Determination and Comparison of Aspartame Level in Low Calorie Table Top Sweeteners by Ultraviolet Visible Spectroscopy

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Abstract: The analysis of aspartame was done in table top sweeteners using Ultraviolet visible Spectroscopy. The method is based on the formation of manganate ion (MnO_4^{2-}) when potassium permanganate oxidizes aspartame. The analysis was carried out at a fixed wavelength of 600nm exactly after 48 minutes of sample preparation using 1mL of 0.01M $KMnO_4$ and 2mL of 1.0M NaOH. A linear calibration graph was obtained with the regression coefficient of 0.9999 and the percentage recovery was in the range of 92.7-101.6%. The Relative standard deviation for the method was found to be 1.03%.

Keywords: Aspartame, UV/Visible spectroscopy, Sweetener, $KMnO_4$.

INTRODUCTION

Aspartame is the most consumed and popular sweetener compared to other non-nutritive sweeteners. The empirical formula of aspartame is $C_{14}H_{18}N_2O_5$ and molecular weight is 294.3g/mol. Aspartame is about 200 times as sweet as sugar (sucrose) but chemically it is different from natural sugar. It is slightly soluble in alcohol and water but is insoluble in fats and oils. It is stable in dry form but degrades at high temperatures and over time [1]. It is the derivative of dipeptide which is L-aspartyl-L-phenyl-alanine methyl ester and it metabolize to three components: phenylalanine, aspartic acid, and methanol [2].

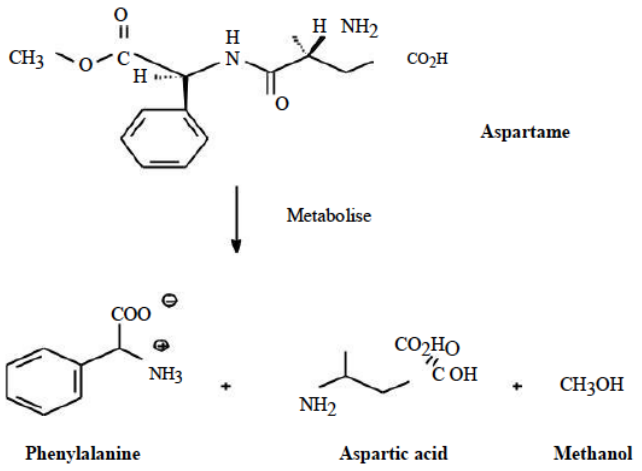

Figure 1: Metabolism of Aspartame.

Aspartame was approved after 16 years of its discovery by Food and Drug Administration [3]. Thousands of researches have condemned the use of aspartame in food items due to number of possible harmful effects. However it is used in more than 6000 products and the Acceptable Daily Intake set by FDA is 50mg/kg body weight [4]. In 2008, Spice Williams-Crosby reported an extensive study on neurodegenerative diseases and showed association between aspartame and serious diseases like Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral

Sclerosis, Multiple Sclerosis, and Huntington's chorea [5]. In 1998, Trocho showed that aspartame ingestion by rodents leads to formaldehyde accumulation which causes irreversible genetic damage for long-term, infertility, seizures and neurobehavioral impairment, headaches, skin problems and low birth weight [6]. In 1997 Blumenthol reported case studies suffering from migraines who consumed chewing gums containing aspartame. In all cases, migraine was relieved after cessation of aspartame product. It was also noted that migraines were reproducible by reintroducing the product [7].

Aspartame was determined in finished bulk and dosage forms by a method based on ion pair high performance liquid chromatography [8]. Several high performance liquid chromatography methods have been reported for the analysis of aspartame [9-11]. A spectrofluorimetric method based on labeling with fluorescamine to determine the concentration of aspartame and glutamate in different food stuffs has been reported [12].

Aspartame was determined in commercial sweeteners and cold drinks using efficient biosensors. The sensor developed was based on a bienzyme method composed of alcohol oxidase and carboxyl esterase [13].

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The aim of the study is to create awareness among the consumers of artificial sweeteners and to check the labeled content of aspartame in table top sweeteners.

MATERIALS AND METHODS

Chemicals and Reagents

Aspartame was purchased from Nabha Market, Lahore. Sodium Hydroxide and Potassium Permanganate was provided by the university. Water used for preparation of solutions was double distilled. 15 different samples of table top sweeteners sold under different brand names were purchased from local markets sampled from Tablet 1 to Tablet 15.

Apparatus

Hitachi U-2800 Ultraviolet visible spectrophotometer was used for the analysis of analytes. Electronic weighing balance and glass ware like measuring cylinders, Viles, pipette, measuring flasks, Whattman filter paper, funnels and beakers were used.

Preparation of Solutions

Preparation of 0.01M $KMnO_4$

0.158grams of solid Potassium permanganate was weighed and transferred to 100mL measuring cylinder and the volume was made up to mark using distilled water.

Preparation of 1.00M NaOH

4 grams of solid NaOH was weighed, transferring it into 100mL measuring cylinder making volume up to mark with distilled water.

Standard Solution

Stock solution was prepared by dissolving 0.1g of standard aspartame in 1000mL of distilled water. Further dilutions were prepared form this stock solution. 1.0mL of 0.01M $KMnO_4$ and 2.0mL of 1.0M NaOH was taken in 10mL measuring cylinder with accurate volume of the stock solution of aspartame with following concentrations 0.5. 1.0, 1.5, 2.0, 2.5mL and each was then made up to mark with distilled water.

Sample Preparation

1 tablet containing 18mg aspartame was grinded well and was dissolved in l00mL of distilled water followed by filtration using a Whattman filter paper. 1mL of the filtered solution was taken in 10mL measuring cylinder and made up to mark with distilled water. 1mL of 0.01M $KMnO_4$ and 2mL of 1.0M NaOH was added to 10mL measuring cylinder with 1mL of working solution and made up to mark with distilled water. The contents were shaken well. The same procedure was repeated for every other tablet.

Procedure

After sample preparation, the absorbance of each sample was measured exactly after 48 minutes by taking samples in quartz cuvettes and subjecting them to Ultraviolet visible spectrophotometer at a fixed wavelength of 600nm. The concentrations in parts per million were determined by plotting a calibration graph between concentration on x-axis and absorbance on y-axis.

RESULTS AND DISCUSSION

Ultraviolet visible spectroscopy proved to be an effective, simple and rapid method for the determination of aspartame. Optimum conditions were applied for the accuracy of results which include optimum volume of $KMnO_4$ and NaOH, fixed wavelength to measure absorbance and time required for complete oxidation of aspartame by $KMnO_4$.

Optimum Absorption Wavelength

Oxidizes aspartame in alkaline medium, resulting in formation of manganate ion (MnO_4^{2-}) which showed a strong absorption peak at 600nm. Wavelength scan was done from 400-800nm and maximum absorption was found at 600nm. All the samples were then analyzed at a fixed wavelength of 600nm.

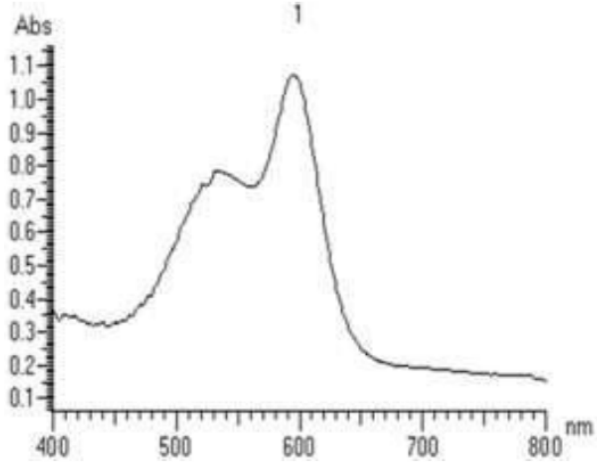

Figure 2: Spectral scan to obtain wavelength of maximum absorption.

Optimum $KMnO_4$ Volume Used

Varying volumes of $KMnO_4$ ranging from 0.2 to 1.4mL were added to the 10ppm aspartame solution one by one and noted the absorbance at 600nm and at fixed time of 48minutes. It was found that the absorbance became constant at 1mL. So 1mL of $KMnO_4$ was used as an optimum volume.

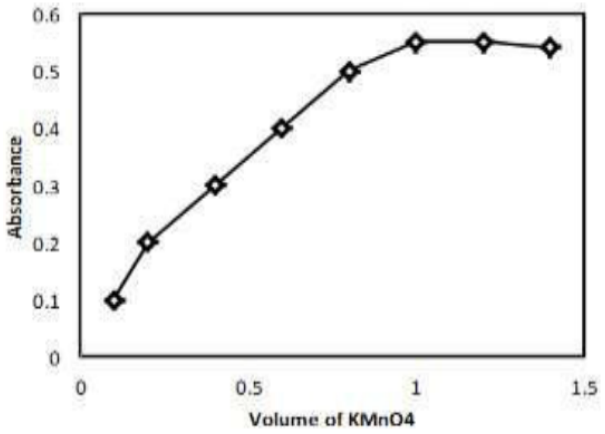

Figure 3: Graph between volume of KMnO4 and absorbance.

Optimum NaOH Volume Used

Using different volumes of NaOH ranging from 0.2 to 2.6mL added to 10ppm aspartame solution, the maximum absorption at 2mL was noted.

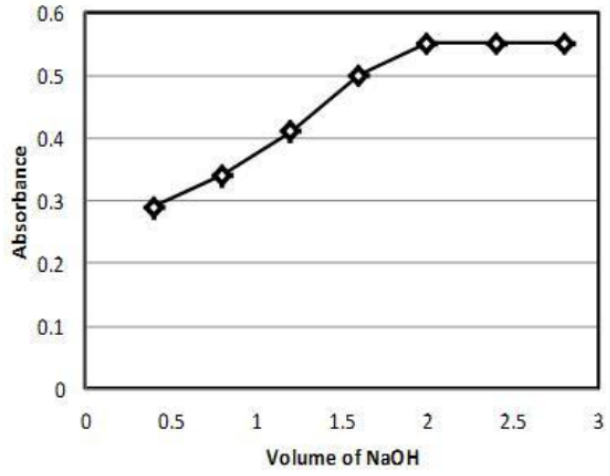

Figure 4: Graph between volume of NaOH and absorbance.

Optimum Oxidation Time

Absorbance at different time intervals i.e. 6, 12, 18, 24 and 48 minutes were noted and the maximum absorption was found to be after 48 minutes. After sample preparation, the intensity of color changes from magenta to navy blue and to dark greenish blue at 48 minutes which was the indication of complete oxidation and gave maximum absorption.

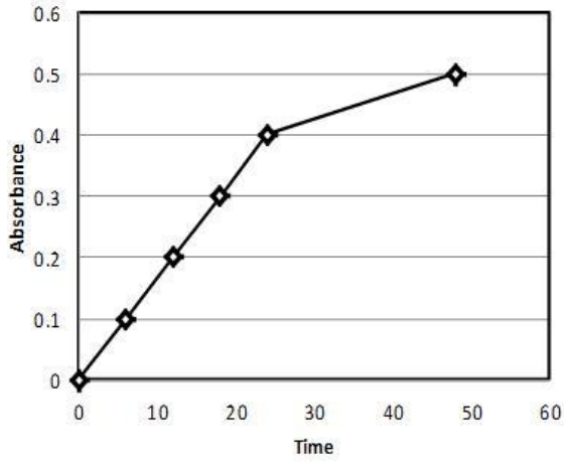

Figure 5: Absorbance measurements at different time intervals.

Calibration Graph

A calibration graph was plotted between concentration on x-axis and absorbance on y-axis. The concentration plotted was in the range of 5-25ppm. As a result a linear calibration graph was obtained with the regression coefficient of 0.9999.

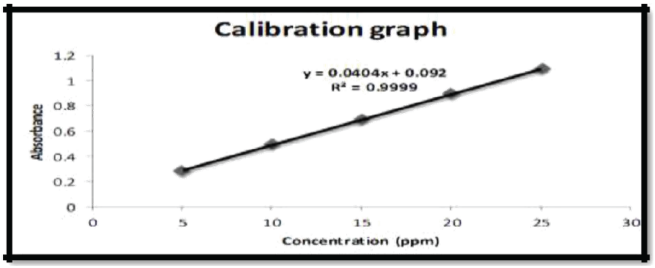

Figure 6: Linear Calibration Curve.

Calculations

The concentrations were calculated from calibration curve

Relative Standard Deviation

The method validation was done by checking the repeatability, which was done by calculating the relative standard deviation (RSD) based on 6 replicate determinations.

DISCUSSION

The analysis was done using the most versatile analytical technique, the Ultraviolet visible Spectroscopy. Optimum conditions were employed to

Table 1: Calculation of Aspartame

Sr. No.	Sample detail	Labeled Amount	Observed Amount (mg)	Difference	% Recovery
1	Tablet 1	18mg tablet	16.7	1.3	92.7
2	Tablet 2	18mg tablet	17.2	0.8	
3	Tablet 3	18mg tablet	17.1	0.9	95.0
4	Tablet 4	18mg tablet	17.6	0.3	
5	Tablet 5	18mg tablet	17.3	0.7	96.1
6	Tablet 6	18mg tablet	17.2	0.8	
7	Tablet 7	18mg tablet	16.9	1.1	93.8
8	Tablet 8	18mg tablet	17.1	0.9	
9	Tablet 9	18mg tablet	17.8	0.2	98.8
10	Tablet 10	18mg tablet	17.7	0.3	
11	Tablet 11	18mg tablet	17.5	0.5	97.2
12	Tablet 12	18mg tablet	17.9	0.1	
13	Tablet 13	18mg tablet	17.4	0.6	96.6
14	Tablet 14	18mg tablet	16.8	1.2	
15	Tablet 15	18mg tablet	17.3	0.7	96.1

obtain accurate results. Wavelength scan was done from 400-800nm which showed that maximum absorption took place at 600nm. So the analysis was carried at a fixed wavelength of 600nm. The absorbance at different time intervals after sample preparation indicated that the solution would show maximum absorption after 48 minutes.

Table 2: Calculation of mean, standard deviation and RSD

Replication	Observed
1	17.2
2	17.4
3	17.2
4	17.1
5	17.6
6	17.3
Mean	17.3
Std. Deviation	0.17
R.S.D	1.03%

15 samples were analyzed and were in concentration range of 16.7-18.3mg. Almost all concentrations obtained were in close agreement with the labeled amount except for tablet 1, 7 and 14 that showed a difference of ±0.5. A linear calibration graph was obtained with a linear regression equation of y=0.0404x+0.092 with regression value of 0.9999. The recovery values for the method were found to be better than 95%. The repeatability of method was also indicated by RSD which was 1.03% for the method.

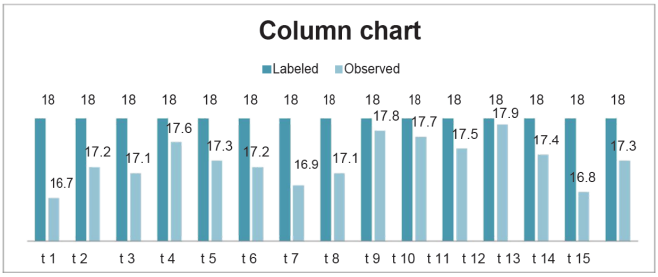

Figure 7: Chart showing observed and labeled amounts.

CONCLUSION

A simple, rapid and low reagent method for the determination of aspartame by Ultraviolet visible Spectroscopy has been successfully employed. The samples were analyzed without pretreatment and obtained results were validated by calculating Relative standard deviation. The results obtained were in good agreement with the labeled amounts.

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