DOI: https://doi.org/10.21009/JRSKT.121.01

Revealing the Role of Mobile Phase Composition and pH in Benzoic Acid Analysis of Beverages and Processed Foods: Implications for SDG 3

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Received: 09 February 2026
Revised: 30 April 2026
Accepted: 29 May 2026
Online: 23 June 2026
Published: 30 June 2026

Jurnal Riset Sains dan Kimia Terapan
p-ISSN: 2302 - 8467
e-ISSN: 2303 – 0720

Abstract

To prevent health risks in excessive consumption, monitoring benzoic acid as a food additive is necessary in line with SDG 3 principles. Conventional methods for determining benzoic acid levels have limitations in selectivity and sensitivity due to matrix interference contained in food products. This study aims to determine benzoic acid levels in support of food safety monitoring in Jakarta by optimizing the mobile phase in a reversed-phase HPLC system (RP-HPLC. Reverse-phase high-performance liquid chromatography with a UV detector, investigated in this research, operated at 225 nm. A C-18 or octadecyl silica (ODS) column stationary phase (250 mm x 4,6 mm, 5 µm particle size) was used, which is classified as nonpolar. The mobile phase used is a polar methanol-phosphate buffer with a ratio 4:96 at pH 6.8. Optimization of mobile phase composition and pH was necessary to control the ionization degree of benzoic acid, improve analyte–stationary phase interactions, and produce optimal retention time, peak shape, and resolution. The test results for benzoic acid levels in carbonated drinks, jelly drinks, and mayonnaise were 140.973 mg/kg, 232.294 mg/kg, and 710.695 mg/kg, respectively. It can be concluded that the benzoic acid levels in the sample remain below the maximum limit set by BPOM RI.

Keywords: benzoic acid, high-performance liquid chromatography, preservative, RP-HPLC

Introduction

Food security is an important pillar of the Sustainable Development Goals (SDGs), which are 17 global goals set by the United Nations to ensure sustainable development in the social, economic, and environmental fields by 2030. One of the goals, SDG 3, is to ensure healthy lives and improve well-being for all people at all ages. Food safety plays a crucial role because it directly affects health risks. Regulation by the Indonesian National Agency of Drug and Food Control (BPOM) No. 11 defines food as anything derived from agricultural, plantation, forestry, fishery, livestock, aquatic, and water products, whether processed or unprocessed, for drink, including food additives, raw food materials, and other materials used in the preparation, processing, and/or manufacture of food or drink.

Food additives are substances added to food as preservatives, coloring agents, or stabilizers (Niu et al., 2025; Savin et al., 2022). The types and levels of food additives are regulated by the Indonesian National Agency of Drug and Food Control (BPOM RI, 2019) and the European Food Safety Authority (EFSA) to avoid potential health risks. Benzoic acid is one of the food additives used as a preservative and is classified as Generally Recognized as Safe (GRAS) under Indonesian Minister of Health Regulation No. 722/88. Benzoic acid is commonly used in various food products, including snacks, carbonated drinks, and mayonnaise. Despite safe preservatives and benzoic acid possessing a tendency to be harmless, excessive amounts for sensitive individuals can pose health risks (Guo et al., 2020).

In a study conducted by Leesuraplanon et al. (2022) and Yepes-Calderón et al. (2025), a combination of benzoic acid with vitamin C in beverages has the potential to pose cancer risk because the interaction between the two compounds provokes benzene production. Benzene is generally recognized as a group 1 carcinogen. Furthermore, risk of mortality due to cardiovascular disease and respiratory disorders may occur even at low concentrations of benzene in prolonged exposure (Wang et al., 2024).

Sodium benzoate is a common salt form of benzoic acid. Studies with animal-based models have shown that patients with kidney disorders have decreased the ability to excrete hippuric acid as a metabolite with declining kidney function (Huang et al., 2018; Khodaei et al., 2019; Sun et al., 2020; Yassien et al., 2022), even though the metabolism of sodium benzoate in the liver remains relatively normal. This condition leads to the accumulation of hippuric acids that cause an increase in the number of free radicals in the body, and it’s hard for antioxidant systems to neutralize the metabolite (Sun et al., 2020). This condition is called oxidative stress. Furthermore, prolonged oxidative stress initiates cell and tissue damage (Chandimali et al., 2025; Mustafa, 2024). For those health risk reasons, the Indonesian National Agency of Drug and Food Control (BPOM RI) has established the maximum limit for benzoic acid. Based on regulation, benzoic acid used in carbonated beverages, jelly drinks, and mayonnaise are 400 mg/kg, 600 mg/kg, and 1000 mg/kg, respectively (BPOM RI, 2019).

The classical titration method is frequently used for qualitative analysis of sodium benzoate as a salt form of benzoic acid (Mishra, 2021; Sri K et al., 2023). In titration methods, endpoint determination depends on naked-eye observation of indicator color transition, rendering the measurements subjective and susceptible to sample color interference. Therefore, its applicability in food regulatory analysis, demanding high accuracy and sensitivity, is limited (Liv, 2021). Another method, a direct UV-Vis spectrophotometric measurement, can be applied (Elferjani et al., 2024; Elmanfe, 2020; Hettiarachchi et al., 2021); however quantitatively has a high limit of detection (LOD) compared to an initial separation step conducted using HPLC prior to analysis (Akbar et al., 2023; Alabyadh, 2023; Spirić et al., 2025). In food analysis, many matrices can interfere with quantitative analysis. Eliminating matrix effects in food samples is an important consideration when determining analyte concentrations (Tungkijanansin et al., 2020). Therefore, in this study, benzoic acid was analyzed in several food samples, including various types of food and beverage products, using the HPLC method with a limit of detection (LOD) of 1.8 μM (Guo et al., 2020).

Two principal separation modes are recognized in high-performance liquid chromatography (HPLC): normal-phase and reversed-phase. Normal-phase systems employ a non-polar mobile phase and a polar stationary phase, commonly silica (Snyder et al., 2009). Despite its applicability for non-polar analytes, this mode is less appropriate for benzoic acid analysis in complex food matrices. The relatively polar and ionizable nature of benzoic acid (Ogbadu, 2014) limits its retention on polar stationary phases, resulting in insufficient resolution from polar matrix interferences. In addition, the reliance on non-polar mobile phase solvents presents compatibility limitations with aqueous food extracts, thereby diminishing chromatographic selectivity and separation efficiency (Zarad et al., 2017).

In contrast, reversed-phase HPLC employs a non-polar stationary phase in combination with a relatively polar mobile phase, rendering it more suitable for the separation of benzoic acid (Astuti et al., 2019). Under appropriately controlled conditions, benzoic acid can be maintained in its non-ionized form, thereby enhancing hydrophobic interactions with the stationary phase and improving analyte retention and chromatographic resolution. Nevertheless, achieving optimal separation performance, mobile phase optimization is required (Žuvela et al., 2019). This optimization includes adjusting the composition of the solvent and buffer as well as the pH of the mobile phase, as both affect the degree of ionization of the analyte and the strength of its interaction with the stationary phase. Therefore, this study conducted mobile phase optimization in a reversed-phase HPLC system for the analysis of benzoic acid in food products.

Method

The instrument used in this research consisted of analytical balance (Precisa), analytical microbalance (Sartorius), volumetric flasks (20 mL and 50 mL), erlenmeyer flask (200 mL), micropipette 20-200 μm and 100-1000 μm, membrane filter (0.45 μm pore size) sample vials, ultrasonic batch (Branson), shaker (Torta), ultrasonic, homogenizer and centrifuge. The pH measurement was conducted with a pH meter (Mettler Toledo). A HPLC Shimadzu LC-20AD was employed for analysis. The system was equipped with SPD-20A Prominence UV-Visible detectors set at 225 nm. The chromatographic separation was performed with a C18 column (Phenomenex) 250 × 4.6 mm, particle size of 5 μm.

The materials used in this research were HPLC-grade methanol (Supelco), and phosphate buffer (Merck). Methanol and water solvents with a ratio of 40:60 are used for diluting standard solutions (benzoic acid). The samples analyzed in this study included carbonated beverages, jelly drinks, and mayonnaise that were obtained from a local market in the DKI Jakarta region.

Preparation of Buffer and Mobile Phase Liquid.

The mobile phase employed in this study is a methanol-phosphate buffer system. Optimization of the mobile phase composition was performed to obtain optimal chromatographic performance by evaluating methanol-to-buffer ratios of 1:99, 2:98, 3:97, 4:96, and 5:95.

Preparation of Working Standard Solution.

Standard solutions (benzoic acid) were prepared with concentrations of 0.2 µg/mL; 10 µg/mL; 20 µg/mL; 30 µg/mL; 40 µg/mL; 50 µg/mL using methanol and water solvents with a ratio of 40:60. The chromatogram obtained from each standard solution was used to create a relationship curve between benzoic acid levels and the area under curve (AUC) value and determine the regression equation and correlation coefficient.

Chromatographic Condition.

The chromatographic analysis was carried out using an HPLC system equipped with a UV-Vis detector. Separation was achieved on a C18 reversed-phase column (250 x 4.6 mm) with an isocratic mobile phase consisting of the optimum mobile phase composition and pH. The mobile phase was filtered through a 0.45 µm membrane and degassed prior to use. The flow rate was maintained at 1.0 mL/min, and detection was performed at 225 nm.

Preparation of Sample Solution.

The carbonated beverage samples were prepared by degassing them for 15 minutes. Thereafter, 2 grams of the sample were dissolved in 50 mL of distilled water and filtered using a membrane filter with a pore diameter of 0.45 µm. The jelly drink sample was prepared by homogenizing the sample and taking 2 grams to be dissolved in 50 mL of distilled water. The prepared sample solution was then filtered using a 0.45 µm pore diameter membrane filter. The mayonnaise sample was prepared by homogenizing the sample in a 100 mL glass beaker. Then, 2 grams of the sample were taken and dissolved in distilled water to 50 mL. It was then centrifuged at 6,000 rpm at 5°C for 10 minutes. The prepared sample solution was filtered through a 0.45 µm pore membrane. Each sample was prepared in duplicate. The benzoic acid content in each sample was calculated by entering the AUC value into the standard curve equation obtained. The results were then interpreted using **Equation 1**.

$$Concentration\ \left({}^{mg}/_{Kg}\right) = \frac{C_{sp} \times F}{w} \tag{1}$$

Where:
C_{sp} = benzoic acid concentration obtained from calculations using the linear equation y = bx + a (µg/mL)
F = dilution factor
w = sample weight

Results and Discussion

The analytical method used to determine benzoic acid levels is reversed-phase HPLC. In this method, polar compounds will elute earlier. In reversed-phase, for achieving good selectivity separation, the strength of the organic solvent must not be too strong to prevent a very low-capacity factor (Santikasari et al., 2025; Wulandari et al., 2024). A nonpolar C18 or octadecyl silica (ODS) column measuring 250 mm x 4.6 mm and 5 µm was chosen for its ability to separate compounds based on their polarity. The mobile phase used was polar methanol-phosphate buffer. The mobile phase transported the components to be separated through the stationary phase, thereby achieving optimal retention times.

Selection of Mobile Phase and Buffer.

Through reverse-phase HPLC, the interaction between analyte, stationary phase, and mobile phase affects the selectivity and sensitivity of the method. To achieve optimal separation, which is indicated by high peak resolution, it is necessary to adopt the mobile phase based on physicochemical properties and optimize the composition (David & Moldoveanu, 2024; Peiró-Vila et al., 2024). As reported in previous studies (Salamah et al., 2025; Shelar et al., 2024; Tesoro et al., 2022), the mobile phase combination consisting of organic solvent and buffer solution has been confirmed to provide optimal separation. The combination is based on the polarity, degree of ionization, and impact of affinity on the stationary phase.

Methanol and buffer phosphate were used as mobile phases in this study. The selection of methanol is considered by its polar protic properties, arising from the hydroxyl functional group characteristic of alcohols. In addition, the presence of a single carbon atom as a short-chain alcohol confers a certain degree of hydrophobic character to methanol, even if the hydrophobic character is relatively weak (Huffman et al., 2012). The dual characteristic of polar protic and weak hydrophobic properties permits methanol to balance interaction between semi-polar analytes, such as benzoic acid, and the nonpolar stationary phase (Kalisz et al., 2025), such as C-18/ODS (Obradović et al., 2022).

During chromatographic analysis, the mobile phase pH must be maintained under controlled conditions to preserve the ionization degree of benzoic acid. Therefore, the use of a buffer solution with reliable buffering capacity within the acidic to neutral pH conditions is essential. Phosphate buffer was selected not only due to its effective buffering performance but also because of its chemical stability when combined with organic solvents such as methanol, allowing the formation of a homogeneous mobile phase system (Pardhi et al., 2020). In addition to buffering of pH, phosphate buffer also prevents undesirable electrostatic interactions between residual silanol groups on the stationary phase surface and the analyte, thereby enhancing peak symmetry and method reproducibility (Carda-Broch et al., 2018).

Optimization has been carried out to find the optimum composition ratio of methanol:phosphate buffer, in a 4:96 ratio. Optimization of the mobile phase composition was carried out by varying the methanol-phosphate buffer ratio at 1:99, 2:98, 3:97, 4:96, and 5:95. At lower methanol proportions, which are 1:99 to 3:97, the organic content was insufficient, resulting in a highly polar mobile phase. Under these conditions, hydrophobic interactions between the analyte and the nonpolar stationary phase became excessively strong, leading to prolonged retention times and significant peak broadening (Kazmouz et al., 2021). Moreover, the proportion of methanol influences both the apparent pH and the buffering capacity of the mobile phase, which in turn affects the ionization degree of benzoic acid. Increasing the organic fraction to 4:96 and 5:95 enhanced the elution strength of the mobile phase, facilitating analyte desorption from the column as interactions with the stationary phase were progressively weakened (Heering et al., 2024). Nevertheless, the 4:96 ratio was selected as the optimal condition, as it resulted in sharper and better-resolved peaks than those obtained at 5:95.

After establishing the optimum mobile phase composition, the study proceeded with pH optimization. A methanol-phosphate buffer mixture at a ratio of 4:96 was evaluated across a pH range of 4-9. In reversed-phase HPLC, the effect of mobile phase pH is closely associated with the pKa value of benzoic acid, where ionized and nonionized species are present in equal proportions, 50:50 (Ogbadu, 2014). **Figure 1** illustrates the ionization transition between the neutral and dissociated form of benzoic acid. In its non-ionized state, benzoic acid is electrically neutral and relatively more hydrophobic, whereas in the ionized form it carries a negatively charged carboxylate (COO^{-}) group and becomes more polar.

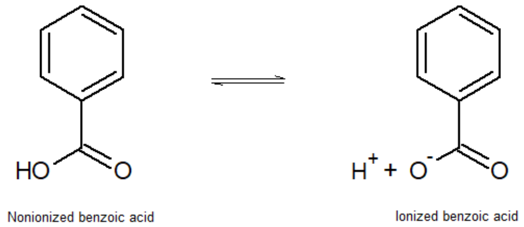

Figure 1. ionization transition for benzoic acid with pKa 4.2

Given that the pKa of benzoic acid is approximately 4.2 (Abiman et al., 2007), the compound predominantly exists in its protonated (neutral) form at pH values below the pKa, whereas at pH values above the pKa it dissociates to form the more polar benzoate anion (Preikša et al., 2023). As described by Ogbadu (2014), at pH 4, slightly below the pKa, the equilibrium shifts toward the neutral species, with neutral species accounting for approximately 60%, while the remaining fraction (40%) exists in the ionized form. Under such conditions, the coexistence of protonated and deprotonated species during migration results in non–uniform analyte velocity, which in turn leads to peak broadening and asymmetry.

When the mobile phase pH is adjusted to 5–6, which is higher than the pKa, the proportion of nonionized (neutral) benzoic acid is reduced to about 1.5% (Ogbadu, 2014). This phenomenon occurs due to the equilibrium shifting toward the ionized form of benzoic acid as benzoate ions (COO^{-}). Under these conditions, the polarity of benzoic acid slightly increases. The relatively small decline (1.5%) in non-ionized benzoic acid is not substantial enough to minimize the hydrophobic interactions with the nonpolar stationary phase. A considerable proportion of neutral benzoic acid remains available to interact with residual silanol groups, potentially leading to silica surface degradation (Steinhoff et al., 2025a, 2025b). Acidic environments promote hydrolytic cleavage of siloxane bonds within the silica-based stationary phase, ultimately reducing the effectiveness of the column and chromatographic performance.

At the optimum pH of 6.8, which is close to neutral conditions, benzoic acid exists in a favorable equilibrium between its ionized and non-ionized forms. The predominance of the ionized species increases analyte polarity, thereby weakening the hydrophobic interactions with the stationary phase and promoting faster elution with reduced retention. At the same time, the residual non-ionized fraction contributes to regulating analyte migration by maintaining a controlled level of hydrophobic interaction (Poole & Lenca, 2017). This balanced interplay prevents excessive retention while preserving sufficient analyte-stationary phase interaction, thereby ensuring chromatographic separation runs optimally and resulting in high retention time producibility (Žuvela et al., 2019).

At pH 8–9, benzoic acid is predominantly present in its ionized form, resulting in markedly weakened hydrophobic interactions with the C18 stationary phase. Consequently, the analyte elutes rapidly, often accompanied by diminished separation resolution (Steinhoff et al., 2025a). Under alkaline conditions, residual silanol groups on the silica surface undergo deprotonation, increasing the likelihood of secondary electrostatic interactions with the negatively charged benzoate ions. Such interactions are chromatographically undesirable, as they can distort analyte migration and compromise peak symmetry. Moreover, exposure to elevated pH accelerates dissolution of the silica matrix through alkaline hydrolysis, ultimately impairing column stability and performance. For these reasons, highly alkaline mobile phase conditions are generally unsuitable for the separation of benzoic acid in silica-based reversed-phase HPLC systems (Sirhan, 2018).

The Quantitative Analysis of Benzoic Acid in Some Samples.

The experimental results show that there is a chromatogram peak for benzoic acid at a retention time of 6.92 minutes. The sharp chromatogram peak indicates that the separation occurred well and there were no impurities or interfering matrices (Ismail, 2024).

Benzoic acid levels were tested using the HPLC method by preparing a standard solution and then creating a regression line equation and correlation coefficient. The solvent composition of the standard benzoic acid solution was a 60:40 ratio of methanol to water. This solvent composition was the optimum composition with the highest average recovery (Uddin et al., 2025).

The area under the curve data for the standard benzoic acid solution in relation to the concentration of the standard solution is presented in the following table. From this data, a calibration curve can be created to determine the level of benzoic acid in the sample.

Based on the calibration curve (**Figure 2**), a linear regression equation was obtained $y = 93233x - 48953$, with a correlation value (R^2) = 0,9997 so it can be inferred that the analysis of benzoic acid using the HPLC method is a valid analysis and meets the linearity requirements with an R^2 value $\geq$ 0,995. Determination of benzoic acid levels in samples can be done by calculating the area data obtained from the analysis using HPLC into the linear regression equation of the standard solution, which was $y = 93233x - 48953$ where the y value is the area and the X value is the concentration. The data for determining the benzoic acid levels in the test samples are then compared with the maximum benzoic acid level limit set by the Indonesian National Agency of Drug and Food Control No. 11 of 2019. The interpretation of the benzoic acid levels in the test samples is listed in **Table 1**.

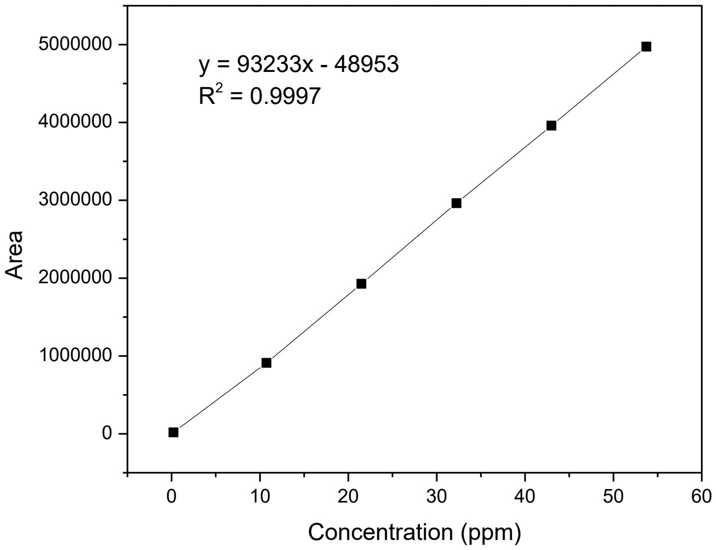

Figure 2. The calibration curve of benzoic acid

Based on the test results, it was found that the levels of benzoic acid in two carbonated beverage samples (1A and 1B) were 140.973 mg/kg and 139.758 mg/kg, respectively. Compared to the maximum limit recommended by the BPOM RI, these results indicate that the carbonated beverage samples (1A and 1B) complied with the regulatory limit of benzoic acid. Therefore, samples 1A and 1B are considered safe for consumption. The benzoic acid levels of jelly drink samples (2A and 2B) were 232.294 mg/kg and 230.877 mg/kg, respectively. The jelly drink samples do not exceed the maximum usage limit as recommended by BPOM RI due to the benzoic acid in the samples is lower than 600 mg/kg. The last samples analyzed in this study were mayonnaise samples. BPOM RI set the maximum levels allowed for benzoic acid in mayonnaise at 1000 mg/kg. In the tested mayonnaise samples (3A and 3B), levels of benzoic acid were obtained at 710.695 mg/kg (sample 3A) and 694.107 mg/kg (sample 3B). This confirms that the tested mayonnaise samples allow standard specifications of BPOM RI. Based on the comparison of benzoic acid levels in carbonated beverages, jelly drinks, and mayonnaise, it can be concluded that these food products are verified to comply with food safety standards in Indonesia.

Table 1. The comparison of benzoic acid in samples and the permittable limit

Food products	Sample	Area	Maximum permittable limit of benzoic acid (mg/kg)	Benzoic acid levels in the sample (mg/kg)
Carbonated beverages	1A	480358	400	140.973
	1B	473113		139.758
Jelly drinks	2A	840322	600	232.294
	2B	812126		230.877
Mayonnaise	3A	2611291	1000	710.695
	3B	2549154		694.107

Conclusion

Based on the experimental results in this study, the optimization of mobile phase composition and pH was carried out at a ratio methanol:phosphate buffer of 4:96 and an optimum pH of 6.8. A linear regression $R^2 \geq 0{,}9995$ was obtained, indicating that the reversed-phase HPLC analysis method is valid for quantitatively testing benzoic acid levels in food product samples. Qualitatively, the sharp chromatogram peaks, which are not adjacent to other peaks, indicate that this method is selective and sensitive to benzoic acid without interference from other matrices. Testing was conducted on test samples that had been prepared according to the sample type. The results showed that the food products, in the form of carbonated drinks (samples 1A and 1B) contained 140.973 mg/kg and 139.758 mg/kg of benzoic acid; jelly drinks (samples 2A and 2B) contained 232.294 mg/kg and 230.877 mg/kg of benzoic acid; and mayonnaise (samples 3A and 3B) contained 710.695 mg/kg and 694.107 mg/kg of benzoic acid. All samples of these food products were below the threshold set by BPOM RI, specifically 400 mg/kg, 600 mg/kg, and 1000 mg/kg for carbonated beverages, jelly drinks, and mayonnaise, respectively. In other words, all food product samples tested were consumable, safe, and in accordance with the United Nations' goal, that is SDG 3 concerning food safety for a healthy life and improving well-being for all people at all ages.

Acknowledgments

The authors sincerely acknowledge the contributions of all individuals and institutions who supported this research, particularly the National Center for Drug and Food Testing Development (PPPOMN) for providing facilities and instrumentation used in this study.

Reference

Abiman, P., Crossley, A., Wildgoose, G. G., Jones, J. H., & Compton, R. G. (2007). Investigating the Thermodynamic Causes Behind the Anomalously Large Shifts in pK_a Values of Benzoic Acid-Modified Graphite and Glassy Carbon Surfaces. *Langmuir*, *23*(14), 7847–7852. https://doi.org/10.1021/la7005277

Akbar, W. H., Pulungan, A. F., & Daulay, A. S. (2023). Analysis of Sodium Benzoate Content in The Market Soft Beverages Using Uv-Vis Spectrophotometry Method. *Indonesian Journal of Science and Pharmacy*, (1), 25–30. https://doi.org/10.63763/ijsp.v1i1.15

Alabyadh, M. (2023). HPLC Method for the Determination of Benzoic acid in samples of commercial children's food in the Markets of Aden -Yemen. *Journal of Science and Technology*, *28*(2), 23–28. https://doi.org/10.20428/jst.v28i2.2138

Astuti, E. J., Ilham, R. F. N., & Rahman, J. (2019). Validation method for determining sodium benzoate in fruit juice drinks in Malang. *Farmasains : Jurnal Farmasi Dan Ilmu Kesehatan*, *4*(1), 19. https://doi.org/10.22219/farmasains.v4i1.6622

BPOM RI. (2019). *Badan Pengawas Obat dan Makanan Republik Indonesia*.

Carda-Broch, S., García-Alvarez-Coque, M. C., & Ruiz-Angel, M. J. (2018). Extent of the influence of phosphate buffer and ionic liquids on the reduction of the silanol effect in a C18 stationary phase. *Journal of Chromatography A*, *1559*, 112–117. https://doi.org/10.1016/j.chroma.2017.05.061

Chandimali, N., Bak, S. G., Park, E. H., Lim, H.-J., Won, Y.-S., Kim, E.-K., Park, S.-I., & Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*, *11*(1), 19. https://doi.org/10.1038/s41420-024-02278-8

David, V., & Moldoveanu, S. C. (2024). Retention factor variation on wide range of mobile phase compositions in reversed-phase high-performance liquid chromatography; a short tutorial. *Journal of Chromatography Open*, *6*, 100176. https://doi.org/10.1016/j.jcoa.2024.100176

Elferjani, H. S., Almattardi, F. A., & Ansir, N. H. (2024). *Analysis of Benzoic Acid in Fruit Juice and Some Soft Drinks in Benghazi Market by UV-Spectrophotometric Method*. https://doi.org/10.37591/JoMCCT

Elmanfe, G. M. (2020). Spectrophotometric Determination Of Sodium Benzoate in Some Soft Drinks Collected from Some Local Markets in El-Bieda City – Libya. *EPH - International Journal of Applied Science*, *6*(2), 22–27. https://doi.org/10.53555/eijas.v6i2.99

Guo, X. Y., Mao, Y. J., Yu, C. H., Qiu, F. L., Pei, L. Z., Ling, X. Z., Zhang, Y., Wang, M. C., & Fan, C. G. (2020). Polythiopene/copper bismuthate nanosheet nanocomposites modified glassy carbon electrode for electrochemical detection of benzoic acid. *International Journal of Electrochemical Science*, *15*, 10463–10475. https://doi.org/10.20964/2020.10.29

Heering, A., Lahe, M., Vilbaste, M., Saame, J., Samin, J. P., & Leito, I. (2024). Improved pH measurement of mobile phases in reversed-phase liquid chromatography. *The Analyst*, *149*(5), 1481–1488. https://doi.org/10.1039/D3AN02029K

Hettiarachchi, R. I., Senadheera, S., Wijelath, W. A. G. E., & Thilakarathana, H. M. G. C. (2021). Total fructose, total glucose and benzoic acid contents in commercially available fruit nectars in Anuradhapura town area, Sri Lanka. *Journal of Food Safety and Hygiene*, *7*(4), 226–236. https://doi.org/10.18502/jfsh.v7i4.9286

Huang, M., Wei, R., Wang, Y., Su, T., Li, P., & Chen, X. (2018). The uremic toxin hippurate promotes endothelial dysfunction via the activation of Drp1-mediated mitochondrial fission. *Redox Biology*, *16*, 303–313. https://doi.org/10.1016/j.redox.2018.03.010

Huffman, B. A., Poltash, M. L., & Hughey, C. A. (2012). Effect of Polar Protic and Polar Aprotic Solvents on Negative-Ion Electrospray Ionization and Chromatographic Separation of Small Acidic Molecules. *Analytical Chemistry*, *84*(22), 9942–9950. https://doi.org/10.1021/ac302397b

Ismail, B. P. (2024). Basic Principles of Chromatography. In *Nielsen's Food Analysis* (pp. 167–192). Springer, Cham. https://doi.org/10.1007/978-3-031-50643-7_12

Kalisz, O., Catani, M., & Bocian, S. (2025). Greener and Whiter Analytical Procedure for Theobromine and Caffeine Determination in Tea Using Dimethyl Carbonate as an Extraction

Solvent and Mobile Phase Constituent in Reversed-Phase Liquid Chromatography. *ACS Omega*, *10*(12), 12432–12440. https://doi.org/10.1021/acsomega.4c11625

Kazmouz, M. Y., Rédei, C., & Felinger, A. (2021). The adsorption of methanol on reversed phase stationary phases in supercritical fluid chromatography. *Journal of Chromatography A*, *1653*, 462386. https://doi.org/10.1016/j.chroma.2021.462386

Khodaei, F., Kholghipour, H., Hosseinzadeh, M., & Rashedinia, M. (2019). Effect of sodium benzoate on liver and kidney lipid peroxidation and antioxidant enzymes in mice. *Journal of Reports in Pharmaceutical Sciences*, *8*(2), 217. https://doi.org/10.4103/jrptps.JRPTPS_68_18

Leesuraplanon, C., Jayasena, V., & Karnpanit, W. (2022). Risk assessment of exposure to benzoic acid and benzene from consumption of functional drinks. *International Journal of Food Science & Technology*, *57*(10), 6805–6812. https://doi.org/10.1111/ijfs.16029

Liv, L. (2021). Measurement uncertainty evaluation for titrimetric determination of benzoic acid purity. *Journal of Chemical Metrology*, *15*(1), 38–51. https://doi.org/10.25135/jcm.54.20.11.1881

Mishra, S. P. (2021). Titrimetric Study of Solubility of Solute Benzoic Acid and Their Partition in Water and Benzene Solvents. *Chemical Science International Journal*, 40–45. https://doi.org/10.9734/csji/2021/v30i330223

Mustafa, Y. F. (2024). Harmful Free Radicals in Aging: A Narrative Review of Their Detrimental Effects on Health. *Indian Journal of Clinical Biochemistry*, *39*(2), 154–167. https://doi.org/10.1007/s12291-023-01147-y

Niu, Y., Fan, Y., & Zhang, J. (2025). Progress and Challenge in the Risk Management of Food Additives. *Journal of Food Protection*, *88*(10), 100607. https://doi.org/10.1016/j.jfp.2025.100607

Obradović, D., Kowalska, T., & Agbaba, D. (2022). Mixed-Mode Hydrophilic Interactions/Reversed-Phase Retention Mechanism in Thin-Layer Chromatography. *Journal of Chromatographic Science*, *60*(4), 372–386. https://doi.org/10.1093/chromsci/bmab068

Ogbadu, L. J. (2014). PRESERVATIVES | Permitted Preservatives – Benzoic Acid. In *Encyclopedia of Food Microbiology* (pp. 76–81). Elsevier. https://doi.org/10.1016/B978-0-12-384730-0.00265-2

Pardhi, V., Pant, G., & Flora, S. J. S. (2020). RP-HPLC method development and validation for bedaquiline fumarate to evaluate its forced degradation behaviour and stability in official dissolution media. *Future Journal of Pharmaceutical Sciences*, *6*(1), 42. https://doi.org/10.1186/s43094-020-00061-x

Peiró-Vila, P., Torres-Lapasió, J. R., & García-Alvarez-Coque, M. C. (2024). Global retention models in reversed-phase liquid chromatography. A tutorial. *Journal of Chromatography Open*, *6*, 100192. https://doi.org/10.1016/j.jcoa.2024.100192

Poole, C. F., & Lenca, N. (2017). Reversed-phase liquid chromatography. In *Liquid Chromatography* (2nd ed., pp. 91–123). Elsevier. https://doi.org/10.1016/B978-0-12-805393-5.00004-X

Preikša, J., Petrikaitė, V., Petrauskas, V., & Matulis, D. (2023). Intrinsic Solubility of Ionizable Compounds from pK_a Shift. *ACS Omega*, *8*(47), 44571–44577. https://doi.org/10.1021/acsomega.3c04071

Salamah, M., Sipos, B., Katona, G., Volk, B., Balogh, G. T., & Csóka, I. (2025). Development and validation of a novel isocratic RP-HPLC method using AQbD approach for the quantification of favipiravir. *European Journal of Pharmaceutical Sciences*, *214*, 107276. https://doi.org/10.1016/j.ejps.2025.107276

Santikasari, C., Wulandari, M., Nafisa Ulfa, S., Siregar, M., & Sulaiman, S. A. S. (2025). Identification and Assay of Isoniazid and Pyrazinamide in Fixed-Dose Dispersible Tablets Containing Rifampicin Using High-Performance Liquid Chromatography. *JRSKT - Jurnal Riset Sains Dan Kimia Terapan*, *11*(2), 31–40. https://doi.org/10.21009/JRSKT.112.04

Savin, M., Vrkatić, A., Dedić, D., Vlaški, T., Vorgučin, I., Bjelanović, J., & Jevtic, M. (2022). Additives in Children's Nutrition—A Review of Current Events. *International Journal of Environmental Research and Public Health*, *19*(20), 13452. https://doi.org/10.3390/ijerph192013452

Shelar, O. A., Kurangi, B. K., Sonawane, S. N., Jalalpure, A. A., Palled, M. S., & Jalalpure, S. S. (2024). Development of simple stability indicating RP-HPLC method to estimate ferulic acid in niosomes and marketed products using quality by design approach. *Journal of the Indian Chemical Society*, *101*(10), 101251. https://doi.org/10.1016/j.jics.2024.101251

Sirhan, A. Y. (2018). Optimization and validation of an HPLC-UV method for determination of benzoic acid and sorbic acid in yogurt and dried-yogurt products using a design of experiment. *Indonesian Journal of Chemistry*, *18*(3), 522–530. https://doi.org/10.22146/ijc.27675

Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2009). *Introduction to Modern Liquid Chromatography*. Wiley. https://doi.org/10.1002/9780470508183

Spirić, D., Silađi, Č., & Stefanović, S. (2025). Comparation of Validation Results of HPLC-UV/PDA and LC-MS/MS Methods for the Determination of Sorbates and Benzoates in Food. *Meat Technology*, *66*(3), 104–110. https://doi.org/10.18485/meattech.2025.66.3.16

Sri K, B., S, S., Banu, S., & Mogilisumakanth. (2023). Quantification Of Preservatives In Processed Food Products By Titrimetric Method. *Innovare Journal Health Sciences*, *11*, 36–38. https://doi.org/10.22159/ijhs.2023.v11i1.48322

Steinhoff, A., Höltzel, A., & Tallarek, U. (2025a). Mobile-Phase Contributions to Analyte Retention and Selectivity in Reversed-Phase Liquid Chromatography: 1. General Effects. *The Journal of Physical Chemistry B*, *129*(25), 6385–6400. https://doi.org/10.1021/acs.jpcb.5c01695

Steinhoff, A., Höltzel, A., & Tallarek, U. (2025b). Mobile-Phase Contributions to Analyte Retention and Selectivity in Reversed-Phase Liquid Chromatography: 2. Solute-Specific Effects. *The Journal of Physical Chemistry B*, *129*(25), 6401–6418. https://doi.org/10.1021/acs.jpcb.5c01697

Sun, B., Wang, X., Liu, X., Wang, L., Ren, F., Wang, X., & Leng, X. (2020). Hippuric Acid Promotes Renal Fibrosis by Disrupting Redox Homeostasis via Facilitation of NRF2–KEAP1–CUL3 Interactions in Chronic Kidney Disease. *Antioxidants*, *9*(9), 783. https://doi.org/10.3390/antiox9090783

Tesoro, C., Ciriello, R., Lelario, F., Di Capua, A., Pascale, R., Bianco, G., Dell'Agli, M., Piazza, S., Guerrieri, A., Scrano, L., Bufo, S. A., & Acquavia, M. A. (2022). Development and Validation of a Reversed-Phase HPLC Method with UV Detection for the Determination of L-Dopa in Vicia faba L. Broad Beans. *Molecules*, *27*(21), 7468. https://doi.org/10.3390/molecules27217468

Tungkijanansin, N., Alahmad, W., Nhujak, T., & Varanusupakul, P. (2020). Simultaneous determination of benzoic acid, sorbic acid, and propionic acid in fermented food by headspace solid-phase microextraction followed by GC-FID. *Food Chemistry*, *329*. https://doi.org/10.1016/j.foodchem.2020.127161

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., & Chandra Bhattacharjee, S. (2025). Multi-matrix HPLC investigation of preservatives employing a recent 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

Wang, J., Ma, Y., Tang, L., Li, D., Xie, J., Sun, Y., & Tian, Y. (2024). Long-Term Exposure to Low Concentrations of Ambient Benzene and Mortality in a National English Cohort. *American Journal of Respiratory and Critical Care Medicine*, *209*(8), 987–994. https://doi.org/10.1164/rccm.202308-1440OC

Wulandari, M., Zahratussaadah, Z., Andreas, A., Adriany, R., Nofrizal, N., Saaid, M., & Urraca, J. (2024). Separation Technique of Tannins and Caffeine in Black Tea Using Modified Microwave-Assisted Extraction and High-Performance Liquid Chromatography. *International Journal of Technology*, *15*(6), 2024. https://doi.org/10.14716/ijtech.v15i6.7116

Yassien, E. E., Mohamed, A. M. S., Mahmoud, M. E., & Zaki, A. M. (2022). Sodium benzoate induced toxicities in albino male rats: mitigating effects of Ficus carica and Cymbopogon citratus leave extract. *Environmental Science and Pollution Research*, *29*(60), 90567–90579. https://doi.org/10.1007/s11356-022-22020-0

Yepes-Calderón, M., Doorenbos, C. S. E., Corpeleijn, E., Franssen, C. F. M., Vos, M. J., Touw, D. J., Mariat, C., de Weerd, A. E., & Bakker, S. J. L. (2025). Vitamin C and Benzoic Acid Intake in

Patients with Kidney Disease: Is There Risk of Benzene Exposure? *Nutrients*, *18*(1), 132. https://doi.org/10.3390/nu18010132

Zarad, S. I., Nimkar, N. R., Desai, K. R., Solanki, M. S., Gandhi, D. M., Gandhi, H. M., Patel, M. S., Khimani, A. A., & Suraliwala, M. N. (2017). Analytical Validation and Comparative Study of HPLC, Spectrophotometric and Titration Method for Determination of Benzoic Acid in Food Products. *Asian Journal of Chemistry*, *29*(7), 1565–1568. https://doi.org/10.14233/ajchem.2017.20576

Žuvela, P., Skoczylas, M., Jay Liu, J., Bączek, T., Kaliszan, R., Wong, M. W., & Buszewski, B. (2019). Column Characterization and Selection Systems in Reversed-Phase High-Performance Liquid Chromatography. *Chemical Reviews*, *119*(6), 3674–3729. https://doi.org/10.1021/acs.chemrev.8b00246