DIONEX

Application Update 151

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Determination of Sucralose in Reduced-Carbohydrate Colas using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

INTRODUCTION

Sucralose (trichlorogalactosucrose or 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-deoxy-α-D-galacto-pyranoside) is a non-nutritive sweetener used to manufacture diabetic and dietetic foods and beverages. Detection of sucralose and other carbohydrates is challenging because they lack a strong chromophore and, therefore, cannot be detected at low concentrations with UV detection. Furthermore, sucralose would typically be present in foods containing compounds with strong UV chromophores. Refractive index detection can be used, but the sensitivity is poor. Most carbohydrates are not ionic at pH 7, therefore suppressed conductivity detection is not a suitable option. However, because carbohydrates, amines, glycols, alcohols, and sulfur compounds are easily oxidized at specific applied potentials they can be detected with high sensitivity by pulsed amperometric detection (PAD). Thus, carbohydrates are detected by PAD in basic solutions (pH >12) using a gold working electrode and Waveform A,[1] specifically designed for carbohydrate detection.

Basic eluents (pH >12) are required to ionize carbohydrates to form oxy-anions for separation. These anions are separated by high performance anion-exchange (HPAE) chromatography, using a CarboPac™ PA20 column. Sucralose, with three chlorine atoms, is more electronegative than sucrose and is retained longer on the CarboPac PA20 column, allowing its separation from fructose and sucrose.

This application update discusses the development of a HPAE-PAD method to determine sucralose in reduced-carbohydrate colas. HPAE-PAD has been used to determine sucralose in other sugar-free beverages, after a 50-fold dilution,[2] and foods[3-5]. Reduced-carbohydrate cola samples have high concentrations of fructose and sucrose relative to sucralose, making these samples challenging for chromatographic analysis. In this application update, we optimized the sample dilution and the eluent to separate high concentrations of fructose and sucrose from sucralose, while at the same time injecting enough sample to detect the low concentrations of sucralose in beverages.

EXPERIMENTAL

Equipment

Dionex ICS-2500 system consisting of:
- GP50 Gradient Pump with degas option and gradient mixer (GM-4 for microbore, P/N 049136; GM-3 for standard bore, P/N 042126)
- ED50A Electrochemical Detector with combination pH Ag/AgCl reference (P/N 046333) electrode and Carbohydrate Certified disposable Au working electrodes (Package of 6 electrodes, P/N 060139; Package of 24 electrodes, P/N 060216)
- AS50 Autosampler
- AS50TC Thermal Compartment
- Chromeleon® Chromatography Workstation with Chromeleon 6.6

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

Vacuum pump

Polypropylene sample vials, 1.5 mL with caps and slit septa (Dionex vial kit, P/N 061696) or 0.3 mL

polypropylene sample vials with caps and slit septa (Dionex vial kit, P/N 055428)

Disposable polystyrene 25-mL pipettes

Micropipettor and tips for preparing samples, standards, and pipetting samples into vials

Reagents and Standard

Deionized water, 18 MΩ-cm resistivity or better (used for all eluent and standard preparations)

Sodium hydroxide, 50% (w/w) (Fisher Scientific)

Sodium acetate, anhydrous (Fluka, Microselect)

Sucralose, micronized (McNeil Nutritionals)

Sucrose, D-Fructose, and α-D-Glucose (dextrose) reference standards, > 99% (United States Pharmacopeial Convention, Inc.)

Electrochemical Cell

The Dionex ED50A Product Manual[6] and theDionex Disposable Electrode Installation Guide[7] describe the calibration, handling, and installation tips on the reference electrodes and Carbohydrate Certified Disposable Au working electrodes. Dionex Technical Note 21 (TN 21)[1] contains a detailed discussion of "Waveform A".

CONDITIONS

Columns:	CarboPac PA20 Analytical (P/N 060144) CarboPac PA20 Guard (P/N 060142)
Flow Rate:	0.5 mL/min
Eluent A:	100 mM sodium hydroxide, 90 mM sodium acetate
Temperature:	30 ºC
Inj. Volume:	25 µL, PEEK sample loop (P/N 042857), full loop injection
Detection (ED50A):	Pulsed Amperometric Detection, carbohydrate four-potential waveform, "Waveform A"
Reference Electrode:	Ag mode
Working Electrode:	Carbohydrate Certified disposable gold working electrode
Background:	11–54 nC
System Backpressure:	~2500 psi
Noise:	9–27 pC
Run Time:	30 min

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Preparation

Consistent eluent preparation is critical for reproducible chromatography. Eluent preparation is thoroughly discussed in the Dionex CarboPac PA20 product manual[8] and Dionex Application Note 159.[9] These documents should be reviewed prior to eluent preparation. It is essential to use high quality water (18 MΩ-cm resistivity or better) and it should contain as little dissolved carbon dioxide as possible. It is also important to minimize carbonate and microbial contamination. Carbonate is a divalent anion at pH >12; it will bind to the column and affect resolution and efficiency. Sodium hydroxide pellets are coated with sodium carbonate and should never be used for preparation of sodium hydroxide solutions. Sodium hydroxide based eluents should always be prepared from 50% (w/w) sodium hydroxide reagents. Microbial contamination is a source of carbohydrates and particles, and will cause higher background levels.

Eluent A (100 mM sodium hydroxide/90 mM sodium acetate)

Measure ~800 mL of deionized water into a 1-L graduated cylinder. Add a magnetic stir bar and begin stirring. Slowly pour 14.77 g of anhydrous sodium acetate (82.03 g/mole) into the graduated cylinder. Stir until it is fully dissolved and then remove the magnetic stir bar. Using a rinsed plastic pipette, measure 10.5 mL of 50% (w/w) sodium hydroxide solution and pipette the solution into the graduated cylinder. Fill and dispense (several times) the sodium acetate-sodium hydroxide solution from the pipette to fully rinse the viscous sodium hydroxide into the same graduated cylinder. Add deionized water to the 1000 mL mark and briefly stir to mix the sodium hydroxide addition. In order to remove particles, vacuum filter the solution through a 0.2-µm nylon filter unit into the 2-L eluent bottle. Measure an additional 1000 mL of deionized water with the same graduated cylinder and vacuum filter through a 0.2-µm nylon filter unit into the same 2-L eluent bottle. Connect the eluent bottle to the Eluent A line from the pump 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.

Column Wash (100 mM sodium hydroxide/800 mM sodium acetate)

Measure ~800 mL of deionized water into a 1 L graduated cylinder. Add a magnetic stir bar and begin stirring. Slowly pour 65.62 g of anhydrous sodium acetate (82.03 g/mole) into the graduated cylinder. Stir until it is fully dissolved and then remove the magnetic stir bar. Using a rinsed plastic pipette, measure 5.2 mL of 50% (w/w) sodium hydroxide solution and pipette it into the graduated cylinder. Fill and dispense (several times) the sodium hydroxide/sodium acetate solution from the pipette to fully rinse the viscous sodium hydroxide into the same graduated cylinder. Add deionized water to the 1000 mL mark and briefly stir to mix the sodium hydroxide addition. To remove particles, vacuum filter the solution through a 0.2-µm nylon filter unit into the eluent bottle. Connect the eluent bottle to the column wash eluent line from the pump 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 column wash.

Standard Preparation

The standards were prepared gravimetrically and deionized water was used as the diluent. A stock solution of 100 mM sucralose (397.64 g/mol) solution was prepared from 0.795 g of reference grade crystalline sucralose (Splenda® by McNeil Nutritionals) dissolved in deionized water to a total weight of 20.00 g. The 100 mM sucralose standard was diluted appropriately with deionized water to prepare the 0.1 µM, 0.3 µM, 0.5 µM, 5 µM, and 10 µM sucralose standards.

Fructose (180.16 g/mol), sucrose (342.30 g/mol), and glucose (180.16 g/mol) stock standards were prepared in the same way as the sucralose stock standard. Individual stock solutions of 100 mM fructose, 100 mM sucrose, and 100 mM glucose were prepared from reference grade standards, 0.360 g of fructose, 6.846 g of sucrose, and 0.360 g of glucose, respectively, and dissolved in deionized water to a final weight of 20.00 g. The 100 mM standards were diluted with deionized water to prepare 10 µM standards. All of the standards were stored in the freezer at -5 ºC and thawed prior to use. These standards determined the retention times of the fructose, sucrose, and glucose.

Sample Preparation

The carbonated beverages were degassed by freezing them overnight. The samples were thawed and diluted prior to analysis. Brand C peach citrus beverage was diluted 50-fold, as prescribed for sugar-free beverages in Dionex Application Note 159. The dilution levels of the reduced-carbohydrate colas were established during the course of this work and are described in the next section of this document.

Results and Discussion

According to the manufacturer's label, Brand A reduced-carbohydrate cola contains: carbonated water; high fructose corn syrup and/or sucrose; caramel color; natural flavors; phosphoric acid, potassium benzoate, and potassium citrate; caffeine; and the sweeteners aspartame, potassium acesulfame-K (potassium salt of acesulfame), and sucralose. Brand B reduced-carbohydrate cola contains: carbonated water; high fructose corn syrup and/or sucrose; carmel color; natural flavors; caffeine; sucralose; and phosphoric acid, potassium benzoate, citric acid, and potassium citrate.

The first experiments were designed to determine the optimum dilution level that would minimize the amount of fructose–sucrose on the column while maintaining a measurable amount of sucralose. We tested the following dilutions: 2-, 10-, 50-, 100-, 500-, 1000- and 5000-fold dilutions. A 100-fold dilution minimized column overload while maintaining a measurable sucralose peak for both brands of reduced-carbohydrate colas.

The goal for this project was to establish conditions that would elute the fructose and sucrose, as early as possible, and elute the sucralose a short time later, well resolved from the other sugars. We experimented with isocratic separations, using a fixed concentration of sodium hydroxide with variable sodium acetate concentrations. Using 100 mM concentration of sodium hydroxide ensures sucralose ionization and good detection sensitivity. Acetate is a stronger eluent than hydroxide, hence it was used to control retention. Low concentrations of sodium acetate in the eluent resulted in long sucralose retention times (>25 min using <20 mM sodium acetate), or poor sucralose response (<75 mM sodium acetate). High concentrations of sodium acetate (200 mM) eluted sucralose in ~3 min, but caused it to be poorly resolved from sucrose. A 100 mM so-

dium hydroxide/90 mM sodium acetate eluent achieved a good separation of sucralose from fructose and sucrose in under 10 min. Figure 1 shows an overlay of individual injections of 10 µM fructose, glucose, sucrose, and sucralose standards under the stated conditions. Note that glucose, fructose, and sucrose all elute in under 2 min while sucralose elutes at about 6 min.

We applied our HPAE-PAD method to the analysis of two reduced-carbohydrate colas, Brand A and Brand B (Figure 2). While sucralose eluted at about 6 min, there were a number of unknown compounds in these colas that eluted after sucralose (not shown). To ensure that the compounds did not carry over to the next injection, the method run time was extended to 30 min for the reduced-carbohydrate colas and no late-eluting compounds were observed in subsequent injections. To assess method ruggedness, we alternately analyzed standards (2–4 injections), reduced-carbohydrate colas (5 each), and reduced-carbohydrate colas spiked with 10 µM sucralose standard (3 injections). While Figure 3 shows that the retention time of sucralose dropped slightly during the course of the analysis, presumably as a result of the other components in the two colas, the resolution was still sufficient for quantitative analysis. These other components together with excess fructose

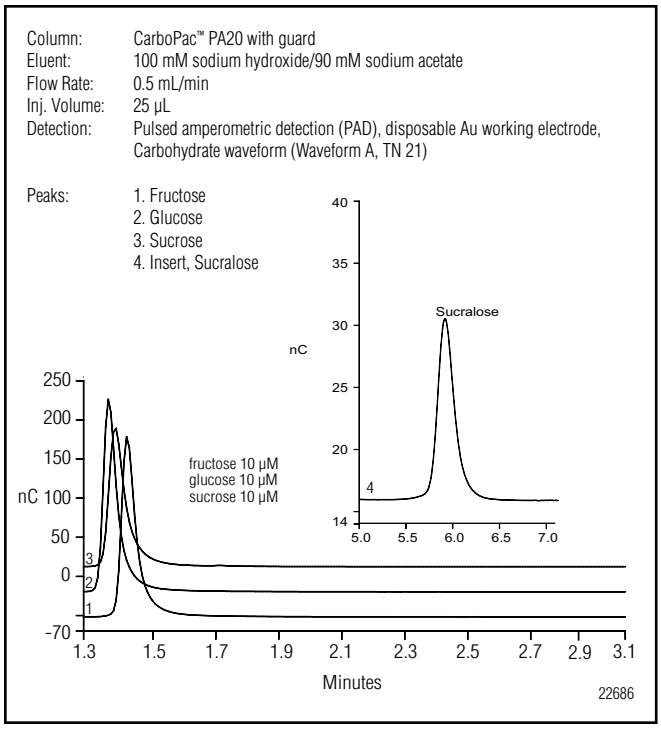

Figure 1. HPAE-PAD analysis of 10-µM standards.

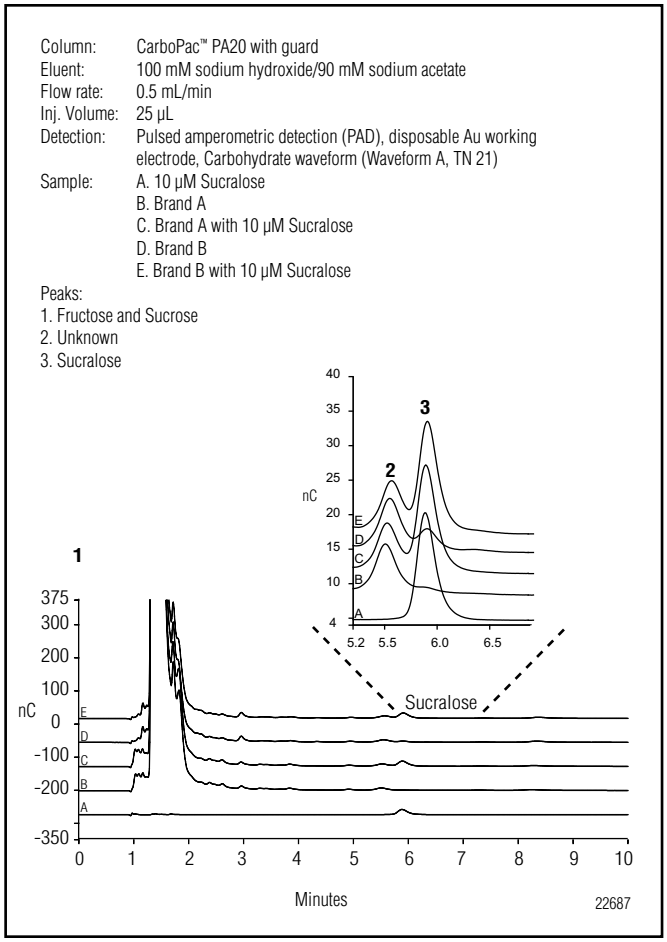

Figure 2. HPAE-PAD analysis of sucralose in a 100-fold dilution of the reduced-carbohydrate colas.

and sucrose also caused sucralose to elute earlier in Brand B reduced-carbohydrate cola compared with Brand A (Table 1). A 30-min column wash of 100 mM sodium hydroxide/800 mM sodium acetate and a 30-min equilibration were added after 24 h of analysis to restore the retention time of sucralose to its original value.

The HPAE-PAD system was calibrated with triplicate injections of sucralose standards (0.1 µM, 0.3 µM, 0.5 µM, 5 µM, and 10 µM sucralose) with 10 min runs (the additional isocratic time was not needed as the standard did not contain late-eluting compounds). This calibration was linear ($r^2 > 0.999$) and was used to determine the sucralose calibrations in the colas. From the sample analyses in Figure 3, we determined the concentration of sucralose in the two reduced-carbohydrate colas. In the 100-fold dilutions measured, Brands A and B reduced-carbohydrate colas had concentrations of 0.26 ± 0.02 µM sucralose and 1.88 ± 0.16 µM sucralose, respectively

(Table 2). To ensure that we were accurately determining these concentrations, we conducted a spike recovery study. Table 2 shows that we observed good recovery for both colas. The spike recovery demonstrates that the observed variability in retention time of the reduced-carbohydrate colas (Table 1) is not interfering with sucralose quantification.

This method was optimized for sucralose and does not fully resolve fructose, sucrose, and glucose for quantification. Fructose, sucrose, and glucose can be determined separately with a 10,000-fold dilution using gradient or isocratic methods.[9] This high dilution (i.e., 10,000-fold dilution) illustrates the sucralose concentration disparity between the diet colas and the reduced-carbohydrate colas, and the difficulty of determining low concentrations of sucralose in a high fructose/sucrose matrix.

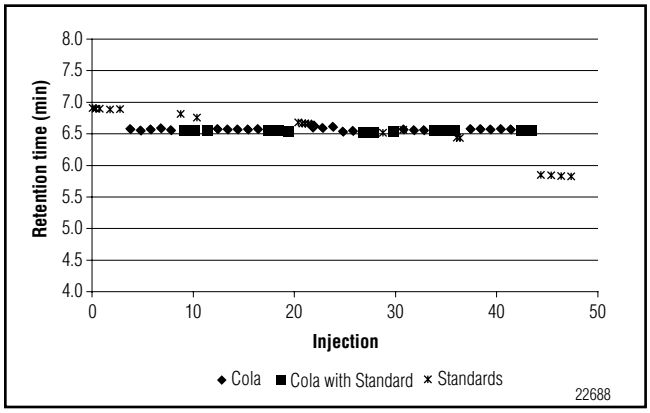

Figure 3. Retention time stability of sucralose during a one-day analysis of reduced-carbohydrate colas.

We also determined sucralose concentration in a low carbohydrate, peach citrus beverage. The peach citrus beverage is a store brand beverage, marketed as a caffeine free, low sodium, low carbohydrate, fruit beverage. According to the manufacturer's label, it contains mostly water with citric acid, potassium citrate, sodium benzoate, potassium sorbate, EDTA, gum acacia, along with sodium hexametaphosphate, natural flavors, the sweeteners sucralose, acesulfame-K, food dye Yellow 6, and food dye Red 40. This sample does not contain sucrose and fructose in high concentrations that can interfere with sucralose determinations. Figure 4 shows that a 50-fold dilution of the peach citrus beverage has no interference from fructose and sucrose. We also found that sucralose in citrus peach beverage did not exhibit the same retention loss with sample injection as observed for the reduced-carbohydrate colas. We analyzed this sample on two separate occasions (21 days apart) and found similar retention times of 6.35 ± 0.02 (n = 6) and 6.23 ± 0.03 min (n = 21). Using the same sucralose method as for the reduced-carbohydrate colas, we determined

Table 1. Retention Time and Area Stability of Reduced-Carbohydrate Colas and Beverages

Beverage	Retention Time (min)	Area (nC-min)
Brand A Reduced-Carbohydrate Cola	6.54 ± 0.05 n = 24	0.08 ± 0.01 n = 24
Brand B Reduced-Carbohydrate Cola	6.02 ± 0.24 n = 21	0.68 ± 0.06 n = 21
Brand C Peach Citrus*	6.23 ± 0.03 n = 21	2.07 ± 0.02 n = 21
9.86 µM Sucralose Standard	6.33 ± 0.46 n = 13	3.44 ± 0.26 n = 13

* Analysis performed on a separate day.

Table 2. Determination of Sucralose in Reduced-Carbohydrate Colas and Beverages

Beverage	Measured Concentration of Diluted Beverage (µM)	Calculated Undiluted Concentration (µM)	Spike Recovery (%)
Brand A Reduced-Carbohydrate Cola	0.26 ± 0.02 n = 24	25.6 ± 1.9 n = 24	101.9 ± 4.3 n = 20
Brand B Reduced-Carbohydrate Cola	1.88 ± 0.16 n = 21	188 ± 16 n = 21	88.1 ± 6.5 n = 23
Brand C Peach Citrus[a]	7.77 ± 0.08 n = 21	389 ± 4 n = 21	—[b]

[a] 50-fold dilution.
[b] not determined

the sucralose concentration in the peach citrus beverage to be 391 ± 4 µM (Table 2). The peach citrus beverage has very low concentrations of fructose and sucrose (compare Figures 2 and 4) and low concentrations of late-eluting unknown compounds (not shown). For this sample, the method can be shortened to 8 min for faster sample throughput. It is recommended that the sucralose retention time be evaluated after 24 h to determine if a column wash is needed.

Conclusion

The HPAE-PAD method described in this application update can be used to determine low concentrations of sucralose in the difficult high fructose and sucrose matrix of reduced-carbohydrate colas. The success of this type of analysis depends on determining the appropriate sample dilution to achieve a sucralose concentration that can be measured accurately.

Precautions

Food and beverage samples can degrade over time, especially after they have been degassed. Reduced-carbohydrate and low carbohydrate beverages should be stored in the refrigerator until sample analysis.

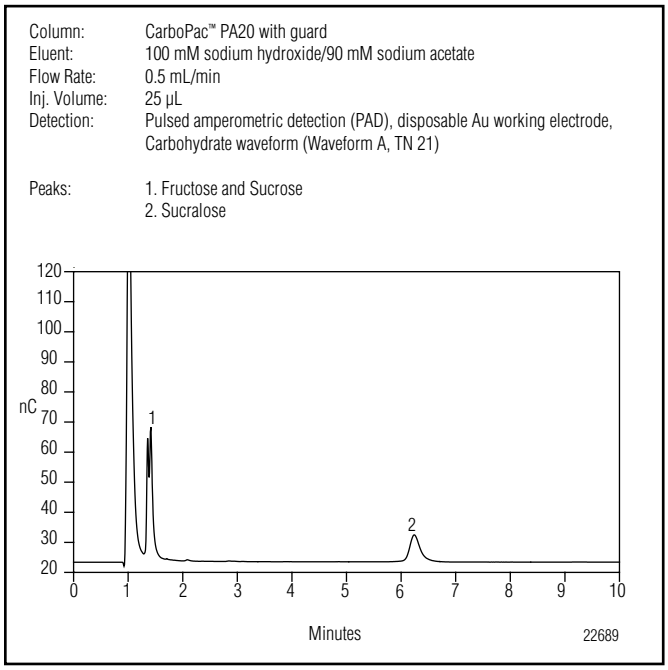

Figure 4. Determination of sucralose in a 50-fold dilution of Brand C peach citrus low-carbohydrate beverage.

References

1. *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector*. Technical Note 21; Dionex Corporation, Sunnyvale, CA, 1998.

2. Hanko, V.; Rohrer, J. Determination of Sucralose in Splenda and a Sugar-Free Beverage Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. *J. Agric. Food Chem.* **2004**, *52*, 4375–4379.

3. Ichiki, H.; Semma, M.; Sekiguchi, Y.; Nakamura, M.; Ito, Y. Determination of Sucralose in Food by Anion-Exchange Chromatography with Pulsed Amperometric Detection. *Jpn. J. Food Chem. Nihon Shoku-hinkagaku Gakkaishi* **1995**, *2*, 119–121.

4. Kobayashi, C.; Nakazato, M.; Yamajima, Y.; Ohno, I.; Kawano, M.; Yasuca, K. Determination of Sucralose in Foods by HPLC. *Shokuhn-Eiseigata-Zasshi* **2001**, *42*, 139–143.

5. Kishi, H.; Kawana, K. Determination of Sucralose in Foods by Anion-Exchange Chromatography and Reversed-Phase Chromatography. *Shokuhn-Eiseigata-Zasshi* **2001**, *42*, 133–138.

6. Operator's Manual for ED50A Electrochemical Detector. LPN 031772; Dionex Corporation, Sunnyvale, CA, 2002.

7. Installation Guide for Disposable Electrodes. LPN 065040; Dionex Corporation, Sunnyvale, CA 2002.

8. Product Manual for CarboPac PA20 Guard Column and CarboPac PA20 Analytical Column. LPN 031884; Dionex Corporation, Sunnyvale, CA, 2003.

9. *Determination of Sucralose Using HPAE-PAD*. Application Note 159; LPN 1572; Dionex Corporation, Sunnyvale, CA, 2004.

Suppliers

Fisher Scientific International Inc., Liberty Lane, Hampton, NH 03842 USA, Tel: 1-800-766-7000, www.fisherscientific.com.

McNeil Nutritionals, LLC, a Johnson and Johnson Company, Rt 2 Box 16M Industrial Rd, McIntosh, AL 36553 USA, Tel: 1-800-777-5363, www.splenda.com.

U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-179, USA, Tel: 1-800-227-8772, www.usp.org.

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

LPN 1766 PDF 09/16