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Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of some fluoroquinolone antibacterials using ammonium reineckate

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Abstract

Three accurate, rapid and simple atomic absorption spectrometric (AAS), conductometric and colorimetric methods were developed for the determination of gatifloxacin (GTF), moxifloxacin (MXF) and sparfloxacin (SPF). The proposed methods depend upon the reaction of ammonium reineckate with the studied drugs to form stable precipitate of ion-pair complexes, which was dissolved in acetone. The pink coloured complexes were determined either by AAS or colorimetrically at λ_{max} 525 nm directly using the dissolved complex. Using conductometric titration, the studied drugs could be evaluated in 50% (v/v) acetone. The optimizations of various experimental conditions were described. Optimum concentration ranges for the determination of GTF, MXF and SPF were 5.0–150, 40–440 μg mL^{-1} and 0.10–1.5 mg mL^{-1} using atomic absorption (AAS), conductometric and colorimetric methods, respectively. Detection and quantification limits are ranges from 1.5 to 2.3 μg mL^{-1} using AAS method or 30–45 μg mL^{-1} using colorimetric method. The proposed procedures have been applied successfully to the analysis of these drugs in pharmaceutical formulations and the results are favourably comparable to the reference methods.

Keywords: Gatifloxacin; Moxifloxacin; Sparfloxacin; Determination; Ion-pair complex; Ammonium reineckate; Dosage forms

1. Introduction

Quinolones have been found to possess an antibacterial property. Fluorinated 4-quinolone derivatives have a broad-spectrum antibacterial activity against many gram-positive and gram-negative bacteria through inhibition of their DNA gyrase.

Gatifloxacin (GTF) [1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid], Moxifloxacin (MXF) [1-cyclopropyl-7-[(1*S*,6*S*)-2,8-diazabicyclo [4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo-3-quinoline-carboxylic acid], and Sparfloxacin (SPF) [rel-5-amino-1-cyclopropyl-7-{(3*R*,5*S*),3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid] are new additions to the class of 4-fluoroquinolones carboxylic acid antibacterials that are widely used in the treating of respiratory trace and urinary trace infections [1]. The structures of the cited drugs are shown in Table 1.

No official (pharmacopoeia) method has been found for the assay of GTF, MXF and SPF in their formulations. However, several methods have been reported for their analysis including UV–vis spectrophotometry [2–12], spectrofluorometry [13–16], atomic absorption spectrophotometry [17], electrophoresis [18–21], chromatography [22–37], flow injection analysis [38,39] and electrochemical methods [40–47].

Reineckate salt is ammonium tetrathiocyanotodiamminochromate (III) monohydrate in which it can be used for quantitative determination of many pharmaceutical compounds applying spectrophotometric, conductometric and AAS [48–51].

The development of AAS, conductometric and colorimetric methods for these drugs is worthwhile. Herein, three different techniques for the simple and accurate determination of these drugs mentioned above were investigated. Ammonium reineckate is used to form ion-pair complexes with the studied drugs with good chromophore. The purpose of the present investigation is to develop a simple, accurate and precise AAS, conductometric, and colorimetric methods for the determination of gatifloxacin (GTF), moxifloxacin (MXF) and sparfloxacin

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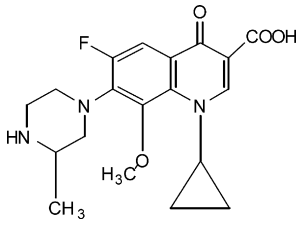
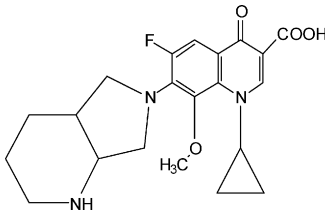
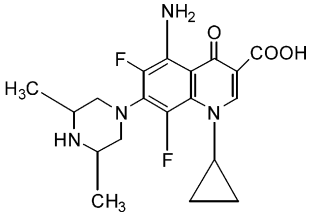
Table 1
Selected fluoroquinolones

Commercial name	Gatifloxacin (GTF)	Moxifloxacin (MXF)	Sparfloxacin (SPF)
Chemical formula			

(SPF) compared to the other reported methods and to apply the procedures to various dosage forms.

2. Experimental

2.1. Apparatus

The pH values of solutions were measured using an Orion research model 601 A/digital ionalyzer pH-meter.

The absorption spectra for all measurements were carried out using Kontron 930 (UV–vis) spectrophotometer equipped with 10 mm quartz cells.

A YSI model 32 M conductance meter (Yellow Springs Instrument Co., Yellow Springs, DH, USA) was used. The measurement range was 1.0–10.0 μS with maximum error of ±0.2%. The YSI model 3417 dip-type cell was used with a cell constant, K_{cell}, of 1.0.

The atomic absorption measurements for the determination of chromium ion were carried out using a Hitachi atomic absorption Z-6100 polarized Zeeman spectrometry. For AAS, the chromium was measured at λ_{max} 357.87 nm, slit width, 0.2 nm, relative noise, 1.0, detection limit, 0.01 μg mL^{-1}, linear dynamic range, 0.01–100 μg mL^{-1}, lamp current, 5.0 mA and integration time, 30 s, the flame used was the acetylene–air mixture.

2.2. Materials and reagents

All solvents and chemicals used are of analytical grade and double distilled water was used to prepare all solution.

- 0.01 M hydrochloric acid aqueous solution (Aldrich).
- Gatifloxacin reference standard was provided by Bristol Myers Squibb Company Egypt, its potency was 99.6 ± 0.70% by HPLC method [25].
- Moxifloxacin reference standard was supplied by Bayer, Germany, its potency was 98.4 ± 0.62% by UV spectrophotometric method [52].
- Sparfloxacin reference standard was supplied by Jedco Company, Cairo, Egypt; its potency was 98.7 ± 0.85% by UV spectrophotometric method [53].

A stock standard solution of 1 mg mL^{-1} was prepared by dissolving an exact weight (0.1 g) of the pure analytical-reagent grade drug in about 70 ml of double distilled water, to which 0.01 M hydrochloric acid was added in a 100 ml measuring flask. The mixture was warmed at 50 °C in a water bath for 5.0 min, agitated by an electrical shaker for another 5.0 min, cooled to room temperature and diluted to volume with double distilled water. The solutions were stable for at least 1 week if they had been stored in a cool (<25 °C) and dark place.

Stock solution, 5×10^{-3} M ammonium reineckate (Aldrich product) solution was also prepared by dissolving appropriate weight in 100 ml double distilled water.

2.3. Formulations

The following commercial formulations were subjected to the analytical procedures:

- Tequin tablets (Bristol Myers Squibb Company, Egypt) labeled to contain 400 mg GTF/tablet.
- Floxin tablets (Global Napi Co., Egypt) labeled to contain 400 mg GTF/tablet.
- Avalox tablets (Bayer, Germany), labeled to contain 400 mg MXF/tablet.
- Parox tablets (Jedco Company, Cairo, Egypt), labeled to contain 200 mg SPF/tablet.
- Sparatec tablets (Uni-Pharm. Co., Egypt), labeled to contain 200 mg SPF/tablet.

2.4. General procedures

2.4.1. Atomic absorption spectral procedure

An aliquot containing 0.5–15.0 mg of the investigated drug was transferred into a 10 ml calibrated flask, 4.0 ml of 5×10^{-3} M of ammonium reineckate and 1.0 ml of 0.01 M HCl were added successively. The mixture was left to stand for 10 min and then the precipitate was filtrated. The precipitate is separated and dissolves in least amount of acetone, and completed to the mark in a 100 ml calibrated flask with water. This solution is then aspirated directly in the atomic absorption spectrometer and measured the chromium ion concentration. Calculate the concentration of the tested drug from the relevant calibration graph.

2.4.2. Conductometric procedure

A volume containing 2.0–21 mg of drug was transferred to a 50 ml calibrated flask and made up to the mark with 50% (v/v) acetone–water mixture. The contents of the calibrated flask were transferred to a beaker and the conductivity cell was immersed. 5×10^{-3} M ammonium reineckate solution was then added from a microburette and the conductance was measured subsequent to each addition of reagent solution and after thorough stirring. The conductance reading, taken 2.0 min after each addition, was corrected for dilution [54] by means of the following equation, assuming that conductivity is a linear function of dilution.

$$\Omega^{-1}_{\text{correct}} = \Omega^{-1}_{\text{obs}} \left[\frac{V_1 + V_2}{V_1} \right]$$

where Ω^{-1}_{obs} is the observed electrolytic conductivity, V_1 the initial volume ad V_2 is the volume of reagent added.

A graph of corrected conductivity versus the volume of added titrant was constructed and the end-point determined. 0.1 ml of 5×10^{-3} M ammonium reineckate is theoretically equivalent to 0.116, 0.114 and 0.12 mg of GTF, MXF and SPF, respectively. The procedure takes 15–30 min in all.

2.4.3. Colorimetric procedure

Proceed as above in AAS procedure "dissolved in acetone" and then completed to the mark in 10 ml calibrated flask. The absorbances of solutions were measured at 525 nm, against a reagent blank solution prepared in the same way without drug. The calibration graph was obtained by applying the procedure, using standard drug solutions.

2.5. Preparation of samples (tablets)

The contents of 20 tablets of each of the studied drugs were thoroughly ground. A quantity equivalent to 200 mg drug was accurately weighed into a 100 ml volumetric flask, completed to volume with bidistilled water, filtered and the procedure was completed as under the previous methods.

3. Results and discussion

According to Babko [55], large number of analytically important complexes consists of the system metal ion–electronegative ligand–organic base. Most of these complexes are extractable in the usual organic solvents such as hydrocarbons and halogenated derivatives. GTF, MXF and SPF are found to react with ammonium reineckate to form stable ion-pair complexes. These complexes are sparingly soluble in aqueous solution, but are readily soluble in acetone.

Investigations were carried out to establish the most favourable conditions for the ion-pair complex formation of GTF, MXF and SPF with ammonium reineckate to achieve sharp end point and/or maximum colour development, in the determination of the drug. The influence of some variables on the reaction has been tested as follow.

3.1. Conditions for conductometric titrations

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below. Three different titrations were attempted: (i) aqueous drug solution with aqueous reagent solution, (ii) acetone drug solution with acetone reagent solution and (iii) drug solution with reagent solution, both in acetone–water (50%, v/v) mixture. Preliminary experiments showed that procedure (iii) were the most suitable for successful results because in procedures (i) and (ii) precipitates were formed which caused some errors. The reagent concentration in each titration must be not less than 10 times that of the drug solution in order to minimize the dilution effect on the conductivity through the titration. The optimum concentration of ammonium reineckate was 5×10^{-3} M to achieve a constant and highly stable conductance reading after 2.0 min mixing. Concentrations less than these led to unstable readings and more time was needed to obtain constant conductance values. On raising the temperature to 40 °C, no change in the conductance reading was observed, whereas above which, the conductance value changed and so changed the shape of the titration curve.

Representative titration curves are shown in Fig. 1. Two straight lines are obtained, intersecting at the end-point, the first branch ascending and the second descending. The increase of conductance may be attributed to the formation of ion-pair in solution as a result of the complexation reaction. After the end-point, the titration curves indicate a continuous decrease of conductance, despite the excess of the reagent. This may be due to further ionic condensation, leading to species of lower mobility.

3.2. Conditions for colorimetric method

3.2.1. Effect of reagent concentration

Experiments was carried out in which the volume was kept constant at 10 ml while the concentration of reagent was increased; revealed that 4.0 ml of 5×10^{-3} M is the optimum concentration (Fig. 2). The excess reagent used is probably as

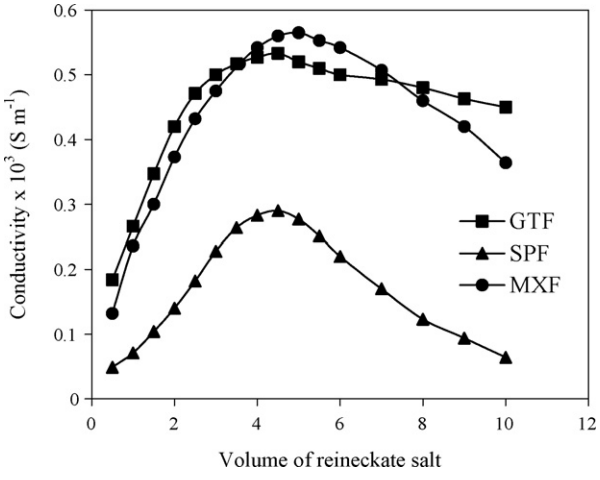


Fig. 1. Conductometric titration of 5.25, 5.70 and 5.40 mg of GTF, MXF and SPF using 5×10^{-3} M ammonium reineckate.

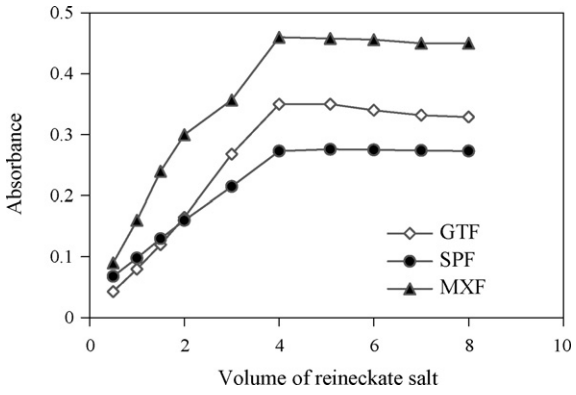


Fig. 2. Effect of 5×10^{-3} M ammonium reineckate on the absorbance of 0.7 mg mL^{-1} of the studied drugs.

a result of dissociation in aqueous medium as fraction of the ion-pair formed.

3.2.2. Effect of acidity

Different acid media was used to increase the colour intensity of the formed precipitated ion-pair. Sulfuric, phosphoric, hydrochloric and acetic acid were tested. The optimum one was hydrochloric acid of 0.01 M concentration, since the results are highly concordant at this media. Moreover, the amount of 0.01 M HCl added to 10 ml was found to be 1.0 ml that gave marginally the best results.

3.2.3. Effect of solvent

Acetone was found to be the best solvent for dissolving the precipitated ion-pair formed in aqueous acidic media. On the other hand, dioxane and propanol are possible substitutes, but methanol, ethanol, benzene and chloroform were unsuitable owing to the limited solubility of ion-pair in these solvents.

3.3. Optimization of AAS measurements

It was not practical to aspirate the dissolved ion-pair in acetone to the atomic absorption spectrometer. It is better to dilute the formed ion-pair with water in a ratio 10% (v/v) acetone aqueous media, which can be aspired directly to the AAS.

3.3.1. Solubility and stability of the precipitated reineckates

Trials to find out the best solvent to dissolve drug-reineckate precipitate were performed using distilled water, acetone, dioxan, methanol, ethanol and propanol. Then the stability of the produced colour in each solvent was examined periodically at different time intervals over 24 h.

3.3.2. Washing liquid and solvent

The washing liquid of choice was ice water and acetone was found to be the best solvent for dissolving the precipitated ion-pair formed in aqueous acidic media. The colour of drug-reineckate acetone solution was stable for at least 24 h.

Table 2
Analytical characteristic of the AAS procedure ($n = 6$)

Parameters	GTF	MXF	SPF
Range of determination (μg mL^{-1})	10–150	5.0–110	10–140
Detection limits (μg mL^{-1})	1.7	1.5	2.3
Quantification limit (μg mL^{-1})	5.67	4.9	7.67
Regression equation[a]			
Slope (a)	0.013	−0.006	0.0003
Intercept (b)	0.0261	0.0266	0.0124
Correlation coefficient (r)	0.9996	0.9996	0.9993
Relative standard deviation (%)	0.92	0.88	1.04
Range of errors (%)	±1.4	±1.2	±1.6

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}.

3.4. Stoichiometric relationships

For the atomic absorption spectrometric method, Job's method of continuous variation [56] indicated a molar ratio of 1:2 drug to reineckate.

3.5. Reaction mechanism

$$\text{Drug} + \text{ammonium reineckate} \overset{\text{HCL}}{\rightleftharpoons} \text{ammonium chloride} + \text{drug-reineckate}$$

$$\text{GTF} + 2NH_4[Cr(NH_3)_2(SCN)_4] \overset{0.01\,\text{M HCl}}{\rightleftharpoons} [\text{GTF}]\,[Cr(NH_3)_2(SCN)_4]_2 + 2NH_4Cl$$

3.6. Method validation

3.6.1. Linearity

At described experimental conditions for GTF, MXF and SPF determination, standard calibration curves for the studied drugs with ammonium reineckate. For AAS method, calibration graphs with good linearity were obtained as recorded in Table 2. The linear regression equations were also calculated. Correlation coefficient, intercept and slope relative standard deviation values for the calibration data calculated using the least squares method. The linear regression equation was applied to the result obtained from conductometric titration (Table 3) to establish whether the proposed method exhibits any fixed or proportional bias. For spectrophotometric method, Beer's law is valid within the concentration range 0.15–1.30, 0.1–1.10 and 0.2–1.5 mg mL^{-1} for GTF, MXF and SPF, respectively. For more accurate analysis, Ringbom optimum concentration range was calculated to be 0.25–1.10, 0.2–0.95 and 0.3–1.25 mg mL^{-1} for GTF, MXF and

Table 3
Linear regression analysis for the studied drugs using reineckate salt

Parameters	GTF	MXF	SPF
Optimum concentration (μg mL^{-1})	40–400	40–440	40–360
Shift or intercept of the regression line[a]	0.038	0.028	0.033
Slope of regression line	0.9985	0.9985	0.9978
Relative standard deviation (%)	1.50	1.52	1.75

[a] Observed vs. theoretical.

Table 4
Spectral characteristics and precision data

Parameters	GTF	MXF	SPF
Beer's law limits (mg mL^{-1})	0.15–1.3	0.1–1.1	0.2–1.5
Ringbom range (mg mL^{-1})	0.25–1.10	0.2–0.95	0.3–1.25
Stability of ion-pair (h)	40	48	48
Molar absorpitivity $\times 10^3$ (L mol^{-1} cm^{-1})	1.09	1.075	1.032
Sandell sensitivity (μg cm^{-2})	0.344	0.373	0.380
Detection limits (μg mL^{-1})	30	45	42
Quantification limit (μg mL^{-1})	100	146	140
Relative standard deviation (%)	1.62	1.80	1.59
Range of errors (%)	±1.2	±1.55	±1.5
Regression equation[a]			
Slope (a)	0.0004	0.0006	0.0005
Intercept (b)	0.0256	0.0384	−0.022
Correlation coefficient (r)	0.9997	0.9996	0.9993

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}.

SPF, respectively. The molar absorptivity and Sandell sensitivity were calculated and recorder in Table 4. The reproducibility of the proposed methods was assessed by running six replicate samples, each containing 70, 200 and 700 μg mL^{-1} using AAS, conductometric, and colorimetric methods, respectively, of the studied drugs in the final assay solution.

3.6.2. Sensitivity

The detection limit (LOD) for the proposed methods were calculated using the following equation [57]:

$$\mathrm{LOD} = \frac{3s}{k}$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were recorded in Tables 2 and 4.

The limits of quantitation, LOQ, defined as [57];

$$\mathrm{LOQ} = \frac{10s}{k}$$

According to this equation, the limit of quantitation is recorded in Tables 2 and 4.

3.6.3. Specificity, precision, and accuracy

Specificity of ion-pair reaction and selective determination of GTF, MXF and SPF, which was the basic nitrogenous compounds with ammonium reineckate reagent could be possible. Percentage relative standard deviation (R.S.D.%) as precision and percentage relative error (Er%) as accuracy of the suggested method was calculated. Precision was carried out by six determinations at five different concentrations in the proposed methods. The percentage relative error calculated using the following equation:

$$\mathrm{Er}\% = \left[\frac{\text{founded} - \text{added}}{\text{added}}\right] \times 100$$

Table 5
The intra-day accuracy and precision of the proposed methods

Method	GTF					MXF					SPF				
	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	R.S.D. (%)	Er%	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery[a] (%)	R.S.D. (%)	Er%	Taken (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	R.S.D. (%)	Er%
Atomic absorption	20	20.1	100.5	0.56	0.5	10	9.997	99.97	0.85	−0.03	20	19.85	99.25	0.26	−0.75
	50	49.86	99.72	0.64	−0.28	30	30.09	100.30	0.47	0.3	40	40.1	100.25	0.83	0.25
	75	74.4	99.20	0.62	−0.8	50	49.625	99.25	0.27	−0.75	60	59.8	99.67	0.94	−0.33
	100	100.03	100.3	0.73	0.03	70	69.622	99.46	0.62	−0.54	80	79.90	99.88	0.73	−0.13
	140	140.5	100.36	0.81	0.36	100	100.07	100.07	0.80	0.07	120	119.5	99.58	0.31	−0.42
Conductometric	75	75.25	100.33	0.46	0.33	50	50.02	100.04	0.33	0.04	60	60.025	100.04	0.66	0.042
	150	148.60	99.07	0.53	−0.93	100	98.94	98.94	0.82	−1.06	120	119.50	99.58	0.57	−0.42
	200	198.54	99.27	0.84	−0.73	200	199.20	99.60	0.73	−0.4	180	179.65	99.80	0.48	−0.19
	250	247.80	99.12	0.90	−0.88	300	298.14	99.38	0.94	−0.62	240	240.36	100.15	0.94	0.15
	350	346.95	99.13	0.43	−0.87	425	424.58	99.90	0.32	−0.1	360	359.14	99.76	0.61	−0.24
Colorimetry	200	200.30	100.15	0.97	0.15	150	148.58	99.05	0.55	−0.95	250	249.75	99.90	0.10	−0.1
	400	396.80	99.20	0.85	−0.8	300	298.05	99.35	0.48	−0.65	500	501.50	100.30	0.89	0.3
	600	593.75	98.96	0.20	−1.04	450	449.55	99.90	0.64	−0.1	750	750.90	100.12	0.78	0.12
	800	800.20	100.025	0.77	0.025	600	600.12	100.02	0.88	0.02	1000	989.50	98.95	0.70	−1.05
	1200	1203		0.94	0.25	1000	997	99.70	0.51	0.3	1500	1499.00	99.93	0.23	−0.07

[a] Average value of six determinations.

Table 6
Application of the proposed method to the determination of the studied drugs in dosage forms

Sample	Official methods	Recovery[a] (%) ± S.D.		
		AAS	Conductometric	Colorimetry
Pure GTF	99.60 ± 0.70	100.03 ± 0.57	99.32 ± 0.83	99.89 ± 0.64
t-value[b]		0.953	0.516	0.612
F-value[b]		1.51	1.41	1.20
Tequin tablets[c] (400 mg GTF/tablet)				
X ± S.D.[a]	99.30 ± 0.30	99.65 ± 0.41	99.72 ± 0.38	99.85 ± 0.35
t-value[b]		1.38	1.73	2.39
F-value[b]		1.87	1.60	1.36
Floxin tablets[c] (400 mg GTF/tablet)				
X ± S.D.[a]	99.85 ± 0.45	99.75 ± 0.68	99.50 ± 0.35	100.05 ± 0.61
t-value[b]		0.245	1.23	0.528
F-value[b]		2.28	1.65	1.84
Pure MXF	98.40 ± 0.62	99.10 ± 0.80	99.20 ± 0.44	99.04 ± 0.94
t-value[b]		1.38	2.11	1.14
F-value[b]		1.66	1.99	2.30
Avalox tablets[d] (400 mg MXF/tablet)				
X ± S.D.[a]	99.55 ± 0.86	100.04 ± 0.69	99.72 ± 0.97	99.90 ± 0.731
t-value[b]		1.12	0.26	0.62
F-value[b]		1.55	1.27	1.38
Pure SPF	98.70 ± 0.85	99.30 ± 0.59	99.08 ± 0.73	99.06 ± 0.81
t-value[b]		1.16	0.68	0.61
F-value[b]		2.08	1.36	1.10
Parox tablets[e] (200 mg SPF/tablet)				
X ± S.D.[a]	99.40 ± 0.60	99.89 ± 0.76	99.75 ± 0.67	99.94 ± 0.53
t-value[b]		1.01	0.78	1.35
F-value[b]		1.60	1.25	1.28
Spara tablets[e] (200 mg SPF/tablet)				
X ± S.D.[a]	99.87 ± 1.08	100.10 ± 0.92	99.70 ± 0.80	99.65 ± 1.12
t-value[b]		0.32	0.25	0.28
F-value[b]		1.38	1.82	1.08

[a] Average of five determinations.
[b] Theoretical values for *t* and *F*-values at five degree of freedom and 95% confidence limit are ($t = 2.78$) and ($F = 6.39$).
[c] (Bristol Myers Squibb Company, Egypt) and (Global Napi Co., Egypt), labeled to contain 400 mg GTF/tablet.
[d] (Bayer, Germany), labeled to contain 400 mg MXF/tablet.
[e] (Jedco Company, Cairo, Egypt) and (Uni-Pharm. Co., Egypt), labeled to contain 200 mg SPF/tablet.

Table 5 shows the values of between-day relative standard deviations for different concentrations of the drugs obtained from experiments carried out over a period of 4 days. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

3.7. Analytical applications

The performance of the proposed methods was assessed by comparison with the reference methods for GTF [25] (based on HPLC determination for GTF) and for MXF and SPF [52,53] (based on the spectrophotometric determination). Mean values were obtained with a Student's *t*- and *F*-tests at 95% confidence limits for five degrees of freedom [57]. The results showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests were less than the theoretical data.

The proposed procedures were applied to determine the studied drugs in their pharmaceutical formulations. The results in Table 6 indicate the high accuracy and precision. As can be seen from Table 6, the proposed method has the advantages of being virtually free from interferences by excipients such as glucose, lactose, and starch or from common degradation products. The results obtained were compared statistically by the student's *t*-test (for accuracy) and the variance ratio *F*-test (for precision) with those obtained by the reference methods [28,52,53] on samples of the same batch (Table 6). The values of *t*- and *F*-tests obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared.

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