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Spectrophotometric simultaneous analytical method validation to determine isoniazid and pyridoxine in pure and 3D printed tablet forms

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ABSTRACT

Introduction: Isoniazid (INH) is the anti-tuberculosis drugs being used to counter tuberculosis since 1952. Patients on INH should be given daily prophylactic pyridoxine (PYR) with 10-50 mg/day to prevent the development of isoniazid-induced neuropathy. Within the framework of this research, the UV-Vis spectrophotometer is used to quantify simultaneously the drug content of INH and PYR. **Methods:** The standard curve for both INH and PYR were plotted using the concentration of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml and tablets were analysed using simultaneous equation method. The proposed method was validated by analytical method validation for the linearity, specificity, accuracy, intermediate precision, limit of detection (LOD), and limit of quantification (LOQ). **Results:** A regression equation of INH standard and sample were found to be y = 0.0279x + 0.0637 and y = 0.0280x + 0.0522 obtained from the calibration curve and linear with correlation coefficient (R2) values of 0.9950 and 0.9964, respectively. A regression equation of PYR standard and sample were y= 0.0267x + 0.0723 and y = 0.0259x + 0.0806 and to be linear with R2 values of 0.9981 and 0.9962, respectively. The result of accuracy obeyed the accepted criteria of percentage recovery in between 98% to 102%. The method exhibited intermediate precision as demonstrated by relative standard deviation <2%. The LOD and LOQ of INH were 0.166 µg/ml and 0.5018 µg/ml while the LOD and LOQ of PYR were 0.122 µg/mL and 0.371 µg/mL, respectively in the pure form. In tablet dosage form, the LOD and LOQ of INH were 0.071 µg/ml and 0.215 µg/m while LOD and LOQ of PYR give the result 0.124 µg/ml and 0.375 µg/ml, respectively. **Conclusion:** This spectrophotometric simultaneous analytical method validation for INH and PYR was successfully conducted with the notion to spearhead the development of INH and PYR in a single dosage form to improve compliance among tuberculosis patients.

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Introduction

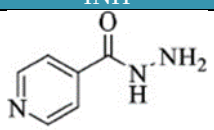
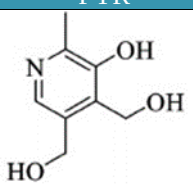
Isoniazid (INH) is a highly effective treatment for *M. tuberculosis* that the World Health Organization recommends (WHO). It serves as the main ingredient of several fixed-dose combination tablets, each of which contains two or more anti-TB drugs and has been in use since 1952 to treat tuberculosis. In addition to extending the tablet's shelf life, antioxidants and INH cocrystals may lessen oxidative stress in TB patients receiving therapy (Mashhadi et al., 2021). Pyridoxine (PYR) species are immediately inactivated by INH metabolites. In people with high-risk conditions, PYR deficiency can result in neurologic adverse effects such peripheral neuropathy. The Clinical Practice Guidelines state that daily prophylactic pyridoxine (vitamin B6) administration to INH patients with 10 to 50 mg/day is recommended to avoid the onset of isoniazid-induced neuropathy.

Pyridoxine (PYR) is a water-soluble vitamin that aids in the metabolism of carbohydrates, lipids, and amino acids. This vitamin has a significant impact on the metabolism of nitrogen-containing compounds such serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and the component of haemoglobin. In addition to encouraging the development of red blood cells, pyridoxine aids in the balance of salt and potassium. Table 1 shows the physicochemical properties of both INH and PYR, respectively (Wishart et al., 2018).

The UV-Vis spectrophotometer's fundamental principle is the absorption of light by a sample. When utilising a UV-Vis spectrophotometer, the purity of the INH and PYR samples may be measured based on how much light and its wavelength are absorbed by the samples. A sample solution is placed in a cuvette, and an Ultraviolet-visible (UV-Vis) spectrophotometer analyses the light's intensity as it passes through the solution and compares it to the light's intensity before the sample. A UV-Vis spectrophotometer's primary components are a light source, a sample holder, a dispersive device to separate the light's various wavelengths, and an appropriate detector. The visual depiction of the UV-Vis spectrum in general is the absorbance as a function of wavelength.

Within the framework of this research, the UV-Vis spectrophotometer is used to quantify simultaneously the drug content in the pure form of INH and PYR by measuring all the absorbance values for each concentration at determined wavelengths which are 263 nm of INH and 290 nm of PYR then will be calculated in the simultaneous equation method. According to Beer's Lambert law, it states that absorbance is proportional to concentration. So, this research study uses two types of modes of the UV-Vis spectrophotometer. The first one is the photometric mode. The photometric mode can help define the known wavelengths of INH and PYR by measuring the absorbance at a single wavelength or at multiple wavelengths. The second type is a spectrum mode. The spectrum mode may obtain sample spectra using wavelength scanning thus resulting in a peak wavelength of each INH and PYR as required. The UV-Vis spectrophotometer interprets the data analysis to provide the necessary information and can subsequently obtain the results.

Table 1: Physicochemical properties of INH and PYR

Properties	INH	PYR
Chemical structure		
Chemical name	Pyridine-4-carbohydrazid	4,5-bis(hydroxymethyl)-2-methylpyridin-3-ol
Molecular formula	$C_6H_7N_3O$	$C_8H_{11}NO_3$
Molecular weight	137.14 g/mol	169.18 g/mol
Melting point	171.4 °C	159 °C to 162 °C
Solubility **(at** 25 **°C)**	1.4 x 10^5 mg/L	2.2 x 10^5 mg/L
Log P	-0.70	-0.77
Half-life	0.5 to 1.6 hours for fast acetylators 2 to 5 hours for slow acetylators	15 to 20 days

Materials

INH standard (99.7% purity), PYR standard (99.9% purity), INH and PYR analytical grade powders were purchased from Sigma-Aldrich (Darmstadt, Germany). INH 300 mg 3D printed tablet and PYR 10 mg tablet were used for analysis purpose. Distilled water was used as a solvent in this experiment.

Methodology

1. Preparation of standard solution

Accurately 10 mg of INH and 10 mg of PYR standard were separately transferred into individual 100 ml volumetric flasks, then dissolved appropriately with distilled water and diluted up to the mark with distilled water to give solutions containing 100 µg/ml of INH and 100 µg/ml of PYR (Figure 1).

2. Preparation of calibration curve

The calibration curve was prepared by using the stock solution to produce six different concentrations of INH and PYR standard which are 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml (Figure 1). The absorbance of each concentration was acquired at the λ max using a fixed wavelength measurement mode. The calibration curve representing concentration versus absorbance was plotted.

3. Determination of Wavelength of Maximum Absorbance (λ max)

A solution containing 15 µg/ml of INH and 15 µg/ml of PYR was scanned separately using full output mode with medium scanning speed for a whole range of dual wavelengths by using a UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) ranging from 400 – 200 nm with distilled water as blank. After acquiring the spectrum, the maximum absorbance was identified.

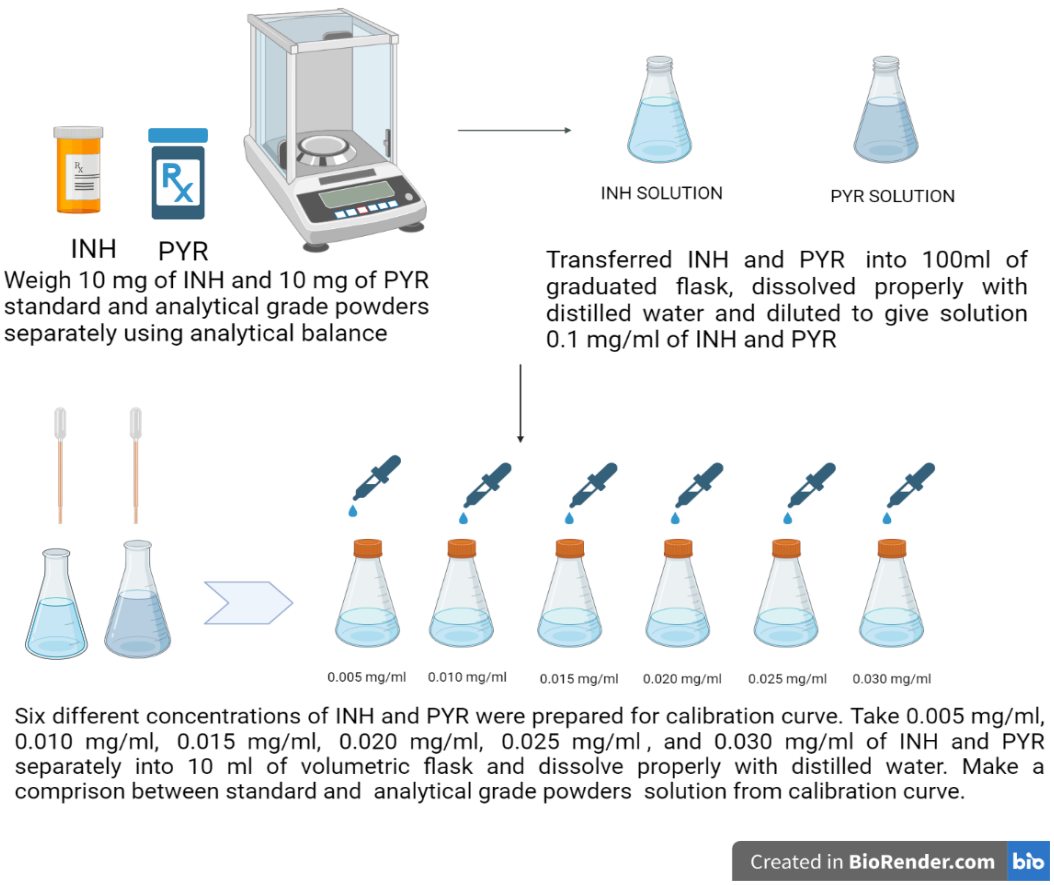


Figure 1. Preparation of stock and calibration curve solutions.

4. Simultaneous equation method

This method of analysis is based on the absorption of INH and PYR at the wavelength maximum of each other (Tilinca et al., 2017). Two wavelengths selected for the development of simultaneous equations were 263 nm and 290 nm which were lambda maximum of INH and PYR respectively. The absorbances of INH and PYR measured at selected wavelengths (Tilinca et al., 2017). Absorptivity values were calculated. The concentrations of both the drugs in mixture can be calculated by using following equations 1 and 2:

$$Cx = \frac{A_2 ay_{1-} A_1 ay_2}{ax_2 ay_{1-} ax_1 ay_2} \quad \text{Eq. 1}$$

$$Cy = \frac{A_1 ax_{2-} A_2 ax_1}{ax_2 ay_{1-} ax_1 ay_2} \quad \text{Eq. 2}$$

Where, A_1 and A_2 are absorbances of mixture at 263 nm and 290 nm, respectively.

ax_1 and ax_2 are the absorptivity of INH at 263 nm and 290 nm, respectively.

ay_1 and ay_2 are the absorptivity of PYR at 263 nm and 290 nm, respectively.

Cx and Cy are concentrations of INH and PYR, respectively.

5. Application of the proposed method for the determination of INH and PYR in tablets

The 3D-printed tablet containing 300 mg INH and 10 mg PYR was analysed by this method. An amount equivalent to 10 mg INH and 10 mg PYR of the selected tablet was weighed and dissolved in 100 ml distilled water to obtain a stock solution containing 100 µg/ml standard solution. The solution was then filtered through Whatman filter paper. INH and PYR were diluted appropriately. The absorbance of the resulting solutions was measured at 263 nm and 290 nm. The concentration of INH and PYR in the sample solution was calculated using the equation constructed from the calibration curve of each drug.Values were substituted in the respective formula to obtain concentrations.

6. Analytical method validation (AMV)

The main objective of performing analytical method validation is to demonstrate that the analytical method which is a UV-Vis spectrophotometer is suitable and adequate for its intended purpose (Patil, Patil, Chalikwar, Surana, & Firke, 2019). The validation of the developed method was carried out in terms of specificity, linearity, accuracy, precision, intermediate precision, the limit of detection (LOD), and limit of quantification (LOQ). It was validated according to the International Conference on Harmonization guidelines.

6.1 Specificity

Specificity is its ability to detect and differentiate the analyte of interest in the presence of other substances, including its related substances to guarantee character of an analyte (Patil et al., 2019). The specificity of the direct spectrophotometric method was assessed by comparing the spectrum obtained from the solvent system alone (placebo), which is distilled water, and of standard INH, PYR solution in the diluent.

6.2 Linearity and Standard Curve

In order to find the line that best fits a provided set of data, the linearity was established. This allowed for a visual representation of the relationship between the data points (Patil et al., 2019). The linearity of this method was established using six different calibration standards. Standard INH and PYR were tested at six known concentrations using a pre-determined wavelength. Every concentration's absorbance was recorded. The linearity was determined by plotting six concentrations (x-axis) of INH and PYR standard and sample against absorbance (y-axis). The equation of $Y = mX + C$ and the R^2 was developed.

6.3 Accuracy

The degree to which test results agree with the genuine value, or how closely the outcomes of the method correspond with the true value, is known as accuracy. In order to minimise potential operating errors, it is often established on samples of the material to be analysed that have been produced with quantitative accuracy. (Patil et al., 2019). Accuracy should be established across the specified range of the analytical procedure. To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels which are 80%, 100% and 120% were subjected to the determined wavelength (nm) in which 263 nm of INH and 290 nm of PYR. The percentage recovery should be in between 98% and 102% to meet the acceptance criteria.

6.4 Intermediate precision

Precision is how close individual measurements are to each other. Intermediate precision is a part of precision in which the method is tested on multiple days, instruments, and analysts to measure of the ruggedness of the method's reliability when performed in different environments (Patil et al., 2019). The intraday precision of INH and PYR was checked by assay the sample solution on same day at an interval of one hour for three hours and interday precision was carried out by estimating the correspondence responses on three different days with different preparations. According to this study, the solutions may be

analysed within 48–72 hours without negatively affecting the drug's chemical stability when urea is present. The wavelength was applied to each concentration in triplicates, and the mean and standard deviation were then calculated. To achieve the acceptance standards, the accuracy percentage of the relative standard deviation (RSD) value must be less than 2.0%.

6.5 Limit of detection (LOD) and limit of quantification (LOQ)

The lowest concentration of an analyte in a sample that can be identified and measured with suitable precision and accuracy under the specified test conditions was used for the evaluation of LOD and LOQ. The following equations 3 and 4 describe the precise calculations to estimate LOD and LOQ, respectively:

$$LOD = (3.3 \times SD)/m \qquad \text{Eq. 3}$$
$$LOQ = (10.0 \times SD)/m \qquad \text{Eq. 4}$$

SD or standard deviation in the formula was referring to the standard deviation of the absorbance values of the blank and m is the slope of the standard curve constructed previously (Ismail et al., 2016). All readings for LOD and LOQ were conducted in triplicates.

Results

1. Specificity

The identification of wavelength with maximum absorbance is needed for quantitative UV analysis. The specificity should not be tested without any blank or matrix spectrum because it does not give any reading or specified wavelength of drug content. The standard solution of INH and PYR with concentration of 15 µg/mL-1 was separately scanned in the range of 200-400 nm. The result showed that the λ_{max} was determined for each drug. The λ_{max} INH and PYR were found to be 263 nm with the absorbance is 0.519 and 290 nm with the absorbance is 0.422, respectively as shown in Figure 2a and Figure 2b. After scanned both drugs separately, then overlap the spectras and obtained the isosbestic wavelength at 280 nm as λ_{max} of common absorbance as shown in Figure 2c. Isosbestic wavelength is used when two substances of equimolar concentration show the same absorbance at particular wavelength and by using isosbestic wavelength it may record the absorbance of formulation or multi wavelength photometric mode of UV-Vis spectrophotometer.

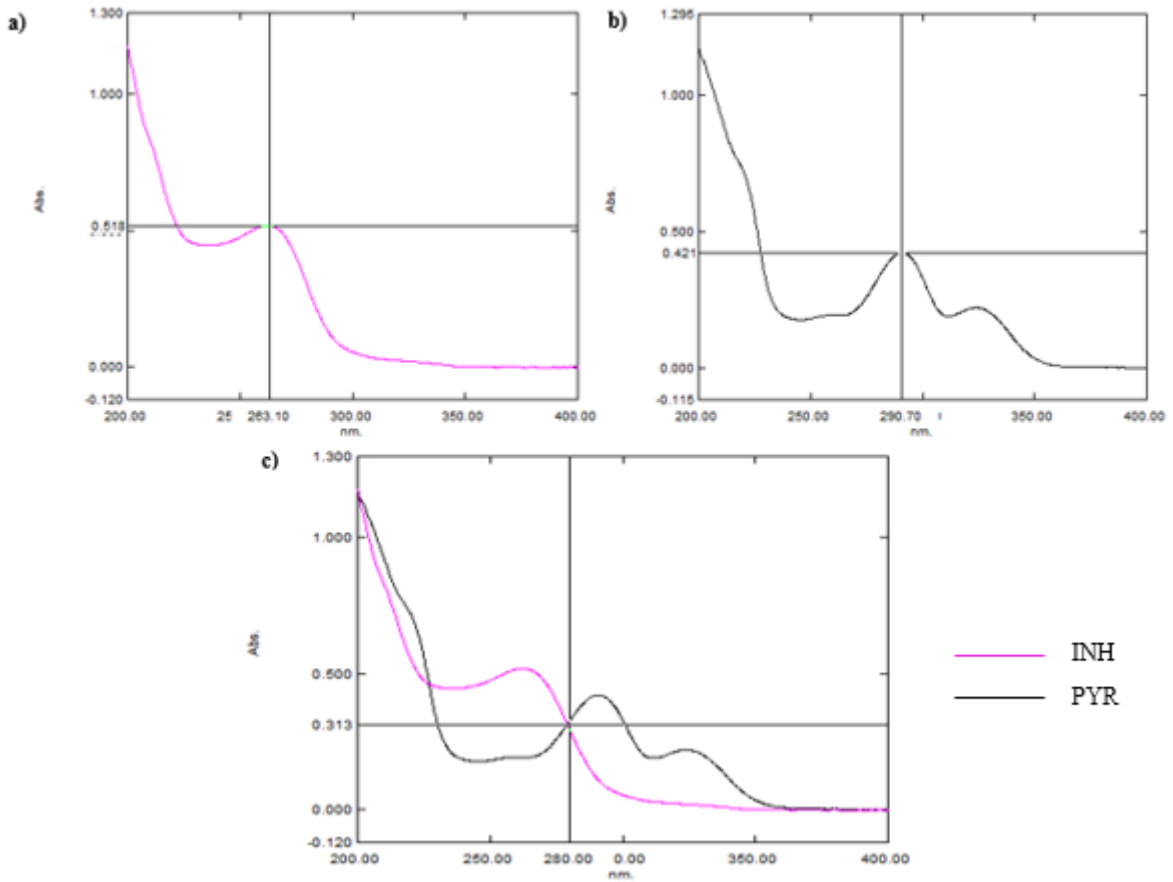


Figure 2. a) UV–Vis spectra of standard INH (λ:263 nm). and PYR (λ:290 nm). c) UV-Vis overlaid spectra of both standards (Isosbestic λ:280 nm).

2. Linearity and calibration curve

The linearity was confirmed using the absorbance values at a constant set wavelength, which is 263 nm, and the direct percentage relationship between the concentration of standard INH and sample INH. In the meantime, the linearity was established using the absorbance values at a set established wavelength, which is 290 nm, and the direct percentage relation between the concentration of standard PYR and sample PYR. Six known concentrations of both standard and sample of INH and PYR were prepared namely are 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, and 30 µg/ml were subjected to 263 nm and 290 nm for INH and PYR to get the absorbance values for each sample. The calibration of the developed UV-Vis spectrophotometer method was in linear form with the equation and the R2 of standard INH is y = 0.0279x + 0.0637 and 0.9950 respectively while the equation and the R2 of standard PYR is y = 0.0267x + 0.0723 and 0.9981 respectively. In addition, the calibration of the developed UV-Vis spectrophotometer method was in linear form with the equation and the R2 of sample INH is y = 0.0280x + 0.0522 and 0.9964 respectively while the equation and the R2 of sample PYR is y = 0.0259x + 0.0806 and 0.9962, respectively.

3. Accuracy

In pure form, to ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels which are 80%, 100% and 120% were subjected to the determined wavelength (nm) in which 263 nm of INH and 290 nm of PYR. Three samples were prepared for each level, and their absorbance was quantified using a UV-Vis spectrophotometer. The concentrations of INH and PYR in the sample solution were determined using an equation derived from the calibration curves of the respective drugs. To acquire concentrations, absorbance values were substituted in the applicable formula. Following that, the concentration was subtracted with 6 ppm, which is stated in the linearity of standard curve for the six concentrations created, and the result was represented as the obtained value. The actual value was determined using the cross-multiplication approach based on the different levels (80%, 100%, 120%), and the actual value was the fixed value for both INH and PYR. Then, the percentage recovery calculation was followed by using Eq. 5.

$$\% \text{ Recovery} = \frac{\textbf{Obtain value}}{\textbf{Actual value}} \times \mathbf{100} \qquad \text{Eq. 5}$$

Futhermore, in 3D printed tablet dosage form, the accuracy was developed by prepared six samples for each different levels which are 80%, 100%, and 120% and the absorbance was recorded to obtained the concentration of the sample. The simultaneous equation method was utilised by applying the absorbance and absorptivity values in the calculation in order to get the true concentration and then dividing it with the initial concentration. Next, it was multiplied by 100 and the result represents the percentage recovery. Overall, the percentage recovery should be in between 98% and 102% to meet the acceptance criteria, and all these accuracy results of INH and PYR for both pure form and 3D printed tablet dosage form were tabulated in Table 2 and Table 3, respectively.

Table 2: Results of accuracy for the simultaneous determination of INH and PYR in pure form

Drug	Level of accuracy	Recovery (%)	RSD (%)
INH	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
	80	98.13	0.17
	100	98.17	0.16
	120	99.17	0.36
PYR	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23
	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23
	80	99.74	0.56
	100	100.33	0.15
	120	102.36	0.23

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Table 3: Results of accuracy for the simultaneous determination of INH and PYR in 3D printed tablet dosage form

Drug	Label claim	Sample (mg)	Actual (mg)	Accuracy (%)	SD	RSD (%)
INH	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
	80	8	8.16	101.96	0.0010	0.40
	100	10	9.91	99.00	0.0030	1.17
	120	12	11.86	98.83	0.0006	0.27
PYR	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42
	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42
	80	4	4.02	100.52	0.0010	0.14
	100	5	4.97	99.50	0.0017	0.23
	120	6	5.99	99.87	0.0020	0.42

4. Immediate Precision

The intraday precision study of INH and PYR in pure form was carried out by estimating the correspondence responses three times on the same day with concentrations of 5 g/ml, 10 g/ml, and 15 g/ml, and the interday precision study of INH and PYR was carried out by estimating the correspondence responses three times the next day with different preparations of 5 g/ml, 10 g/ml, and 15 g/ml had been recorded in Table 4. Meanwhile, the method's intermediate precision was assessed in 3D printed tablet dosage form by assaying the sample solution on the same day at one-hour intervals (intraday precision) for three hours and on three distinct days (interday precision), as shown in Table 5. According to this study, the solutions may be analysed within 48-72 hours without affecting the chemical stability of the drug in the presence of urea. Overall, the percentage of RSD for each concentration of simultaneous INH and PYR that was subjected to the specified wavelengths of 263 nm and 290 nm was less than 2%, which passed the acceptance requirements.

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

The limit of detection represents the lowest concentration of analyte that can be reliably detected. Meanwhile, the limit of quantification represents the lowest concentration of analyte that can be analysed and quantified. The LOD and LOQ of INH were found to be 0.166 µg/ml and 0.5018 µg/ml while the LOD and LOQ of PYR were found to be 0.122 µg/mL and 0.371 µg/ml respectively in pure form as shown in Table 6. Besides that, the LOD and LOQ of INH were obtained to be 0.071 µg/ml and 0.215 µg/ml respectively but however, the LOD and LOQ of PYR were obtained to be 0.124 µg/ml and 0.375 µg/ml respectively in 3D printed tablet dosage form as presented also in Table 6.

6. Assay of 3D printed tablet dosage form

The optimized method was successfully applied for the simultaneous determination of INH and PYR in the 3D printed tablet dosage forms, containing 300 mg INH and 10 mg PYR. Six samples were tested using a UV-Vis spectrophotometer and the absorbance of each sample was measured in triplicate at specific wavelengths of 263 nm and 290 nm. The mean absorbance and absorptivity of the samples were used and the amount found of tablets was calculated using the simultaneous equation method. Satisfactory results were obtained for each compound as the found amounts were in good agreement with the amount taken as indicated in Table 7.

Table 4: Results of intraday and interday precision for the simultaneous quantification of INH and PYR in pure form

Analyte (µg/ml)	SD		RSD (%)	
Intraday (n=6)	INH	PYR	INH	PYR
5	0.0006	0.0006	0.29	0.22
10	0.0010	0.0020	0.31	0.50
15	0.0006	0.0006	0.13	0.13
Interday (n=18)	INH	PYR	INH	PYR
5	0.0030	0.0006	0.37	0.31
10	0.0030	0.0006	0.54	0.26
15	0.0006	0.0010	0.07	0.33

Table 5: Results of intraday and interday precision for the simultaneous quantification of INH and PYR in 3D printed tablet dosage form

Parameters	Drug	Label claim	Sample (mg)	Actual (mg)	SD	RSD (%)
Intraday (n=3)	INH	300	10	9.91	0.0030	1.17
	PYR	10	5	4.97	0.0017	0.23
Interday (n=3)	INH	300	10	10.11	0.003	0.56
	PYR	100	5	4.92	0.004	0.65

Table 6: LOD and LOQ data of the UV-Vis spectrophotometer method for the simultaneous determination of INH and PYR in pure and 3D printed tablet forms.

Drug	Type	LOD (µg/mL)	LOQ (µg/mL)
INH	Pure	0.1660	0.5018
	3D-printed tablet	0.0710	0.2150
PYR	Pure	0.1220	0.3710
	3D-printed tablet	0.1240	0.3750

Table 7: Assay results of INH and PYR determination in tablet dosage form

Drug	Label claim	Sample (mg)	Actual (mg)	Accuracy (%)	SD	RSD (%)
INH	300	10	9.78	98.0	0.0006	0.61
PYR	10	10	10.01	100.1	0.0010	0.23

Discussion

The proposed method that used to determine simultaneous of INH and PYR in this study was the simultaneous equation method or also known as Vierordt's method. The importance of the study of the simultaneous equation method was that it allows the analysis of multicomponent drugs using different analytical techniques such as spectrophotometer, chromatography and electrophoresis. However, this study focuses more on the use of UV-Vis spectrophotometer because it is an applicable method and most scientific work has been done using this technique.

The reason why the author chose Vierord's method is that it has many advantages. For instance, this method can save time and cost effective since the absorption measurement were obtained with ease, the process was fast, and simple. Vierordt's method also had its shortcoming such as the lambda max of two drugs should be reasonable different, there must no chemical interaction between the absorbing components, and they should obey Beers law at their wavelength maximum if used in UV-Vis spectrophotometer.

Based on the result, all the validation parameters that validate the proposed method showed it was specific, linear, accurate, precise, and sensitive. It could be consistent with the other published article, where the author (Tilinca et al., 2017) also obtained the same result in the simultaneous determination of INH and rifampicin (RIF) using the same method. The assay part indicated that found amount of INH (148.84 mg) and

RIF (297.68 mg) were in good agreement with the declared amount of INH (150 mg) and RIF (300 mg). The correlation coefficient (R2) was more than 0.99, the specificity showed that 263 nm (λ_{max} for INH) and 338 nm (λ_{max} for RIF), percentage recovery in the range of 98%-102%, the RSD of an intermediate precision also less than 2%, and LOD of INH (2.60 µg/ml) and RIF (3.50 µg/ml) were showed always lesser than lowest concentration in the standard curve but the LOQ result of INH was (8.58 µg/ml) and PYR (11.70 µg/ml).

In addition, there had one article entitle simultaneous estimation of Salbutamol sulphate (SAL) and Ambroxol HCl (AMB) from their combined dosage form by UV-Vis spectrophotometer using the simultaneous equation method also discussed the same methods (Panchale, Gulhane, Manwar, & Bakal, 2020). As a result, all the validation parameters proved that the proposed method was specific, linear, accurate, precise, and sensitive. The specificity showed had two different maximum wavelength present which are 242 nm for SAL and 272 nm for AMB. The R2 of the linearity in the calibration curve showed that more than 0.99 and the percentage recovery's result still in the range between 98% and 102%.

Moreover, the RSD of intermediate precision give less than 2% in which meet the acceptance criteria and the LOD and LOQ result were 0.95 µg/ml and 0.18375 µg/ml respectively for both SAL and AMB. Finally, the author recommends in the future, the methods can be employed for routine analysis in simultaneous determination of another combination drugs and also quality control analysis.

Conclusion

In a sample that contains of two absorbing drugs like INH and PYR in which each of them absorbs at a maximum wavelength different from the other, it may be possible to determine both drugs in the pure form and 3D printed tablet dosage form by the technique of simultaneous equation method. To conclude, the described method was validated in accordance with the International Conference on Harmonisation guidelines and give a specific, linear, accurate, precise, and sensitive results for the simultaneous determination of INH and PYR from pure form and 3D printed tablet dosage form. As mentioned in the discussion part, all the results got in this work were acceptable and corresponded with the results of other published articles. Hence, the suggested approach may be directly used to quantify INH and PYR simultaneously.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Attala, K., & Elsonbaty, A. (2020). Smart UV spectrophotometric methods based on simple mathematical filtration for the simultaneous determination of celecoxib and ramipril in their pharmaceutical mixtures with amlodipine: A comparative statistical study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 118853. doi:10.1016/j.saa.2020.118853

Atole, D. M., & Rajput, H. H. (2018). Ultraviolet spectroscopy and its pharmaceutical applications- A brief review. Asian Journal of Pharmaceutical and Clinical Resea4rch, 11(2), 59. doi:10.22159/ajpcr.2018.v11i2.21361

Basha, M. A., Hassan, S. A., Abd El-Rahman, M. K., Bebawy, L. I., & Moustafa, A. A. (2020). A comparative Study of two analytical techniques for the simultaneous determination of Amprolium HCl and Ethopabate from combined dosage form and in presence of their alkaline degradation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 118756. doi:10.1016/j.saa.2020.118756

Eissa, M. S. (2017). Simultaneous determination of the brand new two-drug combination for the treatment of hepatitis C: Sofosbuvir/ledipasvir using smart spectrophotometric methods manipulating ratio spectra. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 183, 362–370. doi:10.1016/j.saa.2017.04.052

Ferreira, C. P., Techera Antunes, F. T., Rebelo, I. N., da Silva, C. A., Vilanova, F. N., Silva Corrêa, D., & de Souza, A. H. (2020). Application of the UV-Vis Spectrophotometry Method for the Determination of Glutamate in the Cerebrospinal Fluid of Rats. Journal of Pharmaceutical and Biomedical Analysis, 113290. doi:10.1016/j.jpba.2020.113290

Kamal, A. H., Hammad, S. F., & Kamel, D. N. (2021). Chemometric spectrophotometric methods for simultaneous estimation of metoprolol succinate and amlodipine besylate in their tablet formulation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 254, 119641. doi:10.1016/j.saa.2021.119641

Kavathia, A., & Misra, M. (2017). Development and validation of RP-HPLC and UV-spectrophotometric methods for rapid simultaneous estimation of amlodipine and benazepril in pure and fixed dose combination.

Arabian Journal of Chemistry, 10, S3021–S3028. doi:10.1016/j.arabjc.2013.11.043

Li, Z., Sun, Y., Bar-Shalom, D., Mu, H., Larsen, S. W., Jensen, H., & Østergaard, J. (2021). Towards functional characterization of excipients for oral solid dosage forms using UV–vis imaging. Liberation, release and dissolution. Journal of Pharmaceutical and Biomedical Analysis, 194, 113789. doi:10.1016/j.jpba.2020.113789

Lorêdo de França, M., Separovic, L., Longo Junior, L. S., de Oliveira, D. C., Rebello Lourenço, F., & Calixto, L. A. (2021). Determining uncertainty in a simple UV–Vis spectrometry method employing dimethyl carbonate as green solvent for lead determination in water. Measurement, 173, 108581. doi:10.1016/j.measurement.2020.108581

Mahmood, A., Rapalli, V. K., Waghule, T., Gorantla, S., Dubey, S. K., Saha, R. N., & Singhvi, G. (2020). UV spectrophotometric method for simultaneous estimation of betamethasone valerate and tazarotene with absorption factor method: Application for in-vitro and ex-vivo characterization of lipidic nanocarriers for topical delivery. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 235, 118310. doi:10.1016/j.saa.2020.118310

Mahrouse, M. A., Elwy, H. M., & Salem, E. M. (2020). Simultaneous determination of cefixime and erdosteine in combined dosage form using validated spectrophotometric methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 118647. doi:10.1016/j.saa.2020.118647

Mansour, F. R. (2018). A new innovative spectrophotometric method for the simultaneous determination of sofosbuvir and ledipasvir. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 188, 626–632. doi:10.1016/j.saa.2017.07.066

Mashhadi, S. M. A., Batsanov, A. S., Sajjad, S. A., Nazir, Y., Bhatti, M. H., & Yunus, U. (2020). Isoniazid-Gentisic acid cocrystallization: Solubility, Stability, Dissolution rate, Antioxidant and Flowability Properties Studies. Journal of Molecular Structure, 129388. doi:10.1016/j.molstruc.2020.129388

Mawazi, S. M., Hadi, H. A. B., Al-mahmood Sinan Mohammed Abdullah, & Doolaanea, A. A. (2019). Development and validation of UV-Vis spectroscopic method of assay of carbamazepine in microparticles. International Journal of Applied Pharmaceutics, 11(1), 34. doi:10.22159/ijap.2019v11i1.26256