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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CLINDAMYCIN PHOSPHATE AND CLOTRIMAZOLE IN SOFT GELATIN VAGINAL SUPPOSITORIES

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ABSTRACT

An Isocratic RP-HPLC method for the simultaneous quantitative and qualitative determination of Clindamycin phosphate and Clotrimazole in soft gelatin vaginal suppositories has been developed and validated with U.V detection at 210 nm. The method utilizes a reversed phase C_8 – (250 mm x 4.6 mm, 5µm) Column to analyze samples and mobile phase was prepared in the mixture of Phosphate buffer: Acetonitrile: Methanol in the ratio of 40:30:30 %v/v. Flow rate was maintained at 1.5 ml/min. column temperature was maintained at 40°C.Forced degradation studies are carried out and samples are analysed. The stressed Blank, Placebo and sample solutions shows that there is no interference of Blank and placebo peaks and degradents peaks at the retention time of Clindamycin phosphate and Clotrimazole. Retention time of Clindamycin phosphate and Clotrimazole was found to be 2.39 and 6.57 min respectively. The average recovery of Clindamycin phosphate and Clotrimazole was found to be 100.6 % and 100.3%. The described method of Clindamycin phosphate and Clotrimazole is linear over a range of 59.4µg/ml-178.2 µg/ml and Clotrimazole in range of 50-150 µg/ml and correlation coefficient of both drugs was found to be 0.999.A simple, rapid, precise, stable and accurate liquid chromatographic method (HPLC) was developed. The peak purity index values of standard and sample solutions are within the Acceptance criteria.

Key Words:- RP-HPLC, Clindamycin Phosphate, Clotrimazole.

INTRODUCTION

Clindamycin Phosphate is a Lincosamide antibiotic. It is commonly used in the treatment of Acne and can be useful against some methicillin-resistant *Staphylococcus aureus* infections. Clindamycin phosphate has a bacteriostatic effect. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation. It does so by binding to 50s RNA OF the large bacterial ribosomal unit (Daum RS, 2007). Clotrimazole (Tettenborn D, 1974; Wolfson *et al*., 1981; Tobias Porsbring *et al*., 2009) is an Antifungal drug and used in the treatment of fungal infections of both humans and other animals such as vaginal yeast infections, oral thrush and ringworm, athlete's foot and jock itch. It can also be used to prevent oral thrush. The most commonly noted side effects are redness, stinging, blistering, peeling, swelling, itching, hives or burning at the area of application. Clindamycin phosphate and Clotrimazole combination in Soft gelatin

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vaginal suppositories is used for the treatment of infective leucorrhoea, mixed infection, non-specific vaginitis, vaginal candidaiasis and bacterial vaginosis and trichomonasis (Lamont RF, 2005).

MATERIALS AND METHODS

Equipments and settings

HPLC system (shimadzu L.C-2012 With U.V/P.D.A detector) was used.

An analytical column used is C_8-(250 mmX4.6, 5 µm) zodiac was found to be ideal as it gave good peak shape and good resolution. Degassing of the mobile phase was performed by using sonicator (Fisher scientific FS 220).Instrumental HPLC settings are as follows: Flow rate was maintained at 1.5ml/min. Injection Volume: 20 µl; Column temperature 40°C; and U.V detection at 210nm as this both drugs has good absorbance at 210 nm (Darji RB *et al*., 2012; Tamaddon L *et al*., 2012).

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Reagent and Materials

Clindamycin phosphate working standard and Clotrimazole working standard was supplied from softgel health care private limited. Potassium dihydrogen ortho phosphate, Methanol, Acetonitrile supplied from Rankem chemicals (New Delhi, India). Ortho phosphoric acid was obtained from Fisher scientific. HPLC grade water was used for method of analysis. Clindamycin phosphate B.P equivalent to Clindamycin (dose=100mg) and Clotrimazole (dose=100mg) soft gelatin vaginal suppositories was used for the analysis.

Phosphate buffer with pH of 2.5 was prepared by using 1.36 gm of dihydrogen ortho phosphate into 1000ml of HPLC grade water and then add 300ml of Acetonitrile and then add then 300ml of methanol in the ratio of (40:30:30%v/v) and sonicated for 15 min and then filter through 0.45µm nylon membrane filter. Diluents was prepared in the ratio of 40:20:40% v/v.

Standard preparation

A standard solution having 118.8 ppm and 100 ppm of Clindamycin phosphate and Clotrimazole, respectively was prepared as follows: 118.8 mg of Clindamycin phosphate was dissolved in 30ml of diluents and then made up to mark with diluents in 100 ml of volumetric flask and sonicate it (Solution A), 100 mg of Clotrimazole was dissolved in 30 ml 0f diluents and then made up to mark with diluents in 100 ml of volumetric flask (Solution B). Then, pipette out 10 ml of Solution A and 10 ml of Solution B was diluted to 100 ml with diluents & sonicate it (Chinmoy Roy *et al*., 2012; Useni Reddy Mallu, 2011).

Sample preparation

Accurately weighed and transferred 1600mg of Clindamycin phosphate and Clotrimazole capsule medicament into a 100mL clean dry volumetric flask added about 70mL of Diluents and sonicated to dissolve it completely and made volume up to the mark with the diluents. Filter through whatman-42 filter paper (stock solution). Further pipette 10ml of above solution of the above stock solution into a 100 ml volumetric flask and dilute up to the mark with diluents (Adnan Manassra, 2010; Daniel J Platzer and Brent A White, 2006).

System suitability results

Tailing factor for the peaks due to Clindamycin phosphate and Clotrimazole in Standard solution Should not be more than 2.

Theoretical plates for the Clindamycin phosphate and Clotrimazole peaks in Standard solution should not be less than 2000.

RESULTS AND DISCUSSION

Method development

Reversed-phase LC-method was employed for the chromatographic separation of Clindamycin phosphate and Clotrimazole**.** Trials was performed by using various combination of mobile phase and Column and finally method was optimized C8-(250 mm X4.6,5 µm) zodiac was found to be ideal as it gave good peak shape and good resolution. Retention time of Clindamycin phosphate and Clotrimazole was 2.39 and 6.57min respectively. Graph 1 represents the standard chromatogram. Perfect base line separation was achieved and sharp peaks were obtained with phosphate buffer: Acetonitrile: Methanol with simple isocratic program. Six replicate injections of standard and samples shows the area of Clindamycin phosphate and Clotrimazole was found to % RSD of not more than 2. Table.1 represents the system suitability results.

Method Validation

Precision

System precision of this method was determined by injecting the standard solution of the two analytes six times. The RSD of peak areas of Clindamycin phosphate and Clotrimazole for the six replicates injections were found to be 0.4% and 0.2% for Clindamycin phosphate and Clotrimazole respectively. Method precision was performed by 6 replicate injection of six standard preparation and six sample preparation of Clindamycin phosphate and Clotrimazole capsules .The %RSD of six areas of six sample preparation of Clindamycin phosphate and Clotrimazole Capsules was found to be 1.7 and 1.3 respectively. Precision was evaluated in terms of intra-day and inter-day precision. The intra-day precision was investigated using six replicates of same concentration of standard solutions. The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding concentration six times on the same day and six times on the different days and the results were obtained was R.S.D not more than 2. LOD and LOQ values were calculated from the calibration curves were obtained and the concentration of Clindamycin phosphate for LOD and LOQ was found to be 1.39 ppm and 4.2 ppm and Clotrimazole was found to be 0.5 ppm and 1.5 ppm. Robustness of the method was determined by deliberately varying certain parameters like flow-rate, analytical wavelength and composition of mobile phase etc.

Linearity

Stock standard solution having 118.8 ppm and 100 ppm of Clindamycin phosphate and Clotrimazole respectively was prepared by dissolving 118.8 mg of Clindamycin phosphate and 100 mg of Clotrimazole in 100 ml diluents. Five different concentrations of Clindamycin phosphate and Clotrimazole were prepared from the sock solution as follows: 5 ml of stock solution was diluted to 100 ml with diluents (59.4 ppm of Clindamycin phosphate and 100 ppm of Clotrimazole), 7.5 ml of stock solution was diluted to 100 ml with diluents (89.1 ppm of Clindamycin phosphate and 75 ppm of Clotrimazole), 10 ml of stock solution was diluted to 100 ml with diluents (118.8 ppm of Clindammycin phosphate and 100 ppm of Clotrimazole), 12.5 ml of stock solution was diluted to 100 ml with diluents (148.5 ppm of Clindamycin phosphate and 125 ppm of Clotrimazole) and 15 ml of stock solution was diluted to 100 ml with diluents (178.2 ppm of Clindamycin phosphate and 150 ppm of Clotrimazole.

Accuracy

Accuracy was determined by recovery studies with three standard solutions containing known concentration of drugs and the percentage recoveries of the added drugs were determined. Accuracy was performed by three different preparations of (50%, 100%, and 150%). The average recovery was obtained within limits. For 50%, 100, 150% the average recovery for Clindamycin phosphate was found to be 101.2%, 100.3%, 100.2% respectively and Clotrimazole was found to be 100.4, 100.3 and 100.3 % respectively.

Robustness

The robustness was assessed by altering the chromatographic conditions such as, by changing the flow rate, wave length and mobile phase composition and the results found to be good.

Table 1. Results of system suitability parameters for Clindamycin phosphate and Clotrimazole

S.No	Name	Retention time (min)	Area	USP Resolution	Area%	USP tailing	USP plate count
1	Clindamycin phosphate	2.39	320795		3.25	1.3	5842
2	Clotrimazole	6.57	9558655	23.2	96.75	1.1	12493

Table 2. Linearity Results: (for Clindamycin phosphate)

S.No	Linearity Level	Concentration (in ppm)	Average Area
1	I	59.4	167063
2	II	89.1	244168
3	III	118.8	329297
4	IV	148.5	419466
5	V	178.2	486589
Correlation Coefficient			0.999

Table 3. Linearity Results: (for Clotrimazole)

S.No	Linearity Level	Concentration	Average Area
1	I	50ppm	4975444
2	II	75ppm	7227408
3	III	100ppm	9908588
4	IV	125ppm	12083804
5	V	150 ppm	14386602
Correlation Coefficient			0.999

Table 4. Results for variation in flow of Clindamycin phosphate

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.3	6101	1.3
2	1.5	5842	1.3
3	1.8	4740	1.3

Table 5. Results for variation in flow of Clotrimazole

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.3	13251	1.1
2	1.5	12493	1.1
3	1.8	11171	1.1

Table 6. Results for variation in mobile phase composition Clindamycin phosphate

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	5 % less	5560	1.3
2	*Actual	5469	1.3
3	5 % more	5288	1.3

Table 7. Results for variation in mobile phase composition of Clotrimazole

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	5% less	12224	1.1
2	*Actual	12178	1.1
3	5% more	11947	1.0

Fig 1. Structure of Clindamycin phosphate

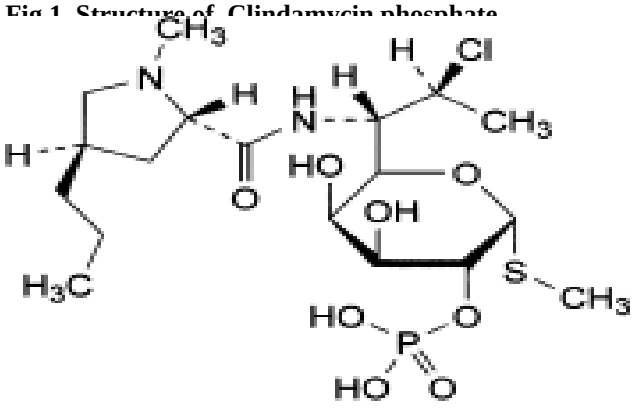


Fig 2. Structure of Clotrimazole

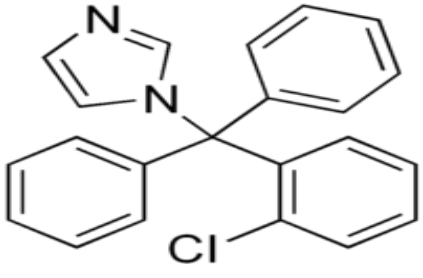


Fig 3. Calibration graph for Clindamycin phosphate at 210 nm

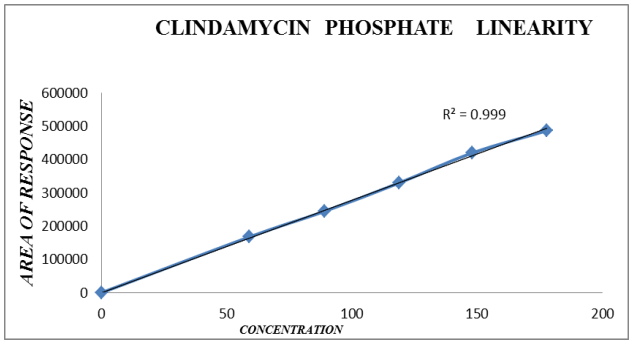


Fig 4. Calibration graph for Clotrimazole at 210 nm

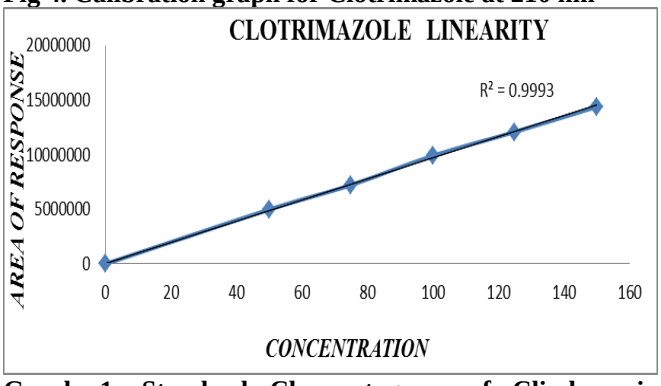
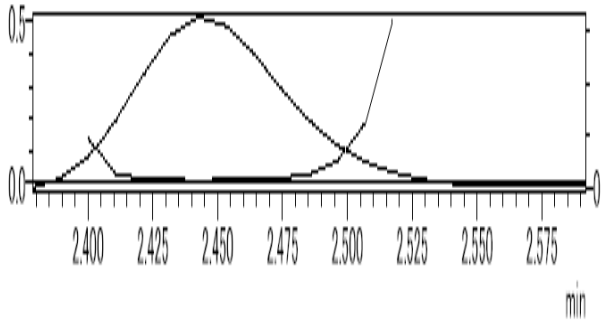
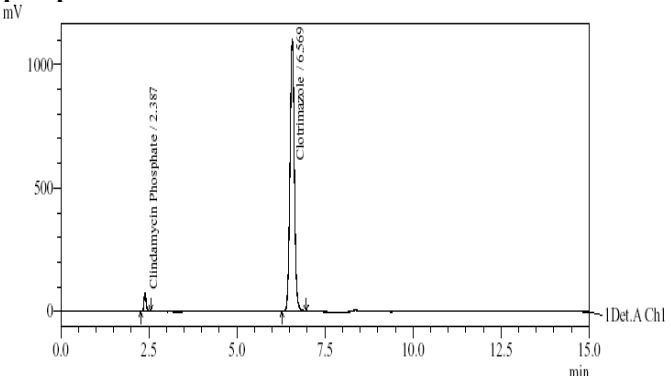


Fig 5. A chromatogram that does not show any interference for Clindamycin phosphate sample solution



Graph 1. Standard Chromatogram of Clindamycin phosphate and Clotrimazole



Specificity

Specificity studies carried out by injecting standard and sample of 118.8 ppm of Clindamycin phosphate and 100 ppm of Clotrimazole Impurity was not detected. Peak purity angle index of Clindamycin phosphate and Clotrimazole was 0.999 and 1.000 for standard and Sample.

Forced degradation study

Acid hydrolysis, Base hydrolysis, Water hydrolysis, oxidation reflux carried out by refluxing sample, placebo for 2hrs at 105°C. Both Sample and placebo was exposed to heat for the period of 3 hours at 105^0C, Exposed to humidity i.e. 90% RH and 25°C in a desicator and Sample was injected. The peak purity angle index of Clindamycin phosphate and Clotrimazole was 0.999 and 1.000 respectively.

CONCLUSION

In this present study, an attempt was made to develop an analytical method for the simultaneous estimation of Clindamycin phosphate and Clotrimazole in capsule dosage form, which is simple and fast and accurate method. The proposed method was subjected to validation parameters as per ICH guidelines and found within the limit.It can be employed as stability-indicating one. The results are found to be complying with the acceptance criteria for each of the parameter. The result shows that there is no interference of Blank and placebo peaks at the retention time of Clindamycin phosphate and Clotrimazole peak. The peak purity index values of standard and sample solutions are within the Acceptance criteria. Hence it is concluded that the method is able to estimate the amount of Clindamycin and Clotrimazole specifically without the interference of blank and placebo peaks.

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