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# Simultaneous spectrophotometric determination of Rifampicin, Isoniazid and Pyrazinamide by first - Derivative UV Spectrophotometry in combined pharmaceutical dosage forms

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**A rapid and simple method for the simultaneous determination of rifampicin (RIF), isoniazid (INH) and pyrazinamide (PYZ) by first - derivative UV spectrophotometry has been developed in combined pharmaceutical dosage forms. RIF is determined by measuring the signal at zero crossing point for INH and PYZ (262.2 and 268.8 nm), INH is determined from the signal at the zero crossing point for RIF and PYZ (254.0 and 268.8 nm) and PYZ is determined from the signal at zero crossing point for INH and RIF (262.2 and 254.0 nm) respectively. Good linearity, precision and reproducibility were found. The proposed method was successfully applied to the determination of drugs in physical mixture and formulation.**

RIFAMPICIN (RIF), isoniazid (INH) and pyrazinamide (PYZ) are effective drugs for the treatment of tuberculosis[1]. These drugs are used either alone or in combination. The simultaneous determination of these three drugs is not possible by direct UV absorption measurement because of spectral overlap of their principal maxima. The present work consists of taking a derivative of convenient order of the analytical signal e.g. absorbance in the wavelength domain.

Besides the official methods for individual drugs (IP[2] and USP[3]), the other analytical methods available in literature and ion pair extraction[4], HPTLC[5] and titrimetry[6] for RIF, HPLC[7], colorimetric[8] and oscillopolarography[9] for INH and polarography[10], spectrophotometry[11] and HPLC[12] for PYZ.

The RIF and INH in combined dosage form is official in USP[3], wherein INH is estimated potentiometrically using bromine solution while RIF is estimated by HPLC using an octylsilane column. Other methods reported for RIF in combination are least squares methods in the matrix form[13] and HPLC[14]. The RIF, INH and PYZ is an unofficial combination and only HPLC method[15] is reported. Analysis of such a matrix through normal phase HPLC method is time consuming. Therefore, an alternative method based on selective derivative UV spectrophotometry is reported and the optimum experimental parameters are described.

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## EXPERIMENTAL

Spectrophotometric analysis was carried out on a Shimadzu UV 160 A recording spectrophotometer in 1 cm matched quartz cells. The amplitudes were obtained directly from the display of the first derivative in the wavelength range 200-350 nm with derivation interval ($\Delta\lambda$5nm) at a fast scan speed for determination of RIF, INH and PYZ.

Table 1
Result of linearity test and calibration graph for RIF, INH and PYZ by proposed method

| Analyte | Method | Regression Parameters | | Coefficient of correlation (r) | RSD % |
|---|---|---|---|---|---|
| | | Slope (1) | Intercept (i) | | |
| **Linearity** | | | | | |
| RIF | $^1$D262.2 | 0.00428 | -0.0018 | 0.9996 | 0.984 |
| n = 5 | $^1$D268.8 | 0.00642 | 0.0028 | 1.000 | 0.657 |
| INH | $^1$D254.0 | 0.00187 | 0.0017 | 0.9995 | 1.97 |
| n = 5 | $^1$D268.8 | 0.00436 | 0.003 | 0.9999 | 1.13 |
| PYZ | $^1$D254 | 0.01832 | 0.001733 | 0.9999 | 1.5 |
| n = 8 | $^1$D262.2 | 0.016634 | 0.025405 | 0.9993 | 2.38 |
| **Calibration** | | | | | |
| RIF | $^1$D262.2 | 0.033962 | 0.011364 | 0.9996 | 1.89 |
| n = 6 | $^1$D268.8 | 0.012838 | -0.00674 | 0.9982 | 2.59 |
| INH | $^1$D254.0 | 0.0635 | 0.0013 | 0.9999 | 1.01 |
| n = 5 | $^1$D268.8 | 0.018 | -0.0038 | 0.9999 | 0.653 |
| PYZ | $^1$D254 | 0.018257 | 0.0004 | 0.9999 | 1.99 |
| n = 4 | $^1$D262.2 | 0.015228 | 0.0055 | 1.000 | 0.725 |

RSD - Reactive Standard Deviation

Authentic specimen of RIF, INH and PYZ were procured from M/s Plethico Pharmaceuticals, Indore. The drugs were used as received. All other chemicals used were of analytical reagent grade.

## LINEARITY

Linearity was investigated by preparing 20 μg/ml solution each of RIF, INH and PYZ in distilled water. The linearity was found to be 0 - 60 μg/ml, 0 - 50 μg/ml and 0 - 40 μg/ml by recording first derivative spectrum over a wavelength range of 200-350 nm respectively for RIF, INH and PYZ. The correlation between measured values and concentration were evaluated by means of least squares method. The results are given in Table 1.

## CALIBRATION

Standard solution of RIF (9.0 μg/ml), INH (6.0 μg/ml) and PYZ (21.0 μg/ml) were prepared in distilled water. The first derivative spectrum were recorded over the wavelength range of 200 - 350 nm and appropriate first derivative amplitudes were measured and plotted against the corresponding concentration to obtain the calibration graph Table 1.

## ANALYSIS OF TABLETS AND CAPSULES

An accurately weighed portion (60 mg) of the powder (mixed contents of 20 tablets) equivalent to about 15 mg of RIF, 10 mg of INH and 35 mg of PYZ and an accurately weighed portion (62.5 mg) of powder obtained from mixed content of 20

Table 2

**Results obtained from authentic and commercial samples for RIF, INH and PYZ by proposed method**

| Authentic sample | | | | | Commercial sample | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analyte | Taken ug/ml | Found ug/ml | Relative Error (%) | $\bar{R} + ts/\sqrt{n}$ | Analyte | Found % | RSD % | $\bar{R} + ts/\sqrt{n}$ | 't' Value |
| RIF | 6.0 | 6.08 | 1.33 | 99.46 ± 2.33 | RIF | | | | |
| | 6.0 | 5.86 | -2.33 | | A | 99.87 | 0.264 | 99.87± 0.33 | 2.75 |
| | 6.0 | 5.88 | -2.0 | | B | 100.23 | 0.641 | 100.23±0.79 | 2.75 |
| | 6.0 | 6.13 | 2.16 | | C | 99.48 | 0.962 | 99.48±1.18 | 2.75 |
| | 6.0 | 5.89 | -1.83 | | D | 99.27 | 0.931 | 99.27±1.14 | 2.75 |
| INH | 4.0 | 4.07 | 1.75 | 99.7 ± 2.52 | INH | | | | |
| | 4.0 | 3.90 | -2.5 | | A | 97.95 | 0.250 | 97.95±0.91 | 2.73 |
| | 4.0 | 3.95 | -1.25 | | B | 100.75 | 0.733 | 100.75±0.91 | 2.75 |
| | 4.0 | 4.1 | 2.5 | | C | 99.80 | 0.689 | 99.80±0.84 | 2.77 |
| | 4.0 | 3.92 | -2.00 | | D | 100.67 | 0.772 | 100.67±0.96 | 2.76 |
| PYZ | 14.0 | 14.17 | 1.21 | 100.54 ± 1.81 | PYZ | | | | |
| | 14.0 | 13.92 | -0.57 | | A | 102.82 | 0.628 | 102.82±0.80 | 2.76 |
| | 14.0 | 13.80 | -1.42 | | B | 98.73 | 0.583 | 98.73±0.71 | 2.77 |
| | 14.0 | 14.36 | 2.57 | | C | 97.22 | 1.032 | 97.22±1.24 | 2.77 |
| | 14.0 | 14.19 | 1.35 | | D | 97.80 | 0.435 | 97.80±0.52 | 2.73 |

$\bar{R}$ = Mean percent recovered A = Tablet sample (labelled content : RIF 150 mg, INH 100 mg and PYZ 350 mg) B = Tablet recovery C = Capsule Sample (labelled content : RIF 150 mg, INH 100 mg and PYZ 375 mg), D = Capsule Recovery.

capsules equivalent to about 15 mg of RIF, 10 mg of INH and 35.5 mg of PYZ were transferred into a 100 ml calibrated flask separately and were extracted by shaking with 50 ml of distilled water for 10 minutes. The resulting solution was filtered. Then 4.0 ml aliquots of the above solution was pipetted out into a 100 ml calibrated flask and the resulting solution was subjected directly to spectrophotometric analysis.

## RESULTS AND DISCUSSION

The Zero order absorption UV spectra of RIF (10 μg/ml, INH (10 μg/ml) and PYZ (10 μg/ml) in the 200-350 nm wavelength region are shown in figure 1.

RIF exhibited three absorption peaks at about 233.0 nm, 256.6 nm and 333.2 nm respectively. INH also absorbed over this wavelength region, with a peak at 263.2 nm and PYZ also exhibited a peak at 268.8 nm.

The extensive overlap of the spectral bands of the three drugs, refrained conventional UV spectrophotometry for their individual determination in a mixture. The first derivative spectra recorded sharp bands of large amplitude of RIF (10 μg/ml), INH (10 μg/ml) and PYZ (10 μg/ml) as shown in figure 2, which permitted more selective identification and determination of these drugs. The choice of the optimum wavelength is based on the fact that the contribution of each component to the over all

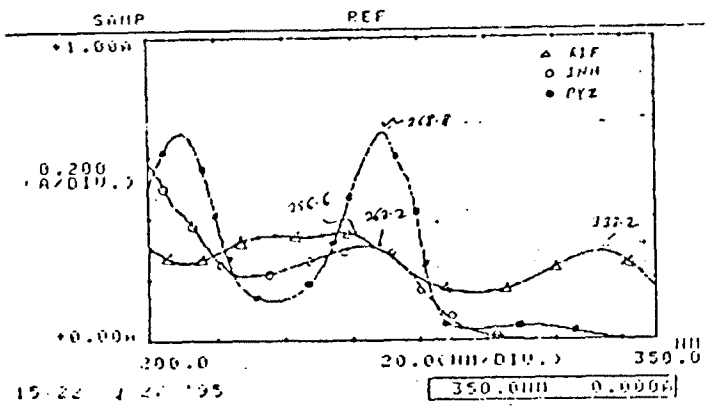

**Fig. 1 Zero order spectrum of RIF (10 ug/ml) INH (10 ug/ml) and PYZ (10 ug/ml) in distilled water**

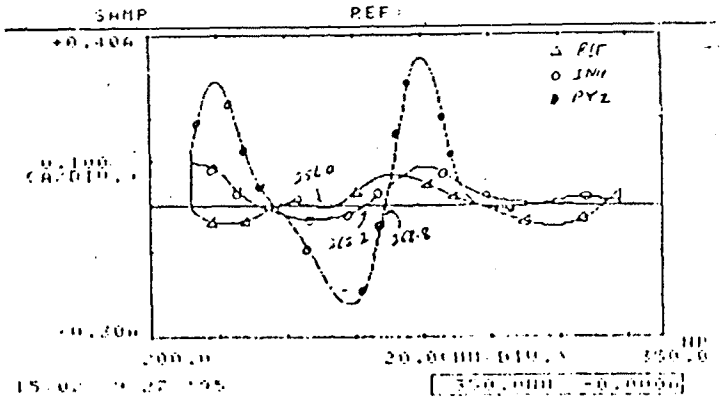

**Fig. 2 First derivative spectrum of RIF (10 ug/ml) INH (10 ug/ml) and PYZ (10 ug/ml) in distilled water**

derivative signal is zero at the wavelength at which other component exhibited maximum absorption. Therefore, the first derivative amplitude at 262.2 nm and 268.8 nm (zero crossing of INH and PYZ) at 254.0 nm and 268.8 nm (zero crossing of RIF and PYZ) and 254 nm and 262.8 nm (zero crossing of RIF and INH) were selected for the simultaneous determination of RIF, INH and PYZ respectively in the mixture form.

Linear relationship between selected amplitudes from first derivative spectra and concentration were presented in Table 1. Least squares regression analysis was carried out on the slope (1), the intercept (i) and the correlation coefficient (r). The relative standard deviation calculated for the separate determination of each drug was 0.657 to 2.38 percent for linearity and 0.653 to 2.59 percent for calibration graph indicating good precision and reproducibility.

To study the recovery of RIF, INH and PYZ, fixed quantity of preanalysed tablet and capsule sample solution was taken and estimated by the proposed first derivative UV spectrophotometry method. The results were found to be accurate and precise as indicated by the recovery (97.8 ± 0.52 to 100.75 ± 0.91 percent) and the relative standard deviation (0.435 to 0.931 percent). The accuracy and the precision as well as the confidence limits of the proposed method were experimentally tested and were found satisfactory.

The results obtained from the determination of RIF, INH and PYZ using the proposed method were statistically compared by means of the students 't' test and were found to be satisfactory.

In conclusion, the derivative UV spectrophotometry appears to be a suitable techniques for the reliable analysis of commercial formulations containing combination of RIF, INH and PYZ. The most striking features of the derivative method are its simplicity, sensitivity and rapidity. It is also an easier and cheaper method than a HPLC separation and does not require the use of an expensive or a toxic reagent. These advantages make it especially suitable for routine quality control.

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## REFERENCES


1. Gilman A.G. and Goodman L.S. In the Pharmacological Basis of Theraputics, 4th Ed. The Macmillan Company, 1970, 1333, 1324, 1330.

2. Pharmacopoeia of India, The controller of publication Govt. of India., 1985, 430, 448.

3. The united states Pharmacopoeia, convention inc. 12601, Twinbroak 1990, 22nd Revision 729, 1192, 1227, 1228.

4. Reddy B.S. and Sastry, C.S.P. **J. Inst. Chem.**, 1983, 55, 69.

5. Jindal, K.C., Chaudhary, R.S., Gangawal, S.S., Singla, A.K. and Khanna, S.C.S., **J. Chromotogr.**, A, 1994, 685, 195.

6. Kilic, E, Koseo, F. and Akay, M.A., **J. Pharm. Biomed. Anal.**, 1994, 12, 347.

7. Butterfield, A.G. Lovering, E.G. and Sears, R.W. **J. Pharm. Sci.**, 1980, 69, 222.

8. Laipanov, A.K., Shulga, T.A. and Obukhova, T.A., **Farmatsiya**, 1983, 32, Through **Anal Abstr.**, 1983, 45, 48, 75.

9. Defileppi, A. and Piancone, G. **J. Chromatogr. Biomed.**, Appl., 1994, 656, 466.

10. Was, T., Akimoto, K. and Yamamoto H, **Bunseki Kagaku.**, 1984, 33, Through **Anal Abstr.**, 1985, 47, 7J30.

11. Rao, E.V. Murthy, S.S.N., and Rao, G.R., **Indian Drugs.**, 1985, 22, 269.

12. Kaka, J.S., **J. Liq. Chromatogr.**, 1994, 17, 3793, 3801.

13. Mahalanabis, K.K., Basu, D. and Roy, D.,**Analyst London.**, 1989, 114, 1311.

14. Ramana Rao, G. and Murthy, S.S.N., **Indian J. Pharm. Sci.**, 1984, 46, 181.

15. Rao, B.E., Raghuveer, S. and Srivastava, C.M.R., **Indian Drug.**, 1992, 29, 412.