



## Food analysis

# Determination of carbohydrates in peanuts by HPAE-PAD

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### Goal

To develop a method to quantitate carbohydrates in peanuts and peanut butters with minimal sample preparation and without requiring pre- or post-column analyte derivatization. The method uses high-performance anion-exchange chromatography with pulsed amperometry detection (HPAE-PAD).

### Keywords

Carbohydrates, peanut butter,  
Dionex CarboPac PA210-Fast-4 $\mu$ m  
column

### Introduction

Peanuts contain the digestible carbohydrates sucrose, fructose, and glucose, but they also often include non-digestible raffinose, stachyose, and verbascose in varying quantities. These non-digestible carbohydrates pass through the human digestive tract unchanged and are subject to fermentation in the lower gut.<sup>1</sup> For determinations of carbohydrates in food, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is widely used because it eliminates the need for pre- or post-column derivatization, simplifying sample preparation steps while providing the required selectivity and sensitivity for carbohydrate detection.

Traditionally, manually prepared sodium acetate mixed with sodium hydroxide eluent is used for HPAE-PAD carbohydrate analysis of food.<sup>2,3</sup> In this experiment, Reagent-Free™ IC (RFIC™) with eluent generation (RFIC-EG) was used to automatically produce high-purity hydroxide eluent, improving method accuracy and ease of operation.

Stronger eluent is required to elute larger carbohydrates and carbohydrates that are negatively charged at neutral pH. While these are not the carbohydrates typically found in peanuts and peanut butter, developing a method using hydroxide as the only eluent it is important to determine if the eluent sufficiently removes sample components that could cause column capacity loss. Although such capacity loss was not observed throughout this experiment, it should be monitored by observing the retention time of standards analyzed before and after samples. If the retention times shorten, the column should be rinsed according to the column manual.

In this experiment, only dry roasted peanuts and peanut butter were tested. However, this method may be useful for the carbohydrate analysis of other legumes.

## Experimental

### Equipment

Any Thermo Scientific™ Dionex™ RFIC systems with ED detector can be used.

Recommended: Thermo Scientific™ Dionex™ Integron™ RFIC system and Thermo Scientific™ Dionex™ ICS-6000 HPIC system.

The Dionex ICS-6000 HPIC system was used in this study, including:

- DP pump module with degas option
- EG eluent generator module
- DC detector/chromatography module with ED electrochemical detector ([P/N 072042](#)) and cell ([P/N 072044](#))
- AS-AP autosampler

### Software

Thermo Scientific™ Chromleon™ 7.3.1 Chromatography Data System (CDS) software or higher

### Consumables

- Thermo Scientific™ Dionex™ CarboPac™ PA210G-Fast-4µm guard column, 4 x 30 mm ([P/N 088955](#))
- Thermo Scientific™ Dionex™ CarboPac™ PA210-Fast-4µm analytical column, 4 x 150 mm ([P/N 088953](#))
- Thermo Scientific™ Dionex™ CR-ATC 600 continuously regenerated anion trap column ([P/N 088662](#))
- Thermo Scientific™ Dionex™ EGC 500 KOH potassium hydroxide eluent generator cartridge ([P/N 075778](#))
- Thermo Scientific™ Dionex™ ED electrochemical detector gold on PTFE disposable electrode ([P/N 066480](#))
- Thermo Scientific™ Dionex™ ED electrochemical detector palladium hydrogen reference electrode ([P/N 072075](#))

### Reagents and standard

- Deionized water (DI), Type 1 reagent grade, 18 MΩ·cm resistivity or better. Eluent water was degassed and blanketed with nitrogen gas.
- Glucose: Sigma-Aldrich, P/N G8270
- Fructose: Sigma-Aldrich, P/N F2543
- Sucrose: Sigma-Aldrich, P/N S5016
- Raffinose: Pfanziehl Laboratories, P/N 13833
- Stachyose: Fisher Scientific, [P/N AC226080010](#)
- Verbascose: Fisher Scientific, [P/N 11-101-4564](#)
- D-(+)-Cellobiose: Sigma-Aldrich, P/N C7252
- Carrez solution I: Fisher Scientific, [P/N SC9101-250](#)
- Carrez solution II: Fisher Scientific, [P/N SC9102-250](#)

### Instrument method

Instrument parameter	Setting
System	Dionex ICS-6000
Columns	Dionex CarboPac PA210-Fast-4µm, analytical, 4 x 150 mm Dionex CarboPac PA210G-Fast-4µm, guard, 4 x 30 mm
Eluent source	Dionex EGC 500 KOH
Gradient	33 mM, 0–16 min; 100 mM, 16–21 min; 33 mM, 21–35 min
Flow rate	0.6 mL/min
Column temperature	30 °C
Injection volume	25 µL
Detection	PAD
Working electrode	Gold on PTFE disposable
Reference electrode	PdH
Waveform	Carbohydrate quad potential waveform for the ED (Figure 1)
System backpressure	~2,950 psi (100 psi = 689.5 kPa)
Background	<42 nC
Noise	~0.06 nC
Run time	35 min

For the analyses of carbohydrates that require manually prepared stronger eluents, detailed information on preparing hydroxide/acetate eluents can be found in Technical Note 71.<sup>4</sup>

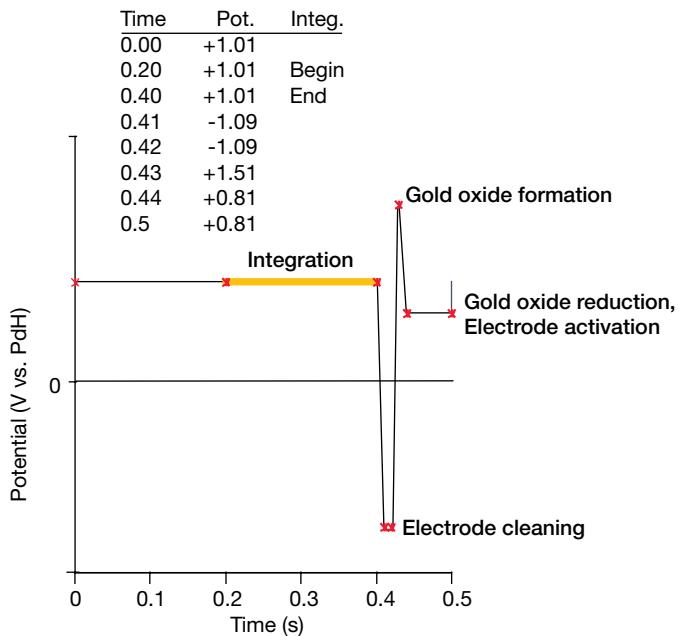


Figure 1. Four-potential waveform for carbohydrate analysis with a PdH reference electrode

### Standard preparation

Each sugar was diluted in DI water to make 10,000 mg/L stock solution and kept refrigerated. The stock solution was further diluted in DI water to make calibration standards. Due to the large disparity in quantity between sucrose and other carbohydrates in peanut samples, both the dilution factor and calibration standard ranges should be determined carefully. In this experiment, all samples were diluted 30 times. Table 1 shows the calibration concentration ranges of each carbohydrate standard and the concentration of the mixed spike standard.

Table 1. Calibration standard ranges and spike standard concentrations

Carbohydrate	Calibration standard range (mg/L)	Mixed spike standard (mg/L)
Glucose	0.004–0.242	0.020
Fructose	0.018–1.36	0.090
Sucrose	1.06–23.35	1.06
Raffinose	0.12–8.82	0.56
Stachyose	0.20–15.24	1.02
Verbascose	0.03–0.90	0.050

### Sample and spiked sample preparation

Peanut samples were ground into fine meals with a food blender prior to the sample preparation below.

1. Add 10 mL DI water to a centrifuge tube. Weigh 0.5 g of sample. Record the weight and place into the tube (water was added first to prevent peanut butter from sticking to the sides of the tube).
2. Add 200  $\mu$ L internal standard (1,000 mg/L cellobiose).\* Mix for 1 min. Add 10 mL DI water (for spike samples, 10 mL mixed standard is substituted for the DI water). Mix for 1 min. Add 10 mL DI water. Mix for 1 min.
3. Add 200  $\mu$ L Carrez I solution. Mix for 1 min.
4. Add 200  $\mu$ L Carrez II solution. Mix for 1 min.
5. Add 19.4 mL DI water (total volume = 50 mL). Mix. Let the solid settle for 2 h.
6. Centrifuge an aliquot of the clear portion at 3,000 rpm for 30 min.
7. Filter the liquid through 0.45  $\mu$ m syringe filter.
8. Dilute 30  $\times$  with DI water (dilution factor = 30).

\*Internal standard was added to monitor column capacity through its retention time change. It was not used for method calibration.

### Amount calculation using a custom variable in Chromleon CDS

The formula below was used to calculate the amount of each carbohydrate in milligrams per gram of peanuts:

$$\text{Amount (mg/g)} = \text{Dilution Factor} \times \text{Result (mg/L)} \times \text{Final Sample Volume (L)} / \text{Sample Weight (g)}$$

In Chromleon CDS, multiple variable options are available to perform complicated calculations automatically. In this experiment, a Custom Injection Variable was created to record sample weight. The dilution factor of 30 was also added (Figure 2). In the processing method, the report column with a formula was set up (Figure 3) to calculate the amount of sugar in milligrams per gram of peanuts as a result (Figure 4).

#	ED_1_Total	Name	Dilution	*sample [g]	Type
55		30 x P1S - 2	30.0000	0.46	Unknown
56		30 x P1S - 3	30.0000	0.46	Unknown
57		30 x P2S - 1	30.0000	0.50	Unknown
58		30 x P2S - 2	30.0000	0.50	Unknown
59		30 x P2S - 3	30.0000	0.50	Unknown
60		water	1.0000	0.00	Unknown

Figure 2. Custom variable for weight of peanuts and dilution factor in the sequence

**Report Column**

Column Management

Column Properties

Formula: `peak.amount*0.05/injection.customVar("sample")`

Header: "Amount"

Unit: "mg/g"

Format: 0.00

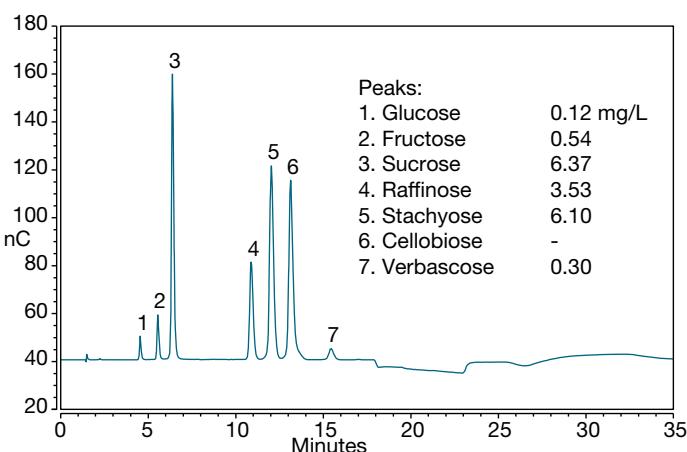
Channel: <Selected Channel>

[Advanced Column Properties](#)

**Figure 3. Calculation formula in the processing method.** The dilution factor is available by default and will be applied automatically; thus, there is no need to include it in the formula.

Peak No.	Peak Name	Ret. Time min	Amount mg/L	Amount mg/g	Area nC*min
1	Glucose	4.557	0.08	0.01	0.0873
2	Fructose	5.577	2.18	0.22	0.3485
4	Sucrose	6.398	580.37	59.22	44.0232
6	Raffinose	10.983	9.92	1.01	0.9168
7	Stachyose	12.161	60.12	6.13	7.5845
8	Celllobiose	13.255	1.00	0.10	0.6424
9	Verbascose	15.601	4.77	0.49	0.7941
Maximum			580.3655	59.22	44.0232
Minimum			0.0777	0.01	0.0873
Sum			658.4260	67.19	54.3969

**Figure 4. Carbohydrate content converted to milligrams per gram of peanuts**



**Figure 5. Separation of carbohydrate standards showing well-resolved peaks**

## Results and discussion

The Dionex CarboPac PA210-Fast-4 $\mu$ m column is a fast, high-resolution column for the separation of mono-, di-, tri-, tetra-, and pentasaccharides in various food samples. Figure 5 shows the separation of seven carbohydrate standards in under 17 min with 33 mM KOH flowing at 0.6 mL/min. A good separation is achieved with resolution values  $>2$  for all components. The total run time of 35 min includes column cleanup and re-equilibration time. Six of the carbohydrates are expected in peanuts, and the seventh, celllobiose, is the internal standard.

## Calibration and estimated method detection limit (MDL)

Standard curves for each carbohydrate were created by injecting standards in triplicate. MDLs were calculated using the formula  $MDL = (t) \times (S)$ , where  $t$  = Student's  $t$  value for a 99% confidence level and a standard deviation estimate with  $n-1$  degrees of freedom ( $t = 6.96$  for three replicates of the MDL standard), and  $S$  = standard deviation of the replicate analysis. Table 2 shows the summary of calibration and MDL results. Calibration curves for sucrose and stachyose were quadratically fit due to their relatively narrower linear range.

## Sample analysis

Figure 6 shows the separation of carbohydrates in peanuts and peanut butter. Celllobiose was added to each sample as an internal standard to evaluate the reproducibility of retention times over multiple injections.

The integrity of the sample preparation and the method accuracy were confirmed by good spike recoveries and comparing total sugar contents per serving listed on the product nutrition labels and the values determined by the HPAE-PAD method.

Columns	Dionex CarboPac PA210-Fast-4 $\mu$ m, analytical, 4 $\times$ 150 mm
Eluent	Dionex CarboPac PA210G-Fast-4 $\mu$ m, guard, 4 $\times$ 30 mm
Gradient	KOH via RFIC eluent generation
Flow rate	33 mM, 0-16 min; 100 mM, 16-21 min; 33 mM, 21-35 min
Column temp.	30 °C
Injection vol.	25 $\mu$ L
Detection	HPAE-PAD
Working electrode	Gold on PTFE disposable
Reference electrode	PdH
Waveform	Carbohydrate quad potential waveform for the ED
System backpressure	~2,950 psi. (100 psi = 689.5 kPa)
Background	<42 nC
Noise	~0.06 nC
Run time	35 min

Table 2. Calibration range and MDLs for carbohydrates found in peanuts

Carbohydrate	Range (mg/L)	Calibration type	Coefficient of determination ( $r^2$ )	MDL standard (mg/L)	Calculated MDL (mg/L)
Glucose	0.004–0.242	Linear	0.9999	0.002	0.004
Fructose	0.018–1.36	Linear	0.9998	0.009	0.006
Sucrose	1.06–23.35	Quadratic	0.9998	0.2	0.007
Raffinose	0.12–8.82	Linear	0.9998	0.6	0.03
Stachyose	0.20–15.24	Quadratic	1.0000	0.1	0.008
Verbascose	0.03–0.90	Linear	0.9997	0.005	0.008

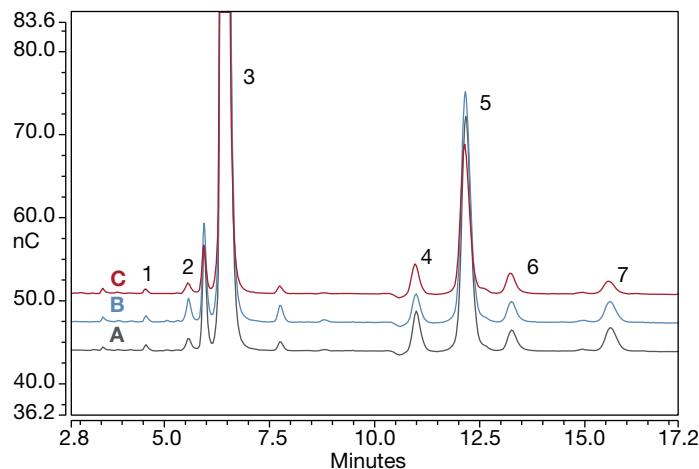


Figure 6. Determination of carbohydrates in peanuts and peanut butter. All peaks are well resolved despite large disparities in quantities.

Known standard amounts were spiked into each sample, and the percent recoveries were calculated. Table 3 shows the recoveries were 85–100%.

The total sugar amount (glucose, fructose, and sucrose combined) was converted to grams per serving to compare to the product label value (Table 4).

Table 3. Spike recoveries from peanuts and peanut butter

	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Verbascose
Peanut butter	99%	96%	99%	98%	99%	98%
Roasted peanut 1	88%	95%	95%	98%	96%	96%
Roasted peanut 2	85%	95%	92%	98%	100%	96%

Table 4. Total sugars (g/serving) compared to the product nutrition facts label

	Label (g/serving)	Result (g/serving)
Peanut butter	2	1.8
Roasted peanut 1	1	1.8
Roasted peanut 2	1	1.2

## Conclusion

Glucose, fructose, sucrose, raffinose, stachyose, and verbascose were separated on a Dionex CarboPac PA210-Fast-4 $\mu$ m column in less than 17 min with good recovery. Carbohydrates were detected without pre- or post-column derivatization of analytes. The total amount of glucose, fructose, and sucrose was in good agreement with the sugar amount listed on each product's nutrition label, demonstrating method accuracy. During this experiment, over 400 injections were made under normal conditions, and the retention times of the internal standard were monitored. No significant column capacity loss was observed.

With careful monitoring of column capacity, using RFIC-EG with HPAE-PAD can simplify the analysis of carbohydrates while maintaining the sensitivity and accuracy needed for carbohydrate detection and quantitation. Chromeleon CDS enables the automatic calculation of carbohydrate concentrations, further streamlining the process.

## References

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