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SIMULTANEOUS ESTIMATION OF MUPIROCIN AND MOMETASONE FUROATE IN PHARMACEUTICAL DOSAGE FORM BY Q-ABSORPTION RATIO METHOD

Arti P Parmar[1] and DilipG.Maheshwari*[1]

[1]Dept. of Quality Assurance, L.J Institute of Pharmacy, Ahmedabad.
[1]Head of the Department, Dept. of Quality Assurance, L.J Institute of Pharmacy, Ahmedabad.

ABSTRACT

A simple, rapid, accurate, precise and economical UV-spectrophotometric method have been developed and validated for simultaneous estimation of Mupirocin and MometasoneFuroate in a Pharmaceutical dosage form. The Q absorbance ratio method, which involves formation of Q-absorbance equation at 226 nm (isoabsorptive point) and also at 220 nm (λmax of Mupirocin).Developed methods were validated according to ICH Q2 (R1) guidelines. The methods were found to be linear between the range of 5 - 25 µg/ml for MometasoneFuroate and Mupirocin. The precision (intra-day, inter-day) of method were found within limits (RSD <2%). Accuracy was determined by recovery studies and showed % recovery between 98 to 102%.

Keywords:MometasoneFuroate, Mupirocin, Method development, Validation, Q-Absorbance Ratio Spectrophotometric method.

INTRODUCTION

MometasoneFuroate [MF]**,** 9, 21 – dichloro-11b, 17 – dihydroxy-16a-methyl-pregnane-1, 4 – diene – 3, 20 – dione 17 – (2 – furoate ester), (Fig. 1) is a synthetic glucocorticoid with anti-inflammatory, anti-allergy effect. It is effective for various skin diseases. MometasoneFuroate is a topical corticosteroid; it has anti-inflammatory, anti-pruritic, and vasoconstrictive properties. Corticosteroids act by the induction of phospholipase A2 inhibitory proteins. MometasoneFuroate is official in IP[5], BP[7], USP[6].

Mupirocin (MUP) is an antibacterial agent produced by fermentation.Chemically it is (*E*)-(2*S*, 3R, 4R, 5S)-5-[(2*S*, 3S, 4S, 5S)-2, 3-epoxy-5-hydroxy-4-methylhexyl] tetrahydro-3, 4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid Mupirocin is official in IP[5], BP[7], USP[6].

Literature survey bare that there are several methods were reported for the estimation of Mupirocin and MometasoneFuroate individually as well as in combination with other drugs. As no method is reported for Mupirocin and MometasoneFuroate in combination, the aim of the present study was to develop accurate, precise and sensitive method for the simultaneous UV spectrophotometric estimation of Mupirocin and MometasoneFuroate in combined dosage form by Q-Absorbance Ratio Spectrophotometric method.

MATERIALS AND METHODS

INSTRUMENTATION

Double Beam U.V. Spectrophotometer (1800 Shimadzu) having two matched quartz cells with 1 cm light path, 1.8 mm bandwidth and 2mm wavelength accuracy was used to measure absorbance of the resulting solutions. MometasoneFuroate (West-coast Pharma. Ahmedabad), Mupirocin (West-coast Pharma. Ahmedabad) and Methanol were used in the study.

REAGENTS AND CHEMICALS

Analytical pure samples of MometasoneFuroate and Mupirocin were obtained as a gift samples from West

Corresponding Author:-**Dilip G. Maheshwari** Email:-dgmaheshwari@gmail.com

coast, Ahmedabad. These samples were used without further purification. Semisolid formulation "MATOS-M" manufactured by West coast Pharmaceutical - Ahmedabad, was purchased from the local market containing MF (5 mg) and MUP (100 mg) per ointment (5gm).

PREPARATION OF STANDARD SOLUTIONS:

For Stock solution of MometasoneFuroate: Accurately weigh 10 mg of MometasoneFuroate and transferred to a 100 ml volumetric flask and diluted with Methanol (100 µg/ml).

For Stock solution of Mupirocin: Accurately weigh 10 mg of Mupirocin and transferred to a 100 ml volumetric flask and diluted with methanol (100µg/ml).

Selection of wavelength:

The solution of Mometasonefuroate was prepared in Methanol at a concentration of 10 µg /ml by pipetting out 1 ml from the individual stock solution (100µg/ml) in 10ml volumetric flask and dilute upto the mark with Methanol and Mupirocin was also prepared in methanol at a concentration of 10 µg/ml by pipetting out 1 ml from stock solution (100 µg/ml). Both were scanned in the wavelength range of 200-400 nm.

Preparation of Sample solution

For the analysis of marketed semisolid formulation, 5 g ointment was weighed accurately and a amount equivalent to 0.5 mg of Mometasonefuroate and 10 mg of Mupirocin was weighed and dissolve in 50 mL methanol with the aid of ultrasonicator for 15 min and solution was filtered through Pre-filter + PVDF (0.45µm) into a 100mL volumetric flask and volume was made up to mark with methanol as a diluents. The solution was suitably make up with methanol to get a concentration of 10µg/mL of Mometasonefuroate and 10µg/mL of Mupirocin, by standard addition of standard solution of Mometasonefuroate.

CALIBRATION CURVE FOR MOMETASONE FUROATE AND MUPIROCIN

For Mometasonefuroate:

An aliquots of stock solution of Mometasonefuroate (0.5, 1, 1.5, 2 and 2.5 ml) were pipette out in five different 10ml volumetric flasks and further diluted to attain concentration of about 5, 10, 15, 20 and 25 µg/ml respectively.
Graph of Absorbance Vs Concentration was plotted.

For Mupirocin:

An aliquots of stock solution of Mupirocin(0.5, 1, 1.5, 2 and 2.5 ml) were pipettes out in five different 10ml volumetric flasks and further diluted to attain concentration of about 5, 10, 15, 20 and 25µg/ml respectively.
Graph of Absorbance Vs Concentration was plotted.

METHOD VALIDATION:

LINEARITY AND RANGE

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5-25 µg/ml and 5-25 µg/ml for MUP and MF respectively (n = 5).
The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for MUP and MF were calculated.

PRECISION

(A)Repeatability

Aliquots of 1 ml of working standard solution of MUP (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 1 ml of working standard solution of MF (100 µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10µg/ml solution of MUP and 10 µg/ml solution of MF. The absorbance of solution was measured spectrophotometry six times and % RSD was calculated.

(B) Intraday precision

Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of MUP (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of MF (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10, 15 and 20µg/ml solution of MUP and MF.
Solution was analyzed 3 times on the same day spectrophotometry and % RSD was calculated.

(C) Interday Precision

Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of MUP (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of MF (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10, 15 and 20µg/ml solution of MUP and MF.
Solution was analyzed 3 times on the 3 different days spectrophotometry and % RSD was calculated.

Limit of Detection (LOD)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.
The LOD may be calculated as,
$LOD = 3.3 * SD/Slope$

Where,
SD = the standard deviation of Y- intercept of 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.

Limit of Quantification (LOQ)
The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.
The LOD may be calculated as,
$LOQ = 10 * SD/Slope$
Where, SD = the standard deviation of Y- intercept of 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.

ACCURACY

To study the accuracy of the proposed methods, recovery studies were carried out by standard edition method at three different levels (80%, 100%, 120% of the test concentrations as per ICH guidelines). A known amount of drug was added and percentage recoveries were calculated. The results of recovery studies were satisfactory.

Q-ABSORBANCE RATIO METHOD

Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an Iso-absorptive point and other being the λmax of one of the two components. From the overlay spectra of two drugs, it is evident that MUP and MF show an Iso-absorptive point at 226 nm. The second wavelength used is 220 nm, which is λmax of MUP (fig. 3). Five working standard solutions having concentration 5, 10, 15, 20 and 25 µg/mL for MUP and MF were prepared in methanol and the absorbance at 226 nm (Iso-absorptive point) and 220 nm (λmax of MUP) were measured and absorptivity coefficients were calculated.
The absorbance of the sample solution were recorded at 226 nm (Iso-absorptive point) and 220 nm (λmax of MUP) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1
Relative concentration of two drugs in the sample was calculated using following equations.

$$Cx = \frac{Qm - Qy}{Qx - Qy} * \frac{A}{ax}$$

$$Cy = \frac{Qm - Qx}{Qy - Qx} * \frac{A}{ay}$$

The Q-values and absorptivitie for both drugs were calculated as follows,
$\mathbf{Q_M} = (A_2)/(A_1)$
$\mathbf{Q_X} = (ax_2)/(ax_1)$
$\mathbf{Q_Y} = (ay_2)/(ay_1)$
Where,
A_1 and A_2 are absorbance of mixture at 226 nm and 220 nm;
QxAndQy are Q value of MUP and MF respectively;
ax_1 and ay_1 are absorptivitie of MUP and MF at 220 nm;
ax_2 and ay_2 are absorptivitie of MUP and MF at 226 nm.
The analysis procedure was repeated 3 times with sample solution.

RESULTS AND DISCUSSION:

A reliable Q absorption ratio method was developed for simultaneous estimation of Mupirocin and MometasoneFuroate in Pharmaceutical dosage form by UV Spectrophotometry.

Table 1: Calibration data for (n=5)Mupirocin:

Mupirocin			**Mupirocin**		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**% RSD**	**Conc.** (µg/ml)	**Mean Absorbance ± SD**	**% RSD**
5	0.235±0.0025	0.8474	5	0.169±0.0018	1.0904
10	0.446±0.0011	0.7815	10	0.302±0.0016	0.96833
15	0.624±0.0047	0.8830	15	0.473±0.0041	0.6489
20	0.833±0.0062	0.9171	20	0.649±0.0056	0.7340
25	1.002±0.0058	0.7938	25	0.812±0.0048	0.6913

Table 2: Calibration data for Mometasone(n=5) Furoate:

MometasoneFuroate			**MometasoneFuroate**		
Conc. (µg/ml)	**Mean Absorbance ± SD**	**% RSD**	**Conc.** (µg/ml)	**Mean Absorbance ± SD**	**% RSD**
5	0.143 ± 0.0011	0.8023	5	0.166 ± 0.0020	1.1904
10	0.317 ± 0.0025	0.7880	10	0.297 ± 0.0028	0.9333
15	0.482 ± 0.0047	0.9730	15	0.471 ± 0.0036	0.6329
20	0.636 ± 0.0052	0.8346	20	0.642 ± 0.0047	0.7300
25	0.799 ± 0.0064	0.7999	25	0.802 ± 0.0055	0.6813

PRECISION:

Table No. 3 : Repeatability Data (n=6)

Drug Name	MometasoneFuroate		Mupirocin	
	220 nm	226 nm	220 nm	226 nm
Concentration	10 µg/ml	10 µg/ml	10 µg/ml	10 µg/ml
MEAN±SD(n=6)	0.313 ± 0.0012	0.304 ± 0.0012	0.451±0.0021	0.301±0.0012
%RSD	0.826797	0.933333	0.897436	0.933333

Table No. 4 : Intraday Precision Data for Mupirocin:

Mupirocin (220)			Mupirocin(226)		
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD
10	0.450 ± 0.0040	0.897	10	0.304 ± 0.0049	1.062
15	0.628 ± 0.004	0.619	15	0.474 ± 0.0056	0.851
20	0.834 ±0.0076	0.915	20	0.658 ± 0.0078	1.076

Table No. 5 : Intraday Precision Data for Mometasonefuroate

MometasoneFuroate(220 nm)			MometasoneFuroate(226 nm)		
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD
10	0.320 ± 0.0035	1.0951	10	0.305 ± 0.0028	0.9333
15	0.487 ± 0.0050	1.0328	15	0.479 ± 0.0036	0.6329
20	0.643 ± 0.0064	0.9993	20	0.647 ± 0.0032	0.5961

Table No. 6 :Interday Precision Data for Mupirocin :

Mupirocin (220 nm)			Mupirocin (226 nm)		
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	%RSD	Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD
10	0.450 ± 0.0051	1.1395	10	0.306 ± 0.0053	1.0183
15	0.620 ± 0.0060	0.9810	15	0.478 ± 0.0048	1.2021
20	0.834 ± 0.0085	1.0244	20	0.659 ± 0.0068	1.0631

Table No. 7 :Interday Precision Data MometasoneFuroate:

MometasoneFuroate(220 nm)			MometasoneFuroate(226 nm)		
Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	%RSD	Conc. (µg/ml)	Mean Absorbance ± SD (n=3)	% RSD
10	0.317 ± 0.0045	1.4231	10	0.302 ± 0.0054	1.1615
15	0.482 ± 0.0061	1.2503	15	0.484 ± 0.0067	1.0515
20	0.636 ± 0.0075	1.1745	20	0.662 ± 0.0079	1.2971

Table No. 8 :LOD and LOQ data of Mometasonefuroate and Mupirocin

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
MometasoneFuroate	0.756	2.291
Mupirocin	1.2375	3.75

ACCURACY:

Table No. 9: Accuracy Data

Drug name	Level of addition	Amount spiked (µg/ml)	Total amount (µg/ml)	Total amount obtained (n=3) ±SD	% Recovery±SD
Mupirocin (10 µg/ml)	80 %	8	18	17.97±0.25	99.97±0.7205 %
	100 %	10	20	19.69±0.26	99.83±1.0055 %
	120 %	12	22	22.15±0.22	100.68±0.440 %
MometasoneFuroate	80 %	8	18	18.16±0.25	100.91±1.035%

(10 µg/ml)	100 %	10	20	19.85±0.26	99.25±0.9041%
	120 %	12	22	22.31±0.22	101.4±1.1912 %

Table No. 10: Optical Regression Characteristics And Validation Parameters.

Parameter	**MometasoneFuroate**		**Mupirocin**	
Wavelength (nm)	220 nm	226 nm	220 nm,	226 nm
Beer's Law Limit (µg/ml)	5 – 25	5 - 25	5 – 25	5 - 25
Regression equation (y = mx +c)	y = 0.038x + 0.053	y = 0.032x - 0.008	y = 0.032x - 0.011	y = 0.032x - 0.007
Correlation Coefficient (r^2)	0.999	0.998	0.999	0.998
Repeatability (% RSD, n=6)	0.8267	0.9333	0.8974	0.9343
Interday (n=3) (% RSD) (µg/ml)	1.174-1.423	1.051-1.297	0.981-1.139	1.018-1.202
Intraday(n=3) (% RSD) (µg/ml)	0.993-1.095	0.596-0.933	0.619-0.915	0.851-1.062
LOD(µg/ml)	0.756		1.2375	
LOQ(µg/ml)	2.291		3.75	
Accuracy	99.25-101.04%		99.83-100.68%	

APPLICATION TO PHARMACEUTICAL DOSAGE FORM:

Applicability of proposed method was tested by analyzing the Pharmaceutical dosage form.

Table No. 11 : Applicability to Pharmaceutical dosage form Data:

Drug	**Label claim**	**Amount found(mg) (n=3) ± SD.**	**%Label Claim ± SD.**
Mupirocin	100 mg	99.26 ± 0.99	99.26%±0.99
MometasoneFuroate	5mg	5.03 ± 0.10	100.21%±1.38

Figure No. 3 : Selection of analytical Overlain Spectra of Mupirocin (10 µg/ml) and MometasoneFuroate (10 µg/ml).

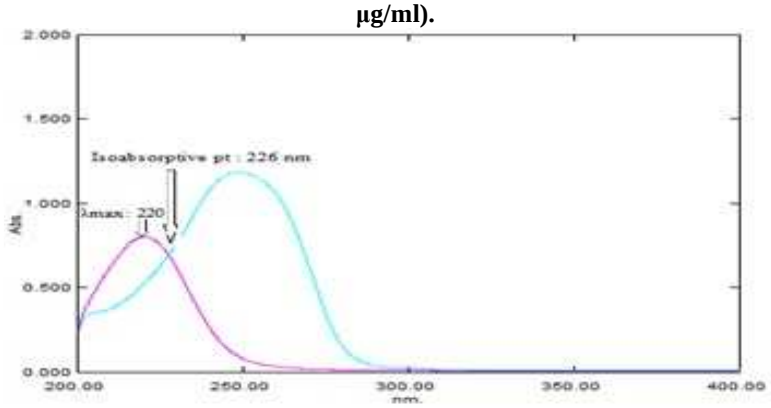
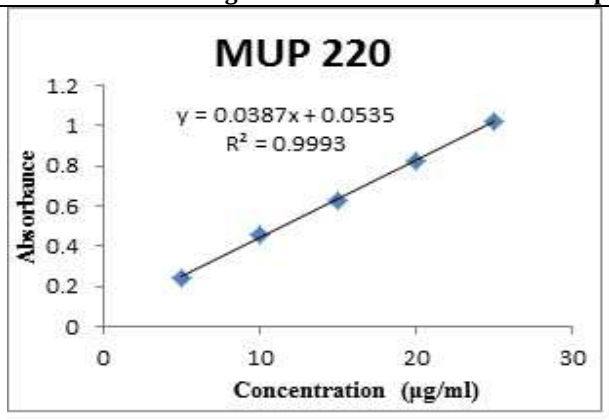


Fig No. 4 : Calibration curve of Mupirocin and MometasoneFuroate at 220 nm



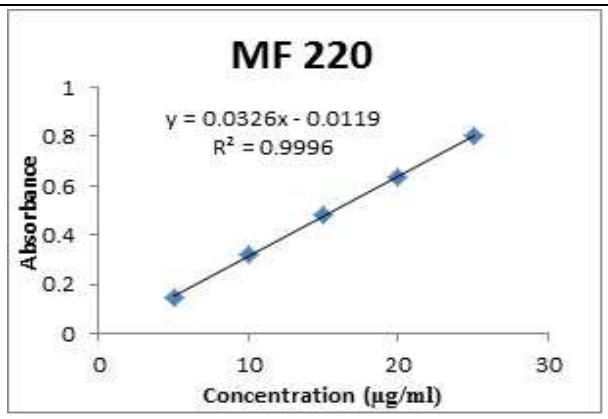
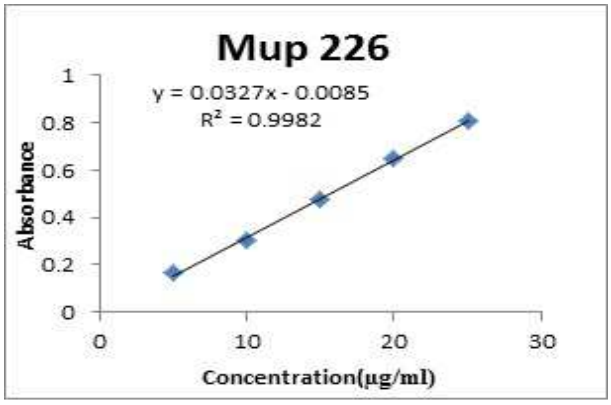


Fig No. 5 : Calibration curve of Mupirocin and MometasoneFuroate at 226 nm



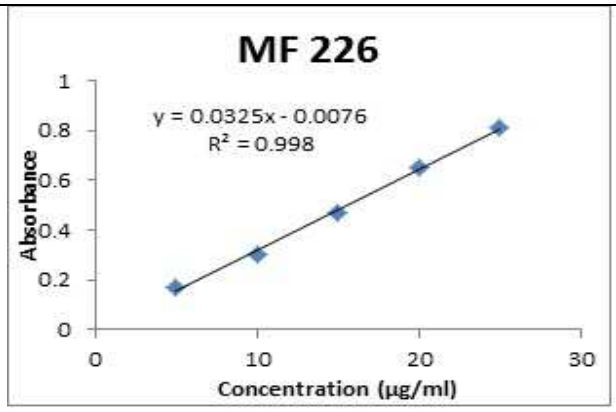


Fig No. 8 : Overlain Spectra of Mupirocin (5-25 µg/ml).

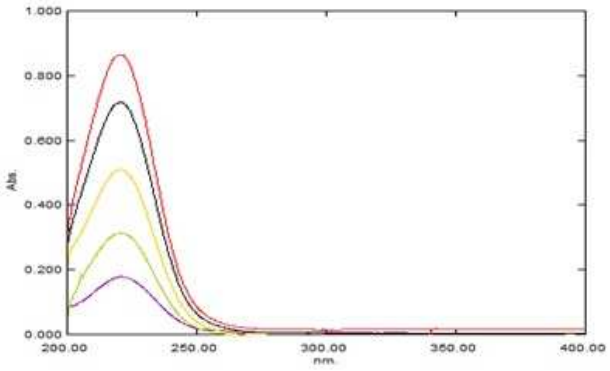
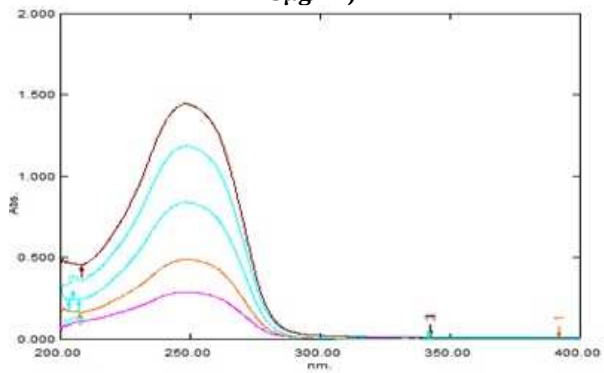


Fig No. 9 : Overlain Spectra of MometasoneFuroate (5-25µg/ml)



CONCLUSION:

Mupirocin and MometasoneFuroate are both commonly prescribed drugs for dermatoses. Mupirocin is bacteriostatic at low concentrations and bactericidal at high concentrations and MometasoneFuroate is a synthetic glucocorticosteroid, anti-inflammatory effect. So, Addition of these two drugs shows synergic effect. Simple, accurate, rapid, and precise Q absorbance ratio method was developed and validated for simultaneous estimation of both these drugs. This method developed in methanol. The plot of absorbance versus respective concentration was found to be linear in the concentration range of 5 – 25 µg/ml for Mupirocin and Mometasone furoate. This method can be successfully applied for the simultaneous estimation of Mupirocin and MometasoneFuroate in pharmaceutical dosage form.

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