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ORIGINAL ARTICLE

Azithromycin assay in drug formulations: Validation of a HPTLC method with a quadratic polynomial calibration model using the accuracy profile approach☆

Dosage de l'azithromycine dans des formulations pharmaceutiques : validation de la méthode CCMHP avec le modèle quadratique en utilisant le profile d'exactitude

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β-expectation tolerance interval

Summary Many different assaying high performance thin layer chromatography (HPTLC) methods have been developed and validated in order to be used in routine analysis in different analytical fields. Validation often starts by the evaluation of the linearity of the calibration curve. Frequently, if the correlation coefficient is close to one, the linear calibration curve model is considered to be proper to predict the unknown concentration in the sample. But is this simple model effective to assess the behavior of the response of an HPTLC method as a function of concentration. To answer this question, a method for the determination of azithromycin by HPTLC has been developed and validated following both the classical approach and that based on the accuracy profile. Silica gel plates with fluorescence indicator F254 and chloroform — ethanol — 25% ammonia 6:14:0.2 (v/v/v) as mobile phase were used. Analysis was carried

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out in reflectance mode at 483 nm. The RF of azithromycin was 0.53. The validation based on the classical approach, shows that the behavior is not linear, even though $r^2 = 0.999$ because the lack of fit test is significant ($P < 0.05$). Validation based on the accuracy profile approach considering both the straight line and the quadratic regression model, show that the former results is a β-expectation tolerance interval outside the acceptance limits, while with the latter, this interval is within the limits of ±5% acceptability for a range which extends from 0.2 to 1.0 μg/zone. With the quadratic model, the method showed to be precise and accurate.

MOTS CLÉS
CCMHP ;
Azithromycine ;
Profil d'exactitude ;
Modèles de calibration ;
Intervalle de β-tolérance

Résumé Plusieurs méthodes de dosage utilisant la chromatographie sur couche mince de haute performance (CCMHP) ont été développées et validées pour leur usage en analyse en routine dans différentes domaines. Souvent, la validation démarre par l'évaluation de la linéarité de la courbe de calibration. Fréquemment, lorsque le coefficient de corrélation est proche de 1, la méthode est utilisée pour quantifier le soluté inconnu dans l'échantillon. Mais la question qui se pose est ce simple modèle est efficace pour évaluer le comportement de la réponse obtenue par la méthode de la CCMHP en fonction de la concentration. Pour répondre à cette question, une méthode de la détermination de l'azithromycine par CCMHP a été développée et validée selon la méthode classique et celle basée sur le profil d'exactitude. La phase stationnaire est constituée de plaques de gel de silice, la phase mobile est mélange de chloroforme-éthanol et ammoniaque (25 %) (6:14:0,2 (V/V/V). Le temps rétention est de 0,53 minutes. La validation par la méthode classique a montré un comportement non linéaire même avec un $r^2 = 0,999$, parce que l'ajustement est significatif. La validation par la méthode basée sur le profile d'exactitude monter que l'intervalle de β-acceptance considérant le modèle simple est en dehors des limites d'acceptations, par contre pour le modèle quadratique, cet intervalle est situé à l'intérieur des limites de ±5 %. L'application du modèle quadratique a montré que la méthode est fidèle et exacte.

Azithromycin [9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin A) (Fig. 1) is a representative of a macrolide group, named azalides, used as antibiotic to treat certain infections with Gram positive as well as Gram negative bacteria [1]. It has been used in treatment of infections of the skin, the respiratory system, and sexually transmitted diseases [2,3].

Generally, the determination of antibiotics, including macrolide antibiotics, is mainly carried out by microbiological methods which suffer from a lack of specificity and sensitivity [4,5]. In order to overcome these problems, azithromycin has been analysed in pharmaceutical dosage forms by high performance liquid chromatography with an UV detector [6—8] or with an electrochemical detector [9] and fluorescence detection after derivatization [10]. The United States Pharmacopoeia recommends a HPLC method with spectrophotometric detection, for its in vitro dissolution studies, which describes the use of high pH mobile phase (pH 11.0) and the use of specific ''gamma-alumina'' column, which is expensive and difficult to procure commercially [11]. While, the European Pharmacopeia recommends HPLC with UV detector for determination of macrolide antibiotics as the bulk drugs which requires the use also an octadecylsilylated vinyl polymer, which is expensive [12].

Due to the evolution of instrumentation, automization, development of new absorbents and supports, and the use of densitometers as detectors, high-performance thin layer chromatography (HPTLC) became a technique widely used in different analytical fields, such as the pharmaceutical, environmental, biological and clinical [13]. Different assaying HPTLC methods have been developed and validated for the estimation of telmisartan and atorvastatin in presence of degradation products [14]. There is also a report on the use of TLC for herbal active ingredient determination [15].

Usually, method validation starts by evaluating the linearity of the calibration curve obtained using the least squares regression model, $y = ax + b$. Often, the quality of this model is assessed by the correlation coefficient. If this parameter is close enough to 1.0, the simple regression model is systematically considered suitable to predict the unknown concentration in the sample. It was demonstrated that a simple regression model with a correlation coefficient close to 1.0 cannot be a reliable indicator of linearity because it has been obtained for a non linear relationship [16]. Even though $r^2 = 0.9996$, the model is not adequate to describe the observed data due to some significant lack of fit [17,18]. However, is this simple straight model always suitable to assess the behavior of the response of an HPTLC method, knowing that this technique used a densitometric detection in reflectance mode, where the Kubelka-Munk equation [19] is applied instead of