

# Development and validation of UV spectrophotometric method for orbifloxacin assay and dissolution studies

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New, simple and cost effective UV-spectrophotometric method was developed for the estimation of orbifloxacin in pharmaceutical formulation. Orbifloxacin was estimated at 290 nm in 0.5 M hydrochloric acid. Linearity range was found to be 1.0-6.0  $\mu\text{g mL}^{-1}$ . The method was tested and validated for various parameters according to main guidelines. The proposed method was successfully applied for the determination of orbifloxacin in tablets. The results demonstrated that the procedure is accurate, precise and reproducible, while being simple, economical and less time consuming. It can be suitably applied for the estimation of orbifloxacin in routine quality control and dissolution studies.

**Uniterms:** Medicines/quality control. Medicines/dissolution. Orbifloxacin/determination. UV spectrophotometry/quantitative analysis/validation.

Um método espectrofotométrico novo, simples e de baixo custo foi desenvolvido para a determinação de orbifloxacino em formulação farmacêutica. O orbifloxacino foi determinado em 290 nm utilizando ácido clorídrico 0,5 M como solvente. O intervalo de linearidade usado foi de 1,0 a 6,0  $\mu\text{g mL}^{-1}$ . O método foi testado e validado em vários parâmetros de acordo com os principais guias. O método proposto foi aplicado com sucesso para a determinação de orbifloxacino em comprimidos. Os resultados demonstraram que este procedimento é exato, preciso e reprodutível, ao mesmo tempo em que é simples, barato e de mais rápida execução e pode ser adequadamente aplicado para a determinação de orbifloxacino na rotina do controle de qualidade e em estudos de dissolução de comprimidos contendo este fármaco.

**Unitermos:** Medicamentos/controle de qualidade. Medicamentos/dissolução. Método analítico. Orbifloxacino/determinação. Espectrofotometria na região do UV/controle de qualidade/validação.

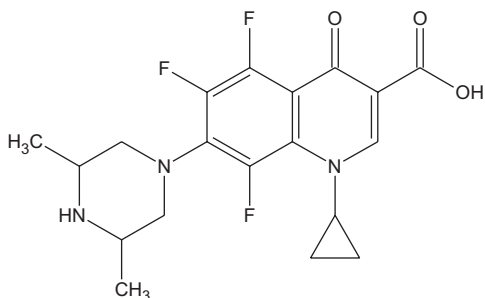
## INTRODUCTION

The fluoroquinolones are a class of compounds that comprise a large and expanding group of synthetic antimicrobial agents. Orbifloxacin (Figure 1, CAS: 113617-63-3), namely 1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, is a new third-generation fluoroquinolone antibacterial drug, developed mainly for the treatment of gastrointestinal and respiratory infections in animals (Nakamura, 1995; Matsumoto *et al.*, 1999a,b; Martinez, McDermott, Walker, 2006). It is also used for the treatment of skin, soft tissue and urinary tract infections

particularly in dogs and cats (Davis, Papich, Weingarten, 2006; Marín *et al.*, 2008). Recent studies have shown that orbifloxacin is effective in the treatment of staphylococcal pyoderma superficial and deep infections (Scott, Peters, Miller Jr, 2006); active against *Pseudomonas aeruginosa* isolated from canine otitis (Mckay *et al.*, 2007), and *Staphylococcus intermedius* responsible for canine skin and ear infections (Ganière, Médaille, Etoré, 2004). Further, research was also being conducted on widening the antibacterial spectrum of orbifloxacin and using it for treatment of infection of other animals, such as horses and rabbits (Davis, Papich, Weingarten, 2006; Marín *et al.*, 2008; Haines *et al.*, 2001).

Fluoroquinolones have become an important group, particularly for the treatment of infections caused by more conventional antibiotic-resistant bacteria (Haines *et al.*, 2001). Antimicrobial resistance in bacteria is a

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**FIGURE 1** - Chemical structure of orbifloxacin.

phenomenon that has been in constant evolution since the introduction of antibiotics. Several factors are known to promote bacterial resistance, including poor compliance with treatment regimen (DiPersio *et al.*, 1998; Grave, Tanem, 1999), prophylactic use of antibiotics (Rantala *et al.*, 2004), and the use of antibiotics as growth promoters (Boerlin *et al.*, 2001; Emborg *et al.*, 2004) emphasizing the importance dosages and treatment appropriate.

For an appropriate dosage of the pharmaceutical form orbifloxacin analytical methods should be developed and validated. To date, methods described in the scientific literature for determination of this analyte in biological and other samples involve liquid chromatography with UV (Matsumoto *et al.*, 1999a,b; Davis, Papich, Weingarten, 2006; Morimura *et al.*, 1995a; Morimura *et al.*, 1995b; Morimura, Nobuhara, Matsukura, 1997; Morimura *et al.*, 1997; Matsumoto *et al.*, 1998a; Matsumoto *et al.*, 1998b; Matsumoto *et al.*, 1999a,b; Hung *et al.*, 2007; British Pharmacopoeia, 2011; Cazedey *et al.*, 2011; United States Pharmacopeia 34, 2011; Yu *et al.*, 2012), fluorimetry (Marín *et al.*, 2008; Hung *et al.*, 2007; García *et al.*, 1999; Kay-Mugford *et al.*, 2002; Schneider, Donoghue, 2002; Schneider, Donoghue, 2003; Schneider *et al.*, 2005; Schneider, Reyes-Herrera, Donoghue, 2007; Marín *et al.*, 2007; Chonan *et al.*, 2008; Abd El-Aty *et al.*, 2009; Elias *et al.*, 2009; Gebru *et al.*, 2009; Cho *et al.*, 2010) and/or detection by mass spectrometry (Matsumoto *et al.*, 1999a,b; Morimura, Nobuhara, Matsukura, 1997a; Morimura *et al.*, 1997; Matsumoto *et al.*, 1998a; Matsumoto *et al.*, 1998b; Matsumoto *et al.*, 1999a,b; Yu *et al.*, 2012; Schneider, Donoghue, 2002; Schneider, Donoghue, 2003; Schneider *et al.*, 2005; Schneider, Reyes-Herrera, Donoghue, 2007; Johnston, Mackay, Croft, 2002; Yamada *et al.*, 2006; Fujita *et al.*, 2008; Kajita, Hatakeyama, 2008; Saito *et al.*, 2008; Li *et al.*, 2009; Santoke *et al.*, 2009; Kantiani, Farré, Barcelo, 2011; Tang *et al.*, 2012).

Other techniques include the determination of orbifloxacin by sequence analysis of samples sensitized with luminescent terbium (Llorent-Martínez *et al.*, 2008)

and by microbiological method (Cazedey, Salgado, 2011).

Also, other ultraviolet analyses have been described for fluoroquinolones, such as sparfloxacin (Marona, Schapoval, 1999), lomefloxacin (Gomes, Salgado, 2005) and gatifloxacin (Salgado, Oliveira, 2005).

The objective of the present study was to develop a simple, precise, accurate and economical analytical method with the better detection range for estimation of orbifloxacin in pharmaceutical formulations and dissolution studies.

## MATERIAL AND METHODS

### Material and reagents

Orbifloxacin reference standard (assigned purity 99.8%) was supplied by Sigma-Aldrich. The pharmaceutical form tablets were commercially obtained and claimed to contain 22.7 mg orbifloxacin (Orbax™ - Schering-Plough). All reagents used were analytical grade.

### Apparatus

A Shimadzu UV-Vis spectrophotometer, model UVmini-1240 was used. UV spectra absorbance of reference and sample solutions were recorded in 10 mm quartz cells at 290 nm. The solutions were prepared in 0.5 M hydrochloric acid.

### Methods

#### Analytical method development

Different media were investigated to develop a suitable UV-spectrophotometric method for the analysis of orbifloxacin in tablets. For selection of media the criteria employed were sensitivity of the method, ease of sample preparation, solubility of the drug, cost of solvents and the applicability of the method to various purposes.

#### Preparation of standard solutions

The orbifloxacin reference standard solution (100.0  $\mu\text{g mL}^{-1}$ ) was prepared by accurately weighing 5.0 mg of orbifloxacin reference in a 50.0 mL volumetric flask. The volume was completed with 0.5 M hydrochloric acid. This flask was sonicated for 30 min. The above solution was diluted in a 10 mL volumetric flask with 0.5 M hydrochloric acid to obtain a final solution containing 3.5  $\mu\text{g mL}^{-1}$  of orbifloxacin.

#### Determination of maximum absorption ( $\lambda_{\text{max}}$ )

From the standard solution (100.0  $\mu\text{g mL}^{-1}$ )

approximately 3.0 mL was taken and scanned from 200 to 400 nm with the UV spectrophotometer. The 0.5 M hydrochloric acid was used as blank. Orbifloxacin presented maximum absorption at 290 nm.

#### *Calibration curve*

The calibration curve was constructed by analyzing six different concentrations of standard solution, prepared on the same day. The range of solutions varied from 1.0 to 6.0  $\mu\text{g mL}^{-1}$ . All determinations were conducted in triplicate.

#### *Assay*

To analyze the concentration of orbifloxacin tablets, 20 tablets were individually weighed and triturated to obtain homogeneous mixture. An amount of powder equivalent to 5.0 mg of drug was transferred to 50.0 mL volumetric flask. The volume was completed with 0.5 M hydrochloric acid. The resulting solution was sonicated for 30 min to ensure proper solubilization. Aliquots of this solution were further diluted with the same solvent, in order to obtain a solution with final concentration of 3.5  $\mu\text{g mL}^{-1}$ . All sample solutions were filtered through a quantitative filter. All determinations were conducted in triplicate.

#### *Chromatographic conditions*

In order to compare the results of the proposed UV spectrophotometric method with a reference method, the same product batches were analyzed by an HPLC technique, which was developed and validated by our research group (Cazedey *et al.*, 2011). The mobile phase consisted of 5% acetic acid: methanol (80:20, v/v). A flow rate of 0.7  $\text{mL min}^{-1}$  was used. Injection volume was set at 20  $\mu\text{L}$ . UV detection of the analyte was carried out at 290 nm.

#### *Dissolution studies*

A dissolution test for evaluation of the dissolution behavior of orbifloxacin tablets was also developed and the profiles were generated. Dissolution samples were analyzed with both HPLC (Cazedey *et al.*, 2011) and the developed UV spectrophotometric method with UV detection at 290 nm. Dissolution studies were carried out in USP dissolution apparatus 2 using 0.05 M phosphate buffer (pH 6.8) as dissolution medium. The dissolution medium was found suitable to ensure sink conditions and chemical stability of orbifloxacin. The rotation speed was kept at 50 rpm and the volume of the dissolution medium was 900 mL. In both methods, the samples were diluted with 0.5 M hydrochloride acid to a final concentration of

2.5  $\mu\text{g mL}^{-1}$ . The results from 60 min, the last time point of the dissolution tests performed, were consistent with the *Assay* results.

### **Analytical Validation**

#### *Selectivity*

One of the several ways to assess selectivity is a comparison between a matrix without analyte and a matrix with the analyte added. In this case, the excipients comprising the matrix should not interfere with the assay result (ICH, 2005). However, if a matrix without the analyte is not available, the selectivity determination can be carried out by comparing the slope of two standard analytical curves. One curve includes the sample matrix, and the other represents a sample without matrix. The selectivity is then assessed by comparing these two linear regression curve slopes. If they are similar, the method is considered selective and the matrix did not cause interference in the method (Bonfilio *et al.*, 2012). Thus, orbifloxacin standard and sample solutions (1.0 to 6.0  $\mu\text{g mL}^{-1}$ ) were prepared in the selected medium and the analytical curves were statistically compared. Additionally, the Student's *t*-test and *F*-test were performed to compare the orbifloxacin standard and sample absorption values.

#### *Linearity*

To establish linearity of the proposed method, six series of orbifloxacin standard solution (1.0–6.0  $\mu\text{g mL}^{-1}$ ) were prepared from the stock solutions and analysed on three consecutive days. Least square regression analyses were done for the obtained data. ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions.

#### *Precision*

Precision was determined by using data from the intraday and interday repeatability studies. Same level of drug concentration (3.5  $\mu\text{g mL}^{-1}$ ), prepared from independent stock solution was used for both the studies. The solutions of orbifloxacin in six replicates were prepared at two different times in a day and studied for intraday variation. Same protocol was followed for three different days to study interday variation ( $n = 18$ ). The method precision was evaluated by calculating relative standard deviation (RSD%).

#### *Accuracy*

To determine the accuracy of the proposed method, standard addition method was performed. In this study,

different concentrations of pure drug (0.8, 1.5 and 2.2  $\mu\text{g mL}^{-1}$ ) were added to a known quantity of sample and the total concentration was determined using the proposed methods ( $n = 9$ ). The percent recovery (%R) of the added pure drug was calculated as shown in equation 1 (AOAC, 2002):

$$\%R = \left[ \left( \frac{C_t - C_u}{C_a} \right) \right] \times 100 \quad (1)$$

where  $C_t$  is the total drug concentration measured after standard addition;  $C_u$  represents the drug concentration in the formulation; and  $C_a$ , the drug concentration added to formulation.

#### Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of orbifloxacin by the proposed methods were determined using analytical curves. LOD and LOQ were calculated as shown in equations 2 and 3 respectively (ICH, 2005):

$$LOD = \frac{3.3\sigma}{S} \quad (2)$$

$$LOQ = \frac{10\sigma}{S} \quad (3)$$

where  $\sigma$  is the standard deviation of y-intercept of regression equation and S is the slope of the calibration curve.

#### Robustness

Robustness of the proposed method was determined by changing wavelength from 290 nm to 289 and 291 nm. The same concentration (3.5  $\mu\text{g mL}^{-1}$ ) was prepared in six replicates for each wavelength. The Student's *t* tests were performed and *t*-values were determined.

## RESULTS AND DISCUSSION

Different media were investigated to develop a suitable UV spectrophotometric method for the analysis of orbifloxacin in formulation. The solvents tested are the following: water, ethanol, ethanol:water (50:50, v/v), methanol, 0.1 M hydrochloric acid (HCl), 0.5 M hydrochloric acid and 1 M sodium hydroxide aqueous (NaOH). All the solutions were sonicated for 30 minutes (Table I). These solvents were tested with respect to the extraction efficiency. The best solvent to extract orbifloxacin was 0.5 M hydrochloric acid with extraction efficiency of 102%.

**TABLE I** - Extraction rate of orbifloxacin from the pharmaceutical dosage form

Solvent	% extracted orbifloxacin*
Water	≈ 78
Ethanol	≈ 68
Ethanol: water (50:50, v/v)	≈ 87
Methanol	≈ 89
0.1 M HCl	≈ 90
0.5 M HCl	≈ 102
0.1 M NaOH	≈ 12

\*All solutions were sonicated for 30 minutes

For media optimization various solvents like water, methanol, 0.1 M sodium hydroxide, 5% acetic acid (HOAc) and 0.5 M hydrochloric acid were investigated. Orbifloxacin showed pH dependent UV absorption spectra (Table II). This implies that the pH of the analytical medium is very important for estimation of orbifloxacin. Orbifloxacin UV absorption spectra in alkali medium showed various other bands that could be due to degradation of the drug. The final decision of using 0.5 M hydrochloric acid as a media was based on criteria like residue generation, sensitivity of the method, cost, ease of sample preparation, solubility, and applicability of the method.

**TABLE II** - Wavelength and absorption of orbifloxacin (10.0  $\mu\text{g mL}^{-1}$ ) in different solvents

Solvent	Wavelength ( $\lambda_{\text{max}}$ ) (nm)	Absorption
Water	290	0.9934
Methanol	291	1.0313
0.1 M NaOH	288	1.4901
5% HOAc	290	1.1724
0.5 M HCl	290	1.1888

The  $\lambda_{\text{max}}$  of orbifloxacin in 0.5 M hydrochloric acid was found to be 290 nm. Apparent molar absorptivity ( $\epsilon$ ) of drug, determined by using Beer's law, was found to be  $4.33 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$  (Table III), this value was calculated according to the standard formulae (Equation 4):

$$\epsilon = \frac{A}{c \times b} \quad (4)$$

where A is the absorbance; c, molar concentration (mol/L) and; b, the cell optical length (cm).

In 0.5 M hydrochloride acid, the linearity range

**TABLE III** - Optical characteristics, statistical data of the regression equations and validation parameters for orbifloxacin

Parameter		
<b>Optical characteristics</b>		
Apparent molar absorptivity ( $L \text{ mol}^{-1} \text{ cm}^{-1}$ )		$4.33 \times 10^4$
<b>Regression analysis</b>		
Slope (S.E. <sup>a</sup> )		0.1107 (0.0006)
Intercept (S.E. <sup>a</sup> )		-0.0028 (0.0008)
Regression coefficient ( <i>r</i> )		0.9999
Calculated <i>F</i> -value (critical <i>F</i> -value) <sup>b</sup>		2215.15 (4.75)
<b>Validation Parameters</b>		
Selectivity	$F_{\text{cal}} (F_{\text{crit}})^c$	0.86 (5.05)
	$t_{\text{cal}} (t_{\text{crit}})^c$	1.74 (1.81)
Linearity ( $\mu\text{g mL}^{-1}$ )		1.0-6.0
LOD ( $\mu\text{g mL}^{-1}$ )		0.04
LOQ ( $\mu\text{g mL}^{-1}$ )		0.12
Accuracy - recovery (RSD%)		99.77 (1.78)
Precision (RSD%) <sup>d</sup>	Intraday	1.23
	Interday	1.31
Robustness - $t_{\text{cal}} (t_{\text{crit}})^c$	290-289 nm	1.61 (2.18)
	290-291 nm	0.36 (2.18)

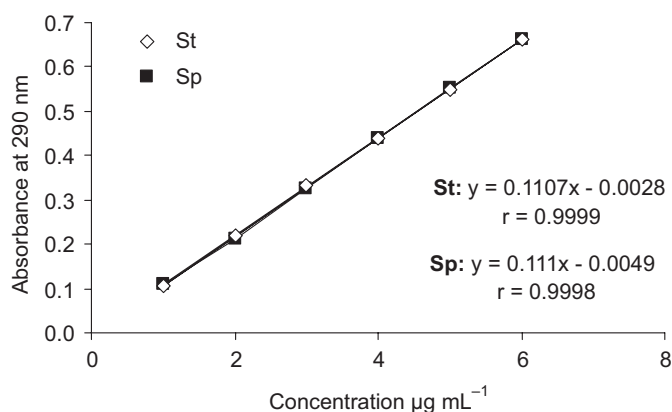
<sup>a</sup>Standard error of mean. <sup>b</sup>Theoretical value of  $F(4,45)$  based on one-way ANOVA test at  $P = 0.05$  level of significance. <sup>c</sup> $t_{\text{cal}}$  and  $F_{\text{cal}}$  are calculated values and  $t_{\text{crit}}$  and  $F_{\text{crit}}$  are theoretical values ( $n = 6$ ) based on paired Student's *t*-test and *F*-test, respectively, at  $P=0.05$  level of significance. <sup>d</sup>Relative standard deviation

was found to be 1.0 to 6.0  $\mu\text{g mL}^{-1}$ . The linear regression equation obtained was: absorbance at 290 nm =  $[0.1107 \times \text{concentration in } \mu\text{g mL}^{-1}] - 0.0028$ ; with a regression coefficient of 0.9999 (Table III).

Absorption spectrum of pure drug sample was matching with the marketed formulation sample in the selected medium. The calculated Student's *t*-values and *F*-values were found to be less than that of the critical values, indicating that statistically there was no significant difference between mean absorbance of solutions prepared from pure drug sample and marketed formulation sample (Table III). Moreover, the analytical curves of orbifloxacin standard and sample solutions were compared and presented no significant statistical difference (Figure 2). Therefore, proposed method is considered selective for the orbifloxacin.

Accuracy ranged from 98.04% to 101.58%. The excellent mean % recovery values (nearly 100%) and its low relative standard deviation values ( $\text{RSD}\% < 2.0$ ) represent accuracy (Table III) of the UV spectrophotometric method.

Precision was determined by studying repeatability and intermediate precision. Repeatability results indicate



**FIGURE 2** - Statistical comparison of orbifloxacin standard (St) and sample (Sp) analytical curves in the range of 1.0 to 6.0  $\mu\text{g mL}^{-1}$ .

the precision under the same operating conditions over a short interval of time and inter-assay precision (intraday). Intermediate precision expresses the results in different days (interday). The precision RSD% values were not more than 1.5% in all the cases (Table III). RSD values were within the acceptable range indicating that this method has excellent repeatability and intermediate precision.

**TABLE IV** - Comparison of the proposed UV spectrophotometric method and an HPLC method for the assay and dissolution test of orbifloxacin in tablets

Method	Mean % drug related to label claim	RSD%	Mean % drug dissolved	RSD% <sup>b</sup>
UV <sup>a</sup>	100.74	0.66	96.84	3.14
HPLC <sup>24</sup>	100.34	1.31	94.25	2.04

<sup>a</sup>UV: UV spectrophotometric method. <sup>b</sup>in 60 minutes (n = 6 vessels)

LOD and LOQ, calculated according to the ICH (2005) guidelines, were found to be 0.04 µg mL<sup>-1</sup> and 0.12 µg mL<sup>-1</sup>, respectively.

Variation of wavelengths by ±1 nm did not have any significant effect on absorbance (Table III). The Student's *t*-test values in both cases-variation were not statistically significant ( $t_{\text{cal}} < t_{\text{crit}}$ ), indicating that the developed method was robust.

The proposed analytical method was applied for determination of orbifloxacin in tablets. The assay values of orbifloxacin in tablets ranged from 99.99% to 101.25% with standard deviation not more than 0.66%. The estimated drug content with low values of standard deviation established the precision of the proposed methods. Furthermore, the results, expressed as percentage of drug related to label claim, are shown in Table IV. Moreover, the same product batches were analyzed by an HPLC method (Cazedey *et al.*, 2011) and a Student's *t*-test was applied. The Student's *t*-values did not exceed the tabulated value (for five degrees of freedom) indicating no significant difference between the methods. In addition, the two developed methods were used to evaluate the dissolution behavior of orbifloxacin tablets. In both methods, the drug could be assayed and the methods were compared. Again, no statistical difference was found to the HPLC and UV spectrophotometric methods (Table IV).

## CONCLUSION

An UV spectrophotometric method was developed, validated and applied for the determination of orbifloxacin in pharmaceutical dosage form and dissolution tests. The developed method was validated as per ICH guidelines and was found to be accurate, precise, linear, reproducible, robust and selective. No interference from any components of pharmaceutical dosage form was observed. Furthermore, the method did not present any statistically significant difference as compared to the HPLC method.

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