DETERMINATION OF CLINDAMYCIN PHOSPHATE IN EXTEMPORANEOUSLY PREPARED PESSARIES WITH GLYCEROL-GELATIN BASIS

Vesna Lj. Savić1, Slavica M. Sunarić1, Jelena V. Živković1, Anastasija S. Stamenković1, Ivana Lj. Nikolić2, Ana D. Tačić2, Vesna D. Nikolić2

1Department of Pharmacy, Faculty of Medicine, University of Niš, Niš, Serbia
2Faculty of Technology, University of Niš, Leskovac, Serbia

Pessaries with clindamycin phosphate as active component were prepared by moulding. Glycerol-gelatin basis was used as the hydrophilic basis. The pessaries were made with and without the surface-active agent Tween 80 as an excipient. The content of clindamycin phosphate in all samples was determined by high performance liquid chromatography (HPLC) based on the calibration curve of clindamycin phosphate standard solution. The clindamycin phosphate content in the glycerol-gelatin pessaries with or without Tween 80 was optimal. Preparation of extemporaneous (magistral) medicines provides personalization of therapy and the possibility of selection of the most appropriate basis for the specific active substance, which also influences the effectiveness of the therapy. The active substance content in the magistral preparations is not subject to verification in the everyday pharmacy practice. However, by validating the technological process of manufacture and by controlling the quality of the obtained preparations, the choice of the basis in the magistral medicine preparation can be optimized. In this manner, the guidelines for the galenic preparation, including pessaries, can be given to ensure their optimal therapeutic effect.

Keywords: clindamycin phosphate, pessaries, glycerol-gelatin basis, magistral preparation, HPLC analysis.

Introduction

Vaginal administration route can be used for the application of antiseptic, antymycotic, astringent, antiphlogistic and antibiotic drugs, estrogen and progesterone hormones, spermicides and contraceptives, among others [1,2]. The ideal vaginal preparation should be easy to use, painless for the patient, effective, economical, widely available and safe [3]. Clindamycin is a lincosamide antibiotic with primary bacteriostatic effect on Gram-positive aerobic bacteria and a broad spectrum of action on anaerobic bacteria [4]. Clindamycin phosphate can be applied intravaginally in the form of pessaries or vaginal cream for the treatment of bacterial vaginosis. The equivalent of 100 mg of clindamycin is administered in the evening for 3 to 7 days.

Pessaries (or vaginal suppositories) are solid, single-dose preparations intended for vaginal application [5]. They have various shape, usually ovoid or conical. Pessaries are solid at the room temperature, and they melt and dissolve in vaginal discharge at the body temperature. Pessaries contain one or more active substances dispersed or dissolved in a suitable basis that does not irritate vaginal mucosa. The basis for the pessaries can be liposoluble which melts at the body temperature and hydrosoluble which dissolves in body fluids. The basic requirement to be fulfilled by the selected basis are related to the chemical, physical and microbiological stability of preparations. In addition, the basis should be compatible, physiologically indifferent and contractile on cooling, shapeable by moulding and extrusion, should have a short interval between melting and solidification point, lubricant and emulsifying ability, it should not affect therapeutic effect, should not have polymorphic modification with different melting points, and should dissolve or melt in vagina while rapidly releasing the drug substance [6]. Surface active agents are added to allow better wetting of dispersed particles, emulsification of the liquid components and to improve the active substance release [1]. Various types of surfactants can be used, but nonionic surface-active agents are generally recommended for the preparation of pessaries. For this study, pessaries with clindamycin phosphate incorporated into the glycerol-gelatin basis, with or without Tween 80, are prepared by moulding. From the pharmaceutical and technological point of view, the prepared pessaries represent a type of solutions, considering the hydrophilic properties of active substance and used basis. This work aims to determine the influence of the composition of basis and excipient Tween 80 on the clindamycin phosphate content in the prepared pessaries.

*Author address: Vesna Savić, Department of Pharmacy, Faculty of Medicine, University of Niš, Bulevar Zorana Djinđića 81, 18000 Niš, Serbia
E-mail: vesna.savic@medfak.ni.ac.rs
The manuscript received: November, 07, 2019.
Paper accepted: December, 02, 2019.
Experimental

Chemicals and reagents
Clindamycin phosphate, gelatin and glycerol (Sigma Aldrich, Darmstadt, Germany), Tween 80 (Polysorbate 80) (Fagron Hellas, Trikala-Larisa, Greece), Paraffinum liquidum (Fagron Hellas, Trikala-Larisa, Greece) were used.

Preparation of pessaries
Glycerol-gelatin pessaries
Glycerol-gelatin basis represents a mixture of glycerol, gelatin and water. The dissolution rate of basis and the release of an active substance depend on the ratio of glycerol to gelatin. Gelatin is a polypeptide produced by hydrolytic degradation of collagen. At 80 °C, the gelatin is colloidal dissolved (sol state) and at the lower temperatures, it cools and transits to a gel state. Glycerol-gelatin basis creates a 3D mesh structure made of gelatin with the incorporated liquid phase of glycerol and water (Figure 1). Pessaries made with glycerol-gelatin basis have a high water content, so antimicrobial preservatives use is recommended [1,2].

![Figure 1. Structure of: a) glycerol, b) gelatin and c) schematic representation of glycerol-gelatin basis (adapted from Kommareddy et al. (2005) and Ramos et al. (2016)) [7,8].](image)

| Substance          | Mass of substance (g) |
|--------------------|-----------------------|
| Clindamycin phosphate | 0.86                  |
| Glycerol           | 11.22                 |
| Gelatin            | 2.25                  |
| Water              | 4.50                  |
| Tween 80           | /                     |

The composition of the prepared glycerol-gelatin pessaries with and without the excipient Tween 80, is shown in Table 1.

Using the same manufacture procedure, placebo and standard sample of pessaries were prepared too. Placebo sample did not contain clindamycin phosphate while standard sample contained 120 mg of clindamycin phosphate. Placebo and standard samples were essentials for determination of selectivity and accuracy of applied HPLC method.

High performance liquid chromatography (HPLC) analysis
The content of clindamycin phosphate in the prepared pessaries has been examined by using HPLC method [10] with slight modifications. The analysis was performed on the chromatography system Agilent 1200 Series Diode Array and Multiple Wavelength detector (Agilent Technologies, USA) under the following conditions: Column: Zorbax Eclipse Plus C8 (3 x 150 mm; 3.5 µm) (Agilent Technologies, USA); mobile phase: acetonitrile:phosphate buffer (pH=2.5) 20:80, v/v; flow
rate: 0.8 cm³/min; column temperature: 40 ºC; detection wavelength: 210 nm; the injected sample volume: 0.01 cm³.

The samples for the analysis were prepared by dissolution of the whole pessary in the 50 cm³ of phosphate buffer (pH=2.5). The volume of 0.83 cm³ of the obtained solution was measured and filled with phosphate buffer to 25 cm³. The obtained samples were filtered through a 0.45 µm membrane filter and analyzed. The main standard solution of clindamycin phosphate concentration of 1000 µg/cm³ was made and used for the preparation of standard solutions of the following concentrations: 120 µg/cm³, 100 µg/cm³, 80 µg/cm³, 60 µg/cm³ and 40 µg/cm³. The calibration curve was constructed and used for the determination of clindamycin phosphate content in the prepared pessaries.

Results and discussion

The weight of the prepared pessaries with and without Tween 80 may vary and can be determined using the gravimetrical method. The measured masses of the pessaries are shown in Table 2.

Table 2. Masses of pessaries with clindamycin phosphate in glycerol-gelatin basis, with and without Tween 80

| Pessary number | Mass of pessaries (g) Glycerol-gelatin | Glycerol-gelatin with Tween 80 |
|---------------|--------------------------------------|-----------------------------|
| I             | 2.42                                 | 2.45                        |
| II            | 2.51                                 | 2.47                        |
| III           | 2.46                                 | 2.47                        |
| IV            | 2.55                                 | 2.47                        |
| V             | 2.42                                 | 2.46                        |
| VI            | 2.53                                 | 2.47                        |
| *AV           |                                      | 2.46                        |

*AV – average value

Based on the weight of the prepared pessaries it can be concluded that mass variations are within limits prescribed by Ph. Jug. V [11], because non pessary mass deviates more than 5% from the average value.

Figure 2 shows the calibration curve of clindamycin phosphate with the equation for the linear part of the curve. The obtained equation was used to calculate the content of clindamycin phosphate in the prepared pessaries.

Figure 3 shows the chromatogram of the clindamycin phosphate standard solution concentration 80 µg/cm³. The peak at the retention time 3.534 min corresponds to clindamycin phosphate.

To determine the basis influence on the active substance content in the pessaries, HPLC analysis of placebo sample (Figure 4), standard sample (Figure 5) and prepared pessaries (Figure 6) was performed. The placebo sample (without active substance) was analyzed to determine the selectivity of the applied HPLC method. The method proved to be selective because the chromatogram of placebo sample did not show peaks at the retention time of clindamycin phosphate. Figure 4 shows the chromatogram of placebo sample with glycerol-gelatin basis.

To prove the accuracy of the applied HPLC method, standard pessary sample was analyzed. In chromatogram of standard pessary sample peak at retention time 3.168 min corresponds to clindamycin phosphate in glycerol-gelatin basis.

Standard pessary sample was prepared with an accurate amount of clindamycin phosphate (120 mg). Recovery value represents the ratio between the obtained and the expected concentration of clindamycin phosphate (Table 3).

The obtained recovery value indicates the satisfactory accuracy of HPLC method used to determine the content of clindamycin phosphate.

To determine the content of clindamycin phosphate, each prepared pessary was analyzed by HPLC method and chromatograms were recorded. The chromatogram of one randomly selected pessary with glycerol-gelatin basis is shown in Figure 6. In the chromatogram, the clindamycin phosphate peak occurs at 3.5 min, which is consistent with the retention time of clindamycin phosphate standard (Figure 3). Based on the obtained clindamycin phosphate peak areas in glycerol-gelatin pessaries, the mean clindamycin phosphate concentration
was determined. It is in the range from 111.96 to 130.5 for glycerol-gelatin pessaries (Table 4).

The chromatogram of clindamycin phosphate in glycerol-gelatin pessaries with the addition of Tween 80 is shown in Figure 7. The peak of clindamycin phosphate is found at the retention time of 3.56 min. The addition of Tween 80 and the basis composition did not affect the change in the retention time of clindamycin phosphate in the prepared pessaries. Namely, the obtained values for the retention time are consistent with the retention time of clindamycin phosphate standard (Figure 3). Based on the obtained peak areas of clindamycin phosphate in glycerol-gelatin basis with Tween 80, clindamycin phosphate concentration in different samples was determined (Table 5). The presented results show that clindamycin phosphate content in glycerol-gelatin pessaries with Tween 80 varies from 106.04 to 147.3 mg/pessary, which is a greater deviation than in the same pessaries without Tween 80.

![Figure 3](image3.png)

**Figure 3.** The chromatogram of the clindamycin phosphate standard solution concentration 80 μg/cm³

![Figure 4](image4.png)

**Figure 4.** Chromatogram of placebo sample with glycerol-gelatin basis

![Figure 5](image5.png)

**Figure 5.** Chromatogram of standard pessary sample with glycerol-gelatin basis

| Mean retention time (min) | Mean peak area (mAU’s) | Obtained concentration (mg/pessary)±SD | Recovery (%) |
|--------------------------|------------------------|----------------------------------------|--------------|
| 3.168±0.004             | 200.7±4.8              | 125.1±4.7                              | 102.6        |
Table 4. Chromatographic parameters, concentration and recovery values for pessaries with glycerol-gelatin basis

| Sample | Retention time (min) | Peak area (mAU*s) | Obtained concentration of clindamycin phosphate (mg/pessary) | Recovery (%) |
|--------|----------------------|-------------------|----------------------------------------------------------|--------------|
| 1      | 3.572                | 180.50            | 111.96                                                   | 92.33        |
| 2      | 3.579                | 186.49            | 115.75                                                   | 95.39        |
| 3      | 3.587                | 191.13            | 118.71                                                   | 97.76        |
| 4      | 3.595                | 181.72            | 112.73                                                   | 92.95        |
| 5      | 3.605                | 209.67            | 130.50                                                   | 107.25       |
| 6      | 3.606                | 186.07            | 115.50                                                   | 95.18        |

*AV 3.59±0.014 189.3±10.70 117.5±6.8 96.81±5.47

*AV – average value

Table 5. Chromatographic parameters, concentration and recovery values for pessaries with glycerol-gelatin basis in the presence of Tween 80

| Sample | Retention time (min) | Peak area (mAU*s) | Obtained concentration of clindamycin phosphate (mg/pessary) | Recovery (%) |
|--------|----------------------|-------------------|----------------------------------------------------------|--------------|
| 1      | 3.559                | 171.20            | 106.04                                                   | 87.57        |
| 2      | 3.560                | 188.67            | 117.14                                                   | 96.51        |
| 3      | 3.571                | 188.50            | 117.03                                                   | 96.42        |
| 4      | 3.576                | 175.60            | 108.80                                                   | 89.82        |
| 5      | 3.576                | 236.12            | 147.30                                                   | 120.77       |
| 6      | 3.578                | 176.30            | 109.30                                                   | 90.18        |

*AV 3.57±0.008 189.4±23.9 117.6±15.2 96.9±12.27

*AV – average value
Conclusion

Two sets of pessaries with clindamycin phosphate as the active compound, with and without the addition of Tween 80 as a surfactant, incorporated in a glycerol gelatin basis were prepared extemporaneously. Studies have shown that the content of the active compound in both sets of pessaries meets the pharmacopoeial requirements. The use of Tween 80 did not affect the content of clindamycin phosphate.

In pharmacy conditions, pessaries with clindamycin phosphate in a glycerol gelatin basis can be made by moulding method.

Acknowledgments

This research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. TR 34012).

References

[1] Z. Đurić, Farmaceutska biotehnologija sa biofarmacijom I deo, Nijansa, Zemun, 2004. (In Serbian)
[2] M. Jovanović, Praktikum iz farmaceutske tehnologije sa biofarmacijom, Nijansa, Zemun, 2004. (In Serbian)
[3] J. das Neves J, M. F. Bahia, Gels as vaginal drug delivery systems, International Journal of Pharmaceutics 318 (1-2) (2006)1-14.
[4] Martindale: The Complete Drug Reference, 36th ed., Sweetman C. S. editor, Pharmaceutical Press, London, 2009.
[5] The European Pharmacopoeia, 9th edition, Council of Europe, Strasbourg, 2017.
[6] D. Krajniškin, S. Grbić, J. Petrović, Lj. Dekić, D. Vasiljević, A. Kovačević, B. Čalić, B. Farmaceutska tehnologija II - Praktikum, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, 2013. (In Serbian)
[7] S. Kommaredy, D. B. Shenoy, M. M. Amiji, M. M., Gelatin Nanoparticles and their Biofunctionalization. Biofunctionalization of Nanomaterials, WILEY-VCH Verlag GmbH &Co. KGaA, Weinheim, 2005, 330-352.
[8] M. Ramos, A. Valdés, A. Beltrán, M. C. Garrigós, Gelatin-Based Films and Coatings for Food Packaging Applications, Coatings, 6(4) (2016) 41.
[9] G. Vuleta G, J. Milić editors. Magistral formula, Pharmaceutical Society of Serbia, Belgrade, 2008. (In Serbian)
[10] M. Stanković, V. Savić, V. Marinković, Determination of Clindamycin Phosphate in Different Vaginal Gel Formulations by Reverse Phase High Performance Liquid Chromatography, Acta Facultatis Medicae Naissensis, 30(2) (2013) 63-71.
[11] Jugoslovenska Farmakopeja (Ph.Jug. V), Savremeni zavod za zaštitu i unapređenje zdravlja, Savremena administracija, Beograd, 2000. (In Serbian)