Utility of Oxidation-Reduction Reaction for Determination of Gemifloxacin Mesylate and Moxifloxacin HCl in Pure Form and in Pharmaceutical Formulations using N-Bromosuccinimide

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Abstract

Three sensitive spectrophotometric methods are presented for the assay of gemifloxacin mesylate (GMF) and moxifloxacin HCl (MXF) in bulk drug and in pharmaceutical formulations using N-bromosuccinimide (NBS) and three dyes, methyl orange, amaranth and indigo carmine, as reagents. The methods involve the addition of a known excess of NBS to drug in acid medium, followed by determination of unreacted oxidant by reacting with a fixed amount of methyl orange and measuring the absorbance at 510 nm (method A), amaranth and measuring the absorbance at 528 nm (method B) or indigo carmine and measuring the absorbance at 610 nm (method C). In all methods, the amount of NBS reacted corresponds to the amount of drug and the measured absorbance is found to increase linearly with the concentration of drug which is corroborated by the correlation coefficients of 0.9992-0.9998. The systems obey Beer's law for 0.1-4.8 and 0.2-4.0 µg mL^{-1} for GMF and MXF, respectively. The limits of detection and quantification are also reported. Intra-day and inter-day precision and accuracy of the methods have been evaluated. The methods were successfully applied to the assay of GMF and MXF in tablet preparations and the results were statistically compared with those of the reference methods by applying Student's t-test and F-test. No interference was observed from the common tablet excipients. The accuracy of the methods was further ascertained by performing recovery studies via standard-addition method.

Keywords: Spectrophotometry; Gemifloxacin mesylate; Moxifloxacin HCl; N-bromosuccinimide; Pharmaceutical formulations

Introduction

Fluoroquinolones are the second-generation members of quinolone antibiotics fluorinated in position 6 and bearing a piperazinyl moiety at position. They are considered to be the most effective Gram-positive and Gram-negative pathogens to combat infection caused by microorganisms that are resistant to other microbials, such as tetracyclines. Also, they have some activity against mycobacteria, mycoplasmas, rickettsias, and the protozoan *Plasmodium falciparum* [1-3]. There is a substantial body of literature related to both the mechanism of their action as DNA gyrase inhibitors, and the influence of systematic structural modifications on their biological activity. Gemifloxacin mesylate (GMF), is (R,S)-7- [(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid methanesulfonate and moxifloxacin (MXF) {1-cyclopropyl-7-[2, 8-diazobicyclo (4.3.0) nonane]-6-fluoro-8-methoxy-1, 4 dihydro-4-0xo-3-quinolone carboxylic acid}. GMF and MXF are fourth-generation a synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial drug derivatives. Due to its clinical advantages, GMF and MXF are receiving a great interest and there was an increase in number of its pharmaceutical dosage forms in the market in recent past. For routine analysis of the studied drugs, a simple, rapid and cost effective analytical method was required. The chemical structure of the studied fluoroquinolones is shown in Figure 1.

No official (pharmacopoeia) method has been found for the assay of GMF and MXF in their pharmaceutical formulations. Several methods have been reported for the determination of fluoroquinolones either in pure forms, in dosage forms, or in biological fluids like chromatography [4-11], microchip electrophoresis [12,13], chiral counter-current chromatography [14], capillary zone electrophoresis [15,16], electrochemistry [17-21], atomic absorption spectrometry [22,23] and spectrofluorimetry [24-27]. However, these methods are expensive and not available at most quality control laboratories.

The spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure, pharmaceutical formulations and in biological samples, for its simplicity and reasonable sensitivity with significant economical advantages. Spectrophotometric methods reported for the assay of GMF [27-42] and MXF [22,43-50]. These methods were associated

Figure 1: The chemical structure of the studied drugs.
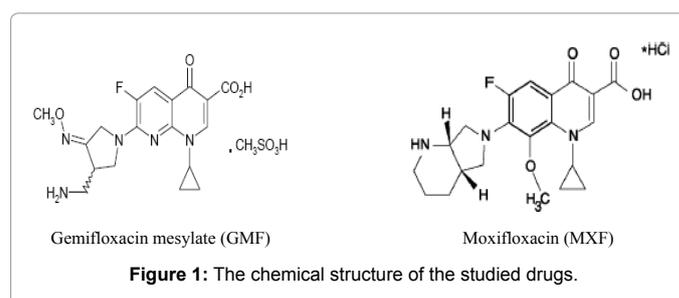
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with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region and/or decreased simplicity of the assay procedure (e.g. tedious precipitation, heating or liquid–liquid extraction steps in the ion-pair formation-based methods). For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of the studied drugs in their pharmaceutical dosage forms.

N-bromosuccinimide (NBS) is a versatile oxidimetric reagent. Since of its high oxidation potential and excellent solutions stability, they were used for the quantitative determination of many compounds [51-55].

The present investigation aims to develop simple, sensitive and cost-effective methods for the determination of GMF and MXF in pure form and in dosage forms using the visible spectrophotometric technique. The methods utilize NBS, methyl orange (method A), amaranth (method B) and indigo carmine (method C) as reagents. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quality control.

Materials and Methods

Apparatus

All absorption spectra were made using Kontron Unikon 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells.

Materials and reagents

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

Materials

Pharmaceutical grade gemifloxacin mesylate (GMF) was supplied by (Al-Obour Pharmaceutical & Chemical Industries Company, Egypt). Moxifloxacin hydrochloride (MXF) reference standard was provided by Sabaa, Kahira Company, Egypt. All pharmaceutical preparations were obtained from commercial sources in the local market. Factive tablets were obtained from Oscient Pharmaceuticals Corporation, USA, labeled to contain (320 mg GMF per tablet); Flobiotic tablets were obtained from Hikma Pharm. & Chem. Ind. Company, Egypt, labeled to contain (320 mg GMF per tablet). GemiQue tablets were obtained from Obour Pharm. & Chem. Ind. Company, Egypt, labeled to contain (320 mg GMF per tablet). Avelox® was obtained from Bayer, Germany, labeled to contain (400 mg MXF per tablet). Moxiflox tablets were obtained from EVA Pharm. & Chem. Ind. Company, Egypt, labeled to contain (400 mg MXF per tablet). Moxifloxacin tablets were obtained from Sabaa International Company for pharmaceuticals and chemical industries S.A.E., labeled to contain (400 mg MXF per tablet).

Preparation of stock standard solutions

Stock standard solutions of GMF and MXF (100 µg mL^{-1}) were prepared by dissolving an exact weight (10 mg) of pure drugs in 2.0 mL 0.1 M HCl, and further diluted to 100 mL with bidistilled water in a 100 mL measuring flask. The solution was diluted stepwise to get working concentrations of 5.0, 10 and 20 µg mL^{-1} drug for methods A, B and C, respectively. The standard solutions were found stable for at least one week without alteration when kept in an amber coloured bottle and stored in a refrigerator when not in use.

Reagents

N-bromosuccinimide (NBS): An approximately 0.01M NBS solution was prepared by dissolving about 1.8 g of chemical (Sigma-Aldrish) in water with the aid of heat and diluted to one liter with water and standardized iodometrically [56]. The solution was kept in an amber coloured bottle and was diluted appropriately to get 100 µg mL^{-1} NBS for use in all methods. The NBS solution was stored in a refrigerator when not in use. Potassium bromide, KBr (1.0% w/v). Hydrochloric acid (5.0 M): A 5.0 mol L^{-1} of HCl was prepared by diluting 43 mL of concentrated acid (Merck, Darmstadt, Germany, Sp. gr. 1.18, 37%) to 100 mL with bidistilled water and standardized as recommended previously [57] prior to use. Methyl orange (50 µg mL^{-1}): A 500 µg mL^{-1} dye solution was first prepared by dissolving accurately weighed 58.8 mg of dye (Sigma-aldrish, 85% dye content) in water and diluting to 100 mL in a calibrated flask and filtered using glass wool. It was further diluted to obtain a working concentration of 50 µg mL^{-1}. Amaranth (200 µg mL^{-1}): A 1000 µg mL^{-1} stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (Sigma-aldrish, 90% dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of 200 µg mL^{-1}. Indigo carmine (200 µg mL^{-1}): A 1000 µg mL^{-1} stock standard solution was first prepared by dissolving accurately weighed 112 mg of dye (Sigma-aldrish, 90% dye content) in water and diluting to volume in a 100 mL calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of 200 µg mL^{-1}.

Recommended general procedures

Method A (using methyl orange): Different aliquots (0.2-4.0 mL) and (0.4-4.8 mL) of a standard 5.0 µg mL^{-1} GMF and MXF solution, respectively were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 6.0 mL by adding adequate quantity of water. To each flask was added 1.0 mL each of 5.0 M HCl; 1.0 mL of NBS solution (100 µg mL^{-1}) and 1.0 mL of 1.0% KBr were added successively. The flasks were stoppered, content mixed and let stand for 15 min with occasional shaking. Finally, 1.0 mL of 100 µg mL^{-1} methyl orange solution was added (accurately measured) and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 510 nm against a reagent blank after 10 min.

Method B (using amaranth): Different aliquots (0.1 - 2.0 mL) and (0.2-4.0 mL) of a standard 10 µg mL^{-1} GMF and MXF solution, respectively were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5.0 mL by adding adequate quantity of 0.1 M HCl. To each flask 1.0 ml of 5.0 M HCl; 1.0 mL of NBS solution (100 µg mL^{-1}), and 1.0 mL of 1.0% KBr were added successively. The flasks were stoppered; content mixed and allowed to stand for 15 min with occasional shaking. Finally, 1.0 mL of 200 µg mL^{-1} amaranth solution was added (accurately measured) and the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 528 nm against a reagent blank after 5.0 min.

Method C (using indigo carmine): Different aliquots (0.1-2.4 mL) and (0.1-1.3 mL) of standard 20 µg mL^{-1} GMF and MXF solution, respectively were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3.0 mL with 0.1 M HCl. To each flask 1.0 mL each of 5.0 M HCl, 1.0 mL of NBS solution (100 µg mL^{-1}) and 1.0 mL of 1.0% KBr were added successively. The content was mixed well and the flasks were kept

aside for 10 min with intermittent shaking. Finally, 1.0 mL of 200 µg mL^{-1} indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance measured against a reagent blank at 610 nm after 5.0 min.

In all methods, a standard graph was prepared by plotting the absorbance versus the concentration of drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Procedure for pharmaceutical formulations (tablets): Ten tablets of each drug were weighed accurately and ground into a fine powder. A quantity of the powder containing 100 mg of GMF or MXF was accurately weighed into a 100 mL calibrated flask and 50 mL of 0.1 M HCl was added. The content was shaken for about 20 min; the volume was finally 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 suitable aliquot of the subsequent portion (100 µg mL^{-1} drug) was diluted stepwise with 0.1 M HCl to obtain working concentrations of 5.0, 10 and 20 µg mL^{-1} concentrations of GMF or MXF for analysis by spectrophotometric methods A, B and C, respectively. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above. Determine the nominal content of the tablets either from a previously plotted calibration graph or using the corresponding regression equation.

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 pharmaceutical preparations', and then subjected to analysis using the procedures described above.

Procedure for the determination of GMF or MXF in synthetic mixtures: A volume of 100 mg of GMF or MXF 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 pharmaceutical preparations'. The solution was mixed well and filtered using a Whatman No. 42 filter paper. The synthetic mixture solution (1.0 mg mL^{-1} in GMF or MXF) was then diluted stepwise with 0.1 M HCl to obtain working concentrations of 5.0, 10 and 20 µg mL^{-1} of drug for spectrophotometric methods A, B and C, 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.
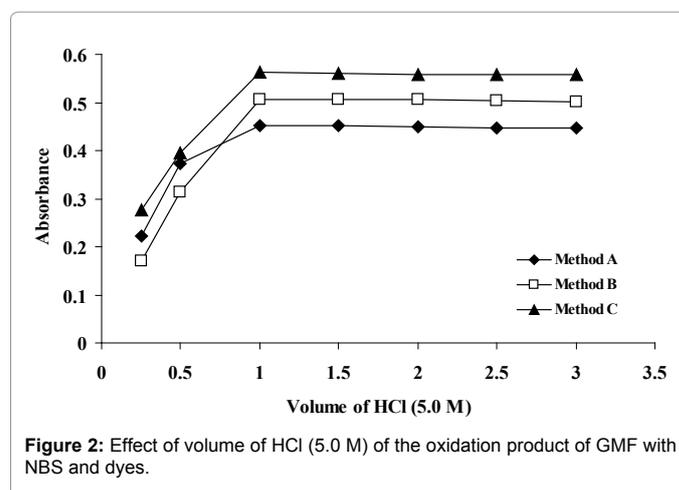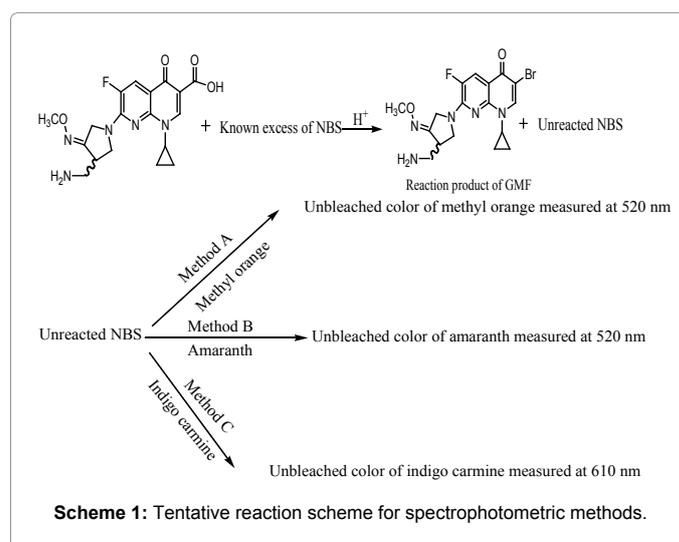
Results and Discussion

A close examination of the literature search presented in the introduction reveals that NBS has not yet been used for the spectrophotometric determination of GMF or MXF. 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 [58]. The present work involves the bromination of GMF or MXF by NBS followed by determination of surplus NBS after allowing the bromination reaction to complete. The ability of NBS to oxidize GMF or MXF and bleach the colors of methyl orange, amaranth and indigo carmine dyes has been used for the indirect spectrophotometric assay of the drugs. In the three methods, the drugs are reacted with a known excess of NBS in acid medium and the unreacted oxidant is determined by reacting with

a fixed amount of dyes and measuring the absorbance at 510, 528 and 610 nm for methods A, B and C, respectively, the absorbance increased linearly with increasing concentration of drug.

GMF or MXF, when added in increasing amounts to a fixed amount of NBS, consumes the latter and there will be a concomitant fall in its concentration. When a fixed amount of each dye is added to decreasing amounts of NBS, a concomitant increase in the concentration of dye results. This is observed as a proportional increase in the absorbance at the respective wavelengths of maximum absorption with increasing concentration of drug as indicated by the correlation coefficients ranged from of 0.9992-0.9998. The tentative reaction scheme of spectrophotometric methods is shown in Scheme 1. The bromination of GMF or MXF will take place in position α to the carbonyl group [54,59].

Effect of acid concentration

The hydrochloric acid was found most appropriate. The effect of HCl was studied and 0.25-3.0 mL of 5.0 M HCl in a total volume of 7.0 mL was found to have constant effect on both reactions (i.e. Drug with NBS, and residual NBS with dyes). The results presented in Table 1 indicated that, at 1.0-3.0 mL of 5.0 M HCl, there was almost same absorbance values were obtained in the presence of GMF or MOX, the

Scheme 1: Tentative reaction scheme for spectrophotometric methods.

Figure 2: Effect of volume of HCl (5.0 M) of the oxidation product of GMF with NBS and dyes.

absorbance values obtained were constant and were almost the same as those of the reagent blank. At the acid volumes less than 1.0 mL, reaction led to go slower and incomplete. Therefore, 1.0 mL of 5.0 M HCl was used though out the study (Figure 2).

Effect of reagents

Preliminary experiments were performed to determine the maximum concentrations of the dyes spectrophotometrically in acid medium, and these were found to be 10, 20 and 20 µg mL^{-1} for methyl orange, amaranth and indigo carmine, respectively. A NBS concentration of 10 µg mL^{-1} was found to irreversibly destroy the red colour of 10 µg mL^{-1} methyl orange, whereas 10 µg mL^{-1} NBS was required to destroy the red and blue colours of 20 µg mL^{-1} amaranth and indigo carmine, respectively in HCl medium. Hence, different concentrations of drugs were reacted with 1.0 mL of 100 µg mL^{-1} NBS in all methods before determining the residual NBS as described

Parameters	GMF			MXF		
	Method A	Method B	Method C	Method A	Method B	Method C
λ max (nm)	510	528	610	510	528	610
Beer's law limits, µg mL^{-1}	0.1-2.0	0.1-2.0	0.2-4.8	0.2-2.4	0.2-4.0	0.2-2.6
Ringboom limits, µg mL^{-1}	0.3-1.8	0.3-1.8	0.5-4.0	0.4-2.0	0.5-3.6	0.4-2.2
Molar absorptivity, x 10^5 L mol^{-1} cm^{-1}	8.25	10.31	6.49	7.17	7.79	10.66
Sandell sensitivity, ng cm^{-2}	5.88	4.71	7.48	6.11	5.62	4.12
Regression equation,[a]						
Intercept (a)	- 0.002	0.0008	0.0045	- 0.0016	0.0004	- 0.0085
Standard deviation of intercept (S$_a$)	0.0055	0.0058	0.0079	0.0067	0.0091	0.0074
Slope (b)	0.1757	0.213	0.1276	0.1669	0.1759	0.2601
Standard deviation of slope (S$_b$)	0.0054	0.0087	0.0094	0.0090	0.0098	0.0086
Correlation coefficient, (r)	0.9997	0.9998	0.9995	0.9997	0.9998	0.9994
Mean ± SD	100.03 ± 0.844	100.18 ± 0.96	100.28 ± 0.76	99.44 ± 1.49	99.90 ± 1.04	99.45 ± 1.15
RSD%	0.844	0.96	0.76	1.50	1.04	1.16
RE%	0.886	1.01	0.80	1.57	1.09	1.21
Limit of detection, µg mL^{-1}	0.026	0.029	0.058	0.059	0.055	0.056
Limit of quantification, µg mL^{-1}	0.087	0.097	0.193	0.197	0.183	0.187
Calculated t-value [b]	0.13	0.39	0.64	0.65	0.07	0.76
Calculated F-value [b]	1.06	1.37	1.16	2.99	1.46	1.78

[a] A = a + bC, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.
[b] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p=0.05).

Table 1: Analytical and regression parameters of spectrophotometric methods.

Drug	Taken (µg mL^{-1})	Intra-day (n=6)				Inter-day (n=5)			
		Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
	GMF								
Method A	0.5	99.80	0.77	-0.20	0.499 ± 0.0004	99.60	0.83	-0.40	0.498 ± 0.004
	1.0	99.90	0.94	-0.10	0.999 ± 0.010	100.10	1.58	0.10	1.001 ± 0.017
	1.5	100.30	1.30	0.30	1.505 ± 0.021	100.50	1.84	0.50	1.508 ± 0.029
Method B	0.5	99.60	0.94	-0.40	0.498 ± 0.005	100.40	0.88	0.40	0.502 ± 0.005
	1.0	99.80	1.25	-0.20	0.998 ± 0.013	99.90	1.36	0.10	0.999 ± 0.014
	1.5	99.70	1.43	-0.30	1.496 ± 0.022	100.60	1.73	0.60	1.509 ± 0.027
Method C	1.0	100.20	0.89	0.2	1.002 ± 0.009	100.20	1.05	0.20	1.002 ± 0.011
	2.0	99.70	1.16	-0.30	1.994 ± 0.024	100.10	1.39	0.10	2.002 ± 0.029
	4.0	99.90	1.28	-0.10	3.996 ± 0.054	99.70	1.86	-0.30	3.988 ± 0.078
	MXF								
Method A	0.5	100.60	0.73	0.60	0.503 ± 0.004	99.75	1.12	-0.25	0.499 ± 0.006
	1.0	99.60	1.15	-0.40	0.996 ± 0.012	99.90	1.47	-0.10	0.999 ± 0.015
	2.0	99.80	1.30	-0.20	1.996 ± 0.027	100.70	1.90	0.70	2.014 ± 0.040
Method B	1.0	100.30	0.87	0.30	1.003 ± 0.009	99.80	0.85	-0.20	0.998 ± 0.009
	2.0	100.80	0.92	0.80	2.016 ± 0.019	100.30	1.40	0.30	2.006 ± 0.029
	4.0	99.80	1.14	-0.20	3.992 ± 0.048	100.90	1.67	0.90	4.036 ± 0.071
Method C	0.5	99.60	0.96	-0.40	0.498 ± 0.005	100.20	0.93	0.20	0.501 ± 0.005
	1.0	100.40	1.45	0.40	1.004 ± 0.015	99.90	1.20	-0.10	0.999 ± 0.013
	2.0	100.20	1.56	0.20	2.004 ± 0.033	99.80	1.46	-0.20	1.996 ± 0.031

[a] RSD%, percentage relative standard deviation; R.E%, percentage relative error.
[b] Mean ± standard error.

Table 2: Evaluation of intra-day and inter-day precision and accuracy for the studied drugs obtained by the proposed methods.

under the respective procedures. 1.0 mL of KBr (1.0%) was chosen as optimum volume in 10 mL total volume to accelerate the oxidation process.

Effect of time and temperature

The reaction time between GMF or MXF and NBS was studied by standing the drug solution after mixing with NBS for different intervals of time in the presence of 1.0 mL of 5.0 M HCl and the results indicated that time of 5.0-10 min was required to complete of the reaction. Therefore, a 10 min reaction time was fixed as optimum after the addition of NBS. A sufficient time to completely bleach dyes due to unreacted NBS was found to be 5.0 min, and the same was fixed in all subsequent studies. Raising the temperature does not accelerate the oxidation process and does not give reproducible results, so the optimum temperature is the ambient (25 ± 1°C). The measured colour was found to be stable for several hours in the presence of the reaction product/s in the three methods.

Effect of sequence of addition

Drug–acid–NBS–KBr-(dye) is the optimum sequence of addition; other sequences gave lower absorbance values under the same experimental conditions.

Method of validation

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

Linearity and sensitivity: Under the optimum conditions a linear correlation was found between absorbance λmax and concentration of GMF in the range of (0.1-4.8 µg mL^{-1}) and of MXF in the range of (0.2-4.0 µg mL^{-1}) (Figure 3). The calibration graph is described by the equation:

$$A = a + b\,C \tag{1}$$

(Where A = absorbance, a = intercept, b = slope and C = concentration in µg mL^{-1}) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [60] and compiled in Table 1. The results attest to the sensitivity of the proposed methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s \tag{2}$$

where σ 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 concentrations of the GMF or MXF were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2. 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:

$$\%R.E. = \left[\frac{found - taken}{taken}\right] \times 100 \tag{3}$$

The assay procedure was repeated six 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 different days to evaluate intermediate precision (inter-day precision).

For the same concentrations of drugs inter- and intra-day accuracy of the methods were also evaluated. The percentage recovery values with respect to found concentrations of each drug were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in (Table 2) shows that the proposed methods are very accurate.

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 spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients present in the placebo mixture. The absorbance obtained in spectrophotometry was the same as those of the respective reagent blanks confirmed the non-interference by the additives.

A separate test was performed by applying the proposed methods to the determination of GMF or MXF in a synthetic mixture. To the placebo blank prepared above, 200 mg of GMF or MXF was added, homogenized and the solution of the synthetic mixture was prepared as done under "assay procedure for tablets". The filtrate was collected in a 100-mL flask. The resulted synthetic mixture extract (2000 µg
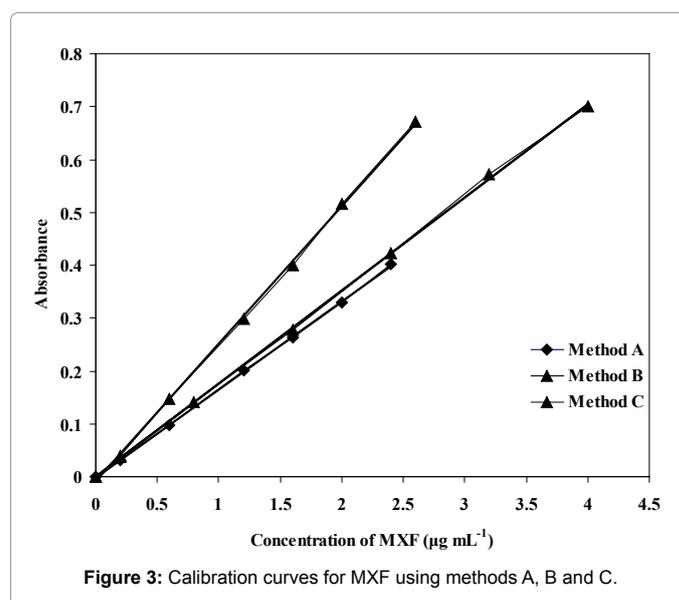

Figure 3: Calibration curves for MXF using methods A, B and C.

Drug	GMF in synthetic mixture taken (µg mL^{-1})	Recovery %^{b} ± SD	MXF in synthetic mixture taken ^{a} (µg mL^{-1})	Recovery %^{a} ± SD
Method A	0.5	98.90 ± 0.69	0.5	98.60 ± 0.55
	1.0	99.20 ± 1.09	1.0	99.00 ± 0.76
	1.5	99.50 ± 1.17	2.0	99.20 ± 0.98
Method B	0.5	98.80 ± 0.73	1.0	99.10 ± 0.70
	1.0	99.30 ± 0.96	2.0	98.50 ± 0.98
	1.5	98.70 ± 1.10	4.0	99.60 ± 1.24
Method C	1.0	99.10 ± 0.60	0.5	99.40 ± 1.07
	2.0	99.40 ± 1.20	1.0	99.10 ± 1.26
	4.0	98.50 ± 0.97	2.0	98.70 ± 1.30

^{a} Mean value of five determinations

Table 3: Recovery of drug from synthetic mixture.

Methods	Nominal amount concentration (µg mL^{-1})	RSD%			
		Robustness		Ruggedness	
		Variable alerted [a]			
		Acid volume (n=3)	Reaction time (n=3)	Different analysts (n=3)	Different instruments (n=3)
GMF					
Method A	0.5	0.87	1.47	1.20	0.90
	1.0	1.14	1.98	1.06	1.10
	1.5	2.10	2.40	0.96	1.82
Method B	0.5	1.27	1.18	0.98	1.03
	1.0	1.65	1.75	1.20	1.30
	1.5	2.04	1.53	1.70	1.92
Method C	1.0	1.39	1.09	1.06	1.45
	2.0	1.56	1.61	1.50	1.70
	4.0	2.20	1.08	2.45	1.86
MXF					
Method A	0.5	1.17	0.93	0.96	1.28
	1.0	1.54	1.05	1.41	1.67
	2.0	1.90	1.90	2.30	2.42
Method B	1.0	1.32	0.88	1.32	1.05
	2.0	1.85	1.42	1.90	1.80
	4.0	2.37	2.05	2.50	2.60
Method C	0.5	1.09	1.16	1.46	1.30
	1.0	1.50	1.75	2.55	1.64
	2.0	1.95	2.09	2.30	2.48

[a]Volume of 5.0 M HCl is (1.0 ± 0.2 mL) and reaction time is (10 ± 2.0 min) (after adding NBS) were used.

Table 4: Results of method robustness and ruggedness (all values in %RSD) studies.

Sample	Recovery% ± SD			
	Reported methods [c]	Proposed methods		
		Method A	Method B	Method C
Factive Tablets				
X ± SD [a]	100.08 ± 0.56	99.84 ± 0.69	100.20 ± 0.72	99.65 ± 0.48
t-Value [b]		0.60	0.29	1.30
F-Value [b]		1.52	1.65	1.36
Flobiotic tablets				
X ± SD [a]	99.94 ± 0.68	100.15 ± 0.82	99.70 ± 0.47	99.80 ± 0.60
t-Value [b]		0.44	0.65	0.35
F-Value [b]		1.45	2.09	0.77
GemiQue tablets				
X ± SD [a]	99.85 ± 0.49	99.45 ± 0.65	100.10 ± 0.53	99.50 ± 0.37
t-Value [b]		1.10	0.77	1.27
F-Value [b]		1.76	1.17	1.75
Avelox® tablets				
X ± SD [a]	99.03 ± 0.97	99.60 ± 1.16	99.70 ± 0.84	99.40 ± 0.76
t-Value [b]		0.84	1.17	0.67
F-Value [b]		1.43	1.33	1.63
Moxiflox tablets				
X ± SD [a]	99.34 ± 0.34	99.54 ± 0.42	99.58 ± 0.50	99.70 ± 0.48
t-Value [b]		0.83	0.89	1.37
F-Value [b]		1.53	2.16	1.99
Moxifloxacin tablets				
X ± SD [a]	99.94 ± 0.92	99.90 ± 0.88	100.20 ± 1.15	100.05 ± 1.20
t-Value [b]		0.07	0.39	0.16
F-Value [b]		1.09	1.56	1.70

[a]Mean for six independent analyses.
[b]Theoretical values for t and F-values at five degree of freedom and 95% confidence limit are (t=2.57) and (F=5.05).
[c]Reference spectrophotometric methods for GMF [39] and MXF [50].

Table 5: Application of the proposed methods for the determination of GMF and MXF in their pharmaceutical preparations.

Formulations	Taken Conc. from tablet ($\mu g\ mL^{-1}$)	Method A			Method B			Method C		
		Pure drug added ($\mu g\ mL^{-1}$)	Total found ($\mu g\ mL^{-1}$)	Recovery% [a] ± SD	Pure drug added ($\mu g\ mL^{-1}$)	Total found ($\mu g\ mL^{-1}$)	Recovery% ± SD	Pure drug added ($\mu g\ mL^{-1}$)	Total found ($\mu g\ mL^{-1}$)	Recovery% ± SD
Factive tablets	0.5	0.5	0.996	99.60 ± 0.98	0.5	0.998	99.80 ± 0.76	1.0	1.503	100.20 ± 0.81
		1.0	1.496	99.70 ± 1.16	1.0	1.523	100.15 ± 1.22	2.0	2.505	100.20 ± 1.10
		1.5	1.998	99.90 ± 1.02	1.5	1.988	99.40 ± 1.19	4.0	4.482	99.60 ± 1.34
Flobiotic tablets	0.5	0.5	0.995	99.50 ± 0.76	0.5	1.001	100.10 ± 0.92	1.0	1.497	99.80 ± 0.68
		1.0	1.497	99.80 ± 0.89	1.0	1.496	99.70 ± 0.09	2.0	2.488	99.50 ± 1.04
		1.5	1.984	99.20 ± 1.25	1.5	1.984	100.10 ± 1.13	4.0	4.464	99.20 ± 1.27
GemiQue tablets	0.5	0.5	0.999	99.90 ± 0.67	0.5	0.993	99.30 ± 1.30	1.0	1.505	100.30 ± 0.86
		1.0	1.496	99.70 ± 0.96	1.0	1.495	99.65 ± 0.40	2.0	2.485	99.40 ± 0.93
		1.5	1.990	99.50 ± 0.74	1.5	1.996	99.80 ± 0.08	4.0	4.482	99.60 ± 1.06
Avelox® tablets	0.5	0.5	1.005	100.50 ± 0.98	1.0	1.503	100.2 ± 0.73	0.5	1.004	100.40 ± 0.67
		1.0	1.484	98.90 ± 1.12	2.0	2.490	99.60 ± 1.15	1.0	1.488	99.20 ± 1.19
		1.5	1.994	99.70 ± 0.65	3.0	3.471	99.17 ± 1.29	1.5	2.002	100.10 ± 1.23
Moxiflox tablets	0.5	0.5	0.987	98.70 ± 0.82	1.0	1.493	99.50 ± 0.87	0.5	0.993	99.30 ± 0.75
		1.0	1.493	99.50 ± 0.96	2.0	2.508	100.30 ± 1.35	1.0	1.498	99.85 ± 1.45
		1.5	1.992	99.60 ± 1.10	3.0	3.472	99.20 ± 1.58	1.5	1.989	99.45 ± 1.60
Moxifloxacin tablets	0.5	0.5	0.998	99.80 ± 0.78	1.0	1.503	100.20 ± 0.97	0.5	0.997	99.70 ± 0.71
		1.0	1.502	100.10 ± 1.10	2.0	2.486	99.45 ± 1.49	1.0	1.491	99.40 ± 1.29
		1.5	1.996	99.80 ± 1.30	3.0	3.490	99.70 ± 1.70	1.5	1.986	99.30 ± 1.70

[a]Mean value of three determinations

Table 6: Results of recovery experiments by standard addition method.

mL^{-1} in GMF or MXF) was diluted stepwise with water to get 10 and 20 $\mu g\ mL^{-1}$ solution and analysed by spectrophotometric methods and the corresponding % recovery of GMF or MXF was ranged from 98.50-99.60% with standard deviation of 0.55-1.30 (n=5). The results of this study shown in Table 3 indicate that the inactive ingredients present in the synthetic mixture did not interfere in the assay. These results further demonstrate the accuracy as well as the precision of the proposed methods. These results complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and ruggedness: For the evaluation of method robustness, volume of HCl and reaction time (between NBS and drug) were slightly varied deliberately. The analysis was performed with altered conditions by taking three different concentrations of drug and the methods were found to remain unaffected as shown by the RSD values in the ranges of 0.87-2.40% and 0.88-2.37% for GMF and MXF, respectively. Methods ruggedness was expressed as the RSD of the same procedure applied by three different analysts as well as using three different instruments (spectrophotometers). The inter-analysts RSD were in the ranges 0.96-2.45% and 0.96-2.55% for GMF and MXF, respectively whereas the inter-instruments RSD ranged from 0.90-1.92% and 1.05-2.60% for GMF and MXF, respectively suggesting that the developed methods were rugged. The results are shown in Table 4.

Application to formulations

The proposed methods were applied to the determination of GMF or MXF in tablets. The results in Table 5 showed that the methods are successful for the determination of GMF and MXF and that the excipients in the dosage forms do not interfere. The results obtained from the assay of GMF or MXF by the proposed methods and reference methods for GMF [39] and MXF [50] for the same batch of material is presented in Table 5. The results agreed well with the label claim and also were in agreement with the results obtained by the reference methods. When the results were statistically compared with those of the reference methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95%

confidence level did not exceed the tabulated values of 2.57 and 5.05, respectively, for five degrees of freedom [61]. Hence, no significant difference existed between the proposed methods and the reference methods with respect to accuracy and precision.

Recovery studies

To study the reliability and accuracy of the proposed methods, a standard addition technique was followed. This study was performed by spiking a fixed amount of tablet preparation to three different levels of pure drug. The total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard (pure drug) was calculated from:

$$\% \mathrm{Re\,cov}\,ery = \frac{[C_F - C_T]}{C_P} \times 100 \qquad (4)$$

where C$_F$ is the total concentration of the analyte found, C$_T$ is a concentration of the analyte present in the tablet preparation, C$_P$ is a concentration of analyte (pure drug) added to tablet preparation. Results of this study presented in Table 6 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets.

Conclusion

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 GMF or MXF. The methods are also useful for their high tolerance limit for common excipients found in drugs 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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