



Research Article

**RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE, CLINDAMYCIN PHOSPHATE AND CLOTRIMAZOLE IN COMBINED PHARMACEUTICAL DOSAGE FORMS**

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**Abstract:** A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for simultaneous determination of metronidazole, clindamycin phosphate and clotrimazole in combined pharmaceutical dosage forms has been developed. The separation was carried out on Hypersil BDS C8 (250 × 4.6mm; 5µm) column using buffer, 13.6g of potassium dihydrogen ortho phosphoric acid in 1000ml of water (adjusted to pH 2.4 with ortho phosphoric acid) : acetonitrile 70:30 v/v as eluent. The flow rate was 2.3 ml/min and effluent was detected at 210 nm. The retention times of metronidazole, clindamycin phosphate and clotrimazole were 4.862 min, 5.712 min and 26.01min respectively. The percentage recovery was within the range between 99.38% and 100.31% for metronidazole, 98.76% and 100.65% for clindamycin phosphate, 99.98% and 99.63% for clotrimazole. The linear ranges were found to be 80-150µg/ml (r<sub>2</sub> = 0.9983) for metronidazole, 80-150µg/ml (r<sub>2</sub> = 0.9993) for clindamycin phosphate and 80-150µg/ml (r<sub>2</sub> = 0.9984) for clotrimazole. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the method could be successfully applied for routine analysis of metronidazole, clindamycin phosphate and clotrimazole in the combined pharmaceutical dosage form.

**Key words:** Metronidazole, Clindamycin phosphate, Clotrimazole, RP-HPLC, Estimation

**INTRODUCTION**

Metronidazole (Fig. 1) chemically, 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol, is used in the treatment of bacterial infections of the vagina, fungating tumors, rosacea and skin ulcers<sup>1</sup>. Clindamycin phosphate (Fig. 2) chemically, ((methyl-7-chloro-6,7,8-trideoxy-6-[[4R)-1-methyl-4-propyl-L-prolyl]amino]-1-thio-L-threo-α-D-galactopyranoside) phosphate, is a semi-synthetic derivative of lincomycin. Clindamycin reveals potent activity against many gram-positive and gram-negative bacterial infections. Topical clindamycin is used for the treatment of acne vulgaris which typically leads to suppression of cutaneous propionibacterium acnes<sup>2</sup>. Clotrimazole (Fig. 3) chemically, 1-[(2-chlorophenyl) (di-phenyl) methyl]-1H-imidazole, is an antifungal agent used primarily in the treatment of superficial fungal infections<sup>3</sup>.

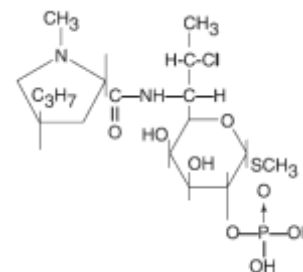


Figure 2: Structure of Clindamycin phosphate

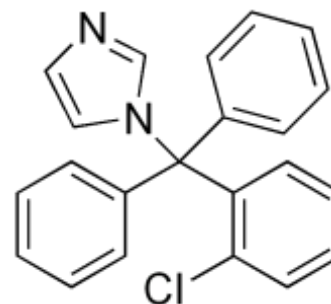


Figure 3: Structure of Clotrimazole

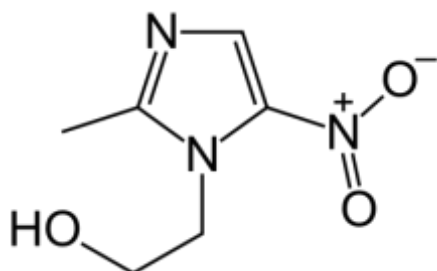


Figure 1: Structure of Metronidazole

The literature survey reveals that a few analytical methods have been reported for the estimation of these drugs individually or in combination with other drugs by spectrophotometry<sup>4-6</sup>, high performance liquid chromatography<sup>7-11</sup> and high performance thin layer chromatography<sup>12</sup> however, no method has been reported for the simultaneous estimation of metronidazole, clindamycin phosphate and metronidazole in combined pharmaceutical

dosage form. Hence, a sample, rapid, precise, accurate RP-HPLC method for the simultaneous estimation of metronidazole, clindamycin phosphate and metronidazole in combined pharmaceutical dosage form is developed and validated.

**MATERIALS AND METHODS**

**Experimental**

**Chemicals and reagents**

Acetonitrile of HPLC grade and Methanol were purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Metronidazole, Clindamycin phosphate and Clotrimazole were a gift sample by Sai Mirra Innopharm Pvt. Ltd., Chennai – 600 098, Tamil Nadu, India. The commercially available vaginal pessary with Metronidazole, Clindamycin phosphate and Clotrimazole, was procured from the local market.

**Instrumentation and chromatographic conditions**

The chromatographic separation was carried out on HPLC system (Shimadzu 1100 Series, Germany) with UV- Visible dual absorbance detector (PDA), Hypersil BDS C<sub>8</sub> column (250 x 4.6mm; 5µm). The mobile phase consisting of 13.6g of potassium dihydrogen orthophosphoric acid in 1000ml water (pH 2.4 adjusted with ortho phosphoric acid) and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 70:30 v/v was pumped into the column at a flow rate of 2.3 ml/min. The detection was monitored at 210 nm. The volume of injection loop was 10 µl prior to the injection of the drug solution; the column was

equilibrated for at least 30 min. with the mobile phase following through the system.

**Preparation of Standard solutions**

**Metronidazole working standard solution:**

40 mg of Metronidazole working standard was weighed and transferred carefully in 100 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 2 ml of above solution was diluted to 50 ml with mobile phase.

**Clindamycin phosphate working standard solution:**

24 mg of clindamycin phosphate working standard was weighed and transferred carefully in 100 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase.

**Clotrimazole working standard solution:**

40 mg of Clotrimazole working standard was weighed and transferred carefully in 100 ml volumetric flask. About 20 ml of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase.

**Analysis of Sample Preparation**

1.6 gm of the sample pessaries was weighed and transferred carefully in a clean and dry 100 ml volumetric flask and make up the volume to 50 ml of mobile phase and sonicate for 30 minutes. Cooled and diluted to volume with mobile phase and mixed well. Filtered through 0.45µ membrane filter. Further 5ml of the above was diluted into 25ml with mobile phase.

**Amount of Metronidazole / Clindamycin phosphate / Metronidazole present in pessaries dosage form**

$$= \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Avg.net content}$$

**RESULTS AND DISCUSSION**

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines<sup>13</sup>.

**System Suitability**

It is essential for the assurance of the quality performance of chromatographic system. Five injections of

standard drug solutions, metronidazole, clindamycin phosphate and clotrimazole were given separately to the system. The system suitability parameters such as retention time, peak area response Number of theoretical plates, Tailing factor and their Mean, Standard deviation & %RSD were also be calculated for the standard drug solutions and mentioned in Table 1, 2 and 3. It was observed that all the values are with in the limits.

**Table 1: System suitability for Metronidazole**

S.No.	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	4.859	3671536	6971	1.300
2.	Standard -2	4.856	3697357	7192	1.297
3.	Standard -3	4.857	3657819	7406	1.297
4.	Standard -4	4.856	3668828	7639	1.294
5.	Standard -5	4.858	3670241	7874	1.120
<b>Mean</b>		<b>4.8572</b>	<b>3673156</b>	<b>7416.4</b>	<b>1.2616</b>
<b>Standard deviation</b>		<b>0.0013</b>	<b>14583.93</b>	<b>356.29</b>	<b>0.079</b>
<b>RSD in %</b>		<b>0.03</b>	<b>0.40</b>	<b>4.8</b>	<b>6.28</b>

**Table 2: System suitability for Clindamycin phosphate**

S.No.	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	5.687	313419	6881	1.240
2.	Standard -2	5.697	316926	7116	1.234
3.	Standard -3	5.705	318314	7275	1.234
4.	Standard -4	5.709	318213	7510	1.223
5.	Standard -5	5.714	318534	7803	1.064
<b>Mean</b>		<b>5.7024</b>	<b>317081</b>	<b>7317</b>	<b>1.199</b>
<b>Standard deviation</b>		<b>0.0106</b>	<b>2141.68</b>	<b>355.56</b>	<b>0.0757</b>
<b>RSD in %</b>		<b>0.919</b>	<b>0.68</b>	<b>4.86</b>	<b>6.31</b>

**Table 3: System suitability for Clotrimazole**

S.No.	Standard	System suitability parameters			
		Retention time (min)	Area	Number of theoretical plates	Tailing factor
1.	Standard -1	24.868	5128123	8807	1.710
2.	Standard -2	24.680	5148069	8991	1.730
3.	Standard -3	24.569	5147360	9208	1.739
4.	Standard -4	24.547	5168614	9381	1.772
5.	Standard -5	24.507	5160630	9622	1.731
<b>Mean</b>		<b>24.6342</b>	<b>5150559</b>	<b>9201.8</b>	<b>1.7364</b>
<b>Standard deviation</b>		<b>0.1455</b>	<b>15388.43</b>	<b>319.77</b>	<b>0.0225</b>
<b>RSD in %</b>		<b>0.59</b>	<b>0.30</b>	<b>3.48</b>	<b>1.30</b>

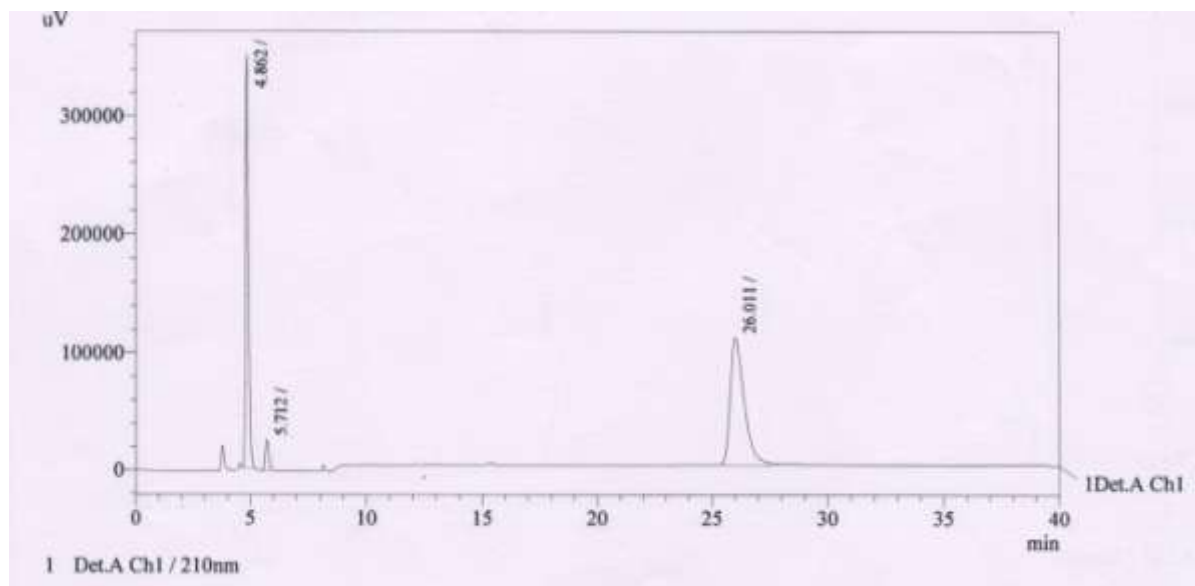
**Specificity**

The specificity of the HPLC method is illustrated in Fig. 4, where complete separations of metronidazole, clindamycin phosphate and clotrimazole were noticed in presence of other inactive excipients used in pessaries. In addition, there was no any interference at the retention time

of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 4.

**Table 4: Specificity for Metronidazole, Clindamycin phosphate, Clotrimazole**

S.No.	Name	No. of Injections	Area		
			Metronidazole	Clindamycin phosphate	Clotrimazole
1.	Blank	1	Nil	Nil	Nil
2.	Placebo	1	Nil	Nil	Nil
3.	Standard	1	3671536	313419	5128123
4.	Sample	1	3636354	319371	5175734



**Figure 4 : Typical HPLC Chromatogram of Sample pessaries**  
(Metronidazole, Clindamycin phosphate and Clotrimazole)

**Linearity and Range**

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for metronidazole, clindamycin phosphate and clotrimazole and it was shown in Table 5, 6 and 7. The plots of peak area of each sample against respective concentration of metronidazole, clindamycin phosphate and clotrimazole were found to be linear (Figure 5, 6 and 7) in the range of 50%– 150% of operating concentrations. Beer’s law was found to be obeyed over this concentration range. The

linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be  $Y= 10492x - 4433.1$ ,  $Y= 1874.6x + 4717.7$  and  $Y= 15190x-15987$  for metronidazole, clindamycin phosphate and clotrimazole respectively and correlation coefficient of the standard curves were found to be 0.9983, 0.9993 and 0.9984 for metronidazole, clindamycin phosphate and clotrimazole respectively. It observed that correlation coefficient and regression analysis are with in the limits.

**Table 5: Linearity of response for Metronidazole**

S.No	Linearity Level	Concentration (µg/ml)	Area
1.	Linearity -1	200	2016477
2.	Linearity -2	320	3420288
3.	Linearity -3	400*	4235135
4.	Linearity -4	480	5062321
5.	Linearity -5	600	6228054

**Table 6: Linearity of response for Clindamycin Phosphate**

S.No	Linearity Level	Concentration (µg/ml)	Area
1.	Linearity -1	100	188823
2.	Linearity -2	160	305889
3.	Linearity -3	200*	385443
4.	Linearity -4	240	453211
5.	Linearity -5	300	564795

**Table 7: Linearity of response for Clotrimazole**

S.No	Linearity Level	Concentration (µg/ml)	Area
1.	Linearity -1	200	3021085
2.	Linearity -2	320	4757504
3.	Linearity -3	400*	6105071
4.	Linearity -4	480	7407095
5.	Linearity -5	600	9009438

\* Operating concentration

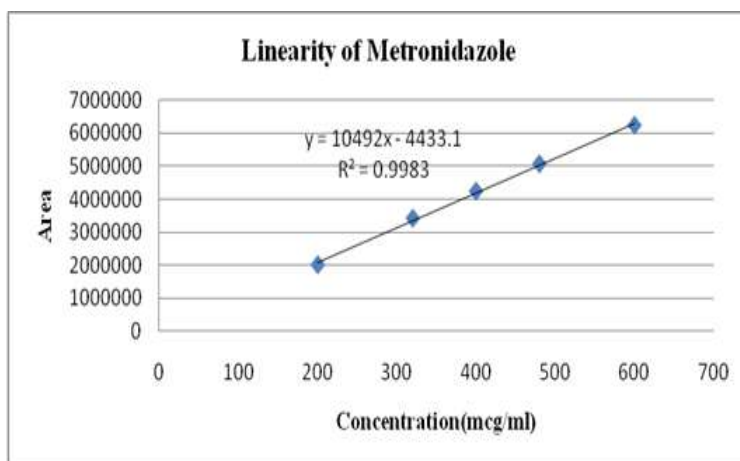


Figure 5: Linearity of response for Metronidazole

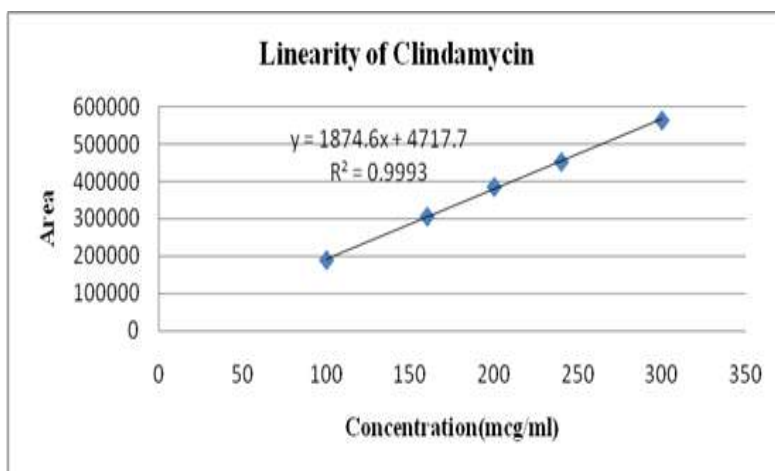


Figure 6: Linearity of response for Clindamycin phosphate

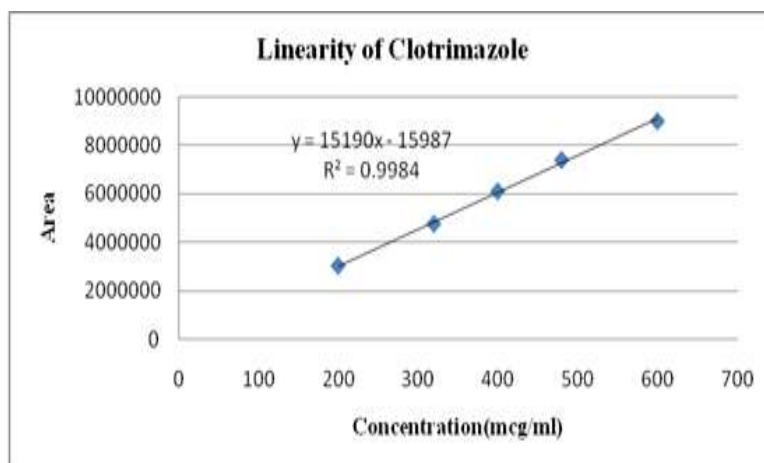


Figure 7: Linearity of response for Clotrimazole

**Accuracy**

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, metronidazole, clindamycin phosphate and clotrimazole were added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed

HPLC method individually. The results of recovery studies were shown in Table 8, 9 and 10. It was observed that the mean percentage recoveries were found to be for metronidazole, clindamycin phosphate and clotrimazole respectively which demonstrated that the method was highly accurate.

**Table 8: Accuracy for Metronidazole**

S.No.	Amount of metronidazole spiked (mg)	Area	Amount recovered (mg)	Recovery (%)
1.	160.89	3609462	159.63	99.22
2.	160.89	3610655	159.69	99.25
3.	160.89	3625403	160.34	99.66
4.	201.12	4557707	201.57	100.22
5.	201.12	4539263	200.76	99.82
6.	201.12	4581338	202.62	100.74
7.	241.34	5462899	241.61	100.11
8.	241.34	5483709	242.53	100.49
9.	241.34	5638800	242.10	100.32
<b>Mean</b>				<b>99.98</b>
<b>Standard deviation</b>				<b>0.5322</b>
<b>RSD in %</b>				<b>0.53</b>

**Table 9: Accuracy for Clindamycin phosphate**

S.No.	Amount of Clindamycin phosphate spiked (mg)	Area	Amount recovered (mg)	Recovery (%)
1.	77.99	324819.5	77.03	98.77
2.	77.99	324102.0	76.86	98.55
3.	77.99	325479.0	77.18	98.97
4.	97.49	418516.0	99.25	101.80
5.	97.49	418156.5	99.16	101.72
6.	97.49	417984.5	99.12	101.67
7.	116.99	494600.0	117.29	100.26
8.	116.99	492701.5	116.84	99.87
9.	116.99	504027.5	119.13	101.83
<b>Mean</b>				<b>100.38</b>
<b>Standard deviation</b>				<b>1.4045</b>
<b>RSD in %</b>				<b>1.40</b>

**Table 10: Accuracy for Clotrimazole**

S.No.	Amount of clotrimazole spiked (mg)	Area	Amount recovered (mg)	Recovery (%)
1.	160.23	5889015	160.42	100.12
2.	160.23	5876934	160.09	99.92
3.	160.23	5876811	160.09	99.91
4.	200.29	7317350	199.33	99.52
5.	200.29	7375077	200.91	100.31
6.	200.29	7322294	199.47	99.59
7.	240.35	8788405	239.41	99.61
8.	240.35	8796520	239.63	99.70
9.	240.35	8786821	239.36	99.59
<b>Mean</b>				<b>99.80</b>
<b>Standard deviation</b>				<b>0.2741</b>
<b>RSD in %</b>				<b>0.27</b>

**Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

**Reproducibility**

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for metronidazole, clindamycin phosphate and clotrimazole were shown in Table 11, 12 and 13. This indicated that method was highly precise.

**Table 11: Precision - Reproducibility for Metronidazole**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.5846	3633182	200.07
2.	Standard -2	1.5874	3624794	199.25
3.	Standard -3	1.5943	3635188	198.96
4.	Standard -4	1.6125	3696057	200.01
5.	Standard -5	1.6079	3690983	200.30
6.	Standard -6	1.6143	3740700	202.20
<b>Mean</b>			<b>3670150</b>	<b>200.13</b>
<b>Standard deviation</b>			<b>46321</b>	<b>1.137</b>
<b>RSD in %</b>			<b>1.26</b>	<b>0.57</b>

**Table 12: Precision - Reproducibility for Clindamycin phosphate**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.5846	319331.5	100.50
2.	Standard -2	1.5874	318446.5	100.04
3.	Standard -3	1.5943	320234.5	100.17
4.	Standard -4	1.6125	328986.5	101.75
5.	Standard -5	1.6079	320499.0	99.40
6.	Standard -6	1.6143	316556.0	97.79
<b>Mean</b>			<b>320675</b>	<b>99.94</b>
<b>Standard deviation</b>			<b>4313.77</b>	<b>1.3089</b>
<b>RSD in %</b>			<b>1.35</b>	<b>1.31</b>

**Table 13: Precision - Reproducibility for Clotrimazole**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.5846	5189564	202.58
2.	Standard -2	1.5874	5173626	201.60
3.	Standard -3	1.5943	5167900	200.51
4.	Standard -4	1.6125	5266524	202.03
5.	Standard -5	1.6079	5193142	199.79
6.	Standard -6	1.6143	5169189	198.08
<b>Mean</b>			<b>5193324</b>	<b>200.76</b>
<b>Standard deviation</b>			<b>37389.9</b>	<b>1.66</b>
<b>RSD in %</b>			<b>0.72</b>	<b>0.83</b>

**Intermediate precision**

Intermediate precision expresses variations within laboratories such as different days, different analysts, different equipments used for its determination. The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same

results once the development phase is over. Intermediate precision for metronidazole, clindamycin phosphate and clotrimazole were shown in Table 14, 15 and 16 by using different analyst on different days. This indicated that method was highly precise.

**Table 14: Precision - Intermediate precision for Metronidazole**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.6054	3718208	200.35
2.	Standard -2	1.6148	3729398	199.78
3.	Standard -3	1.5928	3666145	199.11
4.	Standard -4	1.6112	3722072	199.84
5.	Standard -5	1.5987	3705423	200.50
6.	Standard -6	1.6243	3760013	200.25
<b>Mean</b>			<b>371676</b>	<b>199.97</b>
<b>Standard deviation</b>			<b>30825.8</b>	<b>0.5091</b>
<b>RSD in %</b>			<b>0.83</b>	<b>0.25</b>

**Table 15: Precision - Intermediate precision for Clindamycin phosphate**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.6054	336253.5	101.02
2.	Standard -2	1.6148	337788.5	100.89
3.	Standard -3	1.5928	331166.0	100.28
4.	Standard -4	1.6112	337235.5	100.95
5.	Standard -5	1.5987	335081.0	101.09
6.	Standard -6	1.6243	331620.0	98.47
<b>Mean</b>			<b>334857</b>	<b>100.45</b>
<b>Standard deviation</b>			<b>2840.87</b>	<b>1.0126</b>
<b>RSD in %</b>			<b>0.85</b>	<b>1.01</b>

**Table 16: Precision - Intermediate precision for Clotrimazole**

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	1.6054	5035143	200.50
2.	Standard -2	1.6148	5114221	202.47
3.	Standard -3	1.5928	5003270	200.81
4.	Standard -4	1.6112	5043386	200.11
5.	Standard -5	1.5987	5015111	200.54
6.	Standard -6	1.6243	5166785	203.35
<b>Mean</b>			<b>5062986</b>	<b>201.29</b>
<b>Standard deviation</b>			<b>63900.96</b>	<b>1.29996</b>
<b>RSD in %</b>			<b>1.26</b>	<b>0.65</b>

**Robustness**

Measure of method's capacity to remain unaffected by small, but deliberate variations in method.

**I. Change in the ratio of solvents in the mobile phase**

Two sample preparations were analyzed as per methodology by changing the ratio of solvents in the mobile

phase by means of the robustness data was found for metronidazole, clindamycin phosphate and clotrimazole by changing the ratio of solvents in the mobile phase. It was shown in Table 17, 18 and 19. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

**Table 17: Robustness - Change in the ratio of solvents in the mobile phase ( $\pm 2$ ) for Metronidazole**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (68:32)	
			Area	Amount (mg)
1.	Sample -1	1.6255	3799951	200.63
2.	Sample -2	1.5988	3742317	200.88
<b>Mean</b>			<b>200.75</b>	
<b>Standard deviation</b>			<b>0.1767</b>	
<b>RSD in %</b>			<b>0.09</b>	

**Table 18: Robustness - Change in the ratio of solvents in the mobile phase ( $\pm 2$ ) for Clindamycin phosphate**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (68:32)	
			Area	Amount (mg)
1.	Sample -1	1.6255	348250.0	100.29
2.	Sample -2	1.5988	338217.0	99.03
<b>Mean</b>			<b>99.66</b>	
<b>Standard deviation</b>			<b>0.8909</b>	
<b>RSD in %</b>			<b>0.89</b>	



**Table 19: Robustness - Change in the ratio of solvents in the mobile phase ( $\pm 2$ ) for Clotrimazole**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (68:32)	
			Area	Amount (mg)
1.	Sample -1	1.6255	4538901	200.11
2.	Sample -2	1.5988	4473561	200.53
			<b>Mean</b>	<b>200.32</b>
			<b>Standard deviation</b>	<b>0.2969</b>
			<b>RSD in %</b>	<b>0.15</b>

**II. Change in the pH of buffer in the mobile phase**

Two sample preparations were analyzed as per methodology by changing the pH of the buffer in the mobile phase by means of  $\pm 2$ . The robustness data was found for metronidazole, clindamycin phosphate and clotrimazole by

changing the pH of buffer in the mobile phase. It was shown in Table 20, 21 and 22. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

**Table 20: Robustness - Change in the pH of buffer in the mobile phase (-0.2) for Metronidazole (pH 2.4)**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (70:30) [Buffer pH :4.4]	
			Area	Amount (mg)
1.	Sample -1	1.5846	3671024	201.33
2.	Sample -2	1.5874	3660366	200.39
			<b>Mean</b>	<b>200.86</b>
			<b>Standard deviation</b>	<b>0.6646</b>
			<b>RSD in %</b>	<b>0.33</b>

**Table 21: Robustness - Change in the pH of buffer in the mobile phase (-0.2) for Clindamycin phosphate (pH 2.4)**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (70:30) [Buffer pH :4.4]	
			Area	Amount (mg)
1.	Sample -1	1.5846	331369.5	100.58
2.	Sample -2	1.5874	332193.5	100.65
			<b>Mean</b>	<b>100.61</b>
			<b>Standard deviation</b>	<b>0.0494</b>
			<b>RSD in %</b>	<b>0.05</b>

**Table 22: Robustness - Change in the pH of buffer in the mobile phase (-0.2) for Clotrimazole (pH 2.4)**

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (70:30) [Buffer pH :4.4]	
			Area	Amount (mg)
1.	Sample -1	1.5846	5149873	201.68
2.	Sample -2	1.5874	5118336	200.09
			<b>Mean</b>	<b>200.88</b>
			<b>Standard deviation</b>	<b>1.1242</b>
			<b>RSD in %</b>	<b>0.56</b>

**Ruggedness**

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data for metronidazole,

clindamycin phosphate and clotrimazole were shown in Table 23, 24 and 25. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

**Table 23: Ruggedness data for Metronidazole -Change of analyst**

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg)
1.	Sample -1	1.5943	3713949	200.18
2.	Sample -2	1.5982	3724035	200.23
3.	Sample -3	1.5722	3626993	198.24
4.	Sample -4	1.6023	3761113	201.71
5.	Sample -5	1.5982	3765911	202.49
6.	Sample -6	1.5966	3737131	201.14
<b>Mean</b>				<b>200.66</b>
<b>Standard deviation</b>				<b>1.4800</b>
<b>RSD in %</b>				<b>0.74</b>

**Table 24: Ruggedness data for Clindamycin phosphate -Change of analyst**

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg)
1.	Sample -1	1.5943	344784.0	100.29
2.	Sample -2	1.5982	344729.5	100.03
3.	Sample -3	1.5722	346358.5	102.17
4.	Sample -4	1.6023	345217.5	99.92
5.	Sample -5	1.5982	345353.5	100.21
6.	Sample -6	1.5966	346715.0	100.71
<b>Mean</b>				<b>100.55</b>
<b>Standard deviation</b>				<b>0.8367</b>
<b>RSD in %</b>				<b>0.83</b>

**Table 25: Ruggedness data for Clotrimazole-Change of analyst**

S.No.	Sample Name	Wt. taken (g)	Area	Amount (mg)
1.	Sample -1	1.5943	4540341	202.11
2.	Sample -2	1.5982	4514499	200.46
3.	Sample -3	1.5722	4510750	203.61
4.	Sample -4	1.6023	4530750	200.67
5.	Sample -5	1.5982	4505388	200.06
6.	Sample -6	1.5966	4552596	202.36
<b>Mean</b>				<b>201.54</b>
<b>Standard deviation</b>				<b>1.3708</b>
<b>RSD in %</b>				<b>0.68</b>

## CONCLUSION

The Proposed study describes new and simple RP-HPLC method for the simultaneous estimation of metronidazole, clindamycin phosphate and clotrimazole in combined pessaries dosage form. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of simultaneous estimation of metronidazole, clindamycin phosphate and clotrimazole in combined pessaries dosage form without interference.

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