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NOTE

Determination of Azithromycin by Extractive Spectrophotometry

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Two simple extractive spectrophotometric methods (M_1 and M_2) are developed for the determination of azithromycin (AZ) in bulk and dosage forms. They are based on the formation of coloured species with wool fast blue BL (WFBBL, M_1) and tropileon 000 (TPOOO M_2) reagents exhibiting maximum absorption at 580 and 480 nm, respectively. Methods M_1 and M_2 obeyed Beer's law limits 5-25 μg mL^{-1} and 10-40 μg mL^{-1}, respectively. The proposed methods are simple, selective, sensitive and economical for the routine qualitative and quantitative determination of azithromycin in pharmaceutical formulations.

Key Words: Azithromycin, Extractive spectrophotometry, Wool fast blue BL, TropileonOOO.

Azithromycin (AZ) is a macrolide antibiotic and is chemically known as 1-oxa-6-azocyclopentadecan-15-one, 13-(2,6-dideoxy-3-c-methyl-3-0-methyl-α-lribohexopyranosyl)oxyl]-2-ethyl 3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[(3,4,6-trideoxy-3-(dimethylamino)-β-D-xylohexo-pyranosyl]oxy-[2R(2R*,3S*,4R*,5R*,8R*,10R*,11R*,12S*,14R*)] and is official in USP[1] and matrindale extra pharmacopoeia[2]. It is a drug of choice in the treatment of respiratory tract, throat and skin infections, gonorrhea and other sexually transmitted infections. Reently it has been observed that azithromycin therapy prevents infections in AIDS patients.

Literatiure survey revealed that methods like HPLC[3,4], UV[5], spectroflurometry[6], voltametry[7,8], electronspray ionization mass spectrometry[9] and spectrophotometry[10-15] have been reported. Spectrophotometric techniques continues to be the most preferred methods for the assay of different classes of drugs in pure, pharmaceutical formulations and in biological samples[16-18] because of its simplicity reasonable sensitivity with significant advantages. Some of the methods reported for estimation of azithromycin are time consuming and require expensive equipment[3,6,8,9] and some of the spectrophotometric methods require oxidation, reduction or degradation of the drug. So far there is no report of extractive spectrophotometric method for the determination of azithromycin. This paper describes two visible spectrophotometric methods for the determination of azithromycin based on the formation of an ion association complex with acid dyes wool fast blue BL (WFBBL) and tropeolin OOO (TPOOO) which is extractable into chloroform. The protonated aliphatic tertiary nitrogen (positive charge) of azithromycin in acid medium is expected to attract the oppositely charged portion of the dye ($-SO_3^-$) and behaves as a single unit being held together by electrostatic extraction in methods M_1 and M_2. These methods were simple accurate and can be extended to pharmaceutical formulations containing azithromycin.

A Milton Roy spectronic 1201 and a systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for spectral and absorbance measurements.

All the chemicals were of analytical grade and all solutions were prepared in triply distilled water. Freshly prepared solutions were always used. Aqueous solutions of wool fast blue BL (4.63×10^{-3} M) glycine-HCl buffer of pH 1.3 [prepared by mixing 226 mL of 0.1 M glycine solution (7.507 g of glycine and 5.85 g of sodium chloride dissolved in 1 L distilled water) and 774 mL of 0.1 M HCl and adjusting the pH to 1.3] were prepared for M_1. TropileonOOO (5.709×10^{-3} M) and 0.1 M HCl were prepared for M_2.

Standard drug solution: 1 mg mL^{-1} stock solution of azithromycin was prepared by dissolving 100 mg of the drug initially in 10 mL of 0.1 M HCl and made upto 100 mL with distilled water.

Analysis of azithromycin: Aliquots of standard drug solution ranging from (1-6 mL: 50 μ mL^{-1} for method M_1 1-5 mL 100 μg mL^{-1} for method M_2) were placed in a series of 125 mL separating funnels. 6 mL of pH 1.5 buffer, 2 mL of dye for method M_1 and 6 mL of 0.1 M HCl, 2 mL of dye for method M_2 were added successively. The total volume of aqueous phase in each funnel was brought to 15 mL with distilled water then 10 mL of chloroform was added to each funnel

TABLE-2
ASSAY AND RECOVERY OF AZITHROMYCIN IN PHARMACEUTICAL FORMULATIONS

Formulations[a] (capsules)	Labelled amount (mg)	Amount by proposed methods[b] (mg) M_1M_2		Reference method	Recovery by proposed methods[c] (%) M_1M_2	
I	250	250.0 ± 0.56 F = 1.54 t = 0.2	249.6 ± 0.63 F = 1.96 t = 1.00	250.0 ± 0.45	100.01 ± 0.23	99.85 ± 0.25
II	100	99.9 ± 0.16 F = 1.27 t = 0.37	99.9 ± 0.58 F = 4.0 t = 1.99	99.9 ± 0.18	99.97 ± 0.16	99.92 ± 0.58
III	250	250.1 ± 0.42 F = 4.59 t = 0.25	248.92 ± 0.42 F = 1.04 t = 1.21	250.1 ± 0.90	100.0 ± 0.17	99.58 ± 0.40
IV	100	99.8 ± 0.11 F = 1.19 t = 1.9	100.14 ± 0.24 F = 4.0 t = 1.06	100.1 ± 0.12	99.88 ± 0.11	100.14 ± 0.24

a: Different batches for capsules from four different pharmaceutical companies, b: Average ± standard deviation of six determinations, the t- and F-test values refer to companies of the proposed method with the reference method. Theoretical values at 95 % confidence limit, F = 5.05, t = 2.57. c: Recovery of 10 mg added to the prenalysed capsules (average of three determinations).

and the contents were shaken for 2 min the absorbance of the separated chloroform layer was measured at 580 nm (method M_1) or 480 nm (method M_2) against a reagent blank. The coloured species was stable for 1 h. The drug concentration was deduced from a calibration graph.

The optimum conditions for the colour development of the methods were established by varying parameters one at a time and keeping others fixed and developing the effect of produced on the absorbance of the coloured species.

The optical characteristics such as Beer's law limits, molar absorptivity and sandell's sensitivity for these methods were given in Table-1. The precision of each method was found by measuring absorbances of six replicate samples containing known amount of the drug and the results obtained were incorporated in Table-1 regression analysis using the method of least squares was made to evaluate the slope (b) intercept (a) and correlation coefficient (r) for each system (Table-1). The relative standard deviation and percentage range of error at 95 % confidence level were also given in Table-1. The accuracy of the method was ascertained by comparing the results by proposed and reference methods[19] by t- and F-test (Table-2). This comparison shows that there is no significant difference between the results of studied methods and those of reference ones. The similarity of the results was an obvious evidence that during the application of these methods, the excipients that were usually present in pharmaceutical formulations do not interfere in the assay of proposed methods.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameter	Method 1	Method 2
λ_{max} (nm)	580	480
Beer's law limits (μg mL^{-1})	5-25	10-50
Molar absorptivity (1 mol^{-1} cm^{-1})	2.39×10^4	3.44×10^3
Sandell's sensitivity (μg cm^2/0.001 absorbance units)	3.13×10^{-2}	2.17×10^{-1}
Regression equation[a] (y)		
Slope (b)	3.1×10^{-2}	4.68×10^{-3}
Intercept (a)	-2×10^{-4}	-1.4×10^{-3}
Correlation coefficient	0.9999	0.9997
Relative standard deviation[b] (%)	0.45	0.63
Range of error (0.05 level) (%)	0.47	0.65

a: y = a + bC where C = concentration in μg mL^{-1} and y = absorbance unit, b: Six replicate samples.

The higher λ_{max} values of the two methods have a decisive advantage since the interference from the associated ingredients should be generally less at higher wave lengths than at lower wave lengths. Thus the proposed extractive spectrophotometric methodswere simple and sensitive with reasonable precision, accuracy and constitute better alternatives to the existing ones to the routine determination of azithromycin in bulk and dosage forms.

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