HPLC and UV Spectrophotometric Estimation of Pyrazinamide in Pharmaceutical Formulations

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Abstract:

Pyrazinamide is an antituberculosis agent. It works by killing or preventing the growth of certain bacteria that cause tuberculosis. HPLC and UV method was employed for the estimation of Pyrazinamide in pharmaceutical formulations. A mixture of Methanol: Water in the ratio of 80:20 (v/v/v) at a pH of 4.8 in isocratic conditions and separation was achieved on Zodiac C18 column (250 X 4.6 mm, 5µ) at ambient temperature. UV detection was monitored at a wavelength of 240nm. In these conditions, the drug elutes at a retention time of 5.2 min for sample and standard solutions. In UV method, the standard drug was diluted using methanol solvent and maximum absorbance was found to be 271nm and the analysis was carried in this wavelength. Beers law limit was found to be in the range of 25-150µg/ml and 1-6µg/ml for HPLC and UV methods respectively. Macrozide (150mg) brand was used for formulation analysis and the % assay was found to be more than 98% for both the HPLC and UV methods in all the brands in the study.

Key words: Pyrazinamide, UV Spectrophotometer, HPLC, Macrozide

1. Introduction:

Pyrazinamide is a drug used to treat tuberculosis. The drug is largely bacteriostatic, but can be bacteriocidal on actively replicating tuberculosis bacteria, an analogue of nicotinamide, is an anti-tuberculosis agent. It is a white crystalline powder, stable at room temperature, and sparingly soluble in water.

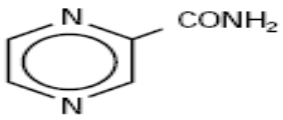

Figure 1: Structure of pyrazinamide

Each pyrazinamide tablet for oral administration contains 500 mg of pyrazinamide and the following inactive ingredients colloidal silicon dioxide, croscarmellose sodium; dibasic calcium phosphate (dihydrate), microcrystalline cellulose, and stearic acid having the IUPAC name pyrazine-2-carboxamide with molecular formula $C_5H_5N_3O$.

2. Materials and Methods:

2.1 Instrumentation

PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, PEAK variable wavelength programmable UV detector UV7000 integrated by PEAK Chromatographic Software version 1.06. Techcomp UV-2301 double beam UV-Visible spectrophotometer by Hitachi software. Sonicator (1.5L) Ultrasonicator Denver electronic analytical balance (SI-234) Systronics digital, pH meter.

2.2 Chemicals and Solvents

Acetonitrile, Methanol and Water are HPLC grade solvents purchased from Merck Specialties Private Limited, Mumbai, India, Spectrophotometric coloring reagent i.e Folin-Ciocalteus reagent (FC reagent) were purchased from merck private limited, Working standard sample Pyrazinamide was obtained from well reputed research laboratory, formulation sample was purchased from local pharmacy.

2.3 Preparation of mobile phase

A mixture of Methanol, Water in the ratio of 80:20 (v/v) was measured accurately. The solution was sonicated till the solvents mixed completely. Then it was filtered through 0.45µm nylon membrane filter paper using vacuum filtration.The final filtrate solution was used as a mobile phase for the estimation of Pyrazinamide.

2.4 Preparation of Standard drug solution:

For analysis Pyrazinamide, weigh 10mg of the standard and is dissolved in 10ml of the diluents and sonicated for one min to dissolve the sample completely. Then it is filtered through 0.2micron meter ultipore filter paper to get a concentration of 1000µg/ml. Then 1ml of the standard solution was taken and make up to 10ml with diluents to get 100 µg/ml standard solution and further required concentrations were prepared from 100 µg/ml solution by proper dilution.

2.5 Preparation of Formulation:

10 tablets from each of the brand selected for assay estimation of Pyrazinamide was grinded to get a fine powder and homogenously mixed using a mortar and pestle. From the powder, amount of the powder equivalent to 10mg of Pyrazinamide was weighed and was dissolved in 10ml of Methanol. The solution was sonicated for 10min to complete extraction of drugs in Methanol. The solution was centrifuged at 4000 rpm for 10 min; the clear supernatant was collected and filtered through 0.45µm nylon membrane filter paper. From this solution selected concentration of 40µg/ml was prepared by proper dilution. Similar procedure was fallowed for the preparation of remaining branded tablets separately. The prepared solutions were used for the assay of Pyrazinamide.

3. Estimation of Pyrazinamide using HPLC method:

HPLC method conditions

PARAMETER	CONDITION
Mobile phase	Methanol : Water 80:20 (v/v/v)
Pump mode	Isocratic
PH	4.8
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	240
Injection Volume	20 µl
Flow rate	1.0ml/min
Run time	10 minutes
Retention Time	5.2minutes

Table 1: Chromatographic conditions for the estimation of Pyrazinamide

3.1Construction of calibration curve:

From the prepared stock solution, a series of calibration standards were prepared at concentrations of 25, 50, 75,100,125 and 150µg/ml using mobile phase as solvent. Each of these drug solutions (20µl) was injected into the column; the peak area and retention times were recorded. The calibration curve for Pyrazinamide was constructed by plotting the mean peak area against the drug concentration.

3.2 Formulation analysis:

From the prepared formulation solution, 20µl of the sample was injected in to HPLC system, peak area response of the prepared formulation solution was used for the assay of Pyrazinamide in the prepared solution. %assay of the method was calculated by considering peak area response of the formulation solution and substituting peak area value in the equation given bellow.

$$\% \textbf{ Assay} = \frac{\textbf{At}}{\textbf{As}} \times \frac{\textbf{Ws}}{\textbf{Ds}} \times \frac{\textbf{Dt}}{\textbf{Wt}} \times \frac{\textbf{P}}{\textbf{100}} \times \frac{\textbf{AW}}{\textbf{LC}} \times \textbf{100}$$

Where

At = Area of the sample
As = Area of the Standard
Ws = Weight of the Standard in mg
Wt = Weight of the Sample in mg
Dt = Dilution of the Sample Solution
Ds = Dilution of the Standard Solution
AW = Average Weight of the Tablet
LC = Label Claim of the Tablet
P = % Purity of the Standard Drug

4. Estimation of pyrazinamide using UV method:

4.1 Selection of solvent for solubility:

The drug pyrazinamide was practically insoluble in Water. Solution is dissolved in methanol and dilutions were prepared with water. We prepare different soluble solvents of pyrazinamide like methanol, Acetonitrile etc in a fixed dilute solution and absorbance of solution was measured. Hence standard drug was dissolved in methanol and necessary required dilutions were prepared with water as diluents for spectrophotometric estimation.

4.2 Selection of wavelength maxima:

Suitable maximum absorbance for the estimation of pyrazinamide was identified by scanning the absorbance in spectrum mode within the wavelength region of 400-200nm in three different dilute solutions. In all the solutions the drug absorbed maximum wavelength at 271nm. Hence 271nm was found to be suitable wavelength for the estimation of pyrazinamide.

4.3 Construction of calibration curve:

From the prepared standard stock solution, a series of calibration standards were prepared by selected dilutions. From the stock solution, 1µg/ml, 2, 3, 4, 5, 6µg/ml was prepared. The absorbance of the prepared solutions was measured at 271nm against a regent blank. At each concentration triplet readings were measured and mean value was used for the Construction of calibration curve. Calibration curve was constructed by taking concentration of the prepared solution on x-axis and corresponding absorbance on y-axis.

4.4 Formulation analysis:

The absorbance of the prepared formulation solution in all the brands was measured at 271nm in triplets separately. The average absorbance value was used for the formulation estimation of pyrazinamide. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the equation given below.

$$\% \textbf{ Assay} = \frac{\textbf{At}}{\textbf{As}} \times \frac{\textbf{Ws}}{\textbf{Ds}} \times \frac{\textbf{Dt}}{\textbf{Wt}} \times \frac{\textbf{P}}{\textbf{100}} \times \frac{\textbf{AW}}{\textbf{LC}} \times \textbf{100}$$

Where

At = Absorbance of the sample
As = Absorbance of the Standard
Ws = Weight of the Standard in mg
Wt = Weight of the Sample in mg
Dt = Dilution of the Sample Solution
Ds = Dilution of the Standard Solution
AW = Average Weight of the Tablet
LC = Label Claim of the Tablet
P = % Purity of the Standard Drug

5. Results and Discussion:

Calibration curve was obtained within a concentration range of 50µg/ml to 100µg/ml. regression equation was found to be y = 3240x + 3154 with correlation of 0.999. And for UV method the concentration range were 1-6µg/ml were selected regression equation was found to be y = 0.049x + 0.070with correlation of 0.999. Linearity results were presented in Table 2 and calibration curve was shown in Figure 3.

Level	HPLC Method		UV Method	
	Concentration	Peak area	Concentration	Absorbance
Level – 1	25µg/ml	87160.9	1µg/ml	0.117
Level – 2	50µg/ml	165674.3	2µg/ml	0.167
Level – 3	75µg/ml	245266.6	3µg/ml	0.221
Level – 4	100µg/ml	321477.3	4µg/ml	0.268
Level – 5	125µg/ml	405463.8	5µg/ml	0.315
Level – 6	150µg/ml	495126.1	6µg/ml	0.362
	Slope : 3240.463 Intercept :- 3154.313 Correlation coefficient: 0.999		Slope :0.049 Intercept :- 0.070 Correlation coefficient: 0.999	

Table 2: Linearity results

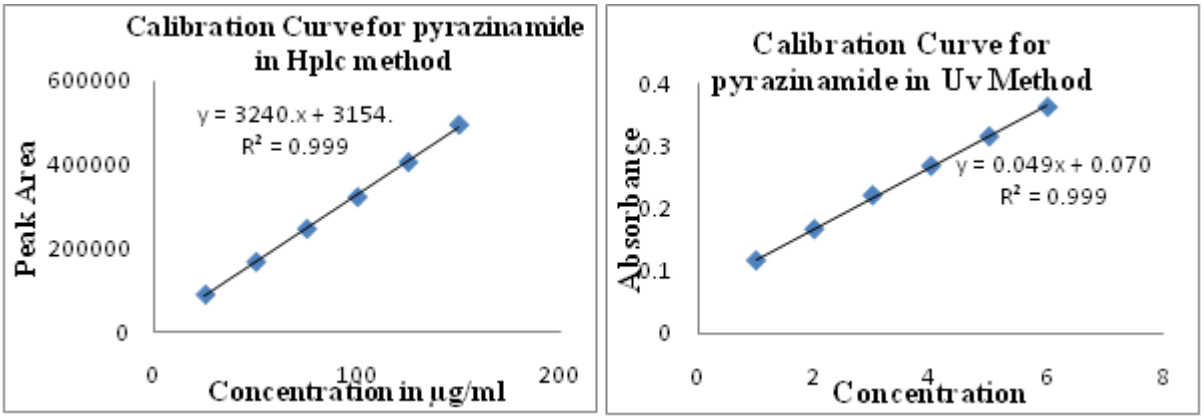

Figure 3: Calibration Curve for Pyrazinamide in HPLC and UV method

Formulation analysis was carried as per the standard procedure and % assay was calculated in both HPLC and UV methods. The formulation chromatogram in HPLC method was given in figure 4. Results found that a % assay of 99.47 for HPLC and 98.49% in UV method. Results were given in table below. The % assay was found to be more than 98% was found in the brand under study by both HPLC and UV methods.

Table 2: Formulation results of Pyrazinamide

Method	Drug	Brand	Dosage	Amount Prepared	%Assay
HPLC	pyrazinamide	Macrozide	150mg	100µg/ml	99.47
UV	pyrazinamide	Macrozide	150mg	10µg/ml	98.89

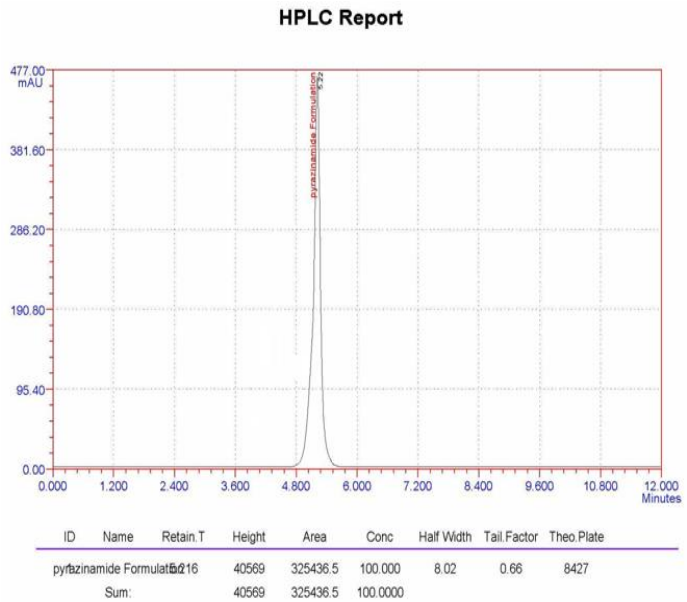

Figure 4 Formulation chromatogram for Pyrazinamide

6.0 Conclusion:

The brand Macrozide (150mg) containing pyrazinamide in the present study show more than 98% assay in the selected method. Both HPLC and UV methods were successfully applied for the estimation of pyrazinamide in pharmaceutical formulation. Both the method does not detect any pharmaceutical excipients used in the formulation, hence no impurities or extra peaks detected in the formulation chromatogram.

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