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Spectrophotometric Determination of Budesonide

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Spectrophotometric methods, including one UV and two colorimetric methods are proposed for the determination of budesonide in pure form and in aerosols. The UV method involves preparation of methanolic extract of the drug and its subsequent dilution. Whereas, the colorimetric methods involve reaction of the drug, with tetrazolium blue (TB) in alkaline medium and with phosphomolybdic acid (PMb) in acidic medium to produce purple and blue coloured complexes respectively. Effects of variables such as reagent concentration, alkalinity, time and temperature have been evaluated to permit selection of the most advantageous technique. Beer's law was followed in the concentration range of 1-40 μg/ml (UV), 5-160 μg/ml (TB) and 10-280 μg/ml (PMb). The molar absorptivity at 241.6 nm was 1.56 x 10^4 mol^{-1} cm^{-1} (UV), 530 nm was 4.04 x 10^3 $mol^{-1}cm^{-1}$ (TB) and at 715 nm it was 2.38 x 10^3 mol^{-1} cm^{-1} (PMb). The relevant Sandell's sensitivity was 0.02 μg cm^{-2}, 0.1 μg cm^{-2} and 0.18 μg cm^{-2} respectively, per 0.001 absorbance unit.

Budesonide, 16α, 17α-butylidene dioxy-11β, 21-dihydroxypregna-1,4-diene-3,20-dione, is a glucocorticoid used by inhalation in the management of asthma and allergic rhinitis[1]. Budesonide is official only in European Pharmacopoeia (EP)[1], which suggests a liquid chromatography method for the estimation of budesonide in bulk. The different analytical methods that are reported for its determination include ELISA[2,3] and High Performance Liquid Chromatography[4,5]. No spectrophotometric methods has been reported till date for the estimation of budesonide. Hence, it was thought worthwhile to develop a spectrophotometric method for quick estimation of pure drug and drug in aerosols.

In the present study, one UV and two colorimetric methods for the determination of budesonide in bulk and in its aerosol formulation are described. The UV method is based on the extraction of budesonide in methanol and its subsequent dilution with methanol before absorbance measurement in UV region. The tetrazolium blue (TB) method is based upon the proportionate reduction of the dye by the C-20 ketal functional group and the phosphomolybdic acid (PMb) method involves the ion-pair formation with budesonide. The absorbance measurements were made at λmax 241.6 nm for UV, 530 nm with TB and 715 nm with PMb. These methods are simple, rapid, sensitive, easy to apply in routine usage and does not need costly instrumentation.

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EXPERIMENTAL

All absorbance measurements were made in the double beam mode on a Hitachi U-2000 UV/Visible spectrophotometer using 1-cm matched quartz cells. All chemicals were of analytical reagent grade and solutions were prepared with double distilled water meeting the specification mentioned in Indian Pharmacopoeia[6]. Stock solution containing 1 mg/ml of budesonide in methanol was freshly prepared. The pharmaceutical grade of budesonide was provided by Astra IDL., Banglore, India and was used without further purification. A solution of tetrazolium blue containing 0.2% w/v of tetrazolium blue

in methanol was prepared and stored in dark and used within seven days. Alcoholic solution of sodium hydroxide containing 12% w/v of sodium hydroxide in 95% v/v of methanol was freshly prepared. Dilute sodium hydroxide solution was also freshly prepared containing 5% w/v of sodium hydroxide in water. A standard solution of phosphomolybdic acid was prepared by dissolving 2 g of phosphomolybdic acid in an mixture of 100 ml of glacial acetic acid, 60 ml of dilute sodium hydroxide and 40 ml of water. It was filtered through a Whatman No. 41 filter paper and used. Inhalation aerosols of Pulmicort (Astra IDL., India) with 200 µg per puff and Rhinocort (Astra IDL., India) with 150 µg per puff, were obtained locally.

Methods of analysis

Method A (UV): Suitable aliquots (0.01-0.4 ml) of the stock solution of budesonide were transferred into 10 ml volumetric flasks. The volume was made upto 10 ml with methanol, the solution shaken well for proper mixing and the absorbance of the solutions measured at 241.6 nm.

Method B (TB): Into a series of 10 ml volumetric flasks, accurately measured aliquots of budesonide solution (0.05-1.6 ml) were pipetted and 1 ml of tetrazolium blue reagent was added followed by 3 ml of alcoholic sodium hydroxide. The volume was made up to 10 ml with methanol and the volumetric flasks were kept in dark for 30 min for colour development. The absorbance of the coloured product is measured at 530 nm against a reagent blank prepared under similar conditions.

Method C (PMb): Similarly, aliquots of budesonide solution (0.1-3.2 ml) were pipetted into a series of 10 ml volumetric flasks. It was evaporated to dryness at 60° using water bath and to it 4 ml of PMb reagent was added. The mixture was allowed to react in a water bath at 95-99° for 5 min; the reaction was then quenched by cooling for about 2 min in an ice bath. It was then diluted to 10 ml with dilute sodium hydroxide solution and the absorbance measured at 715 nm against a reagent blank prepared under similar condition.

Analysis of commercial formulation:

The aerosol canisters were held with their mouth facing inside a 250 ml long-necked borosil beaker. The canisters were held upright and fully actuated ten times at an interval of 30 sec. The deposited budesonide in the inner walls of beaker was rinsed thrice with 2 ml of methanol. Finally its volume was adjusted to 10 ml with methanol and suitable aliquots of solution were subjected to UV/colorimetric methods described earlier.

RESULTS AND DISCUSSION

The effect of different variables were studied in order to develop simple, quick and convenient procedures for the estimation of budesonide. The methods were repeated using different concentrations of budesonide. The results suggest that the variables were independent of the concentration of budesonide.

The effect of reagent concentration on the colour intensity of the complex has been studied. It was found that 1 ml (TB) and 4 ml (PMb) of the reagent, was sufficient for maximum colour development for 100 µg (TB) and 400 µg (PMb) of budesonide respectively. However, the absorbance decreased with higher and lower amounts of the reagent (Fig. 1). The effect of strength (%w/v) of sodium hydroxide on the colour development of budesonide with TB and PMb is shown in figure 2. It was observed that maximum colour intensity was obtained for 12%w/v sodium hydroxide solution (TB) and 5% w/v

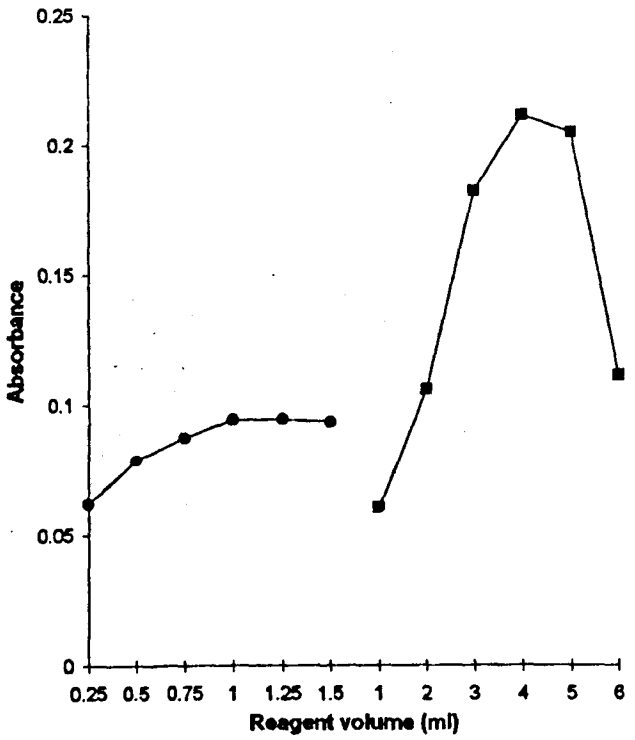

Fig. 1 : Effect of reagent concentration on absorbance of the coloured reaction product.

—●— TB, —■— PMb

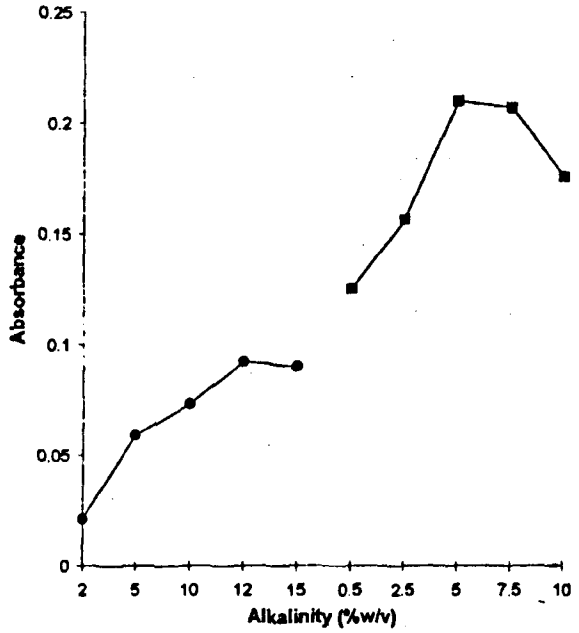

Fig. 2 : Effect of sodium hydroxide concentration on absorbance of coloured reaction product

—●— TB, —■— PMb

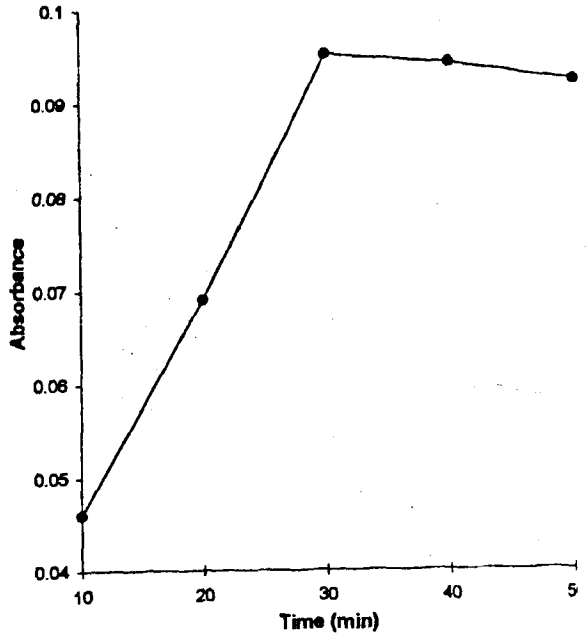

Fig. 3 : Effect of time in dark on absorbance of coloured reaction product

—●— TB

sodium hydroxide solution (PMb) respectively. A higher or lower sodium hydroxide concentration decreased the colour intensity. The effect of time of storage in dark for colour development (TB), on the colour intensity of the complex was studied. The results given in figure 3 show that the maximum absorbance (530 nm) of the coloured complex was attained after 30 min incubation in dark at room temperature. Time below 30 min of incubation in dark was found to be insufficient for adequate colour development. Similarly, absorbance of samples prepared with PMb was measured at 715 nm at various temperature and duration of heating. The blue compound was not formed at room temperature; the reaction rate was enhanced at elevated temperature and maximum absorbance was obtained after heating for 5 min at 95-99° (Table 1). Further heating caused no change in the intensity of the colour; hence a development time of 5 min at 95-99° was kept for all further experiments. The stability of the developed colour was studied over a period of 300 min in light, for both TB and PMb methods. The colour of the solutions is stable for at least 1 h at room temperature (Fig. 4) Methanolic solution of budesonide (UV) was found to be stable for 48 h, after which the study was discontinued. Budesonide in methanol yields a characteristic curve when scanned using ultraviolet spectroscopy. The curve showed absorption maxima at 241.6 nm and its molar absorptivity was 1.56×10^4 $mol^{-1}cm^{-1}$. The purple coloured reduction product of TB with budesonide and the blue colured complex of PMb with budesonide have maxima at wavelength range of 525-533 nm and 710-724 nm respectively. Hence all absorbance measurements were made at 530 nm (TB) 715 nm (PMb) and the molar absorptivity of these solutions were found to be 4.04×10^3 $mol^{-1}cm^{-1}$ and 2.38×10^3 $mol^{-1}cm^{-1}$ respectively. The methanolic solution of budesonide obeyed Beer's law at 241.6 nm in the concentration range of 1-40 µg ml^{-1} with Sandell's sensitivity[7] of 0.02 µg cm^{-2} per 0.001 absorbance unit. Similarly the coloured complex of budesonide with TB and PMb also obeyed Beer's law at 530 nm and 715 nm in the concentration range of 5-160 µg ml^{-1} and 10-320 µg ml^{-1} respectively. The Sandell's sensitivity[7] was 0.10 µg cm^{-2} (TB) and 0.18 µg cm^{-2} (PMb)

TABLE 1 : COLOUR DEVELOPMENT OF BUDESONIDE WITH PHOSPHOMOLYBDIC ACID

Sr. No.	Temperature (time, 5 min)	Absorbance* ±S.D.	Time (min) (temp. 95-99°)	Absorbance* ±S.D.
1	35-45°	0.014 ±0.004	2	0.165 ±0.013
2	55-65°	0.060 ±0.006	3	0.198 ±0.017
3	75-85°	0.178 ±0.003	4	0.203 ±0.009
4	95-99°	0.207 ±0.001	5	0.209 ±0.005
5	—	—	6	0.208 ±0.007
6	—	—	7	0.209 ±0.005

* Mean of five determinations

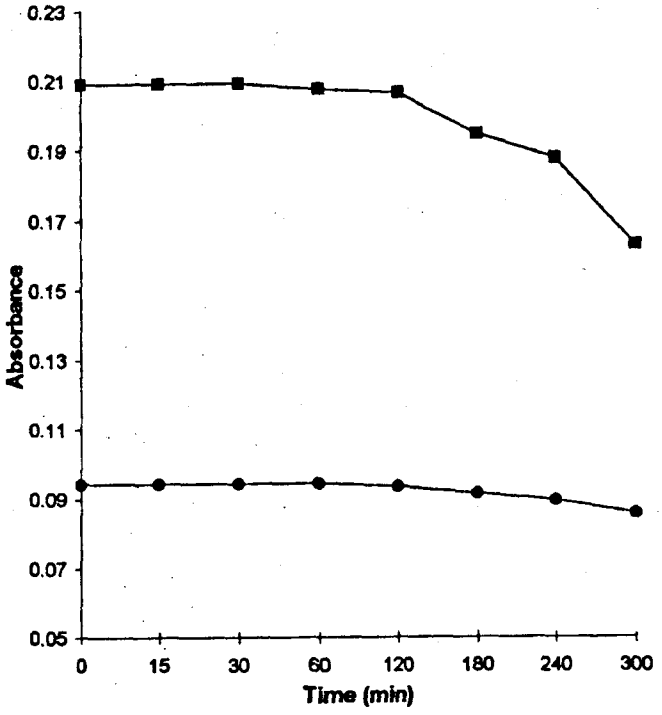

Fig. 4 : Stability of the colour reaction

—●— TB, —■— PMb

per 0.001 absorbance units. Five parallel estimations were made at each concentration level and the regression equations are A = 0.0024+0.034c (UV, n = 35), A = 0.0356+0.0092c (TB, n = 45 and A = 0.0099+0.0045c (PMb, n = 45), where A, absorbance in 1-cm cell; c, concentration in µg/ml. The correlation coefficients are 0.999 (UV), 0.990 (TB) and 0.997 (PMb).

Five replicate determinations of each sample solution were carried out to test the accuracy and precision of the methods for the determination of budesonide in its pure form and in aerosols (after extraction of budesonide as previously described). The results of all the three methods in this investigation appeared to be highly satisfactory and are reported in Table 2 together with the results obtained by the EP method. From statistical analysis (Students t-test) of the findings of the proposed methods and of pharmacopoeial method[1], the calculated t-value at the 95% confidence level (P=0.05) did not exceed the theoretical t_p value (2.78), therefore, the null hypothesis is verified, indicating no significant difference from the results of EP method.

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TABLE 2 : COMPARISON OF DIFFERENT METHODS OF ANALYSIS OF BUDESONIDE

Drugs or proprietary aerosols	Composition	Recovery* ±S.D.				t (calculated)#		
		EP	UV	TB	PMb	UV	TB	PMb
Budesonide (Astra IDL)	100% Budesonide (Absolute)	99.51 ±0.29	99.16 ±0.29	100.20 ±0.54	100.08 ±0.08	1.01	1.69	2.09
Pulmicort (Astra IDL)	200 µg per puff	100.57 ±0.09	100.83 ±0.19	101.06 ±0.23	100.59 ±0.02	1.09	1.93	1.03
Rhinocort (Astra IDL)	150 µg per puff	100.79 ±0.04	101.24 ±0.17	101.72 ±0.94	100.89 ±0.01	1.89	2.09	1.00

* Mean of five determinations, assay as percentage label claim
#Theoretical value of t (P = 0.05) = 2.78

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