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Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Mupirocin and Metronidazole in Pharmaceutical Dosage Form

Arapda Priti *[1], Marvaniya Vanita [2], Patani Pragnesh [3]

1. Student of M.Pharm, Quality Assurance department, A-one Pharmacy College, Ahmedabad, Gujarat, India
2. Assistant Professor, Department of Pharmaceutical Chemistry, A-one Pharmacy College, Ahmedabad, Gujarat, India
3. Professor, Pharmacology Department, A-one Pharmacy College, Ahmedabad, Gujarat, India

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For Correspondence:
Arapda Priti
Student of M.Pharm, Department of Pharmaceutical Chemistry, A-one Pharmacy College, Ahmedabad, Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Metronidazole and Mupirocin in their combined dosage form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Metronidazole and Mupirocin. In their combined dosage form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and buffer (pH 3.5): Methanol (70:30) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 230 nm. Retention time of Metronidazole and Mupirocin were found to be 4.227 and 5.413 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Metronidazole 5-15 µg/ml and for Mupirocin 10-30 µg/ml. Developed method was found to be accurate, precise and rapid. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and thermal degradation, considerable degradation was found in alkaline degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

KEY WORDS: Metronidazole, Mupirocin, Stability indicating RP-HPLC Method, Validation.

INTRODUCTION:

Mupirocin (Fig.1) is chemically 9-[(E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3 hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid. Muciprocin is an antibiotic and used as an antibacterial agent topically. It is effective against the gram positive bacteria. It is mainly used to treat the skin infections. It is an antibiotic that works by stopping the growth of certain bacteria. It works by reversibly binding to bacterial isoleucyl-t-RNA synthetas, an enzyme which promotes the conversion of isoleucine and tRNA to isoleucine t-RNA. Prevention of these enzymes from functioning properly results in the inhibition of bacterial protein and RNA synthesis.[1] Mupirocin is official in Indian pharmacopoeia (IP 2014)[2], The United States Pharmacopeial Convention (USP 2010)[3] and British pharmacopoeia (BP 2009)[4].

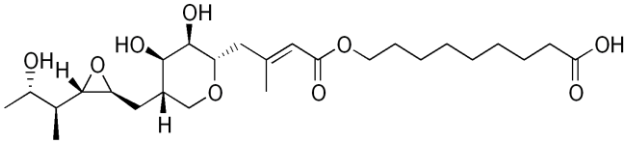

Figure 1: Mupirocin

Metronidazole (Fig.2) is chemically 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol.

Metronidazole is used in the treatment of anaerobic infections and mixed infections, surgical prophylaxis requiring anaerobic coverage, Clostridium difficile-associated diarrhea and colitis, Helicobacter pylori infection and duodenal ulcer disease, bacterial vaginosis, Giardia lamblia gastro-enteritis, amebiasis caused by Entamoeba histolytica, acne rosacea (topical treatment), and Trichomonas infections[5]. METRONIDAZOLE is official in Indian pharmacopoeia (IP 2014)[6], The United States Pharmacopeial Convention (USP 2010)[7] and British pharmacopoeia (BP 2016)[8].

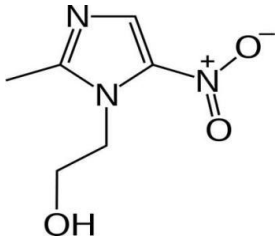

Figure 2: Metronidazole

The Combination of formulation give synergistic effect Combined Dosage form of Mupirocin and Metronidazole used in the Treatment of Skin Disease. Combination of the pharmaceutical formulation is widely used in Non-healing ulcer , Diabetic foot ulcer , Venous ulcer, Pressure ulcer, Surgical Wound & Other infected Wound. Literature review reveals that various methods are reported for the analysis of individual drug and in combination with other drugs but no stability indicating RP-HPLC method has not been reported for simultaneous estimation of Metronidazole and Mupirocin[9-10]. Therefore, the goal of the present work is to develop Stability indicating reverse phase high performance liquid chromatographic method for simultaneous estimation of Mupirocin and Metronidazole in Combined Dosage Form.

MATERIALS AND METHODS:

Chemicals: Mupirocin and Metronidazole powder reference standard (rs) was received as a gift sample. The Co - Mupimet granules containing Metronidazole (1%w/w) and Mupirocin (2%w/w) manufactured and marketed by Fourrts India Laboratories pvt ltd was purchased from local pharmacy. The HPLC grade methanol and Acetonitrile were purchased from Merck specialties pvt, Ltd., Mumbai.

Instrumentation

RP-HPLC instrumentation: Shimadzu LC-20 AT HPLC system, using SPD-20A UV Detector. The column used is C18 (25 cm × 0.46 cm) Hypersil BDS. The temperature of the column was maintained at room temperature and the flow rate 1.0 ml/min. The injection volume is 20µl, 230nm was set as a wavelength and the HPLC run time was selected for 7 minutes.

Mobile phase: Potassium Dihydrogen Phosphate buffer and methanol (adjust to pH 3.5 orthophosphoric acid) in the ration(70:30) v/v was used for separation of this drug. The mobile phase was filttered through the 0.22µ membrane filter after sonication of each solvent for 20 min.

Selection of diluents:-

Diluent : The drug's physical properties from the literature available suggested that the drug is soluble in Methanol and experimental results also showed the same, thus Methanol was tried for the diluent and experimental results produced were good. The same diluent was applied for the formulations available and found satisfactory as the diluent.

RP-HPLC Standard solution:

Mupirocin standard stock solution (200 µg/mL):

Accurately weighted 20 mg of Mupirocin was transferred to a 100 mL volumetric flask. To the volumetric flask 50-60 ml of methanol was added and mixed thoroughly to dissolve. Then the volume was adjusted to make up the volume to 100 ml with methanol.

Metronidazole standard stock solution (100 µg/mL) :

Accurately weighted 10 mg of Metronidazole was transferred to a 100 mL volumetric flask. To the volumetric flask 50-60 ml of methanol was added and mixed thoroughly to dissolve. Then the volume was adjusted to make up the volume to 100 ml with methanol.

Preparation of standard solution of binary mixtures of Metronidazol (10 µg/mL) and Mupirocin (20 µg/mL) :

Transfer 1 mL of the Metronidazole stock solution (100 µg/mL) and 1mL from Mupirocin stock solution (200 µg/mL) to 10 mL volumetric flask and volume was made up to the mark by mobile phase which was used in particular trials.

Preparation of formulation solution:

Weigh the Powder formulation equivalent to 10 mg of Metronidazole and 20 mg of Mupirocin and transfer to a 100 ml volumetric flask. Add 60 ml of Mobile phase and shake for few minutes and made up volume up to the mark with mobile phase. The solution was filtered

through Whatman filter paper no. 42 and first few drops of filtrate were discarded. Pipette out 1 ml of this solution a dilute to 10 ml with mobile phase.

Acid degradation:

One ml of stock solution was transfer in to 10 ml of volumetric flask and to that two ml of 0.1 N HCl solutions was added and mixed well. This solution was kept at room temperature for 4 hrs. The resulting solution is then neutralized using 0.1N NaOH solution. The volume is then made up using the diluent to get 10 µg/ml for Metronidazole and 20 µg/ml for Mupirocin. The above solution thus obtained is filtered and then injected.

Base degradation:

One ml of stock solution was transferred in to 10 ml of volumetric flask and to that two ml of 0.1 N NaOH solutions was added and mixed well. This solution was kept at room temperature for 4 hrs.. The resulting solution is then neutralized using 0.1N HCL solution. The volume is then made up using the diluent to get 10 µg/ml for Metronidazole and 20 µg/ml for Mupirocin. The above solution thus obtained is filtered and then injected.

Oxidative degradation:

The oxidative degradation was carried out using 2 ml of 3% H2O2 which is added to the one ml of stock solution in to 10 ml of volumetric flask. The resultant solution was kept at room temperature for 4 hrs. The volume is then made up using the diluent to get 10 µg/ml for Metronidazole and 20 µg/ml for Mupirocin. The above solution thus obtained is filtered and then injected.

Photo Degradation:

Photo Degradation studies were performed under the UV light. One ml of stock solution was transferred in to 10 ml of volumetric flask and kept under UV Light for approximately 10hrs. Then the volume was adjusted with diluents to get 10µg/ml for Metronidazole and 20µg/ml for Mupirocin.

Thermal degradation:

Thermal Degradation studies were performed by taking one ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 110°C for 10 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Metronidazole and 20µg/ml for Mupirocin.

RESULT AND DISCUSSION

The method was validated for its linearity range, accuracy, precision, sensitivity and specificity. Method validation is carried out as per ICH guidelines.

Linearity

The linearity for Metronidazole and Mupirocin were assessed by analysis of combined standard solution in range of 5-15µg/ml and 10-30µg/ml respectively. Correlation co-efficient for calibration curve Metronidazole and Mupirocin was found to be 0.999 for both.

Table 1 : Linearity data for Metronidazole

Sr. No.	Conc. (%)	Conc. (µg/ml)	Area	Correlation Coefficient > 0.999
1	50%	5	664.935	
2	75%	7.5	998.701	
3	100%	10	1335.143	0.999
4	125%	12.5	1642.816	
5	150%	15	1970.321	
		Regression line equation		y = 130.2x + 20.428
			Slope	130.2

Table 2 : Linearity data Mupirocin

Sr. No.	Conc. (%)	Conc. (µg/ml)	Area	Correlation Coefficient > 0.999
1	50%	10	761.588	
2	75%	15	1143.886	
3	100%	20	1529.221	0.999
4	125%	25	1881.6	
5	150%	30	2229.54	
		Regression line equation		y = 73.472x + 39.72
			Slope	73.472

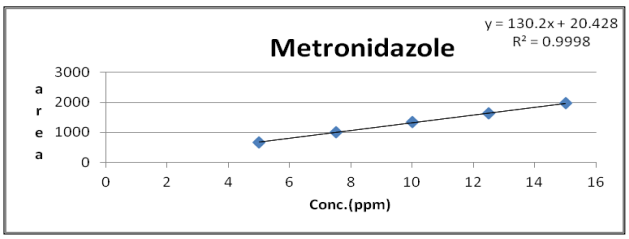

Figure 3 Calibration Curve of Metronidazole (5-15µg/ml)

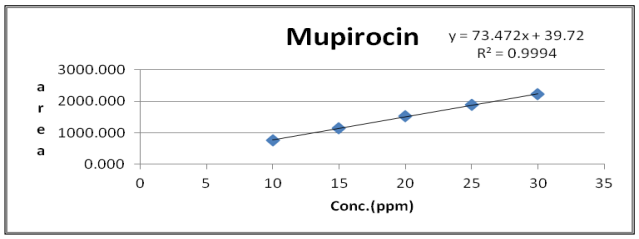

Figure 4 Calibration Curve of Mupirocin (10-30µg/ml)

Repeatability:

The data for repeatability of peak area measurement for Metronidazole and Mupirocin, based on six measurements of same solution Metronidazole and Mupirocin are depicted in table 3,& 4. The %RSD for Metronidazole and Mupirocin was found to be 0.44and 0.36 respectively.

Table 3 Repeatability data for Metronidazole

Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	1327.196 1336.427 1341.744 1328.293 1336.225 1338.852	1334.79±5.82	0.44

Table 4 Repeatability data for Mupirocin

Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1	20	1520.098 1530.7 1527.236 1521.401 1530.514 1533.518	1527.25 ±5.43	0.36

Intraday precision

The data for intraday precision for Metronidazole and Mupirocin is shown in table 5. The % R.S.D. for Intraday precision was found to be 0.213-0.667 for Metronidazole and 0.254-1.40 for Mupirocin.

Interday precision:

The data for intraday precision for Metronidazole and Mupirocin is shown in table 6. The % R.S.D. for interday precision was found to be 0.717-1.408 for Metronidazole and 1.24-1.74 for Mupirocin.

Table 5 Intraday precision data for estimation of Metronidazole and Mupirocin

Sr. No.	Metronidazole			Mupirocin		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	5	668.41 ±4.05	0.605	10	760.58 ±10.64	1.4
2	10	1338.86 ±2.86	0.213	20	1539.33 ±7.26	0.471
3	15	1988.62 ±13.28	0.667	30	2272.30 ±5.78	0.254

Table 6 Interday precision data for estimation of Metronidazole and Mupirocin

Sr. No.	Metronidazole			Mupirocin		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	5	657.40 ±4.71	0.717	10	748.38 ±13.07	1.746
2	10	1316.04 ±13.14	0.998	20	1496.67 ±24.65	1.647
3	15	1961.46 ±27.62	1.408	30	2249.77 ±27.92	1.24

Table 7 Recovery data for Metronidazole

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80%	5	4	3.982	99.56	99.18± 0.84
2		5	4	3.928	98.21	
3		5	4	3.991	99.765	
4	100%	5	5	4.971	99.419	100.63 ± 1.11
5		5	5	5.081	101.61	
6		5	5	5.043	100.869	
7	120%	5	6	5.941	99.019	99.68 ± 0.64
8		5	6	5.984	99.741	
9		5	6	6.017	100.287	

Table 8 Recovery data for Mupirocin

	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80%	10	8	8.145	101.817	101.46 ± 0.54
2		10	8	8.139	101.742	
3		10	8	8.067	100.842	
4	100%	10	10	10.252	102.521	1102.29 ± 0.36
5		10	10	10.187	101.871	
6		10	10	10.248	102.477	
7	120%	10	12	12.225	101.878	100.59 ± 1.14
8		10	12	12.028	100.232	
9		10	12	11.961	99.675	

Table 9 Robustness data for Metronidazole

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase(+2)
1	1305.944	1352.491	1291.306	1361.946	1311.167	1351.095
2	1295.487	1355.169	1305.5	1360.561	1321.634	1345.601
3	1281.211	1334.945	1289.895	1340.385	1312.285	1325.539
% R.S.D	0.959	0.815	0.666	0.891	0.437	1.003

Table 10 Robustness data for Mupirocin

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase(+2)
1	1495.791	1549.082	1479.088	1559.939	1501.787	1547.522
2	1483.765	1552.174	1495.38	1558.346	1485.412	1541.25
3	1460.917	1558.328	1460.723	1567.626	1503.139	1542.782
% R.S.D	1.196	0.303	1.172	0.317	0.659	0.211

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 7 and table 8 Percentage recovery for Metronidazole was 99.18-100.63 %, while for Mupirocin, it was found to be in range of 100.54-101.46 %.

Robustness:

The effect of changes was found to be within the acceptance criteria as shown in table 9 and table 10.The %RSD should Be less than 2%.

LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated.Then LOD and LOQ were calculated as follows:

	Metronidazole	Mupirocin
Limit of Detection :	0.691 µg/ml	0.735 µg/ml
Limit of Quantization :	0.691 µg/ml	2.227µg/ml

CONCLUSION

The study shows that the developed HPLC Method is fast, precise, specific, accurate and stability indicating. The stability -indicating method resolved the drug peak and also the peaks of degradation products formed under variety of conditions.

The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in alkaline degradation. The proposed method was successfully

applied for the simultaneous estimation of both the drugs in commercial Combined dosage form. Hence, the method is recommended for routine quality control analysis of Metronidazole and Mupirocin in presence of degrade product and other pharmaceutical excipients present in dosage form.

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