2.7-Dichlorofluorescein: a Suitable Complexing Agent for the Spectrophotometric Determination of Ofloxacin in Pure and Pharmaceutical Dosage Forms

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Summary: Ofloxacin is a broad spectrum antibiotic effective against many Gram positive and Gram negative bacteria as well as for some key anaerobes. Ofloxacin was reacted with 2,7-dichloroflourescein in an acidified medium at room temperature to give a crimson-red complex which was measured at 430 to 460 nm (working wavelength 430 nm), thus providing a basis for a new spectrophotometeric method of analysis for ofloxacin in pharmaceutical dosage forms. The complex in solution form obeyed the Beer's Law between 0.02-0.25 mg/mL. The limit for detection was 0.02 mg/mL. The relative standard deviation was found to be 0.67 %. Similarly the molar absorptivity for complex was calculated to be 0.01×10^4 $dm^3 mole^{-1} cm^{-1}$. This method was found to be useful for the estimation of ofloxacin in pure as well as in pharmaceutical formulations. This method was easy to execute at laboratory level and required non cumbersome methodology, with a relatively high precision and accuracy.

Introduction

Ofloxacin, (±)9-fluoro-2,3,dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-d,e]-1,4-benzoxazine-6-carboxylic acid, is one of a new generation of fluorinated quinolone structurally related to nalidixic acid [1]. This agent is a broad spectrum antibacterial drug active against most Gram-negative, Gram-positive bacteria, and some anaerobes [2]. Its bacterial action is based on its anti-DNA gyrase activity [3]. The broad spectrum antibacterial activity and widespread distribution to most tissues and body fluids at relatively high concentrations after oral administration have made this drug useful for the treatment of systemic infections including urinary tract, respiratory, and gastro-intestinal infections [4-7]. Ofloxacin has a tricyclic ring structure with a methyl group at the C-3 position of the oxazine ring, which resulting in a chiral center. The *S*-ofloxacin has been reported 8-128 times more potent than the *R*-isomer [8]. The binding properties of both the *S*- and *R*-enantiomers towards DNA were studied and it was found that the binding mode and base specificity of *S*-ofloxacin to DNA was similar to those of norfloxacin, whereas the *R*-ofloxacin did not bind to DNA as efficiently [9, 10]. In order to elucidate the chiral selectivity in the complex formation between ofloxacin and DNA, the structure of the ofloxacin-DNA complex was investigated by molecular modeling and molecular dynamics. In the complex, ofloxacin is located in the minor groove and forms two hydrogen bonds with two consecutive GC base pairs: one between the carbonyl group of ofloxacin and the amine group of guanine and the other between the fluorine of ofloxacin and the amine group of the next guanine at opposite strand [11, 12].

Various analytical methods have been employed for the quantitative analysis of ofloxacin in pharmaceutical dosage forms and biological fluids and tissues. They include as spectrophotometery [13-16], polarography [17], fluorimetry [18, 19], electrophoresis [20, 21], microbial assay [22], high-performance liquid chromatography (HPLC) [23, 24], chemiluminescence [25], and voltammetry [26-28]. Either these methods are of low sensitivity or time consuming, and hence still far from perfection for routine quality assurance for one or another reason. The objective of our investigation was to develop an efficient, precise and simple spectrophotmeteric method for the selective determination of ofloxacin in

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pure and pharmaceutical dosage forms without interferences from the excipients.

Results and Discussion

The present investigation revealed that ofloxacin reacts with 2,7- dichloroflourescein at pH 7-8 to give a crimson-red colored complex. The absorption maximum lied between 430-460 nm, however, the working maxima was 430 nm. This fluctuation in absorption maxima was pronounced in case of pharmaceutical formulations therefore care must be taken, especially during filtration to avoid any unwanted materials interference. The maximum color intensity for the complex was produced when 100 mg/10mL concentration solution of 2,7-dichloroflourescein was used (Fig. 1), however, above and below this concentration the color intensity diminished and the color became unstable, presumably, it was due to deterioration of the complex at lower temperature or due to evaporation of methanol at temperature higher than 65 °C.

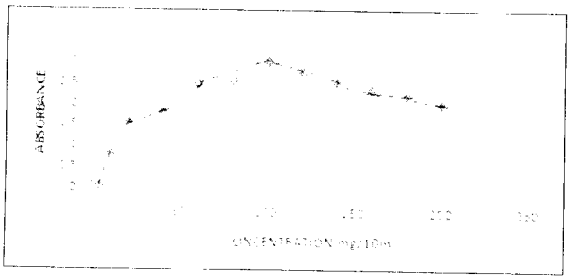

Fig. 1: Concentration effect of 2,7-dichlorofluorescein.

The effect of pH on complex is shown in (Fig. 2). The maximum color intensity was achieved at pH 7-8. The addition of HCl and 2,7-dichlorofluorescein in excess produced yellowish green color and precipitation might also take place. This might be due to protonation of complex by acid resulting in bathochromic shift.

The effect of temperature on complex is shown in (Fig. 3). It was found that color intensity was maximum at 25 °C, but with a rise in temperature higher than 65 °C, the color intensity was decreased and the complex became unstable due to evaporation of methanol.

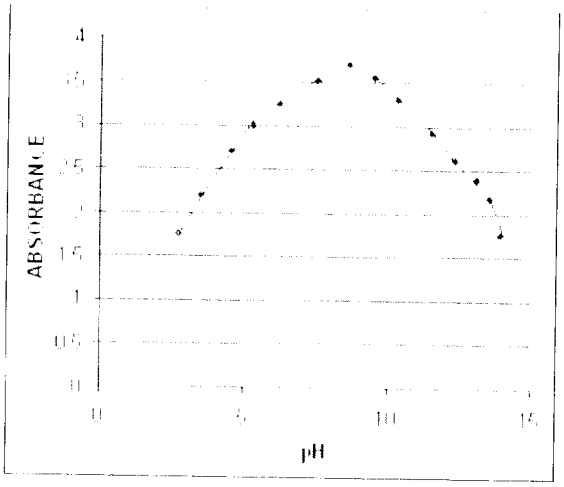

Fig. 2: Effect of pH on ofloxacin-2,7-dichlorofluorescein complex

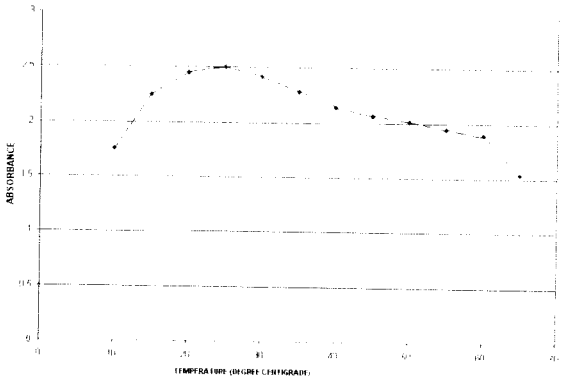

Fig. 3: Effect of temperature on ofloxacin-2,7-dichlorofluorescein complex.

The absorbance of the developed color intensity remain stable for more than 24 hours (Fig. 4). The result of the determination of pure ofloxacin-2,7-dichlorofluorescein complex in acidic medium did not exceed the relative standard deviation, 0.67% which is replicate of five determinations. The apparent molar absorptivity calculated was 0.01×10^{4} $dm^{3}mole^{-1}$ cm^{-1} and the regression equation Y=0.029-0.001, calculated by method of least square from eleven points each of which was average of three determinations. The correlation between absorbance and concentration was 0.999 in terms of correlation coefficient (Fig. 5).

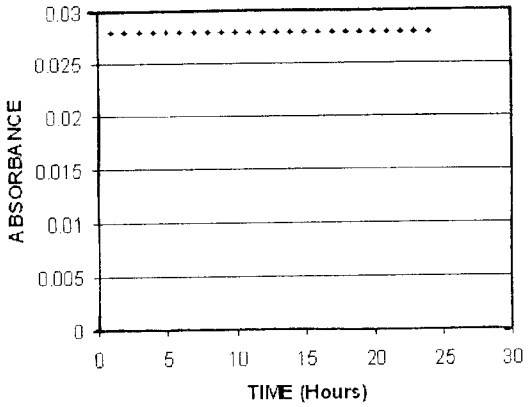

Fig. 4: Stability of complex with time.

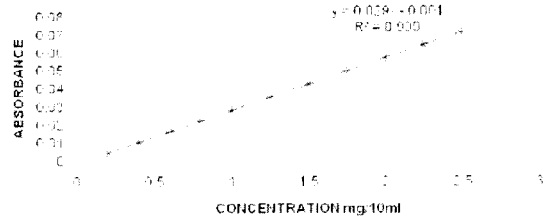

Fig. 5: Calibration curve of ofloxacin complex with 2,7-dichlorofluorescein.

The proposed method is precise, reproducible, robust and non cumbersome for the determination of ofloxacin in pharmaceutical formulations. This method is simple, highly accurate, and gives an advantage of working at room temperature without the use of heat thus favouring less time for quantitative analysis of ofloxacin.

Experimental

Apparatus and Reagents

Schimadzu UV-2300 Spectrophotometer was employed. 0.1M HCl (E. Merck, Germany), 0.1M NaOH (E. Merck, Germany) solutions were prepared in double-distilled water. HPLC grade methanol (E. Merck, Germany) was used. Beckman zerometric pH meter was used.

Preparation of Ofloxacin- 2,7- Dichloroflourescein Complex

Ofloxacin-2,7-Dichloroflourescein complex was prepared by mixing 10 mL of pure ofloxacin solution (0.25 mg/mL in methanol) and 1-2 mL of 2,7-dichlorofluorescein solution (1 mg/mL in methanol). As the solution was acidified with 1-2 drops of 0.1 M HCl, suddenly an exothermic reaction took place and a crimson-red colored complex was formed.

Construction of Calibration Curve

A methanolic aliquot containing the drug was transferred over a working concentration range of (0.02-0.25 mg/mL) into a 10 mL calibrated flask and 1-2 mL of (1 mg/mL) concentrated (methanolic) solution of 2,7-dichlorofluorescein were added. The solution was diluted to volume with methanol and then was acidified with 0.1 M HCl by adding its few drops until an exothermic reaction took place with the formation of crimson-red colored complex. The solution was then allowed to stand for 5 minutes at room temperature (25 + 1°C). The absorbance was measured at optimum λ_{max} (430 nm) after the specified time. A plot was formulated between absorbance and final drug concentration in order to obtain a calibration curve [28].

Assay of Ofloxacin in Pharmaceutical Dosage Forms

i) Tablets

Twenty tablets were weighed and pulverized. An amount equal to one tablet was weighed and extracted with 25 mL methanol for four times and filtered. Necessary amount of filtrate was diluted to 100 mL with methanol and sonicated for 20 min, and a further dilution upto 1 mg/mL was prepared [16]. Aliquots containing 0.2-3 mg/10 mL were prepared from the above mentioned stock solution. Following the above mentioned procedure the absorbance of these dilutions was measured at 430 nm. The quantity per tablet was calculated from standard calibration curve.

ii) Injections

1 mg/mL Parental solution of ofloxacin was prepared directly in methanol. The above mentioned procedure was followed by making aliquots containing 0.2-0.3 mg/mL and the absorbance was calculated at 430 nm. The quantity of ofloxacin was calculated from the standard calibration curve.

Effect of Excipients

In order to apply the proposed method of analysis on pharmaceutical formulations the influence of commonly used excipients (starch, lactose, glucose, sugar, talc, sodium chloride, titanium oxide, magnesium stearate, polyethylene glycol, hydroxyl propyl cellulose) and additives was studied by preparing solution containing 1 mg/mL of ofloxacin and increasing concentrations of the potential interferents upto 1×10^{-3} M [29]. It was seen that these excipients did not exhibit any pronounced effect on the analytical signal of ofloxacin-2,7-dichlorofluorescein complex.

Conclusion

The proposed method is facile, sensitive and suitable to apply for the determination of ofloxacin in bulk and pharmaceutical dosage forms. The projected method offers the advantage of accuracy and time saving as well as simplicity of reagents and apparatus.

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