IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

# RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CORTICOSTEROID DRUG IN BULK AND DOSAGE FORM

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**Abstract:** A novel, precise, accurate, rapid and cost effective isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed, optimised and validated for the estimation of Corticosteroid drug (Halobetasol propionate) in bulk and pharmaceutical dosage forms. The drugs were estimated using Inertsil Sustainswift ODS 3V 250*4.6mm, 5µm particle size column. A mobile phase composed of Acetonitrile: Methanol: Water (40:25:35 v/v/v) at a flow rate of 1.0 ml/min was used for the separation, Detection was carried out at 248 nm. For linearity, R2 value was found to be 0.999 for Halobetasol Propionate. % Assay was found to be 101.4%. Percentage recovery for HP was 99.75%-101.86%. The result of study showed that the proposed RP-HPLC method was found to be simple, sensitive, precise and accurate and also useful for routine analysis of Halobetasol Propionate in dosage form.

**Keywords**: Halobetsol Propionate, RP-HPLC, ICH Guidelines.

## Introduction:

**Name of Drug**: Halobetasol Propionate

**Description:** Some of the medication in Halobetasol Propionate is inevitably absorbed through the skin and into the bloodstream. If applied over a large area, or under an airtight dressing, the drug can cause a number of unwanted side effects, including increased sugar in your blood and urine and a set of symptoms called Cushing's syndrome, characterized by a moon-shaped face, emotional disturbance high blood pressure, weight gain, and growth of body hair in women. Use no more of Halobetasol Propionate than your doctor directs, and do not bandage or wrap the affected area unless the doctor specifically recommends it.

**Chemical structure:**

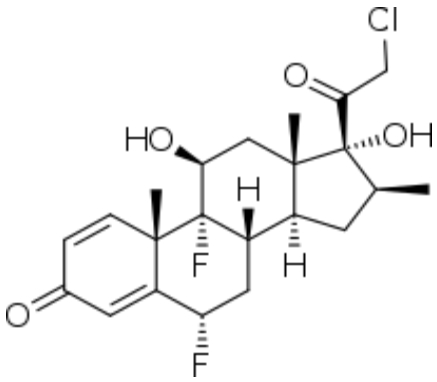


**Fig. No.1- Structure of Halobetasol Propionate**

**Chemical formula:** $C_{22}H_{27}ClF_2O_4$
**Molecular weight:** 428.90 g/mol
**IUPAC Name:** (6S, 8S, 9S, 10S, 11S, 13S, 14S, 16S, 17R)-17-(2-Chloroacetyl)-6,9-difloro-11.17-dihydroxy-10, 13, 16-trimethyl-6, 7, 8,11, 12, 14, 15, 16-octahydrocyclopenta (a) phenanthrene-3-one
**pKa value (Strong basic):** 7.6
**Bioavailability**: >60% (estimated)
**Solubility**: It is practically insoluble in water and freely soluble in dichloromethane and in acetone.
**Pharmacokinetics: Absorption:** Topical, for external use only; not for ophthalmic, oral, or intravaginal use; do not apply to the face; scalp; groin or axillae. Use of occlusive dressings is not recommended unless directed by a health care provider. Apply thin film to affected area and rub in gently and completely. Wash hands after application (unless treating hands). Lotion to the affected skin twice daily for up to two weeks. Rub in gently. Discontinue therapy when control is achieved. If no improvement is seen whin two weeks. Rub in gently. Discontinues therapy when control is achieved. If no improvement is seen within two weeks, reassessment of diagnosis may be necessary. Treatment beyond two weeks is not recommended and the total dosage should not exceed 50 grams (50ml) per week because of the potential for the drug to suppress the hypothalamic pituitary-adrenal (HPA) axis.

**Route of elimination:** Primarily Urine

**Pharmacodynamics:** A vasoconstrictor assay in health subjects with UTRAVATE lotion indicated that the formulation is in the super-high range of potency as compared to other topical corticosteroids; however, similar blanching scores do not necessarily imply therapeutic equivalence. The potential for hypothalamic-pituitary adrenal (HPA) suppression was eluted in a study of 20 adult subjects with moderate to severe plaque psoriasis. A mean dose of 3.5 grams ULTRAVATE lotion was applied twice daily for two weeks and produced HPA axis suppression in 5 of 20 (25%) patients. In this study, the criteria for HPA-axis suppression were a serum cortisol level of less than or equal to 18 micrograms per deciliter 30 minutes after stimulation with cosyntropin (adrenocortropic hormone). These effects were reversible as recovery of HPA axis function was generally prompt with the discontinuation of treatment (see warning and precaution)

**Mechanism of action:** Corticosteroid play a role in cellular signaling, immune function, inflammation and protein regulation; however, the precise mechanism of action in plaque psoriasis is unknown.

**Drug Interaction:**

(Aldesleukin: Corticosteroid may diminish the antineoplastic effect of Aldesleukin. Avoid Combination)

**Precautions:**

- Adrenal suppression: May cause hypercortisolism or suppression of hypothalamic-pituitary adrenal (HPA) axis, particularly in younger children or in patients receiving high doses for prolonged periods. HPA axis suppression may lead to adrenal crisis.
- Contact dermatitis: Allergic contact dermatitis can occur and is usually diagnosed by failure to heal rather than clinical exacerbation. (Discontinue therapy if contact dermatitis develops.
- Kaposi sarcoma: Prolonged treatment with corticosteroids has been associated with the development of Kaposi sarcoma (case reports); if noted, discontinuation of therapy should be considered.
- Local effects: Local adverse reactions may occur (e.g. Skin atrophy, striae, telangiectasias, burning, itching, dryness, folliculitis, acneiform, eruptions, hypopigmentation, perioral dermatitis, allergic contact dermatitis, secondary infection miliaria); may be irreversible. Local adverse reactions are more likely to occur with use of higher potency corticosteroids, occlusive dressings, and prolonged use. If local adverse reaction develops, discontinue use and institute appropriate therapy until skin integrity is restored.
- Ocular effects: topical corticosteroids may increase the risk of posterior subcapsular cataracts and glaucoma. Monitor for ocular symptoms. Avoid contact with eyes.
- Skin infections: use appropriate antibacterial or antifungal agents to treat concomitant skin infections; discontinue treatment if infection dose not resolve promptly.

**Adverse Effect:**

- Headache
- Weakness
- Paresthesia
- Indigestion
- Acne vulgaris
- Local dryness of skin
- Fatigue
- Hyperglycemia
- Telangiectasia
- Herpes zoster infection

**Medical Use:**

It is used to a synthetic corticosteroid with anti-inflammatory, antipruritic and vasoconstrictor activities. Halobetasol, a topical steroid, diffused across cell membranes to interact with cytoplasmic corticosteroids receptors located in both the dermal and intradermal cells, thereby activating gene expressions of anti-inflammatory proteins mediated via corticosteroid receptor response element.

- **Materials and Instruments**

**List of Chemicals:** The following materials were used as supplied by the manufacturer.

**Table No.1: Materials Used and Their Sources**

| Sr. No. | Chemicals | Suppliers |
|---|---|---|
| 1 | Halobetasole Propionate Lotion 0.01% | Aurobindo Pharma Pvt. Ltd. |
| 2 | Acetonitrile | Merk Specialties Pvt. Ltd. |
| 3 | Methanol | Merk Specialties Pvt. Ltd. |
| 4 | Water for HPLC | Lichrosolv (Merck) |

**List of Equipment's:** Following equipment's were used in the present study

**Table No. 2: Equipment's used with Make and Model**

| Sr. No. | Equipment's | Make & Model |
|---|---|---|
| 1 | HPLC | (Agilent Technologies; 1260 Infinity) |
| 2 | Weighing Machine | Metrohm (20mg-200mg) |
| 3 | Volumetric Flask | Borosil |
| 4 | Pipettes | Borosil |
| 5 | Beakers | Borosil |
| 6 | 'Fast Clean'- Ultrasonic Cleaning System | Life Care Equipment's Pvt. Ltd. |

**Solution Preparation:**

**Mobile Phase (Acetonitrile: Methanol: Water (40:25:35v/v/v))**
Using a graduated cylinder, I measured and transferred 2000 mL of acetonitrile, 1250 mL of methanol, and 1750 mL of water separately. I then combined them in a 5000 mL bottle for the mobile phase and thoroughly mixed them. 10 minutes were spent degassing

**Diluent (Methanol: water::80:20 v/v)**
400 mL of water and 1600 mL of methanol were measured and transferred separately using a graduated cylinder, then combined in a suitable container and thoroughly mixed.

**Standard Solution Preparation**

**Halobetasol Propionate Standard Stock Solution Preparation (400 µg/mL)**

weighed 49.85 mg of halobetasol propionate standard material into a volumetric flask with a capacity of 100 mL. About 70 mL of diluent should be added and dissolved using Sonicator. combined thoroughly and diluted with diluent to volume.

**Halobetasol Propionate check Standard Stock Solution Preparation**
Weighed 51.08 mg of halobetasol propionate standard material into a volumetric flask with a capacity of 100 mL. About 70 mL of diluent should be added and dissolved using Sonicator. combined thoroughly and diluted with diluent to volume.

**Working Standard Preparation**
I used a glass pipette to transfer 2.0 mL of the halobetasol propionate standard stock solution into a 100.0 mL volumetric flask. combined thoroughly and diluted with diluent to volume.

**Check Standard Preparation**
I used a glass pipette to transfer 2.0 mL of the Halobetasol Propionate check Standard Stock solution into a 100.0 mL volumetric flask. combined thoroughly and diluted with diluent to volume.

**Precision Sample Preparation-1**

Halobetasol Propionate Lotion 0.01% sample was weighed at 2575.23 mg and placed in a 25 mL volumetric flask. The material was mixed with around 15 mL of diluent in a vortex for 2 minutes. At room temperature,

sonicated for ten minutes with brief shaking combined thoroughly and diluted with diluent to volume.

An aliquot of the sample was transferred to a 15 ml centrifuge tube, centrifuged at 10000 rpm for about 10 minutes, and then the supernatant solution was carefully transferred into a syringe and filtered through a 0.45 m PTFE+Prefilter by filtering out at least 1.0 mL of filtrate.

**Note:** Weighted sample and followed precision sample -1 sample preparation procedure for remaining sample preparation

**Preparation of Drug Solutions for Linearity and Recovery:**

**Standard Solution Preparation**

**Halobetasol Propionate Standard Stock Solution Preparation (400 µg/mL)**

Weighed 49.85 mg of halobetasol propionate standard material into a volumetric flask with a capacity of 100 mL. About 70 mL of diluent should be added and dissolved using a Sonicator combined thoroughly and diluted with diluent to volume.

**Halobetasol Propionate check Standard Stock Solution Preparation**

A 100-mL volumetric flask was filled with a weight of around 50.80 mg of halobetasol propionate standard material. About 70 mL of diluent should be added and dissolved using a Sonicator. combined thoroughly and diluted with diluent to volume.

**Working Standard Preparation**

Pipetted out 2.0 mL of Halobetasol Propionate Standard Stock solution using glass pipette into 100.0 mL volumetric flask. Diluted with diluent and mixed well.

**Check Standard Preparation**

I used a glass pipette to transfer 2.0 mL of the Halobetasol Propionate check Standard Stock solution into a 100.0 mL volumetric flask. mixed well and diluted to volume with diluent

**Recovery Stock Preparation**

In a 100 mL volumetric flask, 49.72 mg of the working standard for halobetasol propionate were weighed. Diluent was added, and it was sonicated to dissolve, adding about 80 ml well-mixed after being diluted with diluent to volume.

**Recovery Spiking Stock Preparation and Linearity Stock**

Used a glass pipette to transfer 10.0 mL of the Recovery Stock solution into a 100.0 mL volumetric flask. combined thoroughly and diluted with diluent to volume.

**Prototype Preparation**

**Recovery 50% Preparation-1**

Weighed 2564.57 mg of placebo into 25 mL volumetric flask. Added approximately 15 ml of diluent vortex for 2 minutes to disperse the sample in diluent and added 2.5 ml of recovery spiking stock solution into the flask. Sonicated for 10 minutes with intermittent shaking at room temperature. Diluted to volume with diluent mixed well. Transferred an aliquot of the sample to a 15 ml centrifuge tube and centrifuge the sample at approximated 10000 RPM for 10 minutes at 5ºC and carefully transferred the supernatant solution into a syringe and filtered through 0.45µm PTFE+Prefilter by filter discarding minimum 1.0 mL of filtrate.

- **VALIDATION METHOD**

The HPLC process has been validate under ICH guidelines.

**Table No. 3- Chromatographic Condition**

| | |
|---|---|
| HPLC ID | SPA-HPLC-006 |
| Mobile Phase | Acetonitrile: Methanol: Water (40:25:35 v/v/v) |
| Flow Rate | 1.0 ml/min. |
| Column Description | Inertsil Sustain Swift ODS 3V 250×4.6 mm, 5µ (C18) |
| Injection Volume | 10.0 µL |
| Column Temperature | 25ºC |
| Run Time | 20 min. |
| Wavelength | 240 nm |
| Diluent | Methanol: Water (80:20) |

**1. Specificity**

**Blank**

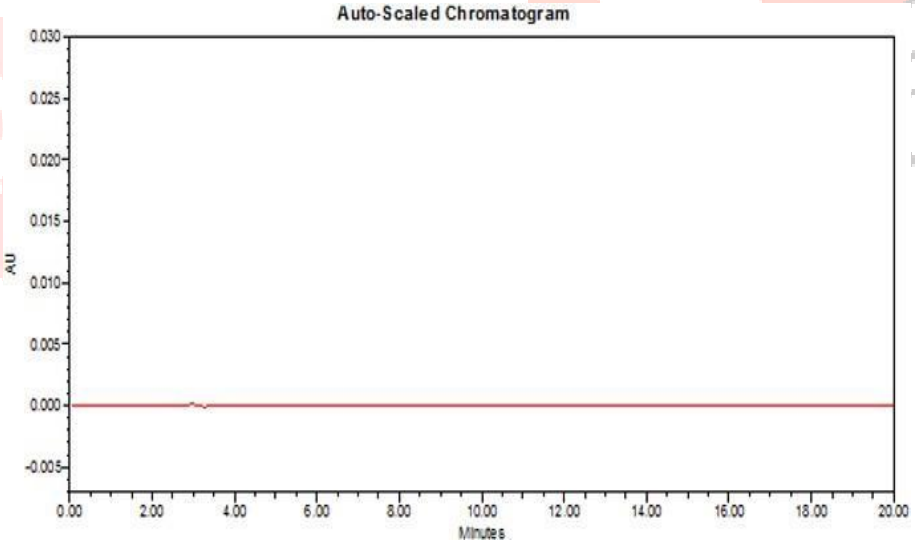


**Fig. No. 2- Chromatogram of Blank**

**Sample**

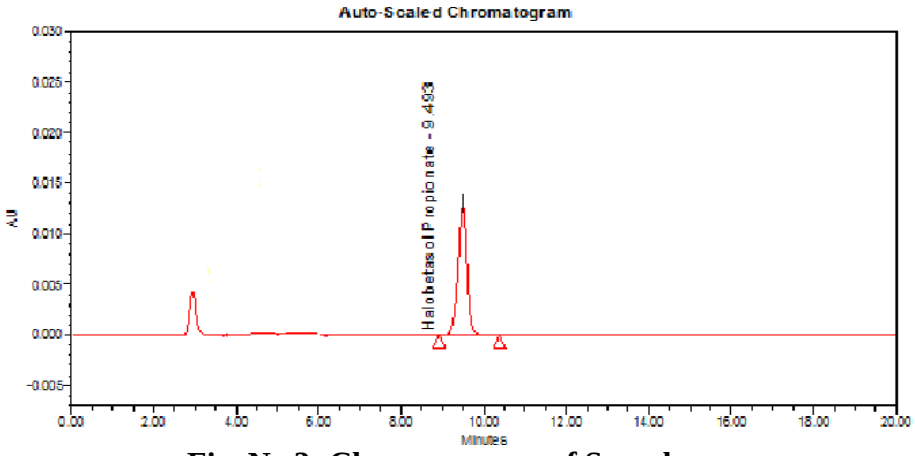


**Fig. No.3- Chromatogram of Sample**

**2. Accuracy:**

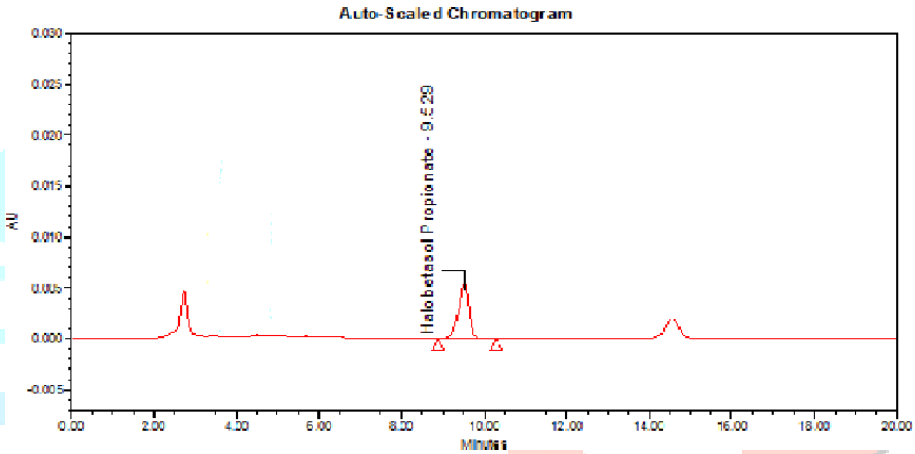


**Fig. No. 4- Chromatogram of Accuracy 50%**

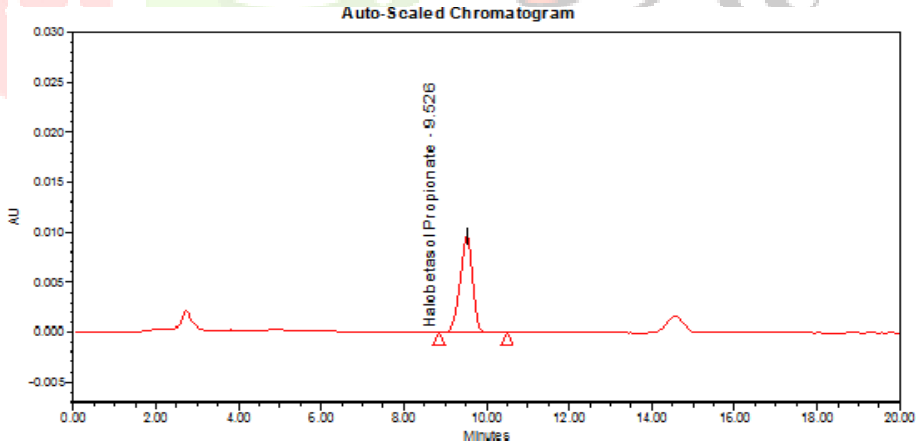


**Fig. No.5- Chromatogram of Accuracy 100%**

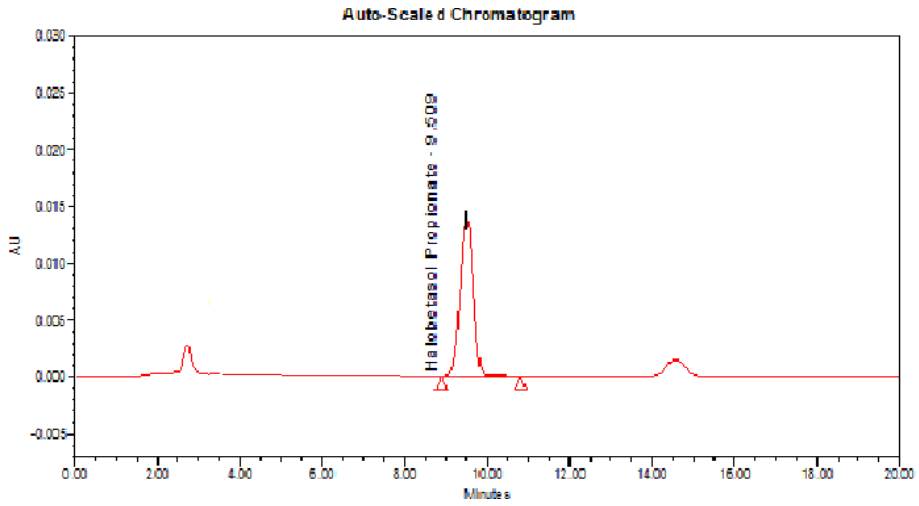


**Fig. No. 6- Chromatogram of Accuracy 150%**

**Table No. 4- Data of Accuracy**

| % Level | Inj No. | Area | Amount Added (µg/ml) | Amount Recovered (µg/ml) | % Recovery | Average | STDV | % RSD |
|---|---|---|---|---|---|---|---|---|
| 50% | 1 | 100271 | 4.9601 | 4.9971 | 100.746 | 100.4 | 0.532 | 0.53 |
| | 2 | 99261 | | 4.9477 | 99.750 | | | |
| | 3 | 100132 | | 4.9901 | 100.605 | | | |
| 100% | 1 | 202762 | 9.9201 | 10.1047 | 101.861 | 101.1 | 0.064 | 0.06 |
| | 2 | 200514 | | 9.9927 | 100.732 | | | |
| | 3 | 200665 | | 10.0002 | 100.807 | | | |
| 150% | 1 | 301153 | 14.8802 | 15.0081 | 100.860 | 100.5 | 0.336 | 0.33 |
| | 2 | 300095 | | 14.9554 | 100.505 | | | |
| | 3 | 299145 | | 14.9080 | 100.187 | | | |

### 3. Method Precision:

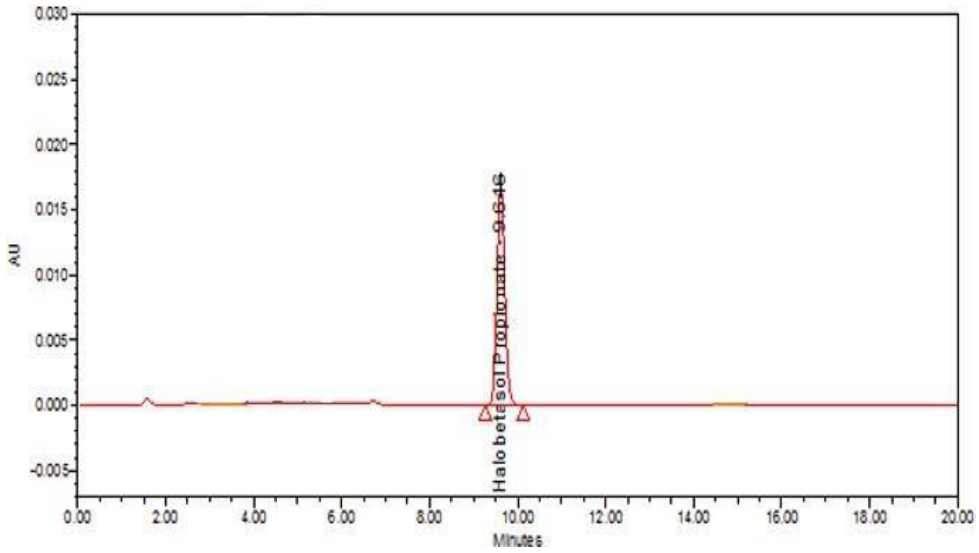


**Fig. No. 7- Chromatogram of Sample 1**

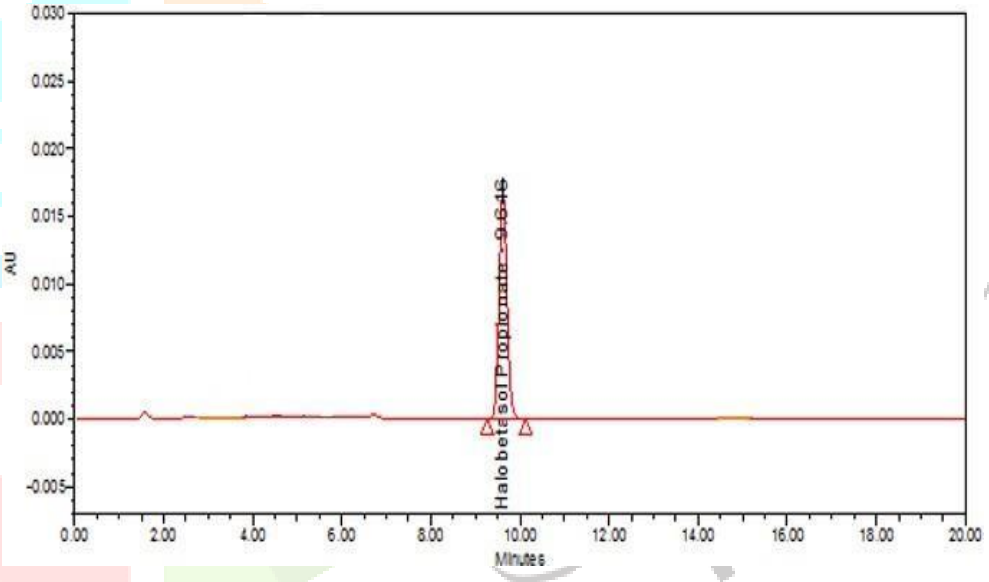


**Fig. No. 8- Chromatogram of Sample 2**

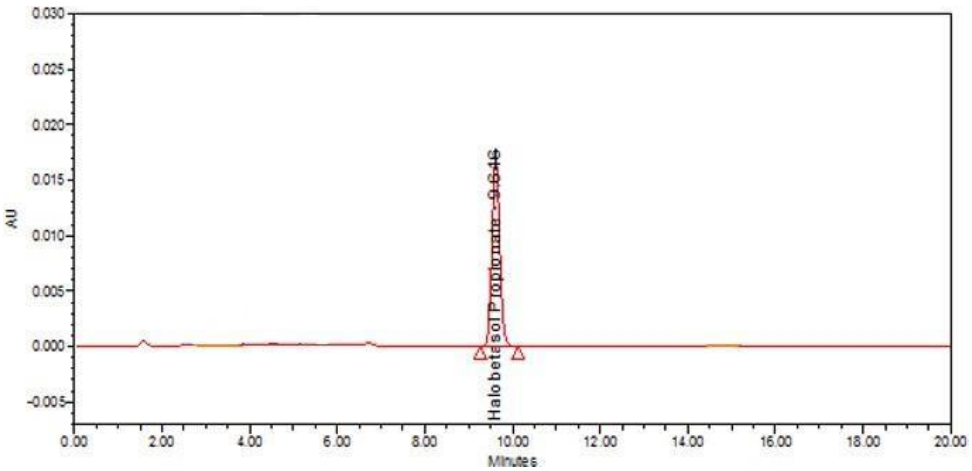


**Fig. No.9- Chromatogram of Sample 3**

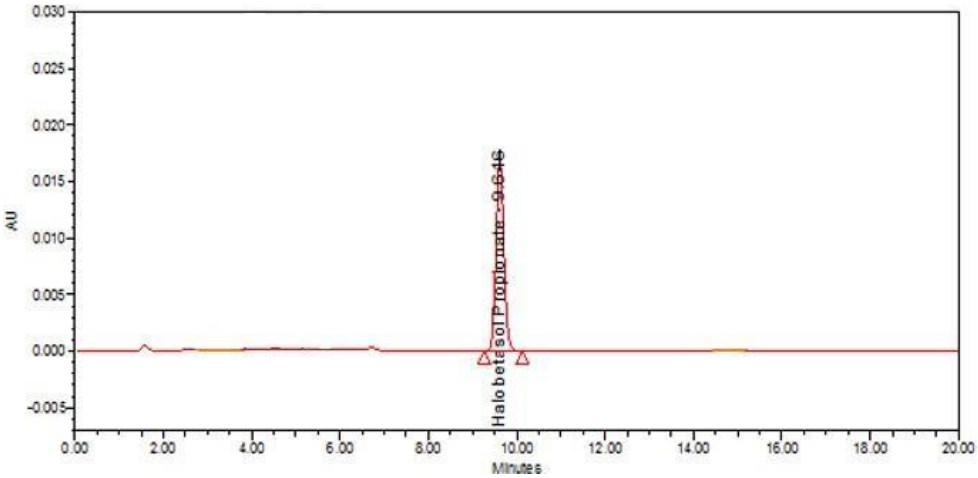


**Fig. No. 10- Chromatogram of Sample 4**

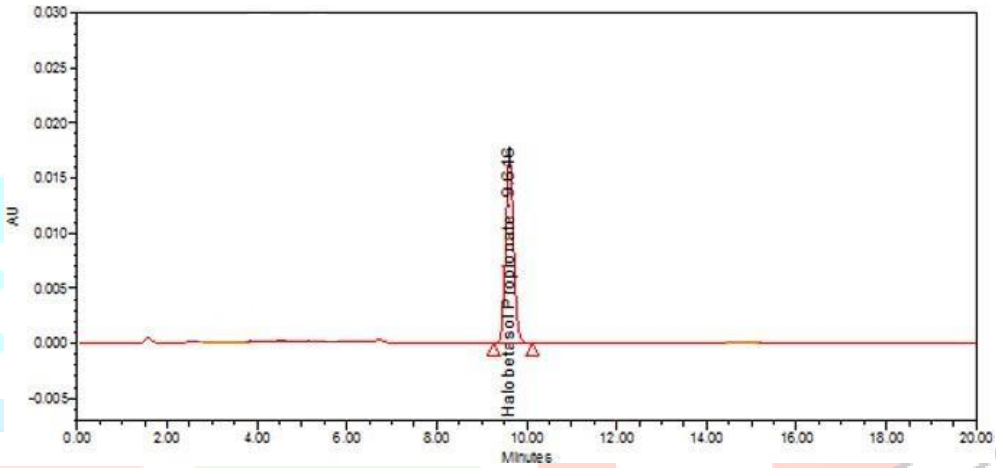


**Fig. No. 11- Chromatogram of Sample 5**

**Table No. 5- Data of Method Precision**

| Sample | RT | Area | Average | % Assay | Average | Std Deviation | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | 9.616 | 212183 | 208055.3 | 102.5 | 101.4 | 2734.05 | 1.31 |
| 2 | | 206627 | | 101.6 | | | |
| 3 | | 205021 | | 101.5 | | | |
| 4 | | 206214 | | 100.0 | | | |
| 5 | | 208165 | | 101.1 | | | |

**4. Linearity:**

**Table No.6- Data of Linearity**

| % Level | Conc. (µg/ml) | RT | Area |
|---|---|---|---|
| 80 | 7.9361 | 9.483 | 158894 |
| 90 | 8.9281 | 9.483 | 177139 |
| 100 | 9.9201 | 9.493 | 197318 |
| 110 | 10.9122 | 9.495 | 216955 |
| 120 | 11.9042 | 9.487 | 237783 |

**Fig. No.12- Linearity Graph**

**5. LOD & LOQ**

$$\text{LOD} = \frac{3.3 \times \text{Standard Derivative of the response}}{\text{Slope of the calibration Curve}}$$

$$= \frac{3.3 \times 791.0947}{19930}$$

$$= 0.13\ \mu g/ml$$

$$\text{LOQ} = \frac{10 \times \text{Standard deviation of the response}}{\text{Slope of the calibration Curve}}$$

$$= \frac{10 \times 791.0947}{19930}$$

$$= 3.969\ \mu g/ml$$

- **CONCLUSION:**

✓ High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The determination of Halobetasol Propionate was done by RP-HPLC.

✓ A Inertsil Sustain swift ODS 3V 250× 4.6 mm, 5µ column particle size or equivalent chemically bonded to porous silica particles was used as stationary phase. The mobile phase composition is Acetonitrile: Methanol: Water (40: 25: 35: v/v/v). The solutions were chromatographed at a constant flow rate of 1.0 ml/min.

✓ The linear regression coefficient was found to be 0.999. The value of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Halobetasol Propionate. The value of LOD and LOQ was 0.13 µg/ml and 3.969 µg/ml respectively.

✓ The results obtained on the validation parameters met ICH requirements.

✓ Hence the partially validated method is Specific, Linear, Precise and Accurate for the determination of Halobetasol Propionate content in Halobetasol Propionate Lotion 0.01%.

✓ The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision

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