www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 3(11): 06-10

www.thepharmajournal.com
Received: 18-11-2014
Accepted: 30-11-2014

Prasanna Kumar Pradhan
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Naimish Raiyani
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Shreya R. Shah
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Grishma H. Patel
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Umesh Upadhyay
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Correspondence:
Prasanna Kumar Pradhan
Sigma Institute of Pharmacy, Bakrol, Vadodara.

Second derivative spectrophotometric method development and validation for simultaneous estimation of gatifloxacin and prednisolone acetate in their combined dosage form

Prasanna Kumar Pradhan, Naimish Raiyani, Shreya R. Shah, Grishma H. Patel, Umesh Upadhyay

Abstract
Gatifloxacin and Prednisolone Acetate combination is used for prevention and treatment of ophthalmic bacterial infections and inflammatory conditions. In Second Derivative Spectrophotometric method, Methanol is used as a mobile phase. linearity range obtained for the method were 1.5 – 4.5 µg/ml and 5 - 15 µg/ml with corresponding correlation coefficient of 0.9990 and 0.9994, for Gatifloxacin and Prednisolone Acetate respectively. Detection of Gatifloxacin and Prednisolone Acetate at 268 nm and 243 nm respectively. The method was found to be rapid, accurate and precise. This method was validated according to ICH guidelines.

Keywords: Gatifloxacin, Prednisolone Acetate, UV spectroscopy, Second Derivative Spectrophotometric method.

1. Introduction
The bactericidal action of Gatifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known tolay a key role in the partitioning of the chromosomal DNA during bacterial cell division. Prednisolone Acetate can inhibit leukocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses. This combination of drugs will be used to treat optical infections.
The literature is enriched with several methods for determination of GATI and PRED in pharmaceutical dosage forms either as a single drug or in combination with some other drugs. The most extensively used technique for estimation of GATI are by UV [3-6], HPLC [7-14] methods and most extensively used technique for estimation of PRED is by UV[16], HPLC [16]. The aim of study is Development and Validation of Analytical Second Derivative Spectrophotometric method for Simultaneous Estimation of Gatifloxacin and Prednisolone Acetate in their combined Dosage form.
The present study was designed to develop a simple, precise, and rapid analytical Second Derivative Spectrophotometric procedure, which can be used for the analysis of assay method for simultaneous estimation of Gatifloxacin and Prednisolone Acetate.

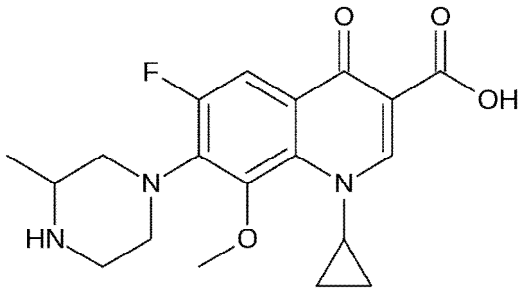

Fig 1: Chemical structure of Gatifloxacin

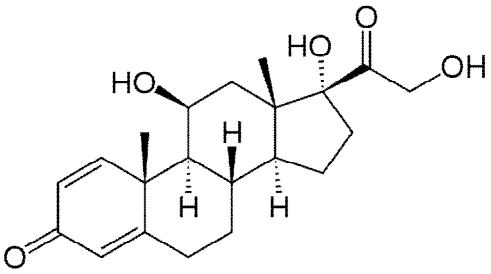

Fig 2: Chemical structure of Prednisolone Acetate

2. Experimental

2.1 Apparatus and Instrument

All absorption spectra and derivatives were recorded with a UV probe version 2.34 software enabled UV-1700 UV/Vis double beam spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells (Shimadzu, Japan). CP224S analytical balance (Sartorius) and ultra sonic cleaner (Frontline FS 4) were used throughout the study.

2.2 Reagents and Materials

Standard samples of Gatifloxacin and Prednisolone Acetate were generous gifts from Provizer Pharma, (Surat, India). Marketed formulation GATSUN P eye drop (SUNWAYS (I) PVT LTD), containing Gatifloxacin 0.3% and Prednisolone acetate 1% procured from local shop. Methanol (S. D. Fine Chemical, Ahmedabad, India) used was of pure analytical grade.

2.3 Solvent

Methanol is used as a solvent because both drugs are soluble in methanol.

2.4 Spectrophotometric conditions

Considering all the orders of derivative spectra of GATI and PRED from first to fourth derivative, the second order derivative spectra was found suitable. The zero crossing point on the second derivative spectra of one drug, the other drug shows substantial absorbance, these two wavelengths can be employed for the estimation of GATI and PRED without any interference from other drug in combined formulations. From the derivatised spectra of prepared mixtures the absorbances were measured at 268 nm for GATI and 243 nm for PRED. These absorbances Vs concentration of standards were plotted in the quantitative mode to obtain the calibration curves from which by extrapolating the value of absorbances of the sample solution, the concentration of the corresponding drugs were determined. Both the drugs obeyed Beer's Law.

2.4.1 Preparation of standard stock solution of GATI (30 µg/ml) and PRED (100 µg/ml)

A 3 mg of standard GATI and 10 mg of standard PRED was weighed and transferred to a 100 ml volumetric flask each and dissolved in 25 ml mobile phase. The flask was shaken and volume was made up to the mark with methanol to give a solution containing 30 µg/ml GATI and 100 µg/ml of PRED.

2.4.2 Preparation of combined working standard solution containing GATI and PRED in ratio of 0.3:1

Accurately weighed 3 mg GATI and 10 mg of PRED were transferred to 100 ml volumetric flask, dissolved in sufficient amount of mobile phase and diluted up to mark with mobile phase to get concentration of 30 µg/ml GATI and 100 µg/ml PRED. This solution was diluted further to get the concentrations in range of 1.2, 2.25, 3, 3.75, 4.5 µg/ml Gatifloxacin and 5, 7.5, 10, 12.5, 15 µg/ml Prednisolone Acetate.

2.5 Method Validation

2.5.1 Precision

i) Repeatability

The Precision of instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for GATI and PRED (3 µg/ml and 10 µg/ml) without changing the parameter of the proposed spectrophotometry method.

ii) Intraday and Interday Precision

Intraday and Interday precision for method were measured in term of % RSD. The experiment was repeated three times in a day for intraday and on three different days for interday precision by taking small, middle and higher concentration of GATI(1.5, 3, 4.5 µg/ml) and PRED(5, 10, 15 µg/ml).

2.5.2 Linearity

The linearity of measurement was evaluated by analyzing standard solutions of GATI and PRED in the range of 1.5-4.5 µg/ml and 5-15 µg/ml for both drugs respectively and calibration plot was constructed.

2.5.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ of GATI and PRED were determined by calibration curve method. Solutions of Gatifloxacin and Prednisolone Acetate were prepared in the range of 1.5-4.5 µg/ml and 5-15 µg/ml for both drugs respectively.

2.5.4 Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method, that is, spiking about 80%, 100%, 120% of GATI and PRED to the standard solutions containing 3 µg/ml of GATI and 10 µg/ml of PRED.

2.5.5 Analysis of Mixture

1 ml of GATSUN-P eye drops was taken in 10 ml volumetric flask and diluted up to 10 ml with methanol. From this stock solution, working standard solution of 3 µg/ml GATI and 10 µg/ml PRED was prepared by taking 1ml and diluted up to 10 ml with methanol. The responses of the sample solution were measured at 268 nm and 244 nm for quantification of GATI nad PRED. The amounts of GATI and PRED present in sample solution were calculated by fitting the responses into regression equation for GATI and PRED in proposed method.

3. Results and Discussion

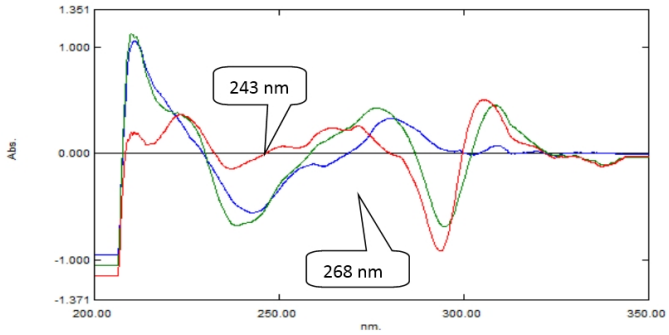

Fig 3: Second derivative UV spectra of GATI and PRED and their Mix. (Red line = GATI, Blue = PRED, Green = MIX)

3.1 Validation

3.1.1 Linearity and Range

The linearity of measurement was evaluated by analyzing standard solutions of GATI and PRED in the range of 25–75 µg/ml and 2.5-7.5 µg/ml respectively for both drugs and calibration plot was constructed.

Table 1: Statistical Parameter for Gatifloxain and Prednisolone Acetate

Statistical Parameter	Gatifloxacin	Prednisolone Acetate
Average Abs.*	0.228	-0.542
	0.428	-1.069
	0.618	-1.555
	0.827	-2.121
	1.026	-2.625
Concentration Range (µg/ml)	1.5-4.5	5-15
Straight line equation	y = 0.265x – 0.171	y = -0.209x + 0.512
Correlation coefficient (R^2)	0.9997	0.9996
LOD (µg/ml)	3.91	13.04
LOQ (µg/ml)	11.85	39.53

*n=5

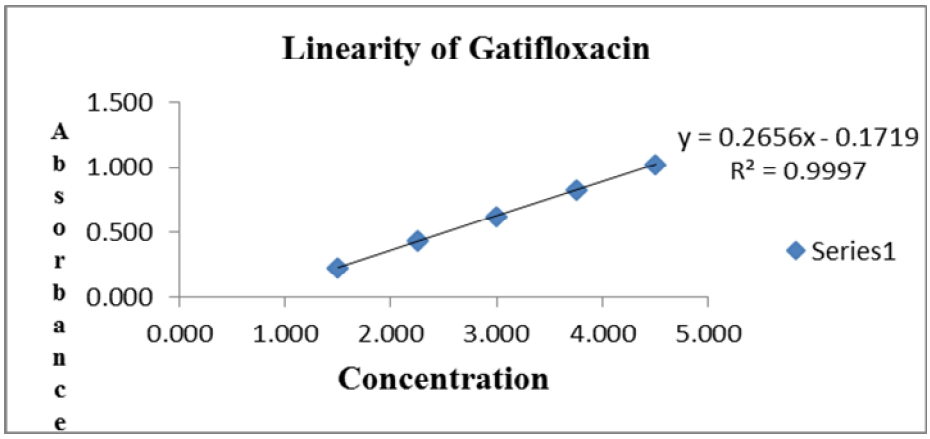

Fig 4: Calibration curve of Gatifloxacin

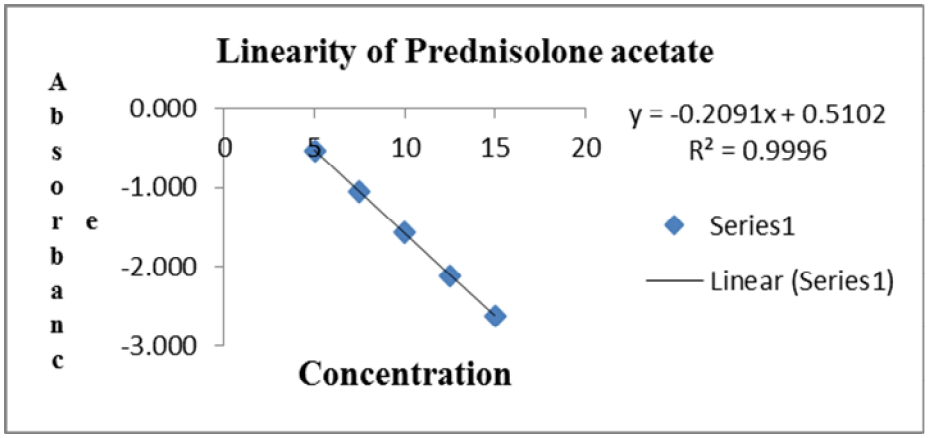

Fig 5: Calibration curve of Prednisolone Acetate

3.1.2 Precision

The Relative Standard Deviation (%RSD) after six determinations was 0.823% at 3 µg/ml for GATI and 1.41% at 10 µg/ml for PRED (see Table 2).

Table 2: Precision data for GATI and PRED

Precision	Concentration found* (µg/ml)		%RSD	
	GATI	PRED	GATI	PRED
Repeatability	3.06	10.05	0.82	1.41
Intraday Precision	1.50	5.00	0.74	0.80
	3.01	10.02	0.91	0.89
	4.53	14.90	1.31	0.97
Interday Precision	1.50	5.00	1.77	1.12
	3.02	9.98	1.06	0.89
	4.51	15.02	1.54	1.32

*n=6

3.1.3 LOD and LOQ
LOD and LOQ of GATI and PRED were determined by calibration curve method. Solutions of GATI and PRED were prepared in the range of 1.5-4.5 µg/ml and -5-15 µg/ml respectively (see Table 1).

3.1.4 Accuracy
Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for GATI and PRED were 101.04% and 100.03%, respectively (see Table 3).

Table 3: Recovery data for GATI and PRED

Drug	Conc. taken (µg/ml)	Conc. Added (µg/ml)	Total conc. found (µg/ml)	Amount recovered (µg/ml)	Mean Recovery* ± SD(%)
Gatifloxacin	3	1.2	2.71	1.21	100.80 ± 1.06
	3	1.5	3.01	1.44	101.18 ± 0.67
	3	1.8	3.32	1.82	101.16 ± 0.94
Prednisolone Acetate	10	4	9.00	3.94	100.00 ± 0.44
	10	5	10.02	5.02	100.42 ± 0.81
	10	6	10.94	5.94	99.10 ± 0.29

***n=3**

Table 4: Summary of Validation Parameter

Sr. No.	Parameters	Results	
		Gatifloxacin	Predprednate
1.	Linearity Range (n=5) (µg/ml)	1.5-4.5	5-15
2.	Regression equation	y = 0.265x – 0.171	y = -0.209x + 0.510
3.	Correlation coefficient (R^2)	0.9997	0.9996
4.	Limit of detection (n=5) (µg/ml)	3.91	13.04
5.	Limit of quantification (n=5) (µg/ml)	11.85	39.53
6.	Precision		
	Repeatability (%RSD) (n=6)	0.82	1.41
	Intraday (%RSD)(n=3)	0.98	0.88
	Interday (%RSD)(n=3)	1.45	1.11
7.	Accuracy (Mean ± SD) (%, n=3)	101.04 ± 0.89	100.03 ± 0.60

Table 5: Analysis of market formulation

Drug	Label claim (%)	Conc. taken for % Assay (µg/ml)	Average Abs.*	Conc. found from Mixture	Assay* ± SD (%)
Gatifloxacin	0.3	2.25	0.438	2.24	99.81 ± 0.18
Prednisolone Acetate	1	7.5	-1.080	7.48	99.89 ± 0.11

*n=3

4. Conclusion
The proposed Second derivative spectrophotometry method was used for the simultaneous estimation of Gatifloxacin and Prednisolone Acetate was found to be sensitive, accurate, precise, simple, and rapid. Hence the present method may be used for routine analysis of the raw materials as well as combined dosage formulations containing Gatifloxacin and Prednisolone Acetate.

5. Acknowledgement
The author is thankful to Provizer Pharma, Surat., India for providing sample Gatifloxacin and Prednisolone Acetate for research. The author is thankful to Sigma Institute of Pharmacy College, Vadodara, Gujarat, India, for providing all the facilities to carry out the work.

6. References
1. Drug Bank, Drug Profile, Gatifloxacin date accessed on 24-12-2013 http://www.drugbank.ca/drugs/DB01044.
2. Key Information on Active Pharmaceutical Ingredients. http://www.betterchem.com/pharmacopeia/USP_109.htm date accessed on 09-12-2013.
3. The British Pharmacopoeia; The Stationary office on behalf of the Medicines and Healthcare products Regulatory Agency, 2011.
4. Drug Bank, Drug Profile, Prednisolone Acetate date accessed on 09-12-2013 http://www.drugbank.ca/drugs/DB00860
5. The International Drug Index. http://www.rxlist.com/gatsun-p-drug.htmldate accessed on 09-12-2013
6. Venugopal K, Saha RN. New simple and validated UV-spectrophotometric methods for the estimation of gatifloxacin in bulk and formulations. Il Farmaco 2005; 60:906–912.
7. Salgado HRN, Lopes CCGO, Lucchesi MBB. Microbiological assay for gatifloxacin in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis 2006; 40:443–446.
8. Tasso L, Costa TD. High performance liquid

chromatography for quantification of gatifloxacin in rat plasma following automated on-line solid phase extraction. Journal of Pharmaceutical and Biomedical Analysis 2007, 205–210.
9. Motwani SK, Khar RK, Ahmad FJ, Chopra S, Kohli K, Talegaonkar *et al.* Stability indicating high-performance thin-layer chromatographic determination of gatifloxacin as bulk drug and from polymeric nanoparticles. Analytica Chimica Acta 2006; 576:253–260.
10. Overholser BR, Kays MB, Sowinski KM. Determination of gatifloxacin in human serum and urine by high-performance liquid chromatography with ultraviolet detection. Journal of Chromatography B 2003; 798:167–173.
11. Al-Dgither S, Alvi SN, Hammami MM. Development and validation of an HPLC method for the determination of gatifloxacin stability in human plasma. Journal of Pharmaceutical and Biomedical Analysis 2006; 41:251–255.
12. Mirza S, Rabindra N, Hassan DM, Huda N, Shaikh F. Simultaneous determination of gatifloxacin and ambroxol hydrochloride from tablet dosage form using reversed phase high performance liquid chromatography. Chinese journal of chromatography 2008; 26(3):358–361.
13. Zhu X, Gong A, Yu S. Fluorescence probe enhanced spectrofluorimetric method for the determination of gatifloxacin in pharmaceutical formulations and biological fluids. Spectrochimica Acta Part A 2008; 69:478–482.
14. Shreef NB. Direct determination of prednisolone by derivative uv spectroscopy. Raf Jour Sci 2008; 19(3):38-46.
15. Zhang Y, Wu H, Ding Y, Xia A, Cui H, Yu R. Simultaneous determination of cortisol and prednisolone in body fluids by using HPLC-DAD coupled with second-order calibration based on alternating trilinear decomposition. Journal of chromatography B 2006; 840:116-123.
16. Katakam P, Sireesha KR. Stability indicating hplc method for simultaneous determination of chloramphenicol and prednisolone acetate in bulk and formulations. Asian journal of pharmaceutical and clinical research 2012; 5(3):182-185.
17. Razzaq SN, Khan IU, Mariam I, Razzaq SS. Stability indicating HPLC method for the simultaneous determination of moxifloxacin and prednisolone in pharmaceutical formulations. Chemistry central journal 2012; 6:94.
18. Fidani M, Gamberini MC, Pompa G, Mungiguerra F, Casati A, Arioli F. Presence of endogenous prednisolone in human serum. Steroids 2013; 78:121-126.
19. Patel SK, Patel HB. Spectrophotometric estimation of Gatifloxacin sesquihydrate and Prednisolone Acetate in combined pharmaceutical dosage form by Q-Absorbance ratio method. International research journal of pharmacy 2013; 4(1):221-224.
20. Patel HB, Patel SK. Dual wavelength spectrophotometric method for simultaneous estimation of Gatifloxacin sesquihydrate and Prednisolone Acetate in combined pharmaceutical dosage form. International Journal of Pharmaceutical, Chemical and Biological Sciences 2013; 4(1):221-224.