Journal of Chromatography A, 1217 (2010) 4127–4134

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight mass spectrometry

Imma Ferrer*, E. Michael Thurman

Center for Environmental Mass Spectrometry, Dpt. Civil, Environmental and Architectural Engineering, University of Colorado, Boulder, CO, USA

ARTICLE INFO

Article history:
Available online 20 February 2010

Keywords:
Sweeteners
Sucralose
LC–TOF-MS
Time-of-flight
Accurate mass
Environmental
Groundwater
Wastewater

ABSTRACT

A methodology for the chromatographic separation and analysis of three of the most popular artificial sweeteners (aspartame, saccharin, and sucralose) in water and beverage samples was developed using liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS). The sweeteners were extracted from water samples using solid-phase extraction (SPE) cartridges. Furthermore, several beverages were analyzed by a rapid and simple method without SPE, and the presence of the sweeteners was confirmed by accurate mass measurements below 2-ppm error. The unambiguous confirmation of the compounds was based on accurate mass measurements of the protonated molecules $[M+H]^+$, their sodium adducts and their main fragment ions. Quantitation was carried out using matrix-matched standard calibration and linearity of response over 2 orders of magnitude was demonstrated ($r > 0.99$). A detailed fragmentation study for sucralose was carried out by time-of-flight and a characteristic spectrum fingerprint pattern was obtained for the presence of this compound in water samples. Finally, the analysis of several wastewater, surface water and groundwater samples from the US showed that sucralose can be found in the aquatic environment at concentrations up to 2.4 μg/L, thus providing a good indication of wastewater input from beverage sources.

1. Introduction

Sweeteners can be divided in two main groups: caloric, or nutritive, and noncaloric or non-nutritive compounds. Nutritive sweeteners are carbohydrates or their derivatives such as glucose, fructose and maltose. Non-nutritive sweeteners do not belong to any particular chemical group and they are usually known as artificial sweeteners. Synthetic sweeteners are steadily increasing in importance with increased public awareness of diabetes and its special dietary requirements, and with more consumers becoming concerned about obesity and dental caries.

The most frequently used synthetic sweeteners are: saccharin, cyclamate, aspartame, and sucralose. Artificial sweeteners are widely used all over the world, and some of them have a long history. For example, saccharin was invented nearly 100 years ago. Artificial sweeteners taste similar to cane sugar, but are low-calorie. They benefit overweight people and those who have problems with sugar metabolism. Artificial sweeteners are also cheaper than natural sugar and can reduce the cost for some foods and beverages. Sucralose is a polar, chlorinated sugar which five hydroxyl groups and three chlorine groups. It is synthetically produced from saccharose by selectively replacing three hydroxyl groups with chlorine atoms. Sucralose has been approved as a sweetener in several countries. In 1991, Canada's Health Protection Branch became the first national regulatory agency to endorse sucralose safety and permit its use in foods and beverages. In 1998, the United States Food and Drug Administration (FDA) approved the use of sucralose in 15 food and beverage categories (the broadest initial approval ever given to a food additive). Later, in 1999, the FDA extended the approval by permitting sucralose use as a general-purpose sweetener in all foods, beverages, dietary supplements and medical foods. In January 2004, the European Union amended its Sweeteners Directive to permit the use of sucralose in a broad range of food and beverage products. Sucralose is now permitted for use in over 60 countries and has been consumed by millions of people worldwide.

However, scientific research has shown that some artificial sweeteners can cause tumors in certain animals, so to prevent potential danger to humans, it is necessary to control the amount of sweeteners in foods and beverages. Regulations set an upper limit on the concentration of artificial sweeteners in foods and beverages. Moreover, the labels of foods and beverages should list what kinds of sweeteners are used. Toxicological data has led to the use of some artificial sweeteners being controlled. For example cyclamate is banned in the United States, the United Kingdom and Japan. The potential toxicity of sucralose has been reviewed as well [1,2].

---

* Corresponding author. Tel.: +1 303 7354147.
E-mail address: imferrer@ono.com (I. Ferrer).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.02.020

Quality control or spot-checking can use a conventional HPLC method to determine the amount of the sweeteners in the samples. To date, artificial sweeteners have been determined by HPLC with reversed phase chromatography using different buffer systems, ion pairing reagents and specific derivatization procedures (aspartame with o-phthalaldehyde [OPA]; cyclamate with 4-fluoro-7-nitrobenzofurazone [NBDF]). Derivatization overcomes detection limitations for these compounds in the low UV range. A number of methods have been published [3,4] for simultaneous determination of aspartame and saccharin. Herrmann et al. [5] reported on a method for the detection of aspartame, cyclamate, dulcin, and saccharin using an ion-pair HPLC separation with indirect photometric detection. However, such varied methods with their differing derivatization protocols make the analysis of artificial sweeteners time consuming and labor intensive. Recently, some methods employing liquid chromatography mass spectrometry have been published for the analysis of sucralose [6] and other artificial sweeteners in water samples [7]. In this work we will explore the capabilities of accurate mass measurements using LC/TOF-MS for the unequivocal identification of three major sweeteners in soft drinks and water samples. We determine the fragment ions of all three compounds with accurate mass measurements. To our knowledge this is the first report for the identification of sweeteners in drinks and water samples using time-of-flight mass measurements.

2. Experimental

2.1. Chemicals and reagents

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 °C. HPLC-grade acetonitrile and methanol were obtained from Honeywell Burdick & Jackson (Muskegon, MI, USA). Formic acid and hydrochloric acid was 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 the HPLC-grade water used during the analyses. For the SPE procedure, Oasis HLB cartridges (500 mg, 6 mL) obtained from Waters (Milford, MA, USA) were used.

2.2. Sample preparation

2.2.1. Beverages and liquid solutions

A simple and quick method was used to analyze liquid samples such as soft drinks and liquid syrups. Soft drinks were juice drinks purchased from a local grocery store. Liquid syrups, such as cough and cold medicines were obtained from a local pharmacy. An aliquot of the sample was taken and filtered through a 0.2 μm membrane to eliminate any particulate present in the solution. Soft drinks were injected directly into the mass spectrometer. Additional dilution with deionized water was performed for those beverages and solutions (i.e. cough syrup) containing high amounts of sweeteners.

2.2.2. Water samples

Water samples (including wastewater, surface, and groundwater) were collected from different locations and states around the US. Wastewater samples were collected from effluent locations downstream from the wastewater treatment plants, surface waters were from several rivers and reservoirs, and groundwaters were collected from wells. An off-line SPE was used for the pre-concentration of the water samples. All the extraction experiments were performed using an automated sample preparation with extraction column system (GX-271 ASPEC, Gilson, Middleton, WI, USA) fitted with a 25-mL syringe pump for dispensing the water samples through the SPE cartridges. Disposable cartridge columns packed with 500 mg of Oasis HLB sorbent were used. The cartridges were conditioned with 4 mL of methanol followed by 6 mL of HPLC water at a flow rate of 1 mL/min. The water samples (200 mL) were loaded at a flow rate of 15 mL/min. Elution of the analytes from the cartridge was carried out with 5 mL of methanol. The solvent was evaporated to 0.5-mL with a stream of nitrogen at a temperature of 45 °C in a water bath using a Turbovap concentration workstation (Caliper Life Sciences, Mountain View, CA, USA).

2.3. LC/TOF-MS analyses

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 × 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: 190 V; skimmer voltage: 60 V; octopole RF: 250 V. LC/MS accurate mass spectra were recorded across the range 50–1000 *m*/*z* at 4 GHz. 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 15,000 ± 500 (*m*/*z* 922).

3. Results and discussion

3.1. Optimization of LC/TOF-MS for the analysis of sweeteners

Analysis of sweeteners has been carried out mainly by tandem mass spectrometric techniques [6–9]. In this work, we exploited the capabilities of high resolution mass spectrometry using time-of-flight detection for the unequivocal identification of the most three common sweeteners in various matrices and determination of their fragmentation ions and pathway of fragmentation. Our approach follows previous work of our group that successfully used mass spectrometry tools such as accurate mass and isotope cluster profiling for the analysis of pesticides in food commodities [10–12]. By using this technique, and specifically for compounds that have characteristic isotope elements (such as sulfur and chlorine), unequivocal identification can be carried out by selecting the accurate mass of both the protonated molecule and a characteristic fragment ion and their respective isotope patterns.

Fig. 1 shows a chromatographic separation of aspartame, saccharin and sucralose using a linear gradient and a C_8 column. The extracted ion chromatograms for each of the analytes are shown. This column provided retention for the three analytes investigated.