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DEVELOPMENT AND VALIDATION OF SPECTROFLUORIMETRIC METHOD FOR THE ESTIMATION OF PAZUFLOXACIN MESYLATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, rapid, precise, accurate & robust spectroflourimetric method has been developed for estimation of Pazufloxacin Mesylate in its pure and pharmaceutical dosage form. Pazufloxacin Mesylate showed good fluorescence intensity in 0.2N HCl and so 0.2N HCl was selected as a solvent for estimation of Pazufloxacin Mesylate by spectroflourimetric method. The optimized excitation (λex) and emission (λem) wavelength were 251 nm and 418 nm respectively with 5nm slit width for Pazufloxacin Mesylate determination. The calibration curves were found to be linear between fluorescence intensity and drug concentration in the range of 8-48 ng/ml with coefficients of determination above 0.994 for the analyte. The method recoveries were higher than 98%. The limit of detection (LOD) and limit of quantitation (LOQ) were found in the range of 0.4803 ng/ml and 1.4556 ng/ml respectively. The % RSD values of intra- and interday variation coefficients were observed less than 2%. The developed method was validated in terms of linearity, precision, accuracy, limit of detection and limit of quantitation, robustness as per International Conference on Harmonization Q2 (R1) guidelines.[1] This method is simple, accurate and rapid therefore can be used for routine analysis of Pazufloxacin Mesylate in quality control laboratories.

KEYWORDS: Spectrofluorimetric; Pazufloxacin Mesylate; Fluorescence; Excitation; Emission; Validation.

INTRODUCTION

Pazufloxacin mesylate is a new fluoroquinolone antibiotics, the broad spectrum antimicrobial products with anti-bacterial activity used in urinary tract infection. Pazufloxacin mesylate is a

fused tricyclic fluoroquinoline antibacterial salt. Chemically it is (3S)-10-(1-Aminocyclopropyl)-9-fluoro-2, 3-dihydro-3-methyl-7-oxo-7H-pyrido[1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid methanesulfonate (Fig. 1).[2] The presence of an aminoacyl group at C_{10} is a unique feature of the molecule, imparting potent broad spectrum activity against gram-positive and gram-negative bacteria including a variety of resistant strains and anaerobic bacteria. Pazufloxacin has shown multimodal mechanism of action and inhibits both DNA gyrase and topoisomerase IV enzyme, leading to increased antibacterial spectrum via DNA gyrase-dependent processes such as DNA polymerization, (ATP-dependent) DNA supercoiling and chromosome fragmentation. Moreover, Pazufloxacin has been shown to have DNA antagonistic actions too. The multimodal mechanism of action is linked to the low potential for the development of resistance in Pazufloxacin. Moreover, it has been shown that Pazufloxacin is not affected by efflux mechanism of resistance.[3] There are lots of analytical methods seen in review of literature. But the aim of the present study was to develop and validate spectroflourimetric method for the estimation of Pazufloxacin mesylate in pharmaceutical dosage form.

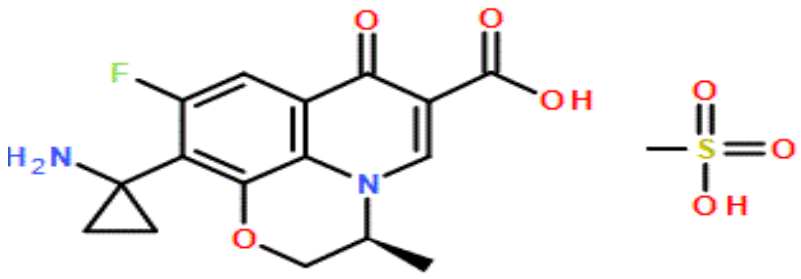

Fig. 1: Chemical structure of pazufloxacin mesylate

MATERIALS AND METHODS

Pharmaceutical grade of Pazufloxacin Mesylate was kindly gifted by Hetero Labs Ltd. (Gujarat, India). The Infusion formulation was purchased from Nusi Wockhardt Hospital (Goa, India) [PAZACE containing Pazufloxacin 500 mg/100 ml]. Ultrapure deionized water processed through BIO-AGE water purification system used for preparing 0.2N HCl. All the reagents used in this method were of analytical grade.

Spectroflourimetric analysis was performed on SHIMADZU RF-5301 PC spectroflourimeter with xenon discharge lamp, photomultiplier tube as detector, software (RFPC) and all weight measurements were taken on WENSAR ELECTRONIC BALANCE MAB 220 at room

temperature. An aliquot of 20 ng/ml sample was assayed for the estimation of Pazufloxacin mesylate in infusion form.

Preparation of stock solution and calibration standards

The Standard solution of Pazufloxacin Mesylate was prepared by weighing 10 mg of drug poured in 10 ml volumetric flask and make up the volume with 0.2N HCl as solvent (1000 µg/ml) and further diluted, 0.1 ml solution in volumetric flask containing 10ml of 0.2N HCl to get concentration of 10 µg/ml. From the above solution take 0.1 ml solution in another 10 ml volumetric flask and make up the volume to 10 ml mark with 0.2N HCl to get stock concentration of 100 ng/ml. Then, this stock solution was used to prepare further required concentrations.

Sample solution was prepared from infusion PAZACE (500 mg/100 ml). A sample volume equivalent to 10 mg was pipetted out from the container and placed in 10ml volumetric flask; volume was made up with solvent to get the concentration of 1000 µg/ml. From this 0.1 ml was transferred to 10 ml volumetric flask and volume was made to get the concentration of 10 µg/ml. From the above flask 0.1 ml was diluted to 10 ml in a volumetric flask to get the concentration of 100 ng/ml. Subsequent dilutions were performed to get optimum concentration of 20 ng/ml.

Six standard concentrations of 8, 16, 24, 32, 40 and 48 ng/ml were prepared from the stock solution of Pazufloxacin mesylate drug (pure form) in 10 ml volumetric flask using 0.2N HCl to form calibration standards.

RESULTS AND DISCUSSION

The literature survey reveals that Pazufloxacin Mesylate can be estimated by various methods like UV-Spectrophotometry,[4] by HPLC,[5-9] & by Spectroflourimetric analysis,[10] individually in bulk drugs & in human plasma. The attempt was made to develop precise & accurate spectroflourimetric method for the estimation of Pazufloxacin Mesylate without derivatization. The present work describes the fast, specific & selective spectroflourimetric method for the estimation of Pazufloxacin Mesylate in pharmaceutical dosage form. Numbers of trials were performed in laboratory using different solvents like distilled water, 0.1N NaOH & HCl. Amongst all, HCl was selected and used as a solvent for the study. Trials were made using different concentrations of HCl likewise 0.1N, 0.2N & 0.3N; but 0.2N of HCl showed good intensity & no interference as that of other solvents so proposed method is

direct & based on the measurement of fluorescence intensity of Pazufloxacin Mesylate in 0.2N HCl at room temperature.

Results were observed on both high intensity mode as well as low intensity mode. But high intensity was used as it shows %RSD>2. Method involves determination of excitation and emission wavelength. For this purpose standard solution of concentration 20 ng/ml was used and scanned over the spectral range of 230 to 600 nm in spectral mode of determination. After autozero with blank the excitation wavelength was determined by keeping emission wavelength constant and vice versa. The solution shows constant excitation and emission wavelength of 251 nm & 418 nm respectively. The slit width of both excitation and emission spectra was selected as 5nm after conducting trial with different combination of slit width such as 1.5, 3, 5, 10 and 15. Observation at slit width of 5nm showed good results within limit (Fig.2, 3). The method was validated as per ICH guidelines.

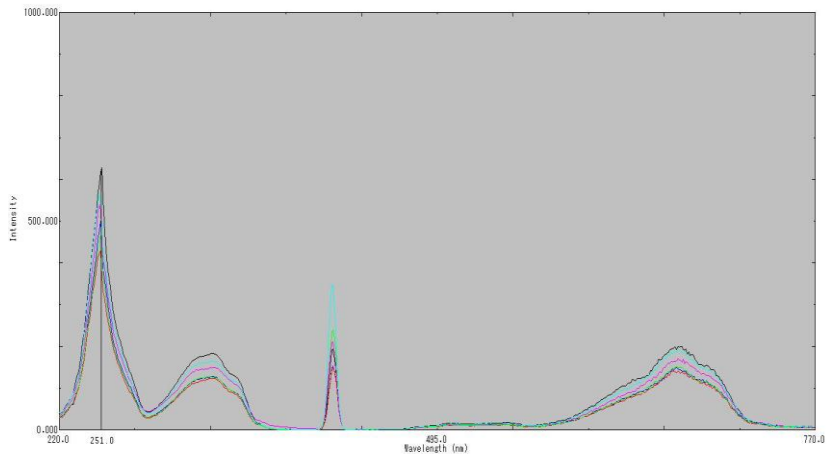

Fig. 2: Excitation spectra of pazufloxacin mesylate (λex 251 nm) slit width 5 nm

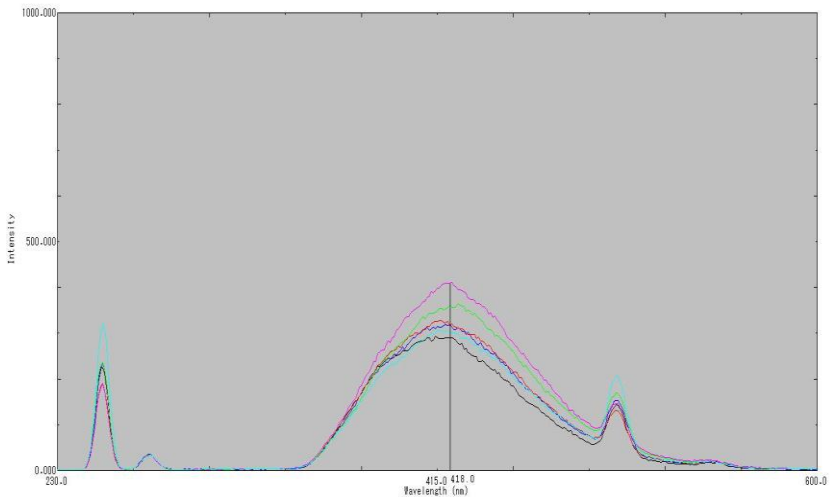

Fig. 3: Emission spectra of pazufloxacin mesylate (λem 418 nm) slit width 5 nm

From the above stock solution 0.8, 1.6, 2.4, 3.2, 4.0, 4.8 ml was pipetted in 10 ml volumetric flask and volume was made up to 10 ml with 0.2N HCl. The calibration graph of the above concentrations was plotted. The R^2 value was found to be 0.9942 (Fig. 4).

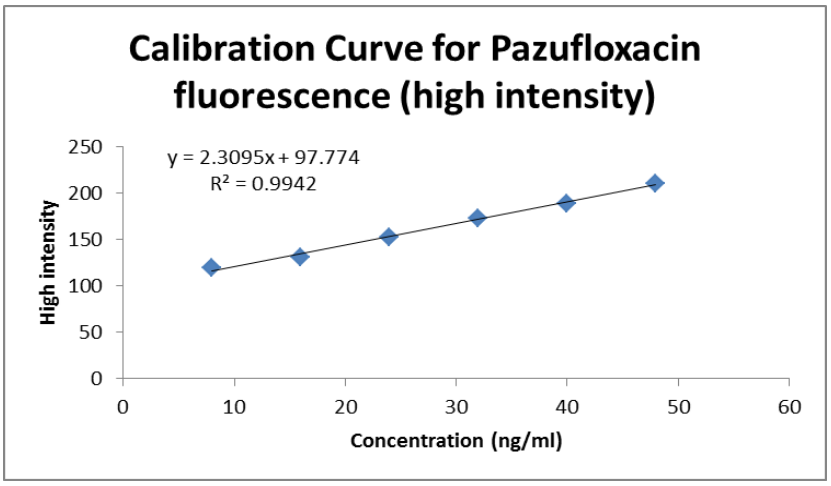

Fig. 4: Calibration curve for pazufloxacin mesylate

VALIDATION PROCEDURE

The method of analysis was validated as per the recommendations of ICH guidelines for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating percentage recovery of the drug.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.[1] The sample solution of concentration ranging from 8-48 ng/ml were prepared and linearity assessed by plotting the graph of concentration (ng/ml) vs. florescence intensity. The regression analysis was accomplished by slope, intercept and correlation coefficient (R^2). The linearity data is shown in (Table No.1).

Table No.1: Linearity data of Pazufloxacin mesylate

Concentration (ng/ml)	Fluorescence Intensity
8	119.910
16	130.674
24	152.343
32	172.475
40	189.040
48	210.197

Accuracy

The accuracy of method was performed by adding 80%, 100% & 120% of the pure drug standard solution to 100% concentration of marketed formulation. 20ng/ml was used as nominal 100% concentration. The % recovery of the same pure drug was than determined. The recovery was found to be ranging from 97.56% - 100.55%.

Precision

Intraday Precision (Repeatability) expresses the precision under the same operating conditions over a short interval of time.[1] The six replicate of prepared 20 ng/ml solution of Pazufloxacin Mesylate were taken from different stock solution and scanned at 230-600 nm for the determination of fluorescence intensity. The relative standard deviation (%RSD) was found to be less than 2 %, which indicates that the proposed method is repeatable.

Interday precision (Intermediate Precision) involves observation of variation of results on two different days.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

Limit of Detection (LOD) was determined on the results of six replicates of 20 ng/ml concentration of pure standard. The LOD for Pazufloxacin Mesylate in 0.2N HCl was found to be 0.4803 ng/ml.

Limit of Quantitation (LOQ) was determined based on the results of six replicates of 20 ng/ml concentration of pure standard. The LOQ for Pazufloxacin Mesylate in 0.2N HCl was found to be 1.4556 ng/ml.

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

Robustness

The robustness of an analytical procedure 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. For this purpose the emission wavelength and excitation wavelength parameters were challenged with changes of ±2 nm i.e. 416 nm & 420 nm and 249 nm & 253 nm respectively and spectra were observed. The results of validation of parameters is been depicted in Table no.1.

Result Table

Table No.2: Results of validation of parameters of pazufloxacin mesylate in 0.2N HCl by spectroflourimetric method

Parameters	Results
Range (ng/ml)	8 - 48
Linearity (ng/ml)	8 - 48
Regression Coefficient (R2 Value)	0.9942
Assay (%)	103
Recovery (%)	97.56 – 100.55
Interday Precision (Intermediate)	0.7800
Intraday Precision (Repeatability)	0.7266
Limit of Detection (ng/ml)	0.4803
Limit of Quantitation (ng/ml)	1.4556
Robustness	Robust

CONCLUSIONS

The developed Spectroflourimetric method for estimation of Pazufloxacin Mesylate is simple, rapid, precise and accurate. The advantage of the present method is used as alternative to reference method (HPLC and other methods) for determination of Pazufloxacin Mesylate in pure form and dosage form in the industrial and research institutional laboratories.

ACKNOWLEDGEMENT

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