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# VALIDATION OF STABILITY INDICATING HPLC METHOD FOR ASSAY OF FUSIDIC ACID, BETAMETHASONE-17 VALERATE AND CHLOROCRESOL CONTENT IN TOPICAL PHARMACEUTICAL FORMULATION

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**ABSTRACT**

A new stability indicating reversed phase HPLC method was developed and validated for assay of fusidic acid, betamethasone -17 valerate and Chlorocresol in Topical pharmaceutical formulation. The chromatographic separation was achieved on Lichrospher RP-18 125 mm x 4.0mm, 5 µm column ,Using a mobile phase comprising of mixture of Acetonitrile, Methanol and 0.05M Ortho-Phosphoric acid in the ratio of 50:5:45 (v/v), at a flow rate of 1.5 ml/min. Injection volume 10 µL, The column temperature was set at 25°C. The detection was carried out at 235 nm. The precision of the method observed in RSD is 0.5 %, 0.7 % and 0.4 %, The Overall RSD of Method Precision and Intermediate Precision are 0.9 %, 1.7 % and 2.3 % , Individual % recovery values observed in the range of 96.1 % to 101.6 %, 96.9 % to 103.9 % and 91.8 % to 99.4 % for Fusidic acid, Betamethasone-17 valerate and Chlorocresol respectively. Sample solution is observed to be stable at least 36 hours at room temperature. The method is found to be robust under the following variable conditions like flow + 10%, column oven temperature + 5°C, organic content in mobile phase + 2%, and wave length + 5 nm. The linearity of response was determined in the range of 289.96 µg/mL to 942.38 µg/mL for Fusidic acid, 19.01 µg/mL to 61.77 µg/mL for Betamethasone-17 valerate and 15.95 µg/mL to 51.85 µg/mL for Chlorocresol, the Correlation coefficient is 0.99886, 0.99898 and 0.99884 respectively. Significant degradation was observed during the Forced degradation studies, in drug product and placebo were exposed to 40°C / 15 minute in hydrolysis Acid, Alkali 1 N NaOH , Peroxide 30 %w/w of Hydrogen peroxide and thermal (105°C / 24 hours 7 minutes) ,photolytic degradation ( 321929 Lux hours & 97.02 Watt hours / sq. m at 25 °C).

**Keywords**: Fusidic acid, Betamethasone -17 valerate, Chlorocresol, Forced degradation, HPLC, Method validation.

## INTRODUCTION

The present study was aimed at developing a simple, specific, accurate, and precise HPLC method in topical formulation in commercially available and for use in stability studies and quality control applications. The HPLC method for assay of Fusidic acid, Betamethasone-17 valerate and Chlorocresol content has been validated to show specificity, linearity, precision, intermediate precision/ruggedness, accuracy, stability in analytical solution and robustness. Fucibet cream contains two active pharmaceutical ingredients (API), fusidic acid and betamethasone 17- valerate. Chlorocresol used as preservative.

Fusidic acid is an antibiotic medicine used to treat infections with bacteria. It works by entering bacterial cells and interfering with the production of proteins that the bacteria need to divide and multiply. It doesn't directly kill the bacteria, but leaves them unable to increase in numbers. The bacteria eventually die or are destroyed by the immune system. Fusidic acid is included in this preparation to treat the bacterial infection that can sometimes occur in eczema.

Betamethasone is a type of medicine called a topical corticosteroid. Corticosteroids are medicines used for reducing inflammation. Skin inflammation in eczema

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occurs due to the irritation of the skin, and is caused by the release of various substances involved in the immune system. These substances cause blood vessels to widen, resulting in the affected area becoming red, swollen, itchy and painful. When betamethasone is applied to the skin it works by acting inside the cells to decrease the release of these inflammatory substances. This reduces swelling, redness and itch. Betamethasone is a potent corticosteroid [1-4]. The combination of betamethasone and fusidic acid is used to treat eczema that is infected with bacteria. The betamethasone reduces the inflammation in the skin while the fusidic acid treats the infection [1-4].

Chlorocresol is a bactericide, closely related to carbolic acid. It is used as a preservative in many pharmaceutical formulations such as creams and ointments. Preservative systems are crucial parts of any cream or ointment formulations. Other preservatives such as propyl paraben and methyl paraben have been used for years in Cream and ointment formulations. The formulator must be fully aware of the normal procedure for preservative systems in a suitable product, which need to be estimated to establish their effectiveness throughout the shelf-life of the product. Knowing the actual concentration of preservative(s) in different formulations is vital in establishing the shelf-life of a product. Apart from this, regulatory agencies often ask for the analytical test result of preservative(s) [5]. Chemical Name and Structure is given in Table 1 [6,7].

## MATERIALS AND METHODS

### Chemical Reagents

Acetonitrile (HPLC grade), Methanol (HPLC grade), Acetic Acid (HPLC grade) Water (HPLC grade), Ortho-Phosphoric acid (85% AR grade), n-Heptane (HPLC grade)

### Instrumentation

Waters 2489 UV/Visible Detector, Waters 2998 UV/Visible Detector, Waters e2695, Separations Module (Alliance), Empower Software version 3, Analytical Balance (LC GC – XA82/220/2X), HPLC Column: Lichrospher RP-18, 5 µm (125 x 4) mm.

### Liquid Chromatography

The chromatographic separation was achieved using Lichrospher RP-18 5 µm (125 x 4) mm column at isocratic mode. The mobile phase consists of a mixture of Acetonitrile, Methanol and 0.05M Ortho-Phosphoric acid in the ratio of 50:5:45 (v/v) and degas by sonication. The flow rate and column temperature was maintained as 1.5 mL $min^{-1}$ and 25ºC respectively throughout the analysis. The injection volume was maintained as 10µL and the detection was carried out at 235 nm.

### Standard and Sample preparation

### Preparation of 1M *Ortho*-Phosphoric acid

Add slowly under swirling 70 mL of 85 per cent *Ortho*-Phosphoric acid to 1000 mL of water in a 2 L flask and mix.

### Preparation of 0.05M *Ortho*-Phosphoric acid

Dilute 50 mL of 1 M *Ortho*-Phosphoric acid in sufficient water to make 100 mL and mix.

### Preparation of Mobile Phase

Prepare a suitable quantity of a mixture of Acetonitrile, Methanol and 0.05M *Ortho*-Phosphoric acid in the ratio of 50:5:45 (v/v) and degas by sonication.

### Preparation of Internal standard Solution

Accurately weigh and transfer about 100 mg of Medroxyprogestrone acetate in a 100 mL volumetric flask. Add 50 mL of Mobile phase, Sonicate to dissolve and make up the volume with Mobile phase and mix to obtain a concentration of 1 mg/mL of Medroxyprogestrone acetate.

### Preparation of standard stock solution A.

Accurately weigh and transfer 33 mg of Chlorocresol working standard and 40 mg of Betamethasone-17 valerate reference substance in a 50 mL volumetric flask. Add 25 mL of Mobile phase, sonicate to dissolve and make up the volume with Mobile phaseand mix.

### Preparation of standard stock solution B

Accurately weigh and transfer 44 mg of Diethanolamine fusidate reference substance in a 50 mL volumetric flask. Add 3 mL of standard stock solution-A and 5 mL internal standard solution and make up the volume with diluent and mix.

### Preparation of standard solution

Mix 10 mL of standard stock solution-B with 3.3 mL of 0.05 M *Ortho*-Phosphoric acid. Filter through 0.45 µm nylon filter.

### Preparation of sample solution

Transfer accurately 2 g of cream, to a 100 mL volumetric flask. Add 5 mL of internal standard solution and 35 mL of Mobile phase. Heat this mixture, until the sample melts. Then add 10 mL of n-heptane and shake vigorously for 15 min. Cool to room temperature and separate the two phase in a separator funnel. Extract the upper layer with another 15 mL of the Mobile phase. Allow the phases to separate. Collect the two lower phase and mix them. Mix 10 mL of this solution with 3.3 mL of 0.05 M *Ortho*-Phosphoric acid. Filter through 0.45 µm nylon filter.

## RESULTS AND DISCUSSION

### Method Development

Thechromatographic separation was achieved

with Lichrospher RP 18 (125 mm x 4 mm x 5 µm) column using the mixture of Acetonitrile, Methanol and 0.05M Ortho-Phosphoric acid as a mobile phase. To specify the LC conditions, different volume fractions were tested and the optimum conditions were obtained using Acetonitrile, Methanol and 0.05M Ortho-Phosphoric acid in the ratio of 50:5:45 (v/v) at the flow rate of 1.5 mL/ min . Throughout the process, 10 µL was maintained as volume of injection and detection was performed at 235 nm with simple UV/Visible detector.

**Method Validation**

This purpose of method validation was to demonstrate the suitability for routine application of the developed methodology in accordance with the ICH guidelines. Method validation was treated as a final verification of the method performance. An important part is system suitability test which covers in the detail number of theoretical plates, peak asymmetry, resolution of individual components and repeatability of injection that was evaluated by retention time and peak area [8-27].

**Specificity**

Placebo solutions (prepared similarly as the sample solution using equivalent amount of placebo) and sample solution were analysed as per the method and the peak purity of Fusidic acid, Betamethasone-17 valerate,Medroxyprogestrone acetate and Chlorocresol peaks were checked. The purity angle and threshold are given in Table 2. The chromatograms indicate that there is no interference from the Excipients with Fusidic acid, Betamethasone-17 valerate and Chlorocresol peaks. The peak purity plots of Fusidic acid, Betamethasone-17 valerate,Medroxyprogestrone acetate and Chlorocresol peaks indicate as purity angle is less than threshold that the peaks are homogeneous and have no co eluting peaks indicating specificity of the method (Fig. 1).

**Degradation studies**

A force degradation study was carried out on Fucibet Cream and common placebo under the following conditions:

**Hydrolytic and oxidative degradation**

Sample and placebo were separately treated with 5 N hydrochloric acid, 1 N sodium hydroxide and 30 % w/w hydrogen peroxide solutions. Solutions of these samples were prepared as per the conditions mentioned in Table 3 and further analysed by the proposed method.

**Thermal degradation**

Sample and placebo were subjected to thermal degradation by keeping at 105°C for 24 hours 7 minutes, followed by analysis by the proposed method.

**Photolytic degradation**

Photolytic degradation study was carried out by exposing the sample and placebo to light at 321929 Lux hours & 97.02 Watt hours / sq. m., followed by analysis by the proposed method.

Using peak purity test, the purity of Fusidic acid, Betamethasone-17 valerate and Chlorocresol peaks were checked at every stage of the degradation study (Fig.2-6). The peak purity plots show that the Fusidic acid, Betamethasone-17 valerate and Chlorocresol peaks were homogeneous and has no co eluting peaks indicating as purity angle is less than threshold that the method is stability indicating and specific. Data is summarized in Table 3.

**Linearity of Response**

The linearity of response for Fusidic acid, Betamethasone-17 valerate, Chlorocresol and was determined in the range of 289.96 µg/mL to 942.38 µg/mL for Fusidic acid, 19.01 µg/mL to 61.77 µg/mL for Betamethasone-17 valerate and 15.95 µg/mL to 51.85 µg/mL for Chlorocresol. Data shown in Table 4 and represented graphically in (Fig. 7-9) indicate that the response is linear over the specified range.

**Precision**

**System Precision**

Six replicate injections of standard solution were given into the HPLC system. Data shown in Table 5 indicate an acceptable level of precision for the analytical system.

**Method Precision**

Six samples of a single batch of Fucibet cream were prepared and analysed as per the proposed method. Data is shown in Table 5. The % RSD values indicate that the method has an acceptable level of precision.

**Intermediate Precision/Ruggedness**

Ruggedness of the method was verified by analyzing six samples of a single batch of Fucibet cream by two different analysts using different instrument and columns on different days. The mean, standard deviation and % RSD for the two sets of data are shown in Table 5. Ruggedness of the method is indicated by % RSD & overall % RSD values between the two sets of data.

**Accuracy**

Known amount of common placebo for Fucibet cream was taken and spiked with known amount of Fusidic acid API, Betamethasone-17 valerate API and Chlorocresol at three different levels, each in triplicate. The solutions were prepared and analysed by the proposed method. Data shown in Table 6 indicate that the method has an acceptable level of accuracy.

**Stability in Analytical Solution**

A fresh sample solution of Fucibet cream was prepared and kept at room temperature. The solution was analysed initially and at different time intervals. The absolute % difference up to 2217 min. meets the acceptance criterion it is concluded that the sample solution is stable in analytical solution for atleast 36 hrs at room temperature.

**Robustness**

Robustness of the method was checked by the system suitability parameters by deliberately varying the instrumental conditions such as flow rate (± 10 %), Methanol content in Mobile phase (± 2 % absolute), Acetonitrile content in Mobile phase (± 2 % absolute), column oven temperature (± 5°C), and wavelength of detection (± 5 nm). Data is presented in Table 7.

Sample solutions were analyzed under each condition and assay (mg/g) of Fusidic acid, Betamethasone-17 valerate and Chlorocresol calculated. The mean, standard deviation and % RSD for each set of data are shown in Table 7 Robustness of method is indicated by the % RSD and overall % RSD values between the data of control and data of at each variable condition.

Under the condition of change in Acetonitrile and Methanol content variation and (keeping buffer content same in mobile phase) by +2% absolute was non robust. Under the condition of change in Acetonitrile content in mobile phase by +2% absolute significant changes in retention time of Betamethasone-17 valerate, Medroxyprogestrone and Fusidic acidpeaks were observed. The condition of change in Methanol content in mobile phase by +2% absolute and flow rate -10% significant changes in retention time of Medroxyprogestrone peak were observed. Hence, it is recommended that the Acetonitrile content, Methanol content in mobile phase and flow rate should be strictly adhered to as per method.

**Fig 1. Specificity**

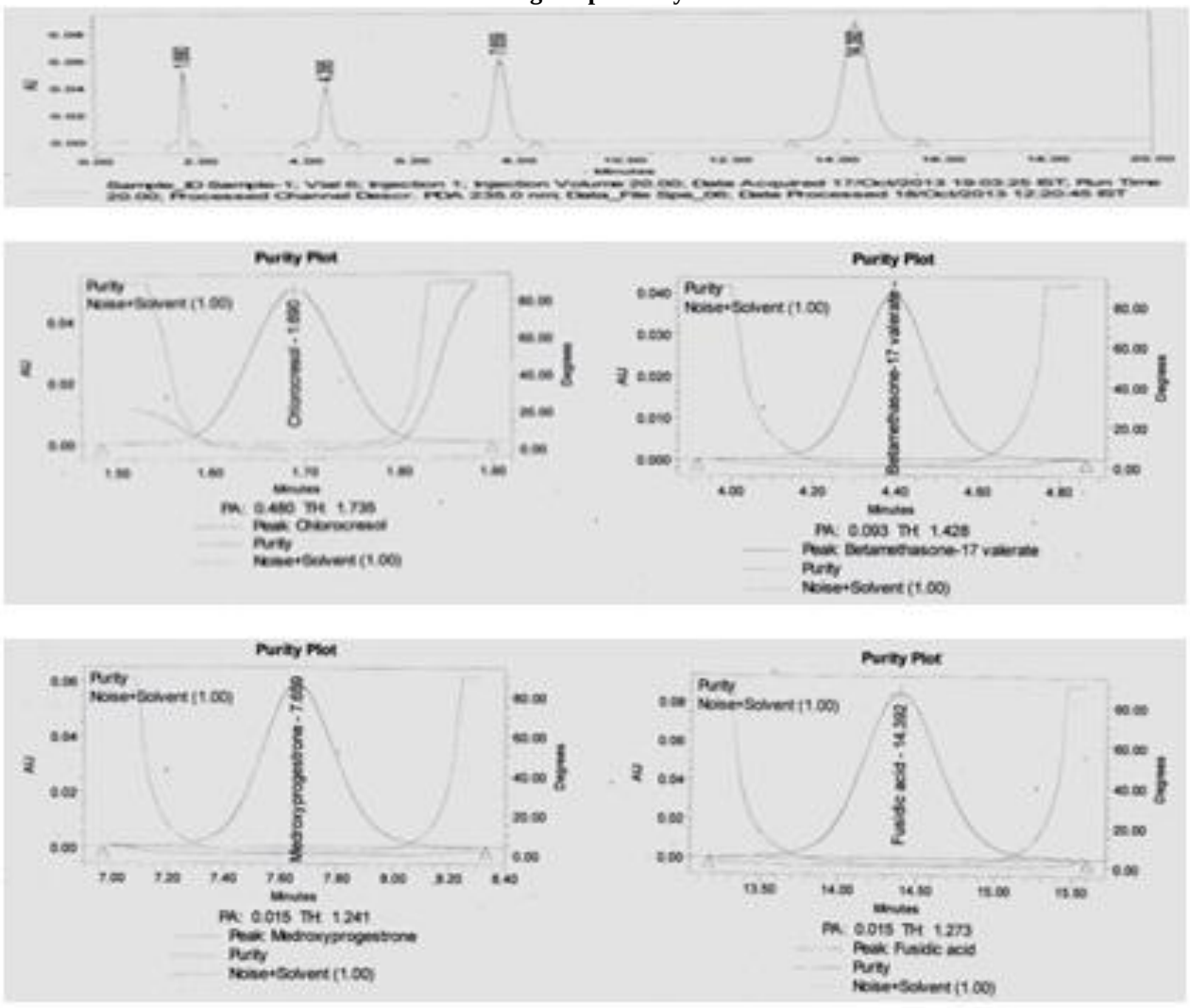


**Fig 2. Chromatogram of Acid degradation Sample**

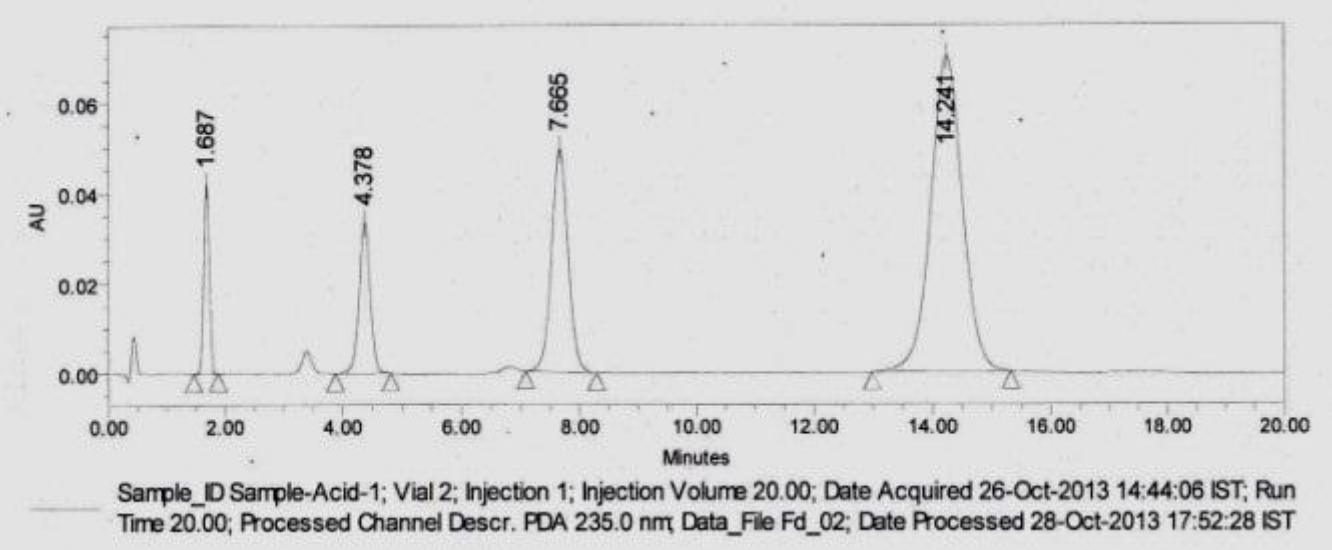



**Fig 3. Chromatogram of Alkali degradation Sample**

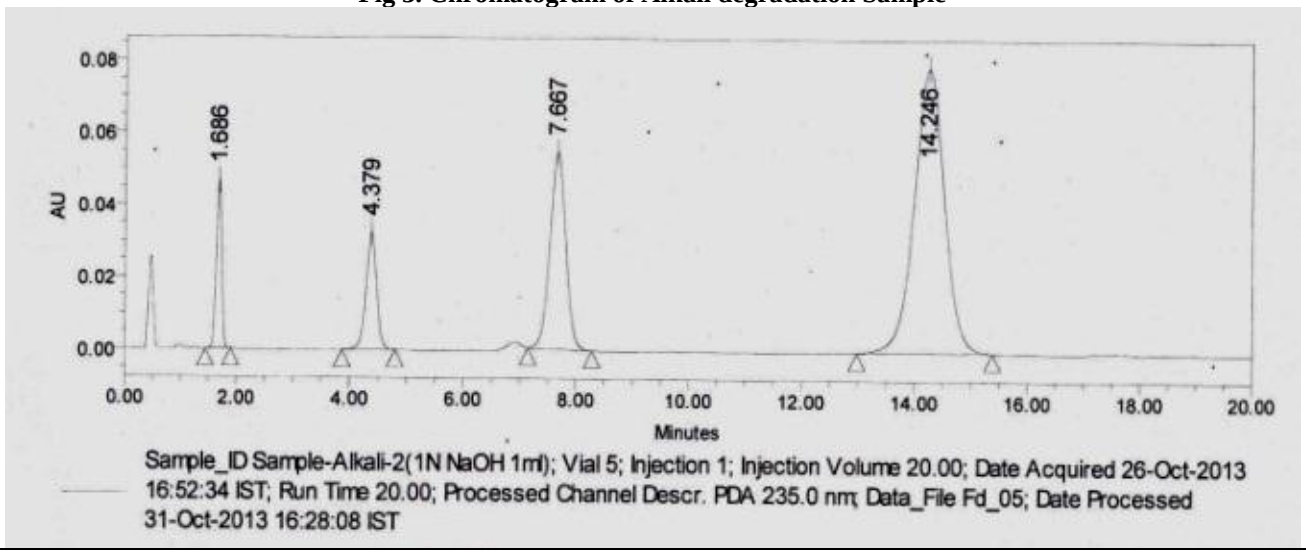



**Fig 4. Chromatogram of Peroxide degradation Sample**

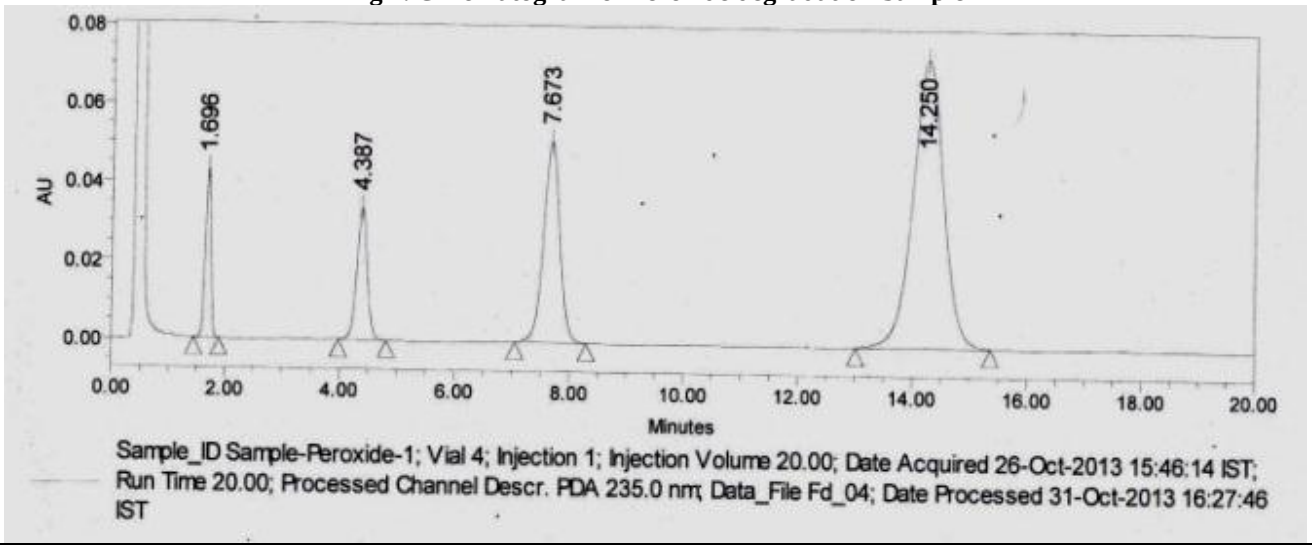


**Fig 5. Chromatogram of Photolytic degradation Sample**

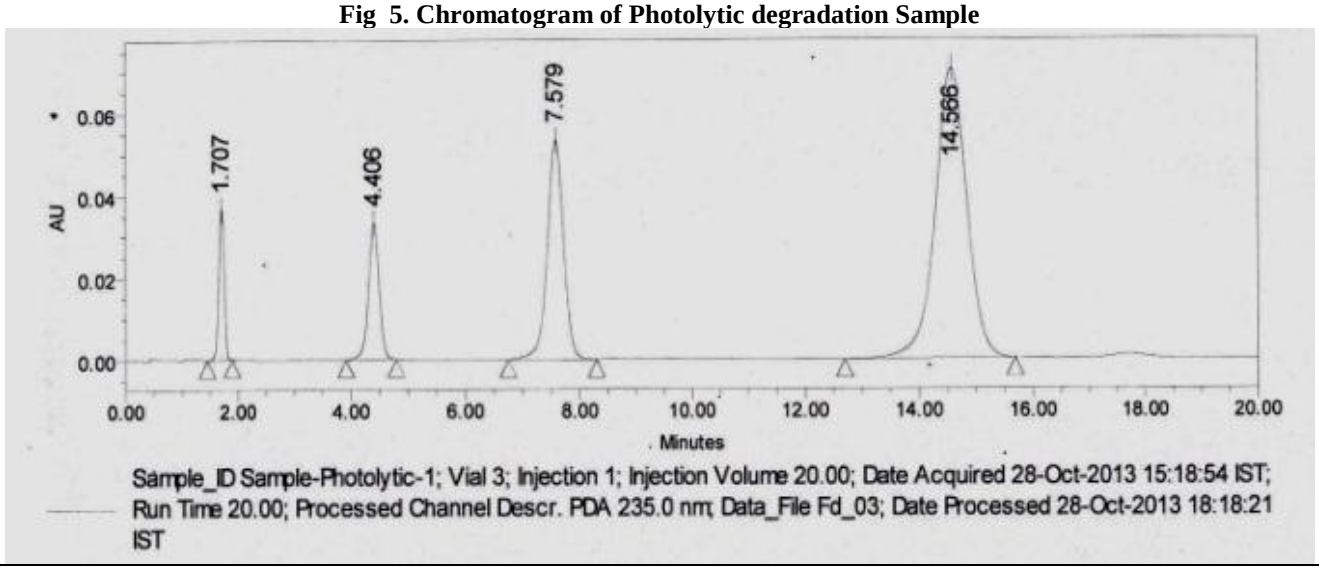



**Fig 6. Chromatogram of Thermal degradation Sample**

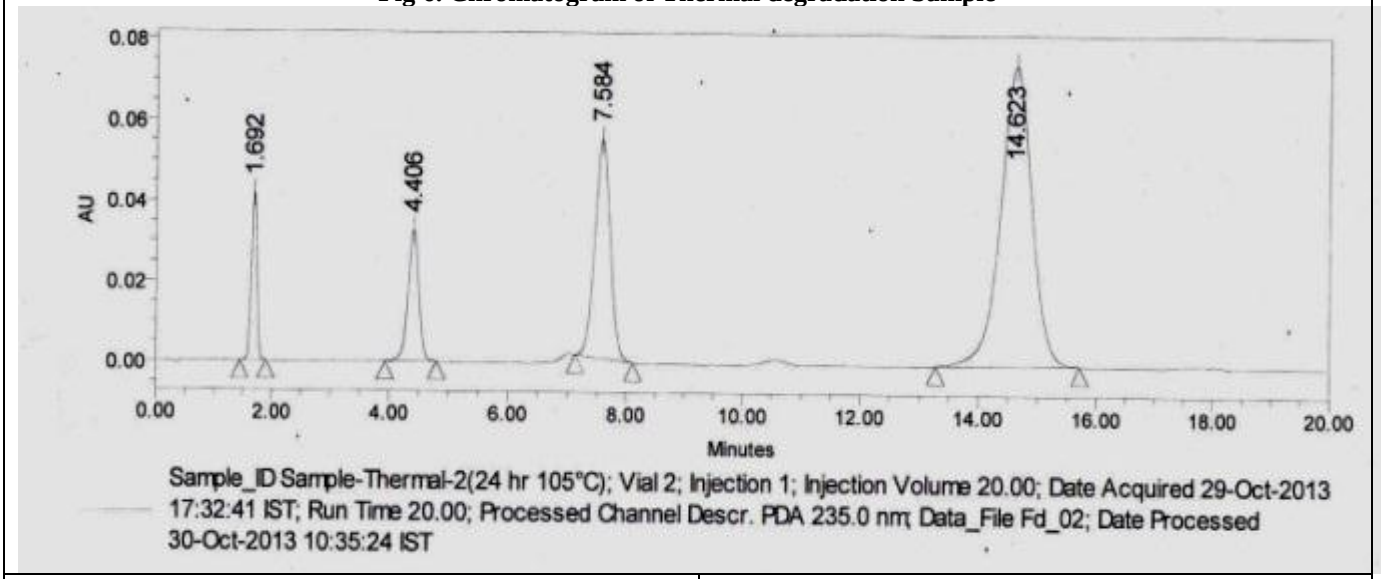



**Fig 7. Linearity plot of Fusidic acid**

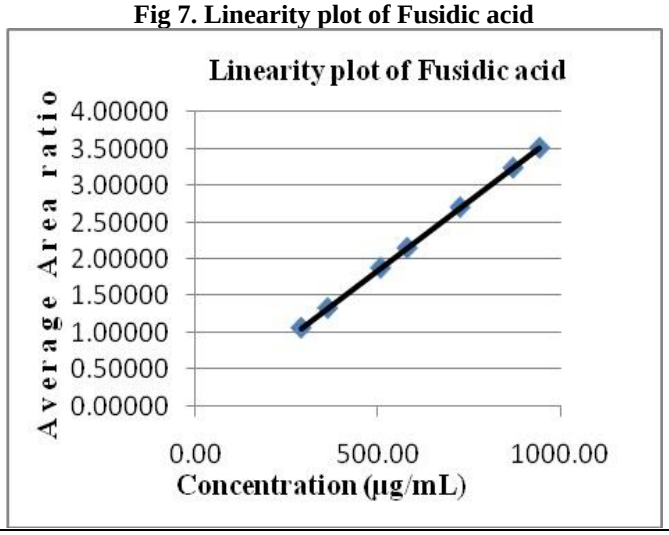



**Fig 8. Linearity plots of Betamethasone-17 valerate**

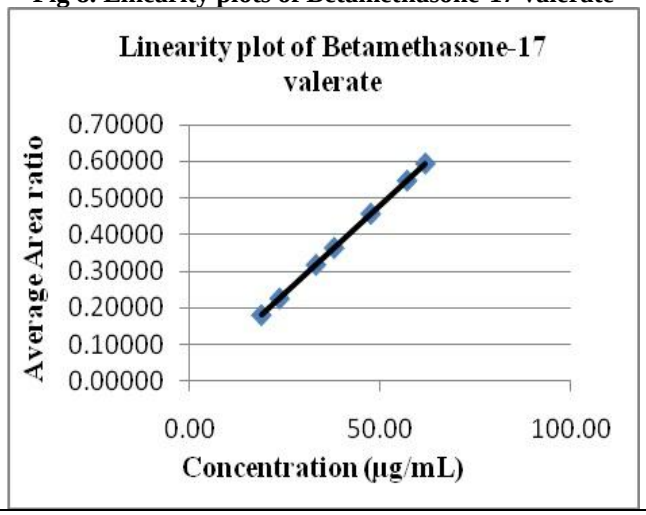


**Fig 9. Linearity plots of Chlorocresol**

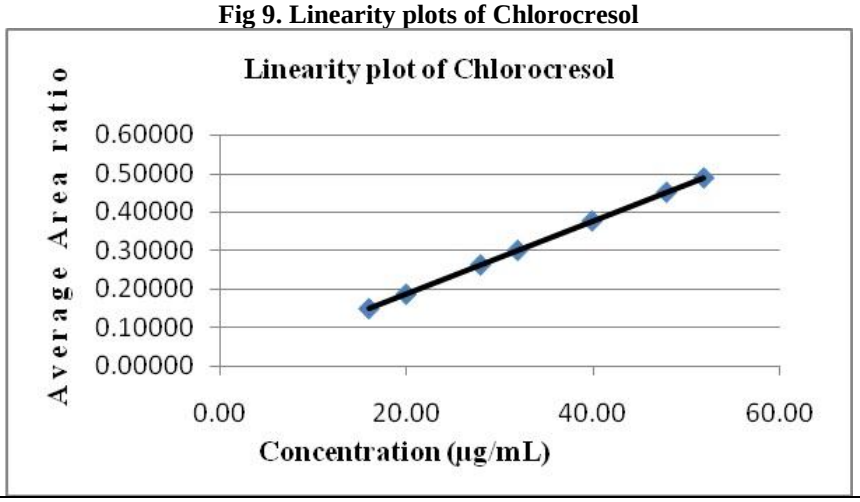



**Table 1.Chemical structure of Fusidic acid, Betamethasone -17 valerate and Chlorocresol**

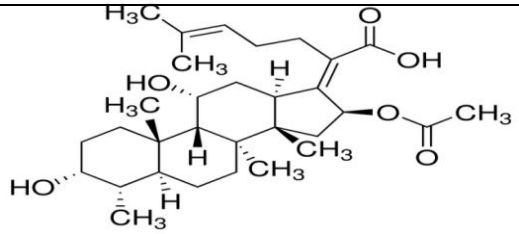
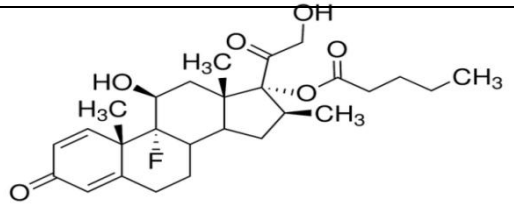
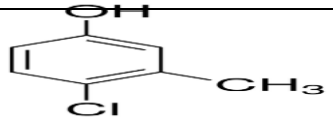
| Name | Chemical Name | Chemical Structure |
|---|---|---|
| Fusidic acid | (2Z)-2-[(3R,4S,5S,8S,9S,10S,11R,13R,14S,16S)-16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-17-ylidene]-6-methylhept-5-enoic acid. |  |
| Betamethasone -17 valerate | [(8S,9R,10S,11S,13S,14S,16S,17R)-9-fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] pentanoate. |  |
| Chlorocresol. | 4-chloro-3-methylphenol |  |

**Table 2. Specificity**

| Name of the sample | Purity angle | Threshold |
|---|---|---|
| Fusidic acid | 0.015 | 1.273 |
| Betamethasone-17 valerate | 0.093 | 1.428 |
| Chlorocresol | 0.480 | 1.735 |
| Medroxyprogestrone acetate | 0.051 | 1.241 |

**Table 3.Summary of Forced Degradation Results**

| Mode of degradation | | Acid | Alkali | Peroxide | Thermal | Photolytic |
|---|---|---|---|---|---|---|
| Fusidic acid | % Degradation | 4 | 1 | 3 | Nil | 3 |
| | Purity Angle | 0.02 | 0.02 | 0.013 | 0.023 | 0.019 |
| | Threshold | 1.15 | 1.13 | 1.772 | 1.057 | 1.731 |
| Betamethasone-17 valerate | % Degradation | Nil | 5 | Nil | 3 | Nil |
| | Purity | 0.13 | 0.06 | 0.034 | 0.057 | 0.039 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Angle | | | | | |
| | Threshold | 1.26 | 1.38 | 2.527 | 1.148 | 2.479 |
| Chlorocresol | % Degradation | Nil | Nil | Nil | 2 | 16 |
| | Purity | 0.23 | 0.13 | 0.986 | 0.13 | 0.304 |
| | Angle | | | | | |
| | Threshold | 1.37 | 1.17 | 2.826 | 1.08 | 2.159 |

**Table 4. Summary of linearity Data**

| Parameter | Fusidic acid | Betamethasone-17 valerate | Chlorocresol |
|---|---|---|---|
| Slope | 12929 | 14608 | 4814 |
| Intercept | -822 | 3742 | -3139 |
| Correlation Coefficient | 0.99886 | 0.99898 | 0.99884 |

**Table 5. System Precision, Method precision and Intermediate Precision Results**

| Substance | System Precision | | | Method precision | | | Intermediate Precision | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | %RSD | Mean | SD | %RSD | Mean | SD | %RSD |
| Fusidic acid | 2.4404 | 0.0021 | 0.1 | 20.3 | 0.27 | 1.3 | 20.2 | 0.18 | 0.9 |
| Betamethasone-17 valerate | 0.4291 | 0.0002 | 0.0 | 1.15 | 0.005 | 0.4 | 1.13 | 0.02 | 1.7 |
| Chlorocresol | 0.2799 | 0.0001 | 0.0 | 1.02 | 0.006 | 0.6 | 0.99 | 0.02 | 2.3 |

**Table 6. Summary of Recovery Results**

| Amount Spiked | % Recovery | | |
|---|---|---|---|
| | Fusidic acid | Betamethasone-17 valerate | Chlorocresol |
| 80% | 99.5 | 99.8 | 97.6 |
| 100% | 0.68 | 0.62 | 1.45 |
| 120% | 0.7 | 0.6 | 1.5 |

**Table 7. Summary of Robustness Results of the HPLC Method**

| Results | Fusidic acid (mg/g) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Set-I | Set-2 | Set-3 | Set-4 | Set-5 | Set-6 | Set-7 | Set-8 | Set-9 | Set-10 |
| Overall mean | 20.3 | 20.2 | 20.3 | 20.3 | 20.3 | 20.3 | 20.1 | 20.1 | 20.1 | 20.2 |
| Overall SD | 0.21 | 0.22 | 0.28 | 0.29 | 0.21 | 0.21 | 0.39 | 0.37 | 0.38 | 0.24 |
| Overall %RSD | 1 | 1.1 | 1.4 | 1.4 | 1 | 1 | 1.9 | 1.8 | 1.9 | 1.2 |
| Results | Betamethasone-17 valerate (mg/g) | | | | | | | | | |
| | Set-I | Set-2 | Set-3 | Set-4 | Set-5 | Set-6 | Set-7 | Set-8 | Set-9 | Set-10 |
| Overall mean | 1.15 | 1.15 | 1.14 | 1.15 | 1.15 | 1.15 | 1.16 | 1.15 | 1.15 | 1.13 |
| Overall SD | 0.005 | 0.005 | 0.005 | 0.007 | 0.006 | 0.006 | 0.017 | 0.006 | 0.005 | 0.021 |
| Overall %RSD | 0.4 | 0.4 | 0.4 | 0.6 | 0.5 | 0.5 | 1.5 | 0.5 | 0.4 | 1.9 |
| Results | Chlorocresol (mg/g) | | | | | | | | | |
| | Set-I | Set-2 | Set-3 | Set-4 | Set-5 | Set-6 | Set-7 | Set-8 | Set-9 | Set-10 |
| Overall mean | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.0 | 1.01 | 1.01 | 1.0 |
| Overall SD | 0.005 | 0.005 | 0.005 | 0.006 | 0.009 | 0.007 | 0.018 | 0.011 | 0.006 | 0.02 |
| Overall %RSD | 0.5 | 0.5 | 0.5 | 0.6 | 0.9 | 0.7 | 1.8 | 1.1 | 0.6 | 2.0 |

## CONCLUSION

Based on the results given in summary report, it is concluded that Isocratic RP-HPLC method was successfully developed for the assay of fusidic acid, betamethasone -17 valerate and Chlorocresol in topical pharmaceutical formulation. The developed method is selective, precise, accurate, linear, and robust. The forced degradation data proved that the method is specific for the analytes and free from the interference of the placebo and degradation products. Moreover, it may be applied for the

individual and simultaneous determination of fusidic acid, betamethasone -17 valerate and Chlorocresol compounds in a pharmaceutical drug productandsubstance. Also, it can be utilized for the determination of an assay, blend uniformity, and content uniformity of pharmaceutical products.

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