Spectrophotometric determination of norfloxacin using bromophenol blue

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Abstract We reported a spectrophotometric method for the determination of norfloxacin. The method is based on the reaction of the drug with bromophenol blue in acetate buffer at pH 4.1 and measurement of the yellow complex formed at 416 nm. Linear calibration graph was obtained from 5 to 150 ppm of norfloxacin with percentage relative standard deviation of less than 2% (n = 6), the correlation coefficient \( r = 0.9998 \). The method was applied successfully to drug analysis.

1. Introduction

Norfloxacin, [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperaziny)quinoline-3-carboxylic acid] (1) is a fluorquinolone carboxylic acid derivative used as a broad spectrum antibacterial used for urinary infections including cystitis and prostatitis.

Many methods have been applied for the determination of norfloxacin such as capillary electrophoresis (Yang et al., 2008), HPLC (Ganti et al., 2012 and Ulu, 2012), TLC (Wang et al., 1997), LC (Lee et al., 2007), Voltammetry (Ghoneim et al., 2001), ISE (Zhang et al., 1999), differential pulse polarography (Jaber and Lounici, 1994), fluorimetry (Vlieiopoulos et al., 1997), flow injection analysis (Yi et al., 2000; Yao-Dong et al., 2004 and Wang et al., 2007) and potentiometric titration.

Spectrophotometric methods have been widely applied to the analysis of norfloxacin. Simple, rapid and accurate method has been described (Amin et al., 1995). The method is based on the reaction of the drug as a \( \pi \) electron donor with 2,3-dichloro-5,6-dicyanobenzoquinone. The absorbance was measured at 460 nm and the method was applied to pharmaceutical analysis.

A simple kinetic spectrophotometric method based on the oxidation of norfloxacin with alkaline potassium permanganate (Rahman et al., 2004). The reaction was followed spectrophotometrically by measuring the rate of change of absorbance at 603 nm, the calibration graph is linear from 2 to 20 \( \mu \)g ml\(^{-1}\). The method was applied successfully to the determination of norfloxacin in dosage forms. (Suliman and Sultan, 1996) used the complex formed with ferric ions to the determination of norfloxacin. Reaction with 2,3,5,6-tetra
chlorobenzoquinone (Darwish et al., 2009) was applied to the drug analysis.

2,4-Dinitrofluorobenzene (El-Walily et al., 1999), tetracyanoethylene (El-brashy et al., 2004), ammonium reineckate (Ragab and Amin, 2004) and ferrous ions in acetate buffer (El-Khateeb et al., 1998) have been applied to the determination of the drug in various samples. Norfloxacin was determined colorimetrically using \( p \)-benzoquinone (Al Khamees, 1995). UV spectrophotometry has been applied for the simultaneous estimation of norfloxacin and tinidazole (Pant et al., 2012). A spectrophotometric method has been described for the determination of norfloxacin in pure and dosage forms by complexation with iron(III) and copper(II) ions (Gaber et al., 2012). \( p \)-Nitrophenol was applied for the determination of some antibacterial drugs such as norfloxacin (Xuan et al., 1998).

2. Experimental

2.1. Chemical reagents

2.1.1. Norfloxacin

Standard solution of norfloxacin (Philip Harris) 1000 ppm was prepared by dissolving 0.1 g in 30 ml of sodium hydroxide with shaking and then completing the volume to 100 ml with deionized water, and working solutions were prepared as needed.

2.1.2. Bromophenol blue

0.1% solution of bromophenol blue was prepared by dissolving 0.1 g of bromophenol blue (BDH) in 50 ml of deionized water and completing the volume to 100 ml with deionized water.

2.1.3. Buffer solution

Acetic acid–sodium acetate buffer was prepared by mixing 0.2 M acetic acid (AnalaR BDH 99.5%) with 0.2 M sodium acetate (BDH) to give the suitable pH value.

2.2. Apparatus

A Perkin–Elmer, Lambda 35 UV–Vis. Spectrophotometer was used for the absorbance measurement.

2.3. Optimization of conditions

When bromophenol blue is added to norfloxacin in the presence of acetate buffer solution, a yellow complex is formed, which can be extracted with chloroform. The complex was found to absorb at 416 nm, Fig. 1.

The absorption of the yellow complex was found to be related to the concentration of norfloxacin, parameters affecting this reaction were studied as follows:

2.3.1. Effect of pH

The effect of pH on the reaction was studied in the range 3.5–4.7 by adding different amounts of the buffer solution to 1 ml of norfloxacin (50 ppm) and 1 ml of 0.1% bromophenol blue. The complex was extracted in 10 ml of chloroform and the absorbance was measured at 416 nm. The absorbance was found to increase with increasing pH until 4.1 as shown in Fig. 2.

2.3.2. Effect of bromophenol blue

Different amounts of bromophenol blue were added to 50 ppm of norfloxacin at pH 4.1, the complex was extracted with 10 ml...
of chloroform and the absorption was measured at 416 nm. 1500 μg of bromophenol blue was found to be enough for the reaction to complete as shown in Fig. 3.

2.3.3. Effect of volume of the buffer
As studied before, the optimum pH value was 4.1, therefore, a buffer solution of 4.1 value was prepared and various volumes were added to 1500 μg of bromophenol blue and 1 ml of norfloxacin (50 ppm). Absorption was found to increase with increasing the volume added, and 0.4 ml gave the highest absorption as shown in Fig. 4.

2.3.4. Effect of shaking time
When the norfloxacin-bromophenol blue complex was formed, it can be extracted by shaking with chloroform. Time of shaking was investigated. No significant change in the absorbance was noticed but 3 min were found to be enough, as shown in Table 1.

After studying the parameters affecting the reaction, we can summarize the optimum conditions in the table below (see Table 2).

2.4. Procedure
In a 10 ml flask, 0.4 ml of the buffer acetate solution is added, followed by 1500 μg of 0.1% of bromophenol blue and 1 ml of 50 ppm norfloxacin, 10 ml of chloroform is added after 30 s of
mixing. Shake well for three minutes and transfer to a separating funnel. Measure the absorbance of the yellow complex at 416 nm against blank.

3. Calibration graph

At the optimized conditions, a linear calibration graph was obtained as shown in Table 3 and Fig. 5. The linearity can be extended up to 150 ppm of norfloxacin, as shown in Fig. 5.

3.1. Stability of the complex

The stability of the complex formed from the reaction of norfloxacin with bromophenol blue at the optimized conditions was studied. The complex was stable for about 60 min as shown in Table 4.

3.2. Analytical application

This method was applied to the analysis of norfloxacin in drugs, 5 tablets containing norfloxacin (MERK) were taken and grinded then 1 g is taken and dissolved in 30 ml of diluted sodium hydroxide with shaking. The volume was completed to 100 ml with deionized water. 1 ml of the stock solution is transferred to a 100 ml volumetric flask followed by 0.4 ml of the buffer and 1500 µg of bromophenol blue, the absorbance is measured at 416 nm as in the procedure described. Very good results were obtained with a recovery of 99.66% as shown in Table 5.

4. Results and discussion

Many organic compounds having basic nitrogen have been determined by reaction with acidic dyes anions at certain pH values to form colored ion-pair complexes. Norfloxacin has a basic nitrogen atom and can therefore similarly be determined. When norfloxacin reacts with bromophenol blue a yellow complex is formed which has a maximum absorbance at 416 nm. The absorbance was found to increase with increasing the drug concentration. This encouraged the development of a spectrophotometric method. Parameters affecting the reaction were investigated and optimized for better sensitivity. The complex formed was found to be stable for about 60 min. At the optimal conditions, the calibration graph was linear from 5 to 150 ppm of norfloxacin. The method was successfully applied to the analysis of drugs containing norfloxacin and gave very good recovery of 99.66%, the method is very simple, rapid only about three minutes needed for the measurement, reproducible with very good accuracy. The relative standard deviation was 2.0%.

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