WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 5, 369-381. Research Article ISSN 2277 – 7105

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OFLOXACIN AND DEXAMETHASONE SODIUM PHOSPHATE BY RP-HPLC

G.N.Renukadevi*, M.Sri lakshmi, V.Prathyusha, SK.Sharmila, K.Shantha Kumari

Department of Pharmaceutical Analysis, Nirmala College of pharmaceutical sciences, Atmakuru (village), Mangalagiri (Mandal), 522503 Andhra Pradesh, India

Article Received on
29 April 2014,

Revised on 07 June 2014,
Accepted on 01 July 2014

***Correspondence for Author**
G.N.Renukadevi
Department of Pharmaceutical Analysis, Nirmala College of pharmaceutical sciences, Atmakuru (village), Mangalagiri (Mandal), 522503 Andhra Pradesh, India

ABSTRACT

A new simple isocratic analytical method has been developed and validated for estimation of Ofloxacin and Dexamethasone sodium phosphate was carried out on Agilent CN, 250mm x 4.6mm, 5µm column in isocratic mode using mobile phase composition of Acetic acid Buffer : Acetonitrile (70 : 30% v/v) with flow rate of 1.0 ml /min at 241 nm. The linearity of the method was demonstrated over the concentration range of 6-180 µg/ml for ofloxacin and 2-60 µg/ml for Dexamethasone sodium phosphate with correlation coefficient of 0.999 & 0.999 respectively. The developed method was validated as per ICH guidelines. The amounts of both the drugs were found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. Hence, this method is used for routine analysis of titled drugs in combination of tablet formulation.

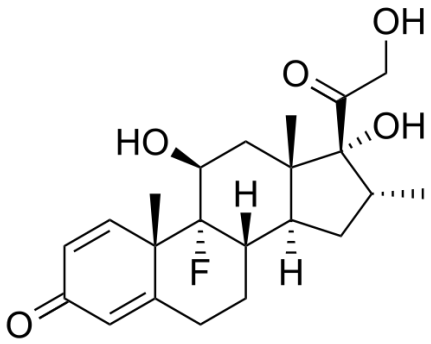
KEYWORDS: Ofloxacin and Dexamethasone sodium phosphate, RP-HPLC, Validation.

INTRODUCTION

Dexamethasone Sodium Phosphate

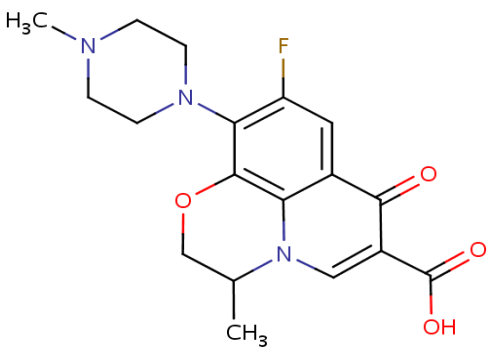
Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs that has anti-inflammatory and immunosuppressant effects. It is 25 times more potent than cortisol in its glucocorticoid effect, while having minimal mineralocorticoid effect. It is also given in small amounts before and/or after some forms of dental surgery, such as the extraction of the wisdom teeth, an operation which often leaves the patient with puffy,

swollen cheeks. Dexamethasone is used in transvenous screw-in cardiac pacing leads to minimize the inflammatory response of the myocardium. The steroid is released into the myocardium as soon as the screw is extended and can play a significant role in minimizing the acute pacing threshold due to the reduction of inflammatory response. Dexamethasone is often administered before antibiotics in cases of bacterial meningitis. It then acts to reduce the inflammatory response of the body to the bacteria killed by the antibiotics (bacterial death releases proinflammatory mediators that can cause a response which is harmful to the patient), thus improving prognosis [1-2]



Ofloxacin

Ofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class considered to be a second-generation fluoroquinolone The original brand, **Floxin.** Ofloxacin is a quinolone/fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria [3-4]



Literature review of simultaneous estimation of ofloxacin and dexamethasone reveals HPLC methods [5-9], Individual UV methods [10] , HPTLC methods [11] . The aim of the present work is to develop and validate a new, simple, better and economical method for the simultaneous estimation of OFLOXACIN and DEXAMETHASONE in Eye Drops Formulation by RP-HPLC with improved conditions and parameters for routine use in the laboratories.

Materials

Instrument	Specifications
HPLC	Waters, 2695 separation module
Software	Empower,
Detector	UV-Visible detector
Analytical balance	Sartorius
UV-Visible spectrophotometer	Shimadzu (UV-2450)
Sonicator	Biotechnics
pH meter	Cyberscan

Chemicals and Reagents

HPLC grade Acetonitrile, HPLC grade Water, are used as solvents. Acetic acid of analytical grade was used in the buffer preparation.

Formulation used

Commercial Pharmaceutical preparations which were claimed to contain 0.3%of Ofloxacin and 0.1% of Dexamethasone were obtained from local market

Liquid Chromatographic Conditions

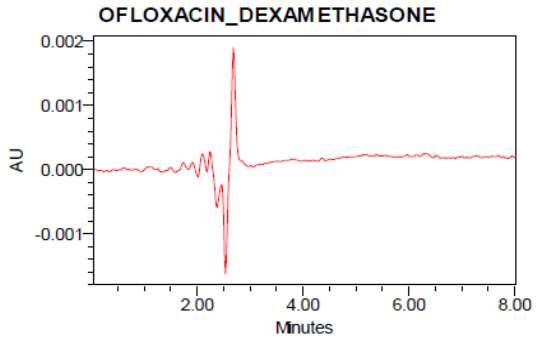
The developed RP-HPLC method for the simultaneous estimation of Ofloxacin and dexamethasone was carried out on Agilent CN, 250mm x 4.6mm, 5µm column in isocratic mode using mobile phase composition of Acetic acid Buffer : Acetonitrile (70 : 30% v/v) with flow rate of 1.0 ml /min at 241 nm.

Preparation of standard stock solution

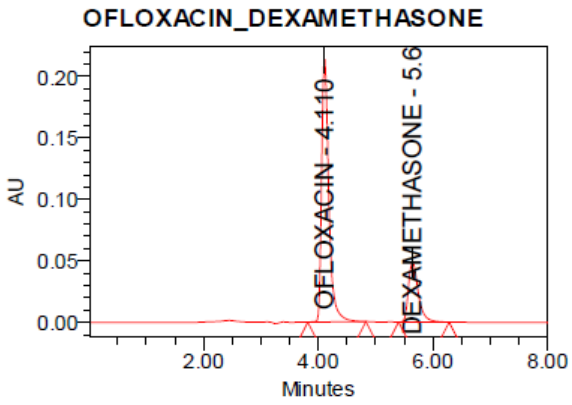
Weigh accurately about 120 mg of Ofloxacin working standard and 40 mg of Dexamethasone working standard into a 100 ml volumetric flask and Add 70 ml of diluent, sonicated to dissolve and dilute to volume diluent. Further dilute 5 ml to 50 ml with the diluent.

Preparation of Sample solution

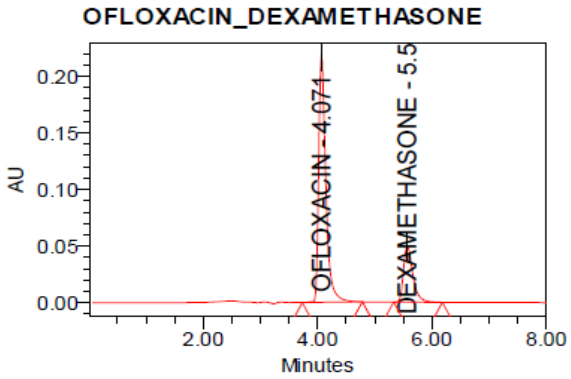
Pipette 4ml of solution into a 100 ml volumetric flask. Add 70 ml of diluent, sonicate to dissolve and dilute to volume with diluent .



Chromatogram of Ofloxacin and dexamethasone (Blank)



Chromatogram of Ofloxacin and dexamethasone (Standard)



Chromatogram of Ofloxacin and dexamethasone (Sample)

Calculation: Calculate the % assay of each drug by using the following formula

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.wt}{L.claim} \times 100$$

Table1: Assay results of ofloxacin and dexamethasone sodium phosphate

Drug	Labeled amount(mg)	Amount present (mg)	% Assay
Ofloxacin	0.3	0.298	99.9
Dexamethasone sodium phosphate	0.1	0.101	100.1

METHOD VALIDATION

The developed analytical method was validated as per ICH guidelines with respect to parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection, limit of quantification, system suitability and solution stability.

Linearity

The linearity of the method was demonstrated over the concentration range of 6 -180 µg/ml for ofloxacin and 2-60 µg/ml for Dexamethasone. Aliquots of the above solutions were prepared from stock solution and labelled as solution 1, 2, 3, 4, 5 and 6 respectively and the solutions were injected into the HPLC system as per test procedure. Calibration curve for Ofloxacin and Dexamethasone was plotted accordingly by taking concentration vs peak area. The chromatograms and results were shown below.

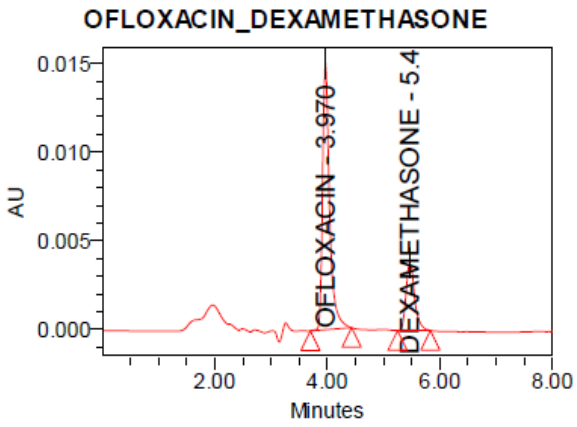
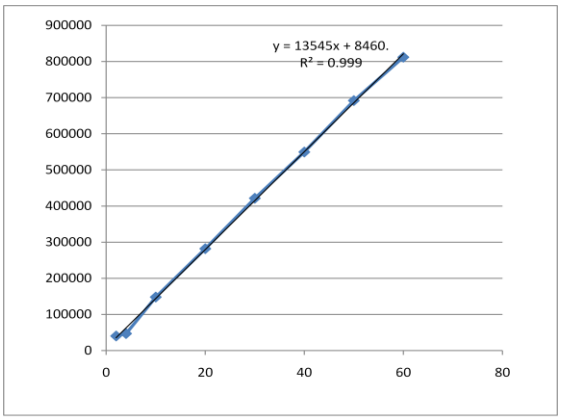


Table 2: Linearity data of ofloxacin and Dexamethasone

S.no	Concentration of ofloxacin µg/ml	Peak area	Concentrationof dexamethasone µg/ml	Peak area
1	6	129751	2	40467
2	12	154697	4	47172
3	30	487982	10	148187
4	60	932126	20	282103
5	90	1388293	30	421694
6	120	1834868	40	549660

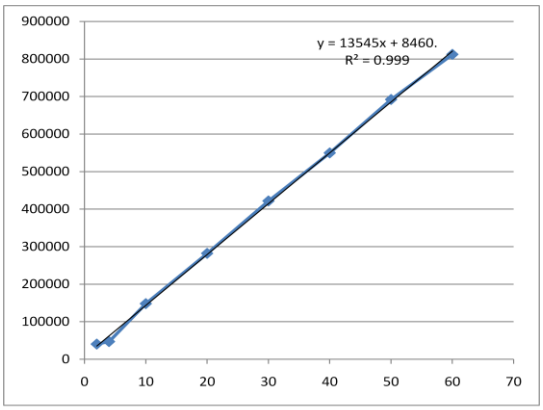
7	150	2302178	50	692022
8	180	2690055	60	812105
	R2=0.999		R2=0.999	

Calibratiaon curve of Ofloxacin



Calibration curve of Ofloxacin

Calibratiaon curve of Dexamethasone



Calibration curve of Dexamethasone

PRECISION

a) System precision : System precision was carried out using six replicate injections of the standard concentration. The chromatograms were recorded and mean, standard deviation and %RSD was calculated. The results and chromatograms were shown below.

Acceptance criteria

The % Relative standard deviation of Peak areas of Ofloxacin and dexamethasone from the six replicate injections should be not more than 2.0.

Table 3: System precision of Ofloxacin and Dexamethasone

S.no	Concentration of ofloxacin µg/ml	Peak area	Concentrationof dexamethasone µg/ml	Peak area
1	3.545	1859262	5.826	550348
2	3.546	1851449	5.831	551025
3	3.546	1858539	5.839	550658
4	3.546	1866024	5.840	552962
5	3.544	1867938	5.840	553025
6	3.547	1846238	5.913	552710
Mean	3.547	1858242	5.848	551788
S.D	0.004816	8317.95	0.0322	1240.26
%RSD	0.1357%	0.448%	0.550%	0.225

Method precision

Method precision was carried out using six different sample preparations from same homogenous blend of marketed sample. The chromatograms were recorded and mean, standard deviation and %RSD was calculated. The results and chromatograms were shown below.

Acceptance criteria

The % Relative standard deviation of Peak areas of Ofloxacin and dexamethasone from the six replicate injections should be not more than 2.0 %.

Table 4: Method precision of Ofloxacin and Dexamethasone

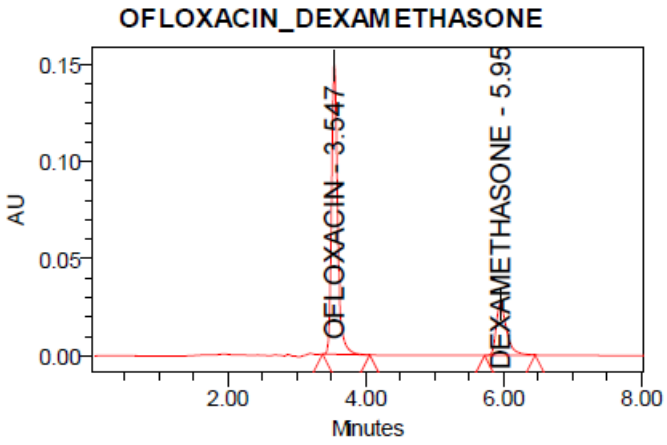
S.no	Concentration of ofloxacin µg/ml	Peak area	Concentrationof dexamethasone µg/ml	Peak area
1	3.545	1859262	5.826	550348
2	3.546	1851449	5.831	551025
3	3.546	1858539	5.839	550658
4	3.546	1866024	5.840	552962
5	3.544	1867938	5.840	553025
6	3.547	1846238	5.913	552710
Mean	3.547	1858242	5.848	551788
S.D	0.004816	8317.95	0.0322	1240.26
%RSD	0.1357%	0.448%	0.550%	0.225

ACCURACY

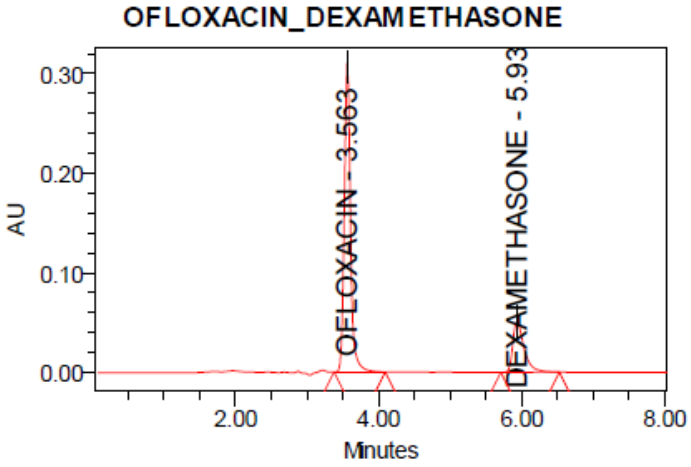
A study of accuracy was conducted by means of recovery studies. Recovery studies were carried out at three different levels. The preanalysed sample was spiked with 50%, 100%, and 150% of mixed standard solution. The mixtures were analysed by the proposed method. The study was carried out in triplicate. The average % recoveries of both the drugs were calculated and the results and chromatograms were shown below.

Acceptance criteria

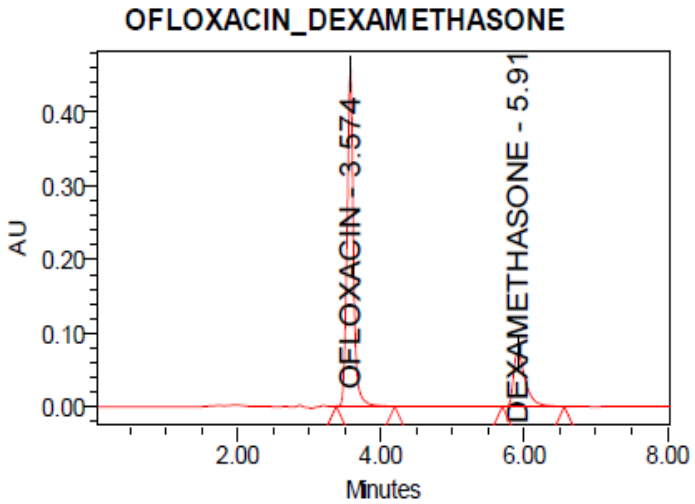
The mean % recovery of the Ofloxacin and dexamethasone at each level should be not less than 98.0% and not more than 102.0%



Chromatogram for Accuracy (50%spike)



Chromatogram for Accuracy (100%spike)



Chromatogram for Accuracy (150%spike)

Table 5: Accuracy of Ofloxacin

Sample No.	Spike Level	Amount (µg / ml) Added	Amount (µg / ml) Recovered	% Recovery	Statistical analysis	
1	50 %	6	5.99	99.8	Mean	100.4
	50 %	6	6.04	100.7	SD	0.48
	50 %	6	5.94	100.7	%RSD	0.480
2	100 %	12	12.04	10.3	Mean	100.1
	100%	12	12.18	100.7	SD	0.73
	100%	12	12.11	99.3	%RSD	0.730
3	150 %	18	18.41	100.1	Mean	99.9
	150 %	18	18.41	99.5	SD	0.33
	150 %	18	18.10	100.1	%RSD	0.330

Table 6 : Accuracy of Dexamethasone

Sample No.	Spike Level	Amount (µg / ml) Added	Amount (µg / ml) Recovered	% Recovery	Statistical analysis	
1	50 %	2	2.09	99.5	Mean	99.4
	50 %	2	2.09	99.5	SD	0.28
	50 %	2	2.08	99	%RSD	0.280
2	100 %	4	4.08	99.5	Mean	99.9
	100%	4	4.1	100	SD	0.37
	100%	4	4.1	100.2	%RSD	0.370
3	150 %	6	6.27	99.5	Mean	99.4
	150 %	6	6.25	99.52	SD	0.18
	150 %	6	6.27	99.5	%RSD	0.180

Observation

The recovery results indicated that the test method has an acceptance level of accuracy. The results were found to be within the limits.

ROBUSTNESS

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

Following optimized conditions were slightly varied.

a. Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates (± 0.2 ml/min) i.e.,

0.8 ml/min and 1.2 ml/min. The effect of variation of flow rate was evaluated.

Table no: 7 Effect of variation of flowrate plus

S.no	Concentration of ofloxacin µg/ml	Peak area	Concentrationof dexamethasone µg/ml	Peak area
1	2.975	1486306	4.965	462561
2	2.980	1478282	4.968	463589
3	2.979	1485742	4.969	463114
4	2,979	1478127	4.968	461381
5	2.979	1479216	4.969	462278
6	2.979	1484319	4.971	464902
Mean	2.978	1482007	4.968	462971
S.D	0.001843	3868.03	0.002	1208.35
%RSD	0.061%	0.261%	0.0402%	0.261%

Table no: 8 Effect of variation of flowrate minus

S.no	Concentration of ofloxacin µg/ml	Peak area	Concentrationof dexamethasone µg/ml	Peak area
1	4.445	2243643	7.434	693918
2	4.446	2254657	7.434	692940
3	4.445	2247758	7.434	696059
4	4.445	2250483	7.433	693708
5	4.446	2246739	7.435	694692
6	4.446	2260062	7.437	698869
Mean	4.445	2250557	7.434	695031
S.D	0.0007745	14065.98	0.001483	2154.59
%RSD	0.0174%	0.625%	0.0199%	0.310%

LOD and LOQ were calculated by the method based on the standard deviation (σ) and slope of the calibration curve, using the formula

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The LOD and LOQ were calculated as per formula and was shown in the table 9

Table 9: Limit of Detection and Limit of Quantification

Sample	LOD (µg/ml)	LOQ (µg/ml)
Ofloxacin	3.215 µg/ml	9.74 µg/ml
Dexamethasone	0.782 µg/ml	2.371 µg/ml

RESULTS AND DISCUSSION

The developed RP-HPLC method for the simultaneous estimation of Ofloxacin and dexamethasone was carried out on Agilent CN, (250mm x 4.6mm, 5µm) column in isocratic mode using mobile phase composition of 1ml of acetic acid in water : Acetonitrile (70 : 30% v/v) with flow rate of 1.0 ml /min at 241 nm. The average retention times for Ofloxacin and dexamethasone was found to be 3-4 and 5-6min respectively. From the results % assay value of Ofloxacin and dexamethasone were found to be 99.9% and 100.1% respectively.

The Linearity of Ofloxacin and dexamethasone was carried out at different concentrations ranging from 6-180 µg/ml and 2-60 µg/ml. Correlation coefficient was found to be 0.999, 0.999, which indicates that the concentration had given good linearity The %RSD values of Ofloxacin and dexamethasone for System Precision was found to be 0.448and 0.225% respectively as shown in the table. As these results are within the acceptance limit of less than 2%, indicates that the proposed method has good reproducibility. The results are good for both method precision and system precision.

From the results shown in accuracy table it was found that the mean percentage recovery values of pure drug were found to be 100.1 % for Ofloxacin and 99.6% for Dexamethasone, and as these results are within the acceptance limit of 98%-102% which indicates that the method was accurate. The robustness of the developed method was evaluated by changing the flow rate and mobile phase composition. All the parameters were within the limits at all variable conditions as shown in table which indicates that the method was robust.

Table 10 : Validation Parameters of Ofloxacin and Dexamethasone by RP-HPLC

S.no	Parameters	Ofloxacin	Dexamethasone
1	Linearity (µg/ml)	6-180	2-60
2	Correlation Coefficient	0.999	0.999
3	Precision(%RSD) (i)Method Precision	0.448%	0.225%
	(ii)System Precision	0.448%	0.225%
4	LOD	3.215	0.782

5	LOQ	9.74	2.371
6	Accuracy (mean % recovery)	100.1%	99.6%
7	Assay (%)	99.9%	100.1%

CONCLUSION

The proposed method was validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of Ofloxacin and Dexamethasone sodium phosphate in bulk and marketed formulations.

ACKNOWLEDGEMENT

The authors are thankful to Nirmala College of Pharmacy for providing gift samples of Ofloxacin and Dexamethasone sodium phosphate. The authors would like to thank, regular counsel and guidance of Prof. K.Shanta Kumari, Prof. S.A.Rahaman Department of Pharmaceutical analysis, Nirmala College of Pharmacy, Nagarjuna University, Mangalagiri, Guntur (Dist.), Andhra Pradesh for providing necessary facilities to carry out the Research work.

REFERENCES

1. en.wikipedia.org/wiki/Dexamethasone
2. www.drugbank.ca/drugs/DB01234
3. en.wikipedia.org/wiki/Ofloxacin
4. www.drugbank.ca/drugs/DB01165
5. Karanam R. Sireesha *, Katakam Prakash, Hplc-Uv Method For Simultaneous Determination Of Ofloxacin And Dexamethasone Sodium Phosphate Vol 4, Issue 1, 2012
6. Garcia CV, Breier AR, Steppe M, Schapoval EE, Oppe TP. Determination of dexamethasone acetate in cream by HPLC.J Pharm Biomed Anal. 2003 Mar 10;31(3):597-600.
7. Huetos O, Ramos M, Martín de Pozuelo M, San Andrés M, Reuvers TB. Determination of dexamethasone in feed by TLC and HPLC. [PubMed - indexed for MEDLINE] 10746317

8. Kumar V, Mostafa S, Kayo MW, Goldberg EP, Derendorf H. HPLC determination of dexamethasone in human plasma and its application to an in vitro release study from endovascular stents. [PubMed - indexed for MEDLINE] 17152981
9. Tan Ruiwei Mo Mingxiu Li Genghao Simultaneous Determination Of Ofloxacin And Dexamethasone Sodium Phosphate In Compound Ofloxacin Nasal Drops By Rp Hplc 《West China Journal Of Pharmaceutcal Sciences》 1999-01.
10. Rossana Barcellos Friedrich; Aline Ravanello; Luiz Carlos Cichota; Clarice Madalena Bueno Rolim; Ruy Carlos Ruver Beck Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablets Quím. Nova vol.32 no.4 São Paulo 2009
11. Jaydeepkumar K. Maradiya, Validated Hptlc Method For Simultaneous determination Of Ofloxacin And Dexamethasone Sodium Phosphate In Eye Drops Volume 2, Issue 2, 593-600.
12. International Conference on Harmonization, Validation of Analytical Procedures, Methodology, Federal Register, Nov. 1996.
13. International Conference on Harmonization, Draft Guideline on Validation of Analytical Procedures, Definitions and Terminology, Federal Register (26), 1995.