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Comparing UV And HPLC Methods For Determining the Preservative Sodium Benzoate in Soft Drinks in the Libyan Market (Alkums City)

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KEYWORDS

Sodium benzoate, Soft drink, concentratin, pH, UVVis spectrophotometric and HPLC method.

Abstract:

Sodium benzoate is a common preservative in food and beverages. This study quantified its levels in 10 soft drink samples from Alkhoms, Libya, using UV-Vis spectrophotometry at 226 nm and HPLC methods, alongside pH measurements. Sodium benzoate concentrations ranged from 2.34–18.21 mg/L (HPLC) and 4.4–30.2 mg/L (UV-Vis). A paired t-test indicated no significant difference between the two analytical techniques, confirming their reliability. All samples were below the tolerable daily intake (5 mg/kg body weight, FAO/WHO) and the permissible limit (150 mg/L) set by USEPA and the EU. Despite safe preservative levels, the measured pH values (2.9–3.6) suggest potential dental effects from prolonged consumption.

1. Introduction

Preservatives are compounds or chemicals that are frequently applied to a variety of items, including food, drinks, paints, biological samples, wood, and medications [1]. Food preservatives work by inhibiting the growth of bacteria, fungi (like yeasts), or other microorganisms, as well as by slowing the oxidation of fats that lead to rancidity. They are used to ensure safety and prevent quality loss caused by microbial, physical, chemical, or enzymatic actions [2, 3]. Foods' nutritional content, color, texture, and edibility can all degrade with time. The principal objective of food preservation is to increase its shelflife retaining original nutritional values, colour, texture, and flavor[4]. It can have a potential harmful effect on our health especially if we consume too much[5]. Preservatives can be chemical, natural (such salt, sugar, vinegar, spices, etc.), or a mix of the two. Chemical preservatives fall into one of several categories, including antimicrobial, antienzymatic, antioxidant, and chelating agents. Their general mode of action is thought to involve interfering with the cell membranes of microorganisms, the enzyme system, acting as a self-reducing agent, or forming a complex with food ingredients [6,7]. Sodium benzoate is still a common food ingredient and was the first preservative approved by the FDA for use in food [8]. It is a sodium salt of benzoic acid (E number E211), an aromatic molecule with the molecular formula $C_7H_5O_2Na$, an odorless substance with a sweet, astringent flavor, and an E number of E211 [9]. It is utilized in the food sector, cosmetics, personal care, and pharmaceutical items because of its antibacterial qualities [10]. Sodium benzoate is widely utilized in a variety of culinary items in the food business, including sauces, margarine, salad dressing, and most notably, carbonated drinks [11]. Because sodium benzoate is highly stable and soluble in water, it is used as a preservative to stop food from spoiling due to dangerous bacteria, yeasts, and molds[12]. In particular, sodium benzoate is regarded by the FDA as generally recognized as safe (GRAS) as long as it is used as directed and its weight percentage in food and drink does not exceed 0.1% [13]. The Joint FAO/WHO Expert Committee on Food Additives indicated the allowable daily intake (ADI) level of benzoic acid and benzoates salts as range of (0-5 mg/Kgbw/day)[14] , and a limit of of 150 mg/L has been set for benzoates (as benzoic acid) if it is found alone in pachaged drinks or it is found conjugate with anothor preservative in a particular drink by European Union[15]. A series of epidemiological studies have evidently identified the role of benzene as a leukomogen and have also revealed that persons exposed to 1-2 ppm of benzene over a 40-year period stands a higher risk of developing leukemia and genotoxicity. It is such mosuse that often present several disease outcomes including DNA

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damage , allergies , hypersensitivity , asthma , urticaria and certain types of cancer[14] . Some researchers as dtermined sodium benzoate and poatassium sorbate UV-Vis method and HPLC in some soft drinks in Nigeria, the results showed the presence of bezoate and sorbate concentrations ranged from not detected to the upper the permissible limits[16].

2.Objectives

The main objective of this atricle is to measure and assess the concentration of the preservative sodium benzoate in Ten samples of Soft Drinks collected from the Libyan markets and then analyzed by using a UV-Visible spetrophotometer analyzer and HPLC Device (Triplicate for each Sample), make sure that sample according within the iternational permissible limits.

3.Methods

Ten samples of soft drinks were gathered from various Libyan markets between the end of 2024 and the end of 2025. Ten different kinds of imported soft drink brands and their places of origin were gathered and examined throughout the expiration dates, as indicated in Table(1).

Table 1. Soft drink samples as country of manufacture product

Sample No.	Country of Manufacture Product	Bottle volume (ml)
1	Libyan	330
2	Saudi	150
3	Libyan	330
4	Libyan	330
5	Libyan	330
6	Libyan	250
7	Libyan	330
8	Algerian	250
9	Egyption	250
10	Egyption	250

3.1Measurements

3.1.1.Measurements pH

As seen in figure (1) , pH meter (JENWAY 3505 pH Meter) was used for the analysis (triplicate for each sample). A clean beaker was filled with 30 milliliters of the sample. Standard buffer solutions with pH values of 4 and 7 were used to calibrate the pH meter. The glass was then submerged in the sample solution's supernatant, and once the reading steadied, the pH value was noted.

Figure 1. pH Meter (JENWAY 3505 PH Meter)

3.1.2Measure the preservative by UV-Vis spectroscopy device

Spectrophotometric measurements of sodium benzoate were carried out by means of a UV/Visble Spectrophotometer type (JENWAY 6305 Spectrophotometer) as shown in Figure 2, produced by JENWAY company, it can attache to a computer, working within the wavelenght range of 198 nm to 1000 nm, and cells of light path length plastic. From the collelation absorance with the calibration graph obtained using standard sodium benzoate solution, the amount of sodium benzoate was determined[16] . An aliquot of 0.4 ml of 6 M hydrochloric acid was added to 5 ml of each of the sample and there after extracted with 45 ml of petroleum ether. For the calibration, working standards solutions of 1 mg/L , 5mg/L and 12 mg/L of sodium benzoate were prepared from stock solution 1000 mg/L of sodium benzoate . 5 ml of each working standard solutions prepared was extracted with 0.4 ml of 6 M hydochloric acid and 45 ml of petroleum ether and the UV absorbance measurements were used to generate the calibration curve at 226 nm[16].

Figure 2. UV-6305 UV-Visble Spectrophotometer

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3.1.3.Measure the preservative by High-Performance liquid chromatography device

HPLC measurements of Sodium benzoate were carried out by means of a HPLC system (Agilent 1100 HPLC System w/ DAD & FLD Detectors) as shown in figure 3. The Agilent 1100 HPLC system as shown in Figure (3) consists of : a quaternary pump, Diode Array Detector, temperature management system ensures optimum baseline stability-even under rough ambient conditions. This detector features superb illumination using combined deuterium and tungsten lamps for the highest intensity and lowest detection limit from 190 to 950 nm, 1024 diodes and 1-nm slit for highest spectral resolution[17]. Twenty microlitre (20 µl) of sample were injected into the injector. Analysis were performed by HPLC-(Agilent 1100) which is composed of a two LC-pumps pump, a UV/Vis detector. C18 column (125 mm × 4.60 mm, 5 µm particle size). Chromatograms were obtained and analyzed using the Agilent ChemStation was used for the chromatographic separation at 37°C. The sodium benzoate analysis was performed with isocratic solvent system using sodium acetate and acetic acid buffer (pH = 4.0)/acetonitrile 80:20 with a flow rate of 1.0 ml/min. Chromatograms were recorded at 254 nm.

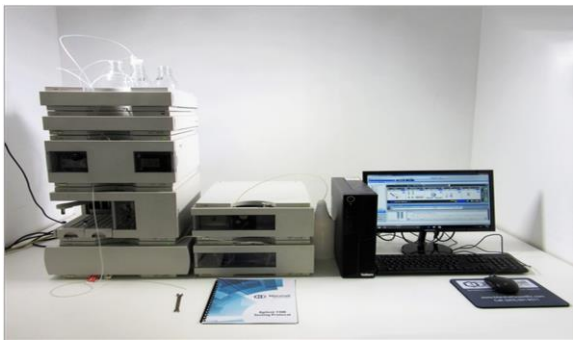

Figure 3. HPLC instrument (Agilent 1100 HPLC System w/ DAD & FLD Detectors)

3.2.Preparation of standard solution of Sodium benzoate

Exactly 50 mg of anhydrous Sodium benzoate was taken in a 50 ml volumetric flask. 20 ml 50% aqueous acetonitrile was added to the volumetric flask and was shaken well. Finally, 50% aqueous acetonitrile was added up to the mark. The solution was filtered with a syringe filter. The standard stock solution-1 was labeled as 1.0 mg/ml. Four ml of stock solution-1 was taken in 50 ml volumetric flask and mobile phase was added up to the mark and was mixed well. The solution-2 was labeled as 80 µg/ml standard solution. Aliquot of 0.0, 62.5, 125, 250 and 500 µl of each standard solution-2 was taken into an Eppendorf tubes and was diluted to volume 1.0 ml with mobile phase and mixed well with a vortex mixer.

3.3.Samples Preparation

Exactly 5.0 ml of each soft drink sample was added in a 50 ml volumetric flask. Aqueous 50% acetonitrile was added up to the mark and mixed well. 10 ml of the solution was taken in another 50 ml volumetric flask and added the same solvent up to the mark. The sample concentration was labeled as 20 µl/ml. Approximately 1.5 ml of the solution was filtered with sample filter (pore size 0.2 µm) to an Eppendorf tube. Exactly 20 µl of this sample mixture was injected onto the HPLC column.

3.4.Analysis of Sodium benzoate

Each 1.0 ml soft drink sample was diluted with mobile phase at a ratio of 1:5, and the dilution was then increased to 1:10. After being diluted, the material was put into a dry Eppendorf tube and centrifuged for fifteen minutes. A PTFE syringe filter was used to filter the transparent aqueous solution. After that, the solution was moved to the dry HPLC vials and injected into the column for quantification and detection.

3.5.Calibration curve

The external standard calibration method was used to get a calibration curve. Then 20 µl of each standard solutions (0.0 5.0, 10.0,20.0 and 40.0 µg/ml) were injected. The peak areas were measured and those of the analytes (y) were plotted against the concentration (µg/ml) of the sodium benzoate (x). Least square linear regression analysis was used to determine the slope, y-intercept and the correlation coefficients of the standard plots.

3.6..HPLC analysis

The precision of the analytical method was evaluated by measuring the chromatographic peak area of sodium benzoate three times for the same sample. Peak areas from HPLC chromatogram were plotted against the known concentrations of stock solutions of varying concentrations[18].

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4.Results and Discussion

According to Table (2), the drink samples' pH levels varied from 2.9±0.047 to 3.6±0.012. Sample 1 of the soft drink samples had the highest pH of 3.6, while sample 3 had the lowest pH of 2.9. According to reports, prolonged exposure to liquids with low pH below 5.5) may cause irreversible damage to dental enamel, dentin exposure, sensitive teeth, and increased cavity formation, even if saliva helps restore the mouth's natural acid content. A beverage with a lower pH will release more calcium from the enamel. According to this investigation, sample 3 showed a higher capacity for releasing calcium than the other soft drinks examined [16, 19]. The majority of canned goods, including soft drinks, are preserved using sodium benzoate. The concentration of standard solutions and absorption were shown to have a linear direct connection based on the calibration curve in Figure(4). The resulting (R2) square value was precisely 0.9968. Table (2) displays the findings of the two techniques used to analyze sodium benzoate. Using the UV-spectrophotometric technique, the results range from 4.4 to 30.2 mg/L; sample 2 had the highest value of 30.2±0.047 mg/L, while sample 6 had the lowest value of 4.4±0.119 mg/L for Soft drink. According to our investigation, all drink samples had sodium benzoate levels within the allowable range of 150 mg/L. The JECFA states that the appropriate daily intake (ADI) of sodium benzoate food preservative is 0–5 mg/kg of body weight. For example, if a consumer weighs 20 kg, their ADI is 100 mg, and if they weigh 50 kg, their ADI is 250 mg. Consequently, all soft drink samples with sodium benzoate levels below ADI limits. Table 2 displays the findings of the HPLC technique for sodium benzoate concentration, which varied from 2.34 to 18.21 mg/L. Sample 1 had the highest value of 18.21±0.117 mg/L, while sample 6 had the lowest value of 2.34±0.013 mg/L. According to our investigation, all drink samples had sodium benzoate levels below the allowable limit of 150 mg/L. The recommended daily intake (ADI) of sodium benzoate food preservative, according to JECFA, is 0–5 mg/kg of body weight. Since all samples had sodium benzoate equivalent to or less than 6.009 mg, they were all within the approved FAO/WHO guidelines. When comparing the HPLC and UV results in Figure(5), it was found that the concentrations obtained from the UV were higher for samples 2, 4, 9, and 10.

This could be because potassium sorbate is present in these samples, which likely interferes with sodium benzoate UV absorbance, as sorbate salts absorb in the ultraviolet region. The results are comparable to those reported by Ogunleye et al., who compared UV and HPLC techniques for the analysis of potassium sorbate and sodium benzoate. Additionally, substances such as vanilla and other flavorings added to foods and beverages may interfere with UV absorption [16]. To statistically verify the agreement between the two analytical methods, a paired t-test was conducted on the sodium benzoate concentrations obtained by HPLC and UV-Vis spectrophotometry. The test revealed no statistically significant difference between the two techniques ($t(9) = 2.151$, $p = 0.05$) at the 95% confidence level, indicating that both methods yield comparable results and can be considered equally reliable for quantifying sodium benzoate in soft drink samples.

Table (2) Comparison of SB concentration between UV and HPLC technique.

Sample No	pH of Soft drink	SB conc (ppm) by UV technique			SB conc (ppm) by HPLC technique		
	Mean±SD	Mean ± SD	RSD %	SB content (mg)	Mean ± SD	RSD %	SB content (mg)
1	3.6±0.012	16.5±0.014	0.08	5.45	18.21±0.117	0.64	6.009
2	3.0±0.051	30.2±0.047	0.16	4.53	15.12±0.093	0.62	2.268

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3	2.9±0.047	14.3±0.113	0.79	4.72	15.22±0.040	0.26	5.023
4	3.5±0.029	11.1±0.157	1.41	3.66	4.75±0.046	0.97	1.568
5	3.4±0.021	10.7±0.185	1.73	3.53	10.54±0.099	0.94	3.478
6	3.5±0.025	4.4±0.119	2.71	1.1	2.34±0.013	0.56	0.584
7	3.2±0.026	14.8±0.111	0.75	4.88	15.03±0.042	0.28	4.96
8	3.0±0.040	6.4±0.125	1.95	1.6	7.00±0.038	0.54	1.75
9	3.0±0.035	16.5±0.104	0.63	4.13	5.13±0.023	0.45	1.28
10	3.1±0.042	22.6±0.244	1.08	5.65	5.56±0.049	0.88	1.39
EU	150±0.00 mg/L						
FAO/WHO	0-5 mg/kg per weight				t-test (t(9) = 2.151, p = 0.05)		

*SB: Sodium Benzoate, Conc: Concentration Sodium Benzoate ,Mean±SD: Values are Means ± Standard Deviation., RSD:Relative standard deviation

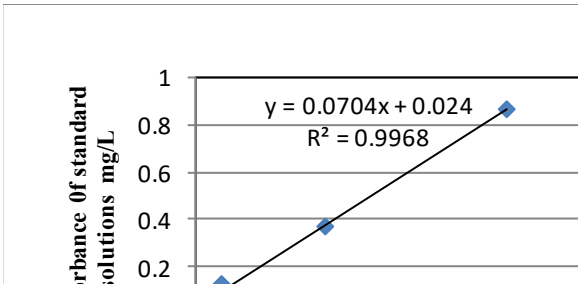

Figure 4. UV Calibration curve of standard solutions concentration versus its absorbance

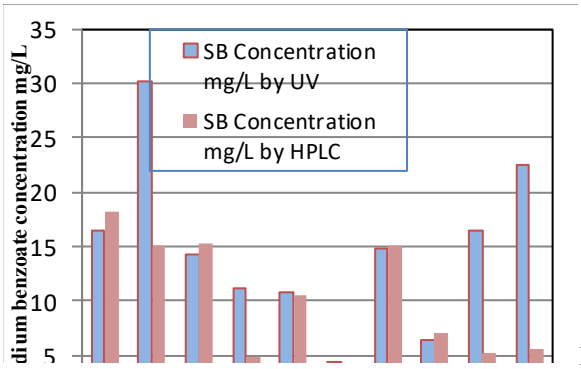

Figure 5. Graphic Comparison between HPLC and UV-Vis methods for sodium benzoate determination

5.CONCLUSION

This study used both UV-visible spectrophotometry and High-Performance Liquid Chromatography (HPLC) methods to successfully measure the sodium benzoate content in 10 distinct soft drink samples marketed in Alkhoms City, Libya. The findings showed that every sample's sodium benzoate concentration was within the EU's and USEPA's allowable limits of 150 mg/L. Furthermore, the values were far lower than the FAO/WHO-recommended average daily intake (ADI) of 0–5 mg/kg body weight. Both methods may be used successfully for the quantitative measurement of sodium benzoate in soft drinks, according to statistical analysis, which showed no significant difference between the findings produced by the UV-Vis and HPLC methods at a 95% confidence level. Slight differences in UV readings, however, could be explained by potential influence from other substances such potassium sorbate or flavoring additives that absorb in the UV spectrum. Despite its compliance with preservative safety regulations, the pH readings 2.9–3.6 indicate that regular use of these acidic drinks may have detrimental impacts on tooth health. Overall, the study supports the dependability of both analytical techniques for regular quality control in the beverage sector and validates the safety of sodium benzoate levels in the tested beverages.

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6.RECOMMENDATION

During our investigation, we suggested that natural preservatives be used rather than artificial ones. Additionally, we suggested that the Food and Drug Standard Authority do a thorough examination of the different beverages available on the market to determine whether the pH and preservatives are within acceptable bounds. To finish our research, we suggested that other researchers look into the detection of sodium benzoate, other preservatives, and other additives in different samples, such as food, cosmetics, or medications.

Refrences

1. Bubai,B.(2023).Different Types of Food Preservatives and its Classification.*Journal of plant pathology & Microbiology.14*(1)
2. Garcia-Garcia,R.&Searle,S.S.(2016).Preservatives:Food Use.*Encyclopedia of food and Health*. 505-509
3. Surwase,G.,Mohan,L.,Kademani,B.S.,& Bhanumurthy,K.(2014).Research Trends on Food Preservation:Ascientometric Analysis.*Journals of Library&Information Technology*.34(3).257-264
4. Amit,S.K.,Uddin,M.,Rahman,R.,Islam,R.,&Khan, M.S.(2017).A review on Mechanisms and Commerical Aspects of Food Preservation and Processing. *Agriculture & food Security*. DOI:10.1186/s40066-017-0130-8
5. Onissiphorou,K.(2023.Mar22).The Effects of Food Additives and Preservatives.*The Science Blog*. Shaikh,S.M.,Doijad,R.C.,Shete,A.S.&Sankpal,P.S.(2016). A Review On: Preservatives Used Pharmaceuticals And Impacts On Health. *Pharma Tutor.4*(5).25-34
6. Gupta,R.&.Yadav,R.K.(2021).Impact of Chemical Food Preservatives on Human Health.*Palarch's Journal of Archaeology of Egypt/Egyptology.18*(15).811-818
7. McCulloch,M.(2023 Jul 03).What Is Sodium Benzoate? Everything You Need to Know. *Healthline blog*.
8. Sodium Benzoate.(2023 Oct18).*The Gale Encyclopedia of Science*.
9. Sodium Benzoate-Uses, Stutcture and properties.(2023 Jan 25). *Turito Blog*.
10. Koh,J & Button,P.(2020 May09). Use of Sodium Benzoate(E211) in food preservation.*Food Microbiology Academy*.
11. Yohannes,A.(2021). *Determination of Benzoic Acid in Packed Fruit Juices and Soft Drink Samples by UV-VIS Spectrophotometryand Titration methods*.(unpublished Master's thesis). Jimma university, Jimma, Ethiopia. Retrieved from:
12. Sodium Benzoate(E211) As A Food Preservative. *The Food Untold*.
13. Azuma,S.L.,Quartey,N.K-A.,& Ofosu,I.W.(2020).Sodium benzoate in non-coholic carbonated (soft) drinks: Exposure and health risks.*Scientific African.10*.
14. EUROPEAN COMMISSION.(2005).Opinion on Benzoic acid and Sodium Benzoate.SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS (SCCP).
15. Ogunleye,D.T.,Oyeyiola,A.O.,Onwordi,C.T.,Falana,T.G.,&Abolade,O.M.(2017).Spectrophotometric and High Performance Liquid Chromatographic Determination of Sodium Benzoate and Potassium Sorbate in some Soft Drinks.Unilag Journal of Medicine, Sciences and Technology.5(1).168-178
16. LabX.Agilent 1100 HPLC System with Diode Array Detector & Fluorescence Detector.
17. Akter,S.,AbuZubair.,Khan,S.,Bari,L.,Huq,A.,&Rashid,M.(2017). Identification and Quantification of Sodium Benzoate in Different Brands of Mango Juices Available in Tangail Region, Bangladesh. *Bangladesh pharmaceutical Journal,20*(1),20-26
18. Manikowski,J.2025.Lincoln Park.The Dangers of Acidity in Beverages. Http://www.lpsdental.com/the-dangers-of-acidity-in-beverages/