METHOD DEVELOPMENT, VALIDATION AND COMPARATIVE STUDY OF GENERIC AND BRANDED FORMULATIONS OF LINEZOLID IN TABLET DOSAGE FORM

[1]Santosh V. Gandhi*, [2]Mohitosh R. Mahajan
Corresponding author:
[1]Dr. Santosh V. Gandhi
[1]Professor
[1]AISSMS College of Pharmacy,
Kennedy Road, Near R.T.O,
Pune -411001, Maharashtra, India.
All India Shri Shivaji Memorial Society's College of Pharmacy, [1]Department of Pharmaceutical Quality Assurance, Kennedy Road, Near R.T.O., Pune, Maharashtra, India-411001

ABSTRACT:

UV Spectrometric and HPLC methods were developed and validated for estimation of Linezolid in tablet dosage form. Developed methods were applied for comparative study of generic vs branded formulations of Linezolid. The main objective is to compare and evaluate the price and quality of branded and generic formulations of Linezolid 600 mg tablet. Linezolid in methanol shows maximum absorbance at 258 nm. The data of linear regression analysis indicated a good linear relationship over the concentration range of 5-30 µg/ml with a correlation coefficient (R^2) 0.998 by both UV- Spectroscopy and HPLC. The evaluation parameters like weight variation, hardness of the tablet, friability, thickness, disintegration test, drug content uniformity and in vitro release studies were performed as prescribed in Indian Pharmacopeia 2010.

Keywords: Branded, Generic, Quantitative and Qualitative Determination, Ultraviolet spectroscopy, HPLC, Comparative studies.

INTRODUCTION:

Introducing generic products from multiple sources into health care systems exist in many countries in an approach aiming to improving the overall healthcare system. The use of generic drugs is steadily increasing internationally as a result of economic pressure on drug budgets. Generic drugs provide the opportunity for major savings in healthcare expenditure since they are usually substantially lower in price than the innovator brands.[1] However, this has been accompanied by a variety of problems, the most critical of which is the widespread distribution of substandard products. Product selection of the same active ingredients from several generic products available in the market is very important step during the course of therapy and cause several concerns to a healthcare practitioner. Therapeutic equivalence must be ensured by ascertaining the biopharmaceutical equivalency of such drug products. Drug products that are therapeutically and chemically equivalent must have the same strength, quality, purity and content uniformity, and disintegration and dissolution rates. The need to ensure that the generic and branded drug products are pharmaceutically and therapeutically equivalent cannot be overemphasized.

Linezolid is the first member of a new class of antibiotics, the oxazolidinones. It is an important therapeutic option for the treatment of infections caused by multiresistant Gram-positive bacteria. Linezolid is active against vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus* (MRSA)and glycopeptide-intermediate *S. aureus*.[2] Fewer methods

have been reported for the quantitative determination of Linezolid, which includes UV [3-7], HPLC [2,8,11], HPTLC [9], bioanalytical method using LC [10].

Literature survey reveals that no comparative study has been reported for the estimation of Linezolid in pharmaceutical tablet dosage. Present work describes a comparative study on generic and branded formulations of linezolid tabletfor the determination of Linezolid in bulk and tablet dosage form.

MATERIALS AND METHODS

REAGENTS AND CHEMICALS

Linezolid Tablets I.P labeled to contain Linezolid600 mg was purchased from local market. Methanol (AR grade), Methanol (HPLC grade), Orthophosphoric Acid (AR grade), Potassium Dihydrogen Ortho-Phosphate (AR grade), Sodium Hydroxide (AR grade), were used in study.

PREPARATION OF STANDARD STOCK SOLUTION:

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From this solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get concentration of solution 100 µg/ml. Further 1 ml of this solution was diluted to 10 ml with methanol to get concentration of solution 10 µg/ml.

SELECTION OF DETECTION WAVELENGTH:

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra were obtained. It was observed that the drug showed linear, stable and considerable absorbance at 258 nm. Representative UV spectrum is shown in Fig. 1.

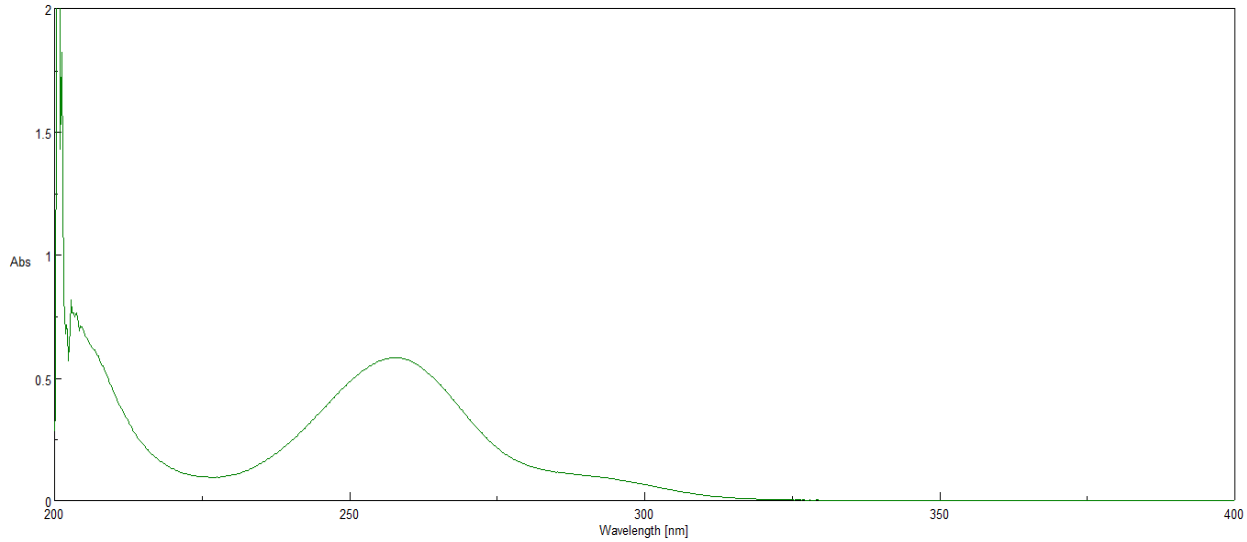

Fig. 1: UV Spectrum of Linezolid (10 µg/ml)

CHROMATOGRAPHIC CONDITIONS:

A rapid high-performance liquid chromatographic method was developed and validated for the determination of linezolid in pharmaceutical dosage forms **[11]**. The method was developed on Jasco HPLC system with HiQSil C8 (150 x 4.6 mm i.d. 3µm) using 10 mM Potassium dihydrogen phosphate buffer (pH 4 adjusted with orthophosphoric acid): Methanol (30:70 v/v) as mobile

phase at a flow rate of 1 mL/min and it was monitored at 258 nm, with a retention time of 3.915-minute. Representative HPLC chromatograph is given in Fig. 2.

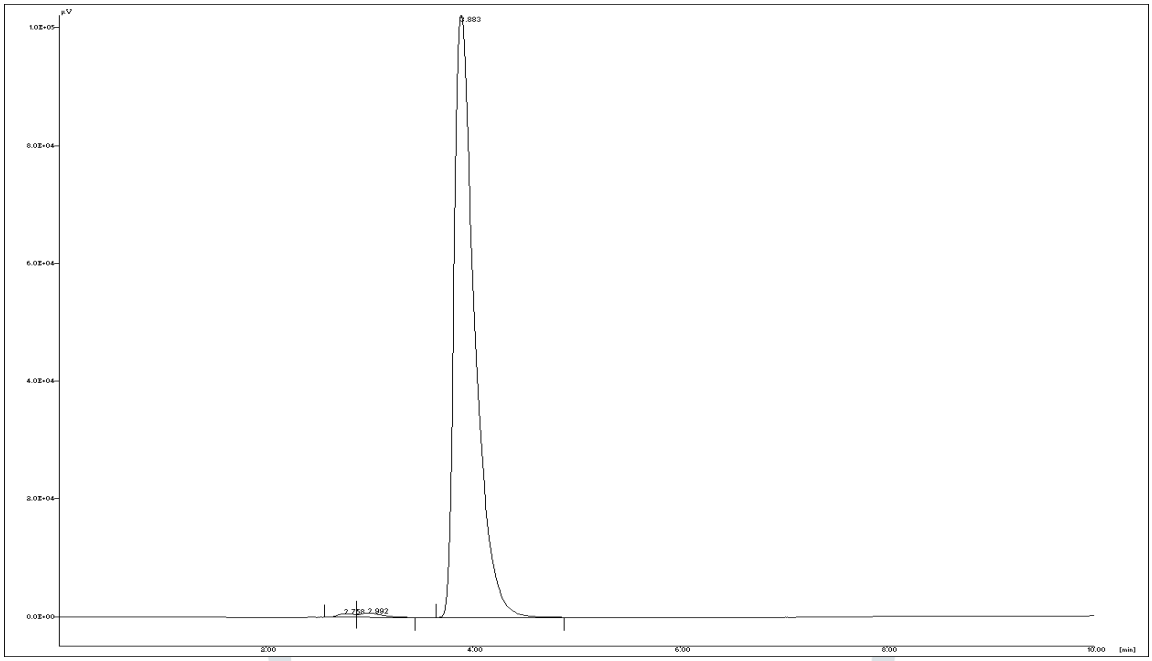

Fig. 2: HPLC Chromatogram of Linezolid (10 µg/ml)

PREPARATION OF SAMPLE SOLUTION:

Twenty tablets were weighed and finely powdered by using mortar and pestle. A quantity of tablet powder equivalent to 10 mg of Linezolid (label claim: 600 mg Linezolid per tablet) was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra-sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. 1.0 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 100µg/ml.

RESULTS AND DISCUSSION:

The present comparative study indicate that both the branded and generic tablets of the drug Linezolid had identical quality and they fulfilled all the criteria prescribed by the pharmacopeia standards. As the patent expired from the formulation; there is no marketing of the products and also due to lack of awareness about generic medications and its significance; people hesitate the use of generic medicines. There is also mindset in the society that lower price means lower the quality so to make aware people that there is no such significant difference between generic medicines and branded medicines, so the following data provides all the comparative values about the tablet formulation of Linezolid 600 mg.

VALIDATION OF ANALYTICAL METHOD:

LINEARITY:

From the standard stock solution (1000 µg/ml) of Linezolid, solution was prepared containing 100 µg/ml of Linezolid in methanol. This solution was further diluted with methanol to get range of solution containing different concentrations5-30 µg/ml. Absorbance (UV) and area (HPLC) was taken at λ_{max}258 nm. The linearity (relationship between absorbance/area and concentration) was determined over the concentration range of 5-30 µg/ml. The equation of calibration curve by UV-Spectroscopy and HPLC was found to be y = 0.0515x + 0.0421 and y = 129147x + 38730, respectively. The absorbance/area of

drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 3 and Fig. 4respectively.

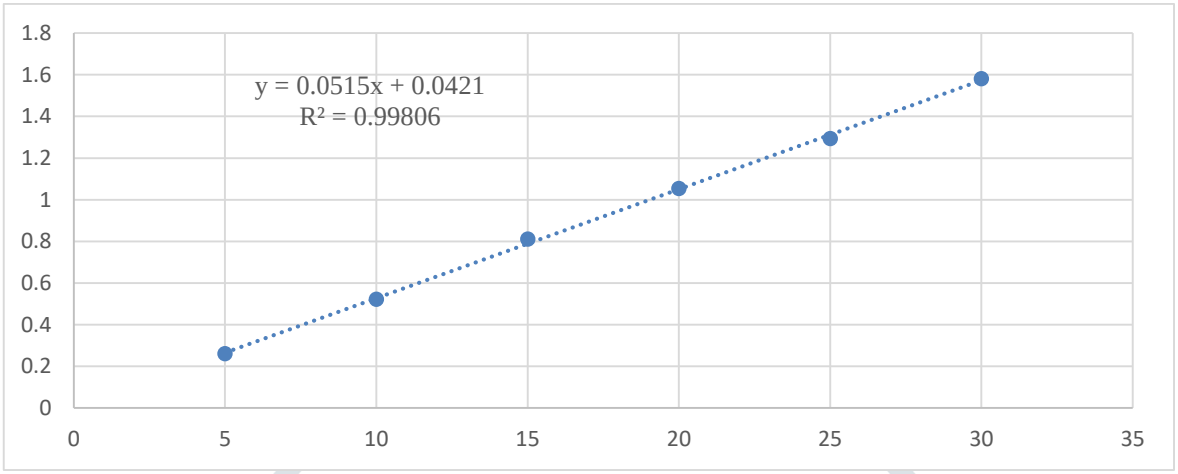

Fig. 3: Linearity curve of Linezolid by UV- Spectroscopy (5-30 µg/ml).

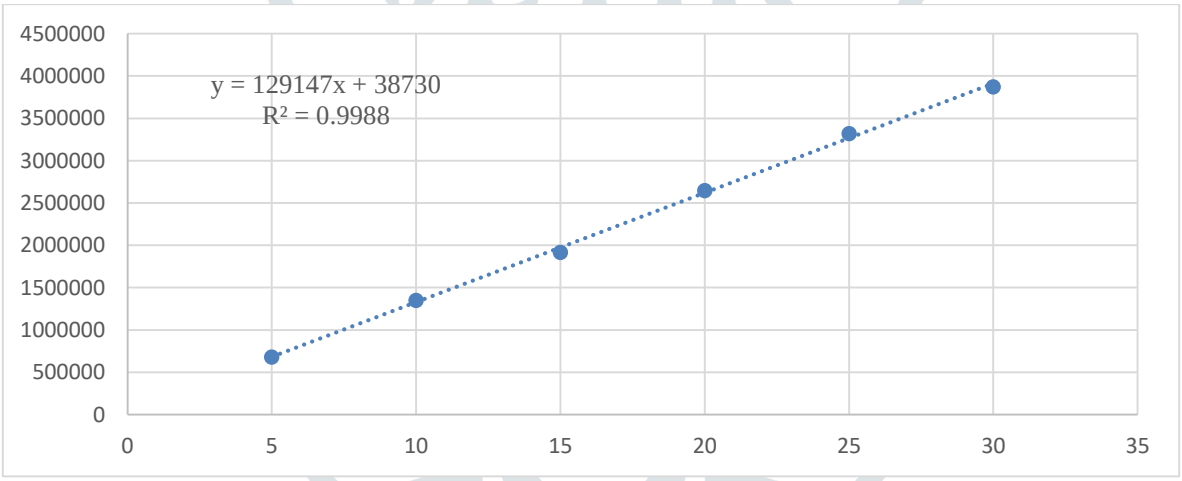

Fig. 4: Linearity curve of Linezolid by HPLC (5-30 µg/ml).

PRECISION:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations by UV-Spectroscopy and HPLC are shown in Table 1 and Table 2 respectively.

Table 1: Intra-day and Inter-day variation studies data for Linezolid by UV-Spectroscopy.

Replicates	Intra-day Precision			Inter-day Precision		
	Conc. (µg/ml)			Conc. (µg/ml)		
	10	15	25	10	15	25
	Abs.	Abs.	Abs.	Abs.	Abs.	Abs.

1	0.582	0.869	1.293	0.583	0.868	1.291
2	0.575	0.853	1.290	0.579	0.852	1.280
3	0.573	0.864	1.291	0.578	0.863	1.290
Mean	0.577	0.862	1.291	0.580	0.861	1.287
SD	0.903	1.071	0.126	0.452	1.071	0.488
%RSD	0.87	1.01	0.13	0.43	1.01	0.51

Table 2: Intra-day and Inter-day variation studies data for Linezolid by HPLC.

Replicates	**Intra-day Precision**			**Inter-day Precision**		
	Conc. (µg/ml)			Conc. (µg/ml)		
	10	15	25	10	15	25
	Area	Area	Area	Area	Area	Area
1	1352836	1916543.12	3317322.01	1379218.08	1915223.44	3280168.96
2	1353631.25	1951447.5	3302650.75	1350402.21	1840095.80	3224430.5
3	1359811	1947681	3368138.5	1353631.25	1951447.5	3302650.75
Mean	1355426.08	1938557.20	3329370.42	1361083.85	1902255.58	3269083.40
SD	0.295	0.988	1.064	1.222	2.931	1.247
%RSD	0.29	1.01	1.04	1.19	3.05	1.25

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ by UV-Spectroscopy were found to be 0.069µg/ml and 0.209µg/ml, respectively and The LOD and LOQ by HPLC were found to be 0.114µg/ml and 0.347µg/ml, respectively.

ACCURACY:

To check accuracy of the method, recovery studies were carried by spiking the standard 600 mg tablet sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 10 µg/ml of Linezolid. % recovery was determined from linearity equation. The results obtained are shown in Table 3 and Table 4.

Table 3: Accuracy of Linezolid by UV-Spectroscopy.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Zyvox			Linid		
			Absorbance	Amount recovered (µg/ml)	% recovery	Absorbance	Amount recovered (µg/ml)	% recovery
50%	10	5	0.8096	14.903	98.822	0.8127	14.963	99.71
			0.8017	14.750		0.7991	14.699	
			0.8052	14.817		0.8252	15.206	
100%	10	10	1.0633	19.829	100.097	1.0872	20.293	98.88
			1.0797	20.148		1.0862	20.274	
			1.0763	20.082		1.0084	18.763	
150%	10	15	1.3225	24.862	99.218	1.3311	25.029	100.8
			1.3248	24.907		1.3292	24.992	
			1.3113	24.645		1.3594	25.579	

Table 4: Accuracy of Linezolid by HPLC.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Zyvox			Linid		
			Area	Amount recovered (µg/ml)	% recovery	Area	Amount recovered (µg/ml)	% recovery
50%	10	5	1989878	15.108	99.547	1999630.259	15.183	101.817
			1992458.23	15.128		2040582.741	15.501	
			1919168	14.560		1993168	15.133	
100%	10	10	2622149.14	20.004	99.918	2698023.5	20.591	100.006
			2639836	20.141		2597851.75	19.816	
			2596708.19	19.807		2569618	19.597	
150%	10	15	3291440.5	25.186	100.817	3190476.75	24.404	99.381
			3319985.58	25.407		3256076	24.912	

			3269879.75	25.019		3295699.5	25.219	

ASSAY:

Linezolid Tablets I.P labeled to contain Linezolid600 mg formulation analysis was carried out as mentioned under section preparation of sample solution and absorbance was recorded. Basic concentration of sample chosen was 10µg/ml from tablet solution. Procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained by UV-Spectroscopy and HPLC are shown in Table 5 and Table 6 respectively.

Table 5: Assay of marketed formulation by UV-Spectroscopy.

Sr. No.	Zyvox			Linid		
	Absorbance	Amount Recovered (µg/ml)	% Recovery	Absorbance	Amount Recovered (µg/ml)	% Recovery
1	0.5671	10.194	101.942	0.5541	9.942	99.417
2	0.5564	9.986	99.864	0.5473	9.810	98.097
3	0.5523	9.907	99.068	0.5603	10.062	100.621
4	0.5673	10.198	101.981	0.5695	10.241	102.408
5	0.5514	9.889	98.893	0.5474	9.812	98.117
6	0.5469	9.802	98.019	0.5506	9.874	98.738
Mean	0.5569	9.996	99.961	0.5549	9.957	99.566
SD	0.0085	0.166	1.656	0.0087	0.168	1.683
%RSD	1.5318	1.657	1.657	1.5617	1.690	1.690

Table 6: Assay of marketed formulation by HPLC

Sr. No.	Zyvox			Linid		
	Area	Amount Recovered (µg/ml)	% Recovery	Area	Amount Recovered (µg/ml)	% Recovery
1	1329540.94	9.995	99.949	1279822.923	9.610	100.726
2	1332540.94	10.018	100.181	1296864.398	9.742	98.624
3	1329651.5	9.996	99.958	1360900.209	10.238	102.377
4	1314076.925	9.875	98.752	1269984.17	9.534	98.564
5	1302174.591	9.783	97.830	1290901.5	9.696	99.389
6	1381025.28	10.394	103.935	1342931.5	10.099	100.986

Mean	1331501.696	10.010	100.101	1306900.783	9.820	99.196
SD	26924.468	0.208	1.085	36515.486	0.283	1.827
%RSD	1.022	1.083	1.083	1.794	1.879	1.879

***IN-VITRO* DISSOLUTION STUDIES:**[12]

The in-vitro dissolution study was carried out by using IP Type-2 dissolution apparatus at 50 rpm. For the dissolution study randomly 6 tablets of both branded and generic formulations are selected. Media used was potassium dihydrogen phosphate buffer at pH 6 adjusted with NaOH solution. Sufficient volume was removed, filtered by Whatman filter paper and diluted up to 10 ml in 10 ml volumetric flask and scanned over the UV-Spectroscopy. The % drug release was plotted against the corresponding time to obtain the curve as shown in Fig. 5.

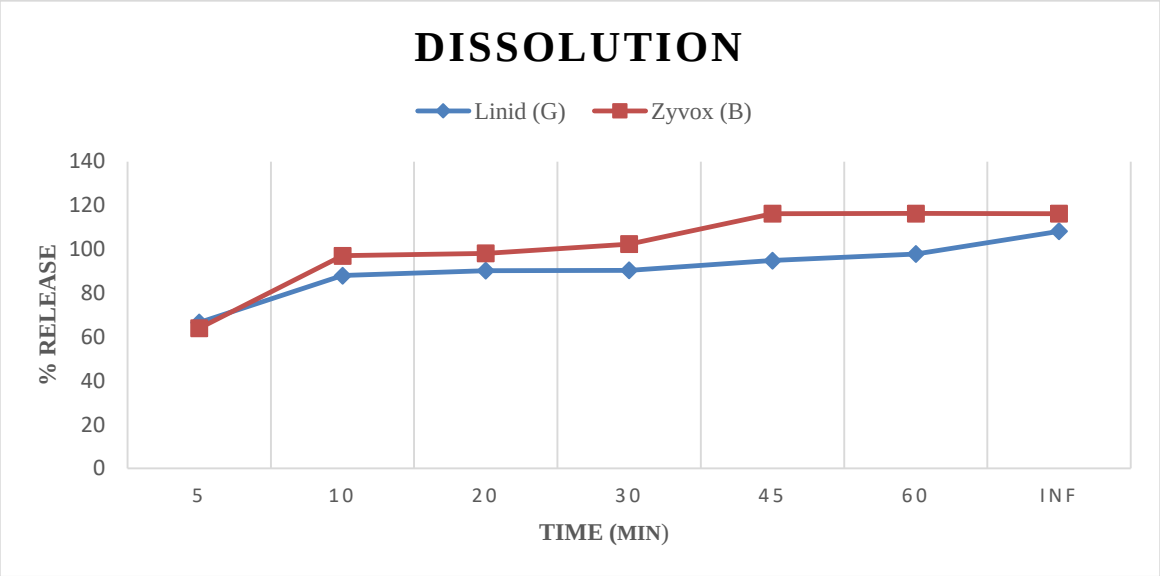

Fig. 5: Comparison of Dissolution Profile of Generic and Branded Tablet

DESCRIPTION (SIZE, SHAPE, COLOUR) [13-15]

The tablets were looking good and non-sticky. The colour, shape of tablets was analysed with naked eye. The diameter and thickness of tablets was performed on 20 tablets from each formulation. Digital Vernier calliper was used for the study, which permits accurate measurements and provides information of the variation between tablets.

WEIGHT VARIATION TEST [13-15]

Weight variation was carried out to ensure that, all individual tablet have nearly same weight. The test was carried out by weighing the 20 tablets individually using analytical balance, then calculating the average weight, and comparing the individual tablet weights to the average.

HARDNESS TEST [13-15]

The hardness test was performed using Monsanto type (Make: Singhla) hardness tester. The instrument measures the force required to break the tablet when the force generated by anvils to the tablet. The tablet was placed between two anvils; force applied to the anvils, and the crushing strength that just causes the tablet to break was recorded.

FRIABILITY TEST [13-15]

For each formulation, the friability of 20 tablets was determined using Roche type friabilator. 20 tablets from each formulation were weighed and tested at a speed of 25 rpm for 4 min. After removing of powder, tablets were re-weighed and friability percentage was calculated.

DISINTEGRATION TEST [13-15]

The disintegration apparatus, described in I.P was used for the study. It contains 2 basket rack assembly. Each basket rack assembly consists of 6 glass tubes that are 3 inches long, open at the top and held against 10 mesh size at the bottom. Each tablet was placed in each tube, and the basket rack was positioned in 1-L beaker of distilled water. The 37±2°C temperature was maintained throughout the study.

Table 7: Identification test: [13-15]

Sr No.	Drug name	Linezolid	
		Branded - Zyvox	**Generic - Linid**
1	**Manufacturer**	Pfizer Ltd.	Zydus Healthcare Ltd.
2	**Shape**	Oval	Oval
3	**Description**	Each tablet contains -600mg of Linezolid	Each tablet contains -600mg of Linezolid
4	**Color**	White	White
5	**Dosage**	As prescribed by the physician	As prescribed by the physician
6	**Storage**	Store in dry and cool place, protect from light and moisture.	Store below 30^0C in dry place, protect from light and moisture.
7	**Mfg. Lic. no.**	97/UA/01	M/446/07
8	**Mfg. date**	10/2017	06/2018
9	**Exp. date**	09/2019	05/2020
10	**Price (PER 10 TABLETS)**	Rs.:847.62	Rs.: 347.31

Table 8: Physiochemical evaluation: [13-15]

Sr No.	Drug name	Linezolid	
		Zyvox	**Linid**
1	Weight Variation (average weight)	1016.00 ± 4.3 mg	839.30 ± 2.15 mg
2	Thickness (mm)	6.018 ± 0.016	5.56 ± 0.013
3	Hardness test (kg/cm^2)	3.4 kg/cm^2	4.56 kg/cm^2
4	Friability test (NMT 1%)	0.1622± 0.009	0.2623± 0.005

5	Disintegration time (min)	3.40 min	30 sec
6	Dissolution test (Not less than 70 per cent of the stated amount)	% release Not less than 90.868 %	% release Not less than 95.568 %

CONCLUSION:

Both versions of tablet were within their permissible range for all the quantitative and qualitative parameters as described in Indian Pharmacopoeia. As there is no significant change between both branded and generic medicines in terms of contents henceforth the data obtain during study is more about same for both. So, the general opinion and doubt about the quality of generic medicines needs change by more such studies and help society to save money. Suitable advertisements about the use of generic medicines and general awareness programs on quality of generic medicines among patients, consumers and medical practitioner need to start. Pharmacists have to take first step to tell consumers about generics and also to made availability of generic medicines.

ACKNOWLEDGEMENT:

Authors are thankful to Dr (Mrs.) A. R. Madgulkar, Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

REFERENCES:

1. Singal G.L., Nanda A., and Kotwani A., A comparative evaluation of price and quality of some branded versus branded–generic medicines of the same manufacturer in India, Indian journal of pharmacology, 2011 Apr; 43(2): 131–136.
2. Swoboda S., Ober M., Anagnostakos K., Geiss H. K., Weigand M. A., and Hoppe-Tichy T., A simple isocratic HPLC assay to determine linezolid concentrations in different biomatrices for in vivo and in vitro studies, Clin Chem Lab Med 2007;45(8):1019–1022.
3. Sushma S. and Latha P., Method development and validation of spectrophotometric method for the estimation of linezolid in pure and tablet dosage form, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 2015; 3(2): 82 - 88.
4. Chavda R. N., Yadav H. N., Hinge M. A., Shing R. D., Patel E. S., Patel D. R., Development and Validation of Analytical Method for Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Tablet Dosage form, International Journal of Pharma Sciences and Research, Apr 2016; 7(4): 172-180.
5. Naik A. D., Pai S.P.N., Spectrophotometric Method for Estimation of Linezolid in Tablet Formulation, Asian Journal of Biomedical and Pharmaceutical Sciences, 2013; 3(21): 4-6.
6. Gadhiya D. T., Bagada H. L., Spectrophotometric Method Development and Validation for Estimation of Linezolid in Tablet Dosage Form, Research in Pharmacy, 2013; 3(3): 06-12.
7. Saikiran B. H., Johnny S. K. and Leela Madhuri P., UV Spectroscopic Method for Estimation Of Linezolid In Tablets, International Journal Of Pharmaceutical, Chemical And Biological Sciences, 2013; 3(3): 729-731.
8. Prasanti K. and Sundar B. S., a validated RP-HPLC method for the determination of linezolid in pharmaceutical dosage forms, International Journal of Pharma and Bio Sciences, 2012 July; 3(3): P 44 – 51.

9. Agrawal H., Mahadik K. R., Paradkar A. R., and Kaul N., Stability Indicating HPTLC Determination of Linezolid as Bulk Drug and in Pharmaceutical Dosage Form, Drug Development And Industrial Pharmacy, 2003; 29(10), 1119–1126.
10. Zhang J, Zhang Y, Wang R, Alvey C, Wang Q, et al. (2014) Pharmacokinetics and Bioequivalence Comparison of 600 mg Single-Dose Linezolid Oral Suspension and Tablet Formulation in Healthy Chinese Subjects, Journal of Bioequivalence & Bioavailability, 2014; 6(5): 153-157.
11. Mohapatra S., Annapurna M., Ravi Kumar B. V. V., Anwar M., Warsi M. H. and Akhter S., Validated stability indicating RP-HPLC method for the estimation of linezolid in a pharmaceutical dosage form, Journal of Liquid Chromatography & Related Technologies, 2011; 34(18): 2185-2195.
12. Reddy K.S., Sahoo L., Dr. Reddy G. K., Krishna L.V., Formulation and Evaluation of Immediate Release Tablets of Linezolid, International Journal of Pharmaceutical & Biological Archives 2011; 2(4):1230-1235.
13. Indian Pharmacopoeia 2010, Government of India Ministry of Health and Family welfare, Ghaziabad, New Delhi, Volume I, II, III: 160, 185, 361, 1590, 1591.
14. Leon Lachman, Herbert A. Lieberman; Granulation properties in "the theory and practice of industrial pharmacy''. Varghese publishing house. 1990; volume-3, 315-317.
15. Remington the Science and Practice of Pharmacy 20th Edition, Lippincott Williams & Wilkins International Student Edition, 2001; 652-658.