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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND CLOTRIMAZOLE IN PESSARIES DOSAGE FORM

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ABSTRACT

Simple reversed phase high performance liquid chromatography may be used to measure Metronidazole and Clotrimazole simultaneously. The system with Buffer (0.1M of monobasic potassium phosphate pH 2.4) (70): Acetonitrile (30) with 2.0 ml / min flow rate was discovered to be best for separation of compounds. The optimum detection wavelength was 210 nm. Metronidazole and clotrimazole were shown to have retention periods of 1.13 minutes and 10.8 minutes, respectively. The method was found to be linear with R2 0.9993 and 0.9958 for metronidazole and clotrimazole respectively. The technique was discovered to be precise and accurate. A system suitability test was also carried out to confirm the chromatographic system's reproducibility. In specificity study, the peaks of metronidazole and clotrimazole were detected separately from blank values demonstrating that the method is specific for the active ingredient.

The stability studies showed that the solutions of metronidazole and clotrimazole was stable for up to 24 hours for both the sample stored at room temperature and stored at 2°C to 8°C. By changing the chromatographic parameters, such as the ratio of solvents in the mobile phase composition, flow rate, change in ratio of solvents in column length and other factors, the robustness of the approach was assessed. The influence of changes of chromatographic parameters on peak parameters was found to significant on theoretical plate and tailing factor but it showed insignificant effect on retention time. Hence, the method was robust except for tailing factor and theoretical plates which demonstrate that the slight changes in chromatographic condition may vary tailing factor and theoretical plates of metronidazole and clotrimazole.

Keywords: Metronidazole, Clotrimazole, RP-HPLC, Pessaries, Potassium dihydrogen phosphate, Acetonitrile

INTRODUCTION

Most of the human illnesses are caused by infection with either bacteria, fungal or viruses. Various Infections are seen in the human body. Vaginal bacterial infections have gained attention due to the increased prevalence of urinary tract infections and sexually transmitted diseases [1]. Bacterial infections are among the most common causes of vaginal discharge during the reproductive age [2].

Metronidazole

The nitroimidazole class of antibiotics includes metronidazole as a member [3]. It is a bacterial, antiprotozoal, and antiameaobic medication [4]. It is frequently used to treat parasite illnesses such trichomoniasis, giardiasis, and ameobiasis as well as gastrointestinal infections. It is widely used to treat skin ulcers, rosacea, fungating tumours, and vaginal bacterial infections [5,6]. It is frequently employed as an antibacterial agent against both gram-positive and gram-negative bacteria, including the lactamase-producing Bacteriode fragilis [7]. The IUPAC name of metronidazole is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol and its Empirical formula is C21H26N2O7. Metronidazole is essentially water soluble and soluble in organic solvent like ethanol, methanol, and chloroform [8].

Clotrimazole

Clotrimazole relates to the imidazole class of medications. Clotrimazole is another form of 1H-imidazole. In instead of the hydrogen attached to the nitrogen, the monochlorotrityl group can be used [9,10]. It performs as a xenobiotic, a contaminants in the environment, and an anti infective agent [5]. It is given as conazole antifungal medication [9]. The IUPAC name of 1-[(2-Chlorophenyl (diphenyl)methyl)] -1H -imidazole and its empirical formula C22H17CIN2and molecular weight is 344.8 g/mol[10]. It is slightly soluble in water, benzene, and toluene and soluble in organic solvent like chloroform, acetone, and ethyl acetate [10].

MATERIALS AND METHODS

Metronidazole and Clotrimazole working standard were gift from Arrow Pharmaceutical Pvt. Ltd. Acetonitrile and Orthophosphoric acid were obtained from Qualigens Fine Chemicals Ltd. Monobasic Potassium Phosphate were obtained from Merck India. Sample was gifted from the Arrow Pharmaceutical Pvt. Ltd.

Instrumentation and chromatographic condition

The chromatographic separation was carried out on HPLC system (Agilent Technologies 1260 Infinity II-DAD). Buffer solution is prepared by dissolving 0.1M Monobasic Potassiun Phosphate in 1000 ml of water and then pH is adjusted to 2.4 with orthophosphoric acid. Mobile Phase is prepared in the ratio of 70:30 volume where 70 volumes is buffer and 30 volumes of acetonitrile. Mobile Phase was filtered with membrane and sonicated for 15 mins. Mobile Phase was used as the solvent for sample and standard preparation as well.

A stainless steel column 25 cm X 4.6 mm, packed with octylsilane bonded to porous silica (5µm) was used with flow rate 2.0 ml/min at wavelength set at 210 nm. The injection volume was 20 µl.

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A stainless steel column 25 cm X 4.6 mm, packed with octylsilane bonded to porous silica (5µm) was used with flow rate2.0 ml/min at wavelength set at 210nm. The injection volume was 20 µl.

Preparation of standard solution

Metronidazole standard stock solution: Weight accurately about 40 mg of Metronidazole working standard gently transfer to 50 ml volumetric flask. Sonicate with 15ml of Acetonitrile. Further dissolve the sample completely with the volume to 50 ml with buffer solution.

Clotrimazole standard stock solution: Weight accurately about 17 mg of Clotrimazole working standard and gently transfer to 100 ml volumetric flask. Sonicate with 30 ml of Acetonitrile, Further dissolve the sample completely with the volume to 100 ml with buffer solution.

2 ml of solution from each standard stock solution is pipetted in 100 ml of volumetric flask, Make volume up to the mark with mobile phase.

Preparation of sample solution: Average weight was determined by weighing 20 sample tablets. The tablets were crushed to obtain fine powder. Weight accurately sample containing 200 mg of Metronidazole and gently transfer to 100 ml volumetric flask. 30 ml of Acetonitrile is added and sonicate to dissolve the sample completely by make up the volume to 100 ml with buffer solution, Mix and filter. Pipette 10 ml of filtrate to 50 ml volumetric flask. Make volume up to the mark with mobile phase. Further pipette 2 ml of the resulting solution to 50 ml volumetric flask with mobile phase. Make volume up to the mark with mobile phase.

RESULT AND CONCLUSION

Specificity

The specificity is determined by injecting the blank solution, matrix solution, standard solution and sample solution to show the non- appearance of interference to the analyte. The outcomes are displayed in the following Table 1 and Figure 1

Table 1. Specificity for metronidazole and clotrimazole.

S.No.	Name of Injection	Area	
		Metronidazole	Clotrimazole
1.	Blank	Nil	Nil
2.	Placebo Solution	Nil	Nil
3.	Standard Solution	2355.0674	5147.77030
4.	Sample Solution	2196.63129	4992.28461

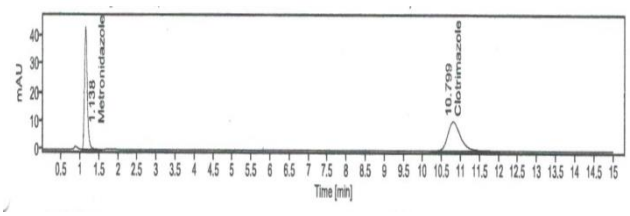

Figure 1. Chromatogram of metronidazole and clotrimazole sample solution.

From the above chromatogram. It is observed that metronidazole and clotrimazole was detected separately from the blank. In the placebo sample there is no peak detection at sample retention time .The Blank and Placebo does not interfere at the retention time of principal peak, thus the Excipient and reagents used does not interfere with the analysis of the targeted analyte, so the Specificity meets the requirement of the analytical method validation specification (limit).

Linearity and range

The Linearity of the method was determined by injecting five replicate of five different concentrations within the range of 80% to 120% of the targeted concentration for Assay by preparing standard of Metronidazole & Clotrimazole. The plots of peak area of each sample against respective weight of Metronidazole and Clotrimazole were found to be linear (Figure 2 and 3) in the range of 80% to 120% of the targeted concentration.
Linearity was determined by calculating the regression line using the mathematical treatment of the sample weight (in mg) (x) versus area obtained (y) and calculating the correlation coefficient (r) by using formulae (Table 2).

$$r = \frac{[n \Sigma xy - (\Sigma x)(\Sigma y)]}{\sqrt{([\{n (\Sigma x^2)\} - (\Sigma x)^2]} \times \sqrt{([\{n (\Sigma y^2)\} - (\Sigma y)^2]}}$$

r2 (for set Metronidazole) = 0.9993,
r2 (for set Clotrimazole) = 0.9958

Table 2. Linearity of response for metronidazole and Clotrimazole.

S. No	Linearity Level	Weight taken (in mg)		Area	
		Metronidazole	Clotrimazole	Metronidazole	Clotrimazole
1	80 %	32.26 mg	13.63 mg	79	187
2.	90 %	36.36 mg	15.36 mg	90	209
3.	100 %	40.59 mg	17.28 mg	99	238
4.	110 %	44.04 mg	18.73 mg	109	256
5.	120 %	48.48 mg	20.45 mg	119	279

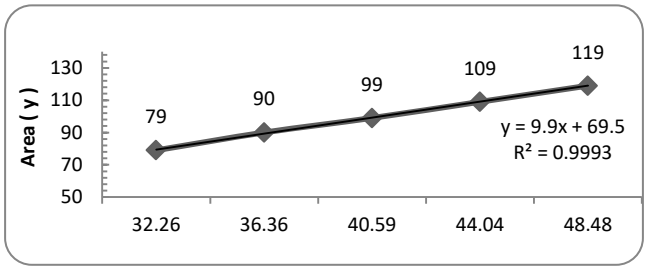

Figure 2. Linearity graph for metronidazole

The square of correlation coefficient (r2) calculated above was 0.9993 for Metronidazole and 0.9958 for Clotrimazole, which lies inside the specified limit. Thus, the Linearity meets the requirement of the analytical method validation specification (limit).

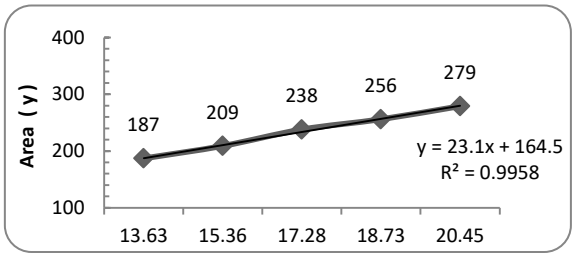

Figure 1. Linearity graph for clotrimazole.

The square of correlation coefficient (r2) calculated above was 0.9993 for Metronidazole and 0.9958 for Clotrimazole, which lies inside the specified limit. Thus, the Linearity meets the requirement of the analytical method validation specification (limit).

Accuracy

Accuracy of the method was found out by recovery study by standard addition method (Spiked). The known amounts of standards, Metronidazole and Clotrimazole were added to pre-analyzed samples and spiking of the standard was carried out in second dilution. Three individually prepared replicates at each concentration had been analyzed (Table 3).

$$\text{Theoretical value} = \frac{\text{spiked standard weight} \times \text{potency of spiked standard} \times 100}{\text{standard weight} \times \text{potency of standard}}$$

$$\text{Assay value} = \frac{\text{area or absorbance of sample} \times 100}{\text{area or absorbance of standard}}$$

The results were illustrated below;

Table 3. Accuracy for metronidazole.

S.No.	Name of Sample	Theoretical Value (mg)	Assay Value (%)	% Recovery
1.	Sample 1 (80 %)	80.22	80.58	99.55
2.	Sample 2 (80 %)	79.31	80.58	98.42
3.	Sample 3 (80 %)	79.75	79.61	100.18
4.	Sample 1 (100 %)	100.22	100.00	100.22
5.	Sample 2 (100 %)	99.70	100.00	99.70
6.	Sample 3 (100 %)	99.07	99.03	100.04
7.	Sample 1 (120 %)	120.05	120.39	99.72
8.	Sample 2 (120 %)	119.56	119.42	100.12
9.	Sample 3 (120 %)	120.05	119.42	100.53
Mean				**99.83**
Standard Deviation				**0.6112**
RSD in %				**0.6122**

From the Table 4, the % Recovery for Metronidazole was calculated and the average percentage recovery was found to be 99.83 %, standard deviation of samples was 0.6112 and relative standard deviation was 0.6122, which lies inside the specified limit, thus, the Accuracy meets the requirement of the analytical method validation specification (limit).

Table 4. Accuracy for clotrimazole.

S.No.	Name of Sample	Theoretical Value (mg)	Assay Value (%)	% Recovery
1.	Sample 1 (80 %)	78.25	78.83	99.90
2.	Sample 2 (80 %)	78.98	78.83	100.19
3.	Sample 3 (80 %)	79.15	79.28	99.84
4.	Sample 1 (100 %)	103.18	102.70	100.47
5.	Sample 2 (100 %)	102.26	102.25	100.01
6.	Sample 3 (100 %)	102.14	102.25	99.89
7.	Sample 1 (120 %)	118.82	118.92	99.92
8.	Sample 2 (120 %)	118.88	118.92	99.96
9.	Sample 3 (120 %)	118.65	118.47	100.15
Mean				**100.04**
Standard Deviation				**0.2010**
RSD in %				**0.2009**

The % Recovery for Clotrimazole was calculated and the average percentage recovery was found to be 100.04 %, standard deviation of samples was 0.0.2010 and relative standard deviation was 0.2009, which lies inside the specified limit, thus, the Accuracy meets the requirement of the analytical method validation specification (limit).

Precision

Repeatability of system and method was performed. For instrument precision, determination of five replicate of standard was injected. Injection of three replicate samples at three different doses were made for precision and the relative standard deviation (RSD) was recorded.

The results were illustrated below Table 5.

The average Assay % for Metronidazole, calculated above was 100.77 %, Standard deviation was 0.7623 and Relative Standard Deviation (RSD) % calculated above was 0.7565, which lies inside the specified limit of Repeatability thus, repeatability meets the requirement of the analytical method validation specification (limit).

Table 5. Repeatability for Metronidazole for method precision.

S.No.	Sample Name	Assay value (mg)	Assay value (in %)
1.	Sample 1 (80 %)	507.0643	101.41
2.	Sample 2 (80 %)	500.6905	100.14
3.	Sample 3 (80 %)	506.9286	101.39
4.	Sample 1 (100 %)	508.2028	101.64
5.	Sample 2 (100 %)	502.3283	100.47
6.	Sample 3 (100 %)	507.9973	101.60
7.	Sample 1 (120 %)	498.6593	99.73
8.	Sample 2 (120 %)	503.3092	100.66
9.	Sample 3 (120 %)	499.2897	99.86
Average Percentage:			**100.77 %**
Standard Deviation:			**0.7623**
RSD:			**0.7565**

The average Assay % for Metronidazole, calculated above was 100.77 %, Standard deviation was 0.7623 and Relative Standard Deviation (RSD) % calculated above was 0.7565, which lies inside the specified limit of Repeatability thus, repeatability meets the requirement of the analytical method validation specification (limit).

The results were illustrated below Table 6.

Table 6. Repeatability for Clotrimazole for method precision.

S.No.	Sample Name	Assay value (mg)	Assay value (in %)
1.	Sample 1 (80 %)	99.0063	99.01
2.	Sample 2 (80 %)	100.6934	100.69
3.	Sample 3 (80 %)	98.9798	98.98
4.	Sample 1 (100 %)	99.7609	99.76
5.	Sample 2 (100 %)	99.1672	99.17
6.	Sample 3 (100 %)	98.3790	98.38
7.	Sample 1 (120 %)	101.1557	101.16
8.	Sample 2 (120 %)	101.2481	101.25
9.	Sample 3 (120 %)	100.9180	100.92
Mean			**99.92**
Standard Deviation			**1.0938**
RSD in %			**1.0946**

From the above table, the average Assay % for Clotrimazole, calculated above was 99.92 %, Standard deviation was 1.0938 and Relative Standard Deviation (RSD) % calculated above was 1.0946, which lies inside the specified limit of Repeatability and meets the requirement of the analytical method validation specification (limit).

Intermediate precision

Intermediate precision of the method was analyse by different analysts, using two different instrument on different day. It helps to compare the relative purity data between the two systems. The experiment's findings are listed in the Table 7 and 8 below.

Table 7. Intermediate precision for operator for metronidazole.

Analyst	Day	Sample No	Assay Value (mg)	Assay Value (%)
(Analyst 1)	1	1	508.2028	101.64
		2	502.3283	100.47
		3	507.9973	101.60
	2	1	503.9221	100.78
		2	505.7688	101.15
		3	498.5128	99.70
(Analyst 2)	1	1	498.4547	99.69
		2	508.8088	101.76
		3	504.0008	100.80
	2	1	500.3703	100.74
		2	501.7638	100.35
		3	498.9002	99.78
Mean				**100.71**
Standard Deviation				**0.7434**
RSD in %				**0.7382**

Table 8. Intermediate precision for instruments for metronidazole.

Instrument ID	Day	Relative Standard Deviation of standard solution
AP/QC/047 (HPLC 1)	1	0.7985
	2	0.0815
AP/QC/013 (HPLC 2)	1	0.4672
	2	0.2434

From the Table 8, the RSD for Metronidazole was 0.7985 and 0.0815 for HPLC 1 on different days, similarly, for HPLC 2 the RSD was 0.4672 and 0.2434 on different days. The obtained value lies inside the specified limit of Intermediate Precision for instrument thus; the Intermediate Precision meets the requirement of the analytical method validation specification (limit).

The results were illustrated below Table 9.

Table 9. Intermediate precision for operator for clotrimazole

.

Analyst	Day	Sample No	Assay Value (mg)	Assay Value (%)
(Analyst 1)	1	1	99.7609	99.76
		2	99.1672	99.17
		3	98.3790	98.38
	2	1	98.9151	98.92
		2	99.9695	99.97
		3	99.5492	99.55
(Analyst 2)	1	1	100.5334	100.53
		2	100.6291	100.63
		3	100.6554	100.66
	2	1	99.0921	99.09
		2	99.5959	99.60
		3	100.0846	100.08
Mean				**99.70**
Standard Deviation				**0.7219**
RSD in %				**0.7241**

From the Table 9, the Mean Assay % calculated for Clotrimazole was 99.70%, Standard Deviation was 0.7219 and Relative Standard Deviation (RSD) % was 0.7241. The finding lies inside the Specified limit of Intermediate Precision, thus; the Intermediate Precision meets the requirement of the analytical method validation specification (limit).

From the Table 10 the RSD for Metronidazole was 0.2758 and

0.0569 for HPLC 1 on different days, similarly, for HPLC 2 the RSD was 0.0732 and 0.0332 on different days. The obtained value lies inside the specified limit of Intermediate Precision for instrument thus; the Intermediate Precision meets the requirement of the analytical method validation specification (limit).

Table 10. Intermediate precision for instruments for clotrimazole.

Instrument ID	Day	Relative Standard Deviation of standard solution
(HPLC1)	1	0.2758
	2	0.0569
(HPLC 2)	1	0.0732
	2	0.0332

Robustness

Mobile Phase, flow rate and column length variations were used to measure how well a method can withstand tiny but intentional changes in the procedure.

Modification of the solvent ratio in the mobile phase: The results were illustrated below in Table 11.

Table 11. Modification of the solvent ratio in the mobile phase for metronidazole.

S.No	Sample Name	Mobile Phase % v/v (Buffer: Acetonitrile)	Assay value (mg)	Assay Value (%)
1	Sample 1	68:32	507.5768	101.52
2	Sample 2	68:32	502.0936	100.42
3	Sample 3	68:32	503.3734	100.67
4	Sample 1	72:28	515.4917	103.10
5	Sample 2	72:28	499.9004	99.98
6	Sample 3	72:28	500.2092	100.04
Mean				**100.96**
Standard Deviation				**1.1896**
RSD in %				**1.1783**

The results were illustrated below Table 12.

Table 12. Modification of the solvent ratio in the mobile phase for clotrimazole.

S.No	Sample Name	Mobile Phase % v/v (Buffer: Acetonitrile)	Assay value (mg)	Assay Value (%)
1	Sample 1	68:32	99.6449	99.65
2	Sample 2	68:32	99.4094	99.41
3	Sample 3	68:32	99.4552	99.46
4	Sample 1	72:28	100.6697	100.67
5	Sample 2	72:28	99.8458	99.85
6	Sample 3	72:28	99.2982	99.30
Mean				**99.72**
Standard Deviation				**0.5029**
RSD in %				**0.5043**

Modification of the solvent ratio in the flow rate: Sample preparations were analyzed as per methodology by changing the flow rate of the mobile phase. Triplicate sample of 100 % concentration was prepared for each condition. The data found was illustrated in below Table 13.

Table 13. Change in flow rate for metronidazole.

S.No	Sample Name	Flow Rate (ml/min)	Assay value (mg)	Assay Value (%)
1	Sample 1	1.8	504.9089	100.98
2	Sample 2	1.8	499.8088	99.96
3	Sample 3	1.8	515.2436	103.05
4	Sample 1	2.2	499.8088	99.96
5	Sample 2	2.2	504.9089	100.98
6	Sample 3	2.2	505.0408	101.01
Mean				**100.99**
Standard Deviation				**1.1284**
RSD in %				**1.1173**

The results were illustrated below Table 14.

Table 14: Change in flow rate for clotrimazole.

S.No	Sample Name	Flow Rate (ml/min)	Assay value (mg)	Assay Value (%)
1	Sample 1	1.8	99.1091	99.11
2	Sample 2	1.8	99.1091	99.11
3	Sample 3	1.8	98.7384	98.74
4	Sample 1	2.2	99.2665	99.27
5	Sample 2	2.2	98.8596	98.86
6	Sample 3	2.2	99.6993	99.70
Mean				**99.13**
Standard Deviation				**0.3377**
RSD in %				**0.3407**

Modification of the solvent ratio in column length: Sample preparations were analyzed as per methodology by changing the column length from 25 cm to 15 cm. Triplicate sample of 100 % concentration was prepared for each condition. The data found was illustrated in below Table 15 and 16.

Table 15. Change in column length for metronidazole.

S.No	Sample Name	Assay value (mg)	Assay Value (%)
1	Sample 1	500.0996	100.02
2	Sample 2	503.0882	100.62
3	Sample 3	511.5182	102.30
Mean			**100.98**
Standard Deviation			**1.1819**
RSD in %			**1.1704**

Table 16. Change in column length for clotrimazole.

S.No	Sample Name	Assay value (mg)	Assay Value (%)
1	Sample 1	99.7086	99.71
2	Sample 2	99.8134	99.81
3	Sample 3	99.8751	99.88
Mean			**99.80**
Standard Deviation			**0.0854**
RSD in %			**0.0856**

It was observed that there were no marked changes in the chromatogram, which demonstrated that the proposed method was robust, thus; the Robustness meets the requirement of the analytical method validation specification (limit).

Solution stability

Solution stability for method was investigated by injecting the freshly prepared standard and sample solutions and storing the sample in auto sampler (24 hours) in room temperature. Triplicate sample of 100 % concentration were prepared as original solution. The calculated mean assay percentage using solutions kept at ambient temperature in auto sampler (for 24 hours) was compared with mean assay percentage obtained from freshly prepared solutions. The obtained data were illustrated below Table 17 and 18.

Table 17: Solution stability for metronidazole.

S.No	Sample Name	Average Assay value (mg)	Average Assay Value (%)
1	Freshly prepared	503.7547	100.75
2	Stored at room temperature in auto sampler (for 24 hours)	496.2648	99.25
98.0 % to 102.0 % in comparison to the freshly prepared solution.			**98.51 %**

Table 18. Solution stability for clotrimazole.

S.No	Sample Name	Average Assay value (mg)	Average Assay Value (%)
	Freshly prepared	100.6060	100.61
	Stored at room temperature in auto sampler (for 24 hours)	98.7014	98.70
98.0% to 102.0 % in comparison to the freshly prepared solution.			**98.10 %**

The result of comparision between freshly prepared solutions with the solution stored at Ambient temperature in auto sampler (for 24 hours) was 98.51 for Metronidazole and 98.10% for Clotrimazole. The obtained assay value lies inside the specified limit.

System suitability

It is performed by injecting five injections of a solution containing Analyte at 100 % of the test concentration. Parameters of system suitability are retention time, theoretical plates, tailing factors, resolution and peak area were recorded.

The obtained data were illustrated below Table 19 and 20.

Table 19: System suitability for metronidazole.

S.No	Sample Name	RSD of RT	RSD of Area	Theoretical Plates	Tailing factor
1	Linearity- 80 %	0.009	0.5337	2307.19407	1.15045
2	Linearity- 90 %	0.029	0.8332	2294.21263	1.17621
3	Linearity- 100 %	0.008	0.7985	2313.17480	1.19703
4	Linearity- 110 %	0.030	0.4023	2230.01468	1.17677
5	Linearity- 120%	0.020	0.4352	2321.00630	1.16867
6	Accuracy	0.039	0.4672	3368.15864	1.20166
7	Repeatability	0.008	0.7982	2313.17475	1.19703
8	Intermediate Precision (Day 1: Analyst 1)	0.008	0.7985	2313.17475	1.19703
9	Intermediate Precision (Day 1: Analyst 2)	0.039	0.4672	3368.15864	1.20166
10	Intermediate Precision (Day 2: Analyst 1)	0.052	0.0815	3420.0028	1.12347
11	Intermediate Precision (Day 2: Analyst 2)	0.009	0.2434	5973.01789	1.06188
12	Robustness-Change in ratio of Mobile Phase (68:32)	0.014	0.4213	2141.60784	1.85789
13	Robustness-Change in ratio of Mobile Phase (72:28)	0.086	0.4065	2396.15810	1.10966
14	Robustness-Change in Flow rate (1.8 ml/min)	0.054	0.3620	4111.29489	1.25280
15	Robustness-Change in Flow rate (2.20 ml/min)	0.062	0.3057	2810.8245	1.21776
16	Robustness-Solution Stability	0.052	0.4495	2865.55051	1.33576

Table 20. System suitability for clotrimazole.

S.No	Sample Name	RSD of RT	RSD of Area	Theoretical Plates	Tailing factor	Resolution
1	Linearity- 80 %	0.046	0.1125	5124.02935	1.31742	27.67850
2	Linearity- 90 %	0.029	0.4042	5174.20670	1.31006	27.77936
3	Linearity- 100 %	0.012	0.2758	5138.21938	1.32256	27.70918
4	Linearity- 110 %	0.003	0.3569	5086.35900	1.32862	27.51164
5	Linearity- 120%	0.007	0.3295	5089.05143	1.32976	27.59580
6	Accuracy	0.054	0.0732	5719.08733	1.16921	26.89230
7	Repeatability	0.012	0.2758	5138.21937	1.32256	27.70918
8	Intermediate Precision (Day 1: Analyst 1)	0.012	0.2758	5138.21937	1.32256	27.70918
9	Intermediate Precision (Day 1: Analyst 2)	0.054	0.072	5719.08733	1.16921	26.89230
10	Intermediate Precision (Day 2: Analyst 1)	0.141	0.0569	3738.99204	0.95074	22.46690
11	Intermediate Precision (Day 2: Analyst 2)	0.008	0.0332	8418.37718	1.10746	33.25757
12	Robustness-Change in	0.025	0.1549	3826.14769	1.32006	17.14248

	ratio of Mobile Phase (68:32)					
13	Robustness-Change in ratio of Mobile Phase (72:28)	0.148	0.2303	2706.65302	1.24878	21.03702
14	Robustness-Change in Flow rate (1.8 ml/min)	0.119	0.2341	5829.0393	1.15266	28.49494
15	Robustness-Change in Flow rate (2.2 ml/min)	0.018	0.1268	4801.28729	1.20278	25.54804
16	Robustness-Solution Stability	0.090	0.5588	4653.71303	1.25520	27.09632

From the above table, the recorded RSD of Retention time, RSD of area, Theoretical plates, tailing factors and resolution at different parameters lies inside the specified limit so the system suitability for both Metronidazole and Clotrimazole, thus; the system suitability meets the requirement of the analytical method validation specification (limit).

CONCLUSION

The suggested method concludes the procedure is simple, accurate, linear, sensitive and precise. Hence, it can be applied for routine estimate of Metronidazole and Clotrimazole in combined pessaries dosage form by reverse phase high performance liquid chromatography.

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REFERENCES

1. KOUMANS EH, KENDRICK JS. Preventing adverse sequelae of bacterial vaginosis: a public health program and research agenda. *Sex. transm. dis*. 2001;292-7.
2. Simoes JA, Discacciati MG, Brolazo EM,et al. Clinical diagnosis of bacterial vaginosis. *Int. J. Gynecol. Obstet*. 2006; 94(1):28-32.
3. Tripathi KD. Essentials of medical pharmacology. *JP Med. Ltd*; 2013 30.
4. Maslarska V, Tsvetkova B, Peikova L et al. RP-HPLC method for simultaneous determination of metronidazole and ofloxacin in synthetic mixture. *InCBU Int. Conf. Proc.* 2016;4: 900-905.
5. Das P, Khatri K, Gangani A, Maity A. A rapid RP-HPLC methodology for the determination of clotrimazole Impurities in topical dosage forms. *Acta Sci., Pharm., Sci*. 2021 5(1):97-106.
6. N. Seethalakshmi, A.Chenthilnathan and K. Rama. 2014 RP-HPLC Method Development and Validation for simultaneous estimation of Metronidazole, Clindamycin Phosphate and Clotrimazole in combination pharacetamol dosgae form. International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS),4(2); 67-77
7. Rajendar L, Potnuri NR. A stability indicating RP-HPLC method for the simultaneous estimation of metronidazole, clindamycin and clotrimazole in bulk and their combined dosage form. *World J. Pharm. Sci*.. 2015 2:93-103.
8. National Center for Biotechnology Information. Compound Summary for CID 4173, Metronidazole. *PubChem*,. 21, 2023.
9. Parmar P, Mehta A. Development and validation of HPTLC method for the estimation of clotrimazole in bulk drug and tablet formulation. *Indian J. pharm. sci*.. 2009;71(4):451.
10. National Center for Biotechnology Information. Compound Summary for CID 2812, Clotrimazole. *PubChem*. 21, 2023.

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