Analysis of Sucralose in Water by Liquid Chromatography/ Time-of-Flight Mass Spectrometry (LC/TOF-MS) after Automated Solid Phase Extraction

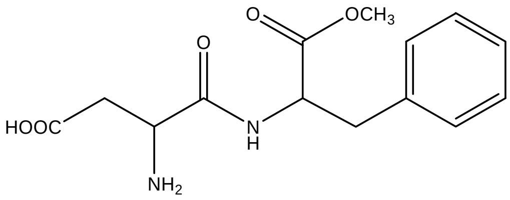
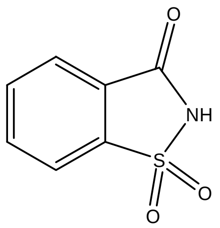
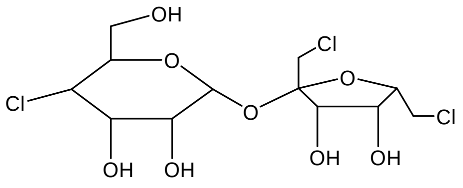
Keywords: Aspartame, Environment, Groundwater, GX-271 ASPEC™, LC/TOF-MS, Saccharin, Solid Phase Extraction, SPE, Sucralose, Surface Water, Water, Wastewater

This study was performed by Imma Ferrer, Ph.D. and E. Michael Thurman, Ph.D. at the Center for Environmental Mass Spectrometry, Dept. of Civil, Environmental & Architectural Engineering, University of Colorado, Boulder, CO, USA.

Introduction

Non-nutritive sweeteners are steadily increasing in importance with increased awareness of diabetes and its special dietary requirements and with more consumers becoming concerned about obesity and dental caries. Some of the most widely used non-nutritive sweeteners include saccharin, aspartame and sucralose (Table 1).

Table 1. Elemental composition, base peak ions, and chemical structures of aspartame, saccharin and sucralose.

Name	Elemental Composition	Base peak Ion	Chemical Structure
Aspartame	$C_{14}H_{18}N_2O_5$	**$[M+H]^+$** $C_{14}H_{19}N_2O_5^+$ 295.1288	
Saccharin	$C_7H_5NSO_3$	**$[M+H]^+$** $C_7H_6NSO_3^+$ 184.0063	
Sucralose	$C_{12}H_{19}Cl_3O_8$	**$[M+Na]^+$** $C_{12}H_{19}Cl_3NaO_8^+$ 419.0038	

Sucralose is a water soluble tri-chlorinated disaccharide that is 600 times sweeter than sugar. It is highly stable and will not readily degrade at high temperatures (McNeil Nutritionals, 2007). In 1991, Canada's Health Protection Branch became the first national regulatory agency to permit the use of sucralose in foods and beverages. In 1999, the U.S. Food & Drug Administration (FDA) approved permitting the use of sucralose as a general-purpose sweetener in a broad range of products. The European Union amended its Sweeteners Directive to permit the use of sucralose in a variety of foods and beverages in January 2004. Sucralose is now permitted for use in over 60 countries and has been consumed by millions worldwide.

Sucralose has a low human toxicity and is not metabolized by the human body. One would expect the presence of sucralose in wastewater effluent. Sucralose does not bioaccumulate in the environment; however, there is a lack of knowledge of the long-term biological effects of sucralose in the aquatic environment. There has been a great deal of interest in monitoring levels of sucralose in water samples (Dye, 2007; Brorström-Lundén, 2007; Mead et al., 2009).

Recently, some methods employing liquid chromatography-mass spectrometry (LC/MS) have been published for the analysis of sucralose (Loos et al., 2009) and other artificial sweeteners in water samples (Scheurer et al., 2009). This study (Ferrer and Thurman, 2010), describes the analysis of several sweeteners in water using liquid chromatography coupled to time-of-flight mass spectrometry (LC/TOF-MS) as well as the automated extraction of these sweeteners from wastewater, surface water and groundwater utilizing the Gilson GX-271 ASPEC System (Figure 1).

Figure 1. Gilson GX-271 ASPEC System with 406 Single Syringe Pump (Part no. 2614007)

Experimental Conditions

Materials

All solvents were distilled in glass suitable for GC, HPLC, pesticide residues analysis and spectrophotometry. HPLC solvents were obtained from Merck (Darmstadt, Germany). Formic acid and hydrochloric acid were obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain HPLC-grade water. Analytical standards were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Supelco (Bellefonte, PA, USA). Individual stock solutions (1000 µg/mL) were prepared in pure methanol and stored at -18 degrees C. The SPE procedure used Oasis™HLB (500mg, 6 mL) obtained from Waters Corporation (Milford, MA, USA).

Sample Preparation of Water Samples

Water samples (including wastewater, surface and groundwater) were collected from different locations around the United States. Wastewater samples were collected from effluent locations downstream from the wastewater treatment plants. Surface water was obtained from several rivers and reservoirs and groundwater was collected from wells. Extraction was performed using the Gilson (Middleton, WI, USA) GX-271 ASPEC System. We noted that aspartame required acidification to pH 3 for complete sorption onto the cartridge. Nevertheless, sucralose showed lower recovery at this low pH (41%), therefore no pH adjustment of the water samples was performed.

SPE Hardware

The Gilson GX-271 ASPEC System was configured as follows:

Description	Part Numbers
GX-271 ASPEC w/ Single 406 Syringe Pump	2614007
25 mL Syringe	25025346
SPE Pressure Reg. Assembly and plumbing package for gas + 25 mL Plumbing Package	25051376, 2644703 and 2644702
221x1.5x1.1 BV Tapered Probe and Guide Assembly for 1.5 mm Probes	27067374 and 26046228
Rinse Stations	26034551 and 26034555
Locator Tray for three 200-series Racks	26041035
Rack Code 211 for 9 – 48 x 113mm (125 mL) bottles	2504611
Rack Code228 for 4 – (500 mL or 700 mL) bottles and pkg of four 700 mL bottles	2504628 and 543701700
Rack Code 306 for 20 – (6 mL) SPE cartridges and Disposable Sealing Caps for 6 mL SPE cartridges	2504306 and 2954730
Glass collection tubes for 6 mL DECs, 10 mL (15 x 85mm), pkg of 1000	2954729

Viton tubing, .313 ID x .438 OD, 20 ft	4701438630
Safety Shield Assembly, GX27X	2604706
TRILUTION® LH Software	21063020, 210630R20 and ORACLE10GXE

Solid Phase Extraction (SPE) Protocol

The SPE procedure used 6 mL Waters Oasis™ HLB (500 mg) Cartridges. The cartridges were sealed using Gilson 6 mL Sealing Caps.

The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system. The SPE steps are summarized with the schematic provided in the GX-271 ASPEC control software, TRILUTION LH (Figure 2).

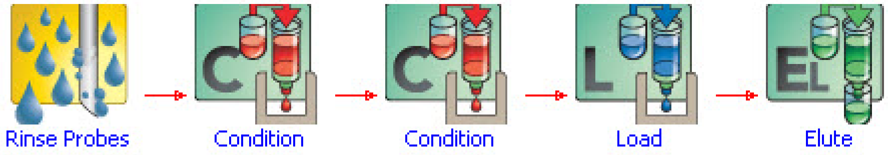


Figure 2. TRILUTION LH SPE Tasks for Extraction of Sucralose from Water

The details of each step are as follows:

- Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 3) and probe rinsed with 10% methanol
- Condition SPE cartridge with 4 mL of methanol at a flow rate of 1 mL/min
- Condition SPE Cartridge with 6 mL of water at 1 mL/min
- Load 200 mL of water sample at a flow rate of 10 mL/min. Follow this with an air push of 25 mL to remove any excess water
- Move the Gilson Mobile SPE Rack over the collection tubes
- Elute the analytes of interest with 5 mL methanol at 1 mL/min
- Evaporate to 0.5 mL with nitrogen at a temperature of 45°C in a water bath using a TurboVap® Concentration Workstation (Caliper Life Sciences, Mountain View, CA, USA)

Figure 3. Gilson Mobile Rack allows for easy method development

LC/TOF-MS Analysis

The separation of the selected sweeteners was carried out using an HPLC system (consisting of vacuum degasser, autosampler and a binary pump) (Agilent Series 1200, Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed phase C_8 analytical column of 150 mm x 4.6 mm and 5 μm particle size (Zorbax Eclipse XDB-C8). Column temperature was maintained at 25 °C. The injected sample volume was 50 μL. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. The optimized chromatographic method held the initial mobile phase composition (10% A) constant for 5 min, followed by a linear gradient to 100% A after 30 min. The flow-rate used was 0.6 mL/min. A 10-min post-run time was used after each analysis. This HPLC system was connected to a time-of-flight mass spectrometer Agilent 6220 MSD TOF equipped with a dual electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 4000 V; nebulizer pressure: 45 psig; drying gas: 9 L/min; gas temperature: 300 °C; fragmentor voltage: 190V; skimmer voltage: 60V; octopole RF: 250 V. LC/MS accurate mass spectra were recorded across the range 50-1000 *m/z* at 4GHz. The data recorded was processed with MassHunter software. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a dual-nebulizer ESI source that introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution (calibrant solution A, Agilent Technologies), which contains the internal reference masses (purine ($C_5H_4N_4$ at *m/z* 121.0509 and HP-921 *[hexakis-(1H,1H,3H-tetrafluoro-pentoxy)phosphazene]* ($C_{18}H_{18}O_6N_3P_3F_{24}$) at *m/z* 922.0098. The instrument worked providing a typical mass resolving power of 15000±500 (*m/z* 922).

Results

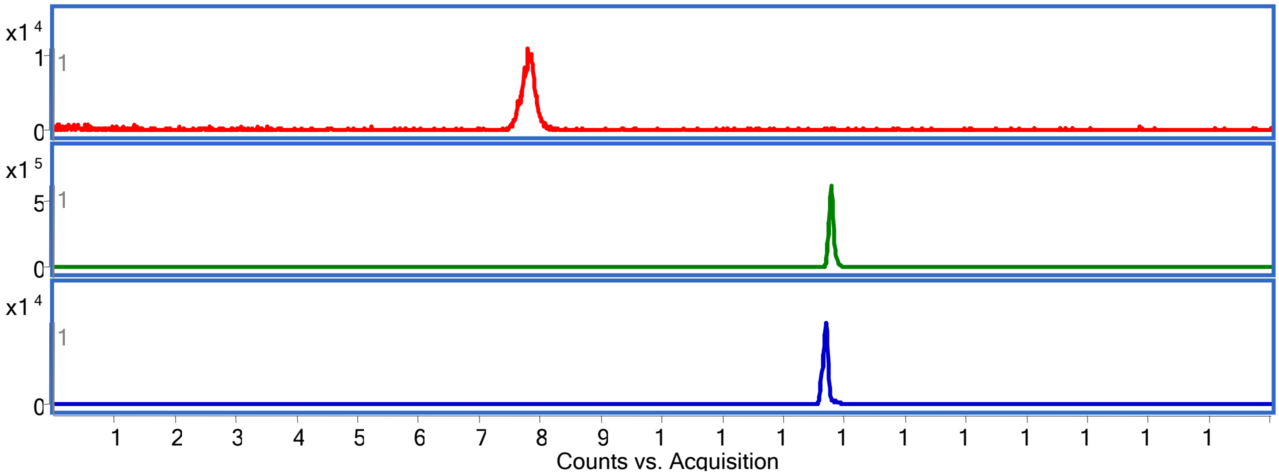


Figure 4. LC/TOF-MS ion chromatograms for saccharin (m/z 184, ret. time = 7.9 min), aspartame (m/z 295, ret. time = 12.9 min) and sucralose (m/z 419, ret. time = 12.8 min). The concentration of the standard is 0.5 µg/mL.

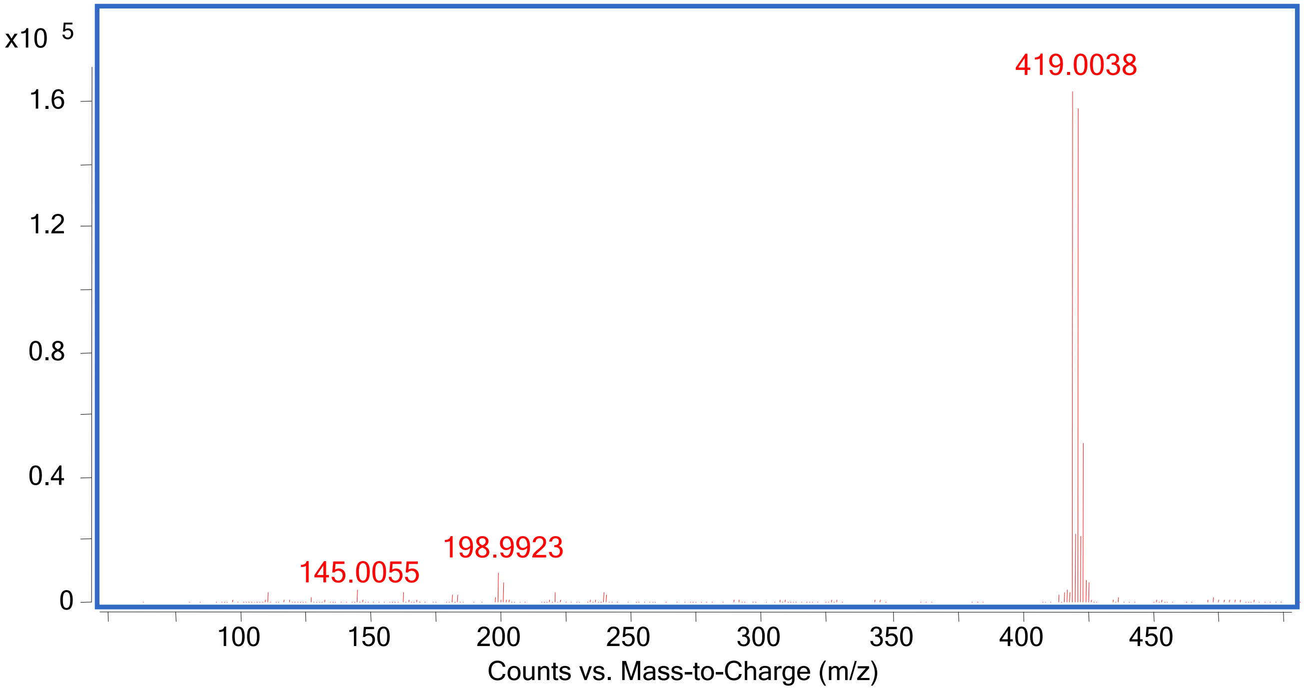


Figure 5. Mass Spectrum for Sucralose

Table 3. Analytical performance parameters for sweeteners in water with SPE followed by LC/TOF-MS.

Compound	**External Calibration range (µg/mL)**	**R^2**	**% Recovery (RSD)**	**Method LODs (µg/L)**	**Method LOQs (µg/L)**
Aspartame	0.05 - 5	0.997	90 (6)	0.02	0.2
Saccharin	1 - 10	0.994	53 (8)	0.5	5
Sucralose	0.1 - 5	0.999	73 (5)	0.05	0.5

Table 4. Analysis of representative wastewater, surface water and groundwater, from different locations in the USA showing concentrations for saccharin and sucralose. No positive findings for aspartame were found. n.d. = not detected (below LOD). n.q. = detected but not quantified (concentration below LOQ).

Samples	Saccharin concentration (µg/L)	Sucralose concentration (µg/L)
Wastewater Location 1(CO)	5	1
Wastewater Location 1(CO)	n.d.	1.8
Wastewater Location 2(CO)	n.d.	0.8
Wastewater Location 3 (OH)	n.d.	1.5
Wastewater Location 3 (OH)	n.d.	1.8
Surface Water Location 2(CO)	n.d.	n.d.
Surface Water Location 2 (CO)	n.d.	n.d.
Surface Water Location 2 (CO)	n.d.	n.d.
Surface Water Location 2 (CO)	n.d.	n.d.
Surface Water Location 4 (CO)	n.d.	n.d.
Surface Water Location 4 (CO)	n.d.	n.d.
Surface Water Location 4 (CO)	n.d.	n.d.
Surface Water Location 4 (CO)	n.d.	n.d.
Surface Water Location 5 (TX)	n.d.	n.d.
Surface Water Location 5 (TX)	n.d.	n.d.
Surface Water Location 5 (TX)	n.d.	n.d.
Surface Water Location 5 (TX)	n.d.	n.d.
Surface Water Location 6 (AL)	n.d.	<LOQ
Surface Water Location 6 (AL)	n.d.	<LOQ
Surface Water Location 6 (AL)	n.d.	<LOQ
Surface Water Location 6 (AL)	n.d.	n.d.
Surface Water Location 7 (CO)	n.d.	0.8

Surface Water Location 7 (CO)	n.d.	1.6
Surface Water Location 7 (CO)	n.d.	1.0
Surface Water Location 7 (CO)	n.d.	1.8
Surface Water Location 8 (MN)	n.d.	<LOQ
Surface Water Location 8 (MN)	n.d.	n.d.
Ground Water Location 9 (CO)	n.d.	0.8
Ground Water Location 9 (CO)	n.d.	2.4
Ground Water Location 9 (CO)	n.d.	1.4
Ground Water Location 9 (CO)	n.d.	2.2
Ground Water Location 10 (CO)	n.d.	0.6
Ground Water Location 10 (CO)	n.d.	1.6
Ground Water Location 10 (CO)	n.d.	2.0
Ground Water Location 10 (CO)	n.d.	2.0

Conclusion

This application note describes the conditions necessary to automate the solid phase extraction of sucralose, saccharin and aspartame from water samples prior to analysis by LC/MS-TOF using the Gilson GX-271 ASPEC System. Extraction recoveries were 90% for aspartame, 53% for saccharin and 73% for sucralose. The RSD for inter-day (n=5) values were between 5 and 8% showing good reproducibility of the methodology. The LC/MS-TOF limit of detection for sucralose was 0.05 µg/L.

Automation of the SPE process allows one to reduce potential errors that may occur during manual extractions, increase lab efficiency, reduce solvent usage and increase sample throughput. Automation also allows one to easily optimize extraction conditions for different matrices and multiple classes of analytes.

Sucralose was the most detected sweetener showing up in wastewater, surface water and groundwater samples. Five wastewater samples from three different locations all showed positive detections for sucralose. Eight surface water samples out of 22 were positive for sucralose and had no detections for the other two sweeteners. Eight alluvial groundwater samples from two locations were positive for sucralose. These are the first reports of sucralose in groundwater, which are most likely the effect of surface water being drawn into these alluvial wells during pumping.

The study data suggest that sucralose may be an excellent tracer of wastewater-contaminated surface water because of its widespread occurrence and its apparent stability in wastewater and surface water. Studies are currently underway to compare sucralose to caffeine as tracers of sewage wastewater in groundwater and drinking water supplies.

References

Brorström-Lundén, E., Svenson, A., Viktor, T., Woldegiorgis, A., Remberger, M., Kaj. L., Dye, C., Bjerke, A. and Schlabach, M. (2007). Measurements of Sucralose in the Swedish Screening Program 2007. **Swedish Environmental Institute (IVL) Report B1769**, January 2008.

Dye, C. (2007). Sucralose – a new persistant pollutant in the environment? **Norwegian Institute for Air Research (NILU).** http://www.nilu.com News Bulletin, 29th May 2007.

Ferrer, I. and Thurman E.M. (2010). Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight-mass spectrometry. **J. Chromatogr. A.** *In Press.* Doi:10.1016/j.chroma.2010.02.020

Loos, R., Gawlik, B.M., Boettcher, K., Locoro, G., Contini, S. and Bidoglio, G. (2009). Sucralose screening in European surface waters using a solid-phase extraction-liquid chromatography-triple quadrupole mass spectrometry method. **J. Chromatogr. A 1216** (7):1126-1131.

Mead, R.N., Morgan, J.B., Avery, G.B., Kieber, R.J., Kirk, A.M., Skrabal, S.A. and Wiley, J.D. (2009). Occurrence of the artificial sweetener sucralose in coastal and marine waters of the United States. **Marine Chemistry 116** (1-4):13-17.

McNeil Nutritionals, LLC (2007). Sucralose: a scientific and safety review. http://www.splendaprofessional.com/splendaprof/pages/safety_fda_approval_science_and_safety_review.jsp

Scheurer, M., Brauch, H.J. and Lange, F.T. (2009). Analysis and Occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). **Analytical and Bioanalytical Chemistry 394** (6):1585-1594.