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DETERMINATION & QUANTIFICATION Of SODIUM SACCHARIN IN BEVARAGES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Javed Iqbal[1], Muhammad Abbas[*2], Muhammad Sohail Anwar[3], Ali Khan[4], Saqib Jahan[5], Abdul Mateen[6], Halima Sadia[7], Hafiz Sajid Akbar[8], Abdus Saboor[9]

[1,*2,9]*Department of Pharmacy, Abdul Wali Khan University, Mardan, KPK, Pakistan*
[3,5,6]*Department of Pharmacy, University of Swabi, KPK, Pakistan*
[4]*The Professional Institution of Health Sciences, Mardan, KPK, Pakistan*
[7]*Department of Pharmacology, Bacha Khan Medical College, Mardan, KPK, Pakistan*
[8]*Vertex College of Pharmacy, Mardan, KPK, Pakistan*

[*1]muhammadabbas@awkum.edu.pk

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Corresponding Author: *
Muhammad Abbas

Abstract

Artificial sweeteners like sodium saccharine and other related products have widely been used as sweeteners in different kinds of drinks and juices. As from the literature survey it is clear that sodium saccharine reported to have some carcinogenic properties when it is used in concentration more than the prescribed amount. The main objective of the study is to detect, determine & quantify the saccharin level in commonly used beverages including carbonated & cola drinks, fruit juices, nectars and energy drinks (non alcoholic) and other commonly used products available in the market.In this study 100 samples of different beverages were analysed. These samples were first degassed for about 30 minutes and then 10ml in 100ml of distilled water was mixed. High Performance liquid chromatographic (HPLC) technique was used for the sample analysis. The analysis was carried out on C18 column in isocratic conditions. The mobile phase contained phosphate buffer of KH_2PO_4 (0.0125mol/L) and acetonitrile (HPLC grade) in composition of 80:20 ratio (80% Phosphate buffer & 20% Acetonitrile) at pH of 2.5 and λ_{max} of 270nm. The results shows that beverages from the renowned companies have shown a very minute and undetectable concentration of Sodium Saccharin. While samples of the beverages especially fruit juices from the locally manufacturing companies shown some higher concentration of sodium saccharin. So this method can be used to accurately determine and quantify sodium saccharine in energy drinks.

INTRODUCTION:

The origin of the name Saccharin, was from the greek word "saccharine," the meaning of this word is "SUGARY". The word "SACCHARIN" is metaphorically used to describe some thing which is very sweet."Saccharin Sodium" (SAC) an artificially used as sweetner of high-intensity. "SAC" is also graded as non-nutritiritional sweetener. Nowadays it is frequently utilized in food pocessng industry. Though in the earlier period, "SAC" was utilized in multiple purposes apart from the use as a sweetening agents. Saccharin Sodium, "SAC" was used firsly as an antiseptic and preservative agent to preserve the food material from fermentation and spoilage. After then, "SAC" was utilized in "Plastic industry" as a tool for antistatic property and modifier. "Saccharin Sodium" was also used for

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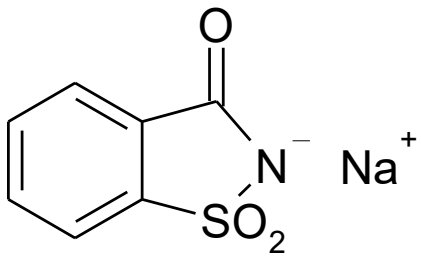
its brightening property in the nickel-plating of automobile bumpers. (Arnold et al., 1983).

Presently, SAC is completely used in the processing and manufacturing of low-caloric, food which enable the people to retain their weight. Medically "SAC" is adivised to diabetics to use foods that is sweetened with "SAC" rather than foods sweetened with sugars.



Chemical structure of saccharin (sodium salt)

The wide use of "SAC' has safety concerns, even today and great controversies are there whether its use is safe or not. (Renwick, 1985, Whysner and Williams, 1996, Cohen-Addad et al., 1986) (Takayama et al., 1998). (Weihrauch and Diehl, 2004) (Maynard, 1997).

The United States, FDA has excluded the compound "SAC" from the list in 1972, as the safety of "SAC", is very uncertain, and the use of "SAC" as sweetening agent in food items & beverages was proposed to be proscribed & banned in 1977. Athough, public has protested against this ban which led to the suspension of this ban and the imposition of suspension on this ban is still present (Pearson, 1991, Kroger et al., 2006).

In 1977, the use of "SAC" as food additive and sweetening agent was banned in Canada. (Arnold et al., 1983). However, the permission was granted that "SAC" can be sold as table-top sweetener in pharmacies only. On the other hand, the selling and use of "SAC" was permitted besides the retrictions on its use to the varying degrees. EU Directives

1. 94/35/EC (European Commission (EC) 1994),
2. 96/83/EC (European Commission (EC) 1996),
3. 2003/115/EC (European Commission (EC) 2003),
4. 2006/52/EC (European Commission (EC) 2006),

has defined the quantity of "SAC", to be used and the foods item in which the "SAC", can be used as food additive and sweetening agent. (Kokotou et al., Asimakopoulos et al., 2012).

The safety of consumer demands that the use and intake of saccharin should be montitored and evaluated at regular basis. A wide range of analytical methods are available for the determination and monitoring of saccharin intake in a variety of food item and food matrices, that are based on variety of monitoring principles. HPLC with UV detection (HPLC–UV) is one of the most commonly used method for the identification and quantification of saccharin in non-alcoholic beverages. The presence of saccharin in non-diet beverages – a fraud commonly used to replace more expensive sucrose – was confirmed by comparing coincident peaks as well as the emission spectra of standards and samples. (Bruno et al., 2014).

Sugars are very common component of our diet which are the major cause of serious health problems like obesity that leads to type II diabetes mellitus, cardiovascular diseases, dental diseases, hypertension and cancers. Companies that are manufacturing non nutritive sweetners (NNS) like Sodium Saccharin etc use this point as an opportunity to sell and market their artificial sweetners claiming them safe for all age groups, but the studies & literature are also indicators of obesity, cardiovascular diseases, cancers and other cardiometabolic activities (Shankar et al., 2013).

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The main objective of the study is to detect, determine & quantify the saccharin level in commly used bevrages including carbonated & cola drinks, fruit juices and other commonly used prodcuts available in the market. To provide mass awareness about the safety of bevarages and to improve public safety & prevent harm of Saccharin to the people using beverages.

MATERIALS AND METHODS

CHEMICALS & REAGENTS

The standard sodium saccharin was obtaind from the Global pharmaceutical (PVT) limited company Islamabad for the preparation of standard solution.

Acetonitrile of HPLC grade manufactured by Merck & Co was purchased from the Syntec chemical distributors Lahore, for the analysis. Potassium dihyrogen phosphate (KH_2PO_4) manufactured by Merck & Co was purchased from the Syntec chemical distributors Lahore. Buffer was prepared by dissolving 1.7gm of Potassium dihyrogen phosphate (KH_2PO_4) in 1000ml (1Liter) of deionized distilled water, which was 0.0125mol/Lt. Deionized water for sample preparation and mobile phase was obtained from a Mill-Q System (Millipore, USA).

INSTRUMENTS

Perkin Elmer 200 series HPLC system with a Flexer LC autosampler, Thermo C18 column (150 x 4.6mm) , Ultrasonic bath Sonicator (Bandelin Sonorex), Potentiometric pH meter (Hanna HI 2211), Digital weighing balance (FR 20 Japan).

SAMPLE COLLECTION

Samples of different beverages from the market including carbonated & cola drinks (regular & diet both), fruit Juices and other beverages commonly used and available in the market are collected. Samples were purchased from the market and 10 Samples from each of the brand was analysed (total 100 samples analysed).

BUFFER PREPARATION

Buffer was prepared by dissolving 1.7gm of Potassium dihyrogen phosphate (KH_2PO_4) in 1000ml (1Liter) of deionized distilled water, which was 0.0125mol/Lt. To make 0.0125molar solution of Potassium dihydrogen phosphate (KH_2PO_4) molecular weight was calculated which is 136.09gm. 1 mole of Potassium dihydrogen phosphate (KH_2PO_4) = 136.09 gm

(Molecular weight in gm/1000ml =1molar sol)

To calculate 0.0125 mole of the Potassium dihydrogen phosphate (KH_2PO_4)

1mole of KH_2PO_4 x 0.0125

136.09 x 0.0125 = 1.7

So, 1.7gm of KH_2PO_4 is dissolved in 1000 ml of deionized water and than filtered to get 0.0125molar sol of KH_2PO_4 (Buffer Solution).

STANDARD PREPARATION

The standard sodium saccharin was obtaind from the Global pharmaceutical (PVT) limited company Islamabad for the preparation of standard solution.

Different concentration of the standard solution were made. First concentrtion made was 5gm /100ml, 5gm of standard sodium saccharin was dissolved in 100 ml of deionized water and filtered through vaccum filter. Second concentration made was 5mg /100ml, 5mg of standard Sodium Saccharin was dissolved in 100 ml of deionized water and filtered on vaccum filter. Third concentration made was of 0.5mg/100ml, 0.5mg of standard Sodium Saccharin was dissolved in 100 ml of deionized water and filtered through vaccum filter. Different working solutions and calibration curve were then prepared from these standard solution

SAMPLE PREPATION

Samples of all the beverages were first degassed for 30 minutes. Then 10 ml from each sample was taken through pipette and put in the 100 ml volumetric flask and the volume was made by using deionized water. Then Shaken well after making up the volume and put in the sonicator for 10 minutes to remove excess of bubbles and homogenous mixing. The solution was filtered

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through the vaccum filteration process to remove any sort of material that can lead to blockade or insufficiency of the column. After filteration the clear liquid sample was subjected to the HPLC analysis through reverse phase chromatography.

HPLC ANALYSIS

High Performance Liguid Chromatrography (HPLC) was used for the sample analysis. Perkin Elmer 200 HPLC with a Flexer LC autosampler. C18 column was used in isocratic conditions at room temperature for analysis. The samples were carefully prepared for each product and then transferred to HPLC vials for analysis into the system. The analysis run time of each sample was 10 minutes. Ultrasonic bath sonicator (Bandelin Sonorex) was used for the proper mixing and degassing of the sample prepared. The prepared standard and sample solutions were placed for 10 minutes in ultrasonic bath sonicator for proper mixing and degassing, bubbles from the solution were removed during this process and then filtered for the analysis. The carbonated drinks samples were also placed for the degassing process in the sonicator for about 15 minutes to remove all the gasesous content from the samples. Mobile phase composed of phosphate buffer of KH_2PO_4 (0.0125mol/L) & Acetonitrile at a ratio of 80:20 (pH of 2.5) and was used in isocratic mode. Potentiometric pH meter was to maintain the pH of the buffer solution made. The flow rate was 1ml/min while the column temperature was kept at ambient temperature. The injection volume was 20 µl. The method was validated according to established guidelines.

RESULTS AND DISCUSSION

Artificial sweeteners like sodium saccharine and other related products have wildely been used as a sweetners in different kinds of drinks and juices. As from the literature survey it is clear that sodium saccharine reported to have some carcinogenic properties when used in concentration than the prescribed amount. There is no such study carried out in Pakistan where quantity of artificial sweeteners were determined. This study aimed to determine the concentration of sodium saccharine commonly available in markets of mardan region of KPK. The consumption of bevereges has increased so much in the last 2 decades especially in young population going to school, colleges and universities as a source of energy or as fashion. This high consumption of beverages containing too much sugar or artificial sweeteners lead to serious health conditions like fatty liver, kidney disease, obesity etc among young population. Thus concentarion of sodium saccharine determination is of prime importance.

The results of analyzing 100 samples of the beverages that are commonly used and available in the market are presented in table 1. The samples were analysed on the HPLC for the detection of the sodium saccharin on C18 Column in isocratic conditions showed that beverages from the renowned companies including carbonated & cola drinks (regular & diet both), fruit Juices, nectars and energy drinks (non alcholic) have shown a very minute and undedetectable concentration of sodium saccharin in the analysis, which indicated that either in those brands saccharine was not used as sweetening agent or the concentration was so low that it was not detected by the HPLC system. On the other hand samples of the beverages especially fruit juices from the locally manufacturing companies shown some higher concentration of sodium saccharin in the analysis, their labels also didn't have any information in their formulation or use of sodium saccharin as sweetening agent. The concentatrion found was in the range of 285 mg/L to 370 mg/L. The permissible limit of sodium saccharine according to Codex GSFA (General Standard for Food Additives) is 300mg/L while some literature reported this value as 400mg/L.

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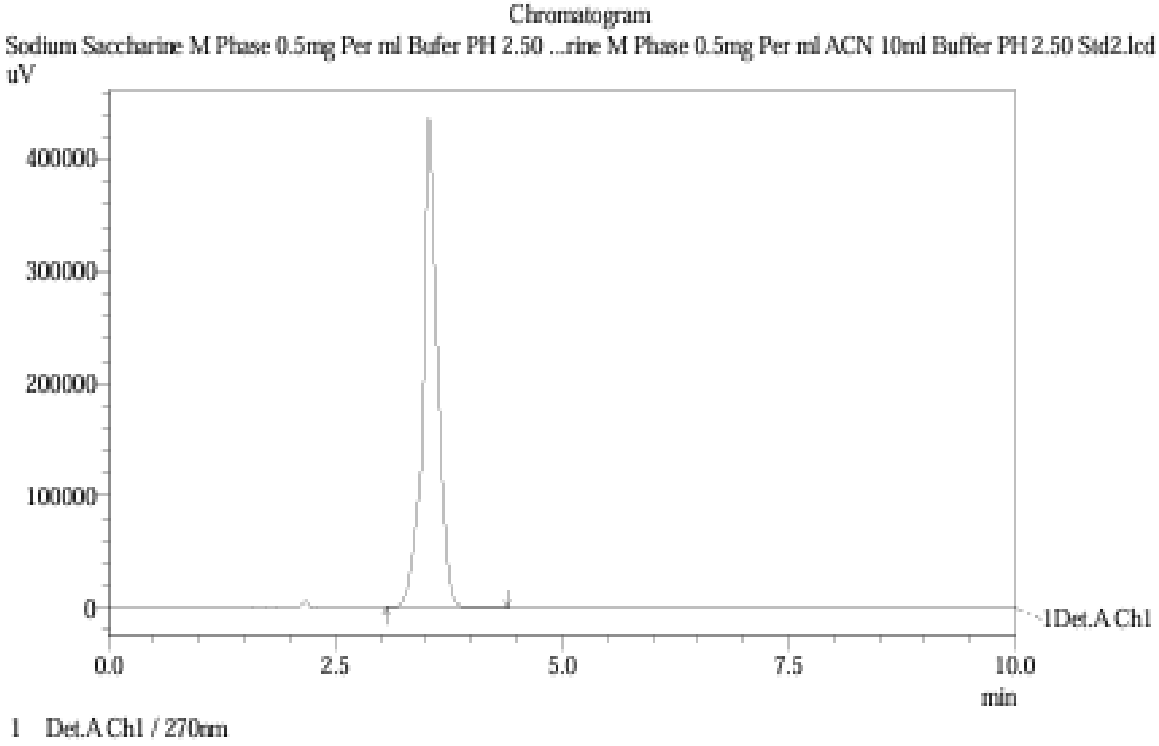

Figure 1: chromatogram of a Standard Solution of Sodium Saccharin at pH 2.5 in buffer 80% and Acetonitrile 20% at 270 nm.

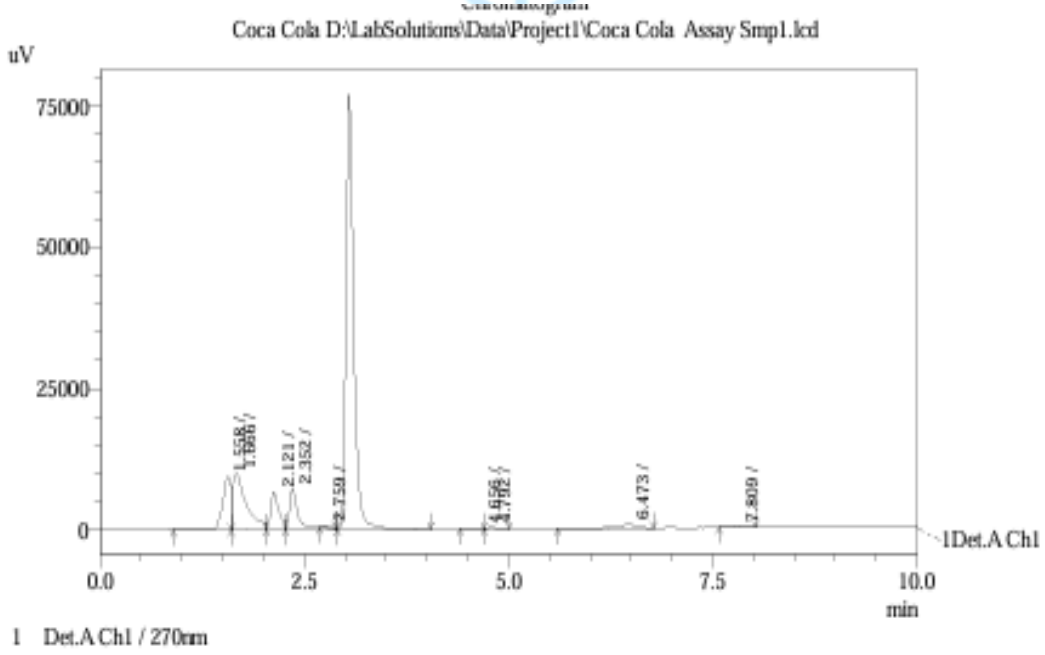

Figure 2: chromatogram of Sodium Saccharin in a sample at pH 2.5 in buffer 80% and Acetonitrile 20% at 270nm.

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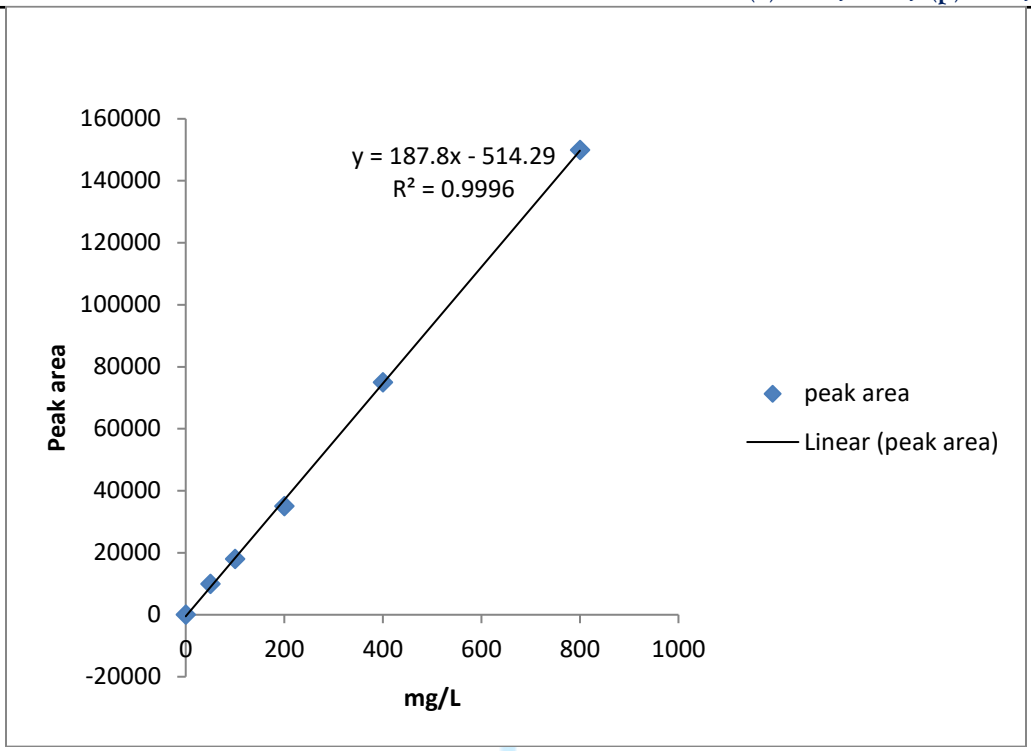

Figure 3: Calibration curve of sodium saccharine

For this analysis reverse phase chromatographic column was used as this compound is apolar in nature therefore shows good separation on C18 column. The chromatoghraphic peaks of the standard preparation and one of the sample were shown in the above figures. Figure 1 which is the clear solution of sodium saccharine showing a sharp peak having retention time of 3.5 minutes. Figure 2 represent peak of sodium saccharine found in one of a sample having similar retention time. The calibration curve was also constructed and shown in figure 3. To validate the method prescion, accuracy and recovery tests was performed. For accuracy and precison the standard samples were prepared in six replicates and were analysed on the HPLC system, similarly recovery test was also performed by the by analyzing the spiked samples in three different concentartions. The results of all the validation tests were within limits.

Conclusion

The significant information from this data is that there is sodium saccharine in many of the commonly used beverages and juices as an alternative to sugar. As it is evident from the table 1 that there is no signifacntly high amount of sodium saccharine found in the collected samples, but the government and regulatory bodies need to make strict laws for the manufacturer to clearly state the amount of sodium saccharine on the packs and bottles, because the consumption of sodium saccharine will creat serious health problems when used for a longer period of time. The government should also make policy for the regular testing of artificial sweetners and if found above the prescribed limit should banned such products.

Table 1: Concentration of sodium saccharine in different brands available in local market of KPK, mardan,the concentration of sodium saccharine is expressed as mean of 10 samples.

S.No	Sample Code	Sodium Saccharine (mean mg/L)
1	*GB 1	200

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2	GB 2	295
3	GB 3	180
4	GB 4	ND
5	GB 5	ND
6	**SD 1	190
7	SD 2	250
8	SD 3	ND
9	SD 4	ND
10	SD 5	ND

*GB= Guice Brand **SD= Soft Drink ND= Not Detected

Conflicts of interest: The authors declare no conflict of interest of any kind.

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