INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Thin-Layer Chromatographic And UV-Spectrophotometric Analysis Of Frequently Utilized Oral Macrolide Antibiotics

Samuel J. Bunu[1], Veronica Aniako[2], Varsharani P. Karade[3], Edebi N. Vaikosen[4], Benjamin U. Ebeshi[5]

[1,2,4,5]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.*

[3]*Dadasaheb Balpande College of Pharmacy, Besa, Nagpur, Maharashtra, India.*

ARTICLE INFO

Received: 31 Aug 2023
Accepted: 03 Sept 2023
Published: 13 Sept 2023

Keywords:
macrolide, analytical method, chromatography, azithromycin, spectrophotometry

DOI:
10.5281/zenodo.8340601

ABSTRACT

Background: Macrolides are bacteriostatic antibiotics used in the management of mild to chronic soft tissues, and upper and lower respiratory and genitourinary tract infections. Misuse of these agents is common among people with infections, thus leading to the development of bacterial resistance. Also, due to high demand, there likelihood of fake and substandard products in the markets, hence the need to develop simple, cost-effective, and rapid methods for the analysis and percentage determination of these antibiotics. The study aimed to quantify various samples of macrolide using thin-layer chromatographic and UV-spectrophotometric analytical methods. Silica gel GF 254 was used as the stationary phase for TLC, while in UV spectrophotometric analysis, samples were identified through absorbance produced at different wavelengths (200 – 350 nm). All the samples were within standard weight (mg), and the spectrophotometry fingerprinting analysis was obtained at a wavelength of 220 nm and 240 nm. The retardation factor (Rf) value calculated for the various macrolide ranged from 0.75 to 0.83 with visible spots on exposure to iodine vapor. The use of appropriate methods of detection and solvent systems permits the identification of the entire macrolide antibiotics samples used in the analysis. TLC and Spectrophotometric fingerprinting procedures can be applied in preparatory and exploratory analytical screening, quality control studies, and therapeutic drug monitoring of macrolide antibiotics.

INTRODUCTION

Antibiotics are classified according to their mechanism of action, chemical structure, and

***Corresponding Author:** Samuel J. Bunu
Address: *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria*
Email ✉: pharmsamuelbunu@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

spectrum of activity (Kapoor et al., 2017). They are further categorized based on their target specificity (Slama et al., 2005). Macrolides are a large group of macrocyclic lactone rings with one or more deoxy sugars, usually cladinose and desosamine, with 14, 15, or 16-member lactone rings. They belong to the polyketide natural compounds, with antibacterial or antifungal properties. In contrast to other antibiotics, macrolides reduce or impede bacterial growth rather than complete eradication (Sonka et al., 1989). Any macrocyclic lactone with more than eight members is generally considered a member of this class. The macrocycle may consist of an oxazole, a thiazole, amino-nitrogen, amide-nitrogen, or both (Omura, 2002). They have basic character and exhibit lipophilicity. After administration, the drugs are excreted from the body in the form of inactive metabolites or unchanged via urine or feces (Lenz et al., 2021).

In combination with other antibacterial agents, they are usually used in the treatment of upper and lower respiratory, and genitourinary tract infections, including bacterial sinusitis (Hauk, 2014), otitis media (Neff et al., 2004), community-acquired pneumonia (Mandell et al., 2007), genital ulcer, pharyngitis or tonsillitis (Randel & Infectious Disease Society of, 2013), trachoma (Burton et al., 2015), chronic obstructive pulmonary disease (Taylor et al., 2015), some skin infections, urethritis, cervicitis, gonorrhea (WHO, 2019). Adverse reactions like acute diarrhea, nausea and vomiting, abdominal pain, mild nervousness, skin, and anaphylactic reactions have been like with the use of many macrolide antibiotics (Mori et al., 2014). By blocking peptidyltransferase from joining the developing peptide linked to the next amino acid and by preventing bacterial ribosomal translation, macrolides act to suppress bacterial protein formation (Kaiser, 2009). Premature peptidyl-tRNA dissociation from the ribosome is another proposed mode of action (Tenson et al., 2003). Macrolide antibiotics bind reversibly to the P site on the bacterial ribosome's 50S subunit. This behavior is regarded as bacteriostatic. Leukocytes actively contain macrolides, which are then transferred to the infection site (Bailly et al., 1991).

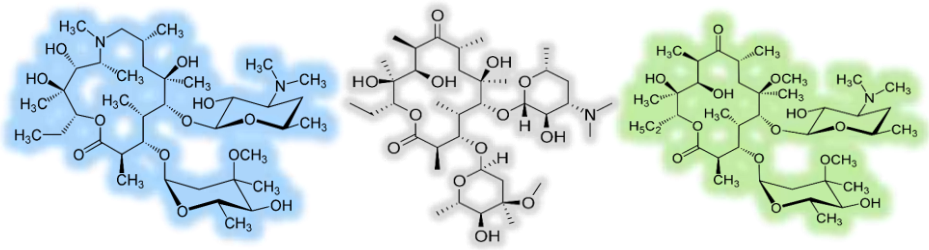


Figure 1: Chemical structure of some macrolide antibiotics: Azithromycin (blue), Erythromycin (olive green), Clarithromycin (orange) (House & Thomson, 1977; Omura, 2002)

Erythromycin is one of the macrolide antibiotics that is rapidly inactivated by gastric acid; thus, it is provided as enteric-coated tablets or more stable salts or esters, such as erythromycin ethyl succinate. It absorbs fast and diffuses into most tissues and phagocytes, and is transported to the site of infection actively (Lebel, 1993). It is metabolized by the hepatic CYP3A4 enzyme, primarily eliminated through bile with little renal excretion, with an elimination half-life ranging between 1.5 and 2.0 hours in patients with end-stage renal impairment and between 5 and 6 hours in individuals with chronic kidney disease. Attains serum peak levels at 4 hours after administration, while ethyl-succinate is between 0.5 and 2.5 hours (Edmunds MW, 2009).

Azithromycin is one of the macrolide antibiotics that is gastric acid-stable and administered orally

without enteric coating. It is absorbed readily, but better without food, and 2.1 - 3.2 hours to peak concentration following oral administration (500mg), actively transported to the site of infection with an elimination half-life of about 68 hours that allows a large single dosage and yet sustains bacteriostatic concentrations for several days (FDA, 2014). Clarithromycin, another active member of the macrolide antibiotic has a fairly rapid first-pass hepatic metabolism, with 50% bioavailability that makes it acquiescent to oral dosing. N-desmethylclarithromycin and 14-(R)-hydroxyclarithromycin are the major inactive and active metabolites, respectively. About 20% - 40% of clarithromycin and 10% - 15% of its active metabolite are eliminated via the urine, with an elimination half-life of approximately 3 to 4 hours following 250 mg administration 12 hourly, while 500 mg has an elimination half-life of 5 - 7 hour at 8 - 12 hourly oral administration. A steady-state concentration of clarithromycin metabolite is attained within 3 to 4 days (Ferrero et al., 1990).

Antibacterial resistance is the development of survival mechanisms by bacteria to withstand antibacterial pharmacotherapy. The overuse or misuse of these antibiotics has been linked to the emergence and spread of microorganisms that are resistant rendering treatment ineffective and posing a serious risk to public health (Larsson & Flach, 2022). Marketing of fake and substandard antibiotics, self-prescription of antibiotics, and incorrect or sub-optimal antibiotics prescribed for certain bacterial infections are common examples of antibiotic misuse. Their effectiveness and easy access led to overuse, especially in livestock raising, promoting bacteria to develop resistance (Buckley GJ, 2013). This has led to widespread problems with antibiotic resistance, prompting the World Health Organization to classify antimicrobial resistance as a serious threat and a prediction for future treatment failure globally (Polianciuc et al., 2020; WHO, 2023).

Several methods have been proposed for the analysis of macrolide antibiotics. HPLC has been used in previous studies to analyze erythromycin and other macrolide antibiotics, phosphate buffer (pH 2.5), and acetonitrile, as mobile phase and monitored at wavelengths 204 – 287 nm. HPLC-MS was also employed for the analysis of seven macrolide antibiotics in fish with a detection limit of 0.01µg/ml (Leal et al., 2001). Also, HPLC erythromycin and benzoyl peroxide in acne gel have been analyzed on an Xterra RP18 column using acetonitrile, 0.2M dipotassium hydrogen phosphate, and water (35:5:60 v/v) as mobile phase and a detection wavelength at 215 nm (Dehouck et al., 2003). Micellar electro-kinetic chromatography (MEKC) was proposed for the determination of β-lactam antibiotics, aminoglycoside, clindamycin phosphate, and erythromycin stearate using borate buffer containing sodium dodecyl sulfate as a background electrolyte (Flurer & Wolnik, 1994). Spectrophotometry using complex formation was also proposed for the analysis of erythromycin in formulations (Amin & Issa, 1996). Other methods like electrochemical detection, fluorescence detection by pre-column derivatization, LC–MS/MS, etc., have been used for the individual quantitative determination of macrolide antibiotics (Chen et al., 2006; Wilms et al., 2005; Xu et al., 2008). Although the authors eulogize the effectiveness of other techniques discussed above, they also acknowledge the demerit of the high cost of equipment and complex operations involved. It is, therefore, pertinent that the utilization of simple cost-effective, precise, and sensitive techniques employed in this study could be useful in an environment with inadequate infrastructure.

Method

Brands of macrolides used in this study

Seven (7) brands of macrolides comprising three brands of erythromycin, three brands of azithromycin, and a brand of clarithromycin

tablets were sourced from community Pharmacies in Yenagoa Bayelsa state, Nigeria. The samples were labelled as follows: erythromycin brands (A1 - A3), azithromycin (B1 - B3), and clarithromycin (C).

Table1. Brands of macrolide tablets utilized in the analysis

Sample Code	Batch No.	Manufacturer	Strength (mg)
A1	5k206	Medopharm, India	500
A2	GT17077	Globella Pharma, India	500
A3	150520	Jiangsu Pharmaceutical Co., Ltd, China	500
B1	J90827	Pfizer Pharmaceutical Inc, USA	250
B2	031015	Saokim Pharmaceutical Vietnam	250
B3	130520	Deshmukh Marg, Mumbai,	250
C	GT17195	Globella Pharma Pvt. Ltd, India	500

Weight uniformity determination

Ten tablets of each brand of macrolide tablets were accurately weighed using an analytical balance and the respective weights were recorded. The average weight, weight variation, standard deviation, and percentage deviation of the respective brands were recorded.

Preparation of Buffer and Stock Solution

1M of sodium dihydrogen orthophosphate was prepared by dissolving 78g of sodium dihydrogen orthophosphate in 500 ml of distilled water while 1M NaOH was prepared by dissolving 20g of sodium hydroxide in 500ml of distilled water, 1M NaOH was used to adjust the pH of the solution to 8 (D.W. Green, 2008). Powdered Erythromycin (A1) tablet equivalent to 2mg was weighed into a beaker where it was dissolved with some dibasic phosphate buffer pH 8 and methanol (1:1). The mixture was filtered and the resultant clear supernatant transferred into a 100ml volumetric flask and was made up to mark with the buffer solution, this gave a stock concentration of 2 mg/ml for the brands A2 to C. From the prepared stock solution of A1 concentration of 1 mg/ml was prepared using a micro-pipette and transferred into a 10 ml volumetric flask. The procedure was repeated for brands A2 to C. From the prepared concentration (1mg/ml) of brand A1, the absorbance was measured from 200 nm - 350 nm. The absorbance was then extrapolated on the calibration curve using Microsoft Excel 2007 version. This procedure was repeated for brands A2 to C.

Thin-Layer Chromatographic Analysis.

An equivalent concentration of 2mg/ml of erythromycin (A1) tablet was prepared using methanol. The mixture was filtered and the resultant clear supernatant was used as a test solution. The test solution of sample A1 was spotted on a TLC plate using a capillary tube and allowed to dry, using methanol, ammonium hydroxide, and glacial acetic acid (50:25:25) as the solvent system. The plate was placed in the TLC chamber until the solvent front had moved to about 8.3 cm, it was removed, carefully marked and allowed to evaporate. The dried plate was placed in an iodine tank, visible spots observed were noted, and Rf value was calculated. This procedure was repeated for drug samples A2, A3, B1, B2, B3, and C. All results obtained were compared with the European Pharmacopeia (EP) official standards (EP, 2008).

RESULTS

Weight Uniformity Test

Table 2. The mean weight uniformities of the various brands used in this study are presented as mean ± SD

Sample Code	Sample weight y(g)	Weight variation (y-ȳ) (g)	$(y-\bar{y})^2$ (g)	Percentage (%) deviation
A1	0.9135 ± 0.02	-0.0004 ± 0.01	0.0001 ± 0.00	-0.0539 ± 1.05
A2	0.8598 ± 0.01	0.0000 ± 0.01	0.0001 ± 0.00	-0.0057 ± 0.80

A3	0.8554 ± 0.02	-0.0006 ± 0.01	0.0003 ± 0.00	-0.0289 ± 1.80
B1	0.4852 ± 0.01	-0.0017 ± 0.01	0.0000 ± 0.00	-0.0156 ± 1.32
B2	0.5244 ± 0.01	0.0000 ± 0.00	0.0013 ± 0.00	0.00003 ± 2.85
B3	0.4876 ± 0.00	0.0000 ± 0.00	0.0000 ± 0.00	-0.0043 ± 0.69
C	0.8679 ± 0.01	0.0000 ± 0.01	0.0001 ± 0.00	0.4988 ± 1.36

The above table shows the mean and standard deviations of the different parameters presented, from the ten (10) tablets of each brand of the macrolide antibiotics used in the analysis.

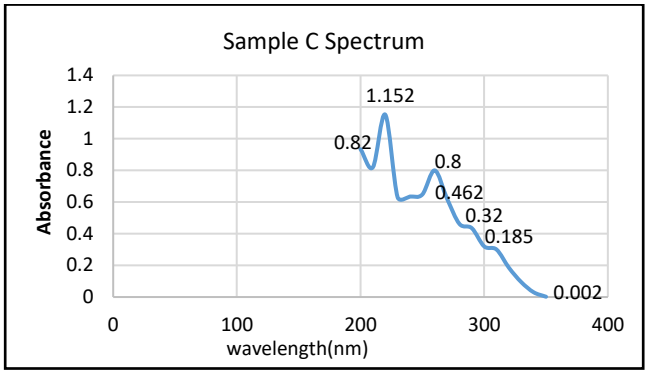
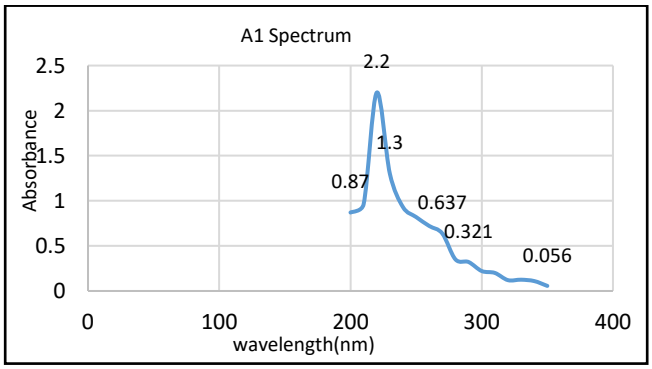
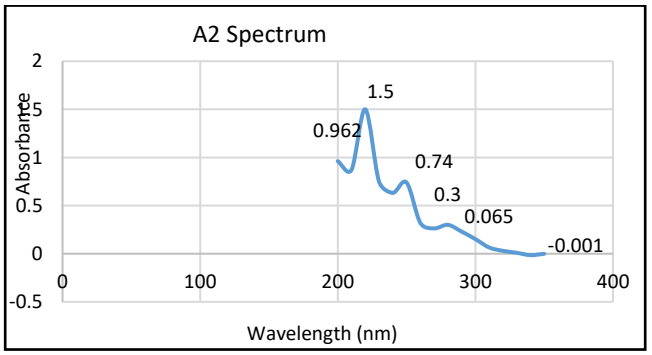


Figure 2. UV spectrum of Sample C

UV-Spectra for Drug Samples





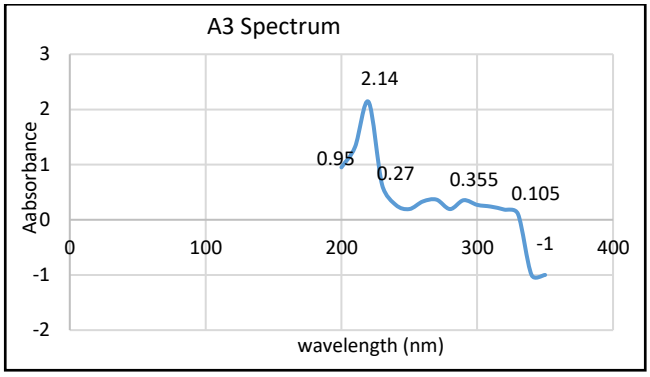
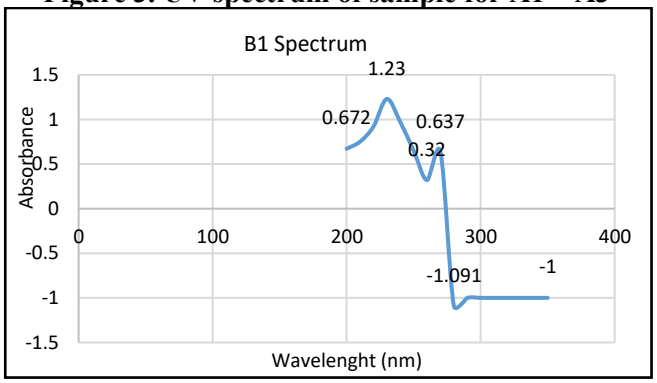
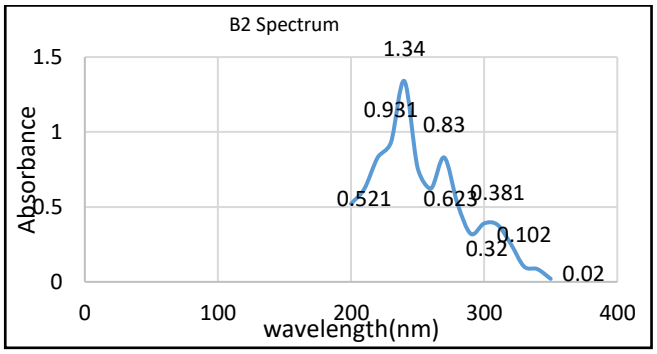


Figure 3. UV spectrum of sample for A1 – A3





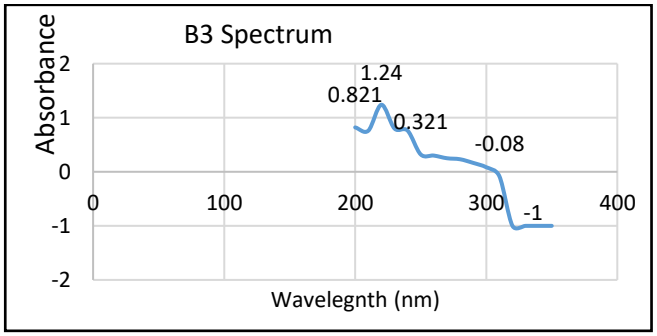


Figure 4. UV spectrum of sample for B1 – B3

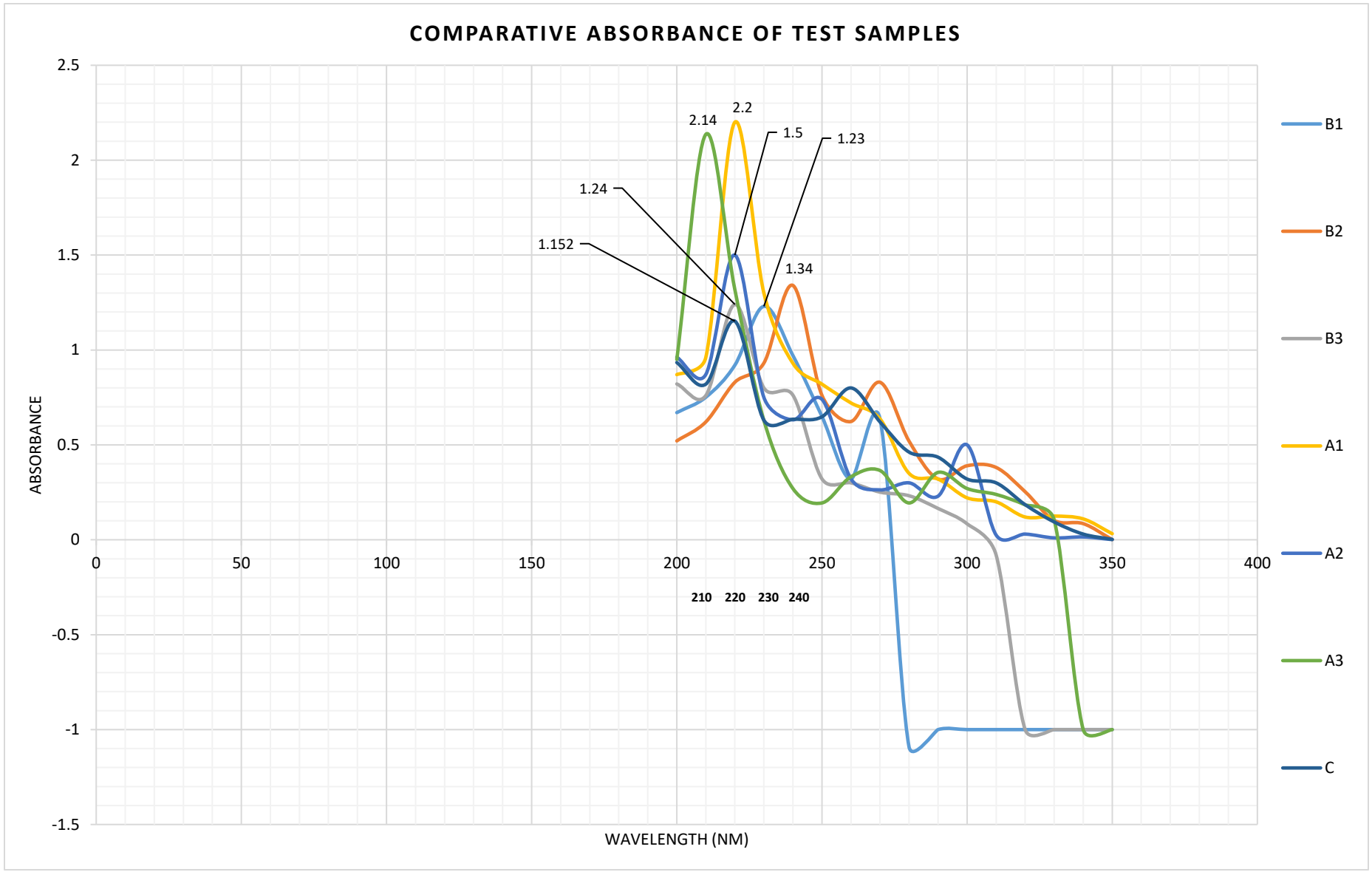


Figure 5. UV-spectrum of all selected brands

Thin-Layer Chromatography

The Rf value, which is calculated as the ratio of the distance moved by the drug sample to the distance moved by the solvent front of test samples A1, A2, A3, B1, B2, B3, and C are shown in the tables below;

Table 3: R_f value for the drug samples for the different solvent systems.

Sample code	S1	S2	S3	S4	Mean ± SD
A1	0.95	0.53	0.71	0.83	0.76 ± 0.18
A2	0.95	0.53	0.71	0.83	0.76 ± 0.18
A3	0.95	0.53	0.71	0.83	0.76 ± 0.18
B1	0.83	0.6	0.82	0.76	0.75 ± 0.11
B2	0.83	0.6	0.82	0.76	0.75 ± 0.11
B3	0.83	0.6	0.82	0.76	0.75 ± 0.11
C	0.9	0.65	0.65	0.78	0.75 ± 0.12

Table 4: R_f value for the drug samples for the S4 (methanol + ammonium hydroxide + acetic acid) solvent system.

Sample code	Distance moved by the solvent front	Distance moved by drug sample	R_F value
A1	8.30	6.90	0.83
A2	8.30	6.90	0.83
A3	8.30	6.90	0.83
B1	8.30	6.30	0.76
B2	8.30	6.30	0.76
B3	8.30	6.30	0.76
C	8.30	6.50	0.78

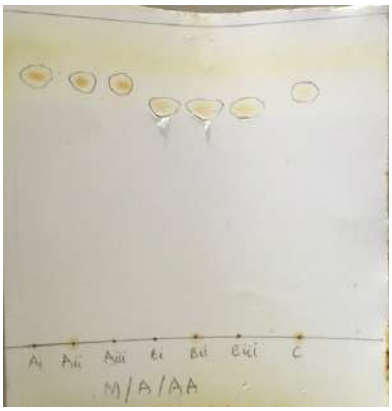


Figure 6: TLC plate on exposure to iodine vapor (Ai, [A1] Aii [A2], Aiii [A3], Bi [B1], Bii [B2], Biii [B3], and C). The spots indicate the pure molecules of macrolide antibiotics.

DISCUSSION

UV spectrophotometry and TLC were employed in analyzing seven brands of macrolide tablets in this study (Table 1, and Figure 1). The standards for uniformity of weight are applied to tablets and capsules, which are supplied in unit dose forms because they are subject to more variation than comparable preparation, supplied in multi-dose forms. For tablets and capsules with an average weight above 250 mg, the percentage deviation from the average weight permissible in the official compendium is + 5% (EP, 2008). The macrolide antibiotics used for the study which comprise three brands of erythromycin (A1 - A3), three brands of azithromycin (B1 - B3), and one brand of

clarithromycin (C) passed the test for uniformity of weight (Tablet 2).

Silica gel, the most widely used stationary phase for the TLC analysis of macrolide antibiotics, silica gel surface has silicon hydroxide (Si-OH) groups capable of initiating hydrogen bonding with polar substances. Polar mobile phases are employed for the identification and separation of macrolide. Acetic acid was added to all the mobile phases to avoid the decomposition. Iodine is more concentrated in the substance zone than in the neighboring polar substance-free silica gel layer, hence suitable in visualizing compounds invisible to the bare eyes. The presence of different macrolides was demonstrated by the appearance of unique colored spots on the chromatoplate on exposure to iodine vapor chamber. The different solvent system produces different forms of spot and some produce tailing on the chromatoplate but S4 (Methanol: Ammonium Hydroxide and Acetic acid; 50: 25: 25% v/v) was observed to produce a fine and significant spot with $R_{f\ value}$ ranging from 0.76 - 0.83 as shown in Tables 3 and 4. Other procedures were repeated for the entire drug sample using S4-solvent system to evaluate its precision and these replicate procedures gave fine spots as well without tailing. All the solvent systems were observed to be adequate as the development of the drug samples on the chromatoplate having high R_f values although some had tailing with fine spots (S2), some had tailing with no fine spot (S1) but S4 was the preferred solvent system due to the fine spots it produced without tailing for the entire drug samples and as such can be employed in the identification of macrolide antibiotics, which will produce similar spots as was observed in the experiment (Table 3). It was observed that the presence of methanol as one of the components in the solvent system (S4) is essential for the proper development of the drug samples (macrolide antibiotics) and also the presence of ammonia in S4 was observed to prevent tailing significantly for the different drug samples. The R_f value calculated using S1 (Methanol: ammonium hydroxide: chloroform) was observed as a result of the relative solubility of the drug sample as they traveled as far as the solvent front (Figure 7). From the good R_f values obtained it can be deduced that polar solvents encourage the development of macrolide antibiotics as was observed in the experiment.

From the UV/Visible spectrophotometry absorbance, all the drug samples were observed at different wavelengths ranging from 200 nm to 250 nm, with specific absorbance of A1, A2, B3 and C at 220 nm with absorbance of 2.2, 1.5, 1.24, and 1.15 respectively. While A3, B1 and B2 absorbed maximally at 210, 230, and 240 nm, with specific absorbance of 2.14, 1.23 and 1.34 respectively (Figures 2 – 5). These absorbances are closely related to previous studies were erythromycin was observed absorbed UV maximally at 205 nm (Wardrop et al., 2000), clarithromycin at 210 nm (Safila Naveed, 2014), and azithromycin at 208 nm (Sandip Bhimani, 2016), in different analytical studies. Also, a fluctuation in the absorbance reading was observed as the readings were high initially at 200 nm, decreased as the wavelength increased, and at a point (270 nm) a sharp increase in absorbance was observed for the different drug samples as shown in Figure 6. Hence all macrolides exhibit a peak absorbance between wavelength of 220 nm to 240 nm as well as a reduction in the absorbance as the wavelength tends to 300 nm.

CONCLUSION

The macrolide antibiotics used in the experiment can be detected in UV/visible spectrophotometer at 220 nm and 240 nm and derivatization using gentian violet helped to reduce detection limit. Therefore, spectrophotometric fingerprinting is essential in the analysis where maximum absorption is obtained at 220 nm. The TLC and UV/Visible identification techniques employed

here are cost-effective, sensitive, precise, simple, yielding good quality reproducible results, and can be applied in preparatory and exploratory analytical screening, quality control studies, therapeutic drug monitoring, purity test, stability control, and in assay procedures of macrolide, and related antibiotics.

REFERENCES

1. Amin, A. S., & Issa, Y. M. (1996). Selective spectrophotometric method for the determination of erythromycin and its esters in pharmaceutical formulations using gentiana violet. J Pharm Biomed Anal, 14(11), 1625-1629. https://doi.org/10.1016/0731-7085(96)01776-1
2. Bailly, S., Pocidalo, J. J., Fay, M., & Gougerot-Pocidalo, M. A. (1991). Differential modulation of cytokine production by macrolides: interleukin-6 production is increased by spiramycin and erythromycin. Antimicrob Agents Chemother, 35(10), 2016-2019. https://doi.org/10.1128/AAC.35.10.2016
3. Buckley GJ, G. L. (2013). The Effects of Falsified and Substandard Drugs. [Committee on Understanding the Global Public Health Implications of Substandard, Falsified, and Counterfeit Medical Products; Board on Global Health; Institute of Medicine]. Countering the Problem of Falsified and Substandard Drugs. https://www.ncbi.nlm.nih.gov/books/NBK202526/
4. Burton, M., Habtamu, E., Ho, D., & Gower, E. W. (2015). Interventions for trachoma trichiasis. Cochrane Database Syst Rev, 2015(11), CD004008. https://doi.org/10.1002/14651858.CD004008.pub3
5. Chen, B. M., Liang, Y. Z., Chen, X., Liu, S. G., Deng, F. L., & Zhou, P. (2006). Quantitative determination of azithromycin in human plasma by liquid chromatography-mass spectrometry and its application in a bioequivalence study. J Pharm Biomed Anal, 42(4), 480-487. https://doi.org/10.1016/j.jpba.2006.05.011
6. D.W. Green, R. H. P. (2008). Densities of Aqueous Inorganic Solutions
7. In Perry's Chemical Engineers' Handbook, (Vol. 2, pp. 99-118,). McGraw-Hill.
8. Dehouck, P., Van Looy, E., Haghedooren, E., Deckers, K., Vander Heyden, Y., Adams, E., Roets, E., & Hoogmartens, J. (2003). Analysis of erythromycin and benzoylperoxide in topical gels by liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci, 794(2), 293-302. https://doi.org/10.1016/s1570-0232(03)00477-x
9. Edmunds MW, M. M. (2009). Macrolides. In M. Saint Louis (Ed.), Pharmacology for the primary care provider (Third ed., pp. 658–662). (Archived from the original on 2022-05-23. Retrieved 2022-03-03.; Kirst HA, Sides GD (1993). "Chapter 28: Erythromycin". In Bryskier A, Butzler JP, Neu HC, Tulkens (eds.). The Macrolides. Oxford UK: Arnette-Blackwell.)
10. EP. (2008). Uniformity of mass of single-dose preparations. In (Vol. 6.0). EUROPEAN PHARMACOPOEIA. http://uspbpep.com/ep60/2.9.%207.%20friability%20of%20uncoated%20tablets%2020907e.pdf
11. FDA. (2014). Zithromax - FDA prescribing information, side effects and use.
12. Ferrero, J. L., Bopp, B. A., Marsh, K. C., Quigley, S. C., Johnson, M. J., Anderson, D. J., Lamm, J. E., Tolman, K. G., Sanders, S. W., Cavanaugh, J. H., & et al. (1990). Metabolism and disposition of clarithromycin in man. Drug Metab Dispos, 18(4), 441-446.

https://www.ncbi.nlm.nih.gov/pubmed/1976065

13. Flurer, C. L., & Wolnik, K. A. (1994). Chemical profiling of pharmaceuticals by capillary electrophoresis in the determination of drug origin. J Chromatogr A, 674(1-2), 153-163. https://doi.org/10.1016/0021-9673(94)85223-5
14. Hauk, L. (2014). AAP releases guideline on diagnosis and management of acute bacterial sinusitis in children one to 18 years of age. Am Fam Physician, 89(8), 676-681. https://www.ncbi.nlm.nih.gov/pubmed/24784128
15. House, A. J., & Thomson, K. R. (1977). Evaluation of a new transthoracic needle for biopsy of benign and malignant lung lesions. AJR Am J Roentgenol, 129(2), 215-220. https://doi.org/10.2214/ajr.129.2.215
16. Kaiser, G. (2009). Protein synthesis inhibitors: macrolides mechanism of action animation. In Classification of agents Pharmamotion.
17. Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol, 33(3), 300-305. https://doi.org/10.4103/joacp.JOACP_349_15
18. Larsson, D. G. J., & Flach, C. F. (2022). Antibiotic resistance in the environment. Nat Rev Microbiol, 20(5), 257-269. https://doi.org/10.1038/s41579-021-00649-x
19. Leal, C., Codony, R., Compano, R., Granados, M., & Prat, M. D. (2001). Determination of macrolide antibiotics by liquid chromatography. J Chromatogr A, 910(2), 285-290. https://doi.org/10.1016/s0021-9673(00)01231-0
20. Lebel, M. (1993). Pharmacokinetic properties of clarithromycin: A comparison with erythromycin and azithromycin. Can J Infect Dis, 4(3), 148-152. https://doi.org/10.1155/1993/168061
21. Lenz, K. D., Klosterman, K. E., Mukundan, H., & Kubicek-Sutherland, J. Z. (2021). Macrolides: From Toxins to Therapeutics. Toxins (Basel), 13(5). https://doi.org/10.3390/toxins13050347
22. Mandell, L. A., Wunderink, R. G., Anzueto, A., Bartlett, J. G., Campbell, G. D., Dean, N. C., Dowell, S. F., File, T. M., Jr., Musher, D. M., Niederman, M. S., Torres, A., Whitney, C. G., Infectious Diseases Society of, A., & American Thoracic, S. (2007). Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis, 44 Suppl 2(Suppl 2), S27-72. https://doi.org/10.1086/511159
23. Mori, F., Pecorari, L., Pantano, S., Rossi, M. E., Pucci, N., De Martino, M., & Novembre, E. (2014). Azithromycin anaphylaxis in children. Int J Immunopathol Pharmacol, 27(1), 121-126. https://doi.org/10.1177/039463201402700116
24. Neff, M. J., American Academy of, P., & American Academy of Family, P. (2004). AAP, AAFP release guideline on diagnosis and management of acute otitis media. Am Fam Physician, 69(11), 2713-2715. https://www.ncbi.nlm.nih.gov/pubmed/15202704
25. Omura, S. (2002). Macrolide Antibiotics. In Chemistry, Biology, and Practice. Academic Press.
26. Polianciuc, S. I., Gurzau, A. E., Kiss, B., Stefan, M. G., & Loghin, F. (2020). Antibiotics in the environment: causes and consequences. Med Pharm Rep, 93(3), 231-240. https://doi.org/10.15386/mpr-1742
27. Randel, A., & Infectious Disease Society of, A. (2013). IDSA Updates Guideline for Managing Group A Streptococcal Pharyngitis. Am Fam Physician, 88(5), 338-340.

https://www.ncbi.nlm.nih.gov/pubmed/24010402

28. Safila Naveed, F. Q. (2014). Simple UV spectrophotometric assay of Clarithromycin. International Journal of Pharma Sciences and Research, 5(9), 582 - 585.
29. Sandip Bhimani, G. S., Trupesh Pethani, Gaurav Dave, Vishal Airao, Tejas Sharma, Navin Sheth, Devendra Vaishnav. (2016). Development of the UV Spectrophotometric Method of Azithromycin in API and Stress Degradation Studies. International Letters of Chemistry Physics and Astronomy, 68, 48-53. https://doi.org/10.18052/www.scipress.com/ILCPA.68.48
30. Slama, T. G., Amin, A., Brunton, S. A., File, T. M., Jr., Milkovich, G., Rodvold, K. A., Sahm, D. F., Varon, J., Weiland, D., Jr., Council for, A., & Rational Antibiotic, T. (2005). A clinician's guide to the appropriate and accurate use of antibiotics: the Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria. Am J Med, 118 Suppl 7A, 1S-6S. https://doi.org/10.1016/j.amjmed.2005.05.007
31. Sonka, J., Schossig, U., Vogt-Schaden, M., & Volm, M. (1989). Initial and early effects of adriamycin in murine sarcoma 180 cannot be restored in a resistant subline by increasing the uptake and external concentration of the drug. Cancer Chemother Pharmacol, 24(1), 33-40. https://doi.org/10.1007/BF00254102
32. Taylor, S. P., Sellers, E., & Taylor, B. T. (2015). Azithromycin for the Prevention of COPD Exacerbations: The Good, Bad, and Ugly. Am J Med, 128(12), 1362 e1361-1366. https://doi.org/10.1016/j.amjmed.2015.07.032
33. Tenson, T., Lovmar, M., & Ehrenberg, M. (2003). The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. J Mol Biol, 330(5), 1005-1014. https://doi.org/10.1016/s0022-2836(03)00662-4
34. Wardrop, J., Ficker, D., Franklin, S., & Gorski, R. J. (2000). Determination of erythromycin and related substances in enteric-coated tablet formulations by reversed-phase liquid chromatography. J Pharm Sci, 89(9), 1097-1105. https://doi.org/10.1002/1520-6017(200009)89:9<1097::aid-jps1>3.0.co;2-d
35. WHO. (2019). World Health Organization model list of essential medicines: 21st list 2019. hdl:10665/325771. WHO/MVP/EMP/IAU/2019.06
36. WHO. (2023). Antimicrobial resistance. Health topics. Retrieved 31st August 2023, from https://www.who.int/health-topics/antimicrobial-resistance
37. Wilms, E., Trumpie, H., Veenendaal, W., & Touw, D. (2005). Quantitative determination of azithromycin in plasma, blood and isolated neutrophils by liquid chromatography using pre-column derivatization with 9-fluorenylmethyloxycarbonyl-chloride and fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci, 814(1), 37-42. https://doi.org/10.1016/j.jchromb.2004.09.058
38. Xu, F., Zhang, Z., Bian, Z., Tian, Y., Jiao, H., & Liu, Y. (2008). Azithromycin quantitation in human plasma by high-performance liquid chromatography coupled to electrospray mass spectrometry: application to bioequivalence study. J Chromatogr Sci, 46(6), 479-484. https://doi.org/10.1093/chromsci/46.6.479

HOW TO CITE: Samuel J. Bunu, Veronica Aniako, Varsharani P. Karade, Edebi N. Vaikosen, Benjamin U. Ebeshi, Thin-Layer Chromatographic And UV-Spectrophotometric Analysis Of Frequently Utilized Oral Macrolide Antibiotics, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 9, 265-274. https://doi.org/10.5281/zenodo.8340601