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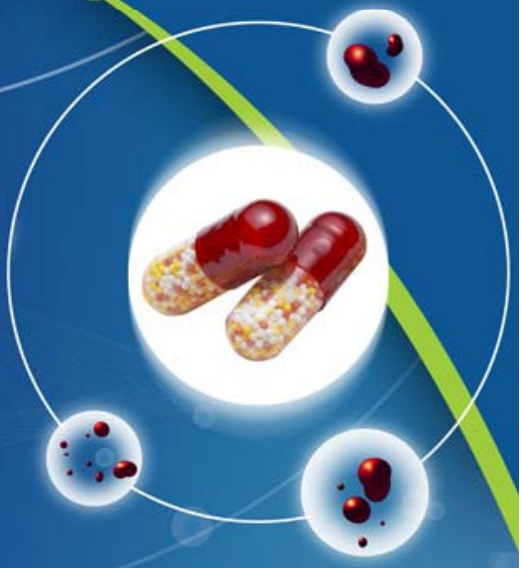

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Research Article

SIMULTANEOUS ESTIMATION OF SPARFLOXACIN AND DEXAMETHASONE IN BULK AND IN THEIR COMBINED DOSAGE FORM BY HPLC METHOD

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ABSTRACT

A sensitive, accurate and precise method was developed and validate for the estimation of SPARFLOXACIN and DEXAMETHASONE in bulk and in combined dosage form.The RP-HPLC method had shown adequate separation for Sparfloxacin and Dexamethasone from its degradation products. The separation was achieved on using a C18 column (250 x 4.6 mmi.d.) gradient mixture of phosphate buffer pH 6 and acetonitrile(70:30%v/v) at a flow rate of 1 ml/min, Injection volume 20 µl with detection wavelength of 245 nm. The retention time for Sparfloxacin and Dexamethasone were 3.493±0.5min and 5.049±0.5min respectively. The described method was linear over a range of 30-90 µg/ml for Sparfloxacin and 10-30 µg/ml for Dexamethasone respectively. The correlation coefficient for Sparfloxacin and Dexamethasone were 0.9998 and 0.9997 respectively. The limit of detection for Sparfloxacin and Dexamethasone were 0.005 µg/ml and 0.03µg/ml respectively. The limit of quantification for Sparfloxacin and Dexamethasone were 0.17µg/ml, 0.36 µg/ml respectively. Proposed method was validated as per ICH guidelines for linearity, accuracy, precision, specificity and LOD and LOQ for estimation Sparfloxacin and Dexamethasone in Ophthalmic dosage form and results were found to be satisfactory. Thus the developed and validated stability indicating method can be used successfully for marketed formulations.

KEY WORDS: *Sparfloxacin and Dexamethasone, RP-HPLC method, Validation.*

INTRODUCTION

Sparfloxacin(SPAR) is 5-amino-1-cyclopropyl-7-[(3R,5S)3,5-dimethylpiperazin- 1-yl]-6,8- difluoro -4-oxo-quinoline-3-carboxylic acid. Sparfloxacinis a Fluoroquinolone Antibiotic used in the treatment of bacterial infections i.e. as an anti-infective.Sparfloxacin,like other quinolones and fluoroquinolones, are bactericidal drugs, actively killing bacteria. Quinolones inhibit the bacterial DNA gyrase or the topoisomeraseIV enzyme, thereby inhibiting DNA replication and transcription.

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Quinolones can enter cells easily 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. Eukaryotic cells do not contain DNA gyrase or topoisomerase IV. Dexamethasone (DEXA) is 8S,9R,10S,11S,13S,14S,16R,17R)-9- Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16- trimethyl-6,7,8,9,10,11,12,13,14,15,16,17- dodecahydro-3H-cyclopenta[a]phenanthren-3-one.Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs. It acts as an anti-inflammatory and immuno- suppressants. Dexamethasone is a glucocorticoid agonist. Unbound dexamethasone crosses cell membranes and binds with high affinity to

specific cytoplasmic glucocorticoid receptors. This complex binds to DNA elements (glucocorticoid response elements) which 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 humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of dexamethasone are thought to involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.

MATERIALS AND METHOD

SPAR is obtained as a gratis sample from Taj Pharmaceuticals Ltd, Mumbai and DEXA as a gratis sample from ShrijiPharmaceuticals,Vadodara. Acetonitrile, Phosphate Buffer and Water (HPLC grade) were used for HPLC method.

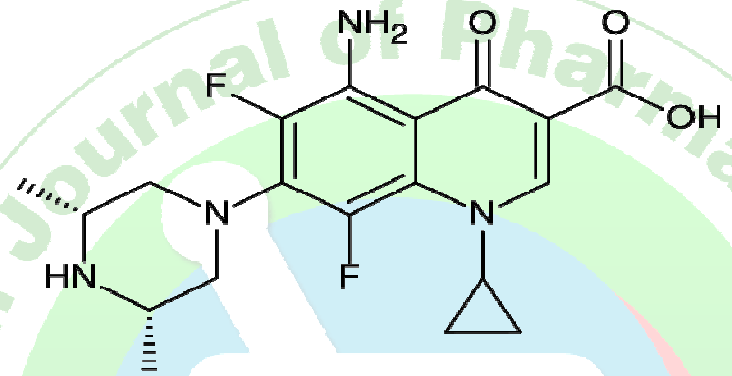

Fig.1: Structure of Sparfloxacin (SPAR)

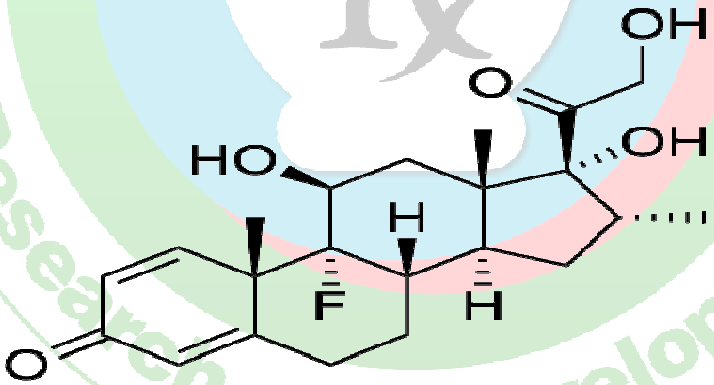

Fig.2: Structure of Dexamethasone (DEXA)

UV Visible Double beamSpectrophotometer with Pair Matched Cuvette(Analytical Spectra 2080), HPLC (Shimadzu LC-20 AT) system used consisted of pump (model Perkin Elmer;Reciprocating Series-200) with universal loopinjector (Hamilton Syringe Perkin Elmer) ofinjection capacity 20µL. Detector consists of Ultravioletdetector SPD 20 A, the column used was Enable C_{18} (5 µm, 25cm X 4.6 mm i.d.), EIE Ultra-sonic Cleaner and Electronic Digital Balance, Shimadzu.

Preparation of Mobile Phase and Stock Solutions:

Mixture of 0.05 M Phosphate buffer (pH 6.0) and Acetonitrile in the ratio of 70:30 was mixed well & filtered through 0.45 µm filter paper, Sonicate for 15 minutes to degas the mixture and used as mobile phase.

SPAR standard stock solution (600µg/ml):

A 60 mg of standard SPAR was weighed and transferred to a 100 ml volumetric flask and dissolved in 25 ml mobile phase. The flask

was shaken and volume was made up to the mark with mobile phase to give a solution containing 600 µg/ml SPAR.

DEXA standard stock solution: (200µg/ml):
A 20 mg of standard DEXA was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 25 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 200µg/ml DEXA.

Chromatographic conditions:
Stationary phase: Octadecylsilane HPLC column (Enable C18, 25 cm × 4.6 mm i.d.)
Mobile phase: Acetonitrile: Phosphate buffer (pH 6.0) (30:70 v/v). The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication.
Flow rate: 1.0 ml/min
Detection: By UV at 245 nm
Injection volume: 20 µl
Run time: 10 min
Pressure:120- 130 psi

Method Validation:

The methods were validated according to International Conference on Harmonization Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Six point calibration curves were generated with appropriate volumes of working standard solutions for HPLC methods. The calibration range was 30-90 µg/ml and 10-30µg/ml for SPAR and DEXA, respectively in the HPLC methods of analysis for two drugs. The linearity was evaluated by the least square regression method using un-weighted data.

Both precision and accuracy were determined with standardquality control samples (in addition to calibration standards)prepared in triplicates at different concentration levels coveringthe entire linearity range. Precision is the degree of repeatabilityof an analytical method under normal operational conditions.The precision of the assay was determined by repeatability(intra-day) and intermediate precision (inter-day) and reportedas %R.S.D. for a statistically significant number of replicatemeasurements. The intermediate precision was studied bycomparing the assays on 3 different days and the results documentedas standard deviation and %R.S.D.Accuracy is the percent of analyte recovered by assay froma known added amount. Data from nine determinations overthree concentration levels covering the specified rangewas determined. The repeatability of the method was determined byassaying six sample solutions of the middle test concentration (60µg/ml for SPAR and 20µg/ml for DEXA for HPLC method)

The limit of detection (LOD) is defined as the lowest concentrationof an analyte that an analytical process can reliablydifferentiate from background levels. The limit of quantification(LOQ) is defined as the lowest concentration of the standardcurve that can be measured with acceptable accuracy, precisionand variability. The LOD and LOQ were calculatedas
$LOD = 3.3\sigma/S$,
And
$LOQ = 10\sigma/S$
Where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

RESULTS AND DISCUSSION

A RP-HPLC method was developed for two anticancer drugs, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The chromatographic conditions were optimized in order to provide a good performance of the assay. The mobile phase for each drugwas selected based on its polarity. Different ratios of methanol–water-acetonitrile combinations were tried for SPAR and DEXA and the final working mobile phase is Acetonitrile: Phosphate buffer (pH-6) in composition of 30: 70 v/v. The flow rate is 1.0 ml/min. Flow rate is critical as it affects the peak symmetry parameters. The optimization of flow rate is critical since the extent of longitudinal broadening is inversely related to flow rate of mobile phase.
The retention times of SPAR and DEXAwere 3.497 and 5.037 min, respectively. The total run time was short for two drugs.

TABLE 1: System Suitability Parameters For HPLC Method

Parameter	*Drug*	
	SPAR	*DEXA*
Retention time (min)	**3.497**	**5.037**
Resolution (Rs)	**6.882**	
Tailing Factor (T)	**1.321**	**1.206**
Theoretical plates	**4453**	**7170**

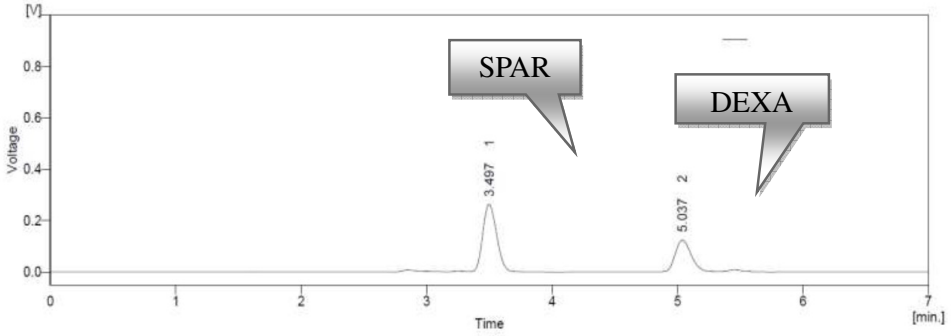

Fig. 3: Typical HPLC chromatogram of SPAR and DEXA

The methods were specific as none of the excipients interfered with the analyte of interest. Hence, the methods were suitably employed for assaying the commercial formulations. A six point calibration curve was constructed with working standards and was found linear ($r2 \geq 0.999$) for each of the analyte over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram.

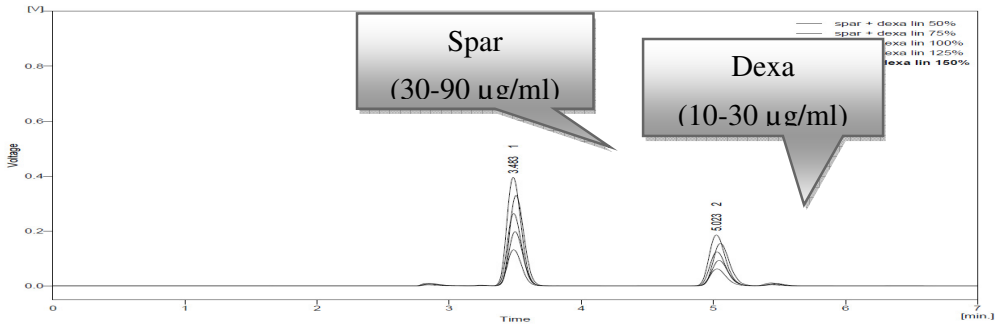

Fig. 4: Overlay chromatogram for calibration curve of SPAR 30- 90µg/ml) and DEXA (10 - 30µg/ml

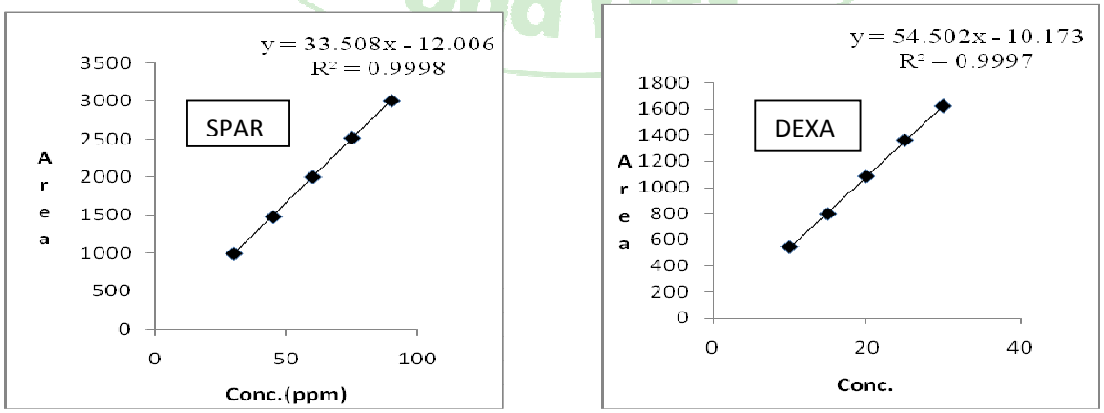

Fig. 5: Calibration curve for SPAR and DEXA

TABLE 2: Linear Regression Analysis of SPAR and DEXA

Statistical Parameter	*HPLC Method*	
	SPAR	*DEXA*
Concentration Range (µg/ml)	**30 – 90**	**10 – 30**
Straight line equation	**y = 33.508x - 12.006**	**y=54.502x - 10.173**
Correlation coefficient (r^2)	**0.9998**	**0.9997**

The developed HPLC methods were accurate, precise, reproducible and very sensitive. All the validation parameters of combination was shown to be within the specified limits.

TABLE 3: Precision by HPLC Method for SPAR and DEXA

Conc. (µg/ml)		*Intraday Precision*				*Interday Precision*			
		*Average area ± SD**		*%RSD*		*Concentration found ± SD**		*%RSD*	
Spar	*Dexa*	*Spar*	*Dexa*	*Spar*	*Dexa*	*Spar*	*Dexa*	*Spar*	*Dexa*
30	10	998.41 ± 0.87	540.51 ± 0.97	1.03	1.15	1000.53 ± 0.77	541.45 ± 0.45	1.03	1.09
60	20	1998.4 ± 0.92	1618.32± 0.84	1.26	1.30	1998.80± 0.82	1081.42± 0.91	1.31	1.33
90	30	2991.5 ± 0.87	2672.54 ± 0.93	1.19	1.21	2982.53± 0.67	1613.5± 0.89	1.82	1.82

(*n = 3)

TABLE 4: Recovery Study for SPAR and DEXA

Drug	*Recovery Level*	*Concentration of Synthetic Mixture (µg/ml)*	*Concentration of Pure Added (µg/ml)*	*Recovery* (µg)*	*Recovery* (%) ± S.D**
SPAR	**80%**	**37.5**	**30.0**	**30.01**	**99.804±0.25**
	100%	**37.5**	**37.5**	**37.33**	**99.098 ± 0.93**
	120%	**37.5**	**45.0**	**45.02**	**99.444 ± 1.15**
DEXA	**80%**	**12.5**	**10**	**9.98**	**100.064±0.80**
	100%	**12.5**	**12.5**	**12.38**	**99.558± 0.86**
	120%	**12.5**	**15**	**14.91**	**100.055±0.70**

(*n=3)

TABLE 5: robustness data of HPLC method for SPAR and DEXA

Parameter	*Variation*	*Average Area*		*% RSD*	
		SPAR	*DEXA*	*SPAR*	*DEXA*
Flow rate	**1.2**	**1909.253**	**1033.046**	**1.2**	**1.2**
	0.8	**2110.776**	**1142.396**	**1.3**	**1.4**
Mobile phase	**31 : 69**	**1993.395**	**1079.594**	**1.0**	**1.2**
	29 : 71	**1991.929**	**1079.364**	**1.2**	**1.5**
Ph	**6.1**	**1998.904**	**1081.773**	**1.2**	**1.3**
	5.9	**1988.399**	**1076.378**	**1.2**	**1.3**

Accuracy and precision were determined by elaboration of two standard calibration curves.The intra- and inter-day precision (%R.S.D.) at differentconcentration levels were found to be less than 5%. All the threedrugs showed 100–102% recoveries from the synthetic mixture when assayed with the developed HPLC methods.

TABLE 6: Assay of dosage form for SPAR and DEXA

Drug	*Concentration as per marketed formulation per ml*	*Concentration taken for % Assay*	*Peak Area of Sample solution*	*Concentration found from dosage form*	*% Assay ± SD**
SPAR	**6 mg**	**60 µg/mL**	**2000.310**	**60.09µg/Ml**	**99.99% ±0.15**
DEXA	**2 mg**	**20 µg/mL**	**1082.066**	**20.10 µg/mL**	**100.25% ±0.98**

(*n=3)

Moreover the %R.S.D. (less variation) shows goodprecision of the developed HPLC methods. The calculated LOQand LOD concentrations confirmed that the methods were sufficientlysensitive. The methods were specific as none of theexcipients interfered with the analyte of interest. Hence, themethods were suitably employed for assaying the synthetic mixture.

The proposed RP-HPLC methods are Simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for SPAR and DEXA make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The recoveries achieved are good by both the methods.

CONCLUSION

The proposed RP-HPLC methods are Simple, reliableand selective providing satisfactory accuracy and precisionwith lower limits of detection and quantification. Moreover theshorter duration of analysis for SPAR and DEXA make these reported methods suitable for routine quantitativeanalysis in pharmaceutical dosage forms. The recoveriesachieved are good by both the methods.

TABLE 7: Summary of Validation parameters for SPAR and DEXA

SR. NO.	*PARAMETER*	*SPAR*	*DEXA*
1.	**Linearity Range (n=6) (µg/ml)**	**30 – 90**	**10 – 30**
2.	**Regression equation**	**y = 33.508x - 12.006**	**y=54.502x - 10.173**
3.	**Correlation coefficient (r^2)**	**0.9998**	**0.9997**
4.	**Limit of detection(n=6) (µg/ml)**	**0.005**	**0.03**
5.	**Limit of quantification (n=6) (µg/ml)**	**0.1762**	**0.3642**
6.	**Precision**		
	Repeatability (%RSD) (n=6)	**1.16**	**0.34**
	Intra-day (%RSD)(n=3)	**1.16**	**1.41**
	Inter-day (%RSD)(n=3)	**1.38**	**1.22**
7.	**Recovery (Mean ± SD)**	**99.70 ± 0.720**	**99.779 ± 0.345**
8.	**Robustness (%RSD)**	**1.22**	**1.54**

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