A Novel UV-VIS Spectroscopic Approach for the Development and Validation of Benzoyl Peroxide Quantification Methods

Nguyen Thi Thuy Khue[1]*, Nguyen Thanh Tam[1], Vu Thuy Dung[1], Pham Thi Phuong Thao[1], Pham Phuong Dung[1], Tran Thi Luong[1]

ABSTRACT

Objective: Benzoyl peroxide (BPO) is a widely used active ingredient in acne treatments, recognized for its antibacterial and exfoliating properties. This study aims to quantitatively determine the concentration of BPO in an acne cream using UV-Vis spectrophotometry. ***Methods:*** the method involves dissolving the cream in a suitable solvent and measuring its absorbance at a specific wavelength. The UV-Vis technique has demonstrated accuracy and reliability quantifying BPO in commercial formulations. This method is suitable for use in quality control and formulation standardization in the pharmaceutical and cosmetic industries. ***Results:*** the UV-Vis method was used to determine the concentration of BPO in the product. This quantitative analytical method was validated according to ICH guidelines at a wavelength of 235nm, range of determination is 4.8 µg/ml to 7.2 µg/ml. ***Conclusion:*** the proposed method for analyzing BPO content is simple, highly reliable and well-suited for use in laboratory settings.

Keywords: *Benzoyl peroxide, BPO, UV-Vis, acne treatments.*

[1] *Hai Phong University of Medicine and Pharmacy, Haiphong, Vietnam*

* **Corresponding author**
Nguyen Thi Thuy Khue
Email: nttkhue@hpmu.edu.vn

Received: December 15, 2024
Reviewed: December 17, 2024
Accepted: December 28, 2024

INTRODUCTION

Benzoyl peroxide (BPO) is a commonly utilized topical medication known for its effectiveness in treating acne and other skin conditions. As an organic compound with strong antibacterial and keratolytic properties, it works by reducing the number of acne-causing bacteria on the skin and promoting the shedding of dead skin cells that can clog pores. Available in various formulations such as gels, creams, and face washes, BPO is commonly used alone or in combination with other acne treatments. While generally well-tolerated, it can sometimes cause dryness, redness, or irritation, particularly when first introduced into a skincare routine. Its accessibility, proven results, and versatility have made BPO a staple ingredient in dermatology for managing mild to moderate acne [1].

Heckmann et al. (2019) found that topical clindamycin, BPO, and their combination do not fully eliminate Cutibacterium acnes from the dermal layer, indicating the need for further research into post-surgical infection control [2]. Jay & Surbhi (2018) introduced a sensitive spectrophotometric method to detect BPO in wheat flour using a potassium iodide reaction, peaking at 580 nm [3]. Ponhong et al. (2015) developed a similar fast and effective spectrophotometric method for food safety monitoring [4]. Xu et al. (2013) formulated a BPO hydrogel targeting bacteria associated with bacterial vaginosis, demonstrating promising antibacterial activity [5]. Kaushik et al. (2012) explored BPO's effect on natural sisal fibers, improving their properties for industrial use via morphological and thermal modifications [6]. Mu et al. (2011), Wang et al. (2010), Abe Onishi et al. (2004), Saiz et

al. (2001) emphasized the need for accurate BPO detection in flour due to potential health risks, using HPLC and UV-Vis spectrophotometry methods [7-10]. Wankhede et al. (2012) validated a method for simultaneously estimating erythromycin and BPO in gel formulations, confirming its accuracy and reliability [11]. Gupta et al. (2009) designed a simple spectrophotometric method for BPO and tretinoin detection in formulations. Nokhodchi et al. (2005) investigated how formulation type affects BPO release using microsponges and HPLC, showing variation in release rates. Leyden et al. (2008) demonstrated that a 6% BPO cleanser reduced antibiotic-resistant P. acnes strains, emphasizing its antibacterial potential [12].

The BPO research landscape in Vietnam is also quite dynamic. In 2020, Van-Ha Nguyen et al reported notable findings. There study established an HPLC method for the simultaneous quantification of Adapalene (AP) and BPO. The sample analyzed a gel formulation containing 0.1% AP and 2.5% BPO), prepared at the Laboratory of the School of Medicine, National University of Ho Chi Minh City [13]. Chromatographic analysis was carried out using a Shimadzu HPLC system with a C18 RP column (250 x 4.6 mm, 5 µm), with an injection volume of 20 µL, detection wavelength 270 nm, flow rate 1 ml/min, column temperature 30°C, and a mobile phase composed of acetonitrile, tetrahydrofuran, and 0.1% formic acid in water (42:32:26). The method satisfied all ICH validation criteria, including system suitability, specificity, linearity, accuracy, and precision. It was applied to determine the concentrations of both active pharmaceutical ingredients in the gel product: 101.38 ± 0.87% for AP and 95.56 ± 0.04% for BO, both falling within the USP 41 specifications.

Building on these preliminary results, we aim to contribute meaningfully to the study of BPO quantification using the UV-Vis spectrophotometric method. This technique offers several advantages, including simplicity, ease of implementation, low cost, and suitability for research in small-scale laboratory settings. This study was carried out with the following objectives: (1) development of analytical methods utilizing UV-VIS spectroscopy; (2) validation of the method for the quantification of BPO.

MATERIALS AND METHOD

Materials and machines

- BPO standard 98%, 10% BPO- containing Acne creams, ethanol 96%, acid acetic and toluene were purchased from sigma- Aldrich and local pharmacies.
- UV-VIS spectrophotometer Aligent Carry 60, Hanna pH meter, Ohaus balance, Memmert thermostatic bath.

Experimental investigation

Preparation test solutions

Mix 10 tubes of cream (almost 500g) purchased from different pharmacies in a 1L beaker; Weigh 600mg of acne cream 10% gel (equivalent to 60mg of BPO) into a 100ml beaker; add 30ml of 96% ethanol, and sonicated for 3mins; then, transfer the resulting solution to a 100ml volumetric flask; dilute to volume with 96% ethanol up to the mark; filter and collect the filtrate.

From this filtrate, take 10ml and transfer it into another 100ml volumetric flask; dilute to volume with 96% ethanol to obtain 60µg/ml solution; to prepare 6µg/ml solution, take 10ml of 60µg/ml solution, transfer it into a separate 100ml volumetric flask, and dilute to volume with 96% ethanol.

Preparation of standard samples

Dissolve 60mg of BPO standard in 96% ethanol and dilute to volume in a 100ml volumetric flask. Then, transfer 10ml solution into another 100ml volumetric flask and dilute to volume with 96% ethanol to obtain standard 60µg/ml solution. To prepare standard 6µg/ml solution, dilute 10 ml of standard 60µg/ml solution to 100 ml with 96% ethanol.

Blank and placebo samples

- Blank sample is ethanol 96%
- Placebo samples: The amount of excipients corresponding to 60 mg of BPO was dissolved in 100 mL of ethanol, and the resulting solution was then diluted 100-fold

Results and discussion

Procedure

- Scan the UV-Vis absorption spectra of the samples (blank, placebo, test solutions and standard solution) over the wavelength range of 220-250nm following the guidelines of the European Pharmacopoeia and the British Pharmacopoeia; determine the peak wavelength (λ_{max}) observed under standard conditions, including pH 7.0, ethanol solvent, and room temperature.
- Measure the absorbance (A) of blank sample at 2 λ_{max}, subtracting the background; perform the same procedure for other samples.
- Calculate the mean value ($\bar{x}$) and the relative standard deviation (RSD%) using the following formulas:

Mean ($\bar{x}$):

$$\bar{x} = \frac{\sum x_i}{n}$$

Where xi is each measured value, and n is the number of measurements.

Standard Deviation (SD)

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

Relative Standard Deviation (RSD%):

$$RSD\% = \frac{SD}{\bar{x}} \times 100\%$$

RESULTS

Determine maximum wavelength (λ_{max})

BPO contains an aromatic ring structure, which gives it strong UV absorption properties, forming the basis for its quantification using UV-Vis spectroscopy. Moreover, UV-Vis spectroscopy is one of the most commonly used techniques in pharmaceutical analysis due to its simplicity, cost-effectiveness and ease of implementation.

Measure the absorbance values of standard and test solutions of concentration 6 µg/ml. The spectral range is 220–250nm. Compare the maximum absorption wavelength (λ_{max}) of the standard and test samples.

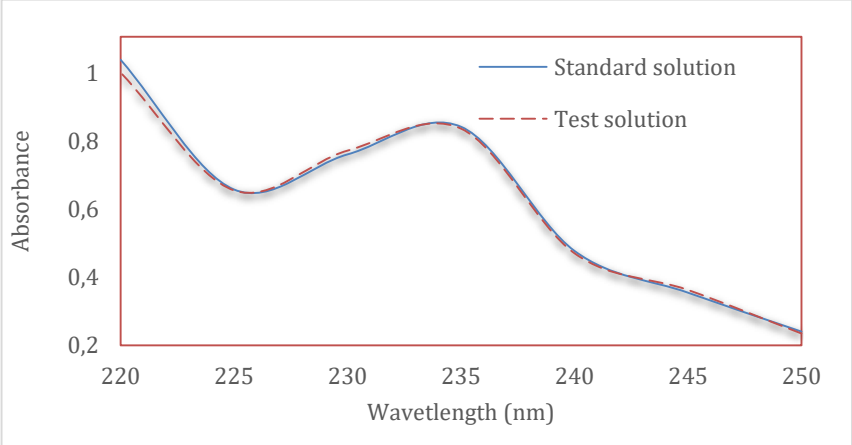

Figure 1. *Graph of absorbance versus wavelength for a BPO solutions 6µg/ml obtained using the UV-Vis spectrophotometry technique*

Based on the results shown in Figure 1, the wavelength maxima are observed at 235nm for solution 6µg/ml.

Validation

Method validation is the process of confirming, through testing and objective evidence, that a method meets its intended requirements (i.e., it is fit for purpose). According to the regulations of the ICH, the parameters that must be validated for chemical analytical methods include: specificity/selectivity, linearity and calibration curve, precision (repeatability and reproducibility). The selection of validation parameters depends on the technique used in the method, the method's intended purpose, and the conditions and resources of the testing laboratory. In each specific case, the appropriate validation parameters may vary [14].

Specificity

Record the UV-Vis absorption spectra of the blank, test, and reference samples across the wavelength range of 220–250nm; record the absorption peaks observed in each of the three spectra, along with the corresponding absorbance values; determine the absorption ratios for the reference sample, test sample, and blank sample; requirement: the UV-Vis spectra of the test sample and the reference sample must correspond to each other. The blank sample should not display any absorption peaks corresponding to those of the test sample.

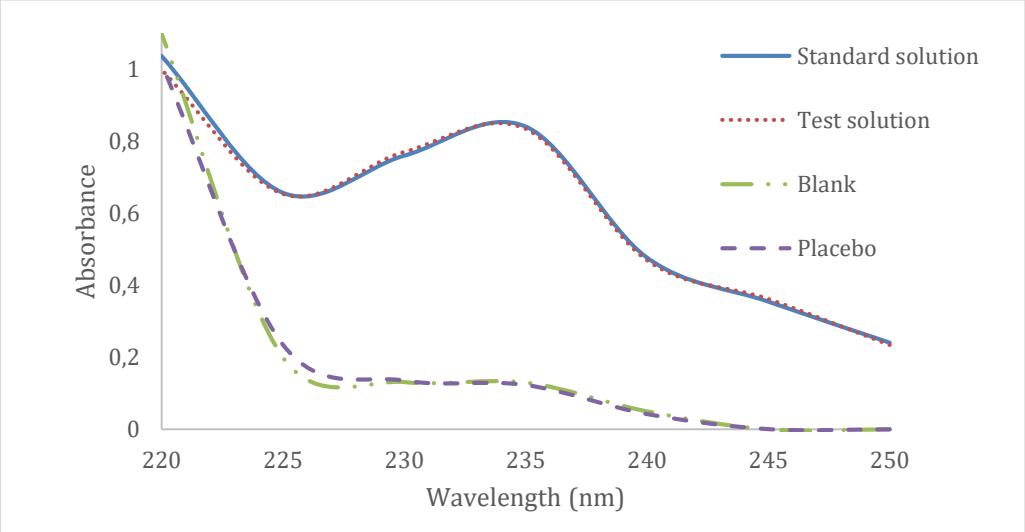

Figure 2. *Graph of absorbance versus wavelength for a 4 samples obtained using the UV-Vis spectrophotometry technique*

Table 1. *Results of specificity test at 235nm wavelength*

No	Samples	A
1	Standard solution	0.8368
2	Test solution	0.8366
3	Blank	-
4	Placebo	-

The results are as follows: the blank and placebo did not exhibit a peak at 235nm, and their absorbance is almost zero (below 2%). The UV-Vis spectra of the test sample and the standard

sample correspond to each other. So conclusion is as the quantitative method meets the specificity requirements.

Linearity

Measure the absorbance (A) of blank sample at the maximum wavelength 235nm, subtracting the background; perform the same procedure for standard samples with concentrations of 4.8; 5.4; 6.0; 6.6 and 7.2µg/ml; construct a linear regression equation between BPO concentration and absorbance and determine the linear concentration range of BPO. Requirement: The correlation coefficient (R^2) must meet the specified acceptance criteria: $R^2 \geq 0.995$ and $Y_{intercept} < 2\%$

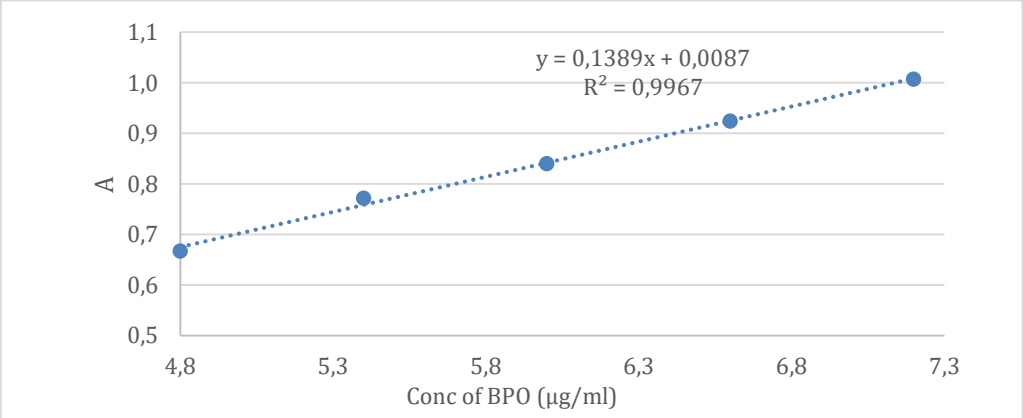

Figure 3. *Linearity*

Table 2. *Results of linearity*

No	Conc of BPO (µg/ml)	A
1	4.800	0.6672
2	5.401	0.7714
3	6.000	0.8400
4	6.600	0.9240
5	7.200	1.0075
	y = 0.1389x + 0.0087	
	R^2 = 0.9967	
	$Y_{intercept}$ = 0.87%	

The relationship between the concentration of the standard solutions and the absorbance was linear, with a correlation coefficient (R^2) ≥ 0.995, and the y-intercept was negligible. The quantitative method meets the linearity requirements. The linear range is from 4.8µg/ml to 7.2µg/ml.

Accuracy

The absorbance (A) values obtained at a wavelength of 235nm correspond to the concentrations of the standard samples, as shown in the Table 3. The linear regression equation y = 0.1389x + 0.0087 was used to determine the recovered BPO content; requirement: the recovery percentage should fall within the range of 98–102% for all tested concentrations.

Calculate the recovery percentage using the following formula:

Recovery (%) = (Conc of BPO recovery / Conc of BPO) × 100%

Table 3. *Result of accuracy*

No	Conc of BPO (µg/ml)	A	Conc of BPO recovery (µg/ml)	Recovery (%)
1	4.801	0.6674	4.742	98.78
2	4.805	0.6679	4.746	98.77
3	4.811	0.6736	4.787	99.73
	Mean	**0.6696**		
	RSD(%)	**0.51%**		
1	6.000	0.8400	5.986	99.76
2	6.010	0.8366	5.961	99.19
3	6.014	0.8414	5.996	99.70
	Mean	**0.8393**		
	RSD(%)	**0.30%**		
1	7.201	1.0177	7.257	100.77
2	7.203	1.0075	7.184	99.73
3	7.209	1.0186	7.263	100.75
	Mean	**1.0146**		
	RSD(%)	**0.61%**		

Results: RSD% = 0.51% (4.8µg/ml), 0.30% (6.0µg/ml), and 0.61% (7.2µg/ml), all <2%; recovery rate range from 98% to 102% for each concentration. The quantitative method satisfies the accuracy requirements, with acceptable accuracy demonstrated across the 4.8µg/ml to 7.2µg/ml.

Precision

Photometrically measure the absorbance (A) of 6 samples at 235nm, subtracting the background. The relative standard deviation (RSD%) must be less than or equal to 2% (RSD ≤ 2%).

Table 4. *Result of precision*

No	Conc of BPO (µg/ml)	A
1	5.995	0.8399
2	5.989	0.8380
3	5.999	0.8388
4	6.000	0.8400
5	6.010	0.8366
6	6.014	0.84144
Mean	6.001	0.8391
RSD(%)		**0.20%**

The result is 0.2% RSD, which is <2%, so the precision is conformed for the 6.0(µg/ml) test solution content.

Range of determination

The range of determination is derived from the combined results of the linear range (3.2.2) and accuracy (3.2.3). Based on the findings from these two sections, the determination range is 4.8µg/ml to 7.2µg/ml.

DISCUSSION AND CONCLUSIONS

To determine the appropriate wavelength for measuring the BPO solution, a scan was performed from 220 to 250nm using ethanol 96% as the blank. The wavelength of maximum absorption for BPO was identified at 235nm. Evaluate the analytical method to meet the following criteria: specificity, linearity and range determination 4.8 µg/ml to 7.2 µg/ml, accuracy with RSD <2% and recovery rates range from 98% to 102%, precision with RSD <2%.

Additionally, we performed a BPO quantification test using titration. The procedure was as follows: A measured quantity of the preparation was mixed with 50mL of acetone, then diluted with additional acetone to a final volume of 100mL and filtered. To the filtrate, a 20% potassium iodide solution was added, the mixture was stirred, covered, and left to stand for 15mins protected from light. Acetone was added again, and the solution was titrated with 0.01M sodium thiosulfate, using starch as an indicator added at the end of the titration. This procedure was repeated twice. Each milliliter of 0.01M sodium thiosulfate corresponds to 1.211mg of $C_{14}H_{10}O_4$. The results obtained are quite promising and can be continued in future studies.

Acknowledgements

The authors thank patients and colleagues who kindly supported this study.

Conflick of interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Sources of funding

None.

Consent

Written consent has been obtained from the collaborators for publication of the accompanying report. A copy of the written consent is available for review by the Editor-in-Chief of this journal upon request.

REFERENCES

1. Taraneh Matin, Marcus B. Goodman. Goodman. Benzoyl Peroxide - StatPearls - NCBI Bookshelf, October 10, 2022.
2. Nethanael Hekmann, M., (209). Cutibacterium acnes persists despite topical clindamycin and benzoyl peroxide. J. of shoulder and elbow surgery, 1-5.
3. Dave, Jay & Benjamin, Surbhi. (2018). Rapid Spectrophotometric Methods for the Determine Benzoyl Peroxide from the Wheat Flour. 10.13140/RG.2.2.10371.14883.
4. Kraingkrai ponhong, s.-a. s. (2015). A rapid and sensitive spectrophometric method for the determination of benzoyl peroxide in wheat flour sample. journal food and drug analysis, 652-659.
5. Xu S, Cavera VL, Rogers MA, Huang Q, Zubovskiy K, Chikindas ML. Benzoyl peroxide formulated polycarbophil/carbopol 934P hydrogel with selective antimicrobial activity, potentially beneficial for treatment and prevention of bacterial vaginosis. Infect Dis Obstet Gynecol. 2013; 2013:909354. doi: 10.1155/2013/909354. Epub 2013 Dec 7. PMID: 24382940; PMCID: PMC3870611.
6. Vijay K. Kaushik, Anil Kumar, Susheel Kall. Effect of Mercerization and Benzoyl Peroxide Treatment on Morphology, Thermal Stability and Crystallinity of Sisal Fibers. international Journal of Textile Science p-ISSN: 2325-0119 e-ISSN: 2325-0100 2012; 1(6): 101-105 doi: 10.5923/j.textile.20120106.07
7. Mu G, Liu H, Gao Y, Luan F. Determination of benzoyl peroxide, as benzoic acid, in wheat flour by capillary electrophoresis compared with HPLC. J Sci Food Agric. 2012 Mar 15;92(4):960-4. doi: 10.1002/jsfa.4677. Epub 2011 Oct 14. PMID: 21997699
8. Qihui Wang, Wenzhen Shi, Caiyun Hou. Determination of benzoyl peroxide

content in wheat products by high-performance liquid chromatography. Journal of Food Processing and Preservation, ISSN: 0145-8892, Vol: 34, Issue: 3, Page: 414-424 Publication Year2010

9. Yukkiko Abo-Onishi, C. Y. (2004, January). Determination of benzoyl peroxide and benzoic acid in wheat flour by high performance liquid chromatography and its identification by high performance liquid chromatography -mass spectrometry. Journal of chromatography A, 209-204
10. A.I.Saiz, G. M. (2001). Determination of benzoyl peroxide and benzoic acid level by HPLC during Wheat flour bleaching process. j.Agric .food Chem, 98-102.
11. Rohini Wankhede, S. B. (2012, may). Analysis of erythromycin and benzoyl peroxide in combined dosage form by uv-visible spectrometry. International journal of pharmacy and pharmaceutical sciences, 4, 527-531.
12. Ankush Gupta, M. G. (2009). A validated uv spectrometric method for simultaneous estimation of tretinoin and benzoyl peroxide in bulk and semi solid dosage form. Rasaj.chem2, 649-654.
13. Nguyen Van Ha, Pham Toan Quyen, et al. Development and validation of a HPLC method for simultaneous quantitative determination of adapalene and benzoyl peroxide in gel products. Science and Technology Development Journal: Health Sciences, 2020.
14. Brianna Cassidy, Timothy Bloomingdale, Judy Carmody, Navigating ICH Q2(R2) compliance in analytical method validation: A gap analysis toolkit to streamline risk assessment and change management, Journal of Pharmaceutical Sciences, Volume 114, Issue 6, 2025, 103749, ISSN 0022-3549.