RESEARCH ARTICLE

Spectrophotometric Determination of Corticosteroids and Its Application in Pharmaceutical Formulation

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ABSTRACT

A simple, sensitive and economically viable spectrophotometric method for the determination of corticosteroids (predinsolone {PSL}, dexamethasone {DEX}, prednisone {PS}, betamethasone sodium phosphate {BEP} and hydrocortisone {HYD} has been developed. The method involves the oxidation of corticosteroids by iron (III) and subsequent complexation of iron (II) with potassium hexacynoferrate (III), forming bluish green colored complex having the maximum absorbance at 780 nm. The method has been applied for the determination of above-mentioned corticosteroids in pharmaceutical formulations. The common excipients do not interfere with the proposed method. A statistical comparison of these results with those of reported method shows good agreement and indicates no significant difference in the precision. The precision of the method developed is implied from the values of standard deviation which are varying from 0.03% to 1.06%. These are remarkable in comparison to the existing visible spectrophotometric studies on steroids. . Recovery was found to be quantitative, and analysis to determine the mass per tablet was obtained with the variation of ±0.25% to 0.85% which implies the success of the method to get rid of the interference from excipients. The studies have shown that the method is fast, reproducible and accurate and can be used in the analysis of marketed formulations. The processed samples were stable up to 2hours minimizing the error in terms of fluctuating absorbance values.

Keywords: *Corticosteroid, Spectrophotometry,Ooxidation*

Synthetic corticosteroids are used in several pharmaceutical preparations. Graham et al. reported a colorimetric procedure for betamethasone benzoate in topical gel preparation that utilized preliminary oxidation of 17-keto function, followed by reaction with blue tetrazolium [1]. The blue tetrazolium reaction is widely used for the analysis of corticosteroids. USP XIX [2] and NF XIV [3] used a slightly modified procedure of Mader and Buck [4] for corticosteroids analysis. Reich et al. have reported the reaction of steroid ketone with 2, 4-dinitrophenylhydrazine [5]. The reaction leading to the formation of dinitrophenylhydrazone is usually carried out in alcoholic solution in the presence of small amounts of mineral acids. Under the condition specified in the experimental part of steroids containing keto group in the 3-, 6-, 7-, 12-, 16-, 17-, 20- positions reacted rapidly with dinitrophenylhydrazine. The 11-keto group is known to be unreactive to all carbonyl reagents. Side reaction such as dehydration was not observed, but keto group acid of the bile acids type was estrified, and the dinitrophenylhydrazones of the corresponding ester were obtained [6]. I.Clark has reported a colorimetric method for the estimation of cortisone, hydrocortisone, aldosterone and related steroids. The diphenylamine reagent, suitably modified to improve the intensity of reaction, was applied to measurement of some steroids [7]. It is worth mentioning that (although not too many) startlingly outdated colorimetric methods based on chemical reactions are still in use for the assay of bulk drug material, for example the blue tetrazolium assay was very popular in 1950s and 1960s for the assay of corticosteroids drug formulations, moreover in their bioassay[8,9,10,11]. However, it would be difficult to find acceptable arguments for the use of this method for the assay of several bulk corticosteroids in the recent addition of US pharmacopeia "Assay of steroids [12]. The specificity of this indirect method based on the natural absorption of corticosteroids and at the same time the advantages of the latter method, i.e. low time and labor consumption as well as high precision is lost. The isoniazide method was reported for the deterrmination of cardioglycosides by classical picrate colour solution having the advantage is that the absorbance maxima is shifted from about 240 nm to about 380 nm [13].

Table 1. Absorbance* replication of proposed procedure

Run	Prednisone	Dexamethasone	Hydrocortisone	Prednisolone	Betamethasone sodium phosphate
1	0.561	0.206	0.248	0.163	0.175
2	0.561	0.206	0.249	0.164	0.177
3	0.563	0.208	0.248	0.164	0.178
4	0.566	0.207	0.246	0.167	0.177
5	0.560	0.206	0.247	0.166	0.173
6	0.561	0.206	0.247	0.166	0.173
7	0.562	0.205	0.248	0.165	0.175
8	0.561	0.206	0.246	0.163	0.175
9	0.562	0.205	0.246	0.164	0.178
10	0.561	0.205	0.245	0.168	0.177
Average	0.561	0.206	0.248	0.160	0.175
S.D.	0.0017	0.0010	0.0012	0.0017	0.0018
R.S.D. (%)	0.30	0.48	0.58	1.06	1.02

Concentration: 20 μg/ml

L. Ayllon et al. [14] have modified the classical blue tetrazolium and porter silber method for the determination of corticosteroids.

The purpose of present work was to provide a simple, sensitive and economically viable spectrophotometric method for the determination of predinsolone, dexamethasone, prednisone, betamethasone sodium phosphate and hydrocortisone. The method is based on the oxidation of corticosteroids with iron (III) in acidic medium and subsequent complexation of iron (II) with potassium hexacynoferrate (III). The method offers the advantage of sensitivity and stability. Common excipients present in pharmaceutical preparation do not interfere in the determination of corticosteroids.

MATERIALS AND METHOD

Experimental

Apparatus: UV-vis double beam spectrophotometer Systronics-2203 with 1 cm stoppered quartz cells was used for the absorbance measurements.

Materials: Analytical reagent grade methanol, prednisone (sigma), hydrocortisone (SRL), prednisolone, betamethasone sodium phosphate and dexamethasone (Mahima Exports, Sonepat (Hr) were used. All other reagents were of analytical grade.

Reagent: Standard solutions of corticosteroids were prepared in methanol. 0.5% (w/v) solutions of iron (III) chloride and potassium hexacyanoferrate (III) were pre-

pared in distilled water.

General procedure: Appropriate volumes of working solutions of corticosteroids were transferred into a series of 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added to each followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^0C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Analysis of tablets: An accurately weighed amount of powdered tablets equivalent to pure form of corticosteroids as dissolved in methanol (~10ml). The solution was filtered through a Whatmann No. 41 filter paper, which was then washed with about 10 ml methanol. The filtrate and washings were collected into a 100 ml standard flask and diluted to mark with double distilled water. A volume of latter solution was diluted with double distilled water to obtain a solution equivalent to 20μg/ml of the corticosteroids and then subjected to analysis as directed under general procedure.

RESULT AND DISCUSSION

The method offers the advantages of simplicity, rapidity and sensitivity without the need for extraction. The method is successfully employed for the determination of corticosteroids in pharmaceutical preparations, and common excipients, used as additives in pharma-

Table 2. Optical characteristics and precision data

Parameter/characteristics	PS	HYD	PSL	DEX	BEP
Color	Bluish green	Bluish green	Bluish green	Bluish green	Bluish green
λmax (nm)	780	780	780	780	780
Stability (h)	2	2	2	2	2
Beer's law range (μg/ml)	10-50	10-50	10-50	10-50	10-50
Molar absorptivity (M^{-1} cm^{-1})	60.0×10^4	0.56×10^4	0.41×10^4	0.55×10^4	0.48×10^4
Regression equation (Y)[a]					
Slope (b)	0.02215	0.001538	0.001321	0.001523	0.001386
Intercept (a)	-0.12167	-0.0827	-0.03247	0.0241	0.0827
Correlation coefficient (r)[b]	0.9998	0.9583	0.9928	0.9967	0.9988

[a] y = bx + a, where x is the concentration in micro gram/millilitre (μg/ml)

[b] n = 5,

[c] Five replicates

Table 3. Results for determination of corticosteroids in pharmaceutical products

Drug	Natural composition (mg)	Steroid found (mg)	Error (%)
Wysolone[a]	5 prednisolone	4.970	-0.6
Dexona[b]	0.5 dexamethasone	0.504	+0.8
Pe-sone[c]	0.5 prednisone	0.496	-0.8
Betnisol[d]	0.5 betamethasone sodium phosphate	0.539	+0.39

[a] Marketed by, Wyeth limited
[b] Marketed by, Cadilla pharmaceuticals
[c] Marketed by, Cadilla pharmaceuticals
[d] Marketed by, Glaxosmithkline India

ceuticals, do not interfere with the proposed method. The applicability of the method for the assay of pharmaceutical preparations was examined. The results of assays of available tablets reported for steroids are reflecting the accuracy and precision in comparison to the existing USP method.

The investigated corticosteroids are oxidized by iron (III) chloride in the acidic medium and produce iron (II). The iron (II) ion reacts with potassium hexacyanoferrate (III) and produces bluish green iron (II) ferricynide complex with an absorbance maximum at 780 nm as shown in Fig. 1. The reactivity of corticosteroids towards reduction of iron (III) appears to be, at least in part, a function of their molecular shape, prednisone possessing C11 carbonyl reacts the fastest with Fe^{3+}. Similar behavior of steroids is reported with terazolium blue reagent. The mechanism involved in the reaction of corticosteroids is a two electron oxidation of ketol side chain using iron (III) as oxidizing agent and may be illustrated as shown in Scheme 1.

The reaction conditions were established by varying one parameter at the time and keeping the others constant. The effect of concentration of iron (III) chloride and potassium hexacyanoferrate (III) on the absorbance, while keeping a fixed concentration of prednisone was investigated. A maximum and constant absorbance was found with 6.0×10^{-3} M concentration of $FeCl_3$ and 7.5×10^{-4} M concentration of $K_4Fe(CN)_6$, which were therefore adopted as optimum. The color reaction occurred at room temperature, though at high temperature the color developed more rapidly (Fig. 2). The maximum absorbance was observed after 30 minutes of heating at 70 ± 2^0C. The temperature of 70 ± 2^0C and a reaction time of 30 minutes were selected for reproducible results. The effect of the studied parameters of prednisolone resembled those of dexamethasone, prednisone,

betamethasone sodium phosphate, and hydrocortisone.

Aliquots of each steroids (20 µg/10ml) were analysed by the proposed method, and the absorbance were determined (Table 1). The relative standard deviation, varied from 0.30 to 1.06 within overall average of 0.68%.

The optimal characteristics and optimal data are given in Table 2. The upper limit of Beer-Lambert's range was determined by a plot of absorbance versus concentration at λmax (Fig.1). Beyond this limit (50 µg/ml); the correlation results were really affected. This means that the current studies were carried out according to the Beer's law range given in Table 2. Hence the absorbance was excluded above these limits to keep the relationship linear. The stability of the products was more than 2 hr. The applicability of the method for the assay of tablets (above mentioned corticosteroids) were tested for the determination of same in their pure form and in proprietary drugs supplied by different companies containing other active ingredients (Table 3). The results of assay of tablets of corticosteroids are summarized in Table 3. The data are reproducible, and the assay of tablets was cross checked by the USP method, which agreed favorably (Table 3). The performance of the proposed method was compared statistically using the Student's t test and variance ratio f test. At the 95% confidence level, the calculated t and F values did not exceed the theoretical values.

The theoretical t value was 2.78 (for n = 5), and the theoretical F value was 6.39 (for n = 5). From the results given in Table 4, it was found that there was no significant difference between the proposed method and the official method, indicating that the proposed method was as accurate and precise as the USP method.

The main advantage of the reported method for the detection of the steroids is low costing and rapid assay
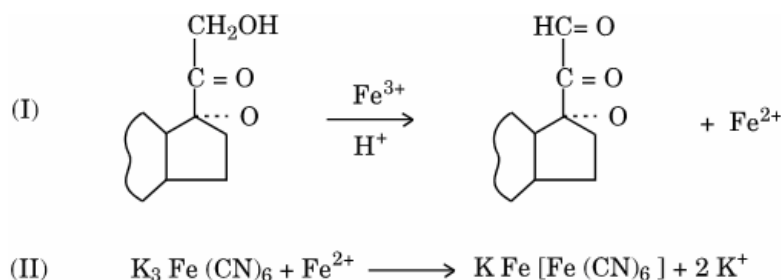
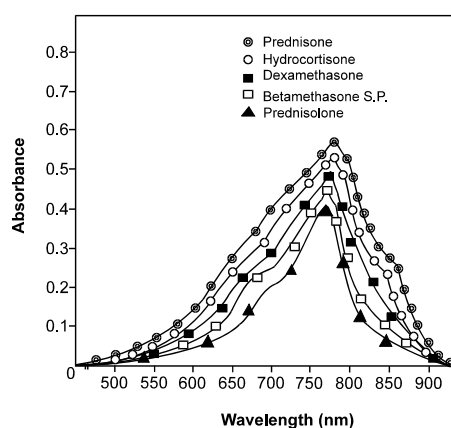
Scheme-1. Reaction sequence for the formation of products.
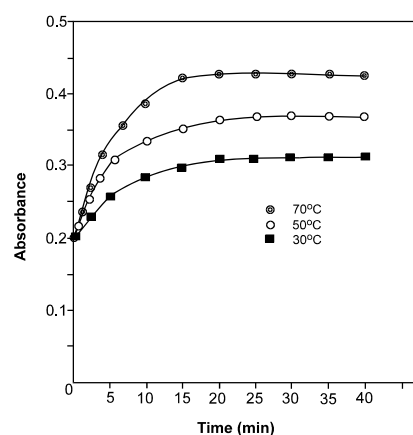
Fig.1 : Absorption spectra of corticosteroids.

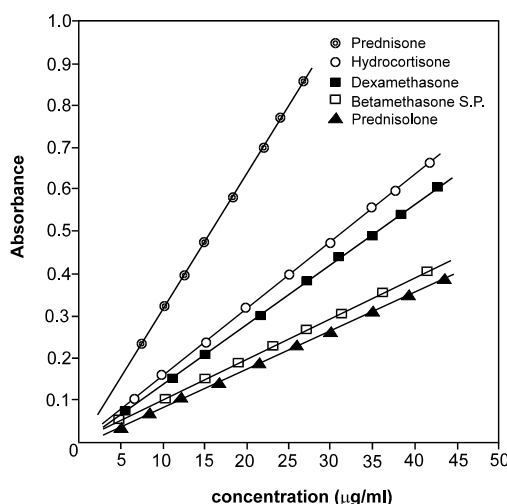**Fig.2 :** Effect of variable on the absorbance of prednisone (20 μg/ml)

Fig.3 : Plots of absorbance versus concentration

without sacrificing the sensitivity and accuracy.

The consumption of very common chemicals like ferric chloride and Conc. Sulphuric acid which you can find even most laboratories make this method very handy cost wise. The usage of such cheaper chemical will definitely reflect on the cost of the method. As far as the time consumption is concerned, the total time to carry out the complete experiment right from the beginning is less than single hour and it will add up in the values of the reported method.

The cost and time is the most serious concern where we have focused while developing method without losing the basics interest like sensitivity and accuracy.

REFERENCES

1. Graham RE, Biehl ER, Kenner CT. Analysis of betamethasone and its organic esters in pharmaceutical products J. Pharm. Sci: 1978; 67: 360-363.

2. The United States Pharmacopoeia, 19th rev., Mack Publishing Co., Easton, Pa. 1975.

3. The National Formulary, 14th ed., Mack Publishing Co., Easton, Pa. 1975.

4. Mader WJ, Buck RR. Colorimetric determination of cortisone and related ketol steroids, Anal. Chem: 1952;24: 666-667.

5. Reich H. Crane KF Sanfilippo SJ. The reaction of steroid ketones with 2, 4 dinitrophenylhydrazine J.Pharm.Sci: 1953; 29: 823-831.

6. Strain H., 3-Nitrophenylhydrazones, 2, 4-dinitrophenylhydrazone and the separation of of hydrazone by adsorption, J. Am. Chem. Soc: 1935; 57: 758-761.

7. Clark I. A colorimetric reaction for the estimation of cortisone, hydrocortisone, aldosterone and related steroids, Nature 1955; 75:123-124.

8. Gorog S. The sacred cow: the questionable role of assay method in characterizing the quality of bulk pharmaceutical, J. Pharm. Biomed. Anal: 2005; 36: 931-937.

9. Gorog S. Quantitative analysis of steroids, Elsevier, Amsterdam, 1983.

10. Blanco M, Coela J, Iturige H, Maspoch S, Villagas N. Kinetic spectrophotometric determination of hydrocortisone acetate in pharmaceutical formation by use of PLS method. Analyst: 1999;124: 911-915.

11. Mostafa AA, Bebawy LI, Refat HH. spectrophotomtric determination of clobetasol propionate, Halobetasol propionate, quinagolide hydrochloride, through Charge transfer complexation., J. Pharm. Biomed. Anal: 2002; 27: 889-899.

12. United State Pharmacopoeia, XXVII ed., The USP convention Inc., Rockville, MD 2004.

13. The European Pharmacopoeia, IV ed., council of Europe, Strasburg 2002.

14. Ayllon L. Improve automatic kinetic method for the determination of various corticosteroids, J. Pharm.Sci. 1994; 83: 1135-1141.

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