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Abstract: Three simple and sensitive spectrophotometric methods are described for the determination of ofloxacin (OFX) in pharmaceuticals and in spiked human urine. First and second methods are based on the measurement of absorbance of OFX in 0.1 M HCl at 293 nm (method A) and 0.1 M NaOH at 287 nm, respectively. The third method is based on the measurement of 2:1 complex formed between OFX and iron(III) in H_2SO_4 medium, the complex peaking at 420 nm (method C). The optimum conditions for all the three methods are optimized. Beer's law is obeyed over the ranges 0.63-12.5 using method A and method B, and 10-120 µg mL^{-1} using method C. The apparent molar absorptivity values are calculated to be 3.5×10^4, 2.76×10^4 and 2.51×10^3 L mol^{-1}cm^{-1} for method A, method B and method C, respectively. The Sandell sensitivity, limit of detection (LOD) and limit quantification (LOQ) values are also reported. All the methods were validated in accordance with current ICH guidelines. The developed methods were employed with high degree of precision and accuracy for the estimation of total drug content in commercial tablet formulations of DOX. The results obtained from human spiked urine are satisfactory and recovery values are in the range 95.5-106.6%.

Keywords: Ofloxacin; determination; spectrophotometry; pharmaceuticals; spiked urine.

Introduction

Ofloxacin (OFX), a new synthetic second generation fluoroquinolone antibiotic with a broad spectrum of activity against gram-positive and gram-negative bacteria [1]. It is chemically known as (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}] trideca-5(13),6,8,11-tetraene-11-carboxylic acid (Fig 1).
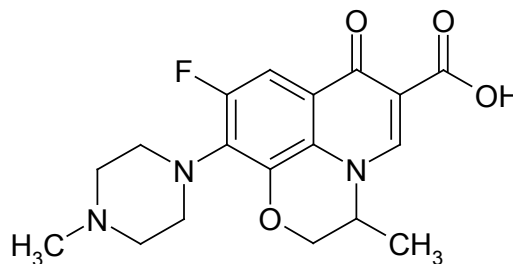

Figure 1. Chemical structure of OFX.

Several methods for the determination of OFX in pharmaceuticals or/and in urine are found in the literature including fluorimetric [2, 3], chromatographic [4-15], electrophoretic [16-18], electroanalytical [19-23], chemiluminescence flow

injection analysis [24-29] and bioassay [30]. In some Pharmacopoeias OFX has been determined [31-33] by non-aqueous titrimetric procedure.

Spectrophotometry is characterized by its speed and simplicity, accuracy and inexpensive instrument needed, and hence it is an important alternative to other analytical methods, with clear advantages in terms of cost of analysis. Inspite of its simplicity and versatility, no UV-spectrophotometric method has ever been reported for OFX.

Several visible spectrophotometric methods [34-40] employing different reactions have been reported for the assay of OFX either alone or with some other therapeutic agents. However, the reported spectrophotometric methods, though sensitive, suffer from one or the other disadvantage such as use of boiling or extraction step, strict pH control, use of organic solvent/expensive chemical and measurement of less sensitive species (Table 1).

Table 1. Comparison of the performance characteristics of the present methods with the published methods.

Sl. No.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg mL^{-1})	Remarks	Ref
1	Citric acid-acetic anhydride	Pink colored chromogen measured	552.5	5-55 ($\varepsilon = 6.04 \times 10^3$)	Boiling for 20 min required.	34
2	a) Bromophenol blue	Yellow chloroform extractable 1:1ion-pair complex complex was measured	410	5-25 ($\varepsilon = 1.04 \times 10^4$)	Ion-pair formation and extraction is pH dependent, extraction step involved, organic solvent is used, narrow linear range.	35
	b) Bromothymol blue		415	2-15 ($\varepsilon = 2.01 \times 10^4$)		
	c)Bromocresol purple		410	2-20 ($\varepsilon = 1.04 \times 10^4$)		
3	a) Tropaeolin 000 (TP 000)	Red chloroform extractable ion-pair complex measured	485	2.5-30 ($\varepsilon = 8.24 \times 10^3$)	Ion-pair formation and extraction is pH dependent, extraction step involved, organic solvent is used, narrow linear range.	36
	b) Supracene Violet 3B (SV 3B)	Chloroform extractable ion-pair complex measured	575	2.5-25 ($\varepsilon = 1.09 \times 10^4$)		
4	Iron(III) nitrate	Ambered coloured complex was measured	370	0-62.5 ($A_{1\%} = 207$)	Buffers used.	37
5	Iron(III) chloride/HCl	Yellow complex measured	410	20-160	Less sensitive, significant blank absorbance.	38
6	Iron(III) alum/H$_2$SO$_4$	Yellow complex measured	420	1.8-289	Flow-injection analysis assembly required.	39
7	Ce(IV)-MBTH	Oxidative coupling reaction product measured	640	1-10	Uses expensive reagent, less stable species measured.	40
8	**a) 0.1 M HCl**	**UV spectrophotometric detection**	**293**	**0.63-12.5** ($\varepsilon = 3.5 \times 10^4$)	**Very simple, sensitive and precise. No heating /extraction involved. Free from critical experimental variables. Highly stable coloured specied measured (visible method), Wide linear dynamic ranges.**	**Present work**
	b) 0.1 N NaOH	**UV spectrophotometric detection**	**287**	**0.63-12.5** ($\varepsilon = 2.76 \times 10^4$)		
	c) iron(III) ammonium sulphate-acid medium	**Yellow complex measured**	**420**	**10-120** ($\varepsilon = 2.51 \times 10^3$)		

ε = molar absorptivity in mol l-1cm-1.

There is a need for methods that are simple, precise, accurate and fast for the routine determination of OFX. This study describes the development and validation of two UV- and one visible-spectrophotometric methods for the determination of OFX in bulk drug, tablets and in spiked human urine. The UV-methods are based on the measurement of the absorbance of OFX solution either in 0.1 N HCl at 293 nm (method A) or in 0.1 N NaOH at 287 nm (method B). The visible spectrophotometric method (method C) is based on the measurement of yellow complex, peaking at 420 nm formed between OFX and iron(III) in H_2SO_4 medium. Different iron(III) salts have previously been employed for the assay of OFX in pharmaceuticals. The method using iron(III) nitrate nanohydrate [37] has not been validated as per the current ICH guidelines. Mathur *et al* [38] have used iron(III) chloride in HCl medium as the chromogenic agent where the reagent blank is found to exhibit significant absorbance at the wavelength of measurement (410 nm). The method using iron(III) alum [39] as a reagent, though has a wide linear dynamic range and has been applied for spiked urine sample lacks the simplicity required for routine analysis since it requires a flow-injection analysis assembly. The proposed method using iron(III) alum overcomes most of the limitations of the existing visible spectrophotometric methods and has been optimized for the experimental variables involved and validated for linearity, sensitivity, selectivity, robustness and ruggedness besides intra-day and inter-day precision and accuracy.

Experimental

Apparatus
Shimadzu Pharmaspec 1700 UV/Visible and Systronics model 106 digital spectrophotometers with 1 cm path length quartz cells were used for absorbance measurements.

Reagents and Solutions
All chemicals used were of analytical reagent grade. Distilled water was used throughout the investigation.

Hydrochloric acid (0.1 M): Prepared by successive dilution of concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.18) with water.

Sodium hydroxide (0.1 N): One g of pure NaOH (S.D. Fine Chem, Mumbai, India) was dissolved in water and diluted to 250 mL.

Sulphuric acid (0.05 and 0.01 M): Concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) was diluted appropriately with water to get 0.05 and 0.01 M solutions.

Iron(III) solution: A 0.5 % iron(III) alum solution was prepared by dissolving 1.25g of pure ammonium iron(III) sulphate (S.D. Fine Chem, Mumbai, India) in 0.05 M H_2SO_4 and diluting to volume with the same acid in a 250 mL calibrated flask.

Urine: Sample was collected from healthy volunteers (around 25-year-old female and 32-year-old male) and kept frozen until use after gentle thawing.

Pure OFX (Pharmaceutical grade) sample was kindly provided by *Cipla India Ltd*, India, as a gift and used as received. Two brands of tablets, namely, OF 400 (J.B Chemicals and Pharmaceuticals Ltd, Mumbai, India) and Zenflox 400 (Mankind Pharma Pvt Ltd, New Delhi, India) were used in the investigation.

Standard drug solutions of 25 µg mL⁻¹ each in 0.1 M HCl and 0.1 N NaOH for method A, and method B, respectively, and 200 µg mL⁻¹ in 0.01 M H_2SO_4 for method C were prepared by dissolving the calculated quantities of pure OFX in the specified solvents.

General Procedures

Method A

Varying aliquots (0.25, 0.5, 1.0, 2,0, 3.0, 4.0 and 5.0 mL of 25 µg mL⁻¹ in 0.1 M HCl) of standard solution corresponding to 0.625-12.5 µg mL⁻¹ OFX were taken into a series of 10 mL volumetric flasks, the content was diluted to the mark with the same solvent and mixed well. The absorbance of each solution was then measured at 293 against 0.1 M HCl.

Method B

Into a series of 10 mL calibration flasks, aliquots of OFX standard solution (25 µg mL^{-1} in 0.1 N NaOH) equivalent to 0.63-12.5 µg mL^{-1} OFX were accurately measured and transferred, and volume was made up to mark with 0.1 N NaOH. After mixing the content, the absorbance of each solution was measured at 287 nm *vs* 0.1 N NaOH.

Method C

Different aliquots (0.0-6.0 mL) of OFX (200 µg mL^{-1}) were accurately measured into a series of 10 mL calibrated flasks by means of microburette and the total volume was adjusted to 6.0 mL with 0.01 M H$_2$SO$_4$. To each flask, 2 mL of 0.5% iron(III) alum solution was added. The content was mixed and allowed to stand for 5 minutes and then diluted to 10 mL with water. After mixing well, the absorbance was measured at 420 nm against the reagent blank.

In all three methods, calibration curves were prepared and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using absorbance-concentration data.

Procedure for tablets

Method A

Twenty tablets were weighed and pulversized. A quantity of tablet powder containing 10 mg of OFX was transferred into a 100 mL volumetric flask. The content was shaken well with about 50 mL of 0.1 M HCl for 20 min. The mixture was diluted to the mark with the same acid. It was filtered using Whatmann No 42 filter paper. First 10 mL portion of the filtrate was discarded. A suitable aliquot of the subsequent portion was diluted to get 25 µg mL^{-1} working concentration and subjected to analysis following the procedure described earlier.

Method B

Tablet powder equivalent to 10 mg of OFX was transferred into a 100 mL volumetric flask. The content was shaken well with about 50 mL of 0.1 N NaOH for 20 min and diluted to the mark with 0.1 N NaOH. It was filtered using Whatmann No. 42 filter paper. First 10 mL portion of the filtrate was discarded and subsequent portion was analyzed after dilution to 25 µg mL^{-1} OFX with 0.1 N NaOH.

Method C

An accurately weighed portion of the tablet powder, equivalent to 20 mg of the drug was shaken with about 50 mL of 0.01 M H$_2$SO$_4$ in a 100 mL standard flask for 20 min. The mixture was diluted to the mark with 0.01 M H$_2$SO$_4$, mixed well and then filtered through a Whatmann No.42 filter paper. First 10 mL portion of the filtrate was discarded and a convenient aliquot of subsequent portion was analyzed by the general procedure described for pure drug.

Procedure for spiked human urine

Method A and Method B

To prepare spiked urine sample, 2.5 mg of the pure OFX and 10 mL of urine sample were transferred into a separating funnel, mixed well till dissolution was complete. The solution was extracted with three 10 mL portion of ethyl acetate and the organic layer was collected in a beaker after drying over anhydrous sodium sulphate. The solvent was evaporated to dryness. The resulting residue was reconstituted either in 0.1 M HCl and diluted to 100 mL or with 0.1 M NaOH before diluting to 100 mL with the same solvent. Then, the analysis was performed as described under general procedure for pure drug by taking 2-3 mL of the above solution.

Method C

Five mg of the pure OFX was transferred into a 25 mL volumetric flask and dissolved in 5 mL of 0.01 M H$_2$SO$_4$. After the addition of 12.5 mL of urine, the volume was brought upto the mark with 0.01 M H$_2$SO$_4$ and mixed well. Two to

three mL portion of aliquot was then subjected to analysis as described earlier.

Results and Discussions

Spectral characteristics

OFX dissolved either in 0.1 M HCl or in 0.1 N NaOH exhibited an absorption peak at 293 nm or 287 nm (Fig 2), and the absorbance at this wavelength was found to be linearly dependent upon the concentration of drug which served as the basis for the quantification of OFX. In both the cases, the corresponding blank solutions showed negligible absorbance. Therefore these wavelengths were used as analytical wavelength throughout the investigation.
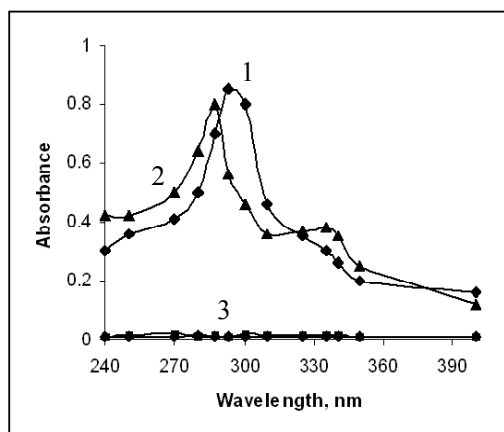

Figure 2. Absorption spectra of: **1.** 8.75 μg mL^{-1} OFX in 0.1 N HCl; **2.** 10.5 μg mL^{-1} OFX in 0.1 N NaOH and **3.** 0.1 N HCl and 0.1 N NaOH.

OFX is reported to react with iron(III) at pH 3.8 to form a water soluble yellow complex [41]. Preliminary spectrophotometric studies concerning the absorbance of iron(III) and OFX-iron(III) complex solutions in different media (methanol, acetic acid, hydrochloric acid and sulphuric acids) were carried out. The greatest difference between the absorbance of OFX-iron(III) complex and iron(III) solution at the wavelength of maximum

absorbance (420 nm) was obtained in H$_2$SO$_4$ medium. This medium was therefore used for developing a spectrophotometric method for OFX.

Figure 3 shows the absorption spectra in H$_2$SO$_4$ medium of OFX-iron(III) complex (40 μg mL^{-1} OFX) (Curve 1) and the blank (Curve 2). As can be seen, iron(III) in H$_2$SO$_4$ medium shows low absorbance at 420 nm at which OFX doesnot absorb. This wavelength was selected for further studies.
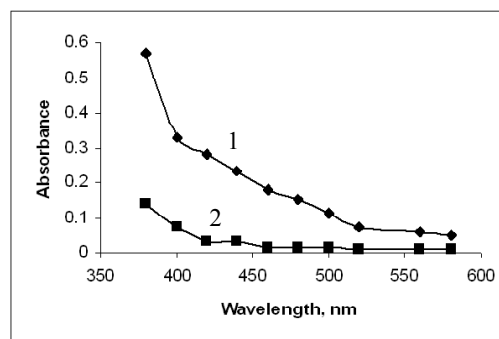

Figure 3. Absorption spectra of: **1.** OFX-iron(III) complex (40 μg mL^{-1} OFX) and **2.** iron(III) in sulphuric acid.

Method optimization

Method C

Effect of iron(III) solution

The effect of iron(III) concentration on the formation of OFX-iron(III) complex was investigated by varying the volume of iron(III) solution, and using a fixed amount of drug. The results revealed that the complex formation was unaffected in the range of 1-4.0 mL of 0.5% iron(III) solution in a total volume of 10 mL. Hence, 2 mL of 0.5% iron(III) solution was used throughout the investigation.

Effect of sulphuric acid concentration on OFX-iron(III) complex formation

The effect of H_2SO_4 concentration on the complex formation was studied by adding various amounts of 2 M sulphuric acid (0 - 4 mL) to a fixed amount of the drug solution before mixing with iron(III) solution. The results revealed that complex formation, sensitivity and stability were unaffected in the concentration range studied. Therefore, no additional sulphuric acid was used in the investigation. This study revealed that pH has no effect on the complex and its sensitivity as well as stability.

Reaction time and stability of the complex

The effect of reaction time after adding iron(III) solution and diluting to the mark with water was studied. The colour formation was complete in 5 min and stable upto 60 min thereafter.

Composition of OFX -iron(III) complex

The composition of the OFX-iron(III) complex was studied using Job's continuous variations method [42]. Drug and iron(III) solutions of 1.36×10^{-3} M each, were prepared in 0.01 M H_2SO_4 and 0.05 M H_2SO_4, respectively, and mixed in various molar ratios (with a total volume of 5 mL) in 10 mL volumetric flasks. After 5 min, the solutions were made upto mark, mixed well and the absorbance was subsequently measured at 420 nm. The graph of the results obtained (fig. 4) gave a maximum at a molar ratio of $X_{max} = 0.666$ which indicated the formation of a 2:1 (OFX:iron(III)) complex as shown in figure 5. The formation constant (K_f) of the complex was also calculated from the continuous variation data using the following equation [43]:

$$K_f = \frac{A/A_m}{\left[1 - A/A_m\right]^{n+2} C_M \left(n\right)^n}$$

where A and Am are the observed maximum absorbance and the absorbance value when all the drug present is complexed, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry with which iron(III) complexes with drug. The log K_f value was found to be 5.65.
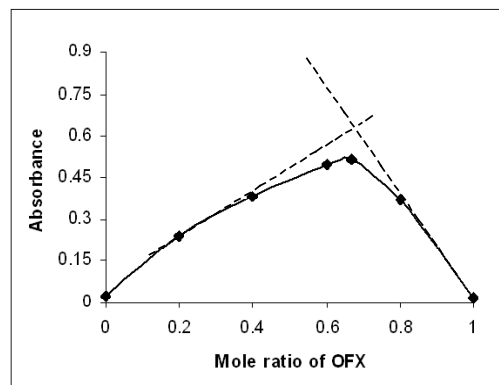

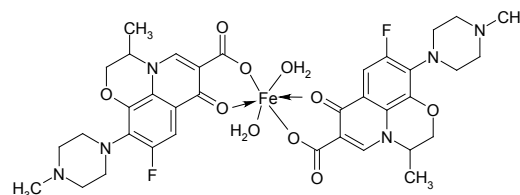**Figure 4.** Continuous variation plot for OFX-iron(III) complex.

Figure 5. Probable structure of the OFX-iron(III) complex.

Method Validation

Linearity, sensitivity, limits of detection and quantification

A linear correlation was found between absorbance at λ_{max} and concentration of OFX in the

ranges given in Table 2. The graphs are described by the regression equation:

$$Y = a + bX$$

(where Y - absorbance of 1-cm layer of solution; a - intercept; b - slope and X - concentration in μg mL^{-1}). Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 2. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values [44] of all the three methods are also given in Table 2. The limits of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines [45] using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 2. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

Table 2. Sensitivity and regression parameters.

Parameter	Method A	Method B	Method C
λmax, nm	293	287	420
Linear range, μg mL^{-1}	0.63-12.5	0.63-12.5	10-120
Molar absorptivity(ε), L mol^{-1} cm^{-1}	3.5×10^4	2.76×10^4	2.51×10^3
Sandell sensitivity*, μg cm^{-2}	0.0103	0.0131	0.1441
Limit of detection (LOD), μg mL^{-1}	0.10	0.12	2.66
Limit of quantification (LOQ), μg mL^{-1}	0.29	0.37	8.05
Regression equation, Y**			
Intercept (a)	0.0033	0.0018	0.0113
Slope (b)	0.10	0.08	0.01
Standard deviation of a (S$_a$)	0.0998	0.0998	0.0998
Standard deviation of b (S$_b$)	0.0089	0.011	0.001
Variance (Sa^2)	0.01	0.01	0.01
Regression coefficient (r)	0.9999	0.9999	0.9996

*Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm2 and l = 1 cm.
**Y=a+bX, Where Y is the absorbance, X is concentration in μg mL-1, a is intercept, b is slope.

Precision and accuracy

The assays described under "general procedures" were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 3. The percentage relative standard deviation (%RSD) values were ≤ 1.58% (intra-day) and ≤ 2.11% (inter-day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for OFX. Bias {bias % = [(Concentration found - known concentration) x 100 / known concentration]} was calculated at each concentration and these results are also presented in Table 3. Percent relative error (%RE) values of ≤ 3.0% demonstrate the high accuracy of the proposed methods.

Table 3. Evaluation of intra-day and inter-day accuracy and precision**.**

Method	OFX taken, μg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
		OFX found ±CL, μg mL^{-1}	%RE	%RSD	OFX found±CL, μg mL^{-1}	%RE	%RSD
A	3.0	3.05±0.02	1.67	0.56	3.09±0.07	3.00	1.80
	6.0	5.93±0.04	1.17	0.67	6.08±0.12	1.33	1.56
	9.0	9.06±0.08	0.67	0.89	9.11±0.22	1.22	1.98
B	3.0	2.92±0.02	2.67	0.62	3.07±0.06	2.33	1.56
	6.0	6.08±0.04	1.33	0.79	6.11±0.09	1.83	1.22
	9.0	8.95±0.07	0.56	0.82	9.10±0.13	1.11	1.11
C	30.0	29.4±0.30	2.00	1.11	30.6±0.56	2.00	1.48
	60.0	60.9±0.69	1.50	1.22	61.3±1.44	2.17	1.89
	90.0	91.0±1.33	1.11	1.58	91.8±2.40	2.00	2.11

%RE. Percent relative error, %RSD. relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√n. (The tabulated value of t is 2.45 and 2.77 for six and four degrees of freedom respectively, at the 95% confidence level; S = standard deviation and n = number of measurements).

Selectivity

A systematic study was performed to determine the effect of matrix by analyzing the placebo blank and synthetic mixture containing OFX. A placebo blank of the composition: starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under 'tablets', and then subjected to analysis. The absorbance of the placebo solution in each case was almost equal to the absorbance of the blank which revealed no interference. To assess the role of the inactive ingredients on the assay of OFX, a synthetic mixture was separately prepared by adding 20 mg of OFX to the placebo mentioned above. The drug was extracted and solution prepared as described under the general procedure for tablets. The solutions after appropriate dilution wherever necessary were analyzed following the recommended procedures. The absorbance resulting from 10 μg mL^{-1} OFX in method A and

method B, and 70 μg mL^{-1} OFX solution method C, were nearly the same as those obtained for pure OFX solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of OFX. Further, the slopes of the calibration plots prepared from the synthetic mixture solutions were about the same as those prepared from pure drug solutions.

Robustness

The robustness of the method (method C) was evaluated by making small incremental changes in the volume of iron(III) and reaction time, and the effect of the changes was studied by calculating the mean RSD values. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (2.66%).

Ruggedness

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using four different instruments. The inter-analysts RSD were within 2.89% whereas the inter-instruments RSD for the same OFX amount was less than about 3% suggesting that the developed methods were rugged. The results are shown in Table 4.

Table 4. Method robustness and ruggedness expressed as intermediate precision (% RSD).

| | | Robustness | | Ruggedness | |
| | | Parameter altered | | Inter-analysts, (%RSD) (n=4) | Inter-instruments, (%RSD) (n=4) |
Method	OFX taken*	Volume of iron(III)* (%RSD)	Reaction time**		
A	10	-	-	1.89	2.98
B	10	-	-	2.89	3.00
C	60	2.66	1.74	2.25	2.55

*Volumes of iron(III) solution used were 1.8, 2.0 and 2.2 mL. **Reaction times studied were 4, 5 and 6 min.

Analysis of pharmaceutical formulations

The described procedures were successfully applied to the determination of OFX in its pharmaceutical formulations (OF 400, Zenflox 400). The results obtained (Table 5) were statistically compared with the British Pharmacopoeial method [31]. The method involved the titration of OFX in anhydrous acetic acid with acetous perchloric acid to a potentiometric end point detection. The results obtained by the proposed methods agreed well with those of reference method and with the label claim. The results were also compared statistically by a Student's t-test for accuracy and by a variance F-test for precision [46] with those of the reference method at 95 % confidence level as summarized in Table 5. The results showed that the calculated t-and F-values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

Table 5. Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method.

Tablet brand name	Nominal amount, (mg/tablet)	Found* (Percent of label claim ± SD)			
		Reference method	Proposed methods		
			Method A	Method B	Method C
OF-400	400	100.2±0.89	98.9±0.98	100.8±1.5	101.8±1.8
			t = 2.19	**t** = 0.79	**t** = 1.88
			F = 1.21	**F** = 2.84	**F** = 4.09
Zenflox 400	400	101.6±0.72	99.2±0.98	102.6±1.57	102.0±1.75
			t = 4.46	**t** = 1.38	**t** = 0.51
			F = **1.85**	**F** = 4.75	**F** = 5.91

*Average of five determinations.
Tabulated t value at the 95% confidence level is 2.77. Tabulated F value at the 95% confidence level is 6.39.

Application to spiked human urine

As another application of the proposed methods, recovery from human urine samples was carried out followed by a prior extraction of drug from urine in method A and method B, and treatment of drug with urine without any extraction step in method C. Recovery studies were performed with the sample containing various amounts of OFX. The results of recovery studies (Table 6) revealed that, other constituents present in the urine did not interfere in the method. The recovery values were in the range 95.5-106.6% with standard deviation of 0.52-1.60. Ofloxacin is rapidly and efficiently absorbed after oral administration and is eliminated principally by hepatic metabolism and subsequent urinary excretion. The normal dose for adults is 400 mg day^{-1}. Ofloxacin undergoes only limited metabolism: nearly 80% of an orally administered dose is excreted unchanged in urine [17]. The concentration of OFX in urine passed during 24 h is within the range of OFX determination by the proposed methods.

Table 6. Application of the proposed methods to the ofloxacin concentration measurements in spiked urine.

Method	OFX added, $\mu g\ mL^{-1}$	OFX found[*], $\mu g\ mL^{-1}$	Recovery of OFX (Percent±SD)
A	4.0	3.82	95.5±0.86
	8.0	7.82	97.8±1.02
	12.0	12.45	103.8±1.58
B	4.0	4.09	102.3±1.55
	8.0	8.44	105.5±1.30
	12.0	12.79	106.6±1.11
C	40.0	38.25	95.62±0.52
	80.0	79.65	99.56±1.25
	120.0	123.8	103.2±1.60

*Mean value of three determinations.

Recovery study

To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analysed tablet powder with pure OFX at three different levels (50, 100 and 150 % of the content present in the tablet powder (taken) and the total was found by the proposed methods. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.7-102.6% with relative standard deviation in the range 0.88-1.5%. Closeness of the results to 100 % showed the fairly good accuracy of the methods. The results are shown in Table 7.

Table 7. Results of recovery study using standard addition method.

Method A			
Tablet studied	OFX in tablet extract, $\mu g \ mL^{-1}$	Pure OFX added, $\mu g \ mL^{-1}$	Total OFX found, $\mu g \ mL^{-1}$	Pure OFX recovered (Percent±SD[*])
OF 400	4.95	2.50	7.42	98.8±1.23
	4.95	5.00	9.96	100.2±1.32
	4.95	7.50	12.35	98.7±1.02
Zenflox 400	4.96	2.50	7.48	100.8±1.50
	4.96	5.00	9.92	99.2±1.45
	4.96	7.50	12.36	98.7±0.99

Method B			
OFX in tablet extract, $\mu g \ mL^{-1}$	Pure OFX added, $\mu g \ mL^{-1}$	Total OFX found, $\mu g \ mL^{-1}$	Pure OFX recovered (Percent±SD[*])
5.04	2.50	7.52	99.2±1.25
5.04	5.00	10.01	99.4±1.22
5.04	7.5	12.60	100.8±1.50
5.13	2.50	7.70	102.6±1.20
5.13	5.00	10.14	100.1±0.89
5.13	7.50	12.75	101.6±0.99

Method C			
OFX in tablet extract, $\mu g \ mL^{-1}$	Pure OFX added, $\mu g \ mL^{-1}$	Total OFX found, $\mu g \ mL^{-1}$	Pure OFX recovered (Percent±SD[*])
40.72	20.0	60.55	99.16±1.45
40.72	40.0	81.04	100.8±0.88
40.72	60.0	101.8	101.8±1.05
40.80	20.0	61.20	102.0±1.33
40.80	40.0	81.44	101.6±0.94
40.80	60.0	101.9	101.9±1.50

*Mean value of three determination.

Conclusion

Three simple, rapid, accurate and precise, and cost-effective spectrophotometric methods were developed and validated for the determination of OFX in pharmaceuticals and in spiked human urine. The proposed spectrophotometric methods do not require any expensive equipment and specialized technicians when compared alongside HPLC, chemiluminescence, FIA, electroanalytical and bioassay techniques. Besides, other characteristics of these methods are short time required for performance and ease of handling. The proposed UV methods are more sensitive than many reported methods, require no additional reagents or organic solvents. Though the sensitivity of method C is less when compared to method A and method B, the OFX-iron(III) complex in sulphuric acid medium has suitable characteristics for the spectrophotometric determination of OFX. The visible spectrophotometric method is the simplest of all the reported spectrophotometric methods in terms reagents used, optimal conditions employed, since no heating or extraction step is involved. The procedure has been demonstrated to be independent of pH of the reaction medium unlike the reported method using iron(III) nitrate which employs buffer medium. The proposed methods are highly

precise compared to most reported methods where the precision is affected by critical experimental variables. The absence of any crucial experimental variable makes the present methods more accurate than many reported methods. The methods are useful for the quality control and routine analysis of OFX in pharmaceuticals since there is no interference from the common excipients that might be found in commercial preparations. Also, these methods allow determination of OFX in human urine samples in the physiological concentration range obtained after the usual therapeutic dose of OFX has been administered.

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References

K. Sat, U. Matsura, M. Inone, T. Ueno, Y. Osada, H. Ogawa, M. Mitshuhashi, Antimicrob. Agents Chemother. 22 (1982) 548.

F.A. El-Yazbi, Spectrosc. Lett. 25 (1992) 279.

Q.J. Gong, J.L. Quiao, L.M. Du, C. Dong, Talanta 53 (2000) 359.

A. Le Coguic, R. Bidault, R. Farinotti, A. Dauphin, J. Chromatogr. 434 (1988) 320.

O. Okazaki, H. Aoki, H. Hakusui, J. Chromatogr. 563 (1991) 313.

G. Carlucci, P. Mazzeo, T. Fantozzi, Anal. Lett. 26 (1993) 2193.

V.M. Shinde, B.S. Desai, N.M. Tendolkar, Indian Drugs 35 (1998) 715.

U.P. Halkar, P.B. Ankalkope, Indian Drugs 37 (2000) 585.

M. S. Ali, M. Ghori, A. Saeed. J. Chromatogr. Sci. 40 (2002) 429.

A.J.N. Groenveld, J.R.B. Brouwers. Pharm. World Sci. 8 (1986) 79-84.

R.R. Kalta, R. Sharma, S.C. Chaturvedi. Ind. J. Pharma. Sci. 70 (2008) 491-494.

K. P. Chan, K. On Chu, W. W-K. Lai, K.W. Choy, C.C. Wang, D. S-C Lam, C. P. Pang. Anal. Biochem. 353 (2006) 30-36.

T. Ohkubo, M. Kudo, K. Sugawara. Anal. Sci. 7 (1991) 741-743.

M.A. Gracia, C. Solans, A. Calvo, M. Royo, E. Hernandez, R. Rey, M.A. Bregante. Chrmatographia, 55 (2002) 431-434.

B. Srividya, R.M. Cardoza, P.D. Amin, Indian drugs, 40 (2003), 41-43.

S.W. Sun, A.L. Wu, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 281.

C. Horstkoetter, G. Blaschke, J. Chromatogr. B 754 (2000) 169.

S.S. Zhang, H. Liu, Y.J. Wu, C.L. Yu, Analyst 126 (2001) 441.

A. Tamer, Anal. Chim. Acta. 231 (1990) 129.

M. Tuncel, Z. Atkosar, Pharmazie 47 (1992) 642.

G. Zhou, J. Pan, Anal. Chim. Acta. 307 (1995) 49.

M. Rizk, F. Belal, F. Aly, N. El-Enany, Talanta 46 (1998) 83.

J. Wu, H. Zhao, L. Wei, T.Z. Ai, X.Z. Dong, Fenxi Huaxue 29 (2001) 1106.

F. Paul, J.L Adcock, Anal. Chim. Acta. 541 (2005) 3.

S. Hanwen, L Liqing, C. Xueyan, Anal. Sci. 22 (2006) 1145.

Y Rao, Y Tong, X.R. Zhang, G. Luo, Baeyens, Willy, J. Fluoresc, 17 (2007) 481.

L. Yi, H. Zhao, S. Chen, L. Jin, D. Zheng, Z. Wu, Talanta 61 (2003) 403.

Y.D. Liang, J.F. Song, X.F. Yang, Anal. Chim. Acta 510 (2004) 21.

B. Li, Z. Zhang, L. Zhao, Ch. Xu, Talanta 57 (2002) 765.

S.E. Lisiane da, E.S.S. Elfrides. J. Pharm. Biomed. Anal, 27 (2002) 91.

British Pharmacopeia. Vol III Her Majesty's Stationery office, London, (2003) p 1357.

United States Pharmacopoeia XXII, National Formulary XVII, Rockville, USA Convention, (1990) p 912.

European Pharmacopoeia. EDQM, Council of Europe, Strasbourg, France. 5 (2005) p 2131.

P.U. Patel, B.N. Suhagia, M.M. Patel, G.C. Patel, G.N. Patel, Indian Pharmacist, 6 (2007) 59.

Y.M. Issa, F.M. Abdel-Gawad, M.A. Abou Table, H.M. Hussein, Anal. Lett. 30 (1997) 2071.

C. S. P. Sastry, Kolli Rama Rao, D. Siva Prasad, Talanta, 42 (1995) 311.

C.J. Eboka, S.O. Aigbavboa, J.O. Akerele, J. Antimicrob. Chemother. 39 (1997) 639.

S.C. Mathur, S. Kumar, N. Murugesan, Y.K.S. Rathore, P.D. Sethi, Indian Drugs, 29 (1992) 376.

M.S. Soledad, M.I. Albero, C. Sanchez-Pedreno, M.S. Abuherba, Eur. J. Pharm. Biopharm. 61 (2005) 87.

C.S.P. Sastry, K.R. Rao, D.S. Prasad, Indian Drugs, 32 (1995) 172.

D. Lee, H. Han, K. Kim, W. Park, J.Cho. J.Kim, J. Pharm. Biomed. Anal. 12 (1994) 157.

Douglas, A. S and Donald. M. W. 1971. "Principels of Instrumental Analysis," Holt, Rinheart and Winston, New York, p 104.

Erk N Anal. Lett. 36 (2003) 1183.

Zavis, H., Ludvik, D., Milan, K., Ladislaw, S. and Frantisck, V. Handbook of Organic Reagents in Inorganic Analysis. Translated by Stanislav, K, Dr. Chalmers (The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester, A Division of John Wiley & Sons IC, New York, London, Sydney. Toronto. (1976) p 364.

International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.

Inczedy, J., Lengyel, T. and Ure, A.M. 1998. IUPAC Compendium of Analytical Nomenclature : Definitive Rules, Blackwell Science Inc., Boston.