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Research Article

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Mupirocin and Methyl Prednisolone in Combined Dosage Form

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Abstract

The present work encompasses the development and validation of UV Spectrophotometric method for the concurrent estimation of Mupirocin (MUP) and Methylprednisolone (MP) in their combined dosage form. The method developed herein involves Second order derivative method. The wavelength selected for quantification were 263 nm for Methylprednisolone (zero-crossing for Mupirocin) and 289 nm for Mupirocin (zero-crossing for Methylprednisolone). The drugs follow linearity in the concentration range 6 - 14 **µg**/ml for MP and 120 - 280 **µg**/ml for MUP respectively. The analytical method was validated as per ICH, Q2, (R1) guideline and all the result were found under the acceptance criteria. The developed method was simple, economic, accurate, precise and can be used for estimation of Mupirocin and Methylprednisolone in their combined dosage form.

Keywords: Mupirocin; Methylprednisolone; UV Spectrophotometry**;** Second-order Derivative Method; ICH Guidelines

Introduction

Mupirocin is a topical antibiotic, a member of group of lipid oxides produced by fermentation of Pseudomonas fluorescens. It has molecular weight of 500.62g/mol and pKa value of 4.83 respectively. Chemically it is 9-[(E)-4-[(2S, 3R, 4R, 5S)3,4-dihydroxy-5-[[(2S, 3S)-3-[(2S, 3S) hydroxylbutan-2-yl] oxiran-2-yl] methyl] oxan- 2- yl]- 3methylbut-2-enoyl] oxynonanoic acid. Mupirocin specifically and reversibly binds to bacterial isoleucyl transfer-RNA (tRNA) synthetase, prevention of these enzyme from functioning properly results in inhibition of bacterial protein and RNA synthesis. It is primarily used in treatment of primary and seconday skin infection, in atopic dermatitis, nasal infection, wound healing [1-3]. Methylprednisolone is a (6S,8S,9S,10R,11S,13S,14 S,17R)-11,17-dihydroxy-17-(2hydroxyacetyl)-6,10,13-trimethyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a] phenanthren-3-one. It is a synthetic corticosteroid medication used to suppress the immune system, thus decreasing inflammation. The anti-inflammatory actions of corticosteroids are thought to involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes [4-6].

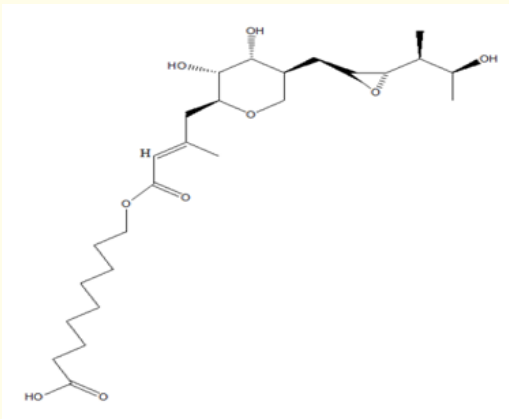

Figure 1: Chemical structure of Mupirocin.

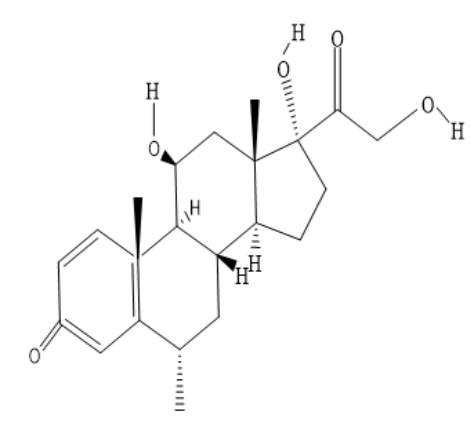

Figure 2: Chemical structure of Methylprednisolone.

No analytical methods are reported for this combination (Mupirocin and Methylprednisolone) in ointment formulation. The estimation of MUP was done alone and in combination with different drugs by UV Spectroscopy, HPLC [7-9] and MP was also detected alone and in combination with different classes of drug by several methods including UV, RP-HPLC [10,11]. Further the detection of both the drugs was also done with HPTLC, Colorimetric methods [12-17].

Material and Methods

Reagents and chemicals: Bills biotech Pvt. Ltd, savli, Vadodara gifted us the drug Mupirocin and Avik Pharma, Vapi gave the Methylprednisolone for the research work. Other chemicals and glass wares were provided by Pioneer Pharmacy Degree College.

Selection of solvent: Solubility of both the drugs were performed in different solvents like Water, Methanol, Acetonitrile, and Ethanol. Thus, both the drugs were found to be completely soluble in Methanol and partially soluble in Water. Thus, cosolvancy method was applied for the estimation and method development.

Instruments used: Absorption spectra of both the drugs were recorded by using Shimadzu UV-1800, Japan with Computer software: UV Probe 2.33. Quartz cell of path length 1 cm was used for estimation.

Preparation of standard stock solution: For UV spectrophotometric methods 25mg each of MUP and MP were weighed accurately and transferred into a 25 ml volumetric flask to which methanol was added up to the mark to produce a primary stock solution stock solution containing 1000 µg/ml of MP and MUP respectively.

Selection of analytical wavelength of MUP and MP: Working solution of both MUP and MP were scanned were scanned at 200 - 400 nm and the analytical wavelength for each method was obtained that is [A] Second order derivative spectroscopy, [B] Absorbance correction method and [C] Dual wavelength method.

Method: Second order derivative spectroscopy

In this method zero order spectra, was derivatized to second order and then on the basis of Zero crossing point (ZCP) of the corresponding drugs the wavelength for analysis was chosen. As per the study, the ZCP of MP 263nm was selected for the estimation of MUP and ZCP of MUP 289nm was selected for the estimation of MP. The parameters delta λ selected was 10.0, and the scaling factor was set to be 500. Shown in figure 3.

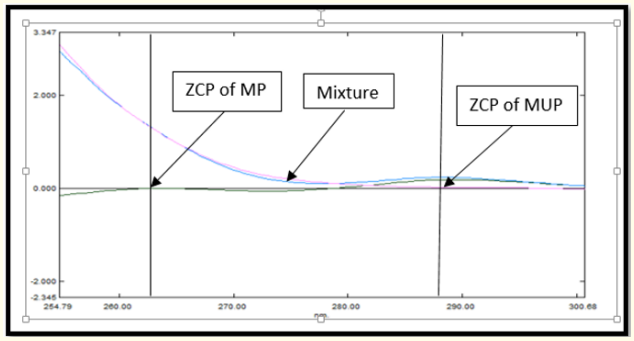

Figure 3: Overlain second derivative spectrum of MP (10 µg/mL), MUP (200 µg/mL), and Mixture.

Method Validation and Results

Validation of the developed method for simultaneous estimation of MUP and MP was performed as per ICH guideline Q2 (R1) validation of analytical procedures: text and methodology. Below mentioned parameters were considered for the validation of method.

Linearity

To evaluate the Linearity, serial dilution of the analyte were prepared from the standard primary stock solution, dilution was done with solvent system selected to get a series of concentration ranging from 6 - 14µg/mL for MP and 120 - 240µg/mL for MUP respec-

tively. Above prepared working solutions of both the drugs were scanned between 200-400 nm to obtain zero order spectra which were further converted to second order derivative. From second order derivative spectra absorbance of MUP and MP were recorded at 263 nm and 289 nm respectively.

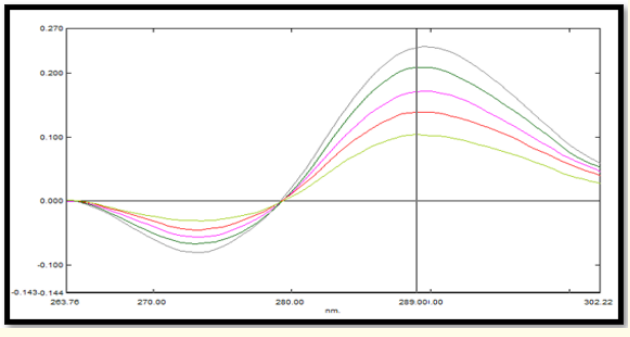

Figure 4: Overlain second derivative spectra of MP (6-14 µg/mL).

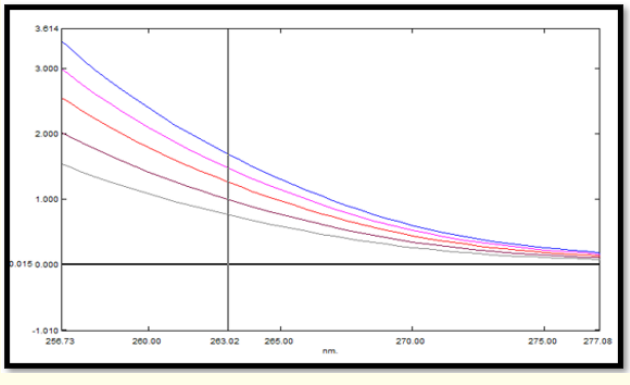

Figure 5: Overlain second derivative spectra of MUP (120-280 µg/mL).

Concentration (µg/ml)		Mean absorbance (ZCP=289 nm and 263 nm) ± SD(n = 3)		%RSD	
MP	MUP	MP	MUP	MP	MUP
6	120	0.115 ± 0.001	0.775 ± 0.004	0.870	0.453
8	160	0.154 ± 0.002	1.006 ± 0.002	1.355	0.199
10	200	0.190± 0.001	1.271 ± 0.003	0.526	0.198
12	240	0.232 ± 0.002	1.487 ± 0.002	0.862	0.140
14	280	0.269 ± 0.001	1.705 ± 0.004	0.372	0.206

Table 1: Calibration curve of MP (6 - 14 µg/ml) and MUP (120-280 µg/ml).

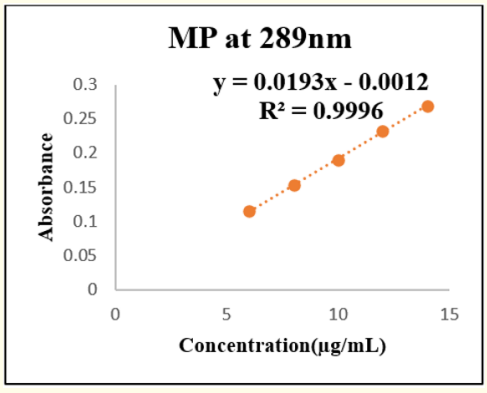

Figure 6: Calibration curve of MP (6-14µg/mL).

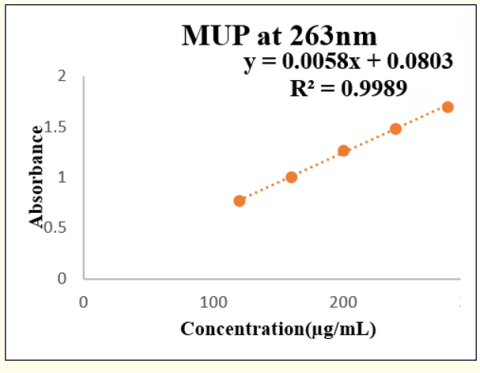

Figure 7: Calibration curve of MUP (120 - 280µg/mL).

Precision

Repeatability

In this middle concentration from calibration curve of MP (10 µg/ml) and MUP (200 µg/ml) was selected. Absorbance was recorded by scanning these solutions six times without changing the spectrophotometric parameters.

Concentration (µg/ml)		Absorbance		Mean ± SD (n = 6)		%RSD	
MP	MUP	MP	MUP	MP	MUP	MP	MUP
10	200	0.189	1.270	0.188 ± 0.002	1.269 ± 0.003	1.063	0.215
		0.187	1.268				
		0.185	1.265				
		0.191	1.267				
		0.190	1.273				
		0.188	1.269				

Table 2: Repeatability of MP and MUP.

Intermediate precision

It includes interday and intraday precision. In interday three concentrations were selected from calibration curve of MP (6, 10 and 14 µg/ml) and MUP (120, 200 and 280 µg/ml) and they were analyzed for 3 different days over a period of 1 week. In intraday three concentrations MP (6, 10 and 14 µg/ml) and MUP (120, 200 and 280 µg/ml) were scanned for 3 times during the same day. The result of these parameters should be reports in terms of relative standard deviation.

Conc. (µg/ml)	Absorbance			Mean ± SD (n = 3)	% RSD
	1 hr	2 hr	3 hr		
MP (at 289 nm)					
6	0.114	0.115	0.112	0.114 **± 0.002**	1.740
10	0.190	0.185	0.188	0.88 **± 0.003**	1.599
14	0.269	0.272	0.265	0.269 **± 0.004**	1.300
MUP (at 263nm)					
120	0.771	0.765	0.767	0.768 ± 0.003	0.398
200	1.273	1.269	1.270	1.271 ± 0.002	0.164
280	1.705	1.698	1.695	1.699 ± 0.005	0.302

Table 3: Intraday precision for MP and MUP (n = 3).

Conc. (µg/ml)	Absorbance			Mean ± SD (n = 3)	% RSD
	Day 1	Day 2	Day 3		
MP (at 289 nm)					
6	0.115	0.114	0.117	0.115 **± 0.002**	1.720
10	0.185	0.188	0.183	0.185 **± 0.003**	1.622
14	0.272	0.268	0.264	0.268 **± 0.004**	1.495
MUP (at 263 nm)					
120	0.775	0.769	0.764	0.769 ± 0.006	0.716
200	1.275	1.269	1.272	1.272 ± 0.003	0.236
280	1.697	1.704	1.694	1.698 ± 0.005	0.302

Table 4: Interday precision of MP and MUP (n = 3).

Accuracy (% Recovery)

This parameter was studied on three different concentration levels of 80%, 100% and 120% by replicate analysis (n = 3). Here the standard addition method was used in which the addition of standard drug solution to preanalysed sample solution and % drug content was calculated. Absorbance of these solutions was recorded at 289 nm for MP and 263 nm for MUP and % recovery of respective drug sample were calculated.

Drugs	Level	Conc. present (µg/ml)	Spiked Conc. (µg/ml)	Total Conc. taken (µg/ml)	Mean of total Conc. found (µg/ml)	Amt recovered (µg/ml)	%Recovery ± SD	%RSD
MP	80	6	4.8	10.8	10.74	4.76	99.3 ± 0.623	0.627
	100		6	12	12.03	6.04	100.74 ± 0.863	0.857
	120		7.2	13.2	13.20	7.21	100.26 ± 1.099	1.096
MUP	80	120	96	216	214.79	95.76	99.75 ± 0.177	0.176
	100		120	240	239.54	120.50	100.42 ± 0.786	0.783
	120		144	264	262.76	143.72	99.81 ± 0.311	0.311

Table 5: Results of recovery study (n = 3).

Limit of detection and limit of quantification

From data of linearity LOD and LOQ were calculated. Slope of the linearity plot was calculated. The y intercept and the standard deviation of the y intercept was calculated for each of the six replicate determinations. LOD and LOQ were calculated by putting these calculated values in response and slope of the regression equation.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma /S$

Parameters	MP	MUP
SD of intercept	0.0014	0.004
Mean of slope	0.0194	0.006
LOD	0.238	2.2
LOQ	0.722	6.667

Table 6: LOD and LOQ of PAR and ETZ.

Assay

Ointment equivalent to 5 mg of MP and 100 mg of MUP was weighed accurately and dissolved up to 100 mL with Methanol to produce 1° stock solution having strength of 1000 µg/mL of MUP and 50 µg/mL of MP, 2 mL was accurately pipetted out and was diluted up to 10 mL with Water, to produce solution having strength of 200 µg/mL of MUP and 10 µg/mL of MP. Test solutions were scanned and transferred to 2nd derivative spectra and absorbance were measured at their respective wavelengths (for MP-289nm and for MU 263 nm).Further, concentration was found out to calculate %Purity. Assay procedure was repeated for 3 times.

Drug	Label claim (mg)	Conc. Taken (µg/ml)	Absorbance	Conc. Found (µg/ml)	% Purity	% Purity ±SD	% RSD	Content found (mg)
MP	5	10	0.189	9.83	98.35	99.55 ±1.657	1.66	4.97
			0.190	9.88	98.86			
			0.195	10.14	101.44			
MUP	100	200	1.269	202.76	101.04	101.04 ±0.129	1.27	101.01
			1.262	201.57	100.78			
			1.263	201.74	100.87			

Table 7: Analysis of MP and MUP in formulation (n = 3).

Discussion

The developed method was successfully applied to the estimation of Mupirocin (MUP) and Methylprednisolone (MP) in their combined dosage form. The drugs follow linearity in the concentration range 6 - 14 µg/ml for MP and 120 - 280 µg/ml for MUP with correlation coefficient value 0.999 and 0.998 respectively. The proposed method was applied to pharmaceutical formulation and % amount of drugs estimated was found to be 99.55% and 101.04% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range of 99.30% - 100.26% for MP and 99.75% and 100.42% for MUP respectively. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than two indicate that the method is precise.

Conclusion

Economical UV methods were developed for the simultaneous estimation of Mupirocin and Methylprednisolone in combined dosage form. During estimation of both the drugs from the formulation another excipients present in the formulation had not shown any interference. Result of all the validation parameters were found within limits. Calibration curve of both the drugs were linear with good correlation coefficient. These methods are very accurate and precise because it indicates low relative standard deviation and high percent of recovery. Hence, this method is simple, accurate, precise and cost effective so it can be used for routine analysis of Mupirocin and Methylprednisolone in combined dosage form.

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