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DEVELOPMENT AND VALIDATION OF AN ASSAY METHOD FOR CIPROFLOXACIN HYDROCHLORIDE DETERMINATION IN COMBINATION EAR DROPS

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Abstract. A simple, precise and accurate UV-Vis spectrophotometric method has been developed and validated for assaying ciprofloxacin hydrochloride in combination ear drops with basil oil (*Ocimum basilicum*). Ciprofloxacin hydrochloride presented an absorption maximum at 278 nm, while the placebo solution showed a very low absorption in the 220-400 nm range. The parameters of validation have been determined according to the International Conference of Harmonization guidelines „Q2R1: For Analytical Procedures and Validation". Linearity was obtained over the concentration range 2-10 µg/mL with a correlation coefficient of 0.999. The value of the limit of detection was of 0.786 µg/mL and of the limit of quantification was of 2.383 µg/mL. The percentages of recovery of ciprofloxacin hydrochloride in combination ear drops exceeded 99.0%. The relative standard deviation values of precision and robustness were less than 2%. Short-term stability results showed that the samples were stable at room temperature for 24 hours.

Keywords: ciprofloxacin hydrochloride, UV-Vis spectroscopy, validation, ear drop, *Ocimum basilicum*.

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Introduction

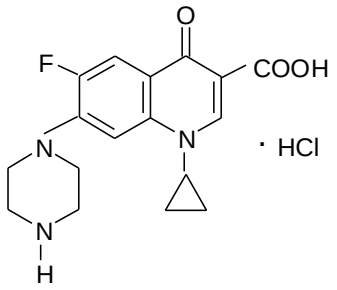
Contemporary pharmacotherapy is indispensable for the use of chemical compounds and active principles of vegetal origin. In this context, the use of polyfunctional combination drugs obtained from known substances, which have demonstrated good clinical efficacy and with polyvalent effects may be considered [1].

Combination drugs involve two or more active substances with different therapeutic actions in the same pharmaceutical dosage form, which can act on the various mechanisms responsible for the onset of the pathological condition. Combination drugs have the ability to increase efficacy through synergistic action of drug substances and reduce side effects [2,3]. Several essential oils have exhibited synergistic activity with antibiotics against microorganisms. Recent studies demonstrate the ability of linalool, which is the basic component of basil volatile oil to potentiate the antibacterial action of antibiotics, including fluoroquinolones, by decreasing the minimum inhibitory concentration of ciprofloxacin by about 4 times [2,3].

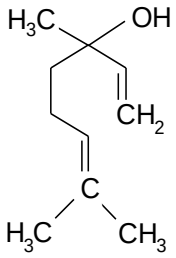
A new combination drug for the treatment of otitis was elaborated within the Scientific Center of Medicine ("Nicolae Testemitanu" State University of Medicine and Pharmacy, Republic of Moldova) under the name CB-12, containing two active substances: ciprofloxacin hydrochloride of 0.15 g/50 mL (0.3%) and volatile basil oil of 0.20 g/50 mL (0.4%). Currently, this dosage form is investigated experimentally for the development of analytical quality control documents.

Ciprofloxacin hydrochloride (Figure 1*(a)*), the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, is a fluoroquinolone antibiotic that possesses antibacterial activity with broad spectrum of action, and active against a large number of pathogenic germs. The fluoride ion from the molecule is responsible for broadening the antibacterial spectrum, while the piperazine cycle makes the drug substance active against *Pseudomonas* sp. [2-5]. Linalool (Figure 1*(b)*), 3,7-dimethyl-1,6-octadien-3-ol, known for its bactericidal and fungicidal properties, is the major

component of essential oils of several plants species, including *Ocimum basilicum* commonly known as basil [6,7]. *Ocimum basilicum* is widely distributed in Republic of Moldova, being used in folk medicines to treat various diseases including upper respiratory tract infections, headaches, eye problems, skin disease, pneumonia, coughs, fevers, and conjunctivitis [6,7].



(a)



(b)

Figure 1. The chemical structures of ciprofloxacin hydrochloride *(a)* and linalool *(b)*.

The assay of drug substances in dosage form is a mandatory requirement for verifying the quality of drugs. European, United States and Indian Pharmacopoeias require the use of the analytical method – reversed phase high performance liquid chromatography, while the British Pharmacopoeia, requires the non-aqueous titrimetry as the official method to determine ciprofloxacin hydrochloride [8-11]. Various other analytical methods such as high-performance liquid chromatography, Fourier transform infrared spectroscopy and differential electrolytic potentiometry have been reported in the literature for the estimation of ciprofloxacin hydrochloride [12-14]. The UV-Vis spectrophotometric method is unavailable as a pharmacopoeial method for quantitative determination of ciprofloxacin hydrochloride [8-15], thus, for routine analysis this simple, rapid and cost-effective analytical method would be preferred [16-18]. A few UV-Vis spectrophotometric methods for determination of ciprofloxacin hydrochloride in combined dosage forms: in tablets with ornidazole and in a combination with metronidazole have been previously reported [17,18]. However, up till now, a UV-Vis spectrophotometric method for the assay of ciprofloxacin hydrochloride in combination with essential oils is not available. Therefore, the development of a simple and accurate UV-Vis spectrophotometric method can provide a very useful alternative for routine analysis of ciprofloxacin hydrochloride in combinations with biologically active compounds from essential oils.

The purpose of this study was to develop and validate a simple, accurate and reproducible UV-Vis spectrophotometric technique to assay ciprofloxacin hydrochloride in CB-12 combination ear drops drug for the treatment of otitis that was elaborated within the Scientific Center of Medicine, "Nicolae Testemitanu" State University of Medicine and Pharmacy.

Experimental

Materials

Ciprofloxacin hydrochloride powder of 99.9%; volatile basil oil (essentially pure basil oil of 100%); polysorbate 20; polyethylene glycol 400 and methylparaben were purchased from Sigma-Aldrich, Germany. Citrate buffer pH 7.8 and 0.1 M hydrochloric acid solution prepared according to European Pharmacopoeia requirements [8]. Double distilled water was used throughout the entire study.

CB-12 combination ear drops, elaborated within the Scientific Center of Medicine, "Nicolae Testemitanu" State University of Medicine and Pharmacy, consisted of 0.15 g ciprofloxacin hydrochloride, 0.2 g volatile basil oil, 2.0 g polysorbate 20, 5.0 g polyethylene glycol 400, 0.02 g methylparaben, citrate buffer pH 7.8 as needed to obtain pH 5.0-7.5 and double distilled water to bring up to 50 mL.

The placebo solution consisted of 0.2 g volatile basil oil, 2.0 g polysorbate 20, 5.0 g polyethylene glycol 400, 0.02 g methylparaben, citrate buffer pH 7.8-8.0 as needed to obtain pH 5.0-7.5 and double distilled water to bring up to 50 mL.

Equipment

The Agilent 8453 UV-Vis spectrophotometer (Germany) and cuvettes with a pathlength of 10 mm were used in this study. The spectra were registered in the 220-400 nm range. The 0.1 M hydrochloric acid was used as blank solution.

The pH meter Consort C 861 model was used in this study.

Methods

The *stock solution A* was prepared by dissolving 0.05±0.0001 g of ciprofloxacin

hydrochloride in 50 mL of 0.1 M hydrochloric acid solution in a volumetric flask.

The *standard solutions* for the calibration curve were prepared by diluting the stock solution A with 0.1 M hydrochloric acid in a quadratic regression. Five solutions with concentration of 2, 4, 6, 8 and 10 μg/mL were prepared, the absorbance values were recorded at 278 nm. The measurements were performed in triplicate and were statistically evaluated.

For the preparation of *the placebo solution*, 2.0 g of polysorbate 20 were transferred into a porcelain cup to which 0.2 mL of volatile basil oil was added and then mixed well until the oil was emulsified. Further, 25 mL of purified water and excipients were added in portions to the obtained primary emulsion: 5.0 g of polyethylene glycol 400 and 0.02 g of methylparaben, which were first dissolved in a minimal amount of purified water (2.0 mL) and thoroughly mixed until a homogeneous emulsion was obtained. The pH of the emulsion was measured and adjusted with citrate buffer pH 7.8 to a value between 5.0-7.5. The obtained emulsion was transferred into a 50 mL volumetric flask and was dissolved in purified water (placebo stock). A volume of 1.0 mL of placebo stock was pipetted into a 250 mL volumetric flask and diluted up to the mark with 0.1 M hydrochloric acid solution. Then, 8 mL of the obtained solution were diluted to 25 mL with 0.1 M hydrochloric acid solution.

The *sample solution* was prepared by dissolving 1.0 mL of CB-12 ear drops solution in 250 mL of 0.1 M hydrochloric acid solution. Further, 8 mL of the obtained solution were diluted to 25 mL with 0.1 M hydrochloric acid solution.

The *accuracy of the method* was determined using three samples consisting of ciprofloxacin hydrochloride and a mixture of excipients in 0.1 M hydrochloric acid in concentrations corresponding to 80-120% of the stated amount. Three samples were prepared for each recovery level. The concentration of ciprofloxacin hydrochloride was determined using the calibration curve. The percentage of regression has been established by using Eq.(1).

$$R = \frac{C_t - C_p}{C_a} \times 100\% \quad (1)$$

where, *R*- recovery, %;

C_t- total concentration of the sample with the addition, μg/mL;

C_p- sample concentration without addition, μg/mL;

C_a- concentration of the standard addition, μg/mL.

The concentration of ciprofloxacin hydrochloride was determined by using Eq.(2).

$$x = \frac{A_1 \times b \times D \times 50}{A_2 \times a} \quad (2)$$

where, *X*- the amount of the active substance in the sample solution, g/50 mL;

A_1- absorbance value of the sample solution;

A_2- absorbance value of the standard solution;

D- dilution, mL;

a and *b*- sample and standard mass, g.

Repeatability was determined using six samples of CB-12 ear drops that were analysed on the same day and under the same conditions.

Intermediate precision was determined using six samples of ear drops, which were analysed on two different days. The concentration of ciprofloxacin hydrochloride from ear drops was measured. The concentration of ciprofloxacin hydrochloride was determined by Eq.(2).

The six samples prepared for the repeatability study were preserved for 24 h at room temperature and analysed on the following day to test the *short-term stability*. The concentration of ciprofloxacin hydrochloride was determined using Eq.(2).

The *robustness of the method* was investigated by varying the maximum absorption wavelength of ciprofloxacin hydrochloride (278 nm) with ±3 nm. The determinations were repeated 3 times for each wavelength.

Results and discussion

In order to determine the absorption maxima, the spectra of 4 μg/mL of standard and sample solutions of ciprofloxacin hydrochloride and placebo solution were recorded in the 220-400 nm wavelength range. The 0.1 M hydrochloric acid solution was used as the reference solution.

The spectra of the standard and sample solutions of ciprofloxacin hydrochloride 4 μg/mL presented the absorption maximum at 278 nm, whilst the spectrum of the placebo solution did not show absorption at 278 nm, which demonstrates the specificity of the method with regards to the placebo solution (Figure 2).

The validation of the method was carried out in accordance with the International Conference of Harmonization (ICH) Guide "Q2R1: For Analytical Procedures and Validation" [19] and the validation parameters such as linearity, limit of detection, limit of quantification, selectivity, accuracy, precision and robustness were determined.

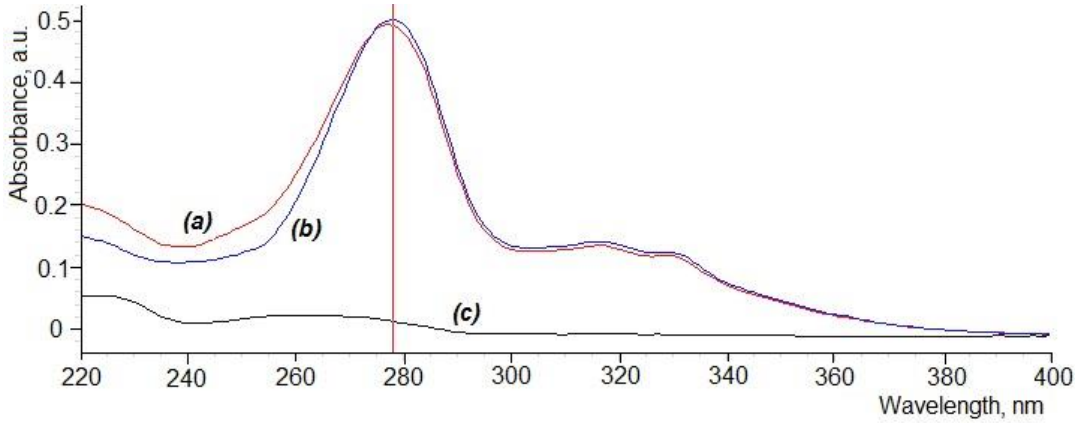


Figure 2. The UV-Vis absorption spectra of the standard solution of ciprofloxacin hydrochloride 4.0 µg/mL *(a)*, sample solution of ciprofloxacin hydrochloride 4.0 µg/mL *(b)* and placebo *(c)*.

A linear relationship was found between the absorbance and the concentration of ciprofloxacin hydrochloride. The linearity of the UV-Vis spectrophotometric method was determined in the concentration range of 2-10 µg/mL of ciprofloxacin hydrochloride, at λ= 278 nm. Based on the obtained results, the calibration curve was drawn and the equation of linear regression was obtained: $y= 0.116x + 0.048$, R^2= 0.999 (Figure 3).

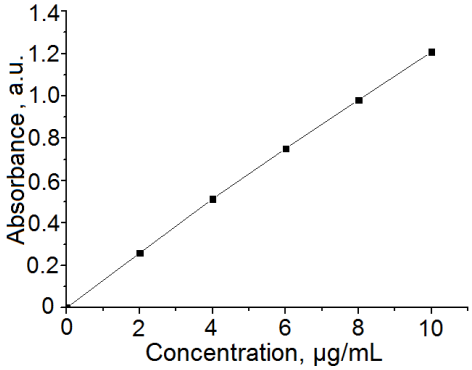


Figure 3. Calibration curve of ciprofloxacin hydrochloride in the concentration range between 2–10 µg/mL, at λ= 278 nm using in cuvettes with a 10 mm pathlength.

The value of the limit of detection calculated in accordance with the ICH guide is 0.786 µg/mL [19]. The value of the limit of quantification calculated in accordance with the ICH guide is 2.383 µg/mL [19].

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted as the reference value and the value found. Accuracy is expressed as the percentage of recovered analyte compared to actual values (Table 1).

The UV-Vis spectrophotometric method developed for the assay of ciprofloxacin hydrochloride in CB-12 combined ear drops is accurate, with an average recovery value ranging from 99.0% to 101.0% (Table 1). The admissibility condition is at least 99.0% [19].

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [19]. Precision is generally expressed by standard deviation and standard relative deviation.

Repeatability of the method expresses the precision under the same operating conditions over a short interval of time [19]. The mean value of the concentration of ciprofloxacin hydrochloride in CB-12 combined ear drops was 0.15492 g/50 mL.

Intermediate precision expresses within-laboratories variations, and in the present study accounted for different days [14]. The mean value of the concentration of ciprofloxacin hydrochloride in the pharmaceutical dosage form for the intermediate precision study was 0.15493 g/50 mL.

The developed UV-Vis spectrophotometric method is precise, as the admissibility condition for precision is relative standard deviation (RSD) ≤2% [19]. The results of the statistical evaluation are presented in Table 2.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [19]. The obtained results indicate that the elaborated method is robust as insignificant changes of absorbance at the examined wavelengths were detected (Table 3), acceptance criterion is RSD≤ 2% [19].

The results of the short-term stability were within the acceptance range. Also, the results show that the samples are stable at room temperature for 24 h, acceptance criterion is RSD≤ 2% [19] (Table 4).

Table 1

Recovery as a condition of admissibility.

No.	*The amount of ciprofloxacin hydrochloride taken into work*		*Amount of ciprofloxacin hydrochloride, μg/mL (average for n= 3)*	*Recovery, %*
	%	*μg/mL*		
1	80	8.00	8.05	100.63
2	100	10.00	9.94	99.40
3	120	12.00	12.08	100.66

Table 2

Results of repeatability and intermediate precision.

No.	*Concentration of ciprofloxacin hydrochloride in ear drops, g/50 mL*	
	Repeatability	*Intermediate precision*
1	0.1552	0.1549
2	0.1547	0.1549
3	0.1548	0.15492
4	0.1549	0.1548
5	0.1549	0.1549
6	0.1550	0.1549
Average	0.1549	0.1549
Standard deviation	0.0002	0.0001
Relative standard deviation, %	0.1159	0.0218

Table 3

Results of the robustness of UV-Vis spectrophotometric method of assay of ciprofloxacin hydrochloride in combination ear drops.

No.	*Absorbance values of ciprofloxacin hydrochloride, 4μg/mL*		
	275 nm	*278 nm*	*281 nm*
1.	0.4587	0.4612	0.4597
2.	0.4586	0.4617	0.4596
3.	0.4588	0.4614	0.4599
Average	0.4587	0.4616	0.4597
Standard deviation	0.0001	0.0002	0.0001
Relative standard deviation, %	0.0310	0.0520	0.0320

Table 4

Results of the short-term stability determined by the proposed method (n= 6).

No.	*Concentration of ciprofloxacin hydrochloride in ear drops, g/50 mL*	
	Declared	*Found*
1	0.1500	0.1542
2	0.1500	0.1541
3	0.1500	0.1541
4	0.1500	0.1539
5	0.1500	0.1548
6	0.1500	0.1540
Average	0.1500	0.1542
Standard deviation		0.0002
Relative standard deviation, %		0.1909

This study presented a simple UV-Vis spectrophotometric method for quantitative determination of ciprofloxacin hydrochloride in a combination dosage (CB-12 ear drops) form compared with another technique, in which second derivative ratio spectrophotometry technique was applied [18]. Close results were obtained with the method presented by Krishna, J.M. *et al*. [17]. The difference is that in the present study 0.1 M hydrochloric acid solution was used as blank to determine the ciprofloxacin hydrochloride in the presence of volatile basil oil in ear drops, whilst in the method presented by Krishna, J.M. *et al*. purified water was used to detect ciprofloxacin hydrochloride and ornidazole in tablets [17].

Conclusions

This study presents the development and validation of a UV-Vis spectrophotometric method of ciprofloxacin hydrochloride assay in CB-12 combination ear drops. The results of the validation of the method demonstrate that the developed method is simple, rapid, accurate and robust over the concentration range 2-10 µg/mL. Linearity was obtained with a correlation coefficient of 0.999. The value of the limit of detection was 0.786 µg/mL and of the limit of quantification was 2.383 µg/mL. The percentages of recovery of ciprofloxacin hydrochloride in CB-12 combined ear drops were more than 99.0%. The relative standard deviation values of precision and robustness were less than 2%. The results of the short-term stability show that the samples are stable at room temperature for 24 h.

The developed and validated UV-Vis spectrophotometric method within the concentration range of 2-10 µg/mL ciprofloxacin hydrochloride could be included in the Analytical Quality Control documents of the combined ear drops containing ciprofloxacin hydrochloride and volatile basil oil.

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