RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CLINDAMYCIN PHOSPHATE AND CLOTRIMAZOLE IN PHARMACEUTICAL DOSAGE FORMS

R. Rajameena^{1*}, K. Rama², C. Muthulakshmi¹

¹Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

²Sai Mirra Innopharm Pvt. Ltd., No. 288, SIDCO Estate, Ambattur, Chennai, Tamil Nadu, India

*Corresponding Author Email: meenagold18@yahoo.com

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ABSTRACT

A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for simultaneous determination of Clindamycin phosphate and Clotrimazole in combined soft gelatin pessaries pharmaceutical formulation has been developed. The separation was carried out on Hypersil BDS C8 (250 × 4.6 mm; 5 μm) column using buffer, 13.6 g Potassium dihydrogen ortho phosphate in 1000 ml of water (adjusted to pH 2.5 ortho phosphoric acid): acetonitrile: 1 in the ratio of 70:30 v/v as eluent. The flow rate was 1.0 ml / min and effluent was detected at 210 nm. The retention times of Clindamycin phosphate and Clotrimazole were 4.47 minutes, 22.06 minutes and 12.03 minutes respectively. The percentage recovery was within the range between 102.2 % and 103.23 % for Clindamycin phosphate, 98.96 % and 100.54 % for Clotrimazole. The linear ranges were found to be 200 mg / ml ($r^2 = 0.9998$) for Clindamycin phosphate, 400 mg / ml ($r^2 = 0.9895$) for Clotrimazole. The percentage relative standard deviation for accuracy and precision was found to be less than 2 %. The linearity was found to be in the range of 80-120 mg / ml and correlation coefficient of were found to be 0.9998, 0.9979, for Clindamycin Phosphate, Clotrimazole respectively. The proposed method is accurate with 102 % recovery and precise (% RSD of Reproducibility repeatability, intra-day and inter-day variations were 0.19, 0.24, 0.44, 0.78, 0.12-0.15, 0.13-0.37, 0.14, 0.22). The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from peccaries excipients were found.

Keywords: Clindamycin, Clotrimazole RP-HPLC, Validation, Acetonitrile, Methanol.

INTRODUCTION

Clindamycin is a lincosamide antibiotic that has been approved by the US Food and Drug Administration (FDA) for the treatment of *anaerobic*, *streptococcal* and *staphylococcal* infections. There has been increased interest in the use of clindamycin because it achieves high intracellular levels in phagocytic cells, high levels in bone and appears to have an antitoxin effect against toxin elaborating strains of *streptococci* and *staphylococci*.

Figure 1: Structure of Clindamycin phosphate

Figure 2: Structure of Triclotrimazole

Clindamycin ($C_{18}H_{34}ClN_2O_8PS$) is a methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galactooctopyranoside (dihydrogenphosphate). Molecular weight 504.9 g / mol. Clindamycin shows broad spectrum activity. It elicits more activity against Gram positive organisms but is also being effective against Gram negative bacteria,

anaerobes and *protozoa*. When used orally, clindamycin is reserved for severe bacterial infections such as female genital infection and lower respiratory infections caused by anaerobic organisms, *staphylococci*, *streptococci*, *pneumococci*. Topically, it is used for acne vulgaris and bacterial vaginosis¹. Clotrimazole ($C_{22}H_{17}ClN_2$) is a 1-[(2-chlorophenyl) (diphenyl)methyl]-1H-imidazole. Molecular weight 344.8 g / mol. Clotrimazole is imidazole derivative that is a broad spectrum fungicidal drug. Clotrimazole is most valuable for the treatment of superficial fungal infection caused by dermatophytes, yeasts and yeast like fungi. Clotrimazole is a topically used azole. The requirement for intravenous administration and the toxicity of the older antifungal agents created a need for antifungal agents with a better therapeutic profile. The relatively non-toxic oral azole medications represent the first major advance in this direction.^{2,3} Literature survey reveals that few HPLC methods have been reported for the estimation of clindamycin phosphate combination with other drugs. Till now, there is no simple RP-HPLC method has been reported using simple solvents. The main objective of the present study is to develop simple, sensitive, accurate and precise RP-HPLC method for estimation of clindamycin phosphate in pharmaceutical dosage forms. The validation has been carried out as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The chromatographic separation was carried out on HPLC system (Shimadzu S.No. 2124404405 Kyoto, Japan) with UV- Visible dual absorbance detector (PDA), Hypersil BDS C8 column (250 × 4.6 mm; 5 μm). The mobile phase consisting of 13.6 ml of Potassium dihydrogen ortho phosphate in 1000 ml water (pH 2.5 adjusted with orthophosphoric acid), acetonitrile and methanol were filtered through 0.45 μ membrane filter before use, degassed and

were pumped from the solvent reservoir in the ratio of 70:30 v/v was then pumped into the column at a flow rate of 1.0 ml / min. The detection was monitored at 210 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 minutes with the mobile phase following through the system.

Reagents and Materials

The reference sample of Clindamycin phosphate and Clotrimazole were supplied by Gujarat Organic Ltd, Lucknow, India. The formulation was procured from the local market. HPLC grade methanol, acetonitrile, OPA was purchased from Alchymars Icm sm Private Limited, Chennai, India.

Selection of Mobile Phase

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peak. The standard solution was run in different mobile phases. From the mobile phase consisting of 13.6 ml of Potassium dihydrogen ortho phosphate in 1000 ml water (pH 2.5 adjusted with orthophosphoric acid), acetonitrile and methanol were filtered through 0.45 µm membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 70:30 v/v was then pumped into the column at a flow rate of 1.0 ml / min with detection at 210 nm.

Preparation of Standard Stock Solution

Clindamycin Phosphate Working Standard Solution

24 mg of Clindamycin Phosphate working standard was weighed and transferred carefully in 50 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 (20 µg / ml).

Clotrimazole Working Standard Solution

40 mg of Clotrimazole working standard was weighed and transferred carefully in 50 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 (500 µg / ml).

Preparation of Calibration Curve

Aliquots of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 ml standard stock solution (100 µg / ml) was transferred to 10 ml of volumetric flasks and made up to the mark with mobile phase to get concentration of 5,10,15,20,25,30,35 µg / ml. An aliquot (20 µl) of each solution was injected under the operating chromatographic conditions and responses were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated. The standard solution was prepared by transferring 1.5 ml of 100 µg / ml to the 10 ml of volumetric flask and made up to the mark with mobile phase to get 15 µg / ml.

Sample Preparation

1500 mg 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. 5 ml of above solution was diluted to 25 ml with mobile phase.

Amount of Clindamycin Phosphate / Clotrimazole / in each of the Pessaris

Sample are	SW	100	25	425	Purity
=	----- x	----- x	----- x	----- x	----- x
Standard area	100	TW	5	505	100

Where,

SW = Standard weight taken in mg. TW = Test weight taken in mg. Av. wt. = Average net content in mg. Purity = Purity of Clindamycin Phosphate

Method Validation

The optimized Chromatographic method^{4,5} was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH,2005)⁶.

System Suitability Test

System suitability parameters are evaluated by following ICH guidelines injecting five replicates of 20 µg / ml, 500 µg / ml and 300 µg / ml concentrations of standards Clindamycin Phosphate, Clotrimazole, respectively. Resolution factor, theoretical plate and tailing factors were evaluated by following ICH guidelines.

Specificity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by comparing the blank chromatogram with the chromatogram obtained for the drug spiked with internal standard (placebo) to trace out the interfering peaks. The specificity of the method was investigated by the analysis of the two blank preparation spiked with two different concentration of standards, Clindamycin Phosphate, Clotrimazole, sample, pessaries and internal standard (placebo) was also added. The mean standard deviation and RSD of specificity were calculated.

Linearity

The linearity of the peak area response was determined by making six measurements a five concentration points in the range of 80 % mg / ml to 120 % mg / ml of operating concentrations of standards, Clindamycin Phosphate, Clotrimazole. Standard area was plotted against the concentration. The linear regression coefficient, correlation coefficient, standard deviation and mean were calculated.

Precision

The precision of an analytical method is the closeness of a series of individual measurements of an analyte when the analytical procedure is applied repeatability to multiple aliquots of a single homogeneous volume of biological matrix. Precision is calculated as coefficient of variation, relative standard deviation. The measure RSD can be subdivided into two categories: repeatability (intra- day precision) and reproducibility.

Reproducibility

Reproducibility of the method assessed by analyzing six times of 100 mg / ml, 200 mg / ml concentrations of standards, Clindamycin phosphate and Clotrimazole respectively. The mean, standard deviation and RSD of reproducibility were calculated.

Repeatability

Repeatability of the peak area response was determined by making six measurements at six different concentration points of sample pessaries and it is compared with the standard drugs Clindamycin phosphate, Clotrimazole. The mean, standard deviation and RSD, of repeatability were calculated.

Accuracy

The accuracy was assessed by using a minimum of three different concentrations of standards, Clindamycin phosphate, Clotrimazole, 80 % mg / ml to 120 % mg / ml of placebo spiked into the standard solution of Clindamycin phosphate, Clotrimazole. The mean, standard deviation and RSD of accuracy were calculated.

Robustness

Robustness was determined by injecting triplicate injection of standards and three sample solutions in single and at different concentration with respect to control condition. Robustness of the method was checked by varying the mobile phase ratio. The sample and standard solutions were injected in each condition, mean, standard deviation and relative standard deviation, of robustness were calculated.

Ruggedness

Robustness is defined as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness of the method was checked by the analysis of sample aliquots from homogeneous lots by a different chemist on a different instrument on a different day.

RESULTS AND DISCUSSIONS

A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for simultaneous determination of Clindamycin phosphate and Clotrimazole in combined soft gelatin pessaries pharmaceutical formulation has been developed. The Separation was carried out on Hypersil BDS C8 (250 × 4.6 mm; 5 µm) column using buffer, 13.6 g Potassium dihydrogen ortho phosphate in 1000 ml of water (adjusted to pH 2.5 ortho phosphoric acid): acetonitrile: 1 in the ratio of 70:30 v/v as eluent. The flow rate was 1.0 ml / min and effluent was detected at 210 nm. The retention times of Clindamycin phosphate and Clotrimazole were 4.47 minutes, 22.06 minutes and 12.03 minutes respectively. The percentage recovery was within the range between 102.2 % and 103.23 % for Clindamycin phosphate, 98.96 % and 100.54 % for Clotrimazole.

Table 1: System Suitability for Clindamycin Phosphate and Clotrimazole

S. No.	Standard		Concentration (µg/ml)		Area	
	Clindamycin	Clotrimazole	Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	Standard -1	Standard -1	100	200	910864	28741520
2.	Standard -2	Standard -2	100	200	913962	28821992
3.	Standard -3	Standard -3	100	200	912694	28910527
4.	Standard -4	Standard -4	100	200	921951	28911919
5.	Standard -5	Standard -5	100	200	912633	28885018
6.	Standard -6	Standard -6	100	200	912273	28790175
Mean					929917	28843525
Standard deviation					1807.768	70147.83
RSD in %					0.194401	0.243201

System suitability parameters	Clindamycin Phosphate	Clotrimazole
Retention time	4.47 minutes	22.06 minutes
Peak area response	929917	28843525

Table 2: Specificity for Clindamycin Phosphate and Clotrimazole

S. No.	Name	No. of Injections	Area	
			Clindamycin Phosphate	Clotrimazole
1.	Blank	1	Nil	Nil
2.	Placebo	1	Nil	Nil
3.	Standard	1	910864	28741520
4.	Sample	1	937463	28257715

Figure 3: Typical chromatogram of Placebo

Figure 4: Typical chromatogram of Clindamycin Phosphate and Clotrimazole

The Linearity of this method was determined at five levels from 80 % – 120 % of operating concentrations for Clindamycin Phosphate, Clotrimazole and it was shown in Table 3. The plot of peak area of each sample against respective concentration of Clindamycin Phosphate, Clotrimazole were found to be linear (Figure 5 and 6) in the range of 80 % – 120 % 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 $Y = 4488.3x - 3906.8$, $Y = 68651x - 245607$, for Clindamycin Phosphate, Clotrimazole, respectively and correlation coefficient of the standard curves were found to be 0.9998, 0.9979, for Clindamycin Phosphate, Clotrimazole, respectively. It observed that correlation coefficient and regression analysis are within the limits.

Table 3: Linearity of response for Clindamycin Phosphate and Clotrimazole

S. No.	Linearity Level	Volume of stock solution (ml)	Volume made up to (ml)	Concentration (µg/ml)		Area	
				Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	Linearity -1	4.0	10	160	320	721835	21708770
2.	Linearity -2	4.5	10	180	360	811658	24457714
3.	Linearity -3	5.0	10	200*	400*	900505	27417966
4.	Linearity -4	5.5	10	110	440	994711	29642489
5.	Linearity -5	6.0	10	120	480	1079140	32846533

*Operating concentration

Figure 5: Linearity of response for Clindamycin Phosphate Figure

Figure 6: Linearity of response for Clotrimazole

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, Clindamycin Phosphate, Clotrimazole, were added to pre-analyzed samples at a level from 80 % mg / ml up to 120 %

mg / ml. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recoveries were found to be for Clindamycin Phosphate, Clotrimazole, respectively.

Table 4: Accuracy for Clindamycin Phosphate and Clotrimazole

S. No.	Area		Amount recovered (mg)		Recovery (%)	
	Clindamycin	Clotrimazole	Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	753295	22457823	23.96	321.76	102.67	100.54
2.	751422	22653066	23.99	324.56	102.41	101.41
3.	750463	22352550	24.00	320.25	102.28	100.07
4.	942503	27967598	29.61	400.70	102.76	100.16
5.	946817	28321138	29.59	405.77	103.23	101.43
6.	946720	28378339	29.55	406.59	103.22	101.64
7.	1129138	33157804	35.32	475.07	102.59	98.96
8.	1124825	33662566	35.47	482.30	102.20	100.47
9.	1125348	33639096	35.39	481.96	102.25	100.40
Mean					102.71	100.51
Standard deviation					0.008	0.003

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

data for Clindamycin Phosphate, Clotrimazole, were shown in Table 5. This indicated that method was highly precise.

Table 5: Precision – Reproducibility for Clindamycin Phosphate and Clotrimazole

S. No.	Sample name	Concentration (µg/ml)		Area	
		Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	Standard -1	100	200	910864	28741520
2.	Standard -2	100	200	913962	28821992
3.	Standard -3	100	200	912694	28910527
4.	Standard -4	100	200	921951	28911919
5.	Standard -5	100	200	912633	28885018
6.	Standard -6	100	200	912273	28790175
Mean				929917	28843525
Standard deviation				1807.768	70147.83
RSD in %				0.194401	0.243201

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple

measurements of the same sample by the same analyst under the same conditions. Repeatability data for Clindamycin Phosphate, Clotrimazole were shown in Table 6. This indicated that method was highly precise.

Table 6: Precision - Repeatability for Clindamycin Phosphate and Clotrimazole

S. No.	Sample Name	Wt. taken (g)		No. of reading	Area		Amount (mg / 5 ml)	
		Clindamycin	Clotrimazole		Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	Sample -1	1.5038	1.5038	1	937463	28257715	99.92	197.83
2.	Sample -2	1.5031	1.5031	1	941506	28941382	100.39	202.71
3.	Sample -3	1.5045	1.5045	1	938216	28654640	99.95	200.51
4.	Sample -4	1.5022	1.5022	1	929704	28673427	99.20	200.95
5.	Sample -5	1.5011	1.5011	1	932806	28646903	99.60	200.91
6.	Sample -6	1.5056	1.5011	1	941953	28684170	100.28	200.57
Mean							99.89	200.58
Standard deviation							0.4414409	1.572151
RSD in %							0.4419318	0.783805

Robustness data Clindamycin Phosphate, Clotrimazole by changing the ratio of solvents in the mobile phase. It was shown in Table 7 and 8. It was observed that there were no

marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 7: Robustness - Change in the ratio of solvents in the mobile phase (± 2) for Clindamycin Phosphate

S. No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg / 5 ml) (Buffer: Acetonitrile)	
			70:30	72:28	70:30	72:28
1.	Sample -1	1.4926	914377	913563	100.14	100.05
2.	Sample -2	1.4942	917031	914115	100.32	100.27
Mean					100.23	100.16
Standard deviation					0.129	0.155
RSD in %					0.12	0.15

Table 8: Robustness - Change in the ratio of solvents in the mobile phase (± 2) for Clotrimazole

S. No.	Sample Name	Wt. taken (g)	Area (Buffer: Acetonitrile)		Amount (mg / 5 ml) (Buffer: Acetonitrile)	
			70:30	72:28	70:30	72:28
1.	Sample -1	12.4014	26649114	27798505	198.47	200.25
2.	Sample -2	12.7135	26628583	27606287	198.11	199.18
Mean					198.29	199.72
Standard deviation					0.25	0.75
RSD in %					0.13	0.37

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data Clindamycin Phosphate, Clotrimazole, were shown in Table 9. It was

observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 9: Ruggedness data for Clindamycin Phosphate - Change of analyst

S. No.	Sample Name	Wt. taken (g)		Area		Amount (mg / 5 ml)	
		Clindamycin	Clotrimazole	Clindamycin	Clotrimazole	Clindamycin	Clotrimazole
1.	Sample -1	1.5022	1.5022	946058	28256527	99.58	200.97
2.	Sample -2	1.5036	1.5036	945896	28338484	99.47	201.37
3.	Sample -3	1.5015	1.5015	945461	28341292	99.57	201.67
4.	Sample -4	1.5038	1.5038	945881	28257280	99.46	200.77
5.	Sample -5	1.5065	1.5065	945909	28341308	99.28	201.00
6.	Sample -6	1.5065	1.5065	945409	28256972	99.23	200.40
Mean						99.43	201.03
Standard deviation						0.14	0.44
RSD in %						0.14	0.22

CONCLUSION

The proposed study describes new and simple RP-HPLC method for the simultaneous estimation of Clindamycin phosphate and Clotrimazole in combined soft gelatin 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 Clindamycin phosphate and Clotrimazole in combined soft gelatin pessaries dosage form without interference.

REFERENCES

- Melissa Johnson. Clindamycin: An overview. Literature review current through: Mar 2013. This topic last updated; 2012.
- Clotrimazole Official FDA information, side effects, and uses. FDA. Retrieved; 2011.
- [http:// Wikipedia, the free encyclopedia.com](http://Wikipedia,the free encyclopedia.com)
- ICH, Guidance on Analytical Method Validation. In: Proceedings of the International Conference of Harmonization, Geneva; 1996.
- ICH, Harmonized Tripartite Validation of Analytical procedure: Methodology (Q2B). International Conference on Harmonization; 1997.
- ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology; 2005.

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