



Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development of UV spectrophotometric methods for estimation of Pazufloxacin in infusion form using Absorbance ratio method

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ABSTRACT

Simple, sensitive and specific spectrophotometric method was developed and validated for quantitation of Pazufloxacin (PAZU) in infusion form. The new analytical method was developed based on the estimation of PAZU in infusion form using Absorbance Ratio method. In graphical absorption ratio method was performed by absorbance at 249 nm and 335nm. PAZU follows Beer-Lambert's law in the range of 2 -10 $\mu\text{g/ml}$ at all the selected wavelength. The percent estimation of pure drug in laboratory was found to be 99.73 ± 0.51 for PAZU. The percent drug estimation in marketed formulation was found to be 99.52 ± 0.72 . The average percent recovery was found to be 99.81 ± 0.51 . The result of the method lies within the prescribe limit of 98-102% shows that method is free from interference from excipients.

Keywords: Pazufloxacin (PAZU), absorption ratio method, recovery, excipients.

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INTRODUCTION

Pazufloxacin (PAZU) chemically is (3*R*)- 10- (1-aminocyclopropyl)- 9- fluoro- 2,3- dihydro- 3 methyl- 7- oxo 7H- pyrido [1,2,3-d,e]1,4 -benzoxazine-6-carboxylicacid [1]. PAZU exhibits antibacterial activity by inhibiting DNA gyrase thus preventing DNA replication and synthesis [2]. PAZU is a fluoroquinolone antibacterial agent given by i.v. infusion as the mesilate in the treatment of susceptible infections in a usual dose equivalent to 1g of PAZU daily in 2 divided doses [3].

Literature survey revealed that various method has been reported for estimation of PAZU viz HPLC [4, 5], UV Spectrophotometric [6, 7, 8]. Since no method has been reported for the adsorption spectrometric estimation of the above mentioned drug, in present project attempt was made to develop alternative simple, rapid, accurate and reproducible UV Spectrophotometric method for estimation of Pazufloxacin in infusion form using absorbance ratio method.

METHODS & MATERIALS

All chemical used in the development of methods are of analytical grades. UV – Spectrophotometer used in this study is Shimadzu UV-1601 double beam spectrophotometer, with 1.0 cm matched pair of rectangular quartz cells.

Standard Solution

Stock standard solution

An accurately weighed quantity of PAZU (~10mg) was taken in a 50.0ml volumetric flask and was dissolved in distilled water and the volume was made up to the mark with distilled water (1000 µg/mL).

Selection of Wavelengths

The aliquots portion of standard stock solution of PAZU was diluted appropriately with distilled water to obtain a concentration (10µg/mL) of drug. The solution was scanned under UV-visible spectrophotometer in the range of 400-200nm in 1.0cm cell against blank.

Study of Beer – Lambert's Law

The aliquot portion of PAZU working stock solution ranging from 1-5mL were taken in a series of 50.0 mL volumetric flask and were diluted upto the mark with distilled water to get concentration in the range of 2-10 µg/mL. The absorbances of each solution were measured at the selected wavelengths.



Additivity Study

5 standard solution of same ratio were prepared an absorbances of each of five solutions were measure at the selected wavelength against blank.

Analysis of Pure drug sample

In order to establish suitability of the propose method for the determination of PAZU in pharmaceutical formulation, the method was first applied for the estimation of drug in the standard laboratory mixtures.

Analysis of Marketed Formulation

Accurately measured quantity of infusion was transferred to 50.0 ml volumetric flask and volume was made to 50.0 ml with distilled water.

Recovery Study

Accurately measured quantity of infusion equivalent to 50 mg of PAZU was taken in volumetric flask (50 mL). The variable amount of standard drug at three different concentration levels was added to volumetric flask. The final dilutions were made by using mobile phase and absorbances were read at 249.0nm.

The validation of proposed method was carried out by recovery studies, adding known quantity of drugs in final dilution and the % recovery was then calculated by using formula.

Validation of Method

Accuracy

Accuracy of each of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

Precision

Precision of the analytical method is expressed as the series of the measurement. It was ascertained by replicate estimation of the drug by the proposed methods.

Specificity

Accurately measured quantities of infusion formulation equivalent to about 50mg of PAZU were taken in a dry 50.0mL volumetric flask. Each sample was then store for 24hrs under the following different conditions.

1. At room temperature (Normal)
2. At 50⁰C after addition of 1ml of 0.1 M NaOH (Alkali)
3. At 50⁰C after addition of 1ml of 0.1 M HCl (Acid)
4. At 50⁰C after addition of 1 ml of 3% H₂O₂
5. At 60⁰C (Heat)

After 24 hrs, each treated sample was dissolved in distilled water and volume was made up to mark. These solutions were filtered. An aliquot portion of each of the filtrate was diluted to get 10µg/mL of PAZU then absorbances of each resulting solution were recorded at two selected wavelength.

Linearity and Range

Accurately measured quantities of infusion formulation, equivalent to 80, 90... 120% labeled claim of PAZU, was taken in five different 50.0mL volumetric flask and were dissolved in distilled water. The volume were made upto the mark with distilled water. The solutions were diluted and absorbances were read at 249.0nm.

Ruggedness

The studies of ruggedness were carried out under different conditions i.e. (i) different elapsed times (intraday and interday) and (ii) different analysts.

RESULT AND DISCUSSION

The attempt was made to develop an alternative and economical method for estimation of PAZU in infusion form using absorbance ratio method.

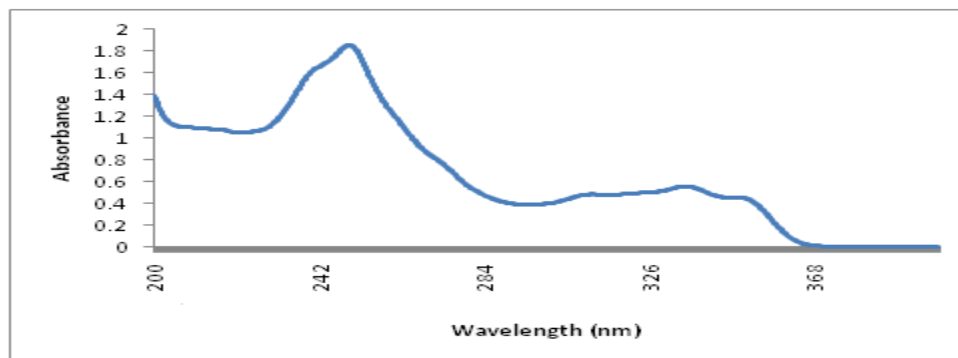


Fig. 1 UV spectra of PAZU

Table 1 Absorbitivity values for PAZU

Sr. No.	E(1%, 1cm)	
	PAZU	
	249nm	335nm
1	1286	480
2	1294	476
3	1291	477
4	1298	475
5	1286	476
6	1265	476
Mean	1286	476
S.D.	1.374	0.76
C.V.	1.152	0.76

Table No. 2: Assay of pure drug sample by proposed method.

Sr. No.	Amount of drug taken (µg/ml)	Absorbance of pure drug sample at 249nm	% Drug estimated at 249nm
1	10.07	0.1292	100.32
2	10.03	0.1278	99.21
3	10.05	0.1289	100.04
4	10.02	0.1236	98.95
5	10.04	0.1279	99.22
Mean			99.73
± SD			0.94
CV			0.94

Table No. 3: Estimation of PAZU in formulation

Sr. No.	Volume of infusion taken for analysis (mL)	Absorbance at 249nm	% Labeled claim
1	2	0.1292	100.21
2	2	0.1278	99.14
3	2	0.1289	100.05
4	2	0.1276	98.99
5	2	0.1279	99.22
Mean			99.522
± SD			0.92
CV			0.92

Table 4 Results of recovery study

Sr. No.	Volume of infusion taken for analysis (mL)	Amount of pure drug added (μg)	Amount of pure drug recovered (μg)	% Recovery
1	2	4	4.052	101.30
2	2	5	5.007	100.14
3	2	6	5.9	98.33
Mean				99.81
SD				1.293
CV				1.295

UV-spectra of PAZU had revealed that it has absorbance at 249.0nm (fig.1). PAZU follows Beer-Lambert’s law in the range of 2 -10 $\mu\text{g}/\text{ml}$ at all the selected wavelength. The percent estimation of pure drug in laboratory was found to be 99.73 ± 0.51 for PAZU (Table 2). The percent drug estimation in marketed formulation was found to be 99.52 ± 0.72 (Table 3). The method was validated according to ICH guidelines. The accuracy of method was validated by percent recovery of the drug. The percent recovery was found to be 99.81 ± 0.51 (Table 4). The result of the method lies within the prescribe limit of 98-102% shows that method is free form interference from excipients. The replicate estimation of PAZU in same infusion was analyzed by the proposed method and showed quite concurrent results indicating reliability of the method. The values \pm SD of RSD and co-efficient and correlation are within the prescribed limit of 2% showing high precision of the method.

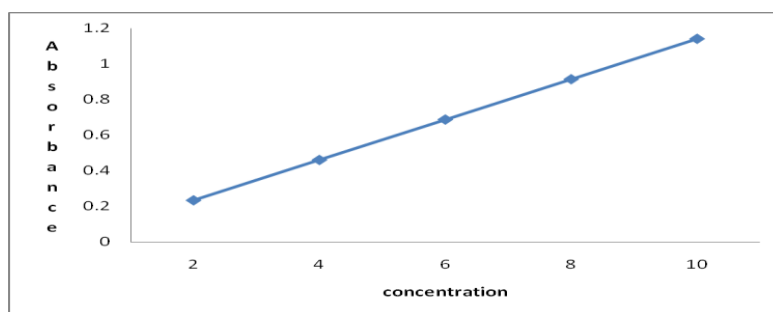


Fig. 2 Calibrations curve for PAZU

Table 5 Result of specificity study

Sr. No.	Conditions	% Labeled Claim
1.	Normal	100.15
2.	Acid	90.89
3.	Alkali	92.37
4.	Oxide	85.15
5.	Heat	98.05
6.	UV	96.33

Table 6 Results for ruggedness study

(A) Different analyst

Sr. No.	Volume of infusion taken for analysis (mL)	Amount of drug estimated in 2 mL (mg)	% of labeled claim
1	2	9.92	99.23
2	2	9.88	98.88
3	2	9.90	99.06
Mean			99.08
SD			0.247
C.V.			0.249

(B) Interday variation

Sr. No.	Volume of infusion taken for analysis (mL)	Amount of drug estimated in 2 mL (mg)	% of labeled claim
1	2	9.89	98.93
2	2	10.00	100.07
3	2	9.93	99.30
Mean			99.43
SD			0.581
C.V.			0.584

(C) Intraday variation

Sr. No.	Volume of infusion taken for analysis (mL)	Amount of drug estimated in 2 mL (mg)	% of labeled claim
1	2	9.98	99.83
2	2	10.00	100.04
3	2	9.86	98.64
Mean			99.52
SD			0.755
C.V.			0.758

The other parameters studied were specificity, linearity and ruggedness. This studied was carried out to check degradation and stability of the drug (Fig 2, Table 5). Overall this method is incapable of finding exact degradation of drugs. The linearity and range study were also carried out over the range of 80-120% of labeled claim for both the drug in formulation the percent labeled claim verses absorbance plots shows a linear relationship with correlation coefficients very closer to 1. Ruggedness studies were carried out under four different conditions i.e., different analyst interday variation, intraday variation, and different instrument shows that the results of estimation by proposed method are very much reproducible under variety of conditions (Table 6).

ACKNOWLEDGEMENT

The authors are thankful to head J. L. Chaturvedi College of Pharmacy, Electronic Zone Building MIDC Hingna Road Nagpur for providing laboratory facilities. Authors are also thankful to Cadila Healthcare Ltd., Ahmadabad for providing Pazufloxacin gift sample.

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