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Physico-chemical and Microbiological Quality Control of Levetiracetam LDM 500mg

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Dedication

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Dedication

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List of Abbreviations

AEDs: Anti-Epileptic Drugs.

USP: United States Pharmacopoeia.

API: Active Pharmaceutical Ingredient.

CFU: Colony Forming Unit.

CTD: Common Technical Document.

CRS: Chemical Reference Standard.

FDA: Food and Drug Administration.

GABA: Gamma-aminobutyric acid**.**

GLPs: Good Laboratory Practices.

GMPs: Good Manufacturing Practices.

HPLC: High-Performance Liquid Chromatography.

ICH: International Conference on Harmonization.

IPQC: In Process Control.

IR: Infrared.

LOD: Loss On Drying.

LOQ: Limits of Quantification.

MCA: MacConkey Agar.

MCB: MacConkey Broth.

MSA: Mannitol-Salt Agar.

NCI: National Cancer Institute.

OSD: Oral Solid Dosage.

pH: Potential of Hydrogen.

Ph. Eur.: European Pharmacopoeia.

Ph. Int.: International Pharmacopoeia.

PVC: Polyvinyl Chloride.

PVdC: Polyvinylidene chloride.

QA: Quality Assurance.

QC: Quality control.

RSD: Relative Standard Deviation.

RRT: Relative Retention Time.

SDA: Sabouraud Dextrose Agar.

SV2A: Synaptic Vesicle protein 2A.

TSA: Tryptone Soy Agar.

TSB: Tryptic Soy Broth.

TVAM: Total Viable Aerobic Mesophiles.

TYM: Total Yeasts and Molds

UV: Ultraviolet.

VRBG: Violet Red Bile Glucose.

WHO: World Health Organization.

XLD: Xylose-Lysine-Deoxycholate.

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Introduction

Introduction

The Pharmaceutical industry is one of the world's most research-intensive industries, generating a continuing steam of new products that save lives and raise the quality of life. The discovery of new drugs has evolved over time from a decidedly empirical process to one based to a considerable degree upon fundamental scientific knowledge.

Nowadays, the industrial production of drugs is increasing considerably throughout the world. The evolution of the pharmaceutical industry requires rigorous measures to ensure good quality of drugs.

The quality, safety and efficacy of pharmaceutical products are stringently regulated all over the world. These aspects are the main objective of the pharmaceutical industry which are achieved by the implementation of excellent manufacturing practices and continuous monitoring of the product's quality (Woodcock, 2018).

Generic drugs like the one in our study are generally distributed around the world, due to cost minimization and providing more affordable options to the patients compared to original drugs. However, this popularization must not be done to the detriment of quality, at the risk of harming the health of the patient or the consumer. Hence the need to ascertain the quality of drugs before they are availed on the market.

Recognized standards are published periodically in the form of pharmacopeias, regulations which provides detailed descriptions, analytical technics and characteristics of a drug to ensure the quality of medicines.

Therefore, the role of quality control laboratories is to validate, through appropriate tests that the drugs meet the required quality standards. Controls are intended for various raw materials all through the process of production and final products. These examinations must be referenced with Pharmacopoeial or Technical File Specifications (Quality Resources , 2024).

In the context of our work, we are interested in executing our research work on the quality control laboratory of Levetiracetam 500mg tablet formulation manufactured by the LDM pharmaceutical industry on the drug.

The dissertation is categorized into 5 chapters which include;

- The first chapter being the bibliographic review where we will discuss on the generalities of drug pharmacology.
- Second chapter where we will be discussing anti-epileptic drugs, their mechanisms of action and later present our drug of interest in particular (Levetiracetam).
- In the third chapter we will discuss the concept of pharmaceutical quality and quality control.
- Subsequently the fourth chapter is the practical part, dedicated for the description of the host company LDM where we realized our study, on physico-chemical and microbiological quality control of raw materials, intermediate product, and the finished product.
- The last section is dedicated to the results obtained from quality analysis of our drug of interest.

The dissertation ends with a conclusion followed by bibliographical references and appendices.

Bibliographic Review

1. Bibliographic Review

1.1 General Pharmacology

Pharmacology is a science that deals with the study of substances that are capable of causing a physiological or biochemical effect or change in the organism's body. There are two main branches of pharmacology which include;

1.1.1 Pharmacodynamics

Pharmacodynamics deals with all that has to do with what "*the drug does to the body*" in other words the drug's physiological or biochemical effect or mode of action in the organism's body. The following are some of the ways in which drugs act or work;

- ✓ Work as enzyme inhibitors.
- ✓ Work as hormones.
- ✓ Work as transmitter substances.
- ✓ Inhibit transport processes.
- ✓ Block transmitter inactivation (Neal, 2020).

1.1.2 Pharmacokinetics

Pharmacokinetics is a branch of pharmacology that deals with what "*the body does to the drug*" which includes;

- Absorption,
- Distribution,
- Metabolism and finally Excretion (Tripathi, 2013).

1.2 Pharmacovigilance

Pharmacovigilance(PV) concerns a combination of activities, measures and systems put in place in the pharmaceutical industry to ensure drug safety (Beninger, 2018).

According to the World Health Organization PV is defined as the "science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems."

Pharmacovigilance was motivated by the historic tragic events as far as drugs are concerned (Beninger, 2018).

1.3 Galenic pharmacy

Galenic formulation is the process of preparing or compounding medicines or drugs to turn an active ingredient into a ready-to-use medicine that can be dosed as required in order to optimize their absorption and forms part of pharmaceutics. In other words, galenic pharmacy is a discipline or science of dosage form design (Eupati, 2015).

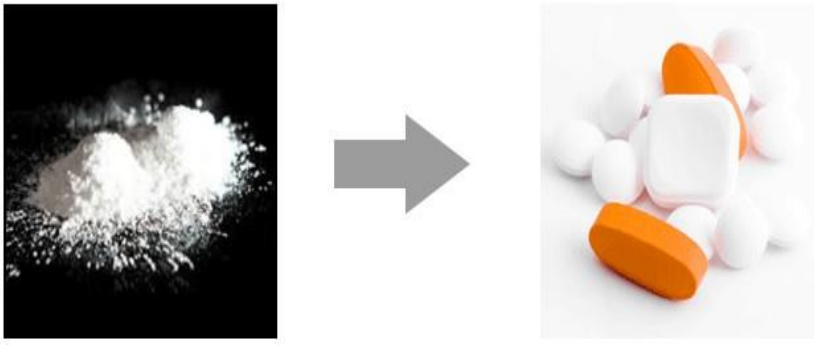


Figure 1 Transformation of the active ingredient into a suitable form of administration (Eupati, 2015).

1.4 Generalities on drugs

1.4.1 Drugs

According to the National Cancer Institute (NCI) of the United States of America, a drug is "any substance other than food that is used to prevent, diagnose, treat, or relieve symptoms of a disease or abnormal condition. Drugs can also affect how the brain and the rest of the body work and cause changes in mood, awareness, thoughts, feelings, or behavior" (NIH NCI, 2024).

1.4.2 Sources of drugs

Sources of different medicaments can be natural, synthetic, and biosynthetic (Alamgir, 2017).

1.4.2.1 Natural medicaments

These drugs are obtained from natural origins such as;

- Plant,
- Animal,
- Microbiological,

- Marine and mineral sources.

1.4.2.2 Synthetic medicaments

Synthetic medicines are made by chemical synthesis to mimic the effects of natural drugs or even improve their potential (Liberty Health Services, 2022).

They are pharmaceutical substances that are produced from chemical reactions between different chemical agents in an industry or laboratory (Rana, 2021).

1.4.2.3 Biosynthetic medicaments

These drugs are obtained by biological synthesis using biotechnological tools (Coudert, 2021).

Such drugs include biologically synthesized vaccines, hormones, antibiotics etc.

1.4.3 Composition of a drug

A drug product is composed of two main constituents or components or ingredients in any pharmaceutical dosage form which are;

1.4.3.1 Active Pharmaceutical Ingredient (API)

An API is a molecule or set of molecules with a therapeutic effect.

According to the European Pharmacopoeia: “an active substance is any substance intended to be used for the manufacture of a medicinal product and which, when used in its production, becomes an active substance of the medicinal product.

1.4.3.2 Excipients

Excipients are substances that make it possible for the medicine or drug to be administered (Les medicaments et les aliments, 2024).

Excipients are used in every pharmaceutical dosage form accordingly depending on the technical needs of that dosage form.

1.4.3.3 Different types of excipients

Excipients are classified basing on their origin, use in any pharmaceutical dosage form and the functions they perform.

Origins or sources of excipients include;

a) **Animal source:** - Lactose, Gelatin, Stearic acid, Bees wax, Honey, Musk, Lanolin etc.
b) **Vegetable source**: - Starch, Peppermint, Turmeric, Guar gum, Arginates, Acacia etc.
c) **Mineral source**: - Calcium phosphate, Silica, Talc, Calamine, Asbestos, Kaolin, Paraffin, etc.
d) **Synthetic source:** - Boric acid, Saccharin, Lactic acid, Polyethylene glycols, Polysorbates, Povidone etc. (Chaudhari & Patil, 2012).

1.4.4 Different Galenic forms of drugs

Pharmaceutical dosage forms are classified according to four criteria which are;

1) Route of administration,
2) Physical properties of dosage form,
3) Type of release pattern,
4) Comprehensive testing strategy (Pharmacopeial Forum, 2009).

1.4.4.1 Route of administration

In this category we have the following forms depending on the respective route of administration of the drug;

- ✓ Dermal forms.
- ✓ Oral forms.
- ✓ Injectable forms through the parenteral route which includes; the intramuscular, intravenous(IV) and subcutaneous routes.
- ✓ Inhalable forms.
- ✓ Mucosal forms.

1.4.4.2 Dosage form physical properties.

Dosage forms under this category include;

- **Solid dosage forms**

These types of dosage forms include;

- o Capsules,
- o Tablets,
- o Dry powder inhalers,
- o Medicated soaps,
- o Shampoos,
- o Effervescence,

- Gummies,
- Pills.

An Oral solid dosage (OSD) product refers to a final drug product therapy that is ingested through the mouth, dissolved in the digestive system, and delivered to the body through absorption into the bloodstream (Dave DiProspero, 2024).

The table below (Table 1) summarizes the other pharmaceutical dosage forms.

Table 1 Other pharmaceutical galenic forms applications (Pharmacopeial Forum, 2009).

Liquid dosage forms	Semi solid or semi liquid dosage forms	Aerosols
-Injectable forms -Syrups for oral administration -Solution for dermal application	Emulsions (creams, lotions) Foams Ointments which are mostly mean for dermal and mucosal applications	Aerosols include sprays, inhalers which are mostly destined for nasal applications

1.4.4.3 Type of release pattern

In this category of dosage forms we have drugs characterized by the release of the API as follows;

- Immediate release.
- Extended release.
- Delayed release (Pharmacopeial Forum, 2009).

1.4.5 Types of drugs or medicines

1.4.5.1 Placebo medicines

These are medicines made up of inactive ingredients such as sugars with no therapeutic or biological effect. However, some of these medicines make patients feel better and some have side effects and bring about what is called the placebo effect (Lynch, 2022).

1.4.5.2 Patented medicines

Patent medicines are defined as medications whose ingredients had been granted government protection for exclusivity for a given period of time. A patented drug can be given a brand name (Hagley, 2024).

1.4.5.3 Generic drugs

According to the European Medicine Agency," a generic medicine is a medicine that is developed to be the same as a medicine that has already been authorized". Its authorization is based on efficacy and safety data from studies on the authorized brand name or patented medicine. A generic medicine can only be marketed once the 10-year exclusivity period for the original medicine has expired (EMA, 2024).

A generic medicine's API is given a non-proprietary or generic name that is decided by an expert committee and is understood internationally, for example, paracetamol/acetaminophen is the non-proprietary name (generic name) while Crocin/Metacin/Meftal/Tylenol etc. are brand names.

A generic drug can be sold under its generic name for example Levetiracetam, or under a brand name (branded generic drug for example Lacetam) but not under the brand name used by the original patent holder (Thakkar1 & Billa, 2013).

1.4.6 Classification of drugs

A drug class is a group of medications with similar properties. According to the U.S. Food and Drug Administration (FDA) three main methodologies or criteria are used to classify drugs. These include the following:

1. **Mechanism of action**: This is how the drug causes specific biochemical changes in the body (pharmacodynamics)
2. **Physiological effect**: This is how an organ such as the skin, brain, or digestive tract responds to the drug (pharmacokinetics).
3. **Chemical structure or chemical makeup**: This is how the molecular makeup of a drug is uniquely structured.

Drugs can also be classified basing on formulary classification, legal definition (Sana Lake Recovery Center, 2023).

1.5 Fabrication of drugs

Pharmaceutical manufacturing is the process after formulation that focuses on the making of a finished drug product (Apollotechnical, 2024).

Pharmaceutical fabrication process focuses on creating different pharmaceutical dosage forms (medications) like tablets and injectables. It must adhere to strict quality and safety standards to ensure effectiveness and compliance with international regulations (Fluidhandling Pro, 2024).

The fabrication process of drugs depends on their pharmaceutical dosage forms.

1.5.1 Fabrication of oral solid dosage (OSD) forms

OSD forms take several different shapes, and with those different forms comes different production techniques and facility designs.

- Tablet forms are made through compression and can either be coated, meaning they have an extra layer to create a smooth surface, or uncoated.
- Capsules are created through a coating process, where the drug substance and dry ingredients layer around a seed material (Dave DiProspero, 2024).

OSD drug products typically consist of a dry powder formulation that includes the drug substance or API, various excipients, and intermediates and fillers. Where they differ is the final form and the individual characteristics of the ingredients such as;

- ✓ Particle size,
- ✓ Bulk density,
- ✓ Flowability among other factors.

As such, different products require different processing methods and platforms. Each of these forms can have variable bioavailability and rate of release. Depending on the therapeutic use of the OSD product, it will have an immediate, sustained, controlled, or extended-release. These factors

influence drug manufacturing platforms and the equipment and technology used in the manufacturing process.

The overall goal for OSD processing regardless of the type of product is to create a formulation that ensures each dose (a single tablet) or (capsule) is consistent. Each one has a repeatable distribution of ingredients, and there is a consistency of dissolution and bioavailability to ensure that the drug product is safe and effective. The final dose form requirements also dictate which processing platform is used (Dave DiProspero, 2024).

1.5.1.1 Granulation

Granulation is the process of creating granules by combining one or more powder particulates through compression activities and/or with a binding agent. The granulation process is categorized as follows;

- ✓ **Dry Granulation:** is the process of converting primary powder particles into granules using the application of pressure without the intermediate use of liquid (Esco Pharma, 2024).
- ✓ **Wet granulation**: is the process of joining powder particles together to create a larger particle, known as a granule. The granules can be composed of particles that are either the same or dissimilar materials depending upon the formulation ingredients.

In the wet granulation process, granules are joined together using a binder solution, often aqueous, that is sprayed into the process (Dave DiProspero, 2024).

1.5.1.2 Direct compression

The direct compression process homogeneously combines ingredients, without directly changing or impacting the starting granules. It is a mixing process that uniformly blends the powders through particle movement and rotation. A tumble blender is the common equipment used for this operation.

1.5.1.3 Tablet coating

Tablet coating according to Science Direct is the process where coating material is applied to the surface of the tablet to achieve the desired properties of dosage form over the uncoated variety.

A liquid and solid suspension is sprayed onto the face of the bead material to achieve the proper coating characteristics. A spray atomization configuration creates a soft and consistent interaction

between the solution and dry particles in a vessel. The most common piece of equipment is a fluid bed coater (Dave DiProspero, 2024).

1.5.1.4 Blending unit operation

The blending unit operation combines the active ingredients and excipients and/or lubricants to achieve a homogenous distribution of ingredients (Dave DiProspero, 2024).

1.6 Pharmaceutical packaging

Effective packaging is an essential element in the pharmaceutical industry. The packaging helps to protect the drug during storage, sale, shipping, and use. For other products, the objectives of packaging include protection, safety, functionality, branding, and attractiveness. For pharmaceutical products, objectives of the pharmaceutical packaging include;

- Chemical protection.
- Portion control.
- Containment.
- Protect the medicines from contamination and all external influences that may alter the drugs' property.
- Security of the drug and therefore the packaging should maintain the drug quality.

The packaging of pharmaceutical products depends on the type of drug as they may react with it (Ship Rocket Packaging, 2024).

Packaging that preserves the integrity of the drug product should be used in addition to correct labelling. The type, quantity, and method of use of the medication that will be prescribed should be taken into consideration when selecting the container. The container should maintain a product's identity, strength, quality, and purity as well as prevent contamination (Kumar, 2023).

There are three types of packing in the pharma industry which include the primary, secondary, and tertiary packing.

1.6.1 Primary packaging

Also known as sales packaging, is in direct contact with drugs and medicines. Therefore, the packaging needs to be inert and should not cause any alteration to the salt in the dosage. The main purpose of primary packaging is product protection and safety from external factors.

Different types of primary packaging include:

- Blister packs,
- Strip packaging,
- Sachet packaging,
- Ampoules,
- Prefilled syringes,
- Vials,
- Plastic bottles (Ship Rocket Packaging, 2024).

1.6.2 Secondary packaging

It refers to the product packaging that is used for storing or grouping multiple primary packages. Examples of secondary packaging include:

- Boxes,
- Cartons,
- Injection trays,
- Shipping containers (Medical Packaging, 2023).

1.6.3 Tertiary Packaging

Tertiary packaging refers to the packaging that is used to ship products in bulk. This type of packaging is used for shipping and handling to facilities that store or distribute large quantities of medication. Different components of tertiary packaging that are used in the pharma industry include:

- Large shipping containers,
- Barrels,
- Edge protectors and other packaging that helps cushion packages as needed (Medical Packaging, 2023).

Antiepileptic Drugs

2 Antiepileptic Drugs

Antiepileptic or anticonvulsant drugs are used to treat epilepsy as well as non-epileptic convulsive disorders.

The antiseizure or antiepileptic drugs are usually used chronically to prevent the occurrence of seizures in people with epilepsy.

These drugs are also, on occasion, used in people who do not have epilepsy to prevent seizures that may occur as part of an acute illness such as meningitis or in the early period following either neurosurgery or traumatic brain injury (Katzung, 2018).

2.1 Epilepsy

Epilepsy is a chronic disorder of brain function characterized by the recurrent and random or unpredictable occurrence of seizures **(**burst of uncontrolled electrical activity between brain cells/neurons or nerve cells) that causes temporary abnormalities in muscle tone or movements (stiffness, twitching or limpness), behaviors, sensations or states of awareness.

Majority of epilepsy cases are as a result of;

- Damage to the brain, as occurs in traumatic brain injury, stroke, or infections,
- Brain tumor or developmental lesion such as a cortical or vascular malformation; these epilepsies are referred to as "symptomatic."
- Genetic factors and in this case genetic epilepsies are often called idiopathic**.**

2.1.1 Mechanisms of Action of Anti-Seizure Drugs

Antiseizure drugs protect against seizures by interacting with one or more molecular targets in the brain. The ultimate effect of these interactions is to;

- Inhibit the local generation of seizure discharges, both by reducing the ability of neurons to fire action potentials at high rate as well as reducing neuronal synchronization.
- Inhibit the spread of epileptic activity to nearby and distant sites, either by strengthening the inhibitory surround mediated by GABAergic interneurons or by reducing glutamate-mediated excitatory neurotransmission.

The specific actions of antiseizure drugs on their targets inhibit the abnormal neuronal discharge are broadly summarized as:

1) Modulation of voltage-gated sodium, calcium, or potassium channel functions by their inhibition;
2) Enhancement of fast GABA-mediated synaptic inhibition;
3) Modification of synaptic release processes;
4) Diminution of fast glutamate-mediated excitation.

The table below (Table 2) show different molecular targets of some of the antiepileptic drugs.

Table 2 Different molecular targets of some of the antiepileptic drugs

Molecular Target	Antiseizure Drugs that Act on Target
Voltage-gated ion channels	
Voltage-gated sodium channels (Na_v)	Phenytoin, fosphenytoin, carbamazepine, oxcarbazepine, eslicarbazepine acetate, lamotrigine, lacosamide; possibly topiramate, zonisamide, rufinamide
Voltage-gated calcium channels (T-type)	Ethosuximide
Voltage-gated potassium channels (K_v7)	Retigabine (ezogabine)
GABA inhibition	
GABAA receptors	Phenobarbital, primidone, benzodiazepines including diazepam, lorazepam, and clonazepam; possibly topiramate, felbamate, ezogabine
GAT-1 GABA transporter	Tiagabine
GABA transaminase	Vigabatrin
Synaptic release machinery	
SV2A	Levetiracetam, brivaracetam
α2δ	Gabapentin, gabapentin enacarbil4, pregabalin
Ionotropic glutamate receptors	
AMPA receptor	Perampanel
Mixed/unknown	Valproate, felbamate, topiramate, zonisamide, rufinamide, adrenocorticotropin

Other mechanisms

Many of the newer antiepileptic drugs were developed empirically on the basis of activity in animal models. Their mechanism of action at the cellular level is not fully understood

Levetiracetam is believed to interfere with neurotransmitter release by binding to synaptic vesicle protein 2A (SV2A), which is involved in synaptic vesicle docking and fusion.

Brivaracetam, a related antiepileptic agent, also binds to SV2A with 10-fold higher affinity (James M. Ritter, 2020).

2.2 Levetiracetam

Levetiracetam is an α-ethyl analog of piracetam and a broad-spectrum antiseizure agent and one of the most commonly prescribed drugs for epilepsy, primarily because of its perceived favorable adverse effect profile, broad therapeutic window, favorable pharmacokinetic properties, and lack of drug-drug interactions.

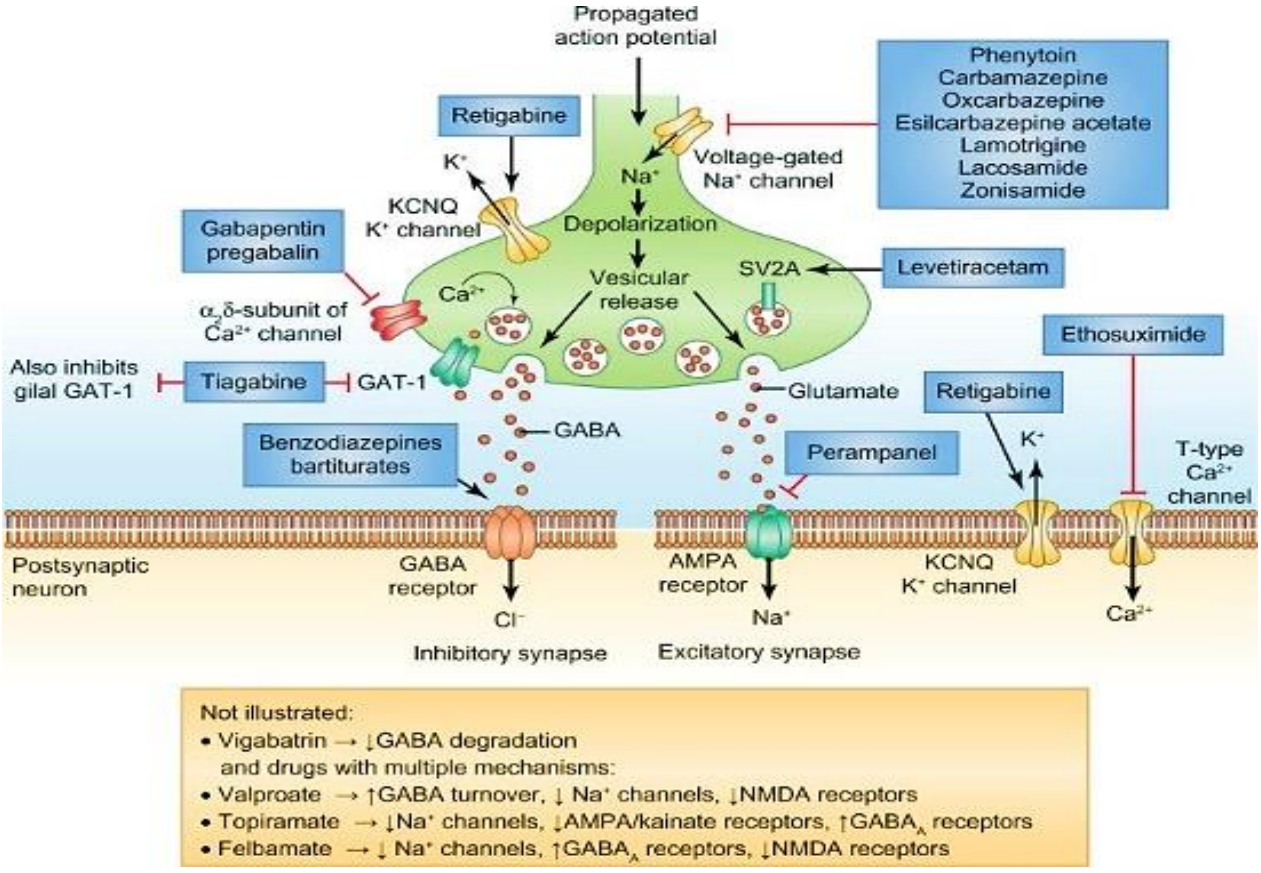


Figure 2 Mechanism of action of Levetiracetam and other antiepileptic drugs.

Levetiracetam can be formulated into different dosage forms such as tablets, oral solutions and injectable in variable concentrations as 250 mg, 500 mg, 750 mg, and 1000 mg tablets and as an clear, colorless, grape-flavored liquid (100 mg/mL) for oral administration (FDA, 2024).

2.2.1 History and Discovery of Levetiracetam

After discovery and adoption of several Antiepileptic Drugs (AEDs) including piracetam, in 1992, a scientist called **Alma Gower**, observed that the (*S*)-enantiomer of the ethyl analogue of piracetam provided more potent protection against sound-induced convulsions in audiogenic seizure susceptible mice than piracetam and this is what we know today as Levetiracetam. Levetiracetam was found to have an activity in a variety of other seizure models, although its spectrum of activity was distinctly different from other AEDs (Rogawski, 2008).

2.2.2 Structure of Levetiracetam

Levetiracetam is a pyrrolidinone and carboxamide that is N-methylpyrrolidin-2-one in which one of the methyl hydrogens is replaced by an amino carbonyl group, while another is replaced by an ethyl group (the S enantiomer) (NCBI, 2024).

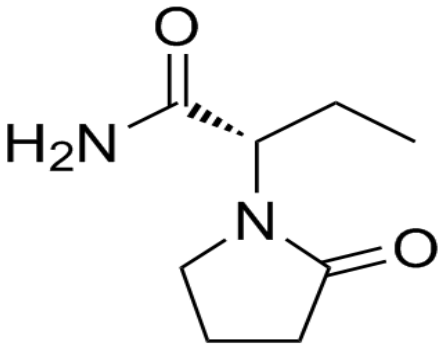


Figure 3 Chemical structure of Levetiracetam

2.2.3 Physical and chemical Properties of Levetiracetam

The different physical and chemical properties of Levetiracetam are summarized in the following table (Table 3).

Table 3 Different identifiers, physical and chemical properties of Levetiracetam

Names and Identifiers	
▪ IUPAC Name	▪ (2*S*)-2-(2-oxopyrrolidin-1-yl)butanamide
▪ InChIKey	▪ HPHUVLMMVZITSG-LURJTMIESA-N
▪ Molecular Formula	▪ $C_8H_{14}N_2O_2$
▪ ChEMBL ID	▪ CHEMBL1286
▪ DrugBank ID	▪ DB01202
Chemical and Physical Properties	
✓ Molecular Weight	✓ 170.21 g/mol
✓ Exact Mass	✓ 170.105527694 g/mol
✓ Monoisotopic Mass	✓ 170.105527694 g/mol
Experimental Properties	
○ Color / Form	○ White to off-white crystalline powder
○ Odor	○ Faint
○ Taste	○ Bitter
○ **Melting Point**	○ 115-119C, 117 °C
○ Solubility	○ 104g/100mL, ○ Very soluble in water (104.0 g/100 mL). ○ It is freely soluble in chloroform (65.3 g/100 mL) and ○ In methanol (53.6 g/mL), ○ Soluble in ethanol (16.5 g/mL), ○ Sparingly soluble in acetonitrile (5.7 g/100 mL) and practically insoluble in n-hexane. (Solubility limits are expressed as g/100 mL solvent)
○ Vapor Pressure	○ $3.5*10^{-6}$ mm Hg at 25 °C
○ Optical Rotation	○ Specific optical rotation: -90 degree at 25 °C/D (c = 1 in acetone)

2.2.4 Pharmacology of Levetiracetam

a) Mechanism of action of Levetiracetam

Levetiracetam binds selectively to a synaptic vesicle protein SV2A, a ubiquitous synaptic vesicle integral membrane protein which may function as a positive effector of synaptic vesicle exocytosis.

Levetiracetam interferes with the release of the neurotransmitter stored within the vesicle. It gains access to the luminal side of recycling synaptic vesicles by vesicular endocytosis after neurotransmitter release as the vesicles are recycled.

Binding to SV2A in the vesicle reduces the release of the excitatory neurotransmitter glutamate during trains of high-frequency activity Thus, it selectively accumulates in, and inhibits, rapidly firing neurons.

Levetiracetam also inhibits potassium and N-type calcium channels (Katzung, 2018).

b) Pharmacokinetics of Levetiracetam

- **Absorption and Distribution**;

Oral absorption of Levetiracetam is rapid and nearly complete, with peak plasma concentrations in 1.3 hours. Food slows the rate of absorption but does not affect the amount absorbed. Protein binding is less than 10%. The oral bioavailability of Levetiracetam tablets is 100% and the tablets and oral solution are bioequivalent in rate and extent of absorption.

- **Metabolism**;

Levetiracetam is not extensively metabolized in humans. The metabolism of Levetiracetam occurs in the blood. There is no metabolism in the liver, and drug interactions are minimal.

The major metabolic pathway is the enzymatic hydrolysis of the acetamide group, which produces the carboxylic acid metabolite (24% of dose).

- **Elimination**;

The plasma half-life is 6–8 or (7 ± 1) hours, but may be longer in the elderly. It is unaffected by either dose or repeated administration. Two-thirds of the drug is excreted unchanged in the urine and the remainder as the inactive deaminated metabolite 2-pyrrolidone-N-butyric acid.

Levetiracetam is eliminated from the systemic circulation by renal excretion as unchanged drug which represents 66% of administered dose. The total body clearance is 0.96 mL/min/kg and the renal clearance is 0.6 mL/min/kg. The mechanism of excretion is glomerular filtration with subsequent partial tubular reabsorption (FDA, 2024).

Below is the summary of the pharmacokinetics of Levetiracetam;

- ✓ Bio-availability ≥95% through the Oral Route.
- ✓ Onset of action <3 days through the Oral Route.
- ✓ Time to peak plasma concentration 1–2h through the Oral Route.
- ✓ Plasma half-life 6–8h.
- ✓ Duration of action 24h.

2.2.5 Indication and usage of Levetiracetam

Levetiracetam is indicated as adjunctive therapy in the treatment of;

- ✓ Partial onset seizures in adults and children 4 years of age and older with epilepsy.
- ✓ Myoclonic seizures in adults and adolescents 12 years of age and older with juvenile myoclonic epilepsy.
- ✓ Primary generalized tonic-clonic seizures in adults and children 6 years of age and older with idiopathic generalized epilepsy (FDA, 2024).

2.2.6 Contraindications of Levetiracetam

This product should not be administered to patients who have previously exhibited hypersensitivity to Levetiracetam or any of the inactive ingredients in Levetiracetam tablets or oral solution.

2.2.7 Undesirable effects of Levetiracetam

- ❖ ***Very common side effects (>10%):*** fatigue, drowsiness, headache.
- ❖ ***Common (<10%, >1%):*** ataxia, hyperkinesia, tremor, dizziness, diplopia, blurred vision, amnesia, abnormal thinking, attention disturbance, behavioral disturbances (emotional lability, irritability, agitation, hostility/aggression, personality disorders), depression, insomnia, anorexia, abdominal pain, diarrhea, dyspepsia, nausea, vomiting, myalgia, rash, pruritus, thrombocytopenia.

Behavioral disturbances occur in 3–4% of patients with epilepsy but only 0.5% of those being treated for other conditions. Risk factors include a history of aggression or psychiatric disturbance.

- ❖ ***Uncommon (<1%, >0.1%):*** suicidal ideation (0.2%).
- ❖ ***Rare (<0.1%):*** psychosis, pancreatitis, hepatic failure, acute kidney injury, bone marrow suppression, hyponatremia, extra-pyramidal symptoms, rhabdomyolysis, severe skin reactions (JPSM, 2018).

2.3 Presentation of Lacetam® (Levetiracetam) LDM 500mg

Levetiracetam LDM 500mg is an antiepileptic generic drug manufactured in LDM with a new generic brand name for LDM, "LACETAM® 500mg" containing 30 yellow, oblong shaped, and film coated tablets in a small box. Each box contains three blisters each containing ten (10) tablets.

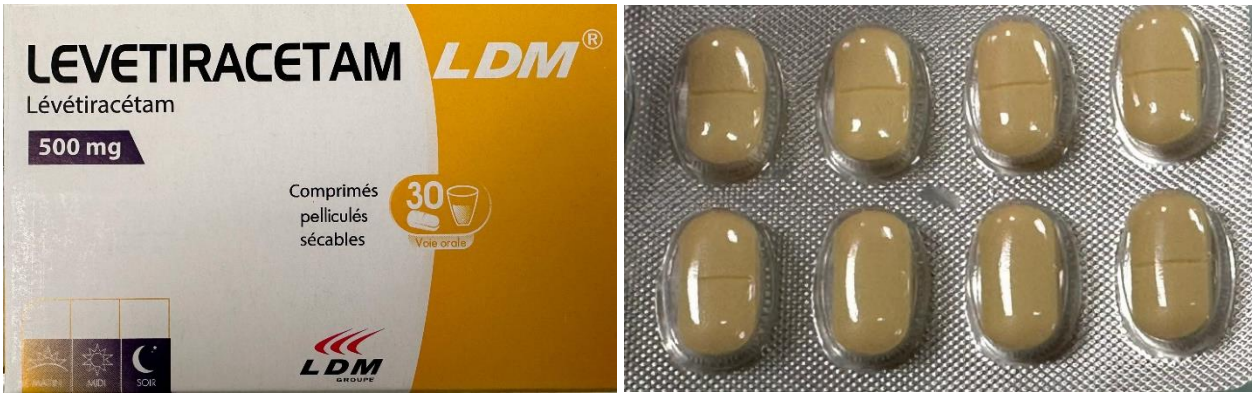


Figure 4 Packaging box and tablet of Levetiracetam 500mg in a blister.

2.3.1 Composition of Levetiracetam LDM 500mg

Levetiracetam 500mg is composed of 500mg of Levetiracetam (Polymorphic form I) as the active ingredient and a number of excipients as demonstrated in the table below (Table 4).

Table 4 Excipients in Levetiracetam 500mg and their functions.

Excipients	**Chemical Formula**	**Roles**
Corn Starch	$C_8H_{10}O_5$	**Diluent.** Acts as a filler in the tablets to increase weight and improve content uniformity.
Croscarmellose Sodium	$C_6H_6NaO_6$	**Disintegrant.** Croscarmellose sodium gives dissolution and disintegration characteristics, thus enhancing the bioavailability of the drug.
Povidone K-30(Kollidon K30)	C_6H_9NO	**Binder.** Polymer and used as a binder in pharmaceutical tablets.
Anhydrous colloidal silica (Aerosol 200)	SiO_2	**Glidant agent** Helps to obtain the optimal powder flow required high speed tablet presses.
Talc extra Superior	$Mg_3Si_4O_{10}(OH)_2$	**Anti-adherent.**

		Acts as a lubricant and diluent which helps to improve the flow properties of the powder mixture and ensure that the tablets are easily swallowed.
Magnesium Stearate	$Mg(C_{18}H_{35}O_2)_2$	**Lubricant.** Capable of forming films on other tablet excipients during prolonged mixing, leading to a prolonged drug liberation time, a decrease in hardness, and an increase in disintegration time.
Purified water	H_2O	Solvent.
Opadry II yellow		Colorant and coating agent.

2.3.2 Fabrication of Levetiracetam (LACETAM® 500mg)

The processes of pharmaceutical production are critical and so rigorous measures and systems are put in place to ensure that Pharmaceutical Good Manufacturing Practices (GMPs) are strictly respected from the reception of raw materials to the final pharmaceutical product to ensure that safe, efficacious and quality products are produced.

The process begins with the reception of raw materials which are then quarantined and cannot continue for production unless validated through tests in the physico-chemical quality control laboratory.

Once the raw materials are declared conform they can then be prepared for production which begins with weighing of raw materials (APIs and the excipients) in required proportions in a designated weighing room (Wehrlé, 2012).

2.3.3 Packaging of Levetiracetam LDM 500mg

Controlled quality packaging materials are used to package Levetiracetam 500mg, once the conformance of these items to the determined specifications and standards is ascertained by the packaging material analysis team they can then be used to condition the tablets.

2.4 Water for Pharmaceutical Use

Water is one of the major utilities used by the pharmaceutical industry. It may be present as an excipient or used for reconstitution of products, during synthesis, during production of the finished

product or as a cleaning agent for rinsing vessels, equipment, primary packaging materials, and for many other pharmaceutical uses (EMA, 2020).

Water for pharmaceutical use is graded depending on its pharmaceutical use as follows;

- Water for Injections.
- Purified Water.
- Water for preparation of extracts (intended for the preparation of Herbal drug extracts).

Each grade of water must comply to given prespecified specifications to be counted in that particular grade of pharmaceutical water (EMA, 2020).

2.4.1 Purified water

Purified Water is water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorized. It is usually produced on-site from potable water, which also must meet stringent quality thresholds (EMA, 2020).

The packaging items must contain specific mandatory information destined for the consumer and for traceability.

The following material (Table 5) is used for its packaging;

Table 5 Different packaging materials, their functions and mandatory information to be mentioned on them.

Component	Function	Mandatory information
Transparent Polyvinyl Chloride (PVC) / Polyvinylidene chloride (PVdC)	Primary Packaging	- Lot number. - Expiry date.
Thermoformed aluminum blisters		
Packaging box	Secondary Packaging	Each packaging box must bear; - Lot number. - Fabrication date. - Expiry date. Each cardboard box must bear; the product name and dosage, lot number, fabrication date, expiry date, package number, case number, date and operator initials.

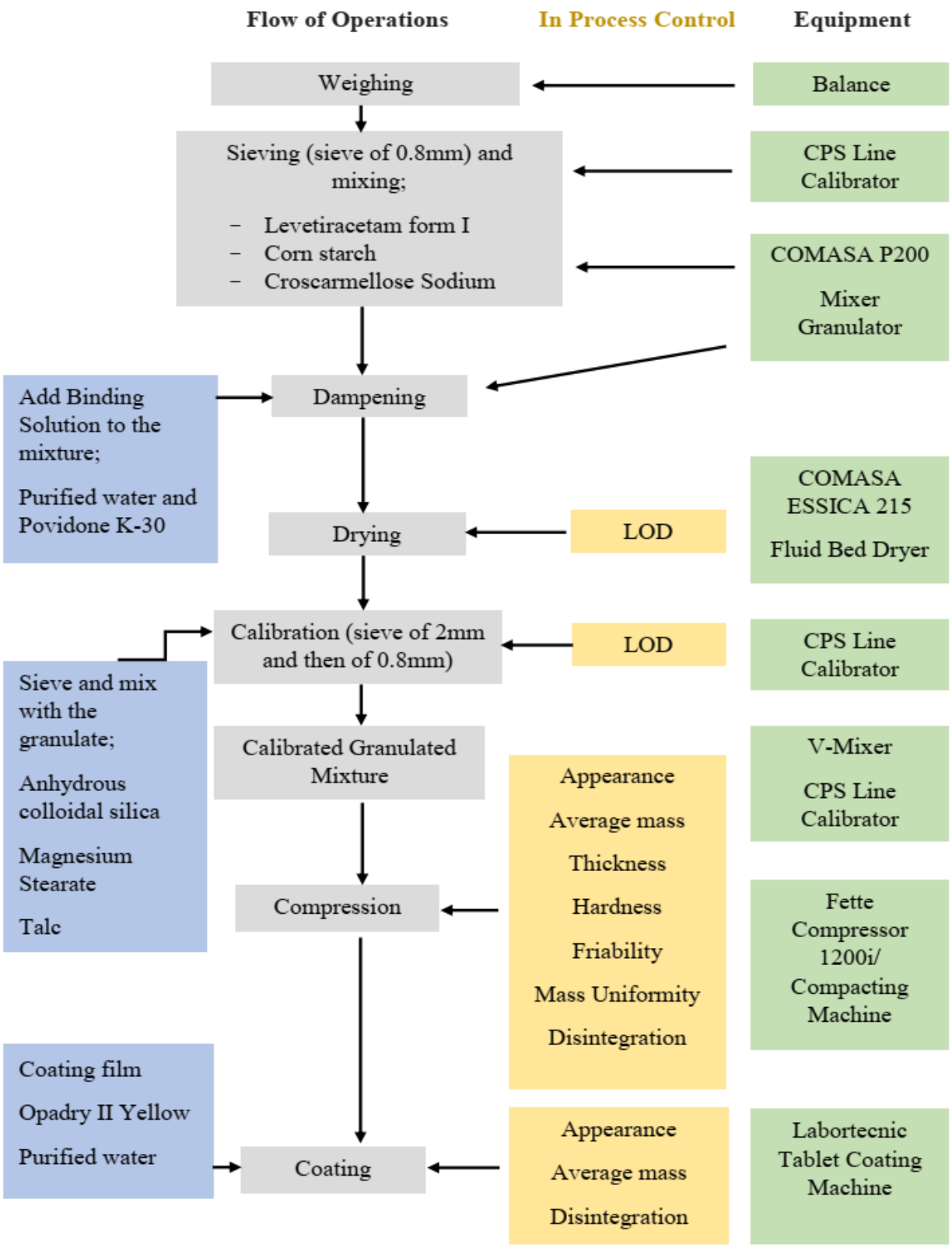


Figure 5 Flow diagram of the production steps of Levetiracetam 500mg and the equipment used.

Quality Control

3 Concept of Quality and Quality Control

3.1.1 Definition of Quality

Quality is a notion that describes specifications to which the product or service's value is attributed (Stephen, Jane, Michael, & Went, 2010).

3.2 Pharmaceutical Quality or Drug Quality

The concept of drug quality describes specifications or features of a drug that make it;

- ✓ Fit for use
- ✓ Meet or exceed customers' (patients and caretakers as well as health professionals) needs.
- ✓ Meet its prespecified quality attributes or regulatory specifications.

A quality drug is also a drug manufactured in compliance with the current Good Manufacturing Practices (CGMPs) (Woodcock, 2018).

3.3 Pharmacopoeia

According to WHO a pharmacopoeia is a legally binding collection of information, standards and quality specifications for medicines that is used as a reference in the pharmaceutical industry in a given country or region. These quality specifications include appropriate tests that confirm the identity and purity of the product, ascertain the amount of the active substance and, the performance characteristics.

The pharmacopoeia may also give general requirements on important subjects such as analytical methods, microbiological purity, dissolution testing, or stability.

There different examples of pharmacopoeia include;

- British Pharmacopoeia (BP)
- United States Pharmacopoeia (USP)
- European Pharmacopoeia (Ph. Eur.)
- Indian Pharmacopoeia (IP)
- International Pharmacopoeia (Ph. Int.) (Ryma & Rayene, Dissertation, 2018).

3.4 Quality Assurance (QA)

Quality assurance is a part of *quality management system* that aims at providing confidence that quality requirements are or will be fulfilled. QA encompasses planned and systematic activities implemented within the quality system to guarantee quality of the pharmaceutical product.

The confidence provided by quality assurance is provided internally to the management and externally to customers, government agencies, regulators, certifiers, and third parties (Quality Resources , 2024).

Therefore, for Quality assurance to guarantee quality, it must englobe all the good practices including;

- ✓ Good Manufacturing Practices (GMPs).
- ✓ Good Laboratory Practices (GLPs)
- ✓ Stability studies.
- ✓ Analytical Validation.
- ✓ Pharmaceutical Development.
- ✓ Risk Management.
- ✓ The Common Technical Document (CTD).
- ✓ Good Distribution Practices (GDPs) (Meriem & Aya, 2021).

Figure 6 Relationship between quality assurance and quality control *(Quality Resources , 2024).*

3.5 Good Manufacturing Practices (GMPs)

GMPs refers to a system that comprises of processes, procedures and documentation that ensures that quality products are consistently manufactured and controlled according to set quality standards. The term **current Good Manufacturing practices** (cGMPs) comes in to ensure continuous improvement in the approach of manufacturers to product quality.

GMPs stand on 5 pillars or components summarized as the five **(5) Ps** which are;

1. People.
2. Products.
3. Processes.
4. Procedures.
5. Premises

Implementing GMP can help to examine and address every aspect of the production process to protect both company and consumer from the risks that can be catastrophic for products such as cross contamination, adulteration and mislabeling (Tarlengco, 2024).

GMP guideline and regulation address areas which directly influence the product quality (PICS, 2023):

- Quality management.
- Sanitation and hygiene.
- Building and facilities.
- Equipment.
- Raw materials.
- Personnel.
- Validation and qualification.
- Complaints.
- Documentation and recordkeeping.
- Inspections & quality audits.

3.5.1 Good Laboratory Practices (GLPs)

The difference between GMP and GLP is that GLPs apply only to the safety testing phase.

GLPs are summarized in the different GLP guidelines as follows (SafetyCulture, 2023);

- Personnel.
- Facility and Equipment.
- Characterization.
- Study plan or Protocol.
- Standard Operating Procedures.
- Final Report.

- Storage of Records.
- Retention of Records.

3.5.2 Validation, Qualification and Verification

Validation is an important part of quality assurance which it involves the systematic study of systems, facilities and processes aimed at determining or confirming whether they will perform their intended functions adequately and consistently as specified.

Validation generally applies to processes or methods and so a validated process is one which has been demonstrated to provide a high degree of assurance that uniform batches will be produced that meet the required specifications and has therefore been formally approved**.** (Sumeet & Gurpreet, 2013)**.**

Therefore, validation generally refers to a process that consists of at least four distinct components or steps:

- Software,
- Instruments,
- Methods or procedures, and
- System suitability to keep the process in check.

3.5.2.1 Types of validation

Validation is divided into following subsections which include;

- ✓ Analytical method validation
- ✓ Process validation
- ✓ Cleaning validation
- ✓ Equipment validation (Jindal, Kaur, Patil, & Patil, 2020)**.**

But while the overall process is called validation, some of the steps also are referred to by that same term, as well as other steps such as qualification and verification.

Verification; refers to regular performance checks to ensure that the instrument to be used is suitable for its intended application.

Qualification; helps to make instruments provide data that is valid and is per the requirements of medical device industries hence ensuring that reliable and valid data is provided.

All equipment used in the production and analysis of products must be properly Validated and Calibrated to demonstrate that it is suitable for its intended purpose as a regulatory requirement, voluntary standards, vendor practices, and industry practices. Analysts use validated methods, system suitability tests, and in-process quality control checks to ensure that the data they acquire are reliable and that there are specific guidance and procedures available to ensure compliance.

The process for instrument qualification follows the 4Qs model approach which includes (Kapoor, Vyas, & Dadrwal, 2018)**;**

- Design qualification (DQ),
- Installation qualification (IQ),
- Operational qualification (OQ),
- Performance qualification (PQ).

3.6 Quality control

Delivering a safe, effective and a quality product is one of the major goal of any pharmaceutical industry hence the significance of quality control in the pharmaceutical industry (PICS, 2023).

Quality Control (QC) involves rigorous testing, inspections, and compliance with regulatory standards to detect and prevent defects or deviations from quality standards or specifications in the manufacturing process. These tests are carried out before the release of products into the market to maintain public health and safety. Quality Control is not just limited to laboratory operations, but involved in all decisions which may concern the quality of the product.

Here are some key pharmaceutical guidelines for quality control (Compliance Quest, 2024):

a) Good Manufacturing Practices (GMP).
b) Good Laboratory Practices (GLP).
c) Pharmacopeial Standards.
d) ICH Guidelines.
e) Validation Guidelines.
f) Stability Testing Guidelines.
g) Data Integrity Guidelines.
h) Quality Risk Management.

3.6.1 Physicochemical Quality Control

This consists assessing the organoleptic characteristics of the different pharmaceutical forms (presentation, color, etc.), identify and dosing of the active ingredient(s), determine the presence of any impurities and carry out their quantification, determine the pharmaco-technical characteristics in relation to the pharmaceutical form (disintegration, dissolution, pH, etc.). This physicochemical quality control consists evaluating;

a) Starting Materials

The main objective of the physicochemical analysis of the raw materials is the identification and then the characterization of the API and excipients before they are integrated into the production process (Hamza & Abderrahim, 2019).

b) Intermediate forms

Intermediate forms differ from one pharmaceutical form to another, such as final mixture before compression, and the bare tablets before coating (for the case of film-coated tablets for example). These intermediate forms must be controlled.

Physicochemical control tests on intermediate forms depend on the pharmaceutical form to be analyzed, for tablets, for example, controls to be carried out may include; friability test, mass uniformity test, content uniformity test, etc. (Hamza & Abderrahim, 2019).

c) Finished product

Physicochemical Quality Controls on the finished product are carried out after packaging. These tests may include; dissolution test, related substances test, API assay etc.

3.6.2 Microbiological Quality Control

Microbiological control is an integral part of quality control in manufacturing of a pharmaceutical product. It is carried out throughout the production chain, from the starting materials to the finished product. A microbiological analysis must make it possible to isolate and identify a specific microorganism (qualitative method) or to quantify a particular flora in a sample (quantitative method) (Ryma & Rayene, 2018).

3.6.3 Stability Studies or Stability Testing

A drug is considered stable when its essential properties do not change, or change in tolerable proportions until its expiration date.

According to the International Conference on Harmonization (ICH), stability is the ability of a drug to retain its chemical, physical, biological and microbiological properties and within specified limits throughout its validity period.

This stability depends on environmental factors which are;

- Temperature,
- Relative humidity,
- Light, and on the other hand, factors linked to the product such as the;

- Physicochemical properties of the active ingredient and excipients,
- The manufacturing process,
- The nature of the container-closure system and the properties of the packaging materials (Ryma & Rayene, 2018) (WHO, 1996).

Stability studies must be done on a new and existing drugs to determine their expiration date or shelf life under specified packaging and storage conditions in controlled artificial climatic conditions. A stability report must be established for internal use, registration purposes, etc., giving details of the design of the study, as well as the results and conclusions (WHO, 1996).

The shelf-life should be established with due regard to the climatic zone in which the product is to be marketed. The four zones into which the world is divided based on the prevailing annual climatic conditions for the purpose of worldwide stability testing, are as follows:

- Zone I: Temperate.
- Zone II: Subtropical, with possible high humidity.
- Zone III : Hot/Dry.
- Zone IV : Hot/Humid (WHO, 1996).

The storage conditions recommended by manufacturers on the basis of stability studies should guarantee the maintenance of quality, safety, and efficacy throughout the shelf life of a product.

There are two types of stability testing as detailed below;

3.6.3.1 Accelerated stability testing

This is a study designed to increase the rate of chemical degradation and physical change of a drug by using exaggerated storage conditions. The data obtained may be used to assess longer-term chemical effects under no accelerated conditions and to evaluate the impact of short-term excursions outside the label storage conditions, as might occur during shipping. The results of accelerated testing studies are not always predictive of physical changes (WHO, 1996).

3.6.3.2 Real-time (long-term) stability studies

This is to experiment on the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a drug, during and beyond the expected shelf-life and storage periods of samples under the storage conditions expected in the intended market. The results are used to establish the shelf-life, to confirm the projected shelf life, and to recommend storage conditions.

3.6.3.3 The frequency of stability testing

The frequency of stability testing depends on the phases of the product and stability of the drug substance. For example, for a product in development phase and for studies in support of an application for registration the frequency is as follows;

- for accelerated studies, at 0, 1, 2, 3 and, when appropriate, 6 months;
- for real-time studies, at 0, 6 and 12 months, and then once a year.

For on-going studies, samples may be tested at 6-month intervals for the confirmation of the provisional shelf-life, or every 12 months for well-established products.

Highly stable formulations may be tested after the first 12 months and then at the end of the shelf-life. Products containing less stable drug substances and those for which stability data are available

should be tested every 3 months in the first year, every 6 months in the second year, and then annually (WHO, 1996).

3.6.3.4 Stability Analytical Methods

All product characteristics likely to be affected by storage, for example assay value or potency, content of products of decomposition, physicochemical properties (hardness, disintegration, particulate matter, etc.), should be determined; for solid or semi-solid oral dosage forms, dissolution tests should be carried out.

Test methods to demonstrate the efficacy of additives, such as antimicrobial agents, should be used to determine whether such additives remain effective and unchanged throughout the projected shelf-life (WHO, 1996).

3.7 In process quality control of solid dosage forms

In Process Quality Control(IPQC) tests which are performed during the manufacturing of product which include thickness, hardness, friability, dissolution time, disintegration time. IPQC tests are necessary to insure the quality and safety of finished pharmaceutical product.

IPQC tests help to identify defective products before the production process is terminated so that they can be corrected by rework since it can be impossible to rework on a finished batch (Savale, 2018).

The common IPQC tests include the following;

3.7.1 Evaluation of tablets General Appearance

It involves assessing the general appearance of a tablet because its identity and general elegance is essential for consumer acceptance, it entails control of *lot-to-lot* uniformity and *tablet-to-tablet* uniformity.

The control of general appearance involves the measurement of size, shape, color, presence or absence of odor, taste etc. (Kala, 2017).

3.7.2 Size and Shape

The shape and dimensions of compressed tablets are determined by the type of tooling during the compression process.

Tablet thickness should be consistent from batch to batch or within a batch only if the tablet granulation or powder blends is adequately consistent in particle size and particle size distribution, if the punch tooling is of consistent length, and if the tablet press is clean and in good working condition. Tablet thickness can be measured by micrometer or by other device. Tablet thickness should be controlled within a ± 5% variation of standard value (Kala, 2017).

3.7.3 Unique identification markings

These marking utilize some form of embossing, engraving or printing. These markings include company name or symbol, product code, product name etc.

3.7.4 Organoleptic properties

Color is a vital means of identification for many pharmaceutical tablets and is also usually important for consumer acceptance. The color of the product must be uniform within a single tablet, from tablet to tablet and from lot to lot. Non uniformity of coloring not only lack esthetic appeal but could be associated by the consumer with non-uniformity of content and general poor product quality. Non uniformity of coloring is usually referred to as mottling. Color distribution must be uniform with no mottling. For visual color comparison compare the color of sample against standard color (Kala, 2017).

3.7.5 Hardness of Tablets

Hardness can be referred to as the *resistance of tablets* to capping, abrasion or breakage under conditions of storage, transportation and handling.

Tablet hardness has been associated with other tablet properties such as density and porosity. Hardness generally increases with normal storage of tablets and depends on;

- The shape,
- Chemical properties,
- Binding agent and,
- Pressure applied during compression.

Hardness test is a non-official quality control method. A hardness tester instrument measures the force required to break the tablet when the force generated by a coil spring is applied diametrically to the tablet. Hardness generally measures the tablet crushing strength (Savale, 2018).

3.7.6 Friability

It is defined as the excessive breakness of tablets during mechanical shocks of handling in manufacture, packaging, and shipping. Friction and shock are the forces that most often cause tablets to chip, break.

In friability test the tablets are prone to abrasion hence enabling us to check for the tablet strength under application of force in different manner.

Why we test friability?

Since tablet hardness is not absolute indicator of strength because some formulations, when compressed into very hard tablets, tend to "cap" on attrition, losing their crown portions.

Therefore, another measure of tablets strength, its friability is often measured (Kala, 2017).

3.7.7 Weight Variation test.

Uniformity of weight is an in process test parameter which ensures consistency of dosage units during compression.

Tablet weight is mainly affected by factors such as tooling of the compression machine, head pressure, machine speed and flow properties of the powder.

Inconsistent powder or granulate density and particle size distribution are common sources of weight variation during compression. Variation between tablet with respect to dose and weight must be reduced to a minimum.

3.7.8 Content Uniformity Test

The content uniformity test is used to ensure that every tablet contains the amount of drug substance intended with little variation among tablets within a batch (Savale, 2018).

3.7.9 Disintegration Test (U.S.P.)

For a drug to be absorbed from a solid dosage form after oral administration, it must first be in solution, and the first important step toward this condition is usually the break-up of the tablet; a process known as disintegration.

The time of disintegration is a measure of the quality. This is because, for example, if the disintegration time is too high; it means that the tablet is too highly compressed or the capsule shell

gelatin is not of pharmacopoeial quality or it may imply several other reasons. And also if the disintegration time is not uniform in a set of samples being analyzed, it indicates batch inconsistency and lack of batch uniformity (Savale, 2018).

3.7.10 Dissolution Test

Dissolution is the process by which a solid solute enters a solution. Dissolution is pharmaceutically defined as the rate of mass transfer from a drug substance into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition. It is a dynamic property that changes with time and explains the process by which a homogenous mixture of a solid or a liquid can be obtained in a solvent. It happens to chemically occur by the crystal break down into individual ions, atoms or molecules and their transport into the solvent. Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence (USP, 2024).

3.8 Marketing Authorization

Marketing Authorization (MA) is the approval assigned by a regulatory body of a given country or region for the commercialization of a pharmaceutical product.

The complete MA application file comprises of four (4) parts;

- Pharmaceutical (galenical and analytical),
- Toxicological,
- Pharmacological and
- Clinical parts which is presented in the “CTD” (Common Technical Document) format which also contains quality, safety and efficacy data of the drug.

The CTD helps to define the product precisely, the composition, the manufacturing conditions and quality control carried out on the raw materials, production process and final products (Hir, J.C.Chaumeil, & D.Brossard, 2009).

Materials And Methods

4 Materials and Methods

Our work was mainly concentrated on quality control of Levetiracetam 500mg within LDM throughout the different processes from the reception of the starting materials to the finished product.

The work was carried out in different laboratories which include; the physico-chemical quality control laboratory, microbiological quality control laboratory and in process control laboratory.

4.1 Laboratoires de Diagnostic Maghrébins – LDM Company Description

Figure 7 LDM company logo

LDM is an industrial and commercial group operating in the pharmaceutical sector, which has a production unit that conforms to international standards with strict compliance with global standards in this area, as well as a performant distribution network.

LDM Group has developed over the two decades 5 main activities:

a) Manufacturing for other parties (Toll or under license)
b) Manufacturing of generic products, contributing to LDM's large generics portfolio.
c) Importation & distribution.
d) Exploitation (Regulatory Affairs & Pharmacovigilance Management)
e) Medical & commercial promotion (Algeria Exporters, 2021).

The history of LDM dates back to 1997 when the ELAMMOUCHI brothers decided to found a medicine import company in Constantine in the EL-Khroub region. With the experience acquired

over the years and a constantly growing number of customers, the company then created its first production unit.

The factory is located in the industrial zone of Oued Hamimime in El Khroub, wilaya of Constantine.

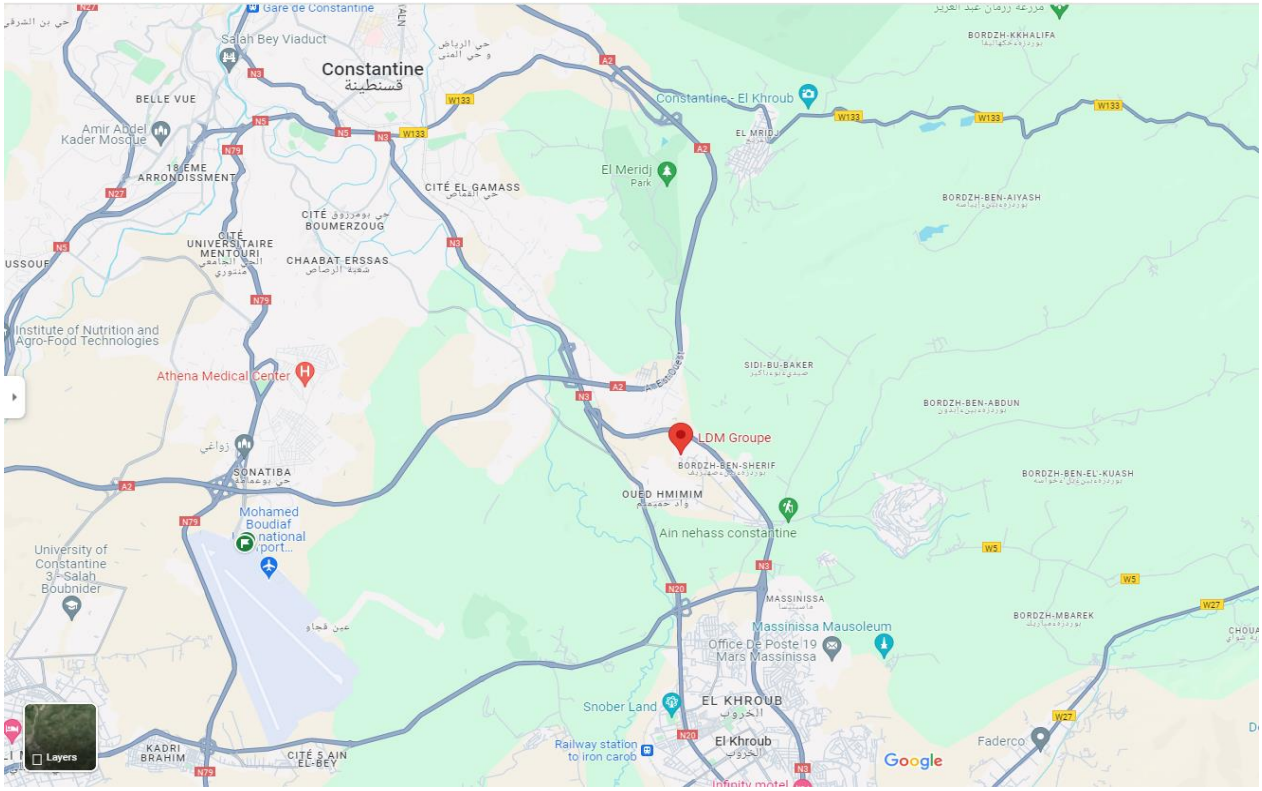


Figure 8 Map showing he location of LDM *(Google Maps, 2024)*

4.1.1 Different therapeutic classes manufactured by LDM

In LDM, all the usual forms are manufactured, namely dry forms (tablets, capsules and sachets), paste forms (gels, creams and ointments) and liquid forms (LDM Groupe, 2024).

These pharmaceutical dosage forms belong to different therapeutic classes such as;

- Antipsychotics,
- Antihypertensive,
- Non-steroidal Anti-inflammatory drugs,
- Antiplatelet agents,
- Antispasmodics,
- Antifungals,

- Lipid-lowering drugs (Atorvastatin LDM 10 mg, 40mg and 80mg),
- Antipyretics,
- Anti-angina drugs,
- Antiepileptic (Levetiracetam 250mg and 500mg) and
- Analgesics (Ryma & Rayene, Contrôle qualité physico-chimique et microbiologique de l'Atorvastatine LDM 10mg, 2018).

4.1.2 Different Departments in LDM

The LDM site is made up of the;

1. Reception and Administrative offices,
2. Production unit,
3. Materials and reagents storage area,
4. Raw and finished products storage area,
5. Quality control laboratories,
6. Water/ waste water treatment plant.

The Quality Control department at LDM is subdivided into several functional units:

- ✓ Microbiological quality(MCQ) control laboratory.
- ✓ In Process Control (IPC) laboratory.
- ✓ Physico-chemical quality(PCQ) control laboratory.

The PCQ control laboratory is subdivided into three teams which are;

The research and development (R&D) team

This team develops and analyses new medicaments that are not available on the market.

Routine team

This team analyses medicaments that are produced by the company and are already established on the market (known drugs).

Raw materials analysis team

This team deals with the analysis of the raw active pharmaceutical ingredients, excipients as wells as the pharmaceutical packaging items such as the PVC, Aluminum, the notice and the package or packaging boxes.

4.2 Physicochemical Quality Control of Levetiracetam LDM 500mg

Physico-chemical quality control of Levetiracetam was carried out on the raw materials, intermediate product through the different steps of the production of Levetiracetam and final on the finished product with the goal of ensuring that a product that conforms to standards or norms as referenced in the Technical File and in the 11th Edition of the European Pharmacopoeia is produced.

4.2.1 Physicochemical Quality Control of Raw Materials

Tests were carried out on the API and the excipients to ascertain their conformance to prespecified specifications according the Technical File and to Eur. Ph and to also ensure that rightful ingredients are used in the formulation.

4.2.1.1 Active Pharmaceutical Ingredient (Levetiracetam)

The following controls were effected to ascertain the conformity of the raw materials before production;

- **Organoleptic Characteristics**

✓ **Appearance**

The appearance of the Levetiracetam (active ingredient) from the batch (several drums) (picking a sample from each drum) was visually examined.

✓ **Solubility**

The solubility of Levetiracetam was studied in different solvents as shown in the Table 6 below.

Table 6 Different solvents used to test for the solubility Levetiracetam

Solvents	Mass of Levetiracetam (mg)	Volume of the Solvent (ml)
Water	100	100
Acetonitrile	10	
Heptane	10	

- **Identification**

The protocol provided several tests on the identification test but we worked with two identification tests as follows:

✓ **Specific rotational power**

The specific rotational power of Levetiracetam was determined using a polarimeter at a temperature of 20°C. To do this, 0.5g of Levetiracetam was dissolved in 25ml of purified water.

The specific rotational power (RP) is calculated according to the following formula:

$$[\alpha] = \frac{d}{c \times l}$$

In which:

[**α**]: Specific Rotation measured in degrees.

d: Observed Optical Rotation angle measured in degrees.

c: Levetiracetam concentration (g/L).

l: Length of the optical cell (cm).

Figure 9 Polarimeter (Kruss)

✓ **Identification by Infra-Red Spectrophotometry**

The identification of the active ingredient was carried out using a Fourier Transform Infrared Spectrophotometer (FTIR) of the Perkin Elmer type (Figure 10). To do this, a sufficient quantity of the active ingredient was placed on the clean sample compartment made of diamond and then pressure was applied in order to record the Infrared spectra.

The background and blank IR absorption is taken beforehand to make sure the instrument provides the correct substance absorption spectrum. The spectrum of Levetiracetam was compared with a Chemical Reference Standard (CRS) of Levetiracetam.

Figure 10 Fourier Transform Infrared Spectrophotometer (FTIR) of the Perkin Elmer type

- **Tests**

✓ **Determination of Water Content**

Principle:

Determination of Water Content in a substance is based on the oxidation of Sulfur dioxide (SO_2) by Iodine (I_2) in presence of water and a base like Pyridine and Methanol. Methanol is used to dissolve the analyte. The reaction produced is;

$$\mathbf{H_2O + I_2 + SO_2 + CH_3OH + 3RN \rightarrow [RNH]SO_4CH_3 + 2[RNH]I}$$

The water content in the active ingredient was determined by the Karl Fischer method. To do this, 0.30g of Levetiracetam and 30 ml of anhydrous methanol were mixed in the Karl Fischer beaker, then the dosage was carried out automatically by the titrator (Figure 11). The water content was displayed on Karl Fischer screen (KF). The water content result must be less than 0.5%**.**

Figure 11 Karl Fischer Titrator

- ✓ **Sulfated Ashes**

Principle:

The Residue on Ignition (Sulfated Ash) test uses a procedure to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid. This test is usually used for determining the content of inorganic impurities in an organic substance.

This test makes it possible to quantify the inorganic substances contained in Levetiracetam. To do this, an empty crucible was heated to 600°C for 30 min in a muffle furnace in the Figure 12 below. After cooling in a desiccator on silica gel, the crucible was weighed (**W1**), then 1g of the Levetiracetam was introduced into the crucible and weighed (**W2**), the substance was moistened with 1 ml to 2 ml of sulfuric acid and heated gently on a hot plate until there was no longer any white fumes, then the substance was calcined at 600°C in the muffle furnace for 2 hours, then the crucible was cooled and weighed again (**W3**). The percentage of Sulfuric Ashes (**SA%)** is calculated as follows;

$$SA\% = [\frac{\mathrm{W2} - \mathrm{W3}}{\mathrm{W2} - \mathrm{W1}}]$$

Figure 12 Muffle furnace

- ✓ **Impurity G test**

The impurity G test is used to detect and quantify the concentration of impurity G in the active ingredient using an HPLC.

Solutions were prepared for the assay performed using HPLC in given chromatographic conditions as shown in (Appendix 1).

Procedure:

We separately injected 50 µl of the Blank, Solution to be examined, Control Solution (b) and Control Solution according to the protocol with the following injection sequence;

Sequence of injections

Table below (Table 7) shows the injection sequence of Impurity G test on the API.

Table 7 Sequence of injections for the Impurity G Assay test

No	**Injections**	**Number of injections**
1	Blank	1
2	Control (c)	2
3	Control (b)	5
4	Solution to examine	2

Identification of impurities: We used the chromatogram obtained with the reference solution (b) to identify impurity G (RRT approximately 3.8).

System Compliance:

Resolution must at least 5.0 between the peaks due to Levetiracetam et impurity G in the control solution (c).

The titer of impurity G was determined by the following formula as shown in Appendix 3.

- ✓ **Related substances**

The test of related substances on the active ingredient at identifying and quantifying known and unknown impurities that would probably be due to its synthesis, its polymorphic forms, degradation etc.

Solutions were prepared for the assay performed using HPLC in given chromatographic conditions in gradient flow mode as shown in (Appendix 1).

Procedure:

We injected separately in gradient mode 10 µl of the Blank, Solution to be examined (a), Control Solution(a), Control Solution (b) and Control Solution (c).

Injection sequence:

Injection sequence for related substance test on the API is mentioned in the table below (Table 8).

Table 8 Injection sequence for the Related Substances test

No	**Injections**	**Number of injections**
1	Blank	2
2	Control (a)	2
3	Control (b)	5
4	Control (c)	2
5	Solution to examine (a)	2

Identification of impurities: We used the chromatograms obtained with the control solution (a) to identify the peaks due to impurities A and E, and the chromatogram obtained with the control solution (c) to identify the peak due to impurity C.

Relative Retention Times

Table below (Table 9) shows the Relative Retention Times for the Related Substances test on the API.

Table 9 Relative Retention Times for the Related Substances test

Substance	Relative Retention Time (RRT)
Levetiracetam	1.0 (around 11min)
Impurity C	0.5
Impurity A	0.7
Impurity E	0.9

System compliance:

Resolution must at least 3.5 between the peaks due to impurity E and Levetiracetam in the control solution (a).

NB: Exclusion limit: ≤ 0.03%, or < 0.19 in RRT except for impurity C.

The titers of the related substances were determined by the following formulas as shown in Appendix 3.

- ✓ **Assay of Levetiracetam by High Performance Liquid Chromatography**

The Assay of API (Levetiracetam) was as indicated in the Related Substances Test with the following modification.

Injection: solution to be examined (b) and control solution (d) as shown in Table 10.

Table 10 Injection sequence for the Assay of the API

No	**Injections**	**Number of injections**
1	Control (d)	5
2	Solution to examine (b)	2

The titer of Levetiracetam was determined by the following formula as shown in Appendix 3.

4.2.1.2 Excipients

Levetiracetam LDM 500mg formulation contains a number of excipients and each of them is required to undergo specific physicochemical quality controls before production to ascertain their conformity to specified standards as referenced in the Technical File or the Pharmacopoeia. Each excipient has its own protocol for the physicochemical quality controls. The tests are generally the same as those of the API and the include the following;

- Organoleptic and characteristics test which include; Appearance, Solubility test.
- Identification tests which include; Identification by IR, Melting Point, Thin Layer Chromatography (TLC).
- Tests which include; acidity and appearance of solution, Conductivity, related substances, Loss On Drying, Sulfated Ashes, Specific Rotation (LDM, LDM Levetiracetam Technical File, 2024).

4.2.2 Physico-chemical Quality Control of Levetiracetam LDM 500mg during manufacturing

Quality controls during manufacturing consist of monitoring and verifying the production cycle. These tests include certain in process controls that are used to that the intermediate mixture conforms to requirements of the Technical File and the 11th edition of the European Pharmacopoeia. These controls also help to validate the manufacturing processes and the efficiency of the different manufacturing equipment.

4.2.2.1 After Blending and Drying

- ✓ **Determination of the active ingredient in the mixture by HPLC**

The test was carried out under the same operating conditions as the dosage of Levetiracetam LDM 500mg as a finished product, with the aim of ensuring the correct distribution of the active ingredient in the mixture.

- ✓ **Loss On Drying (LOD)**

The residual humidity of the intermediate mixture of Levetiracetam and excipients was measured by the loss on drying method using a Mettler Toledo type IR desiccator (figure 13).

To do this, 5g of the powder is put on an aluminum tray. Then, the device automatically measures the mass loss of the powder dried at 105°C to constant weight. Moisture content is calculated by comparing the initial weight of the sample to the final weight of the dried sample.

Figure 13 Mettler Toledo type IR desiccator/ Moisture Analyzer

LOD was determined by the following formula;

$$LOD = \frac{\text{Weight Loss}}{\text{Weight of the Sample}}$$

4.2.2.2 After granulation and calibration

Loss On Drying was also measured after granulation like in the test above and the resulting granules had to be calibrated using a granulometry sieve (Figure 14) which helps to ensure the safety, efficacy, and uniformity of drugs by analyzing and controlling the particle size distribution of materials used in pharmaceutical manufacturing processes.

Figure 14 Granulometry sieve

4.2.2.3 After Compression

A number of Tests were carried out to verify the conformity of the uncoated tablets of Levetiracetam LDM 500mg, these tests include the following;

- ✓ **Appearance**

Levetiracetam LDM 500mg tablets were visually checked to verify the shape, size and color. To do this, a sample tablets were examined by eye to confirm that the tablets were manufactured correctly.

- ✓ **Average Mass and Mass Uniformity**

Twenty (20) tablets of Levetiracetam LDM 500mg tablets were sampled randomly during the compression process and weighed individually using a 0.001g precision balance (Figure 15). The average mass is calculated according to a statistical model which determines the Maximum, the Minimum and the average weight.

Figure 15 Radwag precision balance

- ✓ **Thickness**

The Thickness of the Levetiracetam tablets was measured by using a Vernier caliper (Figure 16). The Vernier calliper gives reading in millimeters (mm).

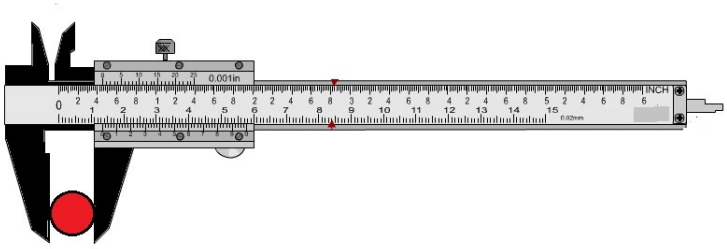


Figure 16 Vernier calliper

- ✓ **Hardness**

Hardness was measured using a Durometer (Tablet Hardness Tester) shown in the figure below (figure 17) on the 21 tablets taken at random. The tablet was placed between the jaws taking into account its shape. At the moment of rupture, the device indicates the force exerted in Newton (N).

Figure 17 Tablet Hardness Tester

Friability

The friability test was carried out (every one hour) during production by a friability meter shown in the figure (figure 18). To do this, 44 tablets were taken, dusted with absorbent paper then weighed to determine the initial mass (M1). Then, they were introduced into the drum of the friability meter. The device was set for a test duration equal to 4min and a number of revolutions equal to 25rpm. After the time had elapsed, the tablets were removed and wiped again and weighed (M2).

Figure 18 Friabilator

The friability rate is expressed as the percentage of mass loss according to the formula below;

$$F = \frac{\mathrm{M1} - \mathrm{M2}}{\mathrm{M1}}\ x\ 100$$

- ✓ **Disintegration**

This test is intended to determine the disintegration time of the tablets (before coating) in a purified water with stirring. To do this, 6 tablets were placed in the 6 cylindrical tubes (one tablet per vessel), then immersed in a water bath at 37.5°C ± 0.5°C (figure 20). Disintegration is achieved when there is no longer any solid residue at a calculated time.

4.2.2.4 After coating

The following controls are realized after their coating;

- ✓ **Appearance**

Coated Levetiracetam LDM 500mg tablets were visually checked to verify the shape, size and color to ensure that the tablets were coated correctly.

- ✓ **Average mass and Mass Uniformity**

Coated Levetiracetam LDM 500mg were sampled randomly after the coating process and weighed individually using a 0.001g precision balance. The average mass is calculated according to a statistical model which determines the Maximum, the Minimum and the average weight.

- ✓ **Disintegration**

This test is intended to determine the disintegration time of the coated tablets in a purified water using the same operating conditions like the previous disintegration test.

4.2.2.5 During and After Packaging

Leak tests were carried out at the end of primary packaging. To do this, the blisters were immersed in the vacuum chamber (Figure 19) filled with water and methylene blue under a pressure of 5 bars for 5 min. The blisters were dried with absorbent paper and then checked visually to confirm the absence of colored water in the cells.

Figure 19 Vacuum leak testing Apparatus

4.2.3 Physico-chemical Quality Control of Levetiracetam LDM 500mg (finished product)

After the manufacturing and packaging of Levetiracetam LDM 500mg tablets, a series of tests were carried out in order to ascertain that the finished product meets and conforms to given specification before the product is cleared to be released in the market. These tests include the following;

4.2.3.1 Appearance

The appearance of Levetiracetam LDM 500mg, was visually examined.

Principle: It's an evaluation carried out to ensure that the appearance of the tablet, including its color, shape, size, markings and the texture of its surface meets the specified standards. It involves visual inspection by trained personnel to detect any defects or deviations from the desired appearance criteria.

These tests are critical for maintaining consistent quality for the tablets.

4.2.3.2 Average Mass and Mass Uniformity

This test involves weighing each tablet in the sample to calculate the average mass of the tablets to determine mass uniformity of the tablets.

Analytical method used:

- ✓ Twenty (20) tablets of Levetiracetam LDM 500mg were picked at random from a sample from the batch to be controlled.

- ✓ The mass of each tablet was weighed individually using a high precision balance (0.001g), and the average mass of the 20 tablets was determined,
- ✓ The individual mass of tablet was then compared.

The average mass of the twenty tablets to must fall in the interval as specified in the norm.

4.2.3.3 Identification by UV-Visible Spectrometry

A UV-Visible Spectrometer was used to determine the presence of the rightful substances and the presence of the correct API in the tablet formulation.

The absorption spectrum of the test solution must correspond to that of the standard solution when the spectra are examined.

Method of operation:

- ✓ Two solutions were prepared; the standard and the test solutions as indicated in Appendix 1
- ✓ The solutions were analyzed using a quartz cuvette because quartz does not absorb in the ultraviolet range, hence not creating any interference.
- ✓ Absorption spectra of the two solutions were recorded using an interval of 200nm-400nm and 0.1N HCl as a blank (for calibration).

4.2.3.4 Disintegration test

A tablet was put in each of the six (6) vessels of the disintegration apparatus (Figure 20) while using purified water as the disintegration medium. All tablets must dissolve before the determined time (minutes or seconds) for the test to be conform.

Figure 20 Disintegration Apparatus

4.2.3.5 Water Content

We tested on a 500 mg sample of the powder tablets using a Karl Fischer titrator (Figure 11). We Placed 30 ml of Anhydrous Methanol in the Karl Fisher beaker and titrated with the Karl-Fisher reagent and then finely grinded the tablets. We quickly transferred a quantity of 500 mg of powder into the Karl-Fisher beaker. The Karl Fischer titrator titrated automatically up to the equivalent point.

The water content in % of Levetiracetam was calculated using the following formula:

$$\%(eau) = \frac{Vx\ F}{P} x\ 100$$

V = Volume of Karl-Fisher reagent necessary for the determination of the test portion.

P = Weight of the test portion. (in grams).

F = Karl-Fisher reagent factor.

4.2.3.6 Dissolution Test

This test was carried out by a paddle dissolution apparatus (Figure 21) in six glass vessels, the aim of which is to evaluate the quantity or concentration of the active ingredient solubilized in an artificial gastrointestinal medium over a given period of time. This test is also used to access and determine the liberation time of the API.

The dissolution is affected by the following factor which must be considered during the test;

- Type of stirrer (paddle or basket),
- Temperature,
- Volume of the medium,
- Rotation rate and,
- pH.

The medium is selected based on the characteristics of the tablet and the intended route of administration.

During the test, samples are withdrawn at a predetermined time interval from the medium and analyzed to obtain the concentration of the tablet released.

The concentration is measured using either HPLC system which is used to construct dissolution profiles that show the percentage of the API released over time. The dissolution profiles are then compared against an acceptation criteria specified in pharmacopeia to ensure that the dosage form releases the drug in a consistent manner.

Operating mode

To realize this test, 6 tablets of Levetiracetam LDM 500mg from the lot were placed in baskets

filled with 900 ml of purified water at pH 7. The temperature of the medium aqueous solution was equilibrated at 37.0°C ± 0.5°C.

The tablets were subjected to dissolution for 30 minutes with a paddle rotation speed of 50 rpm. After 30 minutes a sample of 10 ml was collected from each vessel. The determination of the percentage of the active ingredient released was carried out by HPLC using the same operating conditions as in the Assay.

Acceptance criteria;

- Based on the dissolution profile comparison is; Q = 80% after 30minutes.
- $Q + 5 \geq 85\%$ after 30 minutes.

Figure 21 Dissolution Apparatus (Electrolab)

4.2.3.7 Assay

An assay is an analysis used to determine the presence of the API and its concentration in the tablet formulation.

The assay of the active ingredient present in the Levetiracetam LDM 500mg tablets was carried out by High Performance Liquid Chromatography (HPLC) (Shimadzu) (Figure 22), coupled with UV/visible spectrophotometry. The different solutions prepared as shown in Appendix 1, were filtered, introduced into the vials and placed in the HPLC tray for analysis under specific chromatographic conditions (Appendix 1).

The procedure

20µl of the standard solution (5 injections) and three (3) injections of the test solution were separately injected.

The validity of the HPLC system was established by determining the following parameters;

- Number of theoretical plates(N) should be (greater or equal to) ≥ 3000
- Relative standard deviation(RSD) (%) between the areas should be (less or equal to) ≤ 2%
- Tailing factor should be (less or equal to) ≤ 2

The dosage of Levetiracetam is calculated as shown in Appendix 3.

Acceptance criteria: the content of Levetiracetam must be between [95.0% -105.0%].

Figure 22 High Performance Liquid Chromatograph (Shimadzu)

4.2.3.8 Active ingredient Content Uniformity test

This test is required in order to verify the uniformity of the quantity of API throughout all Levetiracetam LDM 500mg tablets from the same batch. This test was carried out in the same operating conditions as the assay of the active ingredient using the same standard solutions.

Ten (10) tablets from the Average mass test were taken were individually weighed. Each tablet was assayed individually to determine its API content using HPLC.

Acceptance criteria;

The acceptance value should be ≤ 15

4.2.3.9 Related Substances

The main objective of this test is to control, identify, quantify organic impurities that could be as a result of the formulation process, degradation the API or impurities that arise during the synthesis of the API present in the tablet formulation. This test ensures that the tablets meets the purity standard.

Solutions were prepared and the assay on the related substances was carried out under the chromatographic conditions as shown in Appendix 1.

The product must meet the following acceptance criteria to pass this test;

a. LVTIMP01(known impurity) ≤ 0.1%
b. LVTIMP02(known impurity) ≤ 0.15%
c. Unknown impurities ≤ 0.1%
d. Total impurities ≤ 0.5

The procedure

50µl of the diluent, placebo solution, standard solution (5 injections) and the test solution were separately injected into the HPLC system.

The validity of the HPLC system was established by determining the following parameters;

- Number of theoretical plates(N) should be ≥ 3000
- Relative standard deviation(RSD) (%) between the areas should be ≤ 5%
- Tailing factor should be ≤ 2

The dosage for each impurity (known and unknown impurity) is determined as shown in Appendix 3.

Table 11 Relative retention time, Relative response factor and Limit of Quantification (LOQ)% for the known to be considered.

Known impurities	Relative retention time	Relative response factor	Limit of Quantification (LOQ)%
LVTIMP 01	0.65	2.32	0.0010
Levetiracetam	1.00	-	0.0035
LVTIMP02	1.81	1.01	0.0071

4.3 Microbiological Quality Control of Levetiracetam LDM 500mg

These tests depend on the nature of the drug (physicochemical properties) and route of administration.

Microbiological control of Levetiracetam LDM 500mg is carried out on the raw materials and the finished product but our work concentrated on the microbiological control of the finished product because they are generally the same tests.

Microbiological control of Levetiracetam LDM 500mg consists of the;

1. **Microbial enumeration** which encompasses tests which allow the counting of Total Viable Aerobic Mesophilic (TVAM) germs and the counting of Total Yeasts and Molds (TYM).
2. **The search for specified microorganisms** which entails tests that make it possible to verify the absence, or limited presence, of specified microorganisms such as *Escherichia coli*, Salmonella, *Staphylococcus aureus* and *Pseudomonas aeruginosa* that can be detected under determined conditions (LDM, LDM Levetiracetam 500mg Microbiological Control Protocol, 2024).

The microbiological controls of Levetiracetam LDM 500mg using the 11th edition European Pharmacopoeia and the Reference Sheet Specifications: SC PFLDMXXX/XX as the reference.

The purpose of this control is to ensure good hygienic quality and avoid the danger of microbial contamination.

4.3.1 Microbiological quality control of Levetiracetam LDM 500mg (Final Product).

The preparation method of the depends on the physical and chemical characteristics of the product to be analyzed.

Microbiological control of the finished product requires the preparation of the sample as follows:

1) For each batch of LACETAM® 500mg to be analyzed, take 5 samples at random and at different levels of secondary packaging.
2) Briefly flame the blisters with the rising heat of the Bunsen burner flame.
3) Aseptically transfer the equivalent of 10g of the product (18 tablets) of LACETAM® 500mg into a sterile bottle.
4) Dissolve the tablets in 90ml Sodium chloride peptone buffer solution pH=7.0 to ensure complete dissolution of the film-coated tablets, place the bottle in a water bath at a temperature of 40°C for a few minutes, while stirring using the Vortex from time to time.

- **Sample analysis or examination**

Sterility is an important aspect in microbiological controls and so the analysis must be realized under aseptic conditions and all materials involved in the analysis must be sterile.

The prepared sample necessitated according to the protocol to realize the following tests;

4.3.1.1 Enumeration of total viable mesophilic aerobic germs and total yeasts and molds

The enumeration was carried out using the following technique:

- **Deep seeding** which consists:

✓ Prepare 2 petri dishes per medium or each test.

✓ Introduce 1ml of the sample into each petri dish.

✓ Add 40 to 20ml of liquefied agar medium kept super cooled at 45°C, adapted to each test. (Tryptone Soy Agar (TSA) medium for TVAM and Sabouraud dextrose agar (SDA) medium for TYM).

✓ The different culture media were prepared as shown in Appendix 2.

✓ Mix the bottles carefully using "C" and "8" shaped movements.

✓ Invert the dishes and incubate the TSA culture media at 30 to 35°C (median 33°C) for 3 to 5 days and the SDA media at 20 to 25°C (median 23°C) for 5 to 7 days.

✓ Prepare a negative control for each type of enumeration.

LACETAM 500mg is compliant with the test if the test results will meet the following standards**:**

The table below (Table 12) shows the microbiological standards for enumeration of TVAM and TYM in Levetiracetam LDM 500mg.

The counting was done after the incubation period using a colony counter.

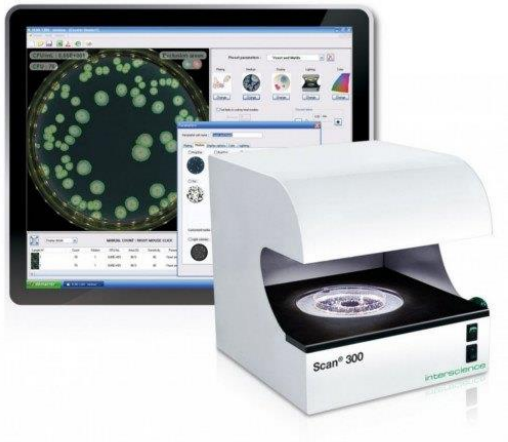


Figure 23 Colony Counter

Table 12 Acceptance criteria for Enumeration of TVAM germs and TYM

Standards Flora Counted	Compliant product	
	Acceptance Limit	Maximum acceptance limit
TVAM	$\leq 10^3$ CFU/g	$\leq 2x10^3$ CFU/g
TYM	$\leq 10^2$ CFU/g	$\leq 2x10^2$ CFU/g

The Colony-Forming Unit (CFU) is calculated using the formula:

$$\textbf{Number of CFU/g} = \frac{N1+N2}{2}$$

Where;

N1: Number of colonies counted on Petri dish 1/dilution.

N2: Number of colonies counted on Petri dish 2/dilution.

- **Search for specific germs**

4.3.1.2 Search for *Escherichia coli*:

Sample preparation and pre-incubation:

- Prepare a sample as described above in sample preparation section.
- Inoculate 100ml of the Tryptic Soy Broth (TSB) liquid medium with 10ml of the sample. TSB is a general purpose liquid enrichment medium used for cultivation of wide range of bacteria, yeasts, and molds.
- Homogenize and incubate at 30 to 35°C (median 33°C) for 18 to 24 hours.

Selection and subculture:

- Shake the container, then transfer 1ml of the contents into 100ml of MacConkey Broth (MCB) medium. MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from non-fermenting gram-negative bacteria.
- Incubate the vials at 42 to 44°C (median 43°C) for 24 to 48 hours.
- Subculture on MacConkey Agar (MCA) medium, incubation is done at 30 to 35°C (median 33°C) for 18 to 72 hours, the boxes turned over in addition to the negative control.

Colony growth indicates the possible presence of *Escherichia coli* which must be confirmed by identification tests.

LACETAM 500mg is compliant if none of the colonies of the types described are observed or if identification confirmation tests are negative.

4.3.1.3 *Staphylococcus aureus* research:

Sample preparation and pre-incubation:

- Prepare a sample as described in sample preparation.
- Inoculate 100 ml of the TSB liquid medium with 10 ml of the 1/10 dilution.
- Homogenize and incubate at 30 to 35°C (median 33°C) for 18 to 24 hours.

Selection and subculture:

- Identify a Petri dish of Mannitol-Salt Agar medium (MSA) (Chapman), previously poured and Solidified. MSA is a semi-synthetic selective culture medium used for the selection of halophilic bacteria, particularly those that ferment mannitol.
- Cultivate onto Chapman medium.
- Invert the plates and incubate at 30-35°C (median 33°C) for 18 to 72 hours with the negative control.

LACETAM® 500mg is compliant if none of the colonies of the types described are observed

or if the identification confirmation tests are negative.

The growth of yellow/white colonies surrounded by a yellow zone indicates the possible presence of *Staphylococcus aureus*, confirmed by identification tests.

4.3.1.4 Search for *Pseudomonas aeruginosa*:

Sample preparation and pre-incubation:

- Prepare the sample as described in sample preparation.
- Inoculate 100ml of the TSB liquid medium with 10ml of the 1/10 dilution.
- Homogenize and incubate at 30 to 35°C (median 33°C) for 18 to 24 hours.

Selection and subculture:

- Identify a Petri dish of Cetrimide medium previously poured and solidified.
- Transplant onto Cetrimide agar medium.
- Invert the plates and incubate at 30-35°C (median 33°C) for 18 to 72 hours with the negative control.

LACETAM 500mg complies with the test if no colonies are observed or if the identification confirmation tests are negative.

Colony growth indicates the possible presence of *Pseudomonas aeruginosa* confirmed by identification tests.

4.3.1.5 Search for Salmonella:

Sample preparation for Salmonella:

- Prepare the 1/10 dilution sample as described in the "sample preparation" section but using TSB medium as diluent.
- Incubate at 30 to 35°C (median 33°C) for 18 to 24 hours.

Selection and subculture:

- Transfer 0.1ml of TSB liquid medium into 10ml of Violet Red Bile Glucose (VRBG). VRBG medium is used to favor and detect the growth of the Enterobacteriaceae group which includes; glucose -fermenting coliforms, lactose-fermenting strains of *E. coli* and lactose-fermenting species such as Salmonella and Shigella.
- Incubate at 30 to 35°C (median 33°C) for 18 to 24 hours.
- Subculture onto Xylose-Lysine-Deoxycholate (XLD) agar medium. XLD agar is a selective growth medium used in the isolation of Salmonella and Shigella species
- Invert the plates and incubate at 30 to 35°C (median 33°C) for 18 to 48 hours with the negative control.

The growth of well-developed red colonies, with or without a black center, indicates the possible presence of Salmonella to be confirmed by identification tests.

LACETAM® 500mg complies with the test if no colonies are observed or if the identification confirmation tests are negative.

The table below (Table 13) shows the microbiological standards for search of different germs in Levetiracetam LDM 500mg.

Table 13 General Acceptance criteria for microbiological quality control

Parameters	Standards
Research for germs	
TVAM germs	$<10^3$ UFC/g
TYM	$<10^2$UFC/g
Research for specific germs	
Escherichia coli	Absence/g
Staphylococcus aureus	Absence/g
Pseudomonas aeruginosa	Absence/g
Salmonella	Absence/10g

Results and Discussion

5 Results and discussion

The results provided in the section below represent the results of the physicochemical and microbiological quality study on Levetiracetam LDM 500mg. All results obtained were compared to the specifications in the Technical File and European Pharmacopoeia in order to determine the product's compliance.

5.1 Physico-chemical Quality Control of Raw Materials

5.1.1 Active Pharmaceutical Ingredient (Levetiracetam)

5.1.1.1 Physical Characteristics

The results of the appearance and the solubility of Levetiracetam are summarized in the Table below (Table 14).

Table 14 Results for the physical Characteristics of the active ingredient (Levetiracetam)

Characteristics	Specifications	Results	Conformity
Appearance	○ White or substantially white powder.	− White powder.	Compliant
Solubility	○ Very soluble in water, ○ Soluble in acetonitrile and ○ Practically insoluble in heptane.	− Very soluble in water, − Soluble in acetonitrile and − Practically insoluble in heptane.	Compliant

The results confirm the compliance of API (Levetiracetam) to the standards of the above examination as indicated in the reference documents hence giving a go ahead to next tests.

5.1.1.2 Identification of Levetiracetam

Specific rotational power

The results of Identification of Levetiracetam by using its Specific rotational power are summarized in the table below (Table 15).

Table 15 Results of the Identification test of Levetiracetam by using its Specific rotational power

	Specifications	Reading	Conformity
Specific rotational power	○ Between -82° to -76°.	− -79°	Compliant

Identification by Infra-Red Spectrophotometry

When experimented by the FTIR Spectrophotometer, the IR spectrum of the test corresponded to that of the standard as shown in the figures below.

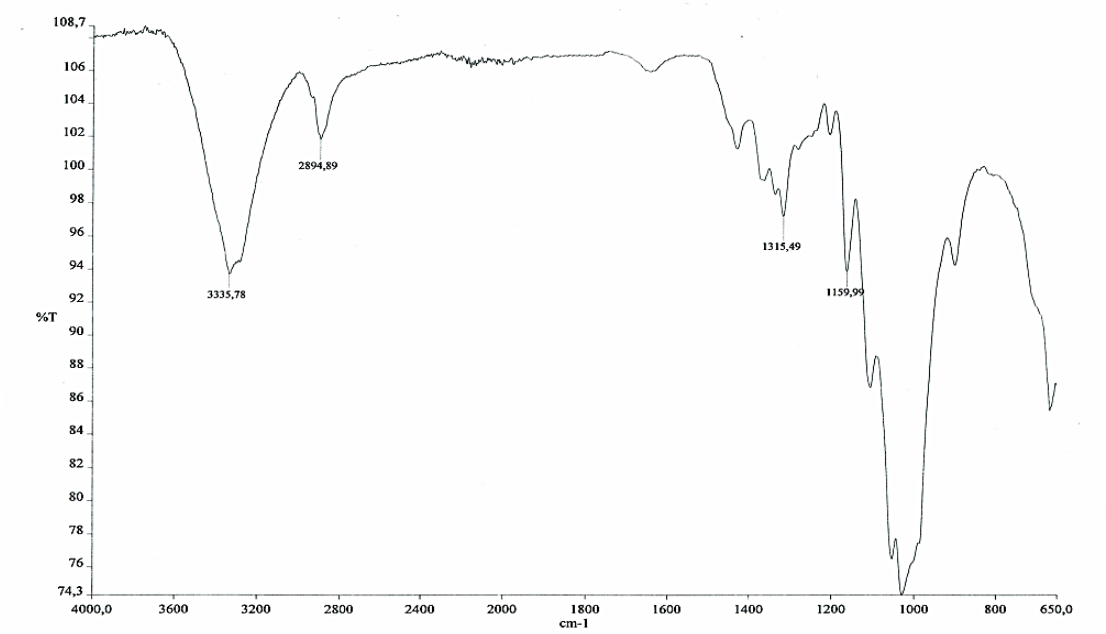


Figure 24 IR Absorption Spectrum of Levetiracetam test sample

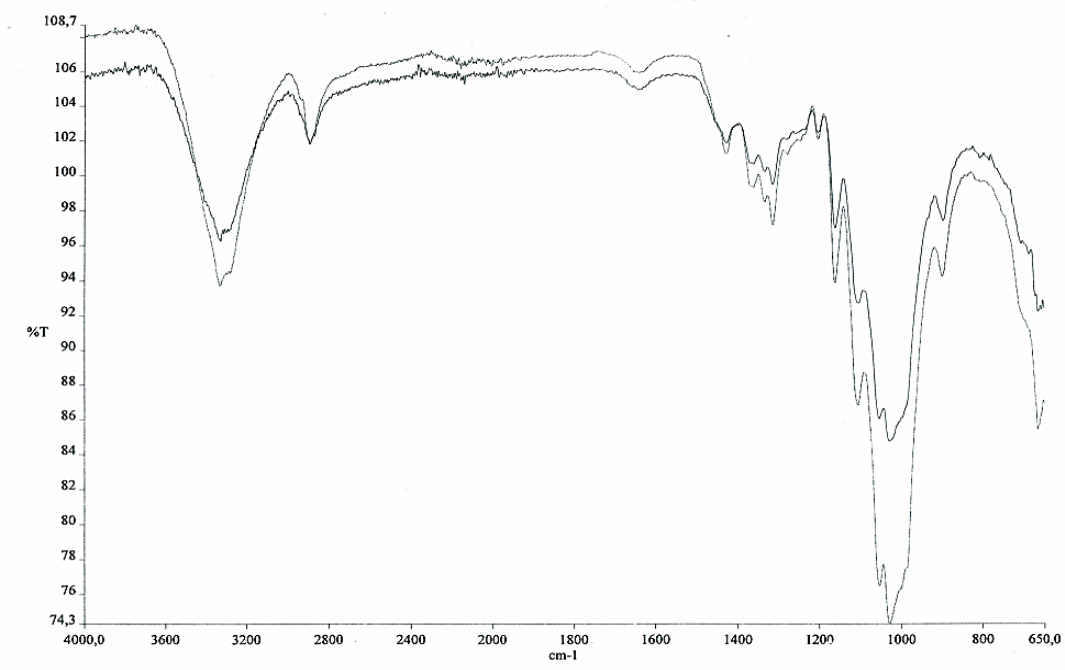


Figure 25 IR Absorption Spectra of Levetiracetam test sample and the standard sample superimposed on each other

The identification test results confirm the identity of the API (Levetiracetam) as indicated in the reference documents hence giving a go ahead to next tests.

5.1.1.3 Tests

Results for the different tests done on the API (Levetiracetam) are summarized in the table below (Table 16).

Table 16 Summary of Results for the different tests done on the API (Levetiracetam)

Test	Standard	Results	Conformity
Water Content	○ Water Content ≤ 0.5%.	− 0.25%.	Compliant
Sulfated Ashes	○ $SA\% \leq 0.1\%$.	− $SA\% = 0.00\%$.	Compliant
Impurity G test	○ ≤ 0.05%.	− 0.00% (Calculation shown in Appendix 3).	Compliant
Related substances	○ Impurity A: ≤ 0.3%. ○ Impurity C: ≤ 250 ppm. ○ Unspecified impurities: ≤ 0.05% (for each impurity). ○ Total impurities: ≤ 0.4%.	− Impurity A = 0.00%. − Impurity C = 0 ppm. − Unspecified impurities = 0.00% (for each impurity). − Total impurities = 0.00%.	Compliant
Assay	○ 98.0% to 102.0% percent (anhydrous substance).	− Levetiracetam % = 99.09%(Appendix 3)	Compliant

According to table 21, the results demonstrate compliance of the API to pharmacopoeial standards hence the API is fit to be used in the Levetiracetam LDM 500mg and so it can be declared compliant and cleared for production.

5.2 Physico-chemical Quality Control of Levetiracetam LDM 500mg (finished product)

5.2.1 Appearance

The results of the appearance of Levetiracetam LDM 500mg, is presented in the table below (Table 17).

Table 17 Appearance results of Levetiracetam LDM 500mg tablets.

Test	Specifications	Results	Conformity
Appearance	Film coated tablet with a yellow score line, oblong.	Film coated tablet with a yellow score line, oblong.	Compliant

5.2.2 Average Mass and Mass Uniformity

These results were obtained after weighing the 20 tablets and are summarized in the table below (Table 18).

Table 18 Mass uniformity results of Levetiracetam LDM 500mg tablets.

Test	Standard	Results	Conformity
Average Mass and Mass Uniformity	**574.0mg ± 3% [556.78mg – 591.22mg]** At most, maximum of 2 tablets may deviate from the average mass by± 5% but no tablet must deviate from Am ± 10%.	Minimum = 570mg Maximum = 581.54mg Average Mass = **572.923mg** (Appendix 3)	Compliant

The results obtained from the test above show that the tablets conform to the mass specifications of the Technical File and Eur. Ph.

5.2.3 Identification by UV-Visible Spectrometry

UV absorption spectrum of the test solution corresponds to that of the standard solution as shown in the figure below. This confirms that tablets contain Levetiracetam as the API.

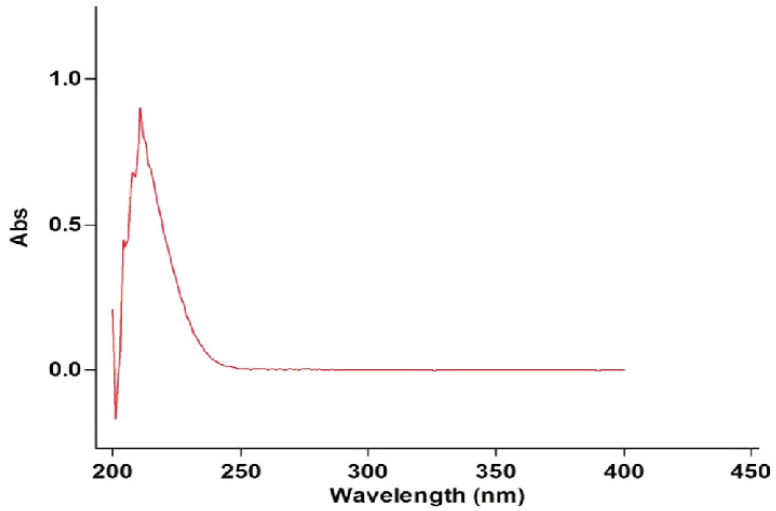


Figure 26 UV absorption spectrum of the Levetiracetam LDM 500mg test solution

5.2.4 Disintegration test

The disintegration results are shown in the table below (Table 19).

Table 19 Levetiracetam LDM 500mg tablets Disintegration test results

Test	**Standard**	**Results**	**Conformity**
Disintegration	Disintegration time ≤ 30 minutes	Disintegration time = 4min 55secs	Compliant

From the results, the disintegration time taken for the Levetiracetam LDM 500mg tablets to completely disintegrate into particles was 4mins 55secs which when compared to the standard provided in the European pharmacopeia is found to be in the acceptance criteria. Hence the dosage form is considered to be compliant.

5.2.5 Water Content

The water content results Levetiracetam LDM 500mg tablets are shown in the table below (Table 20).

Table 20 Water content results of Levetiracetam LDM 500mg tablets

Test	**Standard**	**Results**	**Conformity**
Water content	Water content ≤ 2.5%	Water content = 0.57%	Compliant

The results obtained from the water content test shows that the content of water found in Levetiracetam LDM 500mg tablets is 0.57% which is less that the standard 2.5% stated in the European pharmacopeia, hence the final product is compliant.

5.2.6 Dissolution Test

The Dissolution Test results of six Levetiracetam LDM 500mg tablets are shown in table below.

The results in **table 21** show that the RSD is 0.4%**,** Relative standard deviation is also called percentage relative standard deviation which is the deviation measurement that tells us how the different numbers in a particular data set are scattered around the mean. This formula shows the spread of data in percentage.

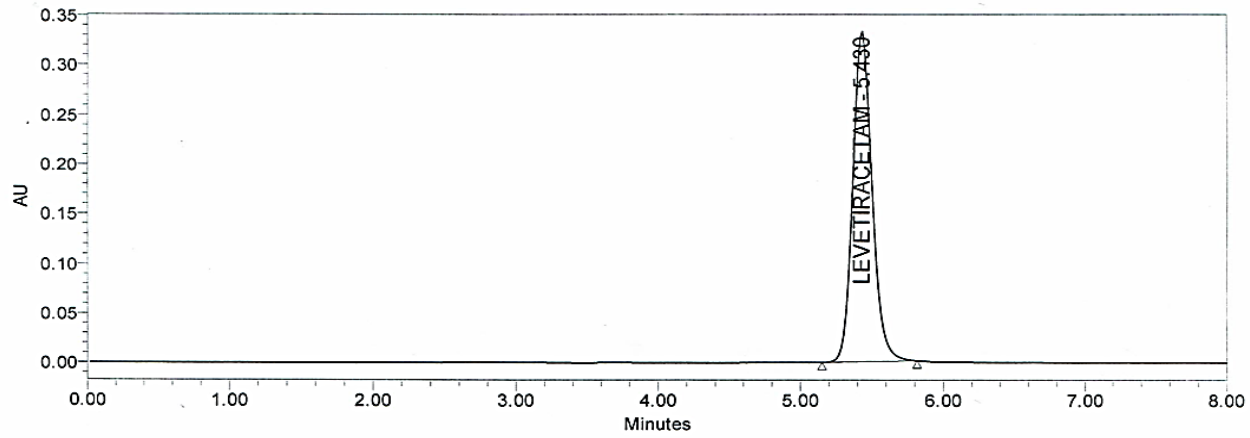
The number of theoretical plates is 7662.46, Theoretical plates are a measuring tool of HPLC column efficiency.

The tailing factor is 1.14, these results affirm the conformity of the HPLC system according to the requirement of the European Pharmacopoeia 11th edition. The average retention time of the six Levetiracetam LDM 500mg tablets is 5.45 minutes, this value is close to that of the standard which is 5.43min which makes it possible to confirm the identity and purity of the active ingredient.

Table 21 Dissolution Test results of six Levetiracetam LDM 500mg tablets

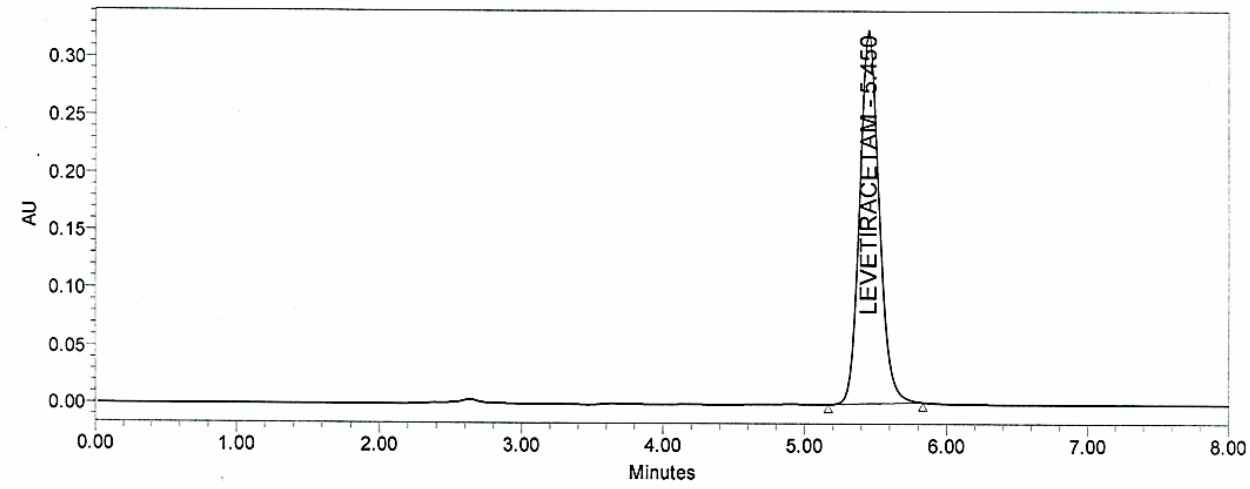
Test	Standard	Results	Conformity
Dissolution	Theoretical plates: ≥ 3000	Average Theoretical plates = 7662.46 (STD)	Compliant
	Tailing factor: ≤ 2	Average Tailing factor = 1.14185783	
	Retention time: Close or identical to the retention time of the standard (5.430 minutes).	Retention time: - Tb1: 5.45min - Tb2: 5.452min - Tb3: 5.452min - Tb4: 5.456min - Tb5: 5.460min - Tb6: 5.462min Average = 5.456min (Appendix 4)	
	Q ≥ 80%	Minimum: 97.46% Maximum: 98.51% Q = 97.93% (Appendix 3)	

The chromatograms of the corresponding tablets and the standards are shown below and results for other tablets from 2 to 6 are shown in Appendix 4.



	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.430	3157374	100.00	7.894724e+003	1.130332e+000

Figure 27 Chromatogram of the Levetiracetam LDM 500mg standard solution for the dissolution test



	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.450	3110857	100.00	7.703520e+003	1.137957e+000

Figure 28 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 1) in the dissolution test

The percentage of API (Q) dissolved in each of the six tablets of Levetiracetam LDM 500mg after the 30minutes is 97.93%, this value is greater than 80%. The calculation of Q is shown in Appendix 3 according to the 9th edition European pharmacopoeia standard, the result is compliant.

All the results show that the finished product (Levetiracetam LDM 500mg) is compliant and

that the tablets dissolve in immediate release.

5.2.7 Active ingredient Content Uniformity test

Since this test was carried out in the same operating conditions as the assay of the active ingredient using the same standard solutions so results for system conformity are the same.

Individual content of each tablet was calculated and the acceptance value determined according to criteria below.

The results of the contents uniformity of Levetiracetam LDM 500mg is shown in the table below (Table 22).

Table 22 Contents uniformity results of Levetiracetam LDM 500mg tablets

Test	**Standard**	**Results**	**Conformity**
Contents uniformity	Acceptance Value (AV) ≤ 15 for 10 tablets.	AV = 1.58	Compliant

The acceptance value is calculated using the following criteria;

- ✓ Their Individual content of the ten (10) tablets was calculated using the formula;

$$\%Xi = \frac{Mi \times T}{Mm} \times 100$$

Where;

Xi: Individual content.

Mm: Average mass of the batch.

Mi: Individual mass of the tablets (mg).

T: Dosage of the batch (%).

$\bar{x}$: Average content of the 10 tablets.

If $98.5\% \leq \bar{x} \leq 101.5\%$, M = $\bar{x}$ **;** VA = kS

$\bar{x} \leq 98.5\%$; M = 98.5 ; VA = 98.5 - $\bar{x}$ + kS

$\bar{x} \leq 101.5\%$; M = 101 ; VA = $\bar{x}$ - 101.5 + kS

AV: Acceptance value

k: Acceptance constant (k = 2.4 for n = 10; k = 2.0 for n = 30)

S: Sample standard deviation

The results show that the acceptance value was found to be 1.58 which complies within the standards of the European pharmacopeia. Hence the product is in compliant.

Assay

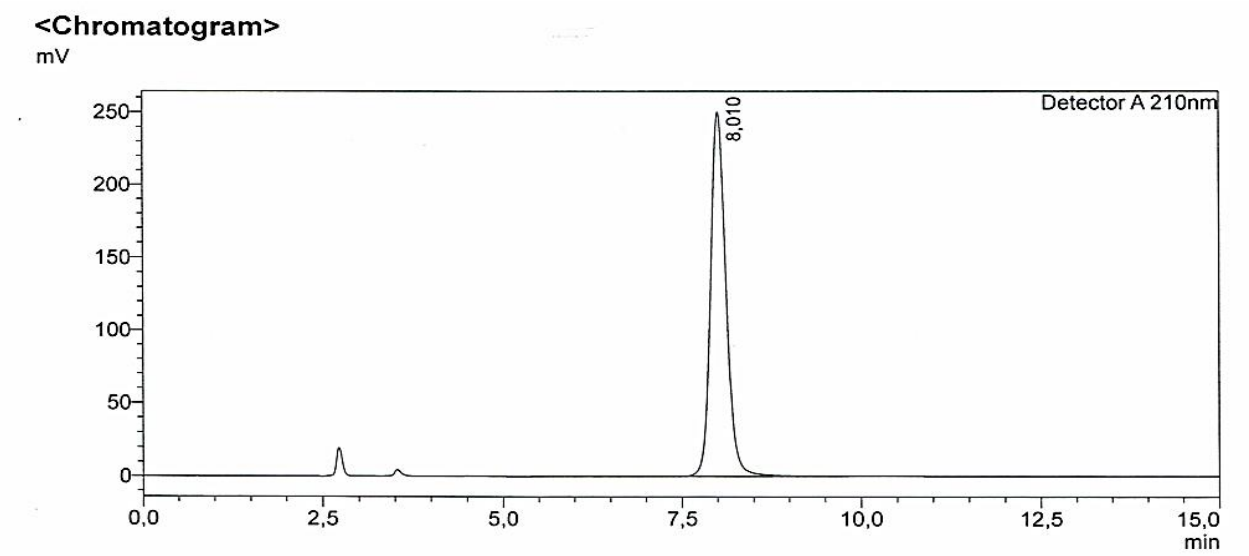
The results of the Levetiracetam LDM 500mg dosage are shown in Table 23.

The results in **Table 23** show that the RSD (0.1%), the tailing factor (1.14) and the number of Theoretical plates (7696) comply with the standards of the European Pharmacopoeia 11th edition. HPLC system is therefore compliant.

Table 23 Assay results of Levetiracetam LDM 500mg

Test	**Standard**	**Results**	**Conformity**
Assay by HPLC	RSD ≤ 2%	0.1%	Compliant
	Theoretical plates: ≥ 3000	7696	
	Tailing factor: ≤ 2	1.14	
	Retention time: Close or identical to the retention time of the standard (**8.010 minutes**).	Retention time = **8.010 minutes.**	
	Percentage of Levetiracetam must be between [95.0% - 105.0%].	Percentage of Levetiracetam = **100.42%** (Appendix 3)	

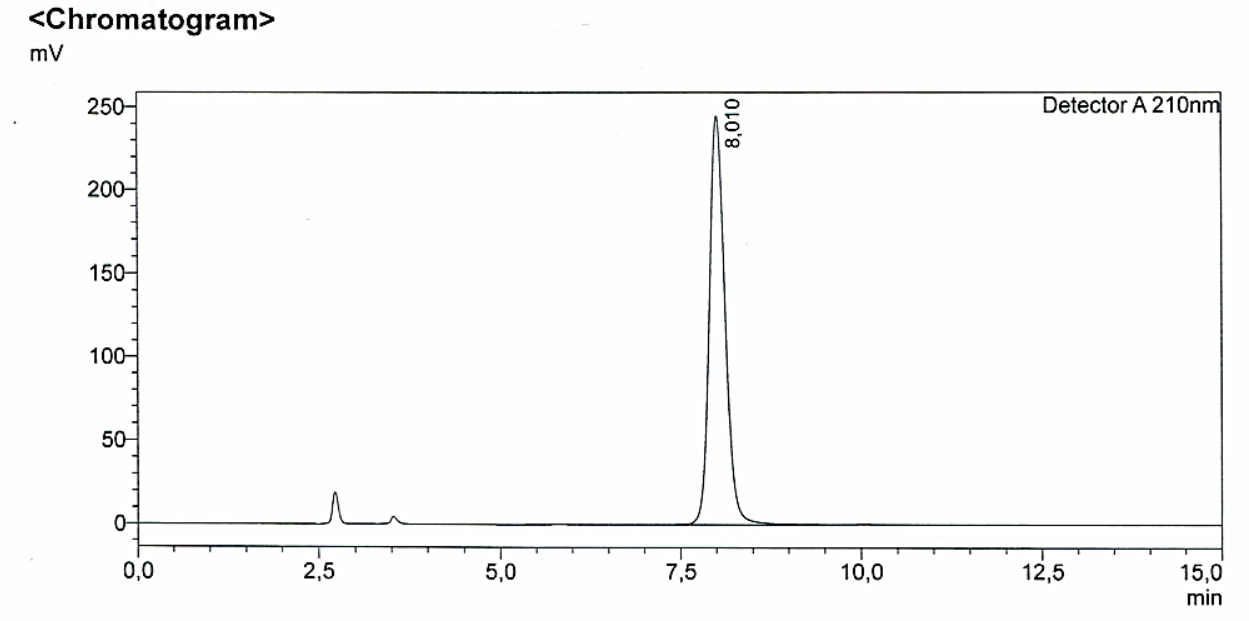
The retention time of the test solution (figure 30) is the same as that of the standard solution (figure 29), which confirms the identity of the active ingredient. The result of the percentage of the active ingredient is 100.42%. This value falls in the interval (95.0% -105.0%) as referenced in the European Pharmacopoeia hence the test is compliant.



<Peak Table>
Detector A 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8,010	3756566	250108	1,000	mg/L		Levetiracetam
Total		3756566	250108				

Figure 29 Chromatogram of the Assay test of Levetiracetam standard solution



<Peak Table>
Detector A 210nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	8,010	3721555	244954	1,000	mg/L	V	Levetiracetam
Total		3721555	244954				

Figure 30 Chromatogram of the Assay test of Levetiracetam LDM 500mg test solution

5.2.8 Related substances

The results of the dosage of related substances of Levetiracetam LDM 500mg tablets

are summarized in table 24.

Table 24 Results for the Dosage of related substances of Levetiracetam LDM 500mg

Test	**Standard**	**Results**	**Conformity**
Assay by HPLC	RSD ≤ 5%	0.74%	Compliant
	Theoretical plates: ≥ 3000	4547	
	Tailing factor: ≤ 2	0.905	
	Retention time: Close or identical to the retention time of the standard (**8.183min**).	Retention time: **7.942min**	
	e. LVTIMP01(known impurity) ≤ 0.1% f. LVTIMP02(known impurity) ≤ 0.15% g. Unknown impurities ≤ 0.1% h. Total impurities ≤ 0.5	- LVTIMP01(known impurity) = 0.00% - LVTIMP02(known impurity) = 0.00% - Unknown impurities = 0.00% - Total impurities 0.00% (Appendix 3)	

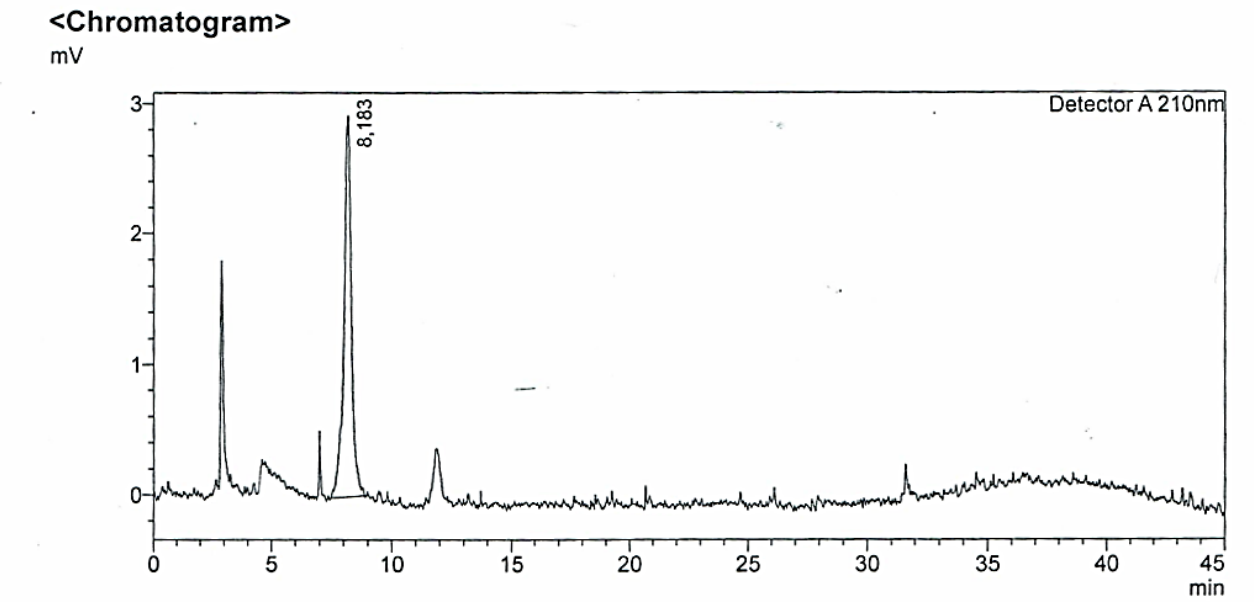
According to the table, the RSD is 0.74%, which is less than 5%. This result affirms the compliance of the HPLC system.

The retention time of the Levetiracetam solution is 7.942 minutes (Figure 32), and the Retention time of the standard Levetiracetam solution is 8.183 minutes (Figure 31), both values are close, which confirms the identity, position of the active ingredient (Levetiracetam) and the position of impurities on the chromatograms.

From the chromatogram's obtained, unknown impurities were not detected in the test solution which signifies that there's no degradation of the API or no impurities arose from the formulation process.

The results obtained also show that 0.00% of the two known impurities (LVTIMP01, LVTIMP02) was detected which leads to 0.00% of the total impurities. All results are in compliance with the standard stated in the European pharmacopeia. Hence the product is said to be compliant.

The peaks in the chromatogram of the placebo and diluents are thereby ignored because they only help to determine if the assumed impurities are not due to the excipients or the diluents used in the preparation of the test.



<Peak Table>

levetiracetam 250 mg + 500 mg R36.lcm_01042024_levetiracetam_std 1_2_009.lcd

Detector A 210nm

Peak#	Ret. Time	Area	Height	Name
1	8,183	62606	2924	levetiracetam
Total		62606	2924	

Number of Theoretical Plate(USP)	Tailing Factor
4547	0,905

Figure 31 Chromatogram for the Standard Solution in the dosage of related substances in Levetiracetam LDM 500mg

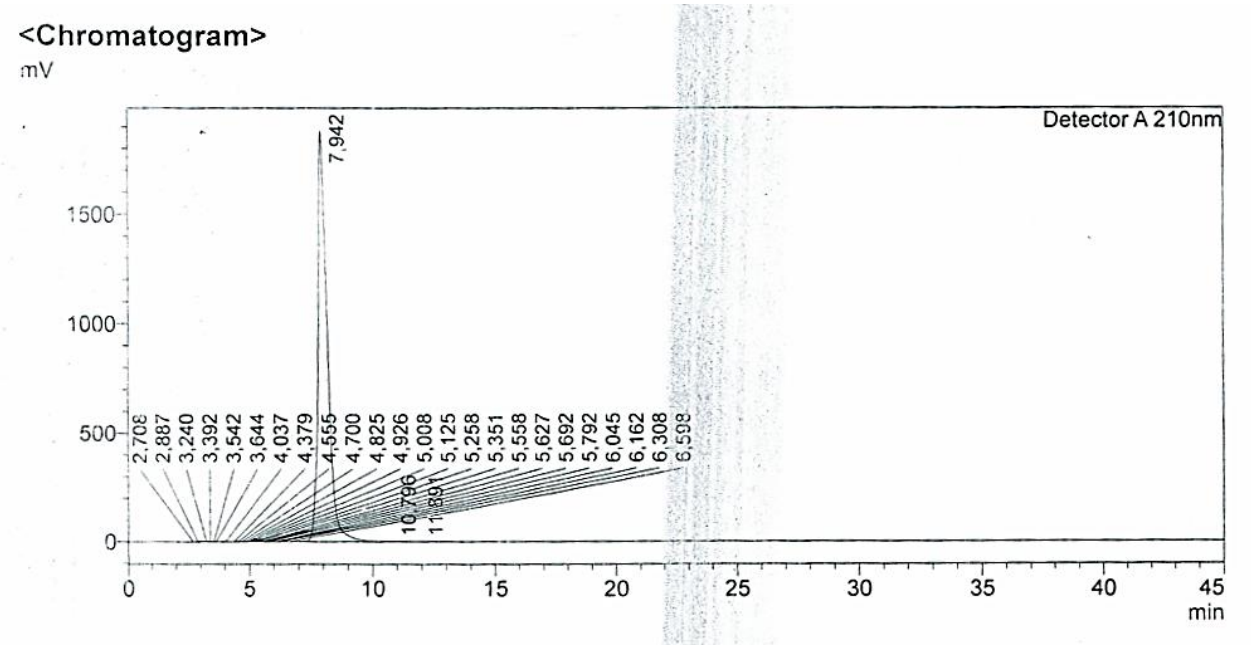


Figure 32 Chromatogram for the test solution in the dosage of related substances in Levetiracetam LDM 500mg

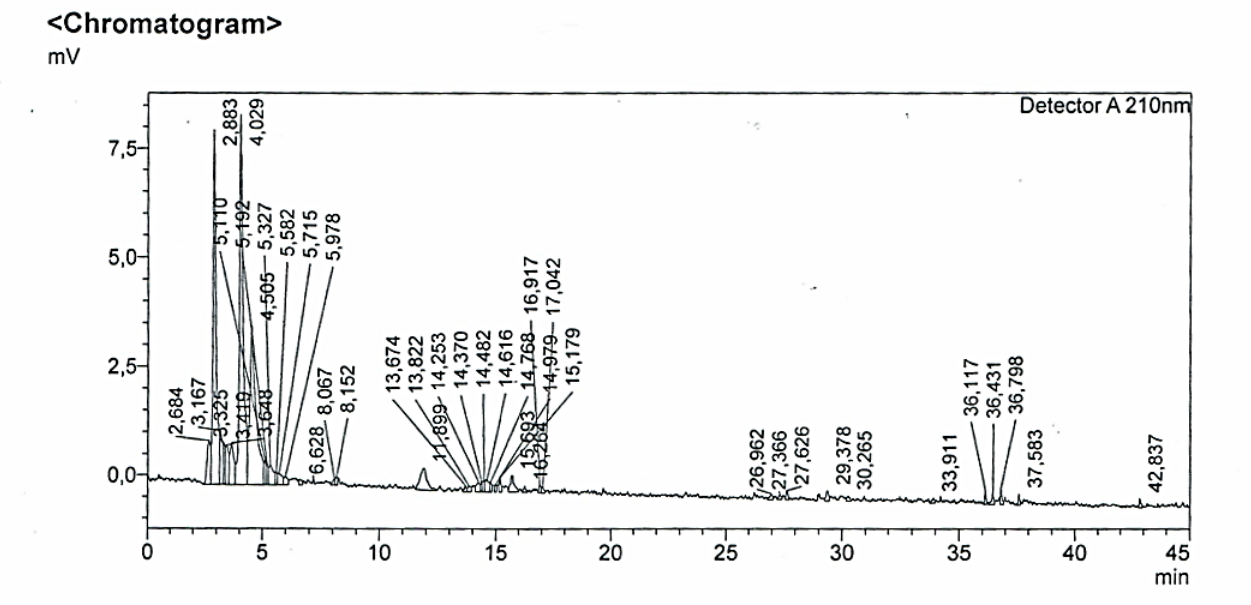


Figure 33 Chromatogram for the Placebo solution in the dosage of related substances in Levetiracetam LDM 500mg

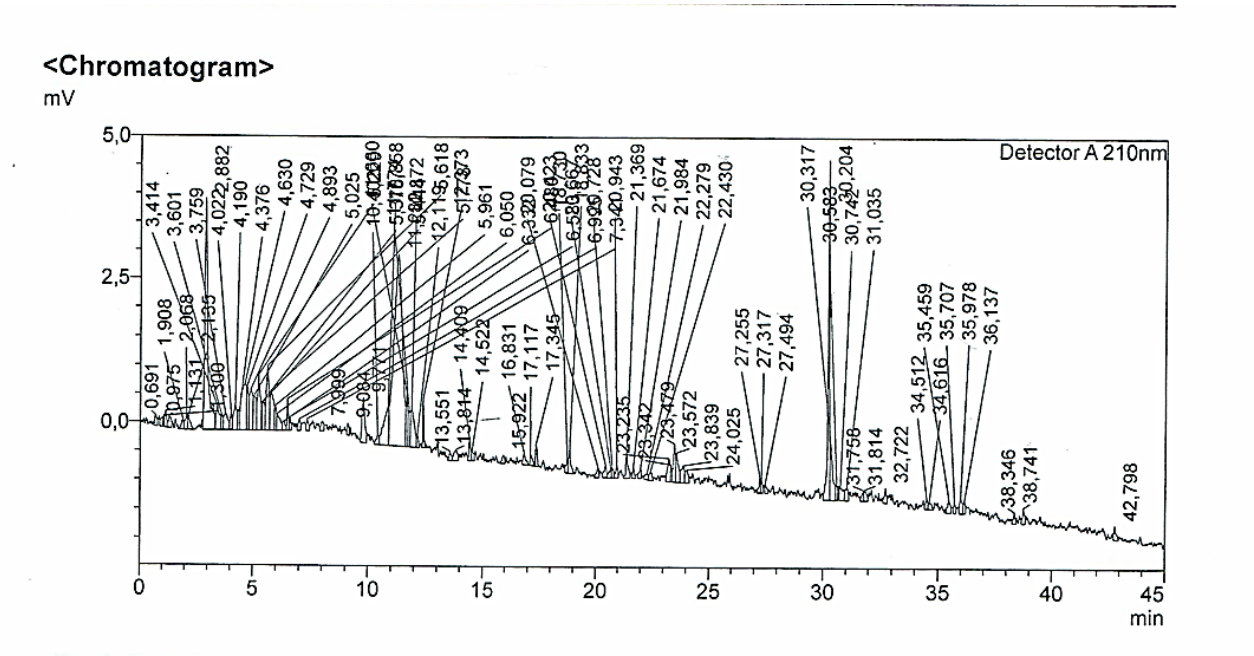


Figure 34 Chromatogram of the diluent in the dosage of related substances in Levetiracetam LDM 500mg.

5.3 Microbiological Quality control of the finished product

The enumeration of TVAM, TYM, was carried out using the colony counter, the results obtained were expressed in Colony Forming Unit per gram (CFU/g) of Levetiracetam LDM 500mg, while the search for *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and Salmonella was carried out by visual examination then confirmed by identification tests if necessary.

The results obtained from the microbiological quality control analysis of the final product of Levetiracetam LDM 500mg are presented in the table below (Table 25).

Table 25 Microbiological quality control results for Levetiracetam LDM 500mg (final product)

Tests	**Standard**	**Results**	**Conformity**
TVAM	$<10^3$ CFU/g	00 CFU/g	**Compliant**
TYM	$<10^2$ CFU/g	00 CFU/g	
Research for *Escherichia coli*	Absence/g	Absence/g	
Research for *Staphylococcus aureus*	Absence/g	Absence/g	
Research for *Pseudomonas aeruginosa*	Absence/g	Absence/g	
Research for *Salmonella*	Absence/10g	Absence/10g	

These results show that there is complete absence of total viable aerobic microorganisms, total molds and yeasts and also the specifically researched germs (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella*) hence the product complies to the microbiological specifications as referenced in the Technical File and European Pharmacopoeia.

This good microbiological quality of the product was achieved due to the rigorous adherence to the GMPs and GLPs.

Conclusion and Outlook

6 Conclusion and perspectives

Our work at LDM pharmaceutical industry allowed us to focus on the essential techniques applied in the physico-chemical and microbiological quality control of Levetiracetam 500mg, a tablet formulation, according to the analytical methods stated in the European Pharmacopoeia and the internal pharmaceutical file (Technical File).

This study on the physicochemical and microbiological control of an Oral Dosage form, was realized to provide us with deeper insights on the subject of Quality Control.

For Quality Control to provide compliant results there is need to have a strong quality assurance system which entails all the good pharmaceutical industry practices. These practices must be adhered to rigorously.

The compliance of a pharmaceutical product and validity of a process must be determined by prespecified norms that can be Pharmacopoeial or found in the Technical File of the product.

Pharmaceutical Quality cannot be compromised due to how significantly it can impact the final consumers' health or wellbeing, the manufacturer but also the integrity of the product its self.

All the results of the tests carried out allow us to conclude that the product is compliant and fit to be availed to the market.

Summary

Summary

This research work discusses drugs in general, their classification, pharmacology, the different stages of production of Levetiracetam 500mg, an anti-epileptic drug a tablet formulation manufactured at LDM pharmaceutical industry.

This work is basically composed of the different physico-chemical and microbiological quality control tests carried out on the above mentioned drug as a finished product, its raw materials, and the tests carried out during the process of its production.

The quality control process begins with the raw materials at the point of reception before they are cleared for use in production. The raw materials are first kept in quarantine before being declared compliant. Test are carried out on each batch of the raw materials to ascertain their identity, composition, physical and chemical characteristics as well as microbiological purity before they are cleared for production.

The microbiological analysis helps to determine the absence or presence of specific germs and also helps to enumerate the total viable aerobic microorganisms, total molds and yeasts whose concentration in case of their presence must be in alignment with pharmacopoeial or Technical File requirements.

The quality control process is followed by in process controls which entail tests which help to validate the processes of manufacturing, efficiency of the equipment and to detect errors that can't be corrected after the product is finished.

Finally, the quality control process ends with tests on the final product to ascertain the conformance of the product's specifications to prespecified norms after which the product is determined either compliant or non-compliant.

The results of this research which explored various tests attest that the final product produced at the company is in full compliance with the standards in European pharmacopeia and the Technical File. Hence Levetiracetam 500mg of the examined batch is considered to be of good pharmaceutical quality and authorized to be distributed on the market.

Keywords: Tablets, Levetiracetam 500mg, Quality control, Microbiological quality control, physico-chemical quality control, European pharmacopeia, Technical File.

Resumé

Ce travail de recherche aborde les médicaments en général, leur classification, leur pharmacologie, les différentes étapes de production du Lévétiracétam 500 mg, un médicament antiépileptique sous forme de comprimé fabriqué dans l'industrie pharmaceutique LDM.

Ce travail est essentiellement composé des différents tests de contrôle de qualité physico-chimiques et microbiologiques effectués sur le médicament mentionné ci-dessus en tant que produit fini, ses matières premières, et des tests effectués au cours du processus de sa production.

Le processus de contrôle qualité commence avec les matières premières au point de réception avant qu'elles ne soient autorisées à être utilisées dans la production. Les matières premières sont d'abord conservées en quarantaine avant d'être déclarées conformes. Des tests sont effectués sur chaque lot de matières premières pour vérifier leur identité, leur composition, leurs caractéristiques physiques et chimiques ainsi que leur pureté microbiologique avant leur autorisation pour la production.

L'analyse microbiologique permet de déterminer l'absence ou la présence de germes spécifiques et permet également de dénombrer la totalité des micro-organismes aérobies viables, les moisissures totales et les levures dont la concentration en cas de présence doit être conforme aux exigences de la pharmacopée ou du dossier technique.

Le processus de contrôle qualité est suivi de contrôles de processus qui impliquent des tests permettant de valider les processus de fabrication, l'efficacité de l'équipement et de détecter les erreurs qui ne peuvent pas être corrigées une fois le produit terminé.

Enfin, le processus de contrôle qualité se termine par des tests sur le produit final pour vérifier la conformité des spécifications du produit aux normes prédéfinies, après quoi le produit est déterminé soit conforme, soit non conforme.

Les résultats de cette recherche qui a exploré différents tests attestent que le produit final fabriqué dans l'entreprise est en totale conformité avec les normes de la pharmacopée européenne et du Dossier Technique. Le Lévétiracétam 500 mg du lot examiné est donc considéré comme de bonne qualité pharmaceutique et sa distribution sur le marché est autorisée.

Mots clés : Comprimés, Lévétiracétam 500mg, Contrôle qualité, Contrôle qualité microbiologique, contrôle qualité physico-chimique, Pharmacopée Européenne, Dossier Technique.

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Appendix

Appendix

Appendix 1

Assay

Impurity G test on the Active Ingredient

The following solutions were prepared as follows;

- Buffer Solution pH 3.0
- **Mobile phase:** Acetonitrile / Buffer Solution (15:85 V/V).
- **Solution to examine:** Levetiracetam.
- **Control solution (a):** Levetiracetam SCR impurity G.
- **Control solution (b):** Dilution of solution (a).
- **Control solution (c):** Mixture of the control solution (a) and the solution to be examined.
- **Blank:** Use mobile phase as blank.

The following Chromatographic conditions were required for this test;

Chromatographic conditions

Table 26 Chromatographic conditions for the Impurity G Assay test

Column	C18 250mm*4.6mm*5µm
Flow	1 ml/min.
Wave length	200nm
Injection volume	50µl
Temperature	27°C
Analysis time	5 times the retention time of Levetiracetam (approximately 4 minutes).

Preparation of solutions:

Buffer Solution:

Dissolve 1.22 g of Sodium decanesulfonate in 850 ml in purified water, add 1.3 ml of Phosphoric acid. Adjust to pH 3.0 with a 200g of Potassium hydroxide solution and make up to 1000 ml with purified water.

Mobile phase:

Acetonitrile / Buffer Solution (15:85 V/V).

Solution to examine:

Dissolve 20 mg of **Levetiracetam** in the mobile phase and make up to 10 ml with the mobile phase.

Control solution (a):

Dissolve 2.0 mg of **Levetiracetam SCR impurity G** in the mobile phase and make up to 100 ml with the mobile phase.

Control solution (b):

Take 1.0 ml of control solution (a) and make up to 20 ml with the mobile phase.

Control solution (c):

Take 1.0 ml of the control solution (a) and 1.0 ml of the solution to be examined and make up to 20 ml with the mobile phase.

Blank:

Use mobile phase as blank.

Related substances test on the Active Ingredient

The following solutions were prepared as follows;

Phosphate Buffer solution pH 5.5

Mobile phase A: Acetonitrile/ buffer solution.

Mobile phase B: Acetonitrile.

Solution to be examined (a): Levetiracetam in the mobile phase (A).

Solution to be examined (b): Solution to be examined (a) and mobile phase (A).

Control solution (a): Mixture of Levetiracetam SCR impurity A, Levetiracetam SCR impurity E, the solution to be examined (a) in the mobile phase (A).

Control solution (b): Dilution of the Solution to be examined (a) in the mobile phase (A)

Control solution (c): Dilution of Levetiracetam SCR impurity C with the mobile phase (A).

Control solution (d): Dilution of Levetiracetam SCR with the mobile phase (A).

Blank: Use mobile phase (A) as blank.

The following Chromatographic conditions were required for this test in a gradient pumping mode as follows;

Chromatographic conditions

Table 27 Chromatographic conditions for the Related Substances test

Column	C18 150mm*4.6mm*5µm
Flow	0.9 ml/min
Wave length λ	205nm
Injection volume	10µl
Temperature	Room Temperature

Table 28 Gradient mode for the Related Substances test

Time (min)	**Mobile phase A**	**Mobile phase B**
0	100	0
3	100	0
20	71	29
25	71	29
26	100	0
30	100	0

Preparation of solutions:

Phosphate Buffer solution pH 5.5

Mix 964 ml of a solution of potassium dihydrogen phosphate (13.61 g/lt) in purified water and 36 ml of a solution of disodium hydrogen phosphate (35.81 g/l) in purified water.

Mobile phase A: Acetonitrile/ buffer solution (5:95) (v/v)

Mobile phase B: Acetonitrile.

Solution to be examined (a): Dissolve 50 mg of Levetiracetam in the mobile phase (A) and make up to 10 ml with the mobile phase (A)

Solution to be examined (b): Take 1.0 ml of solution to be examined (a) and make up to 50 ml with the mobile phase (A).

Control solution (a): Dissolve 5.0 mg of Levetiracetam SCR impurity A and 5.0 mg of Levetiracetam SCR impurity E in the mobile phase (A), add 1.0 ml of the solution to be examined (a) and make up to 100 ml with the mobile phase (A).

Control solution (b): Take 1.0 ml of solution to be examined (a) and make up to 100 ml with the mobile phase (A). Take 1.0 ml of this solution and make up to 20 ml with the mobile phase (A)

Control solution (c): Dissolve 5.0 mg of Levetiracetam SCR impurity C and make up to 100 ml with mobile phase (A). Take 1.0 ml of this solution and dilute to 40 ml with the mobile phase (A).

Control solution (d): Dissolve 50.0 mg Levetiracetam SCR and make up to 10 ml with mobile phase (A). Take 1.0 ml of this solution and dilute to 50 ml with the mobile phase (A).

Blank: Use mobile phase (A) as blank.

Identification of the finished product by HPLC

Preparation of solutions:

Standard solution:

50mg of standard Levetiracetam was weighed in a volumetric flask of 100ml, then dissolved using 60ml of 0.1N HCL in an ultrasonic bath for about 5mins. The volume was then completed using the same solvent and mixed properly for homogeneity.1ml of this solution prepared was diluted with 50ml of the solvent and mixed.

Test solution:

50mg of the crushed tablets(Levetiracetam) was weighed and dissolved in 60ml of 0.1N HCL in an ultrasonic bath for about 15mins, shaking occasionally. The volume was then completed with the same solvent. The solution was filtered through a syringe filter of 0.4µm, eliminating the first ml.1ml of this solution was diluted with same solvent.

Assay of the finished product

Preparation of the solutions

Preparation of the buffer solution

Weigh and transfer a mass of 6.8g of Potassium dihydrogen phosphate in vessel containing 1000ml of purified water. Magnetically stir to dissolve and then using a filter membrane of 0.45µm filter the solution to remove any particles that would block the HPLC column.

Preparation of the mobile phase

The mobile phase was prepared by mixing the buffer solution and Acetonitrile in proportions of 90/10 respectively (v/v).

Preparation of the diluent

The diluent was prepared by mixing purified water and Acetonitrile in proportions of 80/20 respectively (v/v).

Preparation of the standard solution

The standard solution was prepared by weigh 75mg of Levetiracetam Standard and transferred it in a volumetric flask of 100ml and then dissolved and completed the volume with the diluent.

Dilute 5ml of this solution to 25ml with the diluent and mixed.

Preparation of the test solution

Test solution was prepared by weighing and then grinding 20 Levetiracetam LDM 500mg tablets and then weighing an equivalent mass of the powder to 150mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

5ml of the filtrate was diluted to 50ml with the diluent and then mixed.

Related Substances test on the finished product

Preparation of the solutions

Preparation of Orthophosphoric acid (H_3PO_4)

10ml of Orthophosphoric acid was diluted with purified water in a 100ml volumetric flask.

Preparation of Phosphate buffer pH 3.0

Weigh and transfer a mass of 6.8g of Potassium dihydrogen phosphate in vessel containing 1000ml of purified water. Put in an ultrasonic bath to dissolve and adjust the pH to 3.0 ± 0.05 with diluted Orthophosphoric acid and then using a filter membrane of 0.45µm filter the solution.

Preparation of the diluent

The diluent was prepared by mixing the mobile phase and Acetonitrile in proportions of 95:5 respectively (v/v).

Preparation of the standard solution

The standard solution was prepared by weigh 50mg of Levetiracetam Standard and transferred it in a volumetric flask of 50ml and then dissolved and completed the volume with the diluent and the mixture homogenized.

Diluted 5ml of this solution to 100ml with the diluent and mix. Again diluted 2ml of this solution to 100ml with the diluent and mix.

Preparation of the test solution

Test solution was prepared by weighing and then grinding not less than 20 Levetiracetam LDM 500mg tablets and then weighing an equivalent mass of the powder to 100mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

Preparation of a placebo solution

The placebo solution was prepared by weighing and an equivalent mass of the powder to 100mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

Identification of the finished product by HPLC

Preparation of the solutions

Standard solution:
50mg of standard Levetiracetam were weighed in a volumetric flask of 100ml, then dissolved using 60ml of 0.1N HCl in an ultrasonic bath for about 5minutes. The volume was then completed using the same solvent and mixed properly for homogeneity.
1ml of this solution prepared was diluted with 50ml of the solvent and mixed.
Test solution:
50mg of the crushed tablets (Levetiracetam LDM 500mg) was weighed and dissolved in 60ml of 0.1N HCl in an ultrasonic bath for about 15mins, shaking occasionally. The volume was then completed with the same solvent. The solution was filtered through a syringe filter of 0.4µm, saturating the filter with the solution beforehand.1ml of this solution was diluted with same solvent.

Assay of the finished product by HPLC

The following solutions were prepared;

- Potassium dihydrogen phosphate buffer solution.
- **Mobile phase**: Buffer solution and Acetonitrile 90/10 (v/v).
- **Diluent**: purified water and Acetonitrile 80/20 (v/v).
- **Standard solution**: Levetiracetam Standard diluted with the diluent.

- **Test solution**: Levetiracetam LDM 500mg with the diluent.

The Chromatographic conditions for this assay are presented in table below.

Table 29 Chromatographic conditions for the dosage of API in the finished product

Column	Inertsil ODS 2; (250×4.6)mm; 5µm
Debit	1.0ml/min
Wavelength	210nm
Injection volume	20µl
Temperature	Room Temperature
Analysis time	15 Minutes

Preparation of the solutions

Preparation of the buffer solution

Weigh and transfer a mass of 6.8g of Potassium dihydrogen phosphate in vessel containing 1000ml of purified water. Magnetically stir to dissolve and then using a filter membrane of 0.45µm filter the solution to remove any particles that would block the HPLC column.

Preparation of the mobile phase

The mobile phase was prepared by mixing the buffer solution and Acetonitrile in proportions of 90/10 respectively (v/v).

Preparation of the diluent

The diluent was prepared by mixing purified water and Acetonitrile in proportions of 80/20 respectively (v/v).

Preparation of the standard solution

The standard solution was prepared by weigh 75mg of Levetiracetam Standard and transferred it in a volumetric flask of 100ml and then dissolved and completed the volume with the diluent.

Dilute 5ml of this solution to 25ml with the diluent and mixed.

Preparation of the test solution

Test solution was prepared by weighing and then grinding 20 Levetiracetam LDM 500mg tablets and then weighing an equivalent mass of the powder to 150mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

5ml of the filtrate was diluted to 50ml with the diluent and then mixed.

Related Substances of the finished product by HPLC

The following solutions were prepared;

- **Phosphate buffer pH 3.0**
- **Diluent:** Mobile phase and Acetonitrile in proportions of 95:5 (v/v).
- **Standard solution:** Levetiracetam Standard diluted with the diluent.
- **Test solution:** Levetiracetam LDM 500mg diluted with the diluent.
- **Placebo solution:** An equivalent mass of the powder to 100mg of Levetiracetam diluted with the diluent.

These impurities are identified and quantified using HPLC which results are compared with results of a placebo under the following chromatographic conditions;

Table 30 Chromatographic conditions for the Related Substances test in the finished product

Mobile phase	Phosphate buffer pH 3.0
Column	Xterra RP18 (250×4.6)mm, 5µm
Debit	1.0ml/min
Wavelength	210nm
Injection Volume	50µml
Temperature	Room temperature
Analysis time	45 minutes

Preparation of the solutions

Preparation of Orthophosphoric acid (H_3PO_4)

10ml of Orthophosphoric acid was diluted with purified water in a 100ml volumetric flask.

Preparation of Phosphate buffer pH 3.0

Weigh and transfer a mass of 6.8g of Potassium dihydrogen phosphate in vessel containing 1000ml of purified water. Put in an ultrasonic bath to dissolve and adjust the pH to 3.0 ± 0.05 with diluted Orthophosphoric acid and then using a filter membrane of 0.45µm filter the solution.

Preparation of the diluent

The diluent was prepared by mixing the mobile phase and Acetonitrile in proportions of 95:5 respectively (v/v).

Preparation of the standard solution

The standard solution was prepared by weigh 50mg of Levetiracetam Standard and transferred it in a volumetric flask of 50ml and then dissolved and completed the volume with the diluent and the mixture homogenized.

Diluted 5ml of this solution to 100ml with the diluent and mix. Again diluted 2ml of this solution to 100ml with the diluent and mix.

Preparation of the test solution

Test solution was prepared by weighing and then grinding not less than 20 Levetiracetam LDM 500mg tablets and then weighing an equivalent mass of the powder to 100mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

Preparation of a placebo solution

The placebo solution was prepared by weighing and an equivalent mass of the powder to 100mg of Levetiracetam and transferred it into a volumetric flask of 100ml.

60ml of the diluent was added and put in an ultrasonic bath for fifteen (15) minutes mixing from time to time. The volume was completed with the diluent and mixed the solution.

A portion of this solution was filtered using a syringe filter of 0.45µm while making sure to first saturate the filter with the solution.

Appendix 2

Culture medium preparation

Sodium Chloride Peptone Buffer solution pH 7.0 TSE	
Mono potassium phosphate	3.6 g
Di sodium dihydrate	7.2 g
Sodium Chloride	4.3 g
Meat or casein peptone	1.0 g
Purified water	1000ml

Tryptic Soy peptone Agar (TSA) medium	
Pancreatic Casein Peptone	15.0g
Soya peptone (papainic)	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Purified water	1000 ml
The pH is adjusted to 7.3 ± 0.2 at 25 °C after sterilization.	

Sabouraud Dextrose-Agar medium	
Dextrose	40.0g
Peptone	10.0g
Agar	20.0g
Purified water	1000ml
The pH is adjusted to 5.6 ± 0.2 at 25 °C after sterilization.	

Liquid medium with casein and soy peptones (TSB)	
Tryptone (Pancreatic Digest of Casein)	17.0g
Soy tone (peptic digest of soybean)	3.0g
Sodium chloride	5.0g
Glucose monohydrate	2.5g
Dipotassium Phosphate	2.5g
Purified water	1000ml
The pH is adjusted to 7.3 ± 0.2 at 25 °C after sterilization.	

MacConkey Broth	
Pancreatic digest of gelatin	20.0g
Lactose monohydrate	10.0g
Dehydrated Ox Bile	5.0g
Bromocresol purple	0.01g
Purified water	1000ml
The pH is adjusted to 7.3 ± 0.2 at 25 °C after sterilization.	

MacConkey Agar Medium	
Pancreatic Digest of gelatin	17.0g
Peptone from meat and casein	3.0g
Lactose	10.0g
Sodium chloride	5.0g
Bile salts	1.5g
Agar	15.0g
Neutral red	0.03g
Crystal violet	0.001g
Purified water	1000ml
The pH is adjusted to 7.1 ± 0.2 at 25 °C after sterilization.	

Xylose-Lysine-Deoxycholate agar medium	
Yeast extract	3.0g
Lysine	5.0g
Lactose	7.5g
Sucrose	7.5g
Agar	12.5g
Sodium deoxycholate	1.0g
Sodium chloride	5.0g
Sodium thiosulfate	6.8g
Ferric ammonium citrate	0.8g
Phenol red	0.08g
Purified water	1000ml
The pH is adjusted to 7.4 ± 0.2 at 25 °C after sterilization.	

Mannitol salt agar medium (Chapman)	
Pancreatic Digest of Casein	5.0g
Beef Extract	1.0g
Peptic Digest of Animal tissue	5.0g
D-Mannitol	10.0g
Sodium chloride	75.0g
Phenol Red	0.025g
Agar	15.0g
Purified water	1000ml
The pH is adjusted to 7.4 ± 0.2 at 25 °C after sterilization.	

Cetrimide Agar medium	
Pancreatic Digests of Gelatin	20.0g
Magnesium Chloride	1.4g
Potassium Sulfate	10.0g
Centrimide	0.3g
Glycerol	10.0g
Agar	13.6g
Purified water	1000ml
The pH is adjusted to 7.2 ± 0.2 at 25 °C after sterilization.	

Liquid enrichment medium for salmonella Rappaport Vassiliadis (VRBG)	
Soy peptone	4.5g
Sodium Chloride	8.0g
Magnesium chloride hexahydrate	29.0g
Di potassium hydrogen phosphate	0.4g
Potassium di hydrogen phosphate	0.6g
Malachite green	0.036g
Purified water	1000ml
The pH is adjusted to 5.2 ± 0.2 at 25 °C after sterilization.	

Appendix 3

Calculations

Calculation of the content of impurity G

Calculate the content of impurity G, according to the formula below:

$$\% = \frac{AimpG}{Ac} x \frac{Cc}{Ct} x\ 100$$

AimpG = Peak area of impurity G in the solution to be examined.

Ac =Average peak area of impurity G in the control solution (b).

Cc = Concentration of impurity G in the control solution (b).

Ct = Concentration of the test in the solution to be examined.

Calculation of the titers of the related substances

Calculate the content of specified impurities, unspecified impurities and total impurities, according to the formulas below;

A) **Impurity C:**

$$\% = \frac{AimpC}{Ac} x \frac{Cc}{Ct} x\ 100$$

AimpC = Peak area of impurity C in the solution to be examined.

Ac = Average peak area of impurity C in the control solution (c).

B) **Other known impurities and unknown impurities:**

$$\% = \frac{Aimp}{Ac} x \frac{Cc}{Ct} x\ 100$$

Amp = Peak area of each impurity other than impurity C in the solution to be examined

Ac = Average peak area of Levetiracetam in the control solution (b).

C) **Total impurities:**

Total impurities (%) = Impurity C (%) + Total Other specified and unspecified impurities (%)

Calculation of the titers of Levetiracetam

Calculate the content of Levetiracetam according to the following formula:

$$\text{Levetiracetam \%} \left(\frac{\text{m}}{\text{m}}\right) = \frac{At}{Ac} x \frac{Cc}{Ct} x \frac{T}{100} x\, 100$$

In which:

At = Peak area of Levetiracetam in the solution to be examined (b).

Ac= Peak area of Levetiracetam in the = control solution (d).

Cs = Concentration of the solution to be examined (b).

Ce = Concentration of the control solution (d)

T = Standard Levetiracetam titer.

Calculation from the Assay of Levetiracetam LDM 500mg

Calculate the dosage of Levetiracetam in Levetiracetam LDM 500mg according to the following formula:

$$\textbf{Levetiracetam \%} = \frac{\mathbf{AT}}{\mathbf{As}} \times \frac{\mathbf{Cs}}{\mathbf{Ct}} \times \frac{\mathbf{P}}{\mathbf{100}} \times \frac{\mathbf{100}}{\mathbf{500}} \times \mathbf{T}$$

Where;

AT: Average peak area of Levetiracetam in the test solution.

As: Average peak area of Levetiracetam in standard solution.

Cs: Concentration of the standard solution.

Ct: Concentration of the test solution.

P: Standard's purity.

T: Average mass of 20 tablets (mg).

Solution	Area	Concentration	Purity	Average mass	Percentage
Standard	3756566	1	100.2		
Test	3721555	1		**500**	**99.2661412**

Calculation from the Related Substances of Levetiracetam LDM 500mg

The dosage for each impurity is determined as follows;

- ✓ For the known impurities;

$$\%(m/m) = \frac{AT}{As} \times \frac{Ws}{50} \times \frac{5}{100} \times \frac{2}{100} \times \frac{100}{WT} \times \frac{P}{100} \times \frac{T}{500} \times \frac{1}{FRR} \times 100$$

Where;

AT: Average peak area of each known impurity in the test solution.

As: Average peak area of Levetiracetam in standard solution.

Ws: Mass(weight) of standard (mg).

WT: Mass(weight) of test (mg).

P: Content of Standard.

T: Average mass of tablets (mg).

FRR: Relative response factor.

- ✓ For the unknown impurities;

$$\%(m/m) = \frac{Au}{As} \times \frac{Ws}{50} \times \frac{5}{100} \times \frac{2}{100} \times \frac{100}{Wt} \times \frac{P}{100} \times \frac{T}{500} \times 100$$

Where;

Au: Average peak area of the unknown impurity in the test solution.

As: Average peak area of Levetiracetam in standard solution.

Ws: Mass(weight) of standard (mg).

WT: Mass(weight) of test (mg).

***P*:** Standard of content.

T: Average Mass(weight) of tablets(mg).

Calculations from the dissolution test on Levetiracetam LDM 500mg tablets

Calculate the dosage of Levetiracetam in Levetiracetam LDM 500mg according to the following formula:

$$\textbf{Levetiracetam \%} = \frac{\mathbf{AT}}{\mathbf{As}} \times \frac{\mathbf{Ws}}{\mathbf{200}} \times \frac{\mathbf{900}}{\mathbf{1}} \times \frac{\mathbf{10}}{\mathbf{5}} \times \frac{\mathbf{P}}{\mathbf{100}} \times \frac{\mathbf{100}}{\mathbf{500}}$$

Where;

AT: Average peak area of Levetiracetam in the test solution.

As: Average peak area of Levetiracetam in standard solution.

Ws: Weight of the standard (mg).

Ct: Concentration of the test solution.

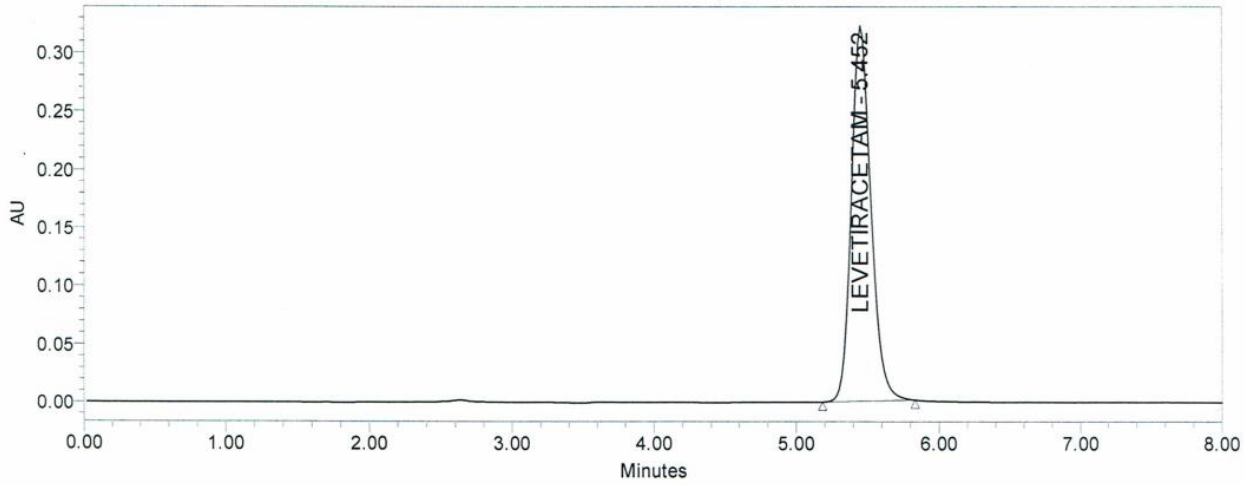
P: Standard's purity.

Table 31 Chromatographic results of Levetiracetam LDM 500mg dissolution test

Tablet	Area of standard	T standard	Standard's weight	Area of Test	Percentage	Average %
1	3157374	100.2	55	3110857	97.7365344	
2	3157374	100.2	55	3102212	97.4649269	
3	3157374	100.2	55	3135479	98.5101055	
4	3157374	100.2	55	3117036	97.9306655	**97.937279**
5	3157374	100.2	55	3118349	97.9719172	
6	3157374	100.2	55	3119546	98.0095244	
Average	**3157374**	**100.2**	**55**	**3117246.5**	**97.937279**	**97.937279**

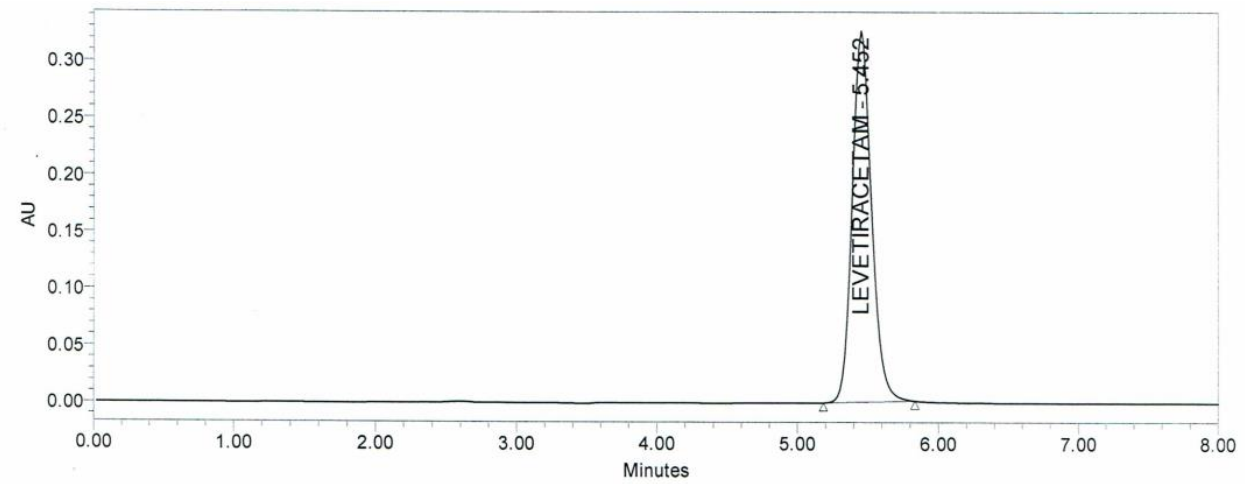
Appendix 4

Dissolution Test chromatograms



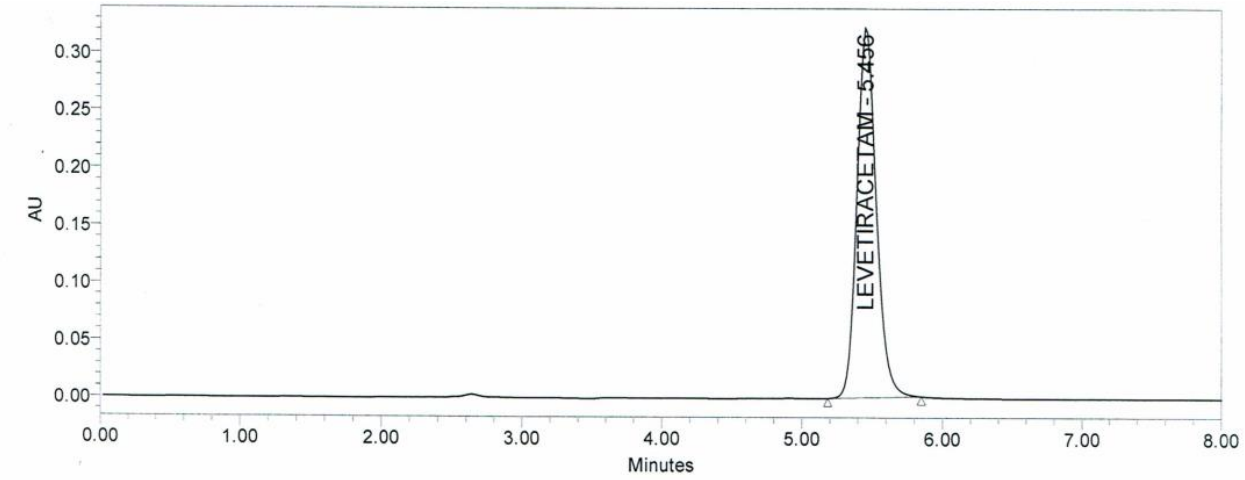
	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.452	3102212	100.00	7.725036e+003	1.139018e+000

Figure 35 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 2) in the dissolution test



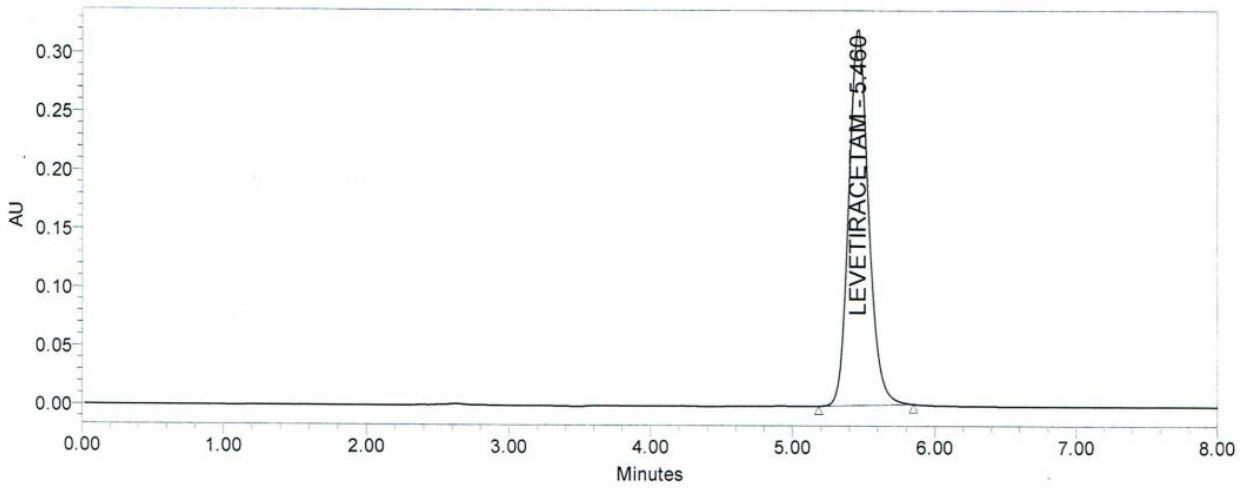
	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.452	3135479	100.00	7.709362e+003	1.140100e+000

Figure 36 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 3) in the dissolution test



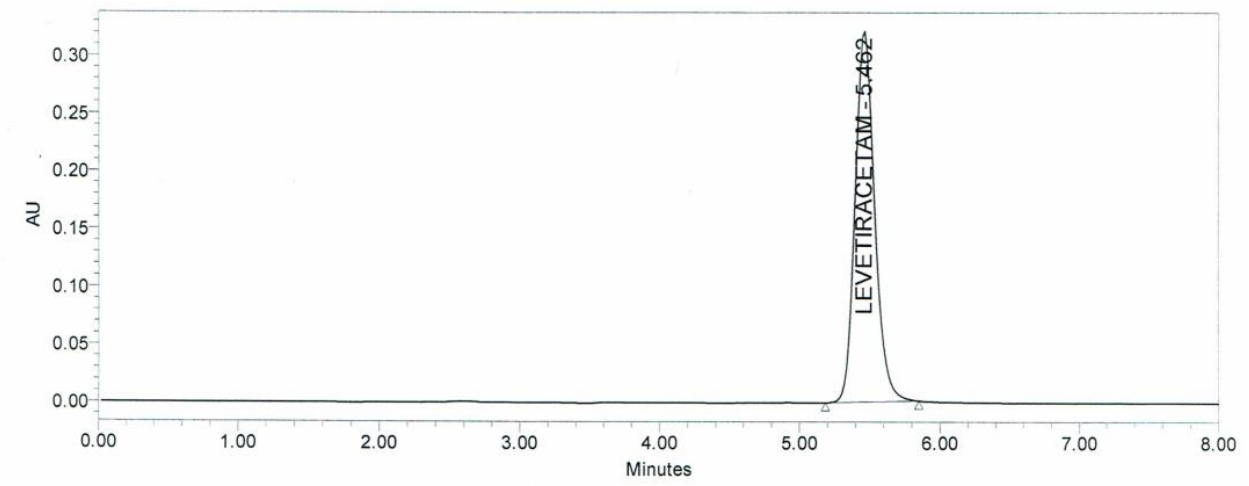
	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.456	3117036	100.00	7.691485e+003	1.144078e+000

Figure 37 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 4) in the dissolution test



	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.460	3118349	100.00	7.572554e+003	1.143407e+000

Figure 38 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 5) in the dissolution test



	Peak Name	RT	Area	% Area	EP Plate Count	USP Tailing
1	LEVETIRACETAM	5.462	3119546	100.00	7.572826e+003	1.146587e+000

Figure 39 Chromatogram of Levetiracetam LDM 500mg test solution (tablet 6) in the dissolution test

University Year: 2023-2024	**Presented by:** KAJJUMBA Julius TUKUR Nafisah Muhammad

Physico-chemical and Microbiological Quality Control of Levetiracetam LDM 500mg

Dissertation presented with the aim of obtaining a Professional Master's degree
In Biotechnology and Quality Control.

Summary;

This research work discusses drugs in general, their classification, pharmacology, the different stages of production of Levetiracetam 500mg, an anti-epileptic drug a tablet formulation manufactured at LDM pharmaceutical industry.

This work is basically composed of the different physico-chemical and microbiological quality control tests carried out on the above mentioned drug as a finished product, its raw materials, and the tests carried out during the process of its production.

The quality control process begins with the raw materials at the point of reception before they are cleared for production. Test are carried out on each batch of the raw materials to ascertain their identity, composition, physical and chemical characteristics as well as microbiological characteristics before they are cleared for production. This quality control process is followed by in process controls.

The microbiological analysis helps to determine the absence or presence of specific germs and also helps to enumerate the total viable aerobic microorganisms, total molds and yeasts whose concentration in case of their presence must be in alignment with pharmacopoeial or Technical File requirements.

Finally, the quality control process ends with tests on the final product to ascertain the conformance of the product's specifications to prespecified norms after which the product is determined either compliant or non-compliant.

The results of this research which explored various tests attest that the final product produced at the company is in full compliance with the standards in European pharmacopeia and the Technical File.

Keywords: Tablets, Levetiracetam 500mg, Quality control, Microbiological quality control, physico-chemical quality control, European pharmacopeia, Technical File.

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