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# STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MUPIROCIN AND FLUTICASONE

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**Abstract:**

*A simple, Accurate, precise method was developed for the simultaneous estimation of the Mupirocin and Fluticasone in ointment dosage form by reverse phase high performance liquid chromatography. Chromatogram was run through Standard Discovery 250 x 4.6 mm, 5µ. Mobile phase containing Buffer Ortho phosphoric acid: Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% Perchloric acid buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 230nm. Retention time of Mupirocin and Fluticasone were found to be 2.146 min and 2.770 min. percentage relative standard deviation of the Mupirocin and Fluticasone were and found to be 0.4 and 0.5 respectively. Percentage Recovery was obtained as 98.75% and 99.42% for Mupirocin and Fluticasone respectively. Limit of detection, Limit of quantitation values obtained from regression equations of Mupirocin and Fluticasone were 0.38, 1.16 and 0.02, 0.05 respectively. Regression equation of Mupirocin is y = 10256.x + 82433, and y= 24529x + 3330 of Fluticasone. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.*

**Key Words**: *Mupirocin, Fluticasone, Quality control, Retention time, Limit of detection (LOD) and limit of quantification (LOQ), Regression equation.*

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## INTRODUCTION:

Analytical methods are used for product research, product development, process control and chemical quality control purposes. Each of the techniques used in chromatographic or spectroscopic, have their own special features and deficiencies, which must be considered. Each step in the method must be investigated to determine the extent to which environment, matrix, or procedural variables can affect the estimation of analyte in the matrix from the time of collection up to the time of analysis. Pharmaceutical analysis require very precise and accurate assay methods to quantify drugs either in Pharmaceutical or biological samples. The assay methods have to be sensitive, selective, rugged and reproducible. Analytical chemistry is the qualitative and quantitative analysis of drug substances in biological fluids (mainly plasma and urine) or tissue [1-4]. It plays a significant role in the evaluation and interpretation of pharmacokinetic data. The main analytical phase comprises method development, method validation and sample analysis (method application).

**Aim:**

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Mupirocin, Fluticasone in bulk and ointment dosage form. The scope for developing and validating an analytical method is to ensure a suitable method for a particular analyte. The main objective was of the present study to improve the analytical conditions for the separation of active ingredient from formulation which could be done in the development and validation.

**Objective:**

Literature survey reveals that there are only a few methods reported so far in the determination of Mupirocin and Fluticasone in markets formulation .Moreover, there is also lack of adequate information regarding stability indicating studies on method developed earlier for the estimation of Mupirocin and Fluticasone in pharmaceutical formulation. So there is need for the development of new method for estimation of Mupirocin and Fluticasone formulation available in market, along with its stability studies in order to determine the degradation products as well as possible pathway of degradation.

Following are the objectives of the present work:

- To develop a new stability indicating High performance liquid chromatography (HPLC) method for simultaneous estimation of Mupirocin and Fluticasone.
- Performing accelerated stability testing for the drug substances as per International Conference on Harmonization (ICH) guidelines.
- Analytical method validation
- To apply the validated method for the simultaneous estimation of Mupirocin and Fluticasone in pharmaceutical formulation.

## MATERIALS AND METHODS:

**Materials:**

Mupirocin and Fluticasone pure drugs (API), Combination Mupirocin and Fluticasone tablets (**Flutibact** ), Distilled water, Acetonitrile, Phosphate buffer, , Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

**Instruments:**

Electronics Balance-Denver, $p^H$ meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software, Ultra violet-Visible (UV-VIS) spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Mupirocin and Fluticasone solutions.

**Methods:**

**Optimisation of chromatographic conditions**

- **Selection of wavelength**

  From the UV-visible spectophotometric results, a detection wavelength of 230nm was selected . Because at this wavelength they showed maximum absorbance with good peak intensity, good peak shape and height was observed.

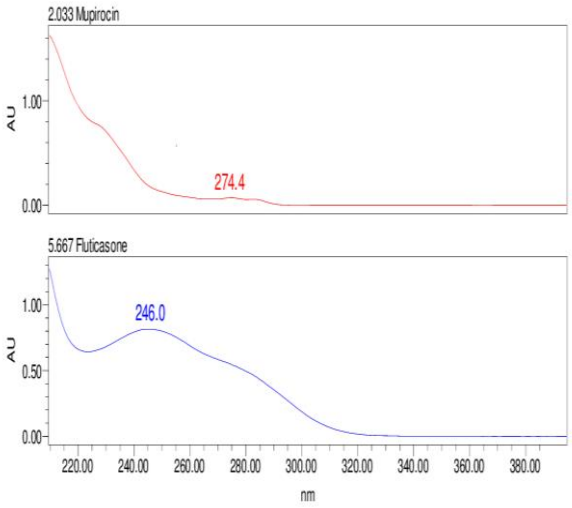


**Fig 1: Individual UV spectra of Mupirocin and Fluticasone**

$\lambda_{max}$ of Mupirocin and Fluticasone was 274.4nm and 246.0nm respectively.

Overlay spectra gave the optimized wavelength for these two drugs.

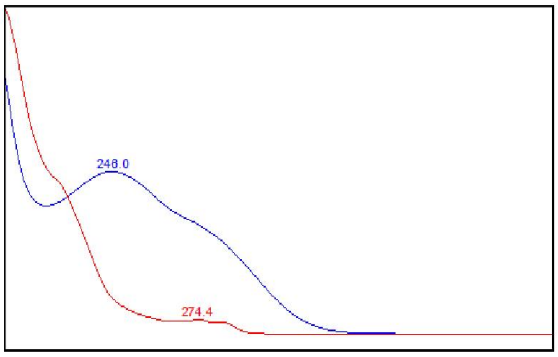


**Fig 2: Overlay UV spectra of Mupirocin and Fluticasone**

Optimized wavelength selected was 230nm.

**Preparation of Solutions:**

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

**Preparation of Standard stock solutions:** Accurately weighed 30mg of Mupirocin, 5mg of Fluticasone and transferred to 10ml and 100ml individual volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. ( 3000µg/ml of Mupirocin and 50µg/ml fluticasone)

**Preparation of Standard working solutions (100% solution):** 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (300µg/ml of mupirocin and 5µg/ml of Fluticasone)

**Preparation of buffer:**

**0.1%OPA Buffer**: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

**Degradation Studies:**

**Oxidation:**

To 1 ml of stock solution of Mupirocin and Fluticasone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at $60^0$c. For HPLC study, the resultant solution was diluted to obtain 300µg/ml&5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies:**

To 1 ml of stock solution Mupirocin and Fluticasone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$c .The resultant solution was diluted to obtain 300µg/ml&5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies:**

To 1 ml of stock solution Mupirocin and Fluticasone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at $60^0$c. The resultant solution was diluted to obtain 300µg/ml& 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies:**

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 300µg/ml & 5µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

**Photo Stability Studies:**

The photochemical stability of the drug was also studied by exposing the 2000µg/ml & Fluticasone µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 300µg/ml& 5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies:**

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 300µg/ml& 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

## RESULTS AND DISCUSSION:

**Method Development:**

Proper selection of the method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. Mupirocin and Fluticasone were dissolved in solvents, so the developed method of estimation was carried out on reverse phase high performance liquid chromatography. To develop a rugged and suitable HPLC method for the quantitative determination of Mupirocin and Fluticasone the analytical conditions were selected after the consideration of different parameters such as diluent, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition, and other chromatographic conditions. Preliminary trials were taken with different composition of buffer and organic phase of mobile phases . The column selection has been done by backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of

the retention time and resolution between Mupirocin and Fluticasone peaks. After evaluating all these factors, a Standard Discovery column was found to be giving satisfactory results. The selection of acetonitrile and buffer were based on chemical structure of both the drugs. The acidic pH range was found suitable for solubility, resolution, stability, theoretical plates, and peak shape of both components. Best results were obtained with 50% OPA: 50% Acetonitrile that improved the peak shapes of Mupirocin and Fluticasone. For the selection of organic constituent of mobile phase, acetonitrile was chosen to reduce the longer retention time and to attain good peak shape. Therefore, final mobile phase composition consisting of a mixture of buffer-pH 2.0 (0.1% OPA): Acetonitrile. Flow rates between 0.5 to1.2ml/min were tried. Flow rate of 1ml/min was observed to be enough to get all the drugs eluted within less than 10min. The column temperature was set at 30oC. Optimized method was providing good resolution and peak shape for Mupirocin and Fluticasone. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

Method development was done by changing mobile phase ratios, buffers etc. Following are the chromatograms of the trails performed:

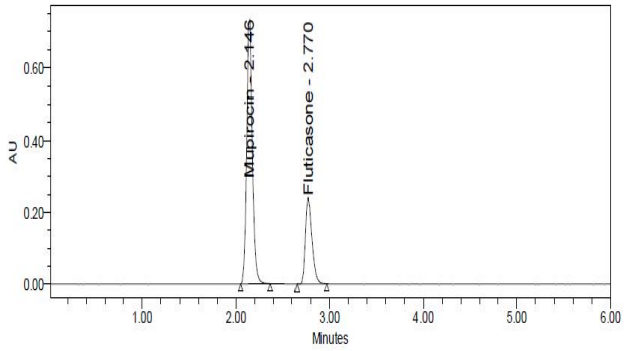


**Fig 3: Optimized Chromatogram**

**System Suitability:**

For all of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Mupirocin and Fluticasone were less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in (Table 1 and Fig. 4). All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

**Table 1: System suitability parameters for Mupirocin and Fluticasone**

| S no | Mupirocin | | | Fluticasone | | |
|---|---|---|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing | RT(min) | USP Plate Count | Tailing |
| 1 | 2.141 | 6364 | 1.25 | 2.765 | 7494 | 1.28 |
| 2 | 2.146 | 6312 | 1.11 | 2.767 | 7944 | 1.27 |
| 3 | 2.146 | 6305 | 1.15 | 2.770 | 7138 | 1.28 |
| 4 | 2.147 | 6713 | 1.16 | 2.771 | 7862 | 1.28 |
| 5 | 2.148 | 6846 | 1.16 | 2.780 | 7801 | 1.30 |
| 6 | 2.153 | 6912 | 1.18 | 2.782 | 7542 | 1.28 |

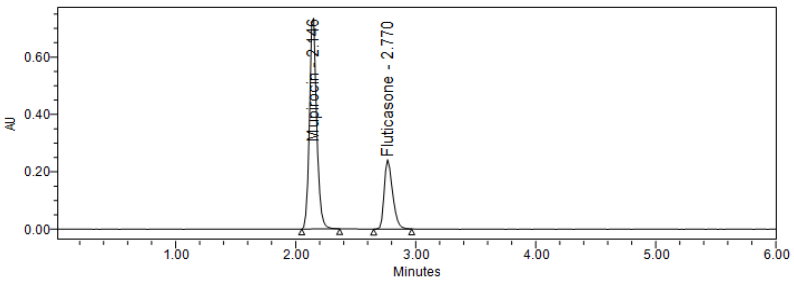


**Fig 4: System suitability Chromatogram**

**Method Validation:**

**Specificity:**

Retention times of Mupirocin and Fluticasone were 2.146 min and 2.770 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Mupirocin and Fluticasone is shown in Fig. 5 clearly shows the ability of the method to assess the analyte in the presence of other excipients.

**Linearity:**

Six linear concentrations of Mupirocin (75-450µg/ml) and Fluticasone (1.25-7.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Mupirocin was y = 10256.x + 82433 and of Fluticasone was y = 24529x + 3330 Correlation coefficient obtained was 0.999 for the two drugs as shown in Table 2 and Figure 6,7.

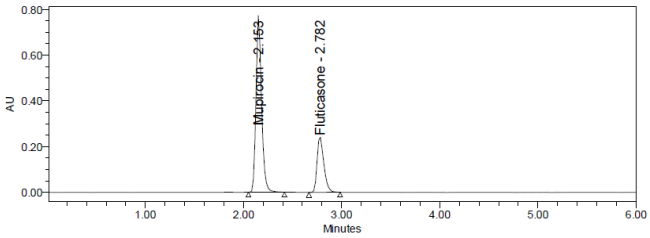


**Fig 5: Typical Chromatogram**

**Table 2: Linearity table for Mupirocin and Fluticasone.**

| **Mupirocin** | | **Fluticasone** | |
|---|---|---|---|
| **Conc (µg/mL)** | **Peak area** | **Conc (µg/mL)** | **Peak area** |
| 0 | 0 | 0 | 0 |
| 75 | 901735 | 1.25 | 36678 |
| 150 | 1631619 | 2.5 | 66491 |
| 225 | 2445171 | 3.75 | 95852 |
| 300 | 3179093 | 5 | 124779 |
| 375 | 3923795 | 6.25 | 156626 |
| 450 | 4648550 | 7.5 | 186781 |

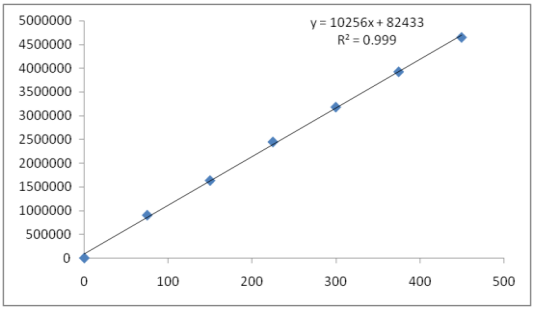


**Fig No. 6: Calibration curve of Mupirocin**

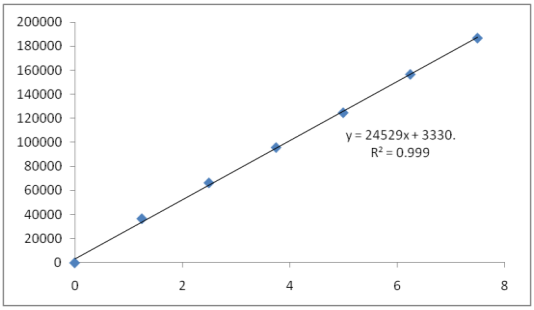


**Fig No. 7: Calibration curve of Fluticasone**

**Precision:**

**System Precision:**

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs.% RSD obtained as 0.8%and 1.0% respectively for Mupirocin and Fluticasone as in Table 3 .As the limit of Precision was less than "2" the system precision was passed in this method.

**Repeatability:**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.4% and 0.5% respectively for Mupirocin and Fluticasone shown in Table 4.

**Table 3: System precision table of Mupirocin and Fluticasone**

| S. No | Area of Mupirocin | Area of Fluticasone |
|---|---|---|
| 1. | 3055697 | 116240 |
| 2. | 3070668 | 116628 |
| 3. | 3040090 | 117554 |
| 4. | 3002213 | 114264 |
| 5. | 3039396 | 116107 |
| 6. | 3020300 | 115160 |
| Mean | 3038061 | 115992 |
| S.D | 24415.6 | 1148.7 |
| %RSD | 0.8 | 1.0 |

**Table 4: Repeatability table of Mupirocin and Fluticasone**

| S. No | Area of Mupirocin | Area of Fluticasone |
|---|---|---|
| 1. | 3018441 | 114459 |
| 2. | 3028350 | 114880 |
| 3. | 3005649 | 115765 |
| 4. | 3037981 | 114442 |
| 5. | 3024676 | 114813 |
| 6. | 3018401 | 115613 |
| Mean | 3022250 | 114995 |
| S.D | 10913.1 | 568.2 |
| %RSD | 0.4 | 0.5 |

**Intermediate precision (Day_ Day Precision):**
Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 1.8% and 1.6% respectively for Mupirocin and Fluticasone specified in Table 5.

**Robustness:**
Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

**Table 5: Intermediate precision table of Mupirocin and Fluticasone**

| S. No | Area of Mupirocin | Area of Fluticasone |
|---|---|---|
| 1. | 3597970 | 136520 |
| 2. | 3504386 | 134614 |
| 3. | 3484832 | 139041 |
| 4. | 3639840 | 140166 |
| 5. | 3500182 | 135033 |
| 6. | 3513339 | 136171 |
| Mean | 3540092 | 136924 |
| S.D | 63145.3 | 2219.7 |
| %RSD | 1.8 | 1.6 |

**Table 6: Robustness data for Mupirocin and Fluticasone.**

| S.no | Condition | %RSD of Mupirocin | %RSD of Fluticasone |
|---|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 0.3 | 0.4 |
| 2 | Flow rate (+) 1.1ml/min | 1.0 | 0.8 |
| 3 | Mobile phase (-) 45B:55A | 0.9 | 0.6 |
| 4 | Mobile phase (+) 55B:45A | 0.6 | 0.7 |
| 5 | Temperature (-) 25°C | 0.5 | 0.4 |
| 6 | Temperature (+) 35°C | 0.3 | 0.5 |

**Accuracy:**
Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 98.75% and 99.42% for Mupirocin and Fluticasone respectively shown in table 7 and table 8.

**Sensitivity:**
The LOD of Mupirocin and Fluticasone were found to be 0.32 and 0.02 respectively. LOQ values of Mupirocin and Fluticasone were found to be 1.16 and 0.05 respectively as shown in Table 9 and Figure 8,9.

**Table 7: Accuracy table of Mupirocin**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 150 | 147.88 | 98.59 | 98.75% |
| | 150 | 148.81 | 99.20 | |
| | 150 | 147.32 | 98.21 | |
| 100% | 300 | 294.57 | 98.19 | |
| | 300 | 295.09 | 98.36 | |
| | 300 | 295.85 | 98.62 | |
| 150% | 450 | 445.17 | 98.93 | |
| | 450 | 443.82 | 98.63 | |
| | 450 | 449.96 | 99.99 | |

**Table 8: Accuracy table of Fluticasone**

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean %Recovery |
|---|---|---|---|---|
| 50% | 2.5 | 2.47 | 98.88 | 99.42% |
| | 2.5 | 2.51 | 100.22 | |
| | 2.5 | 2.51 | 100.23 | |
| 100% | 5 | 4.98 | 99.65 | |
| | 5 | 4.95 | 98.92 | |
| | 5 | 4.94 | 98.75 | |
| 150% | 7.5 | 7.54 | 100.55 | |
| | 7.5 | 7.38 | 98.35 | |
| | 7.5 | 7.44 | 99.19 | |

**Table 9: Sensitivity table of Mupirocin and Fluticasone**

| Molecule | LOD | LOQ |
|---|---|---|
| **Mupirocin** | 0.38 | 1.16 |
| **Fluticasone** | 0.02 | 0.05 |

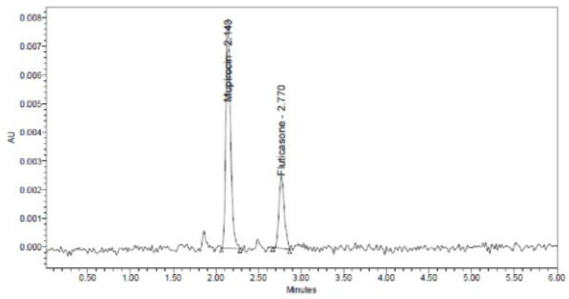


**Fig. No. 8: LOD Chromatogram of Standard**

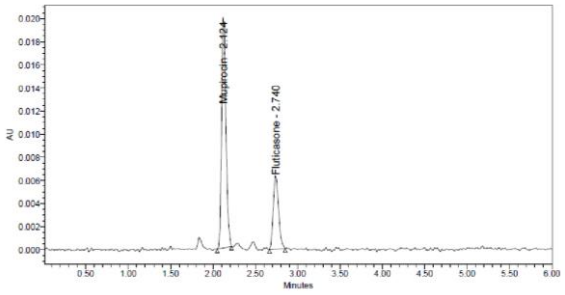


**Fig.No. 9: LOQ Chromatogram of Standard**

**Table 12: Degradation Data of Mupirocin**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 4.86 | 3.930 | 4.380 |
| 2 | Alkali | 2.88 | 0.485 | 0.569 |
| 3 | Oxidation | 1.92 | 0.151 | 0.314 |
| 4 | Thermal | 0.94 | 0.166 | 0.337 |
| 5 | UV | 0.57 | 0.181 | 0.328 |
| 6 | Water | 0.89 | 0.163 | 0.314 |

**Table 13: Degradation Data of Fluticasone**

| S.NO | Degradation Condition | % Drug Degraded | Purity Angle | Purity Threshold |
|---|---|---|---|---|
| 1 | Acid | 4.68 | 0.904 | 1.189 |
| 2 | Alkali | 2.58 | 0.915 | 1.203 |
| 3 | Oxidation | 1.72 | 0.463 | 0.671 |
| 4 | Thermal | 0.53 | 0.635 | 0.930 |
| 5 | UV | 0.50 | 0.602 | 0.811 |
| 6 | Water | 0.70 | 0.467 | 0.693 |

**Degradation studies:**

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali , oxidative , photolytic , thermal and neutral analysis.

Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time.

## DISCUSSION:

From the reported literature review, there were few methods established for the determination of Mupirocin and Fluticasone in individual and in combination with other drugs. It was concluded that there was no method reported for the simultaneous estimation of the above selected duel component dosage form, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for simultaneous estimation of Mupirocin and Fluticasone in combination dosage form.

A simple, accurate, precise method was developed for the simultaneous estimation of the Mupirocin and Fluticasone in ointment dosage form. The developed method was found to be simple and have short run time which makes the method rapid. The robustness of the method was checked in terms of varying Flow rate, Column temperature, Mobile phase composition. The standard was able to give system suitability parameters within limit, which indicates that the method is Robust.Several studies were reported in the literature for the determination of Mupirocin and Fluticasone individually and in combination with other drugs like ketoconazole, itraconazole, azelastine etc.

The present work compiled with our initial research objectives and demonstrated the applicability of HPLC for pharmaceutical analysis of different class of drugs namely Mupirocin and Fluticasone.

## CONCLUSION:

In simultaneous RP-HPLC method development, Waters HPLC grade with UV detector was used. The column used was Discovery C18 (4.6×250mm,

5µm) column. Injection volume of 10µL was injected and eluted with the mobile phase of mixed ortho phosphoric acid and acetonitrile in ration of 50:50. The flow rate was found to be optimized to 1ml/min. detection was carried out at 230nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic conditions. This system produced symmetric peak shape, good resolution and reasonable retention times of Mupirocin and Fluticasone at 2.146 and 2.770 minutes respectively.

Mupirocin and Fluticasone showed linearity in the range of 75-450µg/ml and 1.25-7.5µg/ml respectively. The slope, intercept and correlation coefficients were found to be y=10256.x+82433 and 0.999 respectively for mupirocin and y=24529.x+3330 and 0.999 for Fluticasone respectively. The amount of drug estimated by the proposed method was in good agreement with the label claim.

The % RSD values for precision was found to be within the acceptable limits, which revealed that the developed method was precise. The developed method was found to be robust. The % RSD value for percentage recovery of Mupirocin and Fluticasone was found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the Mupirocin and Fluticasone.

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