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ORIGINAL ARTICLE

Triple-Parameter Optimization of Linezolid 600mg Tablets: Dissolution, HPLC, and UV Spectrophotometric Evaluation

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ABSTRACT

Aims and Objective: The current research's target is to obtain the dissolution profile of various brands of Linezolid 600 mg tablets available in Karachi, Pakistan and the assay was also performed using both the UV Spectrophotometric Technique and High Performance Liquid Chromatographic Techniques.

Methodology: Dissolution profile was conducted using 0.05M phosphate buffer (pH 6.8). UV spectrophotometry and HPLC techniques were employed for assay. The mobile phases used were Methanol: Buffer (55:45) for HPLC and Acetonitrile: Water (50:50) for UV.

Result: The dissolution profiles showed that Brand C-1 had the fastest dissolution rate, achieving 100% dissolution within 20 minutes. The assay results showed that Brand C-1 had an HPLC assay value of 99.5% ± 0.84 and a UV assay value of 104.3% ± 0.14. Therefore, Brand C-1 can be considered as the best brand among the tested brands.

Conclusion: HPLC is recommended as the best analytical method for the estimation of Linezolid due to its high precision and accuracy. However, the UV spectrophotometric method is rather quick and affordable. Complex procedures or treatments (often associated with the chromatographic approach) do not require it. This study fills a research vacuum by comparing the dissolution profile and assay of various brands of 600 mg Linezolid tablets that are available in Karachi, Pakistan. By using both UV Spectrophotometric and High Performance Liquid Chromatographic (HPLC) methodologies, the study specifically aims to close the gap in assessing the bioequivalence and quality consistency of various brands.

Keywords
Linezolid, UV Spectrophotometric, High Performance Liquid Chromatographic Techniques, Oxazolidinone.

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INTRODUCTION

Linezolid is an Oxazolidinone antibiotic, which is newly registered and gives better oral absorption with higher activity against Gram-positive organisms[1]. Linezolid is reversible and non-selective inhibitor of monoamine oxidase. Linezolid can easily cause interference with the agents like adrenergic and serotonergic[2]. Although linezolid has the benefit of being nearly 100% bio available, it may be possible to move from intravenous to oral linezolid (if circumstances allow). In contrast, intravenous administration of Vancomycin, Quinupristin, and Dalfopristin should be taken through intravenous route[3]. Absorption of Linezolid is remarkable after oral dosing.

After giving dose, the maximal concentration of plasma was 1 to 2 hours as well as 100% absolute bioavailability and there is no need to adjust dose because Linezolid can be taken through oral and IV route[2]. Pharmacokinetic research suggested that Linezolid rapidly circulates through well perfused tissues[4]. Linezolid plasma protein binding is 31% which is concentration independent and Linezolid V_D at steady state average about forty to fifty liters in healthy adult[5]. The nonenzymatic chemical oxidation of the morpholine ring of Linezolid is metabolized into two inactive ring-opened carboxylic acid metabolites i.e., hydroxyethyl glycine metabolite (B) as well as aminoethoxyacetic acid metabolite (A)[6]. The percentage of overall clearance that is nonrenal is 65%. About 30% of the dosage, represented by the compounds Linezolid, 40% by substrate B, and 10% by substrate A, are excreted through urine to constant levels[7]. Poor renal clearance is an indicator of Linezolid and suggests net tubular reabsorption[8]. Maintaining the quality and purity of Linezolid 600mg tablets is essential for successful treatment results[9]. Because antibiotics are so important to public health, it is crucial to guarantee the quality, bioavailability, and uniformity of different pharmaceutical formulations[10].

A drug's bioavailability, which in turn affects its therapeutic efficacy, is largely determined by itsrate of dissolution[11]. Consequently, it is crucial to get the dissolution profiles of Linezolid tablets from several brands in order to assess how well they operate in clinical situations. Dissolution profiles and assay values are critical parameters that affect the bioavailability and efficacy of Linezolid[12]. Various analytical techniques, including High-Performance Liquid Chromatography (HPLC) and Ultraviolet (UV) Spectrophotometry, can be employed to evaluate the dissolution and assay of Linezolid tablets[13]. UV Spectrophotometric and High-Performance Liquid Chromatographic (HPLC) methods have been used in a number of studies totest antibiotics, underscoring their significance in guaranteeing precise and trustworthy drug assays[14].The ease, affordability, and speed with which UV Spectrophotometry may yield data for drug quantification make it a popular option for routine pharmaceutical testing[15].

However, HPLC has great sensitivity and specificity, which makes it perfect for complicated research, particularly when working with medications like Linezolid that need to be quantified precisely. While many studies have used these analytical techniques to assess the quality of antibiotics, such as Linezolid, a critical review of previous research shows that little attention has been paid to the dissolution profiles and comparative analysis of different brands that are available in developing nations like Pakistan. The majority of previous research has concentrated on formulations under a single brand or has only examined pharmaceutical product assays without taking dissolution factors into account.

This study closes these gaps by comparing the assay results and dissolution patterns of many brands of 600 mg Linezolid tablets that are available in Karachi, Pakistan. It employs both UV Spectrophotometric and HPLC methods to provide solid and trustworthy results, providing regulatory agencies, medical professionals, and consumers with important information to evaluate the efficacy and therapeutic coherence of these formulations in Karachi, Pakistan.

MATERIALS AND METHODS

Dissolution Analysis

Components and Technique: For the dissolution test, six tablets of each brand were taken and the process was performed following the standard conditions employing Type II apparatus (ERWEKA, DT, HH, and Germany). The dissolution media consisted of 900 ml of 0.05 M phosphate buffer (pH 6.8). The revolving velocity of the apparatus was controlled at 100 rotations per minute and temperature was maintained at 37±0.5 ^{o}C.

Standard Preparation: 22 mg of Linezolid was taken in a 100 ml volumetric flask and dissolved by adding approximately 40 to 60 ml of diluents. The solution was agitated for five minutes; the volume was made up with diluents. 2 ml of this solution was transferred to 50 ml volumetric flask and the volume was made up with diluents and the solution was mixed well.

Sample Preparation: 900 ml of 0.05 M Phosphate buffer (pH 6.8) was taken in 1000 ml measuring cylinder and was converted in to dissolution vessels.

The medium was kept at a temperature of 37^{o}C ± 0.5^{o}C at 50 rpm.

One tablet was placed in each vessel and after different intervals of time, i.e. 10, 20, 30, 45. 60, 90,120 minutes, 10 ml of the sample was withdrawn and replaced with an equal volume of fresh medium and filtered through the Whattman filter paper. The absorbance of the standard as well as the sample was measured[16].

Assessment through the HPLC Method

Components and Technique: HPLC (LC, 10AT, VP, Shimadzu, Corp, Chromatography Japan) assembled with UV detector, auto sampler, HPLC Pump (LC 10A, Shimadzu Corp, Japan), HPLC grade Methanol (Merck, Darmstadt, Germany, Phosphate buffer (Merck, Darmstadt, Germany), HPLC Column (Hi Chrom, 250 x 4.6mm. 5u UK), Linezolid Standard (Fazl e Ellahe Pharmaceuticals, Karachi, Pakistan), Linezolid 600 mg Tablets (Test and Reference) different pharmacies in Karachi, Pakistan. The Buffer 0.23 g/L of ammonium phosphate monobasic was used. Methanol and buffer were taken as mobile phase in the ratio of 55:45. Not less than ten tablets of Linezolid for sample stock solution were taken and crushed finely. From this powder, about 1.2 mg/ml of Linezolid was taken with the proportion of 55:45 methanol and buffer. An appropriate amount of this powder was taken into the volumetric flask. A little amount of buffer was added in order to dissolve the powder. The mixture was shaken for about 10 minutes. Methanol was added to at least 20% of the final volume. It was then sonicated for 10 minutes. The mixture was diluted with methanol to make up the volume.

Procedure: 20µl of each i.e., standard preparation and assay preparation were injected individually in to the chromatograph. The chromatograms were recorded and the peak responses were measured in response to the major peaks.

Limit: 600 mg per tablet (90% to 110 % of label claim)[16].

Assessment through UV Spectrophotometric Method

Components and Technique: The absorbance of the solution was measured using a double-beam Shimadzu 1800 UV-visible spectrophotometer, which has a band range of 1 nm, wavelength uniformity at 243 nm, and two quartz cells that overlap by 1 cm. Spectrum was automatically obtained by UV-Probe 2.0 system software. An analytical balance with an ultrasonic bath was employed.

Standard Preparation: 50 mg of Linezolid working standard was weighed accurately and moved in to volumetric flask (100ml) with the inclusion of 80ml of diluent. The mixture was agitated until dissolved. The volume was made up with the diluent. A 50 ml volumetric flask was pipetted using only one milliliter of the solution, and the remaining content was adjusted with diluents.

Table 1. Dissolution Profile of Different Brands of Linezolid 600mg Tablets at pH 6.8 (0.05 M Phosphate buffer).

Time (min)	C-1 n=6	C-2 n=6	C-3 n=6	C-4 n=6
0	0	0	0	0
5	80.18 ±0.21	81.3±0.55	71.37±1.87	61.26±0.54
10	94.85±0.27	85.11±0.55	79.58±1.52	76.02±0.87
15	98.11±0.11	88.95±0.57	83.27±0.74	78.59±0.59
20	100.14±0.13	93.56±1.17	84.29±0.69	81.15±2.70
25	100.45±2.97	94.62±0.45	88.63±0.69	83.9±2.41
30	101.54±1.97	95.51±4.73	90.7±1.39	85.11±3.15
40	102.63±0.07	95.89±5.57	93±0.01	85.73±1.88
45	103.01±1.01	97.41±1.56	94.9±1.56	87.77±0.98
60	103.21±1.91	99.89±0.78	96.63±0.83	87.97±1.82
90	103.51±2.17	100±1.11	97.95±0.55	88.27±0.82
120	103.56±0.28	101.78±1.61	99.89±0.32	90.54±0.70

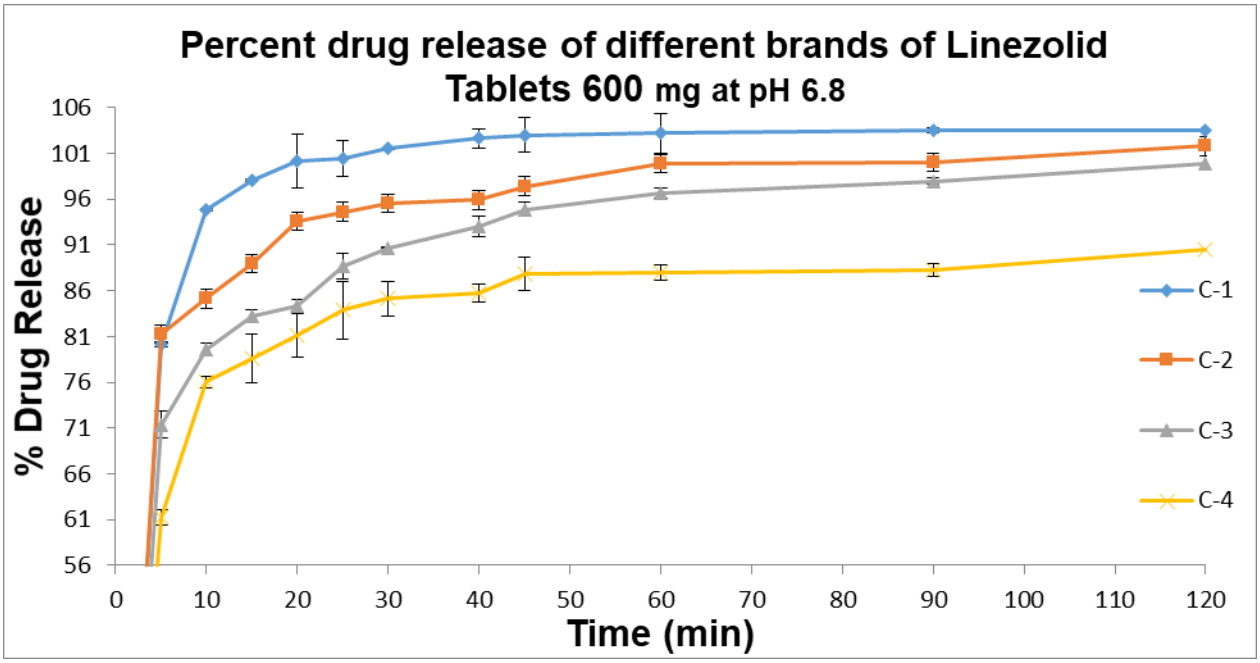

Figure 1. Percent drug release of different brands of Linezolid Tablets 600 mg at pH 6.8.

Table 2. Assay (%) of Different Brands of Linezolid 600mg through HPLC and UV.

HPLC	C-1	C-2	C-3	C-4
	99.5±0.84	98.5±1.05	99.4±1.71	98.7.7±0.93
UV	104.3±0.14	97.8±0.28	104.1±1.6	103.4±2.1

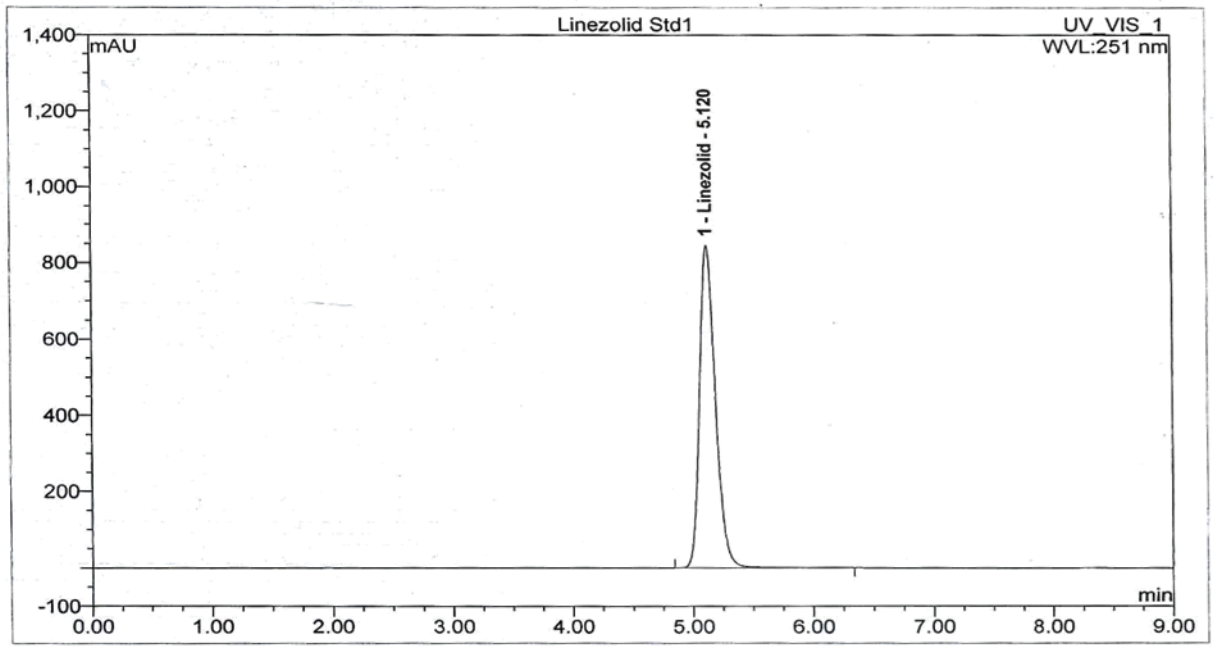

Figure 2. Chromatogram of Linezolid Standard.

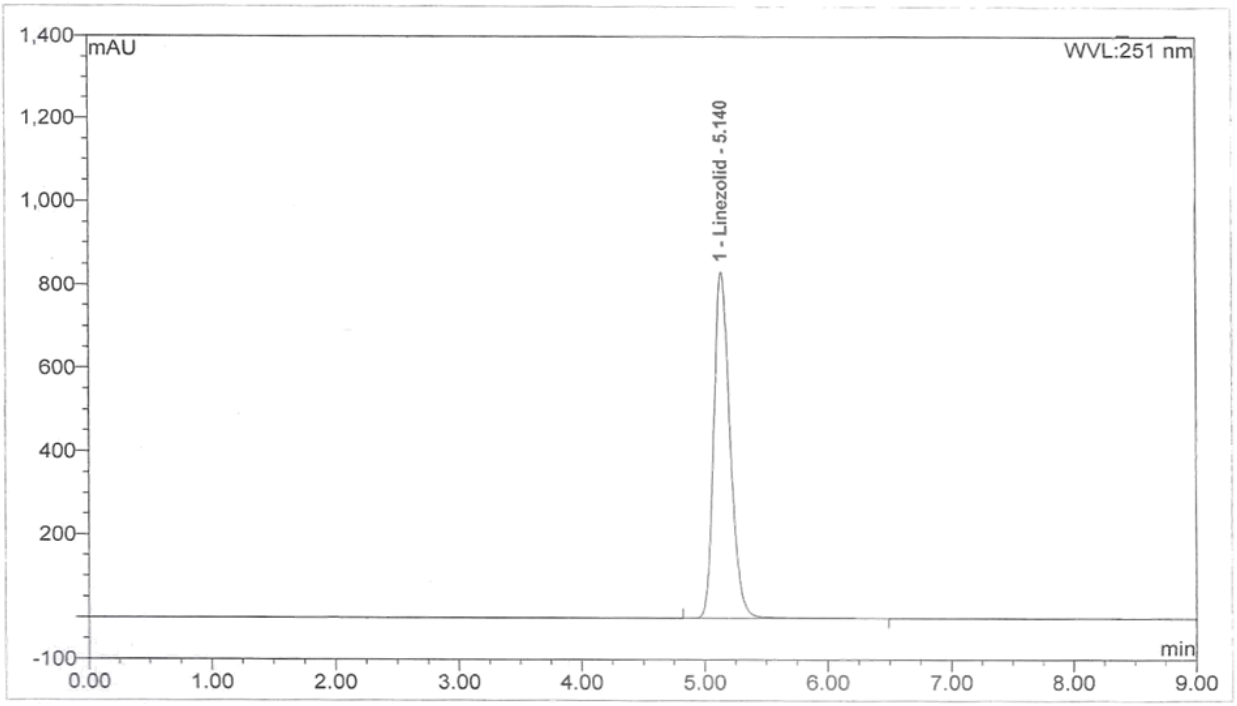

Figure 3. Chromatogram of Linezolid 600 mg Tablet (C-1).

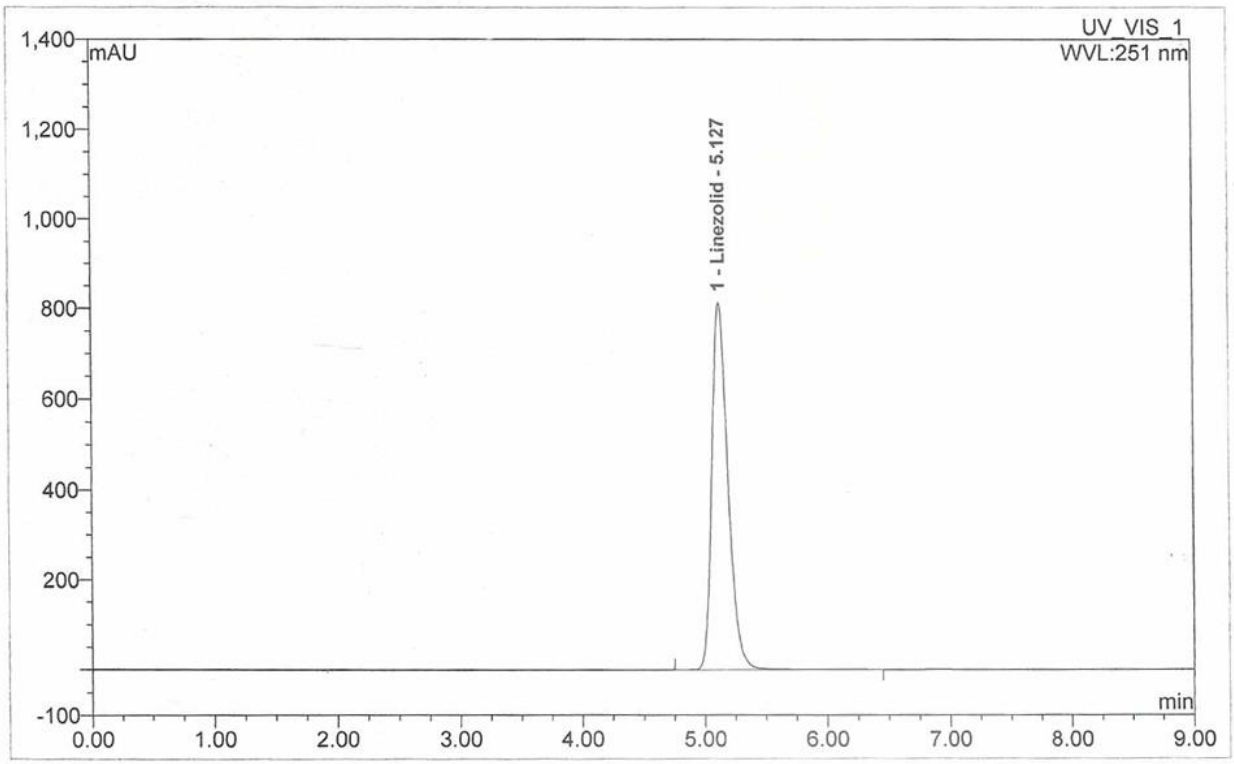

Figure 4. Chromatogram of Linezolid 600 mg Tablet (C-2).

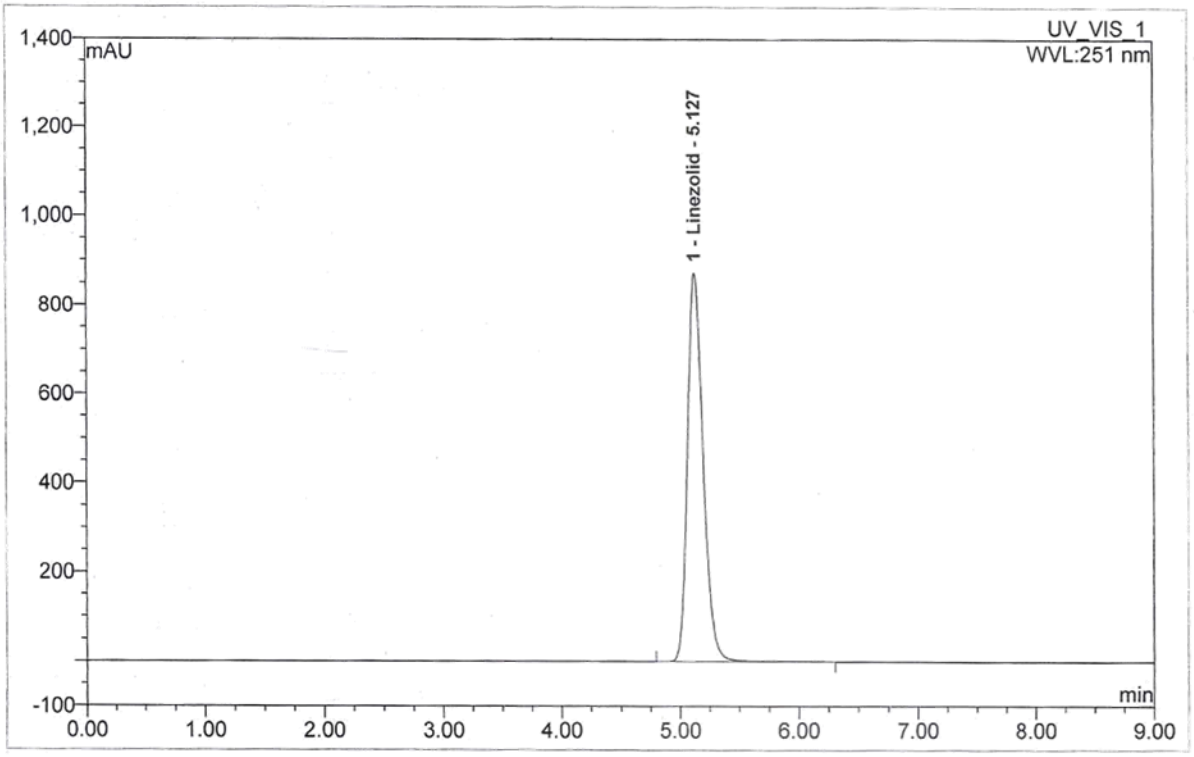

Figure 5. Chromatogram of Linezolid 600 mg Tablet (C-3).

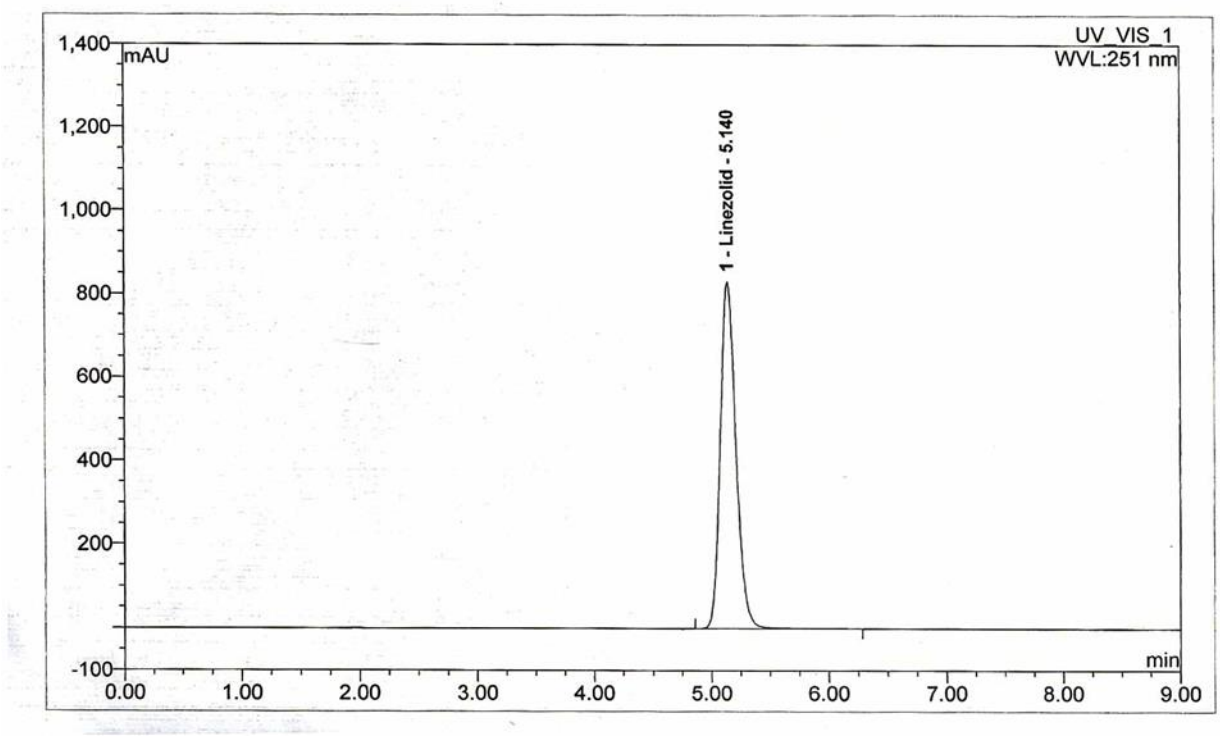

Figure 6. Chromatogram of Linezolid 600 mg Tablet (C-4).

Sample Preparation: The mean weight was measured by weighing twenty tablets. Tablets were going through the crushing stage. Then the material containing 50 mg of Linezolid was transferred into 100 ml volumetric flask and 80 ml of diluent was added. The mixture was agitated until dissolved, and the volume was made up of diluent. 1 ml of this solution was pipetted out into 50 ml volumetric flask and the volume was made with diluent.

Limit: 540.00 mg/ tablet – 660.00mg/ tablet (90.0 to 110% of label claim).

RESULT AND DISCUSSION

Liquid chromatographic and ultraviolet spectrophotometric techniques have been developed to estimate the quantity of Linezolid in tablets and injections, with no significant difference observed between the two methods[17]. Both UV spectrophotometric and High-Performance Liquid Chromatographic (HPLC) methods were used to extensively investigate the dissolution profiles and assay findings for the different brands of 600 mg Linezolid tablets.

Brand C-1 had the quickest rate of dissolution releasing all of the drug in 20 minutes, as seen in Figure **1,** Table **1**. This fast release is significant because it raises the possibility of increased bioavailability and an earlier start to therapeutic activity, both of which could be advantageous in clinical situations where rapid drug action is required. The results of this study have broader implications for quality control in the pharmaceutical industry. The dissolution profiles of Linezolid formulations directly affect their bioavailability, which influences their clinical effectiveness. The faster dissolution rate observed in Brand C-1 suggests that it may be preferable for patients requiring rapid therapeutic action. The dissolution properties of Linezolid formulations have been assessed in a number of investigations. Methanol and ammonium dihydrogen phosphate were used as solvents in one study to create a Reverse Phase High-Performance Liquid Chromatography (HPLC) method for the dissolution test, which has been demonstrated to be appropriate for stability and quality control tests[18]. The dissolution profiles of this study are consistent with previous investigations since they also demonstrate brand-specific variability, with some formulations showing faster rates of dissolution than others. In 2023, Linezolid was estimated in tablet products with the method of reverse-phase high-performance liquid chromatography assay. The column of C18 (250×4.6mm, 5µ) was used to achieve the separation in the mobile phase, which contained methanol and 1% acetic acid (50:50 v/v) and 0.80 mL/min-1 of flow rate. The sample temperature was ambient, the column temperature was 45°C, and the injection quantity was 20µL. The retention time of Linezolid was under acceptance limit[19]. As presented in Table **2**, the assay results for all brands, as determined by UV spectrophotometry and HPLC, fell within the acceptable range of 90-110%. This suggests that all tested brands meet the required standards for drug content, indicating consistency in active pharmaceutical ingredient (API) concentration. Notably, Brand C-1 showed an assay value of 99.5% using HPLC and 104.3% using UV spectrophotometry, which are both well within the acceptable limits. These results are consistent with previous studies where UV spectrophotometry and HPLC were used for the assay of Linezolid, with no significant difference observed between the two methods. Using chromatographic analysis, the retention times for the standard and different brands of Linezolid tablets were 5.120 minutes and 5.140, 5.127, 5.127, and 5.140 minutes, respectively (Figure **2-6**). Minor variances in formulation or manufacturing procedures could be the cause of the little discrepancies in retention times across brands. This is consistent with earlier research that found linezolid from various sources exhibited comparable chromatographic characteristics.

The agreement between UV spectrophotometric and HPLC assay results in this study is also consistent with existing literature, where both techniques were found to yield comparable outcomes for quantifying Linezolid in tablet and injection formulations. This reinforces the reliability of these techniques for quality control in pharmaceutical applications.

CONCLUSION

The product's label claim and the study's findings are fairly consistent. The study suggests that the rate of release of the active through UV Spectrophotometric Technique was found to be under normal limits. HPLC is confirmed as the gold standard for measuring Linezolid levels, ensuring accuracy and reliability. Still, the UV spectrophotometric technique is comparatively fast and economic. It is not required for complex procedures or treatments (frequently

linked to the chromatographic technique), suggesting that both aspects will definitely play a beneficial role obtaining the quantity of linezolid in the composition of tablet.

CONFLICT OF INTEREST

There is no conflict of interest.

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