RESEARCH ARTICLE Am. J. PharmTech Res. 2013; 3(3) ISSN: 2249-3387

AMERICAN JOURNAL OF
PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Simultaneous Estimation of Sparfloxacin and Dexamethasone by Q-Absorbance Ratio Spectrophotometric Method in Eye/Ear Drops

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Sparfloxacin and Dexamethasone. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Sparfloxacin and Dexamethasone show an isoabsorptive point at 271.2 nm in methanol. The second wavelength used is 241.6 nm, which is the λ-max of Dexamethasone in methanol. The linearity was obtained in the concentration range of 3-18 μg/ml for Sparfloxacin and 1-6 μg/ml for Dexamethasone. The concentrations of the drugs were determined by using ratio of absorbances at isoabsorptive point and at the λ-max of Dexamethasone. The method was successfully applied to pharmaceutical dosage form because no interference from the preservative was found. The suitability of this method for the quantitative determination of Sparfloxacin and Dexamethasone was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Sparfloxacin and Dexamethasone in Eye/Ear drops. The results of analysis have been validated statistically and by recovery studies.

Keywords: Sparfloxacin, Dexamethasone, Absorbance ratio method, Isoabsorptive point, Validation

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Received 24 March 2013, Accepted 08 April 2013

Please cite this article in press as: Rathod HJ. *et al.*, Simultaneous Estimation of Sparfloxacin and Dexamethasone by Q-Absorbance Ratio Spectrophotometric Method in Eye/Ear Drops. American Journal of PharmTech Research 2013.

INTRODUCTION

Sparfloxacin (SPAR) is chemically 5-amino-1-cyclopropyl-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-6,8-difluoro-4 oxo-1,4-dihydroquinoline-3-carboxylic acid[3] . It is an anti-bacterial agent, a brad spectrum antibiotic[3,4]. HPLC,UV,HPTLC,RP-HPLC,RP_UPLC and UV-densitometric determination to identify a degradation product of Sparfloxacin in the tablet is also available[13-20] Dexamethasone (DEXA) is chemically (8*S*,9*R*,10*S*,11*S*,13*S*,14*S*,16*R*,17*R*)-9-Flouro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[α] phenanthren-3-one[1-3]. It is official in IP & BP. IP & BP describes spectrometic method for its estimation. Literature survey also reveals UV methods,HPLC,RP_HPLC,SPE-LC-UV with other drugs in combination[5-12]. Literature survey reveals there is no spectrophotometric method (Simultaneous equation) for simultaneous estimation of SPAR and DEXA in synthetic mixture. However, no references have been found for simultaneous estimation of SPAR and DEXA in pharmaceutical formulations by spectrophoto metric method. A successful attempt has been made for SPAR and DEXA and in their combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise spectrophotometric method based on Q-absorption ratio for simultaneous estimation of both drugs in Eye/Ear drops.

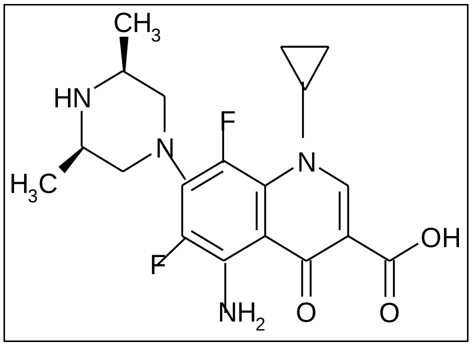

Figure 1 : Chemical structure of Sparfloxacin (SPAR)

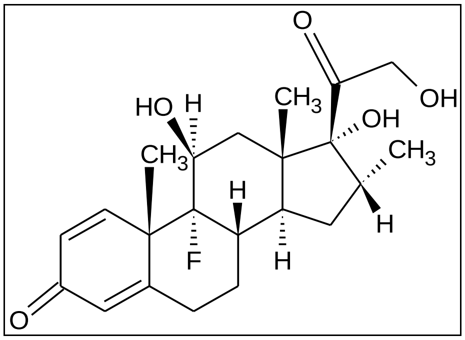

Figure 2 : Chemical structure of Dexamethasone (DEXA)

MATERIALS & METHODS

Apparatus

A Shimadzu model UV-1800 series (Japan) double beam UV/Visible spectrophotometer with spectral width of 1 nm and a pair of 1 cm matched quartz cells was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe version 2.34 system software. An analytical balance (Shimadzu,Japan) , an ultrasonic bath (Toshcon, Toshniwal process instrument PVT. Ltd., Ajmer) was used in the study.

Reagents and Materials

Sparfloxacin(SPAR) was kindly gifted by Dr. reddy's lab, India & Dexamethasone (DEXA) was kindly gifted by Briyosis soft caps Pvt ltd., Vadodara, India. The Eye/Ear drops (Spaxin D-containing Sparfloxacin 0.3%w/v and Dexamethasone 0.1%w/v,Mfd by : Ind-swift Company) was procured from the local market. Methanol (AR Grade, Merck,Rankem) were used in the study. All other chemicals and solvents used were of analytical grade.

Methods

Preparation of standard solutions

An accurately weighed quantity of standard SPAR (100 mg) and DEXA (100 mg) were weighed and transferred to separate 100 mL volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solutions containing 1000 µg/mL each of SPAR and DEXA.10mL from these of each solution was taken and add 50 mL methanol and further diluted to 100mL with water to make 100 µg/mL each SPAR and DEXA.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the ʎ-max of one of the two components. From the overlay spectra of two drugs, it is evident that SPAR and DEXA show an isoabsorptive point at 271.2 nm. The second wavelength used is 241.6 nm, which is the ʎ-max of DEXA. Working standard solutions having concentration 3,6,9,12,15 and 18 µg/mL for SPAR and 1,2,3,4,5 and 6 DEXA were prepared in water and the absorbances at 271.2 nm (isoabsorptive point) and 241.6 nm (ʎ-max of DEXA) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following calculations.

Cx = [(QM-QY) / (QX-QY)] x A1 / ax1……… (1)

Cy = [(QM-QX) / (QY-QX)] x A1 / ay1……… (2)

Where, A1 and A2 are absorbances of mixture at 271.2 nm and 241.6 nm; ax1 and ay1 are absorptivities of SPAR and DEXA at 271.2 nm; ax2 and ay2 are absorptivities of SPAR and DEXA respectively at 241.6 nm; QM = A2/A1, QX= ax2/ax1 and QY= ay2/ay1.

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[21]

Calibration curve (Linearity)

A calibration curves were plotted over a concentration range of 4-24 µg/ml (3-18 µg/ml SPAR and 1-6 µg/ml for DEXA. Accurately measured standard stock solution of SPAR (0.3, 0.6, 0.9, 1.2, 1.5 & 1.8 mL) and standard stock solution of DEXA (0.1, 0.2, 0.3, 0.4, 0.5 & 0.6 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with Water. The absorbances of solutions were measured at both the wavelength 271.2 nm and 241.6 nm against water as blank. Calibration curves were constructed for SPAR and DEXA by plotting absorbance versus concentrations at both wavelengths and the regression equations were calculated.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for mixture (12 µg/ml) without changing the parameters of the proposed spectrophotometry method. Percent relative standard deviation (%RSD) was found and was within limit (Not more than 2%)

Intermediate Precision (Reproducility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of mixture of SPAR and DEXA (4, 12 and 24 µg/ml). Percent relative standard deviation (%RSD) was found and was within limit (Not more than 2%)

Accuracy (Recovery study)

The accuracy of the method was determined by calculating the recoveries of SPAR and DEXA by the standard addition method. Known amounts of standard solutions of SPAR and DEXA were added at 80, 100 and 120 % level to pre-quantified sample solutions of SPAR and DEXA mixture (6 µg/ml for SPAR and 2 µg/ml for DEXA). The amounts of SPAR and DEXA were estimated by applying obtained values to the respective regression line equations and percent recoveries were found .

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs were derived using following equation as per ICH guidelines.

LOD= 3.3 x σ/S

LOD= 10 x σ/S

Where, σ = Standard deviation of the response and

S= slope of the calibration curve.

ANALYSIS OF EYE/EAR DROPS

Spaxin D eye drops (0.2mL, 0.3 % w/v SPAR and 0.1 % w/v DEXA) was transferred in 10 mL volumetric flask and diluted up to mark with methanol. Take 1 mL from above solution in 10 mL volumetric flask and diluted up to mark with water to obtain final concentration of SPAR (6 µg/mL) and DEXA (2 µg/mL). The absorbances of the sample solution i.e. A1 and A2 were recorded at 271.2 nm (isoabsorptive point) and 241.6 nm (ʎ-max of Dexamethasone) respectively, and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample was calculated using above equation (1) and (2). The analytical procedure was repeated 3 times with Eye/Ear drops.

RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 271.2 nm (Isoabsorptive point) and 241.6 nm (ʎ-max of DEXA) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of SPAR (298.3 nm) and DEXA (241.2 nm) showing isoabsorptive point (271.2 nm) in solvent is shown in Figure 3. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table 2).

The method was successfully used to determine the amounts of SPAR and DEXA present in the Eye/ear drops. The results obtained were in good agreement with the corresponding labeled amount. (Table: 3). Optical characteristics and summary of validation parameters for method is given in Table: 1

By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

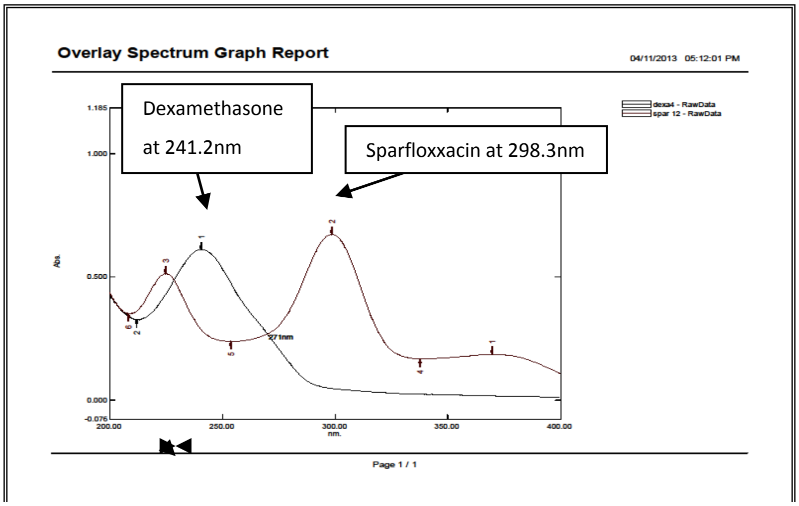

Figure: 3 Overlain spectra of SPAR and DEXA showing Isoabsorptive point at 271.2 nm

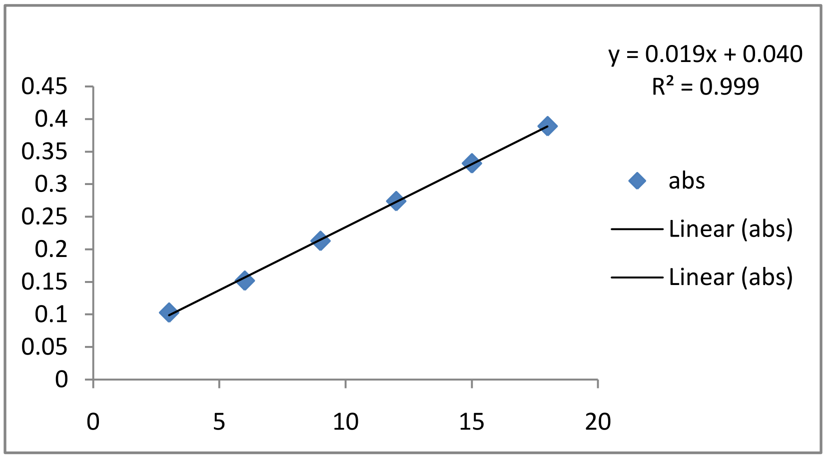

Figure 4: Calibration curve of SPAR at 271.2 nm (ʎ max of DEXA)

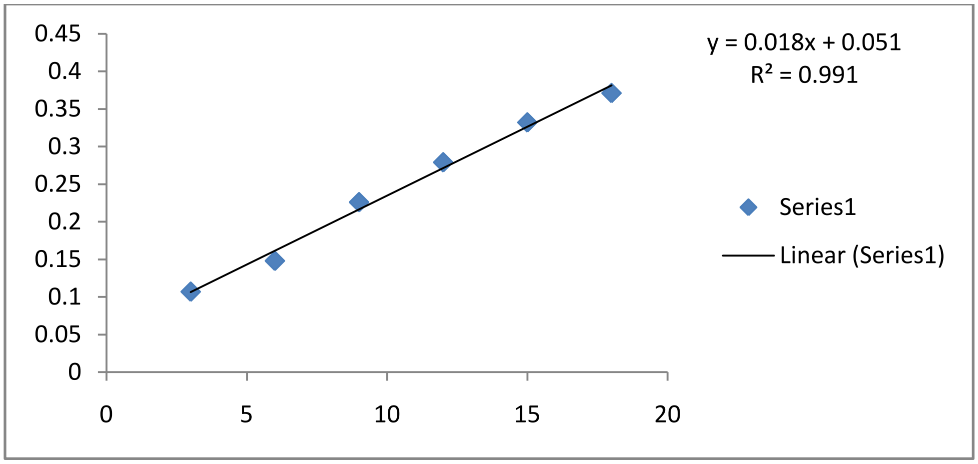

Figure 5: Calibration curve of SPAR at 241.6 nm (Isoabsorptive point)

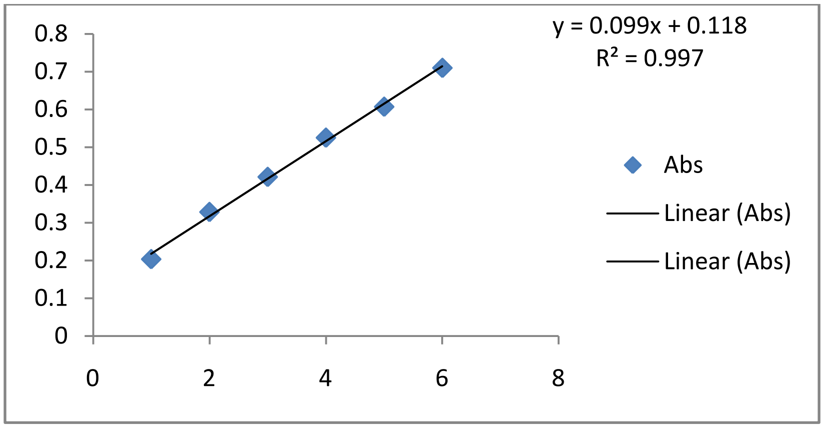

Figure 6: Calibration curve of DEXA at 241.6 nm (ʎ max)

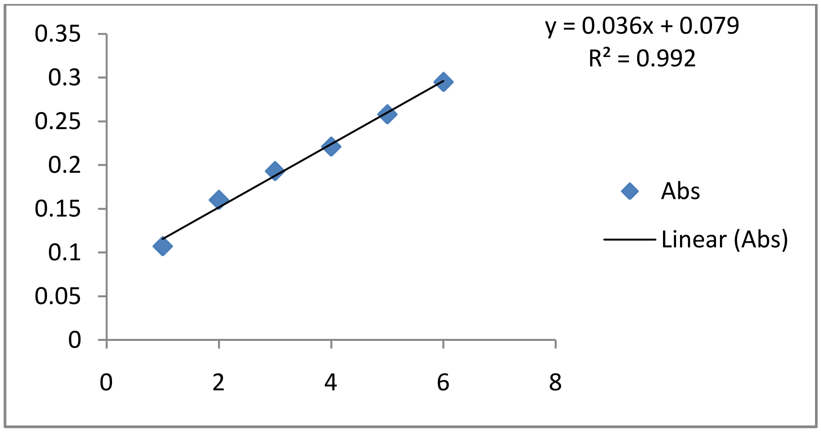

Figure 7: Calibration curve of DEXA at 271.2 nm (Isoabsorptive point)

Table 1: Regression analysis data and summary of validation parameters for SPAR and DEXA

Parameters	SPAR		DEXA	
Wavelength (nm)	271.2	241.6	271.2	241.6
Beer's law limit (µg/ml)	3-18	3-18	1-6	1-6
Regression equ. (y= mx+c)	y = 0.0193x+ 0.0407	y = 0.0183x+ 0.0513	y = 0.0361x+ 0.0795	y = 0.0993x+ 0.1181
Slope (m)	0.0193	0.0183	0.0361	0.0993
Intercept(c)	0.0407	0.0513	0.0795	0.1181
Correlation Coefficient (r^2)	0.9992	0.9912	0.992	0.997
LOD (µg/ml)	0.050		0.215	
LOQ(µg/ml)	0.152		0.65	
Repeatability (% RSD, n = 6)	0.86		0.41	
Precision (% RSD, n=3)				
Interday	0.50		0.90	
Intraday	1.03		0.86	

LOD= Limit of detection, LOQ= Limit of quantification, RSD= Relative standard deviation., SD= Standard deviation

Table:2 Recovery data of SPAR and DEXA

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D. (n=3)
SPAR	6	4.8	10.56	101.86 ± 0.67
	6	6	11.86	98.00 ± 0.042
	6	7.2	13.14	99.58 ± 0.22
DEXA	2	1.6	3.67	99.38 ± 0.58
	2	2	4.06	99.50 ± 0.29
	2	2.4	4.39	99.17 ± 0.75

Table:3 Analysis of SPAR and DEXA in Eye/ear drops.

Formulation	SPAR			DEXA		
	Amount as per marketed formulation(mg)	Amount found (mg)	% Amount found ± S.D. (n=3)	Amount as per marketed formulation(mg))	Amount found (mg)	% Amount found ± S.D. (n=3)
Spaxin-D Eye/ear drops	15 mg	14.825	98.83 ± 0.13	5 mg	4.925	98.5 ± 0.24

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of SPAR and DEXA present in the Eye/ear drops . The method utilizes easily available and cheap solvent; hence the method was also economic for estimation of SPAR and DEXA present in the Eye/ear drops. The common excipients like preservative is usually present in the Eye/ear drops do not interfere in the analysis of SPAR and DEXA in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in Eye/ear drops.

ACKNOWLEDGEMENT

The authors are thankful to Briosis Softcaps Pvt Ltd.,Vadodara, Gujarat for providing all the facilities to carry out the work & also thankful to Dr. Jawed Akhtar, Mr. Pradip Vyas and Mrs Jayshree Patel for providing necessary assistance.

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