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Determination of Sucralose Using HPAE-PAD

INTRODUCTION

Sucralose is used in the manufacture of nonnutritive sweetened food and beverage products. Sucralose, like most carbohydrates, lacks a good chromophore and therefore requires high concentrations to be detected by UV absorbance. Many food and beverage ingredients are chromophoric and can interfere with the direct detection of sucralose by absorbance. Refractive index detection has similar limitations.^{1,2} Carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds can be oxidized and therefore directly detected by amperometry. This detection method is specific for those analytes that can be oxidized at a selected potential, leaving all other compounds undetected. Pulsed amperometric detection (PAD) is a powerful detection technique with a broad linear range and very low detection limits.

High-performance anion-exchange chromatography (HPAE) is a technique capable of separating most carbohydrates and their analogues.^{3,4} For complex samples containing mixtures of sucralose and biologically derived material, such as foods and beverages, the high resolving power of HPAE and the specificity of PAD allow the determination of sucralose with little interference from other ingredients.^{5,6}

This application note describes the use of HPAE-PAD to determine sucralose in food and beverage samples. Red Raspberry Diet Rite® is a soft drink artificially sweetened with sucralose. This beverage also contains natural and artificial flavors, organic compounds (potassium benzoate and citrate), food dye, and another nonnutritive sweetener (acesulfame potassium). Splenda® is a solid artificial sweetener used as a sugar substitute and consists of dextrose, maltodextrin, and sucralose.

These model food and beverage samples present a great challenge for most chromatographic methods. In this application note, the CarboPac™ PA20 anion-exchange column is used to separate sucralose from other ingredients in Red Raspberry Diet Rite and Splenda.

EQUIPMENT

Dionex BioLC® system consisting of:

GP50 Gradient Pump, microbore (2 mm) with degas option.

ED50 Electrochemical Detector with combination pH/Ag/AgCl reference electrode and either or both of the following:

Carbohydrate Certified Disposable Au Working Electrode
(P/N 060139, 6 electrodes;
P/N 060216, 4 packages of
6 electrodes)

AAA-Direct™ Certified Disposable Au Working Electrode
(P/N 060082, 6 electrodes;
P/N 060140, 4 packages of
6 electrodes)

AS50 Autosampler

AS50TC Thermal Compartment

EO1 Eluent Organizer, including three 2-L plastic bottles and pressure regulator

Chromeleon® Chromatography Workstation

Helium; 4.5-grade, 99.995%, <5 ppm oxygen (Praxair)

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

Vacuum pump (Gast Manufacturing Corp., P/N DOA-P104-AA or equivalent)
1.5-mL glass injection vials with caps (Vial Kit, Dionex P/N 055427)

REAGENTS AND STANDARDS

Reagents

Sodium hydroxide, 50% (w/w) (Fisher Scientific and J. T. Baker)
Deionized water, 18 MΩ-cm resistance or higher
Sodium acetate, anhydrous (AAA-Direct Certified, Dionex Corp., P/N 059326)

Standards

Glucose, D- (Dextrose; Pfanstiehl Labs, Reference Grade, Cat #RGG-116)
Sucralose (McNeil Nutritionals, Inc.)

Food and Beverage Samples

Red Raspberry Diet Rite (Dr. Pepper/Seven Up, Inc.; product 29500-85254)
Splenda, (McNeil-PPC, Inc.)

CONDITIONS

Method

Columns: CarboPac PA20 Analytical (P/N 060142)
CarboPac PA20 Guard (P/N 060144)
Flow Rates: 0.5 mL/min
Eluent: A: Water
B: 250 mM NaOH
C: 1 M sodium acetate
Injection Volume: 10 or 25 µL
Temperature: 30 °C
Detection (ED50): Pulsed amperometry, Carbohydrate Certified disposable gold working electrodes (P/N 0600139), or AAA-Direct Certified disposable gold working electrodes (P/N 060082).
Background: 11–54 nC (using the carbohydrate waveform)

Typical System

Operating Backpressure: 2622–2700 psi

Isocratic Method (40 mM NaOH with 75 mM sodium acetate)

<i>Time (min)</i>	<i>%A</i>	<i>%B</i>	<i>%C</i>	<i>Comments</i>
Initial	76.5	16.0	7.5	
0.0	76.5	16.0	7.5	Inject
9.55	76.5	16.0	7.5	End

Gradient Method

<i>Time (min)</i>	<i>%A</i>	<i>%B</i>	<i>%C</i>	<i>Comments</i>
Initial	84.0	16.0	0.0	
0.0	84.0	16.0	0.0	Inject
5.0	84.0	16.0	0.0	Start acetate gradient
15.0	76.5	16.0	7.5	End acetate gradient
20.0	76.5	16.0	7.5	
20.1	4.0	16.0	80.0	Begin column wash
30.0	4.0	16.0	80.0	
30.1	4.0	16.0	0.0	Begin reequilibration
45.0	84.0	16.0	0.0	End run

Periodic Column Wash Method

<i>Time (min)</i>	<i>%A</i>	<i>%B</i>	<i>%C</i>	<i>Comments</i>
Initial	4.0	16.0	80.0	
0.0	4.0	16.0	80.0	No Injection
30.0	4.0	16.0	80.0	
30.1	76.5	16.0	7.5	Reequilibration
45.0	76.5	16.0	7.5	End run

Carbohydrate Waveform for the ED50 (Waveform A, Recommended)*

<i>Time (s)</i>	<i>Potential (V)</i>	<i>Integration</i>
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	–2.0	
0.42	–2.0	
0.43	+0.6	
0.44	–0.1	
0.50	–0.1	

Reference electrode in Ag mode.

* The waveform used for this application note appears in Technical Note 21.7

AAA-Direct Waveform for the ED50 (Alternative, for increased sensitivity)**

Time (s)	Potential (V)	Integration
0.00	+0.13	
0.04	+0.13	
0.05	+0.33	
0.21	+0.33	Begin
0.22	+0.55	
0.46	+0.55	
0.47	+0.33	
0.56	+0.33	End
0.57	-1.67	
0.58	-1.67	
0.59	+0.93	
0.60	+0.13	

Reference electrode in pH mode.

** The alternate waveform used for this application note appears in *Product Manual for the AAA-Direct Amino Acid Analysis System*.⁸

PREPARATION OF SOLUTIONS AND REAGENTS

Eluents

250 mM Sodium Hydroxide

It is essential to use high-quality water of high resistivity (18 M Ω -cm). Biological contamination should be absent. It is important to minimize contamination by carbonate, a strongly eluting divalent anion at high pH that causes changes in carbohydrate retention times. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate (carbonate precipitates at this pH) and is the preferred source for sodium hydroxide.

Dilute 26 mL of 50% (w/w) sodium hydroxide solution into 1974 mL of thoroughly degassed water to yield 250 mM sodium hydroxide. Immediately blanket the NaOH eluent with inert gas.

1 M Sodium Acetate

To prepare 1 L of eluent, dissolve 82 g of AAA-Direct Certified anhydrous sodium acetate in ~800 mL purified water. Adjust the total volume to 1000 mL with additional water. Filter the solution through a 0.2- μ m nylon filter unit and place it under 4–5 psi helium or nitrogen to reduce microbial contamination and carbonate buildup. Note, a sodium acetate that is not AAA-Direct Certified may lead to a loss of detector response due to electrode contamination by impurities when using the recommended AAA-Direct Waveform. To date, no loss in detector response is known to occur when using non-AAA-Direct Certified sodium acetate with Waveform A, the recommended quadruple potential waveform for carbohydrate determinations.

Keep the eluents blanketed under 5–8 psi (34–55 kPa) of inert gas (helium or nitrogen) at all times. On-line degassing is necessary because amperometric detection is sensitive to oxygen in the eluent. Set the pump to degas for 30 s every 4 min. Although not investigated in this note, long-term simple sucralose determinations should be possible using only two eluent channels: (1) mixture of 40 mM NaOH and 75 mM sodium acetate for the separation, and (2) 40 mM NaOH and 800 mM sodium acetate for periodic column washes.

STOCK STANDARDS

Solid sucralose standard was dissolved in deionized water to a 10-mg/mL concentration. This solution was further diluted with water to yield the desired stock mixture concentrations. The solutions were maintained frozen at -5 °C until needed.

SAMPLE PREPARATION

Red Raspberry Diet Rite

Red Raspberry Diet Rite beverage was diluted 50-fold, or as needed, in deionized water. Diluted sample was analyzed directly.

Splenda

Splenda sugar substitute was prepared at a concentration of 100 mg/mL with deionized water. The solution was diluted to 100 μ g/mL and analyzed directly.

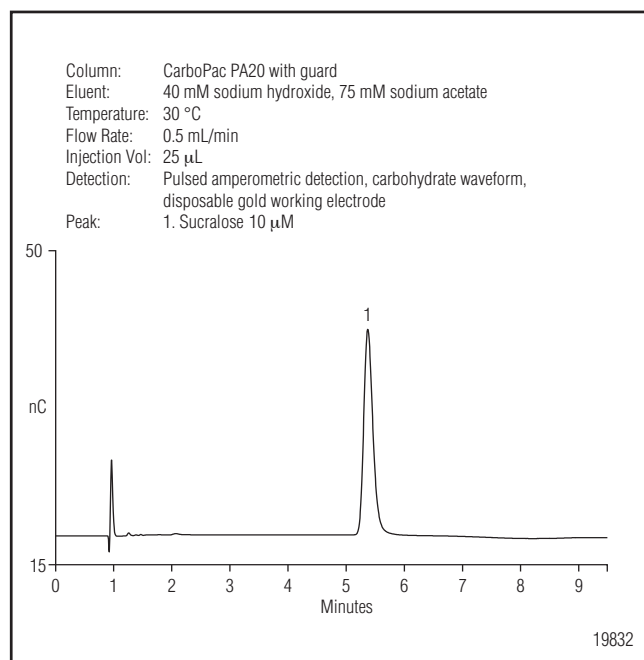


Figure 1. Separation of sucralose using a CarboPac PA20 column set.

RESULTS AND DISCUSSION

Separation

Figure 1 shows the separation of sucralose using a CarboPac PA20 analytical and guard column set with an eluent of 40 mM sodium hydroxide (NaOH) and 75 mM sodium acetate flowing at 0.5 mL/min. This isocratic method was optimized for retention time and detector response. Using this method, sugar alcohols (alditols), glycols, and mono- and disaccharides are not retained and elute near the void, whereas sucralose is retained and typically elutes at about 5.6 min. The retention time of sucralose was determined to vary slightly due to minor variations between different batches of prepared eluent (up to ~4%, including just the replacement of water in eluent A), and the use of different columns. Use of gravimetric measurement in place of volumetric measurement for sodium hydroxide eluent preparation can improve eluent-to-eluent reproducibility.

Run times were optimized on the system to account for the oxygen dip. This dip results from oxygen present in the samples and appears as a function of the column's gas permeation volume. Eluting oxygen produces less background than the eluent, so a dip appears in the baseline where oxygen elutes. The retention time of an

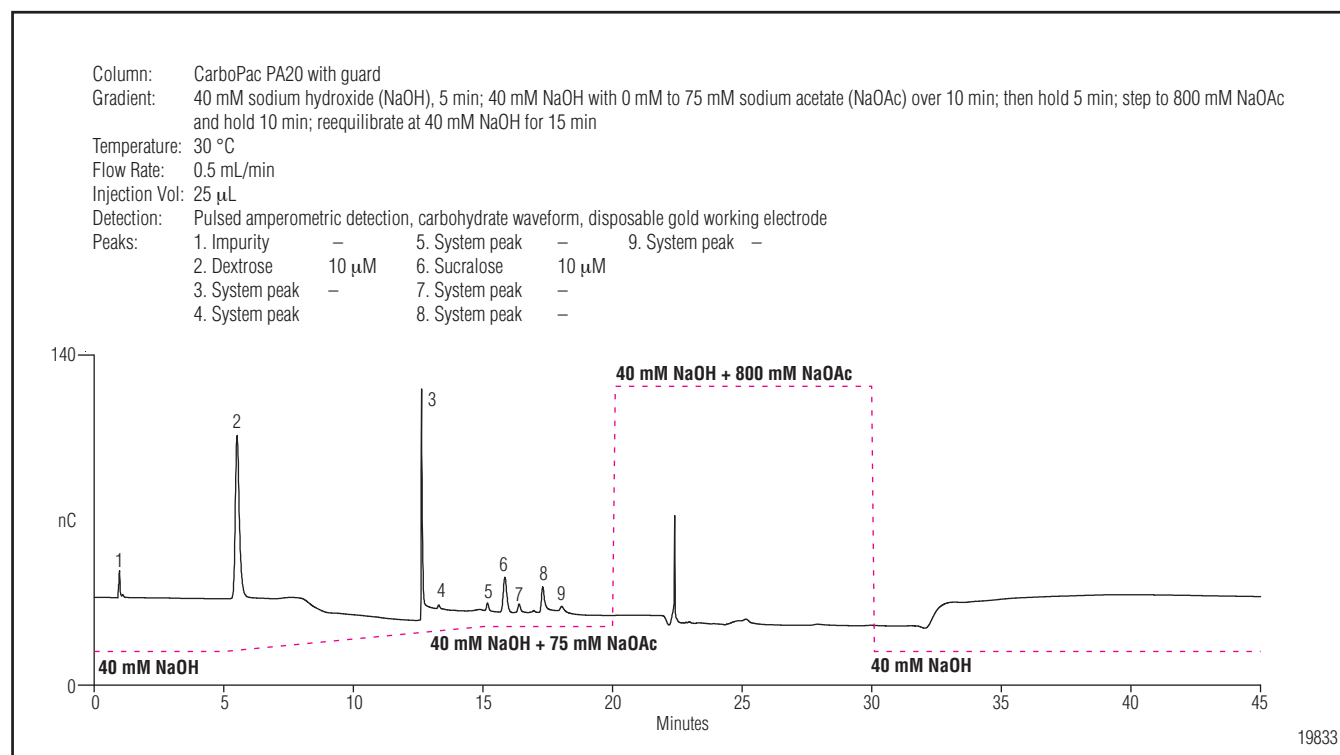


Figure 2. Simultaneous determination of 10 µM dextrose (glucose) and sucralose using a CarboPac PA20 column set with a gradient method.

oxygen dip varies from column to column, and depends on the flow rate but not the eluent strength. Setting the run time to allow two injections to occur before the first oxygen dip elutes doubled the sample throughput. For this system, the oxygen dip at 19.4 min resumed a stable baseline at 20.3 min, and the lag period was 1.22 min (time from the end of the first injection to the beginning of the next injection). With the run time of 9.55 min used in this application note, the oxygen dip was observed in the second and all subsequent injections of a sequence at a retention time of 8.6 min, and did not interfere with the elution of the sucralose peak at 5.6 min. A suitable run time may vary slightly from system to system, and column to column, and depends on the rinse volume selected for the autosampler, as well as whether the sample overlap feature of Chromeleon software is enabled (overlap was enabled for this application note). The oxygen dip and lag period should be determined for each system configuration. Run times longer than the oxygen dip will have no possibility of interferences, but sample throughput will be reduced.

Simultaneous determinations of monosaccharides (e.g., dextrose) and sucralose are possible using a gradient method. Figure 2 shows the separation of both dextrose (glucose) and sucralose using a gradient method where dextrose is first eluted using 40 mM NaOH for 5.0 min, and then a linear sodium acetate gradient is applied (0–75 mM) over 10 min. Dextrose elutes at about 5–6 min (peak 2), and sucralose elutes at about 16 min (peak 6). Peak 1 is the void, and Peaks 3, 4, 5, and 7–9 are unidentified peaks found in a blank (water) injection and result from the accumulation of impurities in the eluent prior to application of the acetate gradient. Peaks eluting during the acetate gradient also occur in the absence of any injection. The source and extent of the impurities can vary with the lag period prior to each run and the quality of reagents used for eluent preparation. An autosampler enables the lag period to be reproducible within a sequence, and therefore makes system peaks reproducible. Although chemical manufacturers have criteria for defining the conformity of the reagents they produce, the tests are not always specific enough to identify suitability for IC or HPLC applications with PAD or IPAD. Therefore, lot-to-lot variability can exist with respect to trace electrochemically active impurities.

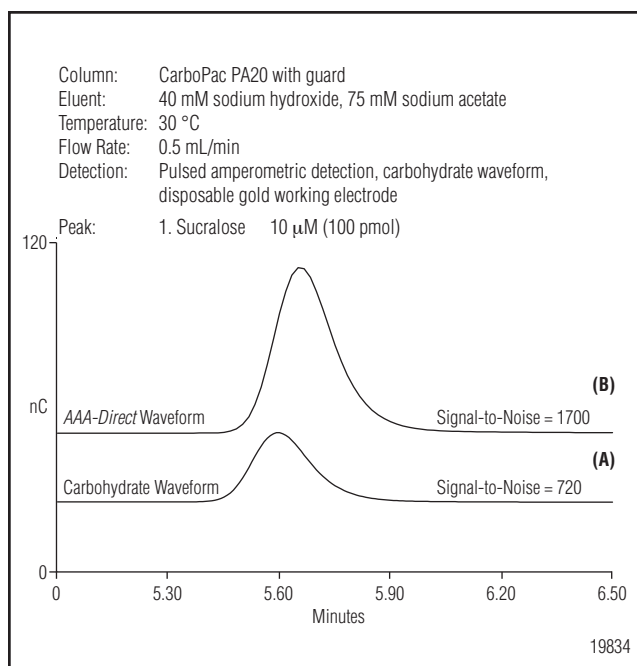


Figure 3. Comparison of 10- μ M sucralose peak height (10- μ L injection) using the carbohydrate waveform (A) and the AAA-Direct waveform (B).

Detection

Figure 3 compares the peak height for 10 μ M sucralose (100 pmol, 10- μ L injection) detected using (A) the carbohydrate waveform, and (B) the AAA-Direct waveform. The use of the AAA-Direct waveform increased signal-to-noise by more than two times. Although the AAA-Direct waveform improved sucralose sensitivity, we recommend the carbohydrate waveform because it allows longer use of each disposable Au working electrode and improves day-to-day peak area reproducibility. The AAA-Direct Certified disposable Au working electrode is guaranteed for 1 week when used with the AAA-Direct waveform, whereas the Carbohydrate Certified disposable Au working electrode is guaranteed for two weeks when used with the carbohydrate waveform. For applications where greater sensitivity is required, the AAA-Direct waveform should be considered.

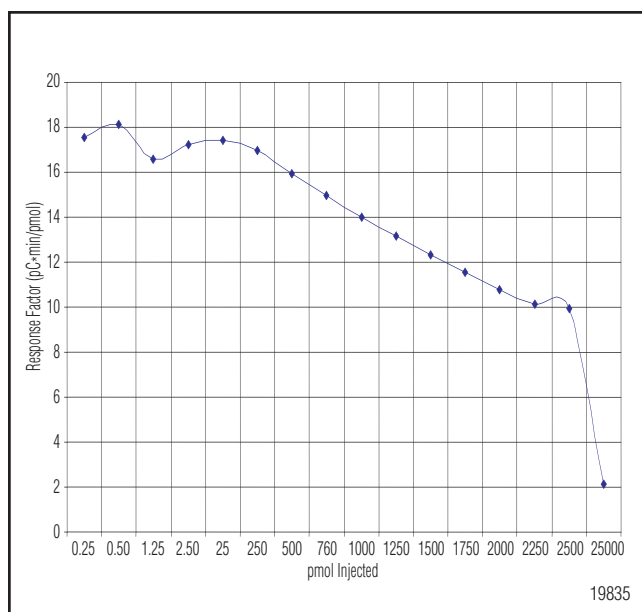


Figure 4. Relationship of sucralose peak area response factors ($\text{pC} \cdot \text{min}/\text{pmol}$ injected) to sucralose concentration injected ($25\text{-}\mu\text{L}$ injection) using the isocratic method (see Figure 1). Note that x-axis is not linear.

Linearity

Figure 4 presents the relationship of sucralose peak area response factors ($\text{pC} \cdot \text{min}/\text{pmol}$ injected) to concentration of the analyte injected ($25\text{-}\mu\text{L}$) using the isocratic separation. The figure shows concentration ranges where the response factor remains unchanged with increasing concentration. In this application note, we consider concentration range to be linear where the response factor remains within a 20% variance from the mean of the plateau region. The plateau region was estimated to extend from 1.24 to 250 pmol, and corresponding mean response factor for this range was $17.0\text{ nC} \cdot \text{min}/\text{pmol}$. Concentrations (pmol) of sucralose injected having response factors below $13.6\text{ nC} \cdot \text{min}/\text{pmol}$ were considered outside the upper linear range. These results show peak area linearity ($r^2 = 0.9958$) up to 1000 pmol ($40\text{ }\mu\text{M}$ for $25\text{-}\mu\text{L}$ injection). The peak height was linear ($r^2 = 0.9942$) to 760 pmol ($30\text{ }\mu\text{M}$ for $25\text{-}\mu\text{L}$ injection).

Lower Limits of Detection and Quantification

The lower limit of detection (LOD) in this application note is based on the average measured baseline noise for 1-min intervals collected over 20 min (9.0 pC). In this study, baseline noise for the standard isocratic method ranged $5.3\text{--}112.5\text{ pC}$ (mean \pm SD; 15.4 ± 11.3 , $n = 1690$ injections) using the carbohydrate waveform.

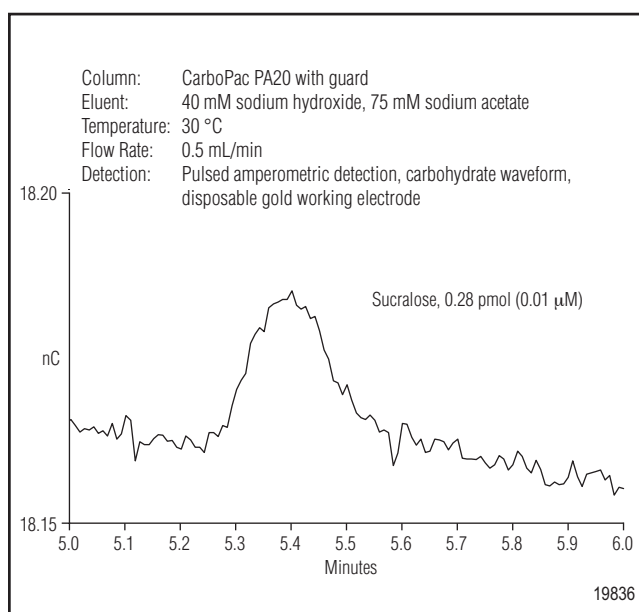


Figure 5. Sucralose at the lower limit of detection of 0.28 pmol ($0.01\text{ }\mu\text{M}$, with a $25\text{-}\mu\text{L}$ injection using the isocratic method (see Figure 1).

The concentration (or mass injected) of sucralose at LOD was calculated from three times the average peak-to-peak noise (a height value), divided by the average peak height response factor for sucralose within its linear region. Similarly, the lower limit of quantification (LOQ) is the concentration (or mass injected) calculated from ten times the average peak-to-peak noise. The estimated LOD was found to be 0.28 pmol ($0.01\text{ }\mu\text{M}$ for $25\text{-}\mu\text{L}$ injection), and the LOQ was 0.93 pmol ($0.04\text{ }\mu\text{M}$ for $25\text{-}\mu\text{L}$ injection). Figure 5 shows the sucralose peak at the LOD where the peak height is about three times above the noise (signal-to-noise ≈ 3).

Precision and Reproducibility

The peak area and retention time RSDs were determined for replicate injections of sucralose standards ($10\text{ }\mu\text{M}$ for $10\text{-}\mu\text{L}$ injection) over 4.7 days (619 injections) using the isocratic method. Over this time, the 250 mM NaOH (eluent B) eluent was replaced once while the (A) water and (C) sodium acetate eluents were unchanged. The sucralose retention time ranged from 5.49 to 5.67 min (mean \pm SD; $5.60 \pm 0.04\text{ min}$, 0.7% RSD). No upward or downward trend was observed. When a relatively pure sucralose sample (e.g., a standard) must be analyzed, the isocratic method can be used without any column regeneration for at least four days. For complex samples such as the Red Raspberry Diet Rite beverage, the same isocratic method can also be

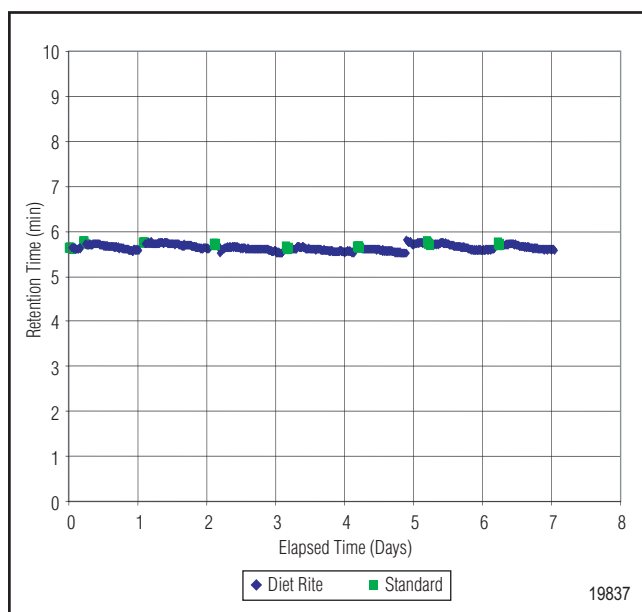


Figure 6. Reproducibility of sucralose retention times for a standard and for sucralose in 50-fold diluted Red Raspberry Diet Rite beverage over seven days (836 injections) using the isocratic method (see Figure 1), with column washes every 24 h.

used, but periodic column washes (e.g., 30 min every 24 h) should be used to regenerate the column to full capacity. Figure 6 shows sucralose retention times over seven days for the following sequence of injections: six injections of sucralose standard (B), followed by 120 injections of a 50-fold diluted beverage (A), followed by a 30 min wash with 40 mM NaOH with 800 mM sodium acetate at 0.5 mL/min. This sequence was repeated seven times. Retention times ranged 5.51–5.83 min with a slight trend to shorter retention times within each day that were restored to original retention times after each 30 min column wash. Table 1 presents the day-to-day precision with an RSD range of 0.1–1.2% for standards within each of the seven 24-h periods (10 μ M), and 0.5–1.2% for the Diet Rite beverage (7.8 μ M measured concentration).

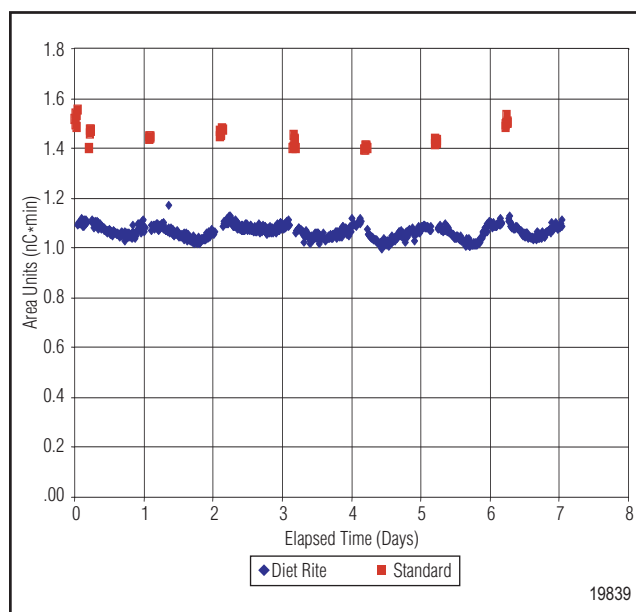


Figure 7. Reproducibility of sucralose peak area for a standard and for sucralose in 50-fold diluted Red Raspberry Diet Rite beverage over seven days (836 injections) using the isocratic method (see Figure 1), with column washes every 24 h.

The peak area precision for a sucralose standard (10 μ M for 10- μ L injection) injected for 4.7 days (619 injections) using the isocratic method ranged from 1.23 to 1.43 nC*min (mean \pm SD; 1.34 ± 0.04 min, 2.7% RSD). Peak height ranged from 6.493 to 7.616 nC (mean \pm SD; 7.093 ± 0.222 min, 3.1% RSD). No increasing or decreasing trending was observed over the 4.7 days. Figure 7 presents the sucralose peak area measured in 50-fold diluted Red Raspberry Diet Rite over 7 days. The peak area RSD was 3.0% for the standard and 2.4% for the beverage. Daily RSDs ranged from 0.3% to 2.9% for the standard and from 1.6% to 2.6% for the sample. The high retention time and peak area reproducibilities indicate that this method is suitably rugged for this application.

Table 1. Sucralose Peak Retention Time and Peak Area Precision Over Seven Days Using the Isocratic Method (See Figure 1)

Days	Sucralose in Red Raspberry Diet Rite Beverage								Sucralose Standard							
	Retention Time (min)				Peak Area (nC*min)				Retention Time (min)				Peak Area (nC*min)			
	Mean	SD	N	RSD	Mean	SD	N	RSD	Mean	SD	N	RSD	Mean	SD	N	RSD
1	5.65	0.05	120	0.8%	1.074	0.022	120	2.0%	5.68	0.07	11	1.2%	1.491	0.044	11	2.9%
2	5.70	0.04	120	0.8%	1.058	0.024	120	2.3%	5.75	0.00	4	0.1%	1.444	0.003	4	0.3%
3	5.62	0.03	113	0.5%	1.082	0.017	120	1.6%	5.71	0.01	6	0.2%	1.464	0.013	11	0.9%
4	5.59	0.04	118	0.7%	1.058	0.019	118	1.8%	5.63	0.02	6	0.4%	1.422	0.022	6	1.5%
5	5.59	0.07	120	1.2%	1.052	0.027	120	2.6%	5.65	0.02	6	0.3%	1.402	0.008	6	0.5%
6	5.68	0.06	120	1.0%	1.054	0.028	120	2.6%	5.73	0.03	6	0.5%	1.428	0.009	6	0.6%
7	5.64	0.05	125	0.8%	1.072	0.022	125	2.0%	5.71	0.02	46	0.3%	1.504	0.015	6	1.0%
Total 7 Days	5.64	0.06	836	1.1%	1.064	0.025	836	2.4%	5.69	0.05	45	0.9%	1.456	0.043	45	3.0%

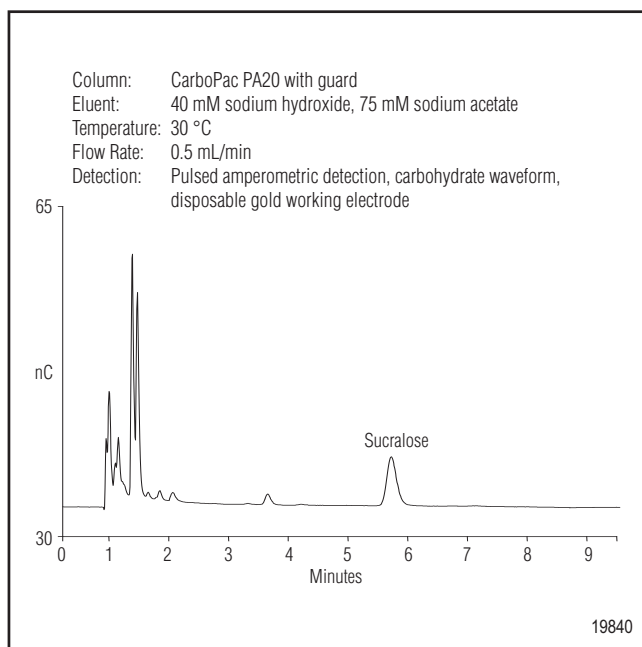


Figure 8. Determination of sucralose in 50-fold diluted Red Raspberry Diet Rite beverage (25 μ L) using the isocratic method (see Figure 1).

Determination of Sucralose in Food and Beverages

Red Raspberry Diet Rite Beverage

The Red Raspberry Diet Rite beverage product contains carbonated water, citric acid, natural and artificial flavors, sucralose (Splenda brand nonnutritive sweetener), caramel color, potassium citrate, potassium benzoate, acesulfame potassium (Sunett® Brand, nonnutritive sweetener), and Red Dye #40. Figure 8 shows the isocratic separation of Diet Rite. Sucralose is well resolved from the other detected ingredients of the soda. Most ingredients, including acesulfame, will not be detected by PAD. The measured concentration of sucralose in a 50-fold dilution of this beverage was 7.8 μ M for a 25- μ L injection, and therefore the concentration of sucralose in the undiluted beverage was calculated to be 390 μ M (155 μ g/mL). Quantitative spike recovery, ranging from 92% to 96%, was observed for 10 μ M sucralose in 50-, 100-, 500-, 1000-, and 10000-fold dilutions of the beverage. The recovery of sucralose can vary with different food and beverage types, and should be determined for each sample type to ensure accurate measurement.

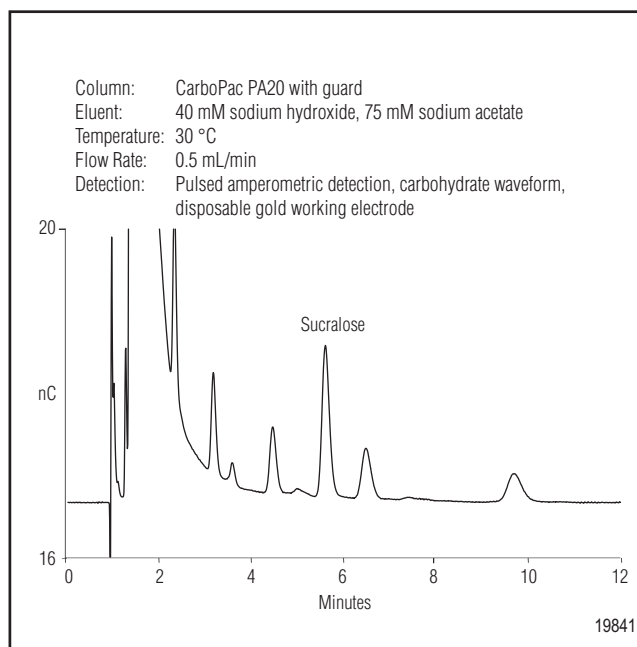


Figure 9. Determination of sucralose in 100 μ g/mL Splenda (25 μ L) using the isocratic method (see Figure 1).

Splenda

Splenda is a granular “no-calorie” sweetener added to food and beverages in a manner similar to granulated table sugar. The ingredients, as listed on the product label, include dextrose, maltodextrin, and sucralose. Dextrose is also known as D-glucose. Maltodextrins are partial hydrolysates of starch. Figure 9 presents the isocratic separation of 100 μ g Splenda/mL (10- μ L injection). The sucralose peak was resolved from other ingredients, whereas the dextrose peak eluted at the void. Maltodextrin peaks elute from the column after sucralose and during the recommended column wash with 800 mM sodium acetate.

Recognizing that a simultaneous determination of dextrose and sucralose could be advantageous, we developed an alternative gradient method that elutes dextrose at a low hydroxide concentration (40 mM), and then elutes sucralose with an acetate gradient. Figure 10 presents the chromatographic separation of both dextrose and sucralose in Splenda using the described gradient method. This figure presents a chromatogram where the dextrose peak (Peak 2) is displayed full scale; and the sucralose peak appears as a minor peak (Peak 6).

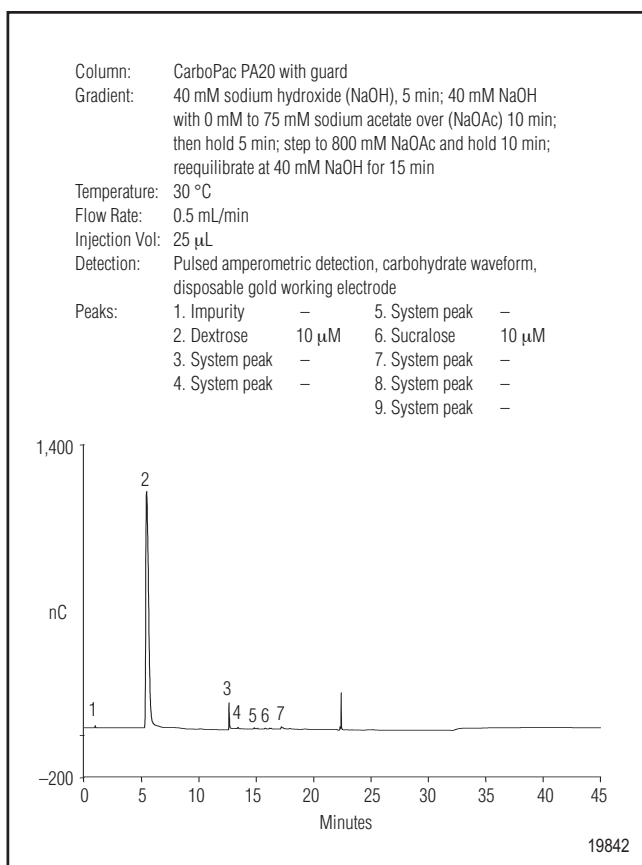


Figure 10. Determination of dextrose and sucralose in 100 µg/mL Splenda using the gradient method (see Figure 2).

Figure 11 rescales the chromatogram to better display the sucralose peak, and overlays it with a gradient without injection (system blank, Panel A), and on a mixed 10-µM dextrose and sucralose standard (Panel B). Peaks 5, 6, 9, 12, 13, 14, and 16 were observed in the system blank. The system peaks were also found in the chromatograms for the standards and Splenda (Panel C). The system blank did not have dextrose (Peak 3) or sucralose (Peak 10). Peaks 1 and 2, found near the void in the dextrose and sucralose standards, were also found in the Splenda sample. Besides dextrose (Peak 3) and sucralose (Peak 10), peaks 4, 7, 8, 11, 15, and 17 were unique peaks—probably maltodextrins—found in Splenda. The dextrose concentration was determined to be 474 µM (85.3 µg/mL; 85% by weight) for a 100-µg/mL solution of Splenda, whereas sucralose was 3.6 µM (1.4 µg/mL; 1.4% by weight) for the same solution of Splenda.

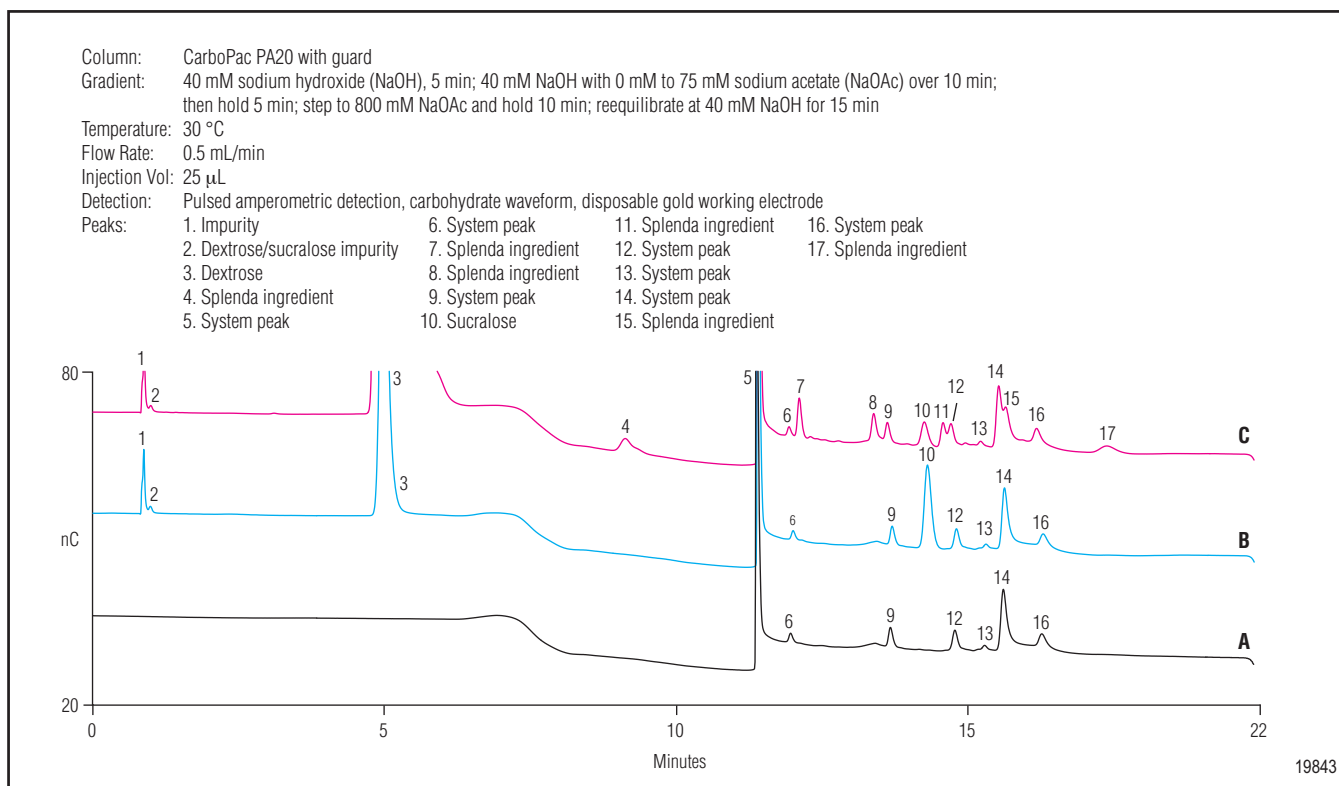


Figure 11. Expanded view of chromatograms using the gradient method (see Figure 2), comparing: (A) blank (without injection), (B) 10 µM dextrose and sucralose standards, and (C) 100 µg/mL Splenda.

CONCLUSION

HPLC-EAD can be used to determine sucralose food and beverages. The linear range of electrochemical response extended over 3 orders of magnitude, from 0.01 μM (LOD) up to 40 μM (16 $\mu\text{g/mL}$; 25- μL injection). High precision, method ruggedness, and high spike recovery are possible for these complex sample matrices. Mixtures of sucralose and other carbohydrates (e.g., dextrose) can be determined simultaneously.

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