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New valid spectrofluorimetric method for determination of selected cephalosporins in different pharmaceutical formulations using safranin as fluorophore

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ABSTRACT

A new validated spectrofluorimetric method has been developed for the determination of some cephalosporins namely; cefepime, cefaclor, cefadroxil, cefpodoxime and cefexime. The method was based on the reaction of these drugs with safranin in slightly alkaline medium (pH 8.0), to form ion-association complexes. The fluorescent products were extracted into chloroform and their fluorescence intensities were measured at 544–565 nm after excitation at 518–524 nm. The reaction conditions influencing the product formation and stability were investigated and optimized. The relative fluorescence intensity was proportional to the drug concentration in the linear ranges of 0.15–1.35, 0.35–1.25, 0.35–1.25, 0.20–1.44 and 0.20–1.25 μg/mL for cefepime, cefaclor, cefadroxil, cefpodoxime proxetil and cefexime, respectively. The detection limits were 40, 100, 100, 60 and 70 ng/mL, respectively. The performance of the developed method was evaluated in terms of Student's t-test and variance ratio F-test to find out the significance of proposed methods over the reference spectrophotometric method. Various pharmaceutical formulations were successfully analyzed using the proposed method and the results were in good agreement with those of the previously reported methods.

1. Introduction

Cephalosporins constitute the second most important β-lactams after penicillin, which are commonly used in the treatment of bacterial infections caused by both gram positive and gram negative bacteria [1]. The wide use of these antibiotics in modern antimicrobial therapy is due to their enhanced intrinsic microbiological activities and favorable safety profile. Chemically cephalosporin antibiotics are derived from the 7-aminocephalosporanic acid (7-ACA) which composed of a β-lactam ring fused with a dihydrothiazine ring (Table 1). The nature of substituents affects either the pharmacokinetic properties or the antibacterial spectrum of the cephalosporin.

Several methods have been reported for the quantitative determination of the cited cephalosporins including spectrophotometry [2–8], spectrofluorimetry [8–14], chemiluminescence [15], high performance liquid chromatography (HPLC) [16–18], thin layer chromatography [19], capillary electrophoresis (CE) [20,21] and voltammetry [22–26]. However, HPLC and CE equipments are expensive. Moreover, these techniques may suffer from some other disadvantages such as high maintenance and acquisition cost, time-consuming analysis, requirement for sample pre-treatment, and in some cases low sensitivity. Furthermore most of the reported spectrometric methods [5–8] are indirect or based on hydrolysis of the parent drug. That is why these methods are time consuming and tedious. Therefore, a new simple spectrofluorimetric method was proposed using safranin as a derivatizing agent.

Safranin, a basic dye, is used mainly as a biological stain and redox indicator. Catalytic effect of metal ions on the oxidation of safranin was utilized for the spectrophotometric or spectrofluorimetric determination of several metal ions such as ruthenium [27], iron [28], mercury [29], and copper [30]. Recently, complex formation of safranin with some pharmaceutical compounds was reported for the spectrophotometric determination of rosuvastatin [31] and spectroflourimetric determination of certain aminoglycoside antibiotic [32].

In the present work a simple and sensitive spectrophotometric method was developed for the determination of some cephalosporins. The method is based on the direct interaction of the basic center of safranin with carboxylic group of the drugs. The method will be applied for the analysis of the cited drugs in their pharmaceutical formulations.

2. Materials and methods

2.1. Apparatus

All spectrofluorimetric measurements were carried out using a Perkin Elmer LS 45 luminescence spectrometer (United Kingdom) equipped with the 150-W xenon arc lamp and 1 cm quartz cell. Slit

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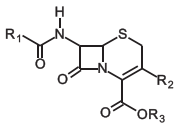
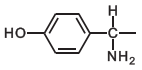
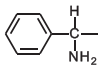
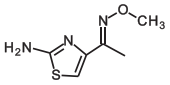
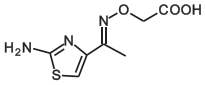
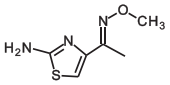
Table 1
Structure of the investigated cephalosporin antibiotics.



No.	Generic name	R_1	R_2	R_3	Generation
1	Cefadroxil monohydrate		CH_3	H	First
2	Cefaclor monohydrate		Cl	H	Second
3	Cefepime hydrochloride			H	Fourth
4	Cefixime		–CH═CH_2	H	Third
5	Cefpodoxime proxetil		–CH_2-O–CH_3	H	Third

width for both monochromators were set at 10 nm. The spectrometer is connected to a PC computer loaded with the FL WINLAB™ software. Milwaukee SM 101 pH meter Portugal was used.

2.2. Reagents

All solvents were of analytical-reagent grade, sodium hydroxide (El-Nasr Chemical Co. Cairo, Egypt) 1 M aqueous solution, 0.1 M hydrochloric acid (El-Nasr Chemical Co. Cairo, Egypt), safranin (3% w/v aqueous solution, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were prepared by dilution with distilled water to obtain 0.004% w/v solution. Cephalosporins were generously supplied by their respective manufacturers and were used as supplied: cefaclor monohydrate (Pharco Pharmaceuticals Co. Amreya, Alexandria, Egypt), cefadroxil monohydrate (Amoun Pharmaceutical Industries Co., APIC, Cairo, Egypt), cefpodoxime proxetil (Hoechst Marion Roussel, S. A. E., Cairo, Egypt) and cefixime (El-Hekma Co., Cairo, Egypt). Teorell and Stenhagen buffer solution [33] of the pH range 5.0–8.5 was prepared in distilled water.

2.2.1. Pharmaceutical formulations

The following pharmaceutical products containing the studied drugs were purchased from local market; curafep 1 g vial (Delta Pharma Egypt, 10th of Ramadan, El Sharkeya, Egypt) was labeled to contain 1000 mg of cefepime per vial, ceclor 125 mg powder for oral suspension (Egyptian Co. for Pharmaceutical & Chemical Industries, EPCI, Beni Suef, Egypt) was labeled to contain 125 mg of cefaclor per 5 mL of suspension, duricef 500 mg capsule (GlaxoSmithKline Egypt, El Salam City, Cairo, Egypt) was labeled to contain 500 mg of cefadroxil per capsule, duricef 500 mg powder for oral suspension (GlaxoSmithKline Egypt El Salam City, Cairo, Egypt) was labeled to contain 500 mg of cefadroxil per 5 mL of suspension, ximacef 100 mg powder for oral suspension (Sigmatec Pharmaceutical industries, 6th of October City Cairo, Egypt) was labeled to contain 100 mg of cefixime per 5 mL of suspension, ximacef 400 mg capsule (Sigmatec Pharmaceutical industries, 6th of October City Cairo, Egypt) was labeled to contain 500 mg of cefixime per capsule, orelox 100 mg tablet (Sanofi Winthrop Industry, Compiegne, France) was labeled to contain 100 mg of cefpodoxime per tablet.

2.3. Preparation of standard solutions

Stock solutions containing 100 μg mL^{-1} of each cephalosporin were prepared in double distilled water (ethanol was used in case of cefpodoxime proxetil and cefixime). Working standard solutions containing 2.0–15 μg mL^{-1} were prepared by suitable dilution of the stock solution with double distilled water. The stock and working standard solutions must be freshly prepared.

2.4. Preparation of sample solutions

2.4.1. Tablets and capsules

Twenty tablets or the contents of 20 capsules were weighed, finely powdered and mixed thoroughly. An accurately weighed amount of the powder equivalent to 25 mg of each drug was transferred into a 25-mL volumetric flask, dissolved in about 10 mL double distilled water (10 mL ethanol was used in case of cefpodoxime proxetil and cefixime), sonicated for 15 min, diluted to the mark with double distilled water (in case of cefpodoxime proxetil and cefixime, dilution was made using ethanol), mixed well and filtered; the first portion of the filtrate was rejected. Further dilutions with the same solvent were made to obtain sample solution containing the specified concentration for each drug as mentioned in Section 2.3.

2.4.2. Vials

An accurately weighed amount of powder equivalent to 25 mg of each drug was transferred into a 25-mL volumetric flask, then the procedure was followed same in Section 2.4.1 (beginning from dissolved in about 10 mL double distilled water).

2.4.3. Powder for oral suspension

The powder was constituted in the required volume of distilled water according the procedure mentioned in the dosage form label. A liquate volume of the suspension containing 25 mg of the studied drug was transferred into a 50-mL calibrated flask, completed to the volume with distilled water and filtered. The filtrate (0.5 mg mL^{-1}) was further diluted with double distilled water (ethanol was used in case of cefixime) to obtain working solutions within the concentration ranges for calibration (1.5–12.5 μg mL^{-1}).

2.5. General analytical procedure

Into 60-mL separating funnel, 0.5 mL of Torell and Stenhagen buffer solution (pH 8.0) was transferred. Aliquot volume of the standard or sample solutions of the drug, within the concentration range and 1.0 mL safranin solution (0.004% w/v) were added. The contents of the