International Journal of Medical and Pharmaceutical Sciences
Open access peer-reviewed Journal
Volume 1, Issue 11, Page 12-27.
https://doi.org/10.5281/zenodo.17510055

Review Article

Analytical Techniques for the Determination of Benzoic Acid in Soft Drinks

Ambati Sireesha*, P. Saisireesha, Dr. M. Suchitra

Rathnam Institute of Pharmacy, Pidathapolur, Nellore, Andhra Pradesh, India

One of the most widely used preservatives in soft drinks is benzoic acid, which prevents the growth of bacteria and increases the product's shelf life. However, in order to guarantee product quality and consumer safety, its concentration must be carefully controlled. According to international standards such as those from the World Health Organization (WHO), the U.S. According to the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA), the maximum amount of benzoic acid that can be present in non-alcoholic beverages is typically between 100 and 150 mg/L. Therefore, keeping benzoic acid levels within regulatory limits necessitates the use of accurate and dependable analytical techniques. For its determination, a number of qualitative and quantitative methods have been developed. Thin-Layer Chromatography (TLC) is frequently utilized for rapid identification, whereas UV–Visible spectrophotometry provides simple and sensitive quantification. For functional group analysis, Fourier Transform Infrared (FTIR) spectroscopy provides a quick and non-destructive method. High-Performance Liquid Chromatography (HPLC) remains the most precise and widely accepted method for routine determination due to its high selectivity and reproducibility. Trace-level and confirmatory studies are carried out with gas chromatography–mass spectrometry (GC–MS). For basic quality control, titrmetric methods are cost-effective alternatives. Using these methods helps to avoid the negative effects of using too many preservatives and ensures that food safety regulations are followed. This review focuses on recent advancements in analytical techniques that help keep the levels of benzoic acid in soft drinks controlled and safe.

Keywords: Benzoic acid, soft drinks, preservative, antimicrobial activity, regulatory limit.

INTRODUCTION

The rapid growth of the global food and beverage industry has increased the demand for preservatives to ensure product safety, stability, and shelf life. Soft drinks, which are frequently consumed by people of all ages, particularly children and young adults, are preserved with preservatives to prevent spoilage and maintain quality. The most widely used of these are benzoic acid and its salts (sodium, potassium, and calcium benzoate) due to their potent antimicrobial properties in acidic environments (pH 2.5–4.0). They prevent bacteria, yeast, and molds from growing in products. However, taking too much benzoic acid can have negative effects on one's health, including hypersensitivity reactions and the possibility of benzene formation when ascorbic acid (vitamin C) is combined with heat and light. Therefore, qualitative and quantitative analysis of benzoic acid in soft drinks is necessary to safeguard public health and ensure compliance with FDA, EFSA, and FSSAI standards. Chemical and biotechnological methods of preservation have replaced the ancient methods of drying, salting, and fermentation. Today, there are five important functions that preservers perform: They stop bacteria from growing (like in sodium benzoate-containing beverages). Increase the shelf life for distribution and storage.

- Use antioxidants like ascorbic acid to maintain the nutritional and sensory quality of the food.
- Keep pathogens under control to guarantee food safety.

- Support innovation in ready-to-eat and functional foods.

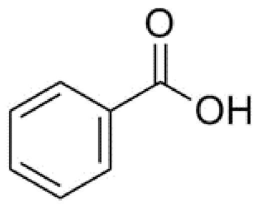
Water, sweeteners, CO2 (for fizziness and microbial inhibition), acidulants (citric or phosphoric acid), flavoring, colorants, and preservatives like benzoic or sorbic acid are typically found in soft drinks. The three main types are cola-based drinks, fruit-flavoured drinks, and energy drinks. Consumption of soft drinks in large quantities, particularly among youth, increases additive exposure. To avoid overuse and maintain safety, constant regulation and monitoring are necessary. Analyzing levels of benzoic acid maintains consumer trust in the beverage industry, prevents the formation of benzene, and ensures compliance with global food safety standards. Common preservatives in Beverages

Preservatives	Chemical formula	Primary use	Effective PH Range
Benzoic acid	C_6H_5COOH	Inhibits mould, yeast, Some bacteria	2.0 - 4.0
Sorbic acid	$C_6H_8O_2$	Prevents mould& yeast growth	3.0 - 6.5
Lactic acid	$C_3H_6O_3$	Acidulant, antimicrobial	3.0 - 5.0
Sulphur dioxide	SO_2	Antimicrobial & antioxidant	<4.0

Chemistry of Benzoic Acid

Molecular Structure of benzoic acid:

Benzoic acid is an aromatic carboxylic acid with the molecular formula C_6H_5COOH. It has a benzene ring, or aromatic moiety, attached to a carboxyl group (–COOH) as its structural unit. Benzoic acid's solubility and reactivity are affected by the combination of an aromatic ring and a carboxyl group, which gives it lipophilic (carboxylic acid) and hydrophilic (aromatic ring) properties. Physical and Chemical Properties of Benzoic Acid



Physical properties of benzoic acid

Property	Description/Value
Molecular formula	C_6H_5COOH ($C_7H_6O_2$)
Molecular weight	122.12 g/mol
Appearance	White crystalline solid
Odour	Faint, Aromatic smell
Melting point	122-123^{0}C
Boiling point	~249^{0}C
Density	1.265g/cm^3
Solubility in water	Low (2.9g/lit at 25^{0}C); Increases in hot water
Crystalline form	Monoclinic
Another physical feature	Sublimes easily (Solid-Vapour without melting).

Chemical properties of Benzoic acid

Property/Reaction	Description
Acidic nature	Weak acid, Pka ~4.20; ionizes to form benzoate ion ($C_6H_5COO^-$)
Salt formation	Forms salts with bases Eg; Sodium benzoate
Esterification	Reacts with alcohols – Benzoate esters
Reaction with Thionyl chloride	Produces benzoyl chloride

Substitution on benzene ring	Undergoes electrophilic substitution; -COOH group is electron-withdrawing, meta-directing
Oxidation	Relatively stable, but under strong conditions can degrade further
Reduction	Can be reduced to benzyl alcohol and other derivatives
Thermal behaviour	Decomposes on strong heating to benzene +CO_2
Antimicrobial Action	In acidic medium, undissociated benzoic acid inhibit microbial growth[8].

Stability in Beverages:

Benzoic acid is generally stable when stored normally; however, when it is combined with ascorbic acid (Vitamin C) in the presence of heat or light, it becomes unstable and gives rise to the carcinogenic compound benzene (C6H6). The primary concern regarding the safety of beverages is this. Nature's Happening: Cranberries, plums, prunes, cinnamon, and cloves all naturally contain benzoic acid, but at much lower concentrations than in commercial beverages.

Purpose as a Preservative:

Due to their high sugar content, moisture content, and lengthy storage, soft drinks are susceptible to microbial spoilage. Sodium, potassium, calcium, and benzoic acid and its salts are added to stop the growth of yeasts, molds, and bacteria. This keeps the flavor, safety, and shelf life of the product intact. Why Benzoic Acid Should Be Used: Effective in Acidic pH (2.5–4.0): Active in its undissociated form, giving strong antimicrobial action.

Low Cost: Produced industrially through the oxidation of toluene, making it affordable for widespread use. It has no effect on color or flavor at regulatory levels (less than 200 mg/L).

Synergistic Action: Combines well with citric acid and sorbates. Proven Safety: Over a century of use; classified as GRAS by FDA and Codex Alimentarius.

Health Concerns & Regulatory Limits:

FDA (USA): Up to 0.1% (1000 ppm); GRAS but monitors benzene risk with Vitamin C.

FSSAI (India): 200–1000 ppm; food products for children should be avoided. Acceptable Daily Intake (ADI): 0–20 mg/kg body weight per day, according to WHO/JECFA. Hyperactivity and allergies can result from excessive exposure. Keeping an Eye on Benzoic Acid Levels: Regulatory Compliance: Ensures that manufacturers adhere to legal limits (0.1 percent in the United States, 200–1000 ppm in India). Protect Vulnerable Populations: Children may exceed the ADI if they consume too much. Prevent Benzene Formation: Monitoring formulations containing Vitamin C, especially under heat/light.

Maintain Quality: Excess preservatives affect taste and appearance.

Support Risk Assessment: Data aids toxicological and exposure studies.

Interactions & Stability Factors:

pH: Most effective below 4.0; loses efficiency above 5.0.

Under light or heat, ascorbic acid can produce benzene. Sugars and sweeteners have minimal pH effects and are typically stable. Metals (Fe, Cu, Mn): Catalyze benzene formation; controlled by PET or coated packaging.

Flavour Agents: Minimal interference; pH adjustment may affect efficiency.

Storage: Stable in cool, dark conditions; heat and prolonged storage increase degradation risk.

AIM AND OBJECTIVES

AIM

A review on analytical techniques for the determination of benzoic acid in soft drinks

OBJECTIVES

1. To summarize and compare various analytical techniques used for the determination of benzoic acid in soft drinks.

2. To evaluate the accuracy, precision, sensitivity, and applicability of methods such as TLC, HPLC, GC–MS, UV–VIS spectro photometry, FTIR, and titrimetric approaches.

3. To discuss the sample preparation and extraction methods employed for soft drink matrices.

4. To assess the regulatory limits of benzoic acid in soft drinks and the compliance of analytical methods with these standards.

5. To provide an overview of the advantages and limitations of different analytical approaches for routine quality control and research purposes.

4. Sample collection and preparation

Types of soft Drinks Tested (Cola, fruit juices, Energy drinks)

Type of Drink	Examples	Key characteristics
Carbonated drinks	Cola, Soda, Sparkling water	High CO_2 content, acidic PH (~3.0), Preservatives like benzoates/sorbates
Fruit juices	Orange juice, Apple juice	Natural sugars, vitamins, susceptible to microbial spoilage
Energy drinks	Red bull, Monster	High caffeine, taurine, sugars, artificial flavours

Sample collection and preparation of soft drinks before Analysis

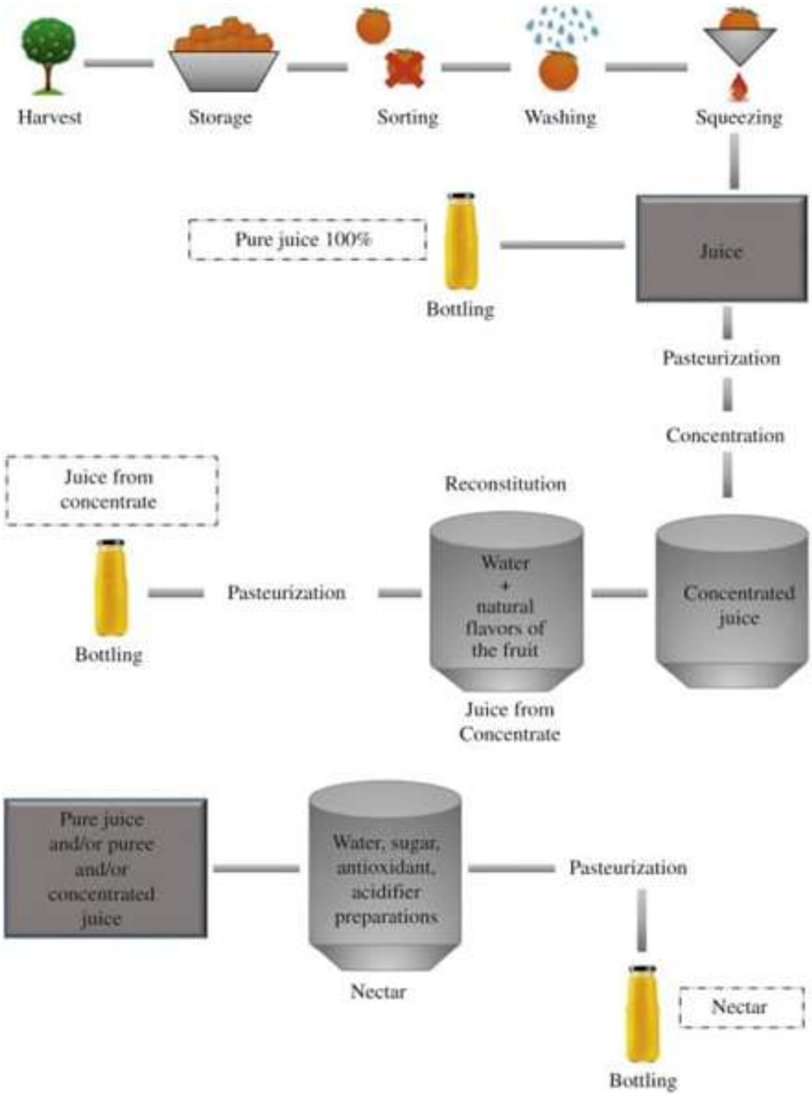
Carbonated drinks like cola and soda, fruit juices, and energy drinks are examples of soft drinks that are commonly consumed. To ensure product quality, safety, and regulatory compliance, laboratory analysis is performed. Sample collection and preparation, which has a direct impact on the results' accuracy and dependability, is an essential step before analysis.

1. Sample Collection

- To prevent contamination, samples are collected in airtight, clean containers that have been sterilized.
- Information about the brand, batch number, date, kind of drink, and storage conditions are listed on the labels of the containers.
- To prevent microbial growth and chemical changes, proper cold storage (4–8 °C) is maintained.

2. Sample Preparation Methods

Before analysis, soft drink samples are treated to remove impurities, standardize conditions, and extract analyses.



(a) Filtration

• Removes suspended particles, sediments, or pulp (in juices).

• Helps avoid clogging of chromatographic instruments.

(b) Acidification

• Adjusts pH to stabilize certain compounds.

• Facilitates the extraction of preservatives (such as benzoic acid and sorbic acid) and prevents the growth of microorganisms.

(c) Extraction

• Used for isolating specific compounds like caffeine, preservatives, or artificial sweeteners.

• Methods like solid-phase extraction (SPE) or liquid–liquid extraction

Sample Preparation of Soft drinks (flowchart)

Sample collection

↓

Labelling & Storage ($4\text{-}8^{0}$c)

↓

Filtration (remove solids, pulp, Sediments)

↓

Acidification (adjust PH for stability)

↓

Extraction (if target analytes need separation)

↓

Prepared sample – sent for Analysis (HPLC, GC, Spectrophotometer, etc)

Importance of Proper Sample Preparation

- Ensures accuracy and reproducibility of results.
- Prevents instrumental damage due to suspended solids.
- Helps in precise quantification of preservatives, caffeine, colorants, and additives[20].

5. ANALYTICAL METHODS

I. Qualitative Methods to Determine Benzoic Acid Levels in Soft Drinks:

A. Thin- layer chromatography (TLC)

1. Principle

TLC separates sample components on a silica gel plate. Using a densitometer or digital imaging, the spots of benzoic acid are measured for quantitative analysis. The amount of benzoic acid in each spot (peak area or height) determines its intensity. Accurate quantification is made possible by calibration standards on the same plate.

Procedure

To keep benzoic acid in its protonated form, soft drink samples are first degassed to remove carbon dioxide and, if necessary, acidified to a pH of 2–3. The benzoic acid is then extracted using ethyl acetate, and the combined extracts are dried over anhydrous sodium sulphate. The residue is redissolved in either methanol or ethyl acetate after the solvent is gently evaporated under nitrogen pressure. Micropipettes are used to spot small volumes of both the sample and benzoic acid standards onto Silica Gel 60 F254 plates, which are prepared by marking an origin line approximately 10–12 mm from the bottom. The mobile phase, typically a 5:4:1 mixture of toluene, ethyl acetate, and formic acid, is used to develop the plate in a pre-saturated development chamber until the solvent front travels approximately 8–9 cm. Benzoic acid is seen as a dark spot on the plate when it is examined under UV light at 254 nm following drying.

The spot intensity is measured using a densitometer or image analysis software, and the concentration of benzoic acid in the sample is determined using a calibration curve constructed from known standards.

$$C_{sample}(mg/l) = \frac{m_{spot}((\mu g) \times V_{final}(ml)}{V_{sample}(ml) \times 1000}$$

Observations

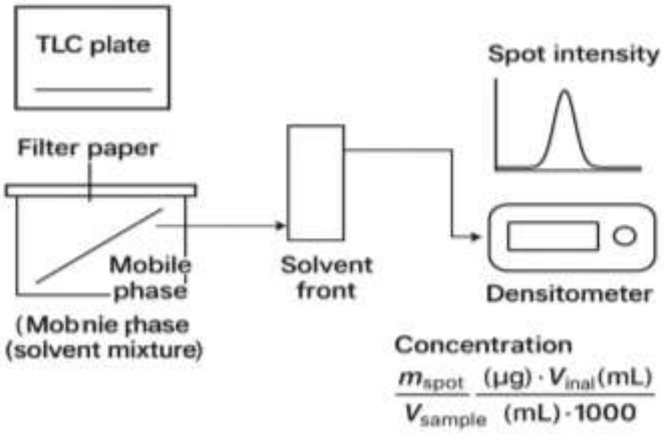
The quantitative TLC method for benzoic acid shows a linear relationship between spot intensity and concentration within the range of 0.05–50 µg per spot. The limit of quantification (LOQ) is typically between 0.03 and 1.0 mg/L, while the limit of detection (LOD) is typically between 0.01 and 0.5 mg/L. The precision of the method is good, with relative standard deviation (RSD) values between 2–8% for intra-plate analysis and 5–15% for inter-plate measurements. Typically, recovery values range from 80 to 110 percent, indicating accurate extraction and quantification. The method's high linearity (r2 0.99) and high accuracy (85–110% recovery) make it suitable for routine quality control of benzoic acid in soft drinks.

Advantages

TLC is a simple, easy, and low-cost method that requires little equipment. It gives quick results, uses only a small amount of sample and solvent, and allows many samples to be analysed at the same time.

Limitations

TLC is less sensitive and accurate than more advanced techniques like HPLC or GC. The outcomes are determined by the operator's skill, and if separation is poor, spots may overlap. Additionally, it can be affected by the environment and is difficult to detect in very small amounts.



B. Colorimetric Method

Principle:

The formation of a coloured complex through the reaction of benzoic acid or its salts with specific reagents is the basis for the colorimetric measurement of benzoic acid. Using a colorimeter or UV–Visible spectrophotometer, the intensity of the color produced is measured at a specific wavelength (typically between 400 and 600 nm). Commonly, benzoic acid reacts with ferric chloride ($FeCl_3$) to produce a pale yellow to brown-coloured ferric benzoate complex. The absorbance of this complex follows Beer–Lambert's law, where absorbance is directly proportional to concentration within a linear range.

Procedure:

First, the sample of soft drink is gently shaken or sonicated to get rid of dissolved carbon dioxide, which can make it hard to get accurate colorimetric readings. A colored ferric–benzoate complex is created by treating a measured portion of the degassed sample with the appropriate reagent, typically ferric chloride. In order to guarantee consistency, a series of standard benzoic acid solutions with known concentrations are prepared and treated concurrently. A colorimeter or UV–Visible spectrophotometer is used to measure the absorbance of the standard and sample solutions at the chosen wavelength, typically 520 nm. Plotting the absorbance against the concentration of the standard benzoic acid solutions yields a calibration curve. By comparing the absorbance value of the unknown sample to the calibration curve, the concentration of benzoic acid can be determined. To guarantee results' reproducibility and accuracy, all measurements are carried out in identical conditions.

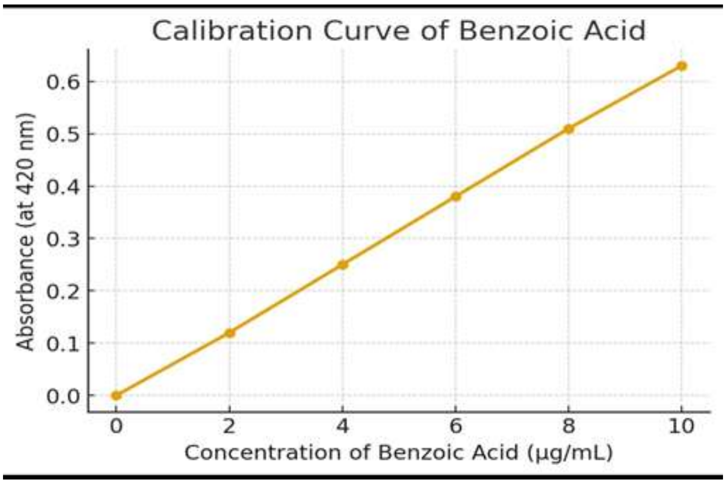
Observation:

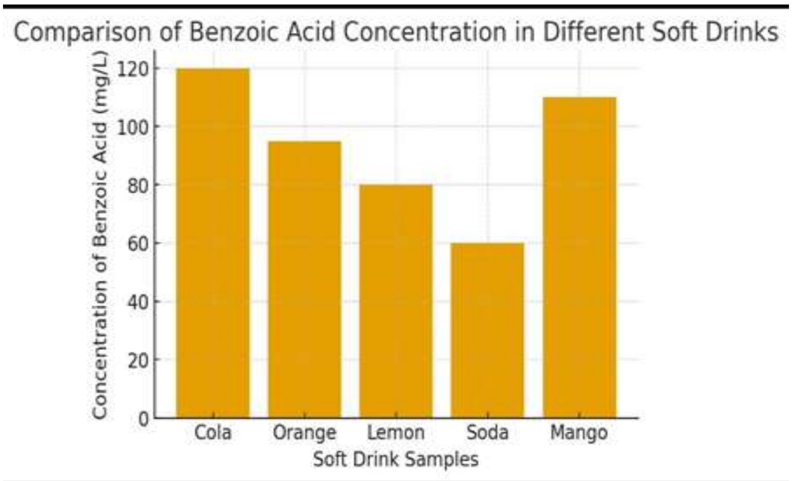
The intensity of the colour developed increases proportionally with the concentration of benzoic acid. The absorbance–concentration relationship remains linear within the working range, and the concentration of benzoic acid in the sample is determined by interpolation from the calibration curve.

Advantages

This method is simple, cost-effective, and suitable for routine quality control. It is ideal for educational and industrial laboratories because it produces quick results and requires minimal sample preparation.

Limitations: The method may suffer from interference caused by other colour-forming compounds such ascorbic acid or caffeine.





It is less specific than chromatographic techniques such as HPLC or GC–MS and requires proper calibration for reliable results

C. FTIR (Fourier-Transform Infrared Spectroscopy) Method for Benzoic Acid

Principle

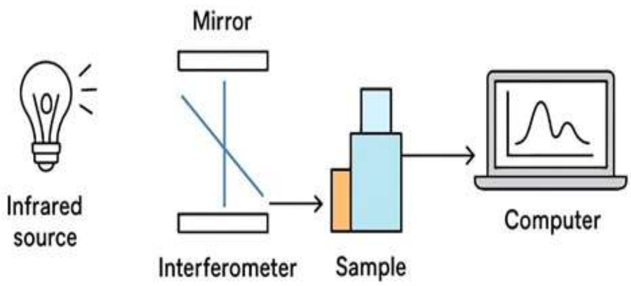
Benzoic acid shows characteristic IR absorption bands, e.g., C=O stretch (~1700 cm⁻¹) and O–H stretch (~2500–3300 cm⁻¹). There is a correlation between the concentration of benzoic acid and the intensity of these bands. Comparison to standard spectra and calibration curves is used in quantitative analysis.

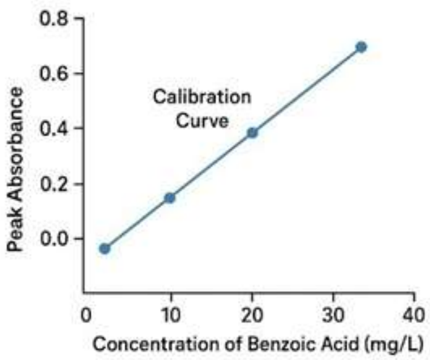
Procedure

First, the sample of soft drink is degassed to get rid of dissolved carbon dioxide, which can mess up spectral measurements. Using a suitable solvent like ethyl acetate or methanol, benzoic acid can be extracted from the beverage and evaporated to dryness if necessary. For solid sample analysis, about 1 mg of the dried benzoic acid residue is finely ground with 100 mg of dry potassium bromide (KBr) using a mortar and pestle, and the mixture is compressed into a transparent pellet using a pellet press. Alternately, the ATR (Attenuated Total Reflectance) method involves placing a small amount of the sample or extract directly on the ATR crystal surface without requiring any additional preparation. The FTIR spectrum is then recorded between 4000 and 400 cm-1, revealing characteristic benzoic acid absorption bands like the C=O stretch close to 1700 cm-1, the O–H stretch between 2500 and 3300 cm-1, and the aromatic C=C stretches between 1600 and 1500 cm-1. The intensity of the C=O band is compared with those of calibration standards, and a calibration curve (peak intensity versus concentration) is used to determine the benzoic acid concentration in the sample.

Observation

The Benzoic acid functional groups are represented by distinct absorption peaks in the FTIR spectra that were recorded. The peak intensity of the C=O stretch increases proportionally with concentration, displaying a linear relationship within the working range of approximately 1–50 mg/L. The method typically has a limit of detection of around 0.5 mg/L, recovery values that range from 85 to 110 percent, and precision (RSD) values that range from 3 to 7 percent. Proper sample preparation and baseline correction are essential to minimize interference from water, sugars, or other beverage components.





Advantages & Limitations

Advantages:

Rapid and non-destructive
Can analyse solids or extracts directly
Minimal solvent use

Limitations:

Less sensitive than HPLC or GC
Matrix interference may affect quantitative accuracy
Requires careful calibration[29].

II. QUANTITATIVE ANALYSIS METHODS

A. UV–Visible Spectrophotometry

Principle

The method is based on the Beer–Lambert law, which states that absorbance is directly proportional to the concentration of an absorbing species in solution. Benzoic acid or its derivative absorbs light at a specific wavelength in the UV region (around 230–275 nm). The sample's concentration is determined by comparing its absorbance to that of standard solutions.

Procedure

Prepare a stock solution of benzoic acid and dilute it to prepare a series of standard concentrations. Plot a calibration curve by measuring the absorbance of each standard at the chosen wavelength. After filtering and degassing the soft drink sample, measure its absorbance under the same conditions. From the calibration curve, determine the concentration.

Observation

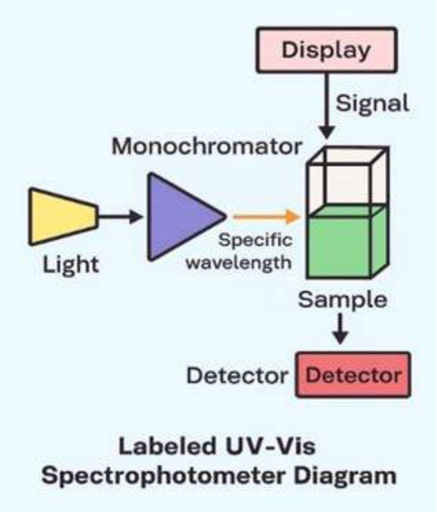
The absorbance of benzoic acid increases linearly with concentration. The sample solution shows a characteristic peak at its λmax. From the calibration plot, the benzoic acid concentration in the soft drink can be quantified accurately.

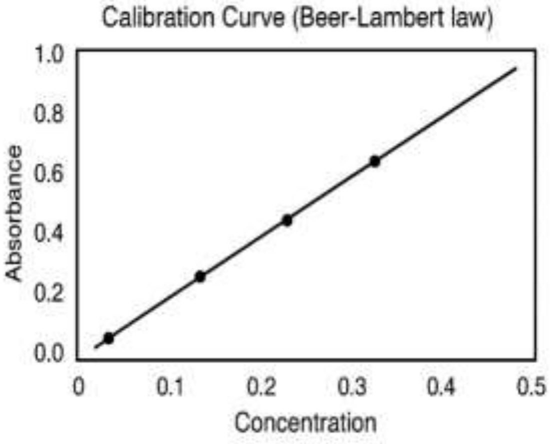
Advantages

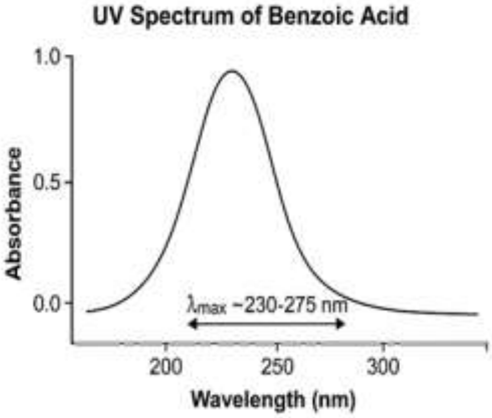
Quick, simple, cost-effective, and accurate for measuring benzoic acid with minimal sample preparation.

Limitations

Less selective, Titrimetric analysis is simple, quick, and cost-effective. It does not require sophisticated[32].







B. High-Performance Liquid Chromatography (HPLC)

Principle

Compounds are separated by HPLC based on how differently they interact with the mobile phase (solvent) and stationary phase (typically a C18 column). Each compound elutes at a specific retention time, and the detector response (peak area) is proportional to its concentration.

Procedure

Prepare mobile phase and degas it. Prepare standard benzoic acid solutions of known concentrations. Filter and inject the sample and standards into the HPLC system. Record chromatograms at a wavelength of 230–254 nm. Utilize the calibration curve that has been plotted between the peak area and concentration to determine the amount of benzoic acid in the sample.

Observation

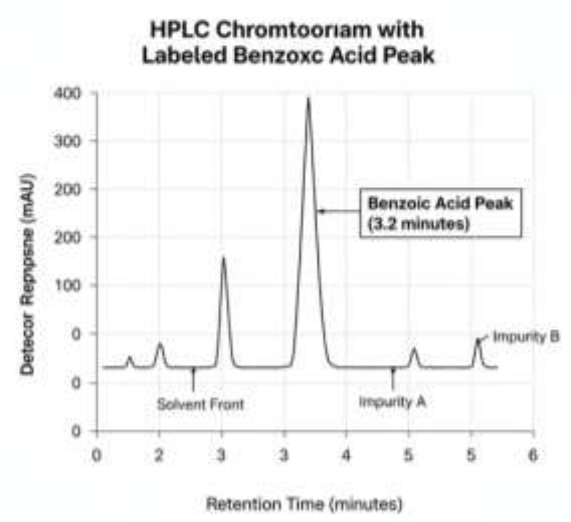
A sharp, well-resolved peak for benzoic acid appears at a characteristic retention time. The peak area of the sample is proportional to the concentration. Accuracy and reproducibility of the method are confirmed by a linear calibration graph.

Advantages

HPLC is highly accurate, sensitive, and selective for determining benzoic acid in soft drinks. It can separate benzoic acid from other compounds in complex mixtures, giving precise quantification even at low concentrations. The method is reproducible and widely used in quality control laboratories.

Limitations

HPLC requires expensive instruments, skilled operators, and longer analysis time compared to simpler methods. Sample preparation can be more complex, and maintenance of the equipment is necessary to ensure consistent results[36].



C. Gas Chromatography-Mass Spectrometry (GC-MS)

Principle

In a gas chromatograph, GC–MS separates volatile components and uses a mass spectrometer to identify them based on their mass-to-charge ratio. Non-volatile benzoic acid may be converted to a volatile derivative (such as methyl benzoate) for analysis.

Procedure

If necessary, derivatize the benzoic acid sample to increase its volatility. Inject a small amount into the GC–MS (between 1 and 2 L). Adjust the temperature program to achieve the best separation. Record chromatograms and mass spectra. Identify benzoic acid by comparing its retention time and characteristic ion fragments with standards.

Observation

A distinct chromatographic peak with matching mass spectral fragments confirms the presence of benzoic acid. The concentration of the peak in the sample is indicated by its intensity. The method provides high sensitivity and specificity.

Advantages

Even in complex soft drink matrices, benzoic acid can be precisely identified and quantified using GC–MS, which is highly sensitive and selective. It provides both qualitative (identification) and quantitative data, and can detect very low concentrations with high precision.

Limitations

GC–MS is costly, necessitates skilled operators, and requires intricate sample preparation. Compared to simpler methods like UV–Vis or HPLC, the equipment is more complicated and needs regular maintenance, making it less convenient for routine analysis.

D. Titrimetric (Acid–Base) Analysis

Principle

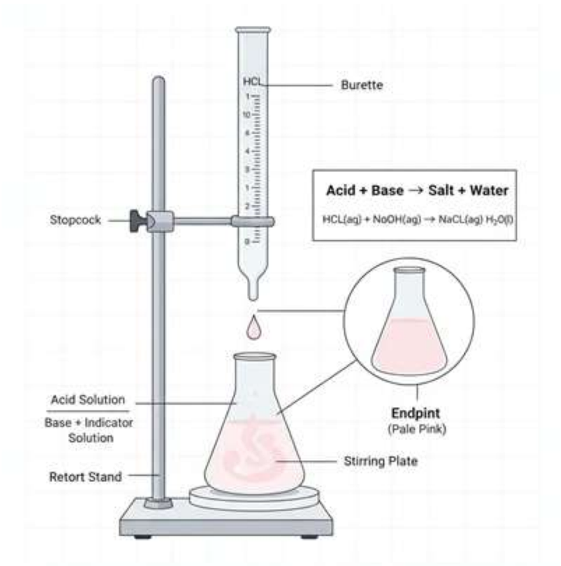
The method is based on acid–base neutralization. Benzoic acid reacts with a standardized sodium hydroxide solution in a 1:1 molar ratio. The endpoint is detected using an acid–base indicator or a pH meter.

Procedure

Pipette a known volume of the degassed and filtered soft drink sample into a conical flask. Add a few drops of phenolphthalein indicator. Titrate with standard NaOH until the endpoint (a faint pink color) remains. Record the volume of NaOH used and calculates the benzoic acid content.

Observation

The solution changes from colourless to pale pink at the endpoint. The volume of NaOH consumed corresponds to the total acidity due to benzoic acid. Accurate titration is indicated by consistent readings.



Advantages

Titrimetric analysis is cost-effective, quick, and simple. It can provide reasonably accurate results for the concentration of benzoic acid and does not require sophisticated instruments. With basic reagents, the procedure is simple to carry out in regular laboratory settings.

Limitations

Because other acids in soft drinks can interfere with the measurement, it lacks selectivity. In comparison to methods like HPLC and GC–MS, the method lacks sensitivity and may not accurately detect very low concentrations of benzoic acid.

Health and Safety Concerns of Benzoic Acid in Soft Drinks

Benzoic acid is widely used as a preservative in soft drinks to inhibit microbial growth and extend shelf life. Even though it is generally accepted as safe (GRAS) at concentrations that have been approved, drinking too much soft drinks on a regular basis can put your health at risk. Benzoic acid can irritate the gastrointestinal tract, resulting in nausea, vomiting, and abdominal discomfort. Sensitive individuals, particularly those with asthma, may experience allergic reactions such as skin rashes or respiratory symptoms43. Moreover, excessive consumption may burden the liver and kidneys due to its metabolism to hippuric acid. Some studies have suggested a potential link between high benzoic acid intake, especially when combined with artificial colorants, and hyperactive behaviour in children. To protect consumers, regulatory authorities typically restrict the amount of benzoic acid in soft drinks to less than 1% to 0.15%44. Continuous monitoring and adherence to these limits are essential to prevent toxicological effects while maintaining the preservative benefits in beverages. Under certain conditions, the benzoic acid in soft drinks can turn into benzene, a known carcinogen. This conversion is typically facilitated by the presence of ascorbic acid (vitamin C) and exposure to heat or light during storage. Despite the fact that benzene formation is generally low, it has raised safety concerns, requiring manufacturers to carefully monitor formulations and storage conditions45. To keep health risks to a minimum, regulatory bodies like the World Health Organization (WHO) and the European Food Safety Authority (EFSA) have set acceptable daily intake (ADI) values for benzoic acid. The ADI is typically set at 0–5 mg/kg body weight per day, ensuring that consumption of soft drinks and other foods containing benzoic acid remains within safe limits. In addition to controlling storage and ingredient combinations, adhering to this guideline helps prevent the toxicological effects of benzoic acid as well as the potential formation of benzene.

CONCLUSION

Accurate determination of benzoic acid in soft drinks is essential for ensuring product safety and regulatory compliance. Among analytical techniques, HPLC offers the highest precision and reproducibility, while TLC, UV–Vis spectrophotometry, and FTIR provide rapid, cost-effective, and non-destructive alternatives. Titrimetric methods are still useful for straightforward analyses, and GC–MS is useful for verifying trace levels. Consumer health is protected by regulatory limits, which typically range from 100 to 150 mg/L. Overall, these techniques play a vital role in monitoring preservative levels to maintain product quality, consumer health, and compliance with international food safety standards. To keep health risks to a minimum, regulatory bodies like the World Health Organization (WHO) and the European Food Safety Authority (EFSA) have set acceptable daily intake (ADI) values for benzoic acid. Typically, the acceptable daily intake is between 0 and 5 mg/kg body weight per day, keeping beverages containing benzoic acid and other foods within safe limits. Adherence to this guideline, along with controlling storage and ingredient combinations, helps prevent both toxicological effects of benzoic acid and the potential formation of benzene.

REFERENCES

1. Lee, S. W., Kim, H. J., & Park, J. M. (2022). Identification of benzoic acid in beverages by GC–MS. Food Chemistry, 386, 132742.
2. Dong, M., Yang, X., & Chen, G. (2021). Influence of ascorbic acid and heat on benzene formation in beverages containing benzoate preservatives. Food Additives & Contaminants: Part A, 38(9), 1551–1560.
3. Gutiérrez, J. M., & Singh, R. K. (2021). Food preservation technologies: Historical development and modern applications. Comprehensive Reviews in Food Science and Food Safety, 20(6), 5743–5762.
4. Russell, N. J., & Gould, G. W. (2003). Food Preservatives (2nd ed.). Springer Science & Business Media.
5. Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research, 5(4), 47–56.
6. Beuchat, L. R. (2018). Microbial stability and spoilage of soft drinks: Role of preservatives. Beverages, 4(4), 88–99.

7. World Health Organization (WHO). (2021). Evaluation of Certain Food Additives: Eighty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: WHO Press.
8. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2020). Introduction to Modern Liquid Chromatography (4th ed.). John Wiley & Sons.
9. Liao, K., Xu, Y., & Wang, J. (2020). Metabolism and toxicological evaluation of benzoic acid in food and beverages. Food and Chemical Toxicology, 146, 111801.
10. Lee, S. W., Kim, H. J., & Park, J. M. (2022). Identification of benzoic acid in beverages by GC–MS. Food Chemistry, 386, 132742.
11. Food and Drug Administration (FDA). (2020). Code of Federal Regulations: 21 CFR §184.1021 — Benzoic Acid. Silver Spring, MD: U.S. FDA.
12. Food Safety and Standards Authority of India (FSSAI). (2022). Food Safety and Standards (Food Products Standards and Food Additives) Regulations. New Delhi: FSSAI.
13. World Health Organization (WHO). (2021). Evaluation of Certain Food Additives: Eighty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: WHO Press.
14. Kümmerer, K. (2009). Antibiotics in the aquatic environment — A review: Occurrence and environmental implications. Chemosphere, 75(4), 417–434.
15. Larsson, D. G. J., & Flach, C. F. (2019). Antibiotic resistance in the environment. Nature Reviews Microbiology, 17(1), 45–56.
16. Oteiza, J. M., & Giannuzzi, L. (2022). Stability of benzoic acid in soft drinks under storage conditions. Food Research International, 153, 110936.
17. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2020). Introduction to Modern Liquid Chromatography (4th ed.). John Wiley & Sons.
18. Poole, C. F. (2019). Sample Preparation: Chromatography and Capillary Electrophoresis. Elsevier.
19. Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. Molecules, 18(2), 2328–2375.
20. Ahmed, M., & El-Kosasy, A. (2021). Optimization of solid-phase extraction for beverage preservatives prior to HPLC analysis. Journal of Food Analysis, 98, 103794.
21. Yadav, R., & Kumar, P. (2023). Quantitative TLC determination of benzoic acid in beverages using densitometric detection. Food Chemistry Advances, 2, 100145.
22. Jain, S., & Patel, R. (2022). TLC determination of food preservatives in soft drinks. Analytical Methods in Chemistry, 2022, 1–7.
23. Sherma, J., & Fried, B. (2019). Handbook of Thin-Layer Chromatography (4th ed.). CRC Press.
24. Ibrahim, N. A., & Khalifa, S. A. (2021). Colorimetric determination of benzoic acid in beverages using ferric chloride reagent. Egyptian Journal of Chemistry, 64(9), 4803–4814.
25. Muntean, E., & Gogoasa, I. (2020). Spectrophotometric determination of benzoates and sorbates in soft drinks. Food Analytical Methods, 13(6), 1449–1456.
26. Sharma, K., & Chauhan, S. (2023). Colorimetric and UV–Vis methods for detection of preservatives in beverages. International Journal of Food Science & Technology, 58(3), 1225–1233.
27. Ferreira, M., & Costa, P. (2020). Quantitative infrared analysis of food additives using FTIR spectroscopy. Vibrational Spectroscopy, 108, 103043. 37. Grube, M., & Reineccius, G. (2021). FTIR in food authentication: Rapid and non-destructive analysis of preservatives. Analytical Methods, 13(2), 188–197.
28. Grube, M., & Reineccius, G. (2021). FTIR in food authentication: Rapid and non-destructive analysis of preservatives. Analytical Methods, 13(2), 188–197.
29. Salem, A. A., & El-Hefnawy, M. (2022). Determination of benzoic acid in beverages by ATR-FTIR spectroscopy. Journal of Applied Spectroscopy, 89(4), 789–797.
30. El-Sheikh, R., & Mahmoud, H. (2022). Spectrophotometric limitations in beverage preservatives. Arabian Journal of Chemistry, 15(11), 104243.
31. Uddin, M. S., & Rahman, M. M. (2023). Quantification of benzoic acid in soft drinks by UV–Visible spectrophotometry. Bangladesh Journal of Scientific Research, 36(1), 25–32.

32. Dong, M., Yang, X., & Chen, G. (2021). Influence of ascorbic acid and heat on benzene formation in beverages containing benzoate preservatives. Food Additives & Contaminants: Part A, 38(9), 1551–1560.
33. Alkhateeb, F. L., Hassan, S., & Malik, R. (2024). HPLC analysis of soft drinks using Alliance™ HPLC system. International Journal of Analytical Chemistry, 2024, 1–7.
34. Abedi, A., Niazi, A., & Afkhami, A. (2024). Efficient extraction and HPLC quantification of benzoic acid in cola beverages. Food Analytical Methods, 17(4), 1292–1304.
35. Alghamdi, S., & Mohammed, A. (2023). Validation of RP-HPLC method for benzoic and sorbic acid determination in carbonated beverages. Saudi Journal of Analytical Chemistry, 11(2), 85–92.
36. Snyder, L. R., & Dolan, J. W. (2020). High-Performance Liquid Chromatography Principles and Practices. John Wiley & Sons.
37. Tranchida, P. Q., Purcaro, G., & Dugo, P. (2023). Challenges in GC–MS quantification of food preservatives. Journal of Chromatography A, 1698, 463011.
38. Lee, S. W., Kim, H. J., & Park, J. M. (2022). Identification of benzoic acid in beverages by GC–MS. Food Chemistry, 386, 132742.
39. Koti, S., Reddy, D., & Singh, P. (2025). Determination of bisphenol A and benzoic acid in soft drinks using MSPE–GC/MS. Food Chemistry, 435, 137526.
40. Okechukwu, P. M., & Adeniyi, O. (2019). Simple titrimetric determination of benzoic acid in beverages. Nigerian Journal of Pharmaceutical Research, 15(2), 123–129.
41. El-Safety, S. A., & Ahmed, M. (2021). Neutralization titration for weak acids in beverages: Application to benzoic acid analysis. Journal of Food Composition and Analysis, 100, 103898.
42. Kumar, R., & Gupta, P. (2019). Effect of carbonation on titrimetric analysis of preservatives in soft drinks. Journal of Analytical Science and Technology, 10(1), 58–65. Health and Safety Concerns of Benzoic Acid in Soft Drinks
43. Liao, K., Xu, Y., & Wang, J. (2020). Metabolism and toxicological evaluation of benzoic acid in food and beverages. Food and Chemical Toxicology, 146, 111801.
44. European Food Safety Authority (EFSA). (2020). Re-evaluation of benzoic acid (E210) and benzoates (E211–E213) as food additives. EFSA Journal, 18(11), e06351.
45. World Health Organization (WHO). (2021). Evaluation of Certain Food Additives: Eighty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: WHO Press.
46. Food and Drug Administration (FDA). (2020). Code of Federal Regulations: 21 CFR §184.1021 — Benzoic Acid. Silver Spring, MD: U.S. FDA.

Cite: Ambati Sireesha*, P. Saisireesha, Dr. M. Suchitra, Analytical Techniques for the Determination of Benzoic Acid in Soft Drinks, Int. J. Med. Pharm. Sci., 2025, 1 (11), 12-27. https://doi.org/10.5281/zenodo.17510055