Asian Journal of Chemistry; Vol. 23, No. 11 (2011), 5092-5094

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Development and Validation for Prednisolone in Tablet Dosage Form by Reverse Phase-HPLC

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(*Received*: 7 February 2011; *Accepted*: 27 July 2011) AJC-10218

A simple, economic, accurate reverse phase isocratic reverse phase-HPLC method was developed for the prednisolone (10 mg) in tablet dosage form. A hypersil ODS C_{18} (250 × 4.6 mm, packed with 5 micron) in an isocratic mode with mobile phase methanol:water (58:42) was used with flow rate (1.0 mL/min) and monitored at 254 nm. The retention times were 7.029 and 1.681 min for prednisolone and dexomethasone (internal standard), respectively. The linearity range was found to be 10-250 µg/mL. The proposed method was validated.

Key Words: Prednisolone, Dexomethasone Tablets, HPLC, Peak area ratio.

INTRODUCTION

Prednisolone is a synthetic corticosteroid drug that is particularly effective as an immunosuppressant and affects virtually all of the immune system[1]. Chemically 11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-3-one. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. A survey of literature reveals that good analytical methods are not available for the drugs like prednisolone. Even though very few methods of estimation of above drugs are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity, simplicity etc. The existing physicochemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for the assay of prednisolone in pharmaceutical dosage forms adapting different available analytical techniques like UV spectrophotometry and HPLC. According to the literature survey it was found that few analytical methods such as HPLC and UV-visible analysis were reported for the estimation of prednisolone[2-5]. The objective of the proposed method is to develop simple and accurate methods for the determination of prednisolone by UV-spectrophotometry as well as RP-HPLC methods in pharmaceutical dosage forms.

EXPERIMENTAL

Prednisolone and dexomethasone were obtained from Biodeal Laboratories Pvt. Ltd., (Himachal Pradesh, India). A commercial sample prednisone tablet containing prednisolone (10 mg) were procured from Biodeal Laboratories Pvt. Ltd., and used within their shelf-life period. The HPLC grade methanol and water from Rankem (New Delhi, India) and all other chemicals used were of pharmaceutical or analytical grade from Rankem. HPLC grade water was prepared using Millipore purification system. Quantitative HPLC was performed on Shimadzu HPLC with LC 10AT VP series pumps besides SPD 10AVP UV-visible detector. Shimadzu Class-VP version 6.14 SPI software was used along with Hypersil ODS C_{18} (4.6 mm × 250 mm, packed with 5 µ) column for the chromatographic separation. Automatic injections (20 µL)were used. The detector wavelength was set at 254 nm. To develop a suitable and robust HPLC method for the determination of prednisolone, different mobile phases methanol:water, were used in different compositions of mobile phases (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5,0.75,1.0, 1.2, 1.5, mL/min). The mobile phase methanol:water in the ratio of 58:42 at a flow rate of 1.0 mL/ min gave peaks good resolution for prednisolone and internal standard dexomethasone. Prednisolone and internal standard dexomethasone were eluted at retention times around 7.029 and 1.681 min, respectively with symmetric peak shape. The data were collected and analyzed with software in a computer system. Mobile phase used as diluents.

Stock solution of prednisolone (1 mg/mL) was prepared by dissolving 25 mg of prednisolone in 25 mL of volumetric flask containing 10 mL of mobile phase. The solution was sonicated for *ca.* 20 min and then made up to volume with mobile phase. The standard solutions of prednisolone was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25 mg of dexamethasone in 10 mL of mobile phase, sonicated for 20 min then made up to the volume with mobile phase. Working standard solutions of prednisolone were prepared by taking suitable aliquots of drug solution from the standard stock solution 1000 µg/mL, spiked with internal standard solution (0.1 mL) and the volume was made upto 10 mL with mobile phase. Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of prednisolone was extracted with mobile phase in a 25 mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in triplicate. The contents of the mobile phase were filtered before use through 0.45 µm filter paper and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the systems. Then, 20 µL of each of standard and sample solutions were injected into the HPLC system for six times to get the chromatograms. The retention time, average peak areas and peak area ratios of drug to internal standard were recorded. Plot a graph of concentration *versus* peak area (Fig. 1). The linearity range was found to be in between 30-250 µg/mL for prednisolone. The linearity range and linearity graphs were shown in Fig. 1. The amount of drug present in pharmaceutical formulation was calculated through peak area ratio of drug to that of internal standard by using the standard calibration curve (concentration in µg/mL was taken on X-axis and peak area ratio on Y-axis). A typical chromatogram of prednisolone in formulation and internal standard was shown in Fig. 2. The described method has been validated for the assay of prednisolone using following parameters[6-8]. Precision was studied to find out variations in the test methods of prednisolone (10 µg/mL) on the same day and on different day by using different column of same dimensions (ruggedness). The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of six replicates of a fixed amount of drug and internal standard. Precision and ruggedness were done on the same day and the different day, respectively and the RSD % was calculated for each. The accuracy of the method was shown by analyzing the model mixtures contained 80, 100 and 120 % of bulk samples of BUP along with internal standard within the linearity ranges were taken. After injected the standard solution, Accuracy-80 %, accuracy-100 % and accuracy-120 % solutions, the amount found, amount added for prednisolone, individual recovery and mean recovery values were calculated. Specificity is the ability to measure

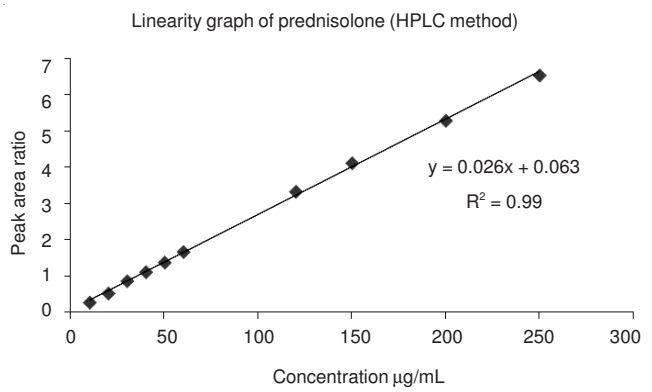

Fig. 1. Linearity curve for prednisolone

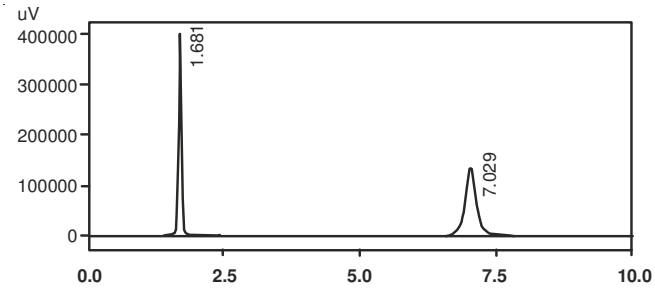

Fig. 2. Typical chromatogram of prednisolone (10 µg/mL) in pure form along with internal standard dexomethasone

accurately and specifically the analyte of interest in the presence of other components that may be expected to present in the sample matrix. It was found that the proposed method was specific as there was no interference of other active ingredients and excipients ensuring that the peak response was due only to a single component. As part of the robustness, deliberate change in the flow rate and mobile phase composition were made to evaluate the impact on the method. The flow rate was varied at 0.9 mL/min to 1.1 mL/min. The organic composition in the mobile phase ratio was varied at 53:47 and 63:37.

RESULTS AND DISCUSSION

A reverse-phase isocratic procedure was proposed as a suitable method for the analysis of prednisolone in tablets. A mixture of methanol:water in the ratio of 58:42 at a flow rate of 1.0 mL/ min was found to be an appropriate mobile phase allowing adequate and rapid separation of prednisolone and internal standard dexomethasone. The retention time was found to be 7.029 and 1.681 for prednisolone and internal standard dexomethasone, respectively. The percentage of purity of prednisolone in tablet dosage form was 99.01 ± 0.077. System suitability for the prednisolone, theoretical plates and tailing factor obtained from the standard injections was 32835.55 and 1.09, respectively. As shown in the Fig. 2 the substances were eluted forming well shaped, symmetrical single peaks, well removed from the solvent front. The precision of the HPLC system was determined using the RSD % of the peak areas for six replicate injections of the drug and internal standard. Precision data were present in Table-1. The RSD % was less than 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures

TABLE-2
DATA FOR ACCURACY

Sample (%)	Concentration (µg/mL)	Recovery (%) of pure drug	Statistical analysis	Sample
S1:80	8	10	98.26405	Mean = 98.30375
S2:80	8	10	98.34365	SD = 0.0398
S3:80	8	10	98.30356	RSD (%) = 0.040487
S4:100	10	10	98.94093	Mean = 99.01725
S5:100	10	10	99.01409	SD = 0.077954
S6:100	10	10	99.09674	RSD (%) = 0.078728
S7:120	12	10	100.2661	Mean = 100.3151
S8:120	12	10	100.2288	SD = 0.118699
S9:120	12	10	100.4505	RSD (%) = 0.118327

TABLE-1
DATA FOR PRECISION

Conc. (µg/mL)	Precision	Intermediate precision (ruggedness)
Peak area ratio	0.3564	0.3465
	0.3421	0.3396
	0.342	0.3356
	0.3423	0.3412
	0.3478	0.3396
	0.3412	0.3358
Mean	0.3453	0.339717
Standard deviation	0.00594	0.004013
RSD (%)	1.720156	1.181333

contained 80, 100 and 120 % of bulk samples of BUP along with internal standard within the linearity ranges. The recovery of prednisolone was evaluated from 80-120 % of the labeled tablet. The mean percentage recoveries were found to be 98.30 ± 0.04, 99.02 ± 0.08 and 100.32 ± 0.12 % for 80 %, 100 and 120 %, respectively. Accuracy data were present in Table-2. The results of robustness indicate that the variation in flow rate affected the method significantly. The method was robust only in less flow condition. Even variation in organic composition in the mobile phase affected the method significantly. Hence it indicates that the method was not robust even by change in the flow rate ± 10 % and change in the mobile phase at 53:47 and 63:37 for prednisolone.

Conclusion

The presented method was precise, sensitive and accurate. The advantages of proposed method were its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of prednisolone in pharmaceuticals.

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