Original Research Article

A NOVEL SYNCHRONIC ESTIMSTION OF METRONIDAZOLE, CIPROFLOXACIN AND DOXYCYCLINE BY RP-HPLC IN BULK AND PHARMACEUTICAL FORMULATION

Aim: To design simple, rapid, new analytical method for estimation of Metronidazole, Ciprofloxacin Doxycycline by using RP-HPLC in bulk and pharmaceutical dosage form.

Study Design: Estimation of Metronidazole, Ciprofloxacin Doxycycline by using RP-HPLC in bulk and pharmaceutical dosage form was planned and executed.

Place And Duration Of Study: Chalapathi Drug Testing Laboratory, Chalapathi Institute Of Pharmaceutical Sciences, Lam, Guntur-522034, Andhra Pradesh, India during the period of November 2019 to February 2020.

Methodology: Metronidazole is an antibiotic and antiprotozoal medication. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis and bacterial vaginosis. Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. Doxycycline is a tetracycline antibiotic that fights bacteria in the body. The study was carried out on SHIMADZU Prominance-i, LC-2030C system equipped with Shim-pack Gist (250 x 4.6 mm, 5µm) column and mobile phase was optimized by using mixture of methanol and 0.25mM potassium phosphate buffer in the ration of 60:40 v/v at a flow rate of 0.8 ml/min. The wavelength was set as 282nm at ambient temperature by injecting 20µl of solution and the run time was fixed for 10 min.

Results: Linearity plot was constructed for concentration range of 5-15µg/ml for Metronidazole,5-15µg/ml for Ciprofloxacin and 1-8µg/ml for Doxycycline standard solutions. It shows best regression coefficient and y/s values. The accuracy of the proposed method was determined by performing recovery studies and was found between 98-102%. The repeatability testing for both sample and standard solutions the %RSD was found as <2.0% which is within the acceptable limits showing that the method is precise as well. The LOD and LOQ were found to be 0.33 and 0.99 µg/ml for Metronidazole, 0.33 and 0.99 µg/ml for Ciprofloxacin, 0.08 and 0.25 µg/ml for Doxycycline respectively. The proposed method was successfully applied for the pharmaceutical dosage form of Metronidazole, Ciprofloxacin Doxycycline and it was as economic, eco-friendly with less retention time around 10.0 min.

Conclusion: The proposed method was validated in terms of linearity, range, Accuracy, precision, Specificity, Robustness. Method was successfully applied to the estimation of Metronidazole, Ciprofloxacin Doxycycline in combined marketed pharmaceutical dosage form.

Novelty of Present Work: As there is no particular combination of these mentioned three drugs, we have formulated one which can be used to treat fungal and bacterial infections and estimated it technically by simultaneous estimation method using RP-HPLC. We Have reported the results in result and discussion section.

1.0 INTRODUCTION

Metronidazole is an antibiotic and antiprotozoal medication. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis and bacterial vaginosis. The chemical name is 2-(2-methyl-5-**nitro**-1H-imidazol-1-yl) ethan-1-ol. Literature survey revealed that only few methods were reported for estimation of Metronidazole by RP- HPLC.

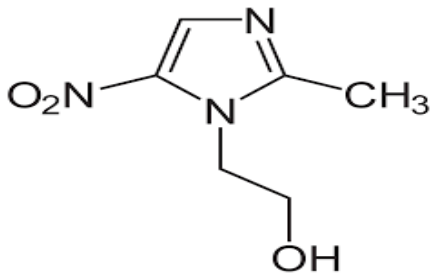

Fig 1: **Chemica**l **Structure of Metronidazole**

Ciprofloxacin is an antibiotic used to treat a number of bacterial infections. This includes bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever and urinary tract infections, among others. For some infections it is used in addition to other antibiotics. It can be taken by mouth, as eye drops, as ear drops, or intravenously. Its chemical name is 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid.

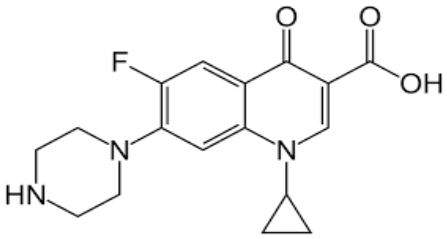

Fig 2: Chemical Structure of Ciprofloxacin

Doxycycline is a tetracycline antibiotic that fights bacteria in the body. Doxycycline is used to treat many different bacterial infections, such as acne, urinary tract infections, intestinal infections, respiratory infections, eye infections, gonorrhea, chlamydia, syphilis, periodontitis and others. There is no proved analytical method in combined dosage form was found by going through the literature.

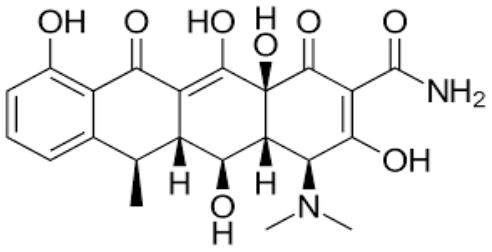

Fig 3: Chemical Structure of Doxycycline

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Gland pharma limited Ltd, Hyderabad, India kindly supplied the pure working standards of known potency of Metronidazole, Ciprofloxacin Doxycycline as gift samples. The reagents like Water, Acetonitrile, Methanol of Merk, Potassium dihydrogen phosphate are Thermo Fisher Scientific India Pvt. Ltd.

2.2 Instrumentation

The HPLC system consists of shimadzu prominence-I, LC-2030C series HPLC consisting quaternary pump, Auto sampler, Auto injector & photo diode array detector, thermostatic column compartment connected with lab solutions software with a Shim-pack GIST C_{18} (250 × 4.6 mm, 5µ) column.

2.3 Preparation of standard solution:

Accurately weighed 10mg of each drug transferred into different 10ml volumetric flasks add 6ml of diluent and sonicated for 15min to dissolve compound and volume made up with diluent to 10ml. Further the concentrations of 10 µg/ml, 10 µg/ml, 4 µg/ml of Metronidazole, Ciprofloxacin Doxycycline were prepared.

2.4 Preparation of Sample solution:

Accurately weighed one tablet equivalent powder and transferred into 250 ml volumetric flask dissolved in diluent and sonicated for 30mins, the volume was made up with diluent, filtered with 0.45µ PVDF filter. Further 1ml diluted 100ml with diluent.

3.0 OPTIMAZATION OF HPLC METHOD

The HPLC method was optimized with shimadzu prominence-I, LC-2030C series HPLC consisting of quaternary pump, Auto sampler, Auto injector & photo diode array detector, thermostatic column compartment connected with lab solutions software and column Shim-pack GIST C_{18} (250 × 4.6 mm, 5µ).Mobile phase 0.025M potassium phosphate buffer and methanol in 70:30 v/v ratio used. The flow rate of the mobile phase was maintained at 0.8 mL/min and the detection was carried out at 260 nm with an injection volume of 20 µl.

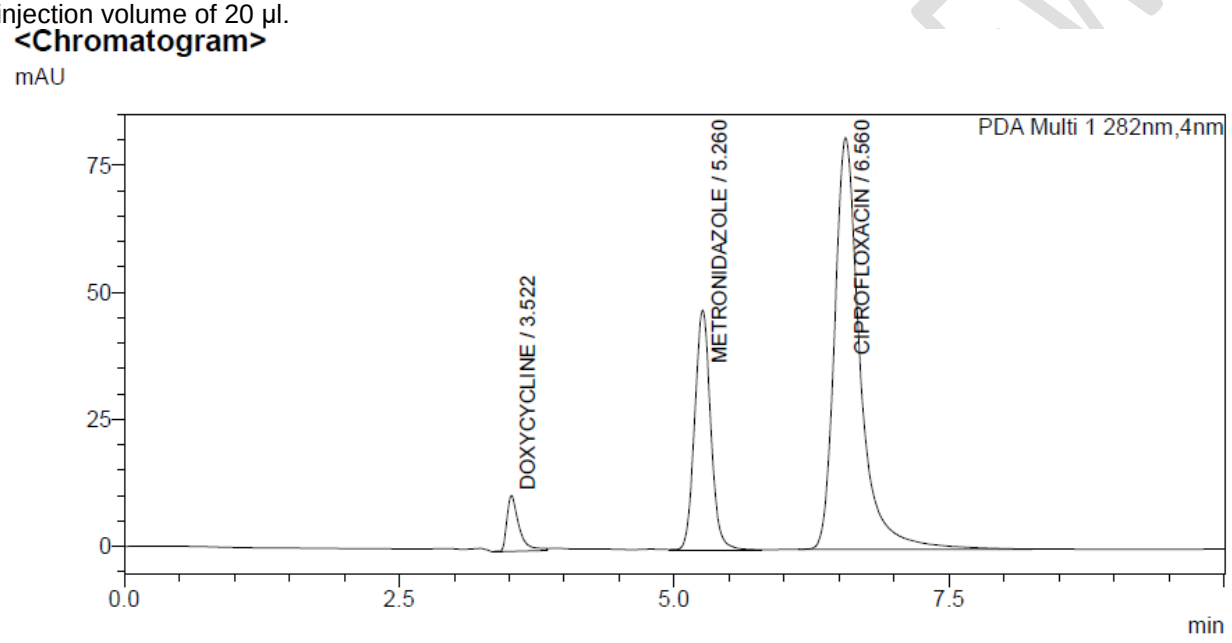

Fig.4: Chromatogram of Formulation

4.0 RESULTS AND DISCUSSION:

After RP-HPLC method development is completed, validation was performed for following parameters.

4.1 System Suitability

System suitability test is performed to determine the suitability and effectiveness of chromatographic system. Chromatographic parameters such as the number of theoretical plates, resolution, asymmetry, detection limit and selectivity were taken into consideration. Standard solution of 10µg/ml, 10µg/ml, 4µg/ml Metronidazole, Ciprofloxacin Doxycycline was prepared and injected into HPLC system and. Observed results were tabulated in table no. 1.

Table: 1 System suitability data

S.No	Injection number	Peak area for Metronidazole	Peak area for Ciprofloxacin	Peak area for Doxycycline	Acceptance criteria
1	01	476958	1412528	84472	The % RSD of peak areas should not be more than 2.0.
2	02	477256	1411954	84859	
3	03	475691	1414368	84762	
4	04	477658	1413568	84389	
5	05	476658	1418456	84581	
6	06	477258	1416189	84656	

Mean	476913	1414511	84620	
%RSD	0.14	0.17	0.21	

System suitability parameters	Observed value			
	Metronidazole	Ciprofloxacin	Doxycycline	Acceptance criteria
Tailing for Metronidazole, Ciprofloxacin and Doxycycline in standard solution	1.21	1.14	1.03	NMT 2.0
Theoretical plates for Metronidazole, Ciprofloxacin and Doxycycline in standard solution	7241	6547	9412	NLT 2000
Resolution Metronidazole, Ciprofloxacin and Doxycycline peaks in standard solution	--	3.65	4.17	NLT 2.0

4.2 Precision

Precision can be defined as the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. The relative standard deviation of individual areas of Metronidazole, Ciprofloxacin and Doxycycline were found to be within limits. (≥2.0)

4.2.1 Intra-day Precision

Table: 2 Intra-day precision for Metronidazole, Ciprofloxacin and Doxycycline

S.NO	Injection Number	Peak area for Metronidazole	Peak area for Ciprofloxacin	Peak area for Doxycycline
1	1	476884	1415825	84514
2	2	476985	1415256	84672
3	3	476879	1414576	84126
4	4	478148	1415487	84681
5	5	476258	1416458	84574
6	6	477369	1417258	84584
Mean		477087	1415810	84525
%RSD		0.13	0.07	0.24

4.2.2 Inter-day Precision

Table: 3 Inter-day precision for Metronidazole, Ciprofloxacin and Doxycycline

S.NO	Injection number	Peak area for Metronidazole	Peak area for Ciprofloxacin	Peak area for Doxycycline
1	1	478416	1411968	84567
2	2	475964	1418569	84897
3	3	476268	1413895	84685
4	4	478562	1416895	84789
5	5	477826	1417259	84562
6	6	476859	1418468	84656
Mean		477316	1416176	84693
%RSD		0.23	0.19	0.15

4.3 Limit Of Detection And Limit Of Quantization

Limit of Detection (LoD) and Limit of Quantitation (LoQ) are terms used to describe the less concentration of a measurand that can be reliably measured by an analytical procedure.

Table: 4 Report of LOD and LOQ

S.NO	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1	Metronidazole	0.33	0.99

2	Ciprofloxacin	0.33	0.99
3	Doxycycline	0.08	0.25

4.4 Linearity

Linearity is the method's ability to obtain test results which are directly proportional to the concentration of analyte in the sample. A series of standard solutions were prepared in the range of 5µg/ml-15µg/ml for Metronidazole, Ciprofloxacin and Doxycycline in range for 1µg/ml-8µg/ml. The mixture of standard solutions was injected into HPLC system and calculated the correlation coefficient value, Y-intercept for area and concentrations of the standard injected.

Table: 5 Report of Linearity

Standard Conc(µg/ml)	Area of Metronidazole	Standard Conc(µg/ml)	Area of Ciprofloxacin	Standard Conc(µg/ml)	Area of Doxycycline
5	238463	5	743057	1	21040
7.5	357694	7.5	1077808	2	41080
10	476925	10	1410013	4	84160
12.5	586156	12.5	1798654	6	125240
15	711388	15	2097774	8	168720

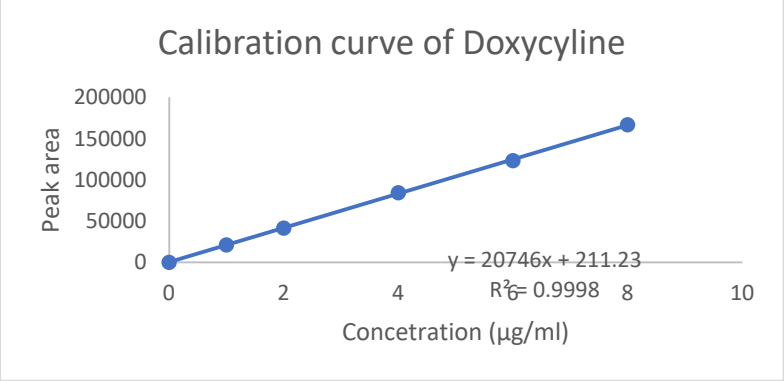

Fig :5 Calibration curve of Doxycyline

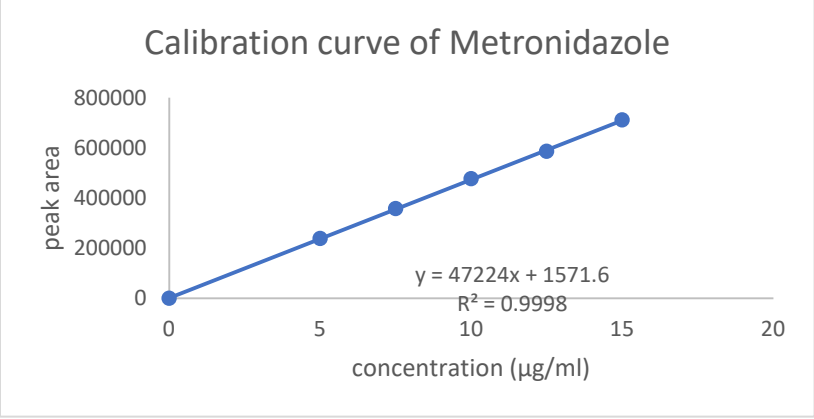

Fig : 6 Calibration curve of Metronidazole

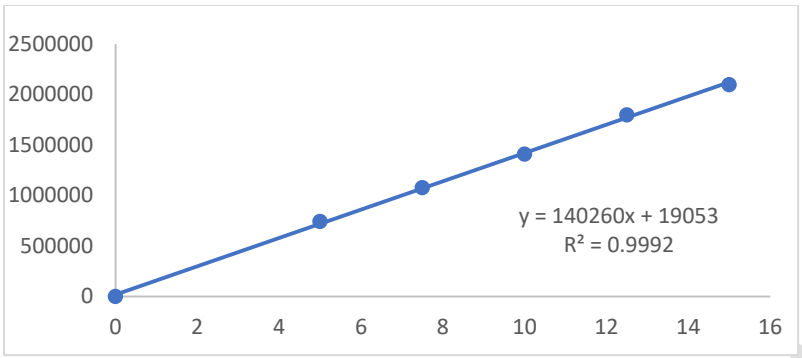

Fig : 7 Calibration curve of Ciprofloxacin

4.5 Accuracy

To determine the Accuracy of the proposed method, recovery studies were conducted. The known amount of pure drug concentrations were spiked in three different levels which were 50%, 100% and 150% and accuracy was calculated and tabulated below in Table 6.

Table: 6 Accuracy data

Level	Peak area			% recovery			Mean % recovery			Over all recovery		
	Met	CIP	Doxy	Met	CIP	Doxy	Met	CIP	Doxy	Met	CIP	Doxy
50	238547	715264	42576	99.72	100.50	100.20	100.01	100.26	100.55	100.14	100.12	100.16
	239562	712458	42872	100.20	100.17	100.96						
	239261	711854	42651	100.11	100.12	100.48						
100	477021	1412856	84589	99.87	99.43	99.71	99.73	99.81	99.87			
	476924	1411965	84750	99.83	99.35	99.88						
	475298	1430589	84890	99.48	100.65	100.03						
150	721895	2120456	126842	100.73	99.45	99.65	100.69	100.29	100.05			
	721245	2152321	127891	100.65	100.96	100.48						
	721516	2141598	127296	100.70	100.47	100.03						

4.6 Robustness

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Metronidazole, Ciprofloxacin and Doxycycline solution (Table 7). The results obtained were compared with that of optimized method. It was confirmed that by making the deliberate changes in the parameters there were no significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor were found.

Table: 7 Report of Robustness - Metronidazole

S.No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP plate count
1	Flow rate by ± 2%	1.0 ml	0.94	0.99	6878
		0.8 ml	0.74	1.05	6695

		0.6 ml	0.65	1.01	6308
2	Column oven temperature by ± 2°C	23°C	0.28	1.02	6603
		25°C	0.19	1.11	6256
		27°C	0.45	1.23	6968
3	Wavelength of analysis ± 2nm	280 nm	0.59	1.10	6965
		282 nm	0.66	1.14	6664
		284 nm	0.80	1.01	6723
4	Organic composition of mobile phase by ± 5%	65:35	0.29	1.23	6527
		70:30	0.45	1.14	6692
		75:25	0.65	1.12	6052

Table: 8 Report of of Robustness - Ciprofloxacin

S.No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP plate count
1	Flow rate by ± 2%	1.0 ml	0.45	1.21	7638
		0.8 ml	0.24	1.23	7410
		0.6 ml	0.19	1.10	7308
2	Column oven temperature by ± 2°C	23°C	0.78	1.24	7603
		25°C	0.65	1.22	7850
		27°C	0.44	1.14	7652
3	Wavelength of analysis ± 2nm	280 nm	0.21	0.91	7921
		282 nm	0.32	0.96	7652
		284 nm	0.71	0.86	7121
4	Organic composition of mobile phase by ± 5%	65:35	0.69	1.23	7542
		70:30	0.58	1.16	7721
		75:25	0.72	1.19	7533

Table: 9 Report of of Robustness - Doxycycline

S.No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP plate count
1	Flow rate by ± 2%	1.0 ml	0.18	1.24	9531
		0.8 ml	0.25	1.15	9456

		0.6 ml	0.15	1.04	9210
2	Column oven temperature by ±2°C	23°C	0.22	1.32	9900
		25°C	0.14	1.21	9533
		27°C	0.17	1.17	9411
3	Wavelength of analysis ± 2nm	280 nm	0.56	1.16	9865
		282 nm	0.72	1.21	9456
		284 nm	0.65	1.09	9741
4	Organic composition of mobile phase by ± 5%	65:35	0.79	1.26	9648
		70:30	0.73	1.15	9315
		75:25	0.75	1.11	9145

ASSAY RESULTS:

Accurately weighed 20 tablets and crushed to fine powder. Accurately weighed powder equivalent to 750mg and transferred into 250 ml volumetric flask dissolved in diluent and sonicated for 30mins, the volume was made up with diluent, filtered with 0.45µ PVDF filter. Further 1ml diluted 100ml with diluent.

Table:10 results of Assay

Drug	Label Claim	%Assay
Metronidazole	250	100.14
Ciprofloxacin	250	100.12
Doxycycline	100	100.16

5.0 CONCLUSION: A simple, specific and reliable isocratic HPLC-PDA method was developed for the estimation of Metronidazole, Ciprofloxacin and Doxycycline in their bulk and pharmaceutical formulation was given. The current method was validated according to Q2 (R1) ICH guidelines in terms of Linearity, Accuracy, Precision, Limit of detection, Limit of quantification and Robustness. Linearity plot was constructed for concentration range of 5-15 µg/ml for Metronidazole, 5-15 µg/ml for Ciprofloxacin and 1-8 µg/ml for Doxycycline as standard solutions. It shows best regression coefficient and y/s values. The accuracy of the proposed method was determined by performing recovery studies and was found between 98-102%. The repeatability testing for both sample and standard solutions was found as %RSD<2.0% which is within the acceptable limits showing that the method is precise as well. The LOD and LOQ were found to be 0.33 and 0.99 µg/ml for Metronidazole, 0.33 and 0.99 µg/ml for Ciprofloxacin, 0.08 and 0.25 µg/ml for Doxycycline respectively. Hence the current developed method can be fruitfully applied for the estimation of Metronidazole, Ciprofloxacin and Doxycycline in drug testing laboratories and pharmaceutical industries.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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