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RAPID AND SENSITIVE ESTIMATION OF ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN IN COMBINED DOSAGE FORM BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid and sensitive method of estimation for isoniazid, pyrazinamide and rifampicin from pharmaceutical dosage was developed. The mobile phase consisting of 80:20, methanol-tetrabutyl ammonium hydroxide (0.005N) pH 3.0 adjusted with phosphoric acid was used with an ODS -CN bonded phase column. The separation of the above drugs was accomplished within 10 min at a flow rate of 1.5 ml/min. For accurate quantitation clofazimine was used as an internal standard with UV detector set at 265 nm. No interference from a variety of excipients present in the dosage forms was observed.

INTRODUCTION

The successful treatment of antitubercular drugs on the tubercular infiltration depends on their bacteriostatic effect in short- or long-course chemotherapy and res-

istance. Despite the large number of drugs available however, five drugs : isoniazid, pyrazinamide, rifampicin, ethambutol and streptomycin sulfate are the principal agents now used in helping patients with pulmonary tuberculosis.

During initial intensive phase in short-course regimens, a combination of isoniazid as bactericidal agent, pyrazinamide and rifampicin were administered in the pharmaceutical dosage form. Many methods for determining isoniazid (1-3), pyrazinamide (4-6) and rifampicin (7) in tablets or capsules have been reported. But these were either time consuming, complex or laborious.

Liquid chromatographic assays (8-13) of isoniazid individually or in combination with other drugs such as rifampicin has also been reported. However, none of these methods comprehend the separation of isoniazid, pyrazinamide and rifampicin in a single step.

We report a rapid reversed-phase liquid chromatographic separation of isoniazid, pyrazinamide and rifampicin from a unit dose. A cyano-bonded phase ODS column was used to separate these drugs with the help of an ion-pairing agent tetrabutyl ammonium hydroxide. Quantitative estimation was accomplished by using clofazimine as an internal standard with UV detection at 265 nm.

EXPERIMENTAL

Apparatus : A liquid chromatographic system (LDC/Milton Roy, Riviera Beach, Florida, U.S.A.) consisting of a dual piston reciprocating pump, ConstaMetric III, UV-visible

variable wavelength detector, SpectroMonitor III 1204D and a Rheodyne injector 7125, 20 ul loop was used. The column used was Excalibar ODS -CN, 250 mm X 4.6 mm, 5 micron (Alltech Associates Inc., Illinois, U.S.A.). Data acquisition was accomplished by using a Computing Integrator-10 (CI-10) (LDC/Milton Roy) with a Sekonics printer plotter (Sekonics Inc., Tokyo, Japan).

Reagents and Chemicals : Analytical reagent grade tetrabutyl ammonium hydroxide (0.1N solution in aqueous media, Sisco Research Laboratories Pvt. Ltd., Bombay, India), orthophosphoric acid (85%) (E. Merck India Ltd., Bombay, India), HPLC grade methanol (S.D. Fine Chemicals Pvt. Ltd., Tarapur, India) and distilled-deionised water prepared in our laboratory was used to prepare mobile phase.

Chromatographic conditions : The mobile phase used consisted of 0.005N tetrabutyl ammonium hydroxide (pH adjusted to 3.0 with phosphoric acid) mixed with methanol (80%). A flow rate of 1.5 ml/min. was used, UV detection being carried at 265 nm with sensitivity 0.05 A.U.F.S. Mobile phase was filtered through a 0.45 micron Millipore filter before use.

Reference Standards : Standards of isoniazid (purity 99.5%), pyrazinamide (purity 99.8%), rifampicin (purity 98.9%) and clofazimine (purity 99.2%) were obtained from Central Drug Laboratory, Calcutta, India and used as received. The stock solutions of isoniazid (1.0 mg/ml), pyrazinamide (0.5 mg/ml) and rifampicin (1.5 mg/ml) with clofazimine (1.0 mg/ml) as

an internal standard were prepared in the mobile phase. The calibration curves for these drugs were prepared in the concentration range 100 to 500 mcg/ml (microgram/ml), with internal standard 500 mcg/ml clofazimine.

Pharmaceutical Dosage Forms : Three commercially available brands of capsules containing isoniazid 100 mg, pyrazinamide 500 mg and rifampicin 150 mg according to the label were used. The three brands were designated as A, B and C respectively.

Sample preparation : (Isoniazid-Pyrazinamide-Rifampicin Capsules) Twenty capsules containing above drugs were emptied, triturated to obtain thoroughly dispersed fine powder and powder equivalent to 100 mg was accurately weighed and transferred into a 100 ml volumetric flask. The contents were dissolved in the mobile phase and made to 100 ml volume. The resulting solution was filtered through a 0.45 micron filter and 5 ml of the filtrate was further diluted to 100 ml with the mobile phase.

Recovery Experiments : The recovery of the added standards isoniazid, pyrazinamide and rifampicin was studied at four different levels. Known amounts of the drug at concentration levels 100, 200, 300, 400 mcg/ml were added to the preanalysed samples and analysed by the present method. Each level was repeated five times. Two different brands A and C were chosen for this purpose.

Calculations : The results were calculated by using internal standard method with the following equation :

$$Conc_i = \frac{(RF)_i \times (Height)_i}{(RF)_{IS} \times (Height)_{IS}} \times IS\ AMT$$

where, $(RF)_i$ is the Response Factor of components calculated during calibration

$(Height)_i$ is the integrated height of components from the analysis

$(RF)_{IS}$ is the Response Factor calculated for the Internal Standard

IS AMT is the amount of Internal Standard added to this sample.

RESULTS AND DISCUSSION

The analysis of the drugs isoniazid, pyrazinamide and rifampicin from the dosage form involved problems of finding : (a) suitable combination of a mobile phase and (b) development of optimum conditions such as pH that would separate the components without overlap.

Preliminary studies on a bonded phase ODS -CN column with different mobile phase combinations such as acetonitrile or methanol-tetrahydrofuran-phosphate buffer (0.01M) were unsatisfactory. This was due to lack of satisfactory resolution of isoniazid, pyrazinamide caused by band spreading of the former on the column. However, addition of tetrabutyl ammonium hydroxide to the mobile phase gave sufficient selectivity to achieve the separation of isoniazid, pyrazinamide and rifampicin.

Table I shows the effect of mobile phase pH on the retention values for isoniazid, pyrazinamide and rifamp-

TABLE I

EFFECT OF MOBILE PHASE pH ON RETENTION (k') VALUES FOR ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN

Mobile phase pH	Isoniazid	Pyrazinamide	Rifampicin
	(k' Values)		
3.0	1.68	4.35	7.23
4.0	1.40	1.97	9.10
5.0	1.12	1.64	12.18
6.0	0.89	1.18	14.30

icin with a mobile phase methanol-tetrabutyl ammonium hydroxide (0.005N) (80:20, v/v). The optimum pH was found to be 3.0 and maintained constant during the analysis. Isoniazid or pyrazinamide were well separated from rifampicin at pH greater than or equal to 3.0. The separation of all the three substances depends on the reversible formation of ion-pairs within the chromatographic system as shown in Figure 1. The mobile phase pH greater than 3.0 was found to be unsuitable for giving adequate selectivity for isoniazid, pyrazinamide and rifampicin as evidenced in Table I.

Figure 2 represents a chromatogram of the separation of internal standard clofazimine from the drugs of intere-

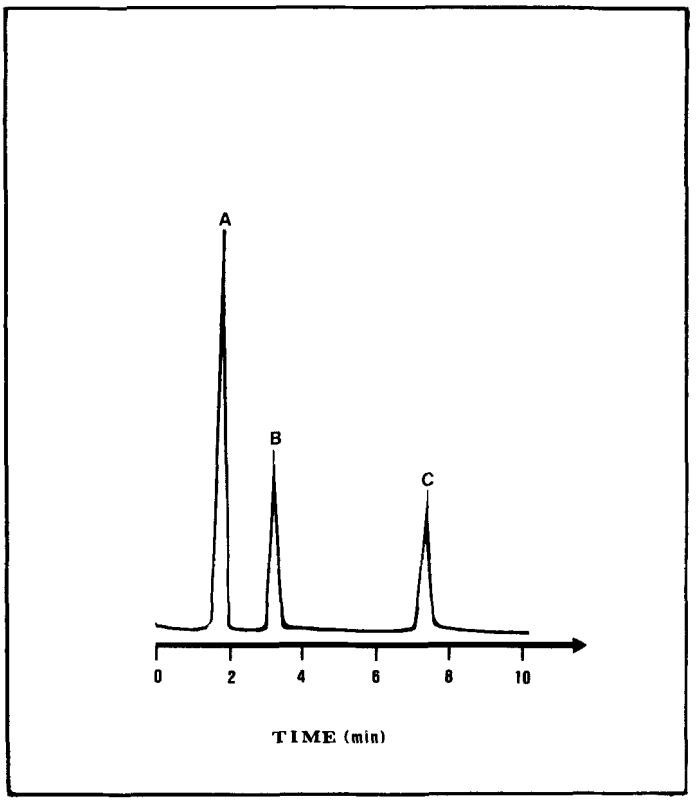

FIGURE 1

A chromatogram of the dosage form solution (20 ul), representing 250 mcg/ml Pyrazinamide (A), 50 mcg/ml Isoniazid (B) and 75 mcg/ml Rifampicin (C). For chromatographic conditions see text.

st. Quantitation was accomplished using internal standard method expressed in terms of a plot of peak height ratio (peak height of the component/peak height of internal standard) versus concentration of the drugs in the range 100 to 500 mcg/ml. The response of the detector was found to be linear with correlation coefficients 0.999, 0.998 and 0.997 for isoniazid, pyrazinamide and rifampicin respectively.

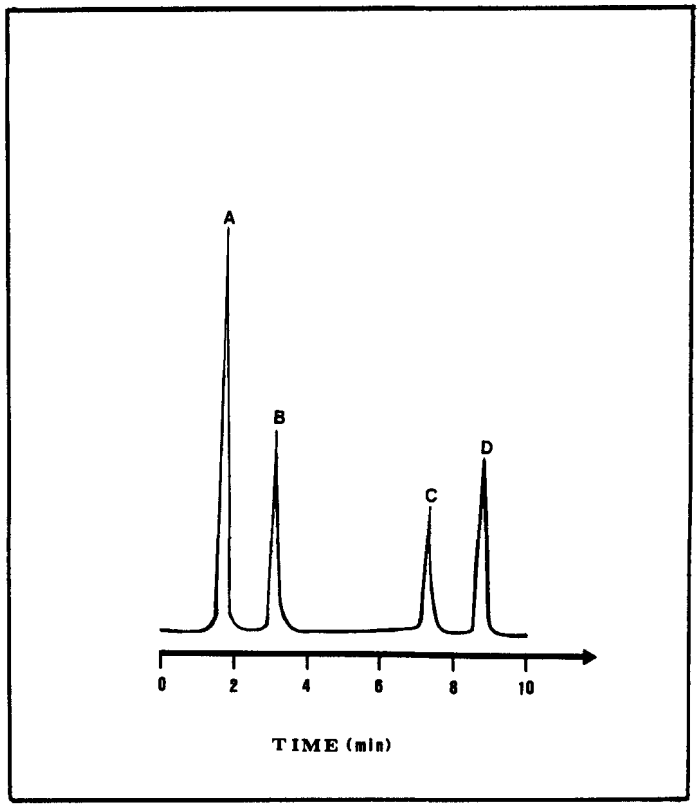

FIGURE 2

A chromatogram of the dosage form solution (20 ul), containing Clofazimine as an Internal Standard (D). Peaks A, B, and C, represents 250 mcg/ml Pyrazinamide, 50 mcg/ml Isoniazid and 75 mcg/ml Rifampicin respectively. For chromatographic conditions see text.

The recoveries of the three drugs was assessed by comparing peak heights obtained from the standard stock solutions of the drugs added to the preanalysed samples. This was performed on two brands A and C using within-day and day-to-day variations as criterion. Tables II and III depicts recovery data for brand A obtained within-day and day-to-day study respectively. An excellent

TABLE II

RECOVERY DATA FOR ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN (BRAND A) OBTAINED DURING WITHIN-DAY STUDY

Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery (mean ± s.d. n = 5)	Coefficient of variation (%)
ISONIAZID			
100	98	98.0 ± 0.68	0.69
200	200	100.0 ± 0.36	0.36
300	299	99.6 ± 0.79	0.79
400	398	99.5 ± 0.85	0.85
PYRAZINAMIDE			
100	99	99.0 ± 0.61	0.61
200	197	98.5 ± 0.42	0.42
300	298	99.3 ± 0.80	0.80
400	399	99.7 ± 0.83	0.83
RIFAMPICIN			
100	100	100.0 ± 0.66	0.66
200	198	99.0 ± 0.38	0.38
300	300	100.0 ± 0.82	0.82
400	398	99.5 ± 0.79	0.79

TABLE III

RECOVERY DATA FOR ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN (BRAND A) OBTAINED DURING DAY-TO-DAY STUDY

Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery (mean ± s.d. n = 5)	Coefficient of variation (%)
ISONIAZID			
100	99	99.0 ± 0.63	0.63
200	200	100.0 ± 0.38	0.38
300	300	100.0 ± 0.77	0.77
400	399	99.7 ± 0.83	0.83
PYRAZINAMIDE			
100	98	98.0 ± 0.59	0.59
200	198	99.0 ± 0.40	0.40
300	300	100.0 ± 0.81	0.81
400	399	99.7 ± 0.80	0.80
RIFAMPICIN			
100	99	99.0 ± 0.64	0.64
200	199	99.5 ± 0.41	0.41
300	301	100.3 ± 0.80	0.80
400	400	100.0 ± 0.81	0.81

TABLE IV

RECOVERY DATA FOR ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN (BRAND C) OBTAINED DURING DAY-TO-DAY STUDY

Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery (mean ± s.d. n = 5)	Coefficient of variation (%)
ISONIAZID			
100	97	97.0 ± 0.64	0.64
200	198	99.0 ± 0.42	0.42
300	299	99.6 ± 0.80	0.80
400	398	99.5 ± 0.79	0.79
PYRAZINAMIDE			
100	99	99.0 ± 0.61	0.61
200	200	100.0 ± 0.39	0.39
300	298	99.3 ± 0.82	0.82
400	399	99.7 ± 0.76	0.76
RIFAMPICIN			
100	100	100.0 ± 0.69	0.69
200	198	99.0 ± 0.45	0.45
300	300	100.0 ± 0.84	0.84
400	399	99.7 ± 0.75	0.75

TABLE V

RECOVERY DATA FOR ISONIAZID, PYRAZINAMIDE AND RIFAMPICIN (BRAND C) OBTAINED DURING WITHIN-DAY STUDY

Amount added (mcg/ml)	Amount found (mcg/ml)	% Recovery (mean ± s.d. n = 5)	Coefficient of variation (%)
ISONIAZID			
100	100	100.0 ± 0.72	0.72
200	198	99.0 ± 0.33	0.33
300	298	99.3 ± 0.80	0.80
400	400	100.0 ± 0.82	0.82
PYRAZINAMIDE			
100	101	101.0 ± 0.57	0.57
200	199	99.5 ± 0.44	0.44
300	300	100.0 ± 0.78	0.78
400	397	99.2 ± 0.85	0.85
RIFAMPICIN			
100	98	98.0 ± 0.60	0.60
200	200	100.0 ± 0.39	0.39
300	298	99.3 ± 0.79	0.79
400	399	99.7 ± 0.83	0.83

precision was achieved by this analytical procedure. The coefficients of variation for four different concentrations of isoniazid, pyrazinamide and rifampicin from dosage forms in the within-day and day-to-day study were varied between 0.36 to 0.86%. Similar experiments were performed on brand C, data of which is shown in Tables IV and V.

CONCLUSION

The method developed from the pharmaceutical dosage form for isoniazid, pyrazinamide and rifampicin is simple, direct and can be adopted for the routine quality control analysis.

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REFERENCES

(1) G.A. Brewer, in "Analytical Profiles Of Drug Substances," vol. 6, Ed. Klaus Florey, Academic Press, New York, 1977, pp. 184-258.

(2) A.N. Nayak, H.S. Yathirajan, S. Manjappa, Curr. Sci., 50, 812 (1981).

(3) K. Kitamura, M. Hattan, S. Fukuyama, K. Hozumi, Anal. Chim. Acta, 201, 357 (1987).

(4) E. Felder, D. Pitre, in "Analytical Profiles Of Drug Substances", vol. 12, Ed. Klaus Florey, Academic Press, New York, 1983, pp. 433-458.

(5) M. Givseppe, C. Claudio, Anal. Chem., 47, 2468 (1975).

(6) E.V. Rao, S.S.N. Murthy, G.R. Rao, Indian Drugs, 22, 269 (1985).

(7) G.G. Gallo, P. Radelli, in "Analytical Profiles Of Drug Substances", vol. 5, Ed. Klaus Florey, Academic Press, New York, 1976, pp. 468-513.

(8) G.R. Rao, S.K. Banerjee, K.K. Ram Mohan, Indian J. Pharm. Sci., 43, 154 (1981).

(9) G.R. Rao, S.S.N. Murthy, Indian J. Pharm. Sci., 46, 181 (1984).

(10) P.S. Mandal, S.P. Tyagi, S.K. Talwar, Indian J. Pharm. Sci., 48, 183 (1986).

(11) G.R. Rao, S.S.N. Murthy, K. Ram Mohan, Indian Drugs, 20, 200 (1983).

(12) S.J. Saxena, J.T. Stewart, I.L. Honigberg, J.G. Washington, G.R. Keene, J. Pharm. Sci., 66, 813 (1977).

(13) J.A. Schmit, R.C. Williams, R.A. Honry, J.F. Dieckman, J. Chromatogr. Sci., 9, 645 (1971).