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## Spectrophotometric methods for the determination of gemifloxacin mesylate in pure form and pharmaceutical formulations

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### ABSTRACT

Three accurate, simple and precise spectrophotometric methods for the determination of gemifloxacin mesylate (GMX) in pure form and tablets are developed. The first method is based on the reaction of ninhydrin reagent with primary amines present in gemifloxacin in *N, N'*-dimethylformamide medium (DMF) producing a colored product which absorbs maximally at 590 nm. The second method is based on the reaction of drug with ascorbic acid in DMF medium resulting in the formation of a colored product, which absorbs maximally at 530 nm. The third method is based on the reaction of gemifloxacin with *p*-benzoquinone (PBQ) to form a colored product with  $\lambda_{\max}$  at 400 nm. Beer's law is obeyed in the concentration ranges 4.0-32, 8.0-40 and 9.0-72  $\mu\text{g mL}^{-1}$  and molar absorptivity of  $9.68 \times 10^3$ ,  $5.58 \times 10^3$  and  $4.98 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$  for ninhydrin, ascorbic acid and PBQ, respectively. The optimum experimental parameters for the reactions have been studied. The validity of the described procedures was assessed. The proposed methods could be used for the determination of gemifloxacin in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision. The procedures were rapid, simple and suitable for quality control application. © 2010 Trade Science Inc. - INDIA

### KEYWORDS

Gemifloxacin;  
Spectrophotometry;  
Ninhydrin;  
Ascorbic acid;  
*p*-Benzoquinone.

### INTRODUCTION

Gemifloxacin mesylate (GMX), 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 (Figure 1). Gemifloxacin, is a new synthetic broad-spectrum quinolone antibacterial agent with activity against both Gram-negative and Gram-positive organisms for

oral administration and is being developed for the treat-

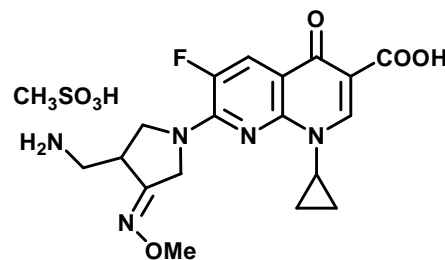


Figure 1 : The chemical structure of gemifloxacin mesylate

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ment of respiratory and urinary tract infections<sup>[1-3]</sup>.

Gemifloxacin is not official in any pharmacopoeia. A literature survey revealed that few analytical methods have been reported for the estimation of GFX; they include high-performance liquid chromatography-tandem mass spectrometry (LC-MS-MS)<sup>[4,5]</sup>, microchip electrophoresis<sup>[6]</sup>, chiral high-performance liquid chromatography<sup>[7]</sup> and chiral counter-current chromatography<sup>[8]</sup>. Only four spectrophotometric methods are cited in the literature<sup>[9-12]</sup>. Extractive method of the drug with safranin O (SFN O) and methylene blue (MB) in basic medium; Naphthol blue 12BR (NB 12BR) and azocaramine G (AG) in acidic medium<sup>[9]</sup> and the charge transfer complexation reaction of the drug as n-electron donor with sigma (s)-acceptor iodine, and the pi (p)-acceptors 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)-7,7,8,8-tetra cyanoquinodimethane (TCNQ) and tetracyanoethylene (TCNE)<sup>[10]</sup>. Three methods, are based on the oxidation of GMX with Fe (III) and the estimation of Fe (II) produced after chelation with either 1,10-phenanthroline or 2,2'-bipyridyl or ferricyanide at 515, 520 and 760 nm, respectively. Another method based on the interaction of GMX with ammonium heptamolybdate tetra hydrate, which resulted in the formation of molybdenum blue with  $\lambda_{\max}$  825 nm<sup>[11]</sup>. Also, method was based on the reaction of GMX with Fe(III) of ferric nitrate and 0.1M hydrochloric acid to produce a orange colored chromogen ( $\lambda_{\max}$  at 471 nm)<sup>[12]</sup>.

In the present manuscript, we developed three simple and sensitive condensation spectrophotometric methods for the analysis of gemifloxacin mesylate in bulk drug and in pharmaceutical formulations. The methods are based on the reaction of primary amino group of gemifloxacin with ninhydrin, ascorbic acid and *p*-benzoquinone (PBQ). The proposed methods have been successfully applied to the determination of GFX in pharmaceutical formulations.

## EXPERIMENTAL

### Instruments

All absorption spectra were obtained using Kontron 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. Hanna pH meter (USA) was used for checking the pH

values of buffer solutions.

### Materials and reagents

All chemicals and materials were of analytical reagent grade and all solutions were freshly prepared in bidistilled water.

Pure grade gemifloxacin mesylate and its tablets (Factive, 320 mg gemifloxacin mesylate per tablet) were obtained from Oscient Pharmaceuticals Corporation, USA. 2% ninhydrin (Merck) solution was prepared in N,N'-dimethylformamide (DMF). 0.2% ascorbic acid was prepared by dissolving 200 mg of ascorbic acid (S.D. Fine) in 10 ml of water in a 100 ml standard flask and diluting to the volume with DMF. 0.5% PBQ solution was prepared in methanol. 0.1 M phosphate (Na H<sub>2</sub>PO<sub>4</sub>) buffer solution was prepared and pH adjusted to 7.5 with NaOH (0.2 M).

### Preparation of standard drug solution

A stock solution 0.1% solution of gemifloxacin mesylate was freshly prepared by dissolving 100 mg of gemifloxacin mesylate in 100 mL of N,N'-dimethylformamide for ninhydrin and ascorbic acid methods and in 100 mL of bidistilled water for PBQ method and then diluted with the same solvent to obtain working standard solutions of 200  $\mu\text{g mL}^{-1}$  (Ninhydrin and Ascorbic acid Methods) and 300  $\mu\text{g mL}^{-1}$  (PBQ Method).

### Analytical procedures

#### Ninhydrin method

Aliquots of 0.2- 1.6 ml GMX standard solution (200  $\mu\text{g mL}^{-1}$ ) were pipetted into a series of 10 mL volumetric flasks. To each flask, 1.5 ml of 2% ninhydrin solution was added, mixed well and heated in a water bath at  $100 \pm 1$  °C for 5.0 min and tubes were cooled to room temperature. The content of the tube was transferred to a 10-mL volumetric flask and dilute to the volume with DMF. The absorbance was measured at 590 nm against reagent blank treated similarly. The calibration curve was constructed by plotting the absorbance versus concentration of GMX. The content of unknown was calculated from regression equation.

#### Ascorbic acid method

Into the 10 mL volumetric flasks, different aliquots of working standard solution (200  $\mu\text{g mL}^{-1}$ ) were trans-

ferred to provide final concentration range 8.0-40  $\mu\text{g mL}^{-1}$ . To each flask, 2.0 ml of 0.2% ascorbic acid solution was added and diluted to volume with DMF. The contents were mixed well and heated in a boiling water bath for 10 min. The solutions were cooled to room temperature. The reaction mixture and their corresponding washings were transferred and collected in a series of 10 mL volumetric flasks and dilute to the volume with DMF. The absorbance of each solution was measured at 530 nm against the reagent blank treated similarly within the stability period of 5.0 h. The calibration curve was constructed by plotting the absorbance versus concentration of GMX. The content of unknown was calculated from regression equation.

### PBQ method

Into 10 mL volumetric flasks, different aliquots (0.3-2.4 mL) of working standard solution (300  $\mu\text{g / mL}$ ) were transferred to provide final concentration range of 9.0–72  $\mu\text{g mL}^{-1}$ . To each flask, 1.5 mL of 0.1 M phosphate buffer solution and 1.5 mL of 0.5 % PBQ reagent were added. The volume was made up to mark with distilled water and the solutions were heated on a boiling water bath for 10 minutes. The solutions were cooled to room temperature and made up to mark with distilled water. The absorbance of each solution was measured at 400 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of GMX. The content of the unknown was calculated from regression equation.

### Analysis of pharmaceutical formulations

Ten tablets were accurately weighed and powdered. A portion equivalent to 50 mg GMX was stirred with 20 ml DMF and let stand for 10 min. The residue was filtered on What man No. 42 filter paper and washed with DMF. The filtrate and washings were diluted to volume in a 50-ml volumetric flask. A suitable volume of this solution was further diluted to give a final concentration of 0.2 mg  $\text{mL}^{-1}$ . An aliquot of this solution was analysed for GMX following the procedure described for the calibration curve.

## RESULTS AND DISCUSSION

### Ninhydrin method

Ninhydrin is a well-established reagent for the de-

termination of certain amines, amino acids and thiophenes<sup>[13]</sup>. It has been extensively used in the determination of the compounds of pharmaceutical importance and in the kinetic studies<sup>[14-16]</sup>. The reaction is usually carried out by heating for a short time in an organic solvent (2-propanol, butanol, DMF) or in a mixture of water and organic solvent. The reaction product is measured between 550 and 600 nm depending on the reaction conditions<sup>[17]</sup>. It has been suggested<sup>[18]</sup> that ninhydrin was converted to *o*-carboxyphenylglyoxal in alkaline medium which would reduce ninhydrin to 2-hydroxyindan-1, 3-dione.

In the present study, it combines with  $-\text{NH}_2$  group of GMX to form amino derivative, which further undergoes condensation with ninhydrin to give diketohydrindylidene-diketohydrindamine (Ruhemann's purple) with  $\lambda_{\text{max}}$  at 590 nm (Figure 2). The reaction between GMX and ninhydrin in DMF medium resulted in the formation of diketohydrindylidene-diketohydrindamine (Scheme 1.).

The different experimental parameters influencing the intensity of the developed colour were extensively investigated to determine the optimal conditions for the assay procedure.

### Effect of heating time

Gemifloxacin was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating the reaction mixture on a boiling water bath at  $100 \pm 1^\circ\text{C}$ . It is apparent that complete colour development was attained after 8.0 min of heating and remained constant up to 15 min. Therefore, the optimum heating time was fixed at 10 min throughout

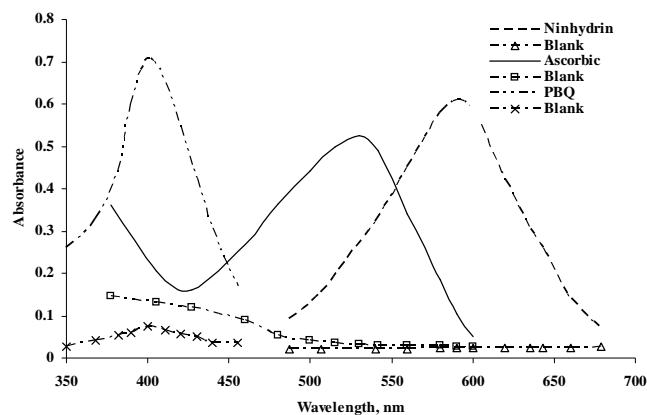
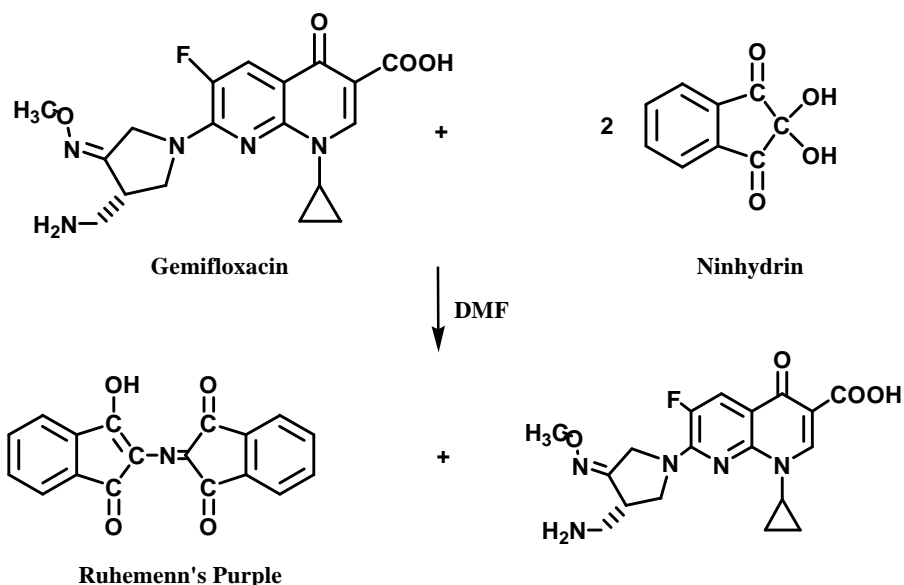


Figure 2 : Absorption spectra of reaction product between GMX ( $30 \mu\text{g mL}^{-1}$ ) and ninhydrin, ascorbic acid and PBQ.

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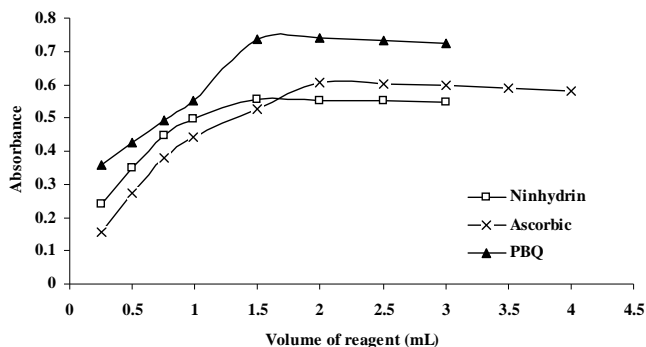


**Scheme 1 : The suggested reaction pathway between GMX and ninhydrin**

the experiment. The developed color was stable for 2.0 h.

### Effect of ninhydrin concentration

The effect of ninhydrin concentration on reaction rate was investigated using 0.5-3.0 mL of 2.0% ninhydrin. The results are presented in Figure 2 which showed that increasing the volume of 2.0% ninhydrin solution would increase the absorbance of the reaction product up to 2.0 mL after which further increase in the volume of ninhydrin resulted in no change in the absorbance of reaction product. Thus 2.0 mL of 2.0% ninhydrin was adopted as the most suitable volume for maximum absorbance (Figure 3).



**Figure 3 : Effect of reagent concentration on the absorbance at  $\lambda_{max}$  of the coloured product (drug = 30  $\mu\text{g mL}^{-1}$ ).**

### Ascorbic acid method

Ascorbic acid has been used as a sensitive reagent for the specific determination of aliphatic amines in

N,N'-dimethylformamide (DMF) medium without elucidating the mechanism of the reaction<sup>[19]</sup>. In this laboratory, we have used ascorbic acid as a reagent for the determination of a pharmaceutical possessing aliphatic amino group and proposed a reaction mechanism after an appropriate study<sup>[20]</sup>. Gemifloxacin, as a primary amine, reacts with ascorbic acid in DMF medium to produce a coloured product, which absorbed maximally at 530 nm (Figure 2). Under the specified experimental conditions, ascorbic acid undergoes oxidation resulting in the formation of dehydroascorbic acid. The carbonyl group of dehydroascorbate reacts with  $-\text{NH}_2$  group of gemifloxacin to form a purple colored condensation product.

The reaction of GMX and ascorbic acid in DMF medium involves two variables *i.e.* heating time and concentration of ascorbic acid.

### Effect of heating time

To study the effect of heating time, 500  $\mu\text{g}$  of GMX was mixed with 1.5 mL of 1% ascorbic acid in a boiling tube and heated on a boiling water bath at  $95 \pm 5$   $^{\circ}\text{C}$ . The absorbance was measured at 530 nm as a function of time. It was observed that the absorbance remained constant between 12 and 20 minutes of heating. Thus, 15 minutes of heating time was selected as an optimum value.

### Effect of ascorbic acid concentration

The influence of volume of 1% ascorbic acid was



critically examined. It was found that increasing the volume of 1% ascorbic acid solution would increase the absorbance of the reaction product up to 1.5 mL and above this volume the absorbance remained unaffected. Therefore, 1.5 mL of 1% ascorbic acid was used throughout the experiment (Figure 3).

### p-Benzoquinone (PBQ) method

PBQ reagent is used for the determination of an amino acid or amino group. The possible reaction pathway predicted from literature<sup>[21-23]</sup> and from results of the present work, where the free primary amine moiety of GMX condenses with carbonyl group of PBQ to form the condensation product. Under the reaction conditions used, which include heating to 95 °C, it was observed that the product of the reaction of PBQ and GMX shows  $\lambda_{\max}$  at 400 nm (Figure 2); for most pharmaceutical compounds<sup>[21-23]</sup> the products of the reaction absorb in the range 370-670 nm, but most of them at about 490-500 nm. On changing the pH to 5.0, 6.0, 7.0 or 0.8, a shift of the maximum absorbance to shorter wavelengths was observed. Zaia *et al*<sup>[24]</sup> observed a similar shift to shorter wavelengths upon reaction of PBQ with proteins and amino acids.

The absorptometric properties of the colored species as well as the influence of different parameters on the color development are extensively studied to determine optimal conditions of the assay procedure. The reaction was studied as a function of the volume of the reagent, selectivity of the solvent, reaction time and stability. The optimum conditions were incorporated into the general procedure.

### Methods of validation

The validity of the methods was tested regarding linearity, specificity, accuracy, precision, repeatability, recovery and robustness according to ICH Q2B recommendations<sup>[25]</sup>.

### Linearity

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard were calculated for all the methods. By using the above procedures, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the analytical response in the three methods to the concentration of GMX over

the ranges cited in TABLE 1. Linear regression analysis of the data gave the following equations,  $A = 0.0014 + 0.0198C$  with a correlation coefficient,  $r = 0.9999$ , for ninhydrin method;  $A = -0.0019 + 0.0116C$  having a correlation coefficient,  $r = 0.9994$ , for ascorbic acid method and  $A = -0.004 + 0.0105C$  with  $r = 0.9999$ , for PBQ method. Where  $A$  is the absorbance,  $C$  is the concentration of the drug ( $\mu\text{g mL}^{-1}$ ), and  $r$  is the correlation coefficient.

**TABLE 1 : Quantitative parameters for the proposed methods for determination of GMX.**

Parameters	Ninhydrin	Ascorbic Acid	PBQ
$\lambda_{\max}$ , nm	590	530	400
Beer's conc. Range ( $\mu\text{g mL}^{-1}$ )	4.0-32	8.0-40	9.0-72
Rinbom conc. Range ( $\mu\text{g mL}^{-1}$ )	3.0-30	6.0-37	7.0-68
Detection limits ( $\mu\text{g mL}^{-1}$ )	0.18	0.44	0.74
Quantification limit ( $\mu\text{g mL}^{-1}$ )	0.60	1.47	2.48
Molar absorptivity $\times 10^3$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	9.68	5.577	4.98
Sandell sensitivity ( $\mu\text{g cm}^{-2}$ )	0.050	0.087	0.098
Regression equation <sup>a</sup>			
Intercept	0.0016	-0.0024	-0.0079
Standard deviation of intercept ( $S_a$ )	0.0012	0.0017	0.0026
Slope	0.0199	0.0116	0.0105
Standard deviation of Slope ( $S_b$ )	0.012	0.006	0.005
Correlation coefficient ( $r$ )	0.9997	0.9994	0.9996
Mean $\pm$ SD%	100.13 $\pm$ 0.61	99.82 $\pm$ 1.49	99.02 $\pm$ 1.22
RSD%	0.61	1.49	1.23
Variance	0.372	2.21	1.50
SE	0.249	0.61	0.50
Student's $t$ -value (2.571) <sup>b</sup>	0.16	0.26	0.16
Variance ratio $F$ -test (5.05) <sup>b</sup>	2.53	2.36	1.58

<sup>a</sup>  $A = a + b C$  where  $c$  is the concentration in  $\mu\text{g mL}^{-1}$ .

<sup>b</sup> Theoretical value for  $t$  and  $F$  at 95% confidence level at  $P = 0.05$ .

The validity of the methods was proved by statistical evaluation of the regression lines, using the standard deviation of the residuals ( $S_{y/x}$ ), the standard deviation of the intercept ( $S_a$ ) and standard deviation of the slope ( $S_b$ ). The results are abridged in TABLE 1.

### Detection and quantification limits (sensitivity)

The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected. The limit of detection was calcu-

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lated by using the following equation<sup>[25]</sup>:

$$\text{LOD} = 3.3s / k$$

where  $s$  is the the standard deviation of the intercept of the regression line and  $k$  is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.35, 0.76 and 0.91  $\mu\text{g mL}^{-1}$  for ninhydrin, ascorbic acid and PBQ methods, respectively.

The limits of quantitation, (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2B and defined as<sup>[25]</sup>;

$$\text{LOQ} = 10 s / k$$

According to this equation, the limit of quantitation were found to be 1.16, 2.53 and 3.03  $\mu\text{g mL}^{-1}$  for ninhydrin, ascorbic acid and PBQ methods, respectively.

### Specificity, precision and accuracy

#### Specificity

The specificity of the condensation reaction and selective determination of GMX with ninhydrin, ascorbic acid and PBQ was investigated by observing any interference encountered from the common tablet excipients, such as talc, lactose, starch, avisil, gelatin, and magnesium stearate. These excipients did not interfere with the proposed methods.

#### Precision

(a) Repeatability (Intra-day precision): The repeatability was tested by applying the proposed methods for the determination of calibration curve of GMX in pure form was prepared six determinations at four different concentrations on the same day. The results are pre-

**TABLE 3 : Determination of GFX in pharmaceutical preparation (Factive tablets) applying the standard addition technique.**

Proposed methods	Amount taken ( $\mu\text{g mL}^{-1}$ )	Amount added ( $\mu\text{g mL}^{-1}$ )	Amount found ( $\mu\text{g mL}^{-1}$ ) $\pm$ S.D. <sup>a</sup>	Recovery (%)	RSD (%) <sup>a</sup>
Ninhydrin	10	10	20.14 $\pm$ 0.054	100.70	0.27
	15	15	30.10 $\pm$ 0.163	100.33	0.54
	20	10	29.91 $\pm$ 0.111	99.70	0.37
Ascorbic acid	10	10	19.98 $\pm$ 0.122	99.90	0.61
	20	20	40.15 $\pm$ 30.11	100.37	0.75
	30	10	39.89 $\pm$ 0.191	99.73	0.48
PBQ	10	10	10.06 $\pm$ 0.093	100.60	0.92
	20	20	40.04 $\pm$ 0.416	100.10	1.04
	30	30	59.70 $\pm$ 0.46	99.50	0.77

<sup>a</sup> Average of six determinations.

sented in TABLE 3.

(b) Intermediate Precision: Intermediate precision was assessed by comparing the assays of GMX in pure form applying the three methods on different days (5 days,  $n = 6$  at each concentration). The results of accuracy and precision summarized in TABLE 3 and shows that the proposed methods have good repeatability and reproducibility.

Precision was expressed as the relative standard deviation (R.S.D. %). Accuracy was expressed as the mean relative error (R.E. %)<sup>[25]</sup>

The percentage mean relative error calculated using the following equation:

$$\text{R. E. \%} = [(\text{founded} - \text{added}) / \text{added}] \times 100$$

#### Accuracy

To test the validity of the proposed methods they were applied to the determination of authentic sample of GMX over the concentration range cited in TABLE 1. The results obtained were in good agreement with those obtained using the reference method. Student's  $t$ -test and the variance ratio  $F$ -test<sup>[26]</sup> revealed no significant differences between the performance of the three methods regarding the accuracy and precision, respectively (TABLE 2).

**TABLE 2 : Evaluation of the accuracy and precision of the proposed methods**

Proposed methods	Amount taken ( $\mu\text{g mL}^{-1}$ )	Recovery (%) $\pm$ S.D. <sup>a</sup>	Precision RSD (%) <sup>b</sup>	Accuracy Er %	SAE <sup>c</sup>	Confidence limit <sup>d</sup>
Ninhydrin	10	99.90 $\pm$ 0.042	0.42	-0.10	0.017	9.99 $\pm$ 0.044
	20	100.15 $\pm$ 0.078	0.39	0.15	0.032	20.03 $\pm$ 0.082
	30	100.27 $\pm$ 0.16	0.53	0.27	0.065	30.08 $\pm$ 0.167
Ascorbic acid	10	100.40 $\pm$ 0.067	0.67	0.40	0.027	10.04 $\pm$ 0.69
	20	99.85 $\pm$ 0.118	0.59	-0.15	0.048	19.97 $\pm$ 0.123
	30	99.53 $\pm$ 0.21	0.71	-0.47	0.087	29.86 $\pm$ 0.224
PBQ	20	99.90 $\pm$ 0.16	0.80	-0.10	0.065	19.98 $\pm$ 0.167
	40	100.20 $\pm$ 0.21	0.52	0.20	0.085	40.08 $\pm$ 0.219
	60	100.25 $\pm$ 0.39	0.65	0.25	0.159	60.15 $\pm$ 0.409

<sup>a</sup> S.D. = Average of six determinations.

<sup>b</sup> RSD (%) = Percentage relative standard deviation

<sup>c</sup> SAE = Standard analytical error

<sup>d</sup> Confidence limit at 95% confidence level and five degrees of freedom ( $P = 0.05$ ) ( $t = 2.571$ ).

#### Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged; the volume of reagents, wavelength range, and time. These minor changes that may take place during the experimental operation did

not affect the absorbance of the reaction products.

Method ruggedness was expressed as RSD% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments suggesting that the developed methods were robust and rugged.

### Recovery

The recovery of the proposed methods, the standard addition technique by adding a known amount of standard at three different levels to the pre-analysed sample. From the amount of drug found, the percentage recovery was calculated by the following equation:

$$\text{Recovery (\%)} = [(C_t - C_u) / C_a] \times 100$$

where  $C_t$  is the total concentration of the analyte found,  $C_u$  the concentration of the analyte present the formulation, and  $C_a$  is the concentration of the pure analyte added to the formulation.

### Analytical applications

The proposed methods were then applied to the determination of GMX in its tablets. The methods were tested for linearity, specificity, accuracy, repeatability, and precision according to ICH Q2B recommendations.<sup>[25]</sup>

### Specificity

The specificity of the methods was investigated by observing any interference encountered from the common tablet excipients, such as magnesium stearate, hydroxypropyl methylcellulose, microcrystalline cellulose, polyethylene glycolate, povidone, titanium dioxide, talc, lactose, gelatin, and starch. These excipients did not interfere with the proposed methods. The re-

**TABLE 4 : Determination of GFX in pharmaceutical preparation (Factive tablets)**

	Proposed method		Reference method <sup>[10]</sup>		$t_{cal}^b$	$F_{cal}^c$
	Recovery (%)	RSD (%) <sup>a</sup>	Recovery (%)	RSD (%) <sup>a</sup>		
Ninhydrin	100.24	0.393			0.523	2.03
Ascorbic acid	100.00	0.613	100.08	0.56	0.216	1.20
PBQ	100.07	0.91			0.021	2.64

<sup>a</sup> Six independent analyses.

<sup>b</sup> Theoretical value for  $t$  at 95% confidence level is 2.571.

<sup>c</sup> Theoretical value for  $F$  at 95% confidence level is 5.05.

sults of the proposed methods were statistically compared with those obtained using the reference method. Statistical analysis of the results, using Student's  $t$ -test and the variance ratio  $F$ -test revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (TABLE 4).

## CONCLUSIONS

The proposed methods are applicable for the assaying of GMX and have the advantage of a wider range under the Beer's law limits. The decreasing order of sensitivity and  $\lambda_{max}$  among the proposed methods are  $A > B > C$ , respectively. The proposed methods are simple, selective and can be used in the routine determination of GMX in bulk samples and formulations with reasonable precision and accuracy.

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