



INVITRO BIOEQUIVALENCE STUDIES OF SIX BRANDS OF ANTI-EPILEPTIC DRUG OF LEVETIRACETAM TABLETS

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Article Received on 23/11/2023

Article Revised on 14/12/2023

Article Accepted on 03/01/2024

ABSTRACT

The purpose of this study was to compare the dissolving profiles of six different pharmaceutical products (Levepil, Levesam, Epitra, Kepra, Levigress, and Epilive) that are registered and sold commercially in India in order to assess their characteristics and determine whether they could be used as indicators of in vitro performance. To carry out the pharmaceutical equivalency medication product's physicochemical property of therapeutic equivalency with a similar reference listed drug. When a drug product is approved and modifications are made to its components, composition, or manufacturing process, bioequivalency studies offer information on the product and its performance. To assess and compare the bioavailability of six distinct levetiracetam brands. Following that, an assessment was conducted to compare the standard values of six brands of levetiracetam tablets with the Indian Pharmacopoeia and the United States Pharmacopoeia. Following assessment, the outcome is reported and compared to the standard values.

1. INTRODUCTION^[1]

An oral medication's bioavailability, or systemic availability, is mostly determined by the degree of hepatic metabolism and absorption. Comparing the Area Under Curve (AUC) obtained from oral administration of a single dose with that obtained from intravenous administration allows one to ascertain the bioavailability of an oral dosage form. The bioavailability of a drug can be calculated as follows: $AUC (oral)/AUC (IV) = \text{Bioavailable dose} / \text{Administered dose}$

Definition

The rate at which a drug enters the biological system in an active state that allows it to exert the intended pharmacological effect—including the timing, severity, and duration of that effect—is referred to as bioavailability.

1.1 The need for bioavailability studies

Bioavailability studies provide an estimate of the fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the bioavailability for a solution, suspension, or intravenous dosage form that is completely available.

Bioavailability studies provide other useful information that is important to establish dosage regimen and to support drug labeling, such as distribution and elimination characteristics of the drug.

Bioavailability studies provide information regarding the performance of the formulation.

1.2 Types of bioavailability^[2]

Absolute bioavailability: The percentage of a drug's identical dose supplied intravenously that reaches the systemic circulation when a formulation is administered extra vascular including orally. $(AUC)_{abs} / (AUC)_{iv}$ equals absolute bioavailability.

Total bioavailability is equal to $(AUC)_{abs} \times \text{Div}$.

Where Div is the dosage size given intravenously and Dabs is the size of the single dose given via the absorption site.

Relative bioavailability: This term refers to the percentage of a medication that enters the systemic circulation after being administered at a certain dose as opposed to a standard dose of the medication that has been clinically demonstrated to work.

Relative bioavailability = $(AUC)_{test} / (AUC)_{STD}$

Evaluation of bioavailability: A medication's rate, degree, and first-pass metabolism must be examined in order to determine its bioavailability, also known as clinical availability.

Quantifying these characteristics is challenging, if not impossible. The technique used to evaluate bioavailability is predicated on the idea that the clinical

availability of a drug can be inferred from the measurement of its concentration in an appropriate bodily fluid, such as blood, plasma, serum, urine, or occasionally saliva. 1.3.4 Bioavailability is determined by following methods:

The pharmacokinetics method is a more useful and selective approach. There are two kinds of pharmacokinetic techniques.

- 1) Calculating the concentration of whole blood, plasma, or serum
- 2) Method of urinary excretion

From dissolving research: The quality of the product is evaluated through in vitro dissolution studies. The rate of in vitro dissolution and in vivo bioavailability ought to be correlated.

Drug bioavailability in vivo is expected to occur quickly in a dose form with a fast rate of dissolution.

The permeability and solubility of the drug material, rather than the medicinal product's dissolution, determine bioavailability.

1.3 Factor effecting bioavailability

There are three primary absorption factors:

The blood level will rise and fall proportionately to the medicine dose that was provided. The same as the first but resulting from a distinct method of drug absorption from a specified dose form. Lowering the dose is the result of just half of the medication being absorbed from a dosage form.

The rate of medication absorption; if the drug is absorbed more quickly from the dosage form than it is absorbed over all the hazardous level may be surpassed. If the dose form is absorbed too slowly, the minimal effective level will not be reached.

1.4 BIOEQUIVALENCE STUDIES

1.4.1 Introduction^[3]

Both bioavailability and bio-equivalence evaluate how well a medication is absorbed into the bloodstream. Bioequivalence is the comparison of a drug product's bioavailability to predefined bioequivalence parameters, while bioavailability refers to an IV formulation, a solution, or a suspension.

When a medicine with a distinct formulation has the same bioavailability, it is said to have bioequivalence.

1.4.2 Definition

Equivalency is a more relative term used to compare two drug products or a set of predetermined norms. There are various ways to define equivalency: Chemical equivalency denotes the presence of the indicated drug concentrations in two or more dosage forms.

When a medication in two or more dosage forms produces the same in vivo effects as determined by a

pharmacological response or by controlling a symptom or condition, this is known as clinical equivalency.

The idea of therapeutic equivalency states that two chemicals with dissimilar structural makeup might have the same clinical outcome.

A medicine is said to be bioequivalent if it enters the general circulation at the same relative rate and extent in two or more dose forms that are comparable.

1.4.3 Important of Bioavailability and Bioequivalence Studies

A global strategy regarding relative bioavailability Whether it is a novel product or a generic, the majority of bioavailability studies share a basic concept. To determine whether a certain product comparison is quantitative in nature, an experiment is carried out. For a novel medication, this comparison might evaluate how well an oral formulation works in contrast to an intravenous dose, or it might evaluate how well a modified release formulation works in contrast to a traditional capsule. It usually compares a rival formulation with a reference drug in the case of a generic product. This shared experience with comparative bioavailability studies points to a consistent experimental methodology.

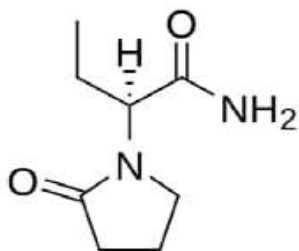
Comparative bioavailability studies for new pharmaceuticals (NDA): Early human safety and efficacy studies of a novel drug are often conducted using the oral formulation. Early oral bioavailability data regarding the drug (and this initial formulation) are frequently obtained through studies that compare the drug with an intravenous dose and a drug solution. These studies use the Universal Approach, where the comparator is either an intravenous dose or possibly a drug solution.

Comparative bioavailability about generic pharmaceuticals (ANDA): The full range of trials required for the initial product need not be conducted when a producer hopes to achieve therapeutic equivalency by bringing a competing generic product to market. By inference, the generic product is considered therapeutically equal to the novel drug product if equivalency has been established in accordance with the specified research requirements and suitably derived metrics.

Testing under fed conditions/Testing under fasting conditions: Bioequivalence studies are used to test a medicine under fasting settings when it does not exhibit the anticipated results. The medication can also be examined while fed to ensure that it satisfies all requirements in accordance with regulatory standards.

2. DRUG PROFILE^[4]

Drug name : Levetiracetam
Molecular weight : 170.209 g/mol
Molecular structure : C₈H₁₄N₂O₂

Chemical structure**Structure 1: Levetiracetam.****Synonyms**

Over a dosing range of 500 to 5000 mg, levetiracetam has linear pharmacokinetics, meaning that the dose is proportionate to the serum concentration. Twice-daily dosing for two days results in a steady-state. Although therapeutic and toxic amounts are not well established, serum level measurement can be used to gauge compliance.

Generic Name: levetiracetam

Brand Name: Levepil, Levigress, Levesam, Epitra, Epilive, Kepra.

Drug Class: Ligands

Medical Author: John P. Cunha, DO, FACOEP

Monoisotopic Mass: 170.105527694 g/mol

Dose: As per physician

Mechanism of Action: The exact processes by which levetiracetam prevents epilepsy remain unclear. Nonetheless, it is thought that binding to a special synaptic vesicle protein 2A (SV2A) is the most significant mode of action. The calcium-dependent vesicular neurotransmitter release is mediated by the SV2A protein, which is a component of secretory vesicle membranes. Levetiracetam's binding to SV2A seems to slow down the release of vesicles.

Pharmacokinetic^[4]

Absorption: Levetiracetam has a very high (96%) bioavailability and is absorbed quickly. After oral treatment, peak plasma concentration is reached around one hour later. Food has no effect on the rate of absorption, although it can cause a thirty-minute delay in the time to maximum concentration. When patients are unable to take oral drugs, the intravenous formulation is employed. When administered intravenously, peak plasma concentration is attained in 5 to 15 minutes. If not, the oral formulation's pharmacokinetic profile is the same.

Distribution: Since levetiracetam binds to proteins to a lesser extent than 10%, it does not compete with other medications for protein binding sites. It is not much

metabolised; the kidneys eliminate over 66% of it unaltered.

Metabolism: The hepatic cytochrome P450 system plays a minor role in the metabolism, accounting for only 2.5%. The primary metabolic pathway is the enzymatic hydrolysis of the acetamide group. Metabolites are eliminated renally and lack pharmacological activity.

Excretion: Glomerular filtration with limited tubular reabsorption is the main excretion mechanism associated with creatinine clearance. Adults' plasma half-lives are normally 6 to 8 hours, however in the elderly, this can be extended by 2 to 3 hours due to increased creatinine clearance with age. Dosage changes are necessary for patients with renal impairment. Because levetiracetam binds very little to proteins and is not metabolised by the liver, pharmacokinetic interactions are minimised. Levetiracetam has linear pharmacokinetics, which means that the dose is proportionate to the serum concentration, over a dosage range of 500 to 5000 mg. A steady-state is reached after two days of twice-daily dosage. While it is unknown what constitutes harmful and therapeutic quantities, adherence can be assessed by tracking serum levels. around 34%.

Route of elimination: Levetiracetam has a plasma half-life of roughly 6 to 8 hours according to several research. It is higher in persons with renal impairment and the elderly (mostly because of reduced renal clearance).

Toxicity: There aren't many documented instances of levetiracetam overdose in clinical studies. Levetiracetam overdose has been associated with drowsiness, somnolence, agitation, respiratory depression, and coma. Levetiracetam does not have an antidote. Patients who have overdosed are given general supportive measures, airway protection, and vital sign monitoring. Doctors ought to get in touch with poison control. Patients with severe renal impairment could require hemodialysis. A four-hour hemodialysis session can eliminate about half of the medication.

Uses

- **Myoclonic seizures:** When treating adults with myoclonic seizures and teenagers aged 12 and older with juvenile myoclonic epilepsy, it is recommended as an adjuvant therapy. Adults with epilepsy and children aged one month or older who experience partial seizures may benefit from supplementary therapy for partial seizures.
- **Primary generalised tonic-clonic seizures:** Levetiracetam is used as an adjuvant treatment for idiopathic generalised epilepsy in adults and children over five years of age who are experiencing primary generalised tonic conic seizures.
- **Off-label (not FDA approved) usage** is occasionally employed for haemorrhage seizure prevention and status epilepticus.

- Off-label uses of levetiracetam include supratentorial neurosurgery and the prophylaxis of traumatic brain injury (TBI). In palliative care, levetiracetam is also used off-label to treat seizures.

Administration: There are oral and intravenous (IV) versions of levetiracetam available. IV levetiracetam is administered at the same total daily dosage as oral. It is given as an infusion lasting fifteen minutes. No data supports the use of a loading dosage. Furthermore, there are immediate-release and extended-release oral preparations available. The extended-release form's metabolism and bioavailability are comparable to those of the immediate-release version. With the extended-release version, however, it takes three hours longer to reach peak plasma levels.

Adult Dosing: A minimum of 500 mg twice a day is advised. Doses as little as 500 mg per day may be effective for certain elderly people. To ensure a clinical response, it should be titrated from a modest starting dose. After a week or two, up the dosage to 250 or 500 mg until the desired clinical response is obtained. A daily intake of up to 3000 mg is advised. Adverse effects may result with a rapid dose increase. Doses for immediate-release and extended-release versions are given twice a day and once daily, respectively. Levetiracetam does not have a defined therapeutic serum concentration; instead, dosage is determined by the patient's response.

The effectiveness of doses greater than 3000 mg per day is not entirely proven. Some recommend raising the dosage to as much as 4000 mg per day for patients who have demonstrated a clear response but occasionally experience breakthrough seizures. The doctor should be aware of the possibility of seizure aggravation while administering greater doses, as there have been some cases.

Off-label use for status epilepticus is also occasionally employed. A single dose of 60 mg/kg or an IV infusion of 1000–3000 mg at a rate of 2 mg/kg per minute are the usual dosages. Because of its acidic composition, levetiracetam is typically administered as an intermittent infusion to reduce the risk of potentially harmful drug responses. A recent study looked at the delivery of undiluted dosages of levetiracetam since the delay in preparing diluted levetiracetam can cause therapy for status epilepticus to be delayed. The findings showed that intravenous levetiracetam given quickly and undiluted is both safe and effective.

3. METHODS AND MATERIALS

Visually, the various brands of Levetiracetam pills differed in size, shape, and colour. Using a Vernier calliper, the dimensions of the pills from each brand were measured.

3.1 Uniformity of Weights^[5]

Twenty sample tablets from each brand were put on a digital analytical scale and weighed separately. Both the average weight and the percentage(%) that each tablet deviated from the mean were calculated. To pass the weight variation test, the tablet must fall inside the I.P.-allowed percentage deviation limits.

3.2 Hardness Test^[6]

Using a Monsanto hardness tester, the hardness of many brands of tablets was assessed and expressed in kg/cm². Ten sample tablets of each brand were obtained, and each tablet was sandwiched between the hardness tester machine's spindle until it broke. The amount of pressure needed to break the tablet was then noted.



Figure 1: Hardness Test Apparatus.

3.3 Friability Test^[7]

Each brand was given ten tablets, which were then weighed and exposed to an abrasive process using a Roche friabilator set to 100 revolutions for four minutes. After meticulously dusting the tablets, they were precisely weighed once more, and the percentage of weight loss was noted. Using the formula, the tablets' friability was determined.

% Friability is equal to [(Start weight - End weight)/Start weight] x 100.



Figure 2: Friability Test Apparatus.

3.4 Disintegration Test^[8]

Six randomly chosen tablets of each brand were tested for tablet disintegration time at 37°C using a disintegration equipment with distilled water used as the test fluid. The point at which there were no more tablet granules on the mesh was considered to be the disintegration time.



Figure 3: Disintegration Test Apparatus.

3.5 Dissolution rate determination^[9]

An eight-compartment Vega dissolution test apparatus with a paddle stirrer at 100 rpm and $37\pm 0.5^\circ\text{C}$ was used to determine the dissolution rate of each brand of tablet. The dissolving fluid utilised was 900 millilitres of pH 6.8 phosphate buffer. For each test, a single 500 mg tablet was used. Ten millilitres of the dissolving fluid sample were taken out every five, ten, fifteen, thirty, forty, and sixty minutes. To keep the sink condition, a new 10 ml dissolving medium was added after each sampling. After filtering each sample that was removed, the filtrate was diluted. Using an Analytical spectroplus 2060 U.V. Visible double beam spectrophotometer, the absorbance was measured at λ_{max} 298nm. The concentration was measured using the same medium and a standard

solution of the medication levetiracetam. Using the concentration and percentage.



Figure4: Dissolution Test Apparatus.

Comparison of price

After comparing and examining the price variations of each brand, it was found that they were all comparable in terms of cost and physicochemical characteristics.

Table 1: Details of Different Brands of Levetiracetam.

S. No	Name of brands	Batch No.	Manufacturing Date	Expiry Date	Price for 10 tablets
1	Levipil	SIE1747A	07\2023	06\2025	Rs.144
2	Levigress	D222334	07\2022	06\2025	Rs.134
3	Levesam	HTJ0049	04\2023	03\2025	Rs. 145
4	Epitra	D2304006	04\2023	03\2025	Rs.127
5	Epilive	1303820	05\2023	01\2025	Rs. 145
6	Keppra	PC182228	11\2022	10\2024	Rs.127



Figure 5: Price Comparison of All Brand.

4. RESULT

The table below displays the consolidated summary of all measured parameters

Table 2: Measured Parameters.

S.NO	Levipil	Levigress	Levesam	Epitra	Epilive	Keppra
%purity	99%	98%	99%	99%	97%	99%
Weight variation**	0.6	0.62	0.58	0.6	0.68	0.6
Length(cm)**	1.8	1.5	1.9	1.7	1.8	1.8
Width(cm)**	0.7	0.9	0.7	0.7	0.8	0.8
Thickness(cm)**	0.56	0.58	0.48	0.52	0.62	0.58
%Friability**	1.73	1.48	0.17	0.30	0.44	0.15
Hardness(kg/cm ²) ***	1.9	1.2	1.5	2.36	1.3	2.3
Disintegration***	4.2	5.12	6.14	4.3	4.28	6.46
Time (minutes)						
Dissolution (%) amount of drug release) #	99%	99%	97%	98%	99%	95%

*Label claim – 500 mg

** - Average measurement of 20 tablets

*** - Average measurement of 10 tablets

**** - Average measurement of 6 tablets

- Measurement of 1 tablet

- Average measurement of 6 tablets

5. CONCLUSION

Levetiracetam tablets come in a variety of brands through India's medicine delivery system. Levetiracetam pills are being used in clinical settings more and more, which necessitates monitoring, quality control evaluations, and generic substitution. Regarding weight homogeneity, hardness, friability, thickness, disintegration, and dissolution profile, all of the products exhibit satisfactory results. According to USP and IP, every test associated with the assessment of levetiracetam 500 mg tablets was successfully completed. Levetiracetam 500 mg tablets IP have well-defined and controlled quality features. There aren't any glaring problems with the product that could throw off the benefit balance. Since these tablets' effectiveness was well-established, patients.

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