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A novel reversed-phase LC method for quantitative detection of azithromycin in bulk drug and tablet formulations in various aqueous media

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A novel reversed-phase HPLC method with UV-detection for separation, identification and quantification of azithromycin (AZH) was developed. The method was validated for the purpose of quantifying AZH in stability, dissolution and solubility studies. The method was validated at two optimum wavelengths where linear regression at both 205 and 210 nm, resulted in correlation coefficients of $r^2 = 0.9999$. This HPLC method proved to be superior to other published methods due to the specificity, resulting in less peak interference and tailing. The pH of the mobile phase, as well as the ratio of buffer to organic component included in the mobile phase, proved to be critical factors in the improved detection of AZH.

1. Introduction

Azithromycin (AZH), a semi-synthetic macrolide, is considered to be one of the world's best-selling antibiotics (Miroshnyk et al. 2006). Previously published HPLC methods for the identification and/or quantification of AZH include electrochemical or UV detection (Kanfer et al. 1998; Miguel and Barbas 2003; Shaikh et al. 2008). Methods using electrochemical (coulometric and amperometric) detection for AZH in biological samples have been reported by Shepard et al. (1991). The USP describes an assay, using an amperometric method for detection (USP 2010). Zubata et al. (2002) described an HPLC method using UV-detection to identify AZH and its impurities present in AZH tablets. These methods utilize equipment not readily available in many laboratories. In this study, an HPLC method with UV-detection was developed. Poor UV absorbance imposes the problem of limited detection of AZH (Kanfer et al. 1998), but the specificity and clear separation of this novel method compensate for and overcome this limitation. Known published HPLC methods (Kanfer et al. 1998; Miguel and Barbas 2003; Shaikh et al. 2008; Shepard et al. 1991; USP 2010; Zubata et al. 2002) for the quantification of AZH were investigated, and all methods used had different constituents for the mobile phase at variable pH, ranging from 3 to 9. The aim of this paper was to develop and validate a reliable HPLC method to identify and/or quantify AZH during product assays, solubility, stability, and dissolution studies.

2. Investigations, results and discussion

During the method development stage it became clear that pH and composition of the mobile phase were critical factors. For a mobile phase with pH < 6 the AZH peak overlapped significantly with mobile phase peaks and poor peak separation was prevalent. This mobile phase also resulted in a very short retention time for the AZH peak. This posed to be problematic, especially for the detection of AZH in solubility and/or dissolution media. The interference of the aqueous media caused very poor peak identification, separation and unreliable peak area integration. For pH > 6 the symmetry of the AZH peak was poor. The absence of symmetrical peaks was due to peak tailing which made peak area integration inaccurate and quantitative repeatability impossible.

Method validation of the developed method was established at two wavelengths, 205 and 210 nm. The proposed method was validated for linearity, range, precision, accuracy, recovery, specificity, robustness, limit of detection (DL) and limit of quantitation (QL). Five calibration standard solutions with concentrations ranging from 0.1 mg/mL to 5.0 mg/mL, were prepared. Accuracy was determined by injecting duplicate injections of the calibration control solutions (100% theoretical concentration). The recovered concentration was compared to the initial theoretical concentration of the control solutions (Table). To determine specificity, a commercial AZH tablet was dissolved in mobile phase. Specificity was also resolved by dissolving AZH at a known concentration in the four general solubility media (acetate and phosphate buffer, 0.1 M HCl and water). Precision of this method was assessed on two levels: repeatability and intermediate precision (robustness). Repeatability was measured through repeated injections (5 replicates) of the calibration standard solutions that represent 100% theoretical concentration. Data recovered from the five replicate injections of the 100 % solution (1.0 mg/mL), at both wavelengths, showed RSD values of 0.16 % and 0.17 % respectively. To establish robustness, this method was performed by two different analysts in the same laboratory on consecutive days. The chromatographic columns and the HPLC systems used were different. The results obtained during the robustness testing proved that this method is accurate in retaining AZH without peak interference. At wavelength 205 nm the equation

Table: Results obtained during the method validation

Wavelength (nm)	Range (μg.ml^{-1})	*r^2	Accuracy (%)	RSD (%)	*DL (μg/ml)	*QL (μg/ml)
205	100.0–5000.0	0.9999	100.68 ± 0.06	0.06	3.88	11.76
210	100.0–5000.0	0.9999	100.74 ± 0.06	0.06	6.87	20.80

*r^2 - Correlation coefficient
*DL - Limit of detection
*QL - Limit of quantitation
The calculated P-values at a confidence level of 95% for the curves obtained at 205 nm and 210 nm were 0.293 and 0.424 respectively

for regression (y = 218.03x + 4213.21) had a correlation coefficient of 0.9983, with percentage recovery (%) of 104.95% (RSD = 0.3%). At wavelength 210 nm the equation for regression (y = 139.89x + 3948.10) had a correlation coefficient of 0.9970, with a recovery of 106.66% (RSD = 0.3%).

During the test for specificity the inactive ingredients included in the commercial AZH tablet did not show any interference, at both wavelengths, towards the AZH peak. Peaks, due to tablet excipients, were detected at retention times of approximately 8.0 and 14.0 min (Fig.). The solution of the commercial AZH tablet was also spiked with azithromycin in order to confirm that the unknown peaks at 8.0 and 14.0 min are not due to azithromycin. A blank mobile phase sample was injected to conclude the presence, but not interference, of small peaks due to the buffer and solvents used in the mobile phase. From the Fig. it is clear that these unknown substances (tablet excipients) cause no interference with the chromatography.

Blank samples containing only solubility media were initially injected to identify peaks due to the buffer contents in each medium. The prepared samples with an AZH concentration of 1.0 mg/mL were injected after the blank samples. Chromatograms (Fig. c–f) revealed the clear separation of the AZH peak from the other peaks (solubility media peaks). A blank water sample was also injected, followed by a sample of AZH dissolved in water (0.1 mg/mL). With previously developed methods, the use of water as solvent made integration and quantification of AZH difficult. With this method there is sufficient separation to integrate the AZH peak for quantification. The peaks that represent water on the chromatogram did not cause any interference with the AZH peak (Fig. c). Despite the poor water solubility of AZH in water and the difficulty it causes to quantify very low concentrations of this API in water, this method still produced clear separation and an AZH peak that can be accurately quantified. Data recovered from the five replicate injections of the 100% solution (1.0 mg/mL), at both wavelengths, showed RSD values of 0.16% and 0.17% respectively. The smallest concentration of analyte in a sample that the method is able to detect, but not essentially quantified, can be described as the limit of detection (DL) (USP 2010). The quantitation limit (QL) can be expressed as the smallest analyte concentration in a sample which the method is capable of quantifying with apt accuracy and precision (USP 2010). An Excel™ spreadsheet (Analysis ToolPak) using linear regression was utilized to calculate the DL and QL.

A novel reversed phase HPLC method using UV-detection has been developed as a multi-purpose method to identify and quantify AZH in drug samples. This method showed to be sensitive but also robust enough so that it can successfully be exploited during stability, solubility and dissolution studies of AZH. From the data obtained during method development, it became clear that the pH of the mobile phase used, and ratio of buffer to organic component, is critical for the optimum detection of AZH.

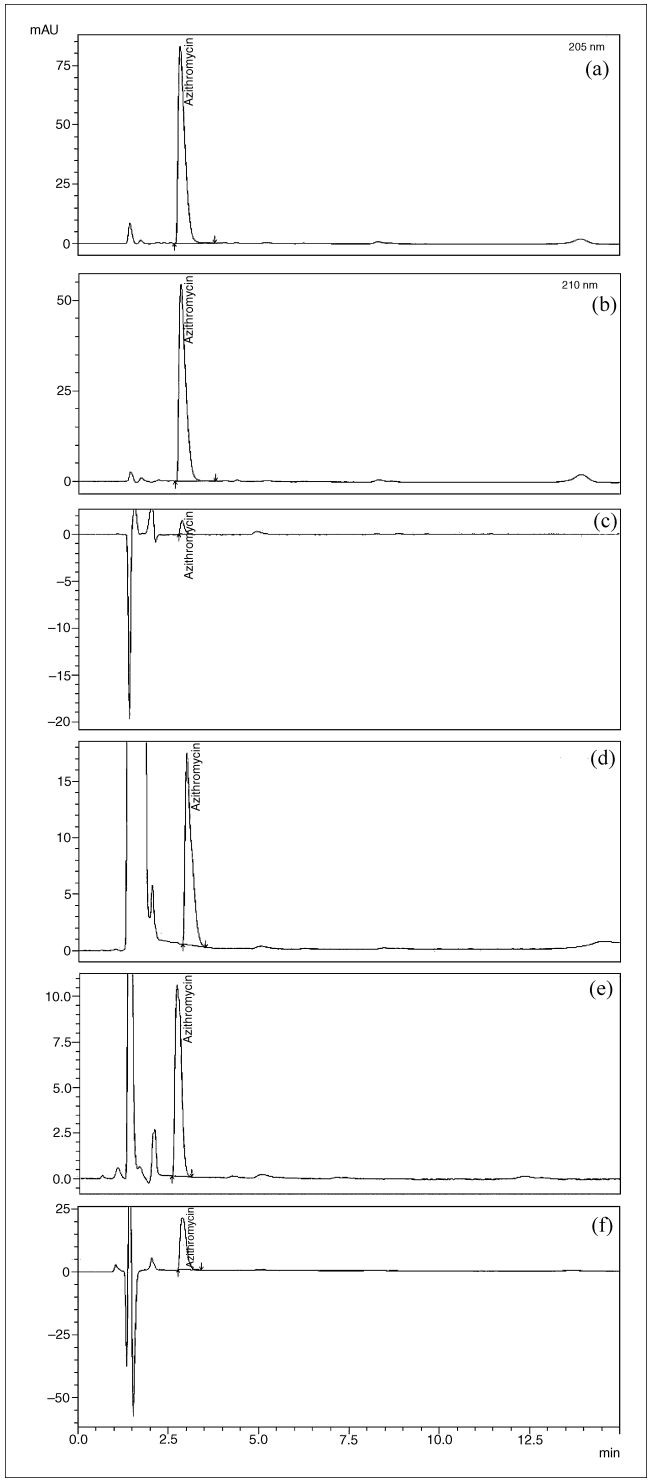

Fig.: A typical chromatogram of azithromycin tablet (5 mg/mL), dissolved in mobile phase, at (a) 205 nm and (b) 210 nm. Mobile phase: phosphate buffer (adjusted with 1.0 M sodium hydroxide to pH 6.0)/acetonitrile (700:300), flow rate 1.0 mL/min, injection volume 15 μL. Column: reversed phase C18, 5 μm, 150 mm x 4.6 mm. UV-detection: 205 nm and 210 nm and chromatograms obtained for azithromycin dissolved in (c) distilled water (0.1 mg/ml), (d) acetate buffer pH 4.5 (1.0 mg/ml), (e) phosphate buffer pH 6.8 (0.5 mg/ml) and (f) 0.1 M HCl (1.0 mg/ml)

3. Experimental

AZH with a purity of 96.5% (on dried basis) was purchased from DB Fine Chemicals (Batch number: IF-AZ080506). A Shimadzu (Kyoto Japan) UFLC (LC-20AD) chromatographic system was utilized. The system consisted of a SIL-20AC auto-sampler fitted with a sampler cooler, a UV/VIS Photodiode Array detector (SPD-M20A) and a LC-20AD solvent delivery module. A Phenomenex® Luna C18 (5 μm) 150 mm × 4.6 mm column was

used as stationary phase. The mobile phase consisted of a combination of 0.06 M phosphate buffer at pH 6.0 (pH adjusted with 1.0 M NaOH), and acetonitrile (700:300). Isocratic elution was used. The flow rate was set at 1.0 mL/min. Photodiode array detection showed that two wavelengths, namely 205 and 210 nm, were most favourable for detection. Injection volume for each sample was 15 μL. The four media utilized to determine the application of this method for solubility and dissolution studies are phosphate buffer (pH 6.8), acetate buffer (pH 4.5), 0.1 M HCl (pH 1.2), and water (BP 2011).

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