Contents lists available at International Journal Press

Journal of Multidisciplinary Sciences, Arts and Humanities

journal homepage: https://ijpress.com/jmsah

Volume 1 · Issue 1 · 2026 · pp. 6-10

HPLC Determination of Benzoic Acid in Commercial Carbonated Beverages

Ritu Gupta[1], Rakesh Kumar Yadav[2*]

[1]Department of Chemistry, Kirodimal Govt. Arts and Science College, Raigarh (C.G), India

[2*] Department of Chemistry, Dr. C.V. Raman University, Kargi Road, Kota, Bilaspur (C.G.) India

* Corresponding Author: rakeshyadav96@gmail.com

ARTICLE INFO

Article history:
Received: 22-07-2026
Received in revised form:30-07-2026
Accepted: 04-08-2026
Available online: 05-08-2026

Keywords:
Benzoic acid
HPLC-UV
Carbonated beverages
Food preservatives
Regulatory compliance

ABSTRACT

Benzoic acid is widely used to control microbial spoilage in acidic beverages, but its concentration requires routine verification. This study quantified benzoic acid in six commercially available carbonated beverage samples by high-performance liquid chromatography with ultraviolet detection. Samples were coded CB-1 to CB-6, extracted in aqueous medium, filtered, and analysed at 230 nm using a phosphate-buffer/acetonitrile mobile phase. External standards covering 10-100 mg/L were used for calibration. Sample concentrations ranged from 235 to 450 mg/kg, with a mean of 358.5 mg/kg, a standard deviation of 79.9 mg/kg, and a median of 360.5 mg/kg. All six samples were below the 600 mg/kg maximum listed by the Food Safety and Standards Authority of India for water-based flavoured drinks. In comparison, only CB-1 was at or below the Codex General Standard for Food Additives level of 250 mg/kg for the same broad food category. The findings provide local market-surveillance data and show that compliance conclusions depend on the regulatory benchmark applied. Larger surveys supported by complete chromatographic validation records are required.

1. INTRODUCTION

Chemical preservatives help maintain the microbiological stability and shelf life of processed foods and beverages. Their use is particularly important in acidic drinks, where yeasts and moulds may remain active during storage. At the same time, preservative concentrations must be monitored because regulatory limits differ by food category and jurisdiction [1].

Benzoic acid and its salts are effective mainly in acidic media because the undissociated form can enter microbial cells and disrupt metabolic activity. Benzoic acid itself is not appropriately described as a direct carcinogen at permitted food-use levels; the principal beverage-related concern is the possible formation of benzene when benzoate coexists with ascorbic acid under favourable processing or storage conditions. Recent reviews therefore recommend exposure assessment and accurate analytical control rather than unsupported general claims about toxicity [2].

Reversed-phase high-performance liquid chromatography with ultraviolet or photodiode-array detection remains a widely applied approach for benzoate determination because it separates preservatives from beverage constituents and permits quantitative comparison with external standards. Recent studies have expanded sample preparation, method validation, and risk-assessment approaches for benzoic acid and related preservatives in drinks and other food matrices [3, 4].

The present work does not propose a new chromatographic method. Its contribution is a focused market-surveillance assessment of six coded carbonated beverage samples and a transparent comparison of the measured concentrations with both Indian and Codex benchmarks. The objective was to determine benzoic acid by HPLC-UV, summarise sample-level variation, and evaluate how the interpretation changes with the regulatory standard applied.

2. MATERIALS AND METHODS

2.1 Study Design and Samples

A cross-sectional laboratory assessment was conducted on six commercially available carbonated beverages obtained from the local retail market. Brand identities were masked, and the samples were coded CB-1 through CB-6. The study involved food products only and did not include human participants, animals, or identifiable personal information.

2.2 Reagents and Laboratory Equipment

HPLC-grade acetonitrile, potassium dihydrogen orthophosphate, concentrated orthophosphoric acid, deionised water, and a sodium benzoate reference standard were used. The equipment included an HPLC system with ultraviolet detection, an analytical balance, pH meter, ultrasonic bath, centrifuge, volumetric glassware, sample vials, microsyringe, and membrane syringe filters [5-7].

2.3 Mobile Phase and Standard Solutions

A 0.05 M potassium dihydrogen orthophosphate buffer was prepared by dissolving 6.8045 g of the salt in 1 L of deionised water. The pH was adjusted to 2.5 with orthophosphoric acid. The mobile phase consisted of phosphate buffer and acetonitrile in a 70:30 (v/v) ratio and was filtered and degassed before use. A 1000 mg/L sodium benzoate stock solution was prepared by dissolving 0.100 g of standard in a 100 mL volumetric flask. Working solutions of 10, 20, 40, 60, 80, and 100 mg/L were prepared by serial dilution with the phosphate buffer [8, 9].

2.4 Sample Preparation

Approximately 25.0 g of each beverage sample was transferred to a beaker and mixed with 50 mL of distilled water. The preparation was mixed thoroughly to release dissolved carbon dioxide, filtered, and centrifuged at 10,000 rpm for 10 min. A 10 mL portion of the supernatant was diluted to 50 mL with phosphate buffer. Before HPLC analysis, the solution was passed through a 0.20 micrometre syringe membrane filter, the initial filtrate was discarded, and the remaining filtrate was transferred to an injection vial.

2.5 Chromatographic Conditions

The working standards and sample extracts were analysed under isocratic reversed-phase conditions. The injection volume was 10 microlitres, and ultraviolet detection was performed at 230 nm. This wavelength lies within the commonly used 225-235 nm region for benzoate determination and provides strong absorbance for quantitative HPLC-UV analysis [4, 10].

The working standards and sample extracts were analysed under isocratic reversed-phase HPLC conditions. The injection volume was **10 µL**, and ultraviolet detection was performed at **230 nm**. This wavelength lies within the commonly used **225–235 nm** region for benzoate determination and provides strong absorbance for quantitative HPLC-UV analysis [4,11]. Chromatographic separation was achieved using a **Shim-pack GIST C18 column (Shimadzu Corporation, Kyoto, Japan; 250 × 4.6 mm i.d., 5 µm particle size)**. The mobile phase was delivered at a **flow rate of 1.0 mL min^{-1}** under isocratic conditions. The **total run time was 10 min**, and **benzoic acid eluted at a retention time of approximately 4.8 min** under the optimised chromatographic conditions.

2.6 Quantification and Statistical Summary

A calibration curve was generated from the peak response of the working standards. The benzoic acid concentration in each original sample was calculated from the concentration measured in the final extract, the final extract volume, the sample mass, and any applied dilution factor:

$$C\ sample\ (mg/kg) = C\ extract\ (mg/L) \times V\ final\ (mL) \times DF / m\ sample\ (g) \quad (1)$$

Where C sample is the concentration in the beverage, C extract is the concentration obtained from the calibration curve, V final is the final extract volume, DF is the additional dilution factor, and m sample is the mass of sample extracted. Because replicate injection data and complete validation records were not supplied, the analysis was limited to sample-level descriptive statistics. The mean, median, range, sample standard deviation, and coefficient of variation were calculated from the six reported concentrations.

3. RESULTS AND DISCUSSION

Benzoic acid was detected in all six beverage samples (Table 1). Concentrations ranged from 235 to 450 mg/kg. The mean concentration was 358.5 mg/kg, the median was 360.5 mg/kg, and the sample standard deviation was 79.9 mg/kg (Table 2). The coefficient of variation was 22.3%, indicating appreciable variation across the products rather than analytical repeatability, because each coded product contributed a single reported value.

Table 1. Benzoic acid concentrations and regulatory classification of the six coded beverages.

Sample	Benzoic acid (mg/kg)	FSSAI 600 mg/kg	Codex 250 mg/kg	Rank
CB-1	235	Complies	Complies	6
CB-2	320	Complies	Exceeds	5
CB-3	395	Complies	Exceeds	3
CB-4	326	Complies	Exceeds	4
CB-5	450	Complies	Exceeds	1
CB-6	425	Complies	Exceeds	2

Note. FSSAI category 14.1.4 lists benzoates at 600 mg/kg as benzoic acid [5]; the Codex GSFA lists 250 mg/kg for water-based flavoured drinks [3].

When the current FSSAI benchmark of 600 mg/kg for water-based flavoured drinks was applied, every sample complied. The original manuscript compared the results with 1000 mg/L; that statement has been corrected because the cited Indian food-category table specifies 600 mg/kg as benzoic acid for the broader water-based flavoured-drink category [5]. The Codex GSFA benchmark is more restrictive at 250 mg/kg [3]. Under that comparison, only CB-1 complied, while CB-2 through CB-6 exceeded 250 mg/kg. These outcomes should not be interpreted as a direct legal non-compliance finding without confirming each product category, formulation, label declaration, and applicable national law; they demonstrate that the selected benchmark materially affects the conclusion [12-14].

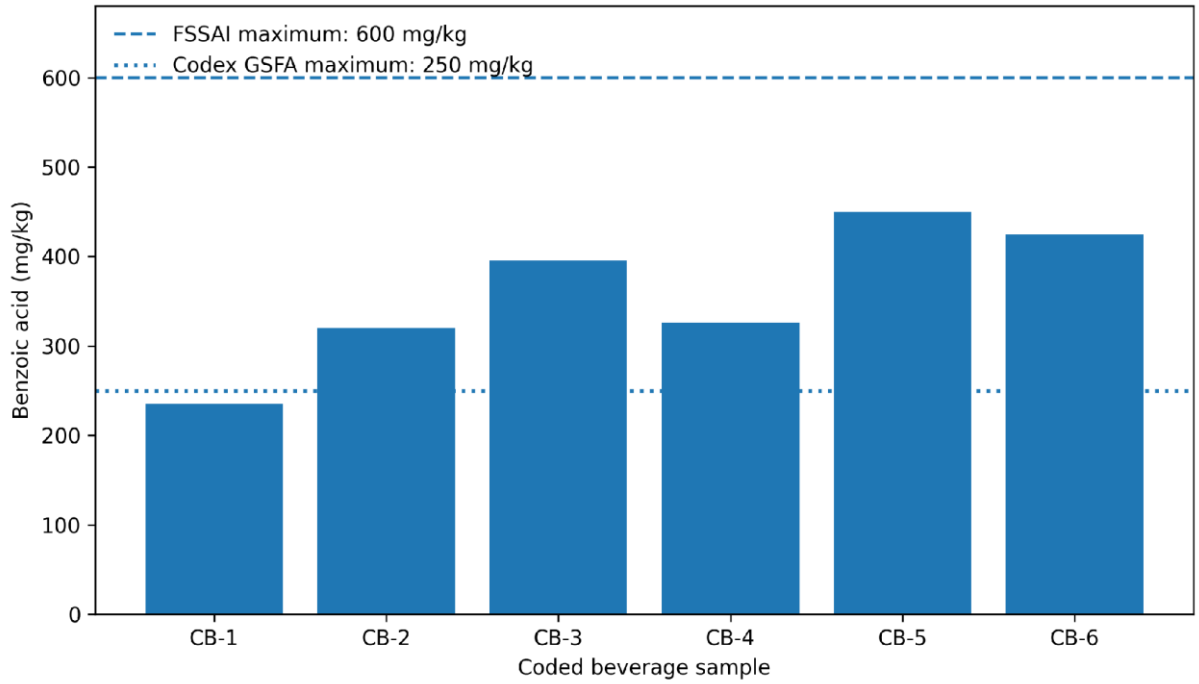

Figure 1. Benzoic acid concentrations in the coded beverages with FSSAI and Codex reference lines.

Table 2. Descriptive statistics for benzoic acid concentrations (n = 6 products).

Statistic	Value
Number of products	6

Minimum	235 mg/kg
Maximum	450 mg/kg
Range	215 mg/kg
Mean	358.5 mg/kg
Median	360.5 mg/kg
Sample standard deviation	79.9 mg/kg
Coefficient of variation	22.3%

The concentration range observed in this small survey is consistent with the wide between-product variation reported in recent studies of commercial beverages and processed foods [4, 13]. Modern HPLC investigations commonly report full validation data, including linearity, selectivity, recovery, precision, limits of detection and quantification, and replicate measurements [1, 4, 7, 11]. Those records were not available in the supplied manuscript [16.17]. Accordingly, the present findings are best interpreted as preliminary product-screening data rather than as a fully validated analytical-method study.

The principal strength of the revised analysis is the direct presentation of all sample-level values together with two clearly identified regulatory benchmarks. Its limitations are the small convenience sample, the absence of manufacturer and batch details, the lack of replicate chromatographic measurements, and incomplete reporting of instrument-specific conditions and validation parameters. These limitations restrict generalisation and prevent formal uncertainty estimation or inferential comparison among product types.

4. CONCLUSION

HPLC-UV analysis showed benzoic acid concentrations of 235-450 mg/kg in six coded carbonated beverages. The sample mean was 358.5 mg/kg, with substantial between-product variation. All samples were below the FSSAI maximum of 600 mg/kg for water-based flavoured drinks, whereas only one sample was at or below the Codex GSFA level of 250 mg/kg. The study therefore supports continued market surveillance and careful identification of the regulatory category used for compliance assessment. Future work should include more brands and production batches, replicate measurements, complete method-validation evidence, and simultaneous determination of benzoate, sorbate, and benzene-related risk factors. No claim of methodological novelty is made.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Chemistry, Dr. C. V. Raman University, Kargi Road, Kota, Bilaspur, Chhattisgarh, for access to academic and laboratory facilities.

FUNDING

This research received no external funding

CONFLICT OF INTEREST

Authors confirm the declaration of no competing interests or disclose any relevant financial/non-financial interests.

DATA AVAILABILITY

The sample-level numerical data are included in Table 1.

ETHICS STATEMENT

Ethical approval was not required because the study analysed commercially available beverage products and did not involve human participants, animals, biological specimens, or identifiable personal data.

AUTHOR CONTRIBUTIONS

Authors confirm that Ritu Gupta and Rakesh Kumar Yadav contributed to the paper.

REFERENCES

[1] Abedi, A., & Khezeli, T. (2024). An easy, low-cost and efficient method for the extraction of benzoic acid and sorbic acid from orange juice and cola soft drink based on PET plastic stir bar sorptive extraction. Journal of Food Composition and Analysis, 136, 106810. https://doi.org/10.1016/j.jfca.2024.106810

[2] AOAC International. (2000). Official Method 994.11: Benzoic acid in orange juice - Liquid chromatographic method. In Official methods of analysis (17th ed.). AOAC International.

[3] Codex Alimentarius Commission. (n.d.). General Standard for Food Additives online database: Food category 14.1.4, water-based flavoured drinks. Food and Agriculture Organisation of the United Nations and World Health Organisation. Accessed July 29, 2026.

[4] Das, S., Uddin, M. N., Khaled, A. S. M., Noyon, M. R. O. K., Chakraborty, D., Mostafa, M., Islam, M. S. M. M., Bhattacharjee, S. C., Das, S. K., & Uddin, M. (2024). Health risk assessment of three preservatives in beverage, cake, ketchup, and therapeutic products available in Bangladesh using the new validated HPLC-PDA method. Journal of Food Composition and Analysis, 126, 105907. https://doi.org/10.1016/j.jfca.2023.105907

[5] Food Safety and Standards Authority of India. (2024). Appendix A: List of food additives (Version 2, 04.11.2024). Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011.

[6] Hejazi, L., Mahboubi-Rabbani, M., Mahdavi, V., Alemi, M., Khanniri, E., & Bayanati, M. (2024). A critical review on sodium benzoate from health effects to analytical methods. Results in Chemistry, 11, 101798. https://doi.org/10.1016/j.rechem.2024.101798

[7] Kefi, B. B., Baccouri, S., Torkhani, R., Koumba, S., Martin, P., & M'Hamdi, N. (2022). Application of response surface methodology to optimise solid-phase extraction of benzoic acid and sorbic acid from food drinks. Foods, 11(9), 1257. https://doi.org/10.3390/foods11091257

[8] Nour, V., Trandafir, I., & Ionică, M. E. (2009). Simultaneous determination of sorbic and benzoic acid in tomato sauce and ketchup using high-performance liquid chromatography. Annals: Food Science and Technology, 10(1), 157-162.

[9] Saad, B., Fazlul Bari, M., Saleh, M. I., Ahmad, K., & Talib, M. K. M. (2005). Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography. Journal of Chromatography A, 1073(1-2), 393-397. https://doi.org/10.1016/j.chroma.2004.10.105

[10] Seetaramaiah, K., Smith, A., Murali, R., & Manavalan, R. (2011). Preservatives in food products: A review. International Journal of Pharmaceutical & Biological Archives, 2(2), 583-599.

[11] So, J. S., Lee, S. B., Lee, J. H., Nam, H. S., & Lee, J. K. (2023). Simultaneous determination of dehydroacetic acid, benzoic acid, sorbic acid, methylparaben and ethylparaben in foods by high-performance liquid chromatography. Food Science and Biotechnology, 32(9), 1173-1183. https://doi.org/10.1007/s10068-023-01264-7

[12] Sultana, A., Islam, R., Islam, M. M., Shoeb, M., & Nahar, N. (2016). Study of preservatives and stimulants in commercial soft drinks. Bangladesh Pharmaceutical Journal, 19(1), 68-74. https://doi.org/10.3329/bpj.v19i1.29241

[13] Uddin, M. N., Das, S., Noyon, M. R. O. K., Islam, M. S. M. M., Khaled, A. S. M., Islam, M. A., Chakraborty, D., Uddin, M., Nabi, M. N., & Bhattacharjee, S. C. (2025). Multi-matrix HPLC investigation of preservatives employing a recently validated method: A Monte Carlo simulation approach to health risks in Bangladeshi processed foods and healthcare. Food and Chemical Toxicology, 197, 115282. https://doi.org/10.1016/j.fct.2025.115282

[14] Werner, J., & Mysiak, D. (2024). Development of thin film microextraction with natural deep eutectic solvents as "eutectosorbents" for preconcentration of popular sweeteners and preservatives from functional beverages and flavoured waters. Molecules, 29(19), 4573. https://doi.org/10.3390/molecules29194573

[15] Willetts, P., Anderson, S., Brereton, P., & Wood, R. (1996). Determination of preservatives in foodstuffs: Collaborative trial. Journal of the Association of Public Analysts, 32, 109-175.

[16] Pandey, J. K., & Kumar, R. (2025). Governing the algorithmic agent: Confronting overt and covert challenges to justice and the future of work. *International Journal of Law Management & Humanities, 8*(4), 1974–1984. https://doij.org/10.10000/IJLMH.1110648

[17] Pandey, J. K., Yadav, A. K., Purushotham, M., Gour, N. I., Gurnule, G. G., & Yadav, K. R. (2022). Colourimetric detection of deltamethrin pesticide from biological samples based on the peroxidase-mimic catalytic activity on MnO_2/SnO_2. *Key Engineering Materials, 928*, 45–51. https://doi.org/10.4028/p-4ti1i0

