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Research Article

Development and Validation of Zero and First Order Derivative Area Under Curve Spectrophotometric Methods for Pyrazinamide in Bulk Material and Pharmaceutical Formulation

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Abstract

Introduction: The aim of this work is to establish a novel, simple, precise and sensitive UV-AUC spectrophotometry method for estimation of Pyrazinamide from bulk and in-Pharmaceutical formulation and validate it according ICH guidelines. Pyrazinamide is used for the treatment of tuberculosis.

Methods and Methodology: Pyrazinamide was estimated by four simple UV-Spectrophotometric methods using double beam UV-Spectrophotometer (UV-2450, Shimadzu). A stock standard solution was prepared by dissolving 10 mg of Pyrazinamide in to 100 mL volumetric flask then volume was made by methanol up to the mark, the obtaining concentration was 100µg/mL. After suitable dilutions, it scans in the UV-visible range 200 - 400 nm. For Method A and Method B zero-order spectrum and area under curve (AUC) recorded at 268 nm and 259.40 - 273.20 nm respectively. While for Method C and for Method D first-order derivative the amplitude and area under curve (AUC) recorded at 279nm and 277.20 - 282.60 nm respectively. For linearity study, series of dilutions were prepared from stock solutions.

Results: In Method A, B, C and Method D, Pyrazinamide followed linearity in the concentration range of 2 - 12 µg/mL with (r^2 > 0.999). The % recovery was found to be in the range of 98 - 102%. Furthermore, the precision of methods were calculated in terms of % RSD less than 2 showed, methods are precise. The developed methods was validate according to ICH guidelines.

Conclusion: The developed methods are simple, rugged, robust and economical. The illustrated methods can routinely be used for determination of Pyrazinamide in bulk and in Pharmaceutical formulation.

Keywords: Area Under Curve Spectrophotometry; Derivative-Spectrophotometry; Pyrazinamide

Introduction

Pyrazinamide (PZA) is an amide of pyrazinoic acid (PC) used in the combination treatment of tuberculosis recommended by WHO. It is used in case of pulmonary and extra pulmonary treatment of tuberculosis. The structure of Pyrazinamide is given in figure 1. The half-life of PZA is 3 - 4h, and after administration absorbed quickly from the gastrointestinal tract with peak serum concentrations of 6-8 µg/ml occurring 1.5 - 2.0h [1-3]. After a literature review, it was found that Pyrazinamide and its degradation product was analyzed by different HPLC methods either alone [4] or in combination with other drugs such as rifampicin, ethambutol hydrochloride and isoniazid [5,6]. The drug was also analyzed along with by using RP-HPLC [7-10], HPLC-MS [11-13], TLC [14], Spectroscopic methods [5,15,16], spectroflourimetric method [17]. On the basis of previ-

ous literature review, there is no reported Spectrophotometric methods available for determination of Pyrazinamide by zero and first order derivative area under curve Spectrophotometry methods. The objective of present work is to establish zero order and first order derivative UV-Spectrophotometry methods by using amplitude and AUC techniques. The current investigation emphasize that it is a simple, sensitive and effective UV-Spectrophotometry method for estimation of Pyrazinamide in bulk material and marketed tablets and validated it according to ICH guidelines.

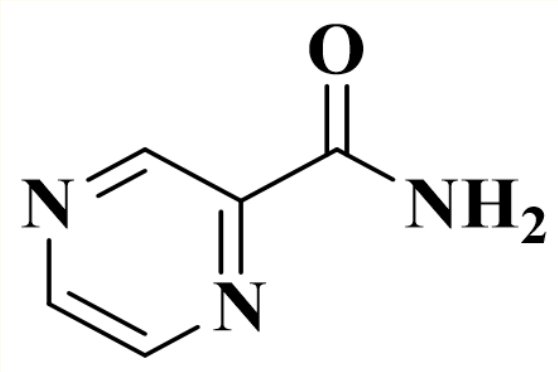

Figure 1: Structure of pyrazinamide.

Instrumentation

A double beam UV-VIS spectrophometer (UV- 2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 1 cm quartz cells was used. An electronic balance (Model Shimaszu AUX 120) was used for weighing purpose.

Materials and Methods

Selection of common solvent

Choice of solvent was made after evaluating solubility of drug in different solvents. Analytical grade Methanol was selected as a solvent for development of spectral characteristics of drug on the basis of its solubility.

Preparation of stock standard solution and determination of λ max

10mg of Pyrazinamide was weighed accurately and it transferred in to 100 mL volumetric flask then volume was made by methanol up to the mark, the obtaining concentration was 100 µg/mL. From the stock standard solution, 1 mL of solution was transferred into 10 ml of volumetric flask and volume was making up with the same to get concentration of 10 µg/mL. The resulting solution was scanned in UV region 400 - 200 nm, the spectrum showed maximum absorption at (λ max).

Methods A (Zero order spectrophotometry) and Method B (Zero order Spectrophotometry–AUC)

Different dilution in the range of 2-12 was prepared from a stock standard solution i.e. 100 µg/mL of Pyrazinamide. In Method A, absorbance was recorded at 268 nm, shown in figure 2A. While in Method B, AUC was selected in the wavelength range of 259.40 - 273.20 nm, shown in figure 2B.

Figure 2A: UV-spectrum of pyrazinamide (Zero order).

Figure 2B: AUC of zero order spectrum of pyrazinamide in wavelength range 259.40 - 273.20 nm.

Figure 2C: Calibration curve of pyrazinamide (Zero order). Y = 0.076 x + 0.078, Correlation Coefficient = 0.999, Slope = 0.076, Intercept = 0.078.

Figure 2D: Calibration curve of pyrazinamide using AUC. Y = 0.092 x + 0.026, correlation coefficient = 0.999, slope = 0.0.092 intercept = 0.026.

Methods C (First order derivative-UV Spectrophotometry) and D (First order derivative-UV Spectrophotometry-AUC)

For Method C and D, spectra were derivatized into first order using software UV-Probe 2.21 with delta lambda 4 and scaling factor 10. In Method C, the amplitude was recorded at 279 nm shown in figure 3A. While in Method D, AUC of the derivative spectrum was selected wavelength range 277.20-282.60 nm shown in figure 3B.

Validation of method [18-20]

As per ICH guidelines presented work was validated with respect to various parameters including linearity, limit of detection and quantification, precision and accuracy.

Linearity study

Aliquots of standard stock solution of Pyrazinamide in the range of 0.2 to 1.2 ml was transferred to six separate 10 ml volumetric flask. To obtain the concentration 2 - 12 µg/mL the volume was adjusted to the mark with methanol and the graph was plotted between concentrations versus absorbance, amplitude and AUC.

Figure 3A: UV-spectrum of pyrazinamide (First derivative).

Figure 3B: AUC of first order derivative spectrum of pyrazinamide in wavelength range 277.20 - 282.60 nm.

Figure 3C: Calibration curve of pyrazinamide using AUC. Y = 0.134 x + 0.304; where, correlation coefficient = 0.999, slope = 0.134 intercept = 0.0.304.

Figure 3D: Calibration curve of pyrazinamide (First derivative). Y = 0.094 x + 0.065 where, Correlation coefficient = 0.999, Slope = 0.094 Intercept = 0.065.

Accuracy/Recovery studies

For the study of accuracy of the predicted methods recovery experiments were performed by the standard addition method. For

this known amount of API was added to the marketed tablet formulation at 80, 100 and 120 % level. Finally, the % recovery was noted.

Precision

Precision of the proposed methods was studied as intra-day and inter-day precision. Intra-day precision was determined by examine the 4, 6 and 8 µg/ml of Pyrazinamide for three times in the similar day. Inter-day precision were determine the concentration of 4, 6 and 8 µg/ml of Pyrazinamide for three days.

Sensitivity

Sensitivity of the anticipated method was studied in terms of limit of detection (LOD) and limit of quantification (LOQ). For it different volume of stock solution in the range 2 - 4 µg/ml was prepared, and analyzed. The procedure was repeated in triplicate. The LOD and LOQ were calculated by using the formula given in ICH guidelines as follows

$$LOD = \frac{3.3 \times N}{B}$$

$$LOQ = \frac{\text{10} \times N}{B}$$

Where, N is average standard deviation of peak height and area of the drug (n= 3), and B is the corresponding calibration curve.

Ruggedness

Ruggedness of the presented methods was determined for 6 µg/mL concentration of Pyrazinamide. For these two analysts using same environmental and operational conditions analyze aliquots from a homogenous slot. The results are in acceptable range that is % RSD values < 2 for all the methods.

Results and Discussion

Pyrazinamide showed a good correlation coefficient for all methods (Table 1) and linear regression data for the calibration curves over the concentration range 2-12 µg/ml for Pyrazinamide. The Linear regression for all the four methods A, B, C, D are obtained by plotting Concentration verses absorbance and AUC of zero order spectrums for method A and B as shown in figure 2C and figure 2D and amplitude and AUC of first order spectrum for Method C and D, respectively, is shown in figure 3C and figure 3D.

Parameters	Method A	Method B	Method C	Method D
Beer-Lambert's range (µg/ml)	02 - 12	02 - 12	02 - 12	02 - 12
λ max (nm)	268	259.40 - 273.20	277.20 - 282.60	279
Intercept	0.078	0.026	0.304	0.065
Correlation coefficient	0.999	0.999	0.999	0.999

Table 1: Linearity study.

Conc µg/mL	Method A		Method B	
	Mean ± SD	% RSD	Mean ± SD	%RSD
2	0.2313 ± 0.0005	0.2380	0.2142 ± 0.0027	1.2672
4	0.3826 ± 0.0023	0.6218	0.3974 ± 0.0015	0.3930
6	0.5384 ± 0.0060	1.1177	0.5870 ± 0.0007	0.1301
8	0.6824 ± 0.0061	0.8968	0.755 ± 0.0004	0.1432
10	0.8371 ± 0.0145	1.7413	0.9593 ± 0.0004	0.0454
12	0.9982 ± 0.0003	0.0351	1.1436 ± 0.0029	0.2603

Table 1.1: Linearity study for method A and method B.

Conc µg/mL	Method C		Method D	
	Mean ± SD	% RSD	Mean ± SD	%RSD
2	0.5693 ± 0.003	0.0564	0.2554 ± 0.0003	0.1174
4	0.8446 ± 0.0003	0.0361	0.4484 ± 0.0003	0.0681
6	1.1042 ± 0.0004	0.0408	0.6374 ± 0.0003	0.0504
8	1.4059 ± 00004	0.0326	0.8134 ± 0.0002	0.0325
10	1.6385 ± 0.0003	0.0220	1.0041 ± 0.0085	0.8556
12	1.9164 ± 0.0008	0.0455	1.0128 ± 0.0036	0.3017

Table 1.2: Linearity study for method C and method D.

Methods	% Amount found	% RSD
A	100.972	0.9943
B	99.946	0.9972
C	100.473	0.9784
D	101.161	1.086

Table 2: Analysis of marketed formulation.

Accuracy/recovery studies

The percentage recovery of Pyrazinamide at three concentration levels 80, 100, and 120 % was calculated and results are shown in table 3.

Precision

Intra-day and inter-day precision was carried out by performing three replicates of three of three different concentration 4, 6 and 8 µg/ml of Pyrazinamide showed % RSD less than 2 was shown in table 4.

Methods	80%		100%		120%	
	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Method A	100.7350	0.5852	100.6286	0.588	101.9437	0.0488
Method B	99.9060	0.0055	100.3562	0.8652	99.6678	0.0455
Method C	100.5827	0.6047	99.2993	0.3991	101.1815	0.3779
Method D	100.5943	0.0149	98.2860	0.3167	98.3451	0.2745

Table 3: Recovery studies.

% RSD	Method A	Method B	Method C	Method D
Intraday				
6	0.6932	0.4794	1.0621	1.1471
8	1.5097	0.0900	0.2103	0.4043
10	0.4267	0.7261	1.0896	1.8406
Intraday				
6	0.5692	0.6026	0.7079	0.3666
8	0.4544	0.6454	0.0684	0.7301
10	1.2375	0.7773	0.9180	0.6415

Table 4: Precision studies.

Sensitivity

The LOD and LOQ of proposed methods were shown in table 5.

g/mL	Method A	Method B	Method C	Method D
LOD	0.2249	0.0033	0.0024	0.0752
LOQ	0.6815	0.0100	0.0074	0.2281

Table 5: Sensitivity studies.

Ruggedness

Ruggedness of the method was determined by performing six-times for the same concentration solution. The % RSD was foundto be less than 2. The result putted in table 6.

% RSD	Method A	Method B	Method C	Method D
Analyst 1	0.8826	0.2668	0.8552	0.0490
Analyst 2	0.7629	0.164	0.8686	0.1681

Table 6: Ruggedness studies.

Limitations of the Study

This technique is depends upon instrumental parameters like speed of scan and the slit width. The instrumental conditions of recording parent zero-order spectrum have strong influence on the shape and intensity of its derivative generations. The acquired spectrum is more or less distorted by instrumental noises and as the consequence the derivative spectrum is distorted too.

Conclusion

For the quantitative analysis of Pyrazinamide in bulk and in marketed tablets all four methods that is zero order, first order and AUC technique of UV Spectrophotometry are established. The developed UV-Spectrophotometric methods are simple, accurate, precise and specific according to statistical boundary and results

obtained. Therefore, proposed methods can routinely be used for estimation of Pyrazinamide in bulk and marketed pharmaceutical formulations.

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