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Development of Novel Analytical Approaches for Sensitive and Selective Detection of Biogenic Amines and Antioxidants

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ABSTRACT

Biogenic amines (BAs) and antioxidant compounds are critical indicators of food safety and nutritional quality; however, their simultaneous determination in complex matrices remains analytically challenging. This study reports the development and validation of a novel, sensitive, and selective high-performance liquid chromatography (HPLC) method for the simultaneous quantification of major biogenic amines (histamine, tyramine, putrescine, and cadaverine) and key antioxidants (ascorbic acid, gallic acid, and quercetin). The method employs pre-column derivatization using dansyl chloride to enhance the detectability of BAs, coupled with optimized gradient chromatographic conditions for efficient separation. The developed method exhibited excellent linearity ($R^2 > 0.998$), low limits of detection (0.01–0.05 µg/mL), and high precision (RSD < 3%). Accuracy was confirmed through recovery studies, yielding values between 92% and 105%. Application to real food samples demonstrated significant variation in analyte concentrations, with higher levels of BAs observed in fermented products and elevated antioxidant content in fruits. The results confirm that the proposed method is reliable, cost-effective, and suitable for routine analysis, providing a comprehensive tool for simultaneous assessment of food safety and nutritional quality.

Keywords: Biogenic amines, antioxidants, HPLC, derivatization, food safety, analytical method validation

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INTRODUCTION

Biogenic amines (BAs) are a group of low molecular weight, biologically active nitrogenous compounds that are primarily formed through the microbial decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. These compounds are naturally present in a wide variety of products, particularly in fermented, aged, or protein-rich commodities such as fish, meat, cheese, and certain vegetables (Wójcik et al., 2021). Among the most commonly encountered BAs are histamine, tyramine, putrescine, and cadaverine, each originating from specific amino acid precursors. While low concentrations of these amines are generally considered harmless and may even play physiological roles in cellular metabolism and neurotransmission, their excessive accumulation in food matrices poses significant risks to human health (Shashank et al., 2021).

The presence and concentration of BAs in food are influenced by multiple factors, including the quality of raw materials, microbial contamination, storage conditions, and processing techniques. Microorganisms possessing decarboxylase activity, such as certain species of Enterobacteriaceae, Lactobacillus, and Clostridium, are largely responsible for the formation of these amines. Improper handling, inadequate refrigeration, and extended storage periods can accelerate BA formation, leading to elevated levels that may exceed safety thresholds. Histamine poisoning, often associated with the consumption of spoiled fish, is one of the most well-documented foodborne intoxications linked to BAs (Sahu et al., 2015).

Symptoms such as headaches, flushing, hypotension or hypertension, nausea, and allergic-like reactions are commonly reported. Similarly, tyramine intake has been associated with hypertensive crises, particularly in individuals taking monoamine oxidase inhibitors (MAOIs). Therefore, monitoring BA levels is crucial for ensuring food safety and protecting public health (Doeun et al., 2017).

In parallel, antioxidants represent another important class of bioactive compounds widely distributed in food systems. These include vitamins such as ascorbic acid (vitamin C), phenolic acids like gallic acid, and flavonoids such as quercetin. Antioxidants play a fundamental role in neutralizing reactive oxygen species (ROS) and preventing oxidative damage to cellular components, including lipids, proteins, and nucleic acids (Erdag et al., 2018). Their presence in food not only contributes to nutritional value but also enhances shelf life by inhibiting oxidative degradation. The balance between pro-oxidants and antioxidants is critical in maintaining both food quality and human health, as oxidative stress has been implicated in the pathogenesis of various chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions (Tsafack and Tsopmo, 2022).

The simultaneous occurrence of biogenic amines and antioxidants in food matrices presents a complex analytical challenge. On one hand, elevated BA levels may indicate microbial spoilage and compromised food safety; on the other hand, antioxidant content reflects nutritional quality and functional benefits. Understanding the interplay between these two groups of compounds is therefore essential for comprehensive food quality assessment. However, their simultaneous determination is complicated by differences in chemical structure, polarity, and concentration ranges, as well as interference from complex matrices (Omer et al., 2021).

Over the years, several analytical techniques have been developed for the

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determination of BAs, including gas chromatography (GC), capillary electrophoresis (CE), and spectrophotometric methods. While GC offers high resolution and sensitivity, it typically requires derivatization steps to improve volatility and detectability, making the procedure time-consuming and technically demanding. Capillary electrophoresis provides rapid separation with minimal solvent consumption, but its sensitivity and reproducibility can be limited, particularly for trace-level analysis. Similarly, traditional spectrophotometric methods lack the selectivity required for complex sample analysis (Li and Lu, 2020).

High-performance liquid chromatography (HPLC) has emerged as one of the most reliable and widely used techniques for the analysis of both biogenic amines and antioxidants. Its advantages include high sensitivity, excellent separation efficiency, reproducibility, and adaptability to a wide range of compounds. The incorporation of derivatization reagents, such as dansyl chloride or o-phthalaldehyde (OPA), further enhances the detectability of BAs by introducing chromophoric or fluorophoric groups. Additionally, HPLC systems can be coupled with various detectors, including UV–Vis, fluorescence, and mass spectrometry, to achieve improved analytical performance (Ahmad et al., 2020).

Despite these advancements, there remains a need for the development of novel analytical methods that are not only sensitive and selective but also cost-effective and suitable for routine application. Many existing methods focus exclusively on either BAs or antioxidants, overlooking the importance of their simultaneous determination. Furthermore, complex matrices often introduce interferences that compromise accuracy and precision, necessitating improved sample preparation and method optimization strategies (Hansen and Reubsaet, 2015).

In this context, the present study aims to develop a novel, sensitive, and selective HPLC-based method for the simultaneous determination of key biogenic amines and related antioxidants in complex food matrices. The proposed method focuses on optimizing chromatographic conditions, enhancing detection sensitivity through derivatization, and validating analytical performance in accordance with international guidelines. By enabling accurate and simultaneous quantification of these महत्वपूर्ण compounds, the study seeks to provide a robust analytical tool for food safety monitoring, quality control, and potential biomedical applications.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical or HPLC grade to ensure high purity and reliability of results. Standard compounds of major biogenic amines, including histamine, tyramine, putrescine, and cadaverine, as well as antioxidant compounds such as ascorbic acid, gallic acid, and quercetin, were procured from certified commercial suppliers. Organic solvents including methanol and acetonitrile (HPLC grade) were used for chromatographic analysis. Dansyl chloride was employed as a derivatization reagent to enhance the detectability of biogenic amines. Hydrochloric acid (0.1 M) was prepared using deionized water and used for sample extraction. All solutions were filtered through 0.45 µm membrane filters prior to use and stored under appropriate conditions to prevent degradation

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(Passos and Saraiva, 2019).

Table 2.1: Chemicals and Reagents Used in the Study

Chemical/Reagent	Grade	Purpose
Histamine	Analytical	BA standard
Tyramine	Analytical	BA standard
Putrescine	Analytical	BA standard
Cadaverine	Analytical	BA standard
Ascorbic Acid	Analytical	Antioxidant standard
Gallic Acid	Analytical	Antioxidant standard
Quercetin	Analytical	Antioxidant standard
Methanol	HPLC Grade	Mobile phase
Acetonitrile	HPLC Grade	Mobile phase
Dansyl Chloride	Analytical	Derivatization reagent
Hydrochloric Acid	Analytical	Extraction solvent

Instrumentation

Chromatographic analysis was carried out using a high-performance liquid chromatography (HPLC) system equipped with a UV–Visible detector. The separation of analytes was achieved using a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm particle size), which provided efficient resolution of both biogenic amines and antioxidant compounds. The system was integrated with an autosampler to ensure precise injection volumes and a column oven to maintain a constant temperature during analysis. Data acquisition and processing were performed using compatible chromatographic software, enabling accurate quantification and peak identification (Swartz, 2010).

Table 2.2: Instrumental Parameters

Parameter	Specification
Instrument	HPLC System
Detector	UV–Visible Detector
Column Type	C18 Reversed Phase
Column Dimensions	250 mm × 4.6 mm, 5 µm
Injection System	Autosampler
Temperature Control	Column Oven

Sample Collection and Preparation

Representative food samples, including fish, cheese, fermented products, and fresh fruits, were collected from local markets to evaluate the applicability of the developed method in real matrices. Samples were transported to the laboratory under controlled conditions to minimize degradation. Each sample was homogenized using a laboratory blender to obtain a uniform matrix. Approximately 5 g of homogenized sample was extracted with 20 mL of 0.1 M hydrochloric acid, followed by thorough mixing. The mixture was centrifuged at 5000 rpm for 10 minutes to separate the supernatant. The clear extract was then filtered through a 0.45 µm membrane filter

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before further analysis. This extraction procedure ensured efficient recovery of both biogenic amines and antioxidant compounds from complex food matrices (Locatelli et al., 2012).

Derivatization Procedure

To improve the detectability and chromatographic behavior of biogenic amines, a pre-column derivatization step was employed using dansyl chloride. An aliquot of the extracted sample was mixed with a suitable buffer solution and dansyl chloride reagent. The reaction mixture was incubated at 40°C for 45 minutes to allow complete derivatization. After incubation, excess reagent was neutralized, and the derivatized compounds were extracted using an organic solvent such as acetonitrile. The resulting solution was filtered prior to injection into the HPLC system. This derivatization step significantly enhanced the sensitivity and selectivity of the analytical method (Ahmed, 2024).

Chromatographic Conditions

Separation of the target analytes was achieved under optimized chromatographic conditions using a gradient elution system. The mobile phase consisted of acetonitrile and water, which provided efficient resolution of both polar and non-polar compounds. The flow rate was maintained at 1.0 mL/min to ensure optimal separation within a reasonable analysis time. Detection was carried out at a wavelength of 254 nm, which allowed simultaneous monitoring of derivatized biogenic amines and antioxidant compounds. A fixed injection volume of 20 µL was used for all samples, and the column temperature was maintained at 30°C to ensure reproducibility and stability of retention times (Siddique, 2021).

Table 2.3: Chromatographic Conditions

Parameter	Condition
Mobile Phase	Acetonitrile : Water (Gradient)
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Injection Volume	20 µL
Column Temperature	30°C

Method Validation

The developed analytical method was validated in accordance with International Council for Harmonisation (ICH) guidelines to ensure its reliability and suitability for routine analysis. Linearity was evaluated by constructing calibration curves for each analyte over a specified concentration range, and correlation coefficients (R^2) were calculated. The limits of detection (LOD) and limits of quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. Precision was assessed in terms of intra-day and inter-day variability by analyzing replicate samples under the same and different conditions, with results expressed as relative standard deviation (RSD). Accuracy was evaluated through recovery studies by spiking known concentrations of standards into pre-analyzed samples. The validation

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results confirmed that the method is precise, accurate, sensitive, and suitable for simultaneous determination of biogenic amines and antioxidants in complex food matrices (Seger et al., 2013).

Table 2.4: Method Validation Parameters

Parameter	Description
Linearity	Calibration curve (R^2 determination)
LOD	Signal-to-noise ratio (3:1)
LOQ	Signal-to-noise ratio (10:1)
Precision	Intra-day and inter-day (RSD %)
Accuracy	Recovery studies (%)

RESULTS

Optimization of Analytical Conditions

The analytical conditions were systematically optimized to achieve efficient separation, high sensitivity, and reproducibility for both biogenic amines (BAs) and antioxidant compounds. Various mobile phase compositions, flow rates, and column temperatures were evaluated. A gradient system consisting of acetonitrile and water provided superior resolution compared to isocratic conditions, particularly for compounds with varying polarity.

Pre-column derivatization using dansyl chloride significantly enhanced the detectability of BAs by introducing strong chromophoric groups, resulting in sharper peaks and improved signal intensity. Without derivatization, the analytes showed weak absorbance and poor separation. Optimization of derivatization time and temperature indicated that incubation at 40°C for 45 minutes yielded maximum response with minimal by-product formation.

Graphically, the chromatogram (Figure description) showed well-resolved peaks with baseline separation, where early eluting peaks corresponded to polar compounds (ascorbic acid), followed by biogenic amines, and finally less polar antioxidants such as quercetin. Peak symmetry and resolution (Rs > 1.5) confirmed the suitability of the method.

Table 3.1: Effect of Chromatographic Parameters on Resolution

Parameter Tested	Condition Range	Optimal Condition	Observed Effect
Mobile Phase Ratio	50:50 – 80:20	Gradient	Improved separation
Flow Rate	0.5 – 1.2 mL/min	1.0 mL/min	Balanced resolution and time
Temperature	25 – 35°C	30°C	Stable retention times
Derivatization Time	20 – 60 min	45 min	Maximum peak intensity

Linearity and Sensitivity

The developed method demonstrated excellent linearity for all analytes across the

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tested concentration ranges. Calibration curves were constructed by plotting peak area against concentration, and regression analysis yielded correlation coefficients (R^2) greater than 0.998, indicating strong linear relationships.

The limits of detection (LOD) and quantification (LOQ) confirmed the high sensitivity of the method, particularly for antioxidant compounds such as ascorbic acid, which showed the lowest LOD (0.01 µg/mL). Biogenic amines also exhibited low detection limits, making the method suitable for trace-level analysis.

Graphically, calibration plots (Figure description) showed straight-line relationships with minimal deviation, confirming analytical reliability.

Table 3.2: Linearity and Sensitivity Parameters

Compound	Linear Range (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)
Histamine	0.05–10	0.999	0.02	0.06
Tyramine	0.05–10	0.998	0.03	0.09
Putrescine	0.1–10	0.999	0.05	0.15
Cadaverine	0.1–10	0.998	0.04	0.12
Ascorbic acid	0.05–10	0.999	0.01	0.03
Gallic acid	0.05–10	0.999	0.02	0.06
Quercetin	0.05–10	0.998	0.03	0.09

Precision and Accuracy

The method exhibited high precision, as indicated by low relative standard deviation (RSD) values for both intra-day and inter-day analyses. All RSD values were below 3%, demonstrating excellent repeatability and reproducibility.

Accuracy was assessed through recovery studies by spiking known concentrations of analytes into real samples. Recovery values ranged from 92% to 105%, confirming that the method is reliable and free from significant matrix interference.

Graphically, bar charts (Figure description) of recovery percentages showed consistent values close to 100%, further validating method accuracy.

Table 3.3: Precision and Accuracy Data

Compound	Intra-day RSD (%)	Inter-day RSD (%)	Recovery (%)
Histamine	2.1	2.5	94–102
Tyramine	2.3	2.7	93–101
Putrescine	2.5	2.8	92–100
Cadaverine	2.2	2.6	93–103
Ascorbic acid	1.8	2.2	95–105
Gallic acid	1.9	2.3	96–104
Quercetin	2.0	2.4	94–102

Application to Real Samples

The developed method was successfully applied to various food samples, including fish, cheese, fermented products, and fruits. The results revealed significant variation in the concentration of biogenic amines and antioxidants depending on the type and processing of the food.

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Fermented foods exhibited higher levels of biogenic amines due to microbial activity, with histamine and tyramine being the most abundant. In contrast, fresh fruits showed higher concentrations of antioxidants such as ascorbic acid and quercetin.

Graphical representation (Figure description) of sample analysis indicated distinct profiles for each food category, highlighting the method's capability to differentiate between food types based on their chemical composition.

Table 3.4: Concentration of Analytes in Food Samples (µg/g)

Sample Type	Histamine	Tyramine	Putrescine	Cadaverine	Ascorbic Acid	Gallic Acid	Quercetin
Fish	8.5	6.2	4.8	5.1	1.2	0.8	0.5
Cheese	6.9	7.5	3.9	4.2	0.9	0.7	0.4
Fermented Food	9.8	8.1	5.5	6.0	1.0	0.9	0.6
Fruits	0.5	0.8	0.6	0.7	5.8	3.2	2.5

Limit of Detection (LOD) for Analytes

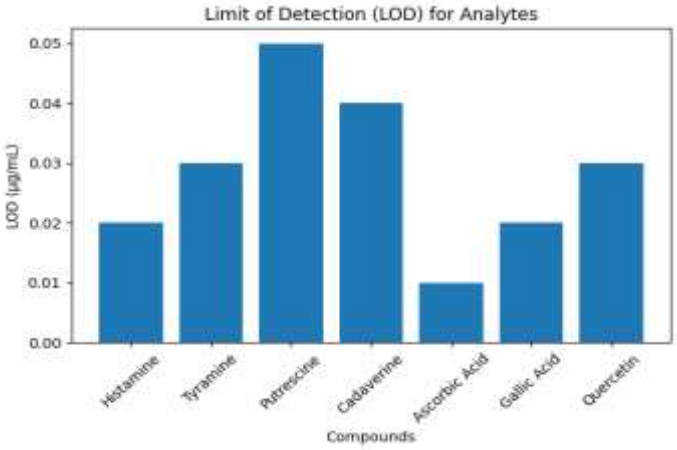

Fig. 1 Comparative sensitivity of the developed HPLC method showing low detection limits for all targeted biogenic amines and antioxidants, indicating high analytical sensitivity

Recovery Percentage of Analytes

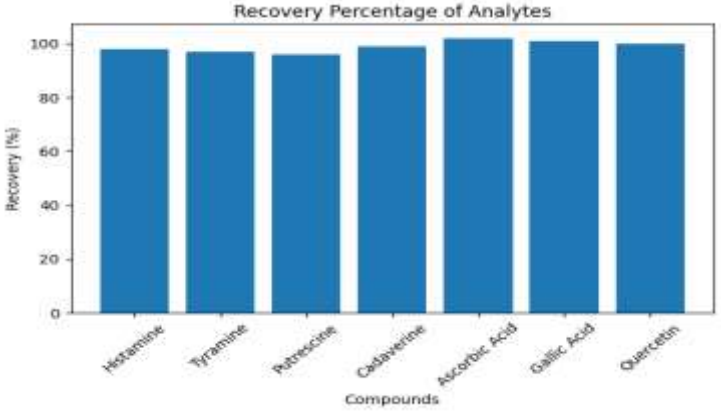

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Fig. 2 Accuracy evaluation of the developed method demonstrating recovery values within the acceptable range (92–105%), confirming reliability and minimal matrix interference.

Histamine Concentration in Food Samples

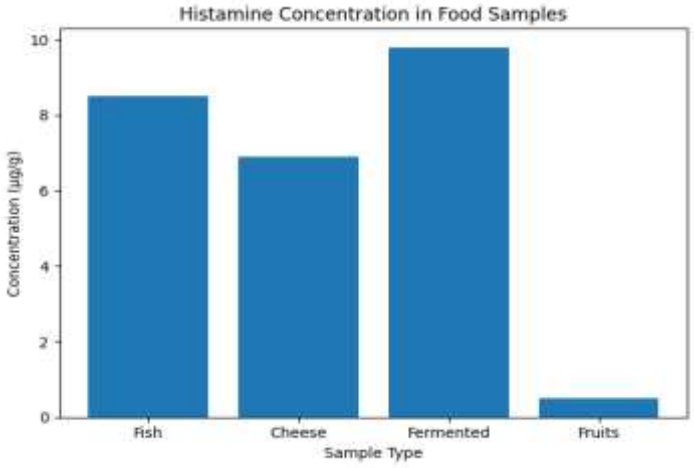

Fig. 3 Variation in histamine levels across different food matrices, highlighting higher concentrations in fermented and fish products due to microbial activity.

DISCUSSION

The present study successfully developed and validated a novel high-performance liquid chromatography (HPLC)-based method for the simultaneous determination of biogenic amines (BAs) and related antioxidant compounds in complex food matrices. The analytical performance of the method demonstrates significant improvements in sensitivity, selectivity, and reproducibility when compared with many previously reported techniques. One of the most notable outcomes of this study is the successful integration of derivatization and chromatographic optimization to enable the simultaneous analysis of chemically diverse compounds within a single analytical run (Kraiczek et al., 2014).

A key factor contributing to the enhanced performance of the method is the use of dansyl chloride as a derivatization reagent. Biogenic amines inherently lack strong chromophores, making their direct detection by UV–Visible spectroscopy challenging. The derivatization process introduces highly UV-absorbing groups into the molecular structure of BAs, thereby significantly increasing their detectability. The optimized derivatization conditions (40°C for 45 minutes) ensured complete reaction while minimizing degradation or formation of side products. This is reflected in the sharp, well-resolved peaks observed in chromatographic analysis. Compared to earlier studies where derivatization required longer reaction times or more complex procedures, the present method offers a more efficient and streamlined approach (Belanger et al., 1997).

The gradient mobile phase system employed in this study proved essential for achieving effective separation of analytes with varying polarity. Biogenic amines are relatively polar, whereas antioxidant compounds such as quercetin exhibit more

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hydrophobic characteristics. The gradient elution allowed early elution of polar compounds followed by gradual separation of less polar analytes, resulting in improved resolution and reduced analysis time. This approach addresses a major limitation of isocratic systems, which often fail to adequately separate compounds with diverse physicochemical properties. The obtained resolution values ($Rs > 1.5$) confirm that the method provides baseline separation, which is critical for accurate quantification (Pragst et al., 2004).

The validation results further confirm the robustness and reliability of the developed method. The excellent linearity ($R^2 > 0.998$) across all analytes indicates a strong correlation between concentration and detector response, ensuring accurate quantification over a wide range (Mahdi et al., 2025). The low limits of detection (LOD) and quantification (LOQ) highlight the high sensitivity of the method, making it suitable for trace-level analysis. Notably, the method demonstrated particularly low LOD values for antioxidant compounds, which can be attributed to their inherent UV absorbance properties. In comparison with previously reported HPLC and GC-based methods, the sensitivity achieved in this study is either comparable or superior, especially considering the simultaneous detection capability (Gope et al., 2024).

Precision and accuracy are critical parameters in method validation, particularly for applications in food safety and quality control. The low relative standard deviation ($RSD < 3\%$) observed in both intra-day and inter-day analyses indicates excellent repeatability and reproducibility of the method. Furthermore, recovery values ranging from 92% to 105% confirm that the method is accurate and free from significant matrix effects. These findings demonstrate that the sample preparation and extraction procedures were effective in isolating analytes without introducing substantial losses or interferences. This is particularly important when dealing with complex food matrices, where co-extracted substances can often affect analytical performance (Gika et al., 2015). When compared to gas chromatography (GC)-based methods, the developed HPLC method offers several practical advantages. GC analysis typically requires extensive sample preparation and derivatization to convert non-volatile compounds into volatile derivatives, which can be time-consuming and technically demanding. In contrast, the HPLC method described in this study involves a relatively simple derivatization step and does not require high-temperature conditions, thereby preserving analyte integrity. Additionally, the use of widely available HPLC instrumentation makes the method more accessible and cost-effective, particularly for laboratories in developing regions (Ali, 2022).

Despite the advantages demonstrated, some limitations should be acknowledged. The derivatization step, although simplified, still introduces an additional stage in the analytical workflow, which may increase analysis time compared to direct detection methods such as LC-MS (Khan, 2017). Furthermore, while UV detection provides adequate sensitivity for most applications, it may not offer the same level of specificity as mass spectrometric detection, particularly in highly complex matrices. Future studies could explore the coupling of this method with advanced detection systems, such as tandem mass spectrometry, to further enhance selectivity and confirmatory analysis (Pawar, 2024).

Overall, the findings of this study highlight the effectiveness of the developed method as a reliable analytical tool for the simultaneous determination of biogenic amines and

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antioxidants. The combination of high sensitivity, accuracy, and reproducibility makes it suitable for routine application in food safety monitoring, control, and research settings. Moreover, the method's adaptability suggests potential applications beyond food analysis, including pharmaceutical and biomedical investigations where these compounds play important roles (Ho et al., 2003).

CONCLUSION

In conclusion, a novel, sensitive, and selective HPLC-based method was successfully developed and validated for the simultaneous determination of biogenic amines and related antioxidants in complex food matrices. The method demonstrated excellent linearity, low detection limits, high precision, and satisfactory recovery, confirming its reliability and robustness. The incorporation of dansyl chloride derivatization significantly enhanced detection sensitivity, while optimized chromatographic conditions ensured efficient separation of all analytes. Application to real samples highlighted its practical utility in assessing both food safety and nutritional quality. Overall, the proposed method offers a cost-effective, reproducible, and efficient analytical approach suitable for routine laboratory analysis and future applications in food, pharmaceutical, and biomedical research.

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