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Research Article

DEVELOPMENT OF SIMULTANEOUS SPECTROPHOTOMETRIC METHOD OF MESALAZINE AND PREDNISOLONE IN SAME DOSAGE FORM

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ABSTRACT

A simple, precise and economical procedure for the simultaneous estimation of Mesalazine and Prednisolone in combined dosage form has been developed. Mesalazine and Prednisolone are widely aimed for bacterial cure and are recommended for patients with inflammation of the digestive tract ulcerative colitis and mild-to-moderate Crohn's disease. The present method involves the solving of simultaneous equations (Vierodt's method). Mesalazine and Prednisolone were found to have absorbance maxima at 320 and 246 nm respectively in phosphate buffer (pH 7.4). Both these drugs obeyed Beer's law in the concentration range of 2-20 µg/ ml. The high values of correlation coefficients (r^2) indicated good linearity of calibration curve for both the drugs. Sandell's sensitivity µg/cm^2/0.001/abs unit of Mesalazine and Prednisolone was found to be sufficient and this show that very less amount of both drugs can be effectively detected by this method. The recoveries of Mesalazine and Prednisolone from the standard mixture solution were found to be 99.04% and 99.92% respectively. The recovery results indicated that Mesalazine and Prednisolone could be quantified by this procedure simultaneously in combined dosage form without the interference of common excipients.

Keywords: Simultaneous estimation, Mesalazine, Prednisolone, Spectrophotometeric method

INTRODUCTION

Spectrometry deals with instruments based on the absorption or emission of electromagnetic radiation as a result of its interaction with matter. Absorption spectrometery is the quantification of electromagnetic radiation absorbed by atoms, molecules or ions of specific wavelength[1]. The amount of absorption depends on the wavelength of radiation and the structure of compound. The absorption of radiation is due to subtraction of energy from the radiation beam when electrons in orbital of lower energy are excited into orbital of higher energy. Since this is a electron transition phenomenon, UV is sometime called electronic spectroscopy[2]. The technique of UV visible spectrophotometery is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380nm) or visible (380-800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV-visible region[3]. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that (a) The absorbance of a solution is the sum of absorbances of individual components or (b) the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combine dosage form include simultaneous equation method, absorbance ratio method, geometric correction method, orthogonal polynomial method, difference spectrophotometery derivative spectrophotometery absorption correction method, multicomponent method of analysis and two wavelength quantation method.

SIMULTANEOUS EQUATION METHOD OR VIERODT'S METHOD

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} different from the other, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method), provided certain criterias apply. The information required is (a) The aborptivities of X at and λ_1 and λ_2 are ax_1 and ax_2 respectively (b) The aborptivities of Y at and λ_1 and λ_2 are ay_1 and ay_2 respectively. (c) The absorbances of the diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ_1 and λ_2, the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At λ_1 $A_1 = a\,X_1\,b\,Cx + aY_1 b\,Cy$ ---------- (1)

At λ_2 $A_2 = a\,X_2\,b\,Cx + aY_2\,b\,Cy$---------- (2)

For measurements in 1 cm cells b=1

Rearrange eq. (2)

$$Cy = \frac{A_2 - a\,X_2\,b\,Cx}{aY_2}$$

Substituting for Cy in eq. (1) and rearranging

$$Cx = \frac{A_2\,aY_1 - A_1\,aY_2}{a\,X_2\,aY_1 - a\,X_1aY_2} \quad \text{----------(3)}$$

$$Cy = \frac{A_1\,a\,X_2 - A_2\,a\,X_1}{a\,X_2\,aY_1 - a\,X_1aY_2} \quad \text{----------(4)}$$

As an exercise one needs to drive modified equation containing a symbol b for path length for application in situations where A_1 and A_2 are measured in cells other than 1 cm path length. Criteria for obtaining maximum precision based upon absorbance ratios have been suggested that place limits on the relative concentration of the components of the mixture[4]. The criteria are that the ratios

$$\frac{A_2/A_1}{aX_2/a\,X_1} \quad \text{and} \quad \frac{A_2/A_1}{aY_2/aY_1}$$

should lie outside the range 0.1-2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λ_{max} of two component are reasonably dissimilar. An additional criterion is that the two components don't interact chemically thereby negating the initial assumption that the total absorbance is the sum of individual absorbances. The additiviy of the absorbance should

always be confirmed in the development of a new application of this techniques[5,6].

MATERIALS AND METHOD

Mesalazine and Prednisolone were generously supplied as a gift samples by M/s Glaxo Smith Kline, Bombay (India) and Torrent Pharmaceuticals, Ahmedabed India respectively. All other chemicals and reagents used were of analytical grade.

Apparatus and conditions

A double beam GBC Cintra-10 UV/Visible spectrophotometer with data processing capacity was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400nm using 1cm quartz cell at a scanned speed of 100 nm /min and fixed slit width of 3n

Preparation of standard stock solution

Stock solutions (1000 µg/ml) of Mesalazine and Prednisolone were prepared by dissolving separately 100mg of drug in minimum quantity of dimethyl formamide (DMF) and finally diluted with PBS (pH 7.4) to make up the volume up to 100 ml. The maximum absorbance (λ_{max}) of Mesalazine and Prednisolone were obtained at 332 nm and 246 nm, respectively for simultaneous estimation of Mesalazine and Prednisolone. A series of standard drug solutions in concentration range of 5-20 µg/ml were prepared by diluting appropriate volumes of the standard stock solutions. The scanning for solution of Mesalazine and Prednisolone were carried out in the range of 200-400 nm against PBS (pH 7.4) solution as blank for obtaining the overlain spectra that was used in the analysis. Absorbance and absorptivity of series of standard solutions were recorded at selected wavelengths.

The molar absorption coefficient equation was determined for the two drugs using calibration curve equations. Further, the molar absorption coefficient was determined by using the equation:

$$(\varepsilon) = E^{1\%}_{1cm} \times \frac{\text{Molecular weight}}{10}$$

$$\text{Sandell's Sensitivity} = \frac{\text{Molecular weight}}{\text{Molar Absorptivity}}$$

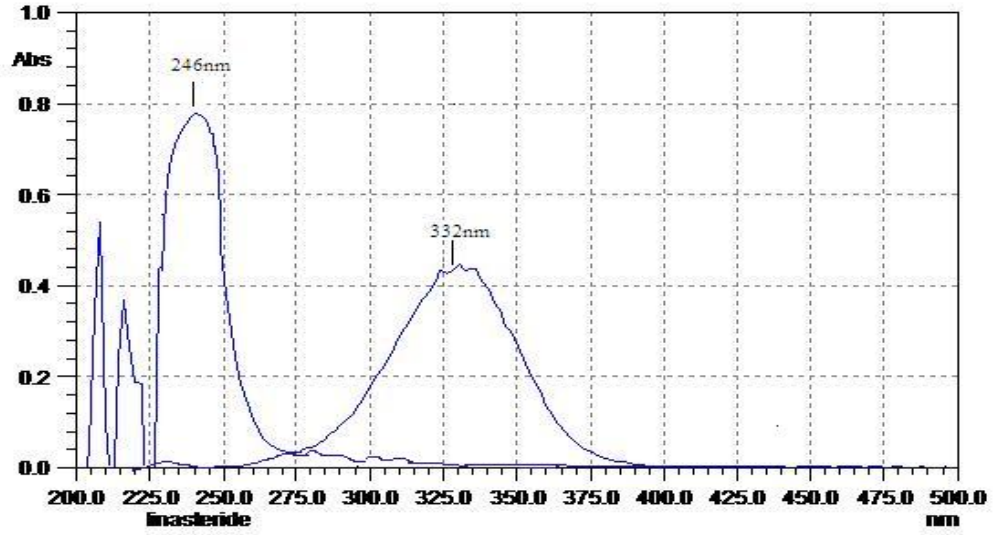

Fig. 1: Overlain spectra of Mesalazine and Prednisolone in PBS (pH 7.4)

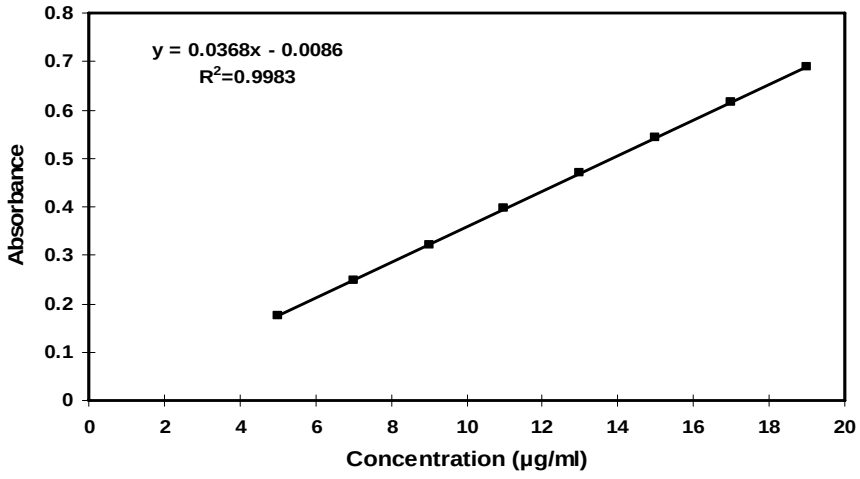

Fig. 2: Regressed standard curve of Mesalazine at λ_{max} 332nm

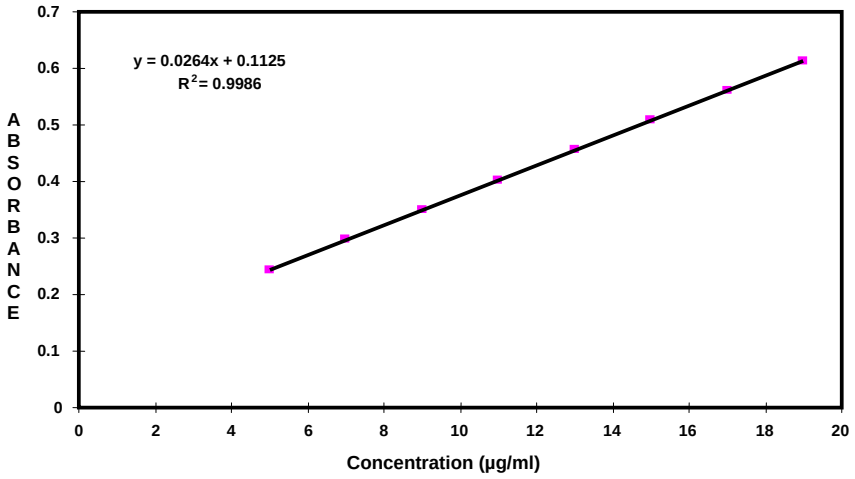

Fig. 3: Regressed standard curve of Prednisolone at λ_{max} 246nm

Development of simultaneous equation

Simultaneous equation was developed using the following set of equations:

At 332 nm $A_1 = a_{x1}bc_x + a_{y1}bc_y$ (1)

At 246 nm $A_2 = a_{x2}bc_x + a_{y2}bc_y$ (2)

Where C_x and C_y are concentration of Mesalazine and Prednisolone respectively.

A_1 and A_2 are absorbance at 332 nm and 246 nm respectively; a_{x1} and a_{x2} are absorption coefficient of Mesalazine at 332 nm and 246 nm respectively; a_{y1} and a_{y2} are absorption coefficient of Prednisolone at 332 nm and 246 nm respectively; b=1 (for measurement in 1cm cells). Substituting the values of a_{x1}, a_{x2}, a_{y1} and a_{y2} the equation could be rearranged as:

$$A_1 = 0.0346c_x + 0.0375cy$$

$$A_2 = 0.0140c_x + 0.0178cy$$

Where C_x and C_y are the concentration in µg/ml.

Recovery studies

In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking standard mixture solution of both Mesalazine and Prednisolone and absorbance was determined at 320 and 246 nm respectively.

Table 1: Absorbance and molar absorption coefficient of Mesalazine and Prednesolone at λmax 332 nm and 246 nm respectively

S.No	Conc. (µg/ml)	Mesalazine				Prednisolone			
		332 nm		246 nm		332 nm		246 nm	
		Absorbance	$E^{1\%}_{1cm}$	Absorbance	$E^{1\%}_{1cm}$	Absorbance	$E^{1\%}_{1cm}$	Absorbance	$E^{1\%}_{1cm}$
1	5	0.1804	360.80	0.0064	0.0129	0.0018	0.0168	0.2397	479.40
2	7	0.2345	335.00	0.0099	0.0141	0.0022	0.0209	0.2922	417.43
3	9	0.3278	364.00	0.011	0.0132	0.0029	0.0267	0.3576	397.33
4	11	0.3966	305.80	0.015	0.0142	0.0033	0.0339	0.4073	370.27
5	13	0.4751	316.30	0.018	0.0145	0.0039	0.0391	0.4591	353.15
6	15	0.5409	360.00	0.021	0.0144	0.0045	0.0401	0.5070	338.00
7	17	0.6225	366.80	0.024	0.0145	0.0050	0.0433	0.5604	329.65
8	20	0.6832	359.80	0.026	0.0141	0.0056	0.0487	0.6101	321.11
	Mean a_{x1}	346.06	Mean a_{x1}	0.0140	Mean a_{y1}	0.0329	Mean a_{y1}	375.79	

Table 2: Optical characteristics of Mesalazine and Prednisolone

Optical characteristics	Mesalazine	Prednisolone
λ_{max} (nm)	320	246
Beer lambert's law limits (µg/ml)	5-50	2-20
Molar absorptivity (L,mol^{-1}cm^{-1})	5.29 x 10^2	13.54 x10^2
Sandell's sensitivity (µg.cm^2/ 0.001 abs unit)	0.2899	0.2661
Regression equation	y = 0.0368x - 0.0086	Y=0.0005X+ 0.0707
Slope (m)	0.0368	0.0005
Intercept (c)	0.0086	0.0707
Correlation coefficient (r)	0.9983	0.9969

Table 3: Recovery studies

Drug in standard mixture (µg/ml)		%Recovery	
Mesalazine	**Prednisolone**	**Mesalazine**	**Prednisolone**
5	4	99.04±0.24	99.30±0.18
10	8	99.72±0.11	99.22±0.21
15	12	98.57±0.39	101.01±0.61
20	16	98.77±0.55	99.92±0.84

* Results are shown as mean ± S.D (n=3)

Sensitivity

Sensitivity of drug *viz* Mesalazine and Prednisolone was separately evaluated by estimating sandell's sensitivity (µg/cm^2/0.001Abs unit) to determine the minimum amount of substance that can be quantified in column of unit cross section.

RESULTS AND DISCUSSION

Absorption maxima and linearity

Overlain spectra of the drugs depicted occurrence of two peaks at 246nm and 332nm. Mesalazine and Prednisolone showed linearity with absorbance in the range 2-20 µg/ml at their respective maxima, which were validated by least square method. Coefficient of correlation was found to be 0.9983 for Mesalazine and 0.9987 for Prednisolone. The observations are presented in Table 1, Fig.2 and Fig.3. The optical characteristics and the statistical analysis of the experimental data, the regression equation from the calibration graphs along with standard error of the slopes and intercepts and regression correlation coefficient for the ultraviolet spectroscopic method are shown in Table 2.

The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law in indicated concentration range. The high values of correlation coefficients (r^2) also indicate good linearity of calibration curve for both the drugs. A study of spectra of Mesalazine and Prednisolone in Phosphate buffer saline (pH 7.4) shows that at 332nm Mesalazine shows maximum absorbance whereas Prednisolone shows almost zero absorbance. Similarly Prednisolone illustrated maximum absorbance at 246nm and Mesalazine depicted almost zero absorbance. This indicates that there is considerable difference in absorbance peaks of both the drugs and hence there is no interference.

Recovery studies and sensitivity

The recovery of Mesalazine and Prednisolone from the standard mixture solution was found to be 99.04 % and 99.92% respectively (Table 3). The recovery results indicated that the method is accurate, reproducible and Mesalazine and Prednisolone could be quantified by this procedure simultaneously. Sandell's sensitivity for Mesalazine was 0.2899 and for Prednisolone was 0.2661 µg.cm^{-2}/0.001 abs respectively (Table 2).

CONCLUSION

The proposed simultaneous equation method is very simple that can be performed by use of any spectrophotometer and dose not require any costly instrument equipped with special package. It also shows good linearity values and sensitivity. The results demonstrated that simultaneous equation method by GBC Cintra-10 UV/Visible spectrophotometer could be useful for technique for determination of Mesalazine and Prednisolone when they are given in same dosage form.

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