Indonesian Journal of Pharmaceutical and Clinical Research
Journal homepage: https://idjpcr.usu.ac.id

OPTIMIZED HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF ARTIFICIAL SWEETENERS, PRESERVATIVES, AND CAFFEINE IN CARBONATED SOFT DRINKS

Sri Wardono[1], Effendi De Lux Putra[2], Siti Morin Sinaga[2], Aminah Dalimunthe[3], Nasri Nasri[2]

[1]*Postgraduate Programe, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[3]*Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia*

*Corresponding Author: wardonosri@gmail.com

ARTICLE INFO

Article history:
Received 26 May 2025
Revised 12 June 2025
Accepted 12 June 2025
Available online 30 June 2025

E-ISSN: 2620-3731
P-ISSN: 2615-6199

How to cite:

Wardono, S., Putra, E. D. L., Sinaga, S. M., Dalimunthe, A., & Nasri, N. (2025). Optimized HPLC method for simultaneous determination of artificial sweeteners, preservatives, and caffeine in carbonated soft drinks. *Indonesian Journal of Pharmaceutical and Clinical Research*, **08**(01), 031–039.

http://doi.org/10.32734/idjpcr.v8i01.20975

ABSTRACT

Sweeteners, preservatives, and caffeine in soft drinks can pose health risks if consumed excessively. This study aimed to develop a method for the simultaneous determination of acesulfame, saccharin, cyclamate, aspartame, caffeine, benzoate, and sorbate in carbonated soft drinks. The analysis was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC) with a dual-wavelength PDA detector (Shimadzu LC-20AD), a C18 column (Shimadzu), and a UV-Vis spectrophotometer (Shimadzu). Standard materials included acesulfame-K, saccharin-Na, cyclamate-Na, aspartame, caffeine, benzoate-Na, and sorbic acid. Samples were obtained from a supermarket in Medan, Indonesia. Optimization parameters included detection wavelength, mobile phase pH, column oven temperature, and mobile phase composition. Validation parameters assessed were linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and selectivity. The optimized method employed a mobile phase of phosphate buffer (pH 3.8) and methanol (80:20, v/v) at a flow rate of 0.55 mL/min and column oven temperature of 40 °C. Detection was carried out at 200 nm for cyclamate, caffeine, aspartame, and benzoate, and at 220 nm for acesulfame, saccharin, and sorbate. Validation results showed the method met all requirements, with recovery rates ranging from 95.21% to 99.82%, system suitability values of 1.0%–1.9%, and precision values between 1.65% and 2.48%. The method also demonstrated good selectivity. The concentrations of acesulfame, saccharin, cyclamate, caffeine, benzoate, and sorbate in the analyzed samples did not exceed the maximum permissible limits.
Keyword: Additional food, High Performance Liquid Chromatography, Optimation, Validation

ABSTRAK

Pemanis, pengawet, dan kafein dalam minuman ringan dapat menimbulkan risiko kesehatan jika dikonsumsi secara berlebihan. Penelitian ini bertujuan untuk mengembangkan metode penetapan kadar asesulfam, sakarin, siklamat, aspartam, kafein, benzoat, dan sorbat secara simultan dalam minuman ringan berkarbonasi. Analisis dilakukan menggunakan kromatografi cair kinerja tinggi fase terbalik (RP-KCKT) dengan detektor PDA dua panjang gelombang (Shimadzu LC-20AD), kolom C18 (Shimadzu), dan spektrofotometer UV-Vis (Shimadzu). Bahan baku standar yang digunakan meliputi asesulfam-K, sakarin-Na, siklamat-Na, aspartam, kafein, benzoat-Na, dan asam sorbat. Sampel diperoleh dari salah satu swalayan di Kota Medan. Parameter optimasi meliputi panjang gelombang deteksi, pH fase gerak, suhu oven kolom, dan komposisi fase gerak. Parameter validasi mencakup linieritas, batas deteksi (LOD), batas kuantitasi (LOQ), akurasi, presisi, dan selektivitas. Metode yang dioptimasi menggunakan fase gerak berupa larutan dapar fosfat (pH 3,8) dan metanol dengan perbandingan 80:20 (v/v), laju alir 0,55 mL/menit, serta suhu oven kolom 40 °C. Deteksi dilakukan pada panjang gelombang 200 nm untuk analisis siklamat, kafein, aspartam, dan benzoat, serta 220 nm untuk asesulfam, sakarin, dan sorbat. Hasil validasi menunjukkan bahwa metode ini memenuhi semua kriteria yang disyaratkan, dengan nilai perolehan kembali (recovery) antara 95,21% hingga 99,82%, kesesuaian sistem sebesar 1,0%–1,9%, dan nilai presisi antara 1,65% hingga 2,48%. Metode ini juga menunjukkan selektivitas yang baik. Kadar asesulfam, sakarin, siklamat, kafein, benzoat, dan sorbat dalam sampel yang dianalisis tidak melebihi batas maksimum yang diizinkan.
Kata kunci: Bahan tambahan pangan, Kromatografi Cair Kinerja Tinggi, Optimasi, Validasi

1. INTRODUCTION

Soft drinks are among the most widely consumed beverages across all age groups, from children to adults. Their practicality, refreshing taste, and wide availability have made them a staple in modern lifestyles. As consumption continues to rise, growing attention is being paid to the composition of food additives used in these beverages[1].

Carbonated soft drinks commonly contain various food additives such as artificial sweeteners, preservatives, and caffeine. Commonly used sweeteners include saccharin, cyclamate, aspartame, and acesulfame-K, while sodium benzoate and sorbic acid are among the typical preservatives[2]. Caffeine is often added as a stimulant to enhance the refreshing effect. Although the use of these additives is permitted under regulations issued by the Indonesian Food and Drug Author, their levels must remain within the established safety limits. For example, the maximum permitted caffeine content in soft drinks is 50 mg per package.

Excessive and prolonged intake of such additives may pose health risks, including allergic reactions, organ damage, and, in some cases, potential carcinogenic effects. Therefore, robust and reliable analytical methods are essential to ensure that the levels of these substances in consumer products remain safe[3].

High-performance liquid chromatography (HPLC) is widely employed for the analysis of food additives due to its high sensitivity and selectivity. However, the simultaneous quantification of multiple additives within a single analytical system presents challenges, particularly when the analytes exhibit differing physicochemical properties such as polarity, pKa, and maximum absorbance wavelengths. To address this complexity, comprehensive optimization of analytical conditions—such as mobile phase pH and composition, column temperature, and detection wavelength—is necessary[4], [5].

This study aims to develop and validate an HPLC method using dual-wavelength PDA detection for the simultaneous determination of acesulfame-K, saccharin-Na, cyclamate-Na, aspartame, caffeine, sodium benzoate, and sorbic acid in carbonated soft drinks. By incorporating a multi-parametric optimization and full validation approach, the proposed method is expected to provide a valuable analytical tool for quality control and food safety monitoring, particularly in widely consumed beverages[6].

Moreover, while several previous studies have focused on the determination of individual or limited groups of additives in beverages, comprehensive methods that allow for the simultaneous analysis of multiple sweeteners, preservatives, and caffeine under a single optimized system remain relatively scarce[7]. Many reported methods lack integrated optimization of critical parameters such as dual-wavelength detection, pH-specific mobile phases, and temperature-controlled elution, which are essential for achieving reliable separation and quantification of structurally diverse analytes. This study addresses these limitations by establishing a robust analytical protocol that not only ensures high sensitivity and selectivity but also complies with standard validation criteria[8]. The proposed method contributes to the advancement of analytical techniques in food safety, offering an effective tool for routine surveillance and regulatory compliance in the beverage industry[9], [10].

2. METHODS

2.1 Materials

HPLC-grade methanol (E. Merck), sodium dihydrogen phosphate monohydrate (E. Merck), orthophosphoric acid (E. Merck), standard reference materials including acesulfame-K, saccharin-Na, cyclamate-Na, aspartame, caffeine, sodium benzoate, sorbic acid, and carbonated soft drink samples.

2.2 Instruments

High-performance liquid chromatography (HPLC) system with PDA detector (Shimadzu LC-20AD), VP-ODS column 150 mm × 2.0 mm (Shimadzu), UV-Vis spectrophotometer (Shimadzu), pH meter (ATC), ultrasonic bath (Bransonic), and analytical balance (Boeco).

2.3 Analytical Procedures

2.3.1 Determination of Detection Wavelengths

This step aimed to determine the optimal detection wavelengths for the HPLC detector. Absorbance measurements were performed on a 200 ppm cyclamate solution and 10 ppm solutions of acesulfame, saccharin, aspartame, caffeine, benzoate, and sorbate. Each solution was scanned from 190–250 nm using a UV-Vis spectrophotometer. The resulting spectra were analyzed to determine the two optimal detection wavelengths for simultaneous analysis[3].

2.3.2 Optimization of Mobile Phase pH

This step aimed to determine the optimal phosphate buffer pH that provides the best separation. The testing conditions included: oven temperature of 30 °C, mobile phase composition of phosphate buffer: methanol (80:20, v/v), flow rate of 0.55 mL/min, and injection of 20 µL of a mixed standard solution (LBC) using two optimized detection wavelengths. LBC consisted of a mixture of 10 ppm each of acesulfame, saccharin, cyclamate, aspartame, caffeine, benzoate, sorbate, and 200 ppm cyclamate. The buffer pH values tested were 3.0, 3.5, 3.8, and 4.0[2], [5].

2.3.3 Optimization of Column Oven Temperature

This step aimed to determine the optimal oven temperature for best separation performance. Conditions were: phosphate buffer: methanol (80:20, v/v) as mobile phase, flow rate 0.55 mL/min, 20 µL LBC injection, using the optimized buffer pH and detection wavelengths. Temperatures tested were 30 °C, 35 °C, and 40 °C[11].

2.3.4 Optimization of Mobile Phase Composition

This step determined the optimal mobile phase composition for maximum separation efficiency. LBC (20 µL) was injected using the previously optimized parameters (pH, oven temperature, and detection wavelengths). The mobile phase ratios tested were phosphate buffer: methanol at 75:25, 80:20, and 85:15 (v/v)[5].

2.4 Method Validation

2.4.1 Linearity

Calibration curves were constructed using mixed standard solutions of acesulfame, saccharin, aspartame, caffeine, benzoate, and sorbate at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ppm, and cyclamate at concentrations of 10, 20, 40, 60, 80, and 100 ppm. Each solution (10 µL) was injected into the HPLC under optimized conditions[12].

2.4.2 Accuracy

Accuracy was assessed using the placebo method. Recovery tests were conducted at three concentration levels: 80%, 100%, and 120% of the expected value. Each solution (10 µL) was analyzed using the optimized HPLC conditions[13].

2.4.3 Precision

Precision was evaluated through system suitability testing and repeatability assessment. System suitability was determined by injecting the same mixed standard solution six times. Repeatability was assessed by analyzing six independently prepared samples of a homogeneous matrix under the same conditions[14].

2.4.4 Selectivity

Selectivity was examined by comparing chromatograms of the standard solution plus placebo and the placebo solution alone, using the optimized HPLC method. The absence of interfering peaks at the analyte retention times confirmed the method's selectivity[15].

2.4.5 Sample Analysis

Analysis was conducted on four commercial brands of carbonated soft drinks. A 50 mL sample was sonicated for 30 minutes, and 5 mL of the solution was diluted to 50 mL with distilled water. A 2 mL aliquot of this dilution was further diluted to 10 mL. The resulting solution was filtered using a 0.2 µm PTFE syringe filter into an autosampler vial, and 10 µL was injected into the HPLC system under the optimized analytical conditions[3], [5].

3. RESULT AND DISCUSSION

3.1 Optimization Stage

3.1.1 Detection Wavelength Optimization

To achieve optimal sensitivity and selectivity in the simultaneous detection of multiple analytes, the selection of appropriate detection wavelengths is critical. Each compound exhibits a distinct absorbance profile in the UV spectrum based on its molecular structure, functional groups, and electronic transitions. Therefore,

UV absorbance spectra were recorded for each analyte to determine the most suitable wavelengths for HPLC detection using a photodiode array (PDA) detector[3].

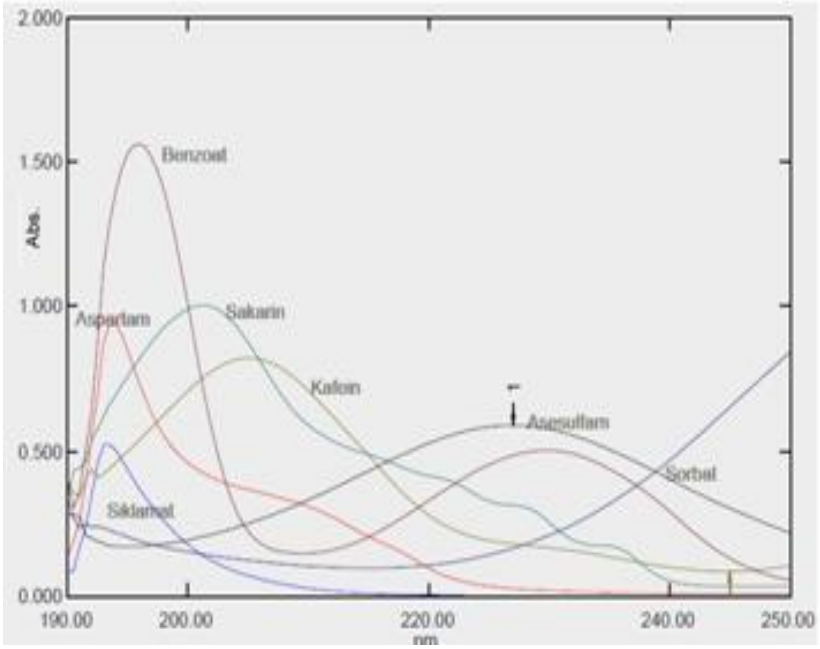


Figure 1. UV Absorption Spectra of All Analytes

Figure 1 shows the UV absorbance spectra of the seven target analytes. Cyclamate demonstrated a sharp absorbance peak in the 195–200 nm range, indicating that this compound is optimally detected at lower wavelengths. In contrast, acesulfame, saccharin, caffeine, aspartame, benzoate, and sorbate exhibited higher absorbance at longer wavelengths, extending beyond 200 nm. These differences reflect the electronic characteristics of each molecule and highlight the necessity of a dual-wavelength detection strategy[16].

Based on the spectral profiles, two detection wavelengths were selected to cover the maximum absorbance ranges of the compounds: 200 nm for cyclamate, caffeine, aspartame, and benzoate, and 220 nm for acesulfame and sorbate. This dual-wavelength setup was implemented using a PDA UV-Vis detector to ensure comprehensive and sensitive detection of all analytes in a single chromatographic run[6].

This approach is in line with previously reported methods that also employed wavelength optimization to enhance method performance, particularly in multicomponent food additive analysis. The use of multiple detection wavelengths improves resolution and peak intensity, ultimately supporting accurate quantification in complex matrices such as soft drinks[2], [3].

3.1.2 Mobile Phase pH Optimization

The pH of the phosphate buffer plays a crucial role in the chromatographic separation of analytes, particularly those with acidic or basic functional groups. Modifying the pH of the mobile phase alters the degree of ionization of each analyte, which subsequently affects their polarity, retention time, and selectivity. An ideal buffer should provide adequate separation between peaks (resolution), reasonable retention times, and efficient analysis duration[17].

Table 1 summarizes the effect of various phosphate buffer pH levels (3.0, 3.5, 3.8, and 4.0) on the retention time (RT) and resolution (Rs) of each analyte. At pH 3.0, most analytes were sufficiently separated except for benzoic acid and sorbic acid, which exhibited overlapping peaks. This result is consistent with the fact that both benzoate and sorbate remain predominantly non-ionized at this acidic pH—far below their respective pKa values of 4.2 and 4.8—leading to strong interactions with the stationary phase and longer retention times.

Table 1. Effect of Phosphate Buffer pH on Retention Time (RT) and Resolution (Rs) of Analytes

No	Analyte	RT (pH 3.0)	Rs (pH 3.0)	RT (pH 3.5)	Rs (pH 3.5)	RT (pH 3.8)	Rs (pH 3.8)	RT (pH 4.0)	Rs (pH 4.0)
1	Acesulfame	1.623	–	1.419	–	1.372	–	1.370	–
2	Saccharin	2.049	3.286	2.018	3.491	1.956	3.563	1.950	3.525
3	Cyclamate	3.417	4.528	3.415	4.975	3.357	5.287	3.350	3.128
4	Caffeine	5.399	5.313	5.440	5.528	5.405	5.713	5.416	5.881
5	Aspartame	9.752	8.382	9.915	8.664	9.949	8.783	9.983	8.285
6	Benzoate	15.542	Overlapping	13.880	5.232	11.543	2.277	10.601	0.873
7	Sorbate	15.548	–	15.452	1.774	14.298	3.433	13.745	4.138

At pH values above 3.0, the separation of benzoate and sorbate improved significantly due to their partial ionization, which enhanced their solubility in the polar mobile phase and reduced their retention. Among the tested pH values, phosphate buffer at pH 3.8 provided the best resolution for all analytes, including critical pairs such as benzoate–sorbate and aspartame–benzoate, while also reducing overall analysis time[18].

Although pH 4.0 showed further shortening of retention times, the resolution between aspartame and benzoate dropped significantly (Rs = 0.873), falling below the acceptable threshold of Rs > 1.5 for baseline separation. Therefore, pH 3.8 was selected as the optimal mobile phase pH, offering a balanced compromise between peak resolution, analysis efficiency, and method robustness. These findings are in agreement with previous research, which emphasized the importance of buffer pH in enhancing analyte resolution in multi-component systems containing both weak acids and artificial sweeteners[5], [19].

3.1.3 Column Oven Temperature Optimization

Column temperature is a critical factor in liquid chromatography that affects the kinetics of analyte migration, the viscosity of the mobile phase, and the interaction between the analytes and the stationary phase. Generally, increasing the column temperature results in reduced viscosity and enhanced mass transfer, which leads to shorter retention times, improved resolution, and more efficient separation[14].

In this study, temperature optimization was conducted by evaluating chromatographic performance at three temperatures: 30°C, 35°C, and 40°C. The separation results showed a direct correlation between increasing temperature and decreasing retention time, while the resolution remained within acceptable limits. This indicates that higher temperatures enhanced elution speed without compromising selectivity[20].

Table 2 presents the retention time (RT) and resolution (Rs) of each analyte under different column temperatures, corresponding to the same mobile phase composition (phosphate buffer pH 3.8 : methanol 80:20, v/v).

Table 2. Effect of Column Temperature on Retention Time (RT) and Resolution (Rs) (Mobile Phase: Phosphate Buffer pH 3.8 : Methanol 80:20, v/v)

No	Analyte	RT (75:25)	Rs (75:25)	RT (80:20)	Rs (80:20)	RT (85:15)	Rs (85:15)
1	Acesulfame	1.125	0.000	1.235	0.000	1.444	0.000
2	Saccharin	1.403	1.957	1.676	2.943	2.212	4.771
3	Cyclamate	2.534	6.266	3.128	6.622	4.117	7.838
4	Caffeine	2.872	1.617	4.277	4.022	7.438	9.309
5	Aspartame	5.269	8.769	7.902	9.854	12.708	9.774
6	Benzoate	6.533	3.548	8.860	1.977	13.379	0.908
7	Sorbate	8.205	3.971	11.416	4.653	16.890	4.179

All analytes showed symmetrical and well-defined peaks across all temperature conditions, indicating their thermal stability and compatibility with the optimized system. While 30°C and 35°C also provided acceptable separation, 40°C demonstrated the shortest analysis time with adequate resolution and the lowest system backpressure due to reduced mobile phase viscosity[5].

Therefore, 40°C was selected as the optimal column oven temperature. It provided a balanced chromatographic environment with efficient elution, stable system pressure, and high-resolution separation. These findings are consistent with previous literature, which highlights the benefits of moderate temperature elevation in improving peak shape and run time in reversed-phase HPLC[11].

3.1.4 Mobile Phase Composition Optimization

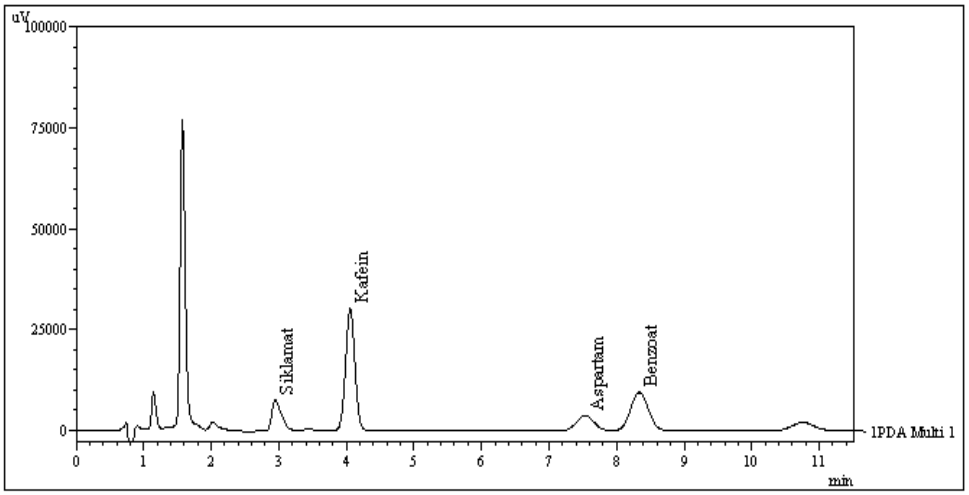
The composition of the mobile phase was optimized using phosphate buffer (pH 3.8) and methanol in three different ratios: 85:15, 80:20, and 75:25 (v/v). The results, as shown in Table 2, indicated that increasing the proportion of methanol significantly reduced the analyte retention time. This effect is attributed to the higher solubility of analytes in methanol compared to the aqueous phosphate buffer, which facilitates faster elution through reduced interaction with the stationary phase[17].

However, higher methanol content also resulted in a notable increase in system backpressure. Furthermore, at 85:15, the chromatograms showed increased baseline noise and interfering peaks, particularly at 200 nm, which could compromise analytical sensitivity and accuracy[14].

Among the tested compositions, the 80:20 phosphate buffer to methanol ratio was selected as optimal. This ratio provided a favorable balance between efficient separation, shorter analysis time, and manageable system pressure. It also minimized spectral interference in the low-wavelength UV region, particularly at the 200 nm detection setting. This composition was therefore considered the most suitable for subsequent analysis, as it ensured both analytical robustness and system stability during routine application[16].

3.1.5 Summary of Optimization Results

Based on the comprehensive optimization of key chromatographic parameters—including detection wavelength, mobile phase pH, column oven temperature, and solvent composition—the final HPLC conditions were established to achieve optimal separation efficiency and detection sensitivity for all target analytes. The optimized mobile phase consisted of phosphate buffer at pH 3.8 and methanol in an 80:20 (v/v) ratio, with a column oven temperature set at 40°C and a flow rate of 0.55 mL/min. To accommodate the varying UV absorbance properties of the compounds, dual-wavelength detection using a photodiode array (PDA) detector was employed. Detection at 200 nm was used for cyclamate, caffeine, aspartame, and benzoate, while 220 nm was selected for acesulfame, saccharin, and sorbate. As shown in the chromatograms (Figure 2 and Figure 3), all seven analytes were baseline-separated under these optimized conditions. Furthermore, the system suitability parameters—including retention time (RT), resolution (Rs), theoretical plate number (N), tailing factor (Tf), and capacity factor (k')—fulfilled the standard acceptance criteria for quantitative analytical methods, confirming the method's reliability and robustness for routine use[2].



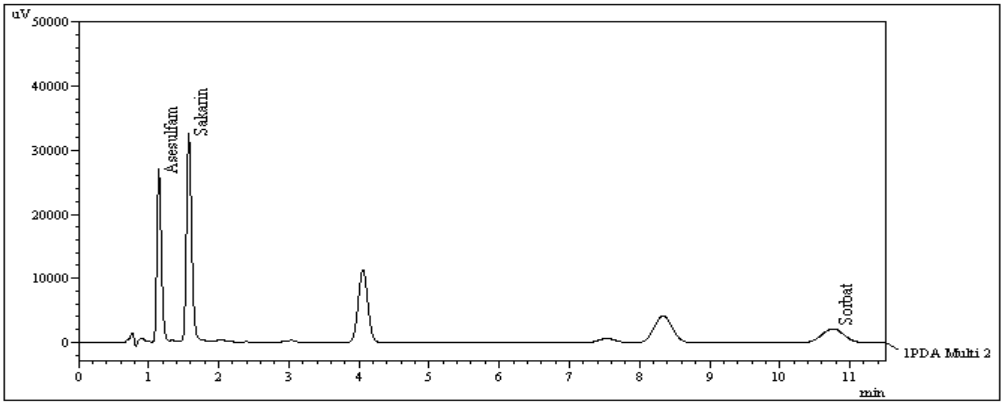
Peak Table

PDA Ch1 200nm 4nm

Name	Ret. Time	Area	Theoretical Plate#	HETP	Resolution	k'	Tailing Factor
Siklamat	2.940	73110	1685.698	88.984	0.000	0.000	1.680
Kafein	4.049	287317	3709.387	40.438	4.014	0.377	1.077
Aspartam	7.533	70553	3754.895	39.948	9.195	1.562	1.039
Benzoat	8.329	174100	4575.969	32.780	1.619	1.833	1.050
		605080					

Figure 2. Chromatogram of Analytes at 200 nm Using the Optimized Method

Cyclamate, caffeine, aspartame, and benzoate showed sharp and symmetrical peaks with retention times ranging from 2.94 to 8.33 minutes. The resolution values between adjacent peaks were all above 1.5, indicating efficient separation. Tailing factors were close to 1, demonstrating excellent peak symmetry.



Peak Table

PDA Ch2 220nm 4nm

Name	Ret. Time	Area	Theoretical Plate#	HETP	Resolution	k'	Tailing Factor
Asesulfam	1.145	118729	1238.045	121.159	0.000	0.000	1.395
Sakarin	1.571	167546	1745.235	85.948	3.036	0.372	1.312
Sorbat	10.752	49352	4938.260	30.375	24.082	8.388	1.036
		335627					

Figure 3. Chromatogram of Analytes at 220 nm Using the Optimized Method

Acesulfame, saccharin, and sorbate were well-resolved, with retention times between 1.14 and 10.75 minutes. High theoretical plate numbers (up to 4983) and resolution values greater than 3.0 further supported the adequacy of the method's selectivity and efficiency.
These chromatograms validate that the developed HPLC method is capable of simultaneously analyzing multiple sweeteners, preservatives, and caffeine in a single run with high resolution, good reproducibility, and robust system performance[3].

3.2 Method Validation

3.2.1 Linearity

All analytes exhibited excellent linearity across the tested concentration ranges, with correlation coefficients (r) greater than 0.99999, except for cyclamate, which showed a slightly lower but still acceptable value of 0.99930. These results indicate a strong correlation between analyte concentration and peak area, confirming the method's linear response. This finding aligns with prior studies, which reported reliable calibration curves for similar food additive analytes in beverage matrices[21].

Table 2. Regression Equations of the Analytes

No	Analyte	Regression Equation	Correlation Coefficient (r)
1	Acesulfame	y = 47.716x – 504	0.99999
2	Saccharin	y = 65.443x + 1.828	0.99999
3	Cyclamate	y = 818.38x + 813	0.99930
4	Caffeine	y = 112.151x – 1.270	0.99999
5	Aspartame	y = 36.798x + 43.73	0.99999
6	Benzoate	y = 133.130x – 1.676	0.99999
7	Sorbate	y = 32351x – 1500	0.99999

3.2.2 Limits of Detection and Quantification

The method demonstrated satisfactory sensitivity, with low values of limit of detection (LOD) and limit of quantification (LOQ) for all analytes. The LOD values were as follows: acesulfame (0.041 ppm), saccharin (0.024 ppm), cyclamate (1.630 ppm), aspartame (0.023 ppm), caffeine (0.017 ppm), benzoate (0.017 ppm), and sorbate (0.019 ppm). The corresponding LOQ values were: acesulfame (0.012 ppm), saccharin (0.081 ppm), cyclamate (5.435 ppm), aspartame (0.075 ppm), caffeine (0.058 ppm), benzoate (0.055 ppm), and sorbate (0.064 ppm). These values meet international analytical requirements for trace detection in food products[5].

3.2.3 Accuracy

Accuracy was evaluated through recovery studies using the placebo method at spiked concentrations. The recovery values ranged from 94.99% to 98.58%: acesulfame (98.22%), saccharin (97.98%), cyclamate (98.58%), caffeine (95.11%), aspartame (94.99%), benzoate (96.82%), and sorbate (98.32%). All results fell within the acceptable range of 90%–107% for analytes at 0.01% concentration levels, demonstrating the method's accuracy. These results are consisten, who applied similar recovery-based validation strategies[14].

3.2.4 Precision

Precision was determined by assessing intra-day repeatability through six replicate injections of homogeneous samples. The relative standard deviation (%RSD) values were within 1.65%–2.02%: acesulfame (1.76%), saccharin (1.84%), cyclamate (2.02%), caffeine (1.76%), aspartame (2.00%), benzoate (1.83%), and sorbate (1.65%). All values complied with the acceptance criterion of RSD < 3.9%, indicating high method repeatability. These results are in agreement with prior work, in similar chromatographic analyses[2].

3.2.5 Selectivity

The selectivity of the method was assessed by comparing chromatograms of standard mixtures with those of placebo and blank (distilled water) samples. The retention times of analytes in the standard plus placebo mixtures matched those of the pure standards, and no interfering peaks were detected in the placebo or blank chromatograms at the respective retention times. These findings confirm that the method is selective and capable of accurately quantifying target analytes in the presence of other matrix components commonly found in soft drinks[12].

3.3 Sample Analysis

Sample analysis was conducted using the finalized HPLC conditions, and each of the four commercial carbonated soft drink samples was analyzed in triplicate to ensure consistency. The results showed that Sample A contained acesulfame-K at 116.68 mg/kg, caffeine at 30.98 mg/serving, and benzoate at 141.45 mg/kg. Sample B was found to contain saccharin-Na (85.17 mg/kg), cyclamate (855 mg/kg), caffeine (42.38 mg/kg),

and benzoate (151.72 mg/kg). Sample C contained sorbate at 118.33 mg/kg, while Sample D contained caffeine (38.82 mg/kg) and benzoate (100.95 mg/kg). Notably, aspartame was not detected in any of the samples, which may suggest a preference by manufacturers for alternative sweeteners such as cyclamate or acesulfame-K, possibly due to their favorable sensory characteristics, stability, or cost-effectiveness. All detected analyte concentrations were within the permissible limits set by relevant food safety authorities, indicating compliance with regulatory standards. These findings confirm the suitability of the developed HPLC method for the accurate and reliable monitoring of multiple food additives in commercial soft drink products[5], [17].

4. CONCLUSION

A validated RP-HPLC method with dual-wavelength detection was successfully developed for the simultaneous analysis of sweeteners, preservatives, and caffeine in soft drinks. The method demonstrated good linearity, sensitivity, accuracy, precision, and selectivity. All analyte levels in tested samples were within regulatory limits, confirming the method's suitability for routine quality control in beverage analysis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] I. Trandafir, V. Nour, and E. Ionică, 'Development and validation of an HPLC method for simultaneous quantification of acesulfame-K, saccharin, aspartame, caffeine and benzoic acid in cola soft drinks', *Scientific Study & Research*, vol. 2, pp. 185–194, 2009.

[2] W. Setyaningsih, A. Rohman, and M. Palma Lovillo, 'Development and Validation of HPLC-DAD Method for Simultaneous Determination of Seven Food Additives and Ca eine in Powdered Drinks', 2020, Accessed: May 26, 2025. [Online]. Available: https://rodin.uca.es/handle/10498/23804

[3] R. Székelyhidi, Z. Ajtony, E. Lakatos, O. Hegyi, and B. Sik, 'Optimization and validation of HPLC–DAD method for simultaneous analysis of sweeteners, preservatives, and caffeine in sugar-free beverages', *Eur Food Res Technol*, vol. 249, no. 11, pp. 2797–2805, Nov. 2023, doi: 10.1007/s00217-023-04328-4.

[4] D. H. Etwaroo *et al.*, 'Level of food additives, caffeine and total sugars in locally manufactured beverages in Mauritius and their compliance with national and international norms', *British Food Journal*, vol. 121, no. 11, pp. 2849–2866, 2019.

[5] M. Shoeb, M. M. Islam, M. S. Reza, N. Nahar, and M. M. Islam, 'HPLC analysis of artificial preservatives, stimulants and sweeteners in carbonated beverages in Bangladesh', *Current Research on Biosciences and Biotechnology*, vol. 3, no. 2, pp. 215–221, 2022.

[6] X.-D. Sun *et al.*, 'Rapid and Sensitive Detection of Multi-Class Food Additives in Beverages for Quality Control by Using HPLC-DAD and Chemometrics Methods', *Food Anal. Methods*, vol. 12, no. 2, pp. 381–393, Feb. 2019, doi: 10.1007/s12161-018-1370-3.

[7] O. Kritsunankul and J. Jakmunee, 'Simultaneous determination of some food additives in soft drinks and other liquid foods by flow injection on-line dialysis coupled to high performance liquid chromatography', *Talanta*, vol. 84, no. 5, pp. 1342–1349, 2011.

[8] M. Grembecka, P. Baran, A. Błażewicz, Z. Fijałek, and P. Szefer, 'Simultaneous determination of aspartame, acesulfame-K, saccharin, citric acid and sodium benzoate in various food products using HPLC–CAD–UV/DAD', *Eur Food Res Technol*, vol. 238, no. 3, pp. 357–365, Mar. 2014, doi: 10.1007/s00217-013-2111-x.

[9] Q.-C. Chen and J. Wang, 'Simultaneous determination of artificial sweeteners, preservatives, caffeine, theobromine and theophylline in food and pharmaceutical preparations by ion chromatography', *Journal of chromatography A*, vol. 937, no. 1–2, pp. 57–64, 2001.

[10] P. Diviš, Z. Jurečková, M. Vespalcová, J. Pořízka, and L. Punčochářová, 'Simultaneous determination of sweeteners and preservatives in beverages by HPLC-DAD-ELSD.', *Slovak Journal of Food Sciences*, vol. 14, 2020, Accessed: May 26, 2025. [Online]. Available: https://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=13389971&AN=148295480&h=w0uVeSLPYqgwl7RjgiNsjwXsPTHOHdRLEwGi2Ryi5iOtc%2FjD3uoEZBHPa%2F%2FF57o6TaWctrSwQnDI2JqzE2SvzQ%3D%3D&crl=c

[11] A. P. Nambiar, M. Sanyal, and P. S. Shrivastav, 'Simultaneous densitometric determination of eight food colors and four sweeteners in candies, jellies, beverages and pharmaceuticals by normal-phase high performance thin-layer chromatography using a single elution protocol', *Journal of Chromatography A*, vol. 1572, pp. 152–161, 2018.

[12] R. C. S. De Sousa *et al.*, 'Optimization and Validation of an Analytical Method for the Determination of Sweeteners in Beverages by HPLC-ELSD', *Food Anal. Methods*, vol. 17, no. 2, pp. 207–225, Feb. 2024, doi: 10.1007/s12161-023-02562-w.

[13] M. Soyseven, B. Sezgin, and G. Arli, 'The development and validation of a novel, green, sustainable and eco-friendly HPLC-ELSD method approach for the simultaneous determination of seven artificial sweeteners in various food products: An assessment of the greenness profile of the developed method with an analytical eco-scale, NEMI, GAPI and AGREE', *Microchemical Journal*, vol. 193, p. 109225, 2023.

[14] M. S. Jankulovska, T. Josimovska, and L. Velkoska-Markovska, 'Development and validation of RP-HPLC method with UV-DAD detection for simultaneous determination of acesulfame K, sodium saccharin and aspartame in beverages', *Acta Chromatographica*, 2024, Accessed: May 26, 2025. [Online]. Available: https://akjournals.com/view/journals/1326/aop/article-10.1556-1326.2024.01239/article-10.1556-1326.2024.01239.xml

[15] L. Wu, C. Zhang, Y. Long, Q. Chen, W. Zhang, and G. Liu, 'Food additives: From functions to analytical methods', *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 30, pp. 8497–8517, Oct. 2022, doi: 10.1080/10408398.2021.1929823.

[16] M. Üstün Özgür and M. Kasapoğlu, 'Development and Validation of a Simple Ultra Fast Liquid Chromatographic Method for the Simultaneous Determination of Aspartame, Acesulfame-K, Caffeine and Sodium Benzoate in Dietic Soft Drinks', *J Anal Chem*, vol. 74, no. 6, pp. 555–564, Jun. 2019, doi: 10.1134/S1061934819060133.

[17] V. Oktavirina *et al.*, 'Analytical methods for determination of non-nutritive sweeteners in foodstuffs', *Molecules*, vol. 26, no. 11, p. 3135, 2021.

[18] N. Ünal and E. Dinç, 'A Novel UPLC Method Development for Quantifying Active and Inactive Ingredients in a Commercial Energy Drink Product Using Chemometric Optimization Methodology', *Food Anal. Methods*, Mar. 2025, doi: 10.1007/s12161-025-02782-2.

[19] S. Suprianto, 'Optimization of Mobile Phase for Simultaneous Determination of Sweeteners, Preservatives and Dyes by UFLC', 2023, Accessed: May 26, 2025. [Online]. Available: https://osf.io/preprints/kj3nt/

[20] J. Werner and D. Mysiak, '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*, vol. 29, no. 19, p. 4573, 2024.

[21] D. F. Oktaviani, S. M. Nursatya, F. Tristiani, A. N. Faozi, R. H. Saputra, and M. D. N. Meinita, 'Antibacterial Activity From Seaweeds Turbinaria ornata and Chaetomorpha antennina Against Fouling Bacteria', in *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, 2019, p. 012045.