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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Sparfloxacin and Dexamethasone in Bulk and Liquid Dosage Form

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Abstract:

Aim The sensitive, precise and reproducible reverse phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of sparfloxacin and dexamethasone in liquid dosage form.

Method The method was developed in reverse phase mode using Phenomenex C18 column using methanol/phosphate buffer (pH 4.5 adjusted with orthophosphoric acid) the mobile phase ratio 70:30(v/v) at a flow rate of 0.8ml/min.

Results and conclusion Quantification of drugs were achieved with ultraviolet detection at 271nm. Sparfloxacin was eluted at 6.23 min and dexamethasone was eluted at 8.41 min with perfect peak properties. The linearity was found to be in the range of 150-750 ng/ml and 50-250 ng/ml, recovery was found to be 99.90% and 99.90% of sparfloxacin and dexamethasone respectively. The limit of detection for sparfloxacin and dexamethasone was found to be 2.99ng/mL and 1.72ng/mL respectively. The limit of quantitation was found at 9.06ng/mL and 5.24ng/mL respectively for sparfloxacin and dexamethasone. The system suitability studies were carried out as specified in ICH guidelines using different parameters. The developed method is eco-friendly and the peaks were more resolved when compared to the previous literatures. The developed method can be applied successfully for the assay of sparfloxacin and dexamethasone in liquid dosage forms available in market.

Key words: Sparfloxacin, Dexamethasone, Orthophosphoric acid, Methanol.

INTRODUCTION

Sparfloxacin (SPAR) (Fig1) is chemically 5-amino-1-cyclopropyl-7-[(3S, 5R)-3, 5-dimethylpiperazin-1-yl]-6, 8-difluoro-4-oxoquinoline-3-carboxylic acid. It is a fluoroquinolone anti-bacterial agent in the treatment of bacterial infection [1]. Dexamethasone(DEXA)(Fig 2) is glucocorticoid class of steroid drugs used to treat any inflammatory and autoimmune conditions and chemically dexamethasone is (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.[2] The combination of sparfloxacin and dexamethasone is used in treatment of steroidal inflammatory condition where risk bacterial population is high. So far, abundant reports applying validated analytical reverse phase-high-performance liquid chromatography (RP-HPLC), ultra-performance liquid chromatography tandem mass spectrometry, fluorescence and spectrophotometric methods for individual estimation of sparfloxacin and dexamethasone[3] and its metabolic products in plasma (rat, rabbit, and human) bulk formulation, and pharmaceutical formulation (capsule, controlled release product, and tablet) by the worldwide researchers have been into applications. While going through the literature available in the standard global databases, not a single report have been found regarding any analytical RP-HPLC method for the routine simultaneous estimation of sparfloxacin[4] and dexamethasone drug combination in bulk and pharmaceutical formulation. Understanding the fact, a simple, robust, precise, economical, and accurate method was developed to meet up the challenge. The present work endeavors development of a RP-HPLC method for simultaneous estimation of sparfloxacin and dexamethasone in liquid formulation [5]. A novel RP-HPLC method was developed and validated for the simultaneous estimation of sparfloxacin and dexamethasone. HPLC method is developed in reverse phase mode using methanol and phosphate buffer (pH 4.5 adjusted with orthophosphoric acid) the mobile phase ratio 70:30(v/v) at a flow rate of 0.8ml/min.

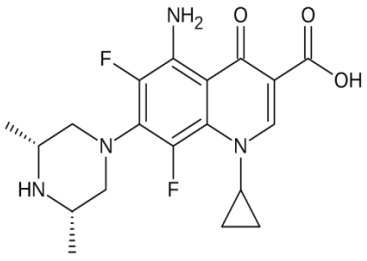

Fig 1: structure of sparfloxacin

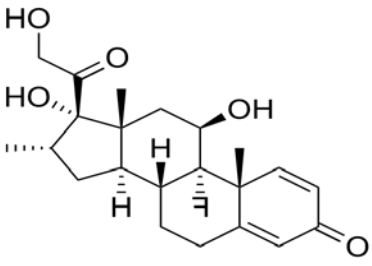

Fig 2: structure of dexamethasone

MATERIALS AND METHODS:

HPLC method:

Chemicals and solvents:

Methanol HPLC grade

Ortho phosphoric acid analytical grade

HPLC grade water was prepared by using Millipore MilliQ water purification system

Potassium di hydrogen orthophosphate

Chromatographic condition:

The chromatographic condition of sparfloxacin and dexamethasone in stationary phase is phenomenex C18 column and mobile phase is methanol and phosphate buffer (pH adjusted to 4.5 with orthophosphoric acid) mobile phase ratio 70:30 (v/v) detection wave length is 271nm and flow rate is 0.8 ml/min.Fig.3

Preparation of standard solution:

Weigh accurately 300mg of sparfloxacin and was dissolved in methanol and made up to 100ml with the same. 20ml of this stock solution was diluted to 100ml to get solution A. similarly 100mg dexamethasone was dissolved in methanol and made up to 100ml with the same and 20ml of this stock solution was diluted to 100ml to get a solution B.

To a 100ml standard flask mix 50ml of each solution A and B to get a final concentration of 0.3mg and 0.1mg per ml solution of sparfloxacin and dexamethasone respectively.

Aliquots of mixed standard solutions were diluted in mobile phase to get a final concentration of 150, 300, 450, 600, 750 ng/ml of sparfloxacin and 50, 100, 150, 200, and 250 ng/ml of dexamethasone. All the solutions were sonicated for 20minutes before injection.

Preparation of sample solution:

Dilute 10ml of the eye drop to 100ml to get the final concentration of 0.3mg and 0.1mg per ml solution of sparfloxacin and dexamethasone respectively.

Validation of the method:

a) Accuracy:

The reference standards of the respective drug were added to the sample solution, (300 ng/ml of sparfloxacin and 100 ng/ml of dexamethasone) at the level of 50%, 100% and 150%. These were further diluted by procedure as followed in the estimation of formulation. The concentration of drugs present in the resulting sample solution was determined by using assay method.table.2.

b) Precision:

The precision of the developed method was determined in terms of intermediate precision (intra-day and inter-day) and repeatability. Sparfloxacin (450 ng/ml) and Dexamethasone (150 ng/ml) were analyzed in six times during the same day (intra-day precision) and three consecutive days (inter-day precision). The %RSD values of intra-day, inter-day, repeatability studies for sparfloxacin and dexamethasone showed that the precision of the method was satisfactory.

c) Linearity and range:

From the standard stock solutions, a suitably mixed standard solution was prepared. Sparfloxacin and Dexamethasone were found to be linear in the range of 150 to 750 ng/ml and 50 to 250 ng/ml respectively. The calibration curve was plotted by using peak area Vs concentration of the standard solution.

d) Limit of detection (LOD) and limit of quantification (LOQ):

The residual standard deviation of the regression line or the standard deviation of y- intercepts of regression lines may be used to calculate LOD and LOQ. LOD=3.3× σ/S and LOQ=10× σ/S, where σ is the standard deviation of y-intercepts of regression line and S is the slope of the calibration curve.

e) Specificity:

The specificity of the RP-HPLC method was determined by complete separation of sparfloxacin and dexamethasone with parameters like retention time(Rt), resolution(Rs), and tailing factor(T), peak purity curve and peak purity index. Tailing factor for peaks of sparfloxacin and dexamethasone less than 2% and resolution was satisfactory. The peaks obtained for were sharp and have clear baseline separation. The peak purity studies were performed to prove that the method is specific in nature.

f) Ruggedness:

It expresses the precision with in laboratory variations like different days, different analyst, and different equipment's. Ruggedness of the method was assessed by spiking the standard concentration of sparfloxacin (450 ng/ml) and dexamethasone (150 ng/ml), 6times in two different days with different analyst.

g) Robustness:

In order to demonstrate the robustness of the method, the following optimized conditions were slightly varied.

1) ±2% in ratio of methanol in mobile phase
2) ±0.2 ml of flow rate
3) ±0.2 units in the pH of buffer
4) ±2 nm wavelength

The separation factor, retention time and peak symmetry were then calculated. The deviation among the results obtained in well within the limits. Hence the method is robust.

h) System suitability studies:

The system suitability studies were carried out as specified in ICH. These parameters include number of theoretical plates, HETP, column efficiency, resolution and capacity factor.

RESULT AND DISCUSSION:

HPLC method:

Analysis of formulation:

The percentage of drug in formulation, mean and relative standard deviation were calculated. The result of analysis showed that the amount of drug present in the formulation is in good correlation with the label claim of formulation.table1.

Linearity:

Sparfloxacin and dexamethasone were found to be linear in the range of 150 to 750 ng/ml and 50 to 250 ng/ml respectively.

The correlation coefficient of sparfloxacin and dexamethasone were found to be 0.994 and 0.994 respectively. The calibration curves were plotted as peak area vs. concentration of standard solution (**Fig. 3 and 4**)

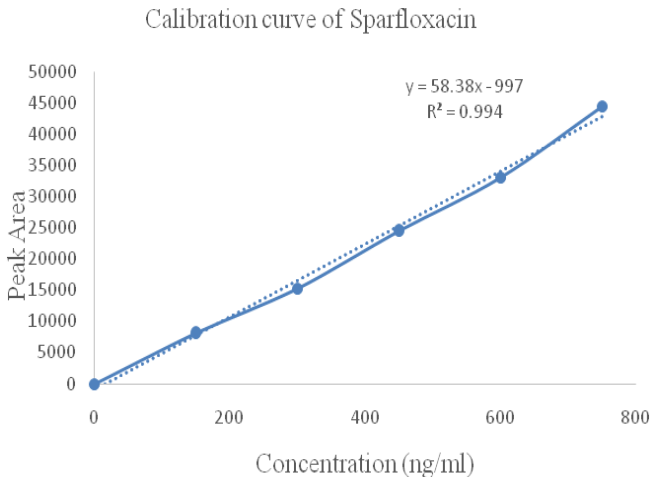

Fig. 4: Calibration curve for Sparfloxacin

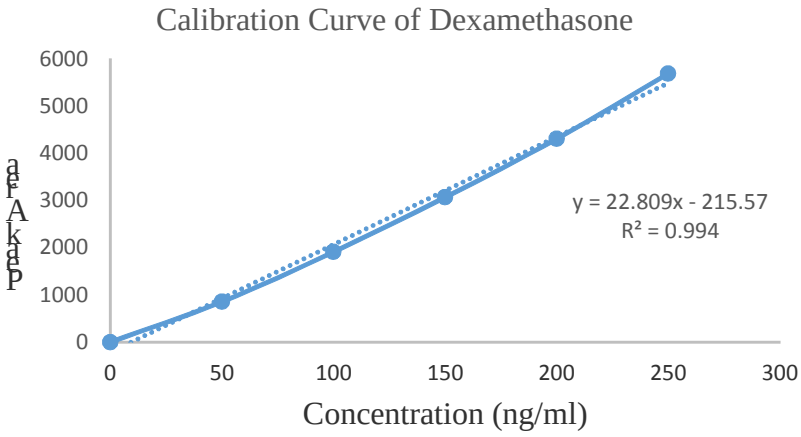

Fig. 5: Calibration curve for Dexamethasone

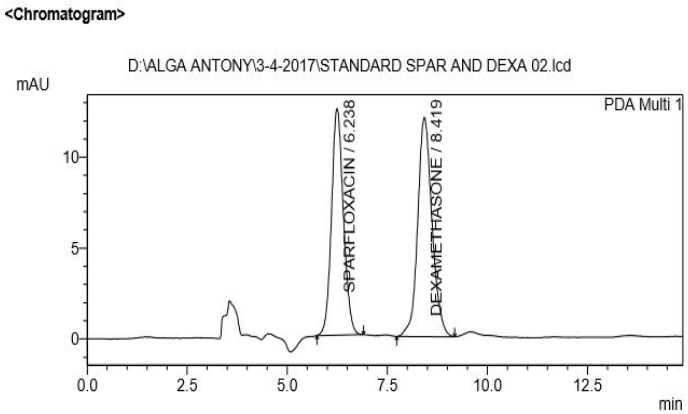

Fig 3.Typical chromatogram obtained for the Sparfloxacin and Dexamethasone

Table 1: analysis of formulation

formulation	Labelled amount (mg)		Amount Found (mg)		Percentage assay (%)		%R.S.D	
Eye drops	SPAR	DEXA	SPAR	DEXA	SPAR	DEXA	SPAR	DEXA
	3	1	2.99	0.98	99.60	99.50	0.17	0.19

Table 2: Accuracy (Recovery studies)

Drug	Amount present in the solution (ng/ml)	Spike Level (%)	Amount of drug added (ng/ml)	Amount of drug recovered (ng/ml)	Percentage Recovery (%)	%RSD
SPAR	300	50	150	149.92	99.90	0.21
		100	300	298.00	99.33	0.24
		150	450	448.70	99.71	0.21
DEXA	100	50	50	49.70	99.40	0.40
		100	100	99.89	99.89	0.43
		150	150	149.85	99.90	0.41

Table 3: Intraday Studies

No. of Injection	Conc. of SPAR (ng/ml)	Peak Area	% RSD	Conc. of DEXA (ng/ml)	Peak Area	% RSD
6	450	24427.83	0.21	150	2953.16	0.40

Table 4: Inter day studies

Day	Conc. of SPAR (ng/ml)	Peak Area	% RSD	Conc. of DEXA (ng/ml)	Peak Area	% RSD
DAY1	450	24440.50	0.18	150	29532.00	0.39
DAY2	450	24445.80	0.17	150	2953.30	0.39
DAY3	450	24451.50	0.19	150	2949.60	0.32

Table 5: Repeatability

Conc. of SPAR (ng/ml)	Peak Area	% RSD	Conc. of DEXA (ng/ml)	Peak Area	% RSD
450	24427.83	0.21	150	2953.16	0.44

Table 6: LOD and LOQ

Parameter	SPAR (ng/ml)	DEXA (ng/ml)
LOD	2.99	1.72
LOQ	9.06	5.24

Table 7: Ruggedness studies

Drug name	Concentration (µg/ml)	Peak area	%RSD
Day-1 analyst-1			
SPAR	450	24360	0.729
DEXA	150	2935.33	1.028
Day-2 analyst-2			
SPAR	450	24367.5	0.759
DEXA	150	2949.33	0.839

Table 8: Robustness Studies

Parameters	Modifications	SPAR Recovery (%)	DEXA Recovery (%)
pH	3.5	98.46	98.53
	4.0	99.92	100.5
Detection wavelength (nm)	274	99.89	99.33
	269	98.89	97.92
Flow rate (ml/min)	1.0	99.93	99.89
	1.2	98.76	98.89

Table 9: System suitability studies

Parameters	SPAR	DEXA
No. of theoretical plates	9492	9394
Tailing Factor	0.99	1.06
HETP	21.07	21.29
LOD	2.99 ng/ml	1.72ng/ml
LOQ	9.06 ng/ml	5.24 ng/ml
Resolution	2.33	
K	3.17	2.57

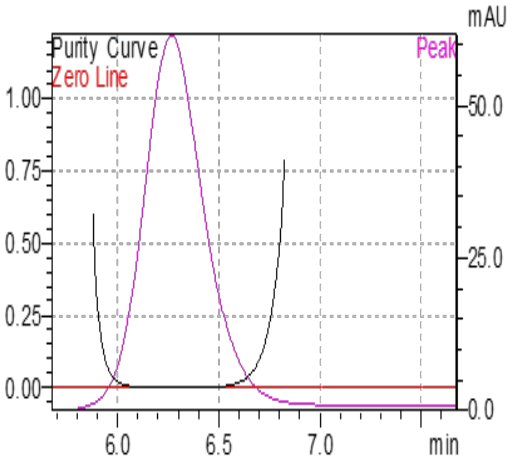

Fig. 6: Peak purity of Sparfloxacin

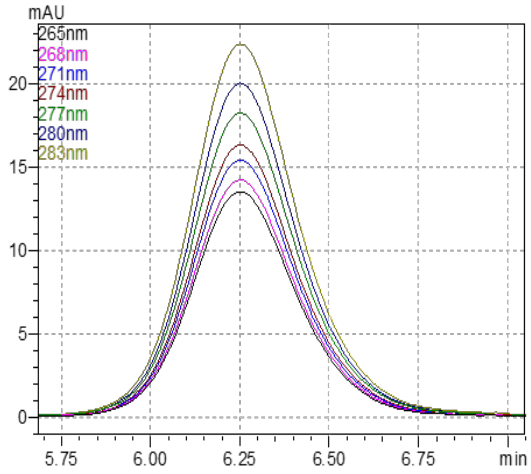

Fig. 7: Peak profile of sparfloxacin

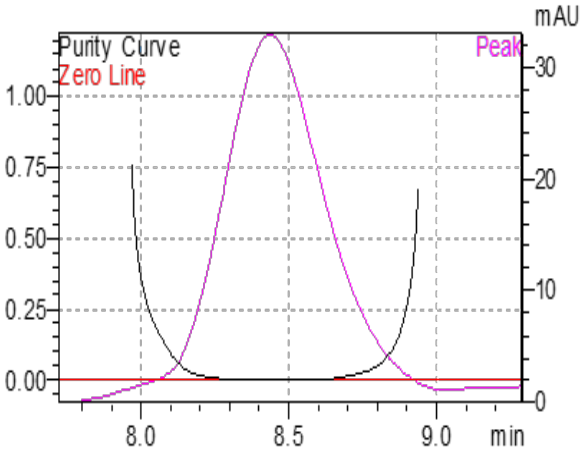

Fig. 8: Peak purity of Dexamethasone

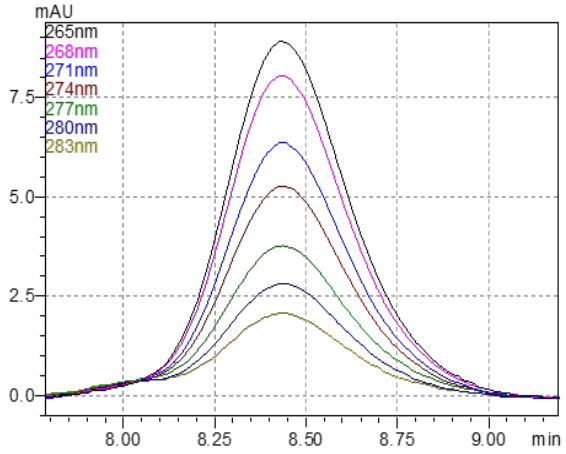

Fig. 9: Peak profile of Dexamethasone

Accuracy (Recovery studies):

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulations at 50%, 100% and 150% levels. The percentage recovery of sparfloxacin and dexamethasone were found to be in the range of 99.33 to 99.90% and 99.4 to 99.9% respectively.

Precision:

The precision of the method was determined by studying reproducibility and repeatability. The results revealed that the developed method was found to be reproducible in nature.

Intraday studies

The results obtained complied with acceptance criteria since percentage relative standard deviation of peak area of SPAR and DEXA were fund to be within the limit i.e., NMT 2%. table.3

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Acceptance criteria

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LOD and LOQ:

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). The LOQ is the smallest concentration of the analyte that gives measurable response (signal to noise ratio 10). Table.6

Ruggedness:

The sample was analyzed by a different chemist and same instruments on different days have been performed. The method is rugged since the deviation among the results obtained by two chemists on a different day was within the limits i.e., NMT 2%.table.7.

Robustness:

The robustness studies were performed for the standard solutions and were presented in the table 8. The assay values were within the limits thus the developed method is robust.

System suitability studies:

The system suitability study was performed for the standard solutions and is presented in table 9. The values obtained demonstrate the system suitability for the analysis of the above drug combination.

CONCLUSION:

The methods were validated by determining system suitability, specificity, precision, linearity, accuracy, stability, LOD, LOQ ruggedness and robustness parameters and found to be satisfactory. There was no other co eluting peak with SPAR and DEXA peaks and hence method is specific for the estimation of both SPAR and DEXA in the presence of other excipient.

The flow rate is reduced (0.8ml/min) as compared with previously reported methods in literatures (1-1.5ml/min). The resolution between increased (2.33) when compared to the reported results in other literatures.

The developed method is eco-friendly as the mobile phase used is biodegradable. It also economic since the solvent used is lower cost when compared with the other literatures already reported. The ruggedness of the RP-HPLC method demonstrated that different operational and

environmental variables had very little influence within the limit on the test results. The method was completely validated showing satisfactory data for all the method validation parameters that were tested. The method can be employed as a stability indicating one, as the degradants were not interfered during the analysis of drug.

It was concluded that, the developed HPLC method is specific, accurate, precise, and it may be used for the routine application for the determination of SPAR and DEXA in the drug formulation in the presence of their degradation product.

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Conflict Of Interest

Conflict of interest declared as none.

Financial Support-None.

Authors Contribution Statement

Author 1: Designed and performed the Analysis.
Author 2: Performed the analysis.
Author 3: Contributed to the analysis of the results and calculations.
Author 4: Contributed for writing of the manuscript.
Author 5: Contributed to the analysis.

REFERENCE:

1. Drug bank, Sparfloxacin, Weblog Available at https://www.drugbank.ca/drugs/DB01208.
2. Drug site trust. Dexamethasone. Weblog Available at https://www.drugbank.ca/drugs/dexamethasone.html.
3. Heda. A. A, Kathiriya. J. M. D, Gadade.D, Puranik, P. K. Development and validation of RP-HPLC method for simultaneous determination of granisetron and dexamethasone. *Ind J.Pharm Sci*. 2011 Nov-Dec; 73(6): 696–699.
4. Najma Sulthana, Saeed Arayne.M, Simultaneous determination of sparfloxacin and commonly used H_2 receptor antagonists by RP-HPLC: application to in vitro drug interactions. *Medicinal Chemistry Research* July 2012, 21(7) 974–98
5. Vibha Gupta, Ajay Deep Kumar Jain, Gill.N.S, Kapil Gupta. Development and validation of HPLC method – a review. *Int. Res J Pharm. App Sci*., 2012; 2(4): 17-25.