Spectrophotometric Estimation of Prothionamide with FC reagent in bulk and Pharmaceutical Formulation

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Abstract-A simple, precise and sensitive spectrophotometric method was developed for the determination of prothionamide in pharmaceutical dosage form. It is based on the formation of blue colour species with folin ciocalteu reagent in alkaline medium. The colored species has an absorption maximum at 718nm. The method was validated according to the ICH guidelines. The absorption was found to increase linearly with the concentration in the range of 8-24µg/ml with correlation coefficient of 0.9997. The limit of detection and limit of quantification was found to be 0.26µg/ml and 0.81µg/ml. The method was precise with %RSD 0.5. The reliability of any method was established by standard addition method and by recovery studies. The proposed method was successfully applied for the determination of prothionamide in pharmaceutical formulations.

KeyWords-Spectrophotometric,method; Prothionamide; Validation; folin ciocalteu reagent.

INTRODUCTION

Prothionamide, 2-propyl pyridine-4-carbothoamide, (Fig.1) with a molecular weight of 180.26g/mol. Prothionamide is a thioamide derivative with ant Tubercular activity. It forms a covalent additive with bacterial nicotinamide adenine dinucleotide (NAD), PTH-NAD, which completely inhibits 2-trans-enoyl-ACP reductase, an enzyme essential for mycolic acid synthesis. This results in increased cell wall permeability and decreased resistance against cell injury eventually leading to cell lysis. Mycolic acids, long chain fatty acids essential mycobacterial cell wall components and play a key role in resistanc3e to cell injury and mycobacterial virulence. The literature survey shows that prothionamide is determined by various analytical methods including UV spectrophotometric method, HPLC in pharmaceutical dosage forms. In the available literature, there is no visible spectrophometric method for determination of prothionamide in pharmaceutical raw and dosage forms. The aim of present work is to develop a simple, sensitive and less expensive spectrophotometric method that rely on the use of cheap chemicals and simple techniques but provide accuracy as compared to costly, sophisticated technique like HPLC.

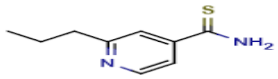

Fig. 1: structure of prothionamide

MATERIALS

All absorption spectra were obtained using Lab India 3000 UV/Visible spectrophotometer equipped with 1cm matched quartz cells.

Chemicals used were of SD fine chemicals (analytical grade) and solutions were prepared with distilled water throughout the procedure. A pure prothionamide (pharmaceutical grade) sample was kindly provided by Hindustan Biosystems, Hyderabad, India. Tablets were obtained from commercial sources (Prithicid 250mg).

Drug and reagent solution

A stock solution of prothionamide (1mg/ml) is prepared by dissolving 100mg of pure drug in 100ml of 0.1N HCl in a volumetric flask.

0.1N HCl solution is prepared by dissolving 8.5ml of concentrated HCl in 100ml of distilled water. Working concentrations of prothionamide were prepared by dilution of the above stock solution with the same (100µg/ml, 10µg/ml). Folin ciocalteu reagent (FC reagent), 10ml dissolved in few ml of distilled water and volume was made up to 25ml with distilled water. NaOH solution (1N) is prepared by dissolving 4g of NaOH in 100ml of distilled water

Parameter fixation

All the parameters were fixed by varying one and fixation in the other parameter in each case.[1].

Effect of volume of 1N NaOH

Effect of volume of 1N NaOH was studied by fixed quantity of drug and reagent. It was done by taking different amount of 1N NaOH 1ml, 1.2ml, 1.4ml, 1.6ml and 1.8ml. The absorbance of colored solution was measured at 718nm against blank and the results were tabulated in the following table1

. **Table 1: Results of effect of 1N NaOH**

S.No.	Volume of drug solution(ml)	Volume of 1M NaOH	Volume of FC reagent	Absorbance
1.	1ml	1.0ml	1ml	0.610
2.	1ml	1.2ml	1ml	0.735
3.	1ml	1.4ml	1ml	0.778
4.	1ml	1.6ml	1ml	0.702
5.	1ml	1.8ml	1ml	0.694

It was observed that maximum absorbance was observed with 1.4ml of 1N NaOH. So 1.4ml of 1N NaOH solution was used in the present study.

Effect of reagent concentration

To the fixed quantities of dry solution, varying quantities of folin-ciocalteu reagent, 0.6,0.8,1.0,1.2,1.4,1.6,1.8ml were added followed by 1.4ml,1.6ml and 1.8ml were added followed by 1.4ml of 1N NaOH to each volumetric flask and shake well for 1min. Absorbance were measured at absorption maximum against the reagent blank. Results were tabulated in the table 2.

Table 2: Results of effect of reagent concentration

S.NO.	**Volume of drug solution**	**Volume of 1M NaOH(ml)**	**Volume of FC Reagent(ml)**	**Absorbance**
1.	1 ml	1.4 ml	0.6 ml	0.445
2.	1 ml	1.4 ml	0.8 ml	0.639
3.	1 ml	1.4 ml	1.0 ml	0.743
4.	1 ml	1.4 ml	1.2 ml	0.827
5.	1 ml	1.4 ml	1.4 ml	0.721
6.	1ml	1.4ml	1.6ml	0.532
7.	1ml	1.4ml	1.8ml	0.492

Maximum absorbance was observed with 1.2ml folin-ciocalteu reagent. So 1.2ml folin- ciocalteu reagent was fixed in the present study.

General analytical procedure

Different aliquots of the working standards of prothionamide (10µg/ml) ranging from 8-24µg/ml were transferred into a series of volumetric flasks. To each of the flask 1.4ml of NaOH solution, 1.2ml of folin-ciocalteu reagent solution was added and the total volume was made up to 10ml with distilled water. The flasks were stoppered; contents were mixed and kept at room temperature for 15min. The absorbance of each solution was measured at 718nm against reagent blank. The results were tabulated in the following table 3. **Table 3: Results of calibration data of the developed method**

S.NO	Concentration µg/ml	Volume of 1N NaOH	Volume of FC reagent	Volume made up to	Absorbance at 718nm
1.	8	1.4 ml	1.2ml	10ml	0.30
2.	12	1.4ml	1.2ml	10ml	0.443
3.	16	1.4ml	1.2ml	10ml	0.596
4.	20	1.4ml	1.2ml	10ml	0.752
5.	24	1.4ml	1.2ml	10ml	0.90

Preparation of sample solution An amount of finely grounded tablet powder equivalent to 25mg of prothionamide was accurately weighed and transferred to 25ml volumetric flask containing 5ml of 0.1M HCl and volume was made up to the mark with 0.1M HCl solution. The solution was then filtered through Whatmann filter paper. The first 10ml portion of the filtrate was discarded and suitable aliquot of the solution was used for the assay as described in the general analytical procedure.[2]

Validation of developed analytical method The developed colorimetric method was validated to linearity, accuracy, precision, LOD,LOQ and robustness.

Linearity:

The linearity of an analytical method is its ability within a define range to obtain results directly proportional; to the concentrations of the analyte in the sample. For linearity study, five solutions at different concentration ranging from 8-24µg/ml were prepared and analyzed at its maximum absorbance wavelength (718nm). The linearity was evaluated by simple linear regression analysis by the least square regression method, which was used to calculate the correlation coefficient, y-intercept and slope of the regression line.

precision:

Precision is defined as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions. Precision was determined with respect to repeatability and intermediate precision. It was expressed as percent of relative standard deviation (%RSD). Intra-day precision (repeatability) was studied by assaying 6 determinations of 12, 16 20µg/ml on the same day under similar experimental conditions. Inter-day precision (intermediate precision) was studied by comparing the same concentrations as above on 3 different days under the similar experimental conditions.

Accuracy:

Standard addition method was used for accuracy study. Here, different known concentrations of prothionamide (12,16 and20µg/ml equivalent to) 80,100 and 120% of the nominal analytical concentration, were added to a pre-analyzed formulation sample and the total concentration was found. The percent recovery was calculated using following formula.

Percent recovery = $[(C_t - C_d) / C_a]^*100$

Where C_t is total drug concentration measured after standard addition, C_d is dug concentration in the formulation sample and C_a is the spiked drug concentration.

Limits of detection and quantitation

The LOD and LOQ values represent the lowest concentrations those could be respectively detected and quantified with sufficient accuracy and precision. LOD and LOQ were determined by the slope of calibration curve and y-intercept of regression equation. LOD was calculated as 3.3σ/S and LOQ was calculated as 10σ/S where σ is the standard deviation of y-intercept f regression equation and S is the slope of the calibration curve.

Robustness

Robustness of analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was performed by taking the concentration of 20 µg/ml, wavelength of ± 2 nm and reagent ± 0.1mi.[3-4]

RESULTS & DISCUSSION

Wavelength of maximum absorption

The wavelength of maximum absorbance was found to be 718nm as shown in Fig.2.

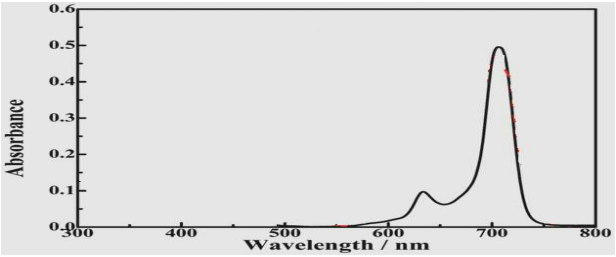

Fig.2: UV-VIS spectra showing λ_{max} of prothionamide

Linearity, LOD and LOQ

The calibration curves were evaluated based on the correlation coefficient value, r^2. The absorbance of the samples in the range of 8-24µg/ml was found to be linear with the r^2 value of 0.999 (Fig.3). The linear equation, y = 0.037x was calculated by least square regression method. The LOD and LOQ were found to be 0.267 and 0.81 µg/ml respectively. The result of LOD indicates that up to 0.267

µg/ml concentration of prothionamide can be detected but not quantized. The result of LOQ indicates that 0.81 µg/ml or more concentration of prothionamide can be quantitatively determined with suitable precision and accuracy.

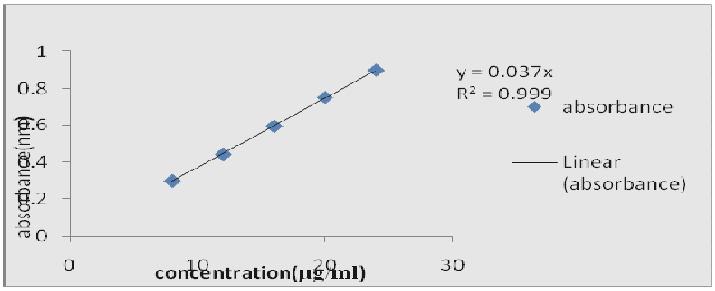

Fig. 2: Calibration curve – Regression analysis for prothionamide

Precision:

The intra-day and inter day relative standard deviation values obtained were found to be less than 1%. The results are shown in the table 4. The RSD value within the acceptable range of less than 2% justifies the precision of the method.

Table 4: Results of precision study (n=6)

Conc(µg/ml	Intra-day precision		Inter-day precision	
	Absorbance	%RSD	Absorbance	%RSD
12	0.434±0.04	0.482	0.432±0.038	0.53
16	0.593±0.03	0.5	0.592±0.03	0.45
20	0.75±0.02	0.491	0.748±0.02	0.61

Accuracy:

The result within the range of 99.83% to 100.7% ensures accuracy of the method as shown in table 5. The low standard deviation value (S.D. less than 2%) represents that any small changes in drug concentration can be accurately determined by the method.

Table-5: Results of accuracy study of the UV spectrophotometric method

Level	Amount added(µg/ml)	Amount Recovered(µg/ml)±SD	% Recovered
80%	12	11.98±0.001	99.83
100%	16	16.02±0.052	100.12
120%	20	20.14±0.078	100.7

Robustness:

Variation in wavelength and volume of FC reagent did not show any significant difference in the percent assay. The variables evaluated for the robustness of the method are shown in the table 6.

Table 6: Robustness study for the estimation of prothionamide

S.NO.	Concentration(20µg/ml)	
	Wavelength ± 2 nm	Reagent ± 0.1ml
1.	716	0.742
2.	720	0.758

Assay: Sample solution was prepared by taking any brand of prothionamide tablets and performed the assay under similar experimental conditions to that of pure drug and amount of drug was estimated and the results were shown in the Table 7.

Table-7: Results of marketed product

S.no	Drug	Concentration (µg/ml)	Amount Found(mg)	% Purity	Label claim
1.	PROTHIONAMIDE	8	248.2	99.3	250 mg
2.		12	249.5	99.8	
3.		16	250.1	100.04	
Average				99.71	

CONCLUSION:

Based on the statistical parameters and results, the validated UV spectrophotometric method was found to be accurate, reproducible and precise for quantitative determination of drug. The low LOD and LOQ suggest that the method can be used for the assay of prothionamide in tablets and bulk. Being simple and inexpensive in comparison to HPLC, the method satisfies the need of a rapid procedure for routine quality control purpose. The validated method can be used for determination of prothionamide in bulk and dosage forms like tablet.

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