IRJSE International Research Journal of Science & Engineering
Int. Res. J. of Science & Engineering, 2018; Special Issue A3:193-198
ISSN: 2322-0015
SJIF Impact Factor 4.11
UGC Approved Journal No. 63628
RESEARCH ARTICLE **OPEN ACCESS**

Estimation of Pazufloxacin Mesylate in bulk and formulation by UV-Spectrophotometric area under Curve Method

Chordiya Bhakti*, Patel SG, Dhobale SM

Department of Quality Assurance Technique, Vishal Institute of Pharmaceutical Education and Research, Ale, Tal- Junnar, Pune (MH) 412411
Corresponding address - bhaktidc24@gmail.com | Contact no. 7843003006

Manuscript Details
Available online on http://www.irjse.in
ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Chordiya Bhakti, Patel SG, Dhobale SM . Estimation of Pazufloxacin Mesylate in bulk and formulation by UV-Spectrophotometric Area under Curve Method, *Int. Res. Journal of Science & Engineering*, January 2018 | Special Issue A3 |: 193-198.

ABSTRACT

The current work is carried out to for estimation of Pazufloxacin mesylate in bulk and pharmaceutical dosage form by utilizing area under curve (AUC) method. For this purpose the wavelength range 229-260nm was selected. Distilled water was used as a solvent throughout the work. Linearity was observed in concentration range 2-12µg/ml (r^2 =0.999) for the method. Recovery studies for area under curve were found to be 99.32%. The method developed was validated for linearity, precision, accuracy, LOD, and LOQ as per ICH guidelines.[1] The present method was found to be simple, linear, precise, accurate and sensitive which can be used for routine quality control analysis for spectrophotometric estimation of Pazufloxacin mesylate in bulk and dosage form.

Keywords: Pazufloxacin mesylate, Area under curve (AUC), and ICH guidelines.

INTRODUCTION

Pazufloxacin mesylate is a newer broad-spectrum fluoroquinolone antibiotic. Chemically is (3R)-10-(1-aminocyclopropyl)-9-fluoro-2,3-dihydro-3-methyl-7-oxo7H-pyrido[1,2,3-de]1,4-benzoxazine-6-carboxylic acid methanesulfonate (Fig. 1) [2]. The potent broad-spectrum activity of the molecule is due to the presence of the aminoacyl group at C10, imparting against gram negative and gram positive bacteria including a variety of resistant strains and anaerobic bacteria.

Mechanism of action shown by Pazufloxacin mesylate is multimodal and it inhibits both DNA gyrase and topoisomerase IV enzyme, leading to increase its antibacterial spectrum via DNA gyrase-dependent process such as polymerization of DNA, (ATP-dependent) DNA supercoiling and chromosome fragmentation. It has also shown DNA antagonistic action. The multimodal mechanism is linked to the low potential for the development of resistance in Pazufloxacin.[3] Many analytical methods have been overviewed in the survey. In the present work, the development and validation of Pazufloxacin mesylate using area under curve (AUC) method has been estimated for pure drug and marketed formulation.

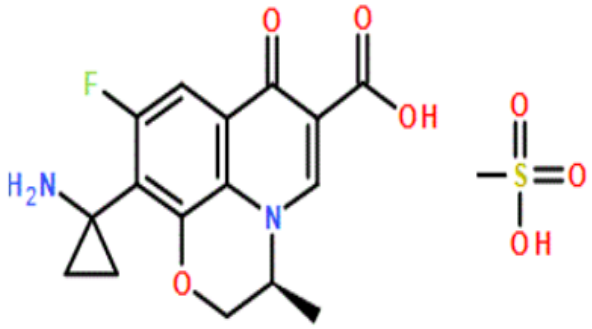

Fig 1. Chemical structure of Pazufloxacin mesylate

METHODOLOGY

1 Chemicals:

Pazufloxacin mesylate was supplied by Macleod's Pharmaceuticals, Sarigam, Gujarat. Pazumac infusion (500mg) was procured from local pharmacy. Methanol (S.D. Fine Chemicals, Mumbai, India) was used. All chemicals and reagents were of analytical reagent (AR) grade.

2 Instrumentation:

A Shimadzu (Kyoto, Japan) model UV-1800 double beam UV-Visible spectrophotometer attached with computer operated software UV probe 2.33 with spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Analytical balance and Mettler Toledo (Model JL1503-C).

3 UV-Spectroscopy Methods

A) Area under curve method:

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above-mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC.[5,6]

4 Experimental Work

a) To check the solubility of Pazufloxacin mesylate:

10 mg of Pazufloxacin mesylate was weighed and solubility of this sample was checked in double distilled water, methanol, ethanol, 1N NaOH, 0.1N HCl.

b) To identify the λmax of Pazufloxacin mesylate:

10 mg of the pure drug was accurately weighed and dissolved small portion of methanol and volume was made up to 10ml using distilled water to give a standard stock solution of 1000µg/ml. Further 2.5ml of 1000ppm solution was withdrawn and was diluted to 25 ml of volumetric flask and 100ppm solution is prepared. Suitable dilutions were made with distilled water to get standard solutions of concentration: 2, 4, 6, 8, 10, 12µg/ml.

C) Sample preparation for analysis of infusion formulation

Sample solution was prepared from infusion PAZUMAC (500 mg/100 ml). A sample volume equivalent to 10 mg was pipetted out from the infusion container and was placed in 10ml volumetric flask; volume was made up with solvent to get the concentration of 1000µg/ml. From this, 2.5 ml of aliquot transferred in 25 ml of volumetric flask containing diluent to form 100µg/ml of Pazufloxacin mesylate stock solution and further dilution of 2, 4, 6, 8, 10, 12ppm and scanned in the range of 200-400nm against water as blank at 249nm and then drug

content of solution was calculated by using standard calibration curve.

5 Analytical Method Development and Validation:
The developed method was validated as per ICH guidelines.

1. Linearity: The linearity of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample.[1]For which it has been demonstrated that the analytical procedure is of linearity, accuracy and precision.

Standard solution of pazufloxacin mesylate (2, 4, 6, 8, 10 and 12µg/ml) was pipette out in to a separated series of 10ml volumetric flask. The volume was adjusted to the mark with distilled water and mixed well. The absorbance maxima and area under curve for the solutions was measured at 249nm and range of 229-260nm for two methods respectively against distilled water as blank.

2. Precision: The precision of analytical procedure expresses closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. It may be considered at three levels: repeatability, intermediate precision, and reproducibility. It is expressed as standard deviation or coefficient of variation.

The repeatability studies were performed by analysis of same solution (2-12µg/ml) on the same day. Intermediate precision of the method was checked by repeating studies on the two different days. The %RSD of both determinations was calculated.

3. Accuracy: It is closeness of the result obtained to the true value. It is often expressed as percentage recovery by analyzing known added amounts of analyte. Also, it can be determined by applying the procedure to quantitatively prepared samples.

4. Sensitivity: The sensitivity of the method was determined in terms of limit of detection (LOD) and limit of quantitation (LOQ).

The LOD and LOQ were calculated by using formula,

LOD= 3.3×σ/S and **LOQ= 10×σ/ S.**
Where, σ is the standard deviation of regression line and S is the slope of line.

RESULTS AND DISCUSSION

1) Linearity & Range:

A] Calibration curve for pure drug:

a) Absorbance maxima method:
Under the experimental conditions described, the graph obtained for the absorbance maxima for pure drug showed linear relationship (Fig.1).Regression analysis was made for the slope, intercept and correlation-coefficient values. The regression equations of calibration curve were Y = 0.071x+0.170(r2 = 0.998) at 249.20nmfor absorption maxima the range was found to be 2-12µg/ml for the UV spectrometry.

Table 1: Calibration curve of Pazufloxacin mesylate

CONC.	ABS
2	**0.325**
4	**0.452**
6	**0.589**
8	**0.729**
10	**0.901**
12	**1.027**

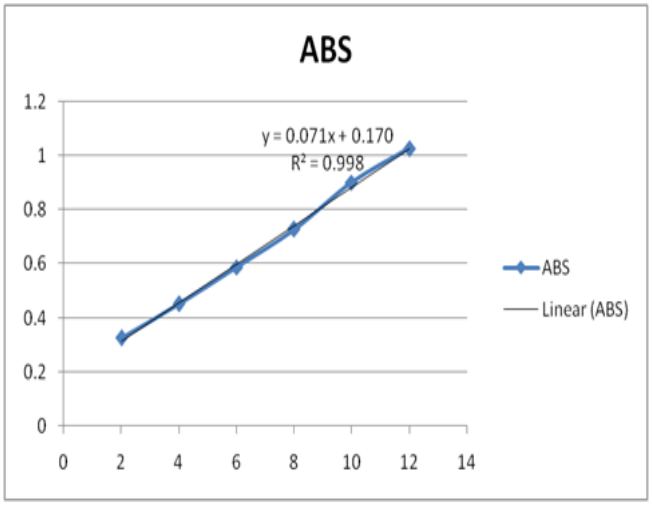

Fig 2: Calibration of pazufloxacin mesylate (Pure Drug)

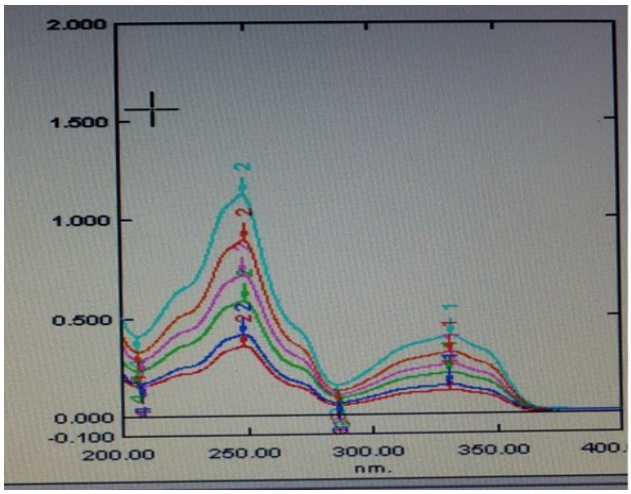

Fig.3: Overlay of spectra of Pazufloxacin Mesylate

Table.2: Calibration curve of Pazumac

Conc.	ABS.
2	0.308
4	0.469
6	0.615
8	0.748
10	0.895
12	1.064

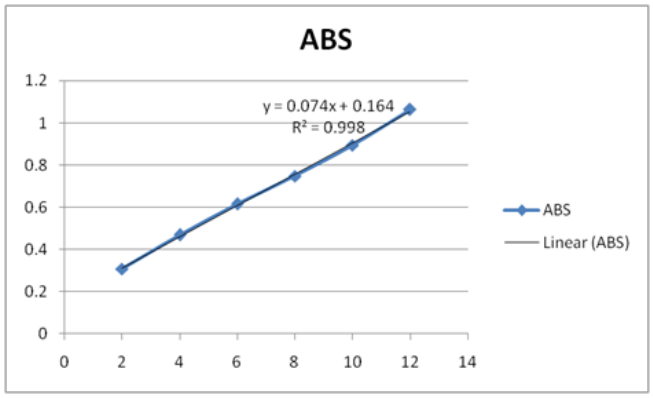

Fig 4: Calibration of formulation of pazufloxacin mesylate (Pazumac)

b) Area under curve method:

Under the experimental conditions described, the graph obtained for area under curve spectra showed linear relationship (Fig.4& Fig.5). Regression analysis was made for the slope, intercept and correlation coefficient values. The equation is y = 0.565x+1.652 (r2=0.999) at 229.20-260.20 nm for area under curve spectrophotometry. The range was found to be 2-12µg/ml for area under curve spectrophotometric analysis.

Table 3: Calibration curve of pazufloxacin mesylate by AUC

Conc.	AUC
2	2.715
4	3.985
6	5.057
8	6.224
10	7.227
12	8.452

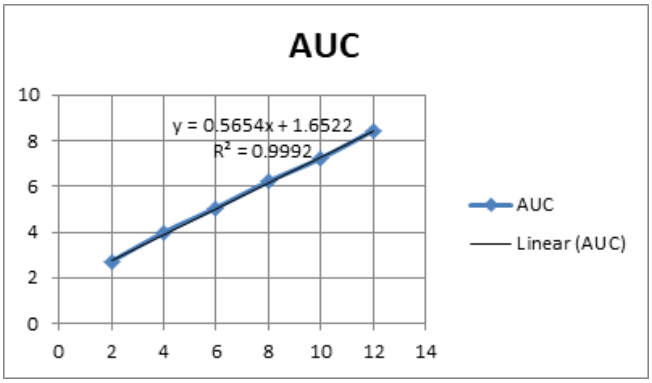

Fig 5: Calibration curve by AUC for pure drug Pazufloxacin mesylate

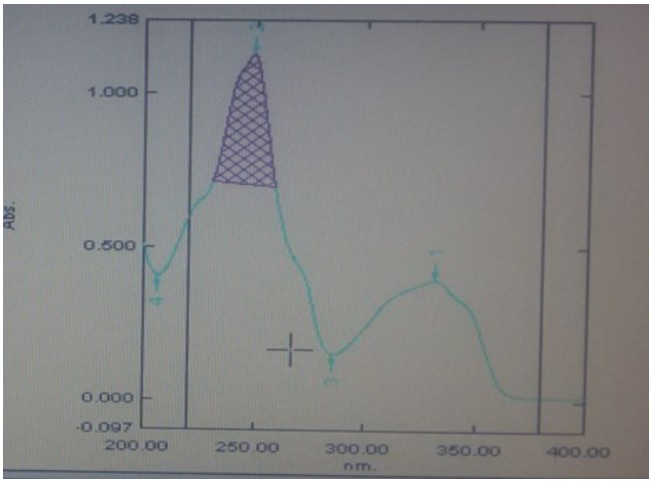

Fig 6: .Area between 229-260nm selected for pazufloxacin mesylate (10µg/ml)

Table 4: Calibration curve by AUC for pazufloxacin mesylate formulation

CONC.	AUC
2	1.363
4	2.824
6	4.239
8	5.678
10	7.264
12	8.671

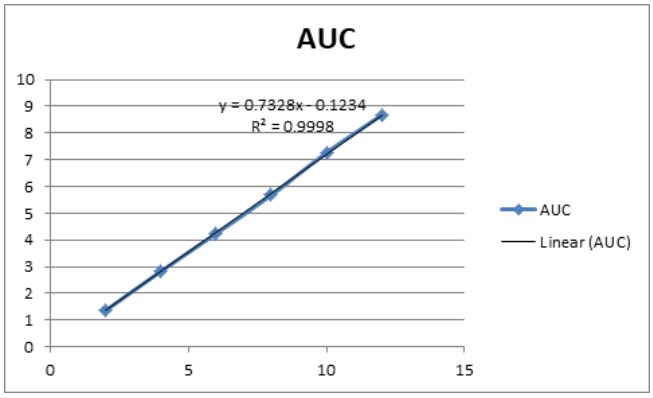

Fig.7. Calibration curve by AUC for pazufloxacin mesylate formulation

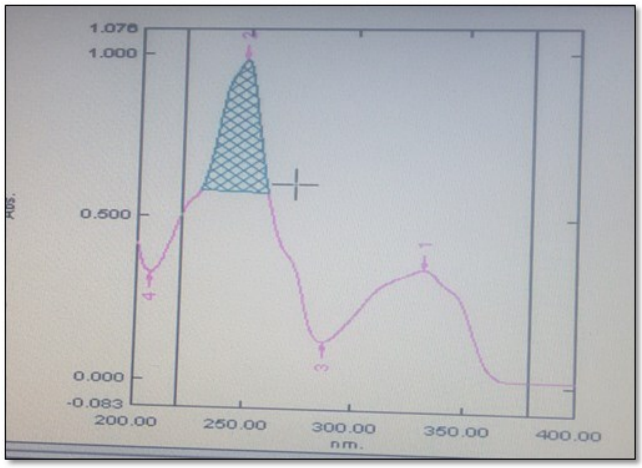

Fig 8: Area between 229.20-260nm selected for Pazufloxacin mesylate infusion formulation (10µg/ml)

Table 4: Regression Analysis Data for Pazufloxacin mesylate by the AUC method

Parameter	AUC
Wavelength range (nm)	229-260
Concentration range (µg/ml)	2-12
Slope(m)	0.565
Intercept(c)	1.652
Correlation coefficient(r2)	0.999

Precision:

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses solution (2-12µg/ml) on the same day. The %RSD of six determinations was calculated. Intermediate precision of the method was checked by repeating studies on two different days. The %RSD of the determinations was calculated.

Table no.5.Result of interday and intraday precision

Parameters	±S.D.*	RSD	%RSD*
Interday	0.0585	0.009	0.975
Intraday	0.0656	0.010	1.00

*n=6

Table 4: Data of Recovery studies

Level of mean recovery (%)	% mean recovery	SD*	%RSD
80%	101.12%	0.0721	1.20
100%	99.32%	0.0595	0.991
120%	100.23%	0.0651	1.08

Table 5: Assay Results for estimation of Pazufloxacin in Pharmaceutical Formulation

Parameter	Label claimed	Amount found	%Label clamed
AUC	10µg/ml	9.93	99.32

Sensitivity:

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the equations LOD= 3.3×σ/S and LOQ= 10×σ/ S, where σ is the standard deviation of the intercept, S is the slope. The LOD and LOQ were found to be 0.0093 and 0.0283 respectively for the area under curve method.

Recovery studies:

To stock solution in 3 different volumetric flask, aliquots of 8ml, 10ml and 12ml of the standard stock solution were added, volume was made upto 10ml with water to give concentration of 8µg/ml (80%), 10(100%) and 12(120%). Absorbance was determined at 249nm. Procedure was repeated 3 times for 80%, 100% and 120% for recovery studies

Table no.6 Summary Data of Validation Parameters

Sr.no	Parameter	AUC Method
1	Linearity	2-12
2	Regression equation	y =0.565x+1.652
3	Correlation Co-efficient	r2=0.999
4	LOD(µg/ml)	0.0093
5	LOQ(µg/ml)	0.0283

6	Precision	
6.1	Interday	0.975%RSD
6.2	Intraday	1.00%RSD

CONCLUSION

Simple UV spectrophotometric methods have been developed and validated for the determination of Pazufloxacin mesylate and infusion. The results of the validation parameters show that the UV spectrophotometric methods were found to be accurate, precise and sensitive. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of pazufloxacin mesylate in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

1. ICH Harmonised Tripartite Guideline: Validation of analytical procedures: text and methodology Q2 (R1).
2. Merck index, Maryadele J.O. Neil Edu. In: 13 Ed, Merck Research Lab NJ, USA. 2001; 868.
3. Chemical structure available online at URL - http://www.chemicalbook.com/ChemicalProductProperty_USCB3188646.aspx; (Accessed January 2015).
4. http://www.aksci.com/item_detail.php?cat=M798; (Accessed January 2015).
5. Michael E. Swartz, Ira S. Krull, Analytical method development and validation, Marcel Dekker, Inc., 1997; 17: 25-2.
6. Christian G.D., Analytical chemistry, sixth edition, John Wiley and Sons, 2003; 1-2,604- 620.
7. www.ukessays.com/essays/biology/estimation-of-pazufloxacin-mesylate-from-pharmaceutical-dosage-form-biology-essay.php; (Accessed October 2014).
8. Sudesh D. Shambharkar , P. D. Hamrapurkar, AbhijeetParate, et.al. Optimization and Validation of RP-HPLC Stability Indicating Method for Determination of Pazufloxacin Mesylate and Its Degraded Product.International Journal of Applied Science and Engineering 2013; 11, 4: 423-432; (Accessed November 2014). http://www.cyut.edu.tw/~ijase/2013/11%284%29/6_024016.pdf(Accessed November 2014).
9. www.researchgate.net/publication/260419864_HPLC_and_Densitometric_TLC_Methods_for_Simultaneous_Determination_of_Pazufloxacin_with_Some_Coadministered_Drugs_in_Human_Plasma;(Accessed November 2014).
10. Nehad A. Abdallah; Validated Stability-indicating HPLC and Thin Layer Densitometric Methods for the Determination of Pazufloxacin; J Chromat Separation Techniq ISSN: 2157-7064 JCGST; Volume5 Issue2 1000218.
11. http://omicsonline.org/open-access/validated-stabilityindicating-hplc-and-thin-layer-densitometric-methods-for-the-determination-of-pazufloxacin-application-to-pharmaceutical-formulation-2157-7064.1000218.pdf; (November 2014). www.wjpr
12. Skoog, Holler, Nieman, Principles of Instrumental Analysis, fifth edition, Thomson Asia Pvt. Ltd., Singapore, 2004; 300-325.
13. Beckett, A. H., Stenlake, J. B., Practical Pharmaceutical Chemistry, 4th edition, CBS Publishers and Distributors, New Delhi, 2002; 2: 275-295.
14. Quin Li, Rui wang, fei pei, Determination of the Pazufloxacin mesilate in human plasma and urine by HPLC, Asian Journel of Drug Metabolism and Pharmaceuticals, 289.
15. Zhefeng Zhang, Gengling Yang, Dexian Wang, Guijian lian, YI chen, Determination of pazufloxacin mesilate by high performance liquid chromatography, Micro chimica acta, 159, 299-301.
16. Chunyan Yang, Zhujun Zhang, Suming Chen, Feng yang, Development of moleculaely imprinted on-solid extraction combined with chemiluminesence for the determination of pazufloxacin mesilate. Spinger, 2007, 159, 299-301.