ISSN : 0974-7419 Volume 12 Issue 5

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(5) 2013 [165-176]

Stability indicating spectrophotometric and TLC densitometric methods for the determination of gemifloxacin mesylate in tablet form

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ABSTRACT

Gemifloxacin mesylate (GEM), a novel fluoroquinolone used for respiratory tract infections, was determined by three simple, accurate and precise spectrophotometric methods and a TLC densitometric method, in presence of its acid degradation product. Method (A) was first derivative technique (^{1}D) which allows the determination of GEM by measuring the peak amplitudes at 280 and 360 nm where the acid degradation product displays zero value. Method (B) was based on second derivative ratio technique (^{2}DD) in which the peak amplitude was measured at 289.3 nm using 10 μg mL^{-1} of the acid degradation product as divisor. Method (C) depends on the formation of a colored product between GEM and *p*-dimethylaminobenzaldhyde (DAB) reagent and the absorbance was measured at 400 nm. Method (D) depends on separation of GEM from its acid degradation product on TLC plates pre-sprayed with EDTA solution (3% w/v), using chloroform:methanol:ammonia solution (6:3:0.5, v/v/v) as developing system, followed by densitometric determination of GEM. The proposed methods were successfully applied to the analysis of GEM in pure and tablet forms, in addition to laboratory prepared mixtures (methods A, B and D). The methods were validated in accordance to ICH guidelines and compared with the reference method, revealing non significant difference.

KEYWORDS

Gemifloxacin mesylate;
First derivative;
Second derivative ratio;
p-dimethylaminobenzaldhyde;
TLC.

INTRODUCTION

Gemifloxacin mesylate (GEM, Figure 1), is a novel fluoroquinolone antibacterial, assigned to third generation because of its increased activity against gram-positive and atypical pathogens, as well as gram-negative organisms. It has excellent activity against multidrug-resistant S. pneumoniae, therefore it is used in the treatment of acute bacterial exacerbations of chronic bronchitis and community-acquired pneumonia[1]. It is chemically 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[2].

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GEM was determined in pharmaceutical formulation by different analytical techniques including UV spectrophotometric[3-7], colorimetric[8-15] and spectro fluorimetric[16,17] methods, either in single form or in combination with ambroxol[18] were reported. In addition, chromatographic methods including capillary electrophoresis[19], HPTLC[20], and HPLC[21-32] were also applied for the determination of GEM in tablets and in plasma.

Derivative and derivative ratio spectrophotometry were suitable tools for resolving closely overlapping spectra. Therefore, the development of sensitive stability indicating spectrophotometric methods for the determination of GEM, without previous separation from the acid degradation product and of lower cost than the reported HPLC methods, were of interest. The acid degradation product was isolated and its structure was suggested. In addition, the analytically useful functional group in GEM includes free primary amino group, which can be exploited for designing suitable spectrophotometric methods and so still offer a scope to develop more colorimetric methods with better selectivity, precision and accuracy. The proposed colorimetric (DAB) method is free from interference of tablet excipients as absorbance measurements are performed at longer wavelength. Moreover, one of the objective of this work was to develop a TLC densitometric method using a simple developing system, composed of easily available inexpensive laboratory solvents. The proposed methods, ^{1}D, ^{2}DD spectrophotometric, colorimetric (DAB method) and TLC densitometric methods were found to be superior to the previously reported methods in terms of their higher sensitivity, simplicity, accuracy and precision.

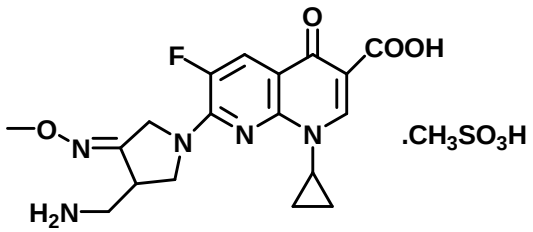

Figure 1 : Chemical structure of gemifloxacin mesylate

EXPERIMENTAL

Chemicals and reagents

Pharmaceutical grade GEM was supplied by El Obour Modern Pharmaceutical Industries Company (Cairo, Egypt) and certified to contain 99.80 %. Factive® tablets, Batch No. 002, contain GEM equivalent to 320 mg GEM base per one tablet, were manufactured and supplied by Hikma Pharma (6th October, Egypt). *p*-Dimethylaminobenzaldehyde (DAB) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and freshly prepared as 0.2 % w/v methanolic solution. Methanol (HPLC grade, Labscan), chloroform, ammonia solution (33 %) and ethylene diamine tetraacetic acid disodium salt (EDTA, 3 % w/v aqueous solution) (EL-Nasr Pharmaceutical Chemicals Co., Egypt) were used. Hydrochloric acid (37 %, Reidel-de-Haën, Germany) and sodium hydroxide (EL-Nasr Pharmaceutical Chemicals Co., Egypt) were prepared as 1 N aqueous solutions. Whatman filter paper No. 42 was used for the preparation of sample solution.

Equipment

A double beam UV/visible spectrophotometer (JENWAY, United Kingdom) model 6800 with quartz cell of 1 cm pathlength, connected to Samsung compatible computer was used. The software was JENWAY model 6800 Spectrophotometer Flight Deck. The spectral band width was 1.5 nm with wavelength scanning speed of 400 nm min^{-1}. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. A Camag (Camag, Muttenz, Switzerland) TLC instrumental set-up consisting of sample applicator Linomat V, 100-µL syringe (Hamilton, Switzerland) and TLC Scanner III operated by winCATS software (V 3.15, Camag, Switzerland) were used. Evaluation was via peak areas with linear regression. Precoated silica gel 60 F_{254} plate, 20 x 10 cm^2 (Fluka Chemie, Buchs, Switzerland) with 200-µm thickness were pre-sprayed with EDTA solution (3 % w/v) and left to dry overnight in dry place. A UV lamp—short wavelength 254 nm was employed for detection of spots. IR charts were obtained using Burker FT-IR spectrophotometer Vector 22, Schimadzu 435, Perkin-Elmer 457 and Jasco FT-IR plus 460 Japan, using potassium bromide discs. Mass spectra were obtained using Hewlett Packard 5988 mass spectrometer at 70 eV.

Preparation of the acid degradation product

An accurately weighed amount of GEM (75 mg)

was dissolved in 50 mL 1 N HCl and the solution was refluxed for 1 hour at 100 ^{0}C. Complete degradation was followed up by diluting 0.2 ml of the solution with methanol and spotting on a TLC plate (pre-sprayed with EDTA solution (3 % w/v) and well dried) next to a spot of intact GEM and allowing the plate to develop using chloroform:methanol:ammonia, (6:3:0.5, v/v/v) as developing system. By examining the TLC plate, two spots were obtained, one for the intact drug (R_f = 0.7) and the other for its acid degradation product (R_f = 0.4). The solution was cooled and neutralized using 1 N sodium hydroxide, evaporated to dryness and purified by dissolving in hot methanol followed by filtration and evaporation to dryness. The acid degradation product was identified and its structure was elucidated using IR and mass spectroscopy.

Stock solutions

GEM stock solutions (1 mg mL^{-1}) and (2.5 mg mL^{-1}) were prepared by dissolving an accurate weight either (100 mg) or (250 mg) in methanol then completing the volume to 100 mL with the same solvent. Further dilution was done to obtain GEM working solutions (50 μg mL^{-1}) for ^{1}D and ^{2}DD and (100 μg mL^{-1}) for DAB method. Acid degradation product solution (200 μg mL^{-1}) was prepared by weighing accurately 20 mg of the acid degradation product into a 100 mL volumetric flask, dissolving in the least amount of distilled water then completing to volume with methanol.

Laboratory prepared mixtures

Accurate aliquots in the ranges equivalent to (40 - 180 μg) of GEM working solution (50 μg mL^{-1}) and (360 – 20 μg) of its acid degradation product solution (200 μg mL^{-1}), were transferred into a series of 10 mL volumetric flasks, completed to volume with methanol and mixed well, in order to obtain different mixtures containing 10 – 90 % of the acid degradation product. While in TLC densitometric method, laboratory prepared mixtures were prepared using accurate aliquots equivalent to (60 -180 μg) of GEM solution (250 μg mL^{-1}) and (140 – 20 μg) of its acid degradation product solution (200 μg mL^{-1}).

Sample preparation

Ten tablets were accurately weighed and powdered in a mortar. Two quantities of the powdered tablets equivalent to 100 mg and 250 mg GEM were transferred, separately, into two 100 mL volumetric flasks, then 25 mL methanol were added. The solutions were stirred for 30 min, completed to 100 mL with methanol, mixed well and filtered on dry funnel and dry filter paper discarding the first few milliliters. Sample solutions of concentrations equivalent to (1 mg mL^{-1}) and (2.5 mg mL^{-1}) of GEM were obtained, for (^{1}D, ^{2}DD and DAB methods) and TLC densitometric method, respectively. Further dilution of sample solution was carried out using methanol to reach the calibration range specified for each method.

Method validation

The methods were validated according to International Conference on Harmonisation (ICH) guidelines[33] for validation of analytical procedures.

(a) Linearity

(A) ^{1}D method

Aliquots from GEM working solutions (50 μg mL^{-1}) equivalent to (25–225 μg) were transferred into a series of 10 mL volumetric flasks and completed to the mark with methanol. The first derivative spectra were recorded using methanol as blank, then the amplitudes of the maxima at 280 and 360 nm (zero ordinate value of the acid degradation product), using smoothing factor 10, were measured. Calibration curves were constructed relating the amplitudes at the selected wavelengths to the corresponding drug concentrations and the regression equations were computed.

(B) ^{2}DD method

The absorption spectra of standard solutions of GEM (2.5 – 27.5 μg mL^{-1}) were recorded against a blank of methanol and stored in the computer. The stored spectra of GEM were divided by the stored spectrum of the acid degradation product (10 μg mL^{-1}). Then, the second derivative of the above ratio spectra were obtained and smoothed at $\triangle\lambda = 20$. The peak amplitudes of the second derivative of the ratio spectra at 289.3 nm were measured, plotted against the corresponding concentrations of GEM and regression equation was computed.

(C) DAB method

Accurately measured aliquots equivalent to (100 – 250 μg) were transferred from GEM working solution

(100 μg mL^{-1}) into a series of test tubes. The aliquots were evaporated to dryness on boiling water bath, 2.5 mL of DAB reagent (0.2 %, w/v) were added to the obtained residue in each test tube and the reaction was allowed to proceed for 10 min at 90 ^{0}C, replacing the volume of evaporated methanol. The contents of the test tubes were quantitatively transferred after cooling into a series of 10 mL volumetric flasks and the volume was completed with methanol. The absorbances were measured at 400 nm against reagent blank prepared similarly, plotted against the corresponding drug concentrations and regression equation was computed.

(D) TLC densitometric method

Accurately measured aliquots of 2.5–25 μL of GEM standard solutions (2.5 mg mL^{-1}) were applied to a TLC plate, pre-sprayed with EDTA solution (3 % w/v) and dried, as band-wise, using Camag autosampler (band length 2 mm, a constant application rate of 0.1 μL s^{-1}, track distance 15 mm, distance from the edge 15 mm). The chromatographic chamber was saturated (lined on the two bigger sides with filter paper) with the developing solvent for 45 min. Plates were developed, by ascending development technique, at room temperature in chloroform:methanol:ammonia (6:3:0.5, v/v/v) as a developing system. The length of chromatogram run was 8 cm and approximately 30 min. Subsequent to the development, TLC plates were removed, dried and the bands were visualized under UV lamp at 254 nm. Densitometric scanning was performed on Camag TLC scanner III in the absorption mode at 270 nm for all measurements. The slit dimension was kept at 6 x 3 μm and 20 mm s^{-1} scanning speed was employed. Each track was scanned thrice and baseline correction was used. The peak areas (AUP $x10^{-3}$) were plotted against the corresponding concentrations to obtain the calibration curve and regression equation was computed.

(b) Accuracy

Recovery experiments were conducted to determine the accuracy of the proposed methods. Different aliquots from GEM working solutions were analyzed using the proposed methods as mentioned under linearity. The concentrations were calculated using the corresponding regression equations then, the mean recovery % and standard deviation (SD) were calculated. In order to check the validity of the suggested methods, the standard addition technique was applied and the accuracy was determined by the addition of known amounts of GEM working standard solution to the sample solution. The % recovery of the added GEM was calculated using the regression equations.

(c) Precision

Repeatability (intraday precision) was evaluated by analyzing three concentrations of GEM in triplicate on the same day, using the suggested procedures. Intermediate precision (interday precision) was investigated by repeating the forementioned procedure in triplicate on three different days for the analysis of GEM. Relative standard deviation (RSD) was calculated.

(d) Specificity

The same procedures mentioned under linearity were applied for the analysis of laboratory prepared mixtures. The concentrations of GEM were calculated using the computed regression equations.

(e) Limit of detection and limit of quantification

The approach based on the SD of the response and the slope was used for determining the limit of detection and limit of quantification.

LOD = 3.3×SD/slope

LOQ = 10×SD/slope

RESULTS AND DISCUSSION

Identification of the acid degradation product of GEM

The literature reveals that GEM undergoes degradation under acidic and basic conditions[16,19]. However, complete degradation and high yield was obtained by refluxing GEM with 1 N HCl for one hours at 100^{0}C. After neutralization and evaporation of the solution, the acid degradation product was obtained by extraction and purification in hot methanol followed by evaporation to dryness. The residue obtained was identified by TLC where a new spot was obtained at R_f = 0.4, which is different from that of the intact drug (R_f = 0.7). IR spectrum (KBr) of the acid degradation product, Figure 2a showed the disappearance of CO and OH carboxylic stretching bands at 1712.79 and 3471.87 cm^{-1}, respectively, which were present in the IR spectrum of the intact GEM, Figure 2b. Moreover, mass spec-

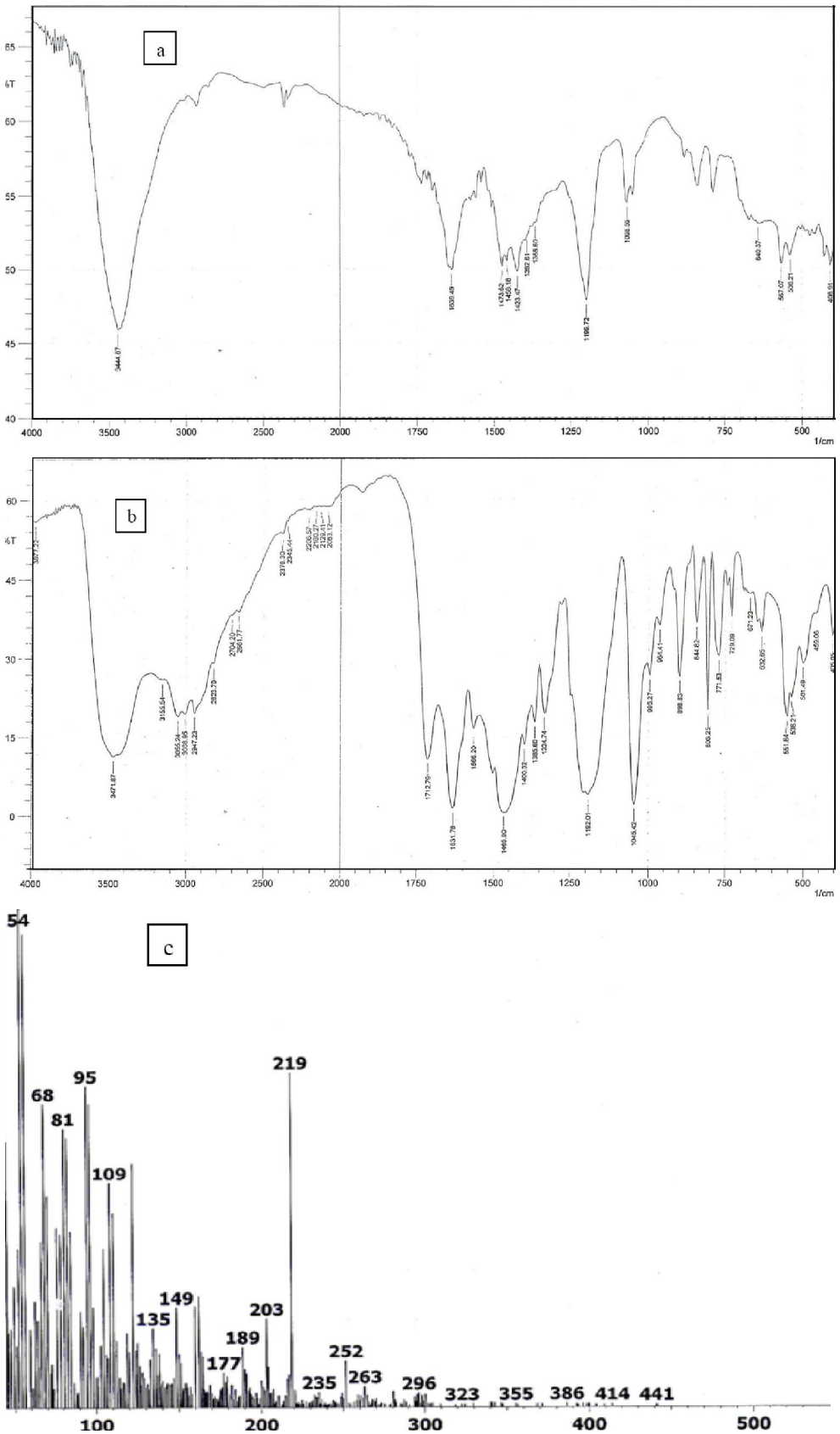

Figure 2 : IR spectrum of the acid degradation product (a), intact GEM (b) and mass spectrum of the acid degradation product (c).

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trum revealed M^+ at 441 which is identical to the molecular weight of the acid degradation product, Figure 2c. These results confirm that acid degradation leads to decarboxylation.

Method optimization

(a) ^{1}D method

Zero–order absorption spectra of the intact GEM and its acid degradation product reveal severe overlap, Figure 3. Upon applying ^{1}D spectrophotometric method, GEM could be determined by measuring the peak amplitudes of ^{1}D spectrum at 280 and 360 nm (where the acid degradation product displays zero value), Figure 4. In order to optimize the ^{1}D method, different solvents were tried and different smoothing factors were tested. Methanol was the solvent of choice, smoothing factor $\Delta\lambda$ = 4 showed a suitable signal-to-noise ratio and the spectra showed good resolution. A linear correlation was obtained between the peak amplitudes at 280 and 360 nm and the corresponding concentrations

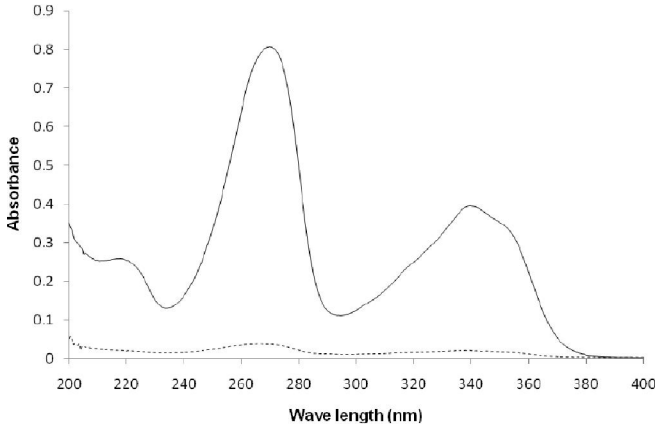

Figure 3 : Zero-order absorption spectra of 10 µg mL^{-1} of GEM (—) and 10 µg mL^{-1} of its acid degradation product (----) in methanol.

of GEM in the range of 2.5–22.5 µg mL^{-1}.

(b) ^{2}DD method

The influence of divisor concentration and smoothing factor was investigated. A divisor concentration of 10 µg mL^{-1} gave the best results, with respect to sensitivity and repeatability. Due to the extent of the noise levels on the ratio spectra, a smoothing function was used and 20 experimental points was found to be suitable, in terms of signal-to-noise ratio and the spectra showed good resolution. For the determination of GEM, the absorption spectra of GEM and that of its laboratory mixture with the acid degradation product were divided by the spectrum of the acid degradation product (10 µg mL^{-1}) to get the ratio spectra, then the second derivative of the ratio spectra were obtained with $\Delta\lambda$ = 20, Figure 5. The ^{2}DD amplitudes at 289.3 nm gave reproducible results. Linearity was obtained over

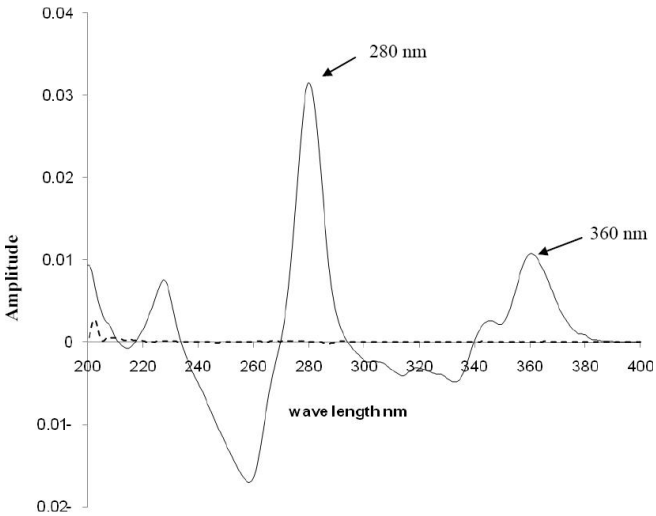

Figure 4 : First derivative absorption spectra of 10 µg mL^{-1} of GEM (—) and 10 µg mL^{-1} of its acid degradation product (-----) in methanol.

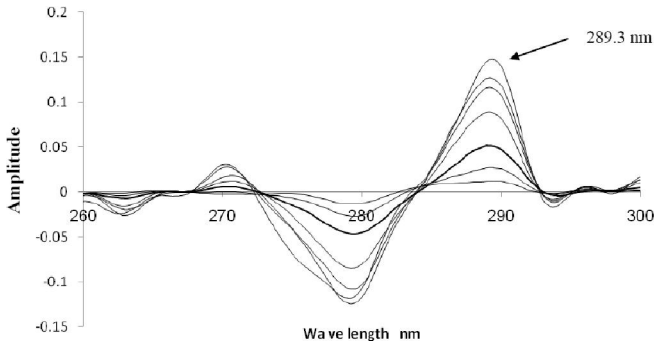

Figure 5 : Second derivative of the ratio spectra for different concentrations of GEM (2.5, 5, 8, 15, 20, 22.5 and 27.5 µg mL^{-1}) using 10 µg mL^{-1} acid degradation product as divisor.

the concentration range of 2.5–27.5 µg mL^{-1}.

(c) DAB method

GEM reacts with DAB to form a colored condensation product, Figure 6, which has a maximum absorbance at 400 nm, Figure 7. The optimum experimental conditions for the color development were investigated by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

(d) Effect of the volume of DAB

The ability of DAB to form Schiff's bases with amines

has been utilized for the spectrophotometric determination of compounds such as ranitidine[34] and metronidazole[35]. DAB (0.2 % w/v, in methanol) is used as coupling agent for the reaction. The increase in the absorbance was tested by adding different volumes of the reagent (1–3.5 mL). It was observed that 2.5 mL of DAB showed maximum absorbance for the reaction, Figure 8a.

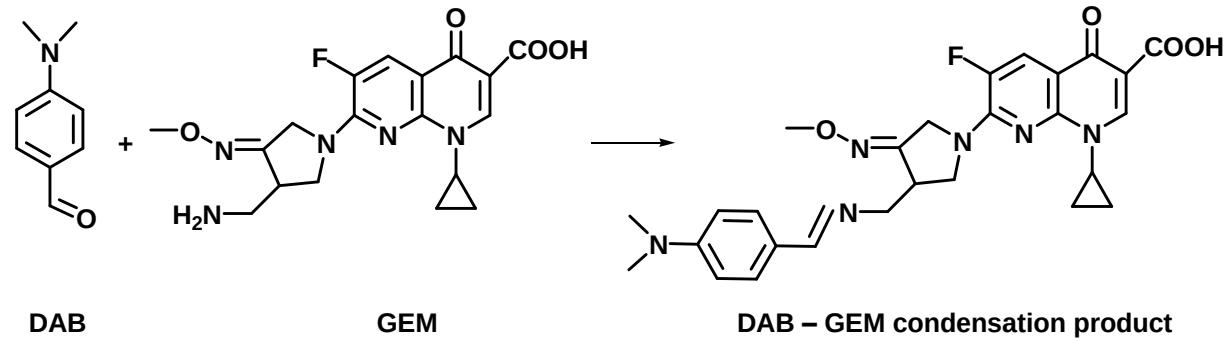

Figure 6 : Condensation reaction between GEM and DAB

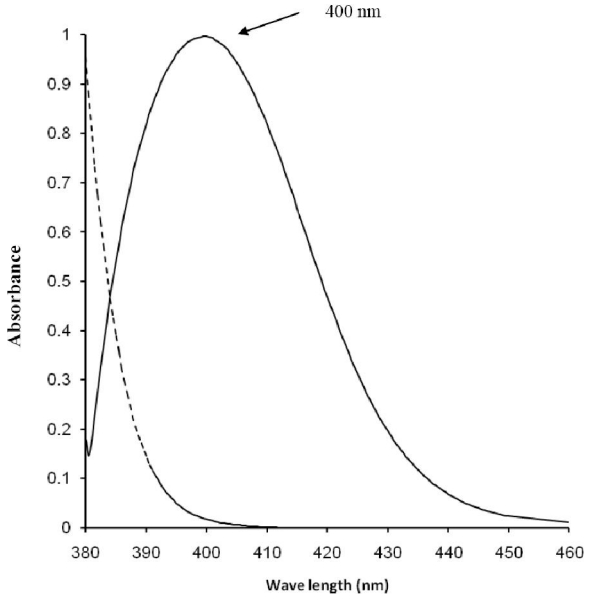

Figure 7 : The absorption spectra of the GEM - DAB condensation product (—) and the corresponding reagent blank (-------).

(e) Effect of the presence of the acid

The absorbance of the colored product decreases, significantly in presence of hydrochloric acid or sulfuric acid. Therefore, the reaction was carried in absence of any acid.

(f) Effect of temperature and heating time

The reaction was carried out at different temperatures, for different time intervals. Maximum absorbance was obtained by heating at 90 ^{0}C for 10 min, Figure 8b and 8c, respectively.

(g) Effect of time on the stability of the formed colored product

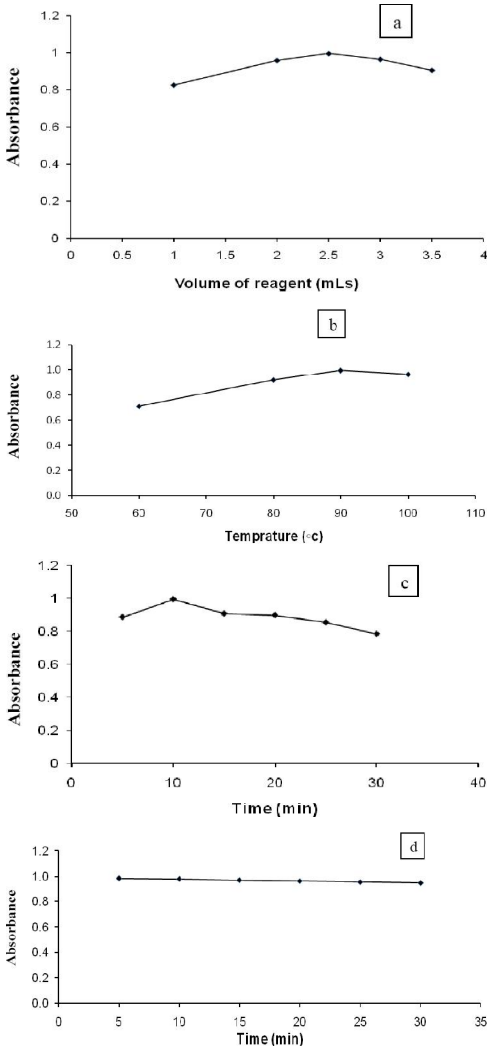

Figure 8 : Effect of reagent volume (a), temperature (b), heating time (c) on the reaction of GEM (25 µg mL^{-1}) with DAB (0.2 % w/v) and effect of time on the stability of GEM - DAB condensation product (d).

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Stability of the formed product was checked by applying the chosen optimum conditions and measuring the absorbance at different time intervals. The colored product was found to be stable for 30 minutes, Figure 8d.

(h) Stoichiometry of the reaction

Job's method of continuous variation[36] was used to determine the stoichiometric ratio at which GEM combines with DAB reagent, using solutions of 11.12 x 10^{-4} M concentration of each. The absorbances of the solutions were measured at 400 nm and plotted against mole fraction of GEM. Figure 9 reveals that GEM and

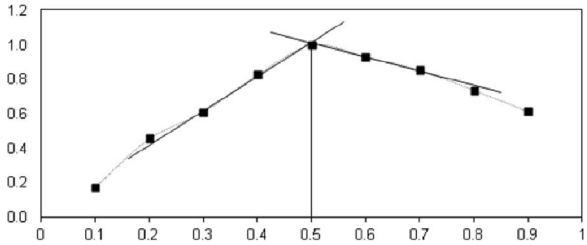

Figure 9 : Determination of the stoichiometry of the reaction of GEM and DAB by the continuous variation method using 11.12 x 10^{-4} M solutions.

DAB form a condensation product in a molar ratio of 1:1, under the optimum experimental conditions attained.

Upon applying the optimum conditions, linear relationship was obtained over the concentration range of 10–25 µg mL^{-1} and the regression equation was computed.

(i) TLC densitometric method

The composition of the developing system for development of TLC chromatographic method was optimized by testing different solvent mixtures of varying polarity. Various developing systems were evaluated. Chloroform and methanol mixture were tried in different ratios. Initially, good separation was obtained using chloroform:methanol (6:3, v/v). However, tailing and undefined spot was an encountered problem as it was difficult to pinpoint and evaluate such spot quantitatively. This problem was thought to be caused by the carboxylic group of GEM molecule which confers high interactivity to the organic molecule. Addition of ammonia to the system decreased such interaction with the sorbent and greatly reduced tailing[37]. In addition, it was found that pre-spraying the TLC plate with EDTA (3 % w/v) solution and drying overnight overcome this problem by decreasing the polarity through complex formation with gypsum (Ca SO_4), used as binder in silica gel G plates and the spot obtained was sharp and well-defined[38]. Finally, chloroform:methanol:ammonia (6:3:0.5, v/v/v) showed good resolution of GEM from its acid degradation product with R_f value of 0.7 and 0.4, respectively, as revealed by densitometric scanning, Figure 10. The suggested method has the advantage of reducing the composition of the developing solvents to three components system as compared to the

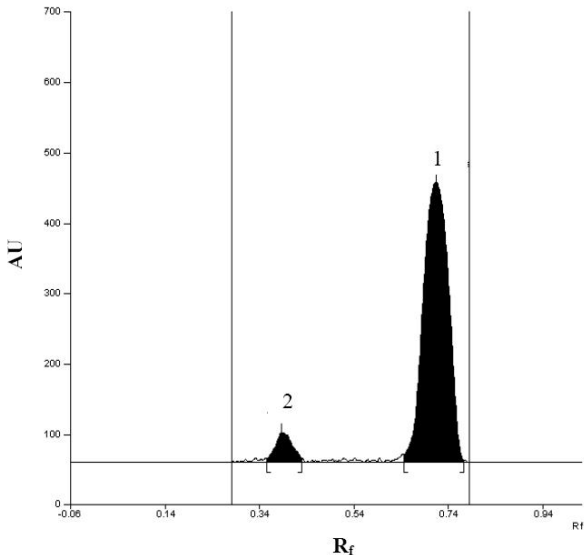

Figure 10 : A typical densitogram of a laboratory prepared mixture of GEM (18 µg $spot^{-1}$), peak 1, R_f = 0.7 ± 0.02 and its acid degradation product (2 µg $spot^{-1}$), peak 2, R_f = 0.4 ± 0.02, in ratio of (9:1) measured at 270 nm, using developing system chloroform:methanol:ammonia (6:3:0.5, v/v/v)

previously reported method which used a five components one (chloroform:methanol:toluene:diethylamine: water, 33.6:33.6:16.8:10.8:6, by volume)[6], thus minimizing time and cost. In addition, water is omitted from the developing system which facilitated quick and uniform drying.

Method validation

Method validation was performed according to ICH guidelines[33] for the proposed methods. Linearity ranges, regression equations, standard deviation of the slope (S_b) and that of intercept (S_a) are indicated in TABLE 1 : Regression equation parameters show good linear relationship for all the methods as revealed by the correlation coefficients. In addition, descriptive statistics of the regression showed low values of standard error of intercept and slope which revealed high accu-

racy with minimum deviations and low scattering of the calibration points[39] .

Results of accuracy and standard addition technique are shown in TABLES 2 and 3, respectively. A good accuracy of the methods was verified by good recoveries and SD values (less than 2) which indicates reproducibility of the results.

The specificity of the proposed ^{1}D, ^{2}DD and TLC densitometric methods was proved by the analysis of laboratory prepared mixtures of GEM and its acid degradation product, as presented in TABLE 4. GEM could be determined in presence of up to 90 % and 70 %, in case of (^{1}D and ^{2}DD) and TLC densitometric methods, respectively. However, since the DAB method was

TABLE 1 : Analytical and validation parameters obtained by applying ^{1}D, ^{2}DD, DAB and TLC densitometric methods for the determination of GEM

Item	^{1}D method		^{2}DD method	DAB method	TLC densitometric method
Measurement Wavelength	280 nm	360 nm	289.3 nm	400 nm	270 nm
LOD[a]	0.731 µg mL^{-1}	0.77 µg mL^{-1}	0.44 µg mL^{-1}	0.89 µg mL^{-1}	0.16 µg mL^{-1}
LOQ[a]	2.23 µg mL^{-1}	2.33 µg mL^{-1}	1.33 µg mL^{-1}	2.71 µg mL^{-1}	0.49 µg mL^{-1}
Range of linearity	2.5-22.5 µg mL^{-1}	2.5-22.5 µg mL^{-1}	2.5-27.5 µg mL^{-1}	10-25 µg mL^{-1}	2.5-25 µg mL^{-1}
Regression equation	y = 3.1549 x - 0.0405	y = 1.1334 x - 0.7438	y = 5.7765x - 1.7914	y = 0.0577 x - 0.4392	y = 1.2157x + 16.0057
Correlation coefficient (*r*)	0.9996	0.9991	0.9999	0.9995	0.9999
S_b	0.0264	0.0149	0.0216	0.0005	0.0074
S_a	0.3629	0.2043	0.3631	0.0092	0.1110
Confidence limit of the slope	3.1549±0.0679	1.1334±0.0383	5.7765±0.0556	0.0577±1.22x10^{-3}	1.125±0.0205
Confidence limit of the intercept	0.0405±0.9330	0.7438±0.5253	1.7914±0.9995	0.4392±0.0225	16.0057±0.3081
Standard error of the estimation	0.4922	0.2770	0.5013	0.0071	0.1502
Intraday[b]					
%RSD	0.428 -0.831	0.417-0.712	0.808 -0.970	0.093-0.865	0.630-1.501
Interday[c]					
%RSD	0.088-1.036	0.639-1.074	0.329 - 0.994	0.495-0.953	0.376-1.412

[a] Limits of detection and quantification are determined via calculations[33]; LOD= 3.3×SD/slope LOQ= 10×SD/slope; [b] The intraday (*n* = 3), average of three concentrations of GEM (12.5,18.75 and 21.25 µg mL^{-1} for ^{1}D, ^{2}DD methods), (15, 19 and 21 µg mL^{-1} for DAB method) and (3, 7 and 11 µg spot^{-1} for TLC densitometric method), repeated three times within the day; [c] The interday (*n* = 3), average of two concentrations of GEM (12.5,18.75 and 21.25 µg mL^{-1} for ^{1}D, ^{2}DD methods), (15, 19 and 21 µg mL^{-1} for DAB method) and (3, 7 and 11 µg spot^{-1} for TLC densitometric method), repeated three times in three successive days

TABLE 2 : Determination of pure samples of GEM by the proposed ^{1}D, ^{2}DD, DAB and TLC densitometric methods

Claimed taken (µg mL^{-1})	^{1}D method at 280 nm Recovery %	^{1}D method at 360 nm Recovery %	^{2}DD method Recovery %	DAB method Claimed taken (µg mL^{-1})	DAB method Recovery %	TLC densitometric method Claimed taken (µg mL^{-1})	TLC densitometric method Recovery %
3.75	100.08	100.56	98.75	13	99.66	3	99.93
8.75	100.74	100.26	98.90	15	100.88	5	98.62
12.5	101.02	99.83	100.39	17	100.38	7	101.70
13.75	101.75	100.38	98.95	19	99.35	9	101.40
18.75	101.83	101.85	100.82	21	99.88	11	100.38
21.25	101.93	100.37	99.62	23	100.23	15	99.01
23	-	-	99.38				
Mean	101.23	100.54	99.54		100.06		100.17
± SD	0.739	0.686	0.793		0.548		1.241

[a]Average of three determinations

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TABLE 3 : Determination of GEM in Factive tablets by the proposed ^{1}D, ^{2}DD, DAB and TLC densitometric methods and application of the standard addition technique

	^{1}D method at 280 nm		^{1}D method at 360 nm		^{2}DD method			DAB method			TLC densitometric method			
Claimed (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery[a] % of tablet	Recovery[a] % of added	Recovery[a] % of tablet	Recovery[a] % of added	Recovery[a] % of tablet	Recovery[a] % of added	Claimed (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery[a] % of tablet	Recovery[a] % of added	Claimed (µg mL^{-1})	Pure added (µg mL^{-1})	Recovery[a] % of tablet	Recovery[a] % of added
6	4.0	101.65	98.58	100.20	102.13	101.53	100.40	11.0	10.0	99.99	98.70	4.0	3.0	100.65	100.07
	6.0		99.73		100.88		99.63		11.0		100.27		4.0		100.35
	8.0		99.01		100.69		99.39		12.0		101.16		5.0		100.84
9	7.0	100.27	99.03	100.42	100.83	100.26	101.44	12.0	11.0	100.89	98.55	8.0	7.0	100.09	101.30
	9.0		100.87		99.70		101.70		12.0		100.15		8.0		101.28
	11.0		100.68		101.86		101.30		13.0		98.30		9.0		100.99
Mean		100.96	99.65	100.31	101.02	100.90	100.64			100.44	99.52			100.37	100.81
± SD		0.976	0.948	0.156	0.877	0.898	0.984			0.637	1.162			0.396	0.501

[a]Average of three determinations

TABLE 4 : Determination of GEM in laboratory prepared mixtures by the proposed the proposed ^{1}D, ^{2}DD and TLC densitometric methods

% of acid degradation product	Claimed taken (µg mL^{-1})	Acid degradation product	^{1}D method at 280 nm	^{1}D method at 360 nm	^{2}DD method	TLC densitometric method
	GEM		Recovery[a] % of GEM	Recovery[a] % of GEM	Recovery[a] % of GEM	Recovery[a] % of GEM
10%	18	2	99.74	99.72	101.15	101.24
30%	14	6	99.14	99.22	99.38	100.32
50%	10	10	101.24	100.09	101.95	98.91
70%	6	14	100.58	100.63	100.67	100.00
90%	4	36	100.18	98.03	99.08	-
Mean			100.18	99.54	100.45	100.12
± SD			0.800	0.988	1.205	0.961

[a]Average of three determinations

TABLE 5 : Statistical comparison of the results obtained by applying the proposed ^{1}D, ^{2}DD, DAB and TLC densitometric methods for the determination of GEM

	Statistical term	^{1}D method at 280 nm	^{1}D method at 360 nm	^{2}DD method	DAB method	TLC densitometric method	Reference method[39]
	Mean ± S.D	101.23 ± 0.739	100.54 ± 0.686	99.54 ± 0.793	100.06 ± 0.548	100.17 ± 1.241	100.27 ± 0.824
	n	6	6	7	6	6	6
Pure	Variance	0.546	0.471	0.629	0.300	1.540	0.679
sample	S.E.	0.302	0.280	0.300	0.224	0.507	0.336
	Student's t test	2.125 (2.228)*	0.617 (2.228)*	1.620 (2.201)*	0.520 (2.228)*	0.164 (2.228)*	
	F ratio	0.804(5.050)*	0.693 (5.050)*	1.080 (4.387)*	2.261 (5.050)*	2.268 (5.050)*	
	Mean ± S.D	101.16 ±0.772	100.09 ± 0.402	101.33 ±0.990	100.91 ± 0.930	100.98±1.093	100.60 ± 1.375
	n	3	3	3	3	3	3
Factive	Variance	0.596	0.162	0.980	0.865	1.195	1.891
tablet	S.E.	0.446	0.232	0.572	0.537	0.631	0.794
	Student's t test	0.615 (2.776)*	0.617 (2.776)*	0.746 (2.776)*	0.323 (2.776)*	0.375 (2.776)*	
	F ratio	0.315 (19.000)*	11.699 (19.000)*	0.518 (19.000)*	0.457 (19.000)*	0.632 (19.000)*	

*** The values in the parenthesis are the corresponding values of t and F at (p=0.05)**

based on the condensation reaction between the aldehyde group in DAB reagent and amino group in GEM, Figure 6, the acid degradation product will interfere in the analysis of GEM by this method.

Satisfactory intraday and interday RSD, as revealed in TABLE 1, indicates that the suggested methods are precise.

Statistical analysis

TABLE 5 shows statistical comparison of the results obtained by the proposed methods and the manufacturer's HPLC method[40], for the determination of pure GEM and factive tablets. The calculated *t* and F-values are less than the theoretical ones indicating that there is no significant difference between the proposed methods and the manufacturer's one with respect to accuracy and precision. In addition, One-way ANOVA was applied for the comparison of these methods showing no significant difference between the proposed methods and the manufacturer one as the p-value is greater than 0.05, TABLE 6.

TABLE 6 : One-way ANOVA testing for the different proposed and manufacturer methods used for the determination of GEM in tablet form

	Source of variation	Degree of freedom	Sum of squares	Mean square	*F*-value	*p-value*
GEM	Between experiment	4	2.770	0.692	0.912	0.494
	Within experiment	10	7.595	0.759		
	Total	14	10.364			

CONCLUSION

The proposed methods were simple, rapid, sensitive and precise. They have the advantage of eliminating the previous separation step. In addition, ^{1}D, ^{2}DD spectrophotometric and TLC densitometric methods are stabiltity indicationg assays. The results demonstrate the usefulness of the suggested methods, which are accurate, inexpensive and non-polluting. Therefore, they could be successfully applied for the routine analysis of GEM in pure bulk powder and in dosage form in quality-control laboratories without any preliminary separation step.

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