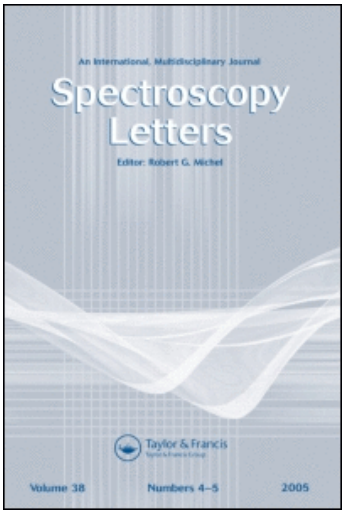

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Fluorometric and Derivative Spectrophotometric Determination of Norfloxacin

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FLUOROMETRIC AND DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF NORFLOXACIN

Key words: fluorometry, derivative spectrophotometry, serum,tablets, norfloxacin analysis

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ABSTRACT

The method for the direct determination of norfloxacin, without prior separation, in serum and pharmaceutical formulations, by means of fluorometry and second derivative u.v. spectrophotometry, was developed. Fluorometric assay of norfloxacin in serum was carried in 0.1 M HCl, with the adition of sodium-dodecylsulphate, at emission wavelength 450 nm (excitation 320 nm). Linear calibration curve was obtained in the concentration range 20 - 320 μg/L with the detection limit 2 μg/L. The second derivative spectrophotometry was used for the determination of the norfloxacin in tablets at 337 nm using 0.05 M NaOH as solvent. Detection limit was 30 μg/L.

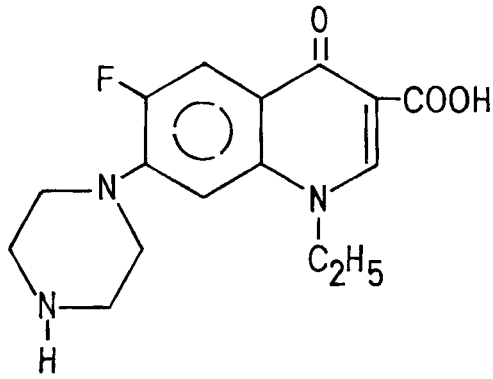

Fig. 1. Chemical structure of norfloxacin

INTRODUCTION

Norfloxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid (Fig.1.) is a fluoroquinolone antibiotic that exhibits strong bactericidal activity against both, Gram-positive and Gram-negative bacteria, in vitro and in vivo.

The mechanism of its action is based on the inhibition of the DNA-gyrase (topoizomeraze II) of bacteria. However its absorption, after oral administration is incomplete thus leading to the reduced bio-availability. So far, there were only few literature data of the direct quantitative assay (without prior extraction) of some fluoroquinolones in biological samples and pharmaceutical formulations based on HPLC [1] and fluorometric [2] determinations.The most commonly employed methods for the determination of norfloxacin are HPLC [4, 5, 8-10, 13, 14], DPP polarography [3], spectrofluorimetry [7, 11] and spectrophotometry [6, 12]. It is therefore of interest to develop fast and simple procedures for quantification of the norfloxacin in bio-fluids and tablets without prior extraction. The need for such procedures is especially important in pharmacokinetic studies of norfloxacin in plasma as well as pefloxacin , since norfloxacin is one of the metabolic products of pefloxacin.

Because the norfloxacin is a highly fluorescent compound and shows intense absorption in the ultraviolet, these properties were used for its direct determination in the serum and in tablets.

EXPERIMENTAL

Apparatus

Fluorescence measurements were made on Aminco SPF - 500 C spectrofluorimeter equipped with a 300 W Xenon lamp and 10 mm silica cells. Slit widths were set to 2.5 nm in both the excitation and emission monochromators; gain was adjusted to 10. UV spectra were made on Shimadzu (Japan) model 2100 double-beam, scaning spectrophotometer interfeced to data Station. Quartz cuvettes of 1 cm pathlength were used. Operating conditions were: scan speed 200 nm/ min.; time responce 0.1 sec and slit width 0.5 nm.

Reagents, solutions and samples

All reagents and solvents were of analytical grade purity. Water was doubly distilled. Norfloxacin standard, purity 100 %, was product of Sigma (USA). Tablets of norfloxacin "Nolicin" (nominally 400 mg) were supplied by "Krka"Novo Mesto (Slovenia). Sodium-dodecylsulphate (SDS) was product of Serva (FRG); 2 % water solution was used. 0.1 M HCl and 0.05 M NaOH were prepared from Merck (Darmstad, FRG) reagents.

Calibration graph and procedure for tablets

Stock solution of norfloxacin (10^{-3} M) was prepared by dissolving of norfloxacin standard in doubly distiled water. Different portions O.1-0.8 mL were pipeted into a 10 mL volumetric flasks and diluted to 10 mL with 0.05 M NaOH. The second -derivative spectrum of each solution was recorded against 0.05 M NaOH as reagent blank.

Ten tablets were accurately weighed and finely powdered. An amount of the powdered tablets , equivalent to 40 mg of norfloxacin ,was weighed and dissolved in water. The solution prepared was filtered and diluted with water up to 100 mL. An aliquot of sample tablet solution (0.2-0.3 mL) was used in the same procedure as described for the calibration graph.

Calibration graph and procedure for serum samples

A human pool serum was mixed with standard solution of norfloxacin given the concentration of drug in the 0.5-8.0 mg/L range. Aliquot of 0.1 mL of prepared solutions

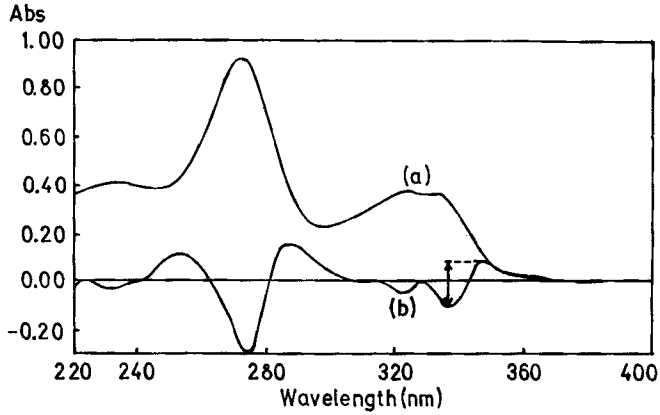

Fig. 2. UV spectrum of $4 \cdot 10^{-5}$ M norfloxacin in 0.05 M NaOH (a) original spectrum, (b) second derivative

was diluted with 0.5 mL of 2% SDS solution and 1.9 mL of 0.1 M HCl. Blanks values obtained by diluting the same volume of serum. The fluorescence of each solution were measured at 455 nm (excitation wavelenght 320 nm).

Analytical recovery

Eight different concentrations of norfloxacin were added to a human serum in order to get concentrations 40-320 μg/L of norfloxacin. These serum samples were treated according to the procedure for the calibration graph.

Results and discussion

The fluorescence excitation and emission spectra of norfloxacin in 0.1 M HCl are shown in Fig. 2. The excitation spectrum shows four maxima at 264, 292, 315 and 325 nm with emission at 450 nm. The emission spectrum shows a maximum at 455 nm with excitation at 320 nm. The excitation at 320 nm was selected in order to minimize the serum emission. The emission at 455 nm was enhanced upon addition of SDS apparently because of the reduced binding of norfloxacin to serum proteins. Therefore SDS was added in each sample in the same concentration (o.4%) which was found, by trial and

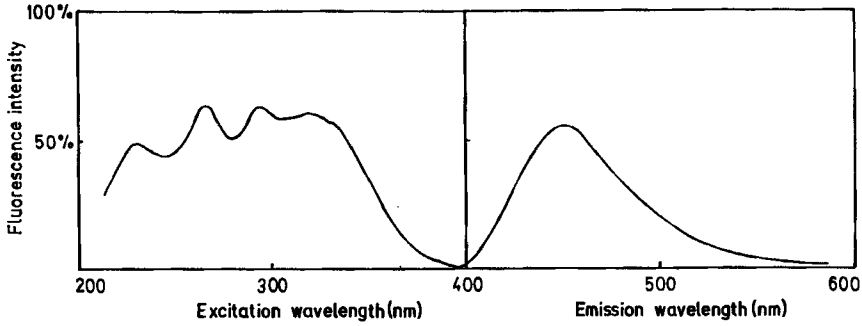

Fig. 3. The fluorescence excitation and emission spectra of $1.6 \cdot 10^{-6}$ M norfloxacin in 0.1 M HCl

error, to be optimal. The emission at 455 nm was chosen for analysis. The fluorescence intenzity was linearly proportional to the concentration of the norfloxacin in the concentration range 20 - 320 μg/L. The regression equation of the calibration line was: Y = 26.16 X + 0.89 (n=10, r= 0.9998) where Y = fluorescence intensity and X = concentration of norfloxacin in μg/L. The detection limit, defined after J.Manes [15] was 2 μg/L. Recovery was 99-101 %.

For the determination of norfloxacin in tablets its strong absorption in ultraviolet was used. In Fig. 3. the zeroth and second derivative spectra are shown. The uv spectrum in 0.05 M NaOH consists of 3 intense absorptions with the maxima at 277, 315 and 330 nm. Sodium hydroxide was chosen because of enhansed solubility of norfloxacin in alkaline medium. In order to avoid interference from other components in tablets, which show absorption bellow 300 nm, the peak at 330 nm was chosen for analysis. Because it is not well separated from the peak at 315 nm the second derivative of the spectrum was used. In second derivation inverse peak apears at 337 nm and its height peak to peak linearly varies with the concentration of the norfloxacin in the concentration range 3-30 μg/mL with the regression equation of the line: $Y = 3.02 \cdot 10^{3} X + 0.85 \cdot 10^{-3}$ (n=10, r=0.9999). Y is the height of the peak, X is the concentration of the norfloxacin in M/L. The proposed method was applied to the determination of norfloxacin in Nolicin tablets. The relative standard deviation was 1.63-2.28 % for norfloxacin concentrations of 0.00798 - 0.01197 mg/mL.

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