IJPSR (2012), Vol. 3, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 June, 2012; received in revised form 21 September, 2012; accepted 27 September, 2012

SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF MESALAMINE AND PREDNISOLONE IN COMBINED ORAL DOSAGE FORM

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Keywords:
Mesalamine (MSM),
Prednisolone (PRD),
Ulcerative Colitis,
Simultaneous equation method,
Validation studies

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QUICK RESPONSE CODE

IJPSR:
ICV- 4.57

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ABSTRACT

The objective of this study was to develop simple, precise, accurate, reproducible and economical vireodt's method for simultaneous estimation of mesalamine (MSM) and prednisolone (PRD) in combined oral dosage form. The method involved measurement of absorbance at two wavelengths, 332nm and 246nm, λmax of MSM and PRD, respectively in phosphate buffer (pH 7.4) with dimethyl formamide (DMF) as cosolvent. The linearity was obtained in the concentration range of 5-50 µg/ml and 2-20 µg/ml for MSM and PRD, respectively. The average percent recovery of MSM and PRD was found to be 99.19±0.78% and 99.71±0.82%, respectively. The accuracy and precision were determined and recovery studies confirmed the accuracy of the developed method that was carried out following the International Conference on Harmonization (ICH) guidelines. The recovery study was carried out by standard addition method. The proposed method was found to be rapid, specific, precise, accurate, and reproducible and can be successfully applied for the routine analysis of MSM and PRD in pharmaceutical dosage form.

INTRODUCTION: Mesalamine (MSM) (**Figure 1**) is chemically (5–amino–2–hydroxy benzoic acid), is an anti-inflammatory drug used to treat inflammation of the digestive tract (crohn's disease) [1, 2] and mild to moderate ulcerative colitis [3]. It is a bowl-specific amino salicylate drug that is metabolized in the gut and has its predominant actions there, thereby having fewer systemic side effects [4].

Prednisolone (PRD) (**Figure 2**) is chemically (11β)-11, 17, 21 trihydroxypregna-1, 4-diene-3, 20-dione), is a typical glucocorticoid has been used for the treatment of ulcerative colitis as the second line drug in the therapy [5, 6]. It has predominant glucocorticoid and low mineral corticoid activity and used for the treatment of a wide range of inflammatory and auto-immune diseases [7].

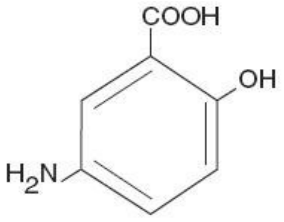

FIGURE 1: CHEMICAL STRUCTURE OF MESALAMINE (MSM)

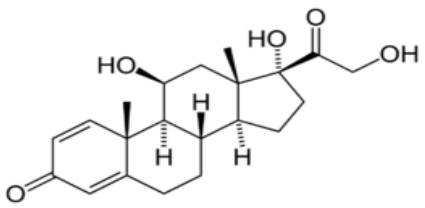

FIGURE 2: CHEMICAL STRUCTURE OF PREDNISOLONE (PRD)

The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of MSM and PRD in their combined dosage forms. Literature survey does not reveal any simple spectro-photometric or chromatographic method for simultaneous estimation of MSM and PRD in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method (vireodt's method) based on simultaneous equation for estimation of both drugs in their combined oral dosage forms.

MATERIALS AND METHOD:

Instrument: A Shimadzu model UV-1800, UV-VIS Spectrophotometer, with matched quartz cell corresponding to 1cm path length.

Reagents and Materials: MSM and PRD were supplied as a gift samples by Cipla Ltd., Ratlam (India) and Kwality Pharmaceuticals Pvt. Ltd., Amritsar (India) respectively. All other chemicals and reagents used were analytical grades.

Preparation of Standard Stock Solutions: Standard stock solutions (1000µg/ml) of MSM and PRD were prepared by accurately weighed 10 mg of MSM and PRD transferred in to 100 ml of volumetric flasks separately. Dissolved and diluted in minimum quantity of dimethyl formamide (DMF) and made up to the volume 10 ml with PBS (pH 7.4).

Study of Spectral and Linearity Characteristics: The standard stock solutions of MSM and PRD were further diluted with minimum quantity of dimethyl formamide (DMF) then finally diluted with PBS (pH 7.4) to get a concentration of 10 µg/ml of each. Both the solutions were scanned in the spectrum mode between the ranges of 200-400 nm against PBS (pH 7.4) as a blank and the overlain spectrum was recorded. The overlain spectra showed isoabsorptive point at 287 nm.

Simultaneous Equation Method: In quantitative estimation of two components by simultaneous equation method, two wavelengths i.e. 332nm, λ_{max} of MSM and 246nm, λ_{max} of PRD were selected from the overlain spectra. A set of two simultaneous equations were framed using absorptivity coefficient at selected wavelengths.

The concentrations of two drugs in the mixture were calculated using the following equations.

$$C_x = \frac{A_1 a_{y_2} - A_2 a_{y_1}}{a_{x_1} a_{y_2} - a_{x_2} a_{y_1}} \quad \text{----------Eq. (i)}$$

$$C_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{y_1} a_{x_2} - a_{y_2} a_{x_1}} \quad \text{----------Eq. (ii)}$$

Simultaneous equation was developed using the following set of equations:

At 332nm $A_1 = ax_1 bc_x + ay_1 bc_y$ ---------- (1)

At 246nm $A_2 = ax_2 bc_x + ay_2 bc_y$ ---------- (2)

Where C_x and C_y are concentration of MSM and PRD, respectively.

A_1 and A_2 are absorbance at 332nm and 246nm respectively; ax_1 and ax_2 are absorption coefficient of MSM at 332nm and 246nm respectively; ay_1 and ay_2 are absorption coefficient of PRD at 332nm and 246nm respectively;

b=1 (for measurement in 1cm cells).

The calibration curves for MSM and PRD were prepared in the concentration range of 5-50 µg/ml and 2-20 µg/ml for MSM and PRD, respectively at all selected wavelengths [8, 9, 10].

Substituting the values of ax_1, ax_2, ay_1 and ay_2 (from table 1 and 2) the equation could be rearranged as:

$A_1 = 0.028c_x + 0.0016c_y$

$A_2 = 0.0334c_x + 0.0595c_y$

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

Statistical Validation of Proposed Method [11, 12]: The method was validated according to ICH Q2 B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analytes.

Linearity (Calibration Curve): Linearity was studied by plotting the calibration curves using the standard solutions in concentration range of 5-50 µg/ml and 2-20 µg/ml for MSM and PRD, respectively and linear regression analysis was carried out.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ of MSM and prednisolone were calculated using the following equations; LOD = 3.3 (σ/S) and LOQ = 10 (σ /S), where σ is standard deviation (SD) of the y-intercept of calibration curve and S is slope of regression equation [12].

Intermediate Precision (Inter-day and Intra-day precision): Intermediate Precision of the method was inter-day and intra-day analysis i.e. the analysis of formulation was repeated six times in the same day and on three successive days. The amount of drug was determined and %RSD (relative standard deviation) were calculated which should be less than 2 % [13].

Accuracy: In order to check the accuracy and reproducibility of the developed method, recovery study was carried out by taking standard mixture solution of both drugs and absorbance was determined at 332nm for MSM and 246nm for PRD.

Sensitivity: Sensitivity of both of these drugs was separately evaluated by estimating Sandell's sensitivity (μg/cm^2/0.0001 Abs unit) to determine the minimum amount of substance that can be quantified in column of unit cross section.

RESULTS AND DISCUSSION: In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The overlain UV absorption spectra of MSM and PRD exhibit λ_{max} of 332 nm and 246 nm for MSM and PRD, respectively which are quite separated from each other (**Figure 3**).

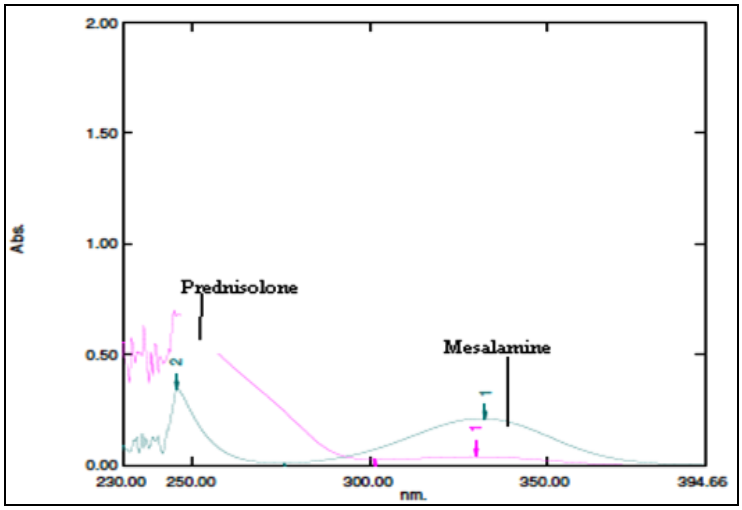

FIG. 3: OVERLAIN UV ABSORPTION SPECTRA OF MSM AND PRD

The two wavelengths were used for the analysis of the drugs were 332nm (λ_{max} of MSM) and 246 nm (λ_{max} of PRD) and at which the calibration curves were prepared for both the drugs. Standard calibration curves for MSM and PRD were linear and obey Beer Lambert's law in concentration range of 5-50 μg/ml and 2-20 μg/ml for MSM (**Table 1, Figure 4**) and PRD (**Table 2, Figure 5**), respectively. The correlation coefficients (r^2) values 0.996 and 0.997 for MSM and PRD, respectively at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.0008 – 0.0059 (**Table 3**).

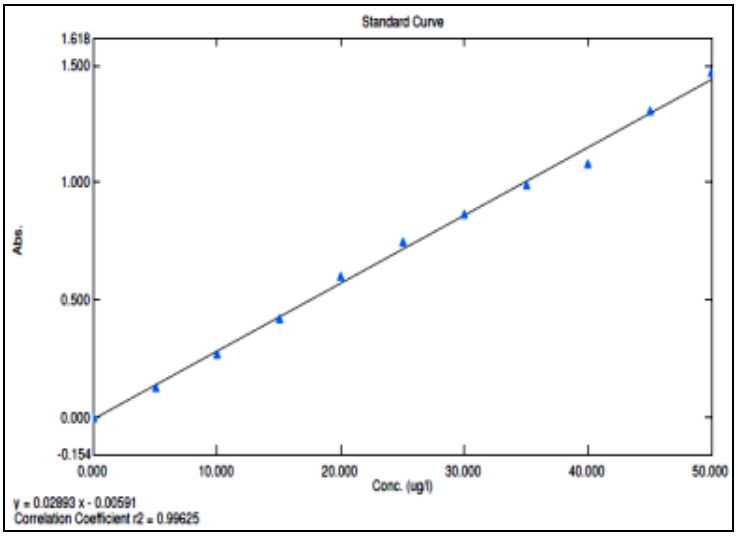

FIGURE 4: CALIBRATION CURVE FOR MSM AT λ_{max} 332nm

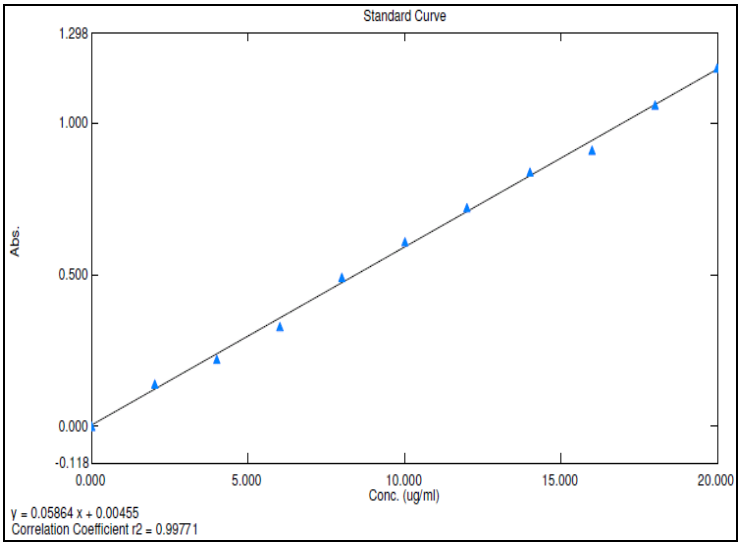

FIGURE 5: CALIBRATION CURVE FOR PRD AT λ_{max} 246nm

TABLE 1: ABSORBANCE AND ABSORBANCE COEFFICIENT OF MSM

Conc. (µg/ml)	332 nm		246 nm	
	Absorbance	$E^{1\%}_{1cm}$	Absorbance	$E^{1\%}_{1cm}$
5	0.13	260	0.18	360
10	027	270	0.35	350
15	0.42	280	0.47	313
20	0.60	300	0.69	345
25	0.75	300	0.91	364
30	0.87	290	1.11	370
35	0.99	280	1.24	354
40	1.08	270	1.27	317
45	1.31	290	1.34	297
50	1.47	290	1.35	270
		Mean ax_1 = 280		Mean ax_2 = 334

* All the values were average of three readings (n=3)

TABLE 2: ABSORBANCE AND ABSORBANCE COEFFICIENT OF PRD

Conc. (µg/ml)	332 nm		246 nm	
	absorbance	$E^{1\%}_{1cm}$	absorbance	$E^{1\%}_{1cm}$
2	0.01	50	0.14	700
4	0.01	25	0.22	550
6	0.01	16	0.33	550
8	0.02	25	0.49	610
10	0.01	10	0.61	610
12	0.02	16	0.72	600
14	0.01	07	0.84	600
16	0.02	12	0.91	560
18	0.01	05	1.06	580
20	0.02	10	1.18	590
		Mean ay_1=16		Mean ay_2 = 595

* All the values were average of three readings (n=3)

LOD values of calibration curves indicates the lowest concentration of analyte(s) in a sample that can be detected under a stated experimental conditions and LOQ values of calibration curves indicates the lowest concentration of analyte(s) in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOD values were found to be 0.67 and 0.047 for MSM and PRD, respectively and LOQ values were found to be 2.04 and 0.14 for MSM and PRD, respectively (**Table 3**).

The precision study carried out by inter and intraday study and % RSD found to be for intra-day were 0.88 and 1.01 and for inter-day 1.0 and 0.73 for MSM and PRD, respectively (**Table 4**).

TABLE 3: LINEAR REGRESSION ANALYSIS OF CALIBRATION CURVES OF MSM AND PRD

Optical characteristics	MSM	PRD
λ_{max} (nm)	332 nm	246 nm
Beer lambert's law limits (µg/ml)	5-50	2-20
Molar absorptivity ($L,mol^{-1}cm^{-1}$)	4.287×10^2	21.44×10^2
Sandell's sensitivity ($\mu g.cm^2$/ 0.001 abs unit)	0.357	0.168
Regression equation	y = 0.02893x - 0.00591	y = 0.05864x + 0.00455
Slope (m)	0.02893	0.05864
Intercept (c)	0.00591	0.00455
Correlation coefficient (r^2)	0.9962	0.9977
LOD (µg/ml)	0.67	0.047
LOQ(µg/ml)	2.04	0.14

TABLE 4: INTERMEDIATE PRECISION STUDY (INTER-DAY AND INTRA-DAY PRECISION)

Intraday precision			Inter day precision		
	% Recovery			% Recovery	
	MSM	PRD		MSM	PRD
After 1hr	100. 14	100.50	First day	100.33	100.10
After 2hr	99.62	98.78	Second day	99.20	98.90
After 3hr	100.32	99.25	Third day	98.35	100.20
After 4hr	98.20	99.10			
After 5hr	98.50	101.20			
After 6hr	98.30	98.30			
Mean	99.18	99.62	**Mean**	99.29	99.73
SD	0.87	1.00	**SD**	0.9900	0.72
% RSD	0.88	1.01	**% RSD**	1.00	0.73

The accuracy of the method was confirmed by recovery studies from synthetic mixtures of standard additions method. The recovery of MSM and PRD from the standard mixture solution was found to be 99.19±0.78% and 99.71±0.82% respectively (**Table 5**). The recovery results indicated that the method was accurate.

TABLE 5: RECOVERY STUDIES OF SYNTHETIC MIXTURES OF MSM AND PRD

Drug in standard mixture (µg/ml)		%Recovery	
MSM	**PRD**	**MSM**	**PRD**
12.5	0.5	100.14± 1.07	100.88±1.48
25	1	98.32±0.41	99.96±0.35
37.5	1.5	98.84±0.52	99.62±0.87
50	2	98.07±0.59	98.58±0.11
62.5	2.5	99.58±0.29	99.55±0.36

* All the values were average of three readings (n=3)

CONCLUSION: The proposed method for simultaneous estimation of MSM and PRD in combined oral dosage forms was found to be simple, accurate, precise, reproducible, economical and rapid. In the method, percentage recovery was close to 100% and % RSD was less than 2% for both the drugs. Hence, it can be employed for routine analysis in quality control.

AKNOWLEDGEMENT: The authors thank Cipla Ltd., Ratlam (India) and Kwality Pharmaceuticals Pvt. Ltd., Amritsar (India) for supplying gift samples of PRD and mesalamine to carry out the study.

REFERENCES:

1. Lichtenstein GR: mesalamine in the treatment of ulcerative colitis: novel therapeutic options. Gast & Hep. 2009; 5(1): 65-73.
2. Mahmud N, Weir DG, Kelleher D: Systemic levels of free 5-aminosalicylic acid depend on the nature of the 5-aminosalicyclic acid derivative and not on disease activity or extent in patients with inflammatory bowel disease. Ir J Med Sci. 1999; 168: 228-32.
3. Naganuma M, Iwao Y, Ogata H, Inoue N, Funakoshi S, and Yamamoto S: Measurement of colonic mucosal concentrations of 5-aminosalicylic acid is useful for estimating its therapeutic efficacy in distal ulcerative colitis: Comparison of orally administered mesalamine and sulfasalazine. Inflamm Bowel Dis. 2001; 7: 221-225.
4. Basturk T, Ozagari A, Ozturk T, Kusaslan R, Unsal A: Crohn's disease and secondary amyloidosis: Early complication? A case report and review of the literature. J Ren Care. 2009; 35: 147-150.
5. Caprilli R, Viscido A, Latella G: Current management of severe ulcerative colitis. Nature Cli Prace Gast & Her. 2007; 4(2): 91-101.
6. Yano H, Hirayama F, Kamada H: Colon-specific delivery of prednisolone-appended a-cyclodextrin Conjugate. Journal of Controlled Release. 2002; 79: 103-112.
7. Czock D, Keller F, Rasche FM, Haussler U: Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoid. Clinical Pharmacokinetics. 2005; 44: 1. 61-98.
8. Indian Pharmacopoeia. Vol II New Delhi. The Controller Publication, Govt. of India, 1996; 4: A-72-76.
9. Beckett AH and Stenlake JB: Practical pharmaceutical chemistry, CBS publishers and distributors. 1998; 3: 2. 275.
10. Jefferey GH, Basset J, Mendham J and Denney RC: Vogel's Textbook of quantitative chemical analysis. 1989; 5: 668-712.
11. Sahu R, Nagar P, Bhattacharya S and Jain D: Simultaneous spectrophotometric estimation of famotidine and domperidone in combined tablet dosage form. Ind. J. Pharm Sci. 2006; 68: 503-506.
12. ICH, Q2 (B) Validation of Analytical Procedures, Proceedings of the International Conference on Harmonization, Geneva. 2005.
13. Bolton S. In Pharmaceutical Statistics: Practice and Clinical Application. 3rd edition, Marcel Dekker Inc., New York. 2000; 80: 30-31.
14. Siddiqui AA, Khar RK, Singh RM, Mathur SC, Singh GN and Shivraj: A simple HPLC method for quantitative determination of nevirapine in tablet dosage form. J. Pharm. Res. 2006; 5: 108-110.
15. Patel SA, Patel NM, Patel MM: Spectrophotometric estimation of cefotaxime and ceftriaxone in pharmaceutical dosage forms, Indian J.Pharm .Sci. 2006; 68: 101-103.

How to cite this article:
Jain S, Jain N, Khambete H and Rawal A: Spectrophotometric Method for Simultaneous Estimation of Mesalamine and Prednisolone in Combined Oral Dosage Form. *Int J Pharm Sci Res*. 3(10); 3707-3711.