AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Spectrophotometric Method for Simultaneous Estimation of Gatifloxacin Sesquihydrate and Prednisolone Acetate in Combined Pharmaceutical Dosage form

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of Gatifloxacin sesquihydrate and Prednisolone Acetate in mixture. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent.Gatifloxacin sesquihydrate has absorbance maxima at 293 nm and Prednisolone Acetate has absorbance maxima at 243 nm in methanol. The linearity was obtained in the concentration range of 2-12 µg/ml and 2-24 µg/ml for Gatifloxacin sesquihydrate and Prednisolone Acetate, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 99.19 ± 0.22 and 99.64 ± 0.37 for Gatifloxacin sesquihydrate and Prednisolone Acetate, respectively. The method was successfully applied to laboratory prepared synthetic mixture because no interference from the mixture excipients was found. The suitability of this method for the quantitative determination of Gatifloxacin sesquihydrate and Prednisolone Acetate was proved by validation. The proposed method was found to be simple and sensitive for the routine quality control application of Gatifloxacin sesquihydrate and Prednisolone Acetate in combination. The results of analysis have been validated statistically and by recovery studies.

Keywords: Gatifloxacin sesquihydrate, Prednisolone Acetate, Recovery, Simultaneous equations method, Validation.

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Received 23 November 2012, Accepted 22 December 2012

Please cite this article in press as Patel HB *et al.*, Spectrophotometric Method for Simultaneous Estimation of Gatifloxacin Sesquihydrate and Prednisolone Acetate in Combined Pharmaceutical Dosage form. American Journal of PharmTech Research 2013.

INTRODUCTION

Gatifloxacin sesquihydrate (GAT) is 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate (Figure 1) is a well known Antimicrobial drug[1]. It is official in Indian Pharmacopoeia (IP). IP[2] describe HPLC method for its estimation. Literature survey reveals HPLC[3] , UV[4] and HPTLC[5] methods for estimation of GAT in single dosage form. Literature survey also reveals HPLC[6-7] and UV spectrophotometry[8] and HPTLC[9] methods for determination of GAT with other drugs in combination. Prednisolone Acetate (PRD) is chemically 11ß,17, 21-Trihydroxypregna-1,4-diene-3, 20-dione 21- acetate[10-11] (Figure 2). Prednisolone Acetate (PRD) is official in USP, JP and BP . USP[11] and JP[12] describes liquid chromatography and BP[13] spectrometric method for its estimation. Literature survey reveals UV[14] methods for determination of PRD in single dosage form. Literature survey also reveals HPLC[15-16] and UV spectrophotometry[17-18] method for the determination of PRD with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of GAT and PRD in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of GAT and PRD in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on simultaneous equations for simultaneous estimation of both drugs in their combined mixture.

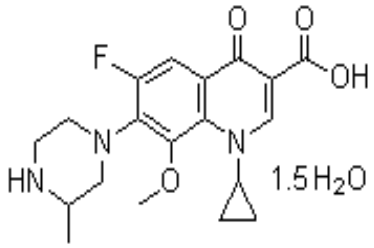

Figure 1: Chemical structure of Gatifloxacin sesquihydrate (GAT)

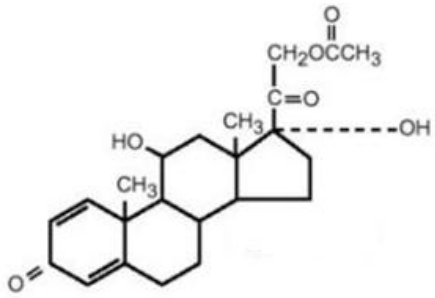

Figure 2: Chemical structure of Prednisolone Acetate (PRD)

MATERIALS AND METHODS

Apparatus

A shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

GAT and PRD bulk powder was kindly gifted by Sun Pharmaceutical Industries Ltd, Halol,Baroda,Gujarat, India and Intas Pharmaceuticals Ltd., Ahmedabad, Gujarat, India respectively. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of standard GAT (10 mg) and PRD (10 mg) powder were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of GAT and PRD.

Methodology

The working standard solutions of GAT and PRD were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm against methanol as blank. Maximum absorbance was obtained at 293 nm and 243 nm for GAT and PRD, respectively. These two wavelengths can be employed for the determination of GAT and PRD without any interference from the other components in their formulations.

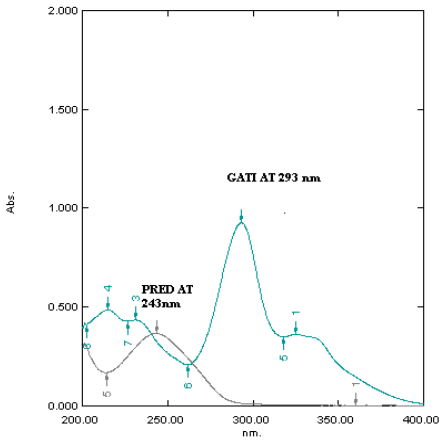

Figure 3: Overlain absorption spectra of GATI (293 nm) and PRED (243 nm) in methanol

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [19]

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 2-12 µg/ml for GAT and 2-24 µg/ml for PRD. Accurately measured standard solutions of GAT (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml) and PRD (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.8, 2.4 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbances of the solutions were measured at 293 and 243 nm against methanol as blank. The calibration curves were constructed by plotting absorbances versus concentrations 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 GAT and PRD (6 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

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 GAT and PRD (2, 8, 12 µg/ml for GAT and 2, 10, 18 µg/ml for PRD). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of GAT and PRD by the standard addition method. Known amounts of standard solutions of GAT and PRD were added at 50, 100 and 150 % level to prequantified sample solutions of GAT and PRD (4 µg/ml GAT and 8 µg/ml PRD). The amounts of GAT and PRD were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines [18]

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

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

Where, σ = the standard deviation of the response and S = slope of the calibration curve

Analysis of GAT and PRD from mixture

Amount of sample equivalent to 3 mg Gatifloxacin and 10 mg prednisolone acetate was transferred in 25 ml volumetric flask ,15 ml of diluents was added , sonicated to dissolve and diluted up to mark. The solution was filtered with Whatman filter paper No.41 and filtrate was taken in 25 ml volumetric flask and dilute to mark with diluent then pipette out 0.25 ml solution in 10 ml volumetric flask and dilute up to mark with diluents to get a final concentration of GAT(3 µg/ml) and PRD (10 µg/ml). The responses of the sample solution were measured at 293 nm and 243nm for quantitation of GAT and PRD, respectively. The amounts of the GAT and PRD present in the sample solution were calculated by solving respective simultaneous equations for GAT and PRD as follows.

Cx = (A2 aY1 – A1 aY2) / (aY1 aX2 - aY2 aX1)

Cy = (A1 aX2 - A2 aX1) / (aY1 aX2 - aY2 aX1)

Where,

A_1 and A_2 are absorbances of mixture at 293 nm and 243 nm;

aX_1 and aY_1 are absorptivities of GAT and PRD respectively at 293 nm;

aX_2 and aY_2 are absorptivities of GAT and PRD respectively at 243 nm.

RESULTS AND DISCUSSION

The standard solutions of GAT and PRD were scanned separately in the UV range and zero-order spectra for GAT and PRD were recorded. Maximum absorbance was obtained at 293 nm and 243 nm for GAT and PRD, respectively. These two wavelengths can be employed for the determination of GAT and PRD without any interference from the other drug in their combined mixture. Overlain zero-order absorption spectrum of GAT and PRD in methanol is shown in (Figure 3). Linear correlation was obtained between absorbances and concentrations of GAT and PRD in the concentration ranges of 2-12 µg/ml and 2-24 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values of GAT were found to be 0.11 and 0.30 % at 293 and 243 nm, respectively. The RSD value of PRD was found to be 1.65 and 0.22 % at 293 and 243 nm, respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.08-0.98% and 0.27-0.89% for GAT at 293 and 243 nm, respectively and 0.27-1.21% and 0.08-0.22% for PRD at 293 and 243 nm, respectively) and intraday (0.02-0.36% and 0.29-0.47% for GAT at 293 and 243 nm, respectively and 0.26-1.21% and 0.09-0.22% for PRD at 293and 243 nm, respectively) variation for GAT and PRD, reveal

that the proposed method is precise. LOD and LOQ values for GAT were found to be 0.02 and 0.05 µg/ml and 0.06 and 0.19 µg/ml at 293 and 243nm, respectively. LOD and LOQ values for PRD were found to be 0.61 and 1.82 µg/ml and 0.05 and 0.16 µg/ml at 293 and 243 nm, respectively. These data show that method is sensitive for the determination of GAT and PRD. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.19 ± 0.22 and 99.64 ± 0.37 for GAT and PRD, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine GAT and PRD in their combined mixture. The results obtained for GAT and PRD were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of GAT and PRD in mixture as well as in pharmaceutical dosage forms.

Table 1: Regression analysis data and summary of validation parameters for GAT and PRD

Parameters	**GAT**		**PRD**	
Wavelength (nm)	293	243	243	293
Beer's law limit (µg /ml)	2-12	2-12	2-24	2-24
Regression equation (y = a + bc)	y = 0.085x + 0.053	y = 0.029x + 0.032	y = 0.043x+ 0.024	y = 0.001x + 0.002
Slope (b)	0.085	0.029	0.043	0.001
Intercept (a)	0.053	0.032	0.024	0.002
Correlation coefficient (R^2)	0.9970	0.9980	0.9970	0.9970
LOD (µg/ml)	0.02	0.06	0.05	0.61
LOQ (µg /ml)	0.05	0.19	0.16	1.82
Repeatability (% RSD , n = 6)	0.06	0.42	0.23	0.84
Precision (% RSD, n = 3)				
Interday	0.08-0.98%	0.27-0.89%	0.08-0.22%	0.27-1.21%
Intraday	0.02-0.36%	0.29-0.47%	0.09-0.22%	0.26-1.21%
Accuracy ± S. D. (% Recovery, n = 3)	99.19 ± 0.22		99.64 ± 0.37	
Assay % Label claim ± S. D. (n=6)	99.72± 0.49		99.07 ± 0.51	

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

[d]S. D. is standard deviation

Table 2: Recovery data of GAT and PRD

Drug	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S. D. (n = 3)
GAT	4	50	98.80 ± 0.24
	4	100	99.38 ± 0.21
	4	150	99.38 ± 0.20
PRD	8	50	99.38 ± 0.59
	8	100	99.57 ± 0.09
	8	150	99.96 ± 0.42

S. D. = Standard deviation, n = Number of determinations.

Table 3: Analysis of GAT and PRD in combined dosage form

Synthetic mixture	Label claim (mg)		Amount found (mg)		% Label claim ± S. D.(n = 6)	
	GAT	PRD	GAT	PRD	GAT	PRD
I	3	10	2.99	9.90	99.72 ± 0.49	99.08± 0.52

S. D. = Standard deviation, n = Number of determinations.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of GAT and PRD in mixture. The method utilizes easily available and cheap solvent for analysis of GAT and PRD hence the method was also economic for estimation of GAT and PRD from mixture. The common excipients and other additives are usually present in the mixture do not interfere in the analysis of GAT and PRD in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

ACKNOWLEDGEMENT

The authors are thankful to Sun pharma, Surat, India and INTAS Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of GAT and PRD, respectively for carry out the research work. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar – 384012, Mehsana, Gujarat, India for providing all the facilities to carry out the research work.

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