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Microbiological assay for the analysis of certain macrolides in pharmaceutical dosage forms

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ABSTRACT

Clarithromycin (CLA) and roxithromycin (ROX) are macrolide antibiotics with an expanded spectrum of activity that are commercially available as tablets. A microbiological assay, applying the cylinder–plate method and using a strain of *Micrococcus luteus* ATCC 9341 as test organism, has been used and validated for the quantification of two macrolide drugs; CLA and ROX in pure and pharmaceutical formulations. The validation of the proposed method was carried out for linearity, precision, accuracy and specificity. The linear dynamic ranges were from 0.1 to 0.5 μg/mL for both compounds. Logarithmic calibration curve was obtained for each macrolide ($r > 0.989$) with statistically equal slopes varying from 3.275 to 4.038, and a percentage relative standard deviation in the range of 0.24–0.92%. Moreover, the method was applied successfully for the assay of the studied drugs in pharmaceutical tablet dosage forms. Recovery from standard addition experiments in commercial products was 94.71–96.91% regarding clarithromycin and 93.94–98.12% regarding roxithromycin, with a precision (%RSD) 1.32–2.11%. Accordingly, this microbiological assay can be used for routine quality control analysis of titled drugs in tablet formulations.

1. Introduction

Macrolide antibiotics have been used clinically for more than 50 years. They are a very important class of antibacterial compounds extensively used to treat a wide range of infections, not only in medical but also in veterinary practices. The macrolides inhibit RNA-dependent protein synthesis by reversibly binding to the 50S ribosomal subunit of a susceptible microorganism (Omura, 2002). In addition to erythromycin A, the prototype macrolide, several other macrolides, clarithromycin and roxithromycin are clinically available (Payne et al., 2007). Besides its antibacterial activity, macrolides exert diverse biological effects including the ability to modulate inflammatory and immune responses without affecting homeostatic immunity (Mencarelli et al., 2011). More recently, macrolides have been also considered for other uses in the areas of neurology, gastroenterology, rheumatology, cardiology, and cancer therapy, facilitating the development of new synthetic methods as well as the design of new therapeutic agents possessing non-antibacterial activities (Miroshnyk et al., 2008).

Clarithromycin is a semi-synthetic 6-*O*-methyl derivative of the 14-member macrolide antibiotic erythromycin, and the *O*-methyl group attached to the position six of the lactone makes it more acid stable than erythromycin (Lu et al., 2009). Roxithromycin-9-{*O*-[(2-methoxyethoxy)-methyl]-oxime}-erythromycin is a semi-synthetic, 14-membered ring macrolide antibiotic, in which the erythronolide A lactone ring has been altered to prevent inactivation in the gastric environment (Głowka and Karaźniewicz-Łada, 2007). These new semi-synthetic erythromycin derivatives have a better bioavailability and a more favorable pharmacokinetic profile than erythromycin. Many dosage forms of both drugs, such as tablets, have been developed based on their wide antibacterial spectrum.

Determination of antibiotics, including macrolides, is mainly carried out by microbiological assays (Horwitz, 2000). These assays excel as a qualitative means by which samples may be screened for residual amounts of antibacterial substances, but they are often lengthy and lack the specificity. Chemical methods such as high-performance liquid chromatography (HPLC) are appropriate alternatives. Some review articles published recently have covered literature on the analysis of macrolide antibiotics (Kanfer et al., 1998; Lahane et al., 2014), and several HPLC methods have been developed.

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A large number of reports in the literature have been published for the quantitative determination of CLA and ROX in raw material, dosage forms and biological fluids. The analytical methods reported include microbiological bioassay, spectrophotometry (Jagtap et al., 2013), and HPLC with fluorescent (Głowka and Karaźniewicz-Łada, 2007; Sastre Toraño and Guchelaar, 1998), ultraviolet (UV) (Głowka and Karaźniewicz-Łada, 2007; Li et al., 2007), electrochemical (Pappa-Louisi et al., 2001), amperometric (Taninaka et al., 2000) and mass spectrometric detection (Shin et al., 2008; Verlag, 2009). A spectrofluorimetric method has been described (Khashaba, 2002) for the analysis of several macrolides including CLA and ROX. Also, UV is the most common detection system. However, macrolides like clarithromycin and roxithromycin lack a suitable chromophore and non-selective low-UV wavelength must be used. Generally, these methods were time-consuming, tedious, and dedicated to sophisticated analytical instruments which is expensive and could not be available in many laboratories.

Among drug quantification methods, the microbiological assays are recommended by different pharmacopoeias (United States Pharmacopeia, 2007; British Pharmacopoeia, 2011) and many publications. This experimental assay was the official method provided by the Japanese Ministry of Health and Welfare (Official Methods for Residual Substances in Livestock Products, 1994). In majority of studies, paper disk procedure was employed for the determination of drug antibacterial activity. However, other methods including standard dilution assay (Ma et al., 2009), standard broth microdilution (Kapić et al., 2011), and monitoring the turbidity (Lange et al., 2006) were used. No official microbiological cylinder–plate assays have been reported for the identification and quantification of CLA and ROX in pharmaceutical formulations. From the different classes of microorganisms frequently used to test the presence of residual antibiotics, *Micrococcus luteus* ATCC 9341, was strong, and its sensitivity of the drug detection was sufficient.

In the study by Breier et al. (2002), investigation of azithromycin concentration present in pharmaceutical formulations was successfully done using cylinder–plate method and *M. luteus* ATCC 9341 as test organism. However, there are few reports applying such as technique described in the literature for macrolides determination in tablet dosage forms and, to our knowledge, none of them includes clarithromycin and roxithromycin.

In the present manuscript, quantitative analysis of the new semi-synthetic 14-membered macrolide antibiotics; clarithromycin and roxithromycin in tablets, as dosage forms of certain local markets from different manufacturers, have been studied. Therefore, a simple, linear, precise and accurate cylinder–plate method based on agar diffusion was performed.

2. Materials and methods

Clarithromycin standard is a kind gift from SAIDAL GROUPE (Algeria). Roxithromycin was kindly obtained from HUP.P.PHARMA laboratories (Algeria) as gift standard, while pharmaceuticals containing these macrolide antibiotics were obtained commercially. Claridar® tablets (Dar Al Dawa, Algeria): labeled to contain clarithromycin as 500 mg/tablet; Clarimed® tablets (Saidal, Algeria): labeled to contain clarithromycin as 500 mg/tablet; Roxid® tablets (Pharmalliance, Algeria): labeled to contain roxithromycin as 150 mg/tablet; Roxithromycine HUP® tablets (HUP.P.Pharma, Algeria): labeled to contain roxithromycin as 150 mg/tablet, respectively. All drugs were used as received and their solutions were prepared freshly every day to be used as working standards.

Dipotassium hydrogen phosphate was of analytical-reagent grade from SIGMA–ALDRICH (Steinheim, Germany). Methanol was of HPLC grade from the same source. All other used reagents were of analytical grade. Doubly distilled water was used throughout.

2.1. Preparation of standard solutions

Stock standard solutions of CLA and ROX were prepared by dissolving an accurately weighted amount of each compound in methanol (1000 μg/mL). Working standard solutions were prepared by diluting the stock solution with 0.025 *M* dipotassium hydrogen phosphate buffer (pH 8.0). The solutions were prepared freshly every day to be used as working standards at concentrations of 0.1, 0.3 and 0.5 μg/mL, which were used in the assay as reference solutions.

2.2. Preparation of the tablet samples

2.2.1. Clarithromycin tablets

The sample preparation was done as follows based on previous papers (Khashaba, 2002; Breier et al., 2002). Ten tablets were weighed and finely powdered. A weighed portion equivalent to the weight of one tablet was transferred to a 100 mL volumetric flask, sonicated for 20 min with about 10 mL methanol then the solution was completed to volume with the same solvent. The mixture was mixed well, allowed any insoluble matter to settle then filtered. A measured volume of the filtrate was diluted quantitatively with the potassium hydrogen phosphate buffer (pH 8.0) solvent to yield a sample solution having a working concentration of 0.1, 0.3 and 0.5 μg/mL. This sample was evaluated in triplicate. This procedure was performed two times.

2.2.2. Roxithromycin tablets

The procedure was followed as mentioned in Section 2.2.1. An amount equivalent to 150 mg of drug was transferred to 100 mL volumetric flask with 10 mL methanol and shaken for 20 min, followed by marking up to volume with the same solvent. After filtration, the dilutions were made, to give the same final concentrations used above. This sample was evaluated in triplicate. This procedure was performed two times.

All preparations were kept at 4 °C, protected from light in amber glass vessels and periodically tested.

2.3. Microbiological assay

A microbiological assay, adapted from the method described in the US Pharmacopoeia applying the cylinder-plate diffusion technique was used to assay CLA and ROX in tablet dosage forms (United States Pharmacopeia, 2007). The parallel-line model was chosen, and differences were considered statistically significant if P was <0.05.

2.3.1. Organism and inoculum

Briefly, the bioanalytical method was implemented, as indicated by Breier et al. (2002), with slight modifications. Therefore, the *M. luteus* strain (ATCC 9341) was grown on selective agar at culture medium (pH 8.0) for 24 h at 35 °C.

2.3.2. Cylinder–plate assay

For each macrolide, nine stainless steel cylinders of uniform size (8 mm × 6 mm × 10 mm) were placed on the solidified surface of the seeded agar. Three alternate cylinders were filled with 200 mL of reference solutions (three concentrations) or problem samples. Each assay was designed to evaluate in the same plate the reference solutions and two different samples. Eight plates were used in each assay. After incubation (24 h at 35 °C) the zone