

Determination of Ethylene Glycol and Diethylene Glycol in a Sorbitol Solution

INTRODUCTION

Glycerin, sorbitol, and propylene glycol (PG) are widely used in pharmaceuticals and personal care products, such as toothpaste, mouth wash, and medicinal syrups.¹⁻⁴ Adulteration of products containing glycerin or PG with the less expensive and highly toxic diethylene glycol (DEG) has been reported in several countries.^{3,5} DEG and ethylene glycol (EG) are known poisons that are commonly used as industrial solvents and in antifreeze solutions, and therefore should not be found in pharmaceutical formulations. Ingestion or application of DEG-contaminated products, such as toothpaste and medicinal syrups (e.g., cough, teething, and acetaminophen syrups)^{1-4, 6} is known to have caused systemic alcohol intoxication, acidosis, and subsequent multiorgan failure, leading to hundreds of fatalities.^{5,6}

Glycerin, sorbitol, DEG, and EG have similar physical properties, such as sweetness and viscosity, which facilitates the adulteration process. Additionally, inconsistent or improper labeling of globally manufactured raw materials (DEG labeled as glycerin, industrial-grade glycerin labeled as pharmaceutical-

grade glycerin) combined with inadequate international regulations^{3,5} and analytical methods^{2,3,8} increase the risk of contamination. To help prevent future adulteration, the U.S. FDA has classified sorbitol solution and its related excipients as being at high risk for contamination and requested the United States Pharmacopeia (USP) to revise the USP 32 NF 26–27 monographs to identify and quantify EG and DEG in PG, sorbitol, and glycerin solutions.^{8,9} Based on FDA recommendations and CDC estimations of potential DEG contamination of 0.1 to 12.7% in pharmaceutical formulations using liquid excipients, the FDA proposed a limit of 0.1% DEG in sorbitol and other excipient solutions.^{1,3,9} Therefore, the USP revised the sorbitol solution monograph to meet this requirement.⁹ In this monograph, gas chromatography with flame-ionization detection is used to assay 0.08 mg/mL EG and DEG in 80 mg/mL sorbitol solution, uncorrected for sorbitol assay i.e., 0.1% EG and DEG in sorbitol solution.^{1,9} To minimize the possibility of false positives, a second confirmatory method is needed to verify the presence of EG and DEG in the sample.

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The application note presented here describes two confirmatory methods to determine 0.1% EG and DEG in sorbitol solutions by liquid chromatography with pulsed amperometric detection (PAD). One method separates EG by high-performance anion-exchange chromatography (HPAE) using sodium hydroxide (NaOH) on a CarboPac® MA1 column set with PAD using a Au on PTFE-working electrode and a four-potential carbohydrate waveform. The second method separates DEG in the sorbitol solution using a mixed-mode separation with an ion-exclusion (ICE) guard column and a cation-exchange analytical column with reversed-phase properties using a methanesulfonic acid (MSA) eluent.¹⁰ The analytes are detected by PAD with a Pt working electrode and glycol waveform. AN 246 presents method qualifications and the analysis of EG and DEG in a sorbitol solution using the described methods. The technique presented here takes advantage of the dual-pump capabilities, which enables the sequential operation of the two methods for the accurate, precise, and direct determination of 0.1% DEG and EG in a sorbitol solution.

EQUIPMENT

Dionex ICS-3000 Ion Chromatography system consisting of:

DP Dual-Gradient Pump module to sequentially run both methods

DC Detector/Chromatography Module

AS Autosampler with Diverter Valve option (P/N 063294) to sequentially run both methods and 1.5 mL sample tray

ED Electrochemical Detector (P/N 061718)

Electrochemical cell (cell and reference electrode, P/N 061756)

Combination pH–Ag/AgCl reference electrode (P/N 061879)

Chromleon® 6.8 Chromatography Data System

Knitted reaction coil, 375 µL (P/N 043700) for each method

Filter unit for vacuum filtration, 0.2 µm nylon (Nalgene® Media-Plus with 90 mm filter, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter

Vortex mixer to mix standard and sample solutions.

Vial Kit, 1.5 mL glass sample vials, with caps and slit septa (vial kit, P/N 055427)

Vacuum pump

PEEK™ Tubing:

Black (0.25 mm or 0.01 in i.d., P/N 052307 for 5 ft) tubing used for liquid line connections from the pumps to the injection valve.

Red (0.127 mm or 0.005 in i.d., P/N 052310 for 5 ft) tubing used for liquid line connections from injection valve to the guard and analytical columns, and cell.

Blue (0.33 mm or 0.013 in i.d., P/N 052303 for 5 ft; in Diverter Valve kit)

25 µL PEEK sample loop (P/N 042857) for each method

Method 1: Determination of Ethylene Glycol in Sorbitol

Au on PTFE disposable working electrode (P/N 066480 package of six)

Method 2: Determination of Diethylene Glycol in Sorbitol

Type GL45, 2 L glass eluent bottle (P/N 045901)

Pt disposable working electrode (P/N 064440 package of six)

REAGENTS AND STANDARDS

Deionized water, Type 1 reagent-grade, 18.2 MΩ-cm resistivity, freshly degassed by ultrasonic agitation and applied vacuum

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

pH 7 (yellow) buffer solution (VWR International, P/N BDH5046)

pH 4 (red) buffer solution (VWR International, P/N BDH5018)

Diethylene glycol (Sigma-Aldrich, P/N 32160)

Ethylene glycol (Sigma-Aldrich, P/N E9129)

Method 1: Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

Method 2: Methanesulfonic acid (Fluka P/N 64280)

Standards for retention time studies only:

Propylene glycol (1, 2 propanediol, Sigma, P/N P6209)

Glycerin (VWR International, P/N JT2140)

SAMPLE

Sorbitol Solution, 67% assay, USP grade (VWR International, P/N 89050, Spectrum Chemical Mfg. Corporation)

CONDITIONS

Method 1: Ethylene Glycol in Sorbitol

Column: CarboPac® MA1 Guard,
4 × 50 mm (P/N 044067),
CarboPac MA1 Analytical
4 × 250 mm (P/N 044066)

Flow Rate: 0.4 mL/min

Eluent: 200 mM Sodium hydroxide

Source: 60% Eluent A, deionized degassed
water; 40% Eluent B, 500 mM
NaOH

Column Temp.: 30 °C

Inj. Volume: 25 µL

Detection: PAD, disposable Au on PTFE

Data Collection Rate: 2.0 Hz

Waveform: See Table 1

Reference Electrode: AgCl mode

Typical Background: 40–60 nC

Typical System

Backpressure: 1250 psi

Typical Noise: 60–80 pC

Typical pH: 12.5–12.9

Run Time: 60 min

**Table 1. Carbohydrate Four-Potential
Detection Waveform¹**

Time (s)	Potential vs Ag/AgCl (V)	Gain Region ^a	Integration	Ramp ^a
0.00	+ 0.10	Off	Off	Ramp
0.20	+ 0.10	On	On (Start)	Ramp
0.40	+ 0.10	Off	Off (End)	Ramp
0.41	- 2.00	Off	Off	Ramp
0.42	- 2.00	Off	Off	Ramp
0.43	+ 0.60	Off	Off	Ramp
0.44	- 0.10	Off	Off	Ramp
0.50	- 0.10	Off	Off	Ramp

^aThe gain and ramp are instrument settings for the ICS-3000 IC electrochemical detector.

Method 2: Diethylene Glycol in Sorbitol

Column: IonPac® ICE-AS1 Guard,
4 × 50 mm (P/N 067842)
IonPac CS14 Analytical,
2 × 250 mm (P/N 044121)

Flow Rate: 0.2 mL/min

Eluent: 100 mM MSA

Column Temp.: 30 °C

Inj. Volume: 25 µL

Detection: PAD, disposable platinum

Data Collection Rate: 0.9 Hz

Waveform: See Table 2

Reference Electrode: AgCl mode

Typical Background: 90–110 nC

Typical System

Backpressure: 900 psi

Typical Noise: 40–70 pC

Typical pH: 0.9–1.1

Run Time: 17 min

**Table 2. PAD Detection Waveform
Optimized for Glycols in Acid Eluents¹²**

Time (s)	Potential vs Ag/AgCl (V)	Gain Region ^a	Integration	Ramp ^a
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

^aThe gain and ramp are instrument settings for the ICS-3000 IC electrochemical detector.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solutions

When preparing eluents, it is essential to use high-quality, Type 1, 18.2 M Ω -cm resistivity deionized water that is free of carbon dioxide. Carbon dioxide dissolved in water produces carbonate, which adversely affects chromatography when using hydroxide eluents. In addition, dissolved gases also cause increased noise. Degas the deionized water before eluent preparation by using vacuum filtration and ultrasonic agitation with applied vacuum for 10 to 20 min.^{13,14} Also, prepare 1 L of degassed Type 1 water weekly for the AS Autosampler flush solution.

Method 1: Eluent A Solution (Degassed Deionized Water)

Connect the eluent bottle to the Eluent A line from the pump and apply ~4–5 psi of head pressure using nitrogen or another inert gas, and prime the pump with the new eluent.

Method 1: Eluent B Solution, 500 mM NaOH

It is essential that high-purity (Fisher) 50% NaOH is used for eluent preparation. Do not use NaOH pellets because they are coated with sodium carbonate; this carbonate will bind to the column and interfere with the column selectivity, resolution, and efficiency.¹³

To prepare 2 L of 500 mM NaOH, add 1947.6 g of degassed, deionized water into a 2 L precleaned eluent bottle. Do not use glass bottles with hydroxide eluents because this can foul the working electrode. Use a top loader balance that is accurate to ± 0.01 g. Using the 25 mL transfer pipette, position the pipette in the center of the bottle and transfer 80 g (52.4 mL) of 50% NaOH solution to the 2 L eluent bottle. Immediately close and cap the hydroxide bottle and eluent bottle. Connect the eluent bottle to the Eluent B line from the pump and place the bottle under ~4–5 psi of head pressure using nitrogen or another inert gas. Swirl the eluent bottle to thoroughly mix the eluent, and prime the pump. For additional information on eluent preparation, please refer to Technical Note 71.¹⁴

Method 1: 2 M NaOH for System Decontamination

Prepare 1 L of 2 M NaOH similar to the 500 mM NaOH eluent using 160 g (105 mL) of 50% sodium hydroxide solution and 895 g of degassed, deionized water into a 1 L precleaned eluent bottle. Connect the eluent bottle to the Eluent C line from the pump and place the bottle under ~4–5 psi of head pressure using nitrogen or another inert gas. Swirl the eluent bottle to thoroughly mix the eluent, and prime the pump.

Method 2: Eluent A, 100 mM MSA

Prepare 2 L of degassed deionized water as previously described. Glass eluent bottles are recommended for this method to minimize noise and contamination from acid-extractable compounds from plastic containers. To prepare 2 L of 100 mM MSA eluent, pipette 13.0 mL (19.2 g) of MSA (FW 96.10) into a 2 L, type GL45 glass eluent bottle containing 1987 g of degassed, deionized water. Immediately cap the bottle, connect it to Pump 2 Eluent A line, place the eluent under ~4–5 psi of head pressure using nitrogen or another inert gas, thoroughly mix the eluent solution, and prime the pump.

Stock Solutions

Prepare the stock solutions monthly. Store at 5 °C.

Method 1: 100 mg/mL EG Stock Standard Solution

To prepare the 100 mg/mL EG stock solution from 99% EG solution, weigh 2 g (1.8 mL) of the 99% EG solution in a 20 mL glass scintillation vial containing 20 mL of deionized water. Mix the standard solution thoroughly using a vortex mixer.

Method 2: 100 mg/mL DEG Stock Standard Solution

Prepare the 100 mg/mL DEG stock solution in the same manner as 100 mg/mL EG stock solution using 99% DEG solution (2 g, 1.8 mL).

Stock Standards for Determining Glycol Selectivity

Prepare individual 100 mg/mL stock standards of PG and glycerin by adding 2 g (1.9 mL) of 99% PG and 2 g (1.6 mL) of 99% glycerin to 20 mL glass scintillation vials. Then, add 20 mL of deionized water to each vial.

Intermediate and Working Standard Solutions

Prepare the working standards daily and the intermediate standard weekly. Store at 5 °C.

Method 1: EG Intermediate and Working Standard Solutions

To prepare a 1.0 mg/mL EG intermediate standard solution, pipette 200 µL of 100 mg/mL EG into 19.8 mL of deionized water on an analytical balance and mix the solution with the vortex mixer. Prepare the 1.3, 2.5, 5.0, 10, and 20 µg/mL EG working standards by pipetting 26, 50, 100, 200, and 400 µL, respectively of the 1.0 mg/mL EG intermediate standard solution in deionized water to a total of 20.0 g. Mix with a vortex mixer. Prepare a 0.5 mg/mL EG spiking standard solution by diluting 5 mL of 1 mg/mL EG stock solution with 5 mL of deionized water, and mixing with a vortex mixer. Prepare the working standards and spiking standard solution daily, the intermediate standard weekly, and the stock standard monthly. Store at 5 °C.

Method 2: DEG Intermediate and Working Standard Solutions

To prepare a 1 mg/mL DEG intermediate standard solution, pipette 200 µL of 100 mg/mL DEG into 19.8 mL deionized water on an analytical balance. Mix with a vortex mixer. Prepare the 0.013, 0.025, 0.050, 0.10, and 0.025 mg/mL DEG working standards in a similar way with 260, 500, 1000, 2000, and 5000 µL, respectively of the 1.0 mg/mL DEG intermediate standard solution and deionized water to a total of 20.0 g, and mixing with a vortex mixer. Prepare a 5 mg/mL spiking standard solution by adding 1 mL of 50 mg/mL DEG stock solution into 9 mL deionized water and mixing the standard solution thoroughly.

SAMPLE PREPARATION

To prepare a stock solution containing 50 mg/mL sorbitol from the 67% sorbitol solution, add 746 mg of the 67% sorbitol solution to 10 mL of deionized water on an analytical balance. Use the vortex mixer to mix the solutions.

Method 1

To prepare the 1.3 mg/mL sorbitol sample for EG determinations, dilute the 50 mg/mL sorbitol solution 40-fold by pipetting 500 µL of the 50 mg/mL sorbitol into 19.5 mL of deionized water on an analytical balance. Mix thoroughly using a vortex mixer. To prepare a spike recovery sample of 1.3 µg/mL EG in 1.3 mg/mL sorbitol (0.1% w/w EG in sorbitol), pipette 52 µL of 0.5 mg/mL EG into 20 mL of 1.3 mg/mL sorbitol, and mix the final solution thoroughly with a vortex mixer.

Method 2

To prepare the 13 mg/mL sorbitol sample for DEG determinations, dilute the 50 mg/mL sorbitol solution four-fold by pipetting 5 mL of the 50 mg/mL sorbitol into 15 mL deionized water on an analytical balance and mix the final solution thoroughly with a vortex mixer. To prepare a spike recovery sample of 0.013 mg/mL DEG in 13 mg/mL sorbitol (0.1% (w/w) DEG in sorbitol), pipette 52 µL of 5 mg/mL DEG into 20 mL of 13 mg/mL sorbitol and mix the final solution thoroughly with a vortex mixer.

SYSTEM PREPARATION AND SETUP

Do not remove or install the ED while the DC is turned on as these power surges may cause internal damage to the ED module. To reduce analysis time, configure the ICS-3000 system to run both methods sequentially under separate timebases.

Configuring Virtual Channel to Monitor pH

The continuous monitoring of pH during sample analyses provides details on reference electrode drift and noise, and also confirms proper eluent preparation. To monitor the pH, follow the instructions in AN 188 to create a Virtual Channel using the Server Configuration program.¹² Each Virtual Channel must be assigned a unique name, such as *VirtualChannel_01* and *VirtualChannel_02* to prevent confusion in programming. Once configured, the pH virtual channel becomes one of the available signal channels.

Plumbing and Configuring the AS Autosampler

To run both systems sequentially, installation of a diverter valve in the AS Autosampler is required. To ensure accurate injections, calibrate the injection port tubing volume that now includes the diverter valve and the blue PEEK tubing according to the calibration instructions (Calibrate IPTV) under the AS Autosampler tab on the Chromeleon panel and in Section 5.9 in the AS Autosampler Operator's manual.¹⁵ After the calibration is complete, verify that the values for each system have been loaded in the AS Autosampler module. Change the mode to sequential and enter the sample loop size for both systems. The additional commands needed to control the position of the diverter valve, use of the AS Autosampler, and control of temperature are thoroughly discussed in Technical Note 64.¹⁶

Plumbing the Chromatography System

Method 1: General Tips

As a precaution to minimize microbial contamination, temporarily install a 1000 psi backpressure loop between the pump and the injection valve and pump 2 M NaOH through the pump and eluent lines at 0.5 mL/min for 1 to 2 h, followed by deionized water for another 1 to 2 h.¹³ Remove the backpressure loop prior to installing the column.

Method 1: Plumbing System 1

To plumb System 1 for EG determinations, install black PEEK tubing between the pump and injection valve and red PEEK tubing for all other eluent lines after the injection valve to the cell inlet. Follow the instructions in the product manual to install the CarboPac MA1 column set on System 1.¹⁷ Once installed, the expected system backpressure of the CarboPac MA1 column set is typically ~ 1300 psi. Install the 375 μ L knitted reaction coil between the outlet of the analytical column and the electrochemical cell as described in AN 188. Allow the column to equilibrate overnight using the method conditions.

Method 2: Plumbing System 2

To plumb System 2 for DEG determinations, install black PEEK and red PEEK tubing, the IonPac ICE-AS1 Guard, IonPac CS14 Analytical column, and 375 μ L knitted reaction coil in a similar way as required for System 1. For more information on the IonPac ICE-AS1 Guard and IonPac CS14 Analytical columns, refer to the product manuals.^{18,19}

Assembling the Electrochemical Cell

To assemble the electrochemical cell, follow the instructions in AN 188, and install a disposable Au on PTFE-working electrode on System 1, and a disposable Pt working electrode on System 2 for the EG and DEG applications, respectively. The Au on PTFE-working electrode was designed for the high base concentrations used in the EG application.²⁰ The disposable Au on PTFE-working electrode can be used with NaOH concentrations up to 750 mM without loss in response. The disposable Pt working electrode has a typical lifetime of two weeks in a strong acid eluent using the waveform optimized for glycol determination.

RESULTS AND DISCUSSION

In preliminary experiments, the authors evaluated the separation of 0.1 mg/mL DEG, EG, sorbitol, glycerin, and PG standards by HPAE, ICE, and by a mixed-mode separation using the IonPac ICE-AS1 guard column with the CS14 cation-exchange column and detected by PAD. In HPAE-PAD, EG was well resolved from sorbitol, however, DEG showed a poor peak response and was not resolved from PG and small unknown peaks in the sorbitol solution. In ICE-PAD, sorbitol was well resolved from glycerin but EG, DEG, and PG eluted at approximately the same time. In the mixed-mode separation, sorbitol, glycerin, and EG eluted near the void. However, DEG was well resolved from the void and was nearly baseline-separated from PG. Therefore, to accurately determine 0.1% EG and DEG in a sorbitol solution, the authors optimized HPAE-PAD and the mixed-mode separation with PAD to separately determine EG and DEG, respectively.

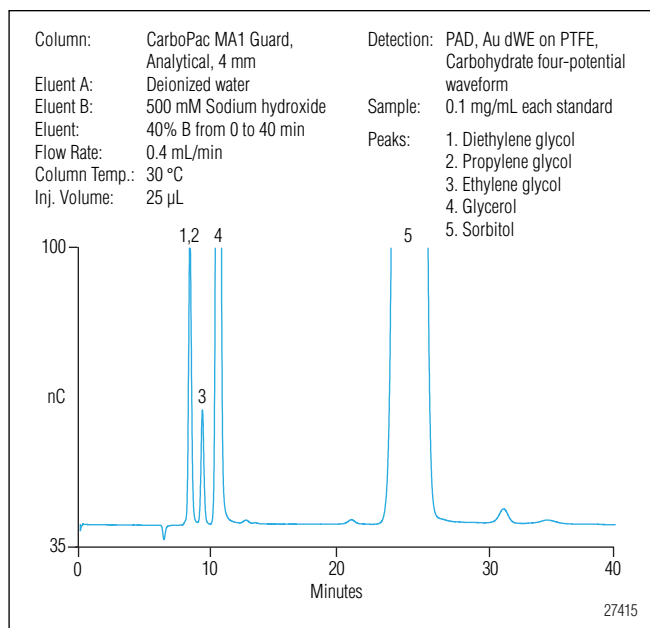


Figure 1. Separation of a 100 mg/mL standard mix by HPAE-PAD.

Method 1: Determination of EG by HPAE-PAD

Figure 1 shows separation of 0.1 mg/mL each of PG, DEG, EG, glycerin, and sorbitol using the CarboPac MA1 column with 200 mM NaOH and detection by HPAE-PAD. The analytes have strong peak responses and good separation from each other with the exception of DEG, which has a poor peak response and coelutes with PG (Peaks 1–2). This column has the high capacity (1450 µEq/column) needed to prevent overloading when injecting high concentrations of sorbitol, and good selectivity for the analytes with the exception of DEG. The large sorbitol peak elutes after the peaks of interest. The separation requires 60 min to elute other late-eluting compounds.

Analyte	Method	LOD (µg/mL)	LOQ (µg/mL)	Linear Range (mg/mL)	Linearity (r ²)
EG	HPAE-PAD	0.25	0.85	0.0013 to 0.010	0.9991
DEG	Mixed Mode and PAD	3.1	10	0.013 to 0.10	0.9993

Limit of Detection, Limit of Quantification, Linear Range, and Precision

To qualify the method, the authors determined the estimated limit of detection (LOD), limit of quantification (LOQ), linear range, and precision. The LOD and LOQ were determined by measuring the peak-to-peak noise in 1-min increments from 20 to 60 min in four replicate runs without a sample injection. Noise averaged 62 ± 0.28 pC for this series of blank runs. The estimated LOD and LOQ were calculated as 0.25 and 0.85 µg/mL EG, respectively, based on the peak response of the standard at 3× and 10× the signal-to-noise (S/N). These values demonstrate the sensitivity of HPAE-PAD for this application. To determine the method linearity, four EG calibration standards from 1.3 to 10 µg/mL in deionized water were injected in triplicate. The results were linear with correlation coefficient (r²) of 0.9991 (see Table 3). The retention time and peak area precisions—based on seven replicate injections of a 1.3 µg/mL EG standard—produced RSDs of < 0.2 and < 3, respectively.

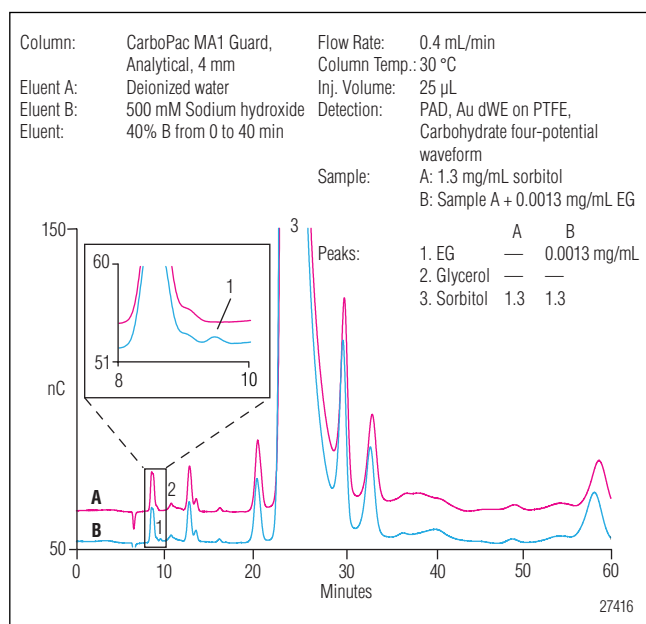


Figure 2. Comparison of 1.3 mg/mL sorbitol (A) without and (B) with 0.0013 mg/mL ethylene glycol (EG) by HPAE-PAD.

EG in Sorbitol Samples

To determine EG in sorbitol, the authors prepared 0.05 mg/mL EG in 50 mg/mL sorbitol (a 0.1% w/w solution of EG in sorbitol) and found low recovery (< 75%) due to possible column overload. Therefore, to improve the accuracy of the method, a 40-fold sample dilution was used. Figure 2 compares the separation of 1.3 mg/mL sorbitol sample with and without 1.3 µg/mL EG. Replicate injections of the spiked sample produced an average recovery of 106% (n = 7) with retention time and peak area RSDs of 0.11 and 1.4, respectively. To determine the limits of the method, the recovery of 1.3 µg/mL EG was determined in 0, 1.3, 1.6, 1.8, and 2.0 mg/mL sorbitol using triplicate injections. The acceptable recoveries of 106–120% of 1.3 µg/mL EG in the sorbitol solutions demonstrated that sorbitol solutions can be diluted up to 1.8 mg/mL without sacrificing accuracy.

Method 2: Determination of DEG by Mixed-Mode Separation and PAD

Figure 3 shows a chromatogram of 0.1 mg/mL each of sorbitol, glycerin, EG, DEG, and PG using a mixed-mode separation on the high-capacity, fully sulfonated ion-exclusion IonPac ICE-AS1 guard column combined with the moderately hydrophobic IonPac CS14 analytical cation-exchange column using 100 mM MSA and PAD.

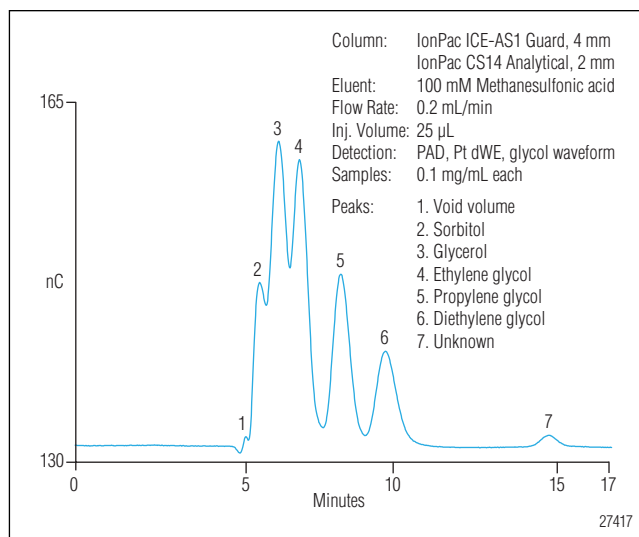


Figure 3. Separation of standards by mixed-mode separation and PAD.

In ICE, undissociated glycols and sorbitol are separated using a strong acid eluent by steric exclusion and thus, elute in order of their decreasing hydroxyl groups.²¹ The ICE-AS1 guard column separates sorbitol from DEG; however, DEG, EG, and PG having two hydroxyl groups, coelute. Combining the IonPac ICE-AS1 guard with the IonPac CS14 Analytical column takes advantage of the ICE properties of the ICE-AS1 Guard column and uses the hydrophobic interaction properties of the CS14 column to resolve PG from DEG and sorbitol.

LOD, LOQ, Linear Range, and Precision

To qualify the mixed-mode separation and PAD method, the authors evaluated the same parameters as discussed in Method 1 using HPAE-PAD. The estimated LOD and LOQ, based on the average peak-to-peak noise of 59 ± 1.1 pC, were 3.1 and 10 µg/mL DEG, respectively. This is approximately 10× higher than with HPAE-PAD for EG determinations. The calibration from 0.013 to 0.10 mg/mL DEG produced a correlation coefficient (r^2) of 0.9993. The retention time and peak area precision of a 0.013 mg/mL DEG (n = 7) standard produced RSDs of 0.17 and 2.2, respectively.

DEG in Sorbitol Samples

To determine an appropriate sample dilution for this method, 0.05 mg/mL DEG added to 50 mg/mL sorbitol [a 0.1% (w/w) solution of DEG in sorbitol] was diluted two-, four-, and eight-fold. The experiments showed that the four-fold dilution was optimum based on recovery

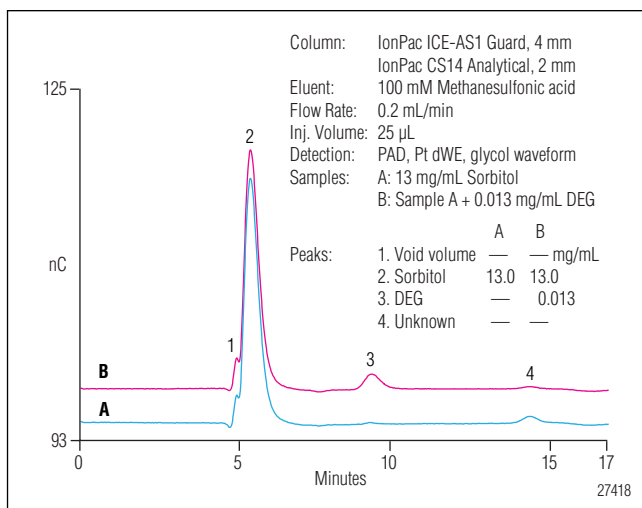


Figure 4. Comparison of 13 mg/mL sorbitol A) without and B) with 0.013 mg/mL diethylene glycol (DEG) by mixed-mode separation and PAD.

of DEG, therefore this dilution was used for the sample analyses. Figure 4 shows a separation of the four-fold diluted sample 0.013 mg/mL DEG resolved from the 13 mg/mL sorbitol. The precision and accuracy were determined by performing seven replicate injections of 0.013 mg/mL DEG added to 13 mg/mL sorbitol. The average recovery was 107% with the retention time and peak area RSDs of 0.23 and 3.9, respectively. To determine the limits of the method, the recovery of 0.013 mg/mL DEG was determined in 0, 6, 8, 13, and 18 mg/mL sorbitol using triplicate injections. Recoveries of 107–112%, of 0.013 mg/mL DEG added to sorbitol concentrations from 0 to 13 mg/mL were achieved. The analysis of sorbitol concentrations > 13 mg/mL by this method is not recommended due to column overload.

CONCLUSION

This work describes two methods to determine 0.1% EG and DEG in diluted sorbitol solutions. EG was determined in 1.3 mg/mL sorbitol on a CarboPac MA1 column by HPAE-PAD with a Au working electrode. DEG was determined in 13 mg/mL sorbitol by mixed-mode separation using an IonPac ICE-AS1 ion-exclusion guard and IonPac CS14 cation-exchange column and detected by PAD and a Pt working electrode. Both methods were run sequentially on an ICS-3000 dual system. These applications provide confirmatory analytical methods to directly, accurately, and selectively determine μ g/mL concentrations of EG and DEG in mg/mL concentrations of sorbitol solution, and meet the USP monograph 0.1% of EG and DEG limits.

LIST OF SUPPLIERS

Sigma-Aldrich Chemical Company
 P.O. Box 2060
 Milwaukee, WA, USA
 Tel: 1-800-558-9160
 www.sigmaaldrich.com

VWR International, Inc.
 1310 Goshen Parkway
 West Chester, PA, USA 19380
 Tel: 1-800-932-5000
 www.vwrsp.com

REFERENCES

- Holloway, G.; Maheswaran, R.; Leeks, A.; Bradby, S.; Wahab, S. Screening Method for Ethylene Glycol and Diethylene Glycol in Glycerin-Containing Products. *J. Pharm. Biomed. Anal.* **2009**, *51*(2010), 507–511.
- Centers for Disease Control. Fatalities Associated with Ingestion of Diethylene Glycol-Contaminated Glycerin Used to Manufacture Acetaminophen Syrup—Haiti, November 1995–June 1996, *MMWR Weekly*. **1996**, *45*, 649–650.
- O'Brien, K. L.; Selanikio, J. D.; Hecdivert, C.; Placide, M-F.; Louis, M.; Barr, D. B.; Barr, J. R.; Hospedales, C. J.; Lewis, M. J.; Schwartz, B.; Philen, R. M.; St. Victor, S.; Espindola, J.; Needham, L. L.; Denerville, K. Acute Renal Failure Investigation Team. Epidemic of Pediatric Deaths From Acute Renal Failure Caused by Diethylene Glycol Poisoning. *JAMA* **1998**, *279* (15), 1175–1180.
- United States Federal Drug Association (FDA). FDA Advises Manufacturers: Test Glycerin for DEG Contamination, Glycerin Contaminated with Diethylene Glycol (DEG) Remains a Potential Health Hazard to Consumers, FDA News Release, P07-81, May 4, 2007. Last updated 06/18/2009.
- Bogdanich, W.; Hooker, J. From China to Panama, a Trail of Poisoned Medicine, *NY Times*, May 6, 2007.
- Increased surveillance of bulk ingredients susceptible to contamination with diethylene glycol. USDA Import Alert, Import Alert 55-02, October 5, 2009.
- Vale, A. Poisonous Substances, Ethylene Glycol and Diethylene Glycol. *Medicine* **2007**, *35* (11), 617–618.

8. United States Pharmacopeia, Press release. New tests for identifying harmful and potentially deadly adulterants in pharmaceutical ingredients part of revised standards. October 30, 2009.
9. United States Pharmacopeia. Sorbitol Solution, *Pharmacopeial Revision Bulletin*. To be printed in USP 33–NF 28 First Supplement, released 1 February 2010, official 1 August 2010.
10. Cheng, J.; Jandik, P.; Liu, X.; Pohl, C. Pulsed Amperometric Detection Waveform with Disposable Thin-Film Platinum Working Electrodes in High Performance Liquid Chromatography. *J. Electroanal. Chem.* **2007**, *608*, 117–124.
11. Dionex Corporation. *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector*, Technical Note 21, LPN 034889, Sunnyvale, CA, 1998.
12. Dionex Corporation. *Determination of Glycols and Alcohols in Fermentation Broths by Ion-Exclusion Chromatography and Pulsed Amperometric Detection*, Application Note 188, LPN 1944, Sunnyvale, CA, 2008.
13. Dionex Corporation. *Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)*, Technical Note 20, LPN 032857. Sunnyvale, CA, 2000.
14. Dionex Corporation. *Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection*, Technical Note 71, LPN 1932, Sunnyvale, CA, 2009.
15. Dionex Corporation. Operator's Manual for AS Autosampler, Section B.3.6 Connecting the Diverter Valve (For Sequential Injections), Section 5.9 Calibrating the Inject Port Volume; Document No. 065051-03. Sunnyvale, CA, 2008, 197–200, 155–160.
16. Dionex Corporation. *Using the AS Automated Sampler in the Simultaneous, Sequential, and Concentrate Modes*, Technical Note 64, LPN 1756. Sunnyvale, CA, 2008.
17. Dionex Corporation. Product Manual for CarboPac MA1, PA1, PA10, PA100 Columns, Document No. 031824. Sunnyvale, CA, 2009.
18. Dionex Corporation. Product Manual for IonPac ICE-AS1 Guard and Analytical Columns, Document No. 031181. Sunnyvale, CA, 2006.
19. Dionex Corporation. Product Manual for IonPac CS14 Guard and Analytical Columns, Document No. 034848. Sunnyvale, CA, 2005.
20. Dionex Corporation. Product Manual for Disposable Gold, Disposable Silver, Disposable Platinum, and Disposable Carbon Electrodes, Document No. 065040. Sunnyvale, CA, 2009.
21. Weiss, J. Ion-Exclusion Chromatography (HPICE). In *Handbook of Ion Chromatography*; 3rd ed.; Wiley-VCH, Weinheim, Germany 2004; pp 359–360.

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