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Novel spectrophotometric method for determination of some macrolide antibiotics in pharmaceutical formulations using 1,2-naphthoquinone-4-sulphonate

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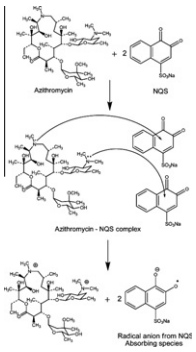
HIGHLIGHTS

- We found that alkaline medium is necessary to form charge transfer complex between azithromycin and erythromycin with NQS.
- Increasing temperature had negative effect on the absorption values.
- The formed complexes were very stable.
- Developed and validated method has been used for azithromycin and erythromycin analysis on real pharmaceutical samples.

GRAPHICAL ABSTRACT



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ABSTRACT

New, simple and rapid spectrophotometric method has been developed and validated for the assay of two macrolide drugs, azithromycin (AZT) and erythromycin (ERY) in pure and pharmaceutical formulations. The proposed method was based on the reaction of AZT and ERY with sodium 1,2-naphthoquinone-4-sulphonate (NQS) in alkaline medium at 25 °C to form an orange-colored product of maximum absorption peak at 452 nm. All variables were studied to optimize the reaction conditions and the reaction mechanism was postulated. Beer's law was obeyed in the concentration range 1.5–33.0 and 0.92–8.0 μg mL^{-1} with limit of detection values of 0.026 and 0.063 μg mL^{-1} for AZT and ERY, respectively. The calculated molar absorptivity values are 4.3×10^4 and 12.3×10^4 L mol^{-1} cm^{-1} for AZT and ERY, respectively. The proposed methods were successfully applied to the determination of AZT and ERY in formulations and the results tallied well with the label claim. The results were statistically compared with those of an official method by applying the Student's *t*-test and *F*-test. No interference was observed from the concomitant substances normally added to preparations.

Introduction

Azithromycin (9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin dehydrate) and erythromycin (3*R**, 4*S**, 5*S**, 6*R**, 7*R**, 9*R**, 11*R**, 12*R**, 13*S**, 14*R**)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-a-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-b-D-*xylo* hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione are widely used macrolide antibiotics indicated for the use of infection with gram positive bacteria such as streptococci, staphylococcus aureus and corynobacteria species as well as gram negative bacteria such as legionella pneumophilia and haemophilius species. Furthermore, the macrolide antibiotics are increasingly used for opportunistic infections in patient with AIDS [1]. Macrolides interfere with RNA dependent bacterial protein synthesis, resulting in a bateriostatics effect on pathogens [2]. In general, the structure of the macrolide antibiotics contains a macrocyclic lactone and a neutral sugar moiety attached to the lactone. Another important structural characteristic is the presence of other sugar moiety containing a

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dimethylamine group, which confers to the macrolides a basic behavior and makes them a potential n-electron donating substances [3].

Several methods have been described for the individual quantitative determination of azithromycin and erythromycin in raw material, dosage forms and biological fluids included high performance liquid chromatography [4–11], spectrophotometry [12–17], capillary electrophoresis [18], NIR spectroscopy [19,20] and voltammetry [21–24]. These methods were time-consuming, tedious, and dedicated to sophisticated and expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Unfortunately, the spectrophotometric methods that have been reported for the determination of macrolides in their pharmaceutical formulations were associated with some major disadvantages, such as lack of selectivity, tedious extraction procedures, and time-consumption. The official procedures in pharmaceutical preparations utilize high performance liquid chromatography [25]. Therefore, the development of new alternative spectrophotometric methods for the determination of macrolides that can overcome the disadvantages of the existing methods is essential.

1,2-Naphthoquinone-4-sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines. It is a popular spectrophotometric reagent due to its efficient reactivity with both primary and secondary amines, and high reaction rate [26–29]. NQS proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group, however the use of NQS for spectrophotometric determination of azithromycin and erythromycin was not reported. Therefore, the present work describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for determining the content of azithromycin and erythromycin in pharmaceutical formulations based on the reaction of NQS with amino group of azithromycin and erythromycin molecules to form orange compounds.

Experimental

Apparatus

Double beam UVD-2960 (Labomed, Inc., USA) ultraviolet–visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 400–600 nm and slit width 0.1 nm. Spectra were automatically obtained by UV-WIN software Ver.5.0.10. Electronic balance (Kern, Germany) was used for weighing the samples.

Materials

Working reference standards of azithromycin dehydrate (AZT) was supplied by Lexandos-Novartis (Switzerland), its purity was 99.8%, and erythromycin (ERY) was supplied by A.T.C Chemical Co. (China), its purity was 98.1%. Sodium 1,2-naphthoquinone-4-sulphonate (NQS), from Aldrich Chemical Co., St. Louis (USA) and methanol was purchased from Labscan (Ireland). All chemicals were of analytical reagent grade. Double distilled water was used in all experiments.

Pharmaceutical formulations

The following commercial formulations were subjected to the analytical procedures:

(1) Zithromax capsules (Pfizer, USA) labeled to contain 250 mg azithromycin/capsule.
(2) Azitrolyd suspension (Al Fares, Syria) labeled to contain 200 mg azithromycin/5 mL.
(3) Azitrolyd tablets (Al Fares, Syria) labeled to contain 500 mg azithromycin/tablet.
(4) Azitrolyd capsules (Al Fares, Syria) labeled to contain 250 mg azithromycin/capsule.
(5) Azithrocine capsules (Alpha, Syria) labeled to contain 250 mg azithromycin/capsule.
(6) Azithrocine tablets (Alpha, Syria) labeled to contain 500 mg azithromycin/tablet.
(7) Erythroma tablets (Thameco, Syria) labeled to contain 250 mg erythromycin/tablet.
(8) Erythroma suspension (Thameco, Syria) labeled to contain 200 mg erythromycin/5 mL.
(9) Erythro-Sol 2 (Domina, Syria) labeled to contain 2 g erythromycin/100 mL solution.

Solutions

Stock standard solutions

An accurately weighed 0.05 g standard sample of AZT and ERY was dissolved in methanol, transferred into a 100 mL standard flask and diluted to the mark with methanol to obtain 0.5 mg mL^{-1}. This stock solution was further diluted to obtain working solutions in the ranges of 1.5–33.0 and 0.92–8.0 μg mL^{-1} for AZT and ERY, respectively.

Sodium 1,2-naphthoquinone-4-sulfonate solution

An accurately weighed 0.2 g of NQS was dissolved in double distilled water, transferred into a 100 ml standard flask and diluted to the mark with double distilled water and mixed well to prepare 0.2% *w/v*. The solution was freshly prepared and protected from light during use.

Alkaline solutions

Sodium hydroxide, disodium hydrogen phosphate, borax and sodium bicarbonate solutions of a concentration range of 0.1–0.6 M were prepared in double distilled water.

General procedure

Aliquots of standard AZT (0.03–0.66 mL, 0.5 mg mL^{-1}) and ERY (0.018–0.16 mL, 0.5 mg mL^{-1}) solutions were transferred into a series of 10 mL calibrated volumetric flasks. Then 1.25 mL and 0.75 mL of 0.2 M sodium hydroxide solution was added for AZT and ERY, respectively, followed by 1.75 mL of NQS 0.2% (*w/v*) and the solutions were allowed to proceed at 25 °C for 20 min for AZT and ERY. After that, the volume was made up to the mark with double distilled water and the absorbance was measured at 452 nm for AZT–NQS and ERY–NQS against reagent blank treated similarly under identical conditions.

Procedures for formulations

Tablet (or capsule) sample solutions

Twenty tablets were weighted accurately and crushed to a fine powder. In the case of capsules, the contents of twenty capsules were completely evacuated from shells. An accurately weighed quantity of powder equivalent to 50 mg of azithromycin or erythromycin were transferred into a 100 mL calibrated flasks, and dissolved in about 50 mL of methanol. The contents of the flask were swirled for 10 min, and then completed to volume with methanol to achieve a concentration of 0.5 mg mL^{-1}. The contents were mixed well and filtered rejecting the first portion of the filtrate.