

## Rapid titrimetric and spectrophotometric determination of ofloxacin in pharmaceuticals using *N*-bromosuccinimide

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One titrimetric and two spectrophotometric methods have been described for the determination of ofloxacin (OFX) in bulk drug and in tablets, employing *N*-Bromosuccinimide as an analytical reagent. The proposed methods involve the addition of a known excess of NBS to OFX in acid medium, followed by determination of unreacted NBS. In titrimetry, the unreacted NBS is determined iodometrically, and in spectrophotometry, unreacted NBS is determined by reacting with a fixed amount of either indigo carmine (Method A) or metanil yellow (Method B). In all the methods, the amount of NBS reacted corresponds to the amount of OFX. Titrimetry allows the determination of 1-8 mg of OFX and the calculations are based on a 1:5 (OFX:NBS) reaction stoichiometry. In spectrophotometry, Beer's law is obeyed in the concentration ranges 0.5-5.0  $\mu\text{g/mL}$  for method A and 0.3-3.0  $\mu\text{g/mL}$  for method B. The molar absorptivities are calculated to be  $5.53 \times 10^4$  and  $9.24 \times 10^4$  L/mol/cm for method A and method B, respectively. The methods developed were applied to the assay of OFX in tablets, and results compared statistically with those of a reference method. The accuracy and reliability of the methods were further ascertained by performing recovery tests via the standard-addition method.

**Uniterms:** Ofloxacin/determination. Titrimetry/quantitative analysis. Spectrophotometry/quantitative analysis. *N*-Bromosuccinimide. Tablets/quantitative analysis.

Descrevem-se métodos, um titulométrico e dois espectrofotométricos, para a determinação de ofloxacino (OFX) na matéria-prima e em comprimidos, empregando a *N*-bromossuccinimida (NBS) como reagente analítico. Os métodos propostos envolvem a adição de excesso conhecido de NBS ao OFX, em meio ácido, seguida de determinação do NBS que não reagiu. Na titulometria, o NBS que não reagiu é determinado iodometricamente e na espectrofotometria, o NBS que não reagiu é determinado pela reação com quantidade fixa de indigo carmim (Método A) ou amarelo de metanila (Método B). Em todos os métodos, a quantidade de NBS que reagiu corresponde à quantidade de OFX. A titulometria permite a determinação de 1-8 mg de OFX e os cálculos se baseiam na estequiometria de reação de 1:5 (OFX:NBS). Na espectrofotometria, a Lei de Beer é obedecida nas faixas de concentração de 0,5-5,0  $\mu\text{g/mL}$ , para o método A, e de 0,3-3,0  $\mu\text{g/mL}$ , para o método B, respectivamente. Os métodos desenvolvidos foram aplicados para o teste de OFX em comprimidos e os resultados foram comparados estatisticamente com aqueles do método de referência. A precisão e a confiabilidade dos métodos foram, posteriormente, verificadas por meio dos testes de recuperação *via* método de adição de padrão.

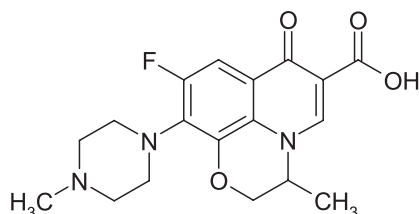
**Unitermos:** Ofloxacino/determinação. Titulometria/análise quantitativa. Espectrofotometria/análise quantitativa. *N*-Bromossuccinimida. Comprimidos/análise quantitativa.

### INTRODUCTION

Ofloxacin (OFX), (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazine-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0<sup>5,13</sup>]

trideca-5(13),6,8,11-tetraene-11-carboxylic acid (Figure 1), is a fluoroquinolone antibiotic considered to be a second-generation fluoroquinolone. It is active against both Gram-positive and Gram-negative bacteria and functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, (Drlica, Zhao, 1997) which is an enzyme necessary to separate replicated DNA, thereby inhibiting

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

cell division. This drug is official in the United States Pharmacopoeia (USP, 2004) and the British Pharmacopoeia (BP, 2003), which recommend non-aqueous titrimetry (USP, 2004; BP, 2003) and HPLC (USP, 2004) techniques for its assay in bulk and dosage forms.

A review of the literature revealed that various analytical methods for the determination of OFX in bulk and pharmaceutical dosage forms have been reported. These include spectrofluorometry (Salem, 2005), atomic absorption spectrometry (Salem, 2005), capillary electrophoresis (Abdalla *et al.*, 2008; Sun, Wu, 1999), HPLC (Arjekar *et al.*, 1996; Carlucci *et al.*, 1993; Kalta, Sharma, Chaturvedi, 2008; Sinde, Desai, Tendolkar, 1998), HPTLC (Srividya, Cardoza, Amin, 2003), chemiluminescence (Francis, Paul, Adcock, 2005), as well as electroanalytical (Tamer, 1990; Tuncel, Atkosar, 1992; Zhou, Pan, 1995) and microbiological methods (Silveria, Schapoval, 2002). Some of these methods have sufficient sensitivity to determine lower concentrations of the drug. However, these methods involve several manipulation steps which are not simple for routine analysis of pharmaceutical formulations and require sophisticated instruments.

Titrimetry and visible spectrophotometry may serve as useful alternatives to many of the aforesaid sophisticated techniques because of their cost-effectiveness, ease of operation, sensitivity, remarkable accuracy, precision and wide applicability. The literature search revealed that no titrimetric methods have yet been reported for the determination of OFX.

Visible spectrophotometric methods based on diverse reaction chemistry have been proposed for the assay of OFX in pharmaceuticals. The method based on the formation of pink colored chromogen by the reaction between OFX and citric acid-acetic anhydride (Patel *et al.*, 2007) has been reported. Extractive spectrophotometric procedures based on the formation of yellow colored ion pair complex (Issa, 1997) with bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP); and red colored ion-pair complex (Sastry, Rama, Siva Prasad, 1995) with tropaeolin and supracene violet 3B, have been used for the assay of OFX. Other visible spec-

trophotometric methods based on reactions such as complex formation reaction with Fe (III) nitrate nonahydrate (Eboka, Aigbavboa, Akerele, 1997), yellow complex with iron (III) chloride in HCl medium (Mathur *et al.*, 1992); and oxidative coupling reaction with Ce(IV)-MBTH (Sastry, Rama, Siva Prasad, 1995), were reported for the assay of OFX. Most of the visible spectrophotometric methods above have one or more inherent disadvantages such as use of heating step, use of organic solvents, close pH control, poor sensitivity and use of expensive reagents, as indicated in Table I.

The aim of the present investigation was to develop simple, sensitive and cost-effective methods for the determination of OFX in pure and dosage forms using titrimetric and spectrophotometric techniques. The methods utilized NBS, indigo carmine and metanil yellow as reagents. The methods developed offer the advantages of simplicity, sensitivity, speed, accuracy and precision without the need for costly equipment/chemicals.

## MATERIAL AND METHODS

### Apparatus

All absorbance measurements were taken on a Systronics model 106 digital single beam spectrophotometer (Ahmedabad, India) equipped with 1-cm matched quartz cells.

### Material and reagents

All chemicals and reagents used were of analytical or pharmaceutical grade and distilled water was used to prepare the solutions.

#### • Standard OFX Solution

Pharmaceutical grade OFX, certified to be 99.98% pure, was received as a gift from Cipla India Ltd, Mumbai, India, and used as received. Standard OFX solutions (1 mg/mL, 10 and 6 µg/mL) were prepared by dissolving a calculated quantity of pure drug in 0.1 M HCl.

Two brands of tablets containing OFX, Zenflox-400 (Mankind Pharma Pvt Ltd., New Delhi, India) and Ofloxin-400 (J. B. Chemicals and Pharmaceuticals Ltd, Mumbai, India), used in the investigation were purchased from local commercial sources.

*N-Bromosuccinimide (NBS)*: an approximately 0.02 M NBS solution was prepared, standardized (Berka, Vulterin, Zyoka, 1965) and used in titrimetry. The NBS solution (0.02 M) was further diluted to a concentration of 150 and 100 µg/mL for spectrophotometric method A and method B, respectively.

**TABLE I** - Comparison of performance characteristics of present methods with published methods

Sl. No.	Reagent/s used	Methodology	$\lambda_{\max}$ (nm)	Linear range ( $\mu\text{g/mL}$ ) ( $\epsilon = \text{L/mol/cm}$ )	Remarks	Ref
1	Citric acid-acetic anhydride	Pink colored chromogen measured	552	5-55 ( $\epsilon = 6.04 \times 10^3$ )	Boiling for 20 min required.	Patel <i>et al.</i> , 2007
2	a) Bromophenol blue	Yellow colored 1:1 ion-pair complex extractable in chloroform was measured	410	5-25 ( $\epsilon = 1.04 \times 10^4$ )	Required close pH control and involved extraction steps organic solvent is used	Issa, 1997
	b) Bromothymol blue		415	2-15 ( $\epsilon = 2.01 \times 10^4$ )		
	c) Bromocresol purple		410	2-20 ( $\epsilon = 1.04 \times 10^4$ )		
3	a) Tropaeolin 000 (TP 000)	Red colored ion-pair complex extractable in chloroform was measured	485	2.5-30 ( $\epsilon = 8.24 \times 10^3$ )	Used of organic solvent, required close pH control and involved extraction steps	Sastry, Rama, Siva prasad, 1995
	b) Supracene Violet 3B (SV 3B)	Chloroform extractable ion-pair complex measured	575	2.5-25 ( $\epsilon = 1.09 \times 10^4$ )		
4	Iron(III) nitrate	Amber colored complex was measured	370	0-62.5 ( $A_{1\%} = 207$ )	Buffers used.	Eboka, Aigbavboa, Akerele, 1997
5	Iron(III) chloride/HCl	Yellow complex measured	410	20-160	Less sensitive, significant blank absorbance.	Mathur <i>et al.</i> , 1992
6	Ce(IV)-MBTH	Oxidative coupling reaction product measured	640	1-10	Uses expensive reagent, less stable species measured.	Sastry, Rao, Prasad, 1995
7	NBS	Increase in absorbance of color measured	610	0.5-5.0 ( $\epsilon = 5.53 \times 10^4$ )	Highly sensitive, no heating or extraction step, inexpensive instrumental setup, use of eco-friendly chemicals, and free from use of organic solvents	Present work
	a) indigo carmine					
	b) metanil yellow	530	0.3-3.0 ( $\epsilon = 9.24 \times 10^4$ )			

- *Sodium thiosulphate (0.04 M)*

This was prepared by dissolving 9.93g of chemical (S. d. Fine Chem. Ltd., Mumbai, India) in one liter of water for use in titrimetry.

- *HCl (1 M, 2 M and 5 M)*

Concentrated HCl (S. D. Fine Chem, Mumbai, India, sp. gr. 1.18) were appropriately diluted with water to attain the required concentrations.

- *Potassium iodide (10%)*

This was prepared by dissolving 10 g of the chemical

(Merck, Mumbai, India) in 100 mL of water and used in titrimetry.

- *Starch indicator (1%)*

One g of the reagent (Merck, Mumbai, India) was made into a paste and poured into 100 mL of boiling water, boiled for 1 min, cooled and used for titrimetry.

- *Indigo carmine (200  $\mu\text{g/mL}$ ) and metanil yellow (50  $\mu\text{g/mL}$ )*

The dye solutions were prepared (S.D. Fine Chem, Mumbai, India, dye content 90%) in water and filtered.

## General procedure

### • Titrimetry

Different volumes (1-8 mL) of standard solution containing 1 mg/mL OFX were accurately measured and transferred into a 100 mL titration flask and the volume was made up to 10 mL with 0.1 M HCl. Two mL of 2 M HCl and 10 mL of NBS (0.02 M) were pipetted into the flask, the content mixed and set aside for 10 min. Subsequently, 5 mL of 10% potassium iodide solution was added, and the liberated iodine was titrated against sodium thiosulphate (0.04 M) using starch indicator. A blank titration was performed under identical conditions.

The amount of OFX in the aliquot was computed from the following formula:

$$\text{Amount (mg)} = V M_w S/n \quad (1)$$

where V = mL of NBS reacted;  $M_w$  = relative molecular mass of drug; S = concentration of NBS, moles/L; n = number of moles of NBS reacting with each mole of OFX.

## Spectrophotometry

### • Method A

Different aliquots, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mL of standard OFX solution (10 µg/mL) were transferred into a series of 10 mL standard volumetric flasks and the total volume in each flask was adjusted to 5 mL with 0.1 M HCl. To each flask, 1 mL of 1M HCl followed by 1 mL of 150 µg/mL NBS were added. The content of each flask was mixed well and set aside for 15 minutes with occasional shaking. Finally, 1 mL of 200 µg/mL indigo carmine was added and the volume was made up to the mark with distilled water after 5 min. The absorbance was measured at 610 nm *Vs* reagent blank prepared in a similar manner.

### • Method B

Different aliquots (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0) mL of standard 6 µg/mL OFX solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a microburette and total volume adjusted to 5 mL with 0.1 M HCl. To each flask, 1 mL of 5 M HCl followed by 1 mL of 100 µg/mL NBS, was added using a microburette. The content of each flask was mixed well and set aside for 10 minutes. Lastly, 1 mL of 50 µg/mL metanil yellow was added to each flask and mixed well. The volume was made up to the mark with distilled water after 5 min and the absorbance was measured at 530 nm *Vs* reagent blank prepared in a similar manner.

Calibration graphs were prepared by plotting the

increasing absorbance values *versus* concentrations of OFX. The concentration of the unknown was read from the respective calibration graph or deduced from the regression equation derived using the Beer's law data.

### *Procedure for the assay of ofloxacin in pharmaceutical preparations*

Twenty tablets were weighed accurately and ground into a fine powder. A quantity of the powder containing 100 mg of OFX was accurately weighed into a 100 mL calibrated flask and 60 mL of 0.1 M HCl was added. The content was shaken for about 20 min; volume diluted to the mark with 0.1 M HCl and mixed, and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded, and a convenient aliquot was taken and the assay completed according to the titrimetric procedure described earlier. The tablet extract containing OFX at a concentration of 1 mg/mL was then diluted stepwise with 0.1 M HCl to obtain working concentrations of 10 µg/mL and 6 µg/mL in OFX for spectrophotometric methods A and B, respectively. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above.

### *Placebo blank analysis*

Based on the approximate composition of excipients normally added in the tablet, a placebo blank of the composition: talc (5 mg), starch (5 mg), acacia (5 mg), methyl cellulose (10 mg), sodium citrate (5 mg), magnesium stearate (5 mg) and sodium alginate (5 mg) was made and its solution prepared as described under 'Procedure for the assay of OFX in pharmaceutical preparations', and then subjected to analysis using the procedures described above.

### *Procedure for the determination of OFX in synthetic mixture*

A volume of 100 mg of OFX was added to the placebo blank of the composition described above, which was then homogenized, transferred to a 100 mL standard flask and solution prepared as described under tablets. The solution was mixed well and filtered using a Whatman No. 42 filter paper. The resulting solution was assayed (n=5) by titrimetry according to the procedure described above. The synthetic mixture solution (1 mg/mL in OFX) was then diluted stepwise with 0.1 M HCl to obtain working concentrations of 10 µg/mL and 6 µg/mL in OFX for spectrophotometric method A and method B, respectively. A convenient aliquot was then subjected to analysis. The analysis was used to study the interferences of excipients such as talc, starch, acacia, methyl cellulose, sodium citrate, magnesium stearate and sodium alginate.

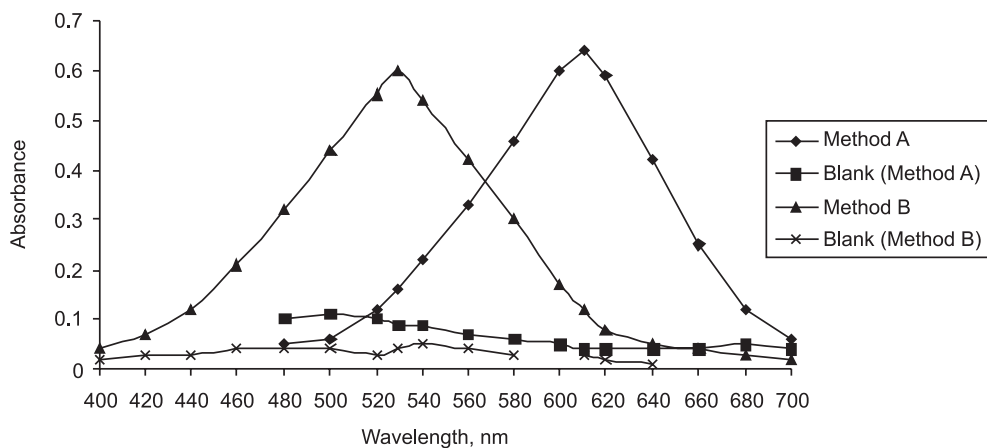
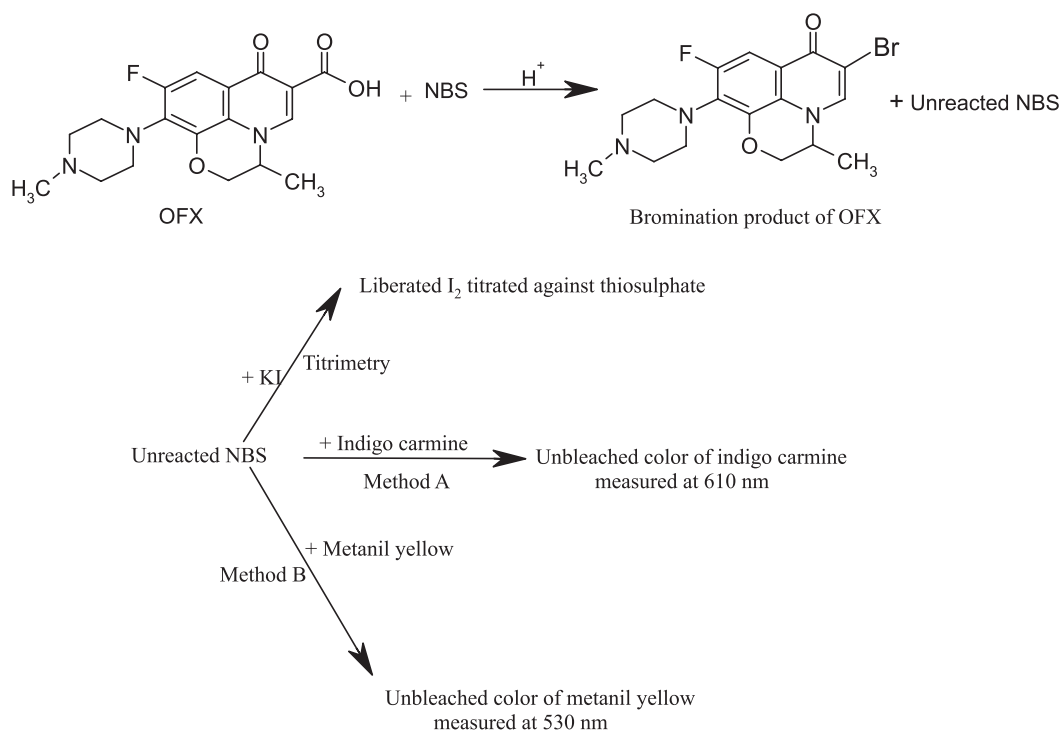


FIGURE 2 - Absorption spectra (4.0 µg/mL OFX in method A and 2.4 µg/mL in method B).

## RESULTS AND DISCUSSION

A close examination of the literature search presented in the introduction reveals that NBS has not yet been used for the spectrophotometric determination of OFX. NBS is a strong oxidizing agent and perhaps the most important positive bromine containing organic compound; it is used for the specific purpose of brominating alkenes at the allylic position (Morrison, Boyd, 2007). The present work involves the bromination of OFX by NBS followed by determination of surplus

NBS after allowing the bromination reaction to complete. In titrimetry, the unreacted NBS is determined iodometrically, while in spectrophotometric methods, it is determined by reacting with a fixed amount of either indigo carmine and measured at 610 nm (Figure 2) or metanil yellow and measured at 530 nm (Figure 2). The spectrophotometric methods make use of the bleaching action of NBS on either of the two dyes, where the discoloration is caused by oxidative destruction of the dye. The tentative reaction scheme of titrimetric and spectrophotometric methods is shown in



SCHEME I - Tentative reaction scheme for titrimetry, spectrophotometric method A and method B.



Scheme 1. The bromination of OFX will take place in position  $\alpha$  to the carbonyl group (Hassan *et al.*, 2007).

### Titrimetry

Direct titration of OFX with NBS in acid medium was not successful. However, a back titrimetric assay was found to be possible when the reactants were allowed to stand for some time in acid medium. The reaction stoichiometry was found to be 1:5 (OFX: NBS). Reproducible and stoichiometric results were obtained when HCl medium was employed. A two mL volume of 2M HCl in a total volume of 22 mL (0.18 M) was found optimal; although the stoichiometry reaction was unaffected in the concentration range of 0.09-0.27 M HCl. The bromination reaction was found to be complete and quantitative in 10 min and contact time up to 20 min had no effect on the stoichiometry or the results. Hence, the reaction time of 10 min was established. A 10 mL aliquot of 0.02 M NBS solution was found adequate for quantitative bromination of OFX in the range determined of 1-8 mg.

### Spectrophotometric methods

The ability of NBS to brominate OFX and bleach the colors of indigo carmine and metanil yellow dyes has been used for the indirect spectrophotometric assay of the drug. Preliminary experiments were performed to determine the maximum concentration of indigo carmine or metanil yellow in the acid medium employed where this was found to be 20  $\mu\text{g/mL}$  and 5  $\mu\text{g/mL}$  for indigo carmine and metanil yellow, respectively. An NBS concentration of 15  $\mu\text{g/mL}$  was found optimum to bleach the blue color produced by 20  $\mu\text{g/mL}$  indigo carmine, whereas in the case of metanil yellow, 10  $\mu\text{g/mL}$  NBS was sufficient to destroy the pink color of 5  $\mu\text{g/mL}$  metanil yellow. Hence, different amounts of OFX reacted with 15  $\mu\text{g/mL}$  NBS in method A, and 10  $\mu\text{g/mL}$  NBS in method B.

Hydrochloric acid was found to be the ideal medium for the bromination of OFX by NBS as well as the latter's determination employing either dye. In both the methods, the reaction between OFX and NBS was unaffected when 0.5-3.0 mL each of 1 M HCl (method A) and 5M HCl (method B) in a total volume of 10 mL was used. Hence, 1 mL each of 1 M HCl in method A and 5M HCl in method B was used for both the steps. For a quantitative reaction between OFX and NBS, contact times of 15 min and 10 min were found necessary in method A and method B, respectively, and constant absorbance readings were obtained when contact times were extended up to 45 min in method A, and 30 min in

method B. A standing time of 5 min was necessary in both the methods for the bleaching of the dye color by the residual NBS. The measured color was found to be stable for several hours in the presence of the reaction products in both methods.

### Method validation procedures

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

#### Linearity and Sensitivity

Over the range investigated (1-8 mg), fixed stoichiometry of 1:5 (OFX: NBS) was obtained in titrimetry, which served as the basis for calculations. In spectrophotometry, under optimum conditions a linear relation was obtained between absorbance and concentration of OFX in the range of 0.5-5.0  $\mu\text{g/mL}$  (method A) and 0.3-3.0  $\mu\text{g/mL}$  (method B) (Figure 3). The calibration graph is described by the equation:

$$Y = a + bX \quad (2)$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in  $\mu\text{g/mL}$ ) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table II. Sensitivity parameters such as apparent molar absorptivity and

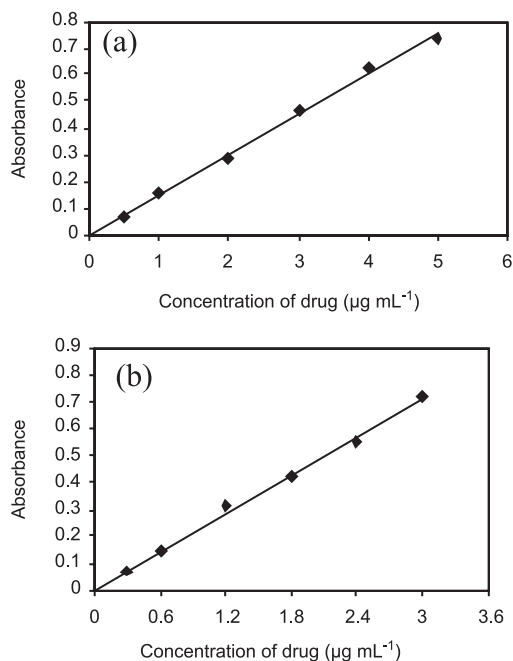


FIGURE 3 - Calibration curves for (a) method A and (b) method B).

Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines (ICH guidelines, 2005) and compiled in Table II. The results attest to the sensitivity of the proposed method. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$\text{LOD}=3.3\sigma/s \text{ and } \text{LOQ}=10\sigma/s \quad (3)$$

where  $\sigma$  is the standard deviation of five reagent blank determinations, and  $s$  is the slope of the calibration curve.

#### Precision and accuracy

In order to evaluate the precision of the proposed methods, solutions containing three different concen-

trations of the OFX were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table III. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% R.E) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\%R.E = \left[ \frac{\text{found} - \text{taken}}{\text{taken}} \right] \times 100 \quad (4)$$

The assay procedure was repeated seven times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision), and over five diffe-

**TABLE II** - Sensitivity and Regression Parameters

Parameter	Method A	Method B
$\lambda_{\text{max}}$ , nm	610	530
Linear range, $\mu\text{g/mL}$	0.5-5.0	0.3-3.0
Molar absorptivity( $\epsilon$ ), L/mol/cm	$5.534 \times 10^4$	$9.242 \times 10^4$
Sandell sensitivity*, $\mu\text{g/cm}^2$	0.007	0.004
Limit of detection (LOD), $\mu\text{g/mL}$	0.03	0.03
Limit of quantification (LOQ), $\mu\text{g/mL}$	0.09	0.08
Regression equation, Y**		
Intercept (a)	0.003	0.003
Slope (b)	0.152	0.237
Standard deviation of a ( $S_a$ )	0.0104	0.0774
Standard deviation of b ( $S_b$ )	0.004	0.046
Regression coefficient (r)	0.998	0.999

\*Limit of determination as the weight in  $\mu\text{g}$  per ml of solution, which corresponds to an absorbance of  $A = 0.001$  measured in a cuvette of cross-sectional area  $1 \text{ cm}^2$  and  $l = 1 \text{ cm}$ . \*\* $Y=a+bX$ , Where Y is the absorbance, X is concentration in  $\mu\text{g/mL}$ , a is intercept, b is slope.

**TABLE III** - Evaluation of intra-day and inter-day accuracy and precision

Method <sup>a</sup>	OFX taken	Intra-day accuracy and precision			Inter-day accuracy and precision			
		OFX found	%RE	%RSD	OFX found	%RE	%RSD	
Titrimetry	2.0	2.03	1.50	1.06	2.05	2.5	1.32	
	4.0	4.05	1.25	1.01	4.11	2.75	0.97	
	6.0	5.94	1.00	0.95	5.85	2.50	1.18	
Spectrophotometry	Method A	2.0	1.96	2.00	0.92	1.94	3.00	1.15
		3.0	3.03	1.00	1.14	3.10	3.33	1.28
		4.0	4.12	3.00	1.02	3.89	2.75	1.11
	Method B	1.2	1.23	2.50	1.14	1.24	3.33	1.35
		1.8	1.76	2.22	1.19	1.75	2.78	1.23
		2.4	2.34	2.50	1.21	2.32	3.33	1.29

RE. Relative error and RSD. Relative standard deviation. <sup>a</sup>In titrimetry, OFX taken/found are in mg, whereas for spectrophotometry values are  $\mu\text{g/mL}$ .

rent days to evaluate intermediate precision (inter-day precision).

### Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution, prepared as described earlier, was subjected to analysis by titrimetry and spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients present in the placebo mixture.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution prepared above yielded percent recoveries which ranged from 97.72-102.9 with standard deviation of 0.95-1.23 in all the cases. The results of this study shown in Table IV indicate that the inactive ingredients present in the syn-

thetic mixture did not interfere in the assay. These results further demonstrate the accuracy, as well as the precision, of the proposed methods.

### Application to formulations

In order to evaluate the analytical applicability of the proposed methods to the quantification of OFX in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method (BP, 2003) by applying Student's *t*-test for accuracy and the *F*-test for precision. The reference method involved the titration of OFX in anhydrous acetic acid with acetous perchloric acid to potentiometric end point detection. The results (see Table V) show that the Student's *t*- and *F*-values at a 95 % confidence level are lower than the tabulated values, thereby confirming good agreement between the results obtained by the proposed methods and the reference method, with respect to accuracy and precision.

**TABLE IV** - Recovery of drug from synthetic mixture

Method	OFX in synthetic mixture taken <sup>a</sup>	OFX recovered <sup>b</sup> (Percent ±SD)
Titrimetry	2.0	102.4 ± 1.23
	4.0	101.7 ± 1.21
	6.0	102.9 ± 1.18
Spectrophotometry Method A	2.0	97.84 ± 1.14
	3.0	98.18 ± 1.09
	4.0	97.72 ± 0.95
Method B	1.2	99.68 ± 1.12
	1.8	100.9 ± 1.07
	2.4	99.17 ± 1.18

<sup>a</sup>mg in titrimetry and µg/mL in spectrophotometry. <sup>b</sup>Mean value of five determinations

### Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet powder was spiked with pure OFX at three concentration levels (50, 100 and 150% of that in tablet powder) and totals then determined by the proposed methods. In all cases, the added OFX recovery percentage values ranged from 98.50-102.5% with a standard deviation of 1.03-1.24 (Table VI), indicating good recovery and absence of interference from the co-formulated substance in the determination.

## CONCLUSIONS

The proposed titrimetric method is a first report on the application of titrimetry for the assay of OFX. The

**TABLE V** - Results of analysis of tablets by proposed methods

Tablet Brand name	Label claim, mg/tablet	Found* (Percent of label claim ± SD)			
		Reference method	Titrimetry	Spectrophotometry	
				Method A	Method B
Zenfloxx-400**	20	100.12 ± 1.09	102.0 ± 1.17	98.12 ± 1.29	98.87 ± 1.15
			<i>t</i> = 2.62	<i>t</i> =2.65	<i>t</i> =1.76
			<i>F</i> = 1.15	<i>F</i> = 1.40	<i>F</i> = 1.11
Ofloxin-400***	20	100.4 ± 1.02	101.7 ± 1.04	98.95 ± 1.32	102.1 ± 1.13
			<i>t</i> = 1.99	<i>t</i> =1.96	<i>t</i> =2.49
			<i>F</i> = 1.04	<i>F</i> =1.67	<i>F</i> =1.23

\*Mean value of five determinations. \*\* Mankind Pharma Pvt Ltd., New Delhi, India; \*\*\* J. B. Chemicals and Pharmaceuticals Ltd, Mumbai, India. The value of *t* (tabulated) at 95 % confidence level and for four degrees of freedom is 2.77. The value of *F* (tabulated) at 95% confidence level and for four degrees of freedom is 6.39.



**TABLE VI** - Accuracy assessment by recovery experiments

Method	Tablet studied	OFX in tablet*	Pure OFX added*	Total found*	Pure OFX recovered** Percent $\pm$ SD
Titrimetry	Ofloxin-400	3.05	1.5	4.57	101.3 $\pm$ 1.03
		3.05	3.0	6.07	100.7 $\pm$ 1.08
		3.05	4.5	7.54	99.78 $\pm$ 1.17
Spectrophotometry Method A	Ofloxin-400	1.98	1.0	2.97	99.00 $\pm$ 1.24
		1.98	2.0	3.95	98.50 $\pm$ 1.07
		1.98	3.0	4.97	99.67 $\pm$ 1.03
Method B	Ofloxin-400	1.22	0.6	1.83	101.6 $\pm$ 1.15
		1.22	1.2	2.45	102.5 $\pm$ 1.04
		1.22	1.8	3.05	101.7 $\pm$ 1.11

\*mg in titrimetry and  $\mu\text{g/mL}$  in spectrophotometry. \*\*Mean value of three measurements.

titrimetric method is straightforward and fast compared to the other methods reported earlier. The proposed methods make use of simple reagent, which an ordinary analytical laboratory can afford and, unlike most currently available spectrophotometric methods, the present methods are free from unwelcome steps such as heating or extraction and also from critical pH conditions. The spectrophotometric methods are the most sensitive reported to date for OFX (Table I). The methods are also useful for their high tolerance limit for common excipients found in drug formulations. These merits, coupled with the use of simple and inexpensive instruments, allow recommendation of the use of these methods in routine quality control Laboratories.

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