JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"Formulation and Evaluation of Ivermectin Cream: Method Development And Validation Consideration By HPLC"

[1*]Chandani Kushwaha, [2]Yogita Tyagi, [3]Hiba Praveen, [4]Praveen Kumar Ashok

Master Scholar, Associate Professor, Assistant Professor, Director

Department of Pharmaceutics

Gyani Inder Singh Institute of Professional Studies, Uttarakhand Technical University, Dehradun, India

Abstract

Rosacea is a recurring, chronic illness that can cause a wide range of cutaneous symptoms. Financially, physically, and psychologically detrimental are the symptoms of centrofacial inflammatory dermatosis. A variety of topical, oral, and systemic therapies are available. However, rosacea therapy is still challenging. Recently, three clinical trials have exhibited the efficiency of topical ivermectin in treating rosacea. Ivermectin exhibits a broad-spectrum anti-parasitic action, specifically targeting Demodex mites residing within the pilosebaceous cells of individuals with papulopustular rosacea. The application of ivermectin cream successfully eradicates these mites. Ivermectin reduces cellular and humoral immune responses and has anti-inflammatory properties as well. But the currently available treatment methodologies like azelaic acid, brimonidine, oxymetazoline & topical metronidazole which are available in market have various problem related to side effect associated with the use of these drugs. Therefore, to combat these problem associated with the rosacea treatment we have prepared topical ivermectin cream. The main reason for choosing ivermectin is that it has very less side effect on skin in comparison to other available medicaments. Ivermectin 1% cream once daily compared to twice-daily metronidazole 0.75% cream, substantially less inflammatory lesions were present, which was proven to increase the number of IGA patients in a separate phase III study. Ivermectin 1% cream significantly increased patient satisfaction compared to metronidazole 0.75% cream. However, the choice to test metronidazole twice daily rather of a similarly efficient once-day regimen may have had an impact on these evaluations. One advantage of using ivermectin 1% cream is the frequency of once-daily dose, which may increase adherence. Ivermectin 1% cream was determined to have a low probability for side effects in vehicle-controlled phase III trials. There were a small number of participants who suffered mild to moderate adverse responses; these reactions were most frequently characterized by dry skin, pruritus, and skin burning. Further the method was validated by HPLC.

1. INTRODUCTION

1.1 Background

Rosacea is a prevalent chronic inflammatory skin condition affecting predominantly individuals over the age of 30 (mostly adults), typically affects women, and gets worse with age [2, 3]. It is a condition that occurs on a regular basis and produces many cutaneous symptoms. The most common locations are cheeks, nose, forehead, chin, and central facial convexities. Telangiectasias, pustules, papules, and erythema are examples of visible skin manifestations. [1]

Rosacea is known to reduce quality of life, is associated with depression, and can make social and professional life more challenging. According to a recent National Rosacea Society study of 1675 individuals, 68% of patients with moderate rosacea felt that their general view on life had been severely influenced. This

proportion increased to 87% and 95%, respectively, for patients with moderate and severe symptoms, demonstrating that patients' emotional effect appears to rise as symptoms worsen. [6]

A review panel and consensus committee made up of 17 worldwide medical experts identified four kinds of rosacea. Similar patterns or clusters of symptoms or indications describe these subgroups. These are some: [4]

- **Subtype 1** –**Erythematotelangiectatic Rosacea:** The erythematotelangiectatic condition manifests with visible blood vessels, accompanied by persistent redness and flushing.
- **Subtype 2** – **Papulopustular Rosacea:** Papules and pustules that appear and disappear on the central part of the face are the primary symptom of this subtype. These lesions can also appear around the mouth, nose, and ear and may resemble acne. Telangiectases or persistent redness may also occur. The face frequently stings, burns, itches, and occasionally becomes red.
- **Subtype 3** – **Phymatous Rosacea:** Plaques, or skin thickening, and skin nodules are the primary symptoms of this subtype. The nose is typically the site of these symptoms, but the chin, forehead, cheeks, ears, and eyelids can also be affected. Telangiectases and an enlargement of the nose known as rhinophyma may also be present. Men are more likely to have this subtype, and if left untreated, it can get worse and cause severe facial disfigurement.
- **Subtype 4** – **Ocular Rosacea:** The primary regions of the eye affected by this particular subtype include the cornea, conjunctiva, and eyelids. Eyelid crusts and scales, watery, bloodshot eyes, burning, stinging, and itching, light sensitivity, blurred vision, the sensation that something is in the eyes, and occasionally eye infections like styes are the primary symptoms.

1.2 PATHOPHYSIOLOGY

It is thought that people with Papulopustular Rosacea (PPR) have a hyperactive innate immune system that is overly sensitive to a variety of triggers, including certain foods, UV rays, temperature changes, heat, and stress. The immune response and lifestyle of individuals are also thought to influence the epidermal barrier effect and the inflammation seen in rosacea patients. It is widely acknowledged in the field that microbiological agents such as "Demodex folliculorum" and "Demodex brevis" may contribute to the development of PPR by triggering an inflammatory or immunological reaction. [5, 6]

Given the multiple etiology of rosacea, combination therapy is usually employed to treat the condition. Each patient receives a treatment plan for rosacea that is tailored to their specific subtype or subtypes. There are medications, procedures, and ways to avoid the environment, all of which have varying degrees of efficacy. [7]. Similar to other chronic illnesses with a wide range of symptoms, rosacea needs continuing, long-term care [5]. For rosacea there are very lees anti-inflammatory treatment choice and very few options available with great effectiveness and once-daily administration [3].

1.3 AVAILABLE TREATMENT [7]

Currently, rosacea can be treated with topical and oral medicines.

- Brimonidine and oxymetazoline can be used to treat refractory erythema.
- Topical azelaic acid, metronidazole, and ivermectin are all first-line therapies for inflammatory lesions such as pustules and papules. Unfortunately, therapeutic failure is common with first-line medications. In certain cases, antibiotic-antibiotic combination treatment may be effective. Doxycycline is the sole FDA-approved therapy for rosacea inflammatory lesions at the moment. Other tetracyclines and macrolides, such as azithromycin, can, nevertheless, be used as adjuncts in the clinic. Isotretinoin is a therapy that can be used as a last resort.
- However, phymatous rosacea should be treated with oral medications like doxycycline, tetracycline, isotretinoin.
- Cyclosporine ophthalmic emulsion or, in more severe cases, oral doxycycline can be used to treat visual rosacea.

1.4 Analytical Method Validation:

Analytical method validation is the process of verifying and documenting that an analytical method is suitable for its intended use. It involves assessing the method's performance characteristics, such as accuracy, precision, specificity, limit of detection, and robustness. Method validation ensures that the method is reliable, provides accurate results, and meets the requirements of regulatory guidelines. It is a critical step in establishing confidence in the analytical data generated and ensuring the validity of the results obtained from the method.

❖ **Purpose of Analytical Method development [15]**

Analytical methods in manufacturing and drug development serve to determine potency, detect impurities, assess bioavailability, evaluate stability, and analyze the impact of manufacturing parameters. These methods ensure the production of safe and effective drugs by providing crucial information on dosage, safety, drug characteristics, degradation products, and manufacturing optimization.

The following are the reasons for the creation of novel drug analysis techniques:

- When a medicine or drug combination is not officially recognized by the pharmacopoeias or listed in the pharmacopoeias.
- When a medicine that is currently on the market cannot be properly analyzed because of patent laws.
- When the excipients used in medication formulation cause interference that limits the development of analytical methods.
- It is found that no analytical techniques exist for measuring the analyte in biological fluids.
- The present analytical procedures could need expensive reagents and solvents. Furthermore, it can entail straining the extraction and separation operations.

1.4.1 Accuracy:

A measurement's accuracy is determined by how closely it resembles the actual value. The measured value is identical to the real value after a sample is evaluated using a high accuracy method.[15]

1.4.2 Precision:

The degree of agreement between a set of measurements obtained from multiple sampling of the same homogeneous sample under the same analytical circumstances is what is referred to as an analytical method's precision. [15]

Three levels can be used to evaluate precision:

- **Repeatability:**
  It should be evaluated using at least nine determinations that span the procedure's stated range, such as three concentrations with three replicates of each or at least six determinations at 100% of the test concentration.[15]

- **Intermediate precision:**
  It conveys variances that occur inside laboratories (often on various days, with various analysts, and with various equipment). A measure of intermediate accuracy is not needed if repeatability is determined.[15]

- **Reproducibility:**
  It conveys accuracy between laboratories. [15]

1.4.3 Specificity:

Specificity is the ability of the analytical technique to identify and quantify the analyte in complicated mixtures. A specificity enquiry must be carried out when identifying contaminants and verifying identification tests. This test is carried out to determine either the analysis method is specific or not.[15]

1.4.4 Linearity:

The ability of an analytical method to generate results that are directly related to the concentration of an analyte in the standard solution is known as linearity.[15]

1.4.5 Range:

The calibration plot is used to determine the operating range. It is the range of analyte concentrations between the upper and lower bounds. The findings for this range show good precision, accuracy, and linearity.[15]

1.4.6 Robustness:

Robustness in analytical techniques means they can handle intentional, small changes to method parameters. In HPLC, factors like flow rate, column/sample temperature, pH, and mobile phase composition may vary. Assessing robustness ensures the method's stability and suitability for routine use.[15]

1.5 High performance liquid chromatography

High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography. It is an analytical technique that separates, identifies, and quantifies components in a mixture. It uses pumps to move a solvent and sample mixture through a column with an adsorbent material. The components interact differently with the material, leading to their separation as they exit the column. HPLC is widely used in various fields for precise analysis and characterization of complex mixtures. [30]

Absorption serves as the fundamental principle of separation. When a mixture of compounds (referred to as the adsorbate) dissolves in the mobile phase (known as the eluent) and passes through a column, their movement is determined by their relative affinities for the stationary phase. Compounds with higher affinities towards the stationary phase exhibit a slower travel rate, resulting in their delayed elution. Conversely, compounds with lower affinity towards the stationary phase travel faster and are eluted earlier.[30]

Advancements in HPLC stationary phase technology have boosted resolving power, while coupling HPLC with MS via electrospray and other ionization methods has broadened its utility and value.[30]

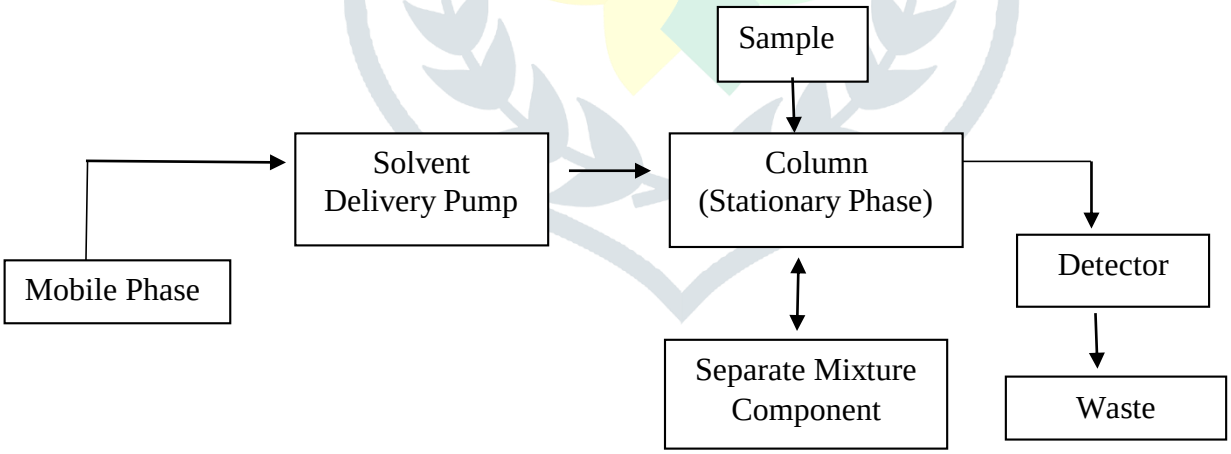


Figure 1.1: Schematic diagram of HPLC

1.6 Reverse Phase High Pressure Liquid Chromatography:

The most frequent method of HPLC is reversed-phase HPLC (RP-HPLC), which is simply the opposite of NP-HPLC in that the stationary phase is more nonpolar than the eluting solvent. RP-HPLC typically employs a nonpolar stationary phase, such as C18 silica, and a moderately polar aqueous mobile phase. In RP-HPLC, there is little attraction between the hydrocarbon chains affixed to the silica (the stationary phase) and the polar molecules in the solution. However, there is substantial attraction between the polar solvent and the polar molecules in the mixture being passed through the column. As a result, the majority of the time in the combination, polar molecules move together with the solvent. Van der Waals dispersion forces lead nonpolar molecules in the mixture to gravitate toward the hydrocarbon group.[30]

1.6.1 Advantages of HPLC [30]

a) The resolution and analysis speed are greater.
b) HPLC columns may be recycled without packaging or regeneration.
c) Greater repeatability as a result of precise control of the variables influencing separation effectiveness.
d) Simple instrument automation and data analysis.
e) The capacity to adapt to extensive, preliminary procedures.

2. DRUG PROFILE

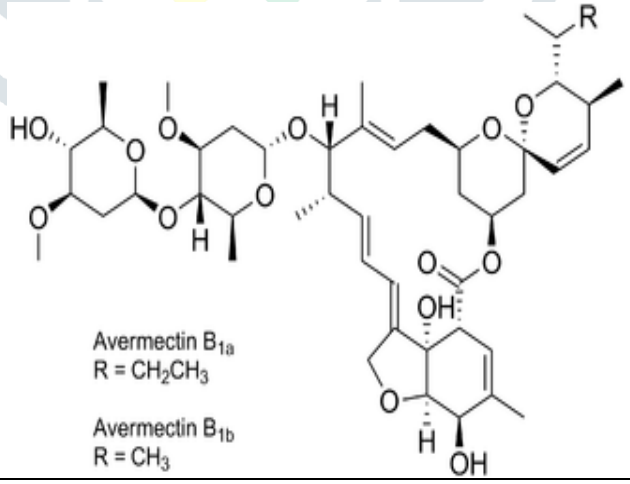
2.1 IVERMECTIN

Ivermectin is a macrocyclic lactone derivative of the avermectin family that is semi-synthetic, which have both broad spectrum anti-parasitic activity & anti-inflammatory properties, may both add to the effectiveness of its treatment of the rosacea family [2]. A cream containing 1% ivermectin is used to treat papulopustular rosacea. By increasing the anti-inflammatory cytokine IL-10 and lowering the pro-inflammatory cytokines IL-1b and TNF-alpha, ivermectin has also been shown to have anti-inflammatory effects, which are produced in response to lipopolysaccharide. Similar to other macrolides, it has anti-inflammatory characteristics that are regarded to be the main cause of its therapeutic impact in rosacea. [3]

2.2 CHEMISTRY

In 1981, it was first introduced. The two B1a and B1b isoforms are the main structural components of ivermectin. Streptomyces avermitilis is the source of both isoforms' macrolides. Because of its structural makeup, ivermectin cannot cross the blood-brain barrier in mammals, shielding people against a variety of possible negative effects. [7]

Table No. 2.2: Chemistry of Ivermectin

Pharmaceutical Name	**Ivermectin**
Therapeutic Classification	Avermectin
Structure	
Molecular Formula	$C_{48}H_{74}O_{14}$, H_2B_{1a} $C_{47}H_{72}O_{14}$, H_2B_{1b}
Molar Mass	875.106 g/mol 861.079 g/mol
Boiling point	940.4 ℃
Density	1.23 g/cm^3

Nature	Ivermectin is yellow or yellowish white crystalline powder, slightly hygroscopic
Solubility	Freely soluble in dichloromethane, soluble in ethanol, practically insoluble in water

2.3 PHARMACOLOGICAL PROPERTIES

2.3.1 Pharmacodynamic properties [7]

Mechanism of action: Ivermectin have both anti-inflammatory as well as anti-parasitic effect thanks to its dual mechanism of action. Prostaglandins, nitric oxide synthesis, and cytokine pathways are all affected by the anti-inflammatory effects, with IL-1b, IL-6, NF-kB, and LPS acting as major inhibitors. Ivermectin works as an agonist on ligand-gated ion channels to suppress parasites. Ivermectin blocks the transmission of GABA or glutamate between synapses after binding. The location of these neurochannels within nerve and muscle cells is unpredictable. Demodex mites are paralyzed as a result of the blockage of chloride channels that results, and the parasite starves to death as a result of digestive failure.

2.3.2 Pharmacokinetic properties [7]

Absorption: Ivermectin can be used orally or topically, however topical ivermectin channels have been investigated the most for the treatment of papulopustular rosacea.

Distribution: It is highly lipid soluble and binds to the plasma protein which results in a widespread dispersion throughout the body. Ivermectin has a half-life of around 6 days and achieves peak plasma concentration within 10 hours following topical administration.

Metabolism: Ivermectin is primarily metabolised by CYP3A4 within liver microsomes.

Elimination: Only about 1% is eliminated by kidneys and most of it is eliminated through feces.

2.4 IVERMECTIN CREAM

Ivermectin 1% cream is a prescription topical medicine used to treat rosacea inflammatory lesions.

2.4.1 MECHANSIM OF ACTION:

Its mode of action is uncertain, although it might involve both its anti-inflammatory and anti-parasitic effects on the Demox mite, which is found on the skin and may be a factor in the symptoms of rosacea. Ivermectin showed anti-inflammatory characteristics in immunopharmacological tests, specifically by suppressing inflammatory cytokine production and increasing the production of interleukin 10, an anti-inflammatory cytokine. [5]

2.4.2 USES OF IVERMECTIN CREAM

Rosacea (Papulopustular) inflammatory lesions in adult patients should be treated with topical ivermectin.

2.4.3 ADVERSE EVENTS

Nasopharyngitis, sinusitis, diarrhea, hypertension, and an increase in aspartate aminotransferase were the most common reported adverse events in patients treated with ORACEA (Doxycycline USP) capsules in controlled clinical studies. [8,9]

3. LITERATURE REVIEW

1. **F.M.N Forton, 2022** have stated that demodicosis and rosacea are frequent facial disorders seen in dermatology practises. Although there is mounting evidence that Demodex mites may play a significant part in the inflammatory process. In fact, rosacea with papulopustules is seen in nearly all instances with high Demodex concentrations (PPR). According to recent research, Demodex triggers two opposing

immune responses in the host: a protective immune response meant to get rid of the mite and an immunosuppressive response meant to encourage its own growth. The data in favour of and against Demodex playing a causal role in rosacea is examined in this review. The results show that Demodex proliferation, which appears to be an essential component at the heart of a causal chain, can reasonably be attributed to PPR. [27]

2. **Carlos Chaccour *et al*., 2021** have revealed that In vitro, ivermectin inhibits SARS-CoV-2 proliferation at concentrations that are difficult to achieve with the dosages that are currently advised. They conducted a pilot research to see if one dose of ivermectin was successful in halting the spread of SARS-CoV-2. Within 72 hours of the initiation of a fever or cough, every enrollee was enrolled. Patients were randomly assigned to receive either ivermectin (400 mcg/kg) or a placebo (placebo) in a 1:1 ratio (n = 12). The primary outcome measure was the percentage of patients having detectable SARS-CoV-2 RNA by PCR from nasopharyngeal swabs at day 7 post-treatment.[16]

3. **Yildiz Hayran MD *et al*., 2021** were examined Serum IL-17 levels in rosacea patients and their relationship to the disease's characteristics. The study included 60 rosacea patients with a diagnosis and 60 healthy controls. ELISA, or enzyme-linked immunosorbent assay, was used to measure the concentrations of serum IL-17. Their study's findings showed a potential function for IL-17 in the development of rosacea and a potential new target for rosacea therapy. Serum IL-17 levels were greater in rosacea patients, and there was a strong association between IL-17 levels and secondary illness symptoms.[27]

4. **Diane Thiboutot *et al*., 2020** noted that rosacea is a chronic inflammatory disease of the face skin that frequently shows remissions and exacerbations, particularly affecting the cheeks, nose, chin, forehead, and eyes. Skin features include things like phymas, papules, pustules, telangiectasia, and facial flushing. Since the rosacea research area has grown so rapidly over the past 15 years, our knowledge of this disease that affects all skin types has significantly improved. [17]

5. **Surajit Das *et al*., 2020** were developed microemulsions and microemulsion gels in order to distribute ivermectin topically. Due to ivermectin's higher solubility in tea tree oil and ethyl butanoate than in the other investigated oils, these two oils were determined to be appropriate for ivermectin loaded microemulsion compositions. Based on these chosen oils and combinations of various surfactant/co-surfactant at various ratios, pseudo-ternary phase diagrams were created. [19]

6. **Barbara M.Rainer *et al*., 2020** reported the Characterization and Analysis of the Skin Microbiota in Rosacea. They conducted a case-control research to compare the skin microbiota of rosacea patients to controls of the same age, gender, and race. Nineteen people with rosacea, erythematotelangiectatic lesions, papulopustular lesions, or both were compared to nineteen people without rosacea. DNA was obtained from skin scrapings taken from the individuals' noses and bilateral cheeks, and it was determined that the skin microbiome of people with rosacea varies from that of healthy skin.[25]

7. **Dalia S. Ashour, 2019** was described that Ivermectin (IVM) is used by individuals every year to treat a number of parasitic diseases, including filariasis, onchocerciasis, strongyloidiasis, scabies, and pediculosis, according to a 2019 report. Its usefulness in treating a variety of illnesses, as well as the right dosage and time of treatment, have been proved by several clinical trials. Additionally, it now includes additional parasitic diseases in its antiparasitic range. IVM has demonstrated excellent efficacy in getting rid of disease-carrying parasite vectors including mosquitoes, sandflies, and tsetse flies. The World Health Organization (WHO) has managed various control programs utilizing IVM to eradicate onchocerciasis, lymphatic filariasis, and decrease malaria transmission. [20]

8. **Mohsena Akhter *et al*., 2019** has assessed oral Ivermectin's effectiveness and safety in treating scabies in comparison to topical Permethrin. Over the course of six months, an out-patient non-randomized study was carried out. According to inclusion criteria, 100 scabies sufferers were included as the study's population. Two groups were created out of them. Ivermectin was administered orally to Group A, and Permethrin 5% cream was administered to Group B. Patients were monitored on days 7, 14, and 15 and

had their efficacy and safety evaluated. They came to the conclusion that topical application of Permethrin 5% cream is more beneficial and secure for treating scabies than taking oral Ivermectin. [21]

9. **E.J. van Zuuren *et al.*, 2019** have revised their systematic assessment of rosacea therapy options. They comprised 20944 people in 152 studies, 46 of which were brand-new. The effects of brimonidine, oxymetazoline, metronidazole, azelaic acid, ivermectin, and other topical therapies were investigated. Topical oxymetazoline and topical brimonidine both exhibited high-to-moderate confidence evidence for minimizing briefly persistent erythema, it was discovered. High certainty evidence supported the use of topical azelaic acid and topical ivermectin for reducing papules/pustules, while moderate to high certainty evidence supported the use of isotretinoin and doxycycline 40 mg modified release (MR), and low certainty evidence supported the use of topical metronidazole, topical minocycline, and oral minocycline as being equally effective to doxycycline 40 mg MR. [22]

10. **Kunlawat Thadanipon MD *et al.*, 2019** have been evaluated the relative efficacy and security of antiscabietic drugs. Randomized controlled studies were thoroughly analyzed. Direct and network meta-analyses on 3 outcomes (cure, persistent itching, and adverse events) were conducted on 13 antiscabietic medications. A network meta-analysis of 52 studies including 9917 patients found that permethrin (the reference therapy) had a significantly higher cure rate than sulfur, malathion, lindane, crotamiton, and benzyl benzoate. When compared to the oral ivermectin and permethrin combination, permethrin alone showed a non-significantly greater cure rate. Permethrin and oral ivermectin were the most effective cures, followed by topical ivermectin for persistent itching and synergized pyrethrins for negative side effects. [23]

11. **Michael Ke Wang *et al.*, 2019** have stated Ivermectin was given on a daily basis to the kidney transplant patient who had crusted scabies. They described a case of crusted scabies in a 65-year-old kidney transplant recipient who presented with recurrent septicemia and was treated with daily ivermectin for seven days following the initial failure of weekly ivermectin treatment. There was a high prevalence of first misdiagnosis in cases of pruritus. The majority of patients received topical medication, whereas one-third were given ivermectin. Three of the seven individuals that had an infection present at the same time died. Due to its rarity, diversity in appearance, and irregular skin distribution, scabies that has been crusted is frequently incorrectly identified in transplant recipients. Given its link with secondary infection and subsequent mortality, it should be taken into consideration in the differential diagnosis of transplant patients presenting with rash and pruritus.[24]

12. **M. Schaller *et al.*, 2019** had updated and expanded prior worldwide ROSCO guidelines in accordance with the most recent data, and continued to support clinical tool development for the phenotypic approach in rosacea uptake. A modified Delphi methodology was utilised by 19 dermatologists and 2 ophthalmologists to agree on key points in the diagnosis, classification, and therapy of rosacea. Electronic and blind voting was used. They concluded the current study provides clinical tools to support a phenotypic approach in practise and improve rosacea patient management. It also updates prior recommendations as a basis for local guideline creation. [26]

13. **Wioletta Baranska – *et al.*, 2019** have described that by applying ivermectin cream to the patient, researchers have evaluated the value and tolerability of ivermectin therapy in mild and moderate perioral dermatitis, SD, and AV. Every patient responded favorably to the therapy. During, inflammatory skin lesions gradually decreased. The combination of PD and topical ivermectin cream resulted in complete or nearly complete clearance in 8 cases. As soon as two weeks after therapy, treatment effects can start to be felt. Overall, "very good" and "excellent" improvement was noted by 19 patients (8 with PD, 8 with SD and 3 with acne). Only one AV sufferer gave the treatment a "good" rating. During their 18-week monitoring periods, they saw no illness flare-ups in any of their patients. They came to the conclusion that topical ivermectin was safe and effective for treating the condition.[18]

14. **P. Lopez Garcia et al., 2005.** HPLC and UV derivative spectrophotometric techniques for determining hydroquinone in gel and cream formulation were developed and validated. This study described and developed an HPLC and Ultraviolet derivative specrophotometric (UVDS) method for quantifying hydroquinone (HQ) in gel and cream as a unit active principle. The validation parameters such as

specificity, Linearity, Precision, Accuracy, Limit of Detection, and Limit of Quantitation were determined, and it was discovered that the Hydroquinone active together with Kojic acid and arbutic causes some interference in both methods, so the method was found to be low cost, non-polluting, simple, rapid, precise, accurate, and sensitive than the existing method.

15. **Tariq Mahmood Ansari et al., 2005.** Tranexamic acid spectrophotometric determination in pharmaceutical bulk and dose forms. This article described and developed a spectrophotometric technique for determining tranexamic acid in bulk and pharmaceutical formulations that is simple, rapid, accurate, and sensitive. The interaction of ninhydrin with the main amino group of tranexamic acid in the basic medium at pH 8.0 constituted the basis for this approach. The reaction generated a bluish-purple hue that was maximum absorbed at 565 nm.

4. AIM AND OBJECTIVE

- **Aim**
  - FORMULATION AND EVALUATION OF IVERMECTIN CREAM: METHOD DEVELOPMENT AND VALIDATION CONSIDERATION BY HPLC.
- **Objectives**
  - To formulate the Ivermectin cream using polymer polyethylene glycol.
  - To perform evaluation of formulated Ivermectin cream by using various analytical method such as pH, Identification by PDA detector, Physical Inspection, Spreadability & Assay.
  - To develop method for evaluation of Ivermectin Cream by HPLC.
  - To develop the method for estimation of Ivermectin in pharmaceutical dosage form (Semi-solid)
  - To verify the interference of placebo on Ivermectin in semi-solid formulation.
  - To determine the stability of Ivermectin cream.

5. MATERIALS AND METHOD

5.1 Formulation Consideration:

The 10 mg/g Ivermectin Cream will be formulated using following materials and instruments.

5.2 Material required:

- Ivermectin was obtained as a gift sample from National Healthcare Pvt. Ltd. Bara, Nepal. All other chemicals were standard.

Table No. 5.2: Material required for formulation

S.No.	Name of Ingredient	Formulation			Used for
		F1	F2	F3	
1.	Ivermectin	1.716 g	1.716 g	1.716 g	Active
2.	Propylene Glycol	20 g	20 g	20 g	Polymer
3.	White soft paraffin	15 g	18 g	22.5 g	Cream base
4.	Cetomacrogol 100	3.75 g	3.75 g	3.75 g	Non-greasy Emollient or Moisturiser
5.	Cetostearyl Alcohol	8 g	10 g	13.5 g	Emulsifying Agent

6.	Hard Wax	3 g	3 g	3 g	Stiffening agent
7.	Light liquid Paraffin	4.5 g	4.5 g	4.5 g	Increases melting pt.
8.	Tween-80	1.5 g	1.5 g	1.5 g	Suspending agent
9.	Methyl Paraben	0.27 g	0.27 g	0.27 g	Preservative
10.	Propyl Paraben	0.03 g	0.03 g	0.03 g	Preservative
11.	Purified Water	79.18 g	79.18 g	79.18 g	Solvent
12	Total	150 g	152.4g	150	-

5.2 Instrument Required:

5.3.1 Weighing balance: The method was developed using a Electronic balance (Schimadzu AP 135W)

5.3.2 pH Meter: pH meter (Pico+ labindia) was used for the determination of pH of ivermectin cream.

5.3.3 Viscometer: The viscosity of the formulated cream was measured by using a Brookfield viscometer. (CAP-2000)

5.3.4 HPLC: The method was developed using a Shimadzu HPLC Prominence I LC-2030 equipped with SPD 20 A detector, isocratic pump system, auto injection, For chromatography (5 µm), a stainless steel column (25 cm 4.6 mm) filled with octadecylsilyl silica gel (Hypersil ODS). Other instruments such as Electronic balance (Schimadzu AP 135 W), pH meter (Pico+ labindia) and vacuum pump (PCI Analytical) is used for analysis procedure.

5.3.5 IR: The identification of Raw Material ivermectin was done by using a Shimadzu Fourier Transform Infrared Spectroscopy (FTIR) having wavelength range 7,800 to 350 cm^{-1}, Resolution 0.25, 0.5, 1, 2, 4, 8, 16 $cm^{-1,}$ Optical system- Single beam.

5.4 Raw Material Identification by IR spectroscopy:

❖ **Operational procedure**

- Turned on the instrument's main power.
- Turned on the computer after hearing a buzzer sound.
- Select the IR solution icon by double-clicking it.
- Typed in the login and password.
- In the menu bar, select Measurement and then select Initialize.
- Checked the instrument status on the left side of the screen using the status monitor. The light source, laser, and beam splitter were all displayed in green.
- Clicked on [BKG] in the measurement file area, a notice will show on the screen.
- Set up the sample compartment for the background scan.
- After clicking OK, the background spectrum was displayed on screen.
- The sample was now prepared and placed in the sample chamber using the spatula.
- Scan the sample by clicking on sample buttom.
- The spectrum was displayed on the screen, and then the [view] tab window was opened to show the measured spectrum in upper and lower windows.
- Opened the IR Library and found the reference spectra for the sample in question.
- Clicked on file, then on print preview, pick the appropriate report template, and print by clicking on the print option.

5.5 Formulation Procedure:

All active and excipients will be weighed.

5.5.1 Step 1: Preparation of Oil Phase

- Melted cetomacrogol 1000, cetostearyl alcohol, white soft paraffin, Light liquid paraffin & hard paraffin in a S.S manufacturing vessel fitted with turbo stirrer & maintained temperature to 65 ± 5°C.

5.5.2 Step 2: Preparation of Water Phase

- Taken pure water in clean vessel & maintained temperature to 65 ± 5°C.

5.5.3 Step 3: Solublization of Active Ingredients

- Taken 19.5g of propylene glycol & Tween-80 (Heat upto 50°C if required) in vessel, dissolve ivermectin to it under stirring till the solution appears clear. And added to the oil phase maintained at 65 ± 5°C.

5.5.4 Step 4: Addition of Preservative

- Heated 3g of propylene glycol in another vessel upto 65 ± 5°C dissolved methyl paraben & propyl paraben in it & added to oil phase.

5.5.5 Step 5: Mixing

- Added water phase in oil phase and maintained temperature of both phase to 65 ± 5°C operating the turbo stirrer at constant speed. Temperature of mixed phase comes down to 40 ± 5°C by the passage of raw water. Continued stirring for next 45 mins at constant speed.

5.5.6 Step 6: Cooling

- Cooled till the mixture attains room temperature by continuously stirring.

5.5.7 Step 7: Filling

- The mixture was transferred to a container using a glass rod.

5.6 Evaluation Parameter:

5.6.1 Description (Visual Appearance): Physical appearance of cream was observed by its colour, roughness.

5.6.2 Identification (By PDA Detector in HPLC):

- **Preparation of Mobile Phase:** 200 ml of water, 270 ml of Methanol & 530 ml of Acetonitrile.
- **Preparation of Std Solution:** Weighed about 50mg of ivermectin working standard into 100 ml of volumetric flask dissolved and diluted upto volume with methanol. Further diluted 5ml to this solution to 25ml of volumetric flask with methanol.
- **Preparation of Test Solution:** Weighed about 1g of cream equivalent to 10mg of ivermectin into 100ml of volumetric flask.
- **Chromatographic Condition:**
  Instrument: HPLC
  Column: 250mm x 4.6mm, 5µm, L1
  Detector: UV 200 - 400 nm
  Flow Rate: 1.5 ml/min
  Inj. Volume: 20 µl
- **Procedure:** Equal volumes of the standard solution and test solution were separately injected, the chromatograms were recorded, and the responses for the principal peak were measured.

5.6.3 Determination of pH:

- **Instrument Required:** pH Meter
- **Sample Solution:** 50 mg/ml mixture of cream in water
- **Procedure:** pH of the sample solution with a pre-calibrated pH meter was determined. Taken reading when the reading on the screen was constant.

5.6.4 Spreadability:

- **Procedure:** The term spreadability refers to the ease with which a product may be applied to the skin. Glass slide apparatus was used to test spreadability. Two glass slides are utilized for this purpose. A second glass plate was put on top of the 0.5g test formulation, which was positioned inside the pre-marked 1cm-diameter circle on the glass plate. A 500mg weight was placed on the upper glass plate for five minutes. Spreadability refers to the area that a specific amount of cream sample on the glass slide covers. It was discovered that the test mixture diffused and produced a diameter increase. On average, three determinations were recorded. Lesser the timetaken to separate the two slides, better will be the spreadability.

5.6.5 Homogeneity & Grittiness:

- **Procedure:** A tiny amount of the prepared cream was tested by pushing it between the thumb and index finger. The consistency of the formulation and the presence of big particles were used to evaluate the homogeneity, grittiness, and texture of the formulations.

5.6.6 Assay (By HPLC):

- **Preparation of Mobile Phase:** 200 ml of water, 270 ml of Methanol & 530 ml of Acetonitrile.
- **Preparation of Std Solution:** Weighed about 50mg of ivermectin working standard into 100 ml of volumetric flask dissolved and diluted upto volume with methanol. Further diluted 5ml to this solution to 25ml of volumetric flask with methanol.
- **Preparation of Test Solution:** Weighed about 1g of cream equivalent to 10mg of ivermectin into 100ml of volumetric flask.
- **Chromatographic Condition:**
  Instrument: HPLC
  Column: 250mm x 4.6mm, 5µm, L1
  Detector: UV 245 nm
  Flow Rate: 1.5 ml/min
  Inj. Volume: 20 µl
- **Procedure:** Separately injected equal volume of standard solution and test solution, recorded the chromatograms, measured the responses for the major peak. Calculated the content of ivermectin (H2B1a + H2B1b) in the cream & the ratio H2B1a (H2B1a + H2B1b).

$$Assay(\%) = \frac{Areaof\ Test}{Avg.area\ of\ Std.} \times \frac{Wt.of\ Std.}{100} \times \frac{5}{25} \times \frac{100}{Wt.of\ Test} \times \frac{Potency\ of\ Std(asisbasis)}{Claim}$$

- **Acceptance criteria:** NLT 90% and NMT 110% of the stated amount of sum of (H_2B_{1a} + H_2B_{1b}) & the ratio of H_2B_{1a} / ((H_2B_{1a} + H_2B_{1b}) is not less than 90%.

5.6.7 Viscosity:

- A CAP-2000 Brookfield viscometer was used to measure the formulation's viscosity. Take the test sample and place it in a dry, clean 250 ml beaker. Utilizing spindle number 4.

- Tighten the connecting screw to join the bottom shaft of the viscometer to the spindle (left-hand thread). Spindle number.
- Turn the motor switch to the "ON" position to test viscosity. As a consequence, the viscometer drive motor is turned on.
- The temperature was set at 20°C and 20 rpm. Give the suggested reading some time to settle.
- 20g of the sample was put in a beaker and given 5 minutes to equilibrate before the dial reading was measured using a spindle rotating at 20 rpm. The dial reading on the viscometer was noted.
- The viscosity in centipoise was obtained by directly multiplying the dial reading by the coefficients listed in the Brookfield viscometer catalogue.

5.7 Method validation:

5.7.1 Specificity:

One gram of placebo and 100 mg of Ivermectin WS were mixed together in a 50 mL volumetric flask and diluted with water to make 25 ml. For 10 minutes, sonicate the ingredients. Shake and add water to make up the volume. It must be shown that the presence of spiking materials (impurities and/or excipients) has no impact on the test.

Demonstrate Specificity by injecting one blank, three replications of placebo and five replications of standard of 100% concentration.

5.7.2 Linearity:

The ICH standards require a minimum of five concentration levels (Such as 80%, 90%, 100%, 110% and 120%) of standard and test solution in addition to some minimum specified ranges. The calibration curve was constructed by plotting graph of peak area VS concentration. The minimal assay range is between 80% and 120% of the intended concentration.

Regression line, $y = ax + b$

Where, a is the slope of regression line and b denotes the y- intercept.

Correlation Coefficient, $$r = \frac{\sum^{n}(x_i - \bar{x})(y_i - \bar{x})}{\left[\sum(x_i - \bar{y})(y_i - \bar{y})\right]^{1/2}}$$

Where x_i is an individual measurement in a collection of n measurement and $\bar{x}$ is the set's arithmetic mean, y_i is an individual measurement in a collection of n measurement and $\bar{y}$ is the arithmetic mean of the set.

Demonstrate Linearity by injecting one blank, three replications of each 80%, 90%, 110%, 120% concentrations of both standard and test, and six replications of 100% concentration of test and standard.

5.7.3 Accuracy:

Three concentrations (Such as 80%, 90% & 100%) prepared sample of each concentration of test sample and standard sample of 100% concentration and the calibration curve was constructed by plotting graph of peak area VS concentration.

The percentage of analyte recovered during assay is used to calculate it. The equation: can be used to calculate the recovery.

Recovery = Analytical Result / True Value x 100%

5.7.4 Precision:

The findings of internal lab differences brought on by chance occurrences such various days, analysts, equipment, etc. are referred to as intermediate precision.

It is the variations within laboratories, such as different days, different analysts, different equipment, and so on. A method intermediate precision may reflect discrepancies in result obtained from:

- Different Analysts
- Different Instruments
- Different Days

5.7.5 Robustness:

The robustness of an analytical technique refers to its ability to withstand small, intentional changes to the procedure without being significantly affected. It helps determine whether the method is suitable for routine use and provides insights into its stability and consistency. Robustness can be assessed while the analytical process is being developed.

Robustness is established by making change in-

a. Flow Rate by ±10%
b. Injection Volume by -2µl & -4µl
c. Wavelength by ± 2 nm (i.e. 243 nm & 247 nm)
d. Column Length (25cm & 15cm)

Demonstrate robustness by injecting one blank, six replications of standard and three replications of test of 100% concentration and calculate as per procedure.

5.7.6 Solution stability:

The solution stability test is performed to check whether or not the temperature condition and time factors affect the result and by what extent. Stability of 100% concentration solution is investigated by changing the following conditions and comparing the result against that of a freshly prepared solution of same concentration:

- Storing at room temperature for 6 hours.
- Storing at room temperature for 24 hours.
- Storing at 2-8ºC, in refrigerator for 24 hours

Demonstrate Solution Stability by injecting one blank, six replications of standard of each condition and three replications of test of each condition of 100% concentration and calculate as per procedure.

6. RESULT AND DISCUSSION

6.1 FT-IR Spectra of Ivermectin:

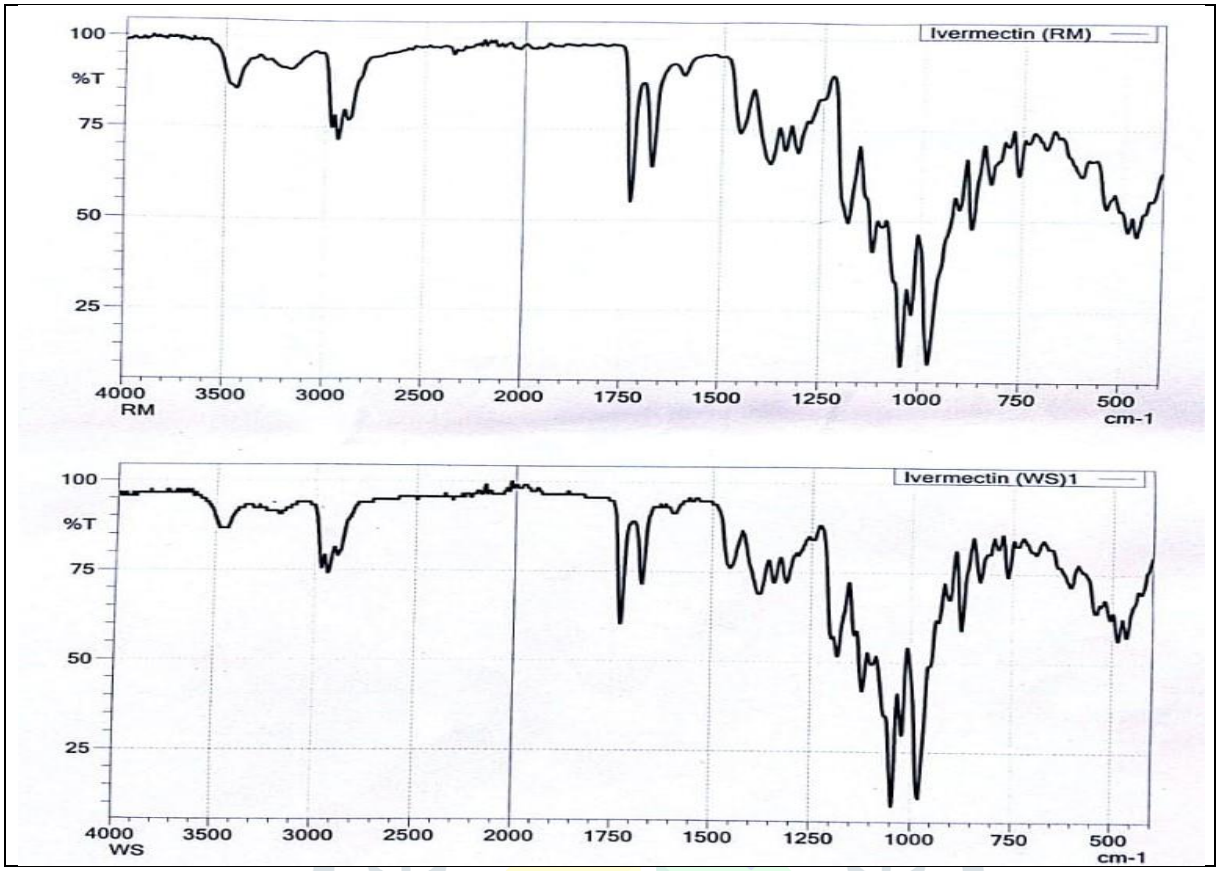


Figure No. 6.1: FT-IR Spectra of Ivermectin Drug

- The IR spectrum obtained from the test sample was concordant to that of the ivermectin reference standard.

6.2 Formulation of Ivermectin Cream:

- The ivermectin cream F1, F2 & F3 was formulated successfully.

6.3 Evaluation Parameter:

6.3.1 Description (Visual Appearance):

S.No.	Formulation	Description
1.	F1	White cream
2.	F2	White cream
3.	F3	White cream

6.3.2 Identification (By PDA Detector):

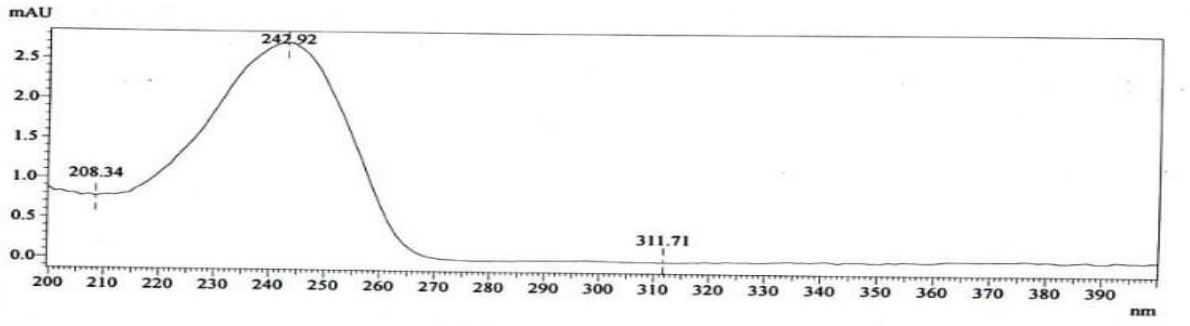


Figure No. 6.3.2.1: Ivermectin Cream Identification Std (1)

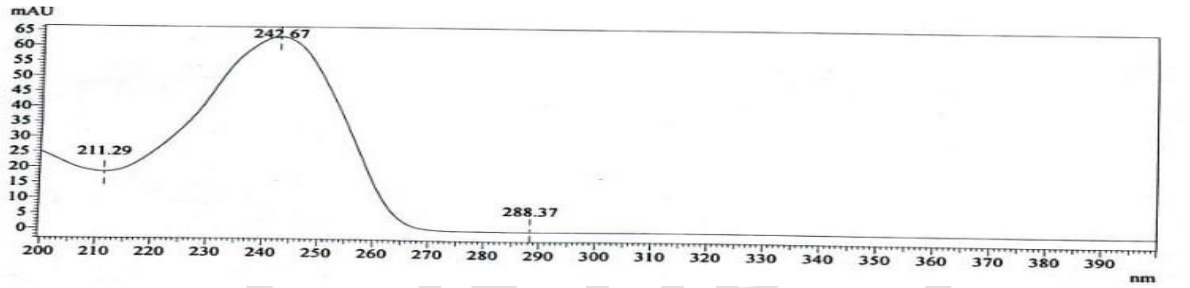


Figure No. 6.3.2.2: Ivermectin Cream Identification Std (2)

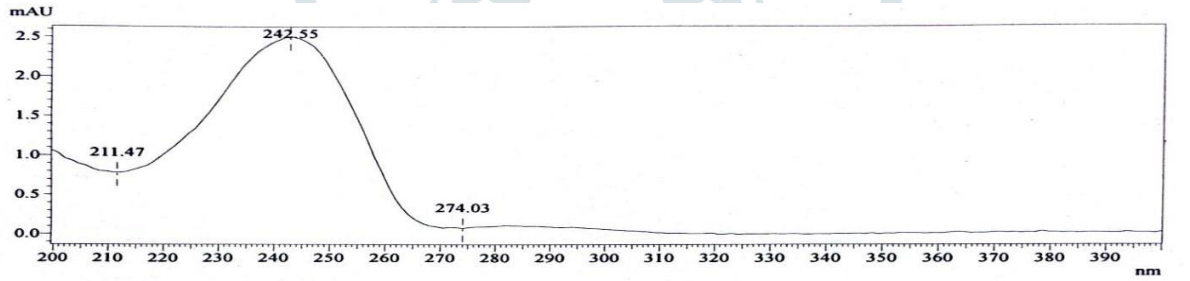


Figure No. 6.3.2.3: Ivermectin Cream Identification TS (1)

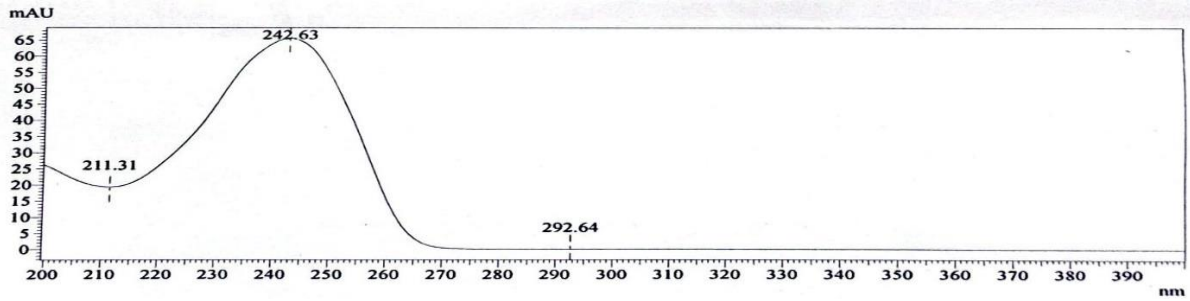


Figure No. 6.3.2.4: Ivermectin Cream Identification TS (2)

- The UV spectrum of the ivermectin peak of the sample solution corresponded to that of the standard solution, as obtained in the Assay.
  - **λmax:** 243 nm
  - **λmin:** 211/293 nm

6.3.3 Determination of pH:

S.No.	Formulation	pH
1.	F1	5.142
2.	F2	5.103
3.	F3	5.214

- pH of formulation were determined and found to be between 4-7 to 5.142, 5.103 & 5.214 that is with in the range.

6.3.4 Spreadability:

S.No.	Formulation	Spreadability
1.	F1	Not so good
2.	F2	Good
3.	F3	Very good

- The spreadability of the formulation was determined and found to be very good (F3) as white soft paraffin coats the skin's surface with an oily coating that prevents water from evaporating from the skin's surface and keeps the skin moisturized.

6.3.5 Homogeneity & Grittiness:

- Homogeneity and Grittiness of the formulation were determined and found that cream was homogenous i.e. no granules were present in F3 formulation as compared to that of F1, F2 (i.e. small particle were observed).

6.3.6 Assay (By HPLC):

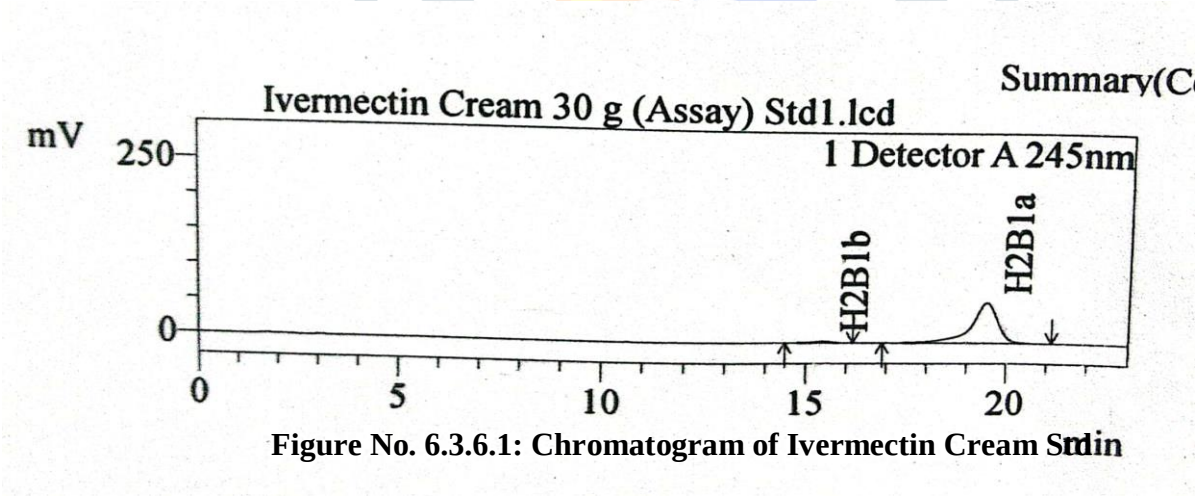


Figure No. 6.3.6.1: Chromatogram of Ivermectin Cream Stdin

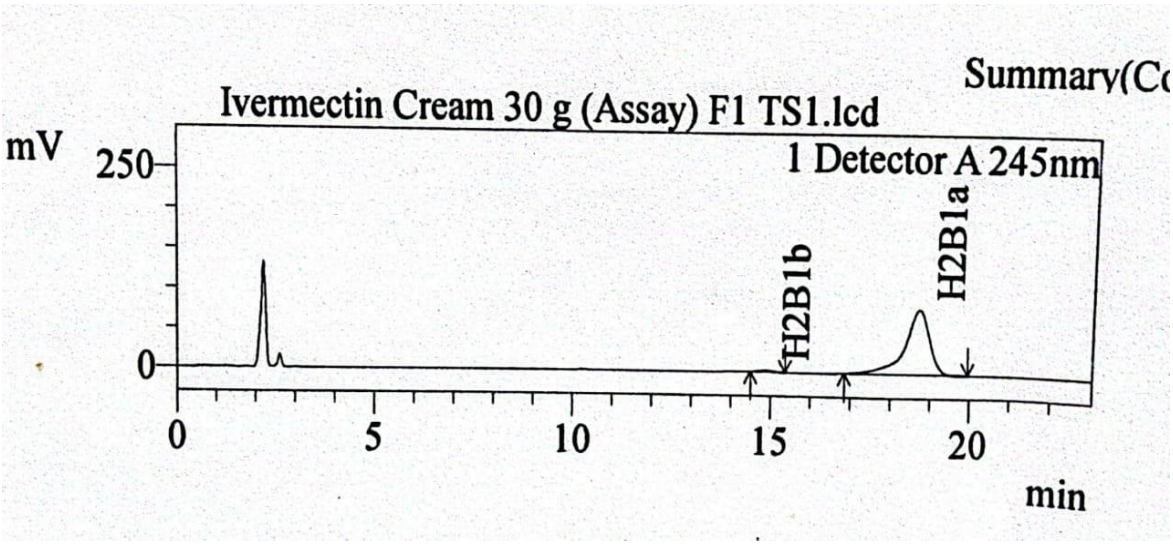


Figure No. 6.3.6.2: Chromatogram of Ivermectin Cream TS (F1)

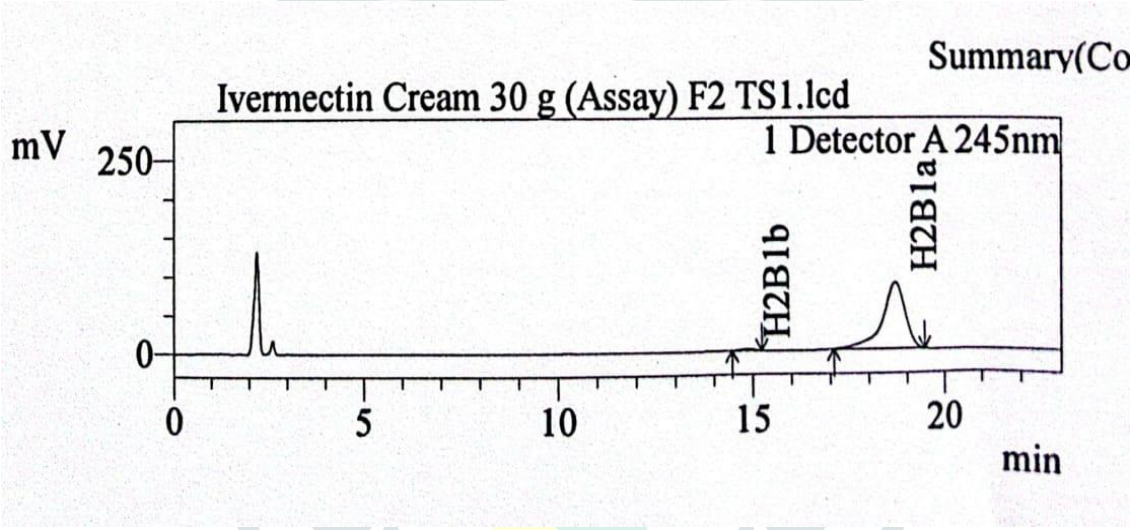


Figure No. 6.3.6.3: Chromatogram of Ivermectin Cream TS (F2)

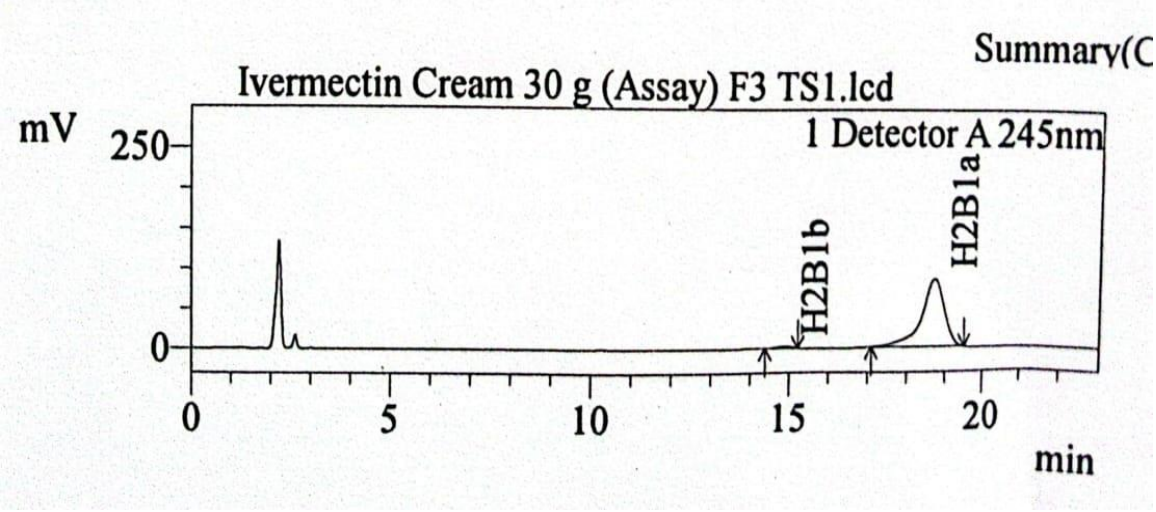


Figure No. 6.3.6.4: Chromatogram of Ivermectin Cream TS (F3)

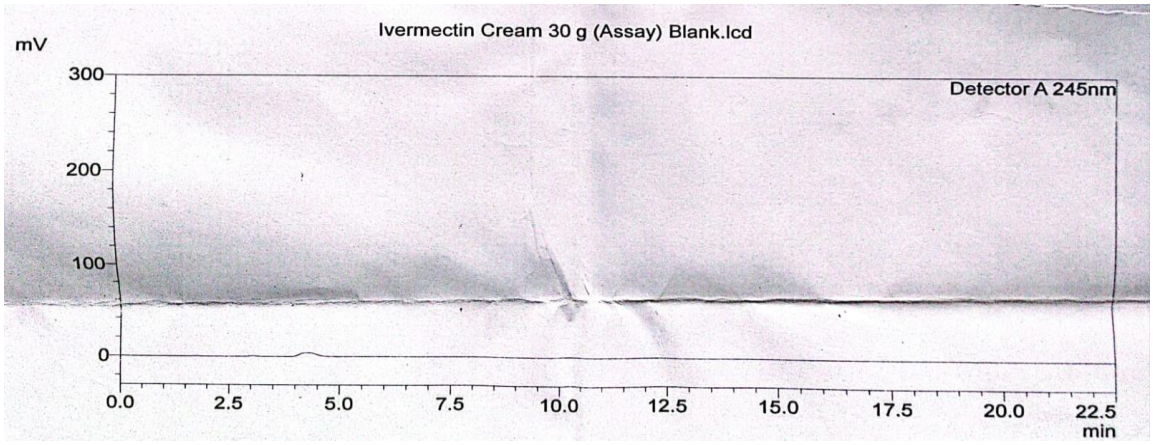


Figure No. 6.3.6.5: Chromatogram of Ivermectin Cream Blank

$$Standard\ solution\colon\ 50.3\ mg/100\ ml \rightarrow\ \ 5ml/25ml$$

Table No. 6.3.6.1: Area of Standard Solution

Title	Area of Standard		
	H2B1b	H2B1a	Sum
Std 1	47445	2498250	2545695
Std 2	47711	2496060	2543771
Std 3	47313	2496057	2543370
Std 4	47672	2494357	2542029
Std 5	47566	2530736	2578302
Std 6	47214	2461541	2508755
Average	47214	2496167	2543654
%RSD	0.347	0.877	0.865
SD	164	21916	22024

$$Standard\ solution\colon\ 50.3\ mg/100\ ml \rightarrow\ \ 5ml/25ml$$

$$Test\ solution\ (TS1)\colon 1.4424\ gm/100ml$$

$$Test\ solution\ (TS2)\colon 1.4529\ gm/100ml$$

Table No. 6.3.6.2: Area of Test Solution (F1)

Title	Area of Test (F1)		
	H2B1b	H2B1a	Sum
TS 1	57009	3534697	3591706
TS 2	57090	3540378	3597468
Average	57049	3537537	3594587
%RSD	0.100	0.114	0.113
SD	57	4017	4074

$$Standard\ solution:\ 50.3\ mg/100\ ml \rightarrow\ 5ml/25ml$$

$$Test\ solution\ (TS1): 1.3424\ gm/100ml$$

$$Test\ solution\ (TS2): 1.4029\ gm/100ml$$

Table No. 6.3.6.3: Area of Test Solution (F2)

Title	Area of Test (F2)		
	H2B1b	H2B1a	Sum
TS 1	56989	3595516	3652505
TS 2	57101	3517943	3575044
Average	57045	3556729	3613775
%RSD	0.139	0.542	1.515
SD	79	54853	54773

$$Standard\ solution:\ 50.3\ mg/100\ ml \rightarrow\ 5ml/25ml$$

$$Test\ solution\ (TS1): 1.2341\ gm/100ml$$

$$Test\ solution\ (TS2): 1.3482\ gm/100ml$$

Table No. 6.3.6.4: Area of Test Solution (F3)

Title	Area of Test (F3)		
	H2B1b	H2B1a	Sum
TS 1	57408	3560299	3617707
TS 2	57907	3526615	3584522
Average	57658	3543457	3601115
%RSD	0.612	0.672	0.651
SD	353	23818	23465

$$Assay(\%) = \frac{AreaofTest}{Avg.areaofStd.} \times \frac{Wt.ofStd.}{100} \times \frac{5}{25} \times \frac{100}{Wt.ofTest} \times \frac{PotencyofStd(asisbasis)}{claim}$$

Then, Calculated ratio H_2B_{1a} / (H_2B_{1a} + H_2B_{1b}) x 100

Table No. 6.3.6.6: Result of all three formulations.

S.No.	Formulation	Assay (%)	Ratio (%)
1.	F1	91.57	98.41
2.	F2	95.5	98.42
3.	F3	101.32	98.39

- The ratio H_2B_{1a} / (H_2B_{1a} + H_2B_{1b}) of Ivermectin was found to be between 90% - 110% to 98.41% (F1), 98.42% (F2), 98.39% (F3) which is in the limit.

6.3.7 Viscosity: A Brookfield viscometer was used to measure the formulation's viscosity. (CAP-2000). 20g of the sample was put in a beaker and given 5 minutes to equilibrate before the dial reading was measured using a spindle rotating at 20 rpm. The viscometer's dial reading was recorded.

- The viscosity in centipoise was obtained by directly multiplying the dial reading by the coefficients listed in the Brookfield viscometer catalogue.

Viscosity in cps = Dial reading x Factor

Table No. 6.3.7: Viscosity of Ivermectin cream

S.No.	Formulations	Dial reading	Factor	Viscosity (cps)
1.	F1	55	10	550
2.	F2	60	10	600
3.	F3	75	10	750

- The viscosity of the formulation was determined and found that viscosity of F3 was good enough to apply and is easily spreadable in comparison to that F1 and F2 formulations.

6.4 Specificity:

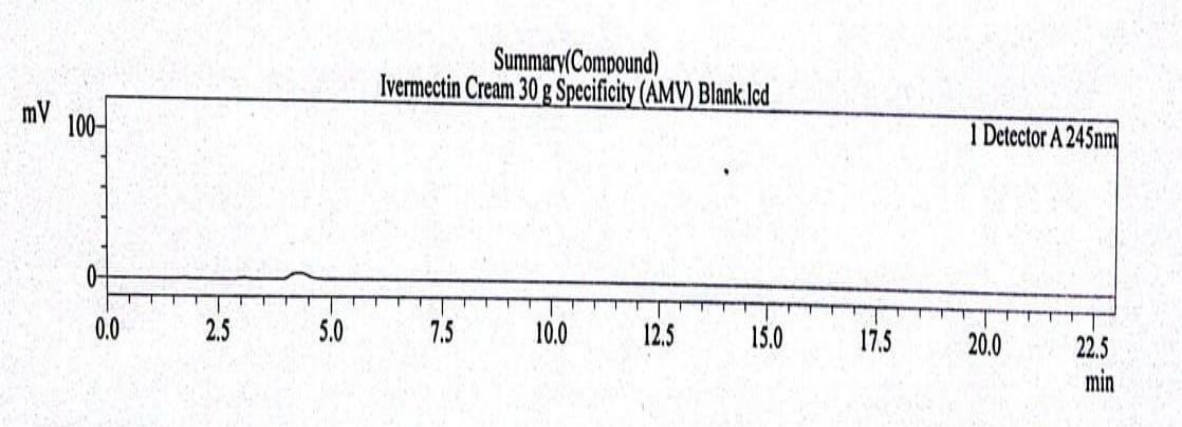


Figure No. 6.4.1: Blank (Mobile phase)

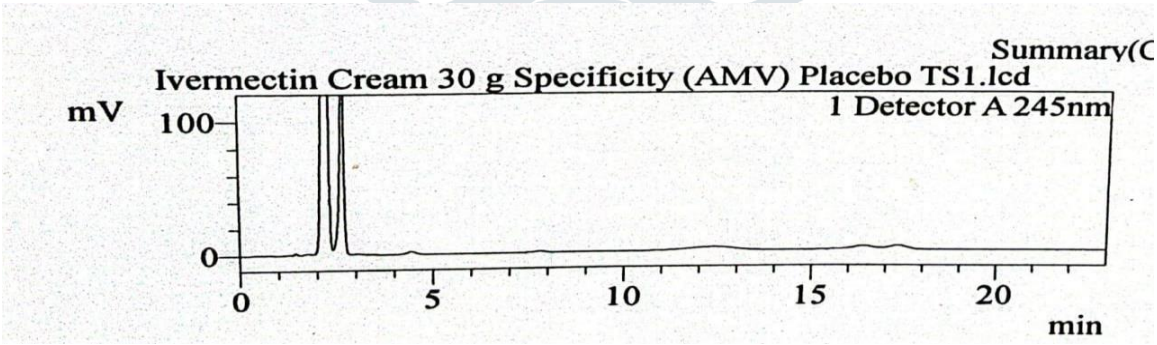


Figure No. 6.4.2: Placebo

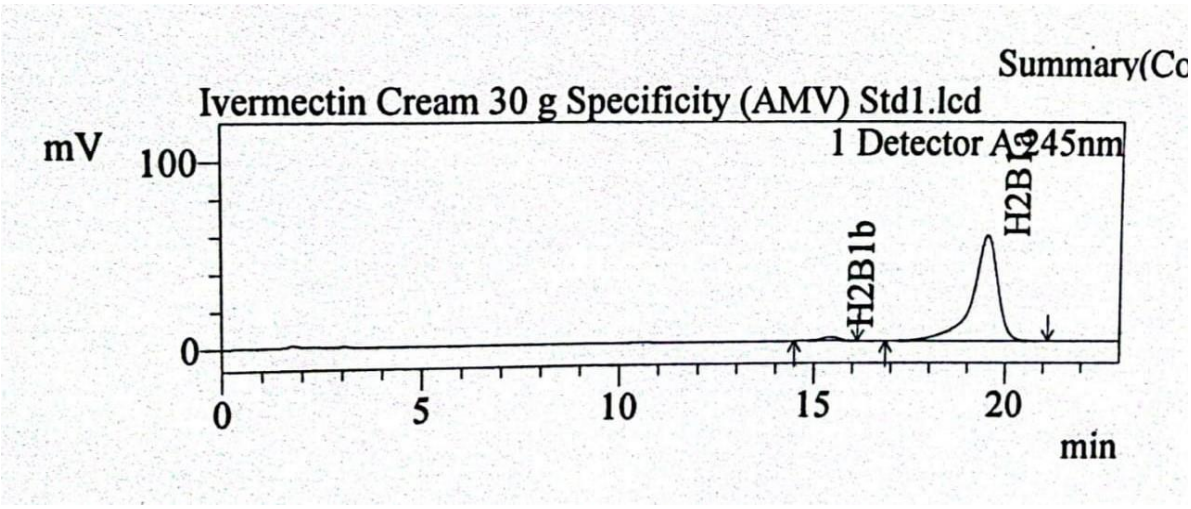


Figure No. 6.4.3: Standard (Ivermectin)

- Fig No.: 6.4.1, 6.4.2, and 6.4.3 show the chromatograms of Blank (Mobile phase), Placebo, and Ivermectin Working Standard. The above chromatogram shows the absence of interference from other substances was confirmed through the evaluation of selectivity using spiked samples. The absence of any significant peaks at the analyte retention time in the chromatogram indicated the specificity of the method.".

6.5 Linearity:

A total of five different concentrations of both the test and standard solutions were prepared, ranging from 80% to 120%. The prepared solutions were then analyzed to obtain the corresponding peak areas using the selected analytical method.

The obtained peak areas and the corresponding concentrations were then used to construct a calibration curve by plotting a graph of peak area versus concentration.

Tab. No. 6.5.1: Concentration and area for linearity of Ivermectin (Standard)

S.No.	Conc. %	Area (H2B1b)	Area (H2B1a)	Sum
1.	80	67766	2079362	2147128
		67497	2089596	2157093
		67588	2089535	2157123
2.	90	75711	2346601	2422312
		75401	2344258	2419659
		75334	2345257	2420591
3.	100	79282	2509859	2589141
		79729	2478383	2558112
		79342	2494319	2573661
		79254	2492486	2571740
		79804	2507699	2587503
		78608	2477672	2556280
4.	110	87682	2752025	2839707
		87754	2752304	2840058
		87772	2753096	2840868

5.	120	90379	3058807	3149186
		90115	2975557	3065672
		90756	2979593	3070349

Table No. 6.5.2: Concentration and area for linearity of Ivermectin (Standard Linearity)

Conc. %	**Area of Standard**								
	Std 1	**Std 2**	**Std 3**	**Std 4**	**Std5**	**Std 6**	**Mean**	**SD**	**%RSD**
80	2147128	2157093	2157123				2153781	5762	0.268
90	2422312	2419659	2420591				2420854	1346	0.056
100	2589141	2558112	2573661	2571740	2587503	2556280	2572740	13955	0.542
110	2839707	2840058	2840868				2840211	595	0.021
120	3149186	3065672	3070349				3095069	46925	1.516

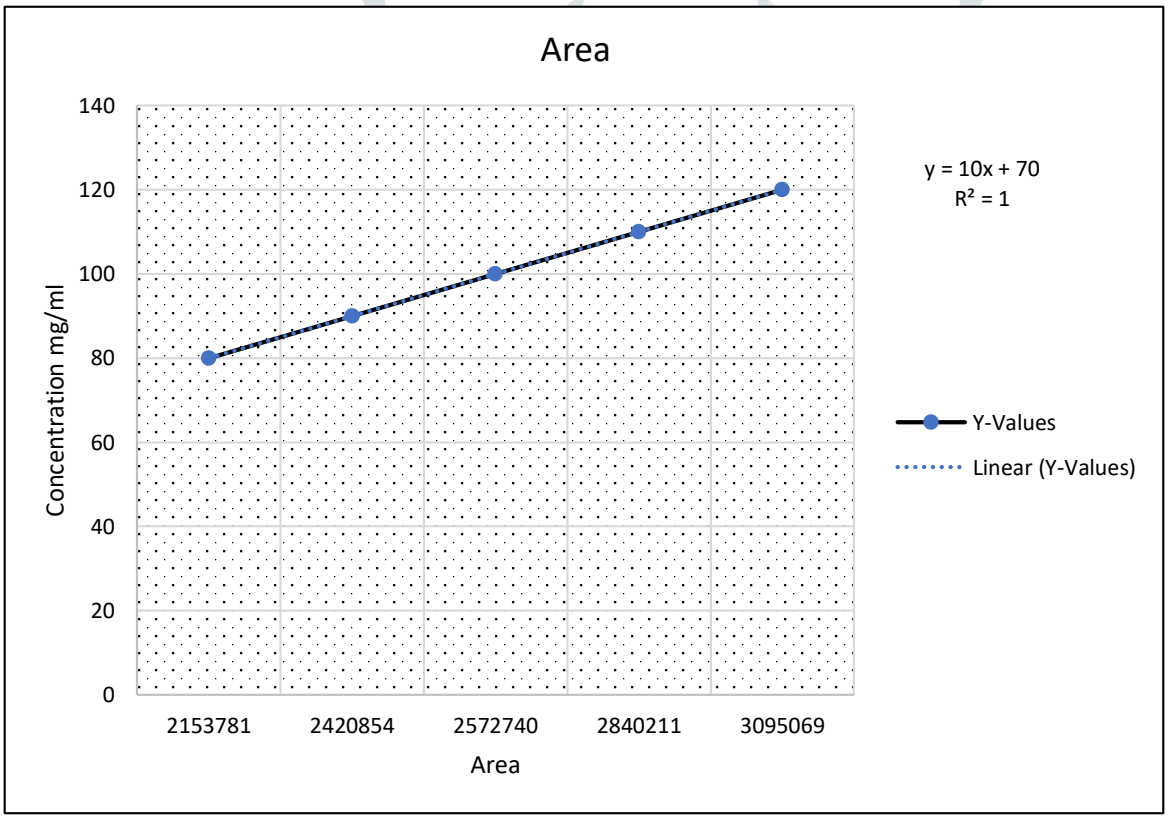


Fig. No. 6.5.2: Calibration curve for area against concentration in mg/ml of Ivermectin (Standard Solution)

Fig. 6.5.2 shows the linear calibration curve with R2 of 1 and y = 10x + 70 as the regression equation for the standard solution (Ivermectin), indicating high accuracy and reliability of the analytical method.

Table No. 6.5.3: Concentration and area for linearity of Ivermectin (Test)

S.No.	Conc. %	Area (H2B1b)	Area (H2B1a)	Sum
1.	80	68935	2563616	2632551
		69231	2564062	2633293
		69156	2562575	2631731
2.	90	75408	2797603	2873011
		75323	2782323	2857646
		75179	2774827	2850006
3.	100	82671	3293652	3376323
		82272	3293373	3375645
		82199	3316537	3398736
		82634	3283191	3365825
		82804	3281462	3364266
		82783	3301077	3383860
4.	110	69868	3617647	3687515
		68769	3610073	3678842
		61570	3600203	3661773
5.	120	128957	3841517	3970474
		122990	3848548	3971538
		129382	3868970	3998352

Table No. 6.5.4: Concentration and area for linearity of Ivermectin (Test Linearity)

Conc. %	Area of Test								
	TS 1	**TS 2**	**TS 3**	**TS 4**	**TS 5**	**TS 6**	**Mean**	**SD**	**%RSD**
80	2632551	2633293	2631731				2632525	781	0.030
90	2873011	2857646	2850006				2860221	11717	0.410
100	3376323	3375645	3398736	3365825	3364266	3383860	3376159	12710	0.376
110	3687515	3678842	3661773				3678842	17351	0.471
120	3970474	3971538	3998352				3980121	15797	1.516

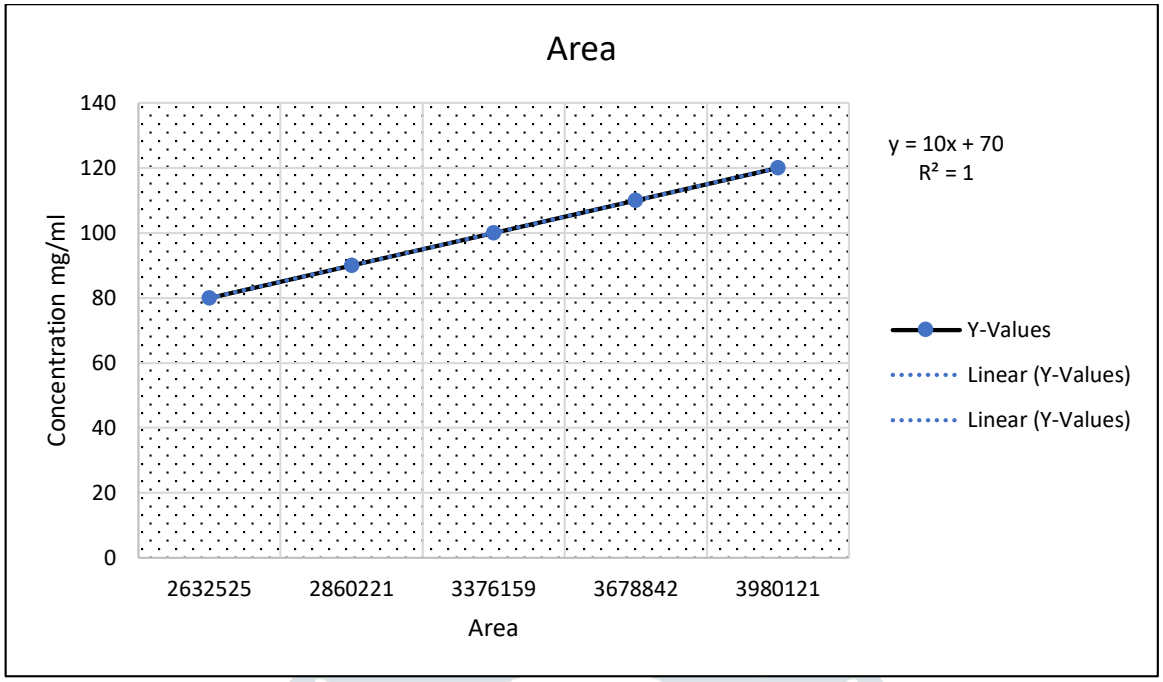


Fig. No. 6.5.4: Calibration curve for area against concentration in mg/ml of Ivermectin (Test Sample)

Fig. 6.5.4 shows the linear calibration curve with R2 of 1 and y = 10x + 70 as the regression equation for the test solution, indicating high accuracy and reliability of the analytical method.

Fig. 6.5.2 and 6.5.4 shows a Linear regression analysis was performed on the data, resulting in a straight line with a slope of 10 and a perfect fit (R2 = 1). The relationship between area and concentration for Ivermectin was found to be linear. The regression equation for the standard solution was y = 10x + 70, and the test sample exhibited a similar relationship.

6.6 Accuracy:

By employing a recovery test, the accuracy of the analysis was assessed. The evaluation encompassed three different concentrations (0.8 mg/ml, 1 mg/ml, and 1.2 mg/ml) for both the sample solution and the standard solution across various drug products. Encouragingly, the recovery percentages obtained at these concentrations exhibited favorable accuracy.

The results are described in the table below.

- ***Standard Accuracy***

$$standard\ preparation = \frac{50.0mg}{100ml} \times \frac{5ml}{25ml} \qquad Conc. = 0.1\ mg/\text{ml} \quad [\text{Note: } 1\text{g} = 10\text{mg}]$$

$$Standard\ preparation\ (80\%) = \frac{41.8mg}{100ml} \times \frac{5ml}{25ml} \qquad C_{U\,80} = 0.08\ mg/ml$$

$$Standard\ preparation(100\%) = \frac{50.0mg}{100ml} \times \frac{5ml}{25ml} \qquad C_{U100} = 0.1mg/ml$$

$$Standard\ preparation(120\%) = \frac{60.0mg}{100ml} \times \frac{5ml}{25ml} \qquad C_{U120} = 0.12mg/ml$$

[Note: Each gram contains 10 mg of ivermectin]

$$Assay\ (\%)\ (80\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 80$$

$$Assay\ (\%)\ (100\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

$$Assay\ (\%)\ (120\ \%) = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 120$$

Where, r_U = Area of test solution
r_S = Area of Standard solution

Tab. No. 6.6.1: Area of Standard Solution

S.No.	Area of Standard		
	H2B1b	H2B1a	Sum
1.	79282	2509859	2589141
2.	79729	2478383	2558112
3.	79342	2494319	2573661
4.	79254	2492486	2571740
5.	79804	2507699	2587503
6.	78608	2477672	2556280
Average			2572740

Table No. 6.6.2: Area of Standard solution (Accuracy)

Area of Standard solution	
Std 1	2589141
Std 2	2558112
Std 3	2573661
Std 4	2571740
Std 5	2577503
Std 6	2556280
Mean	2572740
Standard deviation	13954.91
RSD %	0.542

Table No. 6.6.3: Area of Standard solution (Accuracy)

S.No.	Conc. %	Area of Standard		
		H2B1b	H2B1a	Sum
1.	80	67766	2079362	2147128
		67497	2089596	2157093
		67588	2089535	2157123
3.	100	79282	2509859	2589141
		78608	2478383	2556991
		79342	2494319	25736610
4.	120	90379	3058807	3149186
		90115	2975557	3065672
		90756	2979593	3070349

Table No. 6.6.4: Recovery study of Ivermectin (Standard Accuracy)

Area of Test			
	80%	**100%**	**120%**
TS1	2147128	2589141	3149186
TS2	2157093	2556991	3065672
TS3	2157123	2573661	3070349
Assay %			
TS1	79.86 %	100.64 %	122.41 %
TS2	80.23 %	99.39 %	119.93 %
TS3	80.23 %	100.04 %	119.34 %
Recovery %			
TS1	99.83	100.64	102.00
TS2	100.29	99.39	99.94
TS3	100.29	100.04	99.45
Avg. (%)	**100.21**		
Recovery as per Average %			
TS1	99.62	100.43	101.79
TS2	100.08	99.18	99.73
TS3	100.09	99.83	99.25
		Average	**100.256**

- Table No. 6.6.4 displays a remarkable range of recovery percentages, spanning from 99.18% to 100.79%, with an average recovery of 100.25%. This data attests to the Accuracy's fulfillment of analytical technique validation criteria for Ivermectin Cream.

- ***Test Accuracy***

$$Standard\ preparation = \frac{50.0mg}{100ml} \times \frac{5ml}{25ml} \qquad C_S = 0.1mg/ml$$

$$Test\ preparation(80\%) = \frac{0.8421g}{100ml}$$

$$Test\ preparation(100\%) = \frac{1.1056g}{100ml}$$

$$Test\ preparation(120\%) = \frac{1.2018g}{100ml}$$

[Note: 1g is equals to 10mg]

$$Assay(\%)(80\%) = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency}{Claim}$$

$$Assay(\%)(100\%) = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency}{Claim}$$

$$Assay(\%)(120\%) = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency}{Claim}$$

Table No. 6.6.5: Area of Test solution (Accuracy)

S.No.	Conc. %	Area of Test		
		H2B1b	H2B1a	Sum
1.	80	68935	2563616	2632551
		69231	2564062	2633293
		69156	2562575	2631731
3.	100	82671	3293652	3376323
		82272	3293373	3375645
		82199	3316537	3398736
4.	120	128957	3841517	3970474
		122990	3848548	3971538
		129382	3868970	3998352

Table No. 6.6.6: Recovery study of Ivermectin (Test Accuracy)

Area of Test			
	80%	**100%**	**120%**
TS1	2632551	3376323	3970474
TS2	2633293	3375645	3971538
TS3	2631731	3398736	3998352
Assay %			
TS1	77.82 %	99.78 %	117.70 %
TS2	77.84 %	99.76 %	117.74 %
TS3	77.79 %	100.44 %	118.53 %
Recovery %			

TS1	97.27	99.78	98.09
TS2	97.30	99.76	98.11
TS3	97.24	100.44	98.78
Avg. (%)	**100.21**		
Recovery as per Average %			
TS1	98.72	101.27	99.55
TS2	98.75	101.25	99.58
TS3	98.69	101.94	100.25
		Average	**99.793**

Table No. 6.6.6 displays the real percentage of recovery, ranging from 98.69% to 101.94% with an average recovery of 100.21%. This confirms that the Accuracy of Ivermectin Cream is in line with the required specifications for the analytical technique validation.

6.7 Precision:

The precision (repeatability) of the instrument was evaluated by conducting a series of injections. Over the course of three days, three different analysts performed six injections each using a mixture of Ivermectin standard solution. The injections were carried out at the standard concentration level, ensuring a 100% test concentration.

- **Precision (Analyst-1, Day-1, Instrument-1)**

$$Standard\colon\ (50.4\ mg)/(100\ ml) \times 5ml/25m$$

$$Test\ 1\colon\ 1.2610g/100ml$$

Potency: 91.66%

$$\%\ Assay = \frac{Area\ of\ Test}{Area\ of\ Standard} \times \frac{50.4}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test\ in\ g} \times \frac{Potency}{Label\ claim}$$

Table No. 6.7.1: Results of the precision test solution performed by the first analyst on instrument No. 1 on the first day. (Day 1)

Injection	Area		RT of Ivermectin cream		Tailing Factor		Theoretical Plate	
	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a
1	125280	3762476	15.555	19.664	0.666	0.736	5053	5292
2	127428	3758662	15.517	19.607	0.672	0.737	5025	5326
3	126481	3757108	15.448	19.511	0.675	0.738	5086	5372
4	125772	3750192	15.424	19.485	0.676	0.739	5138	5367
5	125431	3746943	15.426	19.485	0.676	0.739	5164	5406
6	125305	3740978	15.425	19.485	0.675	0.739	5186	5431
Mean	**125949**	**3752727**	**15.466**	**19.540**	**0.673**	**0.738**	**5109**	**5366**
SD	**852**	**8092**	**0.056**	**0.077**	**0.004**	**0.001**	**64**	**51**
% RSD	**0.677**	**0.216**	**0.365**	**0.395**	**0.567**	**0.173**	**1.255**	**0.953**

Table No. 6.7.2: Chromatographic Result of Precision (Analyst-1, Day-1, Instrument-1)

S.No.	Area of Standard			Area of Test			
	H2B1b	H2B1a	Sum		H2B1b	H2B1a	Sum
1.	82615	2664557	2747172	TS1	125280	3762476	3887756
2.	82015	2661976	2743991	TS2	127428	3758662	3886090
3.	82799	2663933	2746732	TS3	126481	3757108	3883589
4.	82670	2667033	2749703	TS4	125772	3750192	3875964
5.	82383	2660963	2743346	TS5	125431	3746943	3872374
6.	82387	2669550	2751937	TS6	125305	3740978	3866283
Average			2747147	Average			3878676
SD			3288	SD			8511
%RSD			0.119	%RSD			0.219

- The first analyst's test solution on Day 1 showed a relative standard deviation of 0.219% (Table 6.7.1), well below the 2.0% threshold. The tailing factor was under 2, and the theoretical plate count exceeded 2000, all meeting the required criteria.

- **Precision (Analyst-2, Day-2, Instrument-2)**

$$Standard\colon\ (50.4\ mg)/(100\ ml) \times 5ml/25m$$

$$Test\ 1\colon\ 1.1194g/100ml$$

Potency: 91.66

Table No. 6.7.3: Results of the precision test solution performed by the second analyst on instrument No. 2 on the second day. (Day 2)

Injection	Area		RT of Ivermectin cream		Tailing Factor		Theoretical Plate	
	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a
1	33278	1488180	15.395	19.481	0.956	0.895	3057	2772
2	33276	1494333	15.410	19.512	0.946	0.884	2889	2712
3	33638	1492222	15.445	19.553	0.941	0.885	2858	2640
4	33521	1491611	15.436	19.564	0.963	0.894	2843	2586
5	34492	1489780	15.432	19.552	0.977	0.904	2703	2572
6	34222	1493507	15.374	19.524	0.952	0.905	2671	2530
Mean	**33738**	**1491605**	**15.415**	**19.531**	**0.955**	**0.894**	**2837**	**2635**
SD	**507**	**2302**	**0.028**	**0.031**	**0.011**	**0.009**	**139**	**92**
% RSD	**1.503**	**0.514**	**0.179**	**0.161**	**1.151**	**0.991**	**4.900**	**3.477**

Table No. 6.7.4: Chromatographic Result of Precision (Analyst-2, Day-2, Instrument-2)

S.No.	Area of Standard			Area of Test			
	H2B1b	H2B1a	Sum		H2B1b	H2B1a	Sum
1.	30015	1175968	1205983	TS1	33278	1488180	1521458
2.	30231	1175862	1206093	TS2	33276	1494333	1527609
3.	30007	1177346	1207353	TS3	33638	1492222	1525860
4.	30490	1176569	1207059	TS4	33521	1491611	1525132
5.	30257	1172190	1202447	TS5	34492	1489780	1524272
6.	30980	1174480	1205460	TS6	34222	1493507	1527729
Average			1205733	Average			1525343
SD			1758	SD			2340
%RSD			0.145	%RSD			0.153

In Day 2's test solution, the second analyst achieved a low relative standard deviation of 0.153%, well below the acceptable limit of 2.0%. Furthermore, the tailing factor was found to be less than 2, indicating good peak symmetry. Additionally, the theoretical plate count exceeded the minimum requirement of 2000, ensuring efficient separation in the analysis.

- **Precision (Analyst-3, Day-3, Instrument-3)**

$$Standard:\ (50.1\ mg)/(100\ ml) \times 5ml/25m$$

$$Test\ 1:\ 1.2010g/100ml$$

Potency: 91.66%

$$\%\ Assay = \frac{Area\ of\ Test}{Area\ of\ Standard} \times \frac{50.4}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test\ in\ g} \times \frac{Potency}{Label\ claim}$$

Table No. 6.7.5 Results of the precision test solution performed by the third analyst on instrument No. 3 on the third day. (Day 2)

Injection	Area		RT of Ivermectin cream		Tailing Factor		Theoretical Plate	
	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a	H2B1b	H2B1a
1	87749	3312477	13.792	17.359	0.750	0.844	6808	6427
2	87071	3304633	13.805	17.377	0.761	0.845	7104	6655
3	87190	3357477	13.599	17.097	0.736	0.844	6049	5533
4	87004	3301376	13.792	17.359	0.753	0.846	6836	6433
5	87456	3425314	13.599	17.097	0.749	0.817	6679	5469
6	87578	3402581	13.607	17.113	0.758	0.837	6618	5463
Mean	**87341**	**3350643**	**13.699**	**17.234**	**0.751**	**0.838**	**6682**	**5997**
SD	**298**	**53518**	**0.107**	**0.144**	**0.008**	**0.011**	**353**	**563**
% RSD	**0.341**	**1.597**	**0.780**	**0.836**	**1.065**	**1.312**	**5.277**	**9.395**

Table No. 6.7.6: Chromatographic Result of Precision (Analyst-3, Day-3, Instrument-3)

S.No.	Area of Standard			Area of Test			
	H2B1b	H2B1a	Sum		H2B1b	H2B1a	Sum
1.	83483	2488115	2571598	TS1	87749	3312477	3400226
2.	83421	2484990	2568411	TS2	87071	3304633	3391704
3.	83230	2481791	2565021	TS3	87190	3357477	3444667
4.	83483	2488115	2594099	TS4	87004	3301376	3388380
5.	83421	2484990	2568411	TS5	87456	3425314	3512770
6.	83230	2481791	2565021	TS6	87578	3402581	3490159
Average			2572094	**Average**			3437984
SD			11060	**SD**			53643
%RSD			0.429	**%RSD**			1.560

On Day 3, the third analyst achieved a test solution area relative standard deviation of 1.560%, meeting the requirement of being less than 2.0%. The tailing factor was found to be less than 2, indicating good peak

symmetry. Moreover, the theoretical plate count exceeded the minimum threshold of 2000, ensuring effective separation during analysis.

Table No. 6.7.7: Final Results of all three Analyst

S.No.	A1, D1, I1	A2, D2, I2	A3, D3, I3
1.	103.65	104.57	103.80
2.	103.58	104.45	103.79
3.	103.58	104.45	103.99
4.	103.38	104.40	103.79
5.	103.28	104.34	104.01
6.	103.12	104.58	104.21
Average	**103.43**	**104.47**	**103.93**
SD	**0.2071**	**0.0944**	**0.1699**
% RSD	**0.200**	**0.090**	**0.1634**

- The analysts' relative standard deviations for A1, D1, I1, A2, D2, I2, A3, D3, I3 on Days 1, 2, and 3, as per Table 6.7.7, were 0.200%, 0.090%, and 0.1634% respectively. These values, deemed less than 2.0%, demonstrated strong consistency and interrelation. Therefore, the procedure is precise.

6.8 Robustness:

Various parameters were modified to assess the robustness, including column length (15 cm, 25 cm), wavelength (220 nm, 218 nm, 222 nm), injection volume (20 µl, 18 µl, 16 µl), and flow rate (1.5 ml/min, 1.35 ml/min, 1.65 ml/min).

❖ ***Column Length – 15cm***

$$Standard:\ (50.8\,mg)/(100\,ml) \times 5ml/25m$$

$$Test\ 1:\ 1.1385g/100ml$$
$$Test\ 2:\ 1.2315g/100ml$$
$$Test\ 3:\ 1.1785g/100ml$$

$$Assay(\%) = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency\ of\ std}{Claim}$$

Table No. 6.8.1: Displaying the results of the test solution's influence on column length

S.No.	Parameter	Test	Assay %	RT of test		Area of test	
				H2B1b	H2B1a	H2B1b	H2B1a
1	Column Length-1 15 cm	TS1	99.14	9.158	11.610	82671	3035198
2		TS2	99.63	9.173	11.628	82272	3050899
3		TS3	99.85	9.170	11.620	82199	3057895
Average			**99.54**	**9.167**	**11.619**	**82381**	**3047997**
Standard Deviation			**0.363**	**0.008**	**0.009**	**254**	**11623**
RSD%			**0.364**	**0.083**	**0.077**	**0.308**	**0.381**
4	Column Length-2 25 cm	TS1	100.34	14.760	18.574	73344	3676565
5		TS2	100.45	14.770	15.591	73905	3680394
6		TS3	100.11	14.763	18.578	73334	3667916
Average			**100.3**	**14.765**	**18.581**	**73528**	**3674958**
Standard Deviation			**0.173**	**0.005**	**0.009**	**327**	**6392**
RSD%			**0.172**	**0.035**	**0.048**	**0.445**	**0.174**

- Table 6.8.1 display Ivermectin outcomes with varying column lengths (15 cm to 25 cm). The Assay (%) RSD for Ivermectin test solution was 0.364% and 0.172% for 15 cm and 25 cm columns, respectively, both below 2.0%.

Table No. 6.8.2: Displaying the Effects of Changing Column Length (Standard Solution)

S.No.	Parameter	Standard	RT of Std.		Area of Std.	
			H2B1b	H2B1a	H2B1b	H2B1a
1	Column Length-1 15 cm	Std 1	9.170	11.620	79282	2509859
2		Std 2	9.174	11.625	79929	2478022
3		Std 3	9.172	11.620	79342	2494319
4		Std 4	9.172	11.621	79254	2492486
5		Std 5	9.175	11.623	79804	2507699
6		Std 6	9.174	11.625	79393	2477672
Average			**9.173**	**11.622**	**79501**	**2493343**
Standard Deviation			**0.002**	**0.002**	**290**	**13863**
RSD%			**0.021**	**0.019**	**0.365**	**0.556**
7	Column Length-2 25 cm	Std 1	14.756	18.574	62784	2531842
8		Std 2	14.756	18.570	62461	2530172
9		Std 3	14.765	18.582	62934	2531655
10		Std 4	14.765	18.586	63068	2531165
11		Std 5	14.765	18.584	63144	2528577
12		Std 6	14.759	18.576	62601	2525582
Average			**14.761**	**18.576**	**62832**	**2529832**
Standard Deviation			**0.005**	**0.006**	**267**	**2404**
RSD%			**0.031**	**0.034**	**0.425**	**0.095**

- Table 6.8.2 displays the impact of column length variations (15 cm to 25 cm) on the effects of Ivermectin. The relative standard deviation (RSD) for the Ivermectin standard solution was found to be 0.365 (15 cm column) and 0.556 (25 cm column), which is less than 2.0%. The table indicates a direct proportionality between column length and retention time.

Table No. 6.8.3: Displaying effects of changing the wavelength

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By IP) 245 nm	TS1	99.69
2		TS2	99.81
3		TS3	99.46
4	Variation in WL by (-2) WL= 243 nm	TS1	99.56
5		TS2	100.13
6		TS3	99.83
4	Variation in WL by (+2) WL= 247 nm	TS1	99.68
5		TS2	99.93
6		TS3	99.79
Average			**99.76**
Standard Deviation			**0.199**
RSD%			**0.199**

- Table No. 6.8.3 reveals its response to altered wavelengths: 245 nm, 243 nm, and 247 nm. Remarkably, the standard deviation and RSD remain below 2.0%, measuring at 1.169 and 1.145, respectively.

Table No. 6.8.4: Displaying the Effects of Changing the Flow Rate

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By IP) FR= 1.5 ml/min	TS1	99.69
2		TS2	99.81
3		TS3	99.46
4	Variation in FR by (-10%) FR= 1.35 ml/min	TS1	99.32
5		TS2	99.43
6		TS3	99.09
4	Variation in FR by (+10%) FR= 1.65 ml/min	TS1	101.50
5		TS2	98.24
6		TS3	98.67
		Average	**98.48**
		Standard Deviation	**0.909**
		RSD%	**0.923**

- In Table No. 6.8.4, Ivermectin results are presented for three different flow rates: 1.5 ml/min, 1.35 ml/min, and 1.65 ml/min. The standard deviation and relative standard deviation (RSD) for all measurements were below 2.0%, specifically 0.909 and 0.923, respectively.

Table No. 6.8.5: Displaying the effects of changing the Injection volume

S.No.	Parameter	Test	Assay %
1	Standard Robustness (By IP) IV = 20 µl	TS1	99.69
2		TS2	99.81
3		TS3	99.46
4	Variation in IV by (-2 µl) IV = 18 µl	TS1	100.41
5		TS2	100.37
6		TS3	100.06
4	Variation in IV by (-4 µl) IV = 16 µl	TS1	99.34
5		TS2	98.84
6		TS3	98.91
		Average	**99.65**
		Standard Deviation	**0.573**
		RSD%	**0.575**

- Table No. 6.8.5 displays the outcomes of Ivermectin by changing its injection volume by 18 µl and 16 µl. The standard deviation and RSD were found to be 0.573 and 0.575 respectively, which were both less than 2.0%.

6.9 Stability:

Sample solution stability:

- Prepare and divide sample solution.
- Store at 25°C for 6 and 24 hours.
- Assess appearance and degradation.
- Analyze and compare with fresh sample solution.

Standard solution stability:

- Prepare and divide standard solution.
- Store at 25°C for 6 and 24 hours.
- Assess appearance and degradation.
- Analyze and compare with fresh standard solution.

Mobile phase stability:

- Prepare mobile phase.
- Store at 25°C for 6, 24, and 48 hours.
- Assess appearance and degradation.
- Analyze and compare with fresh mobile phase.

❖ ***For Mobile Phase Stability:***

$$Stadard\ preparation = \frac{50.4mg}{100ml} \times \frac{5ml}{25ml} \qquad C_S = 0.1\ mg/ml$$

$$Test\ preparation = \frac{1.3025\ g}{100\ ml} \qquad C_U = 0.13\ mg/ml$$

Note: Each gram of cream contains 10 mg of ivermectin.

$$Assay\ in\ \% = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency}{Claim}$$

Where,
r_U = Area of test preparation
r_S = Area of standard preparation

Table No. 6.9.1: Displaying the impact of the Mobile phase's stability

S.No.	Parameter	Sample	Area	Assay %
1	Fresh Sample	Std.	2699653	-
2		TS1	3805514	99.99
3		TS2	3820518	100.39
4		TS3	756849	100.39
Average				**100.26**
5	Store at Real time (6 hours)	Std.	2647872	-
6		TS1	3740331	100.20
4		TS2	3712734	99.46
5		TS3	3711377	99.43
Average				**99.70**
6	Store at Real time (24 hours)	Std.	2580276	-
7		TS1	3679464	101.15
8		TS2	3632016	99.85
9		TS3	3632353	99.86
Average				**100.29**
10	Store at Real time (48 hours)	Std.	2745587	-
11		TS1	3859677	99.72
12		TS2	3806697	98.35
13		TS3	3793770	98.01
Average				**98.69**
RSD%				**0.33**

❖ **For Test and Standard solution:**

$$Standard\ preparation = \frac{50.4mg}{100ml} \times \frac{5ml}{25ml} \qquad C_S = 0.1\ mg/ml$$

$$Test\ preparation = \frac{1.3025\ g}{100\ ml} \qquad C_U = 0.13\ mg/ml$$

Note: Each gram of cream contains 10 mg of ivermectin.

$$Assay\ in\ \% = \frac{r_U}{r_S} \times \frac{wt.of\ std}{100} \times \frac{5}{25} \times \frac{100}{wt.of\ test(g)} \times \frac{Potency}{Claim}$$

Where,
r_U = Area of test preparation
r_S = Area of standard preparation

Table No. 6.9.2: Displaying the outcome of stability of Test and Standard solution

S.No.	Parameter	Sample	Area	Assay %
1	Fresh Sample	Std.	2699653	-
2		TS1	3805514	99.99
3		TS2	3820518	100.39
4		TS3	3820518	100.39
Average				**100.26**
5	Store at Real time (6 hours)	Std.	2647872	-
6		TS1	3740331	100.20
4		TS2	3712734	99.46
5		TS3	3711377	99.43
Average				**99.70**
In comparison to the freshly prepared solution, the efficiency ranges between 98.0% and 102.0%.				**99.44**
6	Store at Real time (24 hours)	Std.	2580276	-
7		TS1	3679464	101.15
8		TS2	3632016	99.85
9		TS3	3632353	99.86
Average				**100.29**
In comparison to the freshly prepared solution, the efficiency ranges between 98.0% and 102.0%.				**100.03**
10	Store at 2 - 8ºC (24 hours)	Std.	2673689	-
11		TS1	3713557	98.52
12		TS2	3726005	98.85
13		TS3	3722084	98.50
Average				**98.62**
In comparison to the freshly prepared solution, the efficiency ranges between 98.0% and 102.0%.				**98.36**

- According to ICH guidelines, the stability tests were conducted on the mobile phase and solution at various time points. The RSD of the mobile phase assay results within 48 hours was 0.33%, well below the acceptable limit of 2.0%. The solution's assay percentage at different storage times was 99.44%, 100.03%, and 98.36% compared to the freshly prepared solution at room temperature. These results indicate that the solution stability meets the analytical technique validation specification.

CONCLUSION

The formulation and evaluation of cream were successfully done. Three formulations, F1, F2, and F3, were prepared, and their properties were studied. Based on the evaluation of viscosity, spreadability, homogeneity, and grittiness, it was found that the F3 formulation was the best among them. The results showed that the F3 formulation had the desired attributes required for an effective cream. It had a reasonable viscosity, which was neither too high nor too low, ensuring easy application and absorption into the skin. The spreadability of the F3 formulation was also ideal, making it easy to apply over a large area, resulting in good coverage. Furthermore, F3 formulation exhibited good homogeneity, with no visible particles present in the cream. Lastly, the grittiness test further confirmed that F3 was the best formulation among the three, as no grittiness was observed. As a result, it can be concluded that F3 formulation is the most effective cream and can be used as a base for developing other creams in the future.

The analytical method validation for Ivermectin was successfully conducted and yielded robust, linear, and accurate results. The method showed no interference between Ivermectin and the mobile phase. The recovery percentage for the experimental Ivermectin cream ranges from 98.69% to 101.94%, while the recovery percentage for standard Ivermectin was discovered to be between 99.18% and 101.79% which are within the acceptable ranges. Intermediate precision and robustness were confirmed through low RSD values. The RSD of intermediate precision was found to be, respectively, 0.219%, 0.153%, and 1.560% which are less than 2%. The tailing factor was found to be less than 2.0% and theoretical plate was found to be not less than 2000 which are within the acceptable ranges. Variations in flow rate, wavelength, injection volume, and column length resulted in acceptable RSD values. It was discovered that the retention time and column length are directly proportional to one another. The peak was reached 15 cm earlier than in a 25 cm column, which is advantageous for the pharmaceutical industry. Stability tests indicated that the mobile phase and sample solution remained stable for specified durations. Overall, the method is reliable for quantifying Ivermectin in cream formulations.

REFERENCE

1. Sahni DR, Feldman SR, Taylor SL. Ivermectin 1%(CD5024) for the treatment of rosacea. Expert Opinion on Pharmacotherapy. 2018 Mar 24;19(5):511-6.
2. Deeks ED. Ivermectin: a review in rosacea. American journal of clinical dermatology. 2015 Oct;16(5):447-52.
3. Taieb A, Ortonne JP, Ruzicka T, Roszkiewicz J, Berth-Jones J, Peirone MH, Jacovella J, Ivermectin Phase III Study Group. Superiority of ivermectin 1% cream over metronidazole 0· 75% cream in treating inflammatory lesions of rosacea: a randomized, investigator-blinded trial. British Journal of Dermatology. 2015 Apr;172(4):1103-10.
4. Abokwidir M, Feldman SR. Rosacea management. Skin appendage disorders. 2016;2(1-2):26-34.
5. Raedler LA. Soolantra (Ivermectin) 1% cream: a novel, antibiotic-free agent approved for the treatment of patients with rosacea. American Health & Drug Benefits. 2015 Mar;8(Spec Feature):122.
6. Taieb A, Khemis A, Ruzicka T, Barańska-Rybak W, Berth-Jones J, Schauber J, Briantais P, Jacovella J, Passeron T, Ivermectin Phase III Study Group. Maintenance of remission following successful treatment of papulopustular rosacea with ivermectin 1% cream vs. metronidazole 0.75% cream: 36-week extension of the ATTRACT randomized study. Journal of the European Academy of Dermatology and Venereology. 2016 May;30(5):829-36.
7. Sahni DR, Feldman SR, Taylor SL. Ivermectin 1%(CD5024) for the treatment of rosacea. Expert Opinion on Pharmacotherapy. 2018 Mar 24;19(5):511-6.
8. IP 2018, page no. 2340-2341
9. Pivotal study: Soolantra (ivermectin) Cream, 1% is indicated for the treatment of inflammatory lesions of rosacea.
10. Indain pharmacopoeia 1996.

11. Chauhan L, Gupta S. Creams: a review on classification, preparation methods, evaluation and its applications. Journal of Drug Delivery and Therapeutics. 2020 Oct 15;10(5-s):281-9.
12. Cardin Julie. " 5 Benefits of a Topical Drug Delivery System"
13. Panchumarthy Ravishankar, Ch. Naga Navya, D. Pravallika, D. Navya Sri, 2015: " A Review on Step-by-Step Analytical Method Validation**.**
14. Chaccour C, Casellas A, Blanco-Di Matteo A, Pineda I, Fernandez-Montero A, Ruiz-Castillo P, Richardson MA, Rodríguez-Mateos M, Jordán-Iborra C, Brew J, Carmona-Torre F. The effect of early treatment with ivermectin on viral load, symptoms and humoral response in patients with non-severe COVID-19: A pilot, double-blind, placebo-controlled, randomized clinical trial. EClinicalMedicine. 2021 Feb 1;32:100720.
15. Thiboutot D, Anderson R, Cook-Bolden F, Draelos Z, Gallo RL, Granstein RD, Kang S, Macsai M, Gold LS, Tan J. Standard management options for rosacea: The 2019 update by the National Rosacea Society Expert Committee. Journal of the American Academy of Dermatology. 2020 Jun 1;82(6):1501-10.
16. Barańska-Rybak W, Kowalska-Olędzka E. New indications for topical ivermectin 1% cream: a case series study. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii. 2019 Feb 1;36(1):58-62.
17. Das S, Lee SH, Chia VD, Chow PS, Macbeath C, Liu Y, Shlieout G. Development of microemulsion based topical ivermectin formulations: Pre-formulation and formulation studies. Colloids and Surfaces B: Biointerfaces. 2020 May 1;189:110823.
18. Ashour DS. Ivermectin: From theory to clinical application. International journal of antimicrobial agents. 2019 Aug 1;54(2):134-42.
19. Yasaswini RS, Annapurna MM. Analytical Methods for the determination of Anti-Scabies drugs-A Review. Research Journal of Pharmacy and Technology. 2019;12(11):5600-4.
20. van Zuuren EJ, Fedorowicz Z, Tan J, van der Linden MM, Arents BW, Carter B, Charland L. Interventions for rosacea based on the phenotype approach: an updated systematic review including GRADE assessments. British Journal of Dermatology. 2019 Jul 1;181(1):65-79.
21. Thadanipon K, Anothaisintawee T, Rattanasiri S, Thakkinstian A, Attia J. Efficacy and safety of antiscabietic agents: A systematic review and network meta-analysis of randomized controlled trials. Journal of the American Academy of Dermatology. 2019 May 1;80(5):1435-44.
22. Wang MK, Chin-Yee B, Lo CK, Lee S, El-Helou P, Alowami S, Gangji A, Ribic C. Crusted scabies in a renal transplant recipient treated with daily ivermectin: a case report and literature review. Transplant Infectious Disease. 2019 Jun;21(3):e13077.
23. Rainer BM, Thompson KG, Antonescu C, Florea L, Mongodin EF, Bui J, Fischer AH, Pasieka HB, Garza LA, Kang S, Chien AL. Characterization and analysis of the skin microbiota in rosacea: a case–control study. American journal of clinical dermatology. 2020 Feb;21:139-47.
24. Schaller M, Almeida LM, Bewley A, Cribier B, Del Rosso J, Dlova NC, Gallo RL, Granstein RD, Kautz G, Mannis MJ, Micali G. Recommendations for rosacea diagnosis, classification and management: update from the global ROSacea COnsensus 2019 panel. British Journal of Dermatology. 2020 May 1;182(5):1269-76.
25. Forton FM. Rosacea, an infectious disease: why rosacea with papulopustules should be considered a demodicosis. A narrative review. Journal of the European Academy of Dermatology and Venereology. 2022 Jul;36(7):987-1002.
26. Hayran Y, Şen O, Fırat Oğuz E, Yücel Ç, Eren F, Külcü Çakmak S, Yalçın B. Serum IL-17 levels in patients with rosacea. Journal of Cosmetic Dermatology. 2022 Mar;21(3):1147-53.
27. Eduarda M.P. Silva, Luisa Barreiros, Paula S, Carlos Afonso, Sibylle Kozek-Langenecker, Marcela A. egundo: Analytical methods for quantification of tranexamic acid in biological fluids: A Review article, 2017, Vol. 134, PP(333-342)
28. Isabel Taverniers, Marc De Loose, Erik Van Bockstaele : Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance: Trends in Analytical Chemistry, Vol. 23, No. 8, 2004
29. National institute of standard and technology (2020)
30. Vijay Gupta, Ajay Deep Kumar Jain, NS Gill, Kapil Guptan, Development and validation of HPLC method –a review: International research journal of pharmaceutical and applied science 2 (4), 17-25, 2012.