

Original article ■

Determination of Clindamycin Phosphate in Different Vaginal Gel Formulations by Reverse Phase High Performance Liquid Chromatography

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SUMMARY

Clindamycin is an antibiotic effective against Gram-positive aerobes and both Gram-negative and Gram-positive anaerobic pathogens. Clindamycin (its phosphate ester) was used as a model drug for our study in order to check the efficacy of the developed vaginal gel formulations.

The aim of this study was to develop an analytical method suitable for the determination of clindamycin phosphate in three different vaginal gel formulations. The main difference between the three formulations was the use of different gelation agents in the formulations (Carbopol® 940, Carbopol® Ultrez 10 and Pemulen® TR 1). A new isocratic high performance liquid chromatography method based on reverse phase separation has been developed for the determination of clindamycin phosphate. The separation was achieved on a Zorbax Eclipse plus C8 column with a mobile phase of acetonitrile and pH 2.5 phosphate buffer and UV detection at 210 nm. A linear response ($r > 0.99$) was observed in the range of 10.0-80.0 $\mu\text{g mL}^{-1}$. The method shows good recoveries and relative standard deviations were less than 1.0%. The method can be applied to assess the stability of clindamycin phosphate in vaginal gel formulations. It can be used for quality control and stability study samples analysis.

Clindamycin phosphate concentration was different in vaginal gel formulations with different gelation agents, so the conclusion is that gelation agents (gel carriers) have a significant influence on the active drug concentration. The obtained results confirm that vaginal gel formulation with Carbopol® 940 shows the maximal stability during the analysis and provides the optimal value of clindamycin phosphate concentration.

Key words: clindamycin phosphate, reversed phase chromatography, vaginal gels, Carbopol® 940

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INTRODUCTION

Clindamycin is an antibiotic effective against Gram-positive aerobes and both Gram-negative and Gram-positive anaerobic pathogens. It is synthesized by chemical modification of lincomycin, an antibiotic produced by microbial fermentation *Streptomyces lincolnensis*, to increase the biological activity (1). Chemically, both antibiotics are weak bases. Clindamycin has greater antibacterial power and is better absorbed than lincomycin orally. Structures of clindamycin and related compounds are shown in Figure 1.

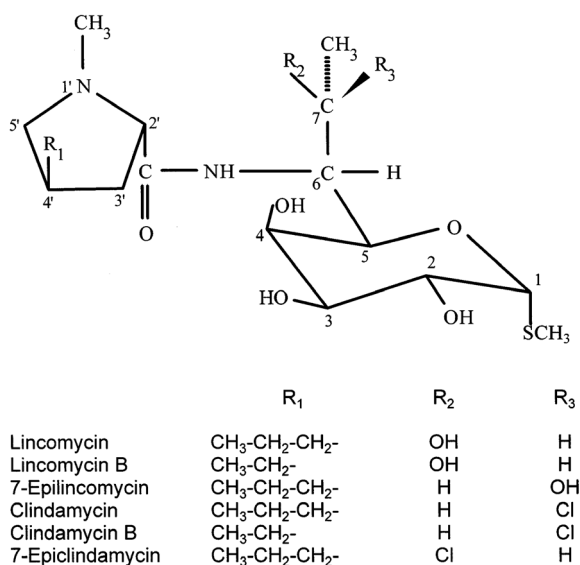


Figure 1. Structures of clindamycin and related compounds

Clindamycin inhibits bacterial protein synthesis at the level of the bacterial ribosome. The effect of clindamycin, which is primarily bacteriostatic, is exerted by its binding to the 50S ribosomal subunit and affects the process of peptide chain initiation (2, 3).

Clindamycin can be found as clindamycin hydrochloride, clindamycin palmitate (used in pediatrics) and clindamycin phosphate (used intramuscularly, intravenously, topically). It can be applied orally (hydrochloride and palmitate), parenterally (phosphate) and locally in the treatment of vaginal infections and acne (hydrochloride, phosphate) (4).

Some clinical trials confirmed that clindamycin is very effective in treating vaginal bacterial diseases. The frequency of adverse effects on the digestive system after the application of clindamycin phosphate vaginal gel is very low (5). The local (vaginal) treatment of clindamycin phosphate has a clear advantage over the systemic one. Treatment is carried out by direct acting on the cause of the infection (6, 7).

Clindamycin is indicated for the treatment of bacterial vaginosis (formerly referred to as *Haemophilus* vaginitis, *Gardnerella* vaginitis, non-specific vagini-

tis, *Corynebacterium* vaginitis or anaerobic vaginosis) in non-pregnant women (2). Clindamycin was used as a model drug for our present study in order to check the efficacy of the developed vaginal gel formulations.

Its phosphate ester is produced by chemical modification of clindamycin (8). Although the ester is not biologically active, the prodrug clindamycin 2-phosphate is rapidly converted *in vivo* to the parent drug, clindamycin, by phosphatase ester hydrolysis. Clindamycin phosphate, unlike clindamycin, is highly water-soluble and does not produce pain upon injection. Aqueous solutions of clindamycin 2-phosphate show a maximum stability at pH 3.5-6.5 (5).

Clindamycin phosphate can be determined using reverse phase chromatography with low wavelength detection along with aqueous acetonitrile or methanol mixtures (5). Several methods have been established for the determination of clindamycin in bulk drugs and formulations. Microbiological and spectrophotometric assays suffer from both the lack of specificity and accuracy. Gas-liquid chromatography (GLC) compensates for the previous, but requires a relatively complicated sample manipulation. High performance liquid chromatography (HPLC) methods are much more accurate and precise. Refractive index, electrochemical and UV detection have been applied. UV detection seems to offer more, in terms of sensitivity and stability (3, 9, 10).

Also, HPLC analytical method for the determination of clindamycin in human plasma and oral solutions has been developed (10, 11).

However, to our knowledge, there are no reports in the literature regarding any analytical method that is able to analyse this drug with UV absorbing characteristics in vaginal gel formulations.

The aim of this study was to develop an analytical method suitable for the determination of clindamycin phosphate in three different vaginal gel formulations.

The main difference between formulations was the use of different gelation agents in the formulations (Carbopol® 940, Carbopol® Ultrez 10 and Pemulen® TR 1). All agents (polymers) show similar organoleptic properties and satisfactory viscosity parameters as vaginal gel carriers and their formulations meet the requirements of the European Pharmacopoeia (12).

In order to assess the chemical stability of the clindamycin phosphate in these vaginal gel formulations, development of stability - indicating analytical method is needed.

MATERIALS AND METHODS

Chemicals and reagents

Carbopol® 940 (2-propenoic acid homopolymer or poly(acrylic acid)), Carbopol® Ultrez 10 (a cross-linked poly(acrylic acid)) and Pemulen® TR 1 (2-methylbutanoic acid homopolymer) were purchased from Noveon

(USA). Propylenglycol and concentrated phosphoric acid were the European Pharmacopoeia 6.0 chemical reference substances. Disodium edetate and sodium dihydrogen phosphate were also the European Pharmacopoeia 6.0 chemical reference substances. HPLC grade acetonitrile was purchased from Merck (USA). Sodium hydroxide solution and purified water were obtained from our laboratory. All other chemicals and solvents used were of analytical grade.

Equipment

The analytical HPLC was performed on an chromatography system Agilent 1200 Series Diode Array and Multiple Wavelength detector (Agilent Technologies, USA). The HPLC system was controlled by the Agilent Chem Station software. The pH value was determined with a Microprocessor pH Meter (HANNA Instruments, USA). For mixing the formulations, laboratory stirrer RW 16 basic (IKA® - Werke, Germany) was used.

Formulations

The clindamycin phosphate hydrophilic vaginal gels were formulated with three different mediums of gelation: Carbopol® 940, Carbopol® Ultrez 10 and Pe-mulen® TR 1 (Table 1). In each of them, clindamycin phosphate is present with the same concentration - 2.4%.

All of the three formulations were made using the same procedure, at room temperature. Disodium edetate was dissolved in a certain volume of purified water (70 mL). Propylene glycol was added to an aqueous solution of disodium edetate. The polymer was dispersed in prepared solution. Dispersion was made by mixing with the laboratory stirrer at 700 r/m (in order to avoid the collapse of polymer gel structure, which results in the loss of viscosity of the Carbopol® gel) (13). The sodi-

um hydroxide solution 10.0% (18 drops) (14) was added to the dispersion and mixed until the homogeneous gel was formed.

Placebo of gel formulation containing all the normal ingredients like formulation F₁, except clindamycin phosphate was also prepared for this study (Table 1). We used placebo of formulation F₁ (with Carbopol® 940) because it is a recommended formulation for vaginal gel carrier in magistral practice (12).

The samples were packed in plastic, tightly closed containers and kept in the dark at 20±5°C until analysis.

Chromatographic conditions

The separation was achieved using a reverse phase Zorbax Eclipse plus C8 column 1.8 μm, 50 mm×4.6 mm (Agilent Technologies, USA).

The mobile phase involved acetonitrile and pH 2.5 phosphate buffer (6.0±0.1 g of anhydrous sodium dihydrogenphosphate was dissolved in 1.0 L of deionized water, and adjusted to pH 2.5 with concentrated phosphoric acid) (15:85, v/v). Then, the mobile phase was filtered through a membrane filter 25/0.45 μm RC (Econofilter, Agilent Technologies, USA). As clindamycin phosphate and its major degradant clindamycin can only be detected at low wavelength (<220 nm), acetonitrile (with low UV cut off) was chosen as organic modifier for the mobile phase. The mobile phase flow rate was 1.0 mL/min.

The injection volume was 10 μL and the column was maintained at 40°C. For quantification, clindamycin phosphate was detected at 210 nm by UV/VIS detector. The chromatogram was derived using the Agilent Chem Station software.

Table 1. Formulations of clindamycin phosphate vaginal gels (F₁, F₂, F₃) and placebo gel (P)

Constituent	F o r m u l a t i o n (g)			
	F ₁	F ₂	F ₃	P
Carbopol® 940	1.00	-	-	1.00
Carbopol® Ultrez 10	-	1.00	-	-
Pemulen® TR 1	-	-	1.00	-
Dinatrii edetas	0.10	0.10	0.10	0.10
Propylenglycolum	10.00	10.00	10.00	10.00
Natrii hydroxydi solutio 10%	1.00	1.00	1.00	1.00
Clindamicyni phosphas*	2.40	2.40	2.40	-
Aqua purificata ad	100.00	100.00	100.00	100.00

* equivalent to 2% clindamycin

Stock and working standard solutions

Clindamycin phosphate stock solution was prepared by adding 10.0 mg clindamycin phosphate to a 10.0 mL volumetric flask, dissolving this quantity in phosphate buffer solution (pH 2.5) and filling to the mark with the same solvent. The concentration of clindamycin phosphate in stock solution was $1000 \mu\text{g mL}^{-1}$. Working standard solutions were prepared by the dilution of the clindamycin phosphate stock solution with the same solvent to obtain five different concentrations within the range of interest, in this case, 10.0, 20.0, 40.0, 60.0 and $80.0 \mu\text{g mL}^{-1}$.

Preparation of sample solution

The method was developed for the assay of three vaginal gel formulations prepared in our laboratory. All three formulations contained 2.4% clindamycin phosphate (equivalent to 2% clindamycin) as active drug in addition to a number of other formulation ingredients.

An accurately weighed portion of gel contents equivalent to 0.60 mg of clindamycin phosphate (sample weight about 25 mg) was transferred to a 25 mL volumetric flask and dissolved in phosphate buffer solution. After it, the flask was shaken vigorously, brought to the volume with the same solvent and filtered through membrane filter 25/0.45 μm RC (Econofilter, Agilent Technologies, USA).

The same procedure was used for the preparation of all formulation samples (placebo - P, F_1 , F_2 and F_3).

RESULTS AND DISCUSSION

The determination of clindamycin phosphate in different vaginal gel formulations was achieved by an isocratic reverse phase HPLC. Isocratic conditions relate to the constant composition of mobile phase during the analysis (pH 2.5 phosphate buffer - eluent A and acetonitrile - eluent B, 85:15, v/v). Clindamycin phosphate lacks a UV absorbing chromophore and can only be detected in the low wavelength UV range.

The selectivity of assay was determined by placebo analysis. Placebo of gel formulation F_1 containing all the normal ingredients except clindamycin phosphate was prepared for this study. It was treated in the same manner as other samples. The obtained chromatogram was used for the study of other ingredient interference on the selectivity of the clindamycin phosphate separation. The chromatograms obtained from samples of placebo P, clindamycin phosphate standard solution (at concentration of 100 ppm) and formulations F_1 , F_2 and F_3 are shown in Figures 2-4.

Figures show that despite the presence of the peaks of pharmaceutical formulation base components

on the obtained chromatograms, the peak of the clindamycin phosphate was properly separated from those ones. There was no interfering peak from placebo sample co-eluting with the clindamycin phosphate.

A linearity relationship was evaluated across the range of analytical procedure. For the linearity experiments, solutions of clindamycin phosphate were prepared at five concentrations. The concentrations of clindamycin phosphate used were 10.0, 20.0, 40.0, 60.0 and $80.0 \mu\text{g mL}^{-1}$. The calibration curve was constructed by analyzing the solutions of clindamycin phosphate (Figure 5). The regression line was calculated as $Y=A +BX$, where X was the clindamycin phosphate concentration ($\mu\text{g mL}^{-1}$) and Y was the response (peak area expressed as mAU*s). The regression data for clindamycin phosphate are presented in Table 2.

Table 3 shows a set of typical system precision results from replicate injections of a standard solution of clindamycin phosphate $100 \mu\text{g mL}^{-1}$. The six replicate injections gave relative standard deviations of less than 1.0% for the peak areas and retention times of this drug, demonstrating that the HPLC system was precise.

Assay results obtained from chromatograms of clindamycin phosphate vaginal gel formulations (Table 4) show that formulation with Carbopol® 940 has an optimal value for clindamycin phosphate concentration (difference between clindamycin phosphate added and clindamycin phosphate recovered is minimal).

All formulations have recovery values that meet the criteria from the European Pharmacopoeia (the preparation complies with the test if not more than one individual content is outside the limits of 85% to 115% of the average content and none is outside the limits of 75% to 125% of the average content; Uniformity of mass of single-dose preparations - Test B) (15). In our case, only the content of the formulation F_3 is outside the limits of 85% to 115% of the average content, but none is outside the limits of 75% to 125% of the average content.

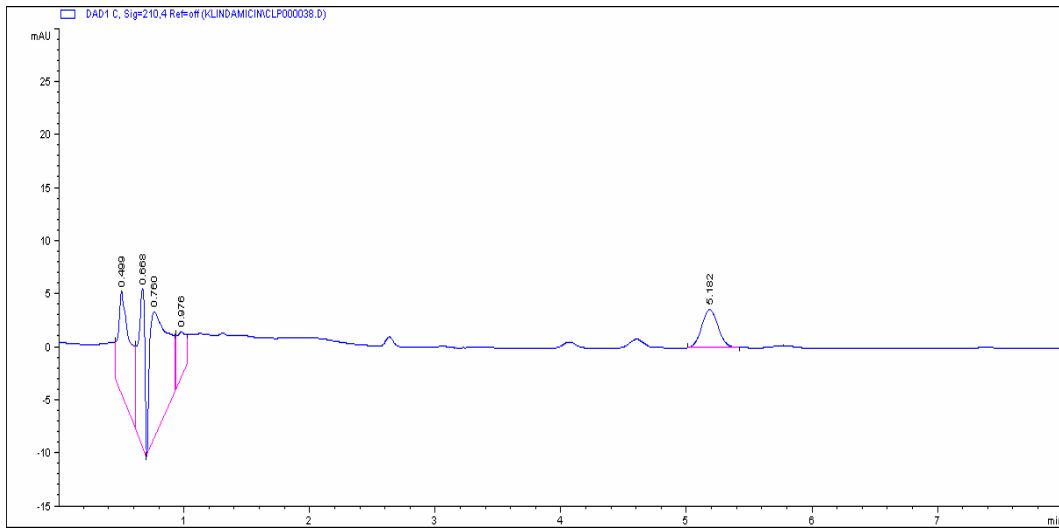


Figure 2. Chromatogram of placebo sample - formulation P, extracted at 210 nm

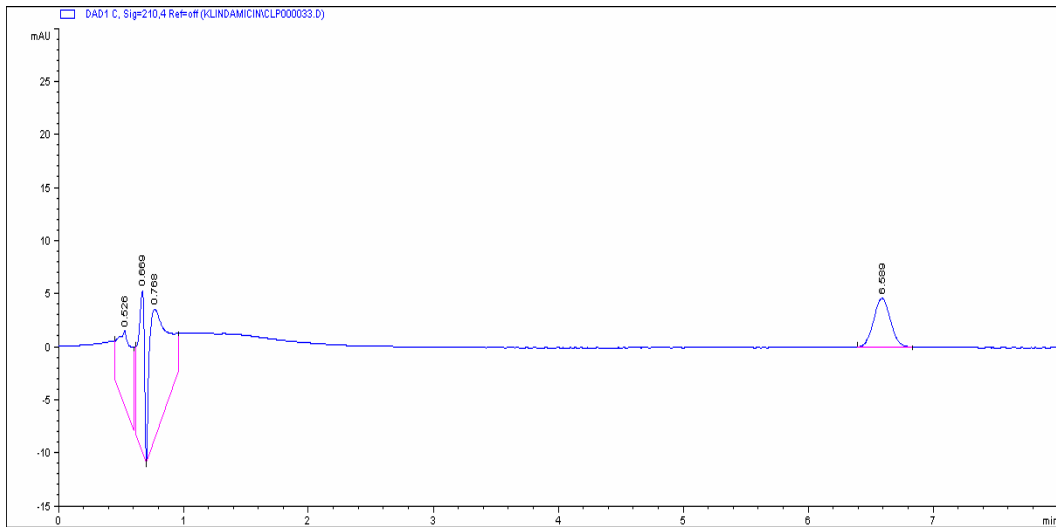
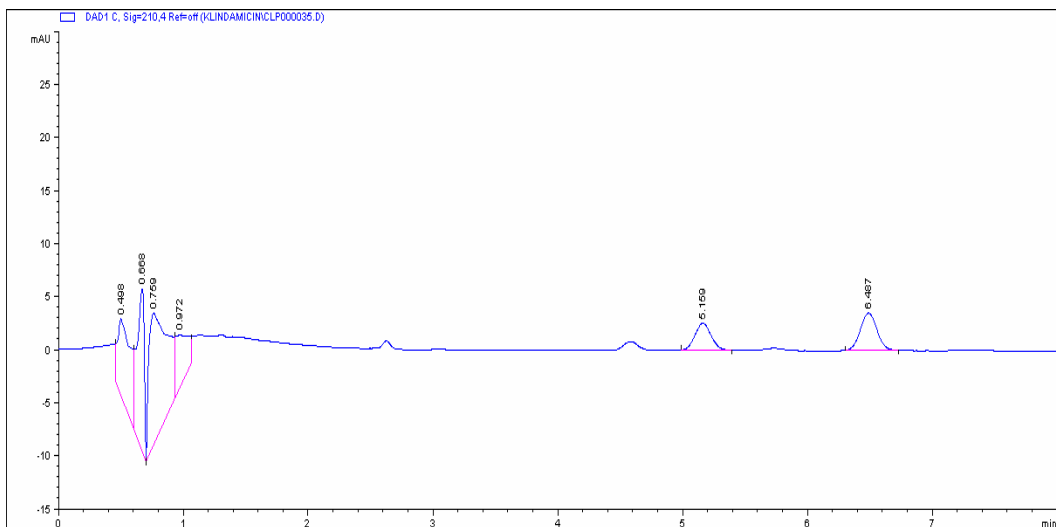
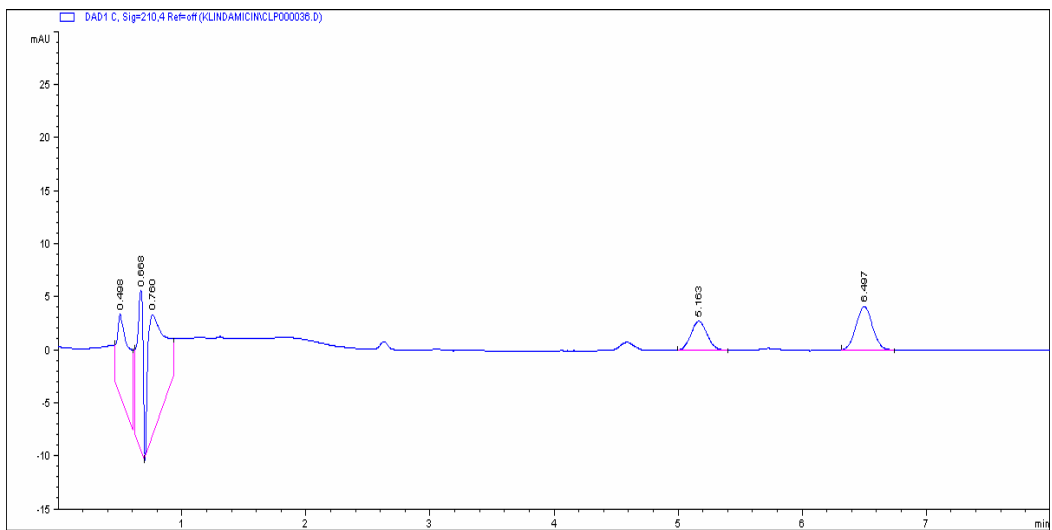


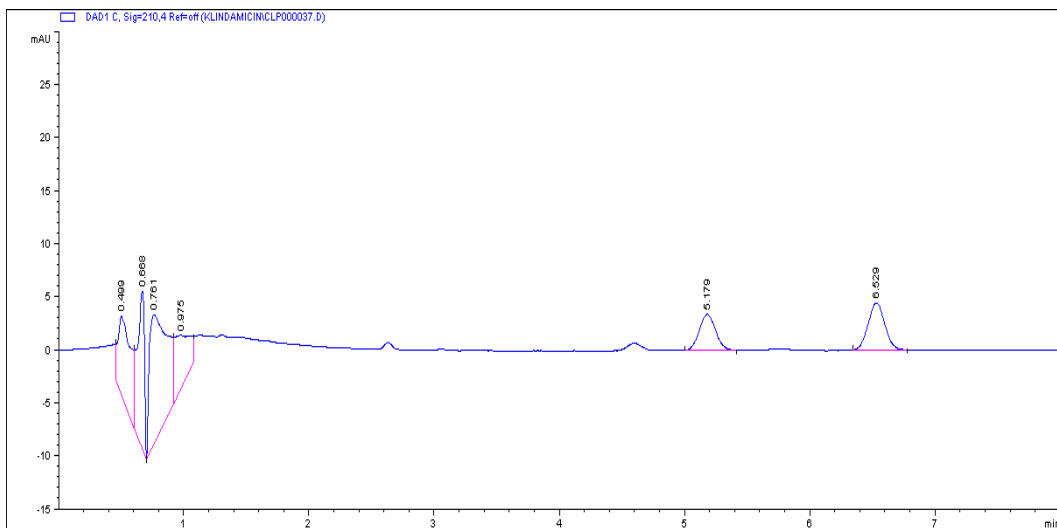
Figure 3. Chromatogram of clindamycin phosphate standard solution ($100 \mu\text{g mL}^{-1}$), extracted at 210 nm



a)



b)



c)

Figure 4. Chromatograms of clindamycin phosphate vaginal gel samples a) formulation F₁, b) formulation F₂, c) formulation F₃, extracted at 210 nm

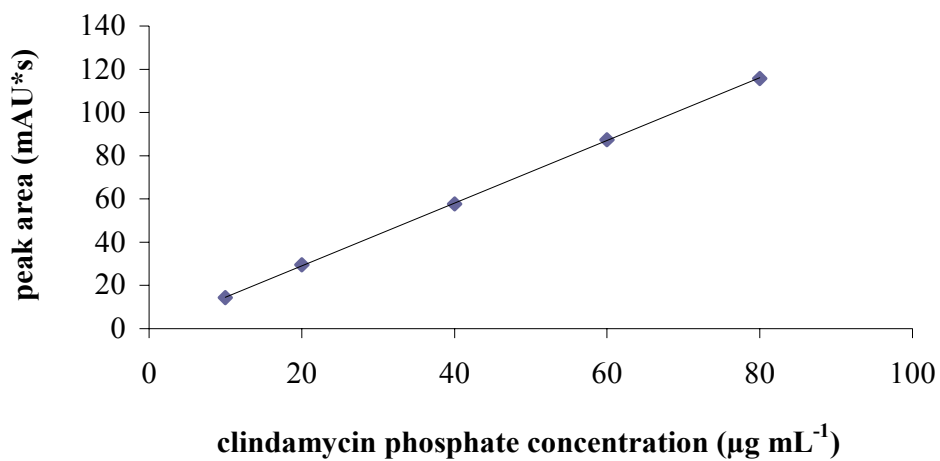


Figure 5. Calibration curve for clindamycin phosphate

Table 2. Linear regression data for clindamycin phosphate

Parameter	Clindamycin phosphate
Concentration range ($\mu\text{g mL}^{-1}$)	10 - 80
Slope	0.0935
Intercept	1.4488
Correlation coefficient (r)	0.9999

Table 3. HPLC system precision for peak areas and retention times of clindamycin phosphate from six replicate injections

Inj. No.	Peak area (mAU*s)	Retention time (min)
1	141.71	6.589
2	142.28	6.588
3	141.61	6.589
4	141.79	6.589
5	141.63	6.588
6	142.69	6.590
Average	141.95	6.589
RSD (%)	0.3083	0.011

Table 4. Assay results from chromatograms of clindamycin phosphate vaginal gel formulations

Formulation sample	Peak area (mAU*s)	Retention time (min)	Clindamycin phosphate added ($\mu\text{g mL}^{-1}$)	Clindamycin phosphate recovered ($\mu\text{g mL}^{-1}$) \pm SD	Recovered (%) \pm SD
F ₁ (Carbopol [®] 940)	33.29	6.487	24.0	22.91 \pm 0.01	95.46 \pm 0.04
F ₂ (Carbopol [®] Ultrez 10)	38.97	6.497	24.0	26.83 \pm 0.02	111.79 \pm 0.08
F ₃ (Pemulen [®] TR 1)	41.96	6.529	24.0	28.90 \pm 0.015	120.42 \pm 0.06

CONCLUSION

The described isocratic HPLC-UV method is suitable for the assay of clindamycin phosphate in the vaginal gel formulations and can be used to assess the chemical stability of the clindamycin phosphate in the formulations. It can be used for quality control and stability study samples analysis for vaginal gel formulations.

The method recovery was shown to be within 75-125% with relative standard deviation of less than 1.0% for clindamycin phosphate in the three different

vaginal gel formulations. All formulations show stability during the analysis.

Clindamycin phosphate concentration was different in vaginal gel formulations with different gelation agents. So, the conclusion is that gelation agents (gel carriers) have a significant influence on the clindamycin phosphate concentration.

The obtained results confirm that vaginal gel formulation with Carbopol® 940 shows the maximal stability and provides the optimal value of clindamycin phosphate concentration.

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ODREĐIVANJE SADRŽAJA KLINDAMICIN FOSFATA U RAZLIČITIM FORMULACIJAMA VAGINALNIH GELOVA METODOM REVERZNO-FAZNE VISOKOEFIKASNE TEČNE HROMATOGRAFIJE

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Sažetak

Klindamicin je antibiotik efikasan protiv Gram-pozitivnih aeroba, kao i Gram-pozitivnih i Gram-negativnih anaerobnih patogena. Klindamicin (njegov fosfatni estar) korišćen je u ovoj studiji u cilju procene efikasnosti formulisanih vaginalnih gelova.

Cilj ove studije bio je da se razvije analitička metoda pogodna za određivanje klindamicin fosfata u različitim vaginalnim gelovima. Glavna razlika između formulacija gelova bila je u primeni različitih gelirajućih sredstava (Carbopol® 940, Carbopol® Ultrez 10 i Pemulen® TR 1). Nova izokratska HPLC metoda reverznih faza razvijena je u cilju određivanja klindamicin fosfata u ovim vaginalnim gelovima. Korišćena je Zorbax Eclipse plus C8 kolona. Mobilna faza se sastojala iz acetonitrila i fosfatnog pufera (pH=2,5), a UV detekcija je rađena na 210 nm. Uočen je linearan odgovor ($r > 0,99$) u opsegu koncentracija klindamicin fosfata od 10,0-80,0 $\mu\text{g ml}^{-1}$. Metoda pokazuje dobre vrednosti za Recovery, a vrednosti za relativnu standardnu devijaciju su manje od 1,0%.

Metoda može da se primeni u proceni stabilnosti vaginalnih gelova sa klindamicin fosfatom, za kontrolu kvaliteta preparata i u studijama stabilnosti uzoraka pri formulaciji vaginalnih gelova.

Koncentracija klindamicin fosfata je različita u formulacijama, pa se zaključuje da sredstvo za geliranje (podloga gela) ima značajan uticaj na koncentraciju aktivne supstance. Dobijeni rezultati potvrđuju da vaginalni gel sa Carbopol-om (Carbopol® 940) pokazuje maksimalnu stabilnost tokom analize i obezbeđuje optimalnu koncentraciju klindamicin fosfata.

Ključne reči: klindamicin fosfat; reverzno-fazna HPLC; vaginalni gel; Carbopol® 940