Derivative Spectrophotometric Determination for Simultaneous Estimation of Isoniazid and Ciprofloxacin in Mixture and Pharmaceutical Formulation

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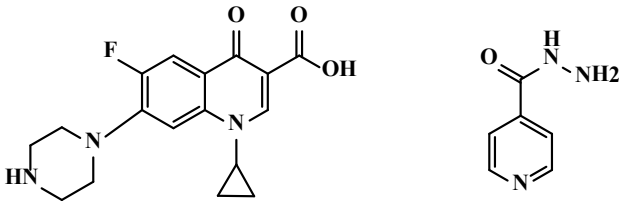
A simple analytical method was used in the present work for the simultaneous quantification of Ciprofloxacin and Isoniazid in pharmaceutical preparations. UV-Visible spectrophotometry has been applied to quantify these compounds in pure and mixture solutions using the first-order derivative method. The method depends on the first derivative spectrophotometry using zero-cross, peak to baseline, peak to peak and peak area measurements. Good linearity was shown in the concentration range of 2 to 24 μg·mL^{-1} for Ciprofloxacin and 2 to 22 μg·mL^{-1} for Isoniazid in the mixture, and the correlation coefficients were 0.9990 and 0.9989 respectively using peak area mode. The limits of detection (LOD) and limits of quantification (LOQ) were measured with first derivative method. The LOD and LOQ were found as 0.45 μg·mL^{-1} and 1.50 μg·mL^{-1} for Ciprofloxacin and 0.68 μg·mL^{-1} and 2.28 μg·mL^{-1} for Isoniazid, respectively. Accuracy and precision were determined by measuring the relative standard deviation and recoveries. The results also showed that the proposed method was successfully applied for direct analysis of ciprofloxacin and isoniazid in the tablet samples.

Keywords: ciprofloxacin, isoniazid, simultaneous determination, pharmaceutical formulations, first derivative spectrophotometry

Antibiotics are the most potent among drugs and have been used for killing or reducing the growth of bacteria [1-3]. Ciprofloxacin is one of the most widely used groups of an antibacterial agent and has been extensively used for the treatment of lung infections [4]. Ciprofloxacin is an important antibiotic for both humans and animals, due to its safety and low cost, however, resistance to this drug makes it less effective [5, 6]. It also used to treat respiratory tract infections [7]. Isoniazid is an antibiotic that can be used for stopping the growth of bacteria. It is a synthesized drug and plays a vital role in the killing of bacterium mycobacterium tuberculosis [8]. On the other hand, isoniazid can be extremely harmful to human beings by may causing liver toxicity and peripheral neuropathy [9]. Chemical structures of ciprofloxacin and isoniazid are shown in Figure 1.



Ciprofloxacin Isoniazid

Fig. 1. The chemical structures of ciprofloxacin and isoniazid.

Several analytical methods have been used to determine the ciprofloxacin and isoniazid in different pharmaceutical forms. Rekha and co-workers have determined the ciprofloxacin in some pharmaceutical preparations using the derivative UV-spectrophotometric method [10]. This method was also developed and used to estimate the ciprofloxacin hydrochloride in the drug-resin complex [11]. In the study by *Sakur et al*., ciprofloxacin was determined in pure and mixed forms of pharmaceutical preparations [12]. In another study, the RP-HPLC method using UV detection has been applied to quantify the ciprofloxacin in various pharmaceutical forms, such as similar, generic and compounded [13]. The UV-Visible method was applied successfully to determine the isoniazid in some pharmaceutical samples including tablets and human serum [14]. Tow UV spectrophotometric methods (employing simultaneous equation and first derivative) were developed by *Tilinca* and co-workers for determination of isoniazid in mixture sample contains isoniazid and rifampicin [15]. HPLC using a diode array detector method was developed for quantification analysis of isoniazid in a mixture of pharmaceutical formulations [16]. In the study by *Rastogi et al*., an electrochemical method using silver nanoparticles has also been used to determine isoniazid in pharmaceutical formulations [17]. A mixture of isoniazid and acetaminophen was also determined by *Zhang et al*., using a voltammetric method [18].

This study aims to use a simple analytical method for the simultaneous determination of Ciprofloxacin and Isoniazid in pharmaceutical preparations. The main advantage of this work is to develop a simple, fast and sensitive derivative spectrophotometric method that can be used to determine the pharmaceutical compounds in a mixture which cannot be determined by other analytical methods due to their spectra interference.

Experimental part

Apparatus. UV-Visible model 1800 (Kyoto-Shimadzu, Japan) double beam spectrophotometer was used. The absorbance of blank and samples was recorded using a 1 mm quartz cell. The wavelength ranges used for all samples were 200-800 nm.

Chemicals and reagents. All solutions were prepared in distilled water. Pure Ciprofloxacin and Isoniazid, and glucose, fructose, lactose, and sucrose were manufactured by the State for Drugs and Medical Appliances (S.D.I.), Samarra, Iraq. Ciprofloxacin tablets containing 500 mg ciprofloxacin per tablet were supplied by the United Arab Emirates (U.A.E.). Isoniazid tablets containing 100 mg Isoniazid per tablet were supplied by India.

Standard and sample preparation. Stock solutions of both Ciprofloxacin and Isoniazid were prepared by dissolving 0.01 g in 100 mL distilled water. From this stock solution, many standard solutions of ciprofloxacin and isoniazid were prepared in the range of 2-24 μg·mL^{-1} and 2-22 μg·mL^{-1}, respectively.

Ten tablets of each compound were powdered and mixed. After that, the average weight equivalent to one tablet was accurately weighed and dissolved in 100 mL distilled water. The solutions then were filtered and diluted to obtain 100 μg·mL^{-1} of each drug.

General procedures. The absorbance of all prepared standard solutions (2-24 μg·mL^{-1} of Ciprofloxacin and 2-22 μg·mL^{-1} of Isoniazid) was measured by the zero-order method using the range of UV-Visible scanning (190-400 nm) to obtain the maximum wavelength for both drugs. Many mixture solutions of both drugs were prepared from the pure standard (100 μg·mL^{-1}). The first set of mixtures were prepared by transferring different volumes of Isoniazid (4, 6, 8, 14, 16, 18, 20 mL) to seven calibrated flasks (5 mL) containing the constant volume of Ciprofloxacin (0.3 mL). The second set of mixtures were prepared by transferring the same range of volumes of Isoniazid to seven calibrated flasks (5 mL) containing another constant volume of Ciprofloxacin (0.7 mL). The third set of mixtures were prepared by transferring different volumes of Ciprofloxacin (2, 4, 6, 8, 10, 12, 14 mL) to seven calibrated flasks (5 mL) containing the constant volume of Isoniazid (0.1 mL). The fourth set of mixtures were prepared by transferring the same range of volumes of Ciprofloxacin to seven calibrated flasks (5 mL) containing another constant volume of Isoniazid (0.6 mL). All these mixtures were diluted by distilled water and measured and then manipulated to obtain first derivative spectra.

Results and discussion

Absorption spectra. The λ_{max} values of ciprofloxacin, isoniazid and the mixture of both compounds were determined at 274, 262 and 270 nm respectively, Figure 2. Absorbance spectra of blank (water) were recorded before any measurement of the sample.

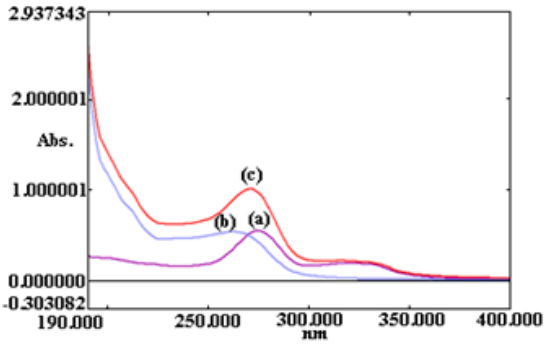


Fig. 2. Zero-order spectra of 6 μg·mL^{-1} Ciprofloxacin (a), 16 μg·mL^{-1} Isoniazid (b) and the mixture of both (c).

First derivative modes. Zero-order absorption was unable to determine the Ciprofloxacin and Isoniazid at the same time in the mixture solution. Therefore, the first-order derivative method was successfully used to show good spectra for individual and mixture compounds (Fig. 3).

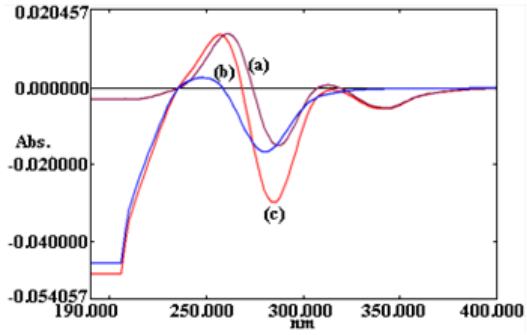


Fig. 3. The first-order derivative spectra of 6 μg·mL^{-1} Ciprofloxacin (a), 16 μg·mL^{-1} Isoniazid (b) and mixture of both (c).

Calibration plots. To find out the derivative spectra values, several graphical modes were used, in particular, zero-cross, peak to baseline, peak to peak and peak area. In this set of analysis, each compound of ciprofloxacin and isoniazid was measured in the individual and mixture solutions. A good linear correlation was shown between the absorbance of zero-order and concentrations of Ciprofloxacin ($y=0.0909 \cdot C+0.0016$, $R^2=0.9992$) and Isoniazid ($y=0.0255 \cdot C+0.132$, $R^2=0.9978$), Figure 4. Analytical parameters for the determination of both compounds using the first derivative method were shown in Tables 1 and 2.

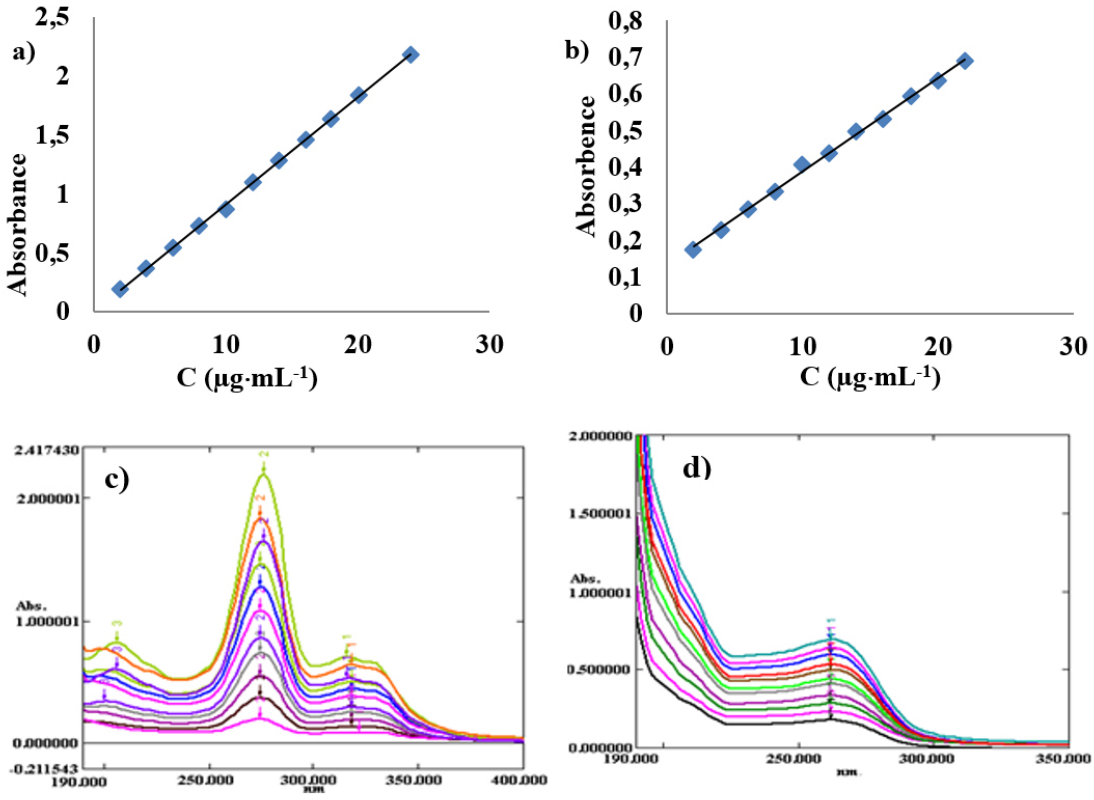


Fig. 4. Calibration curves of and spectra of Ciprofloxacin (2-24 µg·mL^{-1}) (a, c) and Isoniazid (2-22 µg·mL^{-1}) (b, d) using zero-order method.

Table 1. Determination of Ciprofloxacin (2-24 µg·mL^{-1}) in the presence of 18 µg·mL^{-1} Isoniazid using the first derivative method.

Analysis methods	λ (nm)	Regression equation	Correlation coefficient
Zero-cross	236.38	——	——
	274.70	——	——
Peak to baseline	219.87	y = 0.0004 x-0.0200	0.9992
	262.01	y = 0.0023x-0.0033	0.9990
	288.03	y = 0.0023 x-0.0156	0.9984
	314.97	y = 0.0006 x-0.0013	0.9988
Peak to peak	219.87-262.01	y = 0.0027 x-0.0166	0.9992
	262.01-288.03	y = 0.0047 x-0.0123	0.9988
Peak area	239-272	y = 0.0288 x-0. 2190	0.9990
	272-304	y = -0.505x-0. 1332	0.9986
	304-326	y = 0.0057 x-0. 0126	0.9977
	326-360	y = -0.0102 x-0. 0039	0.9984

Table 2. Determination of Isoniazid (2 - 22 µg. mL^{-1}) in the presence of 6 µg·mL^{-1} Ciprofloxacin using the first derivative method.

Analysis methods	λ (nm)	Regression equation	Correlation coefficient
Zero-cross	235.82	——	——
	280.72	——	——
Peak to baseline	249.00	y = 0.0001 x - 0.0069	0.9962
	280.00	y = -0.0008 x - 0.0133	0.9990
Peak to peak	249.02-280	y = 0.001 x - 0. 0202	0.9994
	228- 266	y = 0.0132 x - 0. 0811	0.9989
	266- 300	y = -0.0108 x - 0. 38	0.9996

The results obtained from the analysis of Ciprofloxacin (2-24 μg·mL^{-1}) in the presence of Isoniazid (18 μg·mL^{-1}) and analysis of Isoniazid (2-22 μg·mL^{-1}) in the presence of Ciprofloxacin (6 μg·mL^{-1}) using first derivative spectra are shown in Figure 5.

Validation of method. A simple statistical analysis was used to investigate the accuracy and precision of the proposed method. Several concentrations of each compound were measured five times using the first derivative method. This analysis has shown that the method is suitable for the simultaneous determination of ciprofloxacin and isoniazid in preparation samples (Table 3). Other validation values including the LOD and LOQ were measured. The LOD and LOQ were found 0.45 μg·mL^{-1} and 1.50 μg·mL^{-1} for Ciprofloxacin and 0.68 μg·mL^{-1} and 2.28 μg·mL^{-1} for Isoniazid, respectively. These results were compared to other sensitive method such as HPLC in order to describe the sensitivity characteristics in the suggested method to determine these compounds in pharmaceutical formulations. Elkady and co-worker have determined the Ciprofloxacin hydrochloride in tablet using RP-HPLC method and the LOD and LOQ were found as 0.08 μg·mL^{-1} and 2.43 μg·mL^{-1}, respectively [19]. It is also encouraging to compare the validation method of this study with that found by Kumari and co-workers who found that the LOD and LOQ Isoniazid in pharmaceutical formulations were 0.5 μg·mL^{-1} and 1.75 μg·mL^{-1} respectively using RP-HPLC method [20].

Effect of interferences. Different substances such as glucose, fructose, lactose, and sucrose were used to study the effect of interferences on the quantification of Ciprofloxacin and Isoniazid. These four substances were chosen in this study because they are common used in pharmaceutical industry and they are available in the local laboratory.

Stock solution (1000 μg·mL^{-1}) of interferences was prepared in a 5 mL calibrated flask. 0.5 mL of each interferences was added to the sample solution containing 8 μg·mL^{-1} of Ciprofloxacin. 0.5 mL of each interference was also added to the sample solution containing 16 μg·mL^{-1} of isoniazid. There were no significant differences in interferences on the proposed method (Table 4).

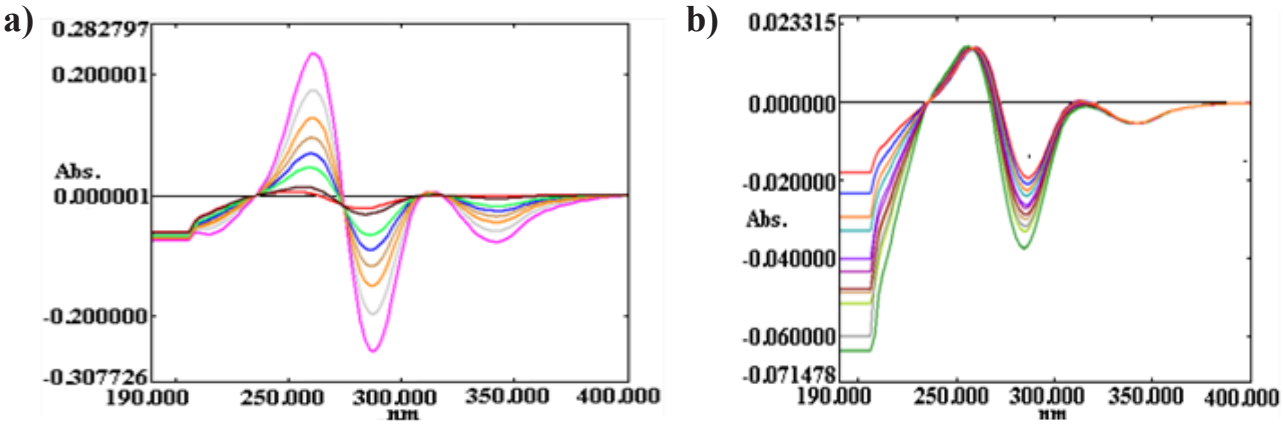


Fig. 5. The first derivative spectra for each drug in the mixture, Ciprofloxacin (2 - 24 μg. mL^{-1}) in the presence of Isoniazid (18 μg·mL^{-1}) (a) and Isoniazid (2-22 μg·mL^{-1}) in the presence of Ciprofloxacin (6 μg·mL^{-1}) (b).

Table 3. Accuracy and precision for the determination of Ciprofloxacin and Isoniazid in a mixture using the first derivative method (n=5).

Drug	Analysis method	λ (nm)	Concentration (μg·mL^{-1})		RSD (%)	Recovery (%)
			Taken	Found		
Ciprofloxacin	Peak to baseline	262	4	4.06	1.70	101.51
			10	9.95	0.45	99.52
	Peak area	272-304	4	4.02	1.06	100.68
			10	9.99	0.02	99.99
Isoniazid	Peak to baseline	280	8	8.06	0.29	100.31
			16	15.96	0.37	99.76
	Peak area	266-300	8	7.99	1.25	99.99
			16	15.90	0.49	0.4992

Analytical applications of the proposed method in pharmaceutical samples. The first derivative method was used in this study to determine the Ciprofloxacin and Isoniazid in their pharmaceutical tablets. The concentrations of both taken and found samples were compared to indicate whether a matrix effect occurs. The results, as shown in Table 5, indicate that the proposed method was successful to analyse the content of these compounds in pharmaceutical samples. The developed method in this study can also be applied to determine the other Isoniazid and Ciprofloxacin tablets which are manufactured by other companies such as Merck, Germany and Sigma-Aldrich, USA [15, 21]. *Tilinca* and co-workers have developed the first derivative method to determine Isoniazid in pharmaceutical solutions and the analytical parameters such as correlation coefficient, LOD, LOQ, recovery and RSD were measured and found to be 0.998, 1.30 μg·mL^{-1}, 4.30 μg·mL^{-1}, 98.90-99.32 % and 0.73-1.04 %, respectively, while the RSD value for Isoniazid in the tablet was 0.87 [15]. In the study by *Hashemi et al*., Ciprofloxacin was determined in tablet samples and LOD, recoveries and RSD were found to be 1.50 μg·L^{-1}, 90.0-96.4 % and 4-5.6 %, respectively [21]. The results, as shown in this study, indicate that the LOQ of active ingredients that can be determined by this method is 1.50 μg·mL^{-1} for Ciprofloxacin and 2.28 μg·mL^{-1} for Isoniazid.

Table 4. Effect of interferences on the determination of Ciprofloxacin and Isoniazid.

Drug	Interferences	Concentration (μg·mL^{-1})		Recovery (%)
		Taken	Found	
Ciprofloxacin	Glucose	8	8.07	100.88
	Fructose		8.10	101.35
	Lactose		8.00	100.07
	Sucrose		8.00	100.01
Isoniazid	Glucose	16	16.01	100.07
	Fructose		16.01	100.07
	Lactose		16.00	100.01
	Sucrose		16.00	100.00

Table 5. First derivative determination of Ciprofloxacin and Isoniazid in pharmaceutical samples.

Sample (Tablets)	Analysis method	λ (nm)	Concentration (μg·mL^{-1})		RSD (%)	Recovery (%)
			Taken	Found		
Ciprofloxacin	Peak to baseline	262.01	8	7.96	0.24	99.52
Isoniazid		249.00	16	16.03	0.01	100.18

Conclusions

The first derivative method was used in this study due it being a simple and direct analysis for the determination of a mixture of pharmaceutical preparation samples. The results of this analysis indicate that the method is able for simultaneous quantification of Ciprofloxacin and Isoniazid in pure form and preparations using the first derivative method. The findings of this study have also shown that the first-order derivative method has a good spectrum for individual and mixture compounds. In the future investigation, the components of drugs in a mixture can be determined directly by this proposed method without the need for separation. This will give more information to confirm the presence of a drug in a mixture. Further research should be carried out to determine the studied drugs using ratio first derivative method.

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