Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 7
ISSN: 2321–3647(online)

Utility of Oxidation–Reduction Reaction for the Spectrophotometric Determination of Azithromycin

Hany A. Omara[1] *, Hamid M. Younis[1]
1 Chemistry Department, Faculty of Science, Sirte University, Sirte, Libya.

ABSTRACT

Two simple, rapid, accurate, sensitive and economical visible spectrophotometric **(**direct, indirect) methods (A and B) for the determination of azithromycin (AZT) in bulk sample and in dosage forms are described. The first method (A) is based on the oxidation of the drug by ammonium metavanadate in sulfuric acid medium and the absorbance is measured at 750 nm. The absorbance concentration plot is linear over the range (0.3-29.7 μg/ml). The second method (B) is based on oxidation of AZT by iodine solution in acidic medium, and determination of the unreacted oxidant by measuring the decrease in absorbance using methylene blue dye (MB) at a suitable λ_{max} (662 nm), respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges (0.4-36.1 μg/ml). The quality control/assurance parameters such as limits of detection (LOD), quantification (LOQ), molar absorptivity and Sandelle's sensitivity values are also reported. The accuracy and precision of the methods were studied on intra-day and inter-day basis. No interference was observed from common pharmaceutical additives. The methods are used successfully to assay AZT in its pharmaceutical dosage forms viz. tablets, capsules and spiked human plasma.

Keywords: Azithromycin, Spectrophotometric, Redox reaction, Ammonium metavanadate, Iodine, Pharmaceutical analysis, Spiked human plasma.

*Corresponding Author Email hanyomara666@yahoo.com
Received 20 June 2014, Accepted 10 July 2014

Please cite this article in press as: Omara HA *et al.*, Utility of Oxidation–Reduction Reaction for the Spectrophotometric Determination of Azithromycin. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Azithromycin, chemically, (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-ethyl-3,4,10-tri-hydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-]oxy}-1-oxa-6-azacyclo-pentadec-13-yl-2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranoside is a semi-synthetic macrolide antibiotic widely used in the respiratory tract infections, like pharyngitis, pneumonia, chronic bronchitis, bronchopneumonia, skin and soft tissue infections and some sexually transmitted diseases, that acts on Gram positive bacteria and Gram negative bacteria[1]. The most commonly used techniques for the determination of azithromycin in pharmaceutical dosage forms are high performance liquid chromatography[2,3], liquid chromatography-mass spectrometry[4], microbiological[5], differential pulse voltammetric[6-8], amperometric[9] diffuse reflectance near infrared spectroscopy[10] and spectrophotometric methods[11-17].

The objective of the present work was to develop a simple spectrophotometric method for the determination of azithromycin in pharmaceutical formulations. The present procedure neither requires any extraction nor any elaborate equipment and the method is less time-consuming.

Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control laboratories[18-22]. However, AZT does not possess any chromophore in its molecule, which is the essential requirement for the direct or indirect spectrophotometric analysis.

The oxidation reaction between ammonium metavanadate or Iodine in acidic medium and AZT have not been investigated yet but for other drugs[23-27]. Therefore, the present study was devoted to explore ammonium metavanadate and Iodine as an oxidant in the development of two (direct and indirect) selective and sensitive spectrophotometric methods for the determination of AZT in tablets and capsules. The present work describes two spectrophotometric methods which are superior to the reported ones, for rapidity, reproducibility, time consuming and high sensitivity. The proposed methods which used are well known for their high absorptivity and they will have been utilized for estimation of oxidants (ammonium metavanadate and iodine) in acidic medium. Where modern and expensive apparatus such as GLC, HPLC and HPTLC are not available.

MATERIALS AND METHODS

Apparatus

All the spectral measurement were made using double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190 –1100 nm, spectral bandwidth 2.0 nm, with

scanning speed 400 nm/min, equipped with 10 mm matched quartz cells. A thermostat water bath, Buchi 461 water bath, Schwiz (France) was used to carry out the temperature studies and Magnetic Mix. 100, Thermo Scientific, UK.

Chemicals and reagents

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were freshly prepared daily in doubly distilled water.

- Pure Azithromycin dihydrate bulk powder was obtained from Egyptian Organization for Control and Pharmaceutical Research-Egypt. AZT working solution was prepared by dissolving 0.01 g of pure AZT in 50 ml of bidistilled water and complete to 100 ml with bidistilled water to obtain the working standard solution of 100 µg/ml, store the prepared solution at room temperature.
- Aqueous solutions of dye, (1.0×10^{-4} M) methylene blue (MB) (Merck) was prepared by dissolving in an appropriate weight in 100 ml bidistilled water.
- A stock solution of 1.0×10^{-2} M ammonium metavanadate (Aldrich Co., Ltd., Gillingham-Dorst, Germany) was freshly prepared by dissolving appropriate weight in a least amount of warm water in a 100 ml measuring flask and then diluted with distilled water to the mark.
- A stock (1.0×10^{-2} M) solution of iodine (Aldrich), was freshly prepared by dissolving an accurate weight in solution containing the same weight of potassium iodide in bidistilled water.
- A solution of 11.0 M H_2SO_4, was prepared by adding exact volume from stock (98%) concentrated acid to bidistilled water in 500 ml measuring flask, and standardized as recorded[28].

General procedure

For the first method, (method A) depends on oxidation of AZT by addition of 0.03-2.97 ml AZT (100 µg/ml) to 1.5 ml of 1.0×10^{-2} M ammonium metavanadate containing 2.0 ml H_2SO_4, 11.0 M. The solution was heated in a water bath at boiling for 10 min, the volume was completed to 10 ml with bidistilled water and measured spectrophotometrically against a blank solution containing the same constituent except drug treated similarly, at λ_{max} 750 nm. For the second method (method B) depends on oxidation of AZT by addition of 0.04-3.61 ml AZT (100 µg/ml) to 1.0 ml of 1.0×10^{-2} M iodine containing 1.2 ml of 0.2 M H_2SO_4 was added. The solution was heated in a water bath at 80±1 ^{o}C for 7.0 min, the mixture of was cooled and 0.9 ml (1.0×10^{-4} M)

of MB was added, the volume was completed to 10 ml with bidistilled water. The decrease in color intensity of dye was measured spectrophotometrically against a blank solution containing the same constituent except drug treated similarly, at their corresponding λ_{max} 660 nm. The concentration range was determined in each case by plotting the concentration of AZT against absorbance at the corresponding maximum wavelengths (Figure 1).

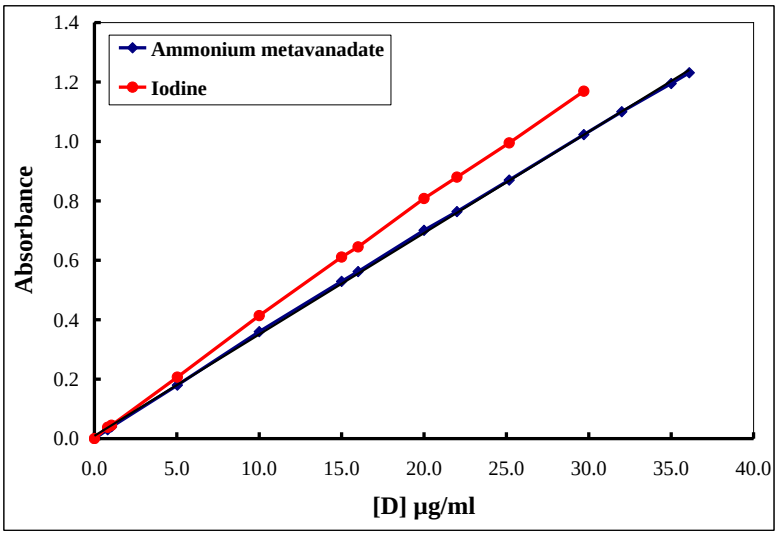

Figure 1. Calibration curve of AZT, using ammonium metavanadate and iodine.

Stoichiometric relationship

The stoichiometry of the reaction between AZT and the oxidant at the selected conditions was established by the molar ratio method. In this method 1.0 ml of 1.0 x 10^{-2} M ammonium metavanadate (method A) 1.0 x 10^{-2} M I_2 (method B) is kept constant and variable concentrations (0.1-4.0 ml) of AZT (1.0 x 10^{-2} M) were added. The absorbance was measured at λ_{max} against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O].

Procedure for dosage forms

Twenty tablets/capsules, were carefully evacuated; their contents were weighed and finely powdered. An accurately weighed quantity of the capsule contents equivalent to 20 mg of AZT was transferred into a 100 ml calibrated flask, and dissolved in about 40 ml of distilled water and sulphoric acid (0.2 ml, 2.0 M). The contents of the flask were swirled, sonicated for 5.0 min, and then completed to volume with water. The contents were mixed well and filtered (through Whatman No. 41 filter paper) rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration for the analysis; the analysis was carried out in triplicate for three pharmaceutical dosage forms i.e. tablets and capsules. The results of analysis of pharmaceutical dosage forms and spiked human plasma are

shown in (Table 2). The good recovery confirmed the accuracy and the specificity of the proposed method, and the lack of interference from the common excipients, and colorant/preservatives, used in the manufacture of tablets and capsules.

Procedure for spiked human plasma samples

Aliquots of 1.0 ml of plasma were spiked with different concentration levels of AZT. The spiked plasma samples were treated with 0.1 ml of 70% perchloric acid and vortexes for 1.0 min. The samples were centrifuged for 20 min at 13000 rpm. The supernatants were transferred into test tubes and neutralized with 1.0 M NaOH solution.

RESULTS AND DISCUSSION

First method (A)

The proposed spectrophotometric methods are based on the reaction between AZT and ammonium metavanadate in acidic medium, and measuring the absorbance at 750 nm. The reaction takes place completely after boiling for 10 min. The absorbance of the colour formed is measured at 750 nm, the color remains constant for at least 24 h.

Second method (B)

The method involves two steps namely:

- Oxidation of AZT with I_2 in acidic medium by heating in water bath 80±1 ^{o}C, 7 min.
- Determination of unreacted oxidant by measuring the decrease in absorbance of MB dye at a suitable λ_{max}.

$$I_2 + H^+ + AZT \rightarrow 2I^- + \text{Oxidation products}$$

(Brownish) (Colorless)

$$I_2 \text{ (excess)} + \text{dye} \rightarrow \text{Oxidative dye (colorless products)}$$

The influence of each of the following variables on the reaction was tested.

Effect of ammonium metavanadate concentration

The influence of ammonium metavanadate concentration was studied in the range from 10^{-4} - 10^{-1} M, as final concentration. The optimum results were obtained with 1.5 ml of 1.0 *x* 10^{-2} M (Figure 2).

Effect of iodine concentration

The influence of iodine concentration was studied in the range from 10^{-4} - 10^{-1} M, as final concentration. The optimum results were obtained with 1.0 ml of 1.0 *x* 10^{-2} M; higher concentration of I_2 caused the color to disturbed (Figure 2).

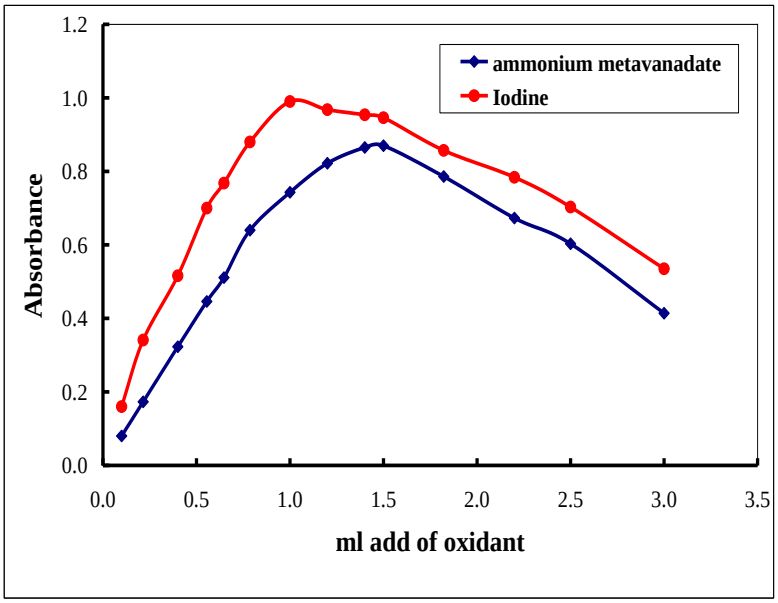

Figure 2: Effect of ml added of ammonium metavanadate (1.0 *x* 10^{-2} M) (method A), I_2 (1.0 *x* 10^{-2} M) (method B) on absorbance of 25.0 µg/ml of AZT.

Effect of medium

The different types of acid were examined (HCl, $HClO_4$, H_2SO_4, H_3PO_4, CH_3COOH and HNO_3). The most suitable acid to achieve maximum yield of redox reaction was found to be 2.0 ml of 11.0 M H_2SO_4 was added for (method A). The most suitable acid to achieve maximum yield of redox reaction was found to be 1.2 ml of 0.2 M H_2SO_4 was added on using MB, for (method B) Figure 3.

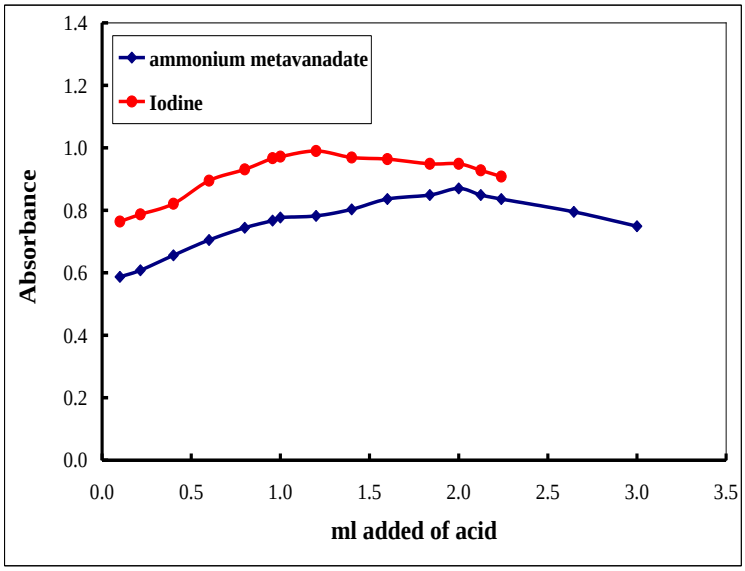

Figure 3: Effect of ml added of H_2SO_4 (method A) (11.0 M) with ammonium metavanadate (1.0 *x* 10^{-2} M), 0.2 M H_2SO_4 (for method B) on absorbance of 25.0 µg/ml of AZT with I_2 (1.0 *x* 10^{-2} M) using MB dye.

Effect of temperature and time

The oxidation process of AZT with ammonium metavanadate in acidic medium (method A) is catalyzed by heating, and the reaction takes place completely after boiling at 10 min. in a thermostat water bath, to produce a water-soluble bluish-green colored species. The oxidation process of AZT for the (method B) I_2 in acidic medium was catalyzed by heating in a thermostat water bath of 80±1 ^{o}C for 7.0. After oxidation process, the solution must be cooled at least for 2.0 min before addition of dye.

Effect of sequence of additions

The effect of sequence of additions on the oxidation process of AZT was studied by measuring the absorbance of solution prepared by different sequence of additions against a blank solution prepared in the same manner. Experiments showed that (Oxidant-Acid-Drug) for both methods, gave the best results.

Effect of MB dye concentration

The optimum volume of dye used for production of maximum color intensity was 0.9 ml of 1.0 *x* 10^{-2} M for (method B). The effect of time after the addition of dye indicated that shaking for 1.0 min was sufficient to give reliable results for all dyes. The color remains constant for at least 24 h.

Stoichiometric ratio

The stoichiometry of the reaction between AZT and the oxidant at the selected conditions was established by the molar ratio method. In this method 2.0 ml of 1.0 *x* 10^{-2} M ammonium metavanadate for (method A), 2.0 ml of 1.0 *x* 10^{-2} M I_2 for (method B) is kept constant and variable concentrations (0.1 - 5.0 ml) of AZT (1.0 *x* 10^{-2} M) were added using micropipette. The absorbance was measured at λ_{max} against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O]. The stoichiometry of [D]/[O] at the selected conditions showed that the inflection of the two straight lines at 0.6 for (method A) 1.13 and 0.88 for (method A, B) respectively.

ANALYTICAL DATA

Beer's law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and recorded. The limits of detection (K=3) and quantitation (K=10) were established according to IUPAC definitions[29] as in Table 1.

The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa / b

Where κ = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. Based on the basis of six replicate measurements, the limit of detection was 0.077 µg/ml, 0.058 µg/ml and the limit of quantification was 0.257 µg/ml, 0.193 µg/ml using method A, B respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods. In order to determine the accuracy and precision of the methods, solution containing three different concentrations of AZT were prepared and analyzed in six replicates. The analytical results obtained from this investigation were summarized in Table 2.

Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 25 µg/ml of AZT (for method A) and (method B) with varying concentration of the additives and excipients such as cellulose, talc powder, lactose, calcium hydrogen phosphate, magnesium stearate, micro-crystalline cellulose and starch. Under the experimental conditions, the effect of excipients frequently found in formulations was evaluated using the proposed method; the excipients in all tablets and capsules are not interfere.

Table 1: Optical and regression characteristics of AZT for the proposed methods.

Parameters	**lethod A)** **nmonium metavanad**	**lethod B)** **dine**
λ_{max} nm	0	0
Stability / h	24	72
Heating time (min.)	.0	)
Beer's law limits (µg/ml)	3 – 29.7	4 - 36.1
Ringbom limits (µg/ml)	4 - 28.9	5 - 35.8
Molar absorptivity L/mol. c	75 *x* 10^4	14 *x* 10^4
Sandell sensitivity (ng/cm)	.57	.00
Detection limits (µg/ml)	)77	)58
Quantitation limits (µg/ml)	257	193
Regression equation*:		
Slope (b)	)35	0.040
RSD% of slope	)065	)047
Intercept (a)	)094	)088
RSD% of intercept	)047	)056
Stoichiometric ratio	1.13	1 : 0.88
Correlation coefficient (r)	)999	)998
RSD** %	59	33

* A = a + bC where C is concentration of drug in µg/ml and A is absorbance.

** Relative standard deviation for six determinations.

Table 2: Evaluation of the accuracy and precision of the proposed methods for AZT

Reagents	Taken µg/ml	Recovery %	RSD [a] %	RE [b] %	Confidence limits [c]
	10.0	100.1	0.78	1.09	10.01 ± 0.1097
(Method A)	20.0	100.05	0.66	0.57	20.01 ± 0.1141
Ammonium metavanadate	25.0	99.96	0.49	0.48	24.99 ± 0.1172
	10.0	99.8	0.69	1.11	9.98 ± 0.1106
(Method B)	20.0	100.1	0.89	0.65	20.02 ± 0.1397
Iodine	30.0	99.93	0.76	0.63	29.98 ± 0.1899

[a] Relative standard deviation for six determinations.

[b] Relative error.

[c] 95 % confidence limits and five degrees of freedom.

Method Validation

The proposed methods were successfully applied to determine AZT in its dosage forms. The accuracy of the proposed methods is evaluated by applying standard addition technique, in which variable amounts of the drug were added to the previously analyzed portion of pharmaceutical preparations. The results recorded in Table 3, were compared statistically with the official method[30] by Student's t-test (for accuracy), and variance ratio F-test (for precision)[31], at 95% confidence level as recorded in Table 4. The results showed that the t- and F- values were lower than the critical values indicating that there was no significant difference between the proposed and official methods. The proposed method was more accurate with high recoveries compared to the official method. So the proposed methods can be recommended for routine analysis of AZT in pure and pharmaceutical forms, i.e. tablets and capsules in the majority of drug quality control laboratories.

Table 3: Determination of AZT in tablets and capsules using standard addition technique.

Samples	Taken µg/ml	Method A			Method B		
		Added µg/ml	Found* µg/ml	Recovery %	Added µg/ml	Found* µg/ml	Recovery %
Zithrokan		0.0	10.02	100.2	0.0	9.88	98.8
500 mg /	10.0	10.0	19.97	99.85	10.0	19.98	99.9
Capsule [a]		15.0	24.95	99.8	15.0	24.96	99.84
Azithromycin		0.0	9.98	99.8	0.0	10.02	100.02
250 mg /	10.0	10.0	19.98	99.9	10.0	19.97	99.85
Tablet [b]		15.0	25.02	100.08	15.0	25.01	100.04
Azithromycin		0.0	10.01	100.1	0.0	10.01	100.1
250 mg /	10	10.0	19.89	99.45	10.0	19.91	99.55
Tablet [c]		15.0	24.93	99.72	15.0	24.96	99.84
Spiked plasma		0.0	9.99	99.9	0.0	10.02	100.2
pimples	10.0	10.0	20.04	99.20	10.0	20.03	100.15
		15.0	25.02	100.08	15.0	25.01	99.04

* Average of six determinations.

[a] Hikma Pharma S.A.E., 6th of October City – Egypt.

[b] Akums Drugs & Pharmaceuticals Ltd. – India.

[c] Fabrique Par Les Laboratoires Ibn Al-Baytar, Carthage, Tunis, Hikma Pharmaceuticals – Jordanie.

Table 4: Determination of AZT in pharmaceutical formulations using the proposed and official methods.

Parameter	Method A	Method B	Official method
	Zithrokan 500 mg / Capsule [1]		
Recovery % [a]	99.8 ± 1.12	100.1 ± 1.1	99.6 ± 1.1
± Standard Deviation	0.86	0.69	1.12
Variance	0.77	0.84	1.17
Student t-value [b]	1.89	1.97	1.57
Variance ratio F-test [b]	1.88	1.86	2.13
	Azithromycin 250 mg / Tablet [2]		
Recovery % [a]	99.9± 1.2	99.8 ± 0.71	99.4 ± 0.71
± Standard Deviation	0.76	0.89	0.97
Variance	0.89	0.77	1.14
Student t-value [b]	0.84	0.79	1.25
Variance ratio F-test [b]	2.2	1.76	2.46
	Zomax 500 mg / Capsule [3]		
Recovery % [a]	99.4 ± 0.52	100.2 ± 1.1	99.5 ± 0.63
± Standard Deviation	1.24	0.98	1.27
Variance	1.07	0.58	1.31
Student t-value [b]	0.66	0.76	0.98
Variance ratio F-test [b]	3.05	3.22	3.22
	Spiked plasma pimples		
Recovery % [a]	99.7 ± 0.42	100.02 ± 1.3	99.2 ± 0.73
± Standard Deviation	1.20	0.96	1.36
Variance	1.27	0.51	1.28
Student t-value [b]	0.79	0.83	0.89
Variance ratio F-test [b]	3.35	2.13	2.92

[a] Recovery %, [b] Theoretical value for t- and F- values for five degrees of freedom and 95 % confidence limits are 2.57 and 5.05, respectively, Number of experiments = 6

[1] Hikma Pharma S.A.E., 6th of October City – Egypt.

[2] Akums Drugs & Pharmaceuticals Ltd. – India.

[3] Fabrique Par Les Laboratoires Ibn Al-Baytar, Carthage, Tunis, Hikma Pharmaceuticals – Jordanie.

CONCLUSIONS

The proposed methods were advantageous over other reported visible spectrophotometric and colorimetric methods, related to their high reproducibility, high sensitivity, less time consuming

and using simple and inexpensive reagents. Moreover, these methods allowed the determination of AZT up to 0.3 μg/ml, in addition to simplicity, rapidity, precision and stability of colored species for more than 48 h. The proposed methods may be applied for routine analysis and in quality control laboratories for the quantitative determination of the AZT in raw materials and in pharmaceutical formulations.

REFERENCES

1. Reynolds JEF, Eds., In; Martindale: The Extra Pharmacopoeia, 32th Edn., The Pharmaceutical Press, London, 1999; 155.
2. Shaikh KA, Patil SD, Devkhile AB., Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form, J. Pharm. Biomed. Anal. 2008; 48: 1481–84.
3. Yang ZY, Wang L, Tang X. Determination of azithromycin by ion-pair HPLC with UV detection, J. Pharm. Biomed. Anal. 2009; 49: 811–15.
4. Debremaeker D, Visky D, Chepkwony HK, Van Schepdael A, Roets E, Hoogmartens J. Analysis of unknown compounds in azithromycin bulk samples with liquid chromatography coupled to ion trap mass spectrometry, Rapid Commun Mass Spectrom. 2003; 17: 342–50.
5. Breier AR, Garcia CV, Oppe TP, Steppe M, Schapoval EE., Microbiological assay for azithromycin in pharmaceutical formulations. J. Pharm. Biomed. Anal. 2002; 29: 957–61.
6. Nigoviæ B., Adsorptive stripping voltammetric determination of azithromycin at a glassy carbon electrode modified by electrochemical oxidation, Anal. Sci., 2004; 20: 639–43.
7. Farghaly OA, Mohamed NA., Voltammetric determination of azithromycin at the carbon paste electrode, Talanta, 2004; 62: 531–38.
8. Nigoviæ B, Simuniæ B. Voltammetric assay of azithromycin in pharmaceutical dosage forms, J. Pharm. Biomed. Anal., 2003; 32: 197–02.
9. Palomeque ME, Ortíz PI., New automatized method with amperometric detection for the determination of azithromycin, Talanta. 2007; 72: 101–05.
10. Ji XD, Wen BZ, Yan CF, Dan QS, Chang QH., Quantitative calibration models for the determination of azithromycin and decladinosylazithromycin in azithromycin injection powders using diffuse reflectance near infrared spectroscopy, J. Near Infrared Spectrosc. 2011; 19: 265–75.

11. Lakshmi S, Arul MM, Jayashankar L, Ramu P, Raja TK., Visible spectrophotometric methods for the determination of azithromycin in tablets, Indian J Pharm Sci. 2004; 66: 249-51.
12. Rachidia M, Elhartia J, Diguab K, Cherraha Y, Bouklouzea A., New spectrophotometric method for azithromycin determination, Anal. Lett. 2006; 39: 1917–26.
13. Carlos ER, Vanessa GK, Ricardo CJ., Spectrophotometric method for the determination of azithromycin in pharmaceutical formulations based on its charge transfer reaction with quinalizarin, J. Braz. Chem. Soc. 2010; 21: 1664–71.
14. Sultana N, Arayne MS, Hussain F, Fatima A., Degradation studies of azithromycin and its spectrophotometric determination in pharmaceutical dosage forms, Pak J. Pharm. Sci. 2006; 19: 98–103.
15. Rufino JL, Pezza HR, Pezza L., Flow-injection spectrophotometric determination of azithromycin in pharmaceutical formulations using p-chloranil in the presence of hydrogen peroxide, Anal. Sci. 2008; 24: 871–76.
16. Jayanna BK, Nagendrappa G and Gowda N., Spectrophotometric estimation of azithromycin in tablets, Indian J. Pharm. Sci., 2012; 74(4): 365–67.
17. Omara HA, Hawa AA, Abeer AE and Salha AM, New Spectrophotometric determination of Azithromycin in pure and dosage forms using N-bromo-succinimide And potassium permanganate as Oxidants. World J. of Pharm. and Pharma. Scie. 2014; 3(4), 100-12.
18. Omara HA and Amin AS., Extractive-spectrophotometric methods for determination of anti-Parkinsonian using sulphonphthalein acid dyes, J. Saudi. Chem. Soci., 2012; 16: 75–81.
19. Omara HA, and Amin AS., New, Simple and validated spectrophotometric method for determination of amikacin in biological samples and its pharmaceutical formulations, IJPBSRD, 2013; 1 (4), 1347-59.
20. Omara HA, Amin AS and Shama SA., Utility of oxidation-reduction reaction for the spectrophotometric determination of antiviral and anti-parkinsonian drug amantadine HCl, WJPR, 2013; 2 (6): 1958–70.
21. Omara HA.,, Spectrophotometric determination of anti-parkinsonian drug in capsules and spiked plasma using iron (III) and potassium ferricyanide, IJBPR., 2014; 5(1): 27-32.
22. Shama SA, Amin AS and Omara HA, Spectrophotometric microdetermination of some antihypertensive drugs in pure form and in pharmaceutical formulations, J. Chil. Chem. Soc., 2010; 55, 4: 431-34.

23. Ibrahim EA, Beltagy YA and Abd El-Khalek MM, Spectrophotometric determination of some penicillins with ammonium vanadate, Talanta. 1977; 24(5): 328-30.
24. Rahman N and Azmi SNH, Spectrophotometric determination of diltiazem hydrochloride with sodium metavanadate. Microchemical J. 2000; 65(1): 39-43.
25. Misiuk W. Spectrophotometry assay of imipramine and desipramine using ammonium metavanadate and its application to pharmaceutical preparations. J. of Pharm. and Biomed. Anal. 2000; 22(1): 189-96.
26. Pupo RF, Mirela NM, Oliveira C and Paterlini WC, Simple and fast spectrophotometric determination of H2O2 in photo-Fenton reactions using metavanadate. Talanta. 2005; 66(1): 86-91.
27. Ahmad S, Sharma RD and Shukla I. C, Microdetermination of oxprenolol hydrochloride and metoprolol tartrate with ammonium metavanadate. Talanta. 1987; 34(2): 296-98.
28. Basset J, Jeffery GH & Mendham J, Vogel's Text Book of Quantitative Inorganic Analysis, 1978; pp. 308.
29. H. M. Irving, H. Freiser, T. S. West, (Eds.). (1981), "IUPAC Compendium of Analytical Nomenclature", Definitive Rules. Pergamon Press, Oxford.
30. The British Pharmacopoeia (BP), Her Majesty's Stationary Office, London, I, 2007.
31. Miller JC and Miller JN, Statistics in Analytical Chemical 3rd Ed. Ellis Horwood Chichester, 1993.