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Process Monitoring, microbiological and physicochemical control of the paste form of Flucidal®3 %.

Présenté par : ***Soutenu le : 24/06/2024***

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From the depths of my heart, I dedicate this work to all those who are dear to me,

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May no dedication be able to express my sincere feelings, for their unlimited patience, their continuous encouragement, their support treasures of goodness, of generousness, in testimony of my deep love and respect for their great sacrifices, God bless you

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Abbreviations

NSAIDs: Non-steroidal anti-inflammatory drug.

COX: Cyclooxygenase enzyme.

IR: Infrared absorption spectrophotometry.

EP: European pharmacopoeia.

SCR: Reference chemical spectrum

HPLC: High performance liquid chromatography.

UV-VIS: Ultraviolet-visible absorption spectrophotometry.

AI: Active ingredient.

TSA : Trypticase soy agar.

SDA: Sabauroud dextrose.

TYMC: The total combined yeast/mold count.

TAMC: The total aerobic microbial count.

CFU: Colony forming unit.

TSB: Tryptic soy broth.

RSD: Relative standard deviation.

Glossary

Inflammation: The immune system protects the body through inflammation, which produces redness, heat, discomfort, and swelling due to leukocyte infiltration and capillary dilatation. Chronic inflammation occurs when the body is exposed to unpleasant stimuli and the inflammatory response is unable to resolve, causing harm to the body. **(AnoushkaRicker M et *al*.,2017)**

Osteoarthritis: Osteoarthritis, a prevalent chronic joint disease, is characterized by joint degeneration leading to pain and function loss, with no current treatment or cure available. **(Assi R et al., 2023**).

Rheumatoid arthritis : Rheumatoid arthritis is a persistent autoimmune disease that can cause damage to various organs, including joints, and can cause significant health issues. **(Radu et Bungau, 2021)**

Pharmacokinetic: To optimize the pharmacodynamic response, pharmacokinetic information is necessary. Pharmacokinetics describes what the body does to the drug, whereas pharmacodynamics describes what the medication does to the body**. (Benet LZ, 1984)**

Eczema: Eczema, often referred to as atopic dermatitis, is a common chronic skin condition that recurs frequently. It is characterized by pruritus, impaired epidermal barrier function, and immunoglobulin E-mediated sensitization to environmental and dietary allergens. **(Andrew et al.,2011)**

Abstract

Our study aimed to verify the compliance of an anti-inflammatory ointment called "FLUCIDAL®3%" with the standards required by the European Pharmacopoeia 2017. FLUCIDAL®3%" is produced by our national pharmaceutical company SAIDAL, located at Dar El Beida, Algiers. The compliance was assessed by controlling its physico-chemical and microbiological properties from raw material (Niflumic Acid (Active ingredient), Sorbic Acid and Nipagine M Sodium (Excipients)) to the finished product.

Physico-chemical quality control of semi-finished, finished, and raw materials that demonstrate various essential parameters such as chemical and chromatographic identification tests. Whereas, microbiological control of the final product, includes identifying harmful microorganisms, enumeration of aerobic germs, yeasts, and molds that might affect the drug's quality.

The results of our present study are completely consistent with the international standards established by the European Pharmacopoeia. The generic drug FLUCIDAL®3% ointment has a good pharmaceutical quality considering the raw materials used, the absence of pathogenic microorganisms and the quality of the semi-finished and final products,

Keywords: Flucidal ®3%, Niflumic Acid, anti-inflammatory drug, physico-chemical quality control, microbiological quality control.

Resumé

Notre étude a pour but de vérifier la conformité d'une pommade anti-inflammatoire appelée « FLUCIDAL®3% » aux normes requises par la Pharmacopée Européenne 2017. Le FLUCIDAL®3% est produit par notre entreprise nationale pharmaceutique SAIDAL située à Dar EL Beida, Alger. La conformité de ce produit a été établie en contrôlant ses propriétés physico-chimiques et microbiologiques depuis la matière première (Acide Niflumique (Principe Actif), Acide Sorbique et Nipagine M Sodium (Excipients)) jusqu'au produit fini.

Le contrôle de la qualité physico-chimique des produits semi-finis, finis et matières premières démontrent divers paramètres essentiels tels que les tests d'identification chimique et chromatographique.

Le contrôle microbiologique du produit final, comprend quant à lui le dénombrement des germes aérobies, des levures et des moisissures susceptibles d'affecter la qualité du médicament et l'identification des micro-organismes nuisibles.

Les résultats de cette étude sont tout à fait conformes aux normes internationales établies par la Pharmacopée européenne. Le médicament générique FLUCIDAL®3% pommade est de bonne qualité pharmaceutique en ce qui concerne la qualité des produits semi-finis et finis, les matières premières utilisées et l'absence de micro-organismes pathogènes.

Mots clés : Flucidal® 3%, Acide Niflumique, médicament anti-inflammatoire, contrôle de qualité physico-chimique, contrôle de qualité microbiologique.

ملخص

تهدف دراستنا إلى التحقق من توافق مرهم مضاد للالتهابات يسمى" فلوسيدال® 3%"مع المعايير المطلوبة في دستور الأدوية الأوروبي 2017 من خلال مراقبة خصائصه الفيزيائية الكيميائية والميكروبيولوجية من المادة الخام (حمض النيفلوميك (المادة الفعالة) وحمض السوربيك ونيباجين م صوديوم (سواغات)) إلى المنتج النهائي، الذي تنتجه شركة صيدال دار البيضاء للأدوية.

مراقبة الجودة الفيزيائية والكيميائية للمواد شبه المصنعة والتامة الصنع والمواد الخام التي تُظهر العديد من المعايير الأساسية مثل اختبارات التحديد الكيميائي والكروماتوغرافي

الرقابة الميكروبيولوجية للمنتج النهائي، والتي تشمل تعداد الجراثيم الهوائية والخمائر والعفن التي قد تؤثر على جودة الدواء وتحديد الكائنات الحية الدقيقة الضارة .

تتفق نتائج هذه الدراسة تمامًا مع المعايير الدولية التي وضعتها دستور الأدوية الأوروبي. الدواء الجنيس فلوسيدال® 3% مرهم بجودة صيدلانية جيدة من حيث جودة المنتجات شبه المصنعة والنهائية، والمواد الخام المستخدمة، وغياب الكائنات الحية الدقيقة المسببة للأمراض.

الكلمات المفتاحية: فلوسيدال® 3%، حمض النيفلوميك، مضاد للالتهابات، مراقبة الجودة الفيزيائية والكيميائية، الميكروبيولوجية.

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Introduction

Medication, seems to be as old as humanity itself. Traces of them can be found in the most ancient civilizations. Since the earlier temples, man has looked to nature for food as well as cures or remedies for his illnesses. He has also learnt to identify toxins. Up until now, Drugs were employed in nature and as complex extracts; medicines were entirely natural and referred to as the three kingdoms (mineral, plant, and animal)**. (Thierry F, 2010)**

The term "generic" refers to a copy of an original medication whose manufacture and distribution are rendered feasible by the patent covering the original active ingredient having expired**. (C. Abelli et *al*.,2001)**

The pharmaceutical industry performs a significant role in global healthcare systems. It is made up of several public and commercial organizations and divisions that research, develop, produce, and distribute medications for the health of people and animals. Research and development (R&D) of medications intended to treat or prevent a variety of illnesses or disorders. **(Gennaro, 1990).**

The concept of "pharmaceutical analysis" is wide and has multiple definitions. It is the set of procedures employed in the development of pharmaceutical products for the purpose of identifying, determining, separating, purifying, and elucidating the structure of a particular chemical. Typically, active pharmaceutical ingredients, pharmaceutical excipients, contaminants found in pharmaceutical preparation, or drug metabolites are the components that are the subject of pharmaceutical analysis. **(Niazi, 2004)**

Samples used in pharmaceutical analysis usually include biological samples, pollutants, impurities, and pharmaceutical raw materials in addition to completed pharmaceutical products. Numerous analytical techniques can be used for pharmaceutical analysis.
In general, pharmaceutical analysis is important for developing and validating the procedures for high-quality pharmaceutical product manufacturing that follow good manufacturing practices.**(Niazi, 2004)**

The establishment of the European Pharmacopoeia in 1970 served as the first step toward that served as the model for other nations to develop their own national pharmacopoeias. In order to improve the quality of pharmaceutical products using various analytical techniques for pharmaceutical analysis, the pharmaceutical industries, principal pharmacopoeias, and registration agencies came together in 1990 to form the International Conference on Harmonization (ICH). Pharmaceutical analysis ensures that drugs are of excellent quality, which directly affects the drug therapy economy and may be evaluated from both a financial and public health perspective.**(Niazi, 2004)**

In Algeria, the pharmaceutical market is clearly on the up, as Algeria intends to aim for the development of local production and become a national production platform, given that a large part of the market relies on imports, with a figure approaching 70%. To achieve this, comprehensive strategic planning is essential to improve the sector, by structuring the regulatory aspects of all pharmaceutical products. **(Bouzabata, 2017)**

The national laboratory for the control of pharmaceutical products was designated for the first time as a WHO collaborating centre for the conformity of medicines in 2003, and is considered to be the reference laboratory for the control of medicines. The LNCPP systematically controls all batches of imported medicines, and validates the control laboratories required by all manufacturers of medicines in Algeria in order to release the product. **(LNCPP, 2012)**

This context establishes the problematic of our study, which has its roots in the following query: Does FLUCIDAL®3% meets the quality standards established in the European Pharmacopoeia?

Thus, here we aimed to achieve a quality control of one of the most used anti-inflammatory drug of Niflumic acid type (active principle of FLUCIDAL®3%) and to describe the manufacturing process of "FLUCIDAL® 3%" ointment, at SAIDAL quality control laboratory, Dar El Beida unit in ALGIERS.

This control consists of analyses of the physico-chemical and microbiological quality of the finished product FLUCIDAL®3%, in order to ensure the conformity of this product and thus enable it to be marketed.

Thus, in order to respond to this query, we will detail three main chapters:

1. Bibliographic research: this section includes general information on the drug and its mechanism of action.

2. Materials and methods: this section highlights physico-chemical and microbiological control of raw materials, semi-finished products and finished products of FLUCIDAL®3%.

3. Results and discussion: this section will compare our product with the standards required by the European Pharmacopoeia.

4.Conclusion: we conclude with a general conclusion based on the main results obtained.

Chapter I Bibliographic part

I.1 Definition of drugs

Drugs are defined as 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 **(lervellec, 1989)**.

I.2 Different types of anti-inflammatory drugs

There are two classes of anti-inflammatory medications:

I.2.1 Nonsteroidal anti-inflammatory drug

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of medications widely used in human and animal health care to reduce persistent inflammation, pain and fever because of their anti-inflammatory, analgesic and antipyretic effects. **(Panchal et Sabina, 2023)**

I.2.2 Steroidal anti-inflammatories

These steroid analogues or precursors of cortisone, naturally secreted by the adrenal glands, possess many pharmacological properties. They influence metabolic processes (particularly lipid, protidial, and glycocidalones), and they induce hypothalamic hypofyse braking, which puts the adrenal glands to rest. In contrast to NSAIDs, glucocorticoids have the ability to block every stage of the inflammatory response **(Muster, 2005)**.

I.3 Mechanism of action of NSAIDS

The main mechanism of action of NSAIDs is the inhibition of the enzyme cyclooxygenase (COX). Cyclooxygenase is required to convert arachidonic acid into thromboxanes, prostaglandins, and prostacyclins. The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids. Specifically, thromboxanes play a role in platelet adhesion, prostaglandins cause vasodilation, increase the temperature set-point in the hypothalamus, and play a role in anti-nociception.

There are two cyclooxygenase isoenzymes, COX-1 and COX-2. COX-1 gets constitutively expressed in the body, and it plays a role in maintaining gastrointestinal mucosa lining, kidney function, and platelet aggregation. COX-2 is not constitutively expressed in the body; and instead, its inducibly expresses during an inflammatory response. Most of the NSAIDs are nonselective and inhibit both COX-1 and COX-2. However, COX-2 selective

NSAIDs (ex. celecoxib) only target COX-2 and therefore have a different side effect profile. Importantly, because COX-1 is the prime mediator for ensuring gastric mucosal integrity and COX-2 is mainly involved in inflammation, COX-2 selective NSAIDs should provide anti-inflammatory relief without compromising the gastric mucosa. **(Schjerning et *al*.,2020)**

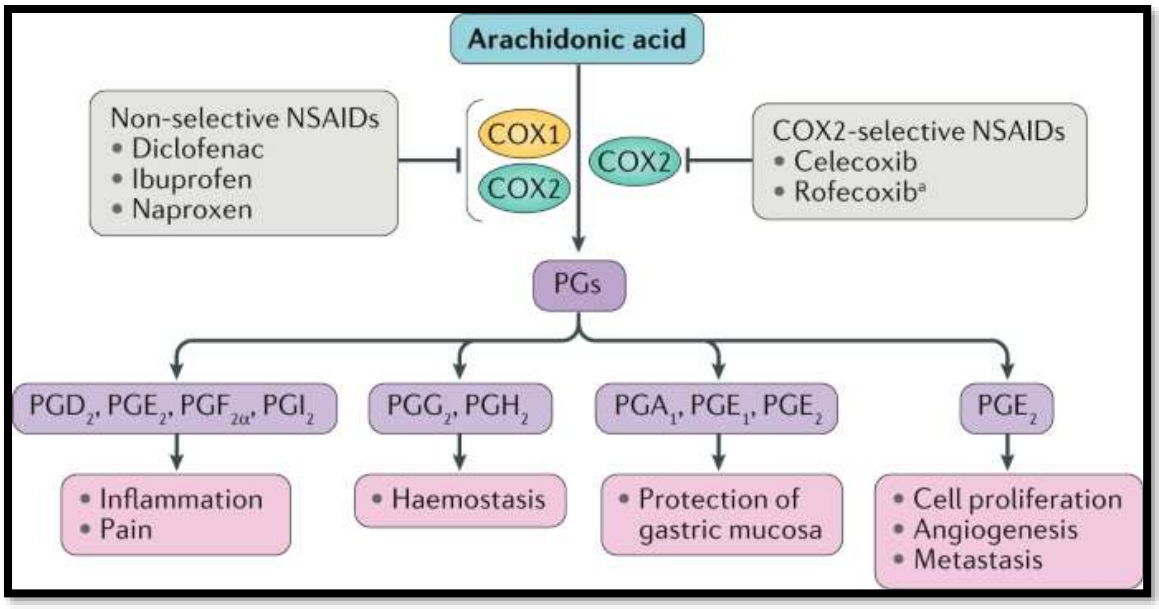

Figure 1. Mechanism of action of NSAIDs ***(*Schjerning *et al*.,2020).**

I.4 Chemical classification

NSAIDs can be categorized based on the molecules they are made of, some of which have structural analogs, they can be classified according to their chemical structure **(Pauline Sivry, 2014);** NSAID Salicylates (ex; Aspirin), NSAIDs Fenamate (ex; Niflumicacid), NSAIDs Heteroarylacetic acids (ex; Diclofenac), NSAIDs Pyrazolone derivatives (ex; Phenylbutazone), NSAIDs Enolic acid derivatives (ex; Piroxicam) …etc.

I.5 Presentation of Flucidal® 3%

The topical application ointment Flucidal® 3% is designed for adults over the age of 15, and used for supplementary treatment of entorses and venitis following sclerotherapy. It contains a non-steroidal anti-inflammatory compound called "Niflumic acid" from the phenamic acid family. **(Saidal ,2023)**

Flucidal® 3% is a generic drug manufactured by the Saidal group, its CID is Niflumic Acid and the princeps is NIFLURIL.

Figure 2. Presentation of the drug package Flucidal® 3%.

I.6 Chemical presentation of Flucidal® 3%

I.6.1 Active components

Niflumic acid (NIF, 2-{[3-(trifluoromethyl)phenyl]amino}-3-pyridinecarboxylic acid) is a widely prescribed NSAID, its mode of action is associated with the reversible, non-competitive inhibition of the enzyme ciclooxygenase-2, is a member of the molecule group fenamate **(Balci et al., 2010)**. It is used in clinics to treat acute or progressive inflammatory diseases including osteoarthritis, as well as rheumatoid arthritis, in order to reduce pain and inflammation **(Acebedo-Martínez et *al*., 2021)**

I.6.2 Chemical structure

Niflumic acid is a derivative of 2- (phénylamino) – benzoic acid, and is also classified as a derivate of N-phenylantranylic acid and its chemical nature belongs to the Mefenamic, Flufenamic and Tolfenamic Acids **(Blaha et *al*., 2021)**.

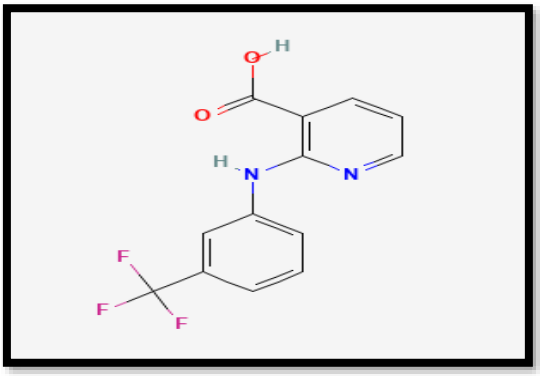

Figure 3. Niflumic acid chemical structure.

I.7 Physico-chemical properties

I.7.1 Niflumic acid (CID)

- IUPAC Name: 2-[3-(trifluoromethyl) anilino] pyridine-3-carboxylic acid Molecular.
- Formula: $C_{13}H_9F_3N_2O_2$.
- Molecular Weight: 282.22 g/mol.
- Physical Description: Solid.
- Melting Point: 203°C.
- Aspect: Light yellow crystalline powder.
- Solubility: Practically insoluble in water, easily soluble in acetone, soluble at 96 percent ethanol and methanol.

I.8 The pharmacokinetic classification

The rate of administration is directly determined by the elimination half-life of NSAIDs. Classified NSAIDs include those with short half-lives (6 hours, such as Profénid®), intermediate half-lives (6 to 24 hours, such as Apranax®), and long half-lives (greaterthan 24 hours, such as Feldene®). Additionally, there are NSAIDs with extended release, such as Voltarene LP®. **(Pauline Sivry ,2015)**

I.9 Pharmacokinetics

Table I. Pharmacokinetics of Niflumic acid. **(Gandin, 2013)**

Properties	Pharmacokinetics
Absorption	Peak plasma concentration 2h after administration.
Distribution	Binding to plasma proteins> 90%.
Metabolism	Conversion to 5-hydroxyniflumic acid and 4-hydroxyniplumic acid, both being inactive.
Elimination	30% fecal. 70% urinary.
Time 1⁄2 life	4–6 hours.

I.10 Excipients

Excipients are components of pharmaceutical products that have no therapeutic effect but are added to their formulation or utilized during manufacturing to enhance certain aspects of the product, such as taste, appearance, or conservatives.

Table II. Flucidal® 3% excipients and their function.

Raw materials	Function
Polyethylene glycol palmitostearate (TEFOSE 1500)	Emulsifying.
LauricMacrogolycerides (LABRAFIL M2130 CS)	Tensile (Co-emulsifying).
Stearic acid	Emulsifying agent, solubilizer.

Fluid Vaseline Oil	Lubricant.
Sodium Methyl Parahydroxybenzoate /Sodium Parabene Methyl (NIPAGINE SODEE)	Conservative.
Sorbic acid	Convervative.
Liquid lemon essence	Aroma (antibacterial).
Lavender essence	Aroma (antibacterial).
Purified water	Solvent.

I.11 Clinical presentation of FLUCIDAL®3% (Saidal, 2023)

I.11.1 Pharmaco therapeutic class

Non-steroidal anti-inflammatory of the phenamic acid class.

I.11.2 Therapeutic indications

According to the therapeutic indications of the leaflet, indicated to adults (over 15 years), FLUCIDAL® 3% is used in the following cases:

- Local treatment of additives.

- Treatment of venitis post-sclerotherapy (venous inflammation that may occur after injection of sclerosis products during treatment of varicose veins).

I.11.3 Contraindication

Flucidal®3% should not be used in the following cases:

- From the sixth month of pregnancy.

- A history of asthma with Niflumic acid or other substances of close activity such as other NSAIDs, aspirin.

- A history of allergy to the other components of the ointment.

- Injured skin, regardless of the lesion: sucking dermatosis, eczema, infected lesion, burn or wound.

I.11.4 Dosage and route of administration

Topical application of FLUCIDAL® 3% can be done in areas where a long, gentle massage is required to allow the ointment to penetrate the painful or inflamed area. Furthermore, one (1) application, three (3) times a day, is the typical dosage. After every use, it's best to give your hands a thorough and prolonged wash. **(saidal, 2023)**

I.11.5 Possible side effects

(Saidal,2023)

FLUCIDAL® 3% ointment, like all medications, may cause side effects, albeit not everyone will experience them.

The following side effects are related to the route of administration:

- Rare skin allergic manifestations, pruritus type or localized erythema.
- Hyper sensitivity reactions: redness, itching, rash.
- Burning sensation and drying of the skin.
- Other systemic effects of NSAIDs: they depend on the transdermal passage of the active substance and therefore the amount of ointment applied, the surface treated, the degree of skin integrity, the duration of treatment and the use or not of occlusive bandage (digestive effects, kidneys).

I.11.6 Conservation of FLUCIDAL® 3%

(saidal,2023)

All medications should be properly preserved, so the following factors need to be kept under control for FLUCIDAL® at 3%:

- Expiry date: it may not exceed the usage limit shown on the box, it refers to the last day of the month.
- Conditions of storage: to be stored at a temperature not exceeding 25°C.
- Shelf life: three (3) years.

Chapter II
Materiel and methods

We conducted our experiment in the quality control laboratory at the Dar El Beida unit, which is the oldest PHARMAL unit.

Previously, this unit's operations were restricted to producing a small number of medications and cosmetics, but today it produces a large variety of medications in a variety of gory forms, including tablets, capsules, syrups (a buzzable solute), pasty forms (salve, gel, and cream), buzzable suspensions, salts, and dermal solutions.

Our work's goal is to confirm, from a physico-chemical and microbiological perspective, the conformance of the non-steroidal anti-inflammatory medicine ointment FLUCIDAL® A 3%.

II Manufacture

II.1 Materials

The materials used in FLUCIDAL® 3% manufacturing processes are displayed in the following table.

Table III : Materials and Raw Materials used for the manufacture of FLUCIDAL® 3%.

Materials	Raw Materials
▪ SARTORIUS type 60 kg and 300 kg loaded weight. ▪ METLER type 60 Kg and 300kg loaded balance. ▪ Stainless steel preparation container equipped with a grinder, mixer, disperser and FRYMA type grinder mixer with a stirrer ▪ IWKATFSA 20 Packing Line Wrapper. ▪ Cartoner and vignetter Type IWCACP150. ▪ Double decalitre.	▪ Niflumic acid<50um. ▪ Polyethylene glycol. Palmitostearate (TEFOSE 1500). ▪ Lauricmacrogolycerides (LABRAFIL M2130 CS). ▪ Stearic Acid. ▪ Vaseline fluid oïl. ▪ Sodium methyl parahydroxybenzoate / Sodium methylparaben (NIPAGINE SODEE). ▪ Sorbicacid. ▪ Liquid lemon oil.

▪ Stainless spatulas. ▪ Decalitre. ▪ PH meter.	▪ Lavandin oil.

- **Weighing of raw materials**

For each batch manufactured, the necessary quantities of raw materials are measured or counted in a location near the central store;

- Weigh the active ingredient and the excipients at the weighing plant**. (Represent in Table of Materials and Raw Materials used for the manufacture of FLUCIDAL® 3%).**
- Fill the weighing labels and attach them to each weighed bag.

- **Verifications**

In the manufacturing room it is necessary to verify the following instructions:

- ✓ Cleaning tanks every 8 batches.
- ✓ Check the cleanliness of equipment and premises before any operation.
- ✓ Attache equipment cleanliness certificates.
- ✓ Check the labelling of equipment and premises.
- ✓ Close the ranges at the time of operation.

II.2 Preparation

The table below describes the different stages of preparation of the two phases of aqueous and oil solution.

Table IV. Preparation of the two phases of aqueous and oil solution.

The aqueous phase	**The oily phase**
▪ Preparation of the mixture 1 “aqueous phase”:	▪ Preparation of the mixture 2 “Oily phase”:

▪ In the pre-mix tank, transfer the purified water, and heat to a temperature of 50 °C. ▪ Insert Nipagine M Sodium and Sorbic Acid. ▪ Shake. ▪ Heating the mixture under agitation. ▪ Under the temperature 70°C. ▪ Agitation time 30 min. ▪ Transfer the aqueous phase to the oily phase by the empty pump for 5 minutes and cooling to 40°C. ▪ Incorporate Niflumic acid under agitation activate, homogenization for 3 minutes. ▪ Shake for 5 minutes. ▪ Add liquid lemon benzene and lavender benzene. ▪ Homogenize for 2 minutes. ▪ Refrigerate the ointment to 30°C, stirring.	▪ Place in the production tank: TEFOSE and LABRAFIL. ▪ Heat to104°C until liquefied and cool to 70°C. ▪ Incorporate Vaseline fluid oil and Stearic acid. ▪ Stir at 70°C. ▪ Stirring speed: 21 rpm. ▪ Mixing time 90 min.

- **Transfer and Storage**

Transfer the mixture to the storage tank using a percentage stirring system. Mixer agitation speed 40rpm.

- **Packing**
  - **Primary packing**

Distribution of the ointment on the IWEKATFS20 packing line intubatorin 40 g painted tubes

Check:

- ✓ The blank line and fill the corresponding sheet.
- ✓ Compliance of packaging materials.
- ✓ Compliance of the label inscription (Name, Batch Number, Manufacturing Date, Pre-emption Date).
- ✓ Filling line rate.
- ✓ The packaging aspect.
- ✓ The batch number marking on the tube.
- ✓ Marking the expiration date on the tube.
- ✓ Check the unit peas of the tubes (40± 2) g and fill in the corresponding sheet.
- o **Secondary packaging**

Feeding the cartoning machine with:

- ✓ Cartons.
- ✓ Leaflets.
- ✓ Stickers.
- ✓ 40 g tubes.

Check:

- ✓ Packaging appearance.
- ✓ Conformity of packaging items.
- ✓ Conformity of markings: product name, lot number, date of manufacture.

Note:

- Semi-finished product in the preparation tank, a sample of the mixture is taken in tubes using a spatula for quality control and drug release.
- Expiry date and shipping decision on labels and cartons.
- Take a sample for physicochemical analysis of the finished product and another for microbiological analysis.

II.3 Material

II.3.1 Non-Biological Material: (see annex 01)

➢ **Materials used**

- Raw material: Niflumic acid.
- IPC: Flucidal® 3%.
- FP: Flucidal® 3%.

➢ **Sampling**

The sampling must be carried out under strict asepsy conditions to avoid any source of contamination. The following information has been given for each sampling:

- the date of collection.
- the quantity collected.
- the batch number and product identification.

II.4 Methods

In addition to describing the operating conditions and acceptance standards of each parameter evaluated, this section will detail all the equipment and methods utilized during the different inspections performed on the product FLUCIDAL® at 3%.

II.4.1 Raw Materials control

❖ **Active ingredient**

➢ **Niflumic acid**

Niflumic acid Purpose: this procedure describes the control technique for Niflumic acid raw material.

- **Area of application:** This procedure applies to the control of Niflumic raw material (acid).
- **Reference document**: European Pharmacopoeia 2020,10éme edition.
- **Definition and abbreviation:** SCR: chemical reference substance.

- **Equipment, materials and systems: (see annex 02)**

- **Definition:**

C H F N O13932 2 -((3-(trifluoromethyl) phenyl) amino) pyridine-3-carboxylic acid.

Content: 98.5 per cent to 101.5 per cent (dried substance).

- **Characters:**

- **Appearance**: pale yellow crystalline powder.

- **Solubility**: practically insoluble in water, freely soluble in acetone, soluble in 96%

ethanol and methanol.

- **F:** approx. 204°C.

- **Identification: Infrared absorption spectrophotometry. Principle:**

Absorption spectroscopy is the study of the absorption of electromagnetic radiation by atoms and molecules.(Heard 2006).The transition between the rotational and vibrational energy levels of the ground (lowest) electronic energy state is facilitated by molecular absorption of electromagnetic radiation in the infrared region of the spectrum. On the other hand, absorption of the energetically stronger visible and ultraviolet radiation causes transitions between various electronic levels' vibrational and rotational energy levels. Molecular vibrations are the main focus of infrared spectroscopy because only the infrared spectra of tiny molecules in the gas phase allow for the measurement of transitions between discrete rotating states.**(Ismail et al., 1997)**

Comparison : Niflumic acid SCR.

- **Loss on drying:** maximum 0.3 per cent, determined in an oven at 105°C on 2,000g of Niflumic acid.
- **Sulfuric ash:** maximum 0.1 per cent, determined in a platinum crucible on 1.0g of Niflumic acid.

❖ **Exipients**

➢ **Sorbic acid**

- **purpose:** This operating procedure describes the control technique for the raw material ASCORBIUM (acid).
- **Field of application:** This operating mode applies to the control of the raw material ASCORBIQUE (acide) used by the Dar El Beida production site.
- **Reference documents:** European Pharmacopoeia 2017, 9th edition.
- **Equipment, materials and systems: (see annex 02)**

- **Definition:**

(5R)-5-(1S)-1,2-Dihydroxyéthyl)-3,4-dihydroxyfuran-2(5H)-one.
Content: 99.0 per cent to 100.5 per cent.

- **Characteristics:**

Table V : The characteristics of Sorbic acid.

Appearance	**Solubility**	**F**
White or approximately white crystalline powder or colourless crystals which colour on exposure to air and moisture.	Freely soluble in water, fairly soluble in 96% ethanol.	About 190°C, with decomposition.

- **Identification :**

o First identification : B, C.
o Seconde identification : A, C, D.

A. Ultraviolet and visible absorption spectophotometry

Dissolve 0.10g of ascorbic acid in water R and immediately make up to 100.0mL with the same solvent. To 10mL of hydrochloric acid R at 10.3 g/L, add 1.0mL of this solution and make up to 100.0mL with water R.

Absorption maximum: at 243 nm, determined immediately after dissolving.

Specific absorption at absorption maximum: 545 to 585.

B. Infrared absorption spectrophotometry

Comparison: ascorbic acid SCR.

C.PH: 2.1 to 2.6 for solution S.

D. Sodium reaction

To 1 ml of solution S, add 0.2 ml of dilute nitric acid R and 0.2 ml of silver nitrate solution R2. A grey precipitate forms.

- **Nipagine M sodium**

- **Definition:**

Sodium 4-(Methoxycarbonyl) phenolate.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

- **Characteristics:**

  Table VI : The characteristics of Nipagine M sodium.

Appearance	Solubility
White or almost white, crystalline powder, hygroscopic.	Easily soluble in water, fairly soluble in 96% ethanol, practically insoluble in methylene chloride.

- **Identification:**

- First identification: B, D.
- Second identification: A,C,D

- **A**. **Melting point**

✓ Dissolve 0.5 g of test substance in 50 ml of water R.

✓ Immediately add 5 ml hydrochloric acid R1.

✓ Filter and wash the precipitate with water R. Dry at 80°C in vacuo for 2 h.

✓ The melting point of the precipitate obtained is 125°C to 128°C.

- **B. Infrared absorption spectrophotometry**

Preparation: precipitate obtained in identification A.

Comparison: methyl parahydroxybenzoate SCR.

- **C. Thin layer chromatography.**

✓ Solution to be examined (a) Dissolve 0.10 g of substance to be examined in 10 ml of water R.
✓ Immediately add 2 ml hydrochloric acid R and shake with 50 ml (1-1-dimethylethyl) methyl ether R. Evaporate the upper phase to dryness and recover the residue with 10 ml acetone R.
✓ Solution (b): Take 1 ml of solution (a) and make up to 10 ml with acetone R.
✓ Solution (b): Take 1 ml of test solution (a) and make up to 10 ml with acetone R.
✓ Control solution (a). Dissolve 10 mg methyl parahydroxybenzoate SCR in 1 ml acetone R and make up to 10 ml with the same solvent.
✓ Control solution (b). Dissolve 10 mg ethyl parahydroxybenzoate SCR in 1 ml test solution (a) and make up to 10 ml with acetone R.

Plate: F254 octadecylsilylated silica gel plate for R TLC.

Mobile phase: glacial acetic acid R, water R, methanol R (1 :30 :70 V/V/V).

Deposition: 5µL of solution to be examined (b) and control solutions (a) and (b).

Development: on 2/3 of the plate.

Drying: air drying.

Detection: examination under ultraviolet light at 254 nm.

System conformity: control solution (b):

- The chromatogram shows 2 clearly separated main spots.

Results: the main spot of the chromatogram obtained with the solution to be examined (b) is similar in position and size to the main spot of the chromatogram obtained with the control solution (a).

D. Sodium reaction

To 1 ml of solution S (**See Test**), add 1 ml of water R. The solution gives the sodium reaction.

TEST

Solution S. Dissolve 5.0 g of test substance in carbon dioxide water R prepared from distilled water R and make up to 50 ml with the same solvent.

Appearance of the solution. Solution S, examined immediately after preparation, is clear and no more strongly colored than control solution JB6.

- **PH.** Take 1 ml of solution S and make up to 100 ml with carbon dioxide-free water R. The PH of the solution is 9.5 to 10.5.

II.5 Physico-chemical control of products Flucidal® ointment to 3%

- **Purpose:** This operator mode describes the control technique of the product Flucidal® 3% ointment (IPC et FP)
- **Scope of application:** this operating mode applies to the control of the product Flucidal 3%. ointment at 3% (IPC and FP) manufactured at the production site of Dar EL BEIDA.
- **Reference document: Technical** documentation of the product Flucidal® 3% internal method.
- **Equipment and equipment and systems: (see annex 02)**.

II.5.1 Intermediate Product Control (IPC)

- **characters:**

- **Appearance:** Bright homogeneous pomegranate with lemon smell and white to slightly yellowish color.
- **pH:** prepare a water solution at 10 and determine its pH (3,5 – 4,5).

❖ **Dosage of AI: By UV-VIS spectroscopy**

Principle:

Based on the principle of light absorption, UV-VIS spectroscopy measures the amount of analyte present in a sample solution in direct proportion to the amount of light absorbed. Light absorption increases linearly with an increase in analyte concentration while light transmission decreases exponentially. The way that radiation is absorbed in the UV-VIS range is determined by the electronic configuration of the absorbing species, which might include atoms, molecules, ions, or complexes.**(Akash et Rehman., 2020)**

- **Operating mode:**

Operating conditions:

- ✓ White: 96% ethanol.
- ✓ 10 mm quartz tank.
- ✓ Wavelength: 290%

Preparation of solutions:

The table below describes the different steps in preparing the two standard solutions and the solution to be examined.

Table VII : Solutions prepared for the dosage of AI by UV-VIS.

Standard solution	Solution Essay
▪ Insert an accurately weighed sample of 30 mg Niflumic acid (titrate draw material) into a 100 ml flask.	▪ Insert an accurately weighed test outlet of 1g of the 3% ointment Flucidal product into a 100 ml flask. ▪ Add 50 ml of 96% ethanol.

▪ Dissolve with 50 ml of 96% ethanol. ▪ Complete to volume with the same solvent. ▪ Shake well insert 1ml of this solution in a flask of 50 ml supplement to the volume with ethanol at 96% (the final concentration obtained is 0.006 mg/ml).	▪ Dissolve by heating the solution in a mary bath for 3 min. ▪ Let cool. ▪ Complete to volume with the solvent meme. ▪ Shake well. ▪ Insert 1 ml of this solution in a flask of 50 ml and supplement the volume with ethanol at 96% (the final concentration in Niflumic acid is 0.006 mg/ml).

- **Calculation formula**

$$T = \frac{ABSE}{ABSst} \times \frac{Pst}{Pe} \times T$$

ABSE: Absorption of Niflumic acid in the solution to be considered.

ABSst: Absorption of Niflumic acid in standard solution.

Pst: Trial of Niflumic acid in the standard solution in mg.

Pe: End product sampling in mg.

T: Substance title expressed in %.

Standard: (2.7 – 3.3) %

II.5.2 Control of the finished product

- **Characters:**
  - **Appearance:** Bright homogeneous pomegranate with lemon smell and white to slightly yellowish color.

- **PH:** Prepare a 10% aqueous solution and determine which PH (3,5-4,5).
- **Average weight:** Conduct an average weighing on 30 sample tubes, compare it to standards taking into account the load of the tube to determine when checking packaging items

Standard: (38 g to 42 g).

- **Identification**

  Proceed as described for the dosage of Niflumic acid PH by HPLC. The retention time of Niflumic acid obtained with the standard solution.

❖ **Dosage of AI: By HPLC.**

Principle:

In high-performance liquid chromatography (HPLC), a stationary phase (the column packing) and a mobile phase (the solvent) are separated (or partitioned). The separation will be influenced by the sample members' capacity to separate themselves between the two phases. Depending on the stationary phase's characteristics.**(Bélanger et al., 1997)**

o **Operating mode:**

The technique adopted is a HPLC chromatographic method that allows both dosing and identification of Niflumic acid.

Operating condition:

- Isocratic diet.
- Moving phase: Phosphoric acid / Water / Acetonitrile: 2,5/500/500 mix and filter the mobile phase on a 0.45 µm membrane filter and then de-gas for 10 min.
- Column: Kinetex C8 (25 cm / 6mm * 5µm) / or equivalent, recommended column for the lowest PH).
- Wavelength: 267 nm.
- Injection volume: 10 µl.

- Output: 2.0 ml/min.
- Column temperature: 25 °C.
- Sample temperature: 25°C.
- System compliance: to be achieved on the standard solution.
- The symmetry factor is not greater than 2.0.
- The relative standard solution standard deviation achieved on 05 injections is not greater than 2.0.

Preparation of solutions:

The following table describes the different preparation stages of the two standard solutions (standard) and the solution to be examined (essay).

Table VIII : Solutions prepared for AI dosage by HPLC.

Standard solution:	**Solution Essay**
▪ Insert an accurately weighed test outlet of 60 mg Niflumic acid (titrated raw material) into a 50 ml flask. ▪ Dissolve with 20 ml of 96% ethanol. ▪ Complete to volume with the same solvent. ▪ Shake well. ▪ Insert 12.5 ml of this solution in a flask of 50 ml, add to the volume with the mobile phase, (the final concentration thus obtained is 0.3 mg/ml).	▪ Insert an accurately weighed sample of 2 g of the product Flucidal 3% ointment in a 50 ml flask. ▪ Add 20 ml of 96% ethanol. ▪ Dissolve by heating the solution in a mary bath for 3 min. ▪ Leave cool. ▪ Complete to volume with the same solvent. ▪ Shake well. ▪ Insert 12.5 ml of this solution in a 50 ml flask, add to the volume with the mobile phase. (the final

	concentration of niflumicacid0.3 mg/ml) ▪ Filter the solution on a 0.45bμm membrane filter.

- **Calculation formula**

$$\textbf{Niflumic acid content in } \% = \frac{\textbf{Se}}{\textbf{Sst}} \times \frac{\textbf{Pst}}{\textbf{Pe}} \times \frac{\textbf{100}}{\textbf{3}} \times \textbf{T}$$

With:

S e: surface of Niflumic acid in the solution to be examined.

S st: surface of Niflumic acid in standard solution.

P st: test sample of Niflumic acid in standard solution, in mg.

P e: test of the finished product, in mg.

T: Rate of the raw material, expressed in %.

Standard: (90-110) %.

❖ **Dosage of conservatives:**

Simultaneous dosage of methyl sodium parahydroxybenzoate (Nipagine M Sodium) and sorbic acid. The technique used for conservatives dosing is a chromatographic method by HPLC.

Preparation of solutions:

- **0.05M ammonium acetate solution:**

  - Insert a 3.88g test outlet in a 1L vial Dissolve in 200ml of purified water and supplement to volume with the same solvent.
  - Adjust the PH to 4.7with glacial acetic acid.

- **Operating conditions:**
  - Isocratic diet.
  - Mobile phase (ammonium acetate solution 0.05 M/acetonitrile (60/40V) mix and filter the mobile phase on a 0.45 micro m membrane filter then decarbonize for 10min.
  - Column: Kinetex C8 (25cm*4.6mm*5micro m) (o equivalent, column recommended for the lowest PH).
  - Wave length: 254nm.
  - Injection volume:20micro L.
  - Flow:1.0ml/min.
  - Column temperature 25C°.
  - Sample temperature:25C°.

Confirmation of the system: To be realized on the standard.

- ✓ The symmetry factor of each analysis is not greater than 2.0.
- ✓ The resolution between sorbic acid and sodium Nipagine M Sodium is not less than 5.0.
- ✓ The relative standard solution standard deviation for each analysis of 05 injections is not greater than 2.0%.

- **Preparation of solutions:**

The following table describes the different preparation stages of the two standard solutions (standard) and the solution to be examined (essay).

Table IX : Solutions prepared for Dosage of conservatives by HPLC.

Standard solution:	**Solution Essay**
▪ insert an accurately weighed injection of 20,0mg sorbic acid and 20.0mg Nipagine M Sodium (titrated raw material) in a 100ml flask.	▪ Introduce an accurately weighed test outlet of 2,0g of Flucidal 3% ointment product in a 50ml flask.

▪ dissolve with 50ml of 96% ethanol. ▪ complement to volume with the same solvent. ▪ well shake. ▪ insert 2.5ml of this solution in a flask of 50ml. ▪ compliment to volume, with the mobile phase, (the final concentrations obtained is 0.01mg/ml in sorbic acid and 0.01 mg/mL in Nipagine M sodium).	▪ Add 20,0ml of ethanol at 96%. ▪ dissolve by heating the solution in a mary bath for 3min. ▪ let cool. ▪ Replenish to volume with the same solvent. ▪ Well stir. ▪ Insert 25ml of this solution into a flask of 50ml. ▪ Supplement to volume with the mobile phase (the final concentration obtained is 0.01mg/ml sorbic acid and 0.01 mg/ml Nipagine M Sodium). ▪ Filter the solution on a 0.45micro m membrane filter.

- **Calculation formula**
  - **SORBIC ACID CONTENT:**

$$\textbf{Sorbic acid content}\,\% = \frac{\textbf{Se}}{\textbf{Sst}} \times \frac{\textbf{Pst}}{\textbf{Dilution st}} \times \frac{\boldsymbol{Dilution\ e}}{\boldsymbol{Pe}} \times \boldsymbol{T}$$

With:

Se: Sorbic acid surface in the solution to be examined.

Sst: Sorbic acid surface in standard solution.

Pst: Test sample of sorbic acid in standard solution in mg dilution.

st: Dilution of the standard solution in ml dilution.

e: Dilution of the solution to be examined in ml

Pe: Test sample of the finished product in the test solution in mg.

T: Raw material title expressed in %.

Standard: 0.020% to 0.055%

- **Nipagine M Sodium content:**

$$\textbf{Nipagine M Sodium contents} = \frac{\textbf{Se}}{\textbf{Sst}} \times \frac{\textbf{Pst}}{\textbf{Dilution}} \times \frac{\boldsymbol{Dilution\ e}}{\boldsymbol{P\ e}} \times \boldsymbol{T}$$

with:

Se: Nipagine M Sodium surface in the solution to be examined.

Sst: Nipagine M Sodium surface in standard solution.

Pst: Test sample of Nipagine M Sodium in standard solution in mg dilution.

st: Dilution of standard solution into ml dilution.

e: Dilution of the solution to be examined in ml.

Pe: Test sample of the finished product in the solution to be examined in mg.

T: Raw material title expressed in %.

Standard: 0.045% to 0.055%.

II.6 Paste preparation microbial purity control

- **Purpose:** This operating mode describes the technique of microbiological control of pasta pharmaceutical preparations for skin administration.
- **Domain of application:** applies to microbiological control of pharmaceutical preparations paste for skin administration ointments.
- **Rreference documents:** European Pharmacopeia 2017 9th edition.
- **Equipment, equipment:(see annex 02)**

Method:

❖ **Enumeration of total aerobic germs and of total molds and yeasts:** TAMC and TYMC:

○ **Plate method:**

➢ **The analyst must:**

- Make the TSA frozen medium and the dextrose-frozen sabouraud medium melt in the mary bath at 100°C by slightly loosing the closures and keeping them in the mary bath in surfusion at 40-45°C.
- Prepare a solution of 10 g of the product to be examined in 90 ml of the buffer solution of pepton to sodium chloride PH 7 in the phosphate buffering solution PH 7.2 (solution A).
- Shake until complete homogenization. Other dilution rates can be adjusted if the product characteristics and sensitivity requirement an appropriate surfactant, such as polysorbate 80 at a concentration of 1g/l, may be added to

facilitate the suspension of hard-to-moist substances. The following dilutions are prepared with the same diluent.

- Take 4 times 1 ml of the solution to prepare and deposit a sample in a 90 nm diameter petri box.
- Pour into 2 and 4 boxes of petri intended for TAMC 15 ml to 20 ml of TSA gelose medium, and in the 2 remaining boxes for TYMC 20 ml dextrose-gelose sabouraud medium.

  Shake the boxes gently with a circular movement to ensure a homogeneous mixture of the sample and the gelose, without boiling and without wetting the covers of the box.
- Incubate the TSA boxes at 30-35°C for 3-5 days and the dextrose-gelose sabouraud boxes to 20-25 °C for 5-7 days.

- **Reading:**

The total aerobic microbial count (TAMC) is considered to be equal to the number of CFU (colony forming unit) obtained with the TSA medium (Trypticase soy agar) , the total combined yeast /mold count (TYMC) are considered equals to the amount of (CFU) obtaining with the frozen sabauroud dextrose medium(SDA), if bacterial columns are detected on this medium, they are compatible in the TYMC if they have predicted that the TYMC may exceed the criterion of acceptance due to the bacteria growth of the freezed sabouroud dextrose medium (SDA) containing antibiotics can be used.

Count the number of columns appearing in each type of box, make the average and deduct the count of colony-forming units per gram of product.

- **Search for pseudomonas aeruginosa and staphylococcus aureus**

  For this purpose, the analyst must:

  - Inoculate 100ml of TSB medium (tryptic soy broth) with 10ml of solution A prepared as described in the TAMC and TYMC of the corresponding quantity of 1g of product.
  - Homogenize and incubate 30-35C° for 18h to 24h.
  - Shake the container, then transfer 0.1ml of TSB liquid medium into cetrimide agar and incubate at 30-35°C for 18-72h to test for pseudomonas aeruginosa.

- Shake the container, then transfer 0.1ml of TSB liquid medium into mannitol salt agar and incubate at 30-35°C for 18-72h to test for staphylococcus aureus.

- **Reading:**

- Growth of colonies on cetrimide gelose indicates possible presence of pseudomonas aeruginosa to be confirmed by identification tests.
- Growth of colonies on mannitol salt gelose indicates the possible presence of *Staphylococcus aureus* to be confirmed by identification tests.
- Product satisfies the test if no colonies are present or if the identification confirmation tests are negative.

- **Negative witness:**

To verify operating conditions, the analyst must:

- ✓ Conduct a test on a prepared negative test by replacing the dilution with the preparation to be examined.
- ✓ Expose the open boxes of medium TSA gelose and sabouraud dextrose-gelose under a light flow hood no microbiological growth should be observed, obtaining a non-conforming result requires investigation.

➢ **European Pharmacopoeia Standards 2017,9th edition:**

The standards chosen are:

- **TAMC(UFC/g):** max. 10^2.
- **TYMC (UFC/ g):** max. $2,10^1$.
- **Pseudomonas aeruginosa/g:** Absence.
- **Staphylococcus aureus/g:** Absence.

NB: The analyst must inform the straw register at each check performed and the analysis bulletin after each check.

➢ **Standards**:

TAMC(UFC/g): at max 10^2

TYMC(UFC/g): at max 10^1

- **Acceptance limits:**

  TAMC(UFC/g): at max $2*10^2$.

  TYMC(UFC/g): at max $2*10^{1.}$

  Pseudomonas aeruginosa /g: absent

  Staphylococcus aureus/g: absent

- **Stability study:**

  According to the ISH (standards)

Carried out in 3 climatic chambers:

- ✓ In real time 25C° and 60% humidity
- ✓ Intermediate time 30C° and 65% humidity T3 T6 T9 T12 T18 T36.
- ✓ In accelerated time 40C° and 75% humidity T3 T6 the study is carried out for up to 6 months.
- ✓ At each date, the analysis is repeated (every 3 months), except for microbiology, to see if the product is in conformity.

- Stability study is for:
- updating a pharmaceutical file.
- See the efficiency of the ointment during the shelf life.

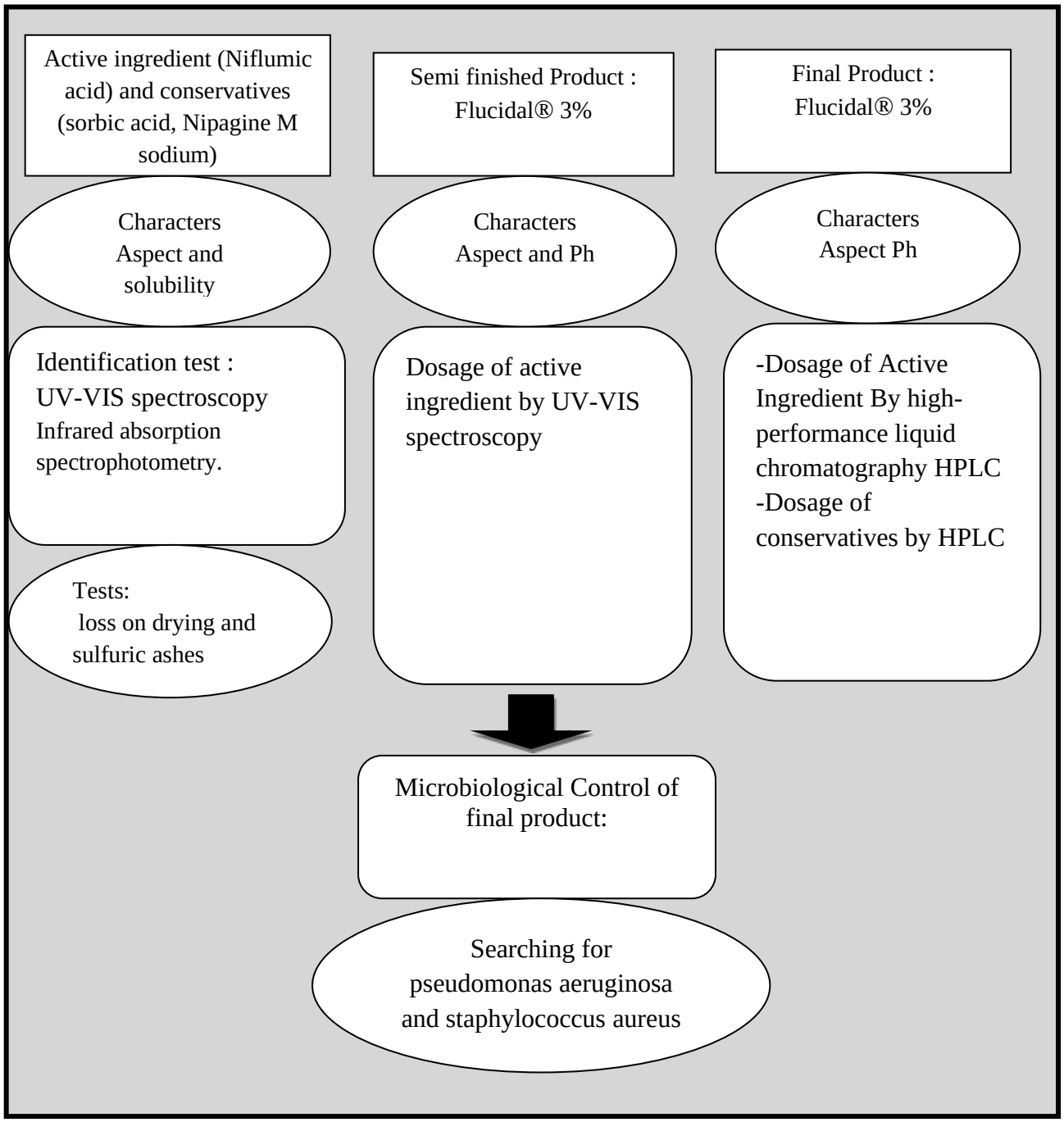

Figure 4. Diagram summarizing physiochemical and microbiological quality controls methods of Flucidal®3% ointment.

Chapter III
Results and discussion

The purpose of This study is to monitor the physicochemical quality control of a non-steroidal anti-inflammatory drug, Flucidal® ointment 3%, using spectrophotometric, chromatographic, and microbiological methods to determine whether it complies with European Pharmacopeia standards both during production and as a finished product.

III.1 Physico-chemical control of raw materials

Table X displays the results of our physico-chemical control over the excipients, sorbic acid and Nipagine M sodium, as well as the active ingredient, Niflumic acid. These controls were carried out through characterization, identification, and testing parameters in compliance with the recommended practices of the European Pharmacopeia.

1.1.1. Quality control of the active ingredient Niflumic acid:

The results obtained from the physico-chemical control of the active substance Niflumic acid are presented in the table below:

Table X : Results of physical and chemical testing of the active substance Niflumic acid.

Tests item	Reading	Specifications PE 2020	Compliance
Character			
Aspect	Pale yellow crystalline powder.	Pale yellow crystalline powder.	
Solubility	insoluble in water, freely soluble in acetone, soluble	Practically insoluble in water, freely soluble in acetone, soluble in 96%	

	in 96% ethanol and methanol.	ethanol and methanol	**Compliant with the specifications described in EP 2020**
Identification			
Melting point	204,4 °C	About 204°C	
Infrared absorption spectrophotometry	Comparable to the SCR Niflumic acid reference spectrum.	Identical to the SCR Niflumic acid reference spectrum.	
Dryingloss (%)	0,09	≤0,3	
Sulphurash (%)	0,059	≤0,1	
Dosage: by C13H9F3N2O2 content potentiometry calculated relative to the dried substance (%)	99,39%	98,5% to 101,5%	

The principal peaks of the spectrum obtained when identifying Niflumic acid are in the figures:

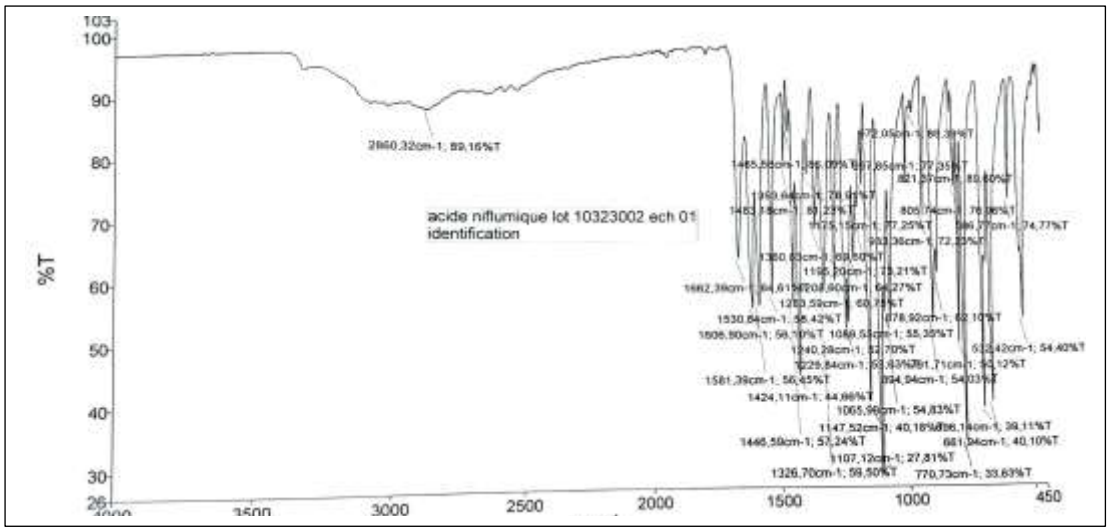

Figure 5. Absorption spectrum of Niflumic acid (Test).

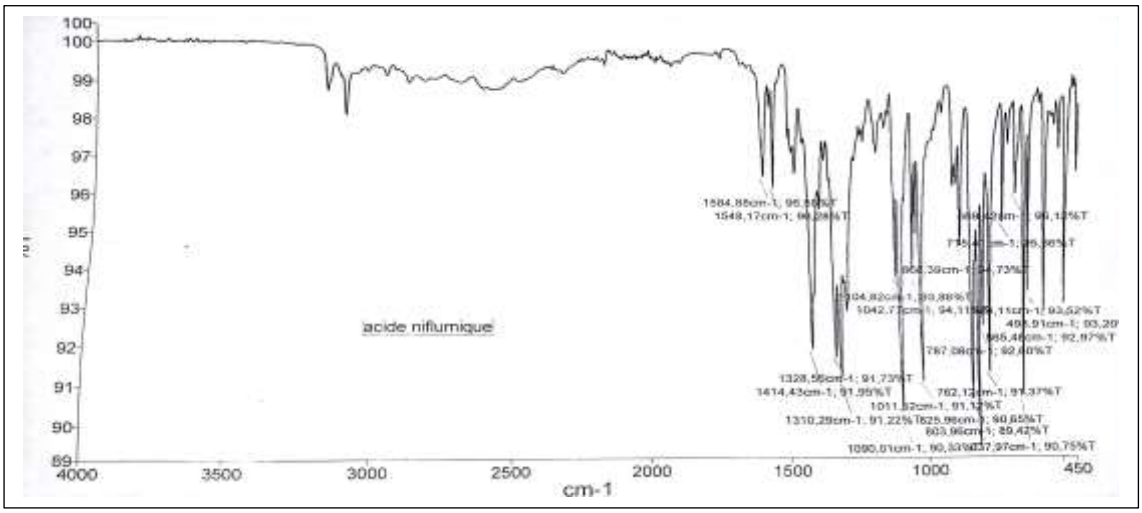

Figure 6. Absorption spectrum of Niflumic acid SCR.

The findings concerning the properties (aspect and solubility) of the active ingredients in raw materials, Niflumic acid, were assessed in accordance with PE 2020 guidelines. Physical characteristics are important, particularly the drug's particle size. The product's compounding process may cause raw ingredients with smaller particle sizes to dissolve more quickly than those with bigger particle sizes. Additionally, the amount of medication in solution and the effectiveness of preservation systems may be impacted by the PH value. **(Niazi ,2004).** Niflumic acid was shown to be the active component by the application of Infrared absorption spectrophotometry. A technique for verifying the identification of non-ionized organic substances that aren't organic acid or basic salts is infrared absorption spectrophotometry. Making use of a material or reference spectrum is essential. **(PE, 2008)**

The SCR reference chemical spectrum and the Niflumic acid (AI) spectrum acquired by infrared absorption spectrophotometry have been compared. The results indicate that the spectrums may be superposed, indicating that the active ingredient meets the standard.

The obtained melting point of 204.4 C° is in line with the 2020 PE standard by around 204 C°, and the drying loss rate of 0.09 C ° is also in accordance with the standard (≤0.3).

Drying loss (determination of water content): this method consists in determining the humidity rate or water content of the active ingredient by steaming at 105C° for 2h (**Sow et al., 2019**)

The resulting Sulphur ash of the active ingredient (0,059%) is below the tolerated limit which is 0.1%, the active substance is compliant.

According to the specifications described in the 2020 European standards, the results obtained for the dosage of the active substance Niflumic acid is 99.39% situated within the standard range (98,5 % to 101,5 %).

The results obtained from the physical chemical control of the active ingredient meet the standards required by the European pharmacopeia 2020, which reflects its good physico-chemical quality.

1.1.2 Quality control of sorbic acid:

The results obtained from the physico-chemical control of the conservative sorbic acid are presented in the table below:

Table XI : Results of the physico-chemical inspection of Sorbic acid.

Test item	Reading	Specifications PE 2017	Compliance
Aspect	White crystalline powder.	White or markedly white crystalline powder.	**Compliant with the**
Solubility	Weakly soluble in water and easy soluble in 96% ethanol.	Weakly soluble in water, easy to solve in 96% ethanol.	
Melting point	134°C	132°C to 136°C	
Identification			
Melting point	134°C	132°C to 136°C	
Infrared absorption spectrophotometry	Comparable to the SCR	Identical to the SCR reference spectrum.	

	reference spectrum.		**specifications described in EP 2017**
Tests :			
Appearance of the solution	S solution is clear and colourless.	S solution is clear and colourless.	
Sulphur ash (%)	0,1	≤0,2	
Volumetric dosage			
Contents of C6H8O2	100,09%	99,0% to 101,0%	

The principal peaks of the spectrum obtained when identifying Sorbic acid are in the figures:

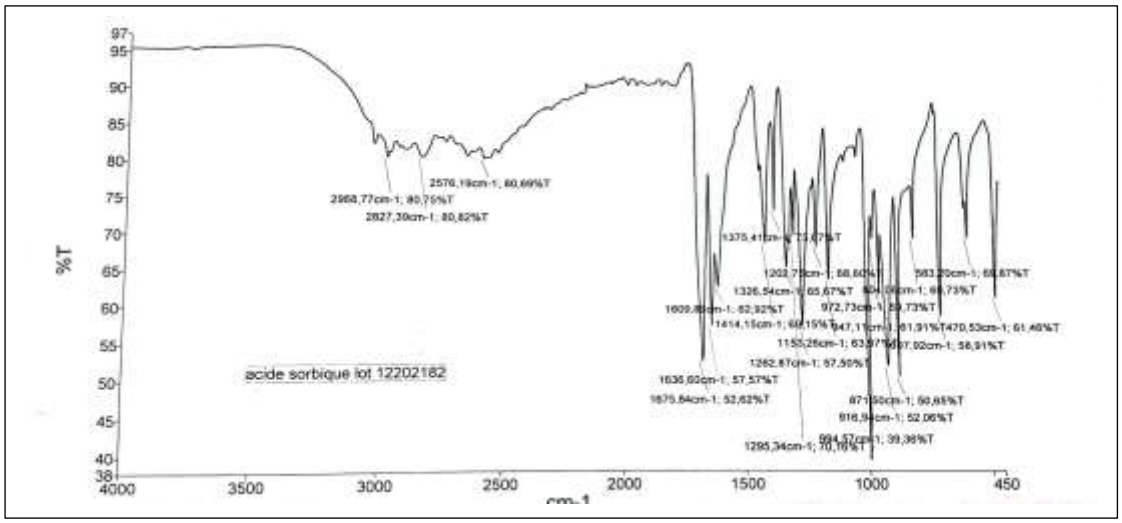

Figure 7. Absorption spectrum of Sorbic acid (Test).

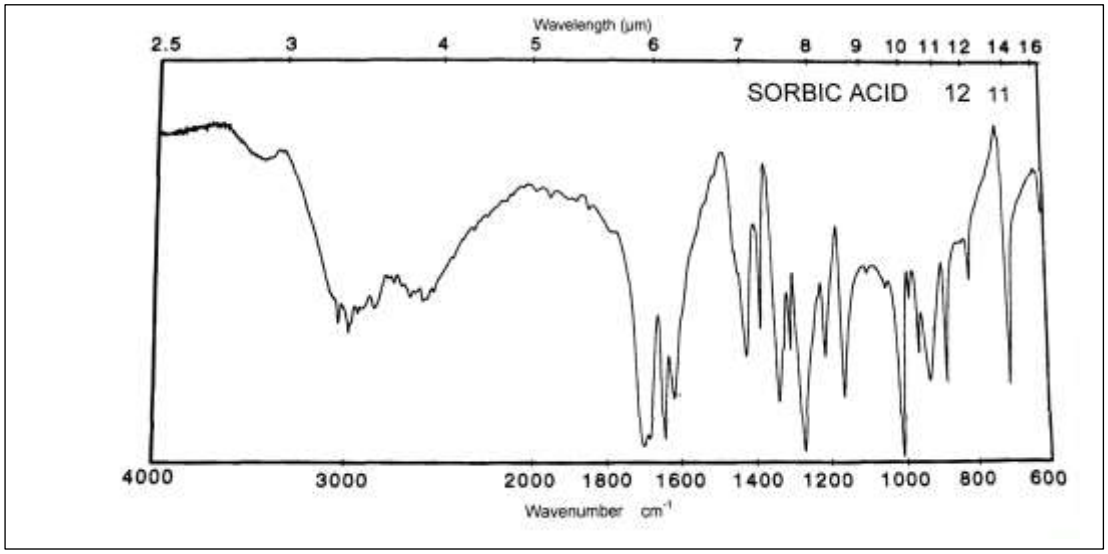

Figure 8. Absorption spectrum of Sorbic acid SCR.

1.1.3 Quality control of Nipagine M Sodium (methyl sodium parahydroxybenzoate):

The results obtained from the physico-chemical control of the conservative Nipagine M Sodium are presented in the table below:

Table XII : Physico-chemical testing results of Nipagine M Sodium.

Test item	Reading	Specifications PE2017	Compliance
Aspect	white or sensibly white crystalline powder.	Hygroscopic white or sensibly white crystalline powder.	**Compliant with the**
Solubility	water soluble and 96% ethanol soluble and practically insoluble in methylene chloride.	water soluble and 96%ethanol soluble and practically insoluble in methylene chloride	
Identification			
First identification : B, D			
B- Infrared absorption spectrophotometry	Comparable to the SCR Sodium methyl parahydroxybenzoate reference spectrum.	identical to the SCR Sodium methyl parahydroxybenzoate reference spectrum.	

D- Sodium reaction(s)	A white,dense precipitation	A white, dense precipitation is formed	**specifications described in EP 2017**
Tests :			
Appearence of the solution	S solution is clear and not more strongly colored than the test solution.	S solution is clear and not more strongly colored than the JB6 test solution.	
PH	9,65	9,5 to 10,5	
Dosage : by HPLC			
Sodium methyl parahydroxybenzoate content calculated in relation to the anhydrous substance (%)	100,1%	95,0% to 102,0%	

The principal peaks of the spectrum obtained when identifying Nipagine M Sodium are in the figures:

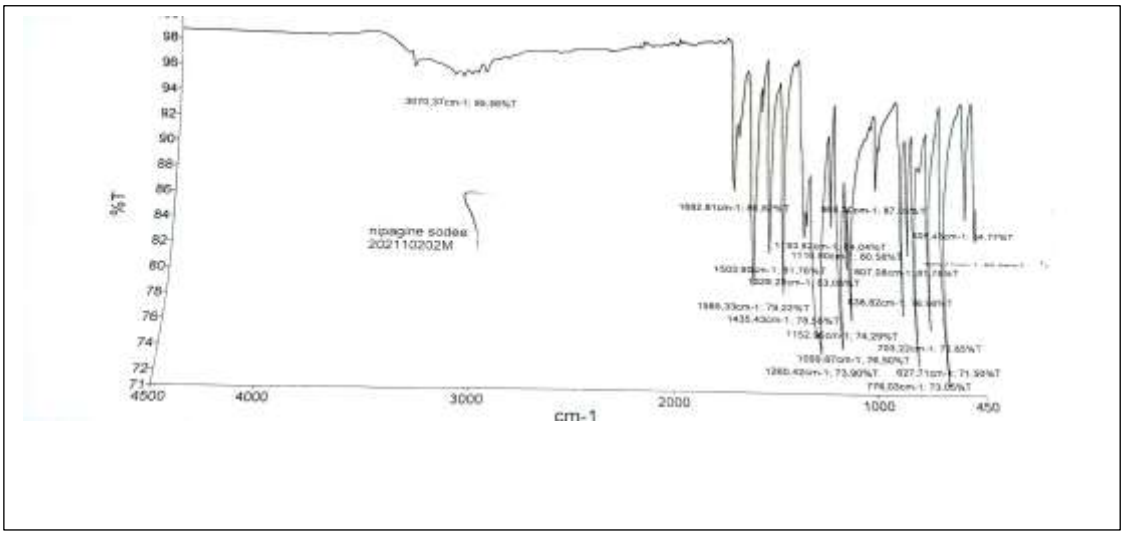

Figure 9. Absorption spectrum of Nipagine M Sodium (Test).

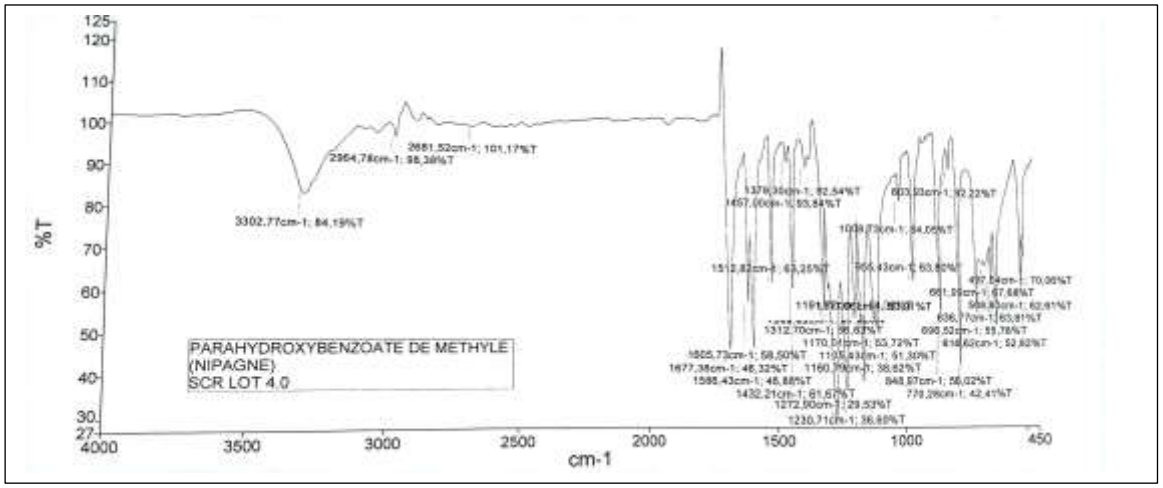

Figure 10. Absorption spectrum of Nipagine M Sodium SCR.

The results about the characteristics (aspect and solubility) of the conservatives in raw materials, Sorbic acid and Nipagine M sodium, in compliance with PE 2017 standards. In all instances a conservative efficacy challenge is needed to prove adequate protection against the growth of microorganisms during the shelf-life and use of the product (**Niazi ,2004)**.

The conservatives, were identified by the use of Infrared absorption spectrophotometry.

The SCR reference chemical spectrum and the conservatives (sorbic acid and Nipagine M sodium) spectrum acquired by infrared absorption spectrophotometry have been compared. The results indicate that the spectrums may be identical, indicating that the conservatives meets the standard of European pharmacopeia.

The obtained melting point of Sorbic acid (134 °C) is in line with the 2017 EP standard by (132 to 136 °C).

About the test of D-sodium reaction the result was a white dense precipitation, the appearance of the solution of sorbic acid was clear and colorless and About the Nipagine M the solution was clear & not more strongly colored than the JB6 test solution those results meets the standard of EP 2017.

The resulting sulfuric ash of sorbic acid (0.1%) is below the tolerated limit of 0.2%

According to the specifications described in the 2017 European standards, the results obtained for the dosage of sorbic acid is 100,09%. This value is within the standard range (99,0% to 101,0%).

The results obtained for the dosage of Nipagine M Sodium is 100,1%, within the standard range (95,0% to 102,0%).

The results obtained from the physic-chemical inspection of the conserver satisfy the standards required by the European pharmacopeia 2017, which reflects its good physico-chemical quality.

III.2 Quality control of intermediate product Flucidal® 3%(IPC)

- **Character control:**

The results obtained from the semi-open product character control (IPC) FLUCIDAL® 3% represent in the table below:

Table XIII : Characteristics of the ointment FLUCIDAL ® 3%.

Product	**Aspect**	**PH**
Flucidal ointment 3%	The ointment is bright, homogeneous with a lemon smell and a slightly yellowish white color.	4,37

Our result showed that the FLUCIDAL® 3% has a white to slightly yellowish color and a lemony scent, which is in line with the required appearance criteria. Regarding the measured PH, it falls within the range specified in the FLUCIDAL® 3% technical file (3,5-4,5).

- **UV measurement**:

A spectral analysis was carried out by the UV spectrophotometer, with a wavelength fixed at 290nm, allowed the detection of absorbances of Niflumic acid in the standard solution, which contains the active compound and the examined solution (essay).

In the light of findings described below (**TABLE XV),** we conclude that the value of the relative standard deviation calculated by the system for the standard solution is equal to 0.03%. Thus, this finding meets the acceptance standard required by the European Pharmacopoeia (<2%)

Table XIV : Absorptions of Niflumic acid in standard solution.

Standard Read Number	Standard Absorbance	Average Absorbances	Relative standard Deviation (RSD)%
Standard solution 01	0,614	0,6142	0,03
Standard solution 02	0,6144		
Standard solution 03	0,6142		

In light of the results presented below **(TABLE XVI)**, the system determined that the relative standard deviation for the test solution is 0.13%. This result, satisfies the European Pharmacopoeia's acceptance requirement (<2%). Using these data, we also aimed to determine the amount of Niflumic acid present.

The calculated value of the Niflumic acid content in the IPC is equal to 2.96%, this value is in the interval required by the protocol (2.7% - 3,3%), Based on the absorption values measured at 290 nm, we can conclude that this product complies with the European Pharmacopoeia.

Table XV: Absorptions of Niflumic acid in essay solution.

Standard Read Number	Standard Absorbance	Average Absorbances	Relative standard Deviation (RSD)%
Test solution 01	0,5922	0,5928	0,13
Test solution 02	0,5933		

III.3 Quality control of finished product (FLUCIDAL ® 3%)

The results obtained from the final product FLUCIDAL® 3% represented according to the methodology recommended by EP 2017 in the table below:

Table XVI : Physico-chemical testing results of finished product (Flucidal® 3%).

Test item	Reading	Specifications PE2017	Compliance
Character			**Compliant with the specifications**
Aspect	Bright homogeneous ointment has a lemon smell and a slightly yellowish white color.	Bright homogeneous ointment has a lemon smell and a slightly yellowish white color.	
Identification :			
Identification of Niflumic acid by HPLC	The Niflumic acid retention time in the test solution is the same as that of the reference solution.	The Niflumic acid retention time in the test solution is the same as that of the reference solution.	
Tests :			
Average tube weight(g)	39.78g	38g to 42g	
PH	3.95	3.5 to 4.5	

Dosage of conservatives by HPLC%			**described in EP 2017**
Sorbic acid	0.044%	0.020% to 0.055%	
Nipagine M sodium	0.054%	0.045% to 0.055%	
Dosage			
Niflumic acid content by HPLC (%)	3.05%	2.7% to 3.3%	

The characterization results of the finished Flucidal® 3 % product shows that it is a bright, homogeneous ointment with a lemon smell and a slightly yellowish white color, identical to the criteria prescribed by SAIDAL's internal pharmaceutical record. **(monographie interne de saidal, 2020).** This quality is in accordance with PE 2017.

The average weight of the tube (g) is 39.78 g this result corresponds well to the standard required by SAIDAL's internal pharmaceutical dossier (**monographie interne de saidal, 2020)** (38g to 42 g) of this declared conformity fact.

The PH of the ointment is 3.95, this value is consistent with SAIDAL's internal record (3.5 to 4.5) (**Monographie interne de saidal, 2020**).

Using HPLC we established the presence of Niflumic acid in the final product FLUCIDAL® 3% and determine its concentration in comparison with standards. Moreover, we identify active compound to estimate the retention time of Niflumic

acid. The value of the Niflumic acid retention time in the test solution (ointment) and standard solution (Niflumic acid) are displayed in the table below.

Table XVII : Peak retention time of standard solution and essay solution.

Retention time of Niflumic acid	**Retention time 01**	**Retention time 02**	**Retention time 03**	**Average retention time**
In the Standard solution	5.193	5.187	5.199	5.193
In the test solution	5.188	5.194		5.191

According to our results, the average retention length of 5.19 minutes for Niflumic acid in the test solution matches that of the Niflumic acid solution, fulfilling the batch file's criteria.

To demonstrate system compliance, two crucial parameters that must be examined are relative standard deviation (RSD) and symmetry factor. The table below displays these findings.

Table XVIII : Comparison of repeatability results and symmetry factor with acceptance criteria.

Settings	**Average result of 3 injections**	**Acceptance critera**
Repeatability RSD (related standard deviation)	0.12	≤2.0%
Symmetry factor	1.521	< 2

Thus, with values less than 2%, the results obtained satisfy the FLUCIDAL® 3 % technical dossier's acceptance standards. This suggests that the HPLC system's

performance is appropriate and compliant for carrying out the quantitative and qualitative studies.

The results shown in the accompanying tables were obtained by dosing the test solution (ointment) with a wavelength of 267 nm and the two standard solutions (Niflumic acid).

Table XIX : HPLC standard solution Niflumic acid dosage results.

Injection number Standard	**Surface standard (air)**	**Average Surface**	**Relative standard deviation (RSD)%**
Injection 01	3036773	3038307.704	3.301
Injection 02	3048138		
Injection 03	3030013		

The system's computed value of the relative standard deviation (RSD) for the Niflumic acid standard solution is 0.301%, which satisfies the EP's necessary acceptance standard of less than 2%.

Table XX : HPLC test solution Niflumic acid dosage results.

Injection number Test	**Surface Test (air)**	**Average Surface**	**Relative standard deviation (RSD)%**
Injection 01	3077489	3085220.185	0.354
Injection 02	3092952		

The value of the relative standard deviation (RSD) calculated by the system for the test solution containing Niflumic acid, is equal to 0.354% so it meets the acceptance standard required by EP (<2%).

Our result reveal that the finished product has a concentration (3.05%) that is within the range of concentrations in the active substance (2.7 to 3.3%) according to SAIDAL's internal pharmaceutical file (**Monographie interne de saidal, 2020**).

The acquired findings, which indicate both the separation and excellent symmetry of the two peaks, fulfill the conditions for acceptance of the FLUCIDAL® 3% internal method product technical file. Consequently, the figure displays well-resolved chromatographic peaks that correspond to the standard solution containing the active ingredient, Niflumic acid:

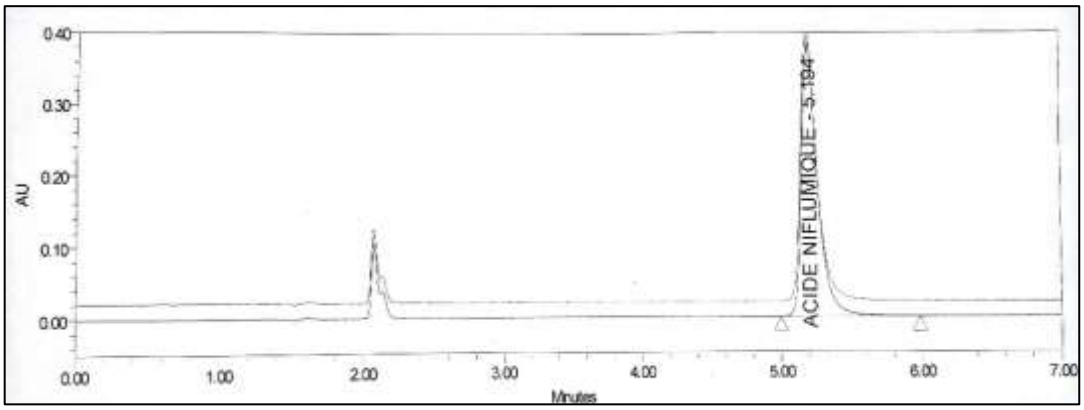

Figure 11. Chromatogram representing the dosage of the active ingredient (Niflumic Acid) in the test solution.

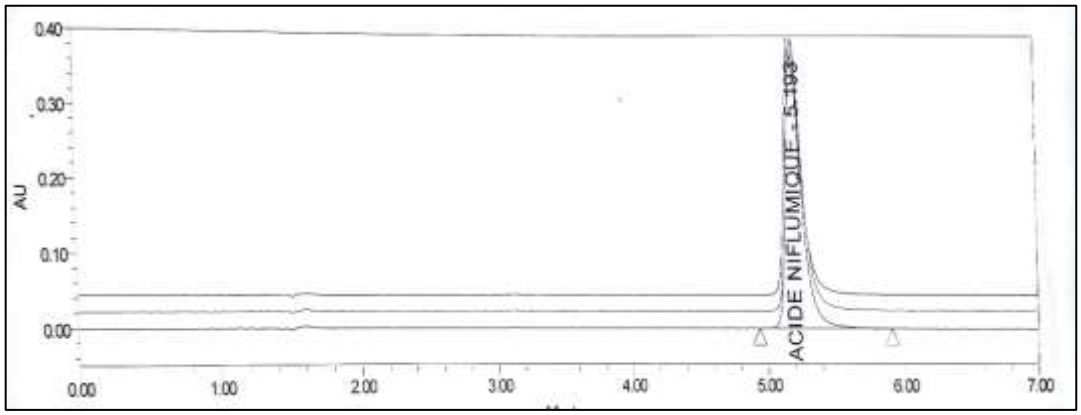

Figure 12.Chromatogram representing the dosage of the active ingredient (Niflumic Acid) in the standard solution.

- **Dosage of conservatives by HPLC**

The conservatives were dosed by HPLC in the finished product in order to detect their presence and to determine their concentrations in comparison with the norms.

- **Sorbic acid dosing by HPLC**

The results of the dosing of sorbic acids of the two solutions (standard solution and test solution) at a wavelength of 267 nm are presented in the tables below.

Table XXI : Results of the sorbic acid dosage of the standard solution by HPLC.

Injection number standard	**Surface standard (air)**	**Average Surface**	**Relative standard deviation (RSD)%**
Injection 01	2874489	2871324.3	0.2
Injection 02	2874653		
Injection 03	2864831		

The sorbic acid standard solution has a sorbic acid content of 0.17%, which satisfies EP's acceptance criterion of less than 2%.

This last result is in accordance with the requirements outlined in EP 2017.

Table XXII : Results of the sorbic acid dosage of the HPLC test solution.

Injection number Test	**Surface Test (air)**	**Average Surface**	**Ecart type relatif (RSD)%**
Injection 01	2788756	2808533.1	1.0
Injection 02	2828310		

The sorbic acid test solution (ointment) has a relative standard deviation (RSD) of 0.38 %, which meets the EP's necessary acceptance criterion of less than 2%.
The measurement of the sorbic acid content is 0.044%, falling between the conservatives concentration range of 0.020% to 0.055%.

- **Dosage of Nipagine M Sodium by HPLC:**

The results of Nipagine M Sodium dosing of the two solutions (standard solution and test solution) at a wavelength of 267 nm are presented in the tables below:

Table XXIII : Results of the dosage of Nipagine M Sodium standard solution by HPLC.

Injection numberstandard	**Surface Standard (air)**	**Average Surface**	**Relative standard deviation (RSD)%**
Injection 01	953382	952873.0	0.4
Injection 02	948551		
Injection 03	956686		

The relative standard deviation value (RSD) calculated by the system for the Standard Nipagine M Sodium solution is equal to 0.25% so it meets the acceptance standard required by EP (<2%).

Table XXIV : Results of the dosage of Nipagine M Sodium from the HPLC test solution.

Injection number Test	**Surface Test (air)**	**Average Surface**	**Relative standard deviation (RSD)%**
Injection 01	1135977	1131387.4	0.6
Injection 02	1126797		

This table demonstrates that relative standard deviation (RSD) for the standard solution of Nipagine M Sodium, as determined by the system, which is equal to 0.39%, meeting the EP's necessary acceptance criterion (<2%).

The internal pharmaceutical record of SAIDAL (**Monographie interne de saidal, 2020**) indicates that the Nipagine M Sodium content value is 0.054, falling within the conservatives concentration interval of 0.045 to 0.055.

Tests on the conservatives standards solution were performed and the results made it possible to examine the system's conformity. Thus, the results of the acceptance criteria for Nipagine M Sodium and Sorbic acid are compared with RSD, symmetry factor, and resolution in the table below:

Table XXV : Repetition results, symmetry factor and resolution between Nipagine M Sodium and sorbic acid with acceptance criteria.

Settings	**Average result of 3 injections**	**Accceptation critera**
Repeatability RSD (related standard deviation)	0.17% (Sorbic acid) 0.25 % (Nipagine M Sodium)	≤ 2.0%

Symmetry factor	1.123(sorbic acid) 1.121(Nipaine M Sodium)	< 2

The acquired findings, which indicate both the separation and excellent symmetry of the two peaks, fulfill the conditions for acceptance of the FLUCIDAL® 3% internal method product technical file. Consequently, the figure displays well-resolved chromatographic peaks that correspond to the standard solution containing the two conservatives, sorbic acid and Nipagine M Sodium:

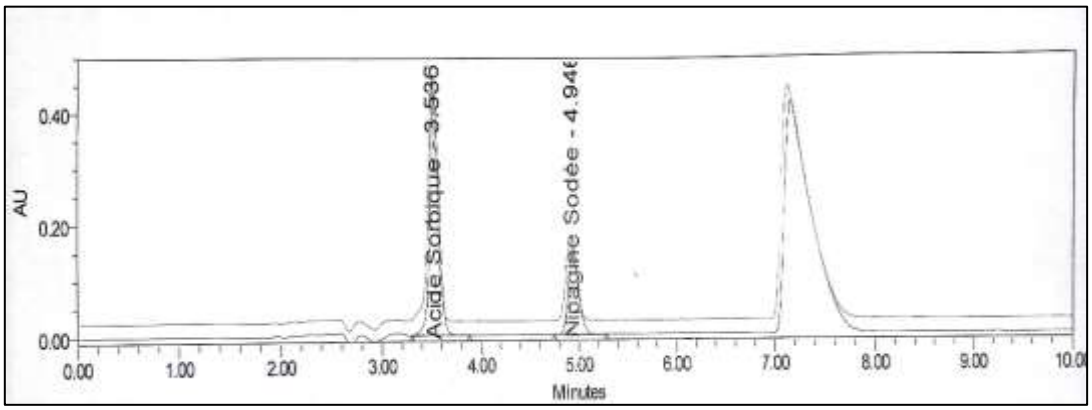

Figure 13. Chromatogram representing the excipients (Nipagine M Sodium, Sorbic Acid) in the test solution.

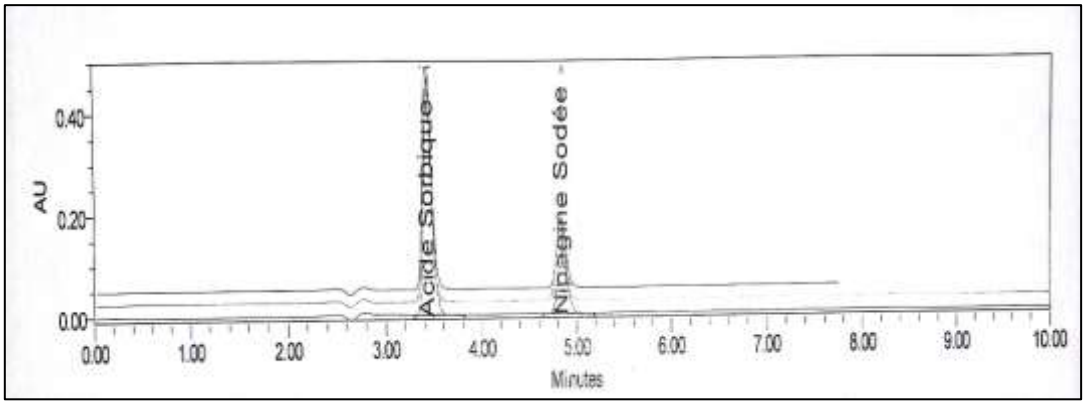

Figure 14. Chromatogram representing the dosage of the excipients (Nipagine M Sodium, Sorbic Acid) in the standard solution.

All physico-chemical analyses prove that the finished product FLUCIDAL® 3% is of a satisfactory and acceptable physical and chemical quality.

The physico-chemical test findings of the FLUCIDAL® 3% finished product satisfied the requirements specified in SAIDAL's internal pharmaceutical record **(Monographie interne de saidal, 2020).** This illustrates the product's efficiency in the production of ointments as well as its adherence to relevant standards and best manufacturing practices recommendations. As a result, we can ensure that FLUCIDAL® 3% is in compliance and has high physico-chemical quality.

III.4 Microbiological control of the finished product (FLUCIDAL® 3%)

The following table displays the findings of the microbiological control of the final product of the FLUCIDAL® 3%, which was conducted in accordance with the PE 2017 suggested methodology:

Table XXVI : Results of microbiological control of the finished product FLUCIDAL® 3%.

Test item	Reading	Specifications PE 2017	Compliance
Microbial Contamination			**Compliant with the specifications described in EP 2017**
Total aerobic germs (UFC/g)	<10	≤100	
Total yeast and harvest (UFC/g)	<10	≤10	

Pseudomonas aeruginosa(/g)	Absence.	Absence.	
Staphylococcus aureus(/g)	Absence.	Absence.	

The testing methods described for microbiological control of non-obligatory sterile products allow the quantification of mesophilic bacteria, molds, and yeasts that have the ability to develop aerobiosis. However, the presence of a specific Pseudomonas sp., or Staphylococcus may indicate raw material contamination, such contamination would be indicative of a deficient process as well as an inadequate conservative system.**(Niazi,2004)**.

In comparison to the PE 2017 standards, the total aerobic germs, total molds, and total yeasts results in the table are acceptable. The results of the search for *staphylococcus aureus* and *pseudomonas aeruginosa* indicate that these bacteria are completely absent from the final product.

FLUCIDAL® 3% is a conforming product due to its high microbiological quality and compliance with the standards outlined in the EP 2017 regulations.

Conclusion

The drug is a very delicate and sensitive product from the point of manufacturing, display, and marketing to the point of usage. Preventive actions are thus required to guarantee the safe use of the drugs.

In order to confirm that all of the substances tested in this study, complied with international standards, we performed a variety of physicochemical and microbiological analyses at SAIDAL DAR EL BEIDA laboratory.

The results of the product's quality control revealed that it was compliant with European Pharmacopoeia and Pharmaceutical Records standards. Additionally, the microbiological quality of the final product's assessed a lack of germs, indicating that the finished product has a good microbiological quality and meets with the international standards.

Consequently, this study indicates that the (FLUCIDAL® 3%) is safe and efficient. Indeed, all the parameters examined as part of the quality control of the product meet the quality standards of the European Pharmacopoeia's requirements, indicating that there is no risk involved in the product's delivery to patients.

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Annexes

Annex01. Non-biologicalmaterial

- Equipment used for physical and chemical control:

Devices:

High-performance liquid chromatography

PH meter

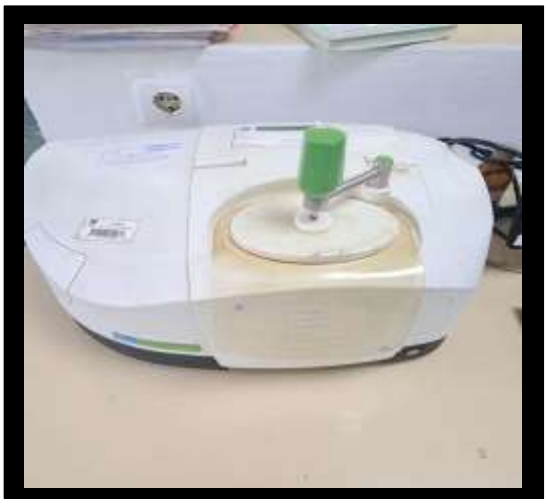
Stove

Magnetic stirrer

Analytical balance

Mary bath

UV-VIS Spectroscopy



Infrared spectroscopy (IR)

Annex02

- Equipment and reagents used for physico-chemical testing:

Equipment	Reagents	Glassware
- Analytical precision balance. - High-performance liquidchromatography.HPLC. - Infrared absorption spectrophotometry. **(PerkinElmer).** - Muffle furnace. - Marie bath. - Oven. - PH-meter. - Polarimeter. - UV/Visible. absorption. spectrophotometry. - Vortex. - Flask	- Acetone. - Distilled water, Water R. - Ethanol R. - Glacial acetic acid. - Hydrochloric acid R. - Methanol. - Methylene chloride. - Nitric acid. - Silver nitrate R2.	- Bulb. - Erlenmeyer. - Graduated glass test tube. - Graduated glass pipettes. - Platinum crucible. - Stainless steel spatula. - Test tubes.

3) Equipment and materials used for microbiological testing

- Cetrimide agar media.
- Mannitol salt agar media.
- Stove adjusted to 30-35°C.
- Stove adjusted to 20-25°C.
- Bath marie adjusted to 100°C.
- Bath marie adjusted to 45°C.
- Paster pipette or platinum anse.
- Bunsen burner.

Annex 03.

- **Physico-chemical control results**

- Raw materials :

  - Niflumic acid

1.Calculation of drying loss

$$Pd\% = (PV + Pe)\text{-}\frac{Pf}{Pe} \times 100 = (0.91000 + 1.0001)\text{-}\frac{1.9084}{1.0001} \times 100 = 0.0016 \times 100$$
$$= 0.169\%$$

$Pd\% \leq 0.3$

Intemediate product control

Calculation formula

$$T\% = \frac{ABSE}{ABSst} \times \frac{Pst}{Pe} \times T$$

$$T\% = \frac{0.5928}{0.6142} \times \frac{30.8}{1004.4} \times 99.92$$

Titre % = 2.96%

NORME (2.7-3.3) %

- Finished product control:

- Dosage of niflumic acid in final product:

  - Niflumic acid content in %:

$$\text{Niflumic acid content in}\% = \frac{Se}{St} \times \frac{Pst}{Pe} \times 100/3 \times T$$

$$\text{Niflumic acid content in\%} = \frac{3085220.2}{3038307.7} \times \frac{0.354}{0.301} \times \frac{100}{3} \times 99.92$$

Niflumic acid content% = 3.05%

NORME (2.7-3.3)

- ❖ Dosage of conservatives
  - Sorbic acid content %

$$\text{Sorbic acid } \% = \frac{\text{Se}}{\text{Sst}} \times \frac{\text{Ps}}{\text{Pe}} \times \text{T}$$

Sorbic acid content %= 0.044%

NORME (0.020%-0.055%)

- Nipagine M Sodium content %:

$$\text{Nipagine M Sodium\%} = \frac{\text{Se}}{\text{Sst}} \times \frac{\text{Ps}}{\text{Pe}} \times \text{T}$$

Nipagine M Sodiumt %= 0.054%

NORME (0.045%-0.055%)