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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CIPROFLOXACIN AND FLUOCINOLONE ACETONIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop and validate a reverse phase high performance liquid chromatographic method for simultaneous estimation of ciprofloxacin and fluocinolone acetonide in bulk and pharmaceutical dosage form.

Methods: The chromatographic separation was achieved on reverse phase Discovery Inertsil ODS3V Column, C18 (250 mm, 4.6 mm, 5 µm). The separation was achieved by employing the mobile phase consists of phosphate buffer (p^H 4) and acetonitrile (40:60). The flow rate was 1.0 ml/min, at a detection wavelength of 295 nm. The proposed method was validated as per the International Council for Harmonisation (ICH) guidelines.

Results: The retention time for ciprofloxacin and fluocinolone acetonide was found at 3.627 min and 5.037 min respectively. The proposed method was validated for specificity, accuracy, precision, linearity, the limit of detection (LOD), limit of quantitation (LOQ) and robustness. All validation parameters were within the acceptable range. The assay method was linear and found in the range from 12.5–37.25 µg/ml for ciprofloxacin and 0.625–1.875 µg/ml of fluocinolone acetonide. The relative standard deviation (RSD) values for ciprofloxacin and fluocinolone acetonide were 0.25 % and 0.18 %, respectively.

Conclusion: A rapid, accurate and precise RP-HPLC method was developed for the simultaneous estimation of ciprofloxacin and fluocinolone acetonide in bulk and ointment formulation. The developed method was validated for specificity, accuracy, precision, linearity, the limit of detection, limit of quantitation and robustness according to ICH guidelines.

Keywords: Ciprofloxacin, Fluocinolone acetonide, RP-HPLC, Method development, Method validation

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INTRODUCTION

Ciprofloxacin (fig. 1) is a broad-spectrum antibiotic of the fluoroquinolone class, which is used to treat wide variety of infections. It is chemically 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid and its molecular weight is 331.347 g/mol.

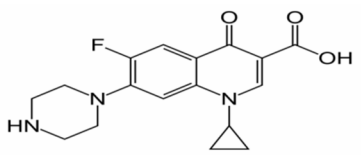

Fig. 1: Structure of ciprofloxacin

Fluocinolone acetonide (fig. 2) is a topical steroid used to treat the inflammation and itching caused by a number of skin conditions such as allergic reactions, eczema, seborrhea and psoriasis. It is chemically 4aS, 4bR, 5S, 6aS, 6bS, 9aR, 10aS, 10bS, 12S)-4b,12-difluoro-5-hydroxy-6b-(2-hydroxyacetyl)-4a,6a,8,8-tetramethyl-4a, 4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho [2',1' :4,5] indeno[1,2-d][1,3]dioxol-2-one and its molecular weight is 452.488 g/mol.

An extensive literature survey revealed that there were analytical methods for the estimation of mentioned drugs alone [1-3] and in combinations [4-11]. No analytical method has been reported for the simultaneous estimation of ciprofloxacin and fluocinolone acetonide. Hence, it was thought appropriate to develop a simple RP-HPLC method for the simultaneous estimation of ciprofloxacin and fluocinolone acetonide.

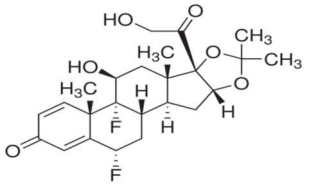

Fig. 2: Structure of fluocinolone acetonide

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutically pure samples of ciprofloxacin and fluocinolone acetonide were obtained as gift samples from Chandra labs, Hyderabad, India. Orthophosphoric acid of analytical reagent grade was purchased from Rankem, New Delhi, India. Dihydrogen sodium phosphate, disodium hydrogen phosphate, acetonitrile, Milli-Q water and methanol of HPLC grade was purchased from Rankem, Maharashtra, India.

Instrument

The chromatographic technique performed on a Waters 2695 with PDA detector and Empower2 software, reversed-phase Discovery Inertsil ODS3V C18 column (250 mmx4.6 mmx5 µm), sonicator (LMUC-2, Labman scientific), p^H meter (AD 1020, ADWA) and analytical balance (ER-200A, AFCOSET) were used.

Chromatographic conditions

The chromatographic separation was achieved on a Discovery Inertsil ODS 3V C18 column (250×4.6 mm, 5 µm particle size) using a mobile phase mixture of phosphate buffer (p^H4) and acetonitrile (40:60), which was filtered and degassed prior to use. The flow rate was 1.0 ml/min and PDA detection at 295 nm. The injection volume was 10 µl at ambient temperature.

Preparation of phosphate buffer

Accurately measured 39 ml of dihydrogen sodium phosphate (0.05 M) was mixed with 61 ml of disodium hydrogen phosphate (0.05 M) and was made up to 200 ml with distilled water**.** The p^H of the resulted solution was adjusted to 4 by using orthophosphoric acid. The buffer solution was sonicated for 10 min to remove gases and filtered through 0.45 µm membrane filters.

Preparation of the mobile phase

A mixture of phosphate buffer and acetonitrile in the ratio 40:60 % v/v was used as mobile phase.

Preparation of diluent

The diluent was prepared by taking water and acetonitrile in the ratio of 50:50 % v/v.

Preparation of standard solution

The standard stock solution of ciprofloxacin and fluocinolone acetonide was prepared by accurately weighing and transferring 25 mg of ciprofloxacin and 1.25 mg of fluocinolone acetonide standards into a 100 ml clean dry volumetric flask, 50 ml of diluent was added, sonicated for 10 min and made up to the final volume with diluent. From the above standard stock solution, 1 ml was taken into a 10 ml volumetric flask and made up to the volume with diluent so as to get 25 µg/ml of ciprofloxacin and 1.25 µg/ml of fluocinolone acetonide.

Preparation of sample solution

The sample stock solution of ciprofloxacin and fluocinolone acetonide was prepared by accurately weighing and transferring 5 g of ointment into a 100 ml volumetric flask. To this 10 ml of glacial acetic acid was added and stirred for 40 min on a magnetic stirrer and made up to the mark with methanol and then it was centrifuged for 20 min. The supernatant was collected and filtered through 0.45 µm filters. From this 1 ml was transferred to 10 ml of volumetric flask and made up to the volume with diluent so as to get 25 µg/ml of ciprofloxacin and 1.25 µg/ml of fluocinolone acetonide.

Validation of the developed method

The developed method was validated for parameters such as specificity, linearity, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines for analytical procedures Q2 [R1] [12].

Specificity

The specificity of the method was confirmed by observing the interferences of blank and placebo at analyte peaks. The blank and placebo were prepared as per test method and injected into the chromatographic column and checked for the interfering peaks at the retention times.

Precision

The precision of method was verified by repeatability. Repeatability was checked by injecting six individual homogenous preparations of standard solution under the same operating conditions over a short interval of time (method precision). The RSD values were determined.

Accuracyz

Accuracy of the method was determined in triplicate at three concentration levels 50 %, 100 % and 150 % of target assay concentration. Known quantities of drug substances corresponding to the specified level of the label claim were added to the pre-analyzed sample. Each set of the addition was repeated thrice. The recovery values were determined for each level.

Linearity and range

Linearity was studied by analyzing five standard solutions covering the range of 12.5–37.25 µg/ml for ciprofloxacin and 0.625–1.875 µg/ml of fluocinolone acetonide. A calibration curve was plotted by considering concentration against the corresponding peak area and the correlation coefficient was determined using least square regression analysis.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined based on the standard deviation of the y-intercept and the slope of the calibration curve.

$$LOD = 3.3\ \delta/S$$

$$LOQ = 10\ \delta/S$$

Where,

δ = the standard deviation of the response

S = the slope of the calibration curve

Robustness

Robustness is the measure of a method which remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength. The detection wavelength was varied by±2 nm and the flow rate was varied by±0.1 ml/min.

RESULTS AND DISCUSSION

Method development

Initially, various combinations of mobile phase and stationary phase were tested in an attempt to obtain the best resolution for ciprofloxacin and fluocinolone acetonide, which lead to the optimization of chromatographic conditions for the estimation of ciprofloxacin and fluocinolone acetonide in the pharmaceutical dosage form. The mobile phase consisting of phosphate buffer whose p^H adjusted to 4 with orthophosphoric acid and acetonitrile (40:60 %v/v), pumped at a flow rate of 1.0 ml/min, was chosen for method development and validation of ciprofloxacin and fluocinolone acetonide by RP-HPLC method. The detection was selected at 295 nm, using reverse-phase Discovery Inertsil ODS 3V C18 column (250 x 4.6 mm x 5 µm), the retention time of ciprofloxacin and fluocinolone acetonide was found at 3.627 min and 5.037 min respectively, with a total run time of 6 min which shows rapid elution time when compared to the retention times of mentioned drugs in reported methods [1,3,9]. The parameters such as theoretical plate count, tailing factor and resolution between the peaks were taken into consideration for testing system suitability and they were presented in table 1. From the results of system suitability it was found that the resolution between two peaks was greater than 2 and the tailing factor for both drugs was less than 2. The theoretical plate count was greater than 2000 for ciprofloxacin and fluocinolone acetonide respectively. The typical chromatogram for simultaneous determination of ciprofloxacin and fluocinolone acetonide from standard preparation was shown in fig. 3.

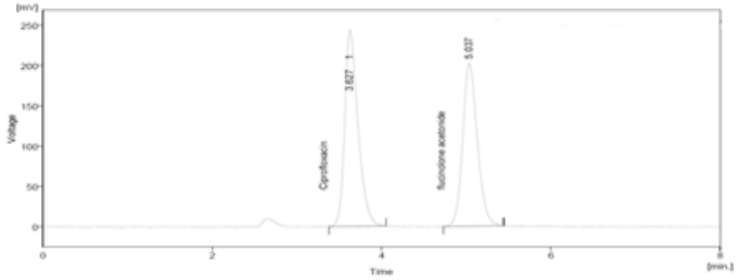

Fig. 3: Chromatogram of ciprofloxacin and fluocinolone acetonide standard preparation

Table 1: Results of system suitability study

S. No.	Drug name	Retention time	Resolution	Plate count	Tailing factor	Peak area
1.	Ciprofloxacin	3.627 min	-	2623	1.703	2606.236
2.	Fluocinolone acetonide	5.037 min	4.741	8592.7	1.298	2436.532

Method validation

Specificity

The results of specificity shows that there was no interference of blank and placebo peaks at the retention times of analyte peaks, hence it was proved that the developed method was specific for the estimation of ciprofloxacin and fluocinolone acetonide.

Precision

The precision of the method was verified by repeatability. The standard solution of ciprofloxacin and fluocinolone acetonide was prepared at working concentration and injected six times into the chromatographic system. The results of precision were tabulated in table 2. The RSD of precision studies were calculated and found that the values were<2 %, hence can be concluded that the method gives consistently reproducible results assuring the repeatability of the developed method and also the method has shown good repeatability of ciprofloxacin and fluocinolone acetonide when compared to the reported methods [1-3, 10]

Accuracy

The results of accuracy were expressed as the percentage of analytes recovered by the assay. The recoveries of the drugs from a series of spiked concentrations were presented in table 3. According to statistical data, the recoveries of drugs were found to be within the specified range of 98-102 %. Hence, it can be concluded that the results of recovery studies specifies that all observed data were within the required range, which indicates good recovery values, confirming the accuracy of the developed method for the determination of ciprofloxacin and fluocinolone acetonide. On comparison of the reported method for estimation of fluocinolone acetonide [3], this method has shown excellent recovery.

Table 2: Results of precision studies

N	Ciprofloxacin	Fluocinolone acetonide
	Peak area	Peak area
Injection 1	2654	2436
Injection 2	2651	2428
Injection 3	2657	2436
Injection 4	2666	2438
Injection 5	2654	2428
Injection 6	2646	2431
Mean	2654.66	2432.8
SD	6.68331	4.40075
RSD (%)	0.25	0.18

RSD = relative standard deviation; SD = standard deviation; Rt = retention time

Table 3: Results of accuracy (recovery studies)

Analyte	Spiked level of standards	Recovery (%)	*Mean recovery
Ciprofloxacin	50 %	99.84	99.87
		99.92	
		99.84	
	100 %	100.04	99.91
		99.76	
		99.92	
	150 %	99.97	99.99
		100.02	
		99.97	
Fluocinolone acetonide	50 %	99.84	99.95
		100.16	
		99.84	
	100 %	99.92	99.97
		99.92	
		100.08	
	150 %	99.94	100.00
		100.05	
		100	

*Average of triplicate determinations

Linearity and range

The calibration curves were plotted for ciprofloxacin and fluocinolone acetonide in fig. 4 and 5, with concentration verses peak areas by injecting the prepared solutions and the obtained data were subjected to regression analysis using the least squares method. The correlation coefficient (r^2) was found to be 0.999 for both the drugs which show good linearity between absorbance and concentration and hence the results of linearity confirms that the method was said to be linear for the specified concentration range as it met the method validation acceptance criteria.

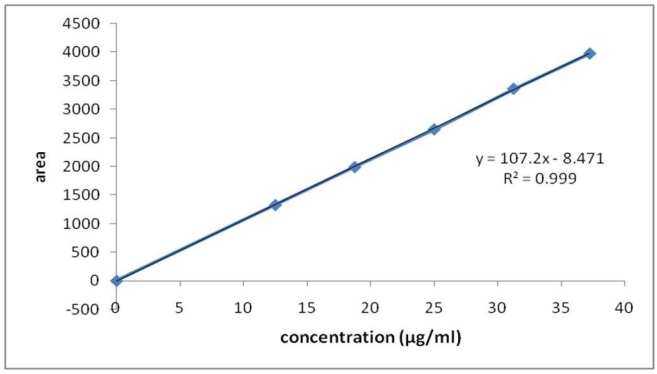

Fig. 4: Linearity chart of ciprofloxacin

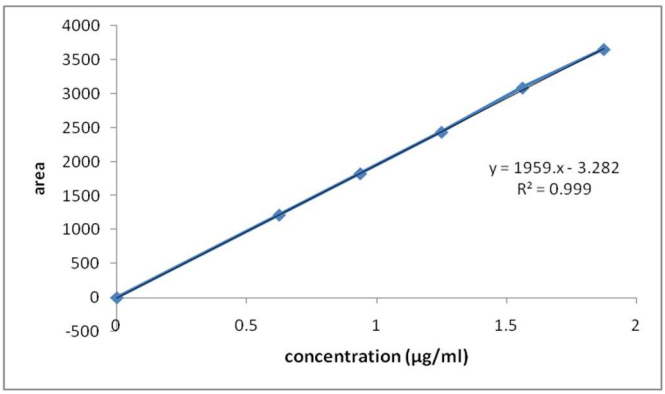

Fig. 5: Linearity chart of fluocinolone acetonide

Table 4: Results of LOD and LOQ

	Ciprofloxacin	Fluocinolone acetonide
LOD	0.09 µg/ml	0.17 µg/ml
LOQ	0.27 µg/ml	0.52 µg/ml

Table 5: Results of robustness

Parameter	Ciprofloxacin		Fluocinolone acetonide	
	Tailing	Plate count	Tailing	Plate count
Less flow rate (0.9 ml/min)	1.750	2543	1.321	8499
Actual flow rate (1.0 ml/min)	1.703	2623	1.298	8592
More flow rate (1.1 ml/min)	1.576	2456	1.268	8486
Less wavelength (293 nm)	1.676	2578	1.326	8554
Actual wavelength (295 nm)	1.703	2623	1.298	8592
More wavelength (297 nm)	1.632	2543	1.271	8476

Limit of detection and limit of quantitation

The detection limits and quantitation limits of the drugs were presented in table 4. From the results of LOD and LOQ it was concluded that the developed method has good sensitivity when compared with reported method [9].

Robustness

The results of robustness were presented in table 5. In all the varied chromatographic conditions no significant differences have been observed in system suitability parameters and were found to be within the limits. The results indicate that the method was unaffected due to deliberate changes in method parameters, which were expressed by system suitability parameters such as tailing and plate count which were found to be within the acceptance criteria and hence the method was found to be robust.

CONCLUSION

From the above experimental results, the newly developed method for the simultaneous estimation of ciprofloxacin and fluocinolone acetonide was found to be simple, precise, accurate, robust and cost-effective and can be effectively applied for routine analysis in research institutions, quality control department in pharmaceutical industries, approved testing laboratories.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Authors declare that no conflicts of interest exist in this research work.

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