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Estimation of Pyrazinamide, Isoniazid and Rifampicin in Pharmaceutical Formulations by High Performance Liquid Chromatography Method

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A simple, rapid, sensitive high performance liquid chromatography (HPLC) method has been adopted for the estimation of pyrazinamide, isoniazid and rifampicin in bulk and pharmaceutical formulations. Simultaneous estimation of pyrazinamide and isoniazid in their pharmaceutical dosage form has been developed using RP-C18 column. The mobile phase containing water, monobasic potassium dihydrogen orthophosphate and acetonitrile buffer was pumped at a flow rate of 1.5 mL/min and the UV detection was carried out at 254 nm. Similarly, estimation of rifampicin was carried out with a mobile phase containing water, acetonitrile, methanol, citric acid and sodium periodate using RP-C8 column. The mobile phase was pumped at a flow rate of 1.2 mL/min and the UV detection was carried out at 254 nm. Retention times for pyrazinamide, isoniazid and rifampicin are about 6.307, 4.763 and 5.124 min, respectively. The study shows that the method is accurate, linear, simple and reproducible for checking the quality of the drug.

Key Words: HPLC, Pyrazinamide, Isoniazid, Rifampicin, Assay, Recovery, Precision.

INTRODUCTION

Pyrazinamide (PZA), isoniazid (INH) and rifampicin (RIF) are first-line anti-tuberculosis drugs[1]. Combined Formulations of these drugs are available in tablet form (Macox-ZH). Pyrazinamide is pyrazine 2-carboxamide with molecular formula $C_5H_5N_3O$. Isoniazid (also called isonicotinyl hydrazine or isonicotinic acid hydrazide) is pyridine-4-carbohydrazide with molecular formula $C_6H_7N_3O$. Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semi-synthetic compound with molecular formula $C_{43}H_{58}N_4O_{12}$. Number of excellent HPLC methods are available for the determination of PZA, INH and RIF in Plasma[2-4]. Also HPLC determination of PZA, INH and indomethacin in pharmaceutical preparations was done by Khuhawar *et al.*[5]. Determination of rifampicin and its main metabolite in plasma and urine in presence of pyrazinamide and isoniazid by HPLC method was done by Panchagnula *et al.*[6]. Some more works were done on plasma concentration of INH, PZA and RIF in tuberculosis patients using HPLC[7-12]. In the present work a rapid, simple, sensitive HPLC method was developed for assaying PZA, INH simultaneously and RIF separately in the pharmaceutical dosage forms.

EXPERIMENTAL

Quantitative HPLC was performed on a gradient high performance liquid chromatograph (Shimadzu HPLC prominence)with single LC-20AT pump, variable wavelength programmable UV-Visible detector SPD-20A prominence equipped with the spinchrome software to monitor and integrate the output data. A 20 µL Rheodyne 7725 loop injector was used for the injection of the samples. The analysis was carried out at Dr. Ceeal Lab, C.L. Baid Mehta Pharmaceutical College, Chennai.

All chemicals used were of analytical or HPLC grade. Dihydrogen potassium orthophosphate buffer (Rankem), acetonitrile (Merck), methanol (Merck), citric acid (Rankem) and sodium periodate (Rankem) were used.

Chromotographic conditions: At first, for the simultaneous estimation of pyrazinamide and isoniazid, the separation was performed on a Phenomenex (Gemini) C18, 110A column of dimension 250 mm × 4.6 mm with particle size 5 µm. A mixture of water, monobasic potassium dihydrogen orthophosphate and acetonitrile in the ratio 900:60:40 v/v was used as mobile phase with flow rate of 1.5 mL/min at an operating pressure of 245-250 kg/cm^2. Similarly for the estimation of rifampicin the separation was performed on a Phenomenex (Gemini) C8, 110A column of dimension 250 mm × 4.6 mm with particle size 5 µm. A mixture of water, acetonitrile, methanol, citric acid and sodium periodate in the ratio 510:350: 100:20:20 v/v was used as mobile phase with flow rate of 1.2 mL/min. The mobile was filtered through a 0.45 µm millipore membrane, filtered and degassed. A 20 µL Rheodyne 7725 loop injector was used for the injection of the samples.The column was maintained at room temperature and the detection was carried out by UV detector at 254 nm.

Procedure: Standard solutions of isoniazid and pyrazinamide were prepared by dissolving 51.4 mg of INH and 251.9 mg of PZA in 100 mL of mobile phase in separate 100 mL volumetric flasks. The same mobile phase was not suited for rifampicin. Hence, RIF was taken separately with different mobile phase. 495.5 mg of RIF was taken in 100 mL of mobile phase in separate 100 mL volumetric flask. The solution was sonicated for 15 min and then diluted up to the mark with further quantity of the mobile phase again. Subsequent dilutions of this solution ranging from 41-62 µg/mL for INH and 202-302 µg/mL for PZA were prepared in 50 mL flasks. For RIF the dilution range was 396-595 µg/mL. The solutions were prepared as above were filtered through 0.45 µm membrane filter and then 20 µL of filtrate was injected each time into the column at a flow rate of 1.5 mL/min. Evaluation of the drug was performed with UV-Visible detector at 254 nm. Peak area was recorded for all the peaks. The plot of peak area *versus* the respective concentration gives the calibration curve. The regression of the drug concentration over the peak area was computed for all the three drugs. These regression curves were used to quantify the drugs present in pharmaceutical formulations.

Estimation of INH, PZA and RIF in tablet form: Twenty tablets of the antitubercular drug, Macox-ZH (Macleods Pharmaceuticals Pvt. Ltd.) were pulverized and the powder equivalent to 50 mg of INH and 250 mg of PZA were taken in 100 mL volumetric flask and 100 mL mobile phase was added. For RIF, 500 mg equivalent was taken in 100 mL flask and 100 mL mobile phase was added. The solution was sonicated for complete solubility of the drug and filtered througth 0.45 μm membrane filter to remove the insoluble portions. 5 mL of the filterate was taken in a 25 mL standard flask and made up to the volume with the suitable mobile phase and mixed well. Each of these solutions of 20 μL was then injected 5 times in to the column and the chromatograms were recorded. The area under the curves due to the drugs indicates the respective quantity in the tablet formulations. The drug contents in the tablet were estimated by using the regression curve obtained from the spectral recordings of the standard drug solutions.

RESULTS AND DISCUSSION

HPLC is extensively used in drug development and design, specifically to find dosage levels, purity and quality of the products. The present study was to develop and validate a simple, specific and precise high performance liquid chromatography method for the estimation of pyrazinamide, isoniazid and rifampicin in their pharmaceutical formulations. As shown in Fig. 1, under the chromatographic conditions described, PZA and INH were well resolved on the reverse phase column, eluting at 6.307 and 4.763 min respectively. Rifampicin was eluted at 5.124 min and there was no interference from other components.

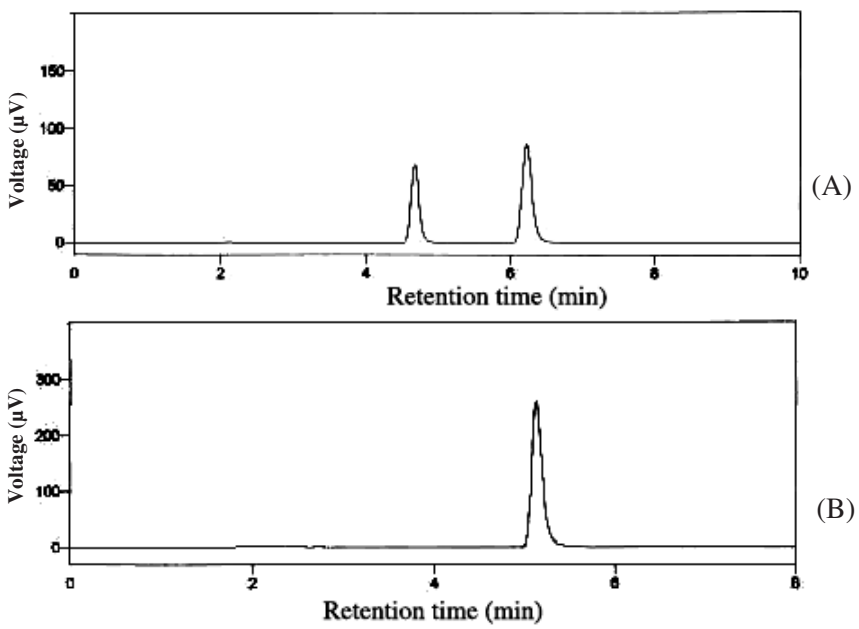

Fig. 1. Chromatogram of (A) pyrazinamide, isoniazid and (B) rifampicin

The linearity study was carried out simultaneously for the drugs at 5 different concentrations. The peak areas for different concentrations and the optical characteristics along with the regression analysis are shown in Tables 1and 2, respectively. A good linear relationship was observed between the concentrations and the peak areas.The linear regression equation obtained for PZA is Y = 2.752X-0.104 (r = 0.9999) where the peak area is given by Y and the concentration of the drug by X, confirm the good linear relationship between the parameters involved. The reproducibility data was obtained with six replicate analyses of same concentration.

TABLE-1
CALIBRATION OF THE HPLC METHOD

Conc. (µg/mL)	Peak area for pyrazinamide	Conc. (µg/mL)	Peak area for isoniazid	Conc. (µg/mL)	Peak area for rifampicin
202	557	41	343	396	1452
227	624	46	398	446	1661
252	692	51	437	496	1873
277	762	57	479	545	2050
302	832	62	530	595	2203

TABLE-2
OPTICAL AND REGRESSION CHARACTERISTICS OF PROPOSED METHOD

Parameters	Pyrazinamide	Isoniazid	Rifampicin
Detection wavelength	254 nm	254 nm	254 nm
Linearity range	202-302 µg/mL	41-62 µg/mL	396-595 µg/mL
Slope	2.752	8.568	3.806
Intercept	-0.104	-2.973	-38.049
Correlation coefficient	0.9999	0.9969	0.9978
Regression equation	Y = 2.752X-0.104	Y = 8.568X-2.973	Y = 3.806X-38.049

The drug contents in the tablet formulation were evaluated and are shown in Table-3. Recovery studies were performed using standard addition technique by spiking working solution from the pharmaceutical formulations to the pre analyzed sample. The results of the recovery studies and precision for the method were shown in Tables 4 and 5. A low percentage of coefficients of variation showed the precision of this method. The high recovery results ranging between 96 and 102 % shows the accuracy and reliability of the proposed method.

TABLE-3
ASSAY OF PHARMACEUTICAL FORMULATIONS BY THE METHOD FOLLOWED (TABLET USED-MACOX-ZH)

Compounds present	Label claim (mg)	Mean ± standard (mg)	Mean ± standard (%)
Pyrazinamide	750	750.59 ± 3.32	100.08 ± 0.44
Isoniazid	150	150.23 ± 1.44	100.15 ± 0.96
Rifampicin	225	227.22 ± 0.52	100.99 ± 0.23

TABLE-4
RESULTS OF RECOVERY STUDY

Conc. level (%)	Recovery from tablet formulation (mg)			Mean recovery (%)		
	PZA	INH	RIF	PZA	INH	RIF
80	741.47	152.10	227.22	98.86	101.40	100.99
100	722.52	152.90	229.74	96.33	101.93	102.10
120	748.84	154.12	225.18	99.84	102.74	100.84

TABLE-5
PRECISION FOR THE METHOD

Replicate	Amount found (mg)		
	Pyrazinamide	Isoniazid	Rifampicin
1	756.69	157.33	224.41
2	763.88	155.56	227.27
3	766.67	156.34	224.76
4	757.98	155.80	222.35
5	769.21	158.39	222.50
6	771.06	157.40	226.94
Mean	764.24	156.80	224.71
Standard deviation	5.89	1.08	2.10
Coefficient of variation (%)	0.77	0.69	0.93

Conclusion

Combination of pyrazinamide, isoniazid and rifampicin in pure and pharmaceutical formulations are quantified using HPLC. The results show that the proposed method is simple, rapid, accurate and highly reproducible and hence can be used for routine analysis of the drug and quality control. The tablet Macox-ZH is found to contain 100.08 to 101.49 % of the labeled amount of drugs. The low percentage of relative standard deviation indicates the reproducibility of the work. It can be concluded that this method can be applied for qualitative and quantitative determination of pyrazinamide, isoniazid and rifampicin in bulk and pharmaceutical formulations.

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ERRATUM

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Heavy Metal Uptake by *Aptenia cordifolia* as Utility for Sewage Sludge Compost Recuperation using Leachate

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All the units given as g kg^{-1} (g/kg) should be mg kg^{-1} (mg/kg) through the page 1085-1088, including in Tables 3 and 4 also.