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Validation of UV spectrophotometric method of analysis of some marketed perfloxacin in Nigeria

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ABSTRACT

Background: Acquiring sophisticated LC instruments by most third world laboratories is capital intensive. Literatures on simple spectrophotometric analytical methods for pefloxacin are scarce.

Objectives: The present study was undertaken to develop and validate a simple and economic UV spectrophotometric method for estimating pefloxacin mesylate (PFM) in dosage preparations

Methods: Using a JENWAY spectrophotometer at predetermined λmax of 277nm with 1% v/v aqueous glacial acetic acid as blank, the method was validated for linearity, accuracy, precision, reproducibility, and specificity as per International Conference on Harmonization (ICH) guidelines and used to determine the content of pefloxacin in seven marketed brands in Nigeria.

Results: The method exhibited good linearity over a range of 0.40–12.0 µg/ml (regression equation: y = 0.0859x+ 0.0211 ; r=0.999). Mean recovery accuracy (99.183%) and assay result in the range of 100.5- 110.17% for the selected brands were not significantly different at p=0.05. The % coefficient of variation (CV) for both intra and inter-day were below 7 %. The method was specific for pefloxacin in the presence of common excipients

Conclusion: The method gave good validation results and could be employed for routine analysis of PFM in commercial formulations.

Key words: Pefloxacin mesylate, spectrophotometric method, validation, and assay

INTRODUCTION

Quinolones comprise an interesting group of antibacterial drugs whose action is based on their anti-DNA gyrase and topoisomerase IV activities. Their antibacterial activity is greatly increased by the addition of 6-fluoro and 7-piperazinyl groups to the molecule giving rise to the novel 6-fluoroquinolones commonly referred to as the fluoroquinolone antibacterial agents. They are the second-generation members of Quinolones and are greatly effective against both gram-negative and gram-positive pathogens that are resistant to other antibacterials [1-3]. Newer members like levofloxacin are used in the treatment of Multi Drug Resistance (MDR) tuberculosis. Pefloxacin mesylate was introduced in1985 a new chemical entity [4].Chemically, it is 1-ethyl-6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1, 4-dihydoquinoline-3-carboxylic acid methane sulphonate. Bacterial resistance to antibiotics is an emerging public health crisis. The prevalence of pathogens resistant to currently available antibiotics is on the increase. Nigeria being an integral part of the global village is not insulated from this trend. This has resulted in the influx of newer and more potent antibacterial agents among which the fluoroquinolones are inclusive, into the Nigerian health community. At present, over seven brand of pefloxacin generic are manufactured and marketed in Nigeria in addition to the innovator peflacine®. Ascertaining the quality of these several brands of the pefloxacin generic has become an imperative. Being a third world economy, acquisition of sophisticated LC instruments by most laboratories in Nigeria is a capital intensive venture. This makes the

development of a simple, rapid and cost effective UV spectrophotometric method for the determination of active pharmaceutical ingredients (API) in formulated drug products an imperative. Several liquid chromatographic methods have been reported for the determination of pefloxacin and other fluoroquinolones in dosage preparations and body fluids. Nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and its hydrochloride are in official compendia 5-6. Both USPXXIV and BP 1998 recommend HPLC methods for the determination of ciprofloxacin in raw material and in dosage forms. The USP XXIV recommends non-aqueous titration methods for determination of nalidixic acid, norfloxacin and ofloxacin in raw material, while HPLC methods are described for analysis of their dosage forms. The BP 1998 recommends a nonaqueous titration method for determination of nalidixic. The spectrophotometric determination of some fluoroquinolones have been reported3,7-11. However there is no pharmacopoeia UV spectrophotometric method for the determination of pefloxacin either in pure form or dosage form. The BP 2003 recommended a complex LC method for the determination of its related substances and titrimetric method for the assay of the raw material. 5 The quality assessment of some flouroquinolone generics in Nigeria have been reported 7-10. However literature on spectrophotometric methods of assay and quality assessment of pefloxacin generics are scarce. In this present study, a simple UV spectrophotometric method was developed and validated as per International Conference on Harmonization (ICH) guidelines10-11. The method was also used in the determination of the content of pefloxacin in seven marketed brands of the pefloxacin generic in the Nigerian market.

MATERIALS & METHODS

Materials

Pure PFM and the common excipients; sodium starch glycolate (SSG), maize starch, lactose, and avicel PH101 were kindly received as a gift from May and Baker Nigeria PLC Ikeja Lagos. Glacial acetic acid (Analytical grade), silica gel HF254 (MERCK), ammonia, n-butanol, and acetone were purchased from Sigma Aldrich. Distilled water was used for all analysis. A JENWAY spectrophotometer model number: 6500/6505 was used for the analysis.

Methods

Method Development and Validation

Determination of wavelength of maximum absorption

PFM standard (0.1g) was dissolved in 50 ml of 1% aqueous acetic acid and diluted to 100 ml mark with the same solvent. 1 ml of this solution was further diluted to 100 ml with the same solvent to obtain a 0.01mg/ml pefloxacin solution which was scanned in the region 190 to 800 nm to determine the wavelength of maximum absorption observed to be 277 nm

Linearity Study

PFM standard (40 mg)was dissolved in 50 ml of 1% aqueous acetic acid and diluted to 100 ml mark with same solvent to obtain a 400 µg/ml pefloxacin stock solution for linearity study. Aliquots range of 0.1 to 3.0 ml of this stock solution were taken and diluted to 100 ml with the 1% aqueous acetic acid to obtain thirteen different concentrations within the range 0.4 –12 µg/ml used for the linearity calibration plot.

Intra day precision study

0.5, 1.0 and 2.0 ml of the 400 µg/ml pefloxacin stock solution for linearity study were taken and respectively diluted to 100ml with the 1% aqueous acetic acid to obtain three concentrations of 2, 4 and 8 µg/ml respectively. Triplicate absorbance measurements of each were taken and the mean, standard deviation and RSD calculated.

Inter day precision study

The selected concentrations for the intra day precision study were yet analysed the second day and the mean, standard deviation and RSD calculated.

Recovery accuracy study

This was done using pre-formulated granules containing 74.4%w/w pure pefloxacin mesylate dihydrate, and common excipients like Sodium starch glycolate SSG, maize starch, lactose and avicel. Triplicate 120 mg of this powdered granule was then transferred into a separate 200 ml volumetric flask. 100ml of the 1% aqueous acetic acid was then added and shaken for 15mins using a vortex mixer and then diluted to 200ml mark with same solvent. This was then filtered to obtain sample stock solution (Po). 1ml of the filtrate Po was further diluted to 100ml with the same 1% aqueous

acetic acid this was then assayed for the content of pefloxacin using the method with a solution containing 4ug/ml of pure pefloxacin mesylate dihydrate as standard for comparison. Also another 1ml of the filtrate Po was spiked with 1ml of the 4ug/ml pefloxacin standard solution (4ug) and diluted to 100ml mark and the absorbance read along side. All analyses were done in triplicate.

Specificity in the presence of excipients

This was done using common excipients like SSG, Lactose, maize starch and avicel. Dummy granules devoid of the pure pefloxacin were prepared as in recovery study above and the absorbance reading at 277nm taken and compared with that of the blank 1% acetic acid and that obtained for the recovery study.

Quality control assessment methods for selected marketed generics Assay of Content of pefloxacin in selected marketed brands This was done using the developed and validated method as follows.

Sample preparation

Accurately weighed 75mg of the respective powdered tablets was transferred into a 100ml volumetric flask. 40ml of the 1% aqueous acetic acid was added and vortex mixed for 15mins. This was then made up to the 100ml mark shaken and filtered. 1ml of the filtrate was further diluted to 100ml mark with the same 1% aqueous acetic acid to obtain the sample preparation for the assay of pefloxacin determination

Reference standard preparation

PFM standard (40mg) was accurately weighed and dissolved in 100ml of 1% aqueous acetic acid.1ml of this solution was further diluted to 100ml mark with the same 1% aqueous acetic acid to obtain the reference pefloxacin standard solution. The absorbance of the sample preparation and reference standard solution were taken using the 1% aqueous acetic acid as blank and the content of anhydrous pefloxacin in the marketed brands determined by comparison using the expressions

%w/w assay of anhydrous pefloxacin

$$(P) = \frac{33350\ As\ Wr\%}{465.5\ ArWs}$$

and % stated or labeled anhydrous pefloxacin claimed = $\frac{Pw_{20}\%}{8}$

Where As and Ar are absorbance of sample and reference standard preparation respectively, Wr and Ws are weight of pure pefloxacin reference and powdered tablets taken respectively, W20 is the weight of twenty pooled tablets, the factor 333.5/465.5 is a correction factor between the anhydrous pefloxacin and its mesylate dihydrate derivative, while the factor 8 is a correction factor taken into consideration the labeled claim of 400mg anhydrous pefloxacin per tablet for the selected market brands.

TLC Examination

As part of identification test to ascertain the presence of pefloxacin in the selected marketed brands, TLC examination was done on silica gel HF254 with water: conc. ammonia: n-butanol: acetone 5:10:20:65v/v/v/v as mobile phase over a path length of 15cm as prescribed in the pharmacopoeia 7. PFM standard was used for the reference chromatogram.

Weight uniformity test

This was done on 20 tablets by taking their individual weights and pooled weight. The mean was then calculated and the percentage deviation of each individual weight from the mean calculated

Statistical Methods the student t test at p =0.05 and one way ANOVA were used.

RESULTS

The method exhibited good linear relationship (r = 0.999) over a concentration range of 0.40 –12.0µg/ml. The linear regression equation was y = 0.0859x+ 0.0211.The intra day and inter day precision result (table 1) showed also good precision with RSD less then 7% the upper allowable limit being 15% 12-14. The result of the inter day study showed a no significant difference between day 1 and day 2 at p=0.05 this indicates stability of sample preparations

Table 1: Intra and inter -Day Precision Result

Conc µg/ml	Mean absorbance± standard deviation.		Relative standard deviation RSD	
	Day 1	Day 2	Day 1	Day 2
2.0	0.173±0.0066	0.167±0.0259	3.8 %	15.6 %
4.0	0.340±0.0120	0.333±0.0201	3.6 %	6.2 %
8.0	0.662±0.0040	0.658±0.0200	0.6 %	3.0 %

Note; (n= 3, p=0.05)

The recovery accuracy result of the method in table 2, showed excellent recovery of 99.183 % with RSD of 1.106 %. The assay results were unaffected by the presence of excipients as shown by the excellent recoveries indicating that the method is specific. When applied to determine the content of pefloxacin in seven marketed brands (table 2), the method showed excellent reproducibility of recovery and specificity with all except one brand having stated dose assay between 90% to 110% limit as recommended by pharmacopoiea6. This variation was however insignificant from the ANOVA data in table 5 (p= 0.098 > 0.05).

Table 2: Physicochemical characteristics of the selected generics in Nigerian market

Brand	TLC Exam. (Reference Rf = 0.56)	Av. wt. per tablet± SD	Uniformity of weight	Description	% stated dose assay± SD (n=3)
A	Similar Rf as reference	0.8208g±0.0151	-4.2 to +2.4%	White oblong film coated caplet	110.2 ±9.4
B	Similar Rf as reference	0.7990g±0.0161	-4.3% to +4.5%	White oblong film coated caplet	101.7±0.2
C	Similar Rf as reference	0.7287g±0.0310	-1.4% to +1.8%	White oblong film coated caplet	102.0±0.6
D	Similar Rf as reference	0.7294g±0.0073	-2.2% to +2.0%	TiO_2 coloured oblong film coated caplet	105.7±4.4
E	Similar Rf as reference	0.6488±0.0162	-4.3% to +4.2%	White oblong film coated caplet	101.6±0.3
F	Similar Rf as reference	0.7920±0.0069	-1.6% to +1.3%	White oblong film coated caplet	101.8±0.3
G*	Similar Rf as reference	0.7705±0.0215	-5.2% to +3.8%	White oblong film coated caplet	100.5±0.9

*** Innovator peflacine (R)**

Table 3: ANOVA data for assay of pefloxacin in selected generics

Sources of variations	sum of squares	Df	Mean squares	F-value	Significance (p)	F-critical
Between groups	212.100	6	35.350	2.258	0.098	2.85
Within groups	219.174	14	15.655			
Totals	431.274	20				

DISCUSSION

Irrespective of the manufacturer, every brand of any given generic should contain the amount of drug substance equivalent to its label claim. Monitoring this requires the use of accurate and precise methods of assay. This is to checkmate and control the influx of sub standard generics into the health system. This will ensure maximum safety of health care products. From the assay of content of pefloxacin in the seven selected brands, it could be inferred that although the different manufacturers formulates the different brands by different methods there is no significant variation in the content of active pefloxacin in their dosage forms as shown from the ANOVA data (p= 0.098 > 0.05). This corroborates the excellent recovery and selectivity obtained from the validation result. The apparent variations could be associated to overages. Similar observations associated with various formulation factors that vary from manufacturer to manufacturer have been reported for some marketed fluoroquinolones generics and other formulated products 7-11,15-17.

CONCLUSION

The proposed UV spectrophotometric method is simple, rapid, specific, accurate, precise and highly sensitive. Therefore it could be used for determination of the pefloxacin either in bulk or in their corresponding dosage forms without interference from commonly used excipients and could be easily used in a quality control laboratory for its analysis.

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