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Quantification Of Rifaximin And Ornidazole In Bulk And Combined Tablet Dosage Formulation By Validated UV- Spectrophotometric Method.

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ABSTRACT

A simple, precise and economical procedure for the simultaneous estimation of Rifaximin and Ornidazole in combined dosage form has been developed. The proposed method involves the solving of simultaneous equations (Vierodt's method). Rifaximin and Ornidazole were found to have absorbance maxima at 294 and 319 nm respectively. Linearity was observed in the concentration range of 5-15µg ml^{-1} (0.9997) for Rifaximin and 6.40 – 19.20 µg ml^{-1}(0.9998) for Ornidazole. The recovery results indicate that Rifaximin and Ornidazole could be quantified by this procedure simultaneously in combined dosage form without the interference of common excipients. The developed method was validated and the results of validation prove the suitability of the method for the quantification of the selected drugs in pharmaceutical formulation.

Keywords: Simultaneous equation, Rifaximin, Ornidazole, Spectrophotometry, Validation.

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INTRODUCTION

Ornidazole(ONZ) is a 5-nitroimidazole derivative [1].Chemically ONZ is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5 nitroimidazole **(Fig.1).**It is used in the treatment of amoebic dysentery, bacterial vaginosis, amoebiasis, giardiasis and trichomoniasis [2]. It destroys the trophozolites of *Entamoebahistolytica* and thus kills the parasite.

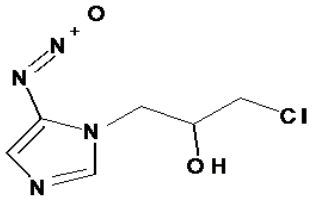

Fig 1: Structure of Ornidazole

Rifaximin (RFX) is a benzimdazole derivative [3].Chemically it is 2S, 16Z, 18E, 20S, 21S, 22R, 24R,25S,27S,5,6,21,23,25-pentahydroxy-27-methoxy 2,4,11,16,20,22,24,26,-octa methyl-2,7-epoxy penta deca-(1,11,13)trienimino)benzofuro(4,5-e)pyrido(1,2-a)-benzimidazole-1,15(2H)-dione,25-acetate**(Fig.2).** It is used in the treatment of Travelers' diarrhea caused by noninvasive strains of *Escherichia coli*. It is an inhibitor of bacterial DNA-directed RNA polymerase alpha chain and thereby reduces protein synthesis and causes cell death [4].

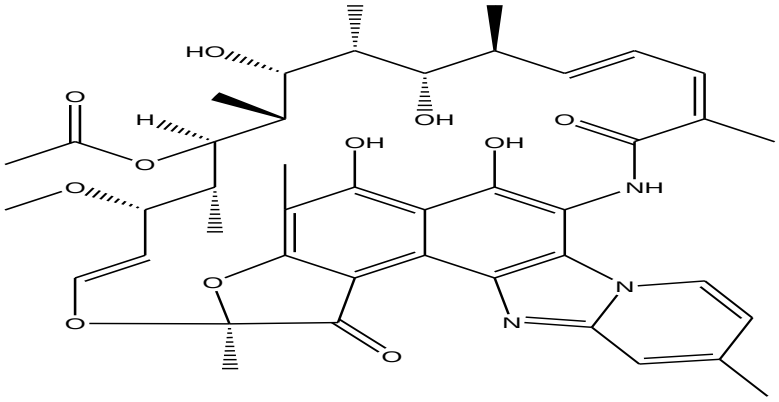

Fig 2: Structure of Rifaximin

Literature review reveals that UV method has been reported for estimation of ONZ [5] and UV and HPLC methods have been reported for estimation RFX [6-9] as a single component and few methods for combination with other drugs[10-12]. From the literature review it is evident that no UV method has been developed for the simultaneous estimation of ONZ and RFX in combined dosage form. The present work is aimed to develop a UV-Spectrophotometric method for simultaneous estimation of both these drugs in combined solid oral dosage form.

MATERIALS AND METHODS

Chemical and Reagents

ONZ and RFX standard (Purity ≥99.0%) were obtained from SaimirraInnopharm Pvt.Ltd, India).All chemicals used were of Analytical grade.

Instrumentation

Spectrophotometric data was achieved by using a UV-VIS Spectrophotometer, SHIMADZU UV-1601.

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EXPERIMENTAL

Preparation of Standard solution

RFX stock solution (100µg ml^{-1}) and ONZ stock solution (125µg ml^{-1}) was prepared by accurately weighing 20mg of RFX and 25mg of ONZ in a 200ml volumetric flask and making up to volume with acetonitrile.10ml of the above solution was further diluted to 100ml with acetonitrile. The spectrum showing the absortion maximum of ONZ, RFX and Mixed sample is shown in **Fig.3.**

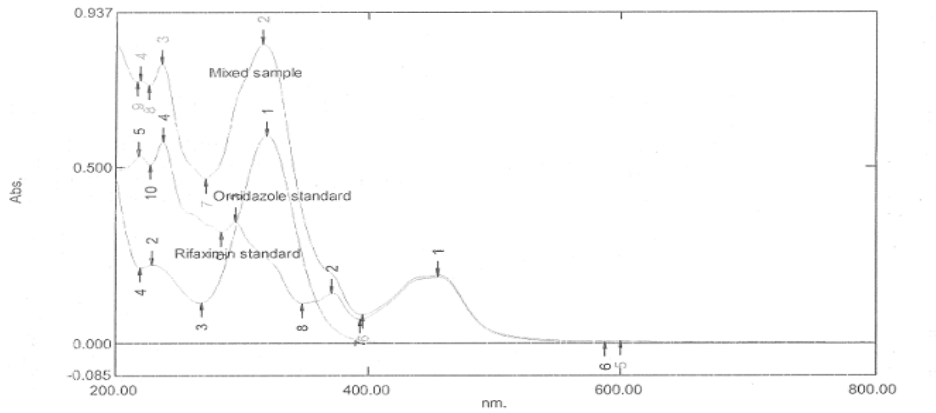

Fig 3: λ_{max} of ONZ, RFX and Mixed sample

Quantification of Pharmaceutical Formulation

20 tablets were weighed and powdered.1.3g of powdered drug was weighed accurately, transferred into a 200mL volumetric flask, about 120mL of diluent was added, Swirled to disperse, and sonicated for about 10 min. It was allowed cool to room temperature, diluted with diluent to volume and mixed. The resulting solution was passed through a membrane filter of 0.45-µm pore size. 5 ml of this solution was diluted to 100 ml with diluent. And then 10ml of above solution was diluted to 100ml with diluent.The absorbance was recorded and the amount of drug was calculated.

Method Validation [13,14]

Linearity: The stock solution was diluted suitably to get various concentrations. The absorbance of the various dilutions were recorded and the analytical curve was constructed by plotting absorbance versus concentration.

Precision: Method Precision of the assay was evaluated by carrying out 6 independent assays of test sample (10 µg ml^{-1} of RFX and 12.5µg ml^{-1} of ONZ) (n = 3) against a qualified reference standard. The % RSD at three different concentration levels was calculated. The Intermediate Precision study was performed on different days and different instruments and the % RSD was calculated.

Accuracy: Accuracy of the proposed method was checked by carrying out recovery experiments. The accuracy of the method was evaluated in triplicate at three concentration levels (50,100 and 150%) and the percentage recoveries were calculated.

RESULTS AND DISCUSSION

Specificity

Acceptance Criteria: Any absorbance observed from the placebo solution should not interfere with the absorbance maxima at 294 and 319 nm respectively of Rifaximin and Ornidazole. The results of specificity is shown in table-1.

ISSN: 0975-8585

Table-1: Specificity

Injection	Response of the peak with Retention time	Influence of placebo
1.Blank	No absorbance observed	-
2.Placebo	No absorbance observed	-
3.Standard solution	The absorbance maxima at 294nm and 319 nm respectively of Rifaximin and Ornidazole.	-
4.Test solution	The absorbance maximum at 294nm and 319nm in test solution which corresponds to Ornidazole and Rifaximin maximum absorbance in standard solution.	No influence of placebo

Remarks: There is no interference of placebo in the analysis of Ornidazole and Rifaximin.

Accuracy

Acceptance Criteria: The recovery at various levels is between 98.0% and 102.0% of added value. The RSD for recovery of triplicate samples at various levels is not more than 2.0%.The results of accuracy study is furnished in table-2.

Table- 2: Accuracy

	Rifaximin					
Level	**Amount Recovered 'mg'**	**Added Value 'mg'**	**% Recovery**	**Average**	**SD**	**%RSD**
50%	197.77	200	98.89	99.88%	1.35	1.35%
50%	198.67	200	99.33			
50%	202.83	200	101.41			
100%	402.82	400	100.71	100.29%	0.57	0.57%
100%	402.14	400	100.54			
100%	398.56	400	99.64			
150%	600.67	600	100.11	100.03%	0.24	0.24%
150%	601.31	600	100.22			
150%	598.54	600	99.76			
Ornidazole						
50%	254.14	250	101.66	100.71%	1.37	1.36%
50%	253.36	250	101.34			
50%	247.84	250	99.14			
100%	492.72	500	98.54	99.02%	1.24	1.26%
100%	490.39	500	98.08			
100%	502.14	500	100.43			
150%	747.70	750	99.69	99.73%	0.35	0.35%
150%	745.50	750	99.40			
150%	750.68	750	100.09			

Remarks: The recovery at various levels and %RSD for recovery of triplicate samples at each level passes the acceptance criteria.

Precision

The results of Method precision is shown in table-3.

ISSN: 0975-8585

Table-3: Method Precision

S.No	Content of Rifaximin mg/tablet	Content of Ornidazole mg/tablet
1	403.24	505.97
2	400.17	506.16
3	397.51	507.53
4	397.89	509.31
5	402.34	508.17
6	403.49	501.63
Average	**400.77**	**506.46**
RSD	**0.66%**	**0.53%**

Remarks: The % RSD is within the limit. Hence the method is precise.

Intermediate Precision

Acceptance Criteria: The % RSD for the 6 assay values is NMT 2.0%. The results of Intermediate Precision is shown in table-4.

Table-4: Intermediate Precision

S.No	Rifaximin 400mg mg/tablet			Ornidazole 500mg mg/tablet		
	Inter day precision	Intra day precision	Reproducibility	Inter day precision	Intra day precision	Reproducibility
1	401.93	403.24	400.18	501.68	505.97	500.65
2	399.19	400.17	400.99	502.31	506.16	501.49
3	396.41	397.51	399.79	503.54	507.53	500.99
4	396.51	397.89	401.13	508.29	509.31	504.56
5	401.14	402.34	400.88	504.02	508.17	500.68
6	402.03	403.49	400.46	497.19	501.63	503.18
Avg	**399.53**	**400.77**	**400.57**	**502.84**	**506.46**	**501.93**
RSD NMT 2.0%	**0.65%**	**0.66%**	**0.13%**	**0.72%**	**0.53%**	**0.32%**
Overall RSD NMT 2.0%	**0.51%**			**0.63%**		

Remarks: Relative standard deviation between the assay values and overall Relative standard deviation between the two sets are within acceptable limits.

Linearity and range

Linearity

Acceptance Criteria: The correlation coefficient is not less than 0.995 and y-intercept is not more than ± 2.0%.The results of linearity is shown in table-5.

Table-5: Linearity

Sample ID	Rifaximin		Ornidazole	
	Concentration	Absorbance	Concentration	Absorbance
50% of operating concentration	5	0.175	6.40	0.294
80% of operating concentration	8	0.275	10.24	0.47
100% of operating	10*	0.346	12.50*	0.587

concentration				
120% of operating concentration	12	0.415	15.36	0.704
150% of operating concentration	15	0.532	19.20	0.881

Report: On plotting the concentration against the area obtained, the graph is found to be linear in the range of 50%-150% of the operating concentration. The y-intercept and correlation coefficient are with acceptable limits.

Range

Acceptance Criteria: The relative standard deviation for peak response at the two concentration levels is not more than 2.0%.The results are shown in table-6.

Table-6: Range

S.No	Absorbance of Rifaximin		Absorbance of Ornidazole	
	50% Std	150% Std	50% Std	150% Std
1	0.173	0.531	0.293	0.879
2	0.173	0.532	0.294	0.878
3	0.174	0.531	0.293	0.879
4	0.174	0.532	0.293	0.878
5	0.173	0.531	0.294	0.879
6	0.173	0.532	0.293	0.880
Average	**0.173**	**0.532**	**0.293**	**0.879**
% RSD	**0.30%**	**0.10%**	**0.18%**	**0.09%**

Remarks: The relative standard deviation for peak response at the two concentration levels passes the acceptance criteria.

CONCLUSION

On evaluating the various parameters it is concluded that the results obtained meets the pre-established acceptance criteria. Hence the method adopted for the assay of Rifaximin and Ornidazole in combined dosage form is validated and can be used for routine analysis of the selected drugs.

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