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Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Prednisolone Acetate and Ofloxacin in its Pharmaceutical Dosage Form

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ABSTRACT:

RP-HPLC method has been developed for the estimation of Prednisolone Acetate and Ofloxacin in combined pharmaceutical dosage form. In RP-HPLC method, Chromatographic separation was achieved using BDS Hypersil C18 (250 mm × 4.6 mm i.d. 5µm) analytical column and Buffer Potassium Dihydrogen Phosphate, pH 6.0: Acetonitrile (70:30) as a mobile phase at a flow rate of 1.0 ml/min with detection wavelength 275 nm. Forced degradation study was carried out at a different condition using Buffer Potassium Dihydrogen Phosphate, pH 6.0: Acetonitrile (70:30) as a mobile phase in RP-HPLC method. For RP-HPLC linearity of Ofloxacin and Prednisolone Acetate were found in the range of 10-30 µm/ml and 3-9 µm/ml respectively. The retention time of Prednisolone Acetate and Ofloxacin are found to be 3.593& 5.263 min respectively. In Forced Degradation Study, 24.82 % degradation and 22.18 % degradation were observed in 0.1 N HCL (4 Hours) for Ofloxacin and Prednisolone Acetate respectively. In 0.1 N NaOH (2.5 Hours) 24.29 % degradation and 20.60 % degradation were observed for Ofloxacin and Prednisolone Acetate respectively. 14.97 % degradation and 20.81 % degradation were observed to sunlight exposure for Ofloxacin and Prednisolone Acetate respectively. Data revealed that the developed RP-HPLC method was found to be Simple, Accurate and Precise in accordance with ICH Guidelines. This method can be applied for routine quality control analysis for the estimation of Ofloxacin and Prednisolone Acetate in combined pharmaceutical dosage form.

KEY WORDS: Ofloxacin, Prednisolone Acetate, Stability Indicating RP-HPLC.

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INTRODUCTION:

Conjunctivitis is the inflammation of the conjunctiva, occurs generally due to an infection or an allergic reaction.Ofloxacin is belongs to broad spectrum antibiotic class which is active against both Gram-Positive and Gram-Negetive bacteria. Ofloxacinmainly functions by inhibiting DNA gyrase, a type II topoisomeraseand topoisomerase IV,which is an enzyme necessary to separatereplicated DNA, thus inhibiting bacterial cell division.

Prednisolone Acetate is having glucocorticoid general properties of the corticosteroids class. Prednisolone Acetate can act on the mechanism by inhibit leukocyte infiltration at the site of inflammation, It can act by interference with the mediators of inflammatory response, and humoral immune responses. The anti-inflammatory actions of glucocorticoids are assumed to involve phospholipase A2 inhibitory proteins, lipocortins, which biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

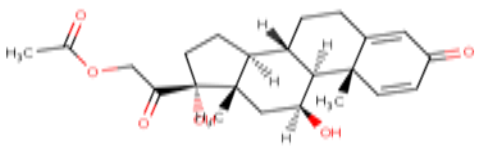

Figure-I-Structure of Prednisolone Acetate

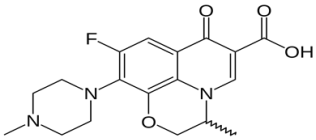

Figure-II-Structure of Ofloxacin

Materials and Method:

Apparatus and Instruments:

HPLC : Young Lin 9101

Column : C18 (250 mm × 4.6 mm i.d. 5µm) Hypersil BDS

Flow Rate : 1.0 ml/ min

Injector : 20 µl fixed loop

Detector : YL9110 Photodiode array (PDA) detector

Selected wavelength : 275 nm

Analytical balance : AUX-200

Reagents and Materials:

Prednisolone Acetate ,Ofloxacin ,Water, Methanol, Acetonitrile,Potassium Dihydrogen Phosphate

Development of RP-HPLC Method:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Prednisolone Acetate (20 ppm) and Ofloxacin (6 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

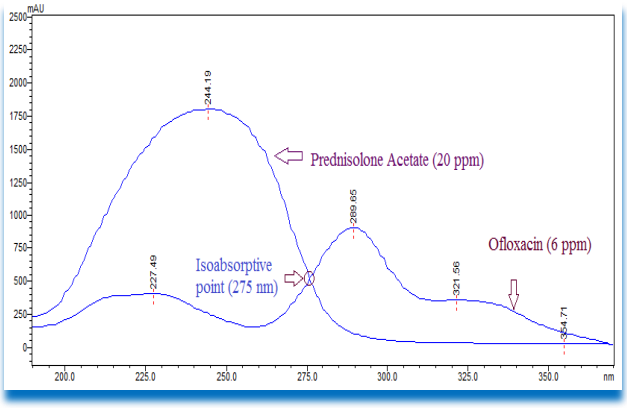

Figure-III-UV Spectra of Prednisolone Acetate (20 ppm) and Ofloxacin (6 ppm) in Methanol

Selection of Mobile Phase:

Trial contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of various trials the mixture of Buffer (Potassium Dihydrogen Phosphate) at pH 6.0 : Acetonitrile (70:30), at 1.0 mL/min flow rate, proved to be better than the other mixture with respect to the peak shape, theoretical plate and asymmetry.

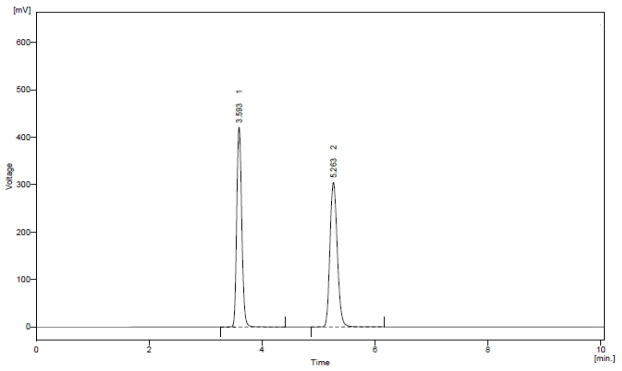

Figure-IV-HPLC Chromatogram of Prednisolone Acetate 20 ppm Buffer (pH 6.0): Acetonitrile (70:30)

Preparation of standard solution of mixtures of Ofloxacin(6 ppm) and Prednisolone Acetate (20 ppm):

(A)Prednisolone Acetate standard stock solution: (200 µg/ml) A 20 mg of Prednisolone Acetate was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase.

(B) Ofloxacinstandard stock solution: (60 µg/ml) A 6 mg of Ofloxacin was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase

(C) Preparation of standard solution of binary mixtures of Prednisolone Acetate (20 µg/ml) and Ofloxacin(6 µg/ml)

Take 1 ml from the Prednisolone Acetate stock solution and 1ml from Ofloxacin stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Validation of RP-HPLC method:

1) Specificity

Specificity of the method was ascertained by analyzing standard drug and sample and blank for the selected drugs. (i.e Prednoisolone Acetate and Ofloxacin).

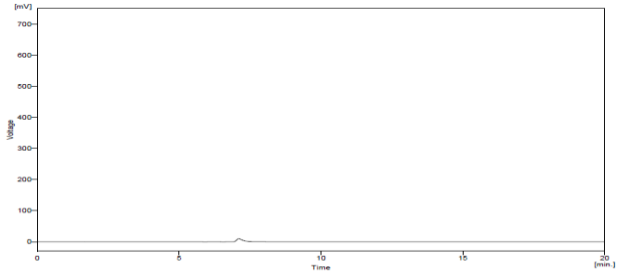

Figure-V-Chromatogram of Ofloxacin and Prednisolone Acetate Blank

The Chromatograms of Ofloxacin and Prednisolone Acetate standards and Ofloxacin and Prednisolone Acetate sample show no interference with the Chromatogram of Ofloxacin and Prednisolone Acetate Blank, so the Developed method is Specific.

2) Linearity:

The linearity for Prednisolone Acetate and Ofloxacin were assessed by analysis of

combined standard solution in range of 10-30 µg/ml and 3-9 µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Prednisolone Acetate (200 µg/ml) andOfloxacin (60 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 10,15,20,25 and 30 µg/ml, and 3,4.5,6,7.5 and 9 µg/ml for Prednisolone Acetate and Ofloxacin respectively.

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve Prednisolone Acetate and Ofloxacinwas found to be 0.996 and 0.999 respectively.

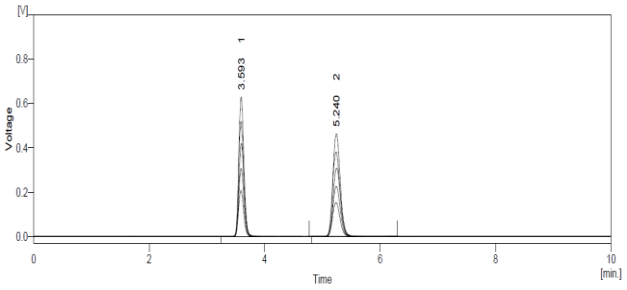

Figure-VI-Overlay chromatogram of different concentrations of mixtures of Prednisolone Acetate and Ofloxacin

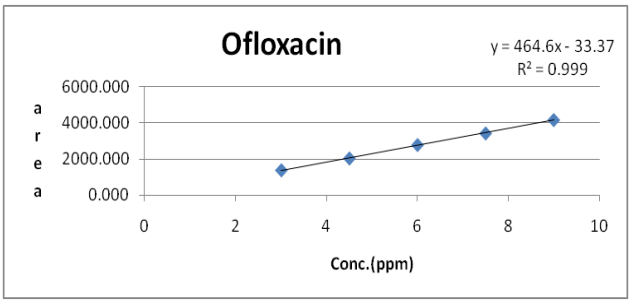

Figure-VII-Calibration Curve of Ofloxacin (3-9 µg/ml)

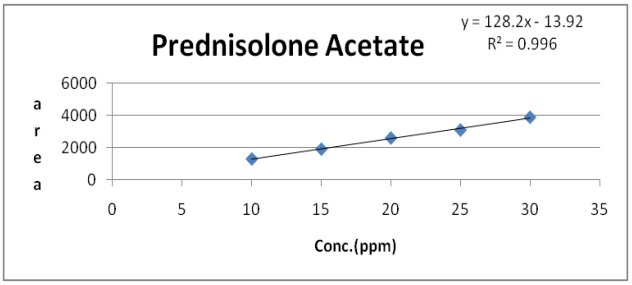

Figure-VIII-Calibration Curve of Prednisolone Acetate (10-30 µg/ml)

3) Precision:

I. Method precision (Repeatability):

The data for repeatability of peak area measurement for Prednisolone Acetate (20 µg/ml) and Ofloxacin (6 µg/ml) based on six measurements of same solution of Prednisolone Acetate (20 µg/ml) and Ofloxacin (6 µg/ml).The % RSD for Prednisolone Acetate and Ofloxacin was found to be 0.42 and 1.49 respectively.

II. Intermediate precision (System precision)

A) Intra-day precision:

Standard solution containing (3,6,9 µg/ml) of Ofloxacin and (10,20,30µg/ml) of Prednisolone Acetate were analysed three times on the same day and % R.S.D. was calculated.

Table-1-Intraday precision data

SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
	Ofloxacin		
1	3	1360.157 ± 16.055	1.180
2	6	2751.935 ± 25.746	0.936
3	9	4127.806± 33.945	0.822
	Prednisolone Acetate		
1	10	1270.066 ± 12.365	0.974
2	20	2569.238 ± 27.825	1.083
3	30	3856.138 ± 34.620	0.898

B) Inter-day Precision

Standard solution containing (3,6,9 µg/ml) of Ofloxacin and (10,20,30µg/ml) of Prednisolone Acetate were analysed three times on the different day and % R.S.D was calculated.

Table-2-Interday precision data

SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
	Ofloxacin		
1	3	1354.395 ± 22.100	1.632
2	6	2750.066± 20.819	0.757
3	9	4121.045± 33.041	0.802
	Prednisolone Acetate		
1	10	1264.626 ± 18.356	1.452
2	20	2567.497± 23.978	0.934
3	30	3856.301 ± 24.087	0.625

4)Accuracy:For Ofloxacin

3 µg/ml drug solution was taken in 3 different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 275 nm. The amount of Ofloxacin was calculated at each level and % recoveries were computed.

For Prednisolone Acetate

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 275 nm. The amount of Prednisolone Acetate was calculated at each level and % recoveries were computed.

Table-3-Recovery data for Ofloxacin

SR. NO.	Conc. (µg/ml)	% Mean Recovery ± S.D
1	80 %	99.805 ± 0.839
2	100 %	99.543 ± 0.299
3	120 %	99.682 ± 0.604

Table-4-Recovery data for Prednisolone Acetate

SR. NO.	Conc. (µg/ml)	% Mean Recovery ± S.D
1	80 %	100.904 ± 1.041
2	100 %	100.485 ± 0.725
3	120 %	100.361 ± 0.713

5) Sensitivity:

The sensitivity measurement of Ofloxacin and Prednisolone Acetate by the use of proposed method was estimated in term of Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Table-5-Limit of Detection data for Ofloxacin and Prednisolone Acetate

Ofloxacin	Prednisolone Acetate
LOD = 3.3 x (SD / Slope) **= 3.3 x (29.756/464.6)** **= 0.211 µg/ml**	LOD = 3.3 x (SD / Slope) = 3.3 x (70.643/128.2) = 1.818 µg/ml

Table-6-Limit of Quantitation data for Ofloxacin and Prednisolone Acetate

Ofloxacin	Prednisolone Acetate
LOQ = 10 x (SD / Slope) **= 10 x (29.756/464.6)** **= 0.640 µg/ml**	LOQ = 10 x (SD / Slope) = 10 x (70.643/128.2) = 5.510 µg/ml

6) Robustness:

Robustness was performed by deliberately changing the chromatographic conditions.The important parameter to be studied was the resolution factor between two peaks. The robustness was checked by changing following parameter one by one tabulated below.

Table-7-Robustness data for Ofloxacin

Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
2864.832	2703.629	2642.628	2834.237	2839.933	2700.930
2875.120	2712.666	2654.498	2819.886	2850.439	2715.545
2898.416	2724.350	2666.089	2864.318	2808.550	2721.617
0.598	0.383	0.442	0.799	0.769	0.392

Table-8-Robustness data for Prednisolone Acetate

Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
2674.587	2524.001	2451.581	2609.190	2632.437	2503.915
2690.263	2534.448	2482.484	2661.675	2667.013	2536.981
2679.691	2537.904	2490.479	2677.345	2659.452	2542.377
0.298	0.286	0.830	1.347	0.685	0.824

System Suitability:

To check system suitability Number of theoretical plate, Resolution, Retention time, Asymmetry and they were determined.

Table-9-System Suitability

Parameters	Prednisolone Acetate	Ofloxacin
Retention Time	3.593	5.263
Theoretical Plates	7153	7470
Asymmetry	1.208	1.229
Resolution	8.077	

Stability Indicating Method for the estimation of Prednisolone Acetate and Ofloxacin By RP-HPLC

1) Acid degradation

Acid decomposition studies were performed by Refluxing 1 ml of stock solution and transferred it into 10 ml of volumetric flask. 2 ml of 0.1 N Hydrochloride solutions was added and mixed well and put for 4 hrs at 70 ºC 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 6 µg/ml for Ofloxacin and 20 µg/ml for Prednisolone Acetate.

24.82 % degradation and 22.18 % degradation were observed in 0.1 N HCL (4 Hours) for Ofloxacin and Prednisolone Acetate respectively.

2) Base degradation

Basic decomposition studies were performed by refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well

and put for 2.5 hrs at 70 ºC 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 6 µg/ml for Ofloxacin and 20 µg/ml for Prednisolone Acetate. In 0.1 N NaOH (2.5 Hours) 24.29 % degradation and 20.60 % degradation were observed for Ofloxacin and Prednisolone Acetate respectively.

3) Oxidative degradation

Oxidative decomposition studies were performed by refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and put for 4 hrs at 70 ºC 250 ml Round bottom flask. After time period the content was cooled to RT. Then the volume was adjusted with diluent to get 6 µg/ml for Ofloxacin and 20 µg/ml for Prednisolone Acetate. In 3% H_2O_2 (4 Hours) 21.96% degradation and 22.30% degradation were observed for Ofloxacin and Prednisolone Acetate respectively.

4) Photo degradation

Photo Degradation studies were performed by refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was keep in presence of Sunlight for 3.5 hrs. Then the volume was adjusted with diluent to get 6 µg/ml for Ofloxacin and 20 µg/ml for Prednisolone Acetate. 24.68 % degradation and 28.26 % degradation were observed to thermal exposure at 110ºC (3 Hours) for Ofloxacin and Prednisolone Acetate respectively.

5) Thermal degradation

Thermal Degradation studies were performed by refluxing 1 ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 110°C for 3 hrs. Then the volume was adjusted with diluent to get 6 µg/ml for Ofloxacin and 20 µg/ml for Prednisolone Acetate. 14.97 % degradation and 20.81 % degradation were observed to sunlight exposure for Ofloxacin and Prednisolone Acetate respectively.

Analysis of marketed formulation by developed method:

Sample Stock Solution (Ofloxacin 60 µg/ml, and Prednisolone Acetate 200 µg/ml):

Take an eye drop equivalent to 6 mg of Ofloxacin, and 20 mg of Prednisolone Acetate and transfer it into 100 ml volumetric flask, Add 60 ml Mobile Phase and Shake it for 15 min and make up the volume with Mobile Phase. The solution is then need to filterthrough Whatman filter paper no. 42.

Working Sample Preparation (Ofloxacin 6 µg/ml, and Prednisolone Acetate 20 µg/ml):

Take 1 ml solution from the standard stock solution and transferred it into 10 ml volumetric flask and make up volume up to the mark with the mobile phase. Inject 20 µl of above solution for assay analysis.

Table-10-Analysis on marketed formulation

Eye Drops	Predfax Eye Drops	
Label claim	**Ofloxacin (0.30 % w/v)**	**Prednisolone Acetate (1% w/v)**
Assay (% of label claim) Mean ± S. D.	98.439±0.886	98.833±0.601

The assay results were comparable to labelled value of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

RESULTS AND DISCUSSION:

For RP-HPLC linearity of Ofloxacin and Prednisolone Acetate were found in the range of 10-30µm/ml and 3-9µm/ml respectively. The retention time of Prednisolone Acetate and Ofloxacin are found to be 3.593&5.263 min respectively. In Forced Degradation Study,24.82 % degradation and 22.18 % degradation were observed in 0.1 N HCL (4 Hours) for Ofloxacin and Prednisolone Acetate respectively.In 0.1 N NaOH (2.5 Hours) 24.29 % degradation and 20.60 % degradation were observed for Ofloxacin and Prednisolone Acetate respectively.In 3% H2O2 (4 Hours) 21.96% degradation and 22.30% degradation were observed for Ofloxacin and Prednisolone Acetate respectively.24.68 % degradation and 28.26 % degradation were observed to thermal exposure at 110ºC (3 Hours) for Ofloxacin and Prednisolone Acetate respectively.14.97 % degradation and 20.81 % degradation were observed to sunlight exposure.

BIBLIOGRAPHY & REFERENCES:

1) Dong W.M., Modern HPLC for Practicing Scientists; A Wiley- Inter Science Publication, USA, 2006, pp 1-9.

2) Kazakevich Y. and Lo Brutto R., HPLC For Pharmaceutical Scientists; A John Wiley and Sons, 2007, pp 1-6.

3) Snyder LR., Kirkland JJ and Glajch LJ., Introduction to Modern Liquid Chromatography; 2nd Edn; A Wiley- Inter Science Publication, NY, USA, 1997, pp 5-42.

4) Snyder LR., Kirkland JJ and Glajch LJ., Practical HPLC Method Development; 2ndEdn.;A Wiley- Inter Science Publication, NY, USA, 1997, pp 3-35.

5) Skoog DA., Holler FJ and Nieman TA., Introduction to UV Spectroscopy; Principle of Instrumental Analysis; 5th Edn; Thomson Brooks/Cole publication, 2005, pp 301.

6) Beckett AH and Stenlake JB., UV-visible Spectrophotometry: Practical Pharmaceutical Chemistry; 4th Edn; Part-II, C.B.S. Publishers, Delhi, 2001, pp. 275-299.

7) FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft Guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.

8) Patel SA and Patel NJ, "Development and Validation of RP-HPLC Method for Simultaneous

Estimation of Cefixime Trihydrate and Ofloxacin in Tablets." Int. J. Pharma. Tech. Res.2011, 3(4), 1958-1962.

9) Dubey A, Pillai S, Sahu S and Keskar N, "Spectrophotometric estimation of Cefixime and Ofloxacin from Tablet Dosage Form." Int. J. Pharma. and Life Sci.2011, 2(3), 329-632.

10) Fegade JD, Bhole RP, Chaudhry RY and Patil VR, "Simultaneous Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form by Reverse Phase High Performance Liquid Chromatography." Asi. J. Chem.2009, 21(5), 3360-3366.

11) Fonte AN and Legro MP, "Simple and Fast RP-HPLC Method for the Determination of Prednisolone Sodium Phosphate, Prednisolone, Atropine, and Homoatropine as Residuals in Cleaning Validation of Industrial Pharmaceutical Equipment." J.liq. chrom. 2012, 36(2).

12) Ashok R, Prakash P and Tamil R, "Development and Validation of Analytical Method for Estimation of Prednisolone in Bulk and Tablets using UV-Visible Spectroscopy." Int. j. of pharm and pharm sci.2011,3, 184.