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Comparative Invitro Studies of Erythromycin Stearate Tablets Commercially Available in Lagos State, Nigeria

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ABSTRACT

Erythromycin is a macrolide antibiotic with a broad spectrum of activity against gram-positive and some gram-negative bacteria. It is unstable at acid pH, this makes its form of presentation have great effect on its activity. In this study, we evaluated the physicochemical properties of the drug using methods described in the BP, content of the active ingredient was evaluated by means of UV/VIS spectrophotometer at 480nm wavelength and dissolution study was done using the USP method. Results showed that 70% of the samples passed the test for content of active ingredient, 100% passed the friability test, 30% failed the weight uniformity test while 90% were harder than the innovator brand. There was a statistically significant difference between disintegration time in acid and buffer solution. Dissolution tests showed that 30% failed to meet the USP standard for erythromycin. The study revealed that 30% of the brands studied were of poor quality.

Keywords: Erythromycin, dissolution, equivalence, quality.

INTRODUCTION

Erythromycin is a leading member of the macrolide group of antibiotics. It is a drug of choice in corynebacterial infections and in respiratory, neonatal, ocular, or genital chlamydial infections. Erythromycin is useful as a penicillin substitute in penicillin-sensitive individuals. Erythromycin has been recommended as prophylaxis against endocarditis during dental procedures in individuals with valvular heart disease.[1] It is prescribed as a first choice for treating *Mycoplasma pneumoniae* infections as well as pneumonia caused by *Legionella pneumophila*.[2]

Several challenges associated with Erythromycin include acid instability, bitter taste and gastrointestinal disturbances. These have made the oral formulation of Erythromycin very challenging. To overcome these challenges, Erythromycin tablet may be protected by enteric coating. This coating protects the drug from the acidic environment of the stomach, but allows the release of the active ingredient in the more favorable alkaline environment of the small intestine.[3]

The drug may also be protected by preparing the salt (Stearate), ester (Ethylsuccinate) or Propionate), or salt of the ester (Estolate); the stearate and succinate being preferred due to decreased side effects.[3]Erythromycin Stearate is the most stable among the various salt and ester forms, however *in vitro* studies have demonstrated that Erythromycin Stearate may dissolve in gastric acid and get rapidly destroyed, providing only 2% antibiotic activity.[4, 5]

On expiration of the patent of an innovator drug, there is usually an upsurge of generic drug products. Due to the lower scrutiny imposed over production of these generic versions, the quality of some generic drug products may become inadequate. Poor quality of drugs has also been linked to counterfeiting of medicines, chemical instability especially in tropical climates, and inadequate quality control processes during production.[6]

These challenges of Erythromycin formulation and those associated with generic products have created a need for careful monitoring of the quality of the brands circulating in the market.

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MATERIALS & METHODS

Equipments

Friability Tester (Erweka-Apparatebau-GMBH®, Heusenstamm, Federal Republic of Germany); Hardness Tester (Erweka-Apparatebau®, Germany); Disintegration Time Tester (Copley®, Erweka-Apparatebau, Germany); pH Meter (Mettler Toledo®, USA); Pre-coated Silica Gel Chromatographic Plates; 20cm ×20cm; Iodine Tank (Simax®); Chromatographic Tank; Ultraviolet/visible Spectrophotometer (PG Instruments Ltd.); Nylon Syringe Filters (0.45µm × 25mm) (Agelas Technologies®, USA);Water Bath ;Plain Sample Bottles (5ml); Dissolution apparatus (USP standard).

Materials

Ten brands of Erythromycin Stearate were purchased from retail outlets in Lagos State and were coded with the alphabets A to K. All the brands had the NAFDAC registration numbers, were within their shelf life and were film coated except brand G which was an uncoated brand. Erythromycin Stearate standard was donated by Fidson pharmaceuticals.

Chemicals and Reagents

Potassium Di-hydrogen Orthophosphate (Surechem® products Ltd, Suffolk, England); Sodium Hydroxide (JT Baker® , USA); Acetonitrile HPLC Grade (BDH® Chemicals Ltd., Poole, England); Sulphuric Acid (Sigma Aldrich®, Germany); Chloroform (May and Baker® Ltd., Dagenham, England); Methanol HPLC Grade (Fischer Scientific® , UK).

Methods

Thin Layer Chromatography

Powdered tablet equivalent to Erythromycin Stearate 30mg of each brand was accurately weighed and transferred into already washed and dried beakers. 10ml of methanol was added and shaken for complete dissolution to obtain a 3mg/ml concentration. To a previously washed and dried chromatographic tank, a mobile phase containing 100ml of a chloroform: methanol mixture (ratio 30:70) was put and left to stand for 30 minutes to allow for saturation of the chromatographic tank. Each brand of Erythromycin Stearate was spotted on the TLC plate leaving about 3cm space on both sides of the spot (vertically) and about 5cm between the spot and the solvent (horizontally). After spotting, the plate was allowed to dry and then it was carefully place in the chromatographic tank.[7]

At 7.5cm solvent front, the chromatographic plate was removed from the tank and allowed to dry. The dried plate was then placed in an iodine tank. The distance moved by the spots was measured using a meter rule and recorded. The following formula was used to calculate the R_f values of each spot;

$$Rf = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent}} \quad (1)$$

Where R_f = Retardation factor

Physicochemical Properties

Uniformity of weight, Hardness, Friability and Disintegration Time tests were carried out using methods described in the British Pharmacopoeia.[8]

Assay

Powdered tablet equivalent to 100mg of Erythromycin Stearate was weighed and transferred into a 100ml volumetric flask. A 1:1 mixture of acetonitrile – water was added to the powder, shaken vigorously for proper dissolution and made up to the 100ml mark to obtain a 1mg/ml stock solution. A syringe filter was used to filter the mixture into a beaker and 200µg/ml concentration for each sample was prepared. To each 1ml volume of the 200µg/ml concentrations, 1ml of concentrated H_2SO_4 was added and heated at 50ºC using a water bath for 30minutes. The mixture was made up to the 10ml mark with acetonitrile-water to obtain a 20µg/ml working concentration and immediately analyzed using a UV/Vis spectrophotometer at 480nm wavelength. The regression equation from the calibration curve was used to calculate the concentration from the absorbance for each sample.[9]

Dissolution

Dissolution studies were carried out using the method in the USP.[10] The conditions were as presented in Table 1 below

Dissolution apparatus	Dissolution tester (USP standard)
Dissolution method	Paddle method
Dissolution medium	900ml of pH 6.8 buffer
Temperature of dissolution medium	37^0C ± 0.5
Rotation rate of paddle	100rpm
Sample volume	5ml with 5ml replacement volume
Sample withdrawal position	Midway between the surface of the dissolution medium and the bottom of the dissolution vessel and halfway between the wall of the vessel and the modified rotating paddle
Sampling times	5minutes, 10minutes, 15minutes, 30minutes, 45minutes, 60minutes, 90minutes and 120minutes.

RESULTS & DISCUSSION

Results

Thin Layer Chromatography was used to confirm the identity of Erythromycin Stearate in the various brands. The spots of the various brands (except brand B) are similar in position, colour and size to that of the innovator brand as shown in Figure 1. The R_f values are included in Table 2

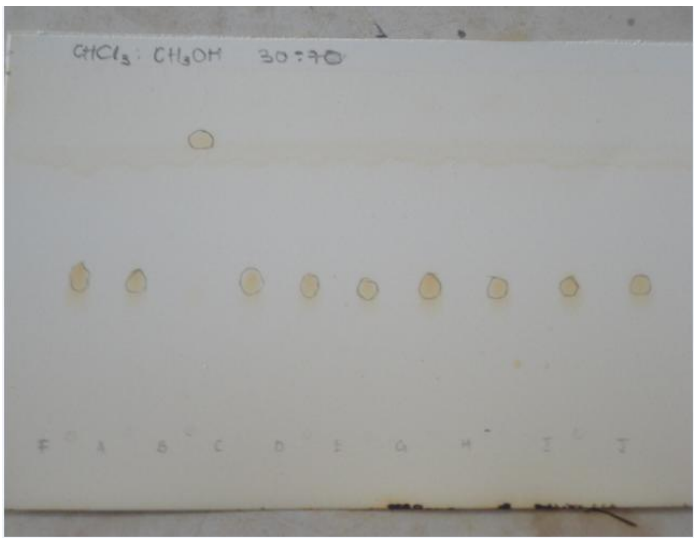

Figure1. *Thin Layer Chromatographic Plate showing spots of all the brands of Erythromycin Stearate Tablets Studied.*

Friability results are presented as percent loss in weight of the tablets while uniformity of weight, hardness and disintegration times in three different media (0.1N HCl, Water pH 7.0 and Simulated Intestinal Fluid) are presented as mean with standard deviation. Summary of the results are presented in Table 2.

Table2. *Summary of Results of the Physico-chemical Properties of the Brands Studied*

Brand Name	R_f Values	Weight Variation	Friability	Hardness (N)	Disintegration Time In 0.1N HCl (Seconds)	Disintegration Time In Water (pH 7.0) (Seconds)	Disintegration Time In Buffer (pH 6.8) (Seconds)
F(innovator)	0.4	910.7 ± 7.7	0.00	7.8 ± 2.0	0.4 ± 0.06	0.6 ± 0.1	0.6 ± 0.1
A	0.4	990 ± 3.4	0.02	9.5 ± 1.2*	>30*	26.3 ± 0.6*	24.5 ± 0.6*
B	0.8	891.2 ± 38.2	0.15	10.9 ± 0.3*	23.7 ±5.9*	15.2 ± 8.9*	2.5 ± 1.4*
C	0.4	869 ± 8.9	0.02	10.9 ± 0.5*	7.7 ± 0.9*	5.8 ± 1.2*	7.1 ± 1.7*
D	0.4	968.3 ± 21.8	0.10	9.7 ± 1.0*	2.3 ±1.4*	1.3 ± 0.5*	2.1 ± 0.9*
E	0.4	812.9 ± 9.8	0.12	9.4 ± 1.3	0.9 ± 0.1*	1.0 ± 0.03*	0.7 ± 0.09
G	0.4	872.2 ± 21.2	0.45	6.4 ± 0.8	0.5 ± 0.2	6.9 ± 0.9*	1.03 ± 0.5
H	0.4	1070.8 ± 13.6	0.02	11.1 ± 0.3*	3.6 ± 0.5*	2.9 ± 0.5*	2.8 ± 0.4*
I	0.4	920.3 ± 26.2	0.08	10.9 ± 0.9*	3.9 ± 3.8	0.8 ± 0.2	1.03 ± 0.3*
J	0.4	911.3 ± 39.1	0.15	10.4 ± 1.3*	>30*	6.2 ± 0.8*	3.3 ± 1.3*
K	0.4	1012 ± 15.9	0.00	11.4 ± 0.2*	13.7 ± 1.7*	7.9 ± 3.2*	9.5 ± 1.5*

*Where: * = Statistically Significant Difference at P<0.05 When Compared with the Innovator Drug (F).*

Five concentrations of standard Erythromycin Stearate were used to plot a calibration curve for the sample analysis as presented in Figure 2 and Table 3 respectively. The linear calibration curve with determination coefficient of 0.9914 is indicative of reproducibility and reliability of the equipment. The regression equation from the calibration curve was used to calculate the concentration from the absorbance of each sample.

Table3. *Standard Solutions of Erythromycin Stearate and their Absorbance Values.*

Concentration (µg/ml)	Absorbance
10	0.071
20	0.15
25	0.213
30	0.26
50	0.411

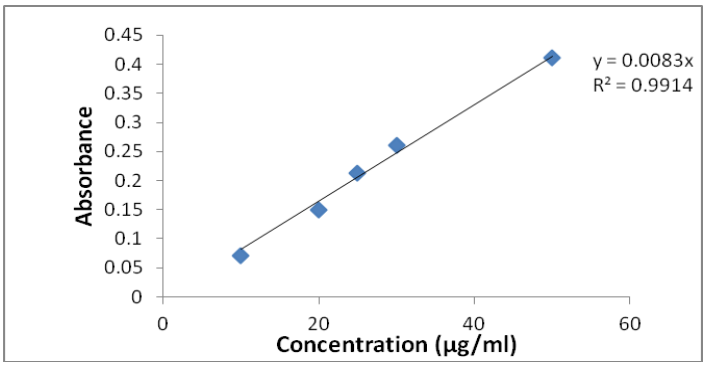

Figure 2.*Calibration Curve for Erythromycin Stearate*

The samples were analyzed by UV/Vis spectrophotometry. Table 4 shows the results of the content of active ingredient for the brands studied.

Table4.*Assay Results of the Various Brands Showing Average Absorbance, Concentration and Percentage Content of Active Ingredient.*

Brand Code	Average Absorbance	Concentration (µg/ml)	Percentage content of active ingredient	Comment
A	0.181	21.807	109.036	Pass
B	0.155	18.635	93.173	Fail
C	0.152	18.273	91.365	Fail
D	0.168	20.201	101.004	Pass
E	0.174	20.924	104.619	Pass
F	0.165	20.000	100	Pass
G	0.171	20.562	102.811	Pass
H	0.174	20.964	104.819	Pass
I	0.179	21.567	107.831	Pass
J	0.16	19.277	96.386	Pass
K	0.179	21.647	108.233	Pass

Drug released at time intervals 5, 10, 15, 30, 45, 60, 90 and 120 minutes have been presented as a percentage of the cumulative in table 5 and their dissolution profile comparatively presented in figure 3.

Table5. *Percentage Cumulative Drug Release at Time Intervals*

Brand	5mins	10mins	15mins	30mins	45mins	60mins	90 mins	120 mins
A	4.5%	22.1%	43.1%	81.2%	94.6%	108.2%	108.9%	111.9%
B	1.2%	4.33%	6.3%	6.4%	7.6%	7.7%	9.7%	10.3%
C	20.2%	32.1%	46.3%	48.8%	67.2%	68.1%	77.2%	84.8%
D	21.7%	26.2%	32.2%	33.6%	40.9%	42.9%	49.1%	51.8%
E	43.5%	54.2%	81.8%	84.2%	97.2%	98.5%	108.1%	108.8%
F	68.2%	82.8%	86.6%	89.4%	96.7%	100.6%	101.8%	108.7%
G	17.8%	27.6%	36.0%	58.0%	58.7%	67.3%	81.6%	87.1%
H	19.5%	32.9%	46.2%	62.4%	75.9%	76.7%	83.1%	84.2%
I	75.4%	83.2%	94.1%	102.7%	107.0%	107.4%	112.0%	111.2%
J	14.2%	34.5%	41.4%	51.9%	60.3%	64.5%	67.6%	74.3%
K	5.2%	26.4%	41.7%	68.9%	81.5%	87.4%	101.0%	105.5%

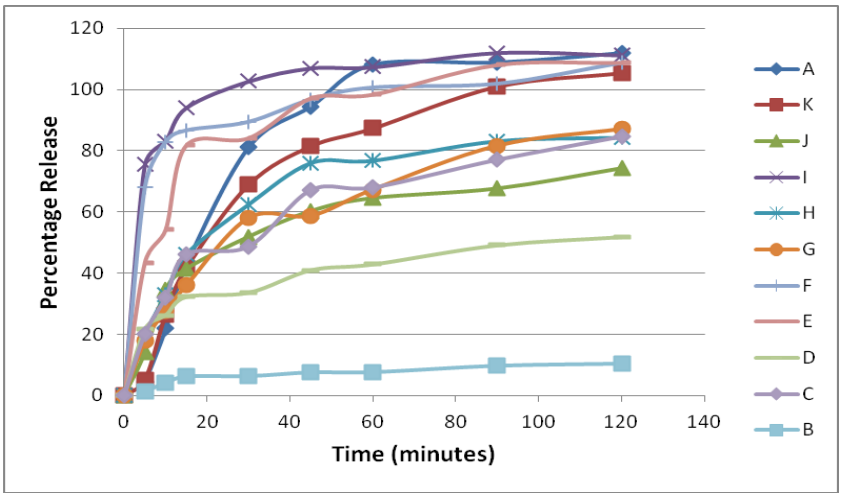

Figure3. *In-vitro Dissolution Profile of Erythromycin Brands Studied.*

DISCUSSION

Identification tests are vital in drug quality assessment and evaluation as the identity of an active ingredient must be ascertained before quantitative tests can be performed. Thin Layer Chromatography of the Erythromycin Stearate tablets showed single spots on the TLC plates with an average R_f value of 0.4 except for brand B which gave an R_f value of 0.8. This implies that the compound present may be an Erythromycin derivative or a different compound altogether. Brand B therefore did not conform with the standard of the British Pharmacopoeia.[8]

The Weight Uniformity test is a test for the degree of conformity of every dosage unit in a production batch to design specifications. By the required standards of the British Pharmacopoeia [8], 30% of the brands failed the uniformity of weight test (Table 2). This result suggests wide variations in the content of the active ingredient present in these brands which may have resulted from factors such as; punch size variation, granulation errors, among other production factors. This could cause variable drug effects (such as toxicity in the high dose tablets or low clinical response in the low dose tablets) as individual dosage units may contain a different amount (higher or less) than is required for biological activity.

Tablets produced should be able to withstand the stress of storage, transportation and handling from the place of manufacture to their point of administration or use if Good Manufacturing Practices are strictly adhered to. The Friability test tries to simulate the stresses experienced by solid oral dosage forms during transportation and storage. Results from this study showed that all brands met the recommended standard of the British Pharmacopoeia [8].

The hardness of a tablet can be said to be inversely proportional to the disintegration as well as the dissolution rate of a drug. Also, the longer the disintegration time of a tablet, the greater the chances of poor bio-availability. The data obtained showed that brands H and K required the largest force to crush them and this may negatively impact on their disintegration time and dissolution profile.

According to the British Pharmacopoeia [8], all film coated and uncoated tablets should disintegrate within 30 minutes. All brands of Erythromycin studied were expected to disintegrate in acidic media and as expected all brands except brands A and J disintegrated in 0.1N HCl. When compared with disintegration time in acid, Brands A and B showed the longest disintegration time in water. Erythromycin Stearate is a salt of Erythromycin designed with the intention to overcome the acid sensitive nature of Erythromycin among other uses. Controversy still exists as to the acid stability of Erythromycin Stearate as several reports have detected the acid degradation of Erythromycin Stearate in-vitro even though it is believed to be the most stable among the various salts and ester forms of Erythromycin.[4,5] These reports have demonstrated that Erythromycin Stearate when dissolved in gastric acid is rapidly destroyed and retains only minimal antibiotic activity after degradation. Hence it is of great importance that, to ensure good oral bioavailability of this form of Erythromycin, Erythromycin oral dosage forms should be protected from the acidic contents of the stomach. An

enteric coating could be used to allow for disintegration in the more alkaline small intestine. On the other hand, a longer disintegration time in acidic medium would indicate that such tablets may be able to survive long enough to move to the more alkaline part of the intestinal tract before complete disintegration. One can deduce that the brands that did not disintegrate completely in acid will have more of the active ingredient available for absorption in the small intestine.

A drug formulation that does not comply with standard in terms of content of active ingredient cannot be bio-equivalent to the innovator brand. The assay results showed that all brands except brands B, C and J passed the assay test according to the United State Pharmacopoeia [10] standard.

Dissolution, being an important process for transfer of solid substance into solution, is affected by pH changes that affect ionization of the drug. The USP recommends dissolution study of Erythromycin Stearate tablets at pH 6.8. With a short half-life of 1- 1.5 hours, Erythromycin needs prompt dissolution (within one hour) for absorption and therapeutic action. The In-vitro dissolution profile of the Erythromycin innovator brand was studied and compared with other brands available in the local market. The mean percentage dissolution of the innovator brand of Erythromycin Stearate tablets showed a steady release profile between 5minutes and 60minutes when about 100% of its active ingredient had been released. The similarity factor (f_2) calculated at time points 5minutes, 15minutes and 30minutes showed that only one brand, I (f_2 = 50.4) had a similar dissolution profile to the innovator brand. Some factors which may have been responsible for this poor dissolution profile of most of the brands studied include physicochemical properties of the tablets such as tablet hardness. Increased compression force and amounts of binders in tablets increases hardness of tablets and prolongs disintegration time and hence slows dissolution. Also, increased amounts of lubricants decrease hydrophilicity and wettability of tablets, thus prolonging disintegration time and slowing dissolution, consequently resulting in slower absorption and poor bioavailability of the drug. Also, much of the undissolved drug may be excreted from the body in feaces, further reducing the chances of late dissolution or absorption.

Results obtained from this study show that some poor quality Erythromycin Stearate tablet brands circulate in Lagos market. In-vitro studies revealed that only one brand (I), was similar to the innovator. A careful perusal of the data obtained in this study showed that this brand had an overall best quality profile than all other brands studied. Hence its good dissolution profile could have been anticipated from results of physicochemical properties such as hardness, friability and disintegration tests.

CONCLUSION

This study has investigated the identity, physico-chemical properties and in-vitro bioequivalence of various brands of Erythromycin Stearate obtained in Lagos, Nigeria. It can be concluded that about 70% of the brands studied passed the test for content of active ingredient. However, 99% of the brands were not statistically interchangeable with the innovator brand (F) based on their dissolution profile.

An in-vivo bioequivalence study would be necessary to determine the full pharmacokinetic profile of these brands and also find the degree of correlation of in-vitro with in-vivo results.

REFERENCES

[1] Betram G. Katzung, (ed.), Basic & Clinical Pharmacology, 10th Ed. McGraw-Hill Companies, USA, 2007.

[2] Margaret Eckman and Diane Labus (eds). "Clinical Pharmacology Made Incredibly Easy", 3rd edition, Lippincott Williams and Wilkins, Philadelphia, 2003.

[3] Terespolsky SA, A Study of the Biopharmaceutics and Pharmacokinetics of the Macrolide Antibiotic, Erythromycin, MSc Thesis Rhodes University USA, 1992.

[4] DiSanto AR and Chodos DJ, Influence of study design in assessing food effects on absorption of erythromycin base and erythromycin stearate, J Antimicrob Agents and Chem, vol. 20, pp. 190-196, 1981.

[5] Periti P, Mazzei T, Mini E, and Novelli A, Clinical pharmacokinetic properties of the macrolide antibiotics - Effects of age and various pathophysiological states, *Clin. Pharmacokinetics*, vol. 16, pp. 193-214, 1989.

[6] Taylor RB, Shakoor O and Behrens RH. Pharmacopoeial quality of drugs supplied by Nigerian pharmacies, *The Lancet* 2001, pp. 357:1933.

[7] Benjamin UE, Adebola O and Paulette IO, Spectrophotometric and bioassay methods for the estimation of erythromycin formulation, *Inter J Bioassays*,vol. 2, pp. 762-768, 2013.

[8] British Pharmacopoeia, Monographs on Medicinal and Pharmaceutical Substances. Her Majesty Stationery office England Pharmacopoeia commission, 2012.

[9] Rohini W., Suhasini B., Hiten P., Aruna P., Ram P. Analysis of erythromycin and benzoyl peroxide in combined dosage form by uv-visible Spectrophotometry. *Intl J. Pharm. and Pharm. Sci*. pp. 527-531, 2012.

[10] The United States Pharmacopoeia Mack Printing Co., Easton, PA. 2007; pg. 519, 843, 1579.

[11] Koch WL. Analytical Profiles of Drug Substances. *Academic Press Inc* New York, 1979; 3: 159.