (n=5), respectively.

Before determining reproducibility and robustness, the 200-, 300-, 400-, 500-, and 1000-fold dilution levels of a heat-quenched, American Wheat *S. cerevisiae* fermentation broth were evaluated. The results showed that the 500- and 1000-fold dilutions had small and poorly defined peaks, while the 200-fold dilutions had column overload. The 300-fold and 400-fold dilutions exhibited the best chromatography, and the 300-fold dilution was selected for the fermentation experiments (Figure 3).

		Peaks:	
Column:	Dionex IonPac ICE-AS1 (4 mm)	1. Exclusion volume	— µM
Eluent:	100 mM methanesulfonic acid	2–3. Unknown	—
Temperature:	30 °C	4. Glycerol	—
Flow Rate:	0.2 mL/min	5–6. Unknown	—
Inj. Volume:	10 µL	7. Ethanol	26
Detection:	PAD, Pt (disposable)	8. Unknown	—
Sample Prep:	Supernatant from heat-quenched, centrifuged broth, diluted 300-fold		

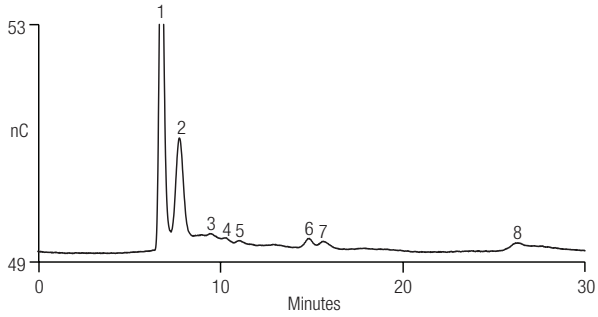

Figure 3. American wheat S. cerevisiae fermentation broth.

The retention time and peak area reproducibilities of glycerol, propylene glycol, and ethanol were measured over 250 injections (~125 h) to determine the reproducibility and robustness of the method. A 300-fold dilution of heat-quenched American Wheat beer *S. cerevisiae* in Bacto YPD growth medium without incubation was spiked with 50 µM glycerol, propylene glycol, and ethanol and was analyzed. One deionized water injection was inserted between groups of six sample injections. The results (Figures 4–5) showed that retention time and peak areas were stable over the entire experiment (125 h). The peak areas for glycerol, propylene glycol, and ethanol had small negative drifts over the five days, -0.2, -4.3, and -1.3%, respectively. The peak areas were also affected when the eluent was changed during the experiment (Figure 5). To determine single day reproducibility, peak areas were averaged for each spiked compound, not including the data immediately after the eluent change. These calculations showed that the peak area reproducibilities were less than 2% RSD for glycerol and ethanol, and less than 8% RSD for propylene glycol (Table 3).

Table 3. Five days peak area reproducibilities for glycerol, propylene glycol, and ethanol spiked into 300-fold diluted sample *S. Cervisiae* incubated in Bacto YPD fermentation broth.

Day	Glycerol (nC-min)	Propylene Glycol (nC-min)	Ethanol (nC-min)
1	0.193 ± 0.003	0.073 ± 0.005	0.457 ± 0.006
2	0.200 ±0.004	0.074 ± 0.005	0.453 ±0.008
3	0.196 ± 0.003	0.073 ± 0.004	0.454 ± 0.006
4	0.195 ± 0.004	0.071 ± 0.006	0.452 ± 0.006
5	0.193 ± 0.004	0.070 ± 0.005	0.451± 0.006

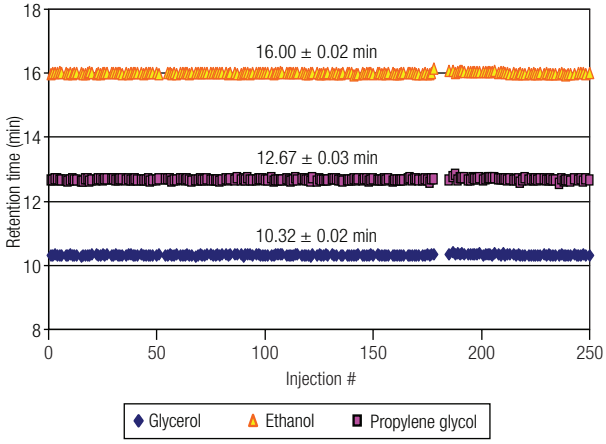

Figure 4. Retention time stability of 50 µM glycerol, propylene glycol, and ethanol spiked into a 300-fold dilution of the supernatant from heat quenched and centrifuged American Wheat S. cerevisiae incubated in Bacto YPD.

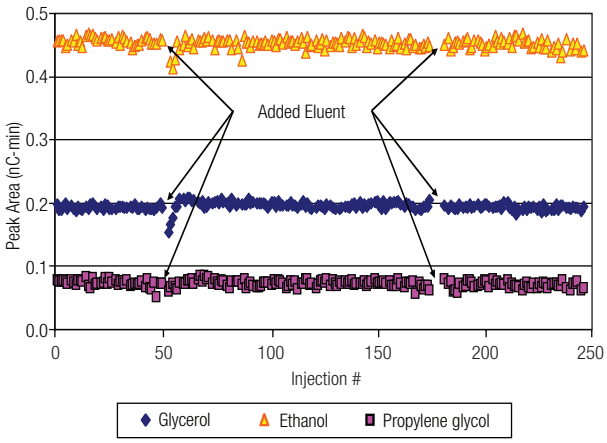

Figure 5. Peak area stability of 50 µM glycerol, propylene glycol, and ethanol spiked into a 300-fold dilution of the supernatant from heat quenched and centrifuged American Wheat *S. cerevisiae* incubated in Bacto YPD.

Disposable Platinum Working Electrodes

To determine the life of the platinum working electrodes with this method and waveform, the peak responses of glycerol, propylene glycol, and ethanol were determined in a 50 µM standard during the course of the experiments. Three disposable platinum working electrodes from the same lot were evaluated. Original response was measured after the newly installed electrode was allowed to equilibrate for 1 h. The working electrode was considered unacceptable and replaced with a fresh electrode when the peak response fell to 80% of the original response. The three working electrodes had similar initial responses for glycerol (0.1931 ± 0.004, 0.1891 ± 0.013, and 0.1940 ± 0.002 nC-min), propylene glycol (0.0720 ± 0.005, 0.0730 ± 0.003, and 0.0720 ± 0.003 nC-min), and ethanol [0.4564 ± 0.007, 0.4558 ± 0.007, and 0.4543 ± 0.006 nC-min (n=5)]. The electrodes showed good reproducibility within the same lot, <1.4% RSD. All three electrodes exceeded the two-week specification.

Fermentation Broth Samples

German Ale and Bohemian Lager *S. cerevisiae* were evaluated for the presence of *meso*-erythritol, glycerol, propylene glycol, and ethanol after incubation for 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 22, 23, 24, 25, 26, 27, and 28 h in Bacto YPD medium at 37 °C. *Meso*-Erythritol and propylene glycol were not detected at any time during the fermentation. Figures 6 and 7 show the total concentration of glycerol and ethanol over the 28 h. Both fermentation broths had similar growth curves for glycerol and ethanol. As expected, the ethanol concentration grew exponentially. The glycerol concentration was below the quantification level of the 400-fold dilution incubation samples from the start of the incubation to 10 h. When the next samples were taken at 22 and 23 h, the glycerol concentration had increased to 21.0 ± 0.2 and 40.4 ± 0.8 µM in the 300-fold dilution German ale and Bohemian lager *S. cerevisiae* fermentation broths, respectively (Figure 8).

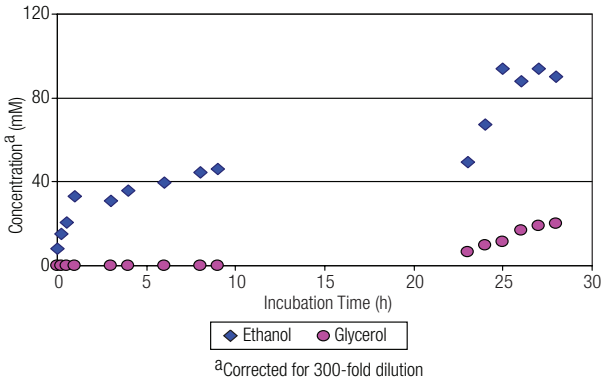

Figure 6. Total ethanol and glycerol concentrations during incubation of German ale S. cerevisae in Bacto YPD growth medium.

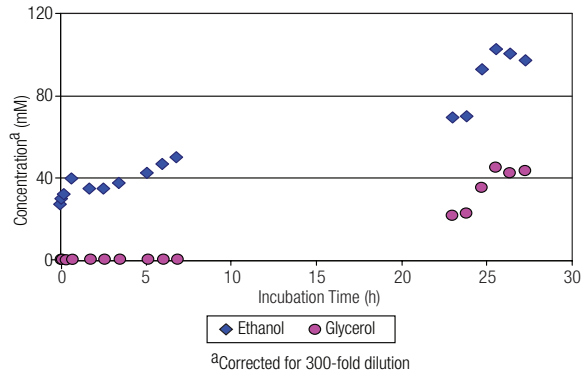

Figure 7. Total ethanol and glycerol concentrations during incubation of Bohemian lager *S. cerevisae* in Bacto YPD growth medium.

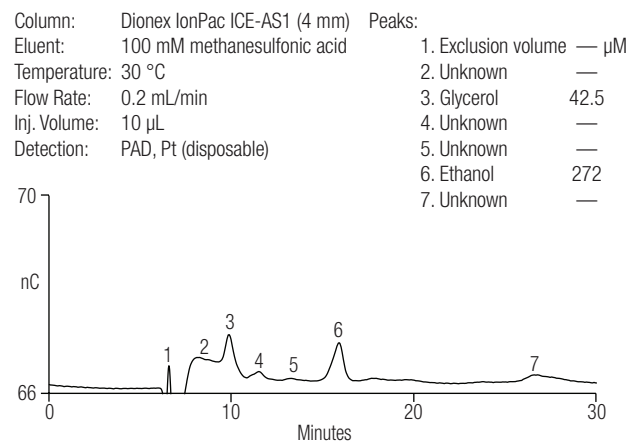

Figure 8. Bohemian lager S. cerevisae incubated in Bacto YPD medium for 22 h.

Table 4. Recovery of glycerol, propylene glycol, and ethanol spiked into 300-fold diluted samples of *S. Cervisiae* Incubated in Bacto YPD fermentation medium.

		Glycerol			Propylene Glycol			Ethanol		
***S. Cerevisiae* Sample**	**Incubation (h)**	**Unspiked (µM)**	**Spiked[a] (µM)**	**Recovered (%)**	**Unspiked (µM)**	**Spiked[a] (µM)**	**Recovered (%)**	**Unspiked (µM)**	**Spiked (µM)**	**Recovered[a] (%)**
Brand A German Ale	5	ND	55.8 ± 1.8	99.8	ND	105.9 ± 2.5	96.2	291.4 ± 0.7	375.4 ± 3.1	94.5
Brand A German Ale	26	55.3 ± 0.1	112.4 ± 1.0	101.1	ND	107.9 ± 1.6	98.0	293.8 ± 6.3	402.3± 0.6	100.6
Brand A Bohemian Lager	7	ND	56.1 ± 0.5	100.4	ND	105.2 ± 2.7	95.5	347.9 ± 1.3	442.9 ± 1.3	97.6
Brand A Bohemian Lager	27	120.3 ± 2.3	178.5 ± 2.4	101.3	ND	104.6 ± 1.0	95.0	358.6 ± 2.6	456.3 ± 1.4	97.4

n = 2 for each sample.

[a]Added 55.9 ± 0.2 µM glycerol, 110.1 ± 1.4 µM propylene glycol, and 106.0 ± 1.3 µM ethanol for spike recovery experiments

To determine recovery of glycerol, propylene glycol, and ethanol in broths that had undergone active fermentation, we spiked 60 mM glycerol and 100 mM propylene glycol and ethanol in 300-fold diluted, centrifuged, and heat-quenched samples from German ale and Bohemian lager (Figure 9) *S. cerevisiae* incubated 5 and 26 h, and 7 and 27 h, respectively. The results (Table 4) show good recoveries for glycerol, propylene glycol, and ethanol in the range of not detected to 180 µM glycerol, not detected to 55 µM propylene glycol, and 300 to 400 µM ethanol.

Beverage Samples

This chromatographic method was also applied to two California wine samples (Chardonnay and Cabernet Sauvignon) and three beer samples (American hefeweizen, German lager, and British ale). To evaluate the optimum dilution, 300-, 400-, 500-, and 1000-fold dilutions of the Chardonnay and British ale samples were tested. Both beverages had a large ethanol peak regardless of dilution. The glycerol was at a measurable concentration in the 300- and 400-fold dilution Chardonnay samples and below the limit of quantification at all tested dilution levels for the British ale. To quantify the glycerol in the wine samples and to minimize column overload, the 400-fold dilution concentration was selected for the beverage samples.

		Peaks:	A	B
Column:	Dionex IonPac ICE-AS1 (4 mm)			
Eluent:	100 mM methanesulfonic acid	1. Exclusion volume	—	— µM
Temperature:	30 °C	2. Unknown	—	—
Flow Rate:	0.2 mL/min	3. Glycerol	120	180
Inj. Volume:	10 µL	4. Unknown	—	—
Detection:	PAD, Pt (disposable)	5. Propylene glycol	ND	105
Sample:	(A) 300-fold dilution broth	6. Ethanol	359	454
	(B) Spiked with 56 µM glycerol, 100 µM propylene glycol and ethanol	7. Unknown	—	—

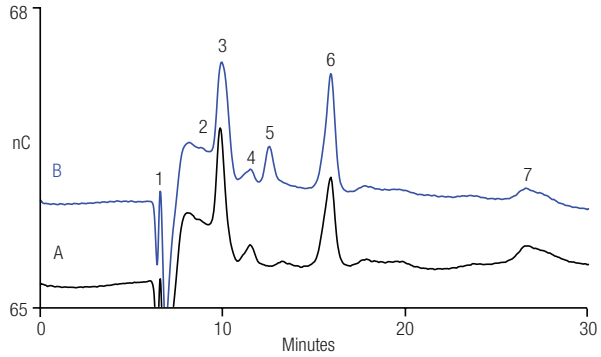

Figure 9. Bohemian lager S. cerevisae incubated in Bacto YPD medium for 27 h.

Table 5. Recovery of 40 µM glycerol and 400 µM ethanol spiked into 400-fold diluted samples of wine and beer.

	Glycerol			Ethanol		
400-fold Diluted Beverage Sample	Unspiked (µM)	Spiked (µM)	Recovered (%)	Unspiked (µM)	Spiked (µM)	Recovered (%)
Domestic Chardonnay	399 ± 4	430 ± 5	97.9	6799 ± 67	7183 ± 51	99.8
Domestic Carbernet Sauvignon	374 ± 6	409 ± 6	98.8	6391 ± 49	6801 ± 114	100.1
German Lager	ND	39.9 ± 2	99.8	2257 ± 32	2671 ± 26	100.5
Domestic Hefeweizen	ND	39.6 ± 1	100.5	2365 ± 78	2765 ± 14	100.0
British Brown Ale	ND	40.5 ± 1	101.3	1924 ± 34	2296 ± 43	98.9

To evaluate the recovery of glycerol and ethanol in the 400-fold dilution of the five wine and beer samples, 40 µM glycerol and 400 µM ethanol were spiked into each and the concentrations of glycerol and ethanol determined in both the unspiked and spiked diluted beverage samples. The results (Table 5) show good recovery for glycerol and ethanol for all samples, ranging from 93.3 to 109.6%. Figures 10 and 11 show the chromatograms of a 400-fold dilution of British ale spiked with 40 µM glycerol and 400 µM ethanol and a 400-fold dilution of Chardonnay without additional glycerol and ethanol.

Conclusion

The method presented in this application note can determine glycols and alcohols in beverages and complex matrices such as fermentation broths using ion-exclusion chromatography and PAD with a waveform optimized for a Pt disposable working electrode. This method offers a sensitive direct detection of alcohols without the need for sample preparation other than dilution and centrifugation.

Many of the compounds of interest in fermentation broths can now be determined using methods described in Thermo Scientific application notes: anions and organic acids [Application Note 123 (AN 123)],[6] carbohydrates and alditols (AN 117 and AN 122),[8,5] amino acids (AN 150),[7] cations using an method developed for water analysis (AN 141),[11] and glycols and alcohols using this note.

Precautions

The Dionex IonPac ICE-AS1 column should not be used with system backpressures greater than 1000 psi. The eluent generator requires a system backpressure greater than 2000 psi and, therefore, cannot be used as the eluent source for this application. Do not remove or install the ED module while the DC module is turned on. These power surges could cause internal damage to the ED module. Glass vials and bottles are required for this application.

		Peaks:		
Column:	Dionex IonPac ICE-AS1 (4 mm)		1. Exclusion volume	— µM
Eluent:	100 mM methanesulfonic acid		2. Unknown	—
Temperature:	30 °C		3. Glycerol	39.9
Flow Rate:	0.2 mL/min		4–6. Unknown	—
Inj. Volume:	10 µL		7. Ethanol	2671
Detection:	PAD, Pt (disposable)			
Sample:	Supernatant of degassed and centrifuged sample			

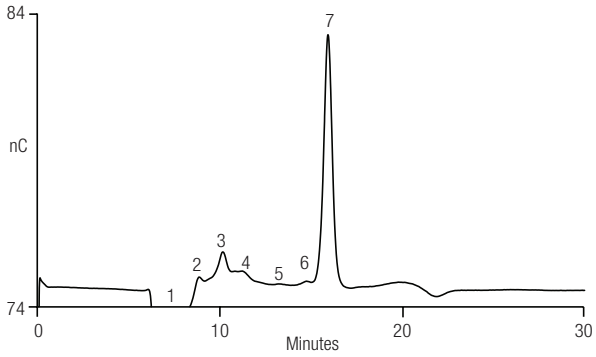

Figure 10. 40 µM glycerol and 400 µM ethanol spiked into a 400-fold dilution of German lager.

		Peaks:		
Column:	Dionex IonPac ICE-AS1 (4 mm)		1. Exclusion volume	— µM
Eluent:	100 mM methanesulfonic acid		2-4. Unknown	—
Temperature:	30 °C		5. Glycerol	399
Flow Rate:	0.2 mL/min		6. Unknown	—
Inj. Volume:	10 µL		7. Ethanol	6799
Detection:	PAD, Pt (disposable)			
Sample:	Supernatant of centrifuged sample, 400-fold dilution			

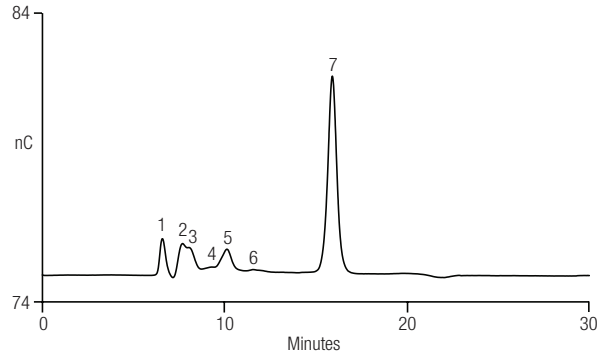

Figure 11. Glycerol and ethanol in Chardonnay.

References

1. Slator, A. The Rate of Fermentation by Growing Yeast Cells. *Biochem. J.,* **1913**, *7* (2), 197–203. [Online] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1276459/ (accessed Aug 7, 2015).
2. Hough, J. S. *The Biotechnology of Malting and Brewing*; Cambridge University Press: New York, 1985. [Online] http://www.cambridge.org/us/academic/subjects/life-sciences/biotechnology/biotechnology-malting-and-brewing (accessed Aug 7, 2015).
3. Fix, G., Ph.D. *Principles of Brewing Science,* 2nd ed.; Brewers Publications: Boulder, CO, 1999. [Online] http://www.wiley.com/WileyCDA/WileyTitle/productCd-3527287019.html (accessed Aug 7, 2015).
4. Weiss, J. Ion Chromatography in the Pharmaceutical Industry. In *Handbook of Ion Chromatography,* 3rd ed.; Wiley-VCH, Weinheim, Germany, 2004; pp 772–781. [Online] http://www.wiley.com/WileyCDA/WileyTitle/productCd-3527287019.html (accessed Aug 7, 2015).
5. Thermo Fisher Scientific. *Determination of Carbohydrates, Alcohols, and Glycols in Fermentation Broths*; Application Note 122, Part Number AN70494, Sunnyvale, CA, 2004. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/AN122-IC-Carbohydrates-Alcohol-Glycol-Fermentation-Broth_AN70494_E.pdf (accessed Aug 7, 2015).
6. Thermo Fisher Scientific. *Determination of Inorganic Anions and Organic Acids in Fermentation Broths*; Application Note 123, Sunnyvale, CA, 2004. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/GC%20HPLC%20and%20UHPLC%20Columns%20and%20Accessories/Chromatography%20Column%20Accessories/4082-AN123_LPN1030_2.pdf (accessed Aug 7, 2015).
7. Thermo Fisher Scientific. *Determination of Amino Acids in Cell Cultures and Fermentation Broths*; Application Note 150, Sunnyvale, CA, 2003. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Columns/4481-AN150_LPN1538.pdf (accessed Aug 7, 2015).
8. Thermo Fisher Scientific. *Quantification of Carbohydrates and Glycols in Pharmaceuticals*; Application Note 117, Part Number AN70233, Sunnyvale, CA, 2000. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Columns/AN-117-IC-Carbohydrates-Glycols-Pharmaceuticals-AN70233-EN.pdf (accessed Aug 7, 2015).
9. *Health Implications of Perchlorate Ingestion*; Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies, National Academies Press: Washington, D. C., 2005. [Online] http://www.nap.edu/openbook.php?isbn=0309095689 (accessed Aug 7, 2015).
10. Cheng, J.; Jandik, P.; Liu, X.; Pohl, C. *New Waveform for Direct and Sensitive Amperometric Detection of Alcohols, Alkenes, Alkynes, Aldehydes, Carboxylic Acids, Ethers, Ketones, Nitriles, Phenols, Thioethers, and Sulfoxides with Disposable Platinum Electrodes*. Paper presented at the 19th Annual International Ion Chromatography Symposium (IICS 2006), Pittsburgh, PA, 2006.
11. Thermo Fisher Scientific. *Determination of Inorganic Cations and Ammonium in Environmental Waters by Ion Chromatography Using the Dionex IonPac CS16 Column*; Application Note 141, Part Number AN70395, Sunnyvale, CA, 2001. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/app/chrom/ic/col/AN-141-IC-Inorganic-Cations-Ammonium-Waters-IonPac-CS16-AN71549-EN.pdf (accessed Aug 7, 2015).
12. Thermo Fisher Scientific. *Operator's Manual for Dionex ICS-3000 Ion Chromatography System*; Document No. 065031: Sunnyvale, CA, 2005. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/Chromatography/Liquid%20Chromatography/Liquid%20Chromatography%20Modules/Column%2Compartments/48428-Man-IC-ICS-3000-System-Operators-2008-01-065031-04.pdf (accessed Aug 7, 2015).

13. Thermo Fisher Scientific. *Installation Manual for Dionex ICS-3000 Ion Chromatography System*; Document No. 065032: Sunnyvale, CA, 2005. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Accessories/57781-Man-IC-ICS3000-Installation-2008-01-065032-04.pdf (accessed Aug 7, 2015).

14. Thermo Fisher Scientific. *Operator's Manual for Dionex AS Autosampler*; Document No. 065051: Sunnyvale, CA, 2005. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Modules/IC%20and%20RFIC%20Autosamplers%20and%20Injectors/57769-Man-IC-AS-Operators-2008-01-065051-03.pdf (accessed Aug 7, 2015).

15. Thermo Fisher Scientific. *Product Manual for Dionex IonPac ICE-AS1*; Document No. 031181: Sunnyvale, CA, 2006. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Columns/4341-031181-07_Manual_ICE-AS1_V30.pdf (accessed Aug 7, 2015).

16. Thermo Fisher Scientific *Product Manual for Disposable Electrodes*; Document No. 065040: Sunnyvale, CA, 2006.

17. Thermo Fisher Scientific. *Installation Guide for Disposable Platinum Electrode for ED (Dionex ICS-3000)*; Document No. 065139: Sunnyvale, CA, 2006. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/quickstart/QS-065139-Disposable-Platinum-Electrode-ED-ICS-3000-QS065139-EN.pdf (accessed Aug 7, 2015).

18. Weiss, J., Ion-Exclusion Chromatography (HPICE). In *Handbook of Ion Chromatography,* 3rd ed.; Wiley-VCH, Weinheim, Germany, 2004; pp 359–391. [Online] http://www.wiley.com/WileyCDA/WileyTitle/productCd-3527287019.html (accessed Aug 7, 2015).

Suppliers

BD Laboratories, 1 Becton Drive, Franklin Lakes, NJ 07417 USA, 1-201-847-6800

www.bd.com

Fermentation Solutions, 2511 Winchester Blvd, Campbell, CA 95008 USA, 1-408-871-1400

www.fermentationsettlement.com

Fisher Scientific, part of Thermo Fisher Scientific, Liberty Lane, Hampton, NH 03842 USA, 1-800-766-7000

www.fisherscientific.com

HopTech Homebrewing Supplies, 6398 Dougherty Road, #7, Dublin, CA 94568 USA, 1-925-875-0246

http://www.hoptech.com

Sarstedt Inc., 1025, St.James Church Road, P.O. Box 468, Newton, NC 28658-0468 USA, 1-828-465-4000

www.sarstedt.com

Sigma-Aldrich Corp., St. Louis, MO 63103 USA 1-800-325-3010

www.sigmaaldrich.com

U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD, 20852-1790 USA, 1-800-227-8772

www.usp.org

VWR International, Inc., Goshen Corporate Park West, 1310 Goshen Parkway, West Chester, PA 19380 USA, 1-800-932-5000

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