

Roxithromycin probably effects the reduction of one, two or three oxygen atoms from tungstate and/or molybdate, there by producing one or more of several possible reduced species which have a characteristic intense blue colour. In conclusion, the proposed methods are simple, sensitive and accurate and can be used for the routine determination of roxithromycin in bulk as well as in its pharmaceutical preparations.

REFERENCES

1. Budavari, S., Eds., In: The Merck Index, 11th Edn., Merck & CO., Inc., Whitehouse Station, NJ, 1989, 8253.
2. Reynolds, E.F.J. and Prasad, B.A., Eds., In; Martindale, The Extra Pharmacopoeia 29th Edn., The Pharmaceutical Press, London, 1989, 294.
3. Sane, R.T., Kulkarni, U.P., Patel, M.K. and Tindokar, V.B., *Indian Drugs*, 1992, 29, 658.
4. Collins, P.G., Diehl, H. and Smith, F.G., *Anal. Chem.*, 1959, 31, 1862.
5. De oliveria, V., Bergold, A.M., Schapoval, E.E.S., *Anal. Lett.*, 1996, 29, 2377.
6. Taninaka, C., Ohtani, H., Hanada, E., Kotaki, H., Sato, H., Iga, T., *J. Chromatogr.*, 2000, 738, 405.
7. Sastry, C.S.P., Rao, S.G. and Srinivas, K.R., *Indian Drugs*, 1998, 35, 594.
8. Peterson, G.L., *Anal. Biochem.*, 1979, 100, 201.

Simultaneous Spectrophotometric Estimation of Isoniazid and Rifampicin from Combined Dosage Forms

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The present communication deals with the development of a simple, specific, sensitive, rapid and economical procedure for simultaneous estimation of rifampicin and isoniazid in a combined dosage form. The method is based on the native ultraviolet absorbance maxima of the two chemotherapeutic agents. As both compounds do not interact chemically in phosphate buffered saline, two wavelengths 263 nm and 333 nm (λ_{max} of isoniazid and λ_{max} of rifampicin, respectively) were used. In addition, rifampicin also shows absorbance at 263 nm in phosphate buffered saline. Both the drugs obey Beer's law in the concentration range that was employed in the method.

Rifampicin (RIF) is a bactericidal antibiotic with a wide spectrum of activity. Though rifampicin is active against gram positive and gram negative bacteria, this drug has been advocated mainly for the treatment of tuberculosis¹. Isoniazid (INH) is bacteriostatic for resting bacilli and chemically it is the hydrazide of isonicotinic acid i.e., 4-pyridine carboxylic acid hydrazide. RIF and INH are official in IP², BP^{3,4} and USP^{5,6}. A combination of 450 mg of RIF and 300 mg of INH is commercially available as tablets and capsules. The IP⁷ suggests a microbiological method

for RIF and a titrimetric method for INH. Few spectrophotometric methods reported in the literature for simultaneous estimation of RIF and INH are tedious and require number of steps and various types of reagents to get the final results^{8,9}. The proposed method is specific, sensitive, rapid, economical and very simple to perform by using very few chemicals and steps.

A Shimadzu UV/Vis recording spectrophotometer (Model: UV-240, Graphiccord) with Spectral band width variable from 0.008 nm to 5 nm in 0.001 nm steps and a resolution of 0.1 nm with Photomultiplier detector R 928-05 was used in the study. The wavelength accuracy of the

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spectrophotometer is ± 0.3 nm with automatic wavelength correction and wavelength reproducibility is ± 0.1 nm. RIF and INH were gift samples from Cadila Pharmaceuticals, Ahmedabad.

Methanol was used as a cosolvent to dissolve RIF in phosphate buffered saline (PBS). Sodium chloride (8.0 g), disodium hydrogen orthophosphate (2.38 g) and sodium dihydrogen orthophosphate (0.19 g) were accurately weighed and dissolved in distilled water. Volume was made upto to 1000 ml with the distilled water and pH adjusted to 7.4. Standard stock solutions containing 100 $\mu\text{g/ml}$ of RIF and 100 $\mu\text{g/ml}$ of INH were made in phosphate buffer saline in separate volumetric flasks. From the standard stock solution of 100 $\mu\text{g/ml}$ of RIF, different concentration of 5, 10, 15, 20, 25, and 30 $\mu\text{g/ml}$ were prepared in PBS and absorbance was recorded spectrophotometrically at 263 nm.

Six mixed standards with different compositions were prepared in PBS and their compositions have been listed in Table 1. As both compounds do not interact chemically in PBS, two wavelengths, 263 and 333 nm were selected for estimations. The absorbance at these two sampling points was used to determine the concentrations of the two drugs in presence of each other i.e., the absorbance of mixed

TABLE 1: COMPOSITION OF MIXED STANDARDS.

Standard Number	1	2	3	4	5	6
Rifampicin (RIF) ($\mu\text{g/ml}$)	5	10	15	20	25	30
Isoniazid (INH) ($\mu\text{g/ml}$)	5	10	15	20	25	30

Mixed standard solutions of RIF and INH ranging from 5 $\mu\text{g/ml}$ were prepared in phosphate buffered saline, pH 7.4.

standard was taken at 263 nm and 333 nm and the absorbance of plain RIF was taken at 263 nm.

Twenty tablets of each of the brands Rimactazid®, Novartis India Limited (T1) and Rifacom®, Indoco Laboratories (T2), were taken at random, weighed accurately and crushed to fine powder. Accurately weighed powder samples equivalent to 10 mg of RIF and 5 mg of INH from each brand were dissolved in PBS in 100 ml volumetric flask separately and filtered and final volume was made up with PBS. The solutions prepared were diluted with PBS to get the final concentration 20 $\mu\text{g/ml}$ of rifampicin and 10 $\mu\text{g/ml}$ of isoniazid. The absorbance of the final solutions was recorded at 263 and 333 nm. The concentrations of rifampicin and isoniazid in the sample solutions were computed from the standard plot of mixed solution at 263 nm and 333 nm and from the standard plot of plain rifampicin at 263 nm. Recovery studies were conducted by addition of different amount of pure drugs into pre-analyzed tablet sample solutions gave satisfactory results which are tabulated in Table 3.

A simple, rapid and sensitive procedure for simultaneous estimation of RIF and INH in combined dosage form was developed. The method is based on the native ultraviolet absorbance maxima of the two chemotherapeutic agents. After several trials, the use of six mixed standards and two-wave lengths was found to reduce interference between the two components. As both compounds do not interact chemically in PBS, two wavelengths were selected 263 and 333 nm. INH has λ_{max} at 263 nm and 333 nm is λ_{max} of RIF in PBS. Since RIF also shows absorbance at 263 nm, the absorbance at 263 nm is the sum of absorbance due to RIF and INH at 263 nm. The absorbance due to RIF at 263 nm was subtracted from the absorbance obtained from combined solution at 263 nm to

TABLE 2: ANALYSIS OF TWO BRANDS COMMERCIAL TABLETS.

Tablet sample	Labeled claim (mg/tablet)		Found (mg/tablet)		Percentage of label claim found	
	RIF	INH	RIF	INH	RIF	INH
T1	450	300	452.87	302.41	100.63	100.80
T2	450	300	451.62	304.12	100.36	101.37

Two brands of tablets, T1 (Rimactazid® from Novartis India Limited) and T2 (Rifacom® from Indoco Laboratories) were analysed for rifampicin (RIF) and isoniazid (INH). All the experiments were repeated for six times (n=6).

TABLE 3: STATISTICAL VALIDATION OF THE METHOD.

Tablet sample	Standard deviation		Coefficient of variation		Standard error		Percentage of recovery	
	RIF	INH	RIF	INH	RIF	INH	RIF	INH
T1	1.68	1.22	0.018	0.014	1.01	0.089	99.62	099.59
T2	1.91	1.63	0.026	0.021	1.19	1.02	99.87	099.96

Above table depicts the statistical validation of analysis of rifampicin (RIF) and isoniazid (INH) from two brand tablets, T1 (Rimactazid® from Novartis India Limited) and T2 (Rifacom® from Indoco Laboratories). All the experiments were repeated for six times (n=6).

get the absorbance of INH at 263 nm and subsequently the concentration of INH. While the absorbance at 333 nm was only due to rifampicin as INH does not show any absorbance at 333 nm. The concentration of RIF was directly computed from the absorbance at 333 nm. By this mathematical manipulation, one could measure the exact amount of RIF and INH in the presence of each other in a combined dosage form. The values of standard deviation and coefficient of variation were satisfactorily low. Recovery study indicates the reproducibility and accuracy of the method. Table 2 and 3 show the results of analysis of commercial tablets of two different bands by reported method and statistical validation of reported method respectively.

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REFERENCES

1. Gaud, R.S., Jain, D.K. and Chaturvedi, S.C., *Indian drugs.*, 1988, 26, 324.
2. The Indian Pharmacopoeia, 3rd Edn., The Controller of Publication, Delhi, 1985, 268.
3. British Pharmacopoeia, International Edn., HMSO, London, 1993, 363.
4. British Pharmacopoeia, International Edn., HMSO, London, 1993, 580.
5. The United State Pharmacopoeia XXI, United States Pharmacopoeial Convention, Rockville, MD, 1985, 566.
6. The United State Pharmacopoeia XXI, United States Pharmacopoeial Convention, Rockville, MD, 1985, 944.
7. The Indian Pharmacopoeia, 3rd Edn., The Controller of Publication, Delhi, 1985, 429.
8. Dahibhate, P.P., Chandwani, O.D., Kadam, S.S. and Dhaneshwar, S.R., *Indian Drugs*, 1997, 34, 95.
9. Rao B.E., Raghuvver, S. and Srivastava, C.M.R., *Indian Drugs*, 1992, 29, 412.