



**A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF
CIPROFLOXACIN AND FLUCIONOLONE IN PHARMACEUTICAL FORMULATION**

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ABSTRACT

A simple, accurate, precise and sensitive RP-HPLC assay method have been validated for the simultaneous estimation of ciprofloxacin and flucionolone in pharmaceutical formulation by RP-HPLC .ciprofloxacin and flucionolone is separated using Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column at a flow rate of 0.8 ml/ min. Here resolution was good, theoretical plate count and symmetry was appropriate .The LOD and LOQ were calculated using statistical methods. The % RSD values were less than 1. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. The method was successfully applied for determination of drug in tablets, wherein no interference from tablet excipients was observed, indicating the specificity of the developed method. The proposed method was found to be simple, precise, accurate, rapid, economic and reproducible for the estimation of ciprofloxacin and flucionolone in pharmaceutical formulation.

KEYWORDS: Ciprofloxacin, Flucionolone, Develosil, Method Development, Validation.

INTRODUCTION

Ciprofloxacin (CIPRO)(1-cyclopropyl-6-fluro-4-oxo-7-(piperazin-1-yl)-3-carboxylic acid.it is a broad spectrum antimicrobial carboxyfluoroquinoline & anti-infective agent. Flucionolone(1S,2S,4R,8S,9S,11S,12R,13S,19S)-12,19-difluoro-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-16-one. Category is anti-inflammtory agent.

The review of literature reveals that no method has been developed so far for simultaneous estimation of ciprofloxacin & Flucionolone by RP-HPLC methods. Further, a good HPLC method for the estimation of of ciprofloxacin & Flucionolone has not yet been cited in literature.

Experimental

Chemicals and reagents

Working standards of pharmaceutical grade CIPRO & FLUC were obtained from cipla pharmaceuticals Ltd. Doubled distilled water, Di potassium hydrogen phosphate, potassium dihydrogen orthophosphate, orthophosphoric acid obtained from Sd fine-Chem ltd; Mumbai.

Instrumentation and chromatographic conditions

Hitachi Lachrome HPLC consists of 1575 model. UV-Visible double beam spectrophotometer UV-1800 model manufacturer Elico India. separation was carried out on Develosil ODS HG-5 RP C₁₈, 5µm, 15cmX4.6mm i.d. column using potassium dihydrogen phosphate buffer+Dipotassium hydrogen phosphate(0.002M,p^H5.0):acetonitrile(40:60) as mobile phase at flow rate of 1.0ml/min.sample was injected using Rheodyne injector with 20 µL loop and detection was carried out at 290 nm. All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of standard solutions

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of each drug in 100 mL of HPLC grade methanol to get concentration of final solution 40µg/ml, 50µg/ml.

Preparation of mix. Standard solution of Flucionolone & Ciprofloxacin

Accurately weighed 10 mg of Flucionolone and 10 mg of Ciprofloxacin were transferred to two different 10 ml volumetric flask. About 4 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 0.5 ml of

ciprofloxacin & 0.4 ml of Flucionolone were taken to a 10 ml volumetric flask & diluted to 10 ml with the solvent system. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique

System suitability

The system suitability was assessed by six replicate injections of the mixture containing 10 $\mu\text{g/ml}$ of both the drugs. The resolution, peak asymmetry, number of theoretical plates and HETP were calculated as represented in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table. 1: System suitability parameters for RPHPLC method.

S. No	Parameters	Limit	Result
1	Resolution	$R_s > 2$	3.15
2	Asymmetry	$T \leq 2$	Flucionolone=0.12 Ciprofloxacin=0.5
3	Theoretical plate	$N > 2000$	Flucionolone= 3246 Ciprofloxacin= 4693

METHOD VALIDATION

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines¹⁵.

Linearity and Range

Method: From the stock solution different concentrations like 16, 24, 32, 40, 48 of Fluocinolone & 20, 30, 40, 50, 60 of Ciprofloxacin has been prepared then injected to HPLC. The calibration curve was obtained by plotting the graph by taking concentrations in X-axis & AUC in Y-axis Linearity range was found to be 0-48 $\mu\text{g/ml}$ for Fluocinolone and 0-60 $\mu\text{g/ml}$ for Ciprofloxacin. The correlation coefficients were found to be 0.993 & 0.992, the slopes were found to be 53030 & 34725 and intercept were found to be 57391 & 53532 for Fluocinolone & Ciprofloxacin respectively.

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Fluocinolone & Ciprofloxacin were taken and injected in three replicates. From that percentage recovery values were calculated from standard graph. The mean recoveries were found to be 96.56, 96.73, 103.57 % for Ciprofloxacin and 95.61, 94.91, 104.74% for Fluocinolone The limit for mean % recovery is 95-105% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision

Repeatability: The precision of each method was ascertained separately from the peak areas obtained by actual determination of five replicates of a fixed amount

of drug. Ciprofloxacin & Fluocinolone. The percent relative standard deviations were calculated for Ciprofloxacin & Fluocinolone. The repeatability study which was conducted on the solution having the concentration of about 40 $\mu\text{g/ml}$ for Fluocinolone and 50 $\mu\text{g/ml}$ for Ciprofloxacin ($n = 5$) showed a RSD of 0.441393% for Fluocinolone and 0.329005123% for Ciprofloxacin. It was concluded that the analytical technique showed good repeatability.

ii Intermediate precision For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration ($n = 3$), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and RSD. Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit ($\leq 2\%$), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

LOD and LOQ: LOD and LOQ were calculated as $3.3 \sigma / S$ and $10 \sigma / S$ respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness: In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase ($0.8 \pm 0.02 \text{ mL/min}$), a wavelength at which the drugs were recorded ($290 \pm 2 \text{ nm}$) and mobile phase percentage with respect to ($\pm 2\%$). One factor at the time was changed to estimate the effect. The solutions containing 16 $\mu\text{g/mL}$ of both the drugs were applied onto the column. A number of replicate analyses ($n = 3$) were conducted at 3 levels of the factor (-, 0, +). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Methanol: 0.025 M Potassium dihydrogen phosphate buffer in ratio of (70:30, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 3.60 ± 0.0525 and $6.24 \pm 0.0619 \text{ min}$ (Mean \pm S.D.) for CEFI and OFLOX respectively. The representative chromatogram of the standard solution of mixture is shown in Fig. 1&2.

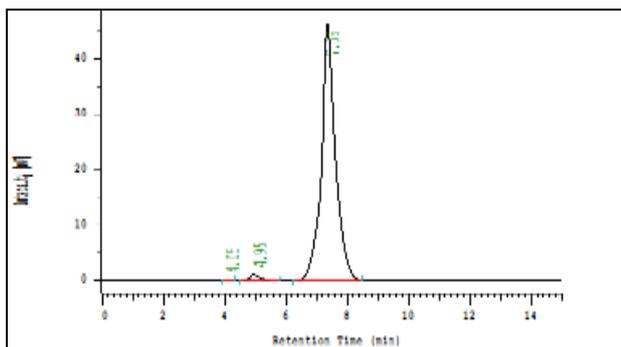


Figure. 1: Running the standard solution of Fluocinolone.

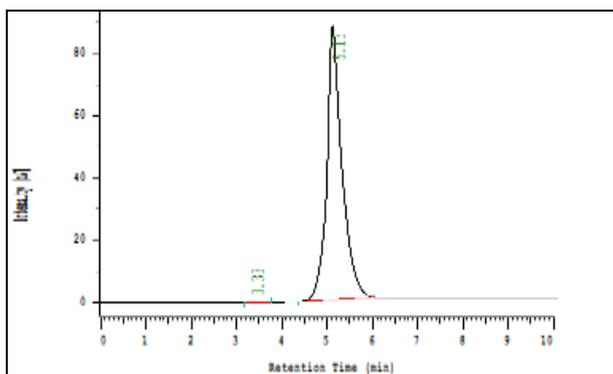


Figure. 2: Running the standard solution of Ciprofloxacin.

Results were found to be linear in the concentration range of 0-48 $\mu\text{g/mL}$ for Fluocinolone, 0-60 $\mu\text{g/ml}$ for ciprofloxacin. The correlation coefficients for the plots were 0.993 & 0.992 for flu & cipro respectively. The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatograms (RSD < 2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in Table 2.

Table. 3: Summary of validation parameters of proposed RPHPLC Method.

Parameter	Fluocinolone	Ciprofloxacin
Linearity	0-48 $\mu\text{g/ml}$	0-60 $\mu\text{g/ml}$
Correlation coefficient	0.993	0.992
Slope	53030	34725
Intercept	57391	53532
LOD ($\mu\text{g/ml}$)	0.32	1.44
LOQ ($\mu\text{g/ml}$)	0.96	4.32
Accuracy (% Recovery)	95.61-104.74%	96.56-103.57%
Precision (% RSD)		
Intraday (n=3) (10 $\mu\text{g/ml}$)	1.05	0.86
Interday (n=3)	0.24	0.87

LOD = Limit of detection; b LOQ = Limit of quantitation; c RSD = Relative standard deviation; dn = Number of determination

CONCLUSION

To develop a precise, linear, specific RP-HPLC method for analysis of Fluocinolone & Ciprofloxacin different chromatographic conditions were applied & the results observed are presented in the thesis.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

4

In case of RP-HPLC various columns are available, but here developed Sil, C-18, V size (150mm*4.6mm \varnothing) column was preferred because using this column peak shape, resolution and absorbance were good.

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1N HCl). Fluocinolone was found to be insoluble in water and soluble in acetonitrile & methanol. Ciprofloxacin was found to be insoluble in water and soluble in methanol & acetonitrile.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of Ciprofloxacin & Fluocinolone it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20 μl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Ciprofloxacin & Fluocinolone in different formulations

A sensitive & selective stability indicating RP-HPLC method has been developed & validated for the analysis of Ciprofloxacin & Fluocinolone API.

Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Ciprofloxacin & Fluocinolone indicated that the developed method is specific for the estimation of Ciprofloxacin & Fluocinolone. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

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