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# Determination of clindamycin hydrochloride content in 1% clindamycin lotion

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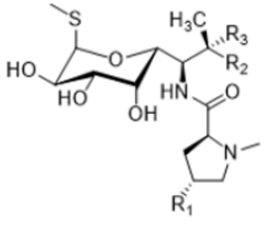
**Abstract:** Clindamycin is a broad spectrum antibiotic that belongs to the lincosamide group. It acts mostly as a bacteriostatic antibiotic, but it also has mild bactericidal activity. The most common clinical conditions in which they are used are: infections in gynecology, gingiva infections, respiratory tract, skin and soft tissue infections, intra-abdominal infections, pneumonia caused by *Pneumocystis jiroveci*, toxoplasmosis, malaria, babesiosis, and acne. Clindamycin is available in several pharmaceutical forms, which can be administered orally, intravenously, intramuscularly or intradermally. It is usually prepared as an *ex tempore* 1% clindamycin lotion that is used dermally, in the treatment of a mild form of acne. The proposed UV-Vis spectrophotometric method allows analyzing the content of clindamycin hydrochloride in the extemporaneous formulation of 1% clindamycin lotion. Clindamycin chloride content analysis was performed on samples of 1% clindamycin lotions purchased in pharmacies in Canton Sarajevo. The results showed that the content of clindamycin hydrochloride in the *ex tempore* prepared preparations varied from 21% to 142%.

The UV-Vis method does not require complicated preparation of the sample, and is therefore fast, reliable and economical, and as such can be used in regular control of the content of clindamycin hydrochloride in *ex tempore* prepared lotion.

## INTRODUCTION

### Clindamycin

Clindamycin (IUPAC name: (2S,4R)-N-[2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-methylsulphanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide) is an antibiotic belonging to the lincosamide group *(Figure. 1).* Clindamycin is formed by the synthesis of linkomycin with thionyl chloride, where at position seven OH groups are replaced with Cl. (Mateja, 2015, Zhou, Zheng, Wu, et al, 2006).




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| lincomycin | $CH_3$-$CH_2$-$CH_2$- | OH | H |
| clindamycin | $CH_3$-$CH_2$-$CH_2$- | H | Cl |

**Figure 1.** Chemical structure of clindamycin [1]

**Mechanism of action**

Clindamycin acts predominantly as bacteriostatic. Although it also has bactericidal activity, it depends on the concentration itself at the site of action and on the type of pathogen.

It acts by binding to the 50S subunit of bacterial ribosomes and inhibiting the formation of a peptide bond, i.e. it inhibits the synthesis of bacterial proteins (Mifsud, Vella, Ferrito, et al, 2014).

**Spectrum of action and clinical use**

Clindamycin acts against gram-positive aerobic and gram-negative and gram-positive anaerobic bacteria. It is especially effective for the treatment of infections caused by *Bacteroides fragilis* in the treatment of most Gram-positive cocci, such as pneumococci, β-hemolytic streptococcus A and B and staphylococci. It also acts on staphylococcal strains that secret beta-lactamase, and especially exhibits action on *Staphylococcus epidermidis*. It is not effective against *methicillin resistant Staphylococcus aureus* (MRSA), and against *Corynebacterium diphteriae*, but does not exhibit anti-*Corynebacterium jeikeum* activity.

Clindamycin does not exhibit an effect on gram negative aerobic bacteria and enterococci, and is less effective in gram negative cocci and coccobacilli, such as *Neisseria spp.* and *Haemophilus influenzae.* However, it exhibits action against *Chlamydia trachomatis.*

The most common clinical conditions in which clyndamicin is used are: infections in gynecology, gingiva infections, respiratory tract, skin and soft tissue, intra-abdominal infections, pneumonia caused by *Pneumocystis jiroveci*, toxoplasmosis, malaria, babesiosis, and acne.

Clindamycin is available in several pharmaceutical forms, which can be administered orally, intravenously, intramuscularly or intradermally. Oral preparations are available in the form of capsules containing clindamycin chloride or as a suspension for use in pediatrics containing clindamycin palmitate hydrochloride.

Clindamycin phosphate ester is used in preparations intended for intravenous or intramuscular administration. It can be given in the form of gel, cream or lotion (Mateja, 2015, Mifsud, Vella, Ferrito, et al, 2014, Rang, Dale, Ritter, et al, 2005, Prakash and Nehal, 2014, Zaenglein, Pathy, Schlosser, et al, 2016).

**Extemporaneous preparation of clindamycin**

Extemporaneous drug is a drug prepared in a pharmacy in accordance with the prescribed prescription for an individual patient (Federalno ministarstvo zdravstva, 2008). According to the European Pharmacopoeia, the extemporaneous medicines belong to a group of pharmaceutical preparations that are made for a patient or group of patients and are issued immediately after manufacturing process , thus are called *extemporaneous preparations*. In the market, the extemporaneous preparation is 1% clindamycin lotion. Its most common indication is acne, i.e., cases of moderately severe acne. Besides lotions, it can be made both in the form of gel and as an aqueous solution (Rohit, Rashmin, Mrunali, 2014, Stanković, Savić, Marinković, 2013, Tamaddon, Mostafavi, 2012, Wang, Kuo, Shu, et al, 2014).

Extemporaneous preparation (*Clindamycini solutio 1%* - Clindamycin, 1% solution for skin) is prepared according to *Formulae Magistrales*, and the *ex-tempore* composition, in certain proportions, include: *Clindamycin hydrochloridum, Propylenglycolum, Ethanolum 70%, Aqua purificata*.

After production of this preparation, 1g of the clindamycin base corresponds to 1.13 g of clindamycin hydrochloride.

The lotion is packed in dark, dry, glass or plastic bottles. The antibiotic preparation as mentioned above, is used in the treatment of acne. The solution is applied 2x daily (Hadžović and Čatić 2012).

**Analytical methods for the determination of clindamycin**

There are many analytical methods for analyzing clindamycin in various pharmaceutical compositions, as well as in biological samples. The most commonly used are high performance liquid chromatography (HPLC), gas chromatography, capillary electrophoresis, micellar electrocinetic chromatography, and spectrophotometry (Mifsud, Vella, Ferrito, et al, 2014, Olbrich and Corbett 2013, Prakash, Nehal, 2014, Rajendar, Potnuri, Rao, 2015).

The objectives of this paper were to: optimize the UV-Vis spectrophotometric method for the determination of clindamycin hydrochloride in the primary preparation of 1% clindamycin lotion and to determine the content of clindamycin hydrochloride in *ex tempore* preparations of 1% clindamycin lotions, which are sold in pharmacies in Canton Sarajevo.

**EXPERIMENTAL**

**Apparatus**

The analysis was carried out on spectrophotometer UV mini 1240, Shimadzu. The water used in the analysis was ultrapure water, obtained on the ultra clean water machine. (Arium mini, Sartorius).

**Chemicals**

$NaH_2PO_4 \cdot H_2O$ (sodium dihydrogen phosphate monohydrate); Merck. $H_3PO_4$ (orthophosphoric acid, 85%); Merck. $C_2H_5OH$ (ethanol 96%). $C_3H_8O_2$ (propylene glycol). Clindamycin chloride (Reference standard), $C_{18}H_3ClN_2O_5 \cdot HCl$; (Purity of 94.8% clindamycin hydrochloride, equivalent to 87.3% clindamycin), Pfizer.

**Terms of analysis**

Solvent: $NaH_2PO_4$ buffer pH 2.5.

Blank test: $NaH_2PO_4$ buffer pH 2.5.

The wavelength at which the analysis was performed is 210 nm.

**Preparation of buffer pH 2.5**

6 g of $Na_2HPO_4$ in a volumetric flask was dissolved in a 1 L solution and dissolved in ultra pure water. The pH was then adjusted with orthophosphoric acid. The buffer solution was used in the preparation of samples and as a blank test in the spectrophotometric analysis.

**1% clindamycin lotion**
1.13 g of *Clindamycin hydrochloridum* was weighed and transerred into a 10 mL volumetric flask and with constant stirring dissolved in a mixture of 10 mL of propylene glycol, 70 mL of 70% ethanol and the flask was filled with ultra pure water to the mark.

**Blind test (blank)**
A blank test of clindamycin lotion was prepared by mixing all the components that were added when using 1% clindamycin lotions, except the active component (clindamycin hydrochloride).

**Preparation of the basic solution of clindamycin chloride**
10 mg standard clindamycin hydrochloride standards was weighed and trasferred into a 10 mL volumetric flask and dissolved in pH 2.5 buffer. The concentration of the solution was 1 mg / mL.

**Clindamycin hydrochloride dilution series for calibration curve**
From the basic solution of concentration 1 mg/mL, concentration dilution series was prepared of 0.05; 0.1; 0.15; 0.2; 0.25 mg/mL The dilutions were prepared with $NaH_2PO_4$ buffer pH 2.5.
Additionally prepared concentrations: 0.1; 0.15; 0.2 mg/mL, were used for repeatability.

**Samples for analysis:**
Samples of 1% clindamycin lotions were purchased in pharmacies in Canton Sarajevo.
According to pharmacists, the extemporaneous preparations were prepared *ex tempore.*
1.5 mL was transferred from each lotion to a 100 mL volumetric flask and filled with a pH 2.5 buffer to the mark. They were analyzed spectrophotometrically with $NaH_2PO_4$ buffer pH 2.5 as a blank test.

## RESULTS AND DISCUSSION

**Optimization of UV-Vis spectrophotometric methods**
These validation parameters were tested: selectivity, linearity, accuracy, repeatability, detection limit and quantification limit.
The selectivity was tested by measuring the absorbance of a blank test at a wavelength of 210 nm and 1% of clindamycin lotions.
No blank test absorbance was observed, while 1% clindamycin lotion showed absorption at mentioned wavelength, suggesting that the proposed method was selective for the determination of clindamycin hydrochloride.
Linearity was carried out on a dilution series (0.05, 0.1, 0.15, 0.2, 0.25 mg/mL), which were prepared as described in the section Material and Methods. Results for the validation parameter linearity are shown in *Table 1*. and *Figure 2.*

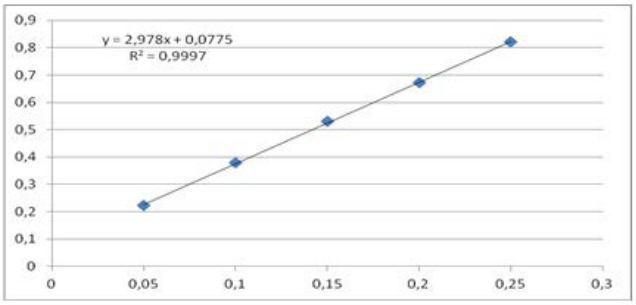



**Figure 2.** Calibration curve for the dilution series of clindamycin hydrochloride

*Table 1* shows the values of the limit of detection and limit of quantification.

**Table 1.** Validation parameter linearity for clindamycin hydrochloride

| Parameter | Value |
|---|---|
| ***Concentration range*** | *0,05- 0,25 mg/mL* |
| ***Curve inclination*** | *2,978* |
| ***Section on the Y axis*** | *0,0775* |
| ***Correlation coefficient*** | *0,9997* |
| ***LOD*** | *7,58E-10* |
| ***LOQ*** | *2,53E-09* |

The repeatability of the method was carried out at 3 different concentrations of clindamycin hydrochloride standards, and the solutions were prepared according to the regulation given in the section Material and Methods. The results are shown in *Table 2.*

Statistical analysis of the results showed that this validation parameter meets the requirements set in the ICH guidelines.

**Sample analysis**
There is no legal regulation that defines the allowed deviation of contents for the extemporaneous and galenical preparations, as these preparations are not subject to registration. There is only a recommendation on the expiry date given by the *Formulae Magistrales*, according to which clindamycin lotion is classified into a group of preparations with preservatives and the recommended shelf life of 6 months.
Consequently, the content of *ex tempore* prepared samples was determined, and the results are shown in *Table 3.*
The content of clindamycin hydrochloride varies from sample to sample and ranges from 21 to 142%. Each extemporaneous preparation should have been prepared in the same way, according to *Formulae Magistrales.*
The absorbance of *sample 6* was not possible to measure due to the turbidity of solution, and according to the declaration, in addition to the components prescribed by *Formulae Magistrales*, other components were detected (2-propanol 85%, aloe vera 2%, eucalyptus oil 1% and chamomile oil 1%).

**Table 2.** Repeatability for clindamycin hydrochloride

| Number of measurements | 0,1 mg/mL | | | 0,15 mg/mL | | | 0,2 mg/mL | | |
|---|---|---|---|---|---|---|---|---|---|
| | Abs. | Conc (mg/mL) | *R (%)* | Abs. | Conc (mg/mL) | *R (%)* | Abs. | Conc (mg/mL) | *R (%)* |
| **1** | 0,378 | 0,10 | 100,91 | 0,531 | 0,15 | 101,52 | 0,671 | 0,20 | 99,65 |
| **2** | 0,378 | 0,10 | 100,91 | 0,531 | 0,15 | 101,52 | 0,671 | 0,20 | 99,65 |
| **3** | 0,378 | 0,10 | 100,91 | 0,530 | 0,15 | 101,30 | 0,670 | 0,20 | 99,48 |
| **4** | 0,378 | 0,10 | 100,91 | 0,530 | 0,15 | 101,30 | 0,670 | 0,20 | 99,48 |
| **5** | 0,378 | 0,10 | 100,91 | 0,530 | 0,15 | 101,30 | 0,670 | 0,20 | 99,48 |
| **6** | 0,378 | 0,10 | 100,91 | 0,530 | 0,15 | 101,30 | 0,670 | 0,20 | 99,48 |
| **<x>** | 0,38 | 0,10 | 100,91 | 0,53 | 0,15 | 101,37 | 0,67 | 0,20 | 99,54 |
| **SD** | | 0,00 | 0,00 | | 0,00 | 0,12 | | 0,00 | 0,09 |
| **RSD (%)** | | 0,00 | 0,00 | | 0,11 | 0,11 | | 0,09 | 0,09 |

**Table 3.** The content of clindamycin and clindamycin hydrochloride in 1% clindamycin lotion

| Samples | Abs | mg/mL | Clindamycin hydrochloride content (%) | Clindamycin content (%) |
|---|---|---|---|---|
| ***SA 1*** | *0,472* | *0,13* | *88* | *76,82* |
| ***SA 2*** | *0,526* | *0,15* | *100* | *87,3* |
| ***SA 3*** | *0,714* | *0,21* | *142* | *123,97* |
| ***SA 4*** | *0,326* | *0,083* | *56* | *48,89* |
| ***SA 5*** | *0,173* | *0,032* | *21* | *18,33* |
| ***SA 6*** | - | - | - | |
| ***SA 7*** | *0,529* | *0,15* | *101* | *88,17* |
| ***SA 8*** | *0,579* | *0,17* | *112* | *97,78* |

*Sample 5* contained the least amount of clindamycin hydrochloride (21%) detected, and the pharmacological effect of such a preparation may be called into question. The question is, what is the content of clindamycin hydrochloride in the raw material, from which the extemporaneous preparation was prepared and how the weighing of the raw material was carried out.

*Sample 3* had the highest content (142%), which is by 42% more then expected. The method was selective and there were no positive interferences, but given that there is no legally defined range of content discrepancies, it can not be said with certainty that this sample is improper, although it is almost impossible that the allowed deviation of + 42% could be considered correct, especially because it is an antibiotic.

*Sample 7* was the only one with the appropriate content (101%), which was also expected, as it was a registered product on the market.

*Sample 8* was prepared in the laboratory according to *Formulae Magistrales* and the analysis of the content of clindamycin hydrochloride was 112%. The prescription states that 1.13 g of weighed clindamycin hydrochloride raw material corresponds to 1 g of clindamycin. Since the complete validation was carried out on the reference standard of clindamycin hydrochloride (purity 94.8%, corresponding to 87.3% clindamycin), which ultimately gives a mathematical ratio of 97,78%. As can be seen from the *Table 3* the content of clindamycin

hydrochloride was within the range of 21% to 142% calculated at the expected concentration. Only the sample 8 had a concentration that should have therapeutic activity. All other tested samples had a lower content than expected, except sample 3 which had an unexpectedly high content of clindamycin hydrochloride.

Theoretically, there are several possible reasons for such results. Given that preparations are not prepared in the same laboratory, the question may be raised as to quality of the raw materials were prepared, its purity, the content of the active substance, the presence of impurities, etc., which could interfere and influence spectrophotometric measurement.

In addition, spectrophotometric determination may also be influenced by the presence of keto group in the clindamycin structure, which under certain conditions may be subject to keto-enol tautomerism.

## CONCLUSIONS

The spectrophotometric method for the determination of clindamycin hydrochloride was optimized and validation of the same through validation parameters (selectivity, linearity, repeatability, detection limit and limit of quantification) was carried out.

This method allows the analysis of the content of clindamycin hydrochloride in the extemporaneous formulations of 1% clindamycin lotion.

The UV-Vis method does not require complicated preparation of the sample, and is therefore fast, reliable and economical, and as such can be used in regular control of the content of clindamycin hydrochloride in ex tempore prepared lotions.

On the basis of the obtained results we can conclude that the content of clindamycin hydrochloride in the ex tempore prepared lotions varied from 21% to 142%. This proved justified preliminary control of the above preparations. Due to the large differences in the content of clindamycin, it is necessary to pay more attention when preparing the extemporaneous formulations.

It is necessary to carry out further studies which will include all critical parameters of preparing of clindamycin lotion, starting from the quality of the active substance, the quality of all substances used in the formulation as well as possible influence other parameters (e.g., temperature, pH) which may affect the active ingredient, solvents and excipiens used in the formulation.

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## Summary/Sažetak

Klindamicin je širokospektralni antibiotik koji pripada grupi linkozamida. Najviše djeluje kao bakteriostatski antibiotik, ali posjeduje i blago baktericidno djelovanje. Najčešća klinička stanja u kojima se koristi su: infekcije u ginekologiji, infekcije gingive, respiratornog trakta, kože i mekog tkiva, intraabdominalne infekcije, pneumonia uzrokovana *Pneumocystis jiroveci*, toksoplazmoza, malarija, babezioza, te akne. Klindamicin je dostupan u nekoliko farmaceutskih oblika, koji se mogu davati oralno, intravenozno, intramuskularno ili dermalno. Na tržištu se najčešće priprema kao *ex tempore* 1% klindamicin losion koji se koristi dermalno, u tretmanu blagog oblika akni. Predložena UV-Vis spektrofotometrijska metoda omogućava analizu sadržaja klindamicin hidrohlorida u extemporaneousnom pripravku 1% klindamicin losionu. Analiza sadržaja klindamicin hlorida se provela na uzorcima 1% klindamicin losiona kupljenih u apotekama u Kantonu Sarajevo. Rezultati su pokazali da je sadržaj klindamicin hidrohlorida u *ex tempore* pripremljenim pripravcima varirao od 21- 142%. UV-Vis metoda ne zahtjeva komplikovanu pripremu uzorka, te je s toga brza, pouzdana i ekonomična i kao takva se može koristiti u redovnoj kontroli sadržaja klindamicin hidrohlorida u *ex tempore* pripremljenom losionu.