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Determination of aspartame and alitame in liquid dairy products and milk-containing beverages in the Chinese market

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PAPER

Abstract

Aspartame and alitame are a type of food additives commonly used in recent years. However, it is difficult to conclude that long-term use of synthetic sweeteners is completely harmless. This study aimed to establish a simple high performance liquid chromatography (HPLC) method to detect these two sweeteners, and to measure the concentrations of sweeteners in liquid dairy products and milk-containing beverages in the Chinese market. In this experiment, aspartame and alitame had a good linearity, and the average recovery values were also good with the relative standard deviations (RSDs) of 0.4–2.2%. The limits of detections (LODs) were 0.52 and 0.48 μg g^{-1} for aspartame and alitame, respectively. According to consumer daily purchase habits, 100 samples were purchased from supermarkets and milk tea shops in Jinan, China. The results showed that the amount of sweeteners added in all samples did not exceed the national standards, but there was a problem that the food label content was incomplete. We hope that the relevant departments would strengthen supervision and management of food labelling, and protect the legitimate rights and interests of consumers.

Keywords: alitame; aspartame; HPLC; liquid dairy products; milk-containing beverages

Introduction

With the development of the food industry, more and more food additives are used in food processing to improve the colour, taste, smell, nutritional value, and shelf-life of food. Food additives are synthetic or natural substances added to food, which can not only improve food quality, colour, aroma and taste but also meet the needs of anti-corrosion, preservation, and processing technology. There are more than 2000 kinds of food additives permitted by the state (GB 2760-2014), which are divided into 22 types according to their functions, mainly including sweeteners, preservatives, acidity regulators, antioxidants, colorants, bleaching agents, etc. Among them, sweeteners are used in all kinds of food to increase the flavour of food. According to different sources, sweeteners can be divided into two categories, one is natural sweeteners such as licorice and stevioside, and the other is synthetic sweeteners. In the past few decades, the global prevalence of obesity has increased rapidly. The survey of lifestyle shows that excessive consumption of sugars such as sugary drinks may lead to obesity, diabetes, and cancer (Malik *et al*., 2010). The World Health Organization recommends that the intake of free sugars should not exceed 10% of the total energy intake (WHO, 2015). In order to reduce the intake of sugar, some countries have begun to levy sugar tax and have successfully reduced sugar consumption and obesity rate (Nakhimovsky *et al*., 2016). Industry is also responding to this policy by reducing sugar in food processing. Sugar is an indispensable ingredient in food processing, because it can improve the structure, colour,

and taste of food. Hence, reducing the sugar content in food may have a negative impact on the taste of food. To ensure sweet taste while reducing sugar, synthetic sweeteners are usually used instead of sugar. The human body does not digest and absorb sweeteners (such as sugar alcohols). The content of sweetener is high and provides little energy, so its dosage is very small. In order to prevent obesity, cardiovascular disease, diabetes, and other chronic diseases, consumers tend to choose foods containing lower calorie sweeteners. As a result, "sugar free" or "no added sugar" products with synthetic sweeteners instead of sugar have become more and more popular. At present, the artificial synthetic sweeteners approved for use in China mainly include aspartame, alitame, saccharin sodium, acesulfame, sodium cyclamate, sucralose, etc.

Aspartame, the chemical name of L-aspartyl-L phenylalanine methyl ester, also known as proteoglycan, sweetener, aspartame essence, aspartame mother, aspartame, etc., belongs to dipeptide derivatives. At room temperature, it is white crystalline powder. The taste is very similar to sucrose, and the sweetness is 100–200 times of sucrose. In China, aspartame can be used as sweetener and flavour enhancer in dairy products, pastries, seasonings, drinks, jellies, puffed foods, etc. After people consume aspartame, it completely decomposes into aspartic acid, phenylalanine, and methanol in the stomach and intestines, and then is absorbed in the blood. Patients with phenylketonuria lack phenylalanine hydroxylase, and their excessive intake of aspartame causes accumulation of phenylpyruvate in the body and damages the nervous system. Thus, foods containing aspartame should be marked as follows: aspartame (containing phenylalanine).

Alitame, the chemical name of L-α-aspartyl-n-(2,2,4,4-tetramethyl-3-sulfotrimethyl)-d-alanamide, also known as aspartame, is a dipeptide derivative, which is generally a white crystalline powder without odour and hygroscopicity. The sweetness of alitame is 2000 times higher than that of sucrose, and its properties are relatively stable, especially for heat and acid. Alitame is easily soluble in water or hydroxyl containing solvents, but it is difficult to dissolve in lipophilic organic solvents. Alitame is often used with other sweeteners and has a good sweetening synergistic effect. China approved the use of alitame in 1994. It can be added to dairy products, frozen beverages, preserves, beverages, gum-based candies, jellies, and other foods as a sweetener (Wang and Shen, 2019).

At present, with the increasing demand for "sugar free" or "low sugar" products, synthetic sweeteners can be an ideal and calorie-free sugar substitute in the food industry. The FDA has approved six synthetic sweeteners (edvantame, aspartame, acesulfame, neotame, saccharin, and sucralose) and two natural non-nutritive sweeteners (stevioside and mogrosides). These sweeteners are considered safe to eat. In the European Union, Australia, and New Zealand, sugar alcohols such as maltitol, lactose alcohol, xylitol, and erythritol are also considered safe to eat. FAO, WHO, and JECFA points out that the use of food additives within the permitted scope will not cause damage to the human body (Praveena *et al*., 2019). China's standard for the use of food additives also refers to the international standards, which clearly stipulates the amount of sweeteners used in all kinds of food.

Nevertheless, the safety of synthetic sweeteners with high sweetness is still controversial. Emerging epidemiological evidence shows that food additives may have non-benign biological effects and have a negative impact on human intestinal flora, leading to the destruction of intestinal mucus layer (Rinninella *et al*., 2019). Eating excessively processed and packaged foods is associated with increased all-cause mortality and increased global incidence of obesity, metabolic syndrome, and inflammatory bowel disease (IBD) (Kim *et al*., 2015; Marion-Letellier *et al*., 2019; Pinget *et al*., 2019; Rico-Campà *et al*., 2019). For example, with the increase in the sales of processed food, the incidence of IBD in China has increased significantly (Ng *et al*., 2013). Recent studies have shown that the metabolic and inflammatory effects of food additives may be induced by changes in intestinal flora. Artificial sweeteners in food can change the number of intestinal bacteria, increase the pathogenicity of intestinal bacteria, and interfere with the normal environment of human and animal intestinal mucosa (Bian *et al*., 2017; Swidsinski *et al*., 2009; Zuo *et al*., 2018). Studies suggest artificial sweeteners such as saccharin, sucralose and aspartame can induce glucose intolerance in rats. Artificial sweeteners have metabolic effects and may lead to type 2 diabetes, obesity, and other diseases (Suez *et al*., 2014).

Consumption of synthetic sweeteners during pregnancy and lactation has adverse effects on the metabolism of infants, which may lead to metabolic disorders in later life (Olivier-Van Stichelen *et al*., 2019). Plows *et al*. find that the intake of synthetic sweeteners during pregnancy can lead to glucose intolerance, hyperglycaemia, shortened pregnancy time, male fetal growth restriction, and female fetal hypoglycaemia (Plows *et al*., 2020). In addition, studies have shown that consumption of aspartame during pregnancy can increase the obesity rate and make offspring more prone to anxiety behavior (Palatnik *et al*., 2020). Long-term consumption of synthetic sweeteners is closely related to memory loss, Alzheimer's disease, and depression (Burke and Small, 2015; Gao *et al*., 2018), in addition to carcinogenicity and genotoxicity (Mao and Song, 2018; Purohit and Mishra, 2018; Zhao and Wang, 2018).

Studies have shown that the effect of aspartame may be related to methanol or its metabolites. As a neurotoxin, methanol is harmful to human health. One litre of beverage containing aspartame can produce about 56 mg of methanol, and a can of beverage containing aspartame can produce about 22.4 mg of methanol. The Environmental Protection Bureau recommends that the daily intake of methanol should not exceed 7.8 mg (Liu *et al*., 2012). Moreover, long-term consumption of aspartame can cause migraine (Sathyapalan *et al*., 2015; Zaeem *et al*., 2016). Some scholars have found that aspartame has become a new type of water pollutant, which can affect the water environment through biochemical reactions (Lin *et al*., 2017; Seo *et al*., 2016). According to national regulations (GB 28050-2011), additives in food must be clearly marked on the label, but there are still phenomena of incomplete and non-standard content of food labels. It is necessary to strengthen the supervision of the use of synthetic sweeteners.

At present, the detection methods of aspartame and alitame mainly include ultra-high performance liquid chromatography (UPLC) (Jin, 2020; Lu *et al*., 2021; Yu *et al*., 2018), ion chromatography (Xie *et al*., 2011; Zhu *et al*., 2005), high performance liquid chromatography (HPLC) (Chen and Li, 2006), and high performance liquid chromatography tandem mass spectrometry (HPLC-MS) (Tang *et al*., 2019; Wang *et al*., 2019; Yang and Chen, 2009), etc. However, most of the above methods require expensive equipment, which is not available in conventional laboratories. In this study, a convenient and reliable HPLC method for the determination of aspartame and alitame was developed and validated. Furthermore, this study measured the concentrations of aspartame and alitame in liquid dairy products and milk-containing beverages collected from Jinan supermarkets and milk tea shops. The results could provide a basis for improving market supervision and regulation in China.

Materials and Methods

Reagents and chemicals

Standards: Aspartame (purity asis for improving market supervision and regulation Solarbio (Beijing, China). Acetonitrile (HPLC grade) was purchased from Damao Chemical Reagent Factory (Tianjin, China), ethanol (superior purity) from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), filter paper (diameter 15 cm) from Hangzhou Special Paper Co., Ltd. (Zhejiang, China), organic filter membrane (0.45 μm) from Beijing high purity Technology Co., Ltd. (Beijing, China), and the experimental water was Milli-Q double distilled water (Bedford, MA, USA).

Sample collection and treatment

All the samples were selected according to the daily purchasing habits of consumers: 100 samples of different types, including 40 liquid dairy products and 60 milk-containing beverages, were collected randomly from supermarkets and milk tea shops in Lixia District of Jinan, China, in August 2019.

An aliquot of 5 g (accurate to 0.0001 g) of thoroughly and adequately mixed sample was put into a 50 mL centrifuge tube (Shimadzu analytical balance AUX120, Kyoto, Japan). Ten milliliters ethanol was added to each sample and then sealed. The centrifuge tube was turned upside down five times without oscillating for milk-containing beverages, or was vortexed for 10 s for liquid dairy products. The resulting mixture was left at room temperature for 1 min, and then centrifuged at 4000 rpm for 5 min (Eppendorf centrifuge 5430R, Hamburg, Germany). The supernatant was transferred to a 25 mL volumetric flask. The residue was dissolved with 8.0 mL ethanol/water (2/1, v/v) and centrifuged at 4000 rpm for 5 min. Subsequently, the supernatant was transferred into the same 25 mL volumetric flask and made up to volume with ethanol/water (2/1, v/v). Finally, the solution was filtered through a 0.45 μm organic filter membrane, and 20 μL aliquot solution was injected into the HPLC system.

Standard solutions

Stock standard solutions of aspartame and alitame were prepared at 205.8 and 200 μg mL^{-1} by dissolving 2.1 and 2.0 mg of powder in 10 mL of water, respectively. All the stock solutions were stored at 4°C for utilization. A mixed standard solution was produced by transferring 2.5 and 1.7 mL of aspartame and alitame stock solutions into a 10 mL volumetric flask and making up to volume with water. Thereafter, a series of mixed standard solutions were obtained by further dilution. The concentrations of aspartame in the mixed standard solutions were 0.51, 1.03, 10.29, 34.99, and 51.45 μg mL^{-1}, respectively; the concentrations of alitame were 0.50, 1.00, 10.00, 34.00, and 50.00 μg mL^{-1}, respectively. The mixed standard solutions were stable for 1 month and were stored at 4°C.

HPLC conditions

A LaChrom Elite HPLC system (Hitachi, Tokyo, Japan) was used for the determination of aspartame and alitame and was quantified by diode array detection using an L-2455 diode array detector (Hitachi, Tokyo, Japan) with the wavelength of 200 nm. The sample was separated on a C18 column (4.6 × 150 mm, 5 μm) (Elite, Dalian, China)

at 30°C, with a mobile phase of 20% acetonitrile and 80% water at a flowrate of 1 mL min^{-1}, and an injection volume of 20 µL.

Method validation

The HPLC method was validated using the following parameters: linearity, recovery, precision, LOD, and limits of quantification (LOQ). Linearity was evaluated from the five concentration levels of mixed standard solution. Recovery was assessed by spiking the sample with two sweeteners at three fortified concentrations of 0.285, 0.355, 0.425 µg mL^{-1} in three replicates, and calculated as the ratio of a measured concentration for spiked sample divided by spiked concentration. The precision tests were conducted using a sample added 71 µg of alitame standard to determine for six times in the same day. The precision was based on the relative standard deviation (RSD%). The LOD and LOQ were defined by signal/noise ratios of 3:1 and 10:1, respectively. Both LOD and LOQ were verified experimentally after injecting blank samples.

Results and Discussion

Optimization of chromatographic conditions

Detection wavelength

The maximum absorption wavelength of aspartame was 195.2 nm, and that of alitame was 198.7 nm. According to the literature, analytes were mostly detected at 200 or 208 nm. In this experiment, the detection wavelength was carried out at 200 nm based on the ultraviolet full-wavelength scanning spectrum.

Mobile phase

The composition of the mobile phase plays an important role in elution and chromatographic separation. At the beginning of this project, we planned to perform the HPLC analysis of aspartame and alitame using two types of mobile phases, methanol/water (40/60, v/v) and acetonitrile/water (20/80, v/v). It was found that the target peaks overlapped, which may be due to the interference of other impurities in the sample, when the mobile phase methanol/water (40/60, v/v) was used for sample detection. Other mobile phases were tested by changing the ratio of acetonitrile and water, but these mobile phases did not deliver a notable improvement to the chromatography. Therefore, acetonitrile/water (20/80, v/v) was used as the mobile phase in terms of peak shape, resolution, and overall analysis time.

Flow rate

The fast flow rate can increase column pressure, while slow flow rate can cause tailing deformation of chromatographic peaks. The flow rate of 0.8, 1, and 1.2 mL min^{-1} was employed for this experiment, and other experimental conditions remained unchanged. When the flow rate was 1.2 mL min^{-1}, the resolution of the chromatographic peak was poor. When the flow rate was 0.8 mL min^{-1}, the retention time (RT) of the chromatographic peak was longer. Considering comprehensively, a flow rate of 1 mL min^{-1} was suitable for sample analysis, and the analysis time was 10 min (Figure 1).

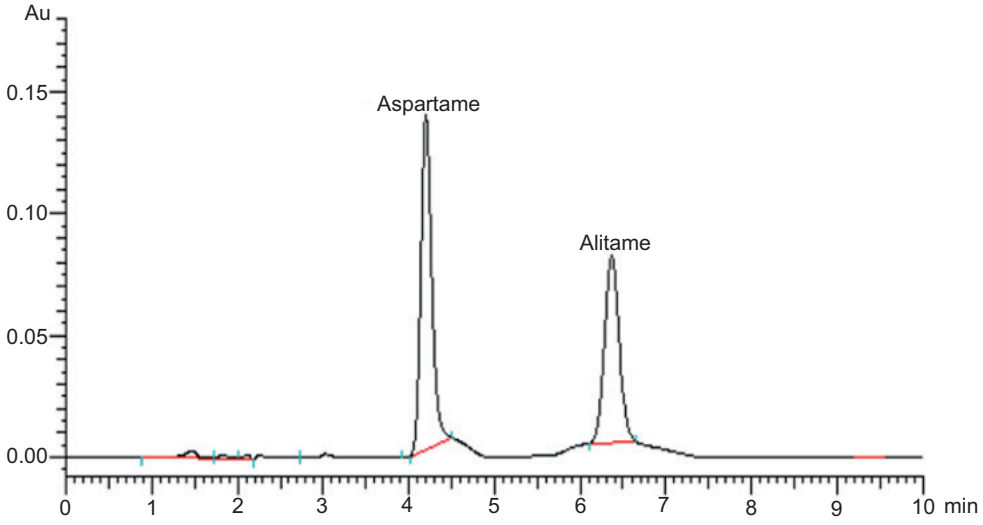

Figure 1. Chromatogram of aspartame and alitame standard solutions.

Method validation

After verification, the standard curve was plotted using the peak area as the ordinate and the concentrations as the abscissa. The linearity was good using the standard solutions at five concentration levels (Table 1). According to the experimental results, the average recovery values of aspartame and alitame were 93.0–107.3% and 97.2–98.2%, respectively. The repeatability expressed as RSD (%) was 1.1% for aspartame and 2.5% for alitame. The data corresponding to the recoveries and precisions were detailed in Tables 2 and 3, and met the needs of analysis with great reliability and repeatability. The LODs were 0.52, 0.48 μg g^{-1} for aspartame and alitame, respectively, and the LOQs for same analytes were 1.72 and 1.58 μg g^{-1}, respectively. Lu Y *et al*. established an analytical method for the detection of nine kinds of sweeteners and preservatives in baked products by UPLC, and the LODs of aspartame and alitame in this method were 3.0 mg kg^{-1}, which were higher than our method (Lu *et al*., 2021). In the study of UPLC detection of sweeteners in beverages, the LODs of aspartame and alitame were 0.75 mg kg^{-1} (Jin, 2020). For the liquid chromatography coupled with mass spectrometry techniques, the LODs of the two sweeteners were sufficient for trace analysis, which were 1.0 and 0.2 ug L^{-1}, respectively (Wang *et al*., 2019). Given the requirements of the equipment and the performance of the method, our method has the advantage of being simple, rapid, and low cost.

Analysis of aspartame and alitame in liquid dairy products and milk-containing beverages

Among the 100 samples in this survey, except for milk drinks on sale, the remaining 82 beverages all have label instructions. Milk tea is immensely popular among young people for its smooth and silky taste, but so far, many businesses lack the label of the ingredient list, especially for aspartame. In our study, under the LOD of this method, no alitame was detected in any sample and it was not identified on the food label. Notably, a total of 21 products were marked with aspartame on the food label, while there were far more than these in actual testing. The samples that detected aspartame accounted for nearly half of the total. Among them, aspartame was detected in 11 samples of 40 liquid dairy products (27.5%), and the concentrations ranged from 2.41 to 103.67 μg g^{-1}; aspartame was detected in 24 samples of 42 milk-containing beverages (57.1%), and the concentrations ranged from 1.81 to 61.49 μg g^{-1}; aspartame was detected in eight samples of 18 milk drinks on sale (44.4%), and the concentrations ranged from 2.14 to 21.11 μg g^{-1}. Table 4 summarized determination results of aspartame and alitame in liquid dairy products and milk-containing beverages from the Chinese market. The national standard stipulates a limit of 600 μg g^{-1} of aspartame in modified milk, protein drinks, and flavored drinks (GB 2760-2014). According to the regression equation, the measured value of aspartame in the sample was calculated, and compared with the national standard, none of them exceeded the standard.

Through the detection of liquid dairy products and milk-containing beverages samples, there was no phenomenon of excessive use of aspartame and alitame. Although alitame was rarely used in the products in this study, most of the products were added with aspartame. As a new type of sweetener in recent years, there is no guarantee that long-term consumption of synthetic sweeteners is safe. Therefore, the type of sweetener added should be identified on the food label, especially aspartame (containing phenylalanine). It is recommended that the market management department implement label

Table 1. Linear equation and linear range of aspartame and alitame.

Analyte	Linear equation	R	Linearity (μg mL^{-1})
Aspartame	y = 11485x + 2613	0.9993	0.51–51.45
Alitame	y = 9200x − 4606	0.9996	0.50–50.00

Table 2. Recoveries and RSD values of aspartame and alitame in samples.

Aspartame			Alitame		
Spiked (μg mL^{-1})	Average recovery %	RSD % n=3	Spiked (μg mL^{-1})	Average recovery %	RSD % n=3
0.285	93.0	2.7	0.285	98.2	1.6
0.355	100.1	2.0	0.355	97.8	0.4
0.425	107.3	1.9	0.425	97.2	1.2

Table 3. Precision test results.

Analyte	Measured value (μg g^{-1})						Average (μg g^{-1})	RSD %
	1	2	3	4	5	6		
Aspartame	595.53	591.05	587.69	581.18	586.44	578.53	586.73	1.1
Alitame	262.58	274.41	267.68	256.39	259.00	262.60	263.78	2.5

Table 4. Concentrations of aspartame and alitame in liquid dairy products and milk-containing beverages.

Group	N[(a)]	Food labels	Sweeteners	Labels containing sweeteners	Detected sample	Concentrations of sweeteners in detected samples (μg g^{-1})				
						Mean	P50[(c)]	P90[(c)]	P95[(c)]	Max
Liquid dairy products	40	40	Alitame	0	–[(b)]	–	–	–	–	–
			Aspartame	8	11	31.66	8.23	72.10	87.88	103.67
Milk-containing beverages (pre-package)	42	42	Alitame	0	–	–	–	–	–	–
			Aspartame	13	24	24.32	16.27	60.64	61.23	61.49
Milk-containing beverages (made-on-site)	18	0	alitame	0	–	–	–	–	–	–
			Aspartame	0	8	5.77	3.00	11.33	16.22	21.11

(a) Number of samples
(b) – indicates did not detect the Acesulfame in these samples
(c) 50th, 90th, and 95th percentiles of the distribution

management on milk drinks, and indicate the types of sweeteners used, which is conducive to protecting the legitimate rights and interests of consumers.

Conclusions

In this paper, a rapid and reliable HPLC method for the simultaneous determination of aspartame and alitame in liquid dairy products and milk-containing beverages from the Chinese market was developed and validated, which was applied for the analysis of 100 products. Since the use of artificial sweeteners has increased, continued monitoring and strengthening the regulation of the sweeteners used in food is essential. In addition, the analysis data showed that 14 samples with food labels were not labelled with aspartame. The mislabelling leads to uncertainty over both the contents and their concentrations, and increases the likelihood of excessive intake of artificial sweeteners. Thus, the results of this survey involve public health issues, and require the attention of the Chinese government to strengthen market supervision and regulation. However, there are still some uncertainties that should be considered. For example, the number of food types involved in this study is not big enough. A more thorough study of the types of artificial sweeteners in different kinds of food is needed.

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