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# SIMULTANEOUS CONTENT ANALYSIS OF RIFAMPICIN, ISONIAZID, AND PYRAZINAMIDE IN TABLET DOSAGE FORM BY SPECTROPHOTOMETRY ULTRAVIOLET WITH DWM AND RSM

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**ABSTRACT**

Treatment of active tuberculosis requires the use of combinations of drugs. One of the common combination of drugs to serve as anti-tubercular medication is rifampin, isoniazid and pyrazinamide. However, a research conducted by the Food Drug Administration (FDA) concluded that the combination of anti-tubercular medication may pose some patients to the risk of sub-optimal drug exposure, which may lead to less optimal treatment. This study aimed to determine the drug level of combination of anti-tubercular medication, namely rifampin, isomiazid, and pyrazinamide and to develop a spectrophotometric method using the dual wavelength method (DWM) and ratio substaction method (RSM) in tablet preparations on the market without separation. During the preparation, methanol was used as the solvent, followed by dilution, determination of calibration curve, determination of wavelength (λ), measurement, data analysis and validity test with several parameters ranging from linearity, accuracy, precision, LOD, and LOQ. The research revealed that the drug levels of rifampin, isoniazid, and pyrazinamide from the ultraviolet spectrophotometric method using sequential DWM were 100.3±1.8785; 99.98±2.5943; 100.03±2.076 and the results of the ultraviolet spectrophotometric method using RSM sequentially were 99.73±0.5437; 99.84±1.7598; 99.91±1.4762. Both methods succeeded in determining the drug level of the combination of rifampin, isoniazid, and pyrazinamide in tablet preparations without separation and the results of the validation parameters met the requirements.

**Keywords:** DWM; Isoniazid; Pyrazinamide; Rifampicin; RSM

## 1. INTRODUCTION

Tuberculosis is an infectious bacterial disease, which requires a treatment of multiple drugs in combination. One of the most commonly used combination to serve as anti-tubercular medication is rifampin, isoniazid and pyrazinamide. These three drugs are Anti-Tubercular Medication drugs in the form of Fixed Dose Combination (FDC). The making of FDC preparations is intended for patients with low levels of drug adherence. This is because patients are required to take many kinds of drugs separately for a long time, and thus many of them do not complete the necessary treatment until the final stage (Peloquin et al., 2008).

Unfinished treatment certainly has a negative impact on the patient's overall treatment. This fact is proven by a research conducted by the Food Drug Administration (FDA), which concluded that the combination of anti-tubercular drugs can lead to sub optimal drug exposure, which may lead to less optimal treatment (Prabowo et al., 2012). The most frequently applied method to analyze FDC preparations of rifampicin, isoniazid and pyrazinamide is the High Performance Liquid Chromatography (HPLC) method (MEM & MA, 2018). However, HPLC is not free from some drawbacks, particularly in terms of difficult sample preparation, lengthy process, and

complicated equipment and maintenance, which thus leads to high cost in the operation of this tool (Ghante et al., 2014). In addition, this method also finds it difficult to identify the complex compounds, which eventually results in poor resolution (Kumar et al., 2018).

Another method to determine drug levels, apart from the High-Performance Liquid Chromatography (HPLC), is ultraviolet (UV) spectrophotometry. Some benefits of Ultraviolet (UV) spectrophotometry are simple operation, good accuracy and precision, and higher efficacy and lower cost when compared to other methods (Viplava & Haritha Pavani, 2012). Rifampin, isoniazid and pyrazinamide have conjugated double bonds, chromophore groups and auxochrome groups with the structure that requires materials that can analyze drug assay using ultraviolet (UV) spectrophotometry (Acharjya et al., 2011).

Spectrophotometric methods have been developed into 2 methods, namely spectrophotometry using the dual wavelength method (DWM) and the ratio substation method (RSM), which does not require any separation to analyze several mixtures of substances, and thus they are easy to apply for routine analysis and do not require derivatization at the beginning, even with adjacent wavelengths. As compared to HPLC, DWM and RSM are much simpler, provide better accuracy and precision, are more effective and incur lower cost (Lotfy et al., 2012). The assay method of rifampin, isoniazid and pyrazinamide needs to be analyzed to determine the three active substances of a preparation. Validation requires parameters of precision, accuracy, sensitivity, linearity and selectivity (Tarigan et al., 2021). On this basis, it is necessary to conduct a simultaneous analysis of the levels of rifampin (RFP), isoniazid (INH), and pyrazinamide (PRZ) without any separation step in tablet preparations by DWM or RSM.

## 2. METHOD

This study used the following tools: a UV-Vis 1800 Spectrophotometer (Shimadzu) and a set of Personal Computer (PC) equipped with UV-Probe 2.34 software, Microsoft Excel and SPSS 20, Matlab® version R2016a, 1 cm cuvette, glassware (Oberoi), mortar and pestle, analytical balance (Boeco), sonicator (Branson 1510), and pH meter (Hanna).

This study used the following materials: all reagents with the grade analysis unless otherwise stated, rifampicin, isoniazid, pyrazinamide, methanol (E-Merck), aquabidestilata (PT. Ika Pharmindo), Whatman filter paper no. 42, parchment paper, and Pro TB 3 Kid® tablet (Phapros).

### 2.1. Preparation of Rifampicin, INH and Pyrazinamide Standard Stock Solutions

50 mg of RFP, INH and PRZ were weighed, put into a 50 mL volumetric flask, made up to reach volume of the mark line with the solvent (1000 g/mL) (LIB I), then pipetted for 2.5 mL from LIB I into 25 mL volumetric flask and added with the solvent up to the mark line (100 g/mL) (LIB II).

### 2.2. Preparation of Rifampicin Absorption Spectrum

Each of 0.6; 0.9; 1,2; 1.5; and 1.8 mL of LIB II was pipetted into a 10 mL volumetric flask, then the volume was filled with solvent until it reached the mark line to obtain a concentration of 6 RFP solution; 9; 12; 15; 18 g/mL consecutively. From these solutions an absorption spectrum was made.

### 2.3. Preparation of the Isoniazid Absorption Spectrum

Each of 0.5; 0.75; 1.0; 1.25; 1.5 mL of LIB II was pippetted into a 10 mL volumetric flask, then the volume was filled with solvent until it reached the mark line to obtain INH concentration of 5; 7.5; 10; 12.5; 15 g/mL consecutively. From these solutions an absorption spectrum was made.

### 2.4. Preparation of Pyrazinamide Absorption Spectrum

Each of 0.6; 0.75; 0.9; 1.05; 1.2 mL of LIB II was pipetted into a 10 mL volumetric flask, then the volume was filled with solvent to the mark line to obtain a PRZ solution of concentration 6; 7.5; 9; 10.5; 12 g/mL consecutively. From these solutions an absorption spectrum was made.

### 2.5. Preparation of Mixed Absorption Spectrum of Rifampicin, Isoniazid and Pyrazinamide

A mixed solution of RFP, INH and PRZ was made by pipetting RFP 1.2 mL LIB II, 1.0 mL LIB II INH and 0.9 mL LIB II PRZ into a 10 mL volumetric flask. Then the volume was filled with solvent to the mark line to obtain a solution containing a mixture of RFP, INH and PRZ with concentrations of 12, 10 and 9 g/mL, respectively. From this solution an absorption spectrum was made.

### 2.6. Creation of RFP, INH and PRZ calibration curves by DWM

The DWM values of RFP, INH and PRZ derived from various concentrations resulting from the absorption spectra at λ, were calculated and plotted to obtain the equation of the regression line.

### 2.7. Creation of RFP calibration curve by RSM

The absorption spectrum of the RFP mixture was added with INH divided by PRZ and to be divided again by the RFP absorption spectrum with a concentration of 12 g/mL as a divisor. Manipulation was carried out with the help of UV Probe 2.34 software to obtain a zero-order RFP spectrum and a regression plot or RFP calibration curve for various drug concentrations.

### 2.8. Making Absorption Spectrum and INH calibration curve by RSM

The absorption spectra of INH, RFP and PRZ mixtures were divided by the previous zero-order INH spectrum, which was a concentration of 10 g/mL as the divisor. Manipulation was carried out with the help of UV Probe 2.34 software to obtain a zero order INH spectrum and a regression plot or INH calibration curve for various drug concentrations.

### 2.9. Preparation of Absorption Spectrum and PRZ calibration curve by RSM

The absorption spectrum of the PRZ mixture was added to the INH divided by the RFP to be divided again by the absorption spectrum of 9 g/mL as the divisor. Manipulation was carried out with the help of UV Probe 2.34 software to obtain a zero order PRZ spectrum and a regression plot or PRZ calibration curve for various drug concentrations.

### 2.10. Determination of Mixed Concentration of Rifampicin, Isoniazid and Pyrazinamide in P Tablets

20 tablets containing 75 mg RFP, 50 mg INH and 150 mg PRZ were weighed and powdered for Tablet P. The amount of tablet powder was carefully weighed equivalent to 50 mg of RFP, INH and PRZ, and was put into a 50 mL volumetric flask, added with solvent, sonicated for 15 minutes, then added up to the dash line. The solution was filtered with Whatman® filter paper no. 42, and the first 10 mL of the filtrate was discarded. 0.4 mL of the filtrate was pipetted into a 25 mL volumetric flask, and added with solvent to the mark line. The absorption was then measured according to the good optimization procedure using the DWM and RSM methods.

### 2.11. Method Validation

#### *2.11.1. Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)*

Solutions with a predetermined concentration are seen to absorb each predetermined substance. The relationship between concentration and absorption was analyzed in terms of each substance based on the following linear regression equation and the correlation value.

$$y = ax + b \quad (1)$$

The LOD and LOQ calculations were also carried out based on the absorbance at λ.

#### *2.11.2. Precision testing*

Precision is usually expressed in terms of the deviation of a set of results, SD or RSD, of a data set. The Relative Standard Deviation (RSD) was calculated using Equation 2:

$$RSD = \frac{SD}{X}\ x\ 100\% \quad (2)$$

Description:

RSD = Relative standard deviation
SD = Standard deviation
X = Averaged data

### *2.11.3. Accuracy*

The accuracy or recovery test was carried out by measuring the percentage of recovery in specific areas, namely: 80%, 100% and 120%, where in each specific area, 70% of the RFP, INH and PRZ samples were used from the analyzed tablets and 30% from the standard to be added. Then the mixture of samples (tablets) and standard were analyzed with the same procedure used for the sample.

## 3. RESULTS AND DISCUSSION

### 3.1. Spectral overlap Studies

### *3.1.1. Dual Wavelength (DWM) Method*

Spectral overlap studies were obtained from a single maximal absorption spectrum of RFP, INH, PRZ and a mixture of RFP, INH, PRZ. The maximum spectral absorption of RFP was obtained from a concentration of 12 µg/ml, INH concentration of 10 µg/ml, and PRZ concentration of 9µg/ml.

This study that examined the spectral overlap started with determining the drug levels using the dual wavelength and ratio subtraction method. This determination process is important in analyzing the wavelength using the dual wavelength and ratio subtraction method, since the study of spectral overlap is expected to produce wavelength points for the dual wavelength method. As for the ratio subtraction method, the initial determination of the drug served as the initial divisor where X or Y was determined to proceed to the next step (Rivai et al., 2021).

A spectral overlap study in the dual wavelength method was carried out for the selection of two selected wavelength points. The rules for determining the wavelength for the dual wavelength method requires the absorbance of the two selected wavelengths to have an absorbance difference of zero and to comply with Lambert-Beer law in the area range of 0.2-0.6.

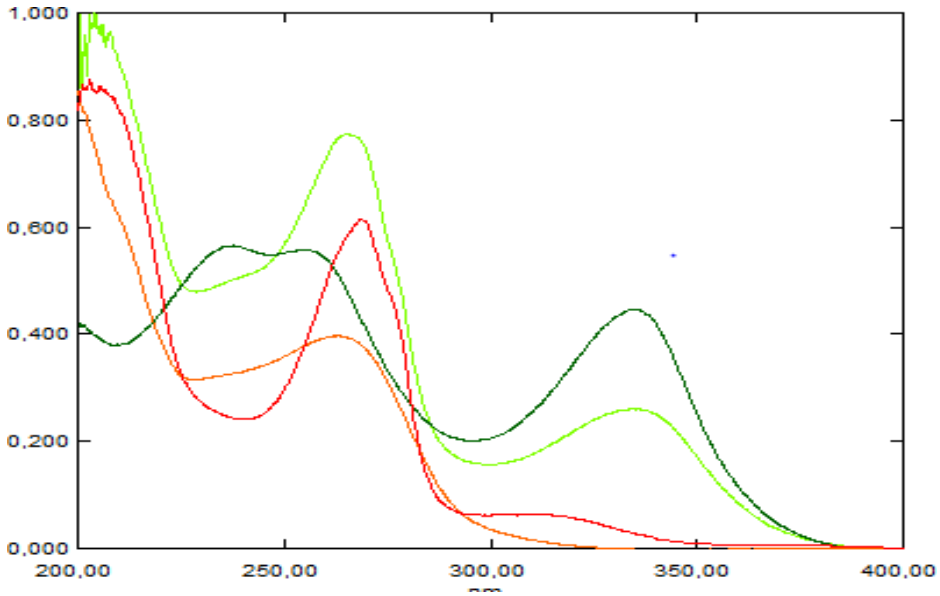


**Figure 1.** Absorption of Spectral Overlap of Raw and Single Mixed Rifampicin, Isoniazid and Pyrazinamide in Methanol Solvent

In **Figure 1,** it can be seen that the maximum spectral absorption of the drug took 2 wavelength points for PRZ drug with a wavelength of 220.99 nm (λ1) to serve as the point of

intersection between RFP and PRZ, after which a straight line was drawn to get the difference in absorbance, which resulted in zero. Thus, the difference of absorbance at the two points of a wavelength of 267 .06 nm (λ2) was zero. In addition, there was a rising absorbance of RFP at that wavelength, which was in the Lambert-Beer range. The INH compound had a wavelength of 225.66 nm (λ3), which indicated a point of intersection between INH and PRZ. Therefore, a straight line was drawn to obtain a zero-absorbance difference for the second wavelength at INH, which was 251.90 nm (λ4). The absorbance decreased and it adhered to the Lambert-Beer law.

The RFP compound had a wavelength of 261.81 nm (λ5) to serve as the point of intersection between RFP and INH. Then, a straight line was drawn to obtain the difference in absorbance, which resulted in zero. As a result, the absorbance difference of a wavelength of 273.47 nm (λ6) at both points was zero. In addition, at this wavelength there was an increasing RFP absorbance, which was in the Lambert-Beer range.

Regression was determined by plotting the difference between the two wavelengths that had been previously selected based on the spectrum of the ratio of the drug mixture of various concentrations made. Thus, the order of zero and so on could be made in a calibration curve to continue with determining drug levels (Darwish et al., 2015).

### *3.1.2. Ratio Subtraction Method (RSM)*

The procedures for ratio subtraction method began by creating a ratio spectrum and selecting the initial divisor concentration (D°). The concentration of the divisor was determined from a concentration range that satisfied the Lambert-Beer law. The spectral overlaps of the ratio spectrum of RFP, INH, and PRZ were obtained from various concentrations (Bachri et al., 2019).

The dividing concentrations used were RFP, INH, and PRZ at concentrations of 12 µg/ml, 10 µg/ml, 9 µg/ml respectively. This dividing concentration was selected since it provided no difference in the location of the maximum wavelength of the substances with divided spectrum. The only difference lied in the absorbance value produced and the concentration at the maximum wavelength (Asadpour-Zeynali & Saeb, 2016).

This study used the initial divisor (D°) based on the study of the spectral overlap, which found that the initial divisor was PRZ. Spectral processing of the raw drug mixture was manipulated using UV probe 2.42 using division operation of PRZ 9 µg/ml. Afterwards, the result was subtracted with a constant of PRZ 9 µg/ml divided by the divisor. The subtraction produced a new spectrum, namely RFP+INH/PRZ°, which was subsequently multiplied by the same divisor to obtain the spectrum of RFP+INH. In subsequence, it proceeded with the second division. The RFP+INH spectrum was divided by RFP 12 µg/ml, which resulted in a subtraction with an RFP constant of 12 µg/ml divided by the divisor. The subtraction produced a new spectrum, namely INH/RFP°, which was multiplied with the same divisor to obtain a single INH spectrum from the mixture (**Figure 2**). The resulted spectra of each stage were plotted in zero order with a regression equation or calibration curve. A brief explanation of the formula is as follows:

Step 1: $\frac{RFP+INH+PRZ}{PRZ°} = \frac{RFP+INH}{PRZ°} + \frac{PRZ}{PRZ°}(Constant)$

Step 2: $\frac{RFP+INH}{PRZ°} + Constant - Constant = \frac{RFP+INH}{PRZ°}$

Step 3: $\frac{RFP+INH}{PRZ°} X\ PRZ° = RFP + INH$

Step 4: $\frac{RFP+INH}{RFP°} = \frac{INH}{RFP°} + \frac{RFP}{RFP°}(Constant)$

Step 5: $\frac{INH}{RFP°} + Constant - Constant = \frac{INH}{RFP°}$

Step 6: $\frac{INH}{RFP°} X\ RFP° = INH$

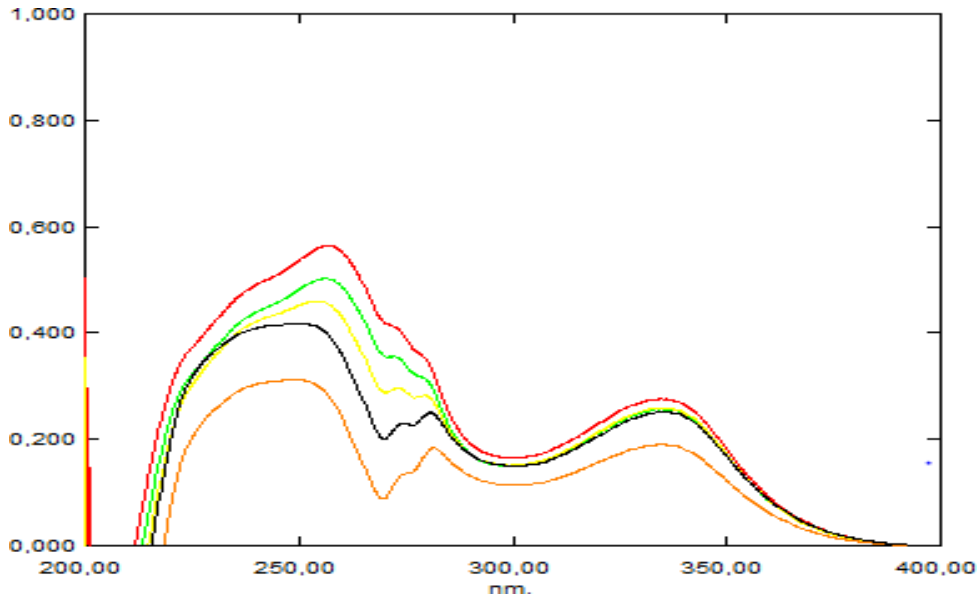


**Figure 2.** Manipulated INH absorption spectrum

The subsequent procedure for determining RFP and PRZ continued by dividing the RFP+INH+PRZ Spectrum from the divisor with PRZ, followed by sharing the spectrum with INH (D°) so as to obtain the RFP+ INH spectrum, which was done by the divisor of each drug to produce a single spectrum from a mixture of the drugs. Spectral absorbance of manipulated INH shows in **Figure 3**.

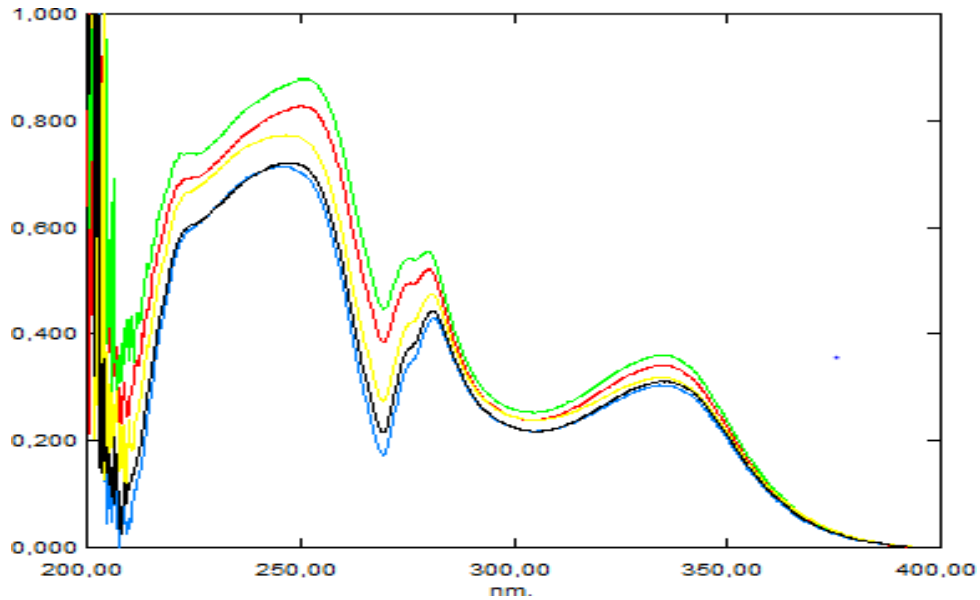


**Figure 3.** Spectral absorbance of manipulated INH

The resulted spectrum was plotted to obtain a linear regression of the absorbance relationship at the maximum versus the measured concentration to obtain a calibration curve. The following is a summary of the process and the image of the manipulated spectrum in **Figure 4**.

The levels of RFP, INH and PRZ in a single form, could be determined by UV spectrophotometry in methanol solvent with RFP at λ 254 nm, INH at λ 266 nm, PRZ and at λ 268 nm (Muhammad et al., 2019). Meanwhile, the method of the maximum wavelength for RSM was 254.65 nm, the maximum wavelength for INH was 266.45 nm and the maximum wavelength for PRZ was 268.60 nm. The resulted wavelengths were used to analyze the wavelength.

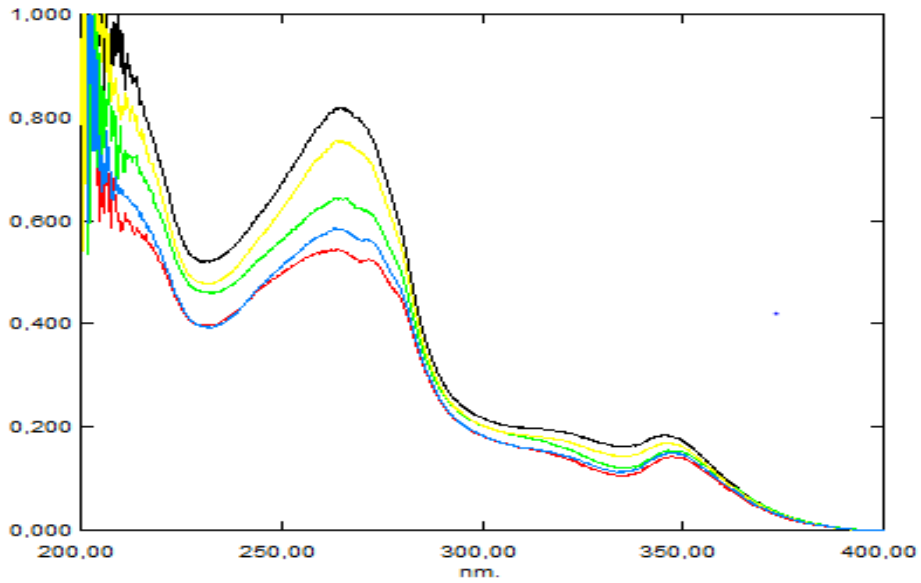


**Figure 4.** Spectral absorbance of the manipulated RFP

**Table 1.** The RFP, INH and PRZ levels in tablet P with DWM and RSM

| Tablet Preparation | Component | DWM | RSM |
|---|---|---|---|
| | | Rate (%) | Rate (%) |
| Tablet P | RFP | 100.32 ± 1.8785 | 99.73 ± 0.5437 |
| | INH | 99.89 ± 2.5943 | 99.84 ± 1.7598 |
| | PRZ | 100.03 ± 2.076 | 99.91 ± 1.4762 |

Based on **Table 1**, the RSM and DWM are applicable methods to determine the levels of RFP, INH and PRZ in tablet preparations on the market because they meet the requirements as proven by the fact that their levels of substances based on the analysis were in the range of 90-110% (Gandhimathi et al., 2012).

The validation of the method using RSM and DWM revealed the values of linearity, accuracy, precision, LOD and LOQ for RFP, INH and PRZ, which can be seen in **Table 2**. It is clear that the RSM and DWM methods meet the validation requirements for linearity, accuracy, precision parameters, detection limit (LOD), and quantification limit (LOQ).

The linearity value described is the correlation coefficient value for both RSM and DWM, which is almost entirely close to one. This result indicates that there is a very good correlation between concentration and absorbance value. This also indicates that the higher the concentration the higher the absorbance.

**Table 2.** Linearity, Accuracy, Precision, LOD and LOQ values

| No. | Parameters | RFP | INH | PRZ |
|---|---|---|---|---|
| 1 | Determination of the analysis wavelength (nm) | 220.99 nm and 267.06 | 225.66 nm and 251.90 | 261.81 nm and 273.47 |
| 2 | Lamber beer (µg/mL) | 6-18 | 5-15 | 6-12 |
| 3 | Regression Equation | Y = 0.0384X + 0.0018 | Y = 0.0465X + 0.0069 | Y = 0.0531X – 0.0087 |
| 4 | Correlation Coefficient | 0.9980 | 0.9996 | 0.9993 |
| 5 | Accuracy (%) | 100.13 | 100.07 | 100.22 |
| 6 | Precision (RSD) (%) | 0.91 | 0.63 | 0.66 |
| 7 | LOD (µg/mL) | 0.27 | 0.18 | 0.32 |
| 8 | LOQ (µg/mL) | 1.04 | 0.72 | 1.22 |

Accuracy parameter, which can be tested using the standard addition method in a certain range of the sample. Both samples were measured, and the added standard was recalculated. The accuracy value in **Table 2** indicates the average return value of three specific ranges with three repetitions. In this case, the three specific ranges used were 80%, 100% and 120%, where the

composition consisted of 70% sample and 30% standard. The accuracy value obtained highlights the fact that this method meets the requirements for method validation (requirements for the accuracy value are from 98% to 102%).

Precision parameter shows the closeness of the analytical results of several repetitions. Precision demonstrates that the method results in similar values even when they are tested in several replications. The precision parameter is reflected in the resulting RSD value, indicating that RSM and DWM meet the RSD validation requirements, with the condition of < 2% (Diani Saraan et al., 2015).

## 4. CONCLUSION

Ultraviolet spectrophotometric methods using RSM and DWM can be used to determine the levels of RFP, INH and PRZ simultaneously and meet the method validation requirements. It can also be used to determine the levels of RFP, INH and PRZ simultaneously in tablet preparations on the market. In further research, it can be developed using different methods and drug samples.

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## 6. CONFLICT OF INTEREST

The author declares that there are no competing conflicts of interest.

## 7. REFERENCES

Acharjya, S. K., Rao, M. E. B., Kumar, B. V. V. R., & Annapurna, M. M. (2011). Journal of Advanced Scientific Research UV-Spectrophotometric Methods For The Determination Of Zolmitriptan in Bulk and Pharmaceutical Dosage Forms. *Journal of Advanced Scientific Research*, *2*(3), 42–47.

Asadpour-Zeynali, K., & Saeb, E. (2016). Simultaneous spectrophotometric determination of rifampicin, isoniazid and pyrazinamide in a single step. *Iranian Journal of Pharmaceutical Research*, *15*(4), 713–723. https://doi.org/10.22037/ijpr.2016.1910

Bachri, M., Masfria, M., Syahputra, S., & Hafid, H. (2019). Quantitative estimation of anti hypertension combination ratio subtraction spectrophotometry method. *Open Access Macedonian Journal of Medical Sciences*, *7*(17), 2940–2946. https://doi.org/10.3889/oamjms.2019.750

Darwish, H. W., Metwally, F. H., & El Bayoumi, A. (2015). Novel ratio subtraction and isoabsorptive point methods for determination of ambroxol hydrochloride and doxycycline in their combined dosage form: Development and validation. *Tropical Journal of Pharmaceutical Research*, *14*(1), 133–140. https://doi.org/10.4314/tjpr.v14i1.19

Diani Saraan, S. M., Sinaga, S. M., & Muchlisyam. (2015). Development method for determination of ternary mixture of paracetamol, ibuprofen and caffeine in tablet dosage form using zero-crossing derivative spectrophotometric. *International Journal of PharmTech Research*, *7*(2), 349–353.

Gandhimathi, R., Vijayaraj, S., & Jyothirmaie, M. P. (2012). Analytical process of drugs by ultraviolet ( UV ). *International Journal of Pharmaceutical Research& Analysis*, *2*(2), 72–78.

Ghante, M. R., Shelar, R. S., Sawant, S. D., & Kadam, M. M. (2014). Development and validation of UV spectrophotometric method for estimation of Darunavir ethanolate in bulk and tablet dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(7), 240–242.

Kumar, C. P., Teja, B. R., Varma, B. K., & Annapurna, M. M. (2018). Derivative and simultaneous equation methods for the determination of fluorometholone and ketorolac in ophthalmic preparations. *Asian Journal of Pharmaceutics*, *12*(12), S640–S646.

Lotfy, H. M., Saleh, S. S., Hassan, N. Y., & Elgizawy, S. M. (2012). A Comparative Study of the Novel Ratio Difference Method versus Conventional Spectrophotometric Techniques for the Analysis of Binary Mixture with Overlapped Spectra. *American Journal of Analytical Chemistry*, *03*(11), 761–769. https://doi.org/10.4236/ajac.2012.311101

MEM, H., & MA, M. (2018). Novel and facile spectrophotometric techniques for the determination of sofosbuvir and ledipasvir in their tablet dosage form. *Journal of Analytical & Pharmaceutical Research*, *7*(2), 92–99. https://doi.org/10.15406/japlr.2018.07.00207

Muhammad, A., Effendy, D. L., & Muchlisyam, P. (2019). Simultaneously Content Analysis of Sulfadoxine and Pyrimethamine in Tablet Dosage Form by Spectrophotometry Ultraviolet with Dual Wavelenght Method. *Asian Journal of Pharmaceutical Research and Development*, *7*(4), 34–37. https://doi.org/10.22270/ajprd.v7i4.555

Peloquin, C. A., Hadad, D. J., Molino, L. P. D., Palaci, M., Boom, W. H., Dietze, R., & Johnson, J. L. (2008). Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. *Antimicrobial Agents and Chemotherapy*, *52*(3), 852–857. https://doi.org/10.1128/AAC.01036-07

Prabowo, M. H., Wibowo, A., & Fauziyah, L. (2012). Pengembangan Dan Validasi Metode Analisis Rifampicin Isoniazid-Pirazinamid Dalam Fixed Dose Combination Dengan Metode Kromatografi Lapis Tipis-Densitometri. *Jurnal Ilmiah Farmasi*, *9*(2). https://doi.org/10.20885/jif.vol9.iss2.art4

Rivai, H., Puspita, R., & Misfadhila, S. (2021). Recent Study on Development and Validation of Loperamide Hydrochloride Tablet Analysis Method with Absorbance and Area under Curve Methods Spectrophotometrically. *Technological Innovation in Pharmaceutical Research Vol. 3*, *5*(2), 48–58. https://doi.org/10.9734/bpi/tipr/v3/1696c

Tarigan, R. E., Muchlisyam, Sinaga, S. M., & Alfian, Z. (2021). Development and validation of area under curve spectrophotometry method for ternary mixture of dextromethorphan HBr, doxylamine succinate and pseudoephedrine HCl in tablet dosage form. *AIP Conference Proceedings*, *2342*(6), 1–4. https://doi.org/10.1063/5.0045551

Viplava, K., & Haritha Pavani, V. (2012). Development and validation of stability-indicating RP-HPLC method for Estimation of Atovaquone. *International Journal of Pharmaceutical and Clinical Research*, *4*(4), 68–72.