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Development and Validation of Analytical Methods for Simultaneous Estimation of Ornidazole and Diloxanide Furoate in their Combined Dosage Form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Ornidazole and Diloxnide furoate in Tablet dosage form. Chromatography was carried on C18 (25 x 0.46)cm ; 5µm) analytical column using mobile phase Buffer(pH 6.0) : Methanol (70:30 v/v) at a flow rate of 01.0 ml/min. The detection was carried out at 277 nm. The retention time of Ornidazole and Diloxanide furoate are found to be 3.400 & 5.747 min respectively. Correlation co-efficient for Ornidazole and Diloxanide furoate was found to be 0.999 & 0.998 over a concentration range of 25-75 µg/ml and 37.5-112.5 µg/ml respectively. The proposed method was validated with respect to linearity, accuracy, precision, selectivity, and robustness. Recovery was found in the range of 99.63 – 100.05% and 99.80 – 100.25% for Ornidazole and Diloxanide Furoate respectively. Statistical Analysis proves that the developed methods were successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEY WORDS: Ornidazole, Diloxanide Furoate, RP-HPLC, Mobile phase, Validation

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1. INTRODUCTION

The IUPAC name of the Ornidazole drug is 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol, with molecular formula and molecular weight $C_7H_{10}ClN_3O_3$ and 219.625 g/mol respectively. The molecular structure of the drug is given in Fig.1. Ornidazole is a nitroimidazole antiprotozoal agent used in ameba and trichomonas infections. It is partially plasma-bound and also has radiation-sensitizing action. Ornidazole has inhibit cell mediated immunity, to induce mutagenesis and to cause radiosensitization.

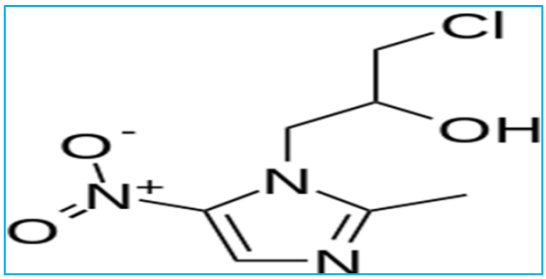

Figure 1: Chemical structure of Ornidazole

The IUPAC name of the Diloxanide Furoate drug is 4-(2,2-dichloro-N-methylacetamido)phenyl furan-2-carboxylate, with molecular formula and molecular weight $C_{14}H_{11}Cl_2NO_4$ and 328.147 g/mol respectively. The molecular structure of the drug is given in Fig.2. Diloxanide inhibits the protein synthesis. Diloxanide is used alone as a primary agent in the treatment of asymptomatic. It highly effective luminal amoebicides. It directly kills trophozoides responsible for production of cysts.

A literature survey reveals there are hardly any method repoted on RP-HPLC method devlopement and validation for simultaneous estimation of Ornidazole and Diloxanide furoate in combined dosage form. The present paper describes the analytical method development and validation of estimation of Ornidazole and Diloxanide Furoate in Pharmaceutical dosage form using UV spectrophotometry. The proposed method are optimized and validated as per ICH guidelines.

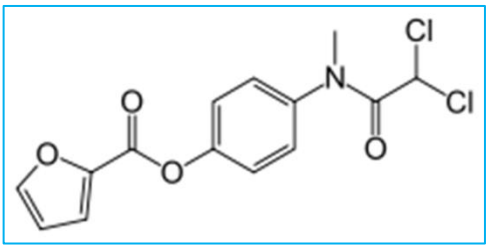

Figure 2: Chemical structure of Diloxanide Furoate

MATERIALS AND METHODS

Materials

HPLC Thermo separation, Product Ornidazole and Diloxanide Furoate was obtained as a gift sample by Gitar Laboratories, Ahmedabad, and Gujarat, India. The commercial fixed dose Amicline Plus (Ornidazole – 250 mg & Diloxanide Furoate – 375 mg) was procured from local market. All solvents (HPLC grade) were obtained from Merck Chemicals.

2.1 Ornidazole standard stock solution: (500 µg/mL)

A 50 mg of Ornidazole was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol

2.2 Diloxanide Furoate standard stock solution: (750 µg/mL)

A 75 mg of Diloxanide Furoate was weighed and transferred to a 100 mL volumetric flask. and volume was made up to the mark with methanol.

2.3 Preparation of standard solution of binary mixtures of Ornidazole (50 µg/mL) and Diloxanide Furoate (75 µg/mL)

Take 1 mL from the Ornidazole stock solution and 1mL from Diloxanide Furoate stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials

2.4 Preparation of Phosphate Buffer:

A 6.8 gm of KH2PO4 is dissolved in 800 ml of water and adjusted pH 6.0 with NaOH and make up volume up to 1000 ml.

2.5 Method validation:

2.5.1 Chromatographic conditions and System Suitability Parameters:

1. Pumps:

Mode of chromatography: Reversed Phase Chromatography

Mode of Elution: Isocratic

Flow Rate: 1.0 ml/min

2. Detector:

SHIMADZU 1800 Double UV- VIS

Wavelength: 277 nm

3. Other parameters:

Column: C18(25 x0. 46) cm ; Hypersil BDS

Sample Volume: 20 µl

Run time: 7 min

Mobile Phase: Phosphate Buffer : Methanol (70:30)

Diluent: Methanol

4. System Suitability Parameters:

Retention time:

- Ornidazole – 3.400

- Diloxanide Furoate -5.747

Asymmetry:

- Ornidazole – 1.364
- Diloxanide Furoate -1.378

Theoretical plates:

- Ornidazole – 7352
- Diloxanide Furoate -7147

2.5.2 Linearity :

The linearity for Ornidazole and Diloxanide furoate were assessed by analysis of combined standard solution in range of 25-75 µg/ml and 37.5-1125 µg/ml respectively 5, 7.5, 10, 12.5, 15 ml solutions were pipette out from the Stock solution of Ornidazole (500 µg/ml) and Diloxanide furoate (750 µg/ml) and transfer to 100 ml volumetric flask andmake up with mobile phase to obtain 25,37.5,50,62.5 and 75 µg/ml and 37.5,56.25,75,93.75 and 112.5 µg/ml for Ornidazole and Diloxanide furoate respectively

2.5.3 Precision

***2.5.3.1* Repeatability**

Standard solution containing Ornidazole (50µg/ml) and Diloxanide furoate (75 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

2.5.3.2 Intraday Precision

Standard solution containing (25, 50, 75 µg/ml) of Ornidazole and (37.5,75,112.5 µg/ml) of Diloxanide furoate were analyzed three times on the same day and % R.S.D was calculated. .

2.5.3.3 Interday Precision:

Standard solution containing (25, 50, 75 µg/ml) of Ornidazole and (37.5,75,112.5 µg/ml) of Diloxanide furoate were analyzed three times on the different day and % R.S.D was calculated.

2.5.4 Accuracy (% Recovery)

For Ornidazole:

50 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 236 nm. The amount of Ornidazole was calculated at each level and % recoveries were computed.

For Diloxanide Furoate:

75 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 236 nm. The amount of Diloxanide furoate was calculated at each level and % recoveries were computed.

2.5.5 Limit of detection and Limit of quantification

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method

linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

2.5.6 Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 6.2 and 5.8.

3.Ratio of Mobile phase was changed(±2) Buffer: Methanol(72:32) and Buffer: Methanol(68:28)

2. RESULT AND DISCUSSION:

2.1 Validation parameters:

2.1.1 Linearity

Linear correlation was obtained between peak area and concentration of Ornidazole and Diloxanide Furoate in the range of 25-75 µg/ml @ 37.5 – 112.5 µg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r). The overlay chromatogram is presented in Figure 2. The linearity data are presented in Table 1 & 2. Calibration curve is presented in Figure 3 & 4.

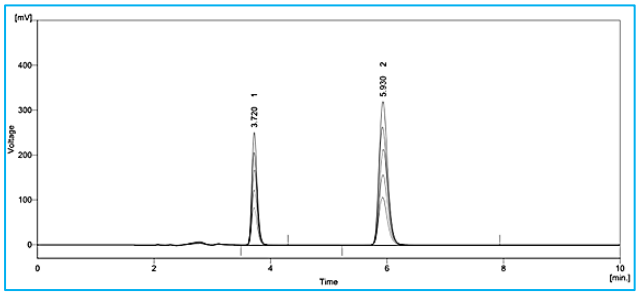

Figure 2: Chromatogram of Linearity Overlay

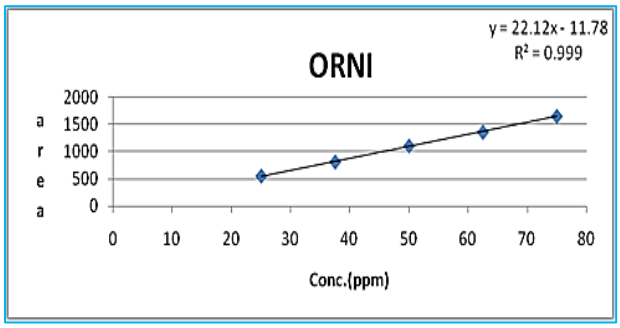

Figure 3: Chromatogram of Linearity Curve for Ornidazole

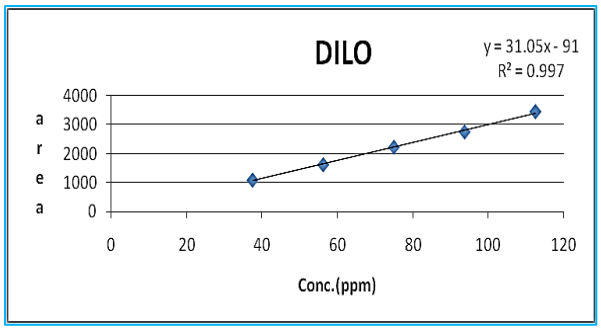

Figure 4: Chromatogram of Linearity Curve for Diloxanide Furoate

Table 1: Data indicating Linearity of Ornidazole

Sample level	Concentration (µg/ml)	AREA
1	25	547.045
2	37.5	807.57
3	50	1104.053
4	62.5	1359.338
5	75	1653.864

Table 2: Data indicating Linearity of Diloxanide Furoate

Sample level	Concentration (µg/ml)	AREA
1	37.5	1106.807
2	56.25	1634.228
3	75	2234.69
4	93.75	2758.353
5	112.5	3456.019

3.1.2 Precision

3.1.2.1 Repeatability

The data for repeatability of peak area measurement for Ornidazole and Diloxanide furoate , based on six measurements of same solution Ornidazole and Diloxanide furoate are depicted in table 3 & 4. The % RSD for Ornidazole and Diloxanide furoate was found to be 0.616 and 0.397 respectively.

Table 3: Repeatability data for Ornidazole from tablet formulation

Conc (µg/ml)	Area	Mean ± S.D (n=6)	% RSD
50	1099.626 1087.164 1104.045 1106.263 1100.73 1102.989	1100.14±6.78	0.616

Table 4: Repeatability data for Diloxanide Furoate from tablet formulation

Conc (µg/ml)	Area	Mean ± S.D (n=6)	% RSD
75	2225.817 2230.188 2212.591 2239.196 2228.052 2232.423	2228.04 ±8.85	0.397

3.1.2.2 Intraday precision

The data for intraday precision for Ornidazole and Diloxanide furoate is shown in table 5. The % R.S.D. for Intraday precision was found to be 0.582-0.855 for Ornidazole and 0.628-1.208 for Diloxanide furoate.

Table 5: Intraday Precision data

	Ornidazole			Diloxanide furoate		
SR. NO.	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	25	543.37 ± 3.16	0.582	37.5	1094.37± 13.23	1.208
2	50	1095.33± 8.59	0.784	75	2111.74± 13.90	0.628
3	75	1640.11± 14.03	0.855	112.5	3323.95±31.45	0.946

3.1.2.3 Interday precision

The data for intraday precision for Ornidazole and Diloxanide furoate is shown in table 6. The % R.S.D. for interday precision was found to be 0.399-0.842 for Ornidazole and 0.725-1.211 for Diloxanide furoate.

Table 6: Intraday Precision data

	Ornidazole			Diloxanide furoate		
SR. NO.	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	25	541.70±4.56	0.842	37.5	1092.54± 13.23	1.211
2	50	1096.10± 4.37	0.399	75	2212.06± 16.04	0.725
3	75	1640.10± 12.40	0.756	112.5	3322.88± 29.43	0.885

3.1.3 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 8. Percentage recovery for Ornidazole was 99.63-100.05 %, while for Diloxanide furoate, it was found to be in range of 99.80-100.25 %.

Table 7: Recovery data for Ornidazole from tablet formulation

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	25	20	19.81	99.07	100.05 ± 0.93
2		25	20	20.18	100.92	
3		25	20	20.03	100.15	
4	100 %	25	25	24.80	99.20	99.70 ± 0.56
5		25	25	25.07	100.30	
6		25	25	24.90	99.60	
7	120 %	25	30	30.03	100.11	99.63 ± 0.49
8		25	30	29.74	99.13	
9		25	30	29.89	99.65	

Table 8: Recovery data for Diloxanide Furoate from tablet formulation

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	37.5	30	29.77	99.23	99.80 ± 0.53
2		37.5	30	29.96	99.88	
3		37.5	30	30.08	100.28	
4	100 %	37.5	37.5	37.57	100.20	100.23 ± 0.37
5		37.5	37.5	37.46	99.88	
6		37.5	37.5	37.73	100.62	
7	120 %	37.5	45	45.04	100.09	100.25 ± 0.29
8		37.5	45	45.03	100.07	
9		37.5	45	45.26	100.58	

3.1.4 Limit of detection and limit of quantifycation

The Limit of detection (LOD) for Ornidazole and Diloxanide Furoate were found to be 1.738 & 5.583 µg/ml. The Limit of quantitation for Ornidazole and Diloxanide Furoate were found to be 5.267 and 16.918 µg/ml respectively.

3.1.5 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%.

Table 9: Robustness data for Ornidazole from tablet formulation

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	1131.357	1062.868	1124.814	1031.597	1119.168	1044.377
2	1144.985	1078.607	1133.93	1056.488	1130.52	1076.442
3	1149.366	1085.222	1140.586	1063.086	1137.16	1084.139
% R.S.D	0.822	1.067	0.699	1.331	0.806	1.974

Table 10: Robustness data for Diloxanide Furoate from tablet formulation

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	2308.535	2169.911	2286.129	2129.472	2283.841	2165.562
2	2283.486	2148.167	2257.099	2108.594	2241.704	2149.973
3	2326.176	2196.688	2308.535	2151.877	2301.605	2194.487
% R.S.D	0.930	1.119	1.129	1.016	1.352	1.040

3.1.6 Applicability of the method

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Amicline plus .The results are shown in table 11.

Table 11: Application of RP-HPLC method

Tablet	Label claim		Assay (% of label claim*) Mean ± S. D.	
	Ornidazole	Diloxanide furoate	% Ornidazole	% Diloxanide
Amiclin e plus	250mg	375mg	98.20 ± 0.978	98.23 ± 0.78

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

4. CONCLUSION

In Estimation of Ornidazole & Diloxanide Furoate in combined tablet dosage form, separation was achieved on C18 (25 x 0.46) cm Hypersil BDS at 30°C temperature by using a mobile phase Buffer : Methanol (70:30) at a

flow rate of 1.0 ml/min and UV detection for both drug was carried out at 277 nm.

Results of the validation for Ornidazole and Diloxanide Furoate of the above method were linear in the range of 25-75 µg/ml and 37.5 – 112.5 µg/ml respectively. The retention time of Ornidazole and Diloxanide furoate are found to be 3.400 & 5.747 min respectively. Correlation co-efficient for Ornidazole and Diloxanide furoate was found to be 0.999 & 0.998 over a concentration range of 25-75 µg/ml and 37.5-112.5 µg/ml respectively. Robustness is performed by making changes in flow rate, Mobile phase composition and temperature. The assay obtained after proposed changes compared with the assay obtained in normal conditions. According to the acceptance criteria difference in the assay should not be more than 2%. The results obtained are well within the acceptance criteria. The % assay results of Ornidazole and Diloxanide Furoate were 98.20 & 98.23 obtained which indicates that the proposed method was successfully utilized for the estimation both drugs in Tablet dosage forms.

Since the results are well within the limit of acceptance criteria for all validation parameters, therefore the method can be considered as validated and suitable for intended use. So, the proposed stability indicating RP-HPLC assay method can be successfully applied in the routine quality control of dosage form in industries

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