Original Research Article

Examining the Artificial Sweeteners in Commonly Consumed Beverages, Chewing Gums, Chocolates, and Mouthwashes using HPLC and TLC Methodology

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Abstract

Background: Artificial sweeteners (AS) are synthetic compounds extensively used in food and beverages industries due to its techno-functional role as alternatives to table sugar. Many studies report the adverse health effect on use of such compounds beyond its permitted limit. Hence, the aim of present study is to analyzed the AS contents in selected commonly consumed beverages and food products.

Methods: High-Performance Liquid Chromatography and Thin Layer Chromatography methodology was used to carry out the analysis.

Results: Concentration of Ace-K ranged from 7 to 14 mg/100 ml, sucralose from 83 to 93 mg/100 ml, and aspartame 11mg/100ml in beverages. In mouthwashes, saccharin ranged from 102 to 140 mg/100g. Among chocolates, saccharine was observed from 9 to 13 mg/100g. Chewing gums contained 9 to 193 mg/100 g of aspartame, 118mg/100g of Ace-K, and 82 to 155 mg/100g of sucralose.

Conclusion: The data could help in public awareness as well as regulatory bodies in monitoring the levels of AS found in food products and beverages with officially permitted limits.

Keywords: Artificial sweeteners, HPLC, TLC, food products, beverages

Introduction

Artificial sweeteners (AS) are synthesized carbohydrate or protein derivatives, which are 200-1300 folds, sweeter than sugars, and used as a sweetening agent in the food and dairy industries. Most of these sweeteners are excreted from the body without metabolization (sucralose and acesulfame potassium), however, some of it is metabolized to respective amino acids as in the case of Aspartame.[1] There is a strong correlation between the usage of sugars in the form of carbonated beverages and various metabolic dyshomeostasis like an increase in blood glucose insulin and triglyceride levels[2] with augmented inflammatory mediators and

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oxygen radicals[3] which increases the risk of diabetes, cardiovascular disease, and other chronic illnesses in humans.[4] India has about 65 million diabetic patients which are predicted to reach around 100 million by 2030, and about 77 million pre-diabetic adults, and children in the age group of 13-14 suffering from diabetes which is mainly due to changes in lifestyle, food choices and of physical activity leading to overweight as well as obesity.[5] Indian diet is a rich source of carbohydrates and free sugars and has become the capital of diabetes in the world and is also leading in obesity and heart diseases, where increase in awareness about free sugars and their risk assessment in the development of metabolic syndrome, has brought about a drastic shift towards the consumption of AS.[6] Of late, AS are extensively used in the preparation of sugar-free bakery products, which can be consumed by diabetic patients.[1] The use of these AS at prescribed levels has been approved by various regulatory bodies like FDA/FAO-WHO, European food safety authorities, and EU, Canadian food inspection agency, Food Standards of Australia, and New Zealand (FSAN) and FSSAI (Food Safety and Standards Authority of India).

According to FDA, aspartame, sucralose, neotame, acesulfame potassium (Ace-K), saccharin, as well as advantame are major highly intense sugar substitutes approved for use as AS in the USA.[7] FDA guides manufacturers and consumers about the daily limits of consuming these highly intense sweeteners measured in Acceptable Daily Intake (ADI) expressed as mg/kg body weight/day (milligrams per kilograms of body weight per day). ADI for sucralose as per USFDA is 5mg/kg body weight/day USFDA.[8] For aspartame it is 50mg/kg body weight/day, Ace-K and saccharin is 15mg/kg body weight/day. According to JECFA (2004),[9] ADI for neotame is 2mg/kg body weight/day, where as in US the ADI is 18mg/person/day.[10] According to the Europe food commission, 8 AS are permitted as tabletop sugars or food additives, which include neotame, cyclamate, steviol glycosides, aspartame-acesulfame salt along with sucralose, neotame, Ace-K, saccharin, and advantame.[10] They have also reported that AS are permitted to be used in a wide range of foods and beverages in the UK and labeled as 'sugar-free' or 'diet, however, is restricted in Infant foods.

Although AS are found to be safe at the level of recommended daily intake from scientific studies, however, few studies exhibited incidences of cancer and psychological impairments in patients who consumed artificial sweeteners for a longer duration.[11] Further, a recent study published in Nature, demonstrated that artificial sweeteners are found to induce glucose intolerance in animal models which also exhibited an alteration in the gut microflora of the exposed animals at doses close to the recommended levels.[10] Therefore, ADI levels for consumption of artificial sweeteners are to be strictly fixed and proper labeling on food products is recommended for helping consumers to maintain ADI levels. Although artificial sweeteners are found to be promising in weight and diabetic management, there is a modest alarm concerning the health status of consumers.[11]

The consumption of AS is gradually increasing in India due to the availability of sugar-free products and tabletop low-calorie sweeteners which are known to regulate the patient's glycemic levels to achieve diabetic management. Amid reports by FDA as well as industry-funded investigations favor on the safety of food-additives, the lack of evidential research is still the matter of concern to draw a conclusion on their application. However, many studies depicted the adverse effect of artificial sweeteners on gut health and neurological imbalances at doses slightly higher than recommended levels in animal and human studies. Therefore, the primary focus is to quantify the levels of high intense sugars such AS present in various food products and beverages available in the local market as a public health concern.

Materials and Methods

Different branded varieties of products such as Mouthwashes, Beverages, Chocolates, and Chewing gums were obtained from local market of twin-city of Hyderabad-Secunderabad, Telangana State, India. Standard AS such as Aspartame, Saccharine, Ace-K, Neotame, and sucralose were procured from Sigma- Aldrich. All other chemicals were of analytical grade. The LC C18 column was procured from Supelco (25cmx4.6mm,5μm), and Thin Layer Chromatography (TLC) plates from Merck (TLC-Silica-gel 60G-F254 Glass Plates). The analysis of AS such as aspartame, saccharine, Ace-K, and neotame was carried out by using High-Performance Liquid Chromatography (HPLC) (Dionex Ultimate 3000 U-HPLC), and sucralose by TLC.

Sample preparation:

For the estimation of sucralose in beverage samples, they were prepared by concentrating 10 times by heating in a water bath for 30min at 80ºC. For chewing gums, about 5 g of the sample was extracted using a water and methanol mixture in a 9:1 ratio. For the estimation of aspartame, saccharine, Ace-K, and neotame, beverage samples were prepared by mixing them with water in the ratio of 1:1 and 20 µl of each sample were injected in HPLC. For chewing gums, about 5 g of sample was extracted using water-methanol (1:1 ratio). For sucralose, about 6µl of the sample was loaded on a TLC plate.

Preparation of Standards:

The stock solution of individual standard such as aspartame, saccharine, Ace-K, and neotame was prepared by dissolving (5 mg) in 10ml of water. From the stock solution, 100µl was taken and diluted with 100µl of Milli-Q water (0.25µg/µl). Standard sucralose was prepared by dissolving 0.1g in 1ml of methanol.

Preparation of Mobile Phase:

For the estimation of sucralose, the mobile phase is prepared by mixing 7 volumes of 5% w/v aqueous solution of sodium chloride and 3 volumes of acetonitrile. For aspartame, the mobile phase was of 75%water, 20% methanol, and 5% acetone. For Ace-K, and saccharine, 70% of 0.02 mol/L ammonium acetate solution and 30% methanol was used and the pH was maintained at 6 using glacial acetic-acid to prepare the mobile phase. For neotame, a mixture of 30% acetonitrile, 70% distilled water, and 0.34% phosphoric acid mobile phase was used.

HPLC Operating Conditions:

HPLC analysis of AS was carried out by the procedure described by de Queiroz Pane et al.[12] The chromatographic system consisted of a Dionex HPLC-DAD system chromatograph equipped with Chromeleon software and an integrator stainless steel Supelco C-18 column (25 cm 4.6 mm, 5µm particle size) as described by Yan et al.[13] Flow rate was set at 1 ml/min with an injection volume of 20µl. The detection wavelength for aspartame was 208nm for 10 min run time, neotame 210 nm for 16 min run time, and Ace-K and saccharine 229 nm for 3 min run time, respectively. The HPLC was calibrated daily by injecting 20 µl of standard solutions of individual AS, the concentration of each AS was 2.5 mg/ml and 0.5 mg/ml for sucralose.

Estimation of sucralose by TLC:

Sucralose was estimated by following the method of the 41st Joint FAO/WHO Expert Committee on Food Additives[9] by the TLC method with slight modification. The commercially available TLC plates (TLC Silica gel 60G F254 Glass Plates) were used for the analysis of sucralose. 5 µl of standard and test solution was applied in triplicate spots to the bottom of the chromatographic plate and dried with a hair dryer. The plate was placed in chromatography chamber containing freshly prepared mobile phase where solvent-front was allowed to mount 15 cm. Then plate was made dried after removing from chamber, after which one spot was sprayed with spraying reagent of 15% v/v solution of conc. sulfuric acid in methanol. The plate was then kept in an oven at 125^0C for 10 min for the color to develop and thereby locate the sucralose. For quantitative estimation, the area (2x3 cm) corresponding to sucralose was scraped and soaked in 3mL of distilled water for 12h. After 12h, the mixture was filtered through Whatman No. 1 filter paper, and the sucralose in 1ml of the filtrate was estimated using the modified anthrone method.[14]

Statistical analysis:

All experimental analysis was repeated 3 times. The results were presented as mean from three replications with standard deviation (SD). The meanvalues were tested for existence of difference by using student's t test.

Results

The HPLC-DAD chromatogram for AS is represented in figure 1, where A and B represents the peak of standard Ace-Kand its presence in Pepsi sample that was eluted at a retention time of 2.8 minutes on a maximum wavelength of 229 nm. C and D show the peak of standard aspartame and its presence in Coke with elution at 9th minutes, at a wavelength of 208nm. E and F represents the standard saccharine and its presencein Close-up mouthwash that was eluted at a retention time of 3.5 minutes, at a wavelength of 229 nm. Similarly, G shows the chromatogram of standard Neotame eluted at a retention time of 9 minutes at a maximum wavelength of 210 nm.

The artificial sweetener content of beverages are summarized in Table 1. From the table, it is evident that the Ace-K was found to be the most commonly present AS in many of the beverages analyzed, where, highest amount was found in coke followed by Pepsi diet, Pepsi black, Red Bull and monster (plain) respectively, however coke was the only beverage containing aspartame. Sucralose content was highest in Red Bull followed by Monster (combo). None of the beverages were detected with Neotame and saccharine.

The AS in mouthwashes such as Close up and Listerine are given in Table 2.Among the AS analyzed, saccharine was found in both the mouthwashes and the highest content in Close up followed by in Listerine respectively.

The AS contents in chocolates are summarized in Table 3. Among the AS analyzed only saccharine was found in chocolates. The highest saccharine content observed in Five-star followed by in Cadbury chocolates, respectively.

The AS contents of Chewing gums are given in Table 4.Most are showed the presence of Aspartame and sucralose and the one chewing gum with Ace-K. Among the chewing gums, aspartame was found highest in mint and lowest in orbit 2, sucralose was highest in center fresh-2 and lowest in Smint, Ace-K was present in Orbit-1 respectively. Neotame was not detected in any of the food products tested.

Table 1 Artificial sweeteners content in Beverages (mg/100ml) [a]

Sample name	Aspartame	Saccharine	Acesulfame Potassium (Ac k)	Sucralose	Neotame
Coke	11± 0.0	ND	14± 0.01	ND	ND
Red Bull	ND	ND	8 ± 0.01	93± 0.00	ND
Monster (combo)	ND	ND	ND	83± 0.00	ND
Monster (plain)	ND	ND	7± 0.00	ND	ND
Pepsi Diet	ND	ND	11 ± 0.00	ND	ND
Pepsi Black	ND	ND	10 ± 0.00	ND	ND
Mirinda	ND	ND	ND	ND	ND
Mountain Dew	ND	ND	ND	ND	ND
Pepsi	ND	ND	ND	ND	ND

[a] Values are expressed as Mean ± SD of triplicates values

ND: Not Detected

Table 2 Artificial sweeteners content in Mouthwashes (mg/100ml) [a]

Sample name	Aspartame	Saccharine	Acesulfame Potassium	Sucralose	Neotame
Close up	ND	140± 0.02	ND	ND	ND
Listerine	ND	102± 0.01	ND	ND	ND

[a]Values are expressed as Mean ± SD of triplicates values

ND: Not Detected

Table 3 Artificial sweetener content in Chocolates (mg/100g) [a]

Sample name	Aspartame	Saccharine	Acesulfame Potassium	Sucralose	Neotame
Dark chocolate	ND	ND	ND	ND	ND
Five star	ND	13± 0.00	ND	ND	ND
Cadbury	ND	9± 0.00	ND	ND	ND
Kopiko	ND	ND	ND	ND	ND
Amul chocomini	ND	ND	ND	ND	ND
Imlitoffee	ND	ND	ND	ND	ND

[a]Values are expressed as Mean ± SD of triplicates values

ND: Not Detected

Table 4 Artificial sweetener content in Chewing gums (mg/100g) [a]

Sample name	Aspartame	Saccharine	Acesulfame Potassium	Sucralose	Neotame
Happy dent (sugar free)	121 ± 0.01	ND	ND	ND	ND
Mentos	131 ± 0.01	ND	ND	ND	ND
Mint	193 ± 0.00	ND	ND	ND	ND
Center fresh 2	9± 0.00	ND	ND	ND	ND
Orbit 2	9 ± 0.00	ND	ND	ND	ND
Orbit 1	ND	ND	118 ± 0.01	ND	ND
Center fresh 1	ND	ND	ND	155± 0.00	ND
Smint	ND	ND	ND	82± 0.00	ND
Double mint	ND	ND	ND	143 ± 0.00	ND

Discussion

James and Claudette, [15] have reported the use of liquid chromatography (LC) as one of the preferred methods for determining AS. Though there are other methods available to determine individual AS, less numbers of study suggests the analysis of multiple AS at a time. Spangenberg et al, [16] have described TLC-method for the determining Sucralose in different food-matrices, which rarely needs the isolation or concentration as sample preparation. Zygler et al, [17] have reported that the sample-preparation is the important step involved in nay analytical-process because of its variance in terms of components present in the sample that can obstruct in analyzing sweeteners. Therefore, the method must be customized with care for determining the determinants, contemplating the instrumental reliability. de Queiroz Pane et al, [12] reported that a combination of AS is used in most food samples, containing up to three compositional determinants. Among soft drinks, aspartame was the most used sweetener, followed by Ace-K. Kubica et al, [18] reported eight simultaneous determination of AS such as Ace-K, saccharine, cyclamate, aspartame, sucralose, alitame, neohesperidin dihydrochalcone, neotame, and five common steviol glycosides in soft and alcoholic beverages using HPLC with tandem mass spectrometry with electrospray ionization (HPLC-ESI-MS/MS). James and Claudette, [15] have analyzed seven artificial sweeteners such as aspartame, saccharin, cyclamate, alitame, Ace-K, sucralose, and dulcin in diets, soft drinks, and table top sweetener preparations by using reverse-phase LC with absorbance Detection. Carloni, [19] has estimated the saccharine from commercial AS using a potentiometric method and suggested the reliability and advantage of the method than existing methodology for analyzing commercial AS. Yan et al, [13] have demonstrated the simultaneous analysis of Ace-K, sodium saccharin, sodium benzoate, and potassium sorbate in ham sausage samples using HPLC-DAD method and concluded that this method is appropriate for monitoring the quality-assurance.

Conclusion

Since level above officially permitted limit might adversely affect the health status, it is high time for consumers to aware of what and how much they are ingesting the AS from markedly available food products and beverages, hence it is pertinent for regulatory body to periodically evaluate the inspection of testing reports of commonly consumed food products and beverages.

Ethical clearance: Not Applicable

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Conflict of Interest: Nil

Abbreviations:

AS: Artificial sweeteners

SD: standard deviation

HPLC: High-Performance Liquid Chromatography

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