Original Article

Identification and Determination Methods of Aspartame

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ABSTRACT

Objective: To evaluate identification and determination methods of a synthetic sweetener, aspartame.

Method: For identification methods, aspartame was tested with ninhydrin solution and with alkaline hydroxylamine solution by using thin layer chromatogram (TLC). TLC was developed with acetonitrile phosphate buffer. For determination methods, colorimetric method and HPLC method were used.

Results: Testing with ninhydrin solution was sensitive (limit of detection was 7.5 μg) but was not specific because this method was also able to detect amino acid with a free α-amino group. Testing with alkaline hydroxylamine solution (limit of detection was 300 μg) was specific method because it was used to detect carbonyl functional group and formed color complex with ferric chloride. The determination methods were colorimetric method (reaction with ninhydrin solution the same as in identification method) and high pressure liquid chromatographic method (HPLC). The relative standard deviation of colorimetric method of simple sugar samples was 5 - 17% and for HPLC method was 3-11%. Percent recovery of these two methods was about 97%. Aspartame was found lower than the labeled amount in many samples and the percent relative standard deviations were large because aspartame was a chiral compound and the samples were kept improperly so aspartame may degrade.

Key words: identification, determination, aspartame

Thai Pharm Health Sci J 2008;3(2):214-218[§]

Introduction

Synthetic sweeteners were used in diabetes patients to control blood sugar and to obese persons to control body weight since sweet taste can be offered with no calories. They are also used to reduce food manufacturing cost. Some synthetic sweeteners can be harmful. For example, cyclamate can exert a carcinogenic effect.

Aspartame is the derivative of dipeptide which is L-aspartyl-L-phenylalanine methyl ester. It has been used in the United States since 1981. Aspartame was metabolized to phenylalanine, aspartic acid and methanol (Figure 1).[1,2] It is contraindicated for the person who lacks enzyme phenylalanine hydroxylase. In the phenylalanine hydroxylase deficient person, phenylalanine was changed to phenylpyruvate and phenylacetate which can cause mental deterioration. Some people who took aspartame might have neurological complaints such as headache, depression and insomnia. Aspartame should not be used in the elderly with diabetes because these patients are more likely to already have dementia and visual deterioration problem. Acceptable daily intake (ADI) for aspartame based on FAO/WHO is 40 mg/kg.[3] To be able to quantify the amount of aspartame in food and beverage products effectively, methods to identify and determine aspartame are needed. Thus it is important to search for the practical identification and determination methods of

[§] 13th year of Srinakharinwirot Journal of Pharmaceutical Science

aspartame as one way to help control its use and promote public safety.[4]

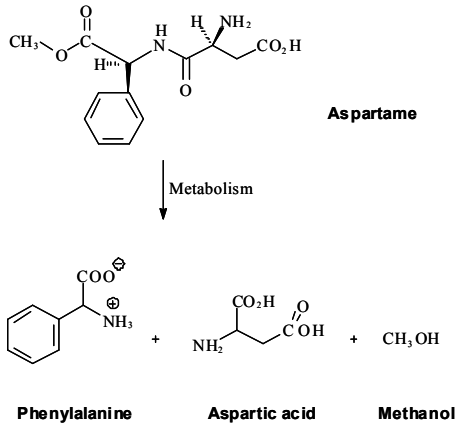

Figure 1 Metabolism of aspartame

Materials and Methods

1. Identification methods

1.1 Test with ninhydrin solution[5-8]

The samples were dissolved in water and spotted on thin layer chromatogram (TLC). TLC was developed in acetonitrile phosphate buffer (10 volumes of acetonitrile R plus 90 volumes of 6.8 g/L solution of potassium dihydrogen phosphate previously adjusted to pH 3.7 with phosphoric acid). TLC was dried, sprayed with ninhydrin solution and warmed in hot air oven. Aspartame gave pink spot (Figure 2). The limit of detection of this method was 7.5 μg.

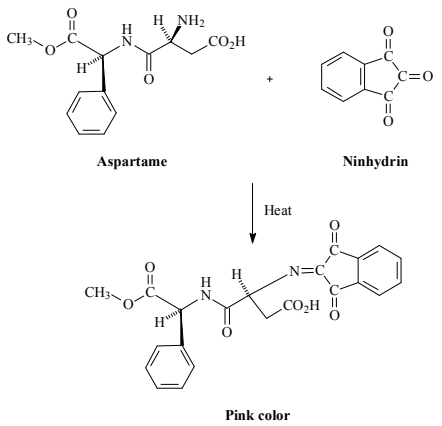

Figure 2 Reaction of aspartame with ninhydrin

1.2 Test with alkaline hydroxylamine solution[7-9]

Thin layer chromatogram of sample aspartame was developed in the same solvent as in ninhydrin method. TLC was dried, sprayed with alkaline hydroxylamine and then with hydrochloric acid (1+1) and dried. Ferric chloride solution was sprayed onto TLC. Positive test was brown spot (Figure 3). Limit of detection of this method was 300 μg.

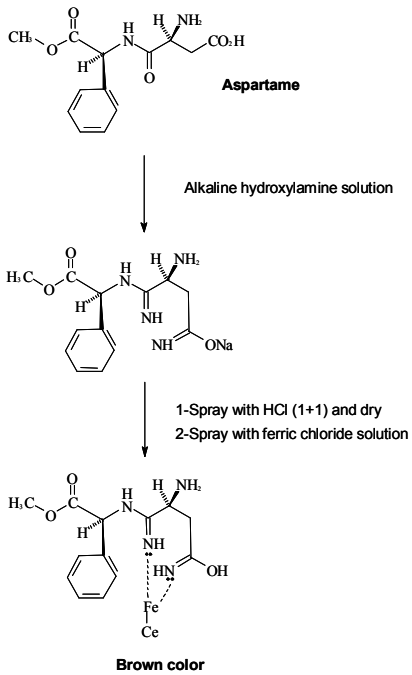

Figure 2 Reaction of aspartame with alkaline hydroxylamine solution

2. Determination methods

2.1 Colorimetric method[5,7-9]

In the test with simple sugar samples, 1 g of sample was dissolved with 40.0 mL distilled water and was diluted to 100.0 mL with absolute ethanol. 1.0 mL of acetate buffer was mixed with 1.0 mL sample solution and 2.0 mL ninhydrin solution. The mixture was heated in boiling water bath for 8 minutes after that the mixture was diluted to 10.0 mL with absolute ethanol. The

absorbance of sample solution was measured at 406 nm.

The reaction time of heating step was at 8 - 10 minutes. Sample solution gave two maximum absorption wavelengths which were 406 and 557 nm. The maximum absorption wavelength at 406 was stable so the absorbance of sample was measured at this wavelength. The purple color of sample solution remained stable for at least one and a half hours. The linearity of calibration curve was 7.5 - 37.5 μg/mL.

2.2 HPLC method[6]

The HPLC method we used followed British Pharmacopoeial (2008) method. HPLC (Agilant 1100), diode array detector was used at 214 nm, the column was Water Sperisorb 5 μm ODS 2 particle size and 4.6 x 250 mm long. The mobile phase was acetonitrile in phosphate buffer (the same solvent as in ninlydrin identification method) and the flow rate was 1.0 mL/min. 25 μL of standard and sample solutions were injected in replicates. Retention time was about 6 minutes as shown in Figure 1 and 2.

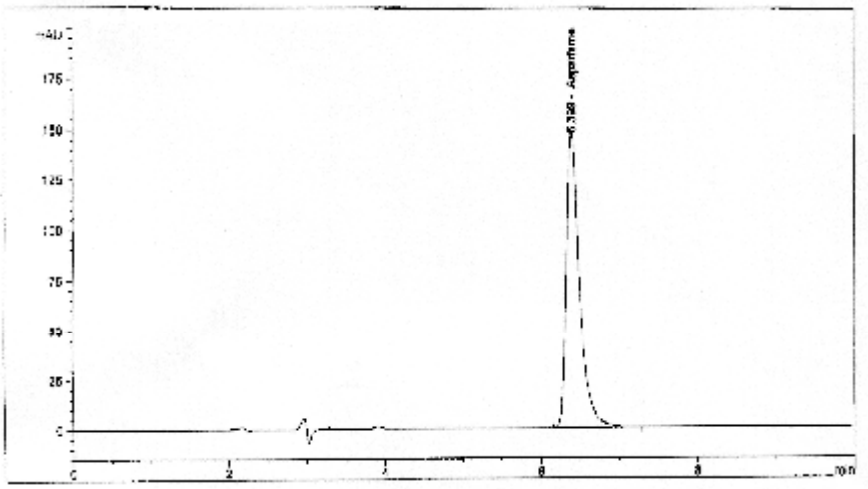

Figure 1 Chromatogram of standard aspartame 50.0 ppm eluted with acetonitrile phosphate buffer. (retention time 6.339 min, peak area 1767.38135 mAU)

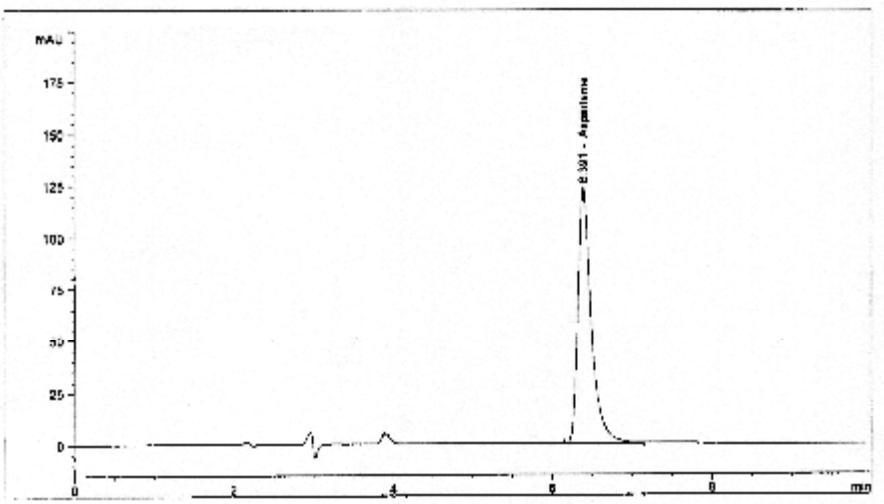

Figure 2. Aspartame in sugar sample eluted with acetonitrile phosphate buffer (retention time 6.391 minutes, peak area 1471.91687 mAU)

Results and Discussions

Identification method[5,7] using ninhydrin solution was sensitive but not specific for aspartame because the detection limit was 7.5 μg and it was also able to detect amino acids having a free α-amino group. The method using alkaline hydroxylamine[7] solution was not sensitive but specific for aspartame since the detection limit was 300 μg and it was used to detect carbonyl functional group where it formed color complex with ferric chloride solution. Colorimetric determination method gave large relative standard deviations (RSD) (Table 1) because aspartame was a chiral compound which was destroyed by heat and the color solution was also decomposed by heat. HPLC determination method also gave large relative standard deviation in some samples because aspartame was a chiral compound if it was kept improperly it could degrade. The relative standard deviations of simple sugar samples 2 - 4 of colorimetric method was about 5 - 6% while HPLC method was found wider (3 - 11%) (Table 1). Mean percent recovery of these two methods was about 97% (Table 2). From these results they showed that these two methods gave no difference. Aspartame was found lower than the labeled amount in many samples (Table 1) probably because if it was kept improperly, aspartame may degrade.

Table 1 Assay results of sugar samples

Samples	%LA	Method	Calculated % LA					Mean	SD	% RSD
			1	2	3	4	5			
Sample 1	3.8	Colorimetric	3.03	2.75	2.33	3.23	2.12	2.69	0.46	17.29
		HPLC	3.12	3.04	3.08	3.00	2.94	3.04	0.06	2.06
Sample 2	3.8	Colorimetric	2.13	2.18	2.08	2.25	2.44	2.22	0.14	6.31
		HPLC	4.09	4.16	3.50	3.17	3.30	3.64	0.41	11.17
Sample 3	3.8	Colorimetric	3.51	3.35	3.20	3.14	3.14	3.27	0.17	5.20
		HPLC	3.82	3.55	3.74	3.67	3.80	3.72	0.10	2.64
Sample 4	3.8	Colorimetric	0.37	0.36	0.36	0.42	0.37	0.38	0.02	5.26
		HPLC	0.38	0.36	0.38	0.31	0.29	0.34	0.04	10.81

Note: % LA = % labeled amount; SD = standard deviation; % RSD = % relative standard deviation

Table 2 % Recovery of sugar sample No.1

Method	Amount added (ppm)	Amount found (ppm)	% Recovery
Colorimetric	200	156.13	78.06
		226.38	113.19
		209.20	104.60
		205.30	102.65
		179.55	89.77
		Mean	97.65
HPLC	200	195.58	97.79
		194.85	97.43
		193.84	96.92
		194.78	97.39
		192.35	96.18
		Mean	97.14

Conclusion

The colorimetric method was simple and with less costly when compared with HPLC method so we could use the former method to determine aspartame in simple sugar samples in small laboratory such as in regional laboratory centers in various provinces. Testing with alkaline hydroxylamine was specific for aspartame identification. Colorimetric method can be a practical method to determine aspartame in simple sugar samples.

References

1. Munay R, Graner DK, Mayes PA, Rodwell VW. Harper's Biochemistry. 24th ed. New Jersey. Appleton & Lange, 1996.
2. Niesink RJM, de Vries J, Hollinger MA. Toxicology: principles and applications. London. CRC Press, 1996.
3. Zhu Y, Guo Y, Ye M, James FS. Separation and simultaneous determination of four artificial sweeteners in food and beverages by ion chromatography. *J Chromatography* A 2005:143-146.
4. Thai Food Act. 2004. Synthetic sweeteners.
5. Lau OW, Luk SF, Chan WM. Spectrophotometric determination of aspartame in soft drinks with ninlydrin as reagent. *Analyst* 1988:765-768.
6. Man MJ. Principles of food chemistry. 2nd ed. London. Chapman & Hall, 1925.
7. British Pharmacopocia Commission. British Pharmacopoeia. London. 2008.
8. Merck Index. 13th ed. New Jersey. Whitehouse Station, 2001.
9. Birch GG, Green LF, Coulson CB. Sweetness and sweeteners. London. Applied Science Publish Ltd. 1971.