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Quantitative Analysis of Ciprofloxacin in an Ophthalmic Solution Using UV Absorption Spectrophotometry and Derivative Spectrophotometry

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Abstract: The quantitative analysis of ciprofloxacin in an ophthalmic solution was performed by UV absorption spectrophotometry and first derivative spectrophotometry. The UV absorption spectra of standard series and samples containing ciprofloxacin were recorded. Then, the derivative of UV absorption spectra was obtained for the derivative spectrophotometric analysis of the related drug. Linear regression equations in the linear concentration range of 3.0-28.0 µg/mL for analysis of ciprofloxacin were obtained by using direct absorbance measurement at 278.9 nm and using the dA/dλ values at 283.2 nm. The recovery assay results were found between 97.8 to 102.0 %. It was observed that the limits of detection and quantification were 0.74 and 2.74 µg/mL for UV absorption spectrophotometry and 0.60 and 2.00 µg/mL for first derivative spectrophotometry, respectively. Analysis results indicated that the proposed methods were precise, accurate and reliable for the determination of ciprofloxacin in commercial samples.

***Keywords**:* Ciprofloxacin, Spectrophotometry, Derivative spectrophotometry, Ophthalmic solution, Quantitative analysis

UV Absorbans Spektrofotometrisi ve Türev Spektrofotometrisi Kullanılarak Oftalmik Çözeltilerde Siprofloksasinin Kantitatif Tayini

Özet: Bu çalışmada oftalmik çözeltide siprofloksasinin kantitatif tayini için UV absorbans spektrofotometrisi ve türev spektrofotometrisi kullanılmıştır. Siprofloksasin içeren standart serinin ve numunelerin UV absorbans spektrumları kaydedilmiştir. Daha sonra türev spektrofotometrisi için, UV absorbans spektrumunun birinci türevi alınmıştır. 3.0-28.0 µg/mL doğrusal çalışma aralığında olmak üzere, 278.9 nm'deki absorbans değerleri, ve 283.2 nm'deki dA/dλ değerleri kullanılarak lineer regresyon eşitlikleri elde edilmiştir. Geri kazanım çalışmaları sonuçları % 97.8 ile % 102.0 arasında hesaplanmıştır. Yakalama ve tayin sınır değerleri, UV absorbans spektrofotometrisi için 0.74 µg/mL ve 2.74 µg/mL; türev spektrofotometrisi için 0.60 µg/mL ve 2.00 µg/mL olarak hesaplanmıştır. Sonuçlar, siprofloksasinin ticari örneklerden analizi için önerilen yöntemlerin kesin, doğru ve güvenilir olduğunu göstermiştir.

***Anahtar kelimeler**:* Siprofloksasin, Spektrofotometri, Türev spektrofotometrisi, Oftalmik çözelti, Kantitatif analiz

1. Introduction

Ciprofloxacin is an anti-infective agent which belonging to the class of fluoroquinolones. Beside its systemic use, it is also indicated for the bacterial eye infections. It affects by

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inhibiting DNA-gyrase [1]. Ophthalmic ciprofloxacin solution is commonly used by the patients, so development of proper analysis methods for ciprofloxacin is an important task in order to perform quality control tests. The aim of this study is to develop a new, rapid, sensitive, accurate and precise spectrophotometric method to quantify ciprofloxacin in its ophthalmic dosage form.

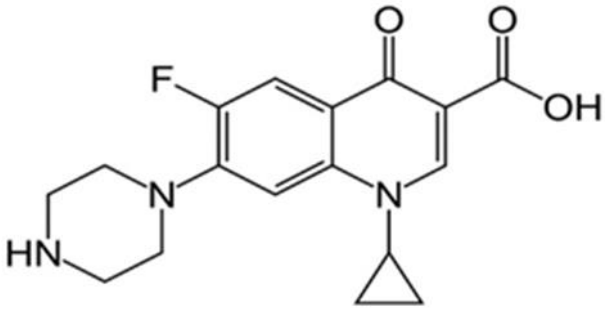

Figure 1. Chemical structure of ciprofloxacin

Some analytical techniques consisting of spectrophotometric method [2, 3], HPLC [4-8] capillary electrophoresis [9], flow injection analysis [8], LC-MS [10], electrochemical analysis [11-13] were reported for the determination of ciprofloxacin in samples. The quantitative determination and quality control of drug formulations have very significant role for the drug industry and human health. Active compounds with a constant amount of excipients, which may give overlapping spectral signals with main active compound, was used in drug formulations. In such case, derivative spectrophotometry is a very important technique to get precise, accurate and reliable results for the analysis of pharmaceutical formulation.

In this presented work, direct absorbance measurement and first derivative spectrophotometry was applied for the analysis of ciprofloxacin in an ophthalmic solution. The proposed direct absorbance measurement and first derivative spectrophotometric methods are based on the use of the measurements of absorbance at 278.9 nm and $dA/d\lambda$ value at 283.2nm. The ability of the methods was tested by analyzing artificial test samples of the related analyte. Successful assay results were obtained by using both direct absorbance measurement and first order derivative spectrophotometry to the analysis of ciprofloxacin in commercial ophthalmic solution.

2. Material and Method

2.1 Apparatus and software

The UV absorption spectra of calibration set, test samples and ophthalmic solution samples were recorded by using a Shimadzu 1601 double beam spectrophotometer connected to a desktop computer loaded with Shimadzu UVPC software. Data treatments and spectral plots were carried out using Microsoft Excel Software and Matlab Software.

2.2 Standard, calibration and validation solutions

Standard stock solution was prepared by dissolving 10.0 mg of ciprofloxacin in 100 mL calibrated flask in methanol. Calibration solutions of ciprofloxacin in the concentration range of 3.0-28.0 μg/mL were obtained from the standard stock solution. For the validation of method, test samples in the working concentration of 3.0-28.0 μg/mL and standard addition samples in three different concentration levels 5.0, 10.0, 15.0 μg/mL were prepared by using the above stock solution of ciprofloxacin

2.3 Pharmaceutical preparation

A commercial ophthalmic solution (Ciloxan® drugs produced by Alcon.Pharm.Ind., Istanbul, Turkey) containing 3.5 mg/mL ciprofloxacin per drug was analyzed.

2.4 Analysis procedure of ophthalmic formulation

For the quantitative analysis of commercial preparation containing 3.5 mg ciprofloxacin per mL, 1 mL of ophthalmic solution sample was taken and dissolved in methanol in 25 mL volumetric flask. The sample solution was diluted by a factor of 10 to get 14 µg/mL which is in the calibration range of analyte. UV absorption spectra were recorded for the application of the proposed methods to the spectral analysis of the related drug.

3. Results and Discussion

In this paper, the UV absorption spectra of ciprofloxacin in the concentration range of 3.0-28.0 µg/mL were recorded between 200-400 nm to get calibration curve. The UV absorption spectra of the analyzed drug in the mentioned concentration range were presented in Figure 2.

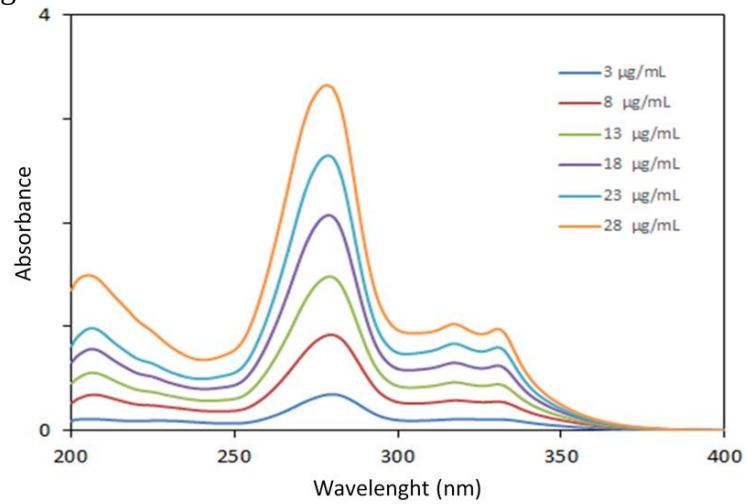

Figure 2. UV absorption spectra of ciprofloxacin in the concentration range of 3.0-28.0 µg/mL

The application of the UV absorption spectrophotometry also named direct absorbance measurement method was proposed for the quantification of ciprofloxacin in ophthalmic solution. In the application of this UV absorption spectrophotometry, the calibration curve for the related drug was computed from least squares regression analysis based on the use of the concentration set and absorbance at 278.9 nm in the UV absorption spectra. The first method gave a linear response to ciprofloxacin from 3.0 to 28.0 µg/mL. Least squares regression analysis and statistical results were displayed in "Table 1". The determination of ciprofloxacin in samples was performed by using the above calibration equation.

Table 1. Linear regression equations and their corresponding parameters for both methods

Parameter	**UV absorbption spectroscopy**	**First order derivative spectroscopy**
Wavelength	278.9 nm	283.2 nm
Concentration range	3.0-28.0 µg/mL	3.0-28.0 µg/mL
Regression equation	A = 0.1177x -0.0275	$dA/d\lambda$ = 0.0607x - 0.0230
Regression coefficient	0.9996	0.9997
Standard deviation of the slope	1.64×10^{-3}	6.87×10^{-4}
Standard deviation of the intercept	2.90×10^{-2}	1.22×10^{-2}
Limit of detection	0.74 µg/mL	0.60 µg/mL
Limit of quantitation	2.74 µg/mL	2.00 µg/mL

In this article, an alternative method is first derivative spectrophotometric method for the quantitative analysis of ciprofloxacin in commercial samples. In the implementation of the derivative method, first derivative spectra of the UV absorption UV spectra of ciprofloxacin and its samples was obtained by using the interval of $\Delta\lambda$=8 nm in the wavelength range of 200-400 nm. First derivative spectra obtained were smoothed by applying the smoothing function of $\Delta\lambda$= 8 nm. First derivative spectra of ciprofloxacin in the concentration range of 3.0 to 28.0 µg/mL were indicated in Figure 3. It was observed that the quantity of ciprofloxacin was proportional to the dA/dλ values at 283.2 nm. Using the relationship between concentration and dA/dλ values, linear regression function and its statistical results were illustrated in "Table 1". Ciprofloxacin in commercial ophthalmic solution was analyzed by using the mentioned linear regression equation.

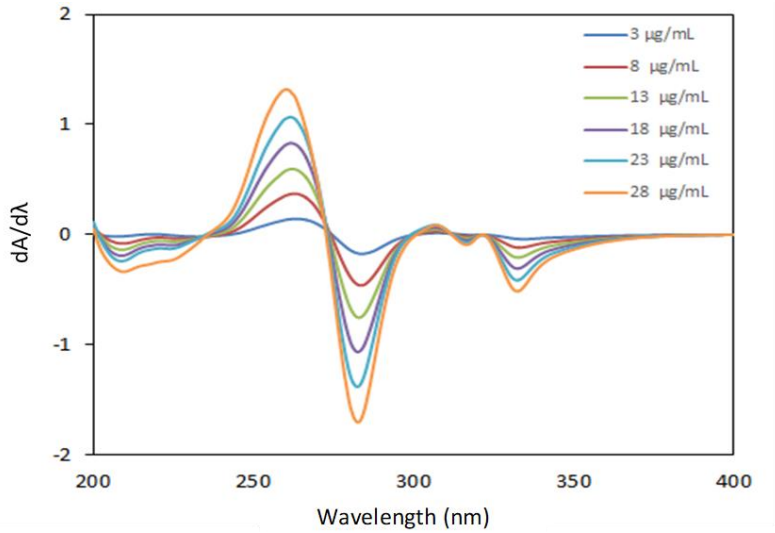

Figure 3. First derivative spectra of ciprofloxacin in the concentration range of 3.0-28.0 µg/mL

3.1 Validation of the method

Before the analysis of ciprofloxacin in an ophthalmic solution, the proposed methods were checked by using validation parameters. Detection and quantitation limits, linearity, accuracy and precision and sample recovery were assessed. An acceptable linearity with high correlation coefficients was reported for the application of UV absorption spectrophotometry and first derivative method to the quantitation of ciprofloxacin in the working concentration range from 3.0 to 28.0 µg/mL. For the calibration curves, the correlation coefficients (r) were found as 0.9996 and 0.9997 indicating good linearity, respectively. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by multiplying the ratio of standard deviation of intercept and the slope by 3 and 10, respectively. The numerical values of the LOD and LOQ were given in "Table 1". In order to visualize the accuracy and precision of the purposed methods, an independent sample set of ciprofloxacin was prepared and analyzed. Recovery results, standard deviation and relative standard deviation were computed for the assessment accuracy and precision for both UV absorption spectrophotometric and first derivative methods. Their analysis results were listed in "Table 2". In the application of both methods, relative standard deviations between five different samples were found to be 2.11 % and 1.70 %, corresponding to method repeatability or precision, respectively.

Table 2. Recovery results of synthetic solutions by UV absorption and derivative spectrophotometry

	UV absorption spectroscopy		**Derivative spectroscopy**	
Added (µg/mL)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)
7	7.14	102.0	7.02	100.3
14	13.69	97.8	13.62	97.3
21	20.91	99.6	21.03	100.1
	Mean	99.8		99.3
	Standard deviation	2.11		1.68
	Relative standard deviation	2.11		1.69

Additionally, to visualize presence or absence of the effect of ophthalmic solution excipients on the analysis of ciprofloxacin, the proposed methods were applied to the analysis of standard addition samples. Added recovery, standard deviation and relative standard deviation were obtained. The analysis results were presented in "Table 3". The recovery results of standard addition samples for both methods were close to 100 %, which means the excipients of ophtalmic solution had no effect on the analysis.

Table 3. Recovery results of standard addition samples for UV absorption and derivative spectrophotometry

		UV absorption spectroscopy		**Derivative spectroscopy**	
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Found (µg/mL)	Recovery (%)
Formulation +	5	4.83	96.5	4.87	97.4
Formulation +	10	9.64	96.4	9.64	96.4
Formulation +	15	14.46	97.0	14.60	97.3
		Mean	96.6		97.0
		Standard deviation	0.36		0.54
		Relative standard deviation	0.35		0.56

3.2 Analysis of ophthalmic formulation

In the application of the methods to real sample analysis, direct absorbance measurement and derivative methods were utilized for the quantitative determination of ciprofloxacin in ophthalmic solution.The label claim for the commercial ophthalmic solution was 3.50 mg/mL. Assay results of ciprofloxacin in ophthalmic solution were shown in "Table 4". As it can be seen from this table, UV absorption spectrophotometry and first derivative method were found suitable for the analysis of ciprofloxacin in commercial ophthalmic samples with high accuracy and precision. In order to evaluate the analysis results, t-test at significance level of 0.05 was performed by using the label claim 3.50 mg/mL and the mean value of the results from ten analyses. Calculated t-values for both methods were smaller than critical value. Statistical comparison of UV absorption spectroscopy and first order derivative spectroscopy was also performed by t-test and these two methods were reported not to be statistically different.

Table 4. Analysis results of ophthalmic fomulation

Nr.	**UV absorbption spectroscopy** mg/mL	**First order derivative spectroscopy** mg/mL
1	3.41	3.43
2	3.48	3.45
3	3.46	3.51
4	3.54	3.52
5	3.48	3.45
6	3.46	3.45
7	3.44	3.46
8	3.41	3.48

9	3.44	3.47
10	3.49	3.46
Mean	3.46	3.47
Standard deviation	0.04	0.03
Relative standard deviation	1.14	0.81

4. Conclusion and Comment

In this study, two different spectrophotometric methods were developed, validated and succesfully applied to the analysis of ciprofloxacin ophtalmic solution. It was concluded that both UV absorption spectrophotometry and first order derivative spectrophotometry were suitable for the quantitation of ciprofloxacin in a commercial ophthalmic solution and were statistially comparable. The applied methods were simpler and faster than the the literature methods involving more sophisticated instruments and were highly reliable for the analysis of ciprofloxacin in ophthalmic solutions. It was observed that the proposed methods can be applied to the quantitative analysis and reliable routine analysis of commercial ophthalmic solution containing ciprofloxacin drug.

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