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Research Article

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Development and validation of a UV spectrophotometric method for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in binary mixture

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ABSTRACT

A new, simple, accurate and sensitive UV spectrophotometric method was adopted and validated for the quantitative determination of ciprofloxacin hydrochloride (CIP) and metronidazole (MET), simultaneously, either in pure form or in pharmaceutical dosage form. Second derivative ratio spectrophotometry technique (^{2}DD) was applied, by measuring the amplitude of the maximum at 253 nm, using a normalized spectrum of MET as divisor, for the determination of CIP. MET was quantified by measuring the amplitude of the minimum at 301 nm, using the normalized spectrum of CIP as divisor. Linearity was obtained over the concentration range 2 – 16 μg mL^{-1} and 4 – 16 μg mL^{-1}, for CIP and MET, respectively. Mean percentage accuracy was found to be 100.39 ± 0.677 and 100.21 ± 0.982, for CIP and MET, respectively. The propose d procedure was successfully applied for laboratory prepared mixtures without prior separation. The percentages recoveries of CIP and MET in tablet were more than 98%. The method was validated in respect to linearity, accuracy, precision and limit of detection and that of quantification, which prove suitability of the developed method for the routine estimation of both drugs in bulk powder and solid dosage form.

Keywords: ciprofloxacin hydrochloride; metronidazole; second derivative ratio.

INTRODUCTION

Ciprofloxacin, (CIP, Fig. 1a) is a second generation fluoroquinolone antibacterial agent. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections. Metronidazole, (MET, Fig. 1b) is a nitroimidazole antibacterial agent used particularly for anaerobic bacteria and protozoa. Combination of CIP and MET in a single dosage form is active against a broad spectrum of obligate anaerobic bacteria [1,2]. Ciprofloxacin was determined in single form by spectrophotometric [3,4], spectrofluorimetric [5,6] and HPLC [7,8] methods. On the other hand, spectrophotometry [9,10] and HPLC [11,12] techniques were applied for the determination of MET. Literature survey reveals few methods for the estimation of both drugs in binary mixture, spectrophotometric [13] and chromatographic [14] methods were developed by the author. The aim of the work was to develop a sensitive, accurate and precise second derivative ratio spectrophotometric (^{2}DD) method for the simultaneous determination of both drugs without prior separation. The use of normalized spectra as divisor eliminates most random noise and therefore, increases the method sensitivity. The proposed method was validated and successfully applied for the estimation of CIP and MET in tablet form, with high percentage recovery, good accuracy and precision.

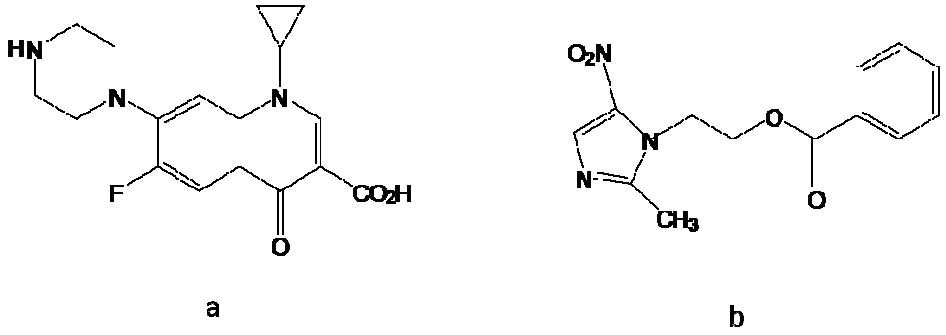

Figure 1: Chemical structures of ciprofloxacin (a) and metronidazole (b).

EXPERIMENTAL SECTION

Instrumentation

A JENWAY double beam UV/visible spectrophotometer (model 6800, United Kingdom) with two matched quartz cells of 1 cm pathlength was used for the present investigation. The slit width was 1.5 nm with wavelength scanning speed of 400 nm min^{-1}.

Materials and solvents

Pharmaceutical grade CIP and MET were kindly supplied by MINAPHARM pharmaceuticals (Cairo, Egypt). The purity of the samples was certified to be 100.70 and 99.44 %, respectively. Ciprodiazole® tablets, Batch No. AKE2558, labeled to contain 500 mg CIP and 500 mg MET per one tablet were manufactured and supplied by MINAPHARM pharmaceuticals (Cairo, Egypt). Methanol (CHROMASOLV® grade) was obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

Standard solutions

CIP and MET stock solutions (1 mg mL^{-1}) were prepared in methanol. Sonication was carried out to dissolve CIP, using an ultrasonic bath for 5 min. Working solutions (40 µg mL^{-1}) were prepared by diluting four mLs of each stock solution to 100 mL with methanol, in two separate volumetric flasks. Light protected volumetric flasks were used in case of MET solutions.

Procedure

Construction of calibration curve

Different aliquots (20-160 µg) and (40-160 µg) were transferred from CIP and MET working solutions (40 µg mL^{-1}), respectively, into two series of 10 mL volumetric flaks and the volume was completed with methanol. The spectrum of each solution was recorded against methanol as blank and stored in the computer. For the determination of CIP and MET, the recorded spectra were divided by the normalized spectrum of MET and CIP and the second derivative of the ratio spectra were obtained using a smoothing factor $\triangle\lambda$ = 20 and 17 nm, respectively. The amplitudes corresponding to the maxima at 253 nm and that of the minima at 301 nm were measured and plotted against the corresponding concentrations of CIP and MET and regression equations were computed.

Analysis of laboratory prepared mixtures

Accurate aliquots equivalent to (50 – 150 µg) were transferred from CIP and MET working solutions (40 µg mL^{-1}) into a series of 10 mL volumetric flasks, completed to volume with methanol and mixed well. The absorption spectra of these solutions were recorded and the same procedure mentioned under construction of calibration curve was applied in order to determine CIP and MET in the laboratory prepared mixtures. The peak amplitudes at 253 nm and 301 nm were measured and the concentrations of the two drugs were calculated from the corresponding regression equations.

Analysis of pharmaceutical dosage form

The contents of twenty tablets were powdered in a mortar and mixed well. A quantity of the powdered tablets equivalent to 100 mg CIP and MET was transferred into a 100 mL volumetric flask, then 50 mL methanol were added. The solution was sonicated for 5 min, completed to 100 mL with the same solvent, mixed well and filtered on dry funnel and dry filter paper discarding the first few milliliters. The sample solution was diluted to prepare a solution equivalent to 40 µg mL^{-1} of each CIP and MET. Different aliquots were separately transferred into 10 mL volumetric flasks and diluted with methanol. The procedure mentioned under construction of calibration curve was followed.

RESULTS AND DISCUSSION

Method development

Spectrophotometry is a widely used technique because of its simplicity, low cost and is time-consuming [15-18]. However, zero-order absorption spectra of CIP and MET reveal severe overlap which interferes with the direct determination of each drug, Fig. 2.

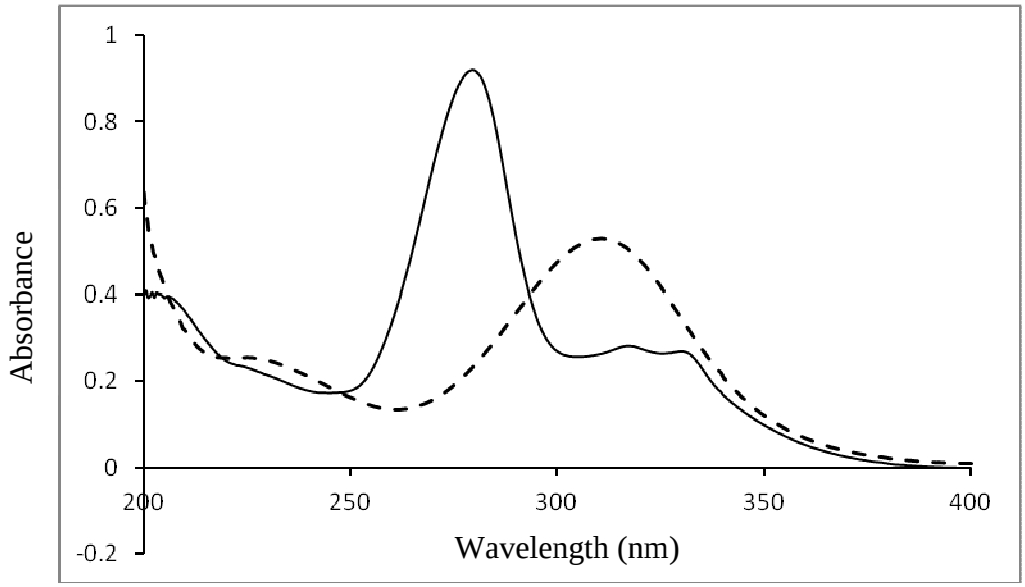

Figure 2: Zero order absorption spectra of 10 µg mL^{-1} of CIP (____) and 10 µg mL^{-1} of MET (- - - -).

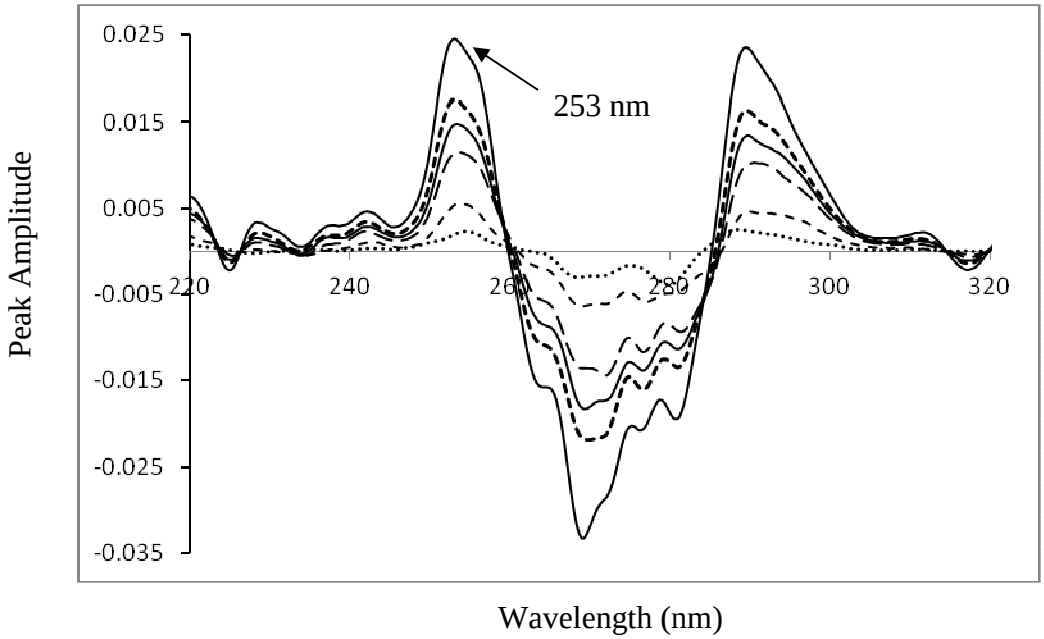

Figure 3: Second derivative of the ratio spectra for different concentrations of CIP (2, 4, 8, 10, 12 and 16 µg mL^{-1}) using normalized spectrum of MET as divisor.

In order to resolve the overlapping of spectra, derivative ratio technique was applied. It allows the determination of each drug in the binary mixture at the wavelengths corresponding to a maximum or a minimum, without previous separation [19-21]. The parameters that affect the shape of the derivative ratio spectra were investigated and optimized. Smoothing factors of 20 and 17 nm were selected, in case of CIP and MET determination, respectively, in order to obtain an adequate signal-to-noise ratio. Method sensitivity was improved upon using the normalized spectra as divisors. These were obtained by dividing the spectra of the standard drug of variable concentrations by their corresponding concentrations and subsequently averaging them, in order to obtain a spectrum of unit

concentration. The use of normalized spectra eliminates most random noise through averaging, therefore assures better sensitivity, repeatability and minimizes quantitation errors [22]. Fig. 3 reveals that CIP can be determined at the maxima at 253 nm while MET was determined by measuring the amplitudes of the minima at 301 nm, as shown in Fig.4.

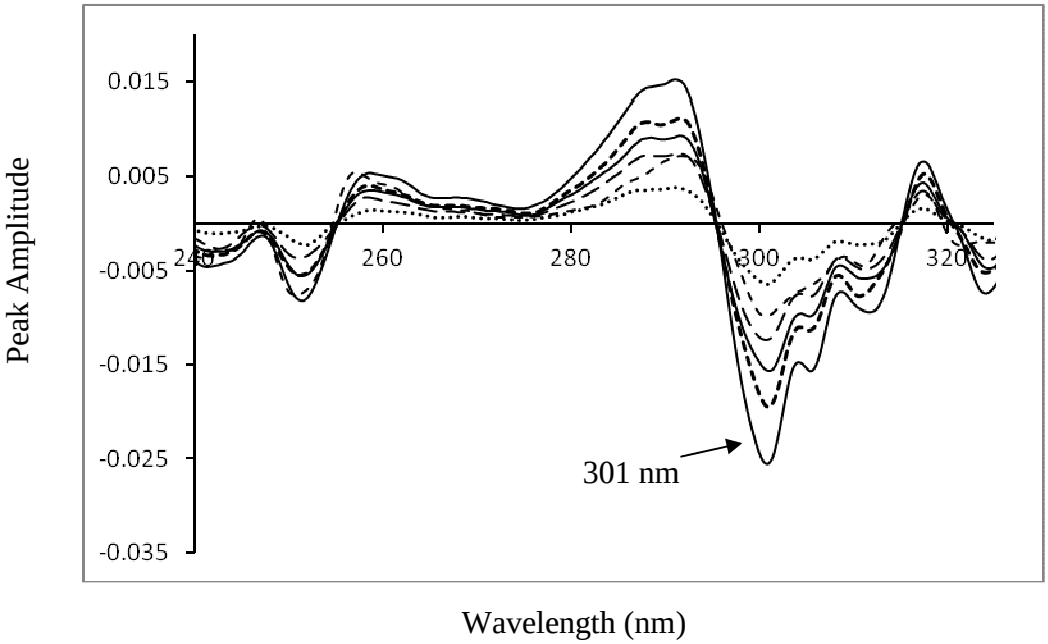

Figure 4: Second derivative of the ratio spectra for different concentrations of MET (4, 6, 8, 10, 12 and 16 µg mL^{-1}) using normalized spectrum of CIP as divisor.

Method validation

Method validation was carried out according to International Conference on Harmonisation guidelines (ICH) [23] for validation of analytical procedures.

Linearity and range

The calibration curve was obtained with six concentrations of each standard solution of CIP and MET (2 - 16 µg mL^{-1}) and (4 -16 µg mL^{-1}), respectively. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. Table 1 reveals the correlation coefficients, standard deviation of the slope (S_b) and that of intercept (S_a).

Table 1: Regression equation parameters and validation of the proposed ^{2}DD spectrophotometric method for the simultaneous determination of CIP and MET in binary mixture

Item	CIP	MET
Measurement Wavelength	253 nm	301 nm
LOD[a]	0.55µg mL^{-1}	0.49 µg mL^{-1}
LOQ[a]	1.66 µg mL^{-1}	1.49 µg mL^{-1}
Range of linearity	2-16 µg mL^{-1}	4-16 µg mL^{-1}
Regression equation	y = 1.571x – 0.832	y = 1.579x + 0.1
Correlation coefficient (*r*)	0.9991	0.9992
S_b	0.0236	0.0230
S_a	0.2325	0.2328
Confidence limit of the slope	1.571±0.0655	1.579±0.0639
Confidence limit of the intercept	0.832±0.6464	0.100±0.6473
Standard error of the estimation	0.2721	0.2220
Intraday[b]		
%RSD	0.781-0.657	0.524-0.693
Interday[c]		
%RSD	0.845-0.731	0.419-0.632

[a] *Limits of detection and quantification are determined via calculations [23]:*
LOD= 3.3×SD/slope *LOQ= 10×SD/slope*

[b] *The intraday (n = 3), average of two concentrations (3, 14 µg mL^{-1}) for CIP and MET, repeated three times within the day.*

[c] *The interday (n = 3), average of two concentrations (3, 14 µg mL^{-1}) for CIP and MET, repeated three times in three successive days.*

Accuracy

The proposed method was applied for the determination of laboratory prepared mixtures containing both drugs. The data shown in Table 2 indicates that the suggested method is accurate over the specified concentration range. In addition, Validity of the method was assessed by spiking the pharmaceutical formulation by known amounts of pure CIP and MET (standard addition technique). The recovery of the added standard was then calculated after applying the proposed method. Good recoveries were obtained, indicating high accuracy and absence of interference from the tablet excipients, as shown in Table 3.

Table 2: Determination of CIP and MET in laboratory prepared mixtures by the proposed ^{2}DD spectrophotometric method

Mixture No.	Claimed taken (µg mL^{-1})		Recovery[a] %	Recovery[a] %
	CIP	MET	CIP	MET
1	5	5	99.71	100.06
2	7	7	99.41	98.62
3	9	9	100.66	101.33
4	11	11	100.87	100.75
5	13	13	100.53	100.84
6	15	15	101.13	99.64
Mean			100.39	100.21
± SD			0.677	0.982

[a]Average of three determinations

Table 3: Determination of CIP and MET in ciprodiazole® tablets by ^{2}DD spectrophotometric method and application of the standard addition technique

	Claimed (µg mL^{-1})	Pure added (µg mL^{-1})		Recovery[a] % of tablet	Recovery[a] % of added	Recovery[a] % of tablet	Recovery[a] % of tablet
		CIP	MET	CIP	CIP	MET	MET
Ciprodiazole® tablets	6	4.8	4.8		99.46		98.98
		6.0	6.0		99.72		99.24
		7.2	7.2	100.06	101.67	99.24	99.42
	7	5.6	5.6	100.32	101.16	99.55	100.68
		7.0	7.0		100.03		100.45
		8.4	8.4		99.27		99.55
Mean				100.19	100.22	99.40	99.72
± SD				0.184	0.975	0.219	0.686

[a] Average of three different determinations.

Precision

The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). Two concentrations of CIP and MET (3, 14 µg mL^{-1}) were analyzed and the percentage relative standard deviation (% RSD) was calculated.

Limit of Detection and limit of quantification

The parameters LOD and LOQ were determined on the basis of standard deviation (SD) of the response and slope of the regression equation.

The validation parameters of repeatability, intermediate precision, LOD and LOQ are presented in Table 1.

CONCLUSION

The present work described a simple and precise UV method which allowed a rapid and economical quantitation of CIP and MET, simultaneously, in pure form and tablet without any time-consuming prior separation. Moreover, the spectrophotometric method involves simple instrumentation compared with other instrumental techniques. A good accuracy of the method was verified and therefore the suggested procedure is recommended for routine and quality control analysis of the investigated drugs in two-component pharmaceutical preparations.

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