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Research Article

# DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF GATIFLOXACIN SESQUIHYDRATE AND PREDNISOLONE ACETATE IN COMBINED DOSAGE FORM

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**ABSTRACT**

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective dual wavelength spectrophotometric method for the simultaneous determination of Gatifloxacin sesquihydrate(GAT) and Prednisolone acetate(PRD)in combined dosage form. The method was based on determination of Gatifloxacin sesquihydrate(GAT) at 346.0 nm and 269.6 nm give same absorbancebut PRD have zero absorbance at 346.0nm so, GAT directly measured at 346.0 nmand Prednisolone acetate(PRD) at the absorbance difference between 269.6 nm and 346 nm. The linearity was obtained in the concentration range of 2.5-40µg/ml for both drugs.LOQ values are found to be 0.51 µg/mL and 1.05 µg/mL for GAT and PRD in method. Percent label claim of the compounds were 99.77±1.16 and 100.22±0.94 in method for GAT and PRD respectively.The method was successfully applied to pharmaceutical dosage form because no interference from excipients was found. The suitability of these methods for the quantitative determination of Gatifloxacin sesquihydrate(GAT) and Prednisolone acetate(PRD)was proved by validation. The proposed methods were found to be simple and sensitive for the routine quality control application of Gatifloxacin sesquihydrate(GAT) and Prednisolone acetate(PRD)in pharmaceutical dosage form. The results of analysis have been validatedstatistically and by recovery studies.

**Keywords:** Gatifloxacin sesquihydrate, Prednisolone acetate and Dual wavelength.

## 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 –(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 1 –(2)). Prednisolone Acetate (PRD) is official in USP, JP and BP. USP[11] and JP[12] describe 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.Present study

involves development and validation of Dual Wavelength Spectrophotometry method for the estimation of GAT and PRD in combination dosage form.

## MATERIALS AND METHODS

### Instrument

A shimadzu model 1700 (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 system software.

### 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 standard PRD and GAT powder (10 mg) 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 of each PRD and GAT. From these stock solutions appropriate dilutions in the range of 2.5-40 µg/ml for GAT and PRD. Mixed standards were prepared in the ratio of 3:10, as the formulation contains GAT and PRD 3 mg and 10 mg, respectively.

## METHOD

The utility of dual wavelength data processing programme is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the components of interest, independent of the interfering components. The principle for dual wavelength method is “the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest”. The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of GAT and PRD. In Dual wavelength method, The diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra , two wavelengths 346.0 nm and 269.6 nm were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of GAT is carried out by measuring the absorbance at 346 nm where PRD have zero absorbance at this wavelength. so, absorbance at 346 nm is directly proportional to concentration of GAT in the mixture. The quantitative determination of PRD is carried out by measuring the absorbance difference value at 269.6 nm and 346.0 nm where GAT have same absorbance at both the wavelength. The difference between 269.6 nm and 346.0 nm is directly proportional to concentration of PRD in the mixture.

### Validation of the proposed method

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

### Linearity (Calibration curve)

Appropriate aliquots from the standard stock solutions of GAT and PRD were used to prepare three different sets of dilutions: Series A, B, and C as follows. Series A consisted of different concentration of GAT (2.5-40 µg/ml). Aliquot from the stock solution of GAT (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2.5-40µg/ml. Series B consisted of varying concentrations of PRD (2.5-40 µg/ml). Appropriate volume of the stock solution of PRD (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol. Series C comprised of mixture of GAT and PRD having varying concentration of GAT (2.5-

40 µg/ml) and PRD (2.5-40 µg/ml). The solutions of GAT and PRD were prepared by transferring 0.25, 0.5, 1.0, 1.5, 20, 30,40 ml equivalent to 2.5, 5, 10, 15, 20, 30,40 µg/ml from the stock solution of GAT and 0.25, 0.5, 1.0, 1.5, 2.0, 3.0,4.0 ml equivalent to 2.5, 5, 10, 15, 20, 30 ,40 ug/ml PRD (100 µg/ml) into a series of 10 ml volumetric flasks and the volume was adjusted up to the mark with methanol. The absorbance of the solutions of series A, B and C were measured at 346.0 nm (1) and 269.6 nm (2). The absorbance at 346 nm is due to the GAT and was plotted against GAT concentration (µg/ml). The difference in absorbance between 269.6 nm and 346.0 nm is due to the PRD and was plotted against PRD concentration (µg/ml) and two different regression equations were obtained.

**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 (15 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 PRD and GAT (10,20,30 µg/ml for PRD and GAT). 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 PRD and GAT by the standard addition method. Known amounts of standard solutions of PRD and GAT were added at 50, 100 and 150 % level to prequantified sample solutions of PRD and GAT (8 µg/ml for PRD and 10 µg/ml for GAT). The amounts of PRD and GAT 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, $\sigma$ = the standard deviation of the response and S = slope of the calibration curve.

**Analysis of 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 346.0nm($\lambda_1$) and 269.6 nm($\lambda_2$) for quantification of GAT and PRD. The amounts of GAT and PRD present in sample solution were calculated by fitting the responses into regression equation for GAT and PRD in proposed method.

**RESULTS AND DISCUSSION**

Dual wavelength method were developed for the simultaneous spectroscopic estimation of GAT and PRDin commercially available dosage forms. Methanol was used as the solvent since both the drugs exhibit good solubility in it and no interference due to excipients of formulation were observed. The overlain spectrum of the drugs suggested that a dual wavelength spectrophotometric method was the most suitable method for simultaneous determination of GAT and PRD. In Dual wavelength method The diluted solutions were scanned over the wavelength range of 200 - 400 nm. From the overlain spectra, Gatifloxacin sesquihydrate(GAT) at 346.0

and 269.6 nm give same absorbance but PRD have zero absorbance at 346.0nm so, GAT directly measured at 346.0 nm and Prednisolone acetate(PRD) at the absorbance difference between 269.6 nm and 346.0 nm. For studying Beer's law, two series of different concentrations in range of 2.5-40 μg/mL for both GAT and PRD were prepared from stock solutions. The calibration curves were constructed at 346.0nm and absorbance difference between 269.6 nm and 346.6 nm respectively. The absorptivities (A1%, 1 cm) of both the drugs at both the selected wavelengths were determined. 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 100.51 ± 0.69and 99.94 ± 0.99for GAT and PRD, respectively (Table 2).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 PRD and GAT in pharmaceutical dosage forms.

## CONCLUSION

The proposed dual wavelength method gives accurate and precise results for determination of GAT and PRDin marketed formulation without prior separation and is easily applied for routine analysis. The most striking feature of the dual wavelength method is its simplicity and rapidity. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision and stability. The developed method has several advantages, as it is simple, accurate, precise and economical. The proposed method was successfully applied to determination of these drugs in pharmaceutical dosage form.

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**Table 1: Data showing linearity of the developed methods**

| PARAMETERS | | GAT | PRD |
|---|---|---|---|
| Wavelength range (nm) | | 346 and 269.6 (same absorbance) | 269.6 |
| Beer's law limit (μg/ml) | | 2.5 - 40 | 2.5 - 40 |
| Regression equation (y = a + bc)<br>Slope (b)<br>Intercept (a) | | y = 0.024x + 0.013<br>0.024<br>0.013 | y = 0.015x + 0.026<br>0.015<br>0.026 |
| Correlation Coefficient ($r^2$) | | 0.999 | 0.998 |
| Molar extinction co-efficient ($l\ mol^{-1}\ cm^{-1}$) | | 14708.5 | 4451.57 |
| Accuracy (Recovery) (n = 3) | Level I | 101.11±0.95 | 100.0±1.67 |
| | Level II | 100.69±0.63 | 99.44 ± 0.48 |
| | Level III | 99.72±0.48 | 100.37 ± 0.85 |
| Method precision (Repeatability) (% RSD, n = 6), | | 0.40 | 0.44 |
| Interday (n = 3) (% RSD[a]) | | 0.24 - 0.39 | 0.55 - 0.75 |
| Intraday(n = 3) (% RSD) | | 0.16 – 0.22 | 0.32 – 0.53 |
| LOD[b] (μg/ml) | | 0.22 | 0.15 |
| LOQ[c] (μg/ml) | | 0.66 | 0.46 |
| Assay ± S. D[d]. (n = 3) | | 99.77 ± 1.16 | 100.22 ± 0.94 |

**Table 2: Recovery data of proposed method**

| Drug | Level | Amount taken (μg/ml) | Amount added (%) | % Mean recovery ± S.D. (n = 3) |
|---|---|---|---|---|
| PRD | I | 8 | 50 | 100.0±1.67 |
| | II | 8 | 100 | 99.44 ± 0.48 |
| | III | 8 | 150 | 100.37 ± 0.85 |
| GAT | I | 10 | 50 | 101.11±0.95 |
| | II | 10 | 100 | 100.69±0.63 |
| | III | 10 | 150 | 99.72±0.48 |

S. D. is Standard deviation and n is number of replicate

**Table 3: Analysis of PRD and GAT in mixture**

| Tablet | Label claim (mg) | | Amount found (mg) | | % Label claim ± S. D. (n = 6) | |
|---|---|---|---|---|---|---|
| | PRD | GAT | PRD | GAT | PRD | GAT |
| I | 10 | 3 | 10.02 | 2.99 | 100.22 ± 0.94 | 99.77 ± 1.16 |

S. D. is standard deviation and n is number of replicate

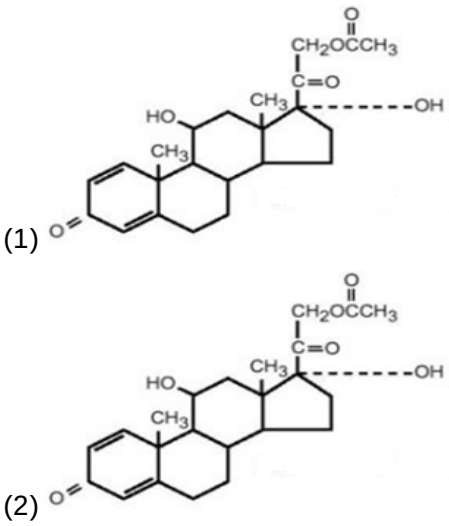


**Fig. 1: Chemical structure of (1) Prednisolone Acetate (PRD) and (2) Gatifloxacin sesquihydrate (GAT)**

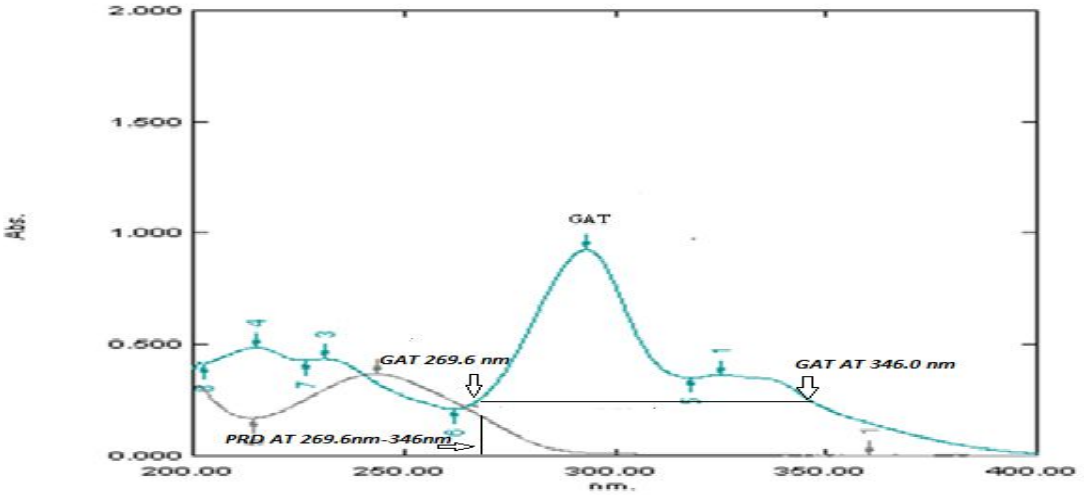


**Fig. 2: Overlain absorption spectra of PRDand GATshowing isoabsorptive point (267.20nm) in methanol**

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