HPLC Method for the Determination of Benzoic acid in samples of commercial children's food in the Markets of Aden -Yemen

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© 2023 جامعة العلوم والتكنولوجيا، المركز الرئيس عدن، اليمن. يمكن إعادة استخدام المادة المنشورة حسب رخصة مؤسسة المشاع الإبداعي شريطة الاستشهاد بالمؤلف والمجلة.

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***Abstract*— The objective of this study was to determine the quantities of benzoic acid in some local and imported food products for the distributed children in Aden, Yemen. Samples of different food products for children (32) using HPLC method, were collected in August 2022, from different supermarkets in Aden, Yemen. Chromatographic separation was achieved using a C-18 column (EXCGL 120 ODS-A) and acetate buffer pH=4.4 - methanol (65:35) as mobile phase, 1 ml/min flow rate and UV detection at 233 nm. The results show that quantities of benzoic acid in studied samples varies greatly starting from 1.778 ppm to 849.295ppm, All samples of group II beverages and Juices samples and group III jam samples were under the study contain concentrations of benzoic acid within the limits allowed by standards of Yemeni and World Health Organization and the estimated maximum allowable benzoic acid in beverages, Juices and jam to 1000 ppm. All samples of the groups I and V cakes and food for children mainly manufacturers of grain, The samples that were under the study contain concentrations of benzoic acid and it is difficult to compare the amount of benzoic acid in samples of two groups with international standards, regional and local non-existence. All samples of the group IV dry milk and cheese samples under study contain concentrations of benzoic acid within the limits allowed according to the standard specifications of Yemen and specifications of the World Health Organization (WHO), which determined the maximum allowable of benzoic acid in dry milk at 50 ppm and the maximum allowed for benzoic acid in the cheese is 2000 ppm, except for sample No. 28 contains a concentration is higher than the allowable limit.**

Keywords— benzoic acid, HPLC, children food, Aden, Yemen.

I. Introduction

Food preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods during its shelf life. They also prevent consumer hazards due to the presence of microbial toxins or pathogenic microorganisms and economic losses due to spoilage (Davidson, 1997). The role of preservatives has become more prominent with the increase in production of processed and convenience foods.

Yemen follows regulations of Yemeni standard on the safe use of food additives, in term of the consuming of benzoic acid is E210 and their salts sodium benzoate E211, potassium benzoate E212 and calcium benzoate E213. According to Yemeni standard, maximum legally allowable limit for benzoic acid in food is set at 1000 ppm in the sample (586/2003). and its presence must be declared on the label.

Previous studies have reported the determination of these preservatives in cakes , Juices and drinks and jams using HPLC (Isabel, et al, 2000; Polypin & Gretter, 2000; Fernando, et al, 2003; Badruddin Saad, et al, 2005 ; Ahmet ,et al, 2008 ; Cornelia and Elena 2009 ; Hong Hong, et al, 2009 ; Khosrokhavar , et al, 2010).

Different eluents were used, including phosphate buffer, acetonitrile, and, in other cases, acetate buffer and methanol. However, some of these methods are not applicable to a wide range of foodstuffs or use lengthy clean up steps prior to HPLC analyses. Efficient sample clean up procedures are essential for eliminating high-molecular mass matrix interferents (e.g. proteins, fats, polysaccharides) and, also, vitamins and food additives. Thus, the aim of our research work was to determine the quantities of benzoic acid in 32 regional and imported children food products distributed in Aden-Yemen, comprising, beverages and Juices, jam, cakes, food for children mainly manufacturers of grain and dry milk and cheese, These products were available on the market that were evaluated after appropriate sample preparation and HPLC separation in order to compare levels of them with respective allowable limits.

II. Experimental part

A. Chemicals and reagents

Chemicals and reagents used were obtained from the (BDH): acetic acid (99.8%), benzoic acid extra pure, ammonium acetate (Analar); methanol (HPLC grade).

B. Preparation of sample

1) Liquid Samples:

5 mL of liquid sample was diluted with 25 mL of methanol into 50mL volumetric flask and mixed well at 50ºC for about 10 min. on sonicator (vibrating heating plate) (BANDELIN

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RK100H, Germany). The volumetric flask was next subjected to vortex mixing (Model ALC, Germany) for 5 min. The contents were filtered through a 0.45 µm syringe filters (Sartorius AG 37070 Gottingen Germany). The content then transferred into another 50mL volumetric flask and diluted with methanol up to the mark. The clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done.

2) Solid Samples

Solid samples were finely ground in an electric mixer prior to the extraction. About 10 g of sample is accurately weighed in a screw capped test tube. 25 mL of methanol was added, and placed in a sonicator that was maintained at 50ºC for 30 min. The test tube was next subjected to vortex mixing for 5 min. The contents were filtered through a 0.45 µm syringe filters. The content then transferred into a 50 mL volumetric flask and diluted with methanol up to the mark. The clear filtrate was injected into the HPLC column. For concentrated samples, prior dilution with the mobile phase was done.

3) Chromatographic conditions

Analytical separation was carried out Perkin Elmer, Germany, Model Series – 200 HPLC unit using EXCGL 120 ODS-A C18 column (15 cm×0.46 mm, 6µm) at room temperature. The detector used a Perkin Elmer, Germany UV-Series – 200, UV–vis spectrophotometer set at 233 nm and the volume of sample injected was 20 µL.

An aqueous phase was prepared by weighing 3.84 g ammonium acetate and dissolving in 1 L water and its pH adjusted to 4.4 using acetic acid. The mobile phase used was methanol–acetate buffer (pH 4.4) (35:65, v/v). The separation was achieved with isocratic elution at a flow-rate of 1 ml/min and 20 µl of sample were injected into the chromatographic system.

4) Preparation of the standard curve

The external standard plot method was used. Duplicate injections of 20 µL benzoic acid standard solutions were used to construct linear regression lines (peak area versus concentration).

The peaks were identified based on the retention time. The standard curves were obtained with twelve points for benzoic acid. Concentrations were 3, 6, 12, 20, 40, 60, 100, 150 and 250 ppm.

5) Food samples

A total of 32 food samples were collected in August 2022, from different supermarkets in the Aden governorate, The analyzed food products comprised of group I cakes (14 samples), group II beverages and Juices (8 samples), group III jams (3 samples), group IV dry milk and cheese (3 samples), and group V food for children mainly manufacturers of grain (4 samples).

6) Statistical analysis

Data of the HPLC analysis were analyzed using Origin program and the values were expressed as mean(RSD%),n=3, for standard solutions of benzoic acid and also for studied samples.

III. Results and Discussion

A. Results

The typical chromatogram resulting from injected standard benzoic acid solution in our work is shown in Fig. 1, resulting chromatogram indicates that retention time of benzoic acid was 7.104± 0.561minutes.

Linearity between the concentration of benzoic acid and the UV absorbance at 233 nanometers was maintained over the concentration range of 3-250 ppm. The calibration curve was obtained by plotting the concentration vs. peak area as shown in Fig. 2.

The limit of detection (LOD) for benzoic acid of the samples was 0.404 ppm. The limit of quantitation (LOQ) for benzoic acid and were 1.348 ppm in the samples. The correlation coefficient for standard curves of benzoic acid were 0.99947.

Peak identification of the preservatives in various foodstuffs was based on the comparison between the retention time of standard compounds and was confirmed by spiking known standard compounds to the sample. Quantification was based on the external standard method using calibration curves fitted by linear regression analysis.

The main concentrations of benzoic acid (ppm) in food products for children were determined and given in table 2, 3, 4, 5 and 6.

A standard solution(100ppm) of benzoic acid was injected 3 times as a test sample. From the area counts, the concentration of benzoic acid were calculated. The accuracy (% deviation) of the calculated concentrations were 1.654% (benzoic acid) corresponding 98.346 recoveries.

According to the results of HPLC analysis, the examined samples, which tested under the same conditions as employed for standard references showed various concentrations in benzoic acid in each sample in the different groups was shown in Tables 2, 3, 4, 5 and 6. The highest concentration of Benzoic acid in group I cake samples 849.295 ppm was found in sample No.14 , while the lowest concentration of benzoic acid 1.778 ppm, was in sample No.1. Table 2.

On the other hand, the highest concentration of benzoic acid in group II beverages and Juices samples was 175.940 ppm in sample No.22, while the lowest 22.942 ppm was found in sample No.15. Table 3.

The result also shows that the highest concentration of benzoic acid in group III jam samples was 519.688 ppm in sample No.25, while the lowest 74.016 ppm was found in sample No.23. Table 4.

Group IV consists of three samples of dry milk and chesses. Benzoic acid means concentrations in these samples' ranges from 2.749 to 94.943 ppm. Sample No.26 and No.27 had a small mean concentration of benzoic acid 2.749 ppm and 11.207 ppm respectively, while sample No.28 had a mean concentration of 94.943ppm, Table 5.

Group V consists of four food samples for food for children mainly manufacturers of grain, the mean concentrations of benzoic acid in these sample's ranges from 84.753 ppm to 180.066 ppm, Table 6.

Bahruddin Saad, *et al*, (2005) observed that jam samples contained concentrations between 1756.3 -1872.1 ppm of benzoic acid. **Bente *et al*.** (2006) found that cake samples contained concentrations between 0.00 -409.700 ppm of benzoic acid. **Cornelia and Elena,**(2009) observed that juice samples contained concentrations between 30.29 -107.75 ppm of benzoic acid. **Fernando *et al*.** (2003) found that Juice samples contained concentrations between 179-198 ppm of benzoic acid. **Mohamed (**2009**)** found that cake samples contained concentrations between 8.84 - 755 ppm of Benzoic acid. and Juice samples contained concentrations between 0.00 - 93.0 ppm of Benzoic acid. **Visti *et al.*** (2003) found that Juice samples contained concentrations between 52-602 ppm of benzoic acid. **Tidke and Solanki, (2014)** observed that Local Market Beverages samples contained concentrations between 360 -1000.3 ppm of benzoic acid. **Amirpour *et al*.** (2015), found that juice and cheese samples contained concentrations between 127.9–288.5 ppm and 18.4–91.2 of benzoic acid, respectively. **Sneha and Preetha ,**(2015) observed that fruit products samples contained concentrations between 96 -674 ppm of benzoic acid. **Salehi *et al*.** (2017) found that cheese brands samples contained concentrations between 239.68 - 534.26 ppm of benzoic acid. Our results are in agreement with previous studies. The concentration of Benzoic acid are acceptable according to Yemen standards.

B. Discussion

The general detections of benzoic acid and sodium benzoate of the most samples, lead to the conclusion that they are commonly used as preservatives in food products. The detections of benzoic acid and sodium benzoate in these food products indicate to ensure compliance with national standards, more cooperation among processors and the Yemeni specifications is essential.

On the whole, the levels of the benzoic acid that were tested in compliance with the Yemen Food Act and Regulations (Food Regulations,1996). no Major violation of the Act, however, contain benzoic acid that is low of the legal limits of 1000ppm. The use of benzoic acid and sodium benzoate should be regulated and used only a mean to control yeast at concentrations not exceeding the actual need.

IV. Conclusion

The method was found to be suitable for the routine determination of benzoic acid in food samples with acceptable parameters such as the limit of detection, limit of quantitation. The levels of benzoic acid in dry milk and cheese samples were of natural occurrences, small amount, and to some extent, similar to those reported in the previous studies. Yemeni specifications No. 860 issued 2004 only allow addition of benzoic acid as preservatives for this food category. The levels of benzoic acid in samples food for children mainly manufacturers of grain, and samples of cake were of natural occurrences, and are a violation of all mentioned specifications. The level of benzoic acid in samples of juices and drinks as well as jams were within the permitted national and international limits, and to some extent, similar to those reported in the previous studies.

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VI. APPENEDEX

Tables

Table 1. Linearity results

Linear Regression for Data1_B: Y = B * X + A			
SD		Value	Parameter
2982.974		152.953	A
324.261		49663.802	B
P	N	SD of YX	R
<0.0001	7	5971.937	0.99989

Y= area. X= concentration. A= Intercept. B=Slope. SD= standard deviation. R=correlation coefficient

Table 2. Concentrations of Benzoic acid (ppm) in group I, cakes samples

No. of Sample	Concentration ppm, n=3, mean ± SD	Mean concentration of acid in the sub-group	Mean concentration range of benzoic acid in the group
1	1.778±0.707	1.778 - 5.492	1.778 - 849.295
2	2.206±0.933		
3	2.587± 0.429		
4	4.689 ± 0.411		
5	5.492 ±0.076		
6	27.220± 1.924	27.220 - 94.670	
7	46.417± 4.677		
8	66.151± 2.185		
9	69.103 ± 5.928		
10	94.670 ± 5.129		
11	108.107 ± 8.484	108.107- 849.295	
12	298.949±5.516		
13	632.029± 4.065		
14	849.295 ± 5.609		

SD= Standard Deviation

Table 3. Concentrations of Benzoic acid (ppm) in group II, beverages and Juices samples

No. of Sample	Concentration ppm, n=3, mean ± SD	Mean concentration of acid in the sub-group	Mean concentration range of benzoic acid in the group
15	22.942± 3.889	22.942 - 77.508	22.942 - 175.940
16	46.053± 5.974		
17	66.621± 0.818		
18	71.001±6.036		
19	77.508±5.230		
20	115.060± 2.218	115.060 - 175.940	
21	125.003± 2.057		
22	175.940± 0.617		

Table 4. Concentrations of Benzoic acid (ppm) in group III, Jam samples

No. of Sample	Concentration ppm, n=3, mean ± SD	Mean concentration of acid in the sub-group	Mean concentration range of benzoic acid in the group
23	74.016 ± 0.252	73.837 - 74.194	74.016 - 519.688
24	357.019±6.092	357.019 - 519.688	
25	519.688± 29.368		

SD= Standard Deviation.

Table 5. Concentrations of Benzoic acid (ppm) in group IV, dry milk and cheese samples

No. of Sample	Concentration ppm, n=3, mean ± SD	Mean concentration of acid in the sub-group	Mean concentration range of benzoic acid in the group
26	2.749± 0.318	2.749- 11.207	2.749 - 94.943
27	11.207± 2.398		
28	94.943± 2.826	94.951- 99.034	

SD= Standard Deviation.

Table 6. Concentrations of Benzoic acid (ppm) in group V, food for children mainly manufacturers of grain samples

No. of Sample	Concentration ppm, n=3, mean ± SD	Mean concentration of acid in the sub-group	Mean concentration range of benzoic acid in the group
29	±0.59184.753	84.753 - 91.245	84.753 - 180.066
30	91.245± 5.818		
31	114.842± 5.546	114.842 - 180.066	
32	180.066± 3.126		

SD= Standard Deviation.

Figures

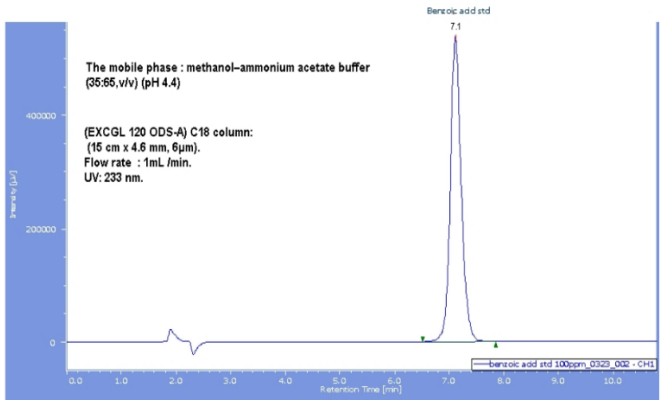

Fig 1. Typical chromatogram of standard containing 100 ppm of Benzoic acid.

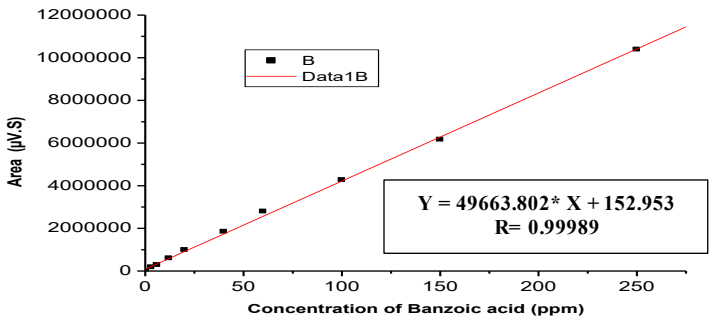

Fig 2. Standard calibration curve of benzoic acid