

SIMULTANEOUS DETERMINATION AND VALIDATION OF ANTI-TUBERCULAR DRUGS IN SIMULATED LUNGS ALVEOLAR MACROPHAGES FLUID BY ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRIC METHOD

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An ultraviolet-visible (UV-Vis) spectrophotometric method was developed for the simultaneous determination of isoniazid (INH) and rifampicin (RIF) using Vierordt's method. In this method, along with methanol, two other solvents such as phosphate buffer pH 7.4 and citro-phosphate buffer pH 5.0 were also selected, the former of which is simulated with the pH of lung epithelial lining fluid and the latter is simulated with the phagolysosomal environment. A 1:1.5 ratio of pure INH:RIF was selected and scanned, which showed similar absorption maxima at three solvents, and on that basis absorption maxima at 263 and 335 nm of INH and RIF, respectively, were selected. A calibration curve within the concentration ranges between 4 and 24 µg/mL of INH and 6–36 µg/mL of RIF follows the Beer–Lambert law. It was validated with respect to linearity, sensitivity, precision, and accuracy according to International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use guidelines.

Keywords: isoniazid, rifampicin, simultaneous determination, UV-Vis spectrophotometry.

УФ-СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОДНОВРЕМЕННОГО ОПРЕДЕЛЕНИЯ ПРОТИВОТУБЕРКУЛЕЗНЫХ ПРЕПАРАТОВ В МОДЕЛИРУЕМОЙ ЖИДКОСТИ АЛЬВЕОЛЯРНЫХ МАКРОФАГОВ ЛЕГКИХ

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Разработан спектрофотометрический метод в ультрафиолетовой и видимой области спектра (UV-Vis) для одновременного определения изониазида (INH) и рифампицина (RIF) с использованием метода Виродта. Наряду с метанолом выбраны два других растворителя — фосфатный буфер с pH 7.4 и цитро-фосфатный буфер с pH 5.0, первый из которых моделирует pH эпителиальной жидкости слизистой оболочки легких, а второй — фаголизосомальной среды. Выбрано соотношение 1:1.5 чистого лекарственного средства INH:RIF. Выявлены схожие максимумы поглощения в трех растворителях, и поэтому выбраны максимумы поглощения при 263 и 335 нм INH и RIF. Калибровочная кривая в диапазоне концентраций 4–24 мкг/мл INH и 6–36 мкг/мл RIF подчиняется закону Ламберта–Бера. Метод проверен с точки зрения линейности, чувствительности и достоверности в соответствии с рекомендациями ИСН.

Ключевые слова: изониазид, рифампицин, одновременное определение, УФ-видимая спектрофотометрия.

INH

RIF

Experimental. Isoniazid and rifampicin were received as gratis samples from Lupin Limited, Chikalthana, Aurangabad, Maharashtra, India. Methanol of analytical reagent grade, and potassium dihydrogen phosphate, sodium hydroxide, and citric acid were purchased from Loba Chemie (India). Milli-Q system (Millipore, USA) water was also used in this study. All the solutions including buffers were prepared fresh and protected from light. A double-beam UV-Vis (Shimadzu UV-1800) spectrophotometer was used to carry

out the experiments. Simultaneous determination of INH and RIF was done using Vierordt's method, which is known as a simultaneous equation method [12]. This method was simple, rapid, and economical.

Phosphate buffer pH 7.4 resembles simulated lung epithelial lining fluid, and for the preparation of the latter we used the chemicals potassium dihydrogen phosphate and sodium hydroxide. We took 50 mL of 0.2 M potassium dihydrogen phosphate in a 200-mL volumetric flask, and then added 39.1 mL of 0.2 M sodium hydroxide and made up the volume with double-distilled water (Milli-Q system water) [13].

The citro-phosphate buffer pH 5.0 resembles lung alveolar macrophage fluid. It was prepared by mixing 48.0 mL of 0.1 M citric acid with sufficient 0.2 M disodium hydrogen phosphate to produce 100 mL [13].

Here, for the simultaneous determination of INH and RIF, a ratio of 1:1.5 was selected. The ratio was decided on the basis of data available from the World Health Organization's international monographs on INH and RIF for this combined dosage form [14]. Ten milligrams of INH and 15 mg of RIF were dissolved separately in different solvents: methanol, phosphate buffer pH 7.4, and citro-phosphate buffer pH 5.0. The solutions were sonicated for 5 min, diluted to a required concentration and scanned separately between 200 and 400 nm for INH and for RIF, the solution was scanned at 200–800 nm against a blank, in order to determine the maximum absorption wavelength of both drugs.

Results and discussion. Spectra of INH and RIF were recorded in methanol, phosphate buffer, and in citro-phosphate buffer, and the obtained absorption maxima were depicted as in Table 1, which is very similar with each solvent.

After scanning at absorption of 200–400 nm for INH, the absorption maxima were observed at 263 nm in methanol. In phosphate buffer pH 7.4, and in citro-phosphate buffer pH 5.0, the absorption maxima were observed at 262 nm, which is similar to methanol. RIF can be determined by UV and visible spectroscopy. RIF was scanned at 200–800 nm. Three peaks were observed at 254, 334.50, and 481 nm in methanol and 254, 335, and 481 nm in both the phosphate buffer pH 7.4 and the citro-phosphate buffer pH 5.0. Also, from Table 1, we can see the similar absorption maxima for three solvents. On that basis, the absorption maxima at 263 and 335 nm for INH and RIF, respectively, were selected for further study. After comparing absorption maxima of methanol, phosphate buffer pH 7.4, and citro-phosphate buffer pH 5.0, we selected citro-phosphate buffer pH 5.0 as a solvent, because it resembles the alveolar macrophage fluid environment. The absorption spectra of INH and RIF in citro-phosphate buffer pH 5.0 are shown in Fig. 1.

TABLE 1. UV Absorption Maxima (λ , nm) of INH and RIF in Methanol, Phosphate Buffer pH 7.4, and Citro-Phosphate Buffer pH 5.0

Analyte	Methanol	Phosphate buffer pH 7.4	Citro-phosphate buffer pH 5.0
INH	263	262	262
RIF	253 334.50 481	253 335 481	252.50 335 481

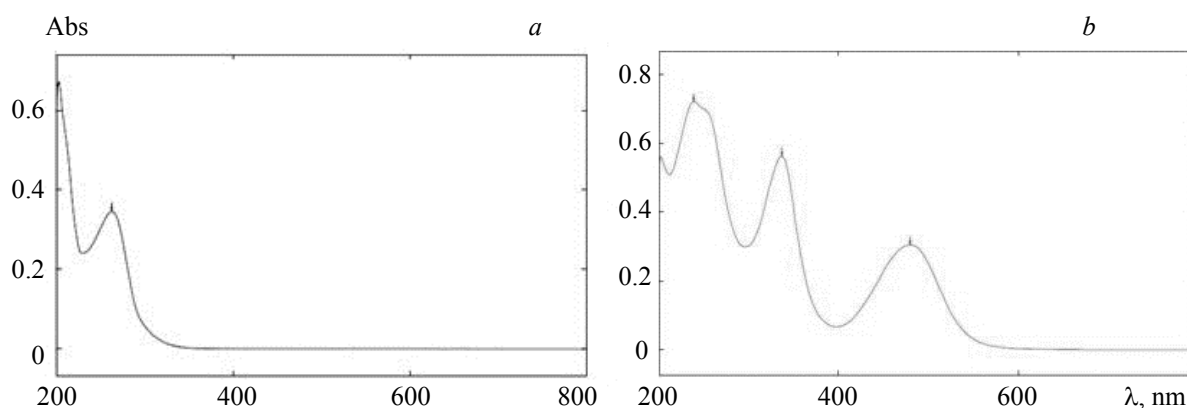


Fig. 1. Absorption spectra of (a) INH and (b) RIF in citro-phosphate buffer pH 5.0.

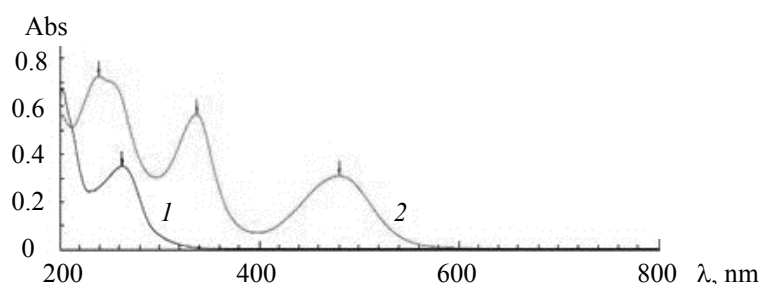


Fig. 2. Overlay spectrum of INH (1) and RIF (2) in citro-phosphate buffer pH 5.0.

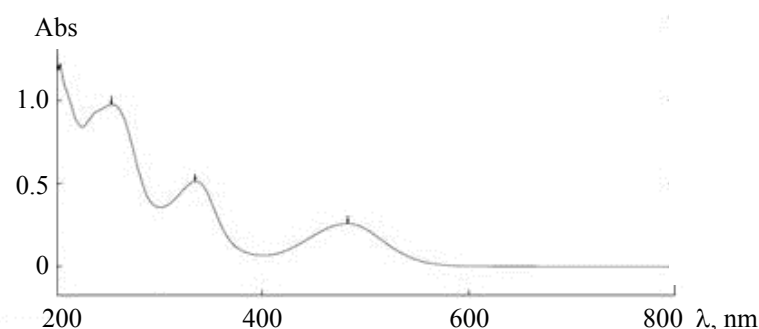


Fig. 3. Combined peak of INH and RIF in citro-phosphate buffer pH 5.0.

The overlaid spectra of INH and RIF were depicted in Fig. 2, and 263 and 335 nm for INH and RIF respectively were selected for further study. A mixture of INH and RIF in the ratio of 1:1.5 was measured by UV-Vis spectrophotometry and it was observed that absorptivity at 263 nm was additive as depicted in Fig. 3.

The overlay spectra of INH and RIF in citro-phosphate buffer pH 5.0 as depicted in Fig. 4 shows the significant increase in absorbance with the increase in the concentration range of 4–24 µg/mL for INH and 6–36 µg/mL for RIF.

Specific absorbance A (1%) was determined at both selected wavelengths λ of each drug as

$$A = A_{at} / C,$$

where C is the concentration, g/100 mL.

Simultaneous determination was calculated from the following:

$$C_{INH} = (A_{mix\ 335nm}A_{RIF\ 263nm} - A_{mix\ 263nm}A_{RIF\ 335nm}) / (A_{INH\ 335nm}A_{RIF\ 263nm} - A_{INH\ 263nm}A_{RIF\ 335nm}),$$

$$C_{RIF} = (A_{mix\ 263nm}A_{INH\ 335nm} - A_{mix\ 335nm}A_{INH\ 263nm}) / (A_{INH\ 335nm}A_{RIF\ 263nm} - A_{INH\ 263nm}A_{RIF\ 335nm}),$$

where C_{INH} and C_{RIF} are the concentrations of INH and RIF, $A_{INH\ 263nm}$ and $A_{INH\ 335nm}$ are the absorptivity of INH at 263 and 335 nm, $A_{RIF\ 263nm}$ and $A_{RIF\ 335nm}$ are the absorptivity of RIF at 263 and 335 nm, and $A_{mix\ 263nm}$ and $A_{mix\ 335nm}$ are the absorbance of mixture 1:1.5 (INH:RIF) at 263 and 335 nm, respectively.

The method was validated according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Q2B guidelines for validation of analytical procedures in order to determine linearity, precision, and accuracy of the analyte [15].

The linearity of measurement was evaluated by analyzing different concentrations of INH and RIF. Here, for the simultaneous determination of INH and RIF by the simultaneous equation method, the Beer-Lambert concentration range was found to be 4–24 µg/mL for INH and 6–36 µg/mL for RIF, as shown in Fig. 5. The linearity data of INH and RIF in citro-phosphate buffer are following: for INH regression equation $y = 0.037x - 0.008$, correlation coefficient $r^2 = 0.999$, and for RIF $y = 0.031x + 0.002$, $r^2 = 0.999$.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated as

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s,$$

where σ is the standard deviation of the Y intercept of the calibration curve, and s is the slope of the regression equation. The sensitivity results of INH and RIF in citro-phosphate buffer pH 5.0 are follows: for INH $LOD = 0.2245$ µg/mL, $LOQ = 0.6803$ µg/mL; for RIF $LOD = 0.3194$ µg/mL, $LOQ = 0.9677$ µg/mL.

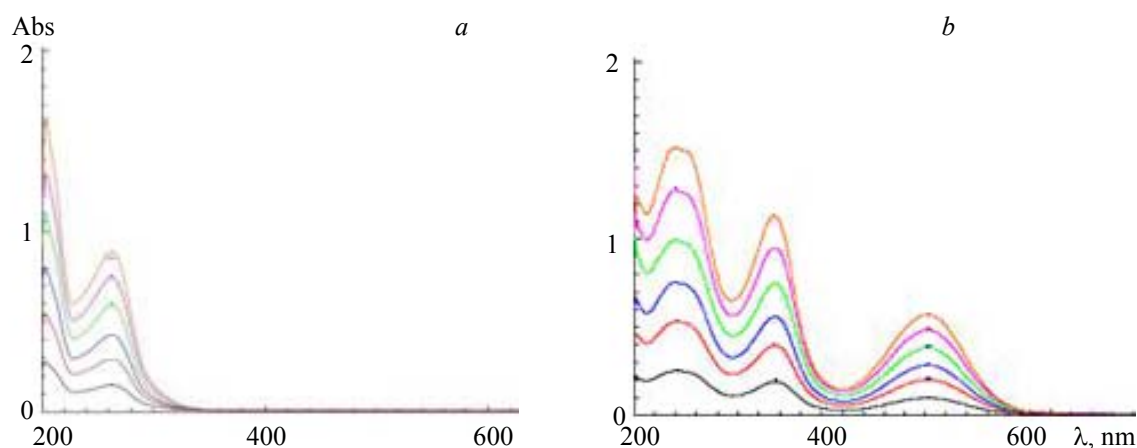


Fig. 4. Overlaid spectra of (a) INH and (b) RIF in citro-phosphate buffer pH 5.0.

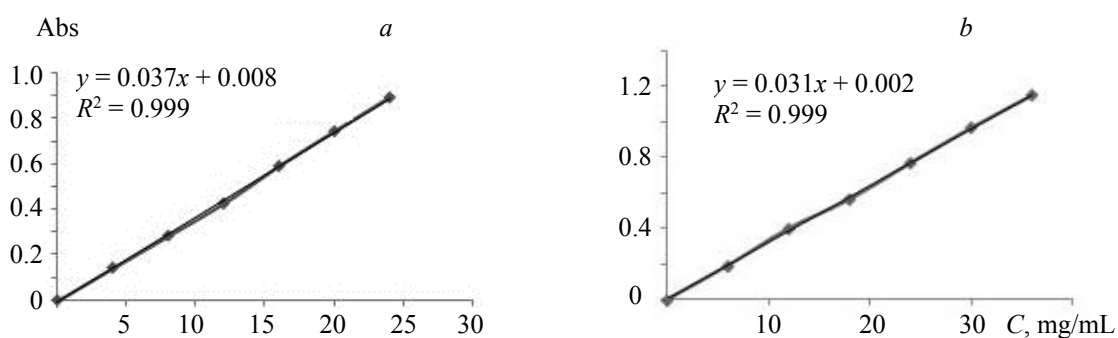


Fig. 5. Calibration curve of (a) INH and (b) RIF in citro-phosphate buffer pH 5.0.

The precision of the method was established by carrying out the analysis of INH and RIF ($n = 6$) by using the proposed developed method. The low value of relative standard deviation (RSD) showed that the method was precise, and its results are shown in Table 2.

Inter-day precision was analyzed by the same analyst on alternate days for 3 days. Intra-day precision was analyzed by the same analyst three times a day at intervals of 1 h. The % RSD is as shown in Table 2.

To determine the accuracy of the simultaneous equation method, percent recovery study was performed, and it was carried out using a standard addition method at 80, 100, and 120% level. The percentage recovery was calculated from the total amount of drug found. The results of recovery studies were depicted in Table 2.

TABLE 2. Recovery and Precision Data of INH and RIF in Citro-Phosphate Buffer pH 5.0.

% Recovery level	Analyte concentration, $\mu\text{g/mL}$	Pure drug added, $\mu\text{g/mL}$	Concentration found, $\mu\text{g/mL}$	% Recovery	% MR ($\pm\text{SD}$)	% RSD*	
						Inter-day	Intra-day
INH							
80	8	6.4	13.99	97.22	96.94 $\pm$ 0.319739	0.0964	0.6029
100	8	8	15.52	97.00			
120	8	9.6	16.99	96.59			
RIF							
80	12	9.6	19.79	91.66	93.23 $\pm$ 1.517641	0.1462	0.1498
100	12	12	22.39	93.33			
120	12	14.4	24.99	94.69			

*Analyte concentration used is 24 $\mu\text{g/mL}$.

Conclusions. The proposed simultaneous UV-Vis spectroscopic method was developed and validated. It was found to be simple, accurate, precise, and an acceptable recovery of isoniazid and rifampicin was observed. It can be concluded that the developed method can be easily applied for routine analysis in quality control of isoniazid and rifampicin.

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