

# Determination of Polydextrose in Foods by AOAC Method 2000.11

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## Introduction

Polydextrose is a synthetic polymer of glucose having mainly  $\alpha$ 1,6 linkages and an average degree of polymerization of 12. This oligosaccharide is recognized as dietary fiber in several countries, including Japan. Unfortunately, existing methods to measure dietary fiber do not measure polydextrose. To overcome this limitation, Stuart et al.<sup>1</sup> developed a method based on high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) to measure polydextrose in foods. The authors organized a successful AOAC collaborative study of this method and it was approved as AOAC Method 2000.11.<sup>2</sup> Polydextrose is separated on a Thermo Scientific™ Dionex™ CarboPac™ PA1 Column and detected by PAD using a triple-potential waveform (Waveform B, Thermo Scientific Technical Note 21<sup>3</sup>). A quadruple-potential waveform (Waveform A, Technical Note 21) provides greater long-term reproducibility of peak area response compared to Waveform B.<sup>4</sup> During our participation in the Method 2000.11 collaborative study, we analyzed samples with both waveforms. This application note shows that Waveform A produced results equivalent to those obtained with Waveform B. Using Waveform A instead of B for Method 2000.11 and other HPAE-PAD methods will yield equivalent results and greater long-term peak area reproducibility.

## Equipment

### Chromatography Equipment

Thermo Scientific Dionex BioLC chromatography system consisting of:

- GP50 Gradient Pump with vacuum degas option
- ED50 Electrochemical Detector
- E01 Eluent Organizer
- AS50 Autosampler with Thermal Compartment
- Chromeleon 6 Chromatography Workstation



### Other Equipment

- Microcentrifuge
- Vortex mixer
- Water bath (preferably two)
- Analytical centrifuge capable of 6000 g (a Spinchron R was used for this study)

### Reagents and Standards

Deionized water, 17.8 M $\Omega$ -cm resistivity or better (used for all eluent and standard preparations)

- Sodium hydroxide, 50% (w/w)
- Sodium acetate, anhydrous
- Polydextrose (available at health food stores)
- Fructanase, 667 U/mL
- Amyloglucosidase, 3260 U/mL (soluble starch)
- Isoamylase, 200 U/mL

### Other Consumables

- Autosampler vials (0.5 mL)
- Microcentrifuge tubes (1.7 mL)
- 0.2  $\mu$ m syringe filters (PTFE, 13 mm diameter)
- Ultrafiltration centrifuge tubes—2 mL tubes with 100,000 dalton cutoff, polyethersulfone membrane

Conditions		
Column:	Dionex CarboPac PA1 Analytical, (4 × 250 mm) Dionex CarboPac PA1 Guard, (4 × 50 mm)	
Expected Operating Pressure:	9.7 MPa (1400 psi)	
Temperature:	30 °C	
Degas:	30 sec every 10 min	
Inj. Volume:	25 µL	
Injection Loop:	100 µL	
Detection:	Pulsed amperometry, gold working electrode, Ag/AgCl reference	
Collection Rate:	2 Hz	
Expected Background:	20–37 nC	
Needle Height:	2 mm	
Cut Segment Volume:	4 µL	
Flush Volume:	400 µL	
Gradient:	Eluent A: 150 mM sodium hydroxide Eluent B: 150 mM sodium hydroxide/ 0.5M sodium acetate	
Flow Rate:	1.2 mL/min	
Time (min)	%A	%B
0.0	70	30
10.0	70	30
10.1	0	100
15.0	0	100
15.1	70	30
25.0	70	30
Autosampler Cycle Time:	26 min	

## Preparation of Solutions and Reagents

### Buffered Enzyme Mix

Dissolve the content of the fructanase bottle (8000 U) in 12 mL of a sodium acetate buffer (pH 4.5). Prepare this buffer by mixing 28 mL of 0.2 M acetic acid with 22 mL of 0.2 M sodium acetate and then bring this solution up to 100 mL with water. Combine 2 mL fructanase, 84 µL amyloglucosidase, and 84 µL isoamylase, and bring to a final volume of 20 mL with the acetate buffer. This mixture is stored at 4 °C and is prepared on the day of use.

### Standard Preparation

The study organizers provided the polydextrose standard. Polydextrose can be purchased at health food stores. The stock standard is 5000 mg/L and is prepared by weighing approximately 500 mg into a preweighed glass container with screw cap (record weight) and adding 100 g of water preheated to 80 °C. Vortex and incubate in an 80 °C water bath until the polydextrose is dissolved. Weigh the container after allowing the solution to cool to room temperature. Prepare the eight intermediate standards by diluting the stock standard to 2500, 2000, 1500, 1250, 1000, 750, 500, and 250 mg/L. These standards are diluted during the standard and sample preparation procedure that follows to yield working standards of 500, 400, 300, 250, 200, 150, 100, and 50 mg/L.

### Sample Preparation

This section has a brief summary of the sample preparation procedure outlined in AOAC Method 2000.11. For more detail, please consult the method.<sup>2</sup> The study organizers provided blind duplicate samples of six food products: milk chocolate candy, iced tea, sugar cookie, grape jelly, soft jellied candy, and a powdered drink mix, as well as blind duplicate samples of polydextrose. Food samples are cut or ground into small particles. If the approximate amount of polydextrose in the sample is

Table 1. Thermo Scientific Dionex ED50 waveforms for carbohydrate analysis.

Waveform A			Waveform B		
Time (sec)	Potential	Integration (V)	Time (sec)	Potential	Integration (V)
0.00	+0.1	—	0.00	+0.05	—
0.20	+0.1	Begin	0.20	+0.05	Begin
0.40	+0.1	End	0.40	+0.05	End
0.41	−2.0	—	0.41	+0.75	—
0.42	−2.0	—	0.60	+0.75	—
0.43	+0.6	—	0.61	−0.15	—
0.44	−0.1	—	1.00	−0.15	—
0.50	−0.1	—	—	—	—

Note: Waveform A should only be used with a new gold working electrode or a gold working electrode that has only been used with this waveform.<sup>3</sup>

known, choose a sample weight within the range of the working standards. This study used 0.5 g of the milk chocolate candy, 5 g of the iced tea, 2 g of the sugar cookie, 2 g of the grape jelly, 0.5 g of the soft jellied candy, and 5 g of the powdered drink mix solution (the dry sample provided was first dissolved in 8 oz water). Add each sample to a preweighed glass container with screw cap and then record the weight. Add 100 g of hot (80 °C) water. Cap the container and treat the same as the standards were treated to dissolve the polydextrose. Weigh the capped vial after allowing it to cool to room temperature.

Treat samples and standards as follows: Pipet 2 mL of solution (freeze the remaining solution) into an ultrafiltration tube and centrifuge at 6400 rpm (5000 g) for 45 min. Add 0.2 mL of the supernatant to a preweighed (accurate to 0.1 mg) 1.7 mL micro-centrifuge tube, record the weight, add 0.8 mL of buffered enzyme mix, and record the weight. Cap, vortex, and incubate the solution at 50 °C for 60 min. After 1 h, remove the tube and place in a boiling water bath to denature the enzymes. Then move the tube to an ice bath to precipitate the enzymes (approximately 5 min). Microfuge for 10 min, and then filter through a 0.2- $\mu$ m syringe filter into an autosampler vial. This solution should be analyzed by HPAE-PAD within 72 h. Analyze each sample in triplicate.

## Eluent Preparation

### 150 mM Sodium Hydroxide

It is essential to use high-quality water of high resistivity (17.8 M $\Omega$ -cm or better) that contains as little dissolved carbon dioxide as possible. Biological contamination should be absent. Sodium hydroxide eluent should be prepared with 50% (w/w) sodium hydroxide. Sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used under any circumstances. To prepare 2 L of 150 mM NaOH, use a 10 mL graduated plastic pipet to deliver 15.6 mL 50% (w/w) sodium hydroxide into 1 L of water. Then add water to reach 2 L. Stir this solution only one to two minutes. Excessive mixing will increase the carbonate ion in the solution by trapping carbon dioxide from the air. After preparation, keep the eluent blanketed under helium at 34–55 kPa (5–8 psi) at all times. If maintained under helium, this eluent remains viable for approximately one week.

### 150 mM Sodium Hydroxide/0.5 M Sodium Acetate

Measure approximately 800 mL of water into a 1 L graduated cylinder. Add a stir bar and begin stirring. Weigh out 41.0 g of anhydrous crystalline sodium acetate. Steadily add the sodium acetate while briskly stirring the water. After the salt dissolves, remove the stir bar with a magnetic retriever and use a 10 mL graduated plastic pipet to add 7.8 mL of 50% (w/w) sodium hydroxide to the sodium acetate solution. Add water to the solution to reach a final volume of 1 L, replace the stir bar, and stir briefly to mix. Vacuum filter this solution through a 0.2  $\mu$ m nylon filter. The vacuuming may be slowed if the filter clogs with insoluble impurities from the sodium acetate. After preparation, keep the eluent blanketed under helium at 34–55 kPa (5–8 psi) at all times. If maintained under helium, this eluent is viable for approximately one week.

## Calculations

Calculate the concentration of the stock standard by dividing the weight of the polydextrose (mg) by the weight of the water (g) added. Multiply this value by 1000 to convert the concentration to mg/g. Calculate the concentrations of the intermediate standards from the known dilutions of the stock standard (this calculation assumes that dilutions were not made to achieve the exact intermediate standard concentrations noted above). Calculate the concentration of each working standard by dividing the weight of the 0.2 mL aliquot of the intermediate standard by the sum of the weight of the 0.2 mL aliquot of the intermediate standard and the 0.8 mL aliquot of the buffered enzyme mix. Then multiply the quotient by the intermediate standard concentration.

To calculate the percent polydextrose in a food sample, multiply the  $\mu$ g/g polydextrose concentration (determined from the calibration curve) by sum of the weight of the 0.2 mL sample aliquot and the 0.8 mL aliquot of the buffered enzyme mix, divided by the weight of the 0.2 mL aliquot. Multiply this value by the sum of the weight of the food and water, divided by the weight of the food. Then multiply by 0.0001 (this factor converts  $\mu$ g polydextrose to g and converts the g polydextrose per g sample to a percent value).

Column: Dionex CarboPac PA1 and guard  
 Eluent: 150 mM sodium hydroxide,  
 150 mM sodium acetate for 12 min  
 150 mM sodium hydroxide,  
 500 mM sodium acetate from 12.1 to 17 min  
 150 mM sodium hydroxide,  
 150 mM sodium acetate from 17.1 to 25 min  
 Temperature: 30 °C  
 Flow Rate: 1.2 mL/min  
 Inj. Volume: 25 µL  
 Detection: PAD (Au), Waveform A (TN 21)

Peaks: 1. Polydextrose 150 mg/L

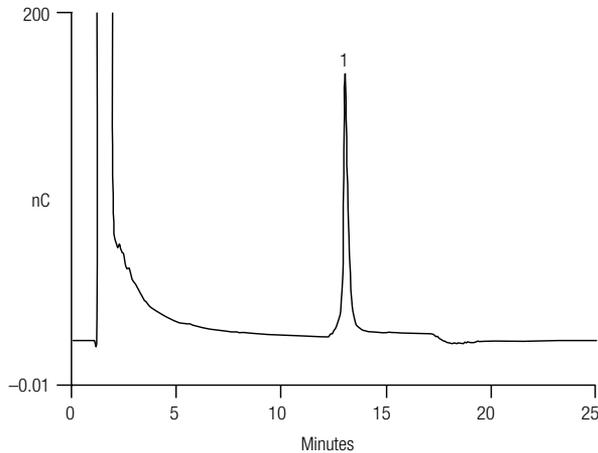


Figure 1. HPAE-PAD separation of 150 mg/L polydextrose using Waveform A.

Column: Dionex CarboPac PA1 and guard  
 Eluent: 150 mM sodium hydroxide,  
 150 mM sodium acetate for 12 min  
 150 mM sodium hydroxide,  
 500 mM sodium acetate from 12.1 to 17 min  
 150 mM sodium hydroxide,  
 150 mM sodium acetate from 17.1 to 25 min  
 Temperature: 30 °C  
 Flow Rate: 1.2 mL/min  
 Inj. Volume: 25 µL  
 Detection: PAD (Au), Waveform A (TN 21)

Peaks: 1. Polydextrose 196 mg/L

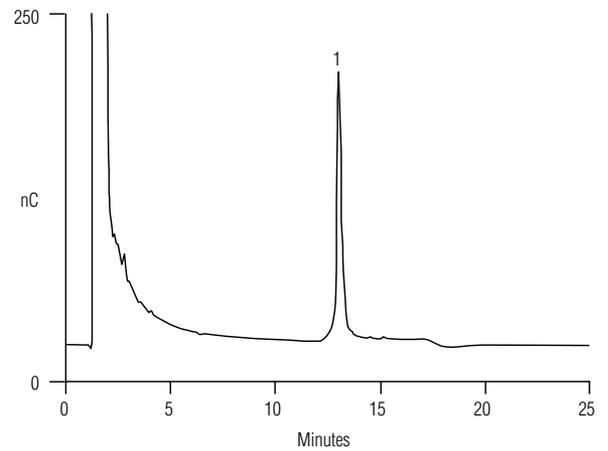


Figure 2. HPAE-PAD determination of polydextrose in milk chocolate candy using Waveform A.

## Results and Discussion

The procedure described in this application note, and in AOAC Method 2000.11, uses hot water to extract polydextrose from foods. Centrifugal ultrafiltration removes the high-molecular-weight components, and then treatment with a mixture of fructanase, amyloglucosidase, and isoamylase removes oligosaccharide interferences. The sample is then analyzed by HPAE-PAD. Figure 1 shows the HPAE-PAD separation of a 150 mg/L polydextrose standard. This separation uses a rapid change in eluent concentration at 12 min (from 150 mM sodium hydroxide, 150 mM sodium acetate to 150 mM sodium hydroxide, 500 mM sodium acetate) to elute all the polydextrose polymers as one peak. To determine the concentration of polydextrose in samples, an eight-point polydextrose standard calibration curve ranging 50–500 mg/L was established. Triplicate injections of

each standard level were made, and the peak areas were fit with a quadratic equation. The correlation coefficient was 0.999. Method 2000.11 states that a polynomial regression of the eight-point calibration should have a correlation  $\geq 0.995$ .

We participated in the collaborative study of what is now AOAC Method 2000.11. This method specified the use of a triple-potential waveform (Waveform B, Thermo Scientific Technical Note 21) for the PAD of polydextrose. We analyzed the seven blind duplicate samples (identities later revealed and are shown here in Table 2, column 1), and reported the percent polydextrose values in the second column of Table 2.<sup>2</sup> These values were consistent with the values determined by the other seven labs participating in the study. Figure 2 shows the HPAE-PAD determination of one of the milk chocolate candy samples.

After the originators of this method finished its development, a new quadruple-potential PAD waveform was described that yields significantly improved, long-term peak area reproducibility for carbohydrate detection.<sup>4</sup> This waveform, Waveform A, was used in the chromatograms in Figures 1 and 2. Immediately after we analyzed the collaborative study samples with Waveform B, we analyzed them with Waveform A. The results using Waveform A are presented in the third column of Table 2. A comparison of Table 2, columns 2 and 3, reveals that Waveform A yields results equivalent to those determined with Waveform B. Because there is greater long-term peak area reproducibility with Waveform A, we recommend its use when following AOAC Method 2000.11.

### Precautions and Recommendations

For best results, the Ag/AgCl reference electrode should be replaced every three to six months.

### References

1. Craig, S. A. S.; Holden, J. F.; Khaled, M. Y. *J. AOAC Int.* **2000**, *83*, 1006–1012.
2. Craig, S. A. S.; Holden, J. F.; Khaled, M. Y. *J. AOAC Int.* **2001**, *84*, 472–478.
3. Thermo Fisher Scientific . Technical Note 21; Sunnyvale, CA.
4. Rocklin, R. D.; Clarke, A. P.; Weitzhandler, M. *Anal. Chem.* **1998**, *70*, 1496–1501.

Table 2. Comparison of percent polydextrose in food samples determined Using AOAC Method 2000.11 with either Waveform A or Waveform B.

Sample	Polydextrose (%) Determined with Waveform B	Polydextrose (%) Determined with Waveform A
Milk Chocolate Candy #1	19.49	20.64
Milk Chocolate Candy #2	20.08	20.20
Iced Tea #1	1.88	1.91
Iced Tea #2	1.94	1.93
Grape Jelly #1	5.58	5.05
Grape Jelly #2	5.42	5.29
Soft Jellied Candy #1	37.78	38.39
Soft Jellied Candy #2	32.25	32.44
Sugar Cookie #1	7.24	7.51
Sugar Cookie #2	8.05	8.20
Powdered Drink Mix #1	1.94	1.96
Powdered Drink Mix #2	1.95	1.94
Polydextrose Sample #1	90.70	91.04
Polydextrose Sample #2	96.50	97.96

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