Available online on 15.08.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

Open Access

Research Article

Determination of Rifampicin, Isoniazid, and Pyrazinamide Simultaneously in Tablet Formulation Using UV Spectrophotometry Method with Mean Centering of Ratio Spectra

Tarigan Rida Evalina[1,*], Siregar Asti Eliani[1], Surbakti Chemayanti[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health, Institut Kesehatan Helvetia, Medan, Indonesia

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

A B S T R A C T

This research aims to simultaneously determine Rifampicin(RI), Isoniazid(IN), andPyrazinamide(PZ) in tablet formulation using the UV spectrophotometry method with Mean Centering Of Ratio Spectra (MCR). The absorption spectra of RI (6-18 µg/mL), IN (5-15 µg/mL), and PZ (6-12 µg/mL) solutions were scanned within the 200-400 nm range. For RI determination, the RI spectra were divided by the IN spectrum at 10 µg/mL, and the resulting ratio spectra were mean-centered. For IN determination, the IN spectra were divided by the PZ spectrum at 9 µg/mL, and the ratio spectra were mean-centered. For PZ determination, the PZ spectra were divided by the RI spectrum at 12 µg/mL, and the ratio spectra were mean-centered.Method validation parameters were assessed, including linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ). The linearity values were 0.9997 for RI, 0.9990 for IN, and 0.9994 for PZ. Accuracy was 100.71% for RI, 100,40% for IN, and 100.31% for PZ. Precision was 0.14% for RI, 0.14% for IN, and 0.11% for PZ. The LOD values were 2.66µg/ml for RI, 1.30µg/ml for IN, and 1.40µg/ml for PZ. The LOQ values were 4.85µg/ml for RI, 3.94µg/ml for IN, and 4.24µg/ml for PZ. All parameters met the International Conference on Harmonization (ICH) requirements.The MCR method effectively for the simultaneous determination of RI, IN, and PZ in the tablet formulation.

Keywords: Rifampicin, isoniazid, pyrazinamide, mean centering of ratio spectra

A R T I C L E I N F O: Received 20 Feb 2024; Review Complete 16 April 2024; Accepted 15 July 2024. ; Available online 15 August 2024

Cite this article as:

Evalina TR, Eliani SA, Chemayanti S, Determination of Rifampicin, Isoniazid, and Pyrazinamide Simultaneously in Tablet Formulation Using UV Spectrophotometry Method With Mean Centering of Ratio Spectra, Asian Journal of Pharmaceutical Research and Development. 2024; 12(4):01-05, DOI: http://dx.doi.org/10.22270/ajprd.v12i4.1434

*Address for Correspondence:

Tarigan Rida Evalina, Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health, Institut Kesehatan Helvetia, Medan, 20124, Indonesia

INTRODUCTION

Tuberculosis (TB) remains a significant global health challenge, with millions of cases reported annually and a high burden of morbidity and mortality worldwide. Rifampicin (RI), Isoniazid (IN), and Pyrazinamide (PZ) are key components of the standard treatment regimen for TB. Ensuring the accurate quantification of these active ingredients in pharmaceutical formulations is essential for effective treatment outcomes and public health efforts to control the spread of the disease[1-3].

Traditionally, the quantification of RI, IN, and PZ in pharmaceutical formulations has relied on various analytical methods, including chromatographic techniques such as High Performance Liquid Chromatography (HPLC)[4,5] and spectrophotometric methods[6,7]. While these methods have proven effective, they often require complex sample preparation procedures and sophisticated instrumentation, making them time-consuming and resource-intensive[6-8].

In recent years, there has been growing interest in exploring alternative analytical approaches that offer simplicity, cost-effectiveness, and practicality without compromising accuracy and reliability. UV spectrophotometry is one such technique that has gained prominence in pharmaceutical analysis due to its ease of use, wide availability, and relatively low cost[7,9].

UV spectrophotometry relies on the principle of measuring the absorbance of light by molecules in solution at specific wavelengths. Each compound exhibits a unique absorption spectrum, allowing for their quantification based on their characteristic absorbance properties[10]. This technique has been widely utilized in pharmaceutical analysis for the quantification of various drug substances, excipients, and impurities[10,11].

Despite its advantages, traditional UV spectrophotometry may face challenges when dealing with complex mixtures or when analytes exhibit overlapping absorption bands. Mean Centering of Ratio Spectra (MCR) is a data processing technique that has been proposed to address these challenges and enhance the selectivity and sensitivity of UV spectrophotometric analysis[12,13].

MCR involves the calculation of ratio spectra, which are obtained by dividing the spectrum of a mixture by the spectrum of a reference compound. The resulting ratio spectra are then mean-centered, whereby the mean value of each wavelength is subtracted from the corresponding data points. This process helps to remove background noise and spectral interferences, thereby improving the accuracy and reliability of quantitative analysis[12-14].

To the best of our knowledge, there has been no previous research that determines RI, IN, and PZ using the UV spectrophotometry method with mean centering of ratio spectra. This research aims to simultaneously determine RI, IN, and PZ in tablet formulation using the UV spectrophotometry method with mean centering of ratio spectra (MCR).

MATERIAL AND METHODS

Instrumentation

A UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), MATLAB software version R2016a, analytical balance (Boeco, Germany), sonicator (Branson 1510).

Chemicals and Reagents

Methanol (E-Merck) analytical grade, raw materials RI, IN, and PZ, tablet Pro TB-3 Kid® containing RI 75 mg, IN 50 mg, and PZ 150 mg (PT. Phapros, Indonesia).

Preparation of Stock Solutions

To prepare the stock solution, 100 mg of each pure drug (RI, IN, and PZ) was accurately weighed and dissolved in methanol in separate 100 mL volumetric flasks. The final volume was adjusted to 100 mL with methanol, resulting in stock solutions with a concentration of 1000 µg/mL for each pure drug.

Preparation of Working Standard Solutions

To obtain working standard solutions in the range of 5-18 µg/mL for each drug, the 1000 µg/mL stock solutions of RI, IN, and PZ were accurately diluted. Specific volumes of the stock solutions were pipetted into 100 mL volumetric flasks and diluted with methanol. These working standard solutions were then used for UV spectrophotometric analysis to ensure accurate and precise measurements across the specified concentration range.

Zero-Order Absorption Spectra of RI, IN, and PZ

In the 200-400 nm region, the zero-order absorption spectra were recorded for RI at a concentration of 12 µg/mL, IN at 10 µg/mL, and PZ at 9 µg/mL

MCR Method

The absorption spectra of solutions with varying concentrations of RI (6-18 µg/mL), IN (5-15µg/mL), and PZ (6-12 µg/mL) were scanned within the 200-400 nm range. For RI determination, the absorption spectra of RI were divided by the absorption spectrum of IN at a concentration of 10µg/mL, and the resulting ratio spectra were mean-centered. For IN, the absorption spectra were divided by the absorption spectrum of PZ at a concentration of 9µg/mL, followed by mean centering of the ratio spectra. Similarly, the mean-centered ratio spectra for PZ were obtained by dividing the absorption spectra by absorption spectrum of RI at a concentration of 12 µg/mL.

Sample Preparation

Grind and pulverize 20 tablets, then take a quantity of the powder equivalent to 75 mg of RI, 50 mg of IN, and 150 mg of PZ. Place the finely ground and homogeneous powder from the Pro TB tablets into a 50 mL volumetric flask, and add methanol to reach the calibration mark. Filter the solution using Whatman® no. 42 filter paper, discarding the first 10 mL of filtrate. Pipette 0.4 mL of the filtrate into a 25 mL volumetric flask and add solvent up to the calibration mark. The absorbance of the solution is then measured according to the optimized procedure using the MCR method.

Method Validation

Linearity

The linearity of the MCRS method was assessed by preparing various concentrations of RI (6-18 µg/mL), IN (5-15 µg/mL), and PZ (6-12 µg/mL). The MCRS regression analysis demonstrated the linearity of the method.

Accuracy

A validation procedure was performed using the standard addition method to verify the reliability and robustness of the proposed methodologies. The recovery test involved assessing the percentage of added standard recovered at three different concentration levels: 80%, 100%, and 120%[15].

Precision

Precision, also known as the Relative Standard Deviation (RSD), is calculated using the regression equation derived from the calibration curve. The formula used is:

$$RSD = \frac{SD}{X} \times 100\%$$

In this equation, RSD represents the Relative Standard Deviation, SD stands for the Standard Deviation, and X denotes the average value of the data[15,16].

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for RI, IN, and PZ were determined using the following equations to evaluate the sensitivity of the

proposed methods, in accordance with the guidelines of the International Conference on Harmonization (ICH):

$$LOD = 3.3\frac{\sigma}{S}$$

$$LOQ = 10\frac{\sigma}{S}$$

Here, σ represents the standard deviation of the response, and S denotes the slope of the linear regression line[17,18].

RESULTS AND DISCUSSION

The zero-order absorption spectra of RI, IN, and PZ

The zero-order absorption spectra of RI, IN, and PZ are presented in Figure 1.

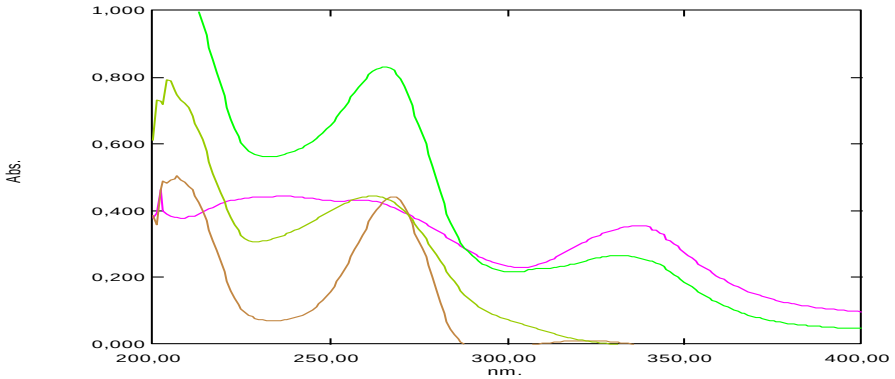

Figure 1: Zero Order Absorption Spectra RI ( ), IN ( ), PZ ( ), and mixture of RI, IN, PZ ( )

Based on Figure-1, these spectra exhibit substantial overlap, posing challenges for direct quantification of RI, IN, and PZ individually. To overcome this spectral overlap issue, the MCR method is applied, enabling accurate determination of the concentrations of RI, IN, and PZ[12,13].

MCR Method

The absorption spectra of each component drug were divided by the spectrum of a selected divisor to obtain the ratio spectra to get MCR Spectrum. The MCR spectrum can be seen in Figure 2–4 below.

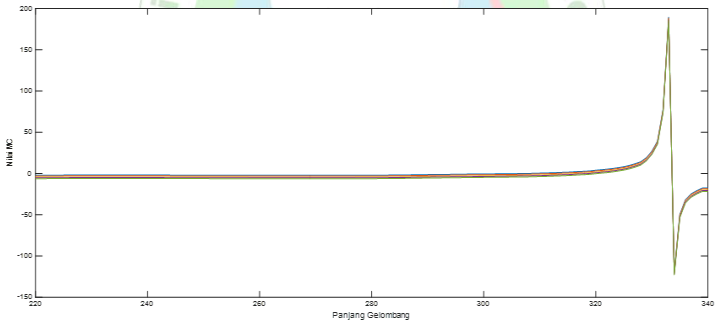

Figure 2: MCR Spectrum of RI (6-18 µg/mL) as Divisor IN 10 µg/mL

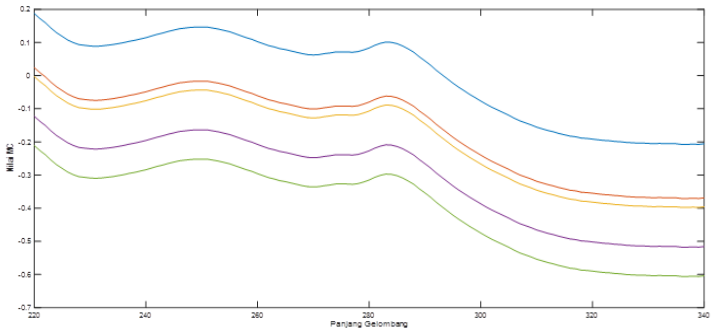

Figure 3: MCR Spectrum of IN (5-15 µg/mL) as Divisor PZ 9 µg/mL

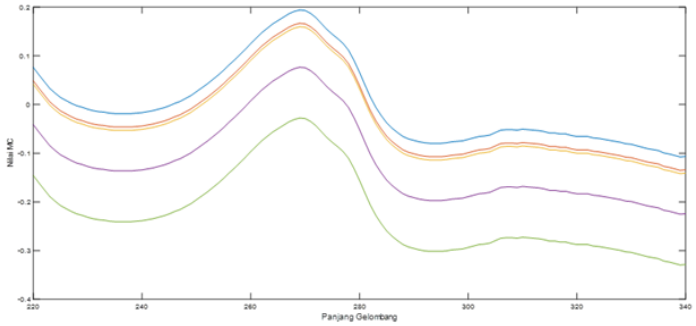

Figure 4: MCR Spectrum of PZ (6-12 µg/mL) as Divisor RI 12 µg/mL

Based on Figures 2-4 above, it is evident that the MCR method can be used for the simultaneous quantification of RI, IN, and PZ without the need for prior separation. This method offers the advantage of eliminating derivative steps, thereby enhancing the signal-to-noise ratio[13,21].

Method Validation

The validation of the method for analyzing RI, IN, and PZ encompasses several critical parameters: linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ). Table-1 compiles the results of these validation parameters for RI, IN, and PZ.

Table 1: Results of The Validation Method

No	Parameter	RI	IN	PZ
1	Linearity (r)	0.9997	0.9990	0.9994
2	Accuracy (%)	100.71	100,40	100.31
3	Precision (RSD) %	0.14	0.14	0.11
4	LOD (µg/mL)	2.66	1.30	1.40
5	LOQ (µg/mL)	4.85	3.94	4.24

Based on Table 1 above, it is evident that the MCR method effectively meets the criteria set for method validation. This method shows a high level of linearity, demonstrated by the correlation coefficients (r) for RI, IN, and PZ, all of which exceed the threshold of $r \geq 0.995$, a standard requirement for method validation[7,19]. Accuracy also meets the acceptable range of 98% to 102%[21]. Precision, another crucial aspect of validation, is indicated by the Relative Standard Deviation (RSD) values being less than 2%[7,22]. LOD and LOQ are additional essential parameters in method validation. The LOD, representing the minimum concentration of an analyte detectable by the method, and the LOQ, indicating the lowest concentration at which the analyte can be quantitatively determined with acceptable accuracy and precision, fall within suitable ranges for RI, IN, and PZ[22,23].

Analysis of RI, IN, and PZ Tablet Formulations

The levels of RI, IN, and PZ in tablet formulation based on the analysis results using the MCR method are presented in Table-2.

Table 2: RI, IN, and PZ in Tablet Formulation

Component	Level (%)	Requirement (%)
RI	99.72	90-110
IN	99.23	90-110
PZ	100.11	90-110

According to the results shown in Table 2, the concentrations of RI, IN, and PZ in the tablet formulation meet the required standards.These values are within the acceptable range of 90.0% to 110.0% of the amount declared on the label[24].

CONCLUSION

The MCR method effectively for the simultaneous determination of RI, IN, and PZ in the tablet formulation.

ACKNOWLEDGMENTS

We gratefully acknowledge the Department of Pharmaceutical Chemistry at Institut Kesehatan Helvetia and research laboratory (Faculty of Pharmacy, University of North Sumatra) for providing the necessary facilities for this research.

REFERENCES

1. Jhun BW, Koh WJ. Treatment of Isoniazid-Resistant Pulmonary Tuberculosis. Tuberc Respir Dis (Seoul). 2020; 83(1):20-30.
2. Putri, O., Salim, M., Gunawan, A., Devin, D., Amaris, E., Alexander, D., Kevin, G., & Febriana, G. Drug-Resistant Pulmonary Tuberculosis. Indonesian Journal of Life Sciences. 2023; 5(01), 10-40.
3. Nathavitharana RR, Cudahy PG, Schumacher SG, Steingart KR, Pai M, Denkinger CM. Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and

meta-analysis. Eur Respir J. 2017; 49(1):1601075. Published 2017 Jan 18. doi:10.1183/13993003.01075-2016
4. Panda, BK., Dikkatwar, M., & Sathiyanarayanan, L. Application of HPLC for simultaneous determination of isoniazid, rifampicin, and pyrazinamide in a fixed dose formulation. JAdv Sci. Res. 2020;11(1)Suppl 1, 248-253.
5. DashtBozorg, B., Goodarzi, A., Fahimi, F., Tabarsi, P., Shahsavari, N., Kobarfard, F., &Dastan, F. Simultaneous Determination of Isoniazid, Pyrazinamide and Rifampin in Human Plasma by High-performance Liquid Chromatography and UV Detection. IJPR, 2019; 18(4):1735–1741.
6. Hawas, S., Parui, S., Dey, S., Mondal, S. K., &Sarkar, S. Simultaneous Spectrophotometric Estimation Of Rifampicin, Isoniazid And Pyrazinamide In Their Pharmaceutical Dosage Form.Asian J Research Chem, 2020; 13(2):1-6.
7. Tarigan, RE, Mendrofa, E., &Surbakti, C. Simultaneous Determination of Isoniazid and Pyridoxine Hydrochloride in Tablet Dosage Forms using Ratio Subtraction Spectrophotometry. JKPK, *2024;* 9(1):1-14.
8. Bulduk, I., &Akbel, E. A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations. J. Taibah Univ. Sci, 2022; 15(1):507–513. https://doi.org/10.1080/16583655.2021.1991737
9. Tarigan, RE., Andry, M., Dewi, RK.,Nasution, MA., &Lubis, MF. Quantitative Analysis of Dextromethorphan-Hbr, Guaifenesin And Diphenhydramine-Hcl In Tablet Dosage Form By Successive Ratio Derivative Spectra Method. Int J App Pharm, 2024; 16(1):71–75.
10. Mukherjee, P., Chakraborty, DD., Chakraborty P., Shrestha B., Bhuyan NR. Different Ultraviolet Spectroscopic Methods: A Retrospective Study On Its Application From The Viewpoint Of Analytical Chemistry. Asian J Pharm Clin Res, 2021; 14(9):1-11.
11. Tarigan, RE., Sianturi, DM.,Nadya, S. Determination Of Pseudoephedrine Hydrochloride And Triprolidine Hydrochloride Simultaneously By Using Area Under Curve Spectrophotometry Ultraviolet. Rasayan J. Chem., 2023; 16(1):223-226.
12. Tarigan, RE., Silitonga, AS., Sari, M. and Nadia, S. Development and validation of mean centering ratio spectra spectrophotometry method for binary mixture of glimepiride and metformin hydrochloride in tablet disage form. AIP Conf. Proc. 2023; 2626(1), 150005.https://doi.org/10.1063/5.0136130.
13. Muchlisyam, Sudarmi, Caroline C. Mean Centering Of Ratio Spectra Method for the Analysis of Theophylline And Ephedrine Hcl Mixture In Tablet. Asian J Pharm Clin Res. 2018; 11(5):218-223.
14. Muchlisyam, Monica, S. Application of Mean Centering Ratio and Multiple Wavelength Methods for Determination of Four Component Level in Multivitamin Syrup by Ultraviolet Spectrophotometry. J. Hunan Univ. Nat. Sci.; 2021, *48*(7):296-303.
15. Gupta, K., andShrivastava, P. Development And Validation Of UV Spectrophotometric Method For Trimethoprim In Pure And Marketed Formulation. Int. J. Health Sci. 2022; 6(S2):10829-10839.
16. Sulasmi, S., Muchlisyam, M., andHaro, G. Simultaneous Analysis of Tablets Content Vitamin C And Zinc In Visible Spectrophotometry. IJSTM. 2023; 4(2):386-392.
17. Nia, NP.,Siti. M.S., Muchlisyam P., Simultaneous Determination of Amlodipine and Valsartan in Tablet By UV Spectrophotometry With Successive Ratio Method. AJPRD. 2021; 9(3):1–5.
18. Amal, L., and A. Prusty. Development And Validation Of Uv-Visible Spectrophotometric Method For Determination Of Duloxetine. Int. J. Pharm. Sci.2019; 11(3):27-31.
19. Saleem, KA., Nabil, NAF., Hemn, HAQ. Simultaneous Evaluation of Triclosan and Methylparaben in Some Personal Care Products Using Green Spectrophotometric Techniques. J. Med. Chem. Sci. 2024; 7(6):858-872.
20. Karan SK, Subhadarshinee S. Validated Method For Estimation Of Ethamsylate By UV-Spectroscopy. Int J Pharm SciDev Res. 2024; 10(1): 001-005.
21. Tarigan, RE., Faisal, H., Lase, IK.,andHafifah, SP. Application Mean Centering Ratio Spectra Method For Ternary Mixture Of Dextromethorphan Hbr, Doxylamine Succinate, And Pseudoephedrine Hcl In Tablet Preparations. Int. J. App. Pharm. 2024; 16(4):244–249.
22. Kale, SA.,&Shaikh, KS. Validated UV-Vis Spectrophotometric Method For The Estimation Of SorafenibTosylate In Bulk And Nanoparticles. Int. J. Health Sci., 2022; 6(S3):4011–4019.
23. Tarigan, RE., Lutvia, L and Sitorus, RMT. Development And Validation Of Area Under Curve Spectrophotometry Method For Binary Mixture Of Paracetamol And Ibuprofen In Tablet Dosage Form. JFI; 2022, 19(2):246–252.
24. Indonesian Health Ministry. Indonesian Pharmacopeia. 6th ed. Director General of Health Care and Medical Devices. 2020.