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Quantitative analytical methods for determination of gemifloxacin mesylate in different matrices: A review

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ABSTRACT: ***Gemifloxacin mesylate (GMF) is a fourth generation fluoroquinolone antibacterial synthetic broad-spectrum agent for oral administration with enhanced affinity for bacterial topoisomerase IV and is being developed for the treatment of respiratory and urinary tract infections. The compound has a broad spectrum of activity against Gram-positive and Gram-negative bacteria. There are different analytical methods available to determine GMF applied in quality control of this medicine in order to ensure its effectiveness and safety. A large number of methodologies including UV-visible spectrophotometry, spectrofluorimetry, high performance liquid chromatography, electrochemical and capillary electrophoresis methods have been developed for the quantitation of GMF in bulk, pharmaceutical, and biological samples. UV-spectrophotometric methods lacked wide linear dynamic ranges and sensitivity. There is some scope for developing methods based on ion-association reactions using many acidic anionic dyes, charge-transfer complexation reactions using substituted p-benzoquinone, polynitro phenols, nitro derivatives, etc., and oxidative coupling reactions involving 3-methyl-2-benzo thiazolinone hydrazone and oxidants like cerium(IV), iron(III), permanganate etc.. This paper will attempt to review several published methodologies and the instrumental conditions, which have been applied to measure GMF and within the last 17 years.***

Keywords: Gemifloxacin mesylate; Analytical methods; Spectroscopy; HPLC; electrochemical and capillary electrophoresis.

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I. INTRODUCTION

Fluoroquinolones, as a group, have shown excellent activity against the most frequently occurring gram-positive and –negative ocular pathogens. Earlier generation fluoroquinolones, such as ciprofloxacin and ofloxacin, havebeen used widely to treat various pathogenic conditions. However, the development of a resistant strain against these fluoroquinolones has been reported. Gemifloxacin is fourth-generation fluoroquinolone, possess an improved antibacterial spectrum, particularly against resistant staphylococcus and streptococcus pathogens, compared with older fluoroquinolones. Gemifloxacin mesylate (GMF) (Fig. 1) is fluoroquinolone antibacterial agent that is effective against both gram-positive and gram-negative bacteria [1, 2].

Chemical properties of GMF

Gemifloxacin mesylate (GMF) (Fig.1) chemically is (R,S)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl] -1cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid mesylate. Its molecular weight is 485.49 g mol^{-1} corresponding to the empirical

formula of $C_{18}H_{20}FN_5O_4.CH_4O_3S$. It melts at 235–237 °C). GMF mesylate salt physically is a white to light brown solid. It is soluble in water, methanol and ethanol [3].

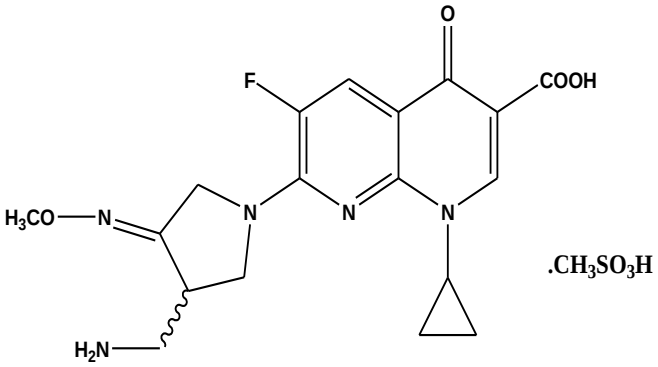

Fig. 1. The chemical structure of GMF

The analytical tools available play an important role helping to identify and separate high-quality products from the others. The development of analytical methods for the qualitative and quantitative quality control of pharmaceuticals should be based on good planning. The methods should allow for complete analysis of the product; considering aspects such as identification and determination of the active substance, the identification and determination of levels of impurity and degradation products, and verification of the stability of the active substance in the formulation is very important.

Because of its therapeutical importance, quantitative determination of GMF in bulk, pharmaceutical, and biological samples is of considerable significance in both quality control of preparations and chemical diagnosis. In the last approximately 17 years, several methods have been reported for the determination of GMF in pharmaceuticals and biological materials including body fluids. The current review surveys the methods developed to determine GMF in bulk, pharmaceutical, and biological samples in bulk, pharmaceutical products and biological materials.

II. Analytical methodologies

UV-Visible spectrophotometric, spectrofluorimetric, electrochemical, chromatographic and capillary electrophoresis methods have been described for quantifying CQP in pure, dosage forms, and biological fluids.

II.1. Pharmacopoeial methods

GMF is an official drug in United States Pharmacopoeia [3]. In the method described in United States Pharmacopoeia, GMF assay was done by using high performance liquid chromatography (HPLC), in which C18 (250 mm×4.6 mm, 5 µm) column was used as stationary phase and the mobile phase was composed of Acetonitrile, water, and trifluoroacetic acid (20: 80: 0.1). Column temperature was set at 40 °C and the flow rate at 1.0 mL/ min with injection volume 5 µL. Column effluent was monitored at 272 nm.

II.2. Densitometric thin-layer chromatography (TLC)

Abdallah, 2014 [4] developed densitometric thin layer chromatography for determination of GMF in human plasma withthree co-administered drugs, theophylline, warfarin and omeprazole. The method was linear over concentration range 0.1 to 3 µg mL^{-1}, 0.5 to 6 µg mL^{-1}, 0.2 to 2.5 µg mL^{-1} and 0.1 to 1.5 µg mL^{-1} of GMF, theophylline, warfarin and omeprazole, respectively. The mobile

phase was selected as mixture of dichloromethane, methanol and ammonia in the ratio of (7: 5.5: 3 v/v/v) for the development of plates. Densitometric analysis was carried out at wavelength 254 nm.

II.3. Spectrophotometric method

A novel univariate and multivariate regression methods along with model-updating technique were developed and validated for the simultaneous determination of quaternary mixture of imatinib (IMB), gemifloxacin (GMF), nalbuphine (NLP) and naproxen (NAP). The univariate method is extended derivative ratio (EDR) which depends on measuring every drug in the quaternary mixture by using a ternary mixture of the other three drugs as divisor. Peak amplitudes were measured at 294 nm, 250 nm, 283 nm and 239 nm within linear concentration ranges of 4.0–17.0, 3.0–15.0, 4.0–80.0 and 1.0–6.0 μg mL^{-1} for IMB, GMF, NLP and NAB, respectively [5]. A spectrophotometric method for the determination of GMF is developed and validated according to ICH guidelines. The analysis of the pure drug was carried out at its λ_{max}270 nm. The method was linear from 0.5-5 μg mL^{-1}, r^2=0.999 and equation is 0.102-0.000. The % RSD for inter-day (0.969%) and intra-day (0.714%) assuring a good precision and accuracy was close to 100%. Limit of detection and Limit of quantification were 0.197 and 0.599 μg mL^{-1}, respectively [6]. Developed and validated dissolution test for GMF tablets using spectrophotometric method by Hajera, 2012 [7]. The dissolution established conditions were: 900 mL of 0.01M HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm for GMF. Validation of UV spectrophotometric method for quantitative determination of GMF in tablets using methanol as solvent was described. The method was specific, linear, precise, exact and robust at 272 and 343 nm. The results confirmed that the method in both wavelengths is valid and useful to the routine quality control of GMF in coated tablets. The validate method was compared to liquid chromatography (HPLC), microbiological assay and visible (VIS) spectrophotometry, which were previously developed and validated to the same drug [8].

Two simple, sensitive, rapid, precise UV spectrophotometric methods have been developed and validated for simultaneous estimation of GMF and ambroxol hydrochloride. Recently this combination of two drugs is approved for the treatment of lower respiratory tract infection mainly pneumonia and bronchitis in adult which is available in tablet dosage form. First method is simultaneous equations method, wavelength selected for estimation of GMF and ambroxol hydrochloride are 271 nm and 209 nm. The second method is first order derivative method based on measurement at zero crossover point of another drug, measurement of GMF and Ambroxol hydrochloride were carried out at 262 and 218 nm respectively. The linearity was obtained in the concentration range of 2-14 μg mL^{-1} for GMF and 1.5-10.5 μg mL^{-1} for ambroxol hydrochloride [9]. Two simple and rapid spectrophotometric methods have been developed for simultaneous estimation of gemifloxacin mesylate (GMF) and ambroxol hydrochloride (AMB) in tablets by Wankhede et. al., 2011 [10]. First method involves solving simultaneous equations based on measurement of absorbance at two wavelengths 272 nm and 249.5 nm λmax of GMF and AMB, respectively. Second method is first order derivative spectroscopy, wavelengths selected for quantitation were 216.0 nm for GMF (zero cross for AMB) and 279.0 nm for AMB (zero cross for GMF). Beer's law was obeyed in the concentration range of 8.0-40 μg mL^{-1} and 6.0-30 μg mL^{-1} for GMF and AMB, respectively. A simple spectrophotometric method has been developed for the estimation of GMF in bulk and marketed tablet dosage form. The proposed method is based on the principle that GMF exhibiting an absorption spectra of wavelength maxima 267 nm in acidic medium. This method has successfully used for the analysis of drug in marketed preparations in the range of 10-70 μg mL^{-1} with correlation coefficient of 0.9987. The percentage of recovery was found to be 99.4-99.5% [11]. Two simple, economic and accurate UV spectrophotometric methods have been developed for determination of GMF in pharmaceutical tablet formulation. The first UV-spectrophotometric method depends upon the measurement of absorption at the wavelength 263.8 nm. In second area under curve method the wavelength range for detection was selected from 268.5-258.5 nm. Beer's law was obeyed in the range of 2 to 12 μg mL^{-1} for both the methods. The proposed methods was validated statistically and applied successfully to determination of GMF in pharmaceutical formulation [12]

Spectrophotometric methods in the visible region for the determination of GMF are based on the reaction of the drug with different reagents, yielding colored compounds. Some reagents are used for determination of GMF through "ion-pairing complex formation" Table 1. Krishna and Sankar, 2008 developed four simple and sensitive ion-pairing spectrophotometric methods for the assay of GMF either in pure form or in pharmaceutical formulations. The developed methods involve formation of colored chloroform extractable ion-pair complexes of the drug with safranin O (SFN O) and methylene blue (MB) in basic medium; napthol blue 12BR (NB 12BR) and azocaramine G (AG) in acidic medium. The extracted complexes showed absorbance maxima at 525, 650, 620 and 540 nm for SFN O, MB, NB 12BR and AG, respectively [13]. Three simple, economical, precise and reproducible Visible Spectrophotometric methods have been developed for the estimation of GMF in pure form. The developed methods are based on the formation of products by the reaction of GMF with Fast Green dye (FGFCF), Brucine and Vanillin. The maximum absorbances observed for the three methods are at 625 nm, 520 nm and 500 nm respectively and linearity in the concentration range of 30-100, 40-80 and 10-40 µg mL^{-1} [14]. Extractive spectrophotometric methods were developed for the determination of GMF in pure form and pharmaceutical formulations. These methods are based on the formation of ion-pair complexes between the basic drugs and acid dyes, namely, bromocresol green (BCG), bromocresol purple (BCP), bromophenol blue (BPB), bromothymol blue (BTB), and methyl orange (MO) in acidic buffer solutions. The formed complexes were extracted with chloroform and measured at 420, 408, 416, 415, and 422 nm for BCG, BCP, BPB, BTB, and MO, respectively. The analytical parameters and their effects are investigated. Beer's law was obeyed in the ranges 1.0-30 µg mL^{-1} [15]. A simple visible spectrophotometric method was developed for the determination of GFM in tablets. The method was based on the formation of a yellow ion-pair complex between the basic nitrogen of the drug and the sulfonphthalein acid dye in phthalate buffer [16]. Sensitive spectrophotometric method was developed for the determination of GMF in pure and pharmaceutical preparation. This method is based on ion pair formation reaction between GMF and rose bengal indicator in universal buffer of pH 5. The formed ion pair is measured at λ_{max} = 575 nm [17]. Simple ion-pair spectrophotometric extraction method for the assay of GMF in pure, tablets and spiked human urine. The method is based upon the reaction of GMF with methyl orange, forming a yellow-colored complex in acidic medium, which is extracted in chloroform and analyzed. The extracted complexes showed absorbance maxima (λ_{max}) found to be at 427 nm. Beer's law was obeyed for a wide concentration range, i.e., 10–80 µg mL^{-1} as the extracted species seemed well defined and stable. [18]

The reactions through "forming a charge transfer complex" are described in Table 1. Krishna and Sankar, 2008 was developed four simple spectrophotometric methods for the determination of GMF in pharmaceutical formulations. The methods were based on the charge transfer complexation reaction of the drug as n-electron donor with sigma (σ)-acceptor iodine, and the pi (π)-acceptors 2, 3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ)-7,7,8,8-tetra cyanoquinodimethane (TCNQ) and tetracyanoethylene (TCNE). The obtained charge transfer complexes were measured at 290 nm for iodine (in 1, 2-dichloroethane), at 470, 840 and 420 nm for DDQ, TCNQ and TCNE (in acetonitrile), respectively. Beer's law is obeyed in the concentration range of 6.0-30, 2.0-10, 2.5-12.5 and 1.0-5.0 µg mL^{-1} for iodine, DDQ, TCNQ and TCNE methods, respectively. [19]

Development and validation of two innovative 96-microwellbased spectrophotometric assay with high throughput for the quality control of GMF. The assay was developed for GMF via the formation of red metal complexes with $FeCl_3$. The reaction of GMF with both $FeCl_3$ reagent was performed in transparent 96-microwell plates, and the absorbance of the colored complexes was

measured at 460 nm using an absorbance microplate reader [20]. A kinetic spectrophotometric method for accurate and sensitive determination of GMF has been described. The method is based on the reaction of the studied drugs with potassium permanganate in the presence of sodium hydroxide to form a water-soluble green product which shows maximum absorbance at 604 nm. The determination of GMF drug by rate constant, fixed-concentration, and fixed time methods was feasible with the calibration equations obtained but the fixed time method had been found to be more applicable. The concentration of the selected drugs is calculated using the calibration equation for the fixed time method. The absorbance–concentration plot is linear over the range of 4–36 μg mL^{-1} with correlation coefficient of 0.9998 [21]. Simple spectrophotometric method was developed for the estimation of GMF in pharmaceutical dosage forms and biological human urine. The method is based on the oxidation reaction between phosphomolybdic acid (PMA) and GMF to form molybdenum blue (Mo^{+5}). Beer's law was obeyed in the concentration range of (5-27 μg mL^{-1}). The correlation coefficient (r^2) was found to be 0.9999 [22]. Simple, sensitive and selective methods are developed for the spectrophotometric determination of GMF. The methods use Chloramine-T oxidant and Rhodamine-B dye as reagents and are based on adding of a known excess of Chloramine-T to GMF in hydrochloric acid medium followed by determination of residual oxidant by reacting with a fixed amount of Rhodamine- B and measuring the absorbance at 557 nm [23].

Simple and sensitive kinetic spectrophotometric method was proposed for the determination of GMF in pure form and pharmaceutical preparations (tablets). The method is based on coupling the studied drugs with 4-chloro-7-nitrobenzo-2-oxa- 1,3-diazole (NBD-Cl) in the presence of alkaline borate buffer. Spectrophotometric measurement was achieved by recording the absorbance at 466 nm, after a fixed time of 20 min on a water bath adjusted at 70 ± 5°C for both drugs. The different experimental parameters affecting the development and stability of the color were carefully studied and optimized. The absorbance-concentration plots were linear over the ranges 0.5-8.0 μg mL^{-1}. The limit of detection of the kinetic method was about 0.12 μg mL^{-1} (2.47×10^{-7} M) [24]. Sensitive kinetic spectrophotometric method was developed for the determination of GMF in bulk and in pharmaceutical preparations. The method is based upon a kinetic investigation of the oxidation reaction of the drugs with alkaline potassium permanganate at room temperature for a fixed time of 20 min. The absorbance of the coloured manganate ion was measured at 610 nm. The absorbance-concentration plots were rectilinear over the ranges of 2.0-20 μg mL^{-1} [25].

Selective methods are developed for the spectrophotometric determination of GMF based on their reactivity towards N-bromosuccinimide (NBS). The method involves the addition of excess NBS of known concentration in the presence of 1M HCl, reactants are allowed to react and the unreacted NBS is estimated by the measurement in the decrease in the absorbance of the Rhodamine-B dye (λ_{max} 557nm) [26]. Spectrophotometric method based on formation of ternary complex with eosin at 543 nm in acetate buffer of pH 4. The absorbance-concentration plot is rectilinear over the range 1-10 μg/mL with LOD of 0.157 μg mL^{-1} and LOQ of 0.476 μg mL^{-1} [27]. Two simple spectrophotometric methods for the determination of the antibiotic GMF in pharmaceutical formulations was described. The first (A) is an indirect method in which oxidation of the drug with a known excess of cerium (IV) sulphate is followed by determination of the residual oxidant by adding excess methyl orange and measuring residual dye at 507 nm. The second (B) is a derivatisation method involving reaction of GMF with 1,2-naphthoquinone-4-sulphonate (NQS) in alkaline medium (pH 11) to form an orange-coloured product exhibiting maximum absorption (λ_{max}) at 411 nm. The methods were linear in the concentration ranges 2-9 and 5-30 mg/mL for methods A and B, respectively, with intra-day precision (as RSD) <1.5% for both [28]. Two simple and sensitive *spectrophotometric* methods (method A and

method B) in the visible region have been developed for the determination of GMF in bulk and in pharmaceutical formulations. Method A is based on the reduction of ferric chloride by GMF and subsequent complexation of ferrous ions with 2,2'-bipyridine reagents to form a red-coloured chromogen with an absorption maximum of 520 nm. Method B is based on the reaction of GMF with ferric alum reagent under acidic conditions to form a yellow-coloured product having an absorption maximum of 460 nm. The colour obeyed Beer's law in the concentration range of 6.4-32.0 and 5.1-25.6 µg mL^{-1} for method A and B, respectively [29]. A simple *spectrophotometric* method was described for the determination of GMF either in pure form or in the tablet. The method is based on chelate formation between GMF and Palladium (Pd II) in aqueous media. The complex showed an absorption maximum at 430 nm, 1st derivative at 480 nm and Second derivative at 500 nm respectively with apparent molar absorpitivities of 1.365×10^4 $L.M^{-1}Cm^{-1}$, 9.37×10^4 $L.M^{-1}Cm^{-1}$ for I st order derivative, 1.59×10^4 $L.M^{-1}Cm^{-1}$ for 2nd order derivative respectively. The solution of the complex obeyed beer's law in the concentration range of 2 to 14 µg ml for zero order,1 to 10 µg mL^{-1} for 1st order and 1to 15 µg mL^{-1} for 2nd order respectively [30]. Accurate spectrophotometric method was described for the determination of GMF a broad either in pure form or in the tablet. The method is based on chelate formation between GFX and Chromium (Cr III) in aqueous media. The complex showed an absorption maximum at 545 nm for zero order, 1st derivative at 620 nm and Second derivative at 660 nm respectively with apparent molar absorpitivities of 1.07×10^4 $L.M^{-1}$ Cm^{-1},,7.01×10^3 $L.M^{-1}$ $Cm^{-1}$for 1st order derivative, 1.04×10^4 $L.M^{-1}$ $Cm^{-1}$for 2nd order derivative respectively. The solution of the complex obeyed beer's law in the concentration range of 2 to 20 µg mL^{-1} for zero order,1 to 15 µg mL^{-1} for 1st order and 1 to 25 µg mL^{-1} for 2nd order respectively [31].

Three accurate and precise spectrophotometric methods for the determination of GMF in pure form and tablets are developed [32]. The first method is based on the reaction of ninhydrine reagent with primary amines present in GMF 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 GMF with p-benzoquinone (PBQ) to form a colored product with λ_{max} at 400 nm. Beer's law is obeyed in the concentration ranges 4.0-32, 8.0-40 and 9.0–72 µg mL^{-1} and molar absorptivity of 9.68×10^3, 5.58×10^3 and 4.98×10^3 l mol^{-1} cm^{-1} for ninhydrin, ascorbic acid and PBQ, respectively. Three simple and reproducible visible spectrophotometric methods (methods A-C) are developed for the determination of GMF in pure and dosage forms. Method A is based on the formation of coloured species on treatment of reduced GMF with Folin-Ciocalteu reagent in presence of 4.0 % NaOH solution. Method B is based on the formation of coloured species on treatment of GMF with 3-methyl-2-benzo thiazolinone hydrazone and ferric chloride. Method C is based on the formation of coloured species on treatment of GMF with $FeCl_3$ and 1,10-phenanthorline [33]. A simple and reproducible spectrophotometric method was developed for the determination of GMF in bulk and its pharmaceutical formulations. This method was based on the reaction of GMF with Fe(III) of ferric nitrate and 0.1M hydrochloric acid to produce an orange colored chromogen (λ max at 471 nm) [34]. Four spectrophotometric methods for the determination of GMF have been proposed by Krishna and Sankar, 2007 [35]. The first three methods. i.e. A, B and C, are based on the oxidation of the drug 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. The beer's law was obeyed in the concentration ranges of 3-15, 4-20 and 2-10 µg mL-1with molar absorptivity of 3.55×10^4, 2.10×10^4 and 3.10×10^4 $L.mole^{-1}cm^{-1}$ for methods A, B and C respectively. The fourth method, i.e. D was based on the interaction of GMF with ammonium heptamolybdate tetra hydrate, which resulted in the formation of molybdenum blue with λ_{max} 825 nm. The linear dynamic range and the molar absorptivity values were found to be 6.0-30 µg mL^{-1} and 1.38×10^3 $L.mole^{-1}cm^{-1}$, respectively.

Sensitive, spectrophotometric method have been developed for the determination of GMF in bulk and tablets. The method was based on formation of ternary complex between palladium (II), eosin and GMF in the presence of methyl cellulose as surfactant and acetate-HCl buffer pH of 4.0. The ternary complex showed absorption maximum at 530 nm. Beer's law was obeyed in the concentration range 2.0-22 µg/mL with a correlation coefficient of 0.9995. The composition of the complex, studied by the limiting logarithmic method shows that the molar ratio GMF: Pd (II): eosin is 1:1:1 [36]

Table 1. Comparison between the reported spectrophotometric methods for determination of GMF.

Reagent	λ_{max} nm	Concentration range (µg mL^{-1})	LOD (µg mL^{-1})	Molar absorptivity L mol^{-1} cm^{-1}	Reference
UV- Spectrophotometry	250	3-15	0.69	NA	[5]
UV- Spectrophotometry	270	0.5-5	0.197	NA	[6]
UV- Spectrophotometry	271	2-14	NA	NA	[9]
UV- Spectrophotometry	272	8-40	NA	NA	[10]
UV- Spectrophotometry	267	10-70	NA	NA	[11]
UV- Spectrophotometry	268.5-258.5	2-12	NA	NA	[12]
Safranin O	525	3.0-15	NA	2.81×10^4	[13]
Methylene blue	650	4.0-20	NA	2.20×10^4	
Napthol blue 12BR	620	2.0-10	NA	4.02×10^4	
Azocaramine G	540	2.0-10	NA	4.15×10^4	
Fast Green dye (FGFCF)	625	30-100	NA	2.37×10^3	[14]
Brucine	520	40-80	NA	0.746×10^3	
Vanillin	500	10-40	NA	1.38×10^3	
BCG	420	1-16	0.23	2.1787×10^4	[15]
BCP	408	1-12	0.26	3.9244×10^4	
BTB	415	2-16	0.52	1.8904×10^4	
BPB	416	1-16	0.28	2.4457×10^4	
MO	422	3-30	0.87	0.9386×10^4	
BCG	417	12-28		NA	[16]
Rose Bengal	575	9.71-53.40	1.90	1.861×10^4	[17]
Methyl orange	427	10-80	0.2563	NA	[18]
Iodine	290	6.0-30	NA	NA	[19]
2, 3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ)	470	2.0-10	NA	NA	
7,7,8,8-tetra cyanoquinodimethane (TCNQ)	840	2.5-12.5	NA	NA	
Tetracyanoethylene (TCNE)	420	1.0-5.0	NA	NA	
$FeCl_3$	460	NA	NA	NA	[20]
$KMnO_4$/ NaOH	604	4.0-36	0.0778	1.21×10^4	[21]
Phosphomolybdic acid	794	5-27	4.49	1.8×10^4	[22]
Chloramine-T/ Rhodamine-B	557				[23]
NBD-Cl/ borate buffer	466	0.5-8.0	0.12	4.0892×10^4	[24]
$KMnO_4$/ NaOH	610	2-20	0.42	1.067×10^4	[25]
N-bromosuccinimide/ Rhodamine-B	557	NA	NA	NA	[26]
Eosin	543	1-10	0.157	NA	[27]
Cerium (IV) sulphate/methyl	507	2–9	0.27	2.117×10^4	[28]

Reagent	λ_{max} nm	Concentration range (µg mL^{-1})	LOD (µg mL^{-1})	Molar absorptivity L mol^{-1} cm^{-1}	Reference
orange					
1,2-naphthoquinone-4-sulphonate (NQS) in alkaline medium (pH 11)	411	5–30	1.04	7.523×10^3	
Fe (III) +2,2'-bipyridyl	520	6.4-32	NA	2.18×10^3	[29]
Ferric alum	460	5.1-25.6	NA	0.550×10^3	
Palladium/ zero order	430	2.0 - 14	NA	1.365×10^4	[30]
Palladium/ Ist derivative	480	1.0 - 10	NA	9.37×10^4	
Palladium/ 2nd derivative	500	1.0- 15	NA	1.59×10^4	
Chromium/ zero order	545	2.0 - 20	NA	1.07×10^3	[31]
Chromium / Ist derivative	620	1.0 - 15	NA	7.01×10^4	
Chromium / 2nd derivative	660	1.0- 25	NA	1.04×10^4	
Ninhydrine (DMF)	590	4.0-32	NA	9.68×10^3	[32]
Ascorbic acid (DMF)	530	8.0-40	NA	5.58×10^3	
p-benzoquinone (PBQ)	400	9.0–72	NA	4.98×10^3	
Folin-Ciocalteu / NaOH	685	10-50	NA	NA	[33]
3-methyl-2-benzo thiazolinone hydrazone/ $FeCl_3$	617	10-100	NA	NA	
$FeCl_3$ / 1,10-phenanthorline	466	40-200	NA	NA	
Ferric nitrate /HCl	471	NA	NA	NA	[34]
Fe (III) +1,10-phenanthroline	515	3.0-15	NA	3.55×10^4	[35]
Fe (III) +2,2'-bipyridyl	520	4.0-20	NA	2.10×10^4	
Fe (III) + ferricyanide	760	2.0 - 10	NA	3.10×10^4	
Ammonium heptamolybdate	825	6.0-30	NA	1.38×10^3	
Palladium (II), eosin /methyl cellulose	530	2-22	NA	NA	[36]

II.4. Spectrofluorimetric method

Spectrofluorimetric method is based on the quantitative quenching effect of GMF on the native fluorescence of eosin at pH 4.0. The quenching of the fluorescence of eosin was measured at 544 nm after excitation at 337 nm. The fluorescence concentration plot is rectilinear over the range 0.6-4 µg mL^{-1} with LOD of 0.108 µg mL^{-1} and LOQ of 0.328 µg mL^{-1} [26]. Accurate spectrofluorimetric method have been developed for the determination of GMF in bulk and tablets. The method was based on a reaction between GMF with zirconium (IV) in presence of acetate buffer of pH 6.0 to give highly fluorescent derivatives. The reaction was monitored spectrofluorimetrically by measuring the emission of the reaction product at 397 nm and excitation maxima at 265 nm. Beer's law was obeyed in the concentration range 0.5-4.0 µg/mL with a correlation coefficient of 0.9997 [36]. Fast and precise spectrofluorometric probe for GMF quantification in authentic drug, commercial tablets and bio-samples. The suggested technique was conducted by complexing GMF with Al ions in the presence of alkaline buffer of pH 8. The FI was enhanced by adding sodium dodecyl sulfate (SDS). Rectilinear relationship was achieved over (0.05-100 ng mL^{-1}) drug samples. The fluorescence spectra were recorded at λex at 268 nm and λem 400 nm. The lowest quantification and detection values were 0.049 ng mL^{-1} and 0.05-100 ng mL^{-1}, respectively [37]. Quinone-based fluorophores and enhanced native fluorescence techniques were applied for a fast quantitative analysis

of GMF in pharmaceutical formulations. For this purpose, two sensitive, accurate and precise spectrofluorimetric methods were developed. GMF, 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 p-electron acceptors, forming charge transfer complexes that exhibit high fluorescence intensity at 441 and 390 nm upon excitation at 260 and 339 nm, respectively. Linearity was obtained over concentration ranges 50–500, and 10–60 ng mL^{-1} for methods A and B, respectively [38]. Novel selective, rapid, simple, sensitive, economic and reproducible spectrofluorimetric method was developed for the determination of GMF in bulk as well as pharmaceutical formulations. GMF, as a primary aromatic amine, reacts fluorescamine, which is highly sensitive fluorogenic reagent used in many investigations. This method was based on the reaction between GMF and fluorescamine in borate buffer solution of pH 8.5 to give a highly fluorescent derivative. This reaction product was measured spectrofluorimetrically at 483 nm after excitation at 353 nm under optimum conditions, linear relationship with best correlation coefficient 0.9999 and the linearity was detected in between the range of 100-1000 ng mL^{-1}. The limit of detection was found to be 0.123 µg/mL and the limit of quantification was 0.369 µg mL^{-1} [39]. A simple, rapid and highly sensitive spectrofluorimetric method was developed for determination of GMF in tablets. The method is based on measuring the native fluorescence of GMF in isopropanol at 400 nm after excitation at 272 nm. The fluorescence–concentration plot was rectilinear over the range of 0.01–0.50 µg mL^{-1} with a lower detection limit of 1.19 ng mL^{-1} and quantification limit of 3.6 ng mL^{-1} [40].

Two new, sensitive and selective spectrofluorimetric methods have been developed for the determination of GMF in tablets and spiked plasma samples. GMF, as a primary amine compound, reacts with 7-chloro-4-nitrobenzofurazon (NBD-Cl) (for method A) and fluorescamine (for method B) which are a highly sensitive fluorogenic reagents used in many investigations. For method A, the reaction product was measured spectrofluorimetrically at 516 nm with excitation at 451 nm. The reaction proceeded quantitatively at pH 8.5, 80 °C in 7 min. For method B, the method was based on the reaction between GMF and fluorescamine in borate buffer solution of pH 8.5 to give highly fluorescent derivatives that were measured at 481 nm using an excitation wavelength of 351 nm. The fluorescence intensity was directly proportional to the concentration over the range 40-200 ng mL^{-1} and 100- 1200 ng mL^{-1} for method A and B, respectively [41].

II.5. Electrochemical methods

Simple conductometric method was developed for the estimation of GMF in pharmaceutical dosage forms and biological human urine. This method is based on formation of an ion associate with phosphomolybdic acid (PMA). It involves direct titration with PMA in the range of 1-20 mg [22]. The voltammetric behavior of GMF on hanging mercury dropping electrode (HMDE) was studied using three different voltammetry modes. A well-defined cathodic peak was obtained in acetate buffer pH 5.0. An irreversible and diffusion controlled peak was characterized. The relationships between the current and the concentration of the investigated drug were plotted and displayed linearity over the concentration ranges of 0.01-0.19, 0.006-0.13 and 0.008-0.27 µg mL^{-1} with minimum detection limits of 1.49, 2.06 and 2.34 ng mL^{-1} using DPV, SWV and CV modes, respectively [42].

A sensitive electroanalytical method for determination of GMF in pharmaceutical formulation has been investigated on the basis of the enhanced electrochemical response at multi-walled carbon nanotubes modified glassy carbon electrode in the presence of CTAB. Solubilized system of different surfactants including SDS, Tween-20 and CTAB were taken for the study of electrochemical behaviour of GMF at modified electrode. The reduction peak current increases in the presence of

CTAB while other surfactants show opposite effect. The modified electrode exhibits catalytic activity, high sensitivity, stability and is applicable over wide range of concentration for the determination of GMF. The mechanism of electrochemical reduction of GMF has been proposed on the basis of CV, SWV, DPV and coulometeric techniques. The proposed squarewave voltammetric method shows linearity over the concentration range 2.47-15.5 µg mL^{-1}. The achieved limits of detection (LOD) and quantification (LOQ) are 0.90 ng $mL^{-1}$and 3.0 ng mL^{-1}, respectively. [43]

A simple, precise, inexpensive and sensitive voltammetric method has been developed for the determination of GMF in the presence of tween 80 in the bulk, pharmaceutical dosage forms and human urine at gold nanoparticles modified carbon paste electrode (GNCPE). The electrochemical behavior of GMF has been investigated by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The electrochemical oxidation of GMF was an irreversible process which exhibited adsorption-diffusion controlled process behavior in Britton-Robinson (BR) buffer over the entire pH range of values from 2 to 9. The adsorptive stripping response was evaluated as a function of some variables such as pH, type of surfactant, scan rate and accumulation time. The anodic peak current varied linearly over the range from 8.0×10^{-7} to 2.8×10^{-5} M. The limits of detection and quantification were 7.32×10^{-8} M and 2.44×10^{-7} M, respectively. The relative standard deviations and the percentage recoveries were found in the following ranges: 0.58-1.35% and 99.37-101.76%, respectively [44].

An ultrasenstive electrochemical probe was suggested for the estimation and quantification of GMF in its pharmaceutical products and biological media. Differential pulse (DPP), Cyclic voltammtery (CV) and Alternative current ACt were exploited to investigate the electrochemical nature of GFX. Over the pH range of 2.6-10, the investigated drug demonstrated significant cathodic peaks. The best polarographic response was achieved in acetate buffer (pH 5), scan rate 15 mV s-1 and pulse amplitude -90 mV. The outcome linearity was 1.59×10^{-6} – 2.70×10^{-5} mol L^{-1} (0.77 – 13.1 µg mL^{-1}). The limits of detection and quantification were determined as 2.89×10^{-7} and 8.76×10^{-7} mol L^{-1}, respectively [45].

Two nanoparticles based potentiometric sensors were fabricated for the selective determination of GMF. The first sensor was based on the formation of molecularly imprinted polymer nanoparticles using methacrylic acid as a functional monomer, trimethylolpropane trimethacrylate as a crosslinker and azobisisobutyronitrile as the initiator. The second sensor was based on the use of Fe_3O_4magnetic nanoparticles as core shells for the molecularly imprinted polymer. The developed sensors showed high selectivity, stability and sensitivity with wide concentration ranges of 1×10^{-3}-1×10^{-8} mol $L^{-1}$and 1×10^{-3}-1×10^{-10} mol L^{-1} for sensors1 and 2, respectively [46].

II.6. Chromatographic methods

Multiple sensitive eco-friendly analytical techniques were applied for the assessment of GMF. GMF was determined by green indicating micellar liquid chromatographic in authentic powder and dosage forms. The separation was performed by using a reversed-phase C18 column; the micellar mobile phase comprised of 12.5% n-propanol, 0.15 M sodium lauryl sulfate, and 0.3% triethylamine in 0.02 M O-phosphoric acid adjusted to pH 3.0 and pumped at 1 ml/min flow rate and detected at 266 nm by UV detection. The method was linear over the range of 4.0–90 µg mL^{-1} with a recovery percentage of 99.81 ± 0.69. The acidic degradation behavior of GMF along with the kinetic investigation was evaluated as recommended by ICH-stress conditions. The separation of the degradation product was performed in good elution time (less than 8 min), and the suggested technique has a good reproducibility (R.S.D. less than 1.0%) and provides an excellent resolution (Rs = 4.5) between GMF and its degradation product [47].

Novel green HPLC and HPTLC chromatographic methods were developed for the concurrent determination of moxifloxacin, levofloxacin, and GMF in bulk and pharmaceutical products. The green HPLC method was used on Thermo C18 (4.6 × 250 mm, 5 µm). By mixing ethanol and 20 mM sodium dihydrogen phosphate dihydrate (pH 5) in a ratio of 25:75, v/v, the mobile phase was created using isocratic elution. The flow rate was 1 mL min^{-1}. The studied antibiotics were separated well within 9.5 min. The green HPTLC method was used on coated HPTLC aluminum sheets with Silica gel 60 F254 using a mobile phase mixture of water: acetone: ammonia (8:1:1, v/v/v) [48].

A simple, accurate, and precise reversed phase (RP)-ultra performance liquid chromatography (UPLC) method was developed and validated for short time analysis of GMF in its bulk and pharmaceutical preparation. The optimum separation was achieved at 0.5±0.03 min using an AcclaimTM RSLC 120 C18 column 2.2 µm (2.1×100 mm) at 30°C by isocratic mobile phase at pH 3.0 composed of acetonitrile: phosphate buffer (25 mM) in a ratio of 75:25 (v/v). The column effluents were monitored at 276 nm using a photodiode array detector at a flow rate of 0.5 mL/min. The linearity of the calibration curve ranged from 0.5 µg/mL to 10 µg/mL and the square of the regression coefficient (r2) was 0.9991. The % relative standard deviation (RSD) of inter-day precision ranged from 0.081% to 1.233%, while for intra-day it ranged from 0.364% to 1.018%. The method was accurate with % recovery ranging from 93.71% to 100.29% and % RSD ranging from 1.054 to 2.722. The limit of detection and the limit of quantification were 0.066 and 0.2 µg/mL, respectively [49].

A liquid chromatography method was developed and validated for the determination of GMF in human plasma using chloramphenicol as internal standard to achieve lower quantification limit. Acetonitrile was used to precipitated and extracted analyte and internal standard from plasma by Protein Precipitation. Analysis was performed isocratically on C18 column using 25% acetonitrile and 75% 0.02 M phosphate buffer as mobile phase. The method was demonstrated to be linear from 0.003 µg/mL to 5 µg/mL with the lower limit of quantitation of 0.003 µg/mL [50].

A validated stability indicating RP-HPLC assay of GMF was developed by separating its related substances on an Inertsil-ODS3V-C18 (4.6 × 250 mm; 5 µm) column using 0.1% trifluoroaceticacid (pH 2.5) and methanol as a mobile phase in a gradient elution mode at a flow rate of 1.0 mL/min at 27°C. The column effluents were monitored by a photodiode array detector set at 287 nm [51].

A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of GMF in bulk and pharmaceutical formulations. The quantification was carried out using cyberlab capcell pak, ODS C_{18} (250 × 4.6 mm i.d., 5 µm particle size) column in an isocratic mode, with mobile phase comprising Buffer (KH_2PO_4 with pH 6.8): acetonitrile in the ratio of 80:20 (%v/v).The flow rate was at 1.2 mL/min and the detection was carried out at 265 nm. The retention time of the drug was found to be 7.47 min and the method produced linear response in the concentration range of 25-150 µg/mL (R~0.99986). The recovery studies were also carried out and % RSD from reproducibility was found to be 0.82 [52].

A high-performance liquid chromatographic method with fluorescence detection was developed and validated for the determination of GMF in human breast milk. The proposed method allows the determination of GMF in breast milk samples without complex sample preparation. The samples were mixed with a mobile phase and filtered with a 0.45 µm polytetrafluoroethylene filter before analysis. Chromatographic separation was carried out on a C18 column (150 × 4.6mm, 5 µmI.D.) usingmethanol:50mMortho-phosphoric acid solution (40:60) as the mobile phase with a 1.0mL/min flow rate. Quantitation was performed using fluorescence detection with an excitation wavelength at 272 nm and an emission wavelength at 395 nm. The linear range was found to be 0.1–2.5 µg/mL [53].

Novel, simple and sensitive high performance thin-layer chromatography (HPTLC) with fluorescence detection has been successfully developed and validated for determination of GMF in plasma samples without prior pretreatment. Montelukast (MK) was used as internal standard. GMF and MK in plasma samples were separated using a mobile phase consisting of a mixture of ethyl acetate: methanol:25% ammonia, (8:4.5:3, v/v/v). The emission intensity was measured using optical

filter K400 after excitation at 342 nm. The Rf values for GMF and MK were 0.45±0.03 and 0.79±0.02, respectively. Under the optimum conditions, a linear relationship with good correlation coefficient (r = 0.9965, n = 6) was obtained in concentration range of 3–180 ng/band. The LOD and LOQ of the proposed method were 0.45 and 1.5 ng/band, respectively. The accuracy of the method was proved as the recovery % of GMF from spiked human plasma was 94.21–101.85% [54].

A simple and rapid reversed phase high performance liquid chromatographic (RP-HPLC) method for the determination of GMF in human urine was developed and validated. GMF was isolated from urine samples after acidification using methylene chloride. Good chromatographic separation was achieved using C18Ultrasphere (250 mm × 4.6 mm, 5 µm.) analytical column maintained at 25 °C. The mobile phase consisted of methanol and 0.1 M phosphate buffer pH 3 in the ratio of (48: 52, v/v), respectively. The analysis time was 10 min at a 1.0 ml/min flow rate. The UV detection was carried out at 272 nm. GMF has been eluted at 7.5 min. Linearity was obtained over a concentration range of 20-200 ng/ml (r^2>0.999). The extraction recovery of GMF from urine samples was 60%. The proposed method demonstrated excellent intra-and inter-day precision and accuracy within 1.19% and 100.65 %, respectively. The limit of detection (LOD) was found to be 6.3 ng/ml. [55]

An isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the determination of GMF in bulk, dosage formulations and human serum at 270 nm. Chromatographic separation was achieved on Purospher STAR C18 (250 × 4.6 mm, 5 µm) column using mobile phase methanol: water (90:10, v/v) adjusted pH 2.8 via phosphoric acid 85% having low rate of 1.5 mL min^{-1} at ambient temperature. Calibration curves were linear over range of 5-100 µg mL^{-1} with a correlation coefficient 0.9998. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.015 and 0.045 µg mL^{-1}, respectively. Intra and inter-run precision and accuracy results were 98.73-100.12% and then correlated through student's t-test [56].

A reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the estimation of GMF in bulk drug and pharmaceutical dosage form. The quantification was carried out using C18 column (250mm × 4.6mm i.d, 5 mm) in an isocratic mode with a mobile phase consisting of methanol: 7% formic acid (80:20v/v), pH was adjusted to 2.1, at a flow rate of 1 ml/min. The separation was performed at ambient temperature and detection was carried out at 260 nm. The retention time of the drug was found to be 2.36min and method produced linear response in the concentration range of 10-60 µg/ml (R~0.9991) [57].

II.7. Capillary electrophoresis methods

A capillary zone electrophoretic (CZE) method has been developed for the determination of GMF in pharmaceutical tablet formulations. The CZE separation was performed using a 75 µm×35 cm fused silica capillary under the following conditions: 25°C; applied voltage, 12 kV; 25 mM H3PO4-NaOH running buffer (pH 8.5). The detection wavelength was 254 nm. Flumequine was used as internal standard. The method was suitably validated with respect to linearity, limit of detection and quantification, accuracy, precision, specificity, and robustness. The calibration was linear from 5 to 50 µg mL^{-1} and the limit of detection and quantification were 2.93 and 4.91 µg mL^{-1} [58].

Sensitive methods for quantitative determination of GMF in tablets by high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE). The HPLC method was carried out on a LiChrospher® 100 RP-8e, 5 µm (125 x 4 mm) column with a mobile phase composed of tetrahydrofuran water (25:75, v/v) with 0.5 % of triethylamine and pH adjusted to 3.0 with orthophosphoric acid. The CZE method was performed using 50 mM sodium tetraborate buffer (pH 8.6). Samples were injected hydrodynamicaly (0.5 psi, 5 s) and the electrophoretic system was operated under normal polarity, at +20 kV and capillary temperature of 18 ºC. A fused-silica capillary 40.2 cm (30 cm effective length) x 75 µm i.d. was used [59].

III. Challenges and conclusions

This review investigated the analysis of GMF from 2007 up to 2023. The review of the analytical methods reported for GMF showed that spectrophotometric, spectrofluorimetric, electrochemical, chromatographic, and capillary electrophoretic techniques have been applied to the determination of GMF in bulk, pharmaceutical, and biological samples. However, the HPLC with UV-detection system has been widely used for pharmaceuticals, and HPLC with mass and tandem mass spectrometric detector system has been largely employed for biological materials. The reviewed UV-spectrophotometric methods lacked wide linear dynamic ranges and sensitivity, which could be tackled by enhancing the aqueous solubility of the drug using strategies such as covalent solubilization and micellar solubilization. Inspite of the drug's ability to form colored products with a number of chromogenic agents, visible spectrophotometric methods based on a few reactions have been reported. There is some scope for developing methods based on ion-association reactions using many acidic anionic dyes, charge-transfer complexation reactions using substituted p-benzoquinone, poly nitrophenols, nitro derivatives, etc., and oxidative coupling reactions involving MBTH and oxidants like cerium(IV), iron(III), permanganate etc. Electrochemical techniques, with low LOD, as a simple, selective, and sensitive method, have been used to measure GMF in pharmaceutical and biological samples. Numerous electrodes have been modified with different nanomaterials such as multiwall carbon nanotubes, silver- and magnetic nanoparticles to increase the efficacy of electrochemical identification. UV-visible spectrophotometry has been only used to determine GMF in pharmaceutical samples.

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