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Investigation of Column Temperature and Validation of an HPLC-PDA Method for Hydrogen Peroxide Analysis in Hair Cosmetics under Tropical Environments

Dinalia Dinalia[1,2*], Husain Sosidi[1], Ahmad Ridhay[1], Hannan Hannan[2], Khairuddin Khairuddin[1], Pasjan Satrimafitrah[1], and Syamsuddin Syamsuddin[1]

[1]Chemistry Department, Faculty of Mathematics and Natural Science, Tadulako University, Palu, Indonesia
[2]Indonesian Food and Drug Authority Regional Office in Palu, Palu, Indonesia
*Corresponding author: Dinalia | Email: dinalia@pom.go.id

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Abstract: Hydrogen peroxide (H_2O_2) is widely used as an oxidizing agent in hair cosmetic products, requiring reliable and validated analytical methods to ensure product safety and regulatory compliance. This study investigated the effect of column temperature and validated a high-performance liquid chromatography–photodiode array (HPLC–PDA) method for H_2O_2 determination in hair cosmetic formulations using triphenylphosphine (TPP) derivatization under tropical laboratory conditions. The triphenylphosphine oxide (TPPO) derivative exhibited a stable maximum absorbance at 222 nm within the concentration range of 20–120 ppm, which was selected as the detection wavelength. System suitability was evaluated at ambient temperature, 35, 40, 45, and 50 °C. Column temperatures between 40 and 50 °C fulfilled all system suitability and validation criteria, demonstrating acceptable efficiency, peak symmetry, retention time stability, and reproducibility. Among these conditions, 40 °C was selected as the optimum column temperature based on overall analytical performance and practical considerations. Method validation showed excellent linearity (correlation coefficient $r \geq 0.995$; residual variance $Vxo \leq 5\%$), acceptable precision (%RSD ≤ 2%), and adequate sensitivity, expressed as limits of detection (LOD) and limits of quantification (LOQ). Accuracy evaluation using matrix-spiked samples yielded recoveries within the AOAC acceptance range (90–107%). Overall, the validated HPLC–PDA method at 40 °C with detection at 222 nm is robust and suitable for routine determination of H_2O_2 in hair cosmetic products under tropical conditions.

Keywords: column temperature, hair cosmetics, HPLC–PDA, hydrogen peroxide, method validation

1. INTRODUCTION

Hair dyes are among the most widely used hair cosmetic products, particularly for masking the appearance of gray hair. The perception of gray hair as a sign of aging has driven a significant increase in the use of hair dye products among both women and men [1]. Along with the growing frequency of use, numerous reports have emerged regarding adverse effects associated with hair dyes, especially concerning scalp irritation and hair health. Reported reactions include inflammation of the skin around the ears, neck, and shoulders, as well as cases of hair loss. Furthermore, concerns have been raised about the potential carcinogenicity of toxic substances that may be absorbed through the skin [2].

Hair dye products generally contain a combination of dye precursors such as p-phenylenediamine (PPD) and oxidizing agents such as hydrogen peroxide (H_2O_2) [3]. Their chemical formulations may also include other compounds, such as ammonia, resorcinol, diaminobenzene, and toluene-2,5-diamine. Long-term exposure to these substances has been associated with various dermatological reactions, including allergic contact dermatitis, irritation characterized by redness and swelling on the scalp and facial areas, and potential carcinogenic risks [4]. In particular, H_2O_2 plays a role in generating reactive oxygen species, especially hydroxyl radicals, which possess high oxidative

capacity. At high concentrations, H_2O_2 is corrosive, capable of causing significant skin tissue damage, and exhibits considerable cellular toxicity due to its strong oxidizing properties [5].

The use of H_2O_2 in cosmetic products is regulated by various international and national authorities, including Indonesian Food and Drug Authority (BPOM). According to the applicable regulation, the maximum allowable concentration of H_2O_2 in hair preparations is 12% [6]. Determination of H_2O_2 content in cosmetics typically refers to standard analytical methods established by organizations such as the United States Pharmacopeia (USP), the Indonesian Pharmacopoeia (FI), the Association of Official Analytical Collaboration (AOAC), the International Organization for Standardization (ISO), and the Indonesian National Standard (SNI), with the most commonly employed techniques being titrimetry [7] and spectrophotometry [8]. Analytical determination of H_2O_2 in cosmetic preparations at the Indonesian Food and Drug Authority Region Palu (Balai POM Palu) follows a titrimetric procedure established by the National Drug and Food Testing Center (PPPOMN BPOM) [9].

Titrimetric methods offer several advantages, including ease of execution, rapid analysis, and minimal instrumentation requirements. Nevertheless, they exhibit significant limitations when applied to complex sample matrices. Titrimetry is often unable to selectively differentiate H_2O_2 from other easily oxidizable species, thereby increasing the potential for analytical interference. The accuracy of titrimetric methods is also generally lower than that of spectrophotometric approaches [10].

To overcome the limitations of titrimetry, the development of more reliable analytical methods for H_2O_2 determination is essential. Several analytical strategies have been explored, including electrochemical methods [11], colorimetry [12], luminescence and spectrophotometry [5] and high-performance liquid chromatography (HPLC) [13]–[15].

Previous studies have developed analytical methods based on the oxidation reaction between triphenylphosphine (TPP) and H_2O_2 to form triphenylphosphine oxide (TPPO), which is subsequently analyzed in teeth-whitening products and hair cosmetic samples. These methods employed a C18 column (150 mm × 4.6 mm, 5 μm), a column temperature of 25 °C, and a gradient elution system using acetonitrile and water [13]. A similar approach was applied to milk matrices using a C8 column (2.1 × 150 mm, 3 μm) [14]. Another study proposed a different technique for determining H_2O_2 in water and wastewater using acidified potassium iodide (KI) as the mobile phase and a capillary column for separation [15].

Most existing methods for H_2O_2 determination continue to employ a fixed column temperature of 25 °C without systematic evaluation. This temperature is commonly treated as "room temperature" in analytical methods developed primarily in subtropical regions such as Europe and North America. However, such conditions may not be representative of laboratories in tropical regions, including Southeast Asia, where ambient temperatures frequently exceed 25 °C [16]. Moreover, many HPLC systems are equipped only with column heaters rather than cooling units, making precise temperature control at 25 °C impractical.

To date, systematic evaluation of column temperature as a critical validation parameter for HPLC–PDA determination of H_2O_2 in cosmetic matrices has not been adequately reported, particularly under tropical environmental conditions. Most existing studies rely on a single temperature setting without assessing its influence on chromatographic efficiency, method sensitivity, and overall validation performance. This lack of investigation represents an important methodological gap, especially for laboratories operating in tropical climates where temperature regulation is inherently constrained.

Adjustment of column temperature can significantly affect key chromatographic parameters, including mobile-phase viscosity, system pressure, analyte–stationary phase interactions, and retention behaviour. Elevated temperatures may reduce solvent viscosity, enhance mass transfer, improve peak symmetry, and shorten analysis time [17]. Therefore, column temperature should be regarded as an integral variable in scientific method optimization rather than a fixed experimental condition.

In this context, the present study introduces a data-driven approach by systematically investigating the effects of column temperature from ambient to elevated conditions and integrating this evaluation into comprehensive method validation. The aim of this study is to develop and validate an HPLC–PDA method for the determination of H_2O_2 in hair cosmetic preparations based on optimized column temperature selection, thereby providing a robust, practical, and regionally relevant analytical method suitable for routine quality control, particularly in tropical laboratories.

2. MATERIALS AND METHODS

2.1. Materials

The instruments used in this study included a High-Performance Liquid Chromatography system equipped with a Photo Diode Array detector (HPLC-PDA, Shimadzu 20AD) with a dual-pump configuration; a Waters X-Bridge C18 column (4.6 mm × 150 mm, 5 μm); an analytical balance (Mettler Toledo); a centrifuge (Nuve NF 800) along with centrifuge tubes; a vortex mixer; micropipettes (Endo); and various glassware, including volumetric flasks, volumetric pipettes, weighing boats, droppers, and beakers (Pyrex).

The materials utilized were a commercial hair cosmetic sample (Merk X); hydrogen peroxide 30% (Merck); acetonitrile (Merck); triphenylphosphine (Sigma-Aldrich); ethanol (Merck) and purified water was produced in-house using a Merck water purification system.

2.2. Methods

2.2.1. Preparation of H_2O_2 Standard Solutions

A 1000 μg/mL hydrogen peroxide stock solution was prepared by diluting 0.167 g of a 30% H_2O_2 solution to 50 mL with water in a volumetric flask. Serial working standards were subsequently prepared from the 1000 μg/mL stock to obtain concentrations of 20, 40, 60, 80, 100, and 120 μg/mL using a diluent consisting of acetonitrile and water (65:35, v/v). To ensure system suitability throughout the analytical sequence, an independent quality control standard at 100 μg/mL was injected repeatedly.

2.2.2. Sample Preparation

Hair cosmetic samples were prepared by weighing 0.25 g of sample into a centrifuge tube, followed by dilution to 25 mL with purified water. The mixture was vortexed for 1 min and centrifuged, An aliquot of 1.0 mL of the supernatant was transferred into a 10 mL volumetric flask and diluted to volume with purified water. Then, 1.0 mL of this diluted solution was subjected to derivatization with triphenylphosphine (TPP) prior to analysis under the validated HPLC–PDA conditions.

2.2.3. Derivatization H_2O_2 with Triphenylphosphine (TPP)

The derivatization procedure was performed following a previously reported method with minor modification [13]. A TPP stock solution (0.01 M) was prepared by dissolving 65.5 mg of triphenylphosphine in acetonitrile and making up to 25 mL in an amber volumetric flask. A 1 mL aliquot of this stock was transferred into a light-protected 10 mL centrifuge tube, followed by the addition of 5 mL acetonitrile and 3 mL ultrapure water. The mixture was vortexed for 1 min. Subsequently, 1 mL of the test solution (either H_2O_2 standard or sample extract) was added and vortexed again. The reaction mixture was allowed to stand in the dark for at least 2 hours. After completion, the solution was filtered through a 0.45 μm membrane filter and injected into the HPLC system for analysis.

2.2.4. HPLC Conditions

The chromatographic system and gradient program were adapted from previously published HPLC methods for H_2O_2 determination [13], with column temperature evaluated as a variable in this study. All standard and sample solutions were injected into the HPLC-PDA system equipped with a Waters XBridge™ C18 column (4.6 mm × 150 mm, 5 μm). Column temperature was evaluated at room temperature, 35 °C, 40 °C, 45 °C, and 50 °C. An injection volume of 10 μL and a flow rate of 1.0

mL/min were used. The detection wavelength (λmax) was examined at 222, 223, 224, 225, 226, and 227 nm, and the wavelength providing the highest peak area was selected for subsequent analyses. A gradient elution program was applied as follows: 0–5.5 min, acetonitrile : water (50:50, v/v); 6.5–9.0 min, 100% acetonitrile; 10–20 min, acetonitrile : water (50:50, v/v).

2.2.5. Method Validation

Method validation parameters were evaluated in accordance with AOAC guidelines and established analytical validation practices reported in the literature [18]–[20]

a. Selectivity/Specificity

Selectivity was evaluated to ensure adequate chromatographic separation between the TPPO derivative and components of the cosmetic matrix. Hair cosmetic samples were prepared and derivatized following the same procedure described previously for sample preparation and TPP derivatization. Briefly, the sample matrix was spiked with the H_2O_2 standard, followed by derivatization with triphenylphosphine (TPP) and HPLC–PDA analysis under the optimized chromatographic conditions. Selectivity was assessed by examining chromatograms for potential interferences at the retention time of TPPO. Adequate selectivity was confirmed based on system suitability test (SST) parameters, including resolution (>2), tailing factor (≤2), theoretical plate count (>2000), and repeatability of peak area and retention time, expressed as %RSD (≤1.0%) [18].

b. Accuracy

Accuracy was evaluated using the same sample preparation and derivatization procedure as described for selectivity. Triplicate hair cosmetic samples (0.25 g each) were spiked at three concentration levels by adding 200 µL, 600 µL, and 1200 µL of the 1000 µg/mL H_2O_2 standard solution, corresponding to low, medium, and high concentration levels within the validated range. Each spiked sample was processed, derivatized with TPP, filtered (0.45 µm), and analyzed under the validated HPLC–PDA conditions. Percent recovery was calculated for each concentration level by comparing the measured concentration with the theoretical spiked concentration. The acceptance criterion for accuracy was a recovery range of 90–107% for analyte concentrations within 30–200 µg/mL, in accordance with AOAC guidelines [19].

c. Precision

Precision was evaluated using the same sample preparation, spiking, derivatization, and analytical procedures as described for the accuracy assessment. Triplicate analyses were performed at three concentration levels corresponding to 200, 600, and 1200 µL additions of the 1000 µg/mL H_2O_2 standard solution. Precision was expressed as the percentage relative standard deviation (%RSD) calculated for each concentration level. The acceptance criterion for precision was set at %RSD ≤ 2%, in accordance with established validation guidelines [20].

d. Limit of Detection (LOD) dan Limit of Quantification (LOQ)

LOD and LOQ were evaluated using matrix-matched samples prepared according to the previously described sample preparation and TPP derivatization procedure. Triplicate sample portions (0.25 g each) were fortified with increasing volumes of the 1000 µg/mL H_2O_2 standard to obtain six concentration levels (corresponding to 200, 400, 600, 800, 1000, and 1200 µL additions). After dilution to volume, derivatization, filtration (0.45 µm), and HPLC–PDA analysis, LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve using the following formulas [21]:

$$LOD = \frac{3.3\ x\ standar\ deviasi}{slope}$$

$$LOQ = \frac{10\ x\ standar\ deviasi}{slope}$$

The standard deviation is subsequently computed according to the formula provided [22]

$$SD = \sqrt{\frac{\sum i(x_i - \bar{x})^2}{n-1}}$$

e. Linearity

Linearity was assessed using matrix-matched samples prepared and derivatized according to the previously described procedure. Triplicate samples (0.25 g each) were fortified with increasing volumes of the 1000 µg/mL H_2O_2 standard to obtain six concentration levels. After dilution, derivatization with TPP, filtration (0.45 µm), and HPLC–PDA analysis, calibration curves were constructed by plotting peak area versus analyte concentration. The regression equation and Vxo were calculated, with acceptance criteria of $r \geq 0.995$ and Vxo ≤ 5% [23].

3. RESULTS AND DISCUSSION

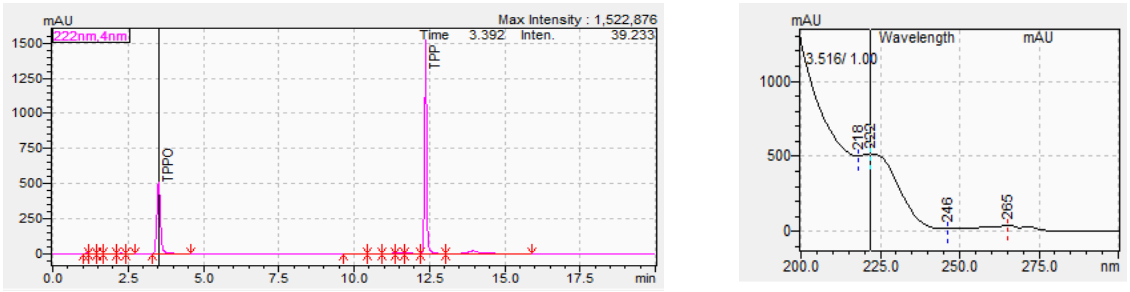
In previous studies, chromatographic conditions were typically maintained at a column temperature of 25 °C [13]–[15]. However, under tropical laboratory environments such as in Palu City, Indonesia, stable operation at this temperature is not feasible since the ambient room temperature averages around 32 °C even with air conditioning. Therefore, the initial experiments were carried out at ambient temperature to maintain chromatographic stability.

Earlier reports indicated that the derivatization product of hydrogen peroxide (H_2O_2) with triphenylphosphine (TPP), triphenylphosphine oxide (TPPO), exhibits a maximum absorbance (λmax) at 225 nm [13], [14]. To replicate these findings, an initial spectral scan was performed following the reported procedure using a 100 ppm H_2O_2 standard solution within the wavelength range of 222–227 nm. The results are presented in the table 1. The chromatogram of TPPO and the corresponding UV spectrum are shown in Figures 1a and 1b, respectively.

Table 1. Optimization of wavelength (λmax) for TPPO detection

Wavelength (nm)	Area
222	2775432
223	2762474
224	2725328
225	2655351
226	2549608
227	2408920

As shown in Table 1, the largest peak area was obtained at 222 nm. Therefore, 222 nm was selected as the optimum detection wavelength for subsequent analyses.



(a) **(b)**

Figure 1. (a) Chromatogram of TPPO and TPP; (b) UV spectrum of TPPO

Using the selected λmax of 222 nm, a calibration curve was initially prepared using H_2O_2 standard solutions at 30, 50, 100, 150, 200, and 250 ppm [13]. A preliminary UV absorbance screening revealed that standard solutions at concentrations ≥150 µg/mL exhibited a shift in the detected maximum wavelength, with the main absorbance peak appearing at approximately 264.97 nm (Table 2). This bathochromic shift was not accompanied by any substantial alteration in the overall absorbance spectral profile, suggesting that it is more likely attributable to instrumental limitations and deviations from the Lambert–Beer law at higher concentrations, such as excessive absorbance, light

scattering, or detector saturation, rather than to intrinsic changes in the electronic properties of the H_2O_2 molecule.

Table 2. Maximum absorbance wavelength (λmax) of TPPO at various H_2O_2 standard concentrations.

H_2O_2 Standard Concentration (ppm)	λmax (nm)
30	222.25
50	222.14
100	221.85
150	264.97
200	264.97
250	264.97

To ensure quantitative stability and a linear detector response under tropical laboratory conditions, spectral evaluation was extended across a broader concentration range. The results indicated that within the 20–120 ppm range, the derivative peak consistently appeared at approximately 222 nm and provided stable, high-intensity responses. Consequently, the calibration standards were adjusted to 20, 40, 60, 80, 100, and 120 ppm, and detection was set at 222 nm. This modification maintained consistency with the theoretical λmax of TPPO while ensuring linearity, reproducibility, and method accuracy under local operational conditions.

3.1. Column Temperature Investigation and Chromatographic Performance Evaluation

Column temperature was evaluated at five settings, ambient, 35 °C, 40 °C, 45 °C, and 50 °C, using six replicate injections of a 100 ppm standard solution. This investigation aimed to identify the most stable analytical condition for laboratories operating under tropical environmental conditions. A summary of the resulting system suitability parameters is presented in Table 3.

Table 3. The System Suitability Test

Parameter	Ambient	35 °C	40 °C	45 °C	50 °C	Acceptance Criteria [18]
Retention time (min)	3.568 ± 0.004	3.516 ± 0.003	3.470 ± 0.005	3.404 ± 0.002	3.354 ± 0.003	
% RSD tR	0.103	0.077	0.132	0.060	0.103	≤ 1%
Area	4360932 ± 2541	4360839 ± 2745	4391532 ± 4105	4367490 ± 5923	4366540 ± 3856	
% RSD Area	0.058	0.063	0.093	0.136	0.088	≤ 1%
Tailing factor	1.062 ± 0.002	1.110 ± 0.002	1.127 ± 0.003	1.201 ± 0.004	1.282 ± 0.008	≤ 2%
Resolution	4.649 ± 0.041	5.050 ± 0.128	4.982 ± 0.071	5.206 ± 0.022	5.357 ± 0.060	≥ 2
Theoritical plate	2073.03 ± 35.03	2657.05 ± 31.44	2558.38 ± 27.47	2837.67 ± 30.54	3090.14 ± 27.03	>2000

Retention time showed a gradual decrease as column temperature increased, which is consistent with the expected enhancement in analyte mass-transfer kinetics and decreased mobile-phase viscosity at elevated temperatures [17]. The %RSD of retention time and area remained well below the acceptance criterion (≤1%) at all temperatures, indicating high repeatability of the chromatographic system and demonstrating stable temporal performance across these settings.

Tailing factor values at all evaluated temperatures remained well within the acceptable limit (≤2), indicating consistently symmetrical peak shapes. The measured values ranged from 1.062 to 1.282,

with relatively small standard deviations (±0.002–0.008), demonstrating excellent reproducibility. Overall, the minimal variation across temperatures confirms that column performance and peak symmetry were not significantly influenced by temperature adjustments within the studied range.

Resolution values exceeded the minimum criterion (≥2) at all temperatures; however, slight temperature-dependent differences were evident. The theoretical plate number exceeded the minimum acceptance requirement (>2000) at all temperatures, confirming adequate column efficiency throughout the study.

3.2. Evaluation of Validation Parameters Across Column Temperatures

Alongside system performance, method validation was conducted at three concentration levels (20, 60, and 120 ppm) to assess accuracy and precision. Linearity, Vxo, LOD, and LOQ were evaluated across the full calibration range (20–120 ppm). A summary is provided in Table 4.

Table 4. Summary of validation parameters at different column temperatures.

Parameter	Conc. Level	Ambient	35 °C	40 °C	45 °C	50 °C	Acceptance Criteria
Recovery (%)	20 ppm	108.15 ± 0.515	116.83 ± 0.625	90.26 ± 0.409	93.04 ± 0.348	97.68 ± 0.447	(90-107)% [19]
	60 ppm	115.51 ± 0.698	105.74 ± 0.438	101.72 ± 0.075	96.20 ± 0.492	104.62 ± 0.636	
	120 ppm	105.06 ± 0.832	100.40 ± 0.194	98.18 ± 0.800	100.19 ± 0.251	104.95 ± 0.235	
Presicion (%RSD)	20 ppm	0.477 ± 0.010	0.535 ± 0.012	0.454 ± 0.008	0.374 ± 0.007	0.458 ± 0.009	≤ 2% [20]
	60 ppm	0.604 ± 0.041	0.414 ± 0.026	0.074 ± 0.004	0.511 ± 0.029	0.607 ± 0.037	
	120 ppm	0.792 ± 0.098	0.193 ± 0.023	0.815 ± 0.093	0.250 ± 0.030	0.224 ± 0.028	
Linearity (r)		0.9962	0.9975	0.9993	0.9996	0.9996	≥ 0.995 [23]
Vxo (%)		4.5386	3.6479	1.9287	1.3861	1.4513	≤ 5% [23]
LOD (ppm)		3.7281	3.6148	3.3361	3.3735	3.4512	
LOQ (ppm)		11.2973	10.9540	10.1094	10.2227	10.4581	

3.2.1. Selectivity

Method selectivity was evaluated through a comprehensive comparison of chromatograms obtained from the mobile phase, solvent blanks (acetonitrile and acetonitrile–water mixture), H_2O_2 standard solutions, cosmetic matrix blanks, and cosmetic samples spiked with H_2O_2 after derivatization with triphenylphosphine (TPP). Chromatographic evaluation showed that neither pure acetonitrile nor the acetonitrile–water solvent mixture produced detectable peaks at the retention time corresponding to TPPO. In addition, no significant response was observed at the TPPO detection wavelength that could potentially interfere with analyte quantification.

Furthermore, the TPPO peak obtained from derivatized spiked cosmetic samples was well resolved and clearly separated from other peaks originating from the sample matrix. No interfering peaks were detected at, or in close proximity to, the TPPO retention time across all investigated column temperatures. The UV spectra of the TPPO peak from spiked samples were also consistent with those of the TPPO reference standard, providing additional confirmation of analyte identity.

Overall, these results demonstrate that the proposed HPLC–PDA method exhibits adequate selectivity for the determination of H_2O_2 in hair cosmetic matrices, and that variations in column temperature within the evaluated range do not compromise the method's selectivity.

3.2.2. Accuracy (Recovery)

The recovery values at ambient temperature exceeded the acceptable range (90–107%), indicating failure to meet the accuracy criterion. Instability in recovery at ambient is likely attributable to uncontrolled thermal fluctuations. Small temperature shifts (1–2 °C) induced by environmental factors, such as air conditioning, instrument heat, or general laboratory conditions can subtly influence the viscosity of the mobile phase and the extent of analyte–stationary phase interactions. For a short-retention analyte like TPPO, even minor variations in elution strength or mobile-phase density can produce notable changes in peak area or shape, particularly at low concentrations [24].

Temperatures around 35 °C also displayed instability, likely because this intermediate range does not provide sufficiently controlled thermal conditions. At this temperature, the column may still experience internal temperature gradients and pressure fluctuations, compromising retention and baseline stability, which is reflected in the inconsistent recovery values. At 40 °C to 50 °C, all recovery values met the acceptance criteria, demonstrating stable and reliable accuracy across concentration levels.

3.2.3. Precision (%RSD)

All temperatures produced %RSD values below 2% across the three concentration levels, demonstrating that the method maintained excellent precision regardless of column temperature. This indicates that temperature variations did not significantly influence analytical repeatability.

3.2.4. Linearity, Vxo, LOD, and LOQ

The method demonstrated strong linearity at all temperatures ($r \geq 0.995$). Vxo values were below 5%, confirming acceptable relative variance of the regression model. Furthermore, the LOD and LOQ values were lowest at 40 °C, and remained lower at 45 and 50 °C compared with ambient and 35 °C, indicating that method sensitivity improved noticeably under higher and more thermally stable column temperatures.

3.3. Determination of the Optimum Column Temperature

All evaluated column temperatures in the range of 40–50 °C satisfied the system suitability requirements and fulfilled all method validation acceptance criteria, including accuracy, precision, linearity, sensitivity, and chromatographic efficiency. These results indicate that the HPLC–PDA method is robust and applicable across this temperature interval.

Although column temperatures of 40 °C, 45 °C, and 50 °C all provided satisfactory analytical performance, the selection of the optimum temperature was based on overall method robustness rather than on isolated performance indicators. To support this evaluation, statistical analysis was applied to assess the influence of column temperature on chromatographic behavior and validation parameters. Factorial analysis of recovery data demonstrated that column temperature had a statistically significant effect on method accuracy ($p < 0.05$), whereas analyte concentration did not show a significant influence within the validated range. Post hoc analysis indicated that recovery values at 40 °C and 45 °C did not differ significantly, while all recovery results obtained at 40–50 °C remained within the accepted interval. These findings confirm that the tested temperature range is analytically acceptable, and that statistical significance should be interpreted in the context of method consistency rather than as an indication of superiority.

From a practical standpoint, 40 °C provided consistently acceptable accuracy, precision, linearity, and sensitivity across all validation tests, without approaching the upper recovery limit at lower concentration levels. Moreover, 40 °C represents the lowest temperature within the validated range (40–50 °C) that achieved full compliance with all system suitability and validation criteria, offering improved energy efficiency compared with higher temperatures. This temperature also aligns well with typical tropical laboratory conditions (32–34 °C), ensuring greater thermal stability with minimal environmental fluctuation. In accordance with green analytical chemistry principles, operation at 40 °C minimizes energy consumption while maintaining analytical performance. Considering chromatographic performance, validation results, statistical evaluation, and practical

applicability under tropical conditions, 40 °C is therefore recommended as the optimum column temperature for H_2O_2 determination using the HPLC–PDA method.

4. CONCLUSION

An HPLC–PDA analytical method using triphenylphosphine (TPP) derivatization was successfully optimized and validated for the determination of hydrogen peroxide (H_2O_2) in hair cosmetic formulations. Wavelength optimization confirmed that the TPPO derivative exhibits a stable maximum absorbance at 222 nm within the working concentration range of 20–120 ppm, establishing this wavelength as appropriate for reliable detection.

Investigation of column temperature under tropical laboratory conditions demonstrated that column temperatures in the range of 40–50 °C fulfilled all system suitability requirements and met the acceptance criteria for method validation parameters, including accuracy, precision, linearity, sensitivity, and chromatographic efficiency. These findings indicate that the developed method is robust across this temperature interval.

Among the validated conditions, 40 °C was selected as the optimum column temperature based on its overall analytical performance. At this temperature, the method provided consistently acceptable separation efficiency, symmetrical peak shapes, stable retention times, and reproducible detector responses, while avoiding unnecessary energy consumption associated with operation at higher temperatures. In addition, 40 °C offers greater practical suitability for routine analysis in tropical laboratories, where ambient temperature control is inherently limited.

Method validation across the 20–120 ppm range demonstrated excellent linearity ($r \geq 0.995$ and $Vxo \leq 5\%$), high precision (%RSD within acceptance limits), appropriate limits of detection and quantification, and satisfactory accuracy as evidenced by recoveries within the accepted range of 90–107%. These results confirm that the method is suitable for quantitative determination of H_2O_2 in cosmetic matrices.

Overall, the HPLC–PDA method employing TPP derivatization, optimized at a column temperature of 40 °C and a detection wavelength of 222 nm, meets all analytical validation requirements and demonstrates stability under tropical laboratory conditions. The method is therefore suitable for routine, accurate, and reproducible determination of hydrogen peroxide in hair cosmetic products.

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