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R E S E A R C H A R T I C L E

RP-HPLC Method for Simultaneous Estimation of Cprofloxacin and Dexamethasone in Bulk &Pharmaceutical Dosage Form

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A B S T R A C T

In the method development for RP-HPLC method for Ciprofloxacin and Dexamethasone in bulk drug dosage form with Acetonitrile and Buffer (Phosphate buffer pH: 3) in the ratio 60:40 used as Diluents 8 mints run time. Method was optimized by varying chromatographic parameters like column, mobile phase composition, mobile phase PH and flow rate to satisfy system suitability testing. Various columns and mobile phase combinations were tried. A satisfactory separation and good peak symmetry was obtained by using Agilent C18 (4.6x250mm) column, 10mM Phosphate buffer (pH 3.0): Acetonitrile (20:80) as mobile phase with Isocratic technique. Quantification was achieved with UV detection at235 nm based on peak area. The assay results obtained by using the proposed method for the analysis of marketed ophthalmic solution containing Ciprofloxacin 5mg and Dexamethasone 50mg were in good agreement with the labeled amounts of Ciprofloxacin and Dexamethasone. The average contents of Ciprofloxacin and Dexamethasone were10mg/ml (99.7 %) and100mg/ml (99.8%) respectively.

Keywords: Ciprofloxacin, Dexamethasone, Accuracy, Precision, Acceptance criteria

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Drug profile

Ciprofloxacin

Chemical Formula: (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid)

Chemical Formula: $C_{17}H_{18}FN_3O_3$

Molecular Weight: 331.347 g/ml

Category: Antibiotic is used to relieve bacterial infections of the eyes, corneal ulcers.

Physical state: Solid

Solubility: Sparingly soluble in water

Storage at: Room temperature

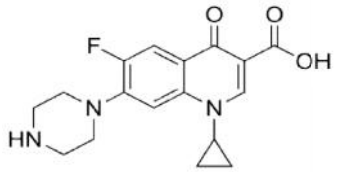

Fig.1.1: Chemical structure of Ciprofloxacin

Dexamethasone

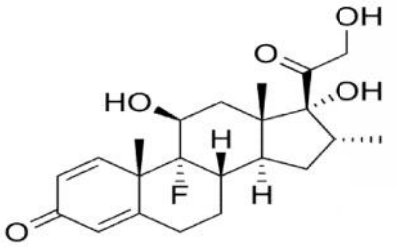

Fig.1.2: Chemical structure of Dexamethasone

Chemical name:
(8*S*, 9*R*, 10*S*, 11*S*, 13*S*, 14*S*, 16*R*, 17*R*)-9-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16- trimethyl-, 7, 8, 9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta [*a*] phenanthren-3-one
Chemical Formula : $C_{22}H_{29}FO_5$
Molecular weight : 392.467 g/mol
Category : Used to treat rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, brain swelling, eye pain following eye surgery.
Physical state : Solid
Solubility : Soluble in ethyl acetate and ethanol; slightly soluble in methanol
Storage at : Room temperature

2. Materials and Methods

Source of drugs: Ciprofloxacin and Dexamethasone working Standards were procured as a gift Sample from Lupin labs Ltd., India.

Apparatus used
HPLC: Equipped with a UV-Visible detector (Agilent).
PH meter: Range from 0-14 (Labindia 352)
Analytical Balance: Accurate to 0.1 mg (Shimadzu)
Graduated Cylinder: 50 ml, 100 ml, and 1000 ml (Borsil)
Volumetric Flasks: 500 ml, 100 ml, 50ml, 10 ml, 5 ml
Volumetric Pipettes: 10 ml, 5 ml, 3 ml, 2 ml, 1 ml (Borsil)
Graduated Pipettes: 10 ml, 5 ml, 2 ml, 1 ml (Borsil)
Water bath Sonicator: (Loba Life)

Chemicals used
Orthophosphoric Acid (OPA): HiMedia Laboratories Pvt. Ltd
Water : Merk
Acetonitrile : Merck
Triethylamine : Merck
Potassium dihydrogen phosphate: Thermo Fisher Scientific India Pvt. Ltd

Instrument used
HPLC: Agilent HPLC 1200 Infinity with UV detector and data was integrated by Ezchrom Elite software.

2.1 Preparation of solutions:

Preparation of Diluent:
Required volume of Acetonitrile and Buffer (Phosphate buffer pH: 3) in the ratio 60:40 was prepared and the prepared mobile phase was mixed, filtered and sonicated for 10 min.

Preparation of 10 mm Phosphate Buffer:
6.056g of Potassium Dihydrogen Ortho Phosphate was dissolved in 445 ml of HPLC water and to this solution 55ml of 0.1M Phosphoric acid was added and pH was adjusted to 3.0 using Triethyl amine.

Preparation of standard stock solutions:

Ciprofloxacin standard stock:
5.0 mg of Ciprofloxacin working standard was accurately weighed into a 10 ml volumetric flask, dissolved it in a few ml of diluent and after it is completely dissolved, the volume was made up to mark with diluent.

Dexamethasone standard stock:
50.0 mg of Dexamethasone working standard was accurately weighed into 10 ml volumetric flask, dissolved it in few ml of diluent and after it is completely dissolved, the volume was made up to mark with diluent.

Working standard stock preparation:
0.1ml from each standard stock solution was collected into 3 individual 10 ml flasks and as a mixture into another 10 ml clean, dry volumetric flask and the volume was made up to mark with diluent and mixed well.

Sample preparation:
A volume of eye drops equivalent to 3 mg and 1 mg of Ciprofloxacin and Dexamethasone respectively was diluted up to 10 ml with methanol. Further dilutions were made with mobile phase to reach the calibration range of each drug and the solutions were treated according to the procedure for linearity of HPLC method.

2.2 Method Development

TRIAL I: Chromatographic conditions
Column : Agilent C 18 (4.6x250mm)
Mobile phase : Acetonitrile: Phosphate buffer (65:35)
Flow rate : 1.2ml/min
Mode : isocratic
Wavelength : 220 nm
Column temperature: Ambient
Injection volume : 20 µL
Run time : 10 min
Observation: By injecting the solution which contains Ciprofloxacin and Dexamethasone unwanted peaks were observed.
Conclusion: Run time was less but unwanted peaks were seen

TRIAL II: Chromatographic conditions
Column : Agilent C 18 (4.6x250mm)
Mobile phase : 10mM Phosphate buffer: Acetonitrile (55:45)
Flow rate : 1ml/min
Mode : isocratic
Wavelength : 240 nm
Column temperature : ambient
Injection volume : 20 µL
Run time : 10 min

Observation: By injecting the solution which contains Ciprofloxacin and Dexamethasone the unwanted peaks were observed.

Conclusion: The response of Ciprofloxacin was not good. Peak shapes of Ciprofloxacin and Dexamethasone were not satisfactory and run time has been increased

Optimized Chromatographic Conditions

Column	: Agilent C 18 (4.6x250mm)
Mobile phase	: 10mM Phosphate buffer (pH 3.0): Acetonitrile (20:80)
Mode	: Isocratic
Flow rate	: 1.0 ml/min
Wavelength	: 235 nm
Column temperature	: ambient
Injection volume	: 20 µL
Run time	:8 min

Observation: This method is finalized, because Ciprofloxacin and Dexamethasone peaks are well separated and have better resolution.

Conclusion: This method is finalized, because Ciprofloxacin and Dexamethasone peaks are well separated without any interference and have better resolution.

Method Validation

After the development of RP-HPLC method for the estimation of drug in a dosage form, validation of the method was performed. This section describes the procedure followed for validation of the developed method.

System Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drugs at a concentration of 16µg/ml.

Observation:

The % Relative standard deviation of individual area response of six replicate injections for Ciprofloxacin and Dexamethasone was found to be 0.42 and 0.84 respectively. The %Relative standard deviation of areas of six replicate injections for Ciprofloxacin and Dexamethasone standard were found to be within limits. The tailing factor for Ciprofloxacin and Dexamethasone peaks was found to be 1.19 and 0.97 respectively. The tailing factor for Ciprofloxacin and Dexamethasone peaks was found to be within limits. The number of theoretical plates for Ciprofloxacin and Dexamethasone were found to be 2227 and 3036 respectively. The resolution was found to be 6.6 respectively which are well within the limits.

Linearity

A series of standard solutions (not less than 5 is recommended) were prepared in the range of 5-25µg/ml containing Ciprofloxacin and Dexamethasone standards and injected. A plot of average peak area versus the concentration in µg/ml or mg/ml is made and from this the correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression line were calculated. The calibration data of Ciprofloxacin and Dexamethasone is given in Table.2.4 and the calibration curve of linearity of is shown in Fig 2.13 and 2.14.

Acceptance Criteria:

The Correlation coefficient should not be less than 0.997.

Observation: The Correlation Coefficient of Ciprofloxacin and Dexamethasone was found to be 0.9973 and 0.9981 respectively.

Precision

The precision of the test procedure was evaluated by injecting the six standard solutions. The Relative Standard Deviation of six injections were calculated. The result of Precision studies is given in Table 3.1 and 3.2.

Specificity

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. Equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy

To validate whether the test method can accurately quantify Ciprofloxacin and Dexamethasone prepare samples in three times for higher and lower levels, in triplicate for other levels by spiking Ciprofloxacin and Dexamethasone active material with equivalent amount of placebo and perform CU as per test procedure. Samples were prepared at levels 50%, 100% and 150% of the target assay concentration i.e., 50% of the lowest strength initial concentration to 150% of the highest strength initial concentration level.

Robustness

Robustness of the method is performed by altering the chromatographic conditions such as pH of the buffer, Wavelength, Mobile phase composition and observed the variation of the results which should be within the acceptance criteria.

Ruggedness: (Intermediate precision):

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

1. Based on Signal-to-Noise for LOD (3:1), LOQ (10:1)
2. Based on the Standard Deviation of the Response and the Slope

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. From the linearity data, the limit of detection and quantitation were calculated using the following formula.

$$\text{LOD} = \frac{3.3\,\sigma}{S}, \quad \text{LOQ} = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response, S = slope of the calibration curve

LOD and LOQ of Ciprofloxacin and Dexamethasone are performed by spiking of known concentrations of the sample into the placebo of formulation and inject the sample.

Assay of marketed formultion:

Assay calculation:

Six replicates of the sample's solutions were injected for quantitative analysis. The amounts of Ciprofloxacin and Dexamethasone estimated were found to 99.7% and 98.01%respectively. A good separation and resolution of both drugs indicate that there was no interference from the excipients commonly present In pharmaceutical formulations. This showed that the estimation of dosage form was accurate within given acceptable level of 95% to 105%.

3. Results and Discussion

Precision

Intraday Precision:

Intraday precision was determined by analyzing same concentration (60µg/ml) of Ciprofloxacin and Dexamethasone for six times in the same day.

Inter-day Precision: Inter-day precision was determined by analyzing the same concentration (60µg/ml) of Ciprofloxacin and Dexamethasone on different days.

Acceptance Criteria:

The Relative standard deviation of individual area of Ciprofloxacin and Dexamethasone from six standard preparations should be not more than 2.0%.

Observation:

The Relative standard deviation of individual area of Ciprofloxacin and Dexamethasone were found to be within limits.

Specificity

The specificity of the method is performed by separately injecting the blank, standard sample containing Ciprofloxacin and Dexamethasone. The interference observed (if any) at the retention times of each analyte in the chromatogram is evaluated.

Acceptance Criteria:

No Interference should be observed at the retention time of standard peaks in the blank.

Observation:

Interference was not observed with the standard peaks and the chromatograms of Standard and Sample were identical with same retention time.

Accuracy:

Acceptance Criteria:

- The mean % Recovery of Ciprofloxacin and Dexamethasone at each level should be not less than 95.0% and not more than 105.0%.
- The %RSD of recovery of Ciprofloxacin and Dexamethasone from the three sample preparations at 50% and 150% levels should not be more than 5.0%.

Observation:

- The mean % Recovery of Ciprofloxacin and Dexamethasone were found to be within limits at each level.
- The % RSD of recovery of Ciprofloxacin and Dexamethasone from the three sample preparations was found to be 0.40 and 1.22 at 50% level and 0.39 and 0.54 at 150% level respectively.

Acceptance criteria:

%RSD should not be more than 2%

Theoretical plates should not less than 2000

Tailing factor should not more than 2.0

Observation:

From the obtained values %RSD was found to be within the range of 0.5%-1.6% which states the method is acceptable.

Acceptance criteria:

%RSD should not be more than 2%

Theoretical plates should not less than 2000

Tailing factor should not more than 2.0

Observation:

From the obtained values %RSD was found to be within the range of 0.4%-1.5% which states the method is acceptable.

Ruggedness: Ruggedness of the method was performed by two different analysts using same experimental environmental conditions. It was performed by injecting the 60µg/ml of Ciprofloxacin and Dexamethasone, respectively. It was found to be rugged and %RSD (less than2) indicating ruggedness of the method

Limit of detection and limit of quantization:

The Limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated.

The Limit of quantification is an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. They can be calculated as

Ciprofloxacin: LOD= $\frac{3.3\,\sigma}{S}$

SD = the Standard deviation of Y-intercept 5 calibrations

Slope = the mean slope of the 5 calibrations

Table.2.1: System suitability parameters of optimized method

Name of Peak	Retention time(mins)	Area	USP plate count	USP Tailing	Injection Volume
Ciprofloxacin	2.3	4152848	8748	1.00	10µl
Dexamethasone	6.2	79949111	11104	1.12	10µl

Table.2.2: System suitability parameters of Ciprofloxacin and Dexamethasone

System suitability parameters	Observed value		
	Ciprofloxacin	Dexamethasone	Acceptance criteria
The Tailing for Ciprofloxacin and Dexamethasone in standard solution	1.19	0.97	NMT 2.0
Theoretical plates for Ciprofloxacin and Dexamethasone in standard solution	2227	3036	NLT 2000

Table.2.3: Linearity data

Standard concentration (µg/ml)	Area of Ciprofloxacin	Standard concentration (µg/ml)	Area of Dexamethasone
10	1768401	100	34894734
12	2211961	120	40069796
14	2601088	140	46861014
16	2892584	160	52952685
18	3123861	180	59847683
20	3567984	200	64595025
22	3871361	220	71995174
Regression R^2= 0.9973		**Regression R^2= 0.9981**	

Table.2.4: Assay data

Sample Name	Peak Area	Retention time(min)	Theoretical plates (USP)	Resolution (USP)	Asymmetry
Ciprofloxacin	3871361	2.31	3211	--	1.11
Dexamethasone	71995174	6.20	3556	2.7	1.22

Table.2.5: Assay calculation

S.No	Drugs	Label claim	Concentration in(µg/ml)	Amount found	% of Assay	% of RSD
1	ciprofloxacin	5mg	10µg/ml	99.85	99.7	1.00
2	dexamethasone	50mg	100µg/ml	99.00	98.01	1.01

Table.3.1: Precision for Ciprofloxacin and Dexamethasone

S.NO	Injection Number	Peak area for Ciprofloxacin	Peak area for Dexamethasone
1	Standard 1	2894916	47944745
2	Standard 2	2849341	47462472
3	Standard 3	2874363	47670552
4	Standard 4	2868064	46880915
5	Standard 5	2899398	47771373
6	Standard 6	2863737	47672412
Mean		2874970	47567078
%RSD		0.66	0.77

Table.3.2: Precision for Ciprofloxacin and Dexamethasone

S.NO	Injection Number	Peak area for Ciprofloxacin	Peak area for Dexamethasone
1	Standard 1	2894916	47944745
2	Standard 2	2919341	47462472
3	Standard 3	2874363	47670552
4	Standard 4	2968064	46880915
5	Standard 5	2899398	47771373
6	Standard 6	2823737	47672412
Mean		291121	47567078
%RSD		1.22	0.86

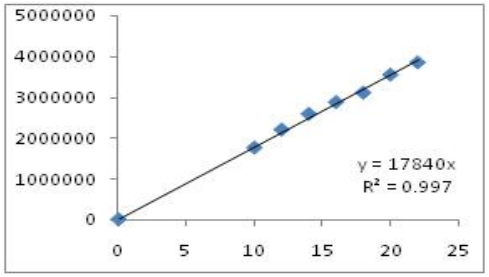

Fig.3 Linearity Plot of Ciprofloxacin

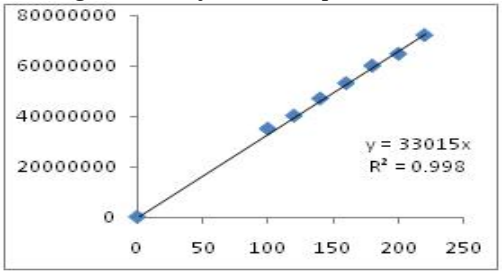

Fig.4 Linearity Plot of Dexamethasone

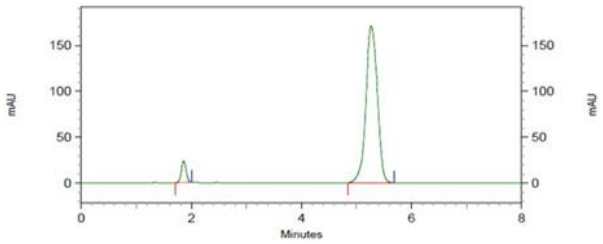

Fig.5: Chromatogram of assay

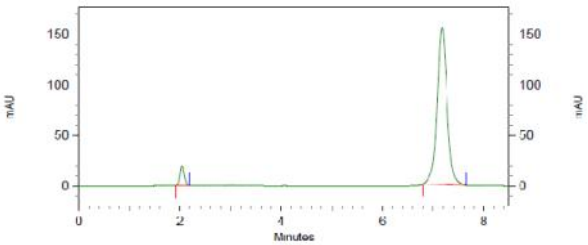

Fig.6: Chromatogram of standard

4. Conclusion

A wavelength of 235nm was selected as a detection wavelength for the estimation of Ciprofloxacin and Dexamethasone in RP-HPLC system. A simple, precise and accurate RP-HPLC method was developed for the analysis of Ciprofloxacin and Dexamethasone in tablet dosage form using the mobile phase consisting of 10mM Phosphate buffer (pH 3.0): Acetonitrile (20:80). The flow rate was found to be optimized at 1.0 ml/min. It reduced the usage of mobile phase. The system suitability parameters like retention time, resolution, efficiency, capacity factor, tailing factor and % RSD was found to be within the limits for the optimized chromatogram.

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