***JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA)**, Vol. 9, No.1, 2024* *pp. 1-14*
Chemistry Education Study Program, Universitas Sebelas Maret *ISSN 2503-4146*
https://jurnal.uns.ac.id/jkpk *ISSN 2503-4154 (online)*

SIMULTANEOUS DETERMINATION OF ISONIAZID AND PYRIDOXINE HYDROCHLORIDE IN TABLET DOSAGE FORMS USING RATIO SUBTRACTION SPECTROPHOTOMETRY

Rida Evalina Tarigan [1,*], Ester Lince Fiani Mendrofa [1], Chemayanti Surbakti [2]

[1] *Departement of Pharmaceutical Chemistry, Faculty of Pharmacy and Health, Institut Kesehatan Helvetia, Medan, Indonesia*
[2] *Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*

ARTICLE INFO

Keywords:
Isoniazid;
pyridoxine hydrochloride;
ratio subtraction;
spectrophotometry;

Article History:
Received: 2023-08-21
Accepted: 2024-01-03
Published: 2024-27-02

**Corresponding Author*
Email:
ridaevalinatarigan@helvetia.ac.id
doi:10.20961/jkpk.v9i1.78154

ABSTRACT

The study focused on the simultaneous quantification of Isoniazid (INH) and Pyridoxine Hydrochloride (PRD) in tablet form, commonly used in antituberculosis treatments. Assessing the accurate concentration of both INH and PRD in tablets is vital to ensure their effectiveness, safety, and quality. Using the ratio subtraction spectrophotometry method, the study analyzed INH and PRD in Pehadoxin forte® tablets (batch no. 36057007, PT. Phapros, Indonesia), with 0.1 N HCl as the solvent. The method involved obtaining ratio absorption spectra by dividing the absorption spectra of INH and PRD, respectively, to derive zero-order spectra for each drug. Method validation parameters included linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ). The results showed linearity values of 0.9985 for INH and 0.9988 for PRD. Accuracy was 98.1838% for INH and 100.0205% for PRD, while precision was 1.8769% for INH and 0.2037% for PRD. LOD and LOQ for INH were 0.8116 µg/mL and 2.7053 µg/mL, respectively, and for PRD, 1.3127 µg/mL and 4.3757 µg/mL. The levels of INH and PRD in the tablets were found to be 102.1157% and 101.3874%, aligning with the Indonesian Pharmacopoeia's standards. This methodological approach provides a reliable analytical tool for the simultaneous assessment of INH and PRD in tablets, potentially extendable to other drug combinations and formulations, thereby contributing to pharmaceutical quality control processes.

How to cite: R.E. Tarigan, E. L. F. Mendrofa, C. Surbakti, " Simultaneous Determination of Isoniazid and Pyridoxine Hydrochloride in Tablet Dosage form by Ratio Subtraction Spectrophotometry" *JKPK (Jurnal Kimia dan Pendidikan Kimia),* vol. 9, no. 1, pp.1 -14, 2024. http://dx.doi.org/10.20961/jkpk.v9i1.78254

INTRODUCTION

Isoniazid (INH) and Pyridoxine Hydrochloride (PRD), available in tablet form, are key medications in the treatment of tuberculosis (TB). INH's effectiveness stems from its unique action mechanism, inhibiting the enzyme enoyl acyl carrier protein reductase (InhA), crucial for mycolic acid biosynthesis in Mycobacterium tuberculosis. This inhibition leads to the weakening and eventual death of the bacterial cell wall, with selective bactericidal activity against mycobacteria. Its use in combination therapies prevents the development of drug-resistant strains, and as a prophylactic, it helps control the spread of TB. The INH mechanism disrupts a critical bacterial process, enhancing its efficacy in TB treatment [1, 2]. Concurrently, PRD administration can reduce the risk of peripheral neuropathy, a common side effect

of INH. PRD's primary function is to replenish low levels of the active vitamin B6 coenzyme, pyridoxal phosphate, thereby offering neuroprotective activity essential for maintaining nerve function and alleviating symptoms caused by INH-induced peripheral neuropathy [3-5].

Determining drug levels is critical in monographs (quality standards) to ensure drug stability. Accurate drug concentration assessment, as stipulated in monographs, is essential for ensuring that each drug dose contains the correct amount of active ingredient. This is pivotal for maintaining drug stability and efficacy, as incorrect dosages could lead to adverse reactions or suboptimal therapeutic outcomes [6,7]. Various methods like HPLC, voltammetry, HPTLC, spectrophotometry, and ultra-fast liquid chromatography are utilized to determine INH and PRD concentrations, individually or in combination. Each method has its drawbacks; for instance, HPLC and HPTLC are costlier [4, 9], voltammetry's sensitivity depends on the electrode properties [8], spectrophotometric measurements can be skewed by interfering substances [10-12], and ultra-fast liquid chromatography is also expensive [13]. The choice of analytical method hinges on specific requirements like sensitivity, selectivity, and cost. In this context, the ratio subtraction spectrophotometry method is favored for its selectivity and cost-effectiveness [14].

The simultaneous determination of Isoniazid (INH) and Pyridoxine Hydrochloride (PRD) in tablet preparations is critical for ensuring the medications' efficacy, safety, and quality. This process provides a detailed understanding of the drug composition, facilitating accurate dosing, treatment success, and improved patient safety in managing tuberculosis and related conditions. Ratio subtraction, a spectrophotometric technique, is valuable for analyzing multiple substances simultaneously without prior separation, even with overlapping adjacent wavelengths. This method is convenient for routine analysis as it doesn't require derivatization, offering practicality and accuracy. It's efficient, needing minimal preparation time, fewer solvents, and straightforward mathematical calculations. The ratio subtraction approach presents a significant alternative to traditional spectrophotometric methods, especially when enhanced selectivity and sensitivity are required [15].

INH and PRD are suitable for spectrophotometric analysis due to conjugated double bonds, chromophores, and auxochromes in their structures, facilitating light absorption at specific wavelengths. This property allows for the quantitative analysis of these compounds [17, 18]. The rationale for employing ratio subtraction spectrophotometry in the simultaneous determination of INH and PRD in tablet form lies in addressing the unique analytical challenges associated with conventional spectrophotometric methods. Potential benefits include improved selectivity, sensitivity, and adaptability to complex formulations. This makes it a promising technique for concurrent analyses. This study aimed to establish a robust analytical method for measuring INH and PRD in tablet dosage forms simultaneously.

METHODS

1. Materials:

The study utilized high-quality raw materials of Isoniazid (INH) and Pyrazinamide (PRD), sourced from the Indonesian Food and Drug Monitoring Agency, ensuring the authenticity and purity of the compounds.

Hydrochloric acid (HCl) supplied by Merck at a concentration of 0.1 N was used as a solvent to prepare the standard solutions of these compounds. Additionally, Pehadoxin forte® tablets, each containing 400 mg of INH and 10 mg of PRD, were chosen for the analysis. PT manufactured these tablets. Phapros Indonesia provides a practical sample for testing the analytical method in a real-world pharmaceutical formulation.

2. Equipment:

The analytical procedures were carried out using state-of-the-art equipment. A Shimadzu Ultraviolet-visible spectrophotometer 1800, renowned for its precision and reliability in spectrophotometric analysis, was employed for measuring the absorbance of the samples. This spectrophotometer was connected to a computer equipped with UV-Probe 2.43 UV-1800 software, facilitating data acquisition and analysis. Additionally, a highly accurate analytical balance from Sartorius, Gottingen, Germany, was used to weigh the samples, ensuring precise measurement of the compounds.

3. Preparation of INH Standard Solution:

The standard solution of INH was prepared by accurately weighing 50 mg of the com pound and dissolving it in a 50-mL volumetric flask with 0.1 N HCl, yielding a stock solution with a concentration of 1000 µg/mL. A working solution was prepared from this stock solution by diluting 2.5 mL in a separate 25 mL volumetric flask to achieve a 100 µg/mL concentration. This dilution process was critical for obtaining a solution concentration suitable for spectrophotometric analysis.

4. Preparation of PRD Standard Solution:

A similar procedure was followed for the preparation of the PRD standard solution. 50 mg of PRD was accurately weighed and dissolved in 0.1 N HCl within a 50-mL volumetric flask, resulting in a 1000 µg/mL stock solution. Subsequently, 2.5 mL of this stock solution was diluted in a 25 mL volumetric flask to prepare a working solution with a 100 µg/mL concentration suitable for the subsequent analytical procedures.

5. Preparation of INH Absorption Spectra:

To analyze Isoniazid (INH), various solutions with varying concentrations were prepared, specifically from 5 µg/mL to 15 µg/mL. These solutions were then subjected to spectrophotometric analysis, where the absorption spectra were recorded across a wavelength range of 200-400 nm. This step is critical for identifying the characteristic absorption peaks of INH and establishing a baseline for further analysis.

6. Preparation of PRD Absorption Spectra:

Similarly, Pyrazinamide (PRD) solutions were prepared with concentrations ranging from 6 µg/mL to 18 µg/mL. The absorption spectra of these solutions were also measured over the 200-400 nm wavelength range. The spectra obtained provide essential data for distinguishing the unique absorption characteristics of PRD.

7. Preparation of Mixed Spectra of INH and PRD:

This step involved the preparation of a solution containing both INH and PRD, allowing for the simultaneous spectrophotometric analysis of both drugs. The mixed solution's absorption spectrum was then measured, crucial for understanding how the two drugs interact spectrally and discerning overlapping absorption peaks.

8. INH Analysis via Ratio Subtraction Spec trophotometry:

For the analysis of INH, the ratio subtraction spectrophotometry method was employed. This sophisticated technique involves

dividing the INH absorption spectrum by the PRD spectrum (used as a divisor), then subtracting and multiplying by the divisor. This method effectively isolates the INH spectrum from the mixture, enabling accurate analysis and quantification. The process is essential for separating overlapping spectral data, facilitating a clearer understanding of INH's spectral characteristics and concentration in the presence of PRD.

9. PRD Analysis via Ratio Subtraction Spectrophotometry:

For the Pyrazinamide (PRD) analysis, the ratio subtraction spectrophotometry technique was similarly employed, but this time using the Isoniazid (INH) absorption spectrum as the divisor. The method involves a series of mathematical operations on the PRD spectrum, using the INH spectrum as a reference, to effectively isolate and analyze the PRD component in the mixture. This precise analytical approach aids in distinguishing the individual spectral characteristics of PRD, even in the presence of INH.

10. Analysis of Tablet Dosage Form:

To analyze the tablet dosage form containing INH and PRD, tablets were first crushed into a fine powder. An aliquot of this powder, equivalent to 400 mg of INH and 10 mg of PRD, was then dissolved in 0.1 N hydrochloric acid (HCl). The resulting solution was subjected to the ratio subtraction spectrophotometry method for quantifying INH and PRD. This analysis is crucial for verifying the actual content of the drugs in tablet form and ensuring compliance with pharmaceutical standards.

11. Method Validation:

a. **Linearity**:

The study evaluated the linearity of the analytical method for both INH and PRD. Concentrations for INH ranged from 5 µg/mL to 15 µg/mL, while for PRD, they varied from 6 µg/mL to 18 µg/mL. The absorbance of these concentrations was measured, enabling the calculation of linear regression equations and correlation coefficients. This step is crucial to ensure the method produces consistent and proportional results over various concentrations.

b. **Accuracy**:

Accuracy was assessed by adding known quantities of standards to the sample mixtures. Three different concentrations of the analytes (80%, 100%, and 120%) were prepared, each containing 70% of the analyte and 30% of the standard. This approach helps verify whether the method can accurately measure the true value of the analyte concentrations [19, 20].

c. Precision:

The precision of the method, an indicator of the reproducibility of the results, was determined through the relative standard deviation (RSD). A method is considered precise if the RSD is less than 2% [21-23]. This low RSD value indicates that repeated measurements under unchanged conditions yield similar results.

d. **Limit of Detection (LOD)**:

The LOD, the smallest concentration of an analyte that can be reliably detected but not necessarily quantified, was calculated using the formula: LOD = 3.3 × (standard deviation/slope) [24, 25]. The LOD is a critical parameter in assessing the sensitivity of the method.

e. **Limit of Quantification (LOQ)**:

The LOQ represents the lowest concentration of an analyte that can be quantitatively determined with acceptable precision and accuracy. It was calculated using the formula: LOQ = 10 × (standard deviation/slope) [26-28]. The LOQ is essential for determining the minimum concentration level at which the analyte can be confidently quantified.

12. Data Analysis:

The data obtained from the experiments were thoroughly analyzed using statistical methods, specifically the t-test. This analysis assessed the statistical significance of the results, with a confidence level set at 99%. Using the t-test allows for a rigorous evaluation of the method's efficacy in measuring the concentrations of INH and PRD, ensuring that the findings are statistically valid and reliable.

RESULTS AND DISCUSSION

1. Absorption spectra of INH, PRD, and mixtures of INH and PRD

The absorption spectra and calibration curves of INH (5-15 µg/mL) and PRD (6-18 µg/mL) measured at λ 200-400 nm are shown in Figures 1a,b, and 2a,b, respectively, while the overlapping spectra of the mixture are shown in Figure 3.

.

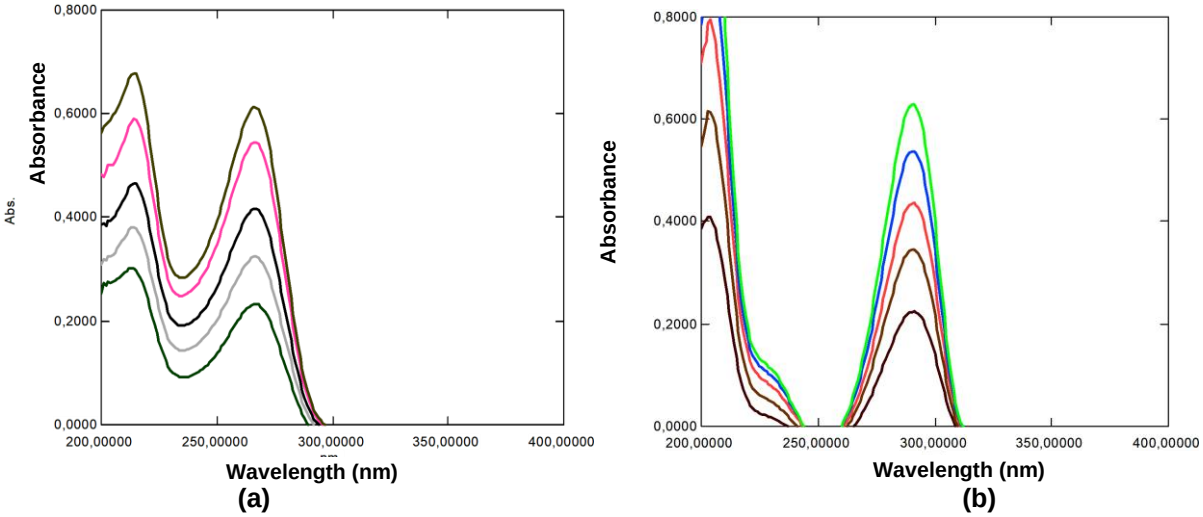

Figure 1. (a) INH Absorption Spectrum (5-15 µg/mL). (b) INH Calibration Curve (5-15 µg/mL).

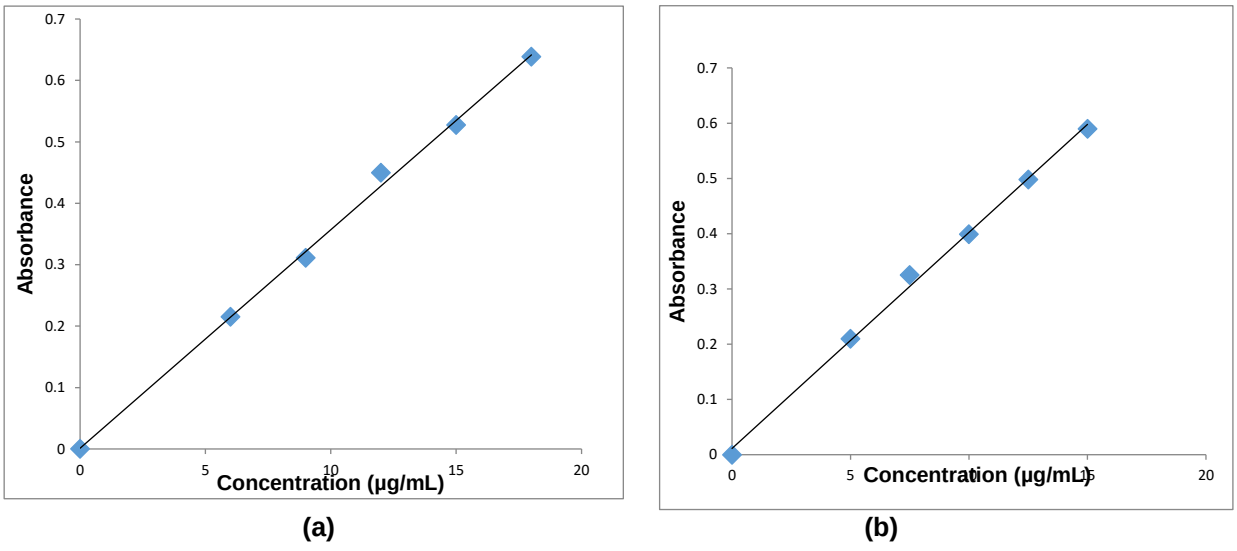

Figure 2. (a) PRD Absorption Spectrum (6-18 µg/mL). (b) PRD Calibration Curve (6-18 µg/mL).

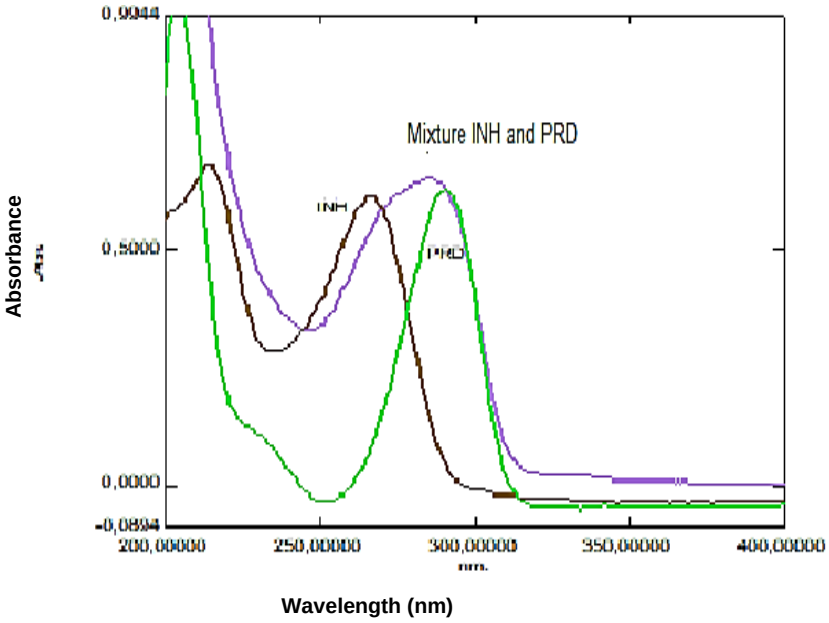

Figure 3. Spectra Overlap INH (15 µg/mL) and PRD (18 µg/mL) and Mixture (INH 15 µg/mL and PRD 18 µg/mL).

Based on Figures 1a and 2a, the absorption spectra and calibration curves of INH (5-15 µg/mL) and PRD (6-18 µg/mL) measured at λ 200-400 nm, respectively, comply with Lambert Beer's law (absorbance 0.2–0.6) [15, 29, 30]. Figures 1b and 2b show that the INH regression equation was Y = 0,0390x + 0,0120 with correlation coefficients 0.9985. The PRD regression equation was Y = 0,0355 + 0,0019, with a correlation coefficient of 0.9988. With a value of r ≤ 1, it can be stated that the relationship between absorbance and concentration is linear [16], meaning the ratio subtraction spectrophotometry method can be used in this study. Figure 3 shows that the spectral overlap of INH (15 µg/mL), PRD (18 µg/mL), and mixture (INH 15 µg/mL and PRD 18 µg/mL) was overcome by using the ratio subtraction spectrophotometry method [15]. The ratio subtraction approach begins with the determination of overlapping spectrum studies. This analysis of wavelength usage for analysis indicates that the initial divisor is used for the ratio subtraction approach, where the initial divisor is employed in the next step [15, 16]

2. INH and PRD Absorption Spectra Using The Ratio Subtraction Spectrophotometry Method

In applying the ratio subtraction technique for spectrophotometric analysis, a crucial step is the creation of a ratio spectrum. This process involves selecting an appropriate dividing concentration, which significantly influences the accuracy and efficacy of the analysis. The divisor concentration is chosen based on the capability to provide the best linear regression, ensuring a reliable separation of overlapping spectral components [15]

Figures 4 and 5 exemplify the outcomes of this technique using specific dividing concentrations for Isoniazid (INH) and Pyrazinamide (PRD). In these figures, INH and PRD were used at concentrations of 15 µg/mL and 18 µg/mL, respectively. These concentrations were selected as the divisors for the ratio spectrum creation.

The selection of 15 µg/mL for INH and 18 µg/mL for PRD as dividing concentrations was based on their effectiveness in providing clear, distinct spectra after the ratio subtraction process. The objective was to achieve spectra that could be easily interpreted and quantified, which is critical for accurate drug analysis in pharmaceutical formulations. By creating ratio spectra at these specific concentrations, the overlapping spectral regions of INH and PRD can be effectively separated. This separation is crucial for accurate spectrophotometric analysis, particularly in complex mixtures where components have closely overlapping spectra. The application of this method facilitates the distinct identification and quantification of each drug component, which is essential for ensuring the quality and efficacy of pharmaceutical products [15].

.

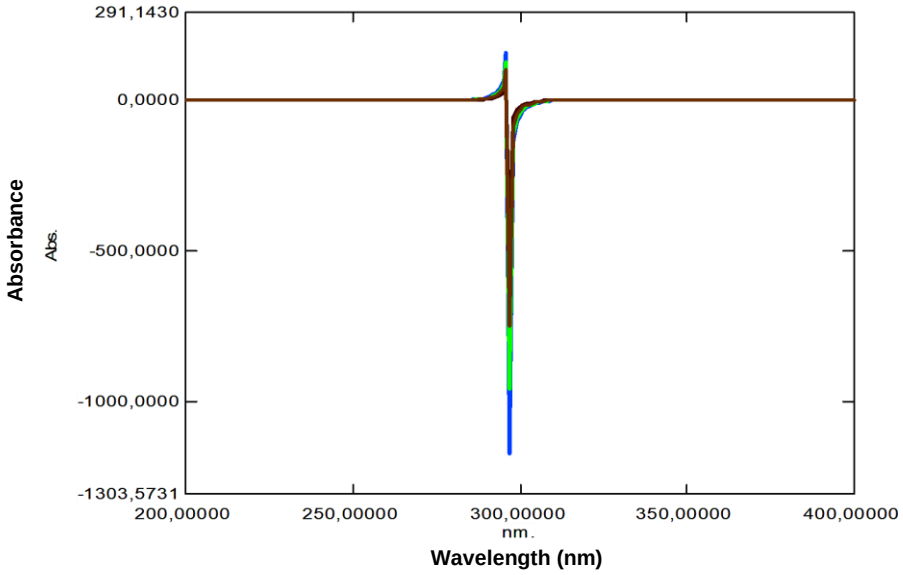

Figure 4. INH Ratio Spectrum as PRD Divider (18 µg/mL).

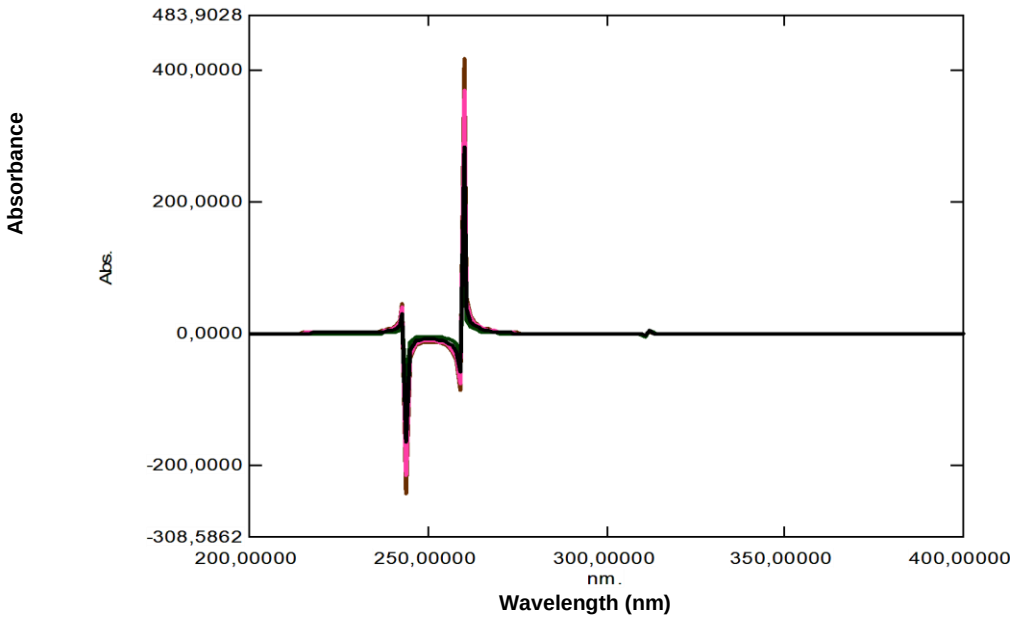

Figure 5. PRD Ratio Spectrum as INH Divider (15 µg/mL).

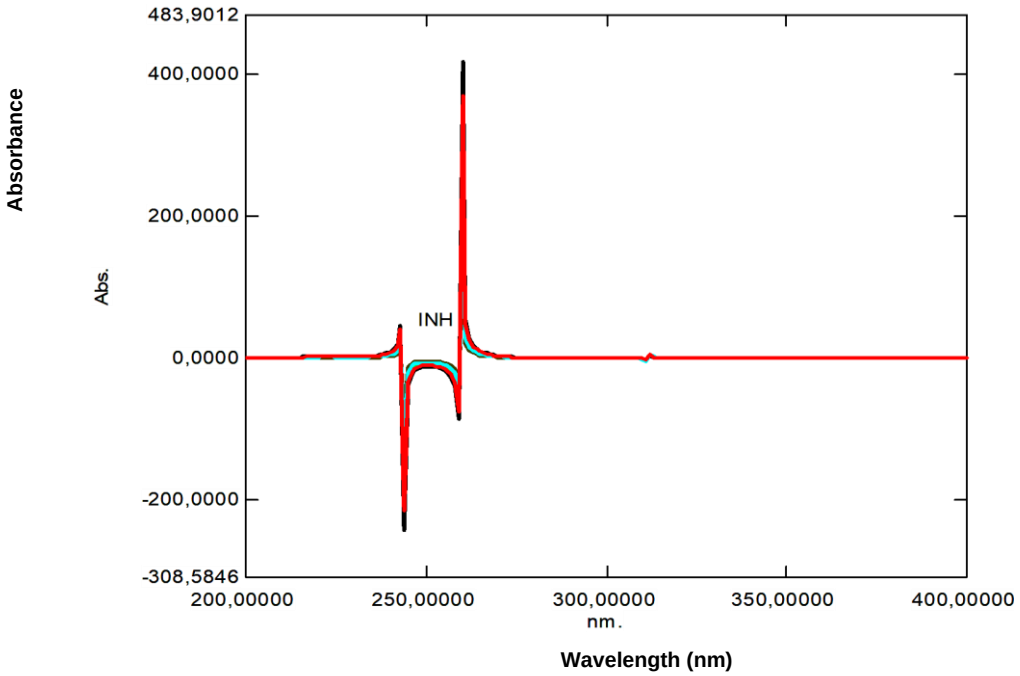

Figure 6. INH Ratio Spectrum as a PRD Divider of 18 µg/mL and after reduction with a PRD divider of 18 µg/mL.

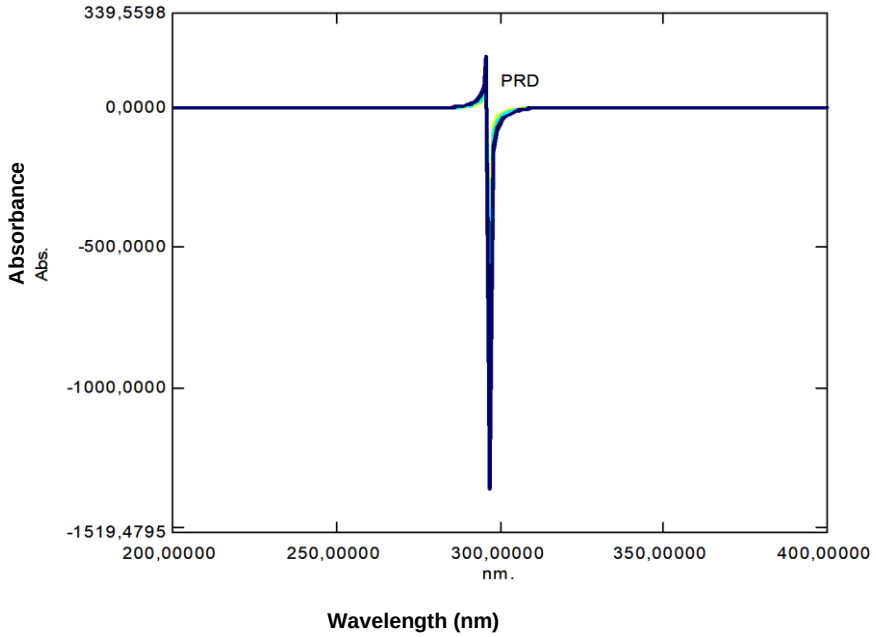

Figure 7. PRD ratio spectrum as a 15 µg/mL INH divider and after reduction with a 15 µg/mL INH divider.

Then, these spectra are reduced by their respective divisors, shown in Figures 6 and 7. After creating the ratio spectra using the ratio subtraction technique, the next step involves multiplying the obtained spectra by the respective dividers. This process is crucial for

converting the ratio spectra back to zero-order spectra, which are more straightforward to analyze and quantify. The zero-order spectrum represents the conventional absorption spectrum of a substance where the absorbance is directly proportional to the concentration.

Figure 8 illustrates the zero-order spectra of INH (Isoniazid) and PRD (Pyrazinamide) obtained through this process. The spectra result from multiplying the ratio spectra by their respective dividers, 15 µg/mL for INH and 18 µg/mL for PRD. This multiplication effectively reverses the initial ratio operation, yielding spectra representative of the original drug compounds in their standard absorption form. The zero-order spectrum is particularly useful in the quantitative analysis of drugs. It allows for the direct correlation of absorbance with concentration, following Beer-Lambert's law. This makes it possible to accurately determine the concentration of INH and PRD in a mixture using the absorbance values obtained from the zero-order spectra. Obtaining zero-order spectra is a critical component of the ratio subtraction spectrophotometry method, enabling precise and accurate analysis of drug compounds with overlapping spectra.

.

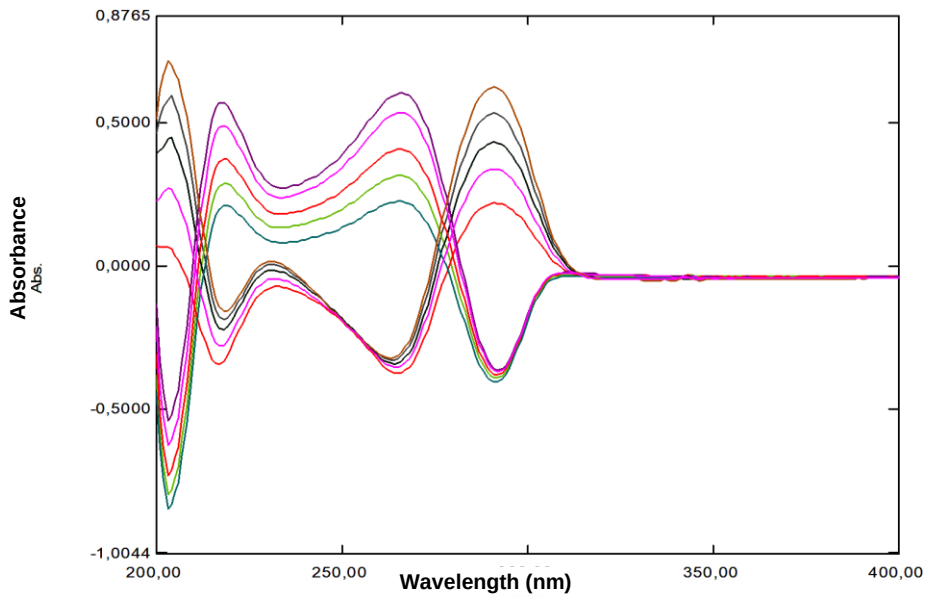

Figure 8. Zero Order Spectrum Of INH and PRD by Subtraction Ratio Method.

The Ratio Subtraction Spectrophotometry method, as depicted in Figures 4-8, represents a systematic approach to analyzing drug mixtures, particularly useful when dealing with components that have overlapping spectra. This method applies to a wide range of pharmaceutical compounds and is crucial for accurately determining the concentration of drugs in a mixture, as exemplified in the analysis of Isoniazid (INH) and Pyrazinamide (PRD). The process begins by dividing the spectrum of the drug mixture by a selected divisor concentration. This step is critical as it sets the stage for the subsequent subtraction. The divisor concentrations for INH and PRD were strategically selected to ensure the best possible resolution and distinction between the two compounds.

After the division, the next step is subtracting the divisor from the divided spectrum. This step is crucial in isolating each component's spectrum from the mixture. It removes the contribution of the divisor from the spectrum, enabling the identification and quantification of the individual components in the mixture.

The final step involves multiplying the subtracted spectrum by the divisor. This multiplication brings the spectrum back to its original scale, but now the overlapping parts are separated. The result of this process is a distinct spectrum for each component, free from the interference of the other.

Figure 8 is particularly significant, presenting this method's INH and PRD zero-order spectra. This figure illustrates the regression line equations for estimating the concentrations of INH and PRD based on their respective zero-order spectra. Specifically, INH is analyzed at a wavelength of 269 nm and PRD at 291 nm. The concentration of each drug in the mixture is then determined by plotting these spectra against a calibration curve [15]. This Ratio Subtraction Spectrophotometry method is vital in pharmaceutical analysis as it allows for the precise quantification of drugs in a mixture, even in cases where their spectra overlap significantly. This accuracy is essential for ensuring pharmaceutical products' correct dosage and efficacy [16].

3. Method Validation

The method validation for the analysis of INH (Isoniazid) and PRD (Pyrazinamide) includes several crucial parameters: linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ). These parameters are essential for assessing the reliability and efficacy of the analytical method used. Table 1 summarizes the results of these validation parameters for both INH and PRD.

Table 1. Method validation

Parameter	INH	PRD
Linearity	0.9985	0.9988
Accuracy (%)	98.1838	100.0205
Precision (%)	1.8769	0.2037
LOD (µg/mL)	0.8116	1.3127
LOQ (µg/mL)	2.7053	4.3757

As detailed in Table 1, the spectrophotometric subtraction ratio method effectively meets the established criteria for method validation, exhibiting both reliability and accuracy in the analytical assessment of INH and PRD. This method demonstrates a high level of linearity, as evidenced by the correlation coefficients for both INH and PRD, which surpass the threshold of $r \geq 0.995$, a standard requirement for method validation [25]. Such linearity is crucial as it ensures a direct and proportional relationship between the concentration of the analytes and the measured response, facilitating accurate quantification.

Regarding accuracy, the method falls comfortably within the acceptable range of 98-102% [21]. This range is essential for ensuring the method consistently yields results that closely align with the true values. The method's accuracy is further enhanced by the inclusion of standards at defined concentrations of 80%, 100%, and 120%, each with a composition of 70% analyte and 30% standard, thus confirming its capability to measure analyte concentrations accurately under varied conditions.

Precision, another critical aspect of validation, is indicated by the Relative Standard

Deviation (RSD) values being under 2% [24, 25]. This level of precision signifies the method's consistency and reproducibility under unchanged conditions, which is vital for reliable analytical testing. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) are additional fundamental parameters in method validation. The LOD, indicating the minimum concentration of an analyte detectable by the method, and the LOQ, reflecting the lowest concentration at which the analyte can be quantitatively determined with acceptable accuracy and precision, are within suitable ranges for INH and PRD [15]. These values highlight the method's sensitivity and accuracy in detecting and quantifying low concentrations of these compounds, further affirming its suitability for analytical applications in pharmaceutical quality control.

4. INH and PRD Levels in Tablet Dosage Form

The levels of INH and PRD in tablets based on the analysis results using the ratio subtraction spectrophotometry method are shown in Table 2.

Table 2. INH and PRD Levels in Tablet Dosage Form

Component	Level (%)	Requirement (%)
INH	102.1157	90.0-110.0
PRD	101.3874	90.0-110.0

The ratio subtraction spectrophotometry method was employed to determine the concentrations of isoniazid (INH) and pyrazinamide (PRD) in tablet forms—the analysis aimed to ensure that these medications adhered to the specified pharmaceutical standards. According to the results presented in Table 2, both INH and PRD concentrations in the tablet dosage forms align with the required standards. The measured concentration of INH in the tablets was 102.1157%, and PRD was 101.3874%. These values fall within the acceptable range of 90.0% to 110.0% of the amount declared on the label. This range is crucial in pharmaceuticals, as it guarantees the medications' effectiveness and safety. Maintaining drug concentrations within this specified range is vital to ensure therapeutic benefits and prevent potential side effects that could result from incorrect dosing.

The successful adherence of INH and PRD concentrations within this range indicates that the manufacturing process of these tablets is consistent and reliable. Such precision in drug formulation is essential for maintaining the standard of care in medical treatments. The results demonstrate the importance of rigorous quality control in pharmaceutical production and underscore the effectiveness of the ratio subtraction spectrophotometry method in ensuring medication compliance with established standards [6].

CONCLUSION

The ratio subtraction spectrophotometry method effectively enables the simultaneous determination of isoniazid (INH) and pyridoxine hydrochloride (PRD) in tablet dosage form. The absorption spectra analysis of INH and PRD confirmed their compliance with Lambert Beer's law, establishing the basis for reliable spectrophotometric measurements. Method validation results, including linearity, accuracy, precision, LOD, and LOQ, demonstrate the robustness and reliability of the proposed method for quantification. The chosen divisor concentrations (15 µg/mL for INH and 18 µg/mL

for PRD) in the ratio subtraction approach effectively overcame spectral overlap and enabled accurate determination. The regression equations derived from the ratio subtraction method provide a mathematical framework for high-precision estimating INH and PRD concentrations. Meeting the content requirements for both INH and PRD levels in tablet dosage form highlights the applicability and accuracy of the proposed method in pharmaceutical quality control. The advantages of the ratio subtraction method, including simplicity, accuracy, and practicality, make it a valuable tool for simultaneous quantification of compounds in complex mixtures. The study's findings contribute to pharmaceutical quality control processes, ensuring tablet formulations meet the required content standards. The proposed method aligns with the study's research objectives by providing a robust analytical approach for quantifying INH and PRD in tablet preparations. The developed analytical strategy can be extended to other drug combinations and complex formulations, enhancing its versatility in pharmaceutical analysis.

ACKNOWLEDGMENT

The author would like to thank the Research Laboratory of the Faculty of Pharmacy at the University of North Sumatra in Medan for providing research facilities.

REFERENCES

[1] H. Y. Vilchèze, and W. R. Jacobs, “The isoniazid paradigm of killing, resistance, and persistence in mycobacterium tuberculosis,” J.Mol.Biol, vol. 431, no. 18, pp. 3450–3460, 2019. doi: 10.1016/j.jmb.2019.02.016.

[2] M. S. Prasad, R. P. Bhole, P. B. Khedekar, and R. V. Chikhale, “Mycobacterium enoyl acyl carrier protein reductase (InhA): A key target for antitubercular drug discovery,” Bioor. Chem., vol. 115, p. 105242, 2021, doi: 10.1016/j.bioorg.2021.105242.

[3] M. Bhargava, and B. Bhargava, “Pyridoxine for patients suffering from drug-susceptible tuberculosis in India,” Public health action, vol. 8, no. 2, pp. 97-97, 2018. doi: 10.5588/pha.18.0017.

[4] T. Benjamin, and D. Ramachandran, “Development and validation of rp-hplc method for simultaneous estimation of isoniazid and pyridoxine in bulk and its pharmaceutical formulations,” Am. J. Pharm. Tech. Res, vol. 9, no. 1, pp. 215–225, 2019. doi: 10.46624/ajptr.2019.v9.i1.015.

[5] C. R. Choudhary, J. Kumari, and M. Advani, “Isoniazid induced early-onset of motor dominant neuropathy and treatment with high dose of pyridoxine,” Indian J Tuberc, vol. 65, no. 2, pp. 175–176, 2018, doi: 10.1016/j.ijtb.2017.04.004.

[6] Departemen Kesehatan Republik Indonesia, “Farmakope Indonesia,” Edisi 6, Kementrian Kesehatan Republik Indonesia. Jakarta, 2020.

[7] S. Umar, T. Saafrida and H. Lucida, “Validasi metode analisis penetapan kadar ketoprofen pada tablet salut enterik secara kromatografi cair kinerja tinggi dan spektrofotometri uv,” JSFK, vol. 8, no. 2, pp. 200–207, 2021. doi: 10.25077/jsfk.8.2.200-207.2021.

[8] N. F. Atta, A. Galal, and R. A. Ahmed, “Voltammetric Behavior and Determination of Isoniazid Using PEDOT Electrode in the presence of Surface Active Agents,” Int. J. Electrochem. Sci., vol. 6, no. 10, pp. 5097–5113, 2011, doi: 10.1016/s1452-3981(23)18391-x.

[9] K. Védaste, K. Egide, K. Claver, and E. Kaale, “Development and validation of high-performance thin-layer chromatographic method for the simultaneous determination of rifampicin, isoniazid, and pyrazinamide in a fixed dosage combination tablet,” JPC-J Planar Chromat, vol. 27, no. 5, pp. 392–397, 2014, doi: 10.1556/jpc.27.2014.5.11.

[10] R. E. Tarigan, and Y. Sarumaha, “Determination isoniazid and pyridoxine hydrochloride levels in tablets with ultraviolet spectrophotometry by successive ratio derivative,” IAMSPH, vol. 3, no. 1, pp. 95–101, 2022.

[11] L. R. Nasution, M. Bachri and E. D. L. Putra, “Simultaneous estimation of isoniazid and pyridoxine hydrochloride levels in tablet dosage form by spectrophotometry ultraviolet with area under curve method,” Asian J. Pharm. Clin. Res, vol. 11, no. 5, pp. 124–126, 2018. doi: 10.22159/ajpcr.2018.v11i5.24314.

[12] Y. Pratiwi, E. V. Nanda, and A. Kistani, “Effectiveness of Using Zn Powder and Spongy Cadmium As Reductor on Free Nitrite and Nitrate Analysis Method Validation in Bread Samples by UV-Vis Spectrophotometry,” JKPK, vol. 6, no. 3, p. 304, 2021, doi: 10.20961/jkpk.v6i3.54583.

[13] S. S. Sanjay, S. G. Alegaon and M. S. Palled, “Development and validation of isoniazid in bulk and pharmaceutical dosage forms by uflc method ,” Int. J. Pharma. Res. Health. Sci, vol. 7, no. 1, pp. 2904–2908, 2019. doi: 10.21276/ijprhs.2019.01.09.

[14] M. M. Sebaiy, “Absorbance Subtraction Method for Simultaneous Determination of Paracetamol and Orphenadrine Citrate in their Combined Pharmaceutical Dosage Forms,” BJSTR, vol. 25, no. 2, 2020, doi: 10.26717/bjstr.2020.25.004176.

[15] M. Bachri, Masfria, and H. Syahputra, “Quantitative estimation of antihypertension combination by ratio subtraction spectrophotometry method,” Open Acces Maced. J. Med. Sci, vol. 7, no. 17, pp. 2940–2946, 2019. doi: 10.3889/oamjms.2019.750.

[16] H. Darwish, F. Metwally, and A. El. Bayoumi, “Novel ratio subtraction and isoabsorptive point methods for determination of ambroxol hydrochloride and doxycycline in their combined dosage form: development and validation,” Trop. J. Pharm. Res, vol. 14, no. 1, pp. 133–140, 2015. doi: 10.4314/tjpr.v14i1.19.

[17] S. M. Riad, M.R. Rezk, G. Y. Mahmoud, and A. A. E. B. A Aleem, “Selective spectrophotometric methods for quantification of sitagliptin and metformin,” Anal. Chem. Indian J, vol. 12, no. 2, pp. 41–47, 2013.

[18] M. Popescu, P. Lancu, V. Plesu, C. S. Bildea, and C. M. Todasca, “Different spectrophotometric method for simultaneous quantification of lycopene and β-carotene from a binary mixture,” LWT Food Science and Technology, vol. 160, pp. 1132–1138, 2022. doi: 10.1016/j.lwt.2022.113238.

[19] H. W. Darwish, A. H. Bakheit and I. A. Naguib, “Comparative study of novel ratio spectra and isoabsorptive point based spectrophotometric methods: application on a binary mixture of ascorbic acid and rutin,” J Anal Methods Chem, vol. 2016, pp. 1–12, 2016. doi: 10.1155/2016/2828647.

[20] R. E. Tarigan, L. Lutvia and R. M. T. Sitorus, “Development and validation of area under curve spectrophotometry method for binary mixture of paracetamol and ibuprofen in tablet dosage form,” JFI, vol. 19, no. 2, pp. 246–252, 2022. doi: 10.31001/jfi.v19i2.1388

[21] S. Umar, Saafrida and H. Lucida, “Validasi metode analisis penetapan kadar ketoprofen pada tablet salut enterik secara kromatografi cair kinerja tinggi dan spektrofotometri uv,” J. Sains Farm. Klin, vol. 8, no. 2, pp. 200–207, 2021. doi: 10.25077/jsfk.8.2.200-207.2021.

[22] R. E. Tarigan, Muchlisyam, S. M. Sinaga and Z. Alfian, “Development and validation of double divisior ratio spectra derivative spectrophotometry method for ternary mixture of dextromethorphan hydrobromide, doxylamine succinate and pseudoepherine hydrochloride in tablet dosage form,” Int J Appl Pharm, vol. 12, no. 6, pp. 249–252, 2020. , doi: 10.22159/ijap.2020v12i6.39037.

[23] D. Ariyanti and M. Syaifuddin, “Au Extraction from Mineral Rocks with Aeration-Cyanidation Hydrometallurgy and Comparative Study of Its Effectiveness in Various Methods and Solvents,” JKPK, vol. 4, no. 2, pp. 115-122, 2019. doi: 10.20961/jkpk.v4i2.29020.

[24] R. E. Tarigan, Muchlisyam, S. M. Sinaga and Z. Alfian, “Simultaneous determination of dextromethorphan hydrobromide, doxylamine succinate and pseudoepherine hydrochloride in cough and cold syrup by area under curve spectrophotometry,” Rasayan J. Chem, vol. 14, no. 01, pp. 629–634, 2021. doi: 10.31788/rjc.2021.1416068.

[25] R. E. Tarigan, D. M. Sianturi and S. Nadya, “Determination of pseudoepherine hydrochloride and triprolidine hydrochloride simultaneously in tablet dosage form,” Rasayan J. Chem, vol. 16, no. 01, pp. 223–226, 2023, doi: 10.31788/rjc.2023.1618072.

[26] D. Hanwar, D. E. Nitoviani, and A. Suhendi, “Validation of Atomic Absorption Spectrometry Method for Contamination Determination of Lead (Pb) and Cadmium (Cd) in Methanol Extract and Product of Curcuma xanthorrhiza Roxb.,” JKPK, vol. 2, no. 3, p. 198-205, 2018, doi: 10.20961/jkpk.v2i3.11968.

[27] G. E. Halim, and M. Tjahjono, “Evaluation Of Color Models For Quantitative Determination Of Food Dyes Using Smartphonebased Digital Image Analysis,” JKPK, vol. 8, no. 1, p. 86-100, 2023, doi: 10.20961/jkpk.v8i1.72928.

[28] N. Dali, S. Dali, A. Chairunnas, H. A. M. Amalia, and S. A. A. Puspitasari, “Highly Selective and Sensitive Determination of Hg(II) Ions Using Ion Selective Electrodes (ISE) Coated with the BEC4ND1 Ionophore as Membranes,” JKPK, vol. 7, no. 2, p. 242-254, 2022, doi: 10.20961/jkpk.v7i2.62213.

[29] A. P. Kumar and D. Kumar, “Determination of Pharmaceuticals by UV-Visible Spectrophotometry,” Curr. Pharm. Anal. vol. 17, no. 9, pp. 1156–1170, 2021, doi:10.2174/1573412917999200909144354.

[30] A. Felix Ojochegbe and I. Adams Udoji, “Dietary Iron Determination by UV-Visible Spectrophotometry in Beans, Egg, Fish, and Pork Sold in Makurdi, Nigeria,” IJSR, vol. 12, no. 10, pp. 1895–1899, 2023, doi: 10.21275/sr231002144612.