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Selective kinetic spectrophotometric method for determination of gatifloxacin based on formation of its N-vinyl chlorobenzoquinone derivative

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ABSTRACT

A selective and simple kinetic spectrophotometric has been developed, for the first time, for the determination of gatifloxacin (GAT) in its dosage forms. The method was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of GAT by its reaction with 2,3,5,6-tetrachloro-1,4-benzoquinone in presence of acetaldehyde. The formation of the colored product was monitored spectrophotometrically by measuring the absorbances at 655 nm. The factors affecting the reaction were studied and optimized. The stoichiometry of the reaction was determined, and the reaction pathway was postulated. Under the optimized conditions, the initial rate and fixed time (at 5 min) methods were utilized for constructing the calibration graphs. The graphs were linear in the concentration ranges of 2–100 and 10–140 μg ml^{-1} with limits of detection of 0.84 and 3.5 μg ml^{-1} for the initial rate and fixed time methods, respectively. The analytical performance of both methods was fully validated, and the results were satisfactory. The proposed methods were successfully applied to the determination of GAT in its commercial dosage forms. The label claim percentages were 99.7–100.5 and 98.2–99.5% for the initial rate and fixed time methods, respectively. Statistical comparison of the results with those of the reference method showed excellent agreement and proved that there was no significant difference in the accuracy and precision between the reference and the proposed methods. The proposed methods are superior to all the previously reported spectrophotometric methods in terms of the procedure simplicity and assay selectivity.

1. Introduction

Gatifloxacin (GAT; 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) is a fourth generation synthetic fluoroquinolone antibacterial agent. It has enhanced *in vitro* activity against extended spectrum of clinically important gram-positive and gram-negative pathogens, with better pharmacokinetics than the older generations. GAT is prescribed for the treatment of acute bacterial exacerbation of chronic bronchitis, acute sinusitis, community-acquired pneumonia, and urinary tract infections. It acts by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV. GAT does not appear to exert phototoxic effects [1].

Due to its clinical advantages, GAT is receiving a great interest and there was an increase in number of its pharmaceutical dosage forms in the market in recent past. For routine analysis of GAT, a simple, rapid and cost effective analytical method was required. A survey of literature revealed that GAT has been determined in its dosage forms by titrimetry [2], voltammetry [3,4], chromatography [5–10], capillary electrophoresis [11], atomic absorption spectrometry [12], chemiluminescence [13], fluorimetry [14,15], and spectrophotometry [12,16–19]. The titrimetry is insensitive and time consuming. The voltammetric, chromatographic, electrophoretic, atomic absorption spectrometric and chemiluminometric methods utilized dedicated and/or expensive instruments that are not available in most quality control laboratories.

Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories [20–25]. However, few spectrophotometric methods were reported for determination of GAT in its pharmaceutical dosage forms [12,16–19]. These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region [16] and/or decreased simplicity of the assay procedure (e.g. tedious precipitation [12] or liquid–liquid extraction [19] steps in the ion-pair formation-based methods). For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of GAT in its pharmaceutical dosage forms.

The kinetic spectrophotometric methods are becoming of a great interest in the pharmaceutical analysis [26–28]. The application of these methods offers some specific advantages such as improved selectivity due to the measurement of the evolution of the absorbance with the reaction time. As well, it provides

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the avoiding of the interference of the colored and/or turbidity background of the samples, and possibility the interference of the other active ingredients present in the combined pharmaceutical formulations. No attempts have been reported for the kinetic spectrophotometric determination of GAT. The present study describes, for the first time, the development and validation of a selective and simple kinetic spectrophotometric method for the determination of GAT.

The proposed method was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of GAT by its reaction with 2,3,5,6-tetrachloro-1,4-benzoquinone (TCBQ) in presence of acetaldehyde (ACD). The development of the color was monitored spectrophotometrically at its maximum absorption peak. The initial rate and fixed time methods, after their full optimization and validation, were adopted for the determination of GAT in its pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

Double beam V-530 (JASCO Co. Ltd., Kyoto, Japan) ultraviolet–visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements.

2.2. Chemicals and dosage forms

Gatifloxacin (GAT; Bristol Myers Squibb, USA). Acetaldehyde (ACD; Sigma Chemical Co., St. Louis, USA) was 70% (v/v), prepared in methanol. 2,3,5,6-Tetrachloro-1,4-benzoquinone (TCBQ; Sigma Chemical Co., USA) was 2×10^{-2} M, prepared in dioxane. All solvents and other chemicals used throughout this study were of analytical grade. Gatiflox® tablets (Bristol Myers Squibb, USA) are labeled to contain 400 mg of GAT per tablet. Tymer® tablets (Jamjoom Pharmaceuticals, Jeddah, Saudi Arabia) are labeled to contain 200 mg of GAT per tablet.

2.3. Preparation of standard and sample solutions

2.3.1. Preparation of stock standard solution

Into a 50-ml calibrated flask, an accurately weighed amount (50 mg) of GAT was dissolved in 40 ml of methanol. For complete dissolution of GAT, the solution was sonicated for 5 min. The resulting solution was completed to volume with the same solvent. This stock solution (1 mg ml^{-1}) was diluted with butanol to obtain working concentrations in the range of 20–1400 μg ml^{-1}.

2.3.2. Preparation of dosage forms sample solution

Twenty tablets were weighed and finely powdered. A quantity of the powder equivalent to 100 mg of GAT was transferred into a 50-ml calibrated flask, dissolved in 25 ml of methanol, swirled and sonicated for 5 min, completed to volume with the same solvent, shaken well for 10 min, and filtered. The first portion of the filtrate was rejected, and 25 ml of the filtrate was diluted with butanol to obtain working concentrations in the range of 20–1400 μg ml^{-1}.

2.4. General analytical procedures and data treatment

One milliliter of the standard or sample solution (20–1400 μg ml^{-1}) was transferred into 10-ml calibrated flasks. One milliliter of the ACD solution (70%, v/v in methanol) and 1 ml of TCBQ (2×10^{-2} M in dioxane) were added. The reaction mixture was mixed and completed to volume with butanol. After dilution and mixing, the reaction mixture was immediately transferred to a spectrophotometric cell and the absorbance was recorded (at 655 nm) as a function of time against reagent blank treated similarly.

The kinetic data that has been recorded were transformed to the Slide Write Plus software, version 5.011 (Advanced Graphics Software, Inc., CA, USA) for curve fitting, regression analysis, and statistical calculations. The initial rate (K) of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance–time curve. The calibration curve was constructed by plotting the logarithm of the initial rate ($\log K$) of reaction versus logarithm of the concentration ($\log C$) of GAT. Alternatively, the calibration curve was constructed by plotting the absorbance measured after a fixed time of 5 min.

2.5. Determination of molar ratio of the reactions

2.5.1. For GAT with ACD

The limiting logarithmic method [29] was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried using increasing ACD concentrations (3×10^{-2} to 1.8×10^{-1} M) at a fixed GAT concentration (1.3×10^{-4} M). The second set of experiments was carried using increasing GAT concentrations (2.49×10^{-5} to 2.49×10^{-4} M) at fixed ACD concentration (0.2 M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the ACD and GAT concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

2.5.2. For GAT with TCBQ

The limiting logarithmic method [29] was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried using increasing TCBQ concentrations (1.3×10^{-4} to 2.0×10^{-3} M) at a fixed GAT concentration (1.3×10^{-4} M). The second set of experiments was carried using increasing GAT concentrations (2.49×10^{-5} to 2.49×10^{-4} M) at fixed TCBQ concentration (2.0×10^{-3} M). The logarithms of the obtained absorbances were plotted as function of the logarithms of the TCBQ and GAT concentration in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

3. Results and discussion

3.1. Involved reaction and absorption spectra

The reaction involved in the present study was based on the formation of a colored N-vinyl chlorobenzoquinone derivative of GAT by its reaction with TCBQ in presence of ACD. The formation of this colored product was monitored spectrophotometrically at its maximum absorption peak (655 nm). The absorption spectrum for the reaction product is given in Fig. 1. The following sections describe the optimization of different factors affecting the reaction, kinetics, and the use of the optimized conditions in the development of the assay procedures.

3.2. Optimization of reaction conditions

The factors affecting reaction conditions (concentrations of ACD and TCBQ reagents, temperature, and the diluting solvent) were studied by altering each variable in turn while keeping the others constant. The intensity of the developed color was recorded as a function of the concentrations of ACD and TCBQ reagent. It was found that the color intensity was dependent on the concentration of both reagents (Fig. 2). The highest color intensity was attained