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Original research paper

Spectrofluorimetric determination of gemifloxacin mesylate and linezolid in pharmaceutical formulations: Application of quinone-based fluorophores and enhanced native fluorescence

BAHIA ABBAS MOUSSA[1]
MARIANNE ALPHONSE MAHROUSE[1]*
MAHMOUD ALI HASSAN[2]
MICHAEL GAMAL FAWZY[2]

[1] *Pharmaceutical Chemistry Department Faculty of Pharmacy, Cairo University Kasr El-Aini St., Cairo 11562, Egypt*

[2] *Pharmaceutical Medicinal Chemistry Department, Faculty of Pharmacy Egyptian Russian University, Badr City Cairo, 11829, Egypt*

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Quinone-based fluorophores and enhanced native fluorescence techniques were applied for a fast quantitative analysis of gemifloxacin mesylate (GEM) and linezolid (LIN) in pharmaceutical formulations. For this purpose, three sensitive, accurate and precise spectrofluorimetric methods were developed. GEM, as an n-electron donor, reacts with 7,7,8,8-tetracyanoquinodimethane (method A) and 2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone (method B) as π-electron acceptors, forming charge transfer complexes that exhibit high fluorescence intensity at 441 and 390 nm upon excitation at 260 and 339 nm, respectively. Method C depends on measurement of enhanced native fluorescence of LIN in phosphate buffer (pH 5) at 380 nm upon excitation at 260 nm. Experimental factors affecting fluorescence intensity were optimized. Linearity was obtained over concentration ranges 50–500, 10–60 and 20–400 ng mL^{-1} for methods A, B and C, respectively. The developed methods were validated and successfully applied for determination of the cited drugs in tablets.

Keywords: gemifloxacin mesylate, linezolid, 7,7,8,8-tetracyanoquinodimethane, 2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone, fluorimetry, charge transfer complex

Gemifloxacin mesylate (GEM) is a broad-spectrum fluoroquinolone antibacterial agent. Its bactericidal activity depends on inhibition of DNA synthesis. This mode of action involves dual targeting of two bacterial enzymes: DNA gyrase and topoisomerase IV, which are essential for bacterial DNA replication and transcription (1). Chemically, it is designated as (±)-7-[3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate (1, 2) (Fig. 1a).

* Correspondence; e-mail: mariannealphonse@yahoo.com

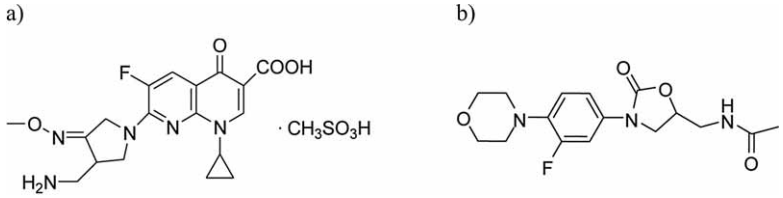

Fig. 1. Chemical structures of: a) gemifloxacin mesylate and b) linezolid.

GEM is not official in any pharmacopoeia. The literature survey revealed several reported analytical approaches for its determination in pharmaceutical dosage forms and in biological fluids. Various spectrophotometric (3–11) and spectrofluorimetric (12, 13) methods were described. Chromatographic methods were also reported and include capillary electrophoresis (14), HPTLC (15) and HPLC (16).

Linezolid [LIN, Fig. 1b, (*S*)-*N*-({3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3--oxazolidin-5-yl}methyl) acetamide], belongs to a new class of antibacterial agents, the oxazolidinones. It exhibits a unique mechanism of action based on inhibition of bacterial protein synthesis. Clinical utility appears in the treatment of serious infections caused by multi-resistant Gram positive bacteria (17).

Only few methods were reported for the determination of LIN in tablets, including spetrophotometric (18), TLC (19) and HPLC (20) methods, either alone or in the presence of alkaline induced degradation products. Estimation of LIN in biological fluids using LC/MS (21) and HPLC (22) was also described.

The widespread use of these antibacterial drugs and the need for clinical and pharmacological studies require fast and sensitive analytical methods for their determination in pharmaceutical formulations. Chromatography, the most common technique applied, generally requires sophisticated instruments and high analysis cost which limit its routine use in analytical laboratories. Therefore, development of alternative methods is desirable. Fluorescence spectroscopy is considered as one of convenient analytical techniques because it possesses good analytical selectivity, improved limit of detection when compared to spectrophotometric methods and wide availability in most laboratories. Therefore, the purpose of the present investigation was to develop sensitive, accurate and precise methods for the determination of GEM and LIN using spectrofluorimetry.

EXPERIMENTAL

Chemicals and reagents

Pure samples of GEM and LIN were kindly provided by El Obour Modern Pharmaceutical Industries Company (Egypt) and their certified purity was 99.8 and 99 %, respectively. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) and 2,5-dichloro-3,6-dihydroxy-*p*--benzoquinone *(p*-chloranilic acid, CLA) were purchased from Sigma-Aldrich Chemie GmbH (Germany) and were freshly prepared as 2.4×10^{-3} mol L^{-1} solutions in acetonitrile. Methanol (HPLC grade, Labscan, Poland) and acetonitrile (HPLC grade, Riedel

de Haën, Germany) were used. Disodium hydrogen phosphate was supplied by EL-Nasr Pharmaceutical Chemicals Co., Egypt. Phosphate buffer was composed of 0.1 mol L^{-1} disodium hydrogen phosphate (EL-Nasr Pharmaceutical Chemicals) in water and adjusted to pH 5 with orthophosphoric acid (Riedel de Haën).

Pharmaceutical formulations

Two pharmaceutical dosage forms were obtained from the local market in Egypt: Factive® tablets (Hikma Pharma, Egypt), labeled to contain GEM mesylate equivalent to 320 mg GEM base per one tablet, and Averzolid® tablets (El Obour Modern Pharmaceutical Industries Company, Cairo, Egypt), labeled to contain 600 mg LIN.

Equipment

Fluorescence spectra were recorded and intensity measurements were made on an RF-1501 spectrofluorimeter (Shimadzu, Japan). A pH meter model 3505 (Jenway, UK) was used for all pH measurements.

Standard solutions

Standard stock solutions of 100 μg mL^{-1} of GEM and LIN were prepared in acetonitrile and in methanol, respectively. Working solutions of GEM, having concentrations of 1 μg mL^{-1} and 0.5 μg mL^{-1}, were prepared from its stock solution by dilution using acetonitrile, for methods A and B, respectively. For method C, further dilution of LIN stock solution was carried out to obtain a working solution of 1 μg mL^{-1} in methanol.

General procedures

Method A (TCNQ method). – Aliquots containing (500–5000 ng) GEM were transferred into a series of 10-mL volumetric flasks. An accurate aliquot of TCNQ solution (2.4×10^{-6} mol L^{-1}) was added, the reaction mixture was mixed and allowed to stand for 40 min. The volume was completed to 10 mL with acetonitrile. After standing for 15 min, fluorescence intensity was measured at the emission wavelength (λ_{em}) of 441 nm upon excitation at 260 nm, against a similarly treated blank. A linear calibration curve was obtained by plotting the fluorescence intensity against the corresponding concentration of the drug and the regression equation was computed.

Method B (CLA method). – Into a series of 10-mL volumetric flasks, accurately measured aliquots of the GEM working standard solution equivalent to 100–600 ng were transferred and an accurate aliquot of CLA solution (1.1×10^{-6} mol L^{-1}) was added. The reaction was left for completion for 5 min before dilution to volume with acetonitrile. After standing for 5 min, fluorescence intensity was measured at λ_{em} 390 nm using the excitation wavelength (λ_{ex}) of 339 nm, against a similarly treated blank. The calibration graph was constructed and the regression equation was computed.

Method C (native fluorescence method). – Varying aliquots of the LIN working standard solution equivalent to 200–4000 ng were accurately measured and transferred into a series of 10-mL volumetric flasks. An accurate aliquot of phosphate buffer (5×10^{-4}

mol L^{-1}, pH 5) was added, the volume was adjusted to the mark with water and mixed well. Fluorescence intensity was measured at λ_{em} at 380 nm (λ_{ex} 260 nm) against a blank similarly prepared without adding LIN. The calibration graph was constructed and the regression equation was computed.

Analysis of pharmaceutical formulations

Ten tablets of each formulation were accurately weighed and finely powdered. A quantity of the mixed powder equivalent to 10 mg of the active component was transferred into a 100-mL volumetric flask, dissolved either in 25 mL acetonitrile (methods A and B) or methanol (method C), stirred for 30 min and then completed to volume with the same solvent. The contents were mixed well and filtered and the first portion of the filtrate was rejected. The filtrate was further diluted quantitatively to obtain suitable concentrations for the analysis by the proposed methods, as described under the general procedures section. The contents of the tablets were calculated using the corresponding regression equations.

Stoichiometry of the reaction

The reaction stoichiometry between GEM and quinone-based reagents (TCNQ and CLA) was investigated by the limiting logarithmic method (23). Two sets of solutions were prepared, the first set had a fixed concentration of GEM and varying concentrations of the reagent while the second one had a fixed concentration of the reagent and varying concentrations of the drug. Logarithms of the obtained fluorescence intensities for the reaction of GEM and each reagent were plotted as a function of the logarithms of the concentrations of the reagent and GEM in the first and second sets of solutions. The slopes of straight lines were computed and the ratios between slopes were calculated.

Methods validation

The developed methods were validated pursuant to the guidelines of the International Conference on Harmonisation (ICH) for validation of analytical procedures (24).

Linearity and range. – Linearity of the methods was evaluated by linear regression analysis, which was calculated by the least square method. Six different concentrations of standard solutions of each of GEM and LIN were analyzed by the developed methods. All measurements were carried out in triplicate. The assays were performed according to the general procedures previously established for the proposed methods. Three calibration curves were obtained by plotting fluorescence intensities *vs.* concentrations of the drugs and regression equations were computed.

Accuracy. – Accuracy of the proposed methods was tested by analyzing different concentrations of GEM and LIN solutions (in triplicate for each concentration). The accuracy was determined in terms of percentage recovery. In addition, the validity of the suggested methods was checked by applying the standard addition technique, in which the recoveries of known amounts of GEM and LIN, added to their sample solutions of known concentrations, were calculated.

Precision. – Method precision was estimated by measuring repeatability (intra-day precision) and intermediate precision (inter-day precision). Intra-day precision was assessed by preparing samples of drug standard solutions at varying concentration levels, covering low, medium and high concentrations of the linearity range and analyzing them in triplicate on the same day using the proposed methods. Inter-day precision was determined by analyzing the same samples on three consecutive days. The precision of the method was expressed as relative standard deviation.

Selectivity. – Selectivity is the ability of the analytical method to measure analyte response in the presence of foreign substances. In the present work, selectivity was ascertained by applying the developed methods to pharmaceutical dosage forms and the resulting emission spectra were checked for the appearance of any new spectra of excipients.

Limit of detection and limit of quantification. – The limit of detection (*LOD*) and limit of quantification (*LOQ*) were determined based on the standard deviation of the intercept of the calibration line (SD) and the slope of regression lines, using the formula: 3.3×SD/slope and 10×SD/slope, respectively.

RESULTS AND DISCUSSION

Sensitive methods for the determination of GEM and LIN using spectrofluorimetry were developed. Two of the proposed methods were based on the formation of fluorescent charge transfer complexes, whilw the third was performed by enhancing the native fluorescence of the drug using phosphate buffer (pH 5).

Method A (TCNQ method) and method B (CLA method)

Excitation and emission spectra. – The reaction of amines as n-electron donors with quinone-based reagents such as 7,7,8,8-tetracyanoquinodimethane (TCNQ) and 2,5-dichloro-3,6-dihydroxy-*p*-benzoquinone (CLA) as π-electron acceptors leads to the formation of charge transfer complexes which have been shown to exhibit sensitive fluorescence (25, 26). Charge transfer spectrofluorimetry is associated with major advantages such as good selectivity, low detection limit and hence high sensitivity. Since GEM structure contains a basic centre, tertiary nitrogen of the pyrrolidine ring, TCNQ and CLA were selected as valuable derivatizing agents to form quinone-based fluorophores. For this reason, the present study was devoted to investigate the reaction between GEM and quinone-based reagents and employ this reaction in the development of two new, simple and sensitive spectrofluorimetric methods for the determination of GEM in tablets. The reaction was performed under optimal experimental parameters and the fluorescence excitation and emission spectra of the charge transfer complexes produced were recorded (Fig. 2).

Optimization of experimental parameters. – Different experimental parameters affecting the fluorescence development of the reaction product and its stability were studied and optimized. Such factors were investigated by varying the parameters, one at a time, keeping the others fixed and observing the effect produced on the fluorescence intensity.

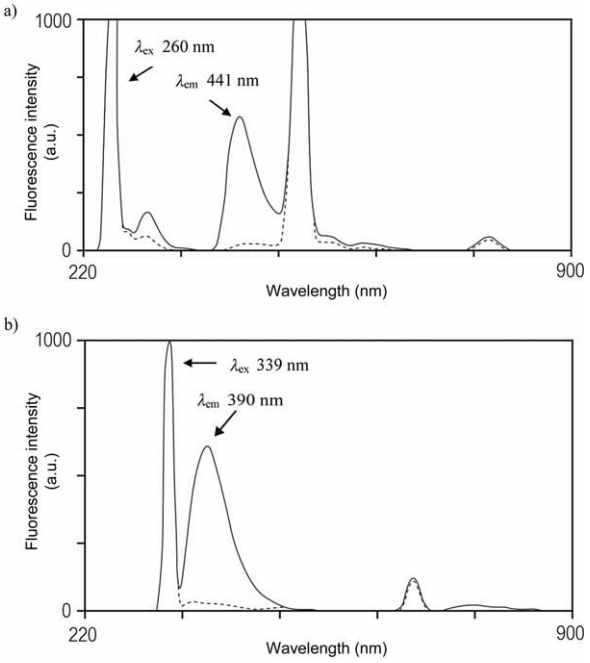

Fig. 2. Fluorescence excitation and emission spectra of the charge transfer complex of: a) GEM (500 ng mL^{-1}) with TCNQ (——) and blank (-----), and b) GEM (60 ng mL^{-1}) with CLA and blank (-----).

The influence of TCNQ and CLA molar concentration was studied. It was found that final molar concentrations of TCNQ and CLA sufficient for the production of maximum and reproducible fluorescence intensity were 2.4×10^{-6} and 1.1×10^{-6} mol L^{-1}, respectively.

Fluorescence spectral characteristics of the charge transfer complex formed in different diluting solvents were compared. The studied solvents involved water, methanol, ethanol, acetonitrile and acetone. Experimental results indicated that acetonitrile afforded the maximum and stable fluorescence emission for both methods (Table I).

Different reaction time intervals (10–45, 2.5–20 min, for methods A and B, respectively) were tested to ascertain the time after which the reaction product attains its highest fluorescence intensity. It was found that the reaction product reached the highest fluorescence within 40 and 5 min, for methods A and B, respectively.

To establish the optimum reaction temperature, GEM (500 and 60 ng mL^{-1}) was allowed to react with TCNQ (2.4×10^{-6} mol L^{-1}) and CLA (1.1×10^{-6} mol L^{-1}), respectively, at different temperatures. Maximum fluorescence intensity was obtained at room temperature (25 °C). Increasing the reaction temperature above room temperature would result in a subsequent decrease in fluorescence intensity of the reaction product (Table I).

Stability of the fluorescent product. – Stability of the formed product was checked by applying the chosen optimum conditions and measuring the fluorescence intensity of the reaction solution (after dilution) at different time intervals. Fluorescence intensity was found to increase and reach a stable value from 15 to 50 min and 5 to 60 min, for me-

Table I. Effects of diluting solvent and temperature on the reactions of GEM with TCNQ and CLA and effects of buffer pH and concentration on the enhancement of native fluorescence intensity of LIN

GEM				LIN					
Solvent	Relative fluorescence intensity[a]		Temperature (°C)	Relative fluorescence intensity[a] (in acetonitrile)		Buffer pH	Relative fluorescence intensity[a]	Buffer concentration (mol L^{-1})	Relative fluorescence intensity[a]
	TCNQ method	CLA method		TCNQ method	CLA method				
Acetone	1	7	25	3	1.5	1	1	2×10^{-4}	1
Acetonitrile	123	10	40	2.5	1	3	2	3×10^{-4}	1
Ethanol	63	2	60	1	1	5	3	4×10^{-4}	1.5
Methanol	46	1	80	1	1	7	1	4.5×10^{-4}	2
Isopropanol	33	2						5×10^{-4}	3
								6×10^{-4}	2

[a] Relative fluorescence intensity is the ratio relative to the lowest intensity, in the respective column.

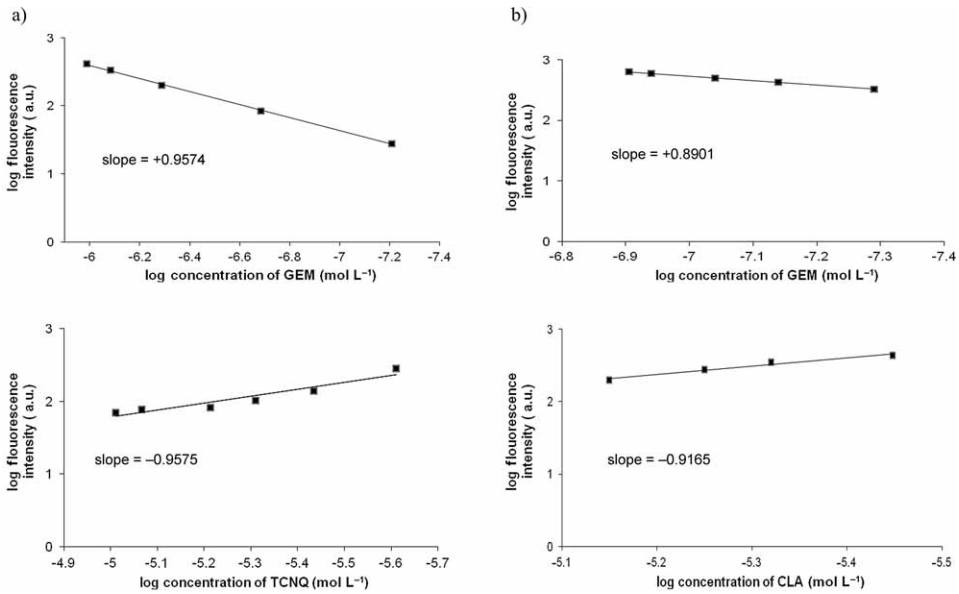

Fig. 3. Determination of the stoichiometry of the reaction of GEM with: a) TCNQ and b) CLA, by the limiting logarithmic method.

thods A and B, respectively. Therefore, measurements were carried out after standing for 15 and 5 min, for methods A and B, respectively. Stability of the fluorescent products up to an hour allows the analysis of a large number of samples in quality control laboratories.

Stoichiometry and mechanisms of reactions. – Molar ratio of the reactants (drug/reagent) in the charge transfer complex was determined by the limiting logarithmic method (23). Two straight lines were obtained by plotting log fluorescence intensity *vs.* log molar concentration of the drug in one plot and log fluorescence intensity *vs.* log molar concentration of the reagent in another one (Figs. 3a and 3b). The values of slopes were 0.9574, –0.9575 and 0.8901, –0.9165 for methods A and B, respectively, confirming the 1:1 molar ratio of the reactants for both methods. This finding was anticipated by the presence of an electron donating centre, tertiary nitrogen of pyrrolidine ring in GEM molecule, which interacts with TCNQ and CLA (strong π-electron acceptors) forming charge transfer complexes of n-π type (Fig. 4).

Method C (enhanced native fluorescence)

Excitation and emission spectra. – LIN was found to emit weak fluorescence in aqueous solution and in methanol. However, this weak native fluorescence was enhanced in phosphate buffer (pH 5). This fact has been used to develop an improved spectrofluorimetric method for the determination of LIN in tablets. Fig. 6 shows the fluorescence

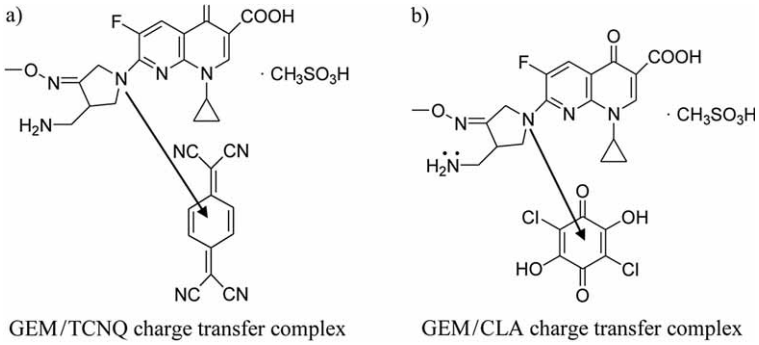

Fig. 4. The suggested structures of: a) GEM/TCNQ and b) GEM/CLA charge transfer complexes.

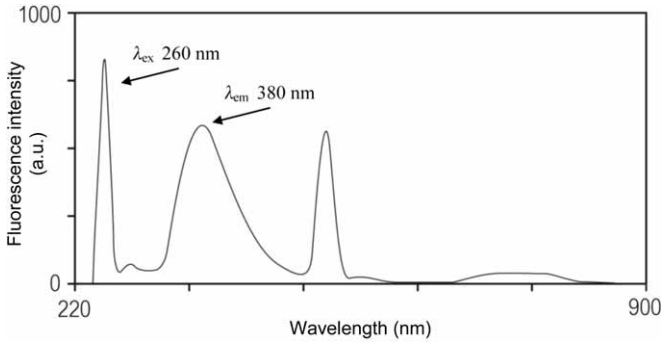

Fig. 5. Fluorescence excitation and emission spectra of LIN (350 ng mL^{-1}) in water.

spectrum of LIN having an excitation maximum at 260 nm and an emission maximum at 380 nm. Various experimental factors that affect the enhancement of fluorescence intensity were investigated.

Optimization of experimental parameters. – The influence of pH on the native fluorescence of LIN was investigated by measuring the fluorescence intensity of the drug using buffer solutions of varying pH values. Maximum fluorescence intensity was obtained upon using phosphate buffer of pH 5. The increase in fluorescence intensity was tested by adding different molar concentrations of the buffer ($2 \times 10^{-4} - 6 \times 10^{-4}$ mol L^{-1}). It was observed that 5×10^{-4} mol L^{-1} phosphate buffer (pH 5) showed the highest fluorescence intensity (Table I).

Upon diluting the solution of the drug in phosphate buffer (pH 5) with different solvents, water was found to be the most suitable solvent. This was attributed to the lowest background noise and satisfactory results obtained over the linearity range of 20–400 ng mL^{-1}.

Methods validation

Linearity and range. – Under the optimum experimental conditions, linear correlations were established over the concentrations ranges of 50–500, 10–60 and 20–400 ng mL^{-1},

Table II. Assay parameters for the determinations of GEM and LIN

Parameter	GEM		LIN
	TCNQ method (method A)	CLA method (method B)	Native fluorescence method (method C)
Excitation wavelength (nm)	260	339	260
Emission wavelength (nm)	441	390	380
LOD (ng mL^{-1})[a]	7.38	0.86	4.28
LOQ (ng mL^{-1})[a]	22.37	2.60	12.95
Range of linearity (ng mL^{-1})	50–500	10–60	20–400
Correlation coefficient (*R*)	0.9998	0.9998	0.9998
SD_b (%)	0.01	0.07	0.01
SD_a (%)	1.81	2.76	1.0
Confidence limits of the slope	0.8079 ± 0.0167	10.6201 ± 0.1971	0.8937 ± 0.0167
Confidence limits of the intercept	3.0371 ± 5.0162	51.2392 ± 7.6645	130.3979 ± 3.6282
Repeatability (RSD, %)[c]	1.063, 1.056, 0.916	1.358, 0.321, 0.473	1.000, 0.425, 0.712
Intermediate precision (RSD, %)[d]	0.280, 0.739, 0.608	0.195, 0.089, 0.338	1.004, 0.561, 0.454

[a] Limits of detection and quantification are determined *via* calculations (24): *LOD* = 3.3×SD/slope; *LOQ* = 10×SD/slope; SD is standard deviation of the intercept of regression line.

[b] SD_b and SD_a are SD_s of calibration line slope and intercept, resp.

[c] The intra-day precision, average of three concentrations of GEM (150, 250 and 350 ng mL^{-1}, in case of TCNQ method; 25, 35 and 55 ng mL^{-1}, in case of CLA method) and of LIN (90, 230 and 350 ng mL^{-1}, in case of native fluorescence method), repeated three times within a day.

[d] The inter-day precision, average of three concentrations of GEM (150, 250 and 350 ng mL^{-1}, in case of TCNQ method; 25, 35 and 55 ng mL^{-1}, in case of CLA method) and of LIN (90, 230 and 350 ng mL^{-1}, in case of native fluorescence method), repeated three times on three successive days.

for methods A, B and C, respectively. The high values of correlation coefficients > 0.9998 for all the proposed methods indicate excellent correlation between fluorescence intensities and analyte concentrations. The analytical data of the calibration curves including standard deviations of the slope and intercept are summarized in Table II.

Accuracy. – The results of accuracy are revealed in Table III. Good recoveries were obtained, confirming that the developed methods are accurate. In addition, the proposed methods were applied successfully to the determination of GEM and LIN in pharmaceutical dosage forms. By applying the standard addition technique, the mean percentage recoveries of the added standard ranged from 99.6 to 100.7 %, indicating good accuracy of the method. The results of analysis of the pharmaceutical dosage forms and the recovery study are shown in Tables IV and V.

Precision. – RSD values were found to be up to 1.4 % in case of repeatability and up to 1.0 % in the case of intermediate precision, for methods A, B and C, as reported in Table II. Values of the percentage relative standard deviation did not exceed 2 %, proving good precision of the suggested methods.

Selectivity. – No interference from any of the excipients was found at the excitation/emission wavelengths of the examined drugs. In addition, the spectrum of each drug in the tablet solution is identical to the spectrum received by the standard solution at the wavelengths applied. Besides, results of the analysis of pharmaceutical dosage forms, as revealed in Tables IV and V, show good recoveries (ranging between 98.5–101.5 %). Therefore, the proposed methods are highly selective for the determination of GEM and LIN, with no interference from the frequently encountered excipients in pharmaceutical formulations.

Limit of detection and limit of quantification. – *LOD* and *LOQ* values, as summarized in Table I, reveal that the proposed methods have adequate sensitivity, which is higher than that in the reported spectrophotometric methods with *LOD*s ranging 21–124 ng mL^{-1}.

Table III. Accuracy of the proposed methods for the determination of GEM and LIN in pure samples

GEM				LIN	
TCNQ method (method A)		CLA method (method B)		Native fluorescence method (method C)	
Claimed taken (ng mL^{-1})	Recovery (%)[a]	Claimed taken (ng mL^{-1})	Recovery (%)[a]	Claimed taken (ng mL^{-1})	Recovery (%)[a]
90.00	101.6	15.00	100.0	90.00	100.9
150.00	99.7	25.00	101.4	150.00	101.1
250.00	98.2	35.00	99.6	230.00	100.8
350.00	100.4	45.00	101.0	250.00	99.3
450.00	100.6	55.00	99.8	350.00	99.6
Mean ± SD	100.1 ± 1.3		100.4 ± 0.8		100.3 ± 0.8

[a] $n = 3$.

Table IV. Determination of GEM in tablets by the proposed methods

TCNQ method (method A)					CLA method (method B)				
Claimed taken (ng mL^{-1})	Pure added (ng mL^{-1})	Conc. found of tablet (ng mL^{-1})	Conc. found of total (ng mL^{-1})	Recovery of total (%)[a]	Recovery of added (%)[a]	Claimed taken (ng mL^{-1})	Pure added (ng mL^{-1})	Conc found of tablet (ng mL^{-1})	Conc found of total (ng mL^{-1})	Recovery of total (%)[a]	Recovery of added (%)[a]
150.00	100.00	149.53	251.49	100.6	101.9	20.00	15.00	20.09	34.89	99.9	99.3
	150.00		299.89	100.0	100.2		20.00		40.05	100.1	99.8
	200.00		350.33	100.1	100.4		25.00		44.82	99.6	98.9
200.00	150.00	202.60	350.76	100.2	98.8	25.00	20.00	25.30	45.32	100.7	100.1
	200.00		406.04	101.5	101.7		25.00		50.56	101.1	101.0
	250.00		454.73	101.1	100.9		30.00		54.85	99.7	98.5
Mean ± SD				100.6 ± 0.6	100.7 ± 1.2					100.2 ± 0.6	99.8 ± 1.0

[a] $n = 3$.

Comparing the *LOD* and *LOQ* values for methods A and B showed that the sensitivity of the CLA method (*LOD* 0.85 ng mL^{-1}) is relatively higher than that of the TCNQ method (*LOD* 8.75 ng mL^{-1}).

Methods application. – Already published HPLC methods (27, 28) and developed methods were applied on pure samples and tablets and the results were statistically compared. It was concluded that with 95 % confidence, there is no significant difference in their accuracy and precision, since the calculated *t*- and *F*-values were less than the theoretical (Table VI) values proving comparable accuracy and precision of determination of GEM and LIN by both methods (Table VI). In addition, ANOVA test was applied, the *p*-value (0.675, 0.968 for GEM and LIN, respectively) are greater than 0.05, indicating that there is no significant difference between the proposed methods together with the reference methods.

In comparison of the developed methods and the reported ones (11–13), the analysis based on fluorescence provides better sensitivity than the spectrophotometric technique by four orders of magnitude. Linearity ranges were found to be 50–500, 10–60 and 20– 400 ng mL^{-1} for TCNQ, CLA methods for GEM and native fluorescence method for LIN, respectively. On the other hand, the linearity ranges for the reported methods were 6–30, 2–10, 2.5–12.5 and 1.5 μg mL^{-1} for iodine, DDQ, TCNQ and TCNE methods (11), 40–200 and 100–1200 ng mL^{-1} (12) and 0.05–3 μg mL^{-1} (13). Lower linearity ranges of the suggested methods ascertained their higher sensitivity. Moreover, the developed methods are less time consuming and simpler than the reported ones, enhancing their application in quality control laboratories.

Table V. Determination of LIN in tablets by the proposed method

Claimed taken (ng mL^{-1})	Pure added (ng mL^{-1})	Conc. found of tablet (ng mL^{-1})	Conc. found of total (ng mL^{-1})	Recovery of total (%)[a]	Recovery of added (%)[a]
100.00	80.00	101.1	180.00	100.8	100.4
	100.00		200.00	100.9	100.6
	120.00		220.00	100.1	99.2
150.00	100.00	151.4	250.00	100.6	99.9
	150.00		300.00	100.4	99.8
	200.00		197.63	99.8	99.8
Mean ± SD				100.4 ± 0.4	100.0 ± 0.5

[a] $n = 3$.

Table VI. Statistical comparison of the results obtained by applying the proposed fluorimetric methods for the determination of GEM and LIN

	Value	GEM			LIN	
		TCNQ method	CLA method	Reference method (27)[b]	Native fluorescence method	Reference method (28)[c]
Pure sample	Mean (%)	100.09	100.38	100.27	100.33	99.95
	RSD (%)	1.270	0.794	0.822	0.803	1.433
	n	5	5	6	5	6
	Student's *t*-test	0.273 (2.262)[a]	0.224 (2.262)[a]		0.553 (2.262)[a]	
	F-ratio	2.378 (5.192)[a]	1.069 (6.256)[a]		3.155 (6.256)[a]	
Tablets	Mean	100.99	100.88	100.60	100.79	100.03
	RSD (%)	1.161	0.372	1.367	0.405	1.266
	n	3	3	3	3	3
	Student's *t*-test	0.374 (2.776)[a]	0.340 (2.776)[a]		0.990 (2.776)[a]	
	F-ratio	1.377 (19.000)[a]	13.411 (19.000)[a]		9.628 (19.000)[a]	

[a] Values in the parentheses are the corresponding values of *t*- and *F*- at $p = 0.05$.
[b] HPLC method (27).

CONCLUSIONS

The present study describes the utility of quinone-based reagents such as TCNQ and CLA for the development of quinone-based fluorophores. In addition, successful evaluation of native fluorescence as an analytical tool for the analysis of drugs was considered. These concepts were explored for spectrofluorimetric determinations of novel fluoroquinolone and oxazolidinone antibacterial agents, gemifloxacin mesylate and linezolid,

with high sensitivity and selectivity. Two of the proposed methods were based on the formation of fluorescent charge transfer complexes, while the third was performed by enhancing the native fluorescence of the drug using phosphate buffer (pH 5). Optimum experimental parameters affecting the reaction of GEM with TCNQ and CLA and those enhancing the native fluorescence of LIN were investigated. Stoichiometric ratio and mechanisms of reactions of GEM were studied and postulated. The suggested methods have the advantages of simplicity, accuracy and high sensitivity and they allowed successful determination of the cited drugs in tablets. Therefore, the methods are valuable for routine application in quality control laboratories.

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