JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Simultaneous RP-HPLC Determination of Sparfloxacin and Dexamethasone in Combined Dosage Form

Kunjal L. Vegad*[1], Noopur H. Dani[2], Dhwani A. Shah[3], Ekta D. Patel[4], Yogesh K. patel[5], Chhaya R. Macwana[6]

1. Assistant Professor, Department of Pharmacognosy, Sharda School of Pharmacy, Gandhinagar, Pethapur. Gujarat, India
2. Student of Master of Pharmacy, Department of Quality Assurance, Sharda School of Pharmacy, Gandhinagar, Pethapur, Gujarat, India
3. Assistant Professor, Department of Pharmaceutical Chemistry, Sharda School of Pharmacy, Gandhinagar, Pethapur, Gujarat, India
4. Assistant Professor, Department of Pharmacology, Sharda School of Pharmacy, Gandhinagar, Pethapur, Gujarat, India
5. Associate Professor, Department of Pharmaceutics, Sharda School of Pharmacy, Gandhinagar, Pethapur, Gujarat, India
6. Assistant Professor, Department of Pharmaceutics, Sharda School of Pharmacy, Gandhinagar, Pethapur, Gujarat, India.

Article history:

Received 03 May 2017
Accepted 02 Jun 2017
Available online 1 Aug 2017

Citation:
Vegad K. L., Dani N.H., Shah D. A., Patel E. D., Patel Y. K., Macwana C. R. Simultaneous RP-HPLC Determination of Sparfloxacin and Dexamethasone in Combined Dosage Form **J Pharm Sci Bioscientific Res**. 2017. 7(4): 270-278

For Correspondence:
Kunjal L. Vegad
Assistant Professor, Department of Pharmacognosy, Sharda School of Pharmacy, Gandhinagar, Pethapur. Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

A precise, simple, accurate and selective method was developed and validate for estimation of SPARFLOXACIN and DEXAMETHASONE in laboratory prepared mixtures as well as in combined dosage form. Reversed phase high performance liquid chromatographic (RP-HPLC) method was developed for routine quantification of Sparfloxacin and Dexamethasone in laboratory prepared mixtures as well as in combined dosage form. Chromatographic separation was achieved on a BDS hypersil C18 (5µ, 250 x 4.6 mm) utilizing mobile phase of 0.1% Potassium dihydrogen phosphate: Acetonitrile (60:40 v/v) at a flow rate of 1 mL/min with UV detection at 247 nm. The method has been validated for linearity, accuracy and precision. In RP-HPLC method, the calibration graphs were linear in the concentration range of 6-36 µg/mL for Sparfloxacin and 2-12 µg/mL for Dexamethasone with mean recoveries of 99.91% and 99.94% for Sparfloxacin and Dexamethasone respectively. The results obtained by RP-HPLC methods are rapid, accurate and precise.

KEY WORDS: RP-HPLC, Sparfloxacin, Dexamethasone, Simultaneous estimation

1. INTRODUCTION

1.1 Sparfloxacin [1-2]

Sparfloxacinum [Latin] is synonym of Sparfloxacin. Sparfloxacin is chemically designated as 5-amino- 1-cyclopropyl- 7- [(3R,5S)- 3,5- dimethylpiperazin-2-yl]- 6,8-difluoro-4-oxo- 1,4-dihydroquinoline-3- carboxylic acid (Figure 1).

Sparfloxacin, trade names Zagam and Zagam Respipac, is a fluoroquinolone antibiotic used in the treatment of bacterial infections. It is not official in IP, BP, USP, EP and JP, but it is widely utilized to treat infections of the lower respiratory system, urinary tract, sinuses, the skin, bones and joints and prostate. Sparfloxacin is also used for inhalational anthrax, STDs, severe bronchial infections, infectious diarrhea, typhoid fever, and pneumonia.

Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. Quinolones can enter cells easily via porins and therefore are often used to treat intracellular pathogens such as Legionella pneumophila and Mycoplasma pneumoniae. For many gram-negative bacteria DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria.

1.2 Dexamethasone [3-6]

Dexamethasone is chemically designated as (8S, 9R, 10S, 11S, 13S, 14S, 16R,17R)-9-fluoro-11,17-dihydroxy-17-(2-

hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16 octahydrocyclopenta [a] phenanthren-3-one, 9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-Dione (Figure 2).

Dexamethasone is a synthetic glucocorticoid and is an isomer of betamethasone. It acts as anti-inflammatory and immunosuppressant. Its potency is about 20-30 times that of hydrocortisone and 4-5 times of prednisone. [16] Unbound Dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic receptors. This results in a modification of transcription and, hence, protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humeral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of Dexamethasone are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

1.3 Introduction to dosage form [7-10] :

Spar D eye drops was manufactured by Biomedica International Ltd. Each eye drops claimed to contain 0.3% w/v of sparfloxacin and 0.1% w/v of dexamethasone. Spar D Drops is used for Pneumonia infections, Tuberculosis, Ear infections, Sinus infections, Throat infections, Bacterial infections and other conditions. Spar D Drops works by inhibiting the bacterial growth and killing the bacteria; preventing the release of chemicals responsible for inflammation.

2. MATERIAL AND METHODS

System suitability parameters

System suitability parameters like average peak area of standards, resolution, tailing factors etc. has been measured and data were shown in Table 1.

2.1. Reagent and Chemicals

2.1.1. Pure Samples

Sparfloxacin working standard was kindly gifted by Astron Research Limited (Ahmedabad, India). Dexamethasone working standard was kindly gifted by Indiana Ophthalmics (Surendranagar, India).

2.1.2. Chemicals used in RP-HPLC method

Acetonitrile (HPLC), Potassium dihydrogen phosphate, Water (HPLC), - Orthophosphoric acid (AR), Triethylamine (AR) and Methanol (HPLC) were procured from Merck, Rankem.

2.2 Wavelength determination

0.2 mL of sparfloxacin and dexamethasone working standard solutions were separately transferred into a 10 mL volumetric flask and diluted to volume with mobile phase. The zero order overlain spectrums of the prepared solutions were recorded from 200 to 400 nm in double beam UV-visible spectrophotometer (Shimadzu, model 1800). The wavelength of maximum absorption for both the drugs sparfloxacin and dexamethasone showed 292 nm (λ1) and 240 nm (λ2) respectively. The isoabsorptive point was found at 247 nm and it was selected as wavelength of determination for both the drugs. The overlain spectrum of sparfloxacin and dexamethasone was shown in Figure 3.

2.3. Chromatographic conditions

The HPLC system consisted of Shimadzu LC-20A system equipped with model LC-20AT pump, SPD- 20AT Shimadzu UV- Vis (Diode array) detector, Hamilton syringe and DGU-20A5 online degasser, and a Rheodyne injection valve. Peak areas were integrated using a Spinchrom. Software program. Experimental conditions were optimized on a BDS Hypersil C 18 column (5µ, 250 x 4.6 mm), Thermo scientific at room temperature using 0.1 % KH2PO4: ACN (60:40, V/V) as mobile phase. Mobile phase was flowed at 1 mL/min and all chromatographic experiments were performed at room temperature (25 ˚C ± 2˚C)

2.3.1 Preparation of mobile phase

Preparation of 0.1 % KH2PO4:

Phosphate buffer was prepared by dissolving 100mg of KH2PO4 in 100 mL of water. Its pH was adjusted to 3.00 ± 0.05 using orthophosphoric acid.

Mobile Phase: Mix 60 mL of the buffer solution with 40 mL of acetonitrile. Sonicate for 30 minutes and filter through 0.45 µm size membrane filter. [0.1 % KH2PO4: ACN (60:40, V/V)]

2.3.2. Preparation of stock solution

2.3.2.1. Standard Stock Solution

Accurately weighed amount of Sparfloxacin equivalent to 60 mg of free base was transferred to 100 mL of volumetric flask. It was dissolved in 10 mL of mobile phase, sonicated for 10 min and diluted up to mark with mobile phase (S1=0.6 mg/mL).

Accurately weighed amount of standard Dexamethasone equivalent to 20 mg of free base was transferred to 100 mL of volumetric flask (protected from light due to photosensitivity). It was dissolved in 10 mL of mobile phase, sonicated for 10 min and diluted up to mark with mobile phase (S2=0.2 mg/mL).

2.3.2.2. Sample solution

SPAR-D eye drops (1 mL) were transferred directly from the container (0.3% w/v Sparfloxacin and 0.1% w/v Dexamethasone) in 10mL volumetric flask, volume was adjusted up to the mark with mobile phase. The solution was sonicated for 30 min and filtered through 0.45µm membrane filter to obtain final concentration about 300 µg/mL of sparfloxacin and 200 µg/mL of dexamaethasone. 0.5 mL of this solution was diluted up to 10 mL with mobile phase to get final concentration of 15 µg/mL and 5 µg/mL of sparfloxacin and dexamethasone respectively.

2.3.2.3. Standard Solutions for linearity

Linearity was studied by preparing standard solutions at 6 different concentrations. Each concentration was repeated 5 times. The linearity range for Sparfloxacin and Dexamethasone were found to be 6—36 µg/mL and 2—12 µg/mL, respectively. Calibration curve were obtained by plotting respective peak area against concentration in µg/mL and the regression equation was computed.

2.3.3. Validation of the developed method:

To be done by using following parameters as per International Conference on Harmonization (ICH Guidelines).[11]

1.Specificity
2. Linearity
3.Range
4.Accuracy
5.Precision
6.Detection limit
7.Quantitation limit
8.Robustness
9.System suitability testing

2.4 Method validation [12]

2.4.1. Linearity and range:

Linearity was studied by preparing standard solutions at 6 different concentrations. Each concentration was repeated 5 times. The linearity range for Sparfloxacin and Dexamethasone were found to be 6—36 µg/mL and 2—12 µg/mL, respectively. Linearity was assessed in the terms of slope, intercept and correlation coefficient for both the drugs. Linearity range was established through consideration of practical range necessary, according to each drug concentration present in the pharmaceutical product to give accurate and precise results. According to ICH, at least 5 concentrations must be used. In this study, 6 concentrations were chosen for each drug.

2.4.2 Precision:

The precision of analytical method is the degree of agreement among individual result when the method is applied to multiple sampling of homogenous samples. It provides an indication of random error in results and was expressed as % Relative standard deviation.

2.4.2.1 Intraday Precision

Intraday precision was determined by assay of sample solution three times in a day for three different concentrations (Combined standard samples of concentrations 12, 18, 24 µg/mL for Sparfloxacin and 4, 6, 8 µg/mL for Dexamethasone).

2.4.2.2 Interday Precision

Interday precision was determined by an assay of sample solution on three different days for three different concentrations (Combined standard samples of concentrations 12, 18, 24 µg/mL for Sparfloxacin and 4, 6, 8 µg/mL for Dexamethasone).

2.4.2.3. Repeatability

Repeatability was checked by repeatedly (n=6) injecting same concentration of Sparfloxacin (18 µg/mL) and Dexamethasone (6 µg/mL) and result was recorded.

2.4.3 Limit of detection (LOD) and Limit of quantitation (LOQ):

LOD is the lowest concentration of the analyte in sample that can be detected, but not necessarily quantitated precisely and accurately. It is expressed in terms of

concentration units. Limit of Detection values are always specific for a particular set of experimental conditions.LOQ is the lowest concentration of analyte in a sample that may be measured in a sample matrix such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. The value of LOQ is almost 3-10 times higher than LOD. According to the ICH recommendation, the approach based on the standard deviation (SD) of the response and slope was use for the determining the LOD and LOQ values.

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where σ = Standard deviation of response and S = Slope of calibration curve.

2.4.4 Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Accuracy of the method is determined by performing the recovery studies. Recovery study was performed by addition of known amounts of standard drugs to a known concentration of commercial pharmaceutical product (standard addition method).

Standard drug was added at three different concentrations (12, 15, 18 µg/mL of Sparfloxacin and 4, 5, 6 µg/mL of Dexamethasone) to pre-analyzed sample (SPAR D Eye drops i.e. 15 µg/mL of Sparfloxacin and 5 µg/mL of Dexamethasone) and mixture were analyzed by proposed method. The experiment was repeated three times.

2.4.5 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. In RP-HPLC method development, following parameters were changed, one by one and their effects were observed on system suitability test and assay.

Change in mobile phase composition by ± 2 ml of organic solvent, change in pH by ± 0.2 unit and change in flow rate by ± 0.2 mL/min.

Change in mobile phase composition:

Inject standard working solution (18 µg/mL of Sparfloxacin and 6 µg/mL of Dexamethasone) three times by change in the mobile phase composition by ± 2.0 ml of organic solvent (62:38 v/v and 58:42 v/v) of developed method. Calculate the %RSD of mean area for change in method parameter.

Change in pH:

Inject standard working solution (18 µg/mL of Sparfloxacin and 6 µg/mL of Dexamethasone) three times by change in the pH by ± 0.2 unit of buffer solution (3.2 pH unit and 2.8 unit) of developed method. Calculate the %RSD of mean area for change in method parameter.

Change in Flow rate:

Inject standard working solution (18 µg/mL of Sparfloxacin and 6 µg/mL of Dexamethasone) three times by change in the flow rate by ± 0.2 ml/min (0.8 mL/min and 1.2 mL/min) from developed method. Calculate the %RSD of mean area for change in method parameter.

2.4.6 Solution stability study

Solution stability was determined by a repeat analysis of same solution after two days to check stability of drug in solution condition.

2.5 Analysis of pharmaceutical formulation

The proposed method was applied for the determination of the two drugs in SPAR-D and validity was further assessed by applying the standard addition technique.

3. RESULT AND DISCUSSION

3.1. Specificity

A method that is able to measure unequivocally the drug(s) in the presence of all degradation products, in the presence of excipients expected to present in the formulation. Results had shown in graph in figure 4, 5 and 6.

3.2 Linearity and Range

The linearity of the response for Sparfloxacin and Dexamethasone was determined by preparing standard solutions with concentration range of 6-36 µg/ml Sparfloxacin and 2-12 µg/ml Dexamethasone. The calibration curve of sparfloxacin was shown in Figure 7. The calibration curve of dexamethasone was shown in Figure 8. It indicates that the response is linear over the concentration range

by correlation coefficient (r) value 0.999 for Sparfloxacin and 0.999 for Dexamethasone.

3.3 Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, three repeated injections of standard solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, three injections of standard solutions were made for three consecutive days and response of drug peaks and percentage RSD were calculated. From the data obtained, the developed RP- HPLC method was found to be precise.

Intraday precision

The data for intraday precision of Sparfloxacin and Dexamethasone was shown in Table 2. The % R.S.D. for intraday precision was found to be 0.676-1.151 % for Sparfloxacin. The % R.S.D. for intraday precision was found to be 0.725-1.175 % for Dexamethasone. The average % R.S.D. for intraday were found 0.963 % and 0.984 % for Sparfloxacin and Dexamethasone respectively.

Percentage R.S.D. of intraday was found to be NMT 2% for both the drugs, indicates that the method is precise.

Interday precision

The data for interday precision of Sparfloxacin and Dexamethasone was shown in Table 3. Sparfloxacin. The % R.S.D. for interday precision was found to be 0.808-1.259**%** for Dexamethasone.

The average % R.S.D. for interday was found to be 0.931% and 0.959% for Sparfloxacin and Dexamethasone respectively.

Percentage R.S.D. of interday was found to be NMT 2% for both the drugs, indicates that the method is precise.

Repeatability

Repeatability of test procedure using theoretical assay concentration of Sparfloxacin (18 µg/mL) and Dexamethasone (6 µg/mL) are shown in Figure 9. Percentage RSD for Sparfloxacin and Dexamethasone were found to be 0.19 and 0.32 respectively.

LOD and LOQ

LOD and LOQ of Sparfloxacin and Dexamethasone were shown in table 4. Limit of Detection was found 0.15 µg/ml for Sparfloxacin and 0.1 µg/ml for Dexamethasone. Limit of Quantitation was found 0.51 µg/ml for Sparfloxacin and 0.3 µg/ml for Dexamethasone.

Accuracy Study

Accuracy of the methods was assured, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The good recoveries prove the good accuracy of the proposed methods. The accuracy data for solution preparation is shown in table 5. The data for accuracy for Sparfloxacin and Dexamethasone is shown in Table 6 and 7. Percentage recovery for Sparfloxacin was found to be 99.07-100.42%**,** while for Dexamethasone, it was found to be in range of 99.94-100.30%. Mean % Recovery for Sparfloxacin and Dexamethasone were found to be 99.91% and 99.94% respectively. Recovery is in the range of 98-102%, indicates that method is accurate.

Robustness

Robustness was carried by varying parameters from the optimized chromatographic condition and it is shown in table 8.

Solution Stability

Solution Stability data was shown in table 9 and sample solution data shown in table 10.

Assay of Pharmaceutical dosage form

Assay results from dosage form obtained within the limit that data shown in table 11.

4. CONCLUSION

The proposed method is suitable for QC laboratories, where economy and time considerations are essential. High recovery shows that the methods are free from the interferences of the commonly used excipients and additives in the formulations of drugs. In addition, the run times are suitable for processing numerous samples on a daily basis.

5. REFERENCES

1. Chemspider, "Sparfloxacin," http://www.chemspider.com/Chemical-Structure.54517.html?rid=73ad04af-7bbb-4ccb-9a58-b5585038657b. Accessed 4 February 2017.
2. Drug Bank, http://www.drugbank.ca/. Accessed 4 February 2017.
3. Chemspider, "Dexamethasone," http://www.chemspider.com/Chemical-Structure.5541.html?rid=bfe92f1c-8f40-4080-b09c-1a2a8dff9843. Accessed 4 February 2017.
4. Indian Pharmacopoeia Commission, Indian Pharmacopoeia, Ghaziabad: Ministry of Health and Family welfare, India, 2007.
5. British Pharmacopoeia, London: The Stationary Office Led, CD-ROM, 2009.
6. United States Pharmacopoeia, Washington DC: United States Pharmacopoeia Commission, 2007.
7. Cagle GD. Combination of quinolone antibiotics and steroids for topical ophthalmic use. WO1990001933A1, 1988.
8. Singh ON and Bhagat HG Topical suspension formulations containing ciprofloxacin and dexamethasone. US6284804B1, 2001.
9. Abelson M. Ophthalmic formulations and uses thereof. CA2603902C, 2013.
10. Wong VG and Wood LL. Sustained release eye drop formulations. US8541413B2, 2013.
11. ICH, Q2 (R1), "Validation of Analytical Procedures: Text and Methodology", 2005.
12. Rathod H and Akhtar J. Simultaneous Estimation of Sparfloxacin and Dexamethasone by Q-absorbance Ratio Spectrophotometric Method in Eye/Ear Drops, *American Journal of Pharmtech Research*, 2013, *3(3)*: 546-555.

Figures

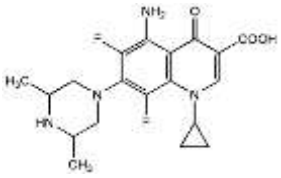

Figure 1: chemical structure Sparfloxacin

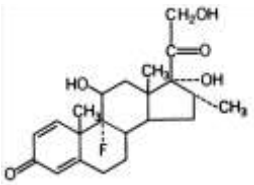

Figure 2: Chemical structure of Dexamethasone.

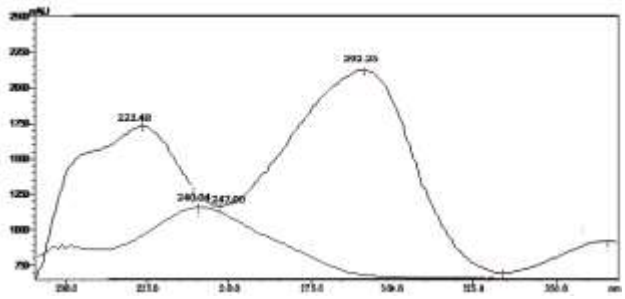

Figure 3: Overlain UV spectra of Sparfloxacin (30 µg/mL) and Dexamethasone (10 µg/mL) in mobile phase

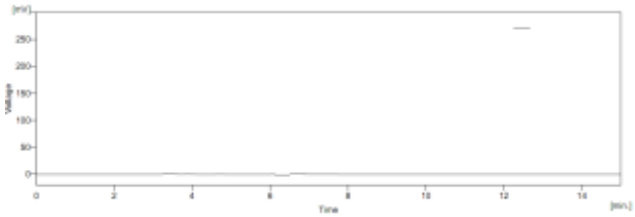

Figure 4: Chromatogram of mobile phase (Blank)

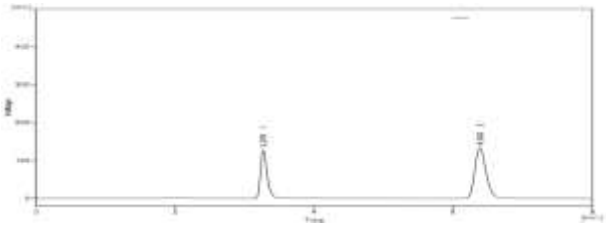

Figure 5: Chromatograms of Sparfloxacin 30 (µg/ml) and Dexamethasone 10 (µg/ml), form Standard solution

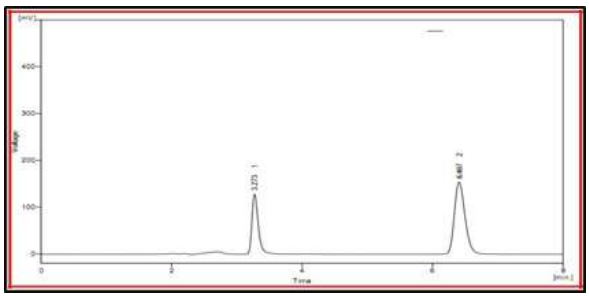

Figure 6: Chromatograms of Sparfloxacin 30 (µg/ml) and Dexamethasone 10 (µg/ml), form sample solution

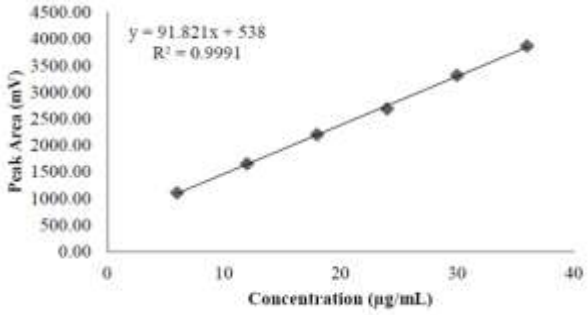

Figure 7: Calibration curve of Sparfloxacin (6-36 µg/mL)

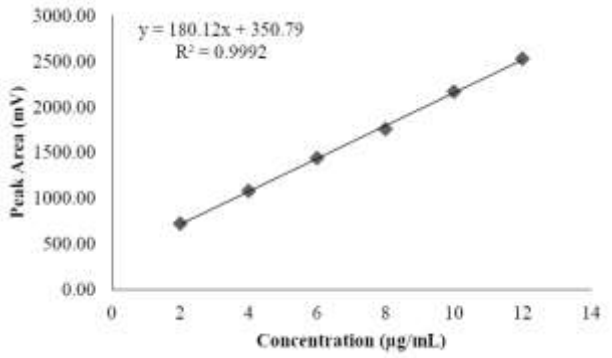

Figure 8: Calibration curve of Dexamethasone (2-12 µg/mL)

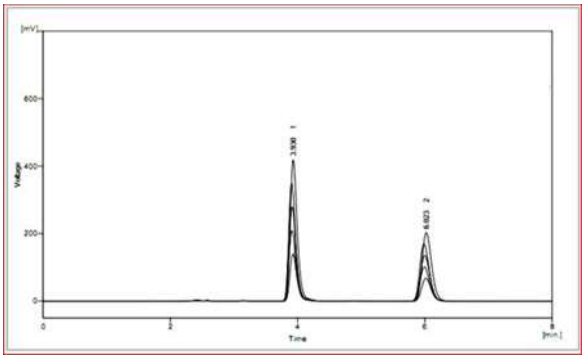

Figure 9: Overlay chromatograms of Sparfloxacin and Dexamethasone

TABLES:

Table 1. System suitability parameters:

System Suitability Parameters	Sparfloxacin	Dexamethasone
Average peak area of standard*	2193.34	1433.43
No. of theoretical plates	5912	7490
Retention Time (min)	3.267 ± 0.10	6.373 ± 0.10
Capacity Factor, (k')	1.11	2.49
Relative Retention (α)	2.26	
Tailing factor	1.719 ± 0.016	1.405 ± 0.011
Asymmetry	1.5	1.3
% RSD (Injection Repeatability)	0.19%	0.32%
Resolution	5.922 ± 0.061	

Table 2: Intraday precision for Sparfloxacin and Dexamethasone

Con. (µg/mL)	Sparfloxacin			Dexamethasone			
	Peak Area Mean*	SD	% RSD	Con. (µg/mL)	Peak Area Mean *	SD	% RSD
12	1101.051	12.677	1.151	**4**	719.370	8.459	1.175
18	2190.589	14.824	0.676	**6**	1431.970	10.383	0.725
24	3305.451	35.031	1.059	**8**	2160.667	22.772	1.053

*** Average of Three determination**

Table 3: Interday precision for Sparfloxacin and Dexamethasone

	Sparfloxacin			Dexamethasone			
Con. (µg/mL)	Peak Area Mean*	SD	% RSD	Con. (µg/mL)	Peak Area Mean *	SD	% RSD
12	1099.65	12.913	1.174	**4**	718.045	9.044	1.259
18	2189.548	17.562	0.802	**6**	1431.02	11.57	0.808
24	3306.718	27.032	0.817	**8**	2161.38	17.47	0.808

*** Average of Three determination**

Table 4: Result of LOD and LOQ

PARAMETER	Sparfloxacin	Dexamethasone
S.D. of Intercept	2.71	3.52
Slope of Calibration Curve	91.82	180.12
LOD (µg/mL)	0.15	0.1
LOQ (µg/mL)	0.51	0.3

Table 5: Preparation of solutions for accuracy

Con. Level (%)	Volume of Sample taken (ml)	Concentration of sample (µg/ml)		Volume of working stock solution (S_3 and S_4) of API spiked (ml)		Concentration of API Spiked (µg/ml)	
		S	D	S	D	S	D
80	1	15	5	2	2	12	4
100	1	15	5	2.5	2.5	15	5
120	1	15	5	3	3	18	6

(Note: S=Sparfloxacin and D=Dexamethasone)

Table 6: Accuracy data for Sparfloxacin

Amount taken (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery ±SD	% RSD
15	**12**	27.126	100.42±0.43	0.433
15	**15**	30.39	99.72±0.59	0.593
15	**18**	33.915	99.81±0.71	0.707

Table 7: Accuracy data for Dexamethasone

Amount taken (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery ± SD	%RSD
5	**4**	8.994	99.94 ±0.37	0.373
5	**5**	10.32	99.59 ±0.47	0.468
5	**6**	11.035	100.30 ±0.43	0.425

***Average of three determination**

Table 8: Results of Robustness parameters

CONDITION	PEAK AREA MEAN *		SD		%R.S.D.	
	SPA	DEXA	SPA	DEXA	SPA	DEXA
Change in the Mobile Phase Composition(± 2 ml organic Phase)						
Change in the -2ml organic phase (58:42 v/v)	2194.638	1434.382	16.45	10.82	0.74	0.75
Change in the + 2 ml organic phase (62:38 v/v)	2192.043	1432.494	27.74	18.05	1.26	1.26
Change pH(±0.2 unit)						
Change in the -0.2 unit pH	2194.637	1434.381	20.93	13.75	0.95	0.96
Change in the +0.2 unit pH	2192.042	1432.49	27.45	17.90	1.25	1.25
Change Flow rate (±0.2 ml/min)						
Change in the -0.2ml/min F.R.	2316.245	1513.378	1433	9.24	0.62	0.61
Change in the +0.2ml/min F.R.	2098.097	1371.476	19.94	12.81	0.95	0.93

***Average of three determination**

Table 9: Results of standard solution stability

Time	ASSAY (%)		%DIFFERENCE	
	SPA	DEXA	SPA	DEXA
Initial	99.55	100.95	---	---
After 6 hours	99.14	100.60	0.41	0.35
After 12 hours	99.05	100.44	0.50	0.51
After 24 hours	98.98	100.23	0.57	0.72

Table 10: Results of sample solution stability

Time	ASSAY (%)		%DIFFERENCE	
	SPA	DEXA	SPA	DEXA
Initial	99.17	101.38	---	---
After 6 hours	98.69	100.98	0.48	0.40
After 12 hours	98.53	100.81	0.64	0.57
After 24 hours	98.47	100.48	0.70	0.90

Table 11. Assay of Pharmaceutical dosage form

Product SPAR-D	Sparfloxacin Label Claim (% w/v)	% Recovery Mean±S.D.[a]	Dexamethasone Label Claim (% w/v)	% Recovery Mean±S.D.[a]
	0.3	100.34±1.29	0.1	99.25±1.08