ISSN : 0974-7419 Volume 13 Issue 1

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 13(1) 2013 [23-32]

Simultaneous spectrophotometric determination of metronidazole and diiodohydroxyquine

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ABSTRACT

Four sensitive and precise spectrophotometric methods were developed and validated for the simultaneous determination of metronidazole (MTR) and diiodohydroxyquinoline (DIQ) in their mixture and in their pharmaceutical formulations. Among the methods adopted were, second-derivative (^{2}D), third-derivative (^{3}D), derivative ratio spectroscopy (^{1}DD) and isosbestic point technique. The selectivity of the proposed methods was checked using laboratory prepared mixtures. The proposed methods were simple, not expensive and applicable that make them suitable for the analysis of MTR and DIQ in their mixture and in pharmaceutical formulations for routine unknown analysis in quality control labs.

KEYWORDS

Metronidazole;
Diiodohydroxyquinoline;
Determination;
Spectrophotometry.

INTRODUCTION

Metronidazole (MTR), is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (Figure 1)[1]. It is used as antibacterial and antiamaebiasis. Diiodohydroxyquinoline (DIQ), 5, 7 -diiodoquinolin-8-ol (Figure 2). It is widely known by the trade name Diodoquin, is a quinoline derivative which can be used in the treatment of amoebiasis[2].

The literature survey reveals several analytical methods for quantitative estimation of MTR in body fluids and in pharmaceutical formulations these methods include ultaviolet spectrophotometry[3-5], high performance liquid chromatography (HPLC)[6,7] and voltammetry[8]. Quantitation of metronidazole and spiramycin in human plasma, saliva and gingival crevicular fluid by LC–MS/MS[9]. Simultaneous multi residue determination of metronidazole and spiramycin in fish muscle was done using HPLC with UV detection[10]. Microsized graphite sensors for potentiometric determination of metronidazole and spiramycin was done[11]. DIQ was determined in pharmaceutical formulations using HPLC[12]. MTR and DIQ were determined by bivariate spectrophotometric method[13].

In modern analytical laboratory, there is always a need for simple, rapid and accurate methods for simultaneous determination of drug combinations that could be used for routine analysis. The present work aimed to develop simple instrumental methods for simultaneous determination of MTR and DIQ in combination.

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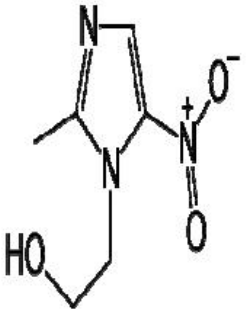
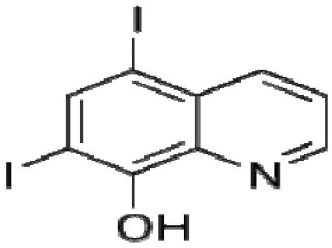


M.W. 171.15 gm

Figure 1: Chemical structure of metronidazole (MTR)



M.W. 396.951 gm

Figure 2 : Chemical structure of Diiodohydroxyquine (DIQ)

EXPERIMENTAL

Instruments

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-1601PC, with 1 cm quartz cells, connected to an IBM-compatible computer was used. The software was UV-PC personal spectroscopy software version 2.32. The spectral band width was 2 nm with wavelength-scanning speed of 2800 nm min^{-1}.

Materials and reagents

Reference metronidazole hydrochloride powder (MTR) and reference diiodohydroxyquine (DIQ) were kindly donated by Al Qahira Pharmaceuticals Co. The potency was certified to contain 1002 μg $mg^{"1}$ for MTR and 1009 μg $mg^{"1}$ for DIQ. Pharmaceutical dosage form (Paramibe compound, 500 mg tablets were kindly supplied by Chemical Industries Development (CID) and were claimed to contain 250 mg of MTR and 250 mg of DIQ per each tablet. Methanol was spectroscopic grade.

Standard solutions

Stock standard solutions of MTR (1 mg mL^{-1}) and DIQ (1 mg mL^{-1}) in methanol were prepared for the proposed spectrophotometric methods. All solutions were freshly prepared on the day of analysis.

Procedures

Direct spectrophotometric method

Spectral characteristics of MTR and DIQ

Two aliquots (1 mL) of each MTR and DIQ were separately transferred into two 100 mL volumetric flasks. Each flask was completed to volume with methanol to obtain final concentration of 10μg mL^{-1} of MTR and 10 μg mL^{-1} of DIQ. The spectrum of each solution was scanned and recorded separately.

Linearity

Portions of MTR standard solution and of DIQ standard solution each was separately transferred to a series of 10 mL volumetric flasks. Each flask was completed to the volume with methanol to reach the concentration range of 2-24 μg $mL^{-1}$and 1-12μg mL^{-1}, respectively. The absorbance was measured at 311 nm and 254 nm. Calibration graphs were constructed by plotting the absorbance versus concentrations. The regression equations were then computed for MTR and DIQ at the specified wavelengths.

Second-derivative (^{2}D) method

Linearity

Standard serial concentrations in the range of 2-24 μg mL^{-1} of MTR and of 1-12μg mL^{-1} of DIQ were prepared as described under section 2.4.1.2. The amplitudes of the second-derivative peaks of MTR were measured at 311 nm and the amplitudes of the second-derivative peaks of DIQ were measured at, 255.3 nm, 238.5 nm with Δ= 8 nm and scaling factor =100.

Calibration graphs were constructed by plotting the peak amplitudes versus concentrations. The regression equations were then computed for MTR and DIQ at the specified wavelengths.

Third -derivative (^{3}D) method

Linearity

Standard serial concentrations in the range of 1-12 μg mL^{-1} of DIQ were prepared as described under section 2.4.1.2. The amplitudes of the third -derivative peaks of DIQ were measured at 260 nm and 267.6 nm

with Δ= 8 nm and scaling factor =1000.

Calibration graphs were constructed by plotting the peak amplitudes versus concentrations. The regression equations were then computed DIQ at the specified wavelengths.

First-derivative of ratio spectra (^{1}DD) method

Linearity

Standard serial concentrations in the range of 2-24 μg mL^{-1} for MTR and 1-12 μg mL^{-1} for DIQ were prepared as under section 2.4.1.2. and accurately 5 μg mL^{-1} of DIQ standard solution to be used as a divisor for MTR and 12 μg $mL^{-1}$of MTR to be used as a divisor for DIQ. The spectra of the prepared standard solutions were scanned (200-400 nm) and stored into the PC.

The stored spectra of MTR were divided (the amplitude of each wavelength) by the spectrum of 5 μg mL^{-1} of DIQ. The first-derivative of the ratio spectra (^{1}DD) with Δ=4 nm and scaling factor of 10 was obtained. The amplitude of the first-derivative peaks of MTR were measured at 286nm and 328.5 nm.

The stored spectra of DIQ were divided (the amplitude of each wavelength) by the spectrum of 12μg mL^{-1} of MTR. The first-derivative of the ratio spectra (^{1}DD) with Δ=4 nm and scaling factor of 10 was obtained. The amplitude of the first-derivative peaks of DIQ were measured at 250 nm and 260.7 nm

Calibration graphs were constructed relating the peak amplitudes of (^{1}DD) to the corresponding concentrations. The regression equations were then computed for MTR and DIQ at the two specified wavelengths.

Isosbestic point

In this method DIQ is determined by derivative ratio technique at 260.7 nm where MTR don't interfere while total is measured at the isosbestic point at 280 nm then concentration of MTR is determined by subtraction.

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of MTR and DIQ were analyzed using the suggested methods, aliquots of MTR and DIQ were mixed to prepare different mixtures and the procedures were followed as mentioned under each method, the concentrations from the corresponding regression equations were calculated.

Assay of pharmaceutical formulations (Paramibe compound, 500mg tablets)

Twenty tablets were weighed from the dosage form and the average weight was calculated, tablets were crushed to furnish a homogenous powder and certain amount of powdered tablets were dissolved in methanol for 15 minutes and filtered. The solutions were diluted to the same concentration of the appropriate working solutions then the procedures were followed as described under each method.

RESULTS AND DISCUSSION

Direct spectrophotometric method

The UV spectra of MTR and DIQ allow direct determination of MTR at 311 nm with interference from DIQ which can be determined directly at 254 nm (Figure 3). A linear relationship was obtained in the range of 2-24μg mL^{-1} for MTR. The corresponding regression equation was computed and found to be: A = 0.05 C - 0.01 (r=0.9997), at 311 nm Where, A is the absorbance of MTR at 311 nm, C is the concentration of MTR (μg mL^{-1}) and r is the correlation coefficient. A linear relationship was obtained in the range of 1-12 μg mL^{-1} for DIQ. The corresponding regression equation was computed and found to be: A = 0.2013 C - 0.042 (r=0.9998), at 254 nm Where, A is the absorbance of DIQ at 254 nm, C is the concentration of DIQ (μg mL^{-1}) and r is the correlation coefficient.

Second-derivative (^{2}D) method

The second-derivative (^{2}D) ultraviolet spectrophotometry was applied for the determination of MTR and DIQ, either in raw material or in pharmaceutical formulations.

The absorption spectra of MTR and DIQ showed overlapping, interference and error probability affect the use of direct spectrophotometry and first-derivative method (^{1}D)for determination MTR and DIQ in presence of each other. When the second derivative spectra were examined (Figure 4), it was found that MTR could be determined at 311nm where DIQ has no contribution (zero crossing) at 311nm the clear zero crossing of DIQ allowed accurate determination of MTR

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in presence of any level of DIQ. A linear relationship was obtained in the range of 2-24 μg mL^{-1} for MTR. The corresponding regression equations were computed and found to be:

^2D = 0.012C +0.0007 (r=0.9999), at 311 nm

Where ^2D is the peak amplitude of the second-derivative curve at the corresponding wavelengths, C is the concentration of MTR (μg mL^{-1}) and r is the correlation coefficient.

The mean percentage recovery was found to be

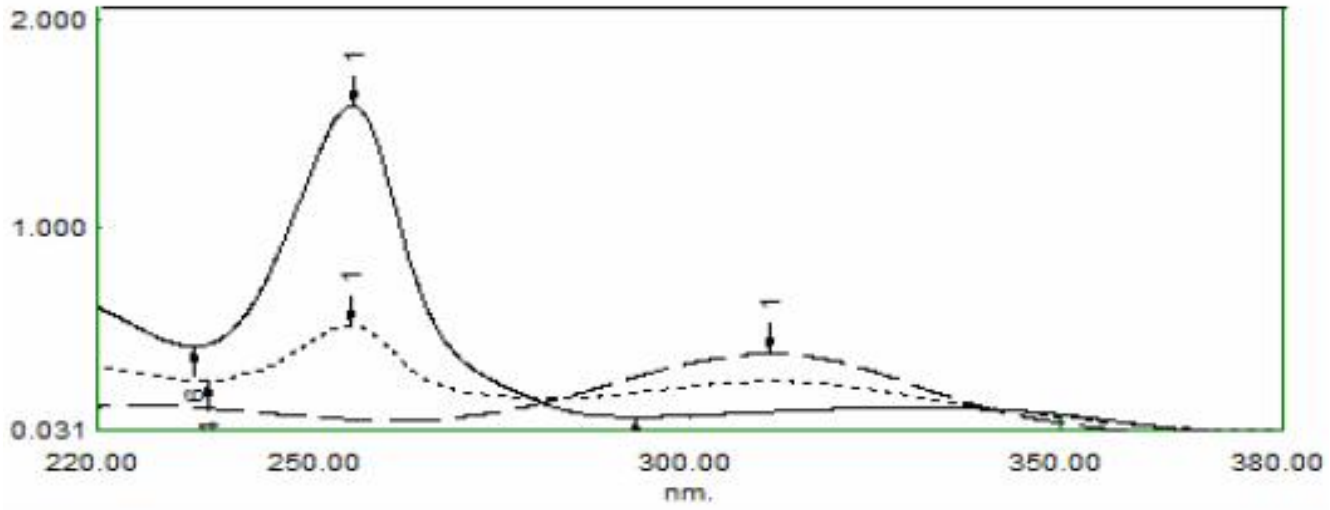


Figure 3 : DIQ 8 μg mL^{-1} (————) showing maximum absorbance at 254 nm and MTR 8μg mL^{-1} (——) showing maximum absorbance at 311 nm and mixture containing 4 μg mL^{-1}(.....) of each showing isosbestic point

100.347 at 311 nm.

When the second derivative spectra for DIQ were examined (Figure 4), it was found that DIQ could be determined at 255.3nm and 238.5nm, where MTR has no contribution, the clear zero contribution of MTR allowed accurate determination of DIQ in presence of any level of MTR. A linear relationship was obtained in the range of 1-12 μg mL^{-1} for DIQ. The corresponding regression equations were computed and found to be:

^2D = 0.0973 C - 0.0229 (r=1), at 238.5 nm

^2D = 0.3023C -0.0911 (r=0.9998), at 255.3 nm

Where ^2D is the peak amplitude of the second-derivative curve at the corresponding wavelengths, C is the concentration of DIQ (μg mL^{-1}) and r is the correlation coefficient.

The mean percentage recoveries were found to be 100.053 at 238.5 nm, 99.929 at 255.3 nm.

Third-derivative (^3D) method

The third derivative (^3D) ultraviolet spectrophotometry was applied for the determination of DIQ, either in raw material or in pharmaceutical formulations.

When the third derivative spectra for DIQ were examined (Figure 5), it was found that DIQ could be determined at 260nm, 267.6nm, where MTR has no contribution (zero crossing), the clear zero contribution of MTR allowed accurate determination of DIQ in presence of any level of MTR. A linear relationship was obtained in the range of 1-12 μg mL^{-1} for DIQ. The corresponding regression equations were computed and found to be:

^3D = 0.8405 C - 0.2326 (r=0.9998), at 260 nm

^3D = 0.1932 C + 0.1467 (r=0.9998), at 267.6 nm

Where ^3D is the peak amplitude of the third-derivative curve at the corresponding wavelengths, C is the concentration of DIQ (μg mL^{-1}) and r is the correlation coefficient.

The mean percentage recoveries were found to be 99.978 at 260nm and 100.041 at 267.6 nm.

Derivative ratio spectrophotometric method

Derivative ratio spectrophotometric method was used to determine MTR in presence of DIQ. The zero-order of the derivative ratio spectra of MTR and the first-order of the derivative ratio spectra were presented in Figure 6 & Figure 7, respectively. The concentration of the divisor was studied. It was found that upon dividing by 5 μg mL^{-1} of DIQ product led to the best results in terms of sensitivity, repeatability and signal to

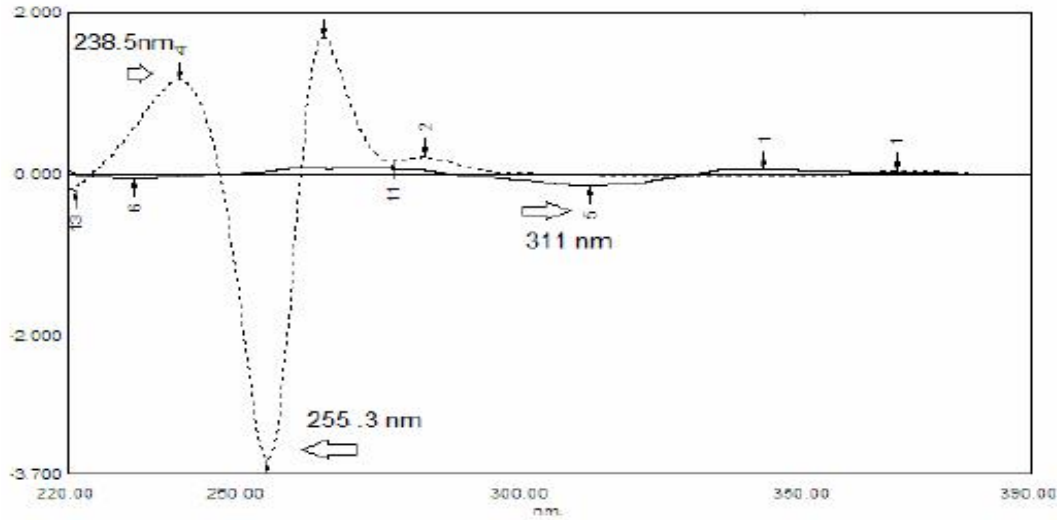
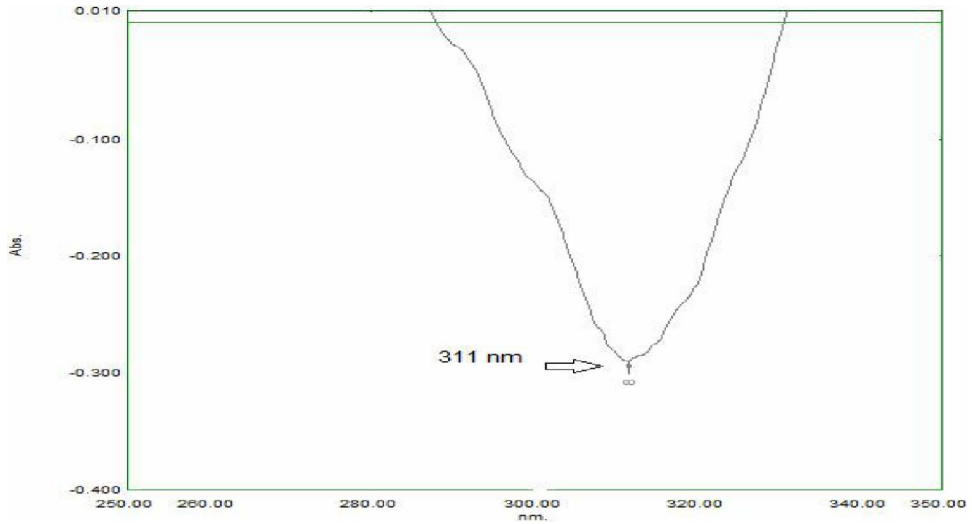


Figure 4 : Second derivative of DIQ 12 µg mL^{-1} (------) and of MTR 12 µg mL^{-1} (——) where MTR could be determined at 311 nm when DIQ has no contribution, DIQ could be determined at 255 .3 nm, 238.5 nm, where MTR has no contribution



Magnification of MTR Peak at 311nm

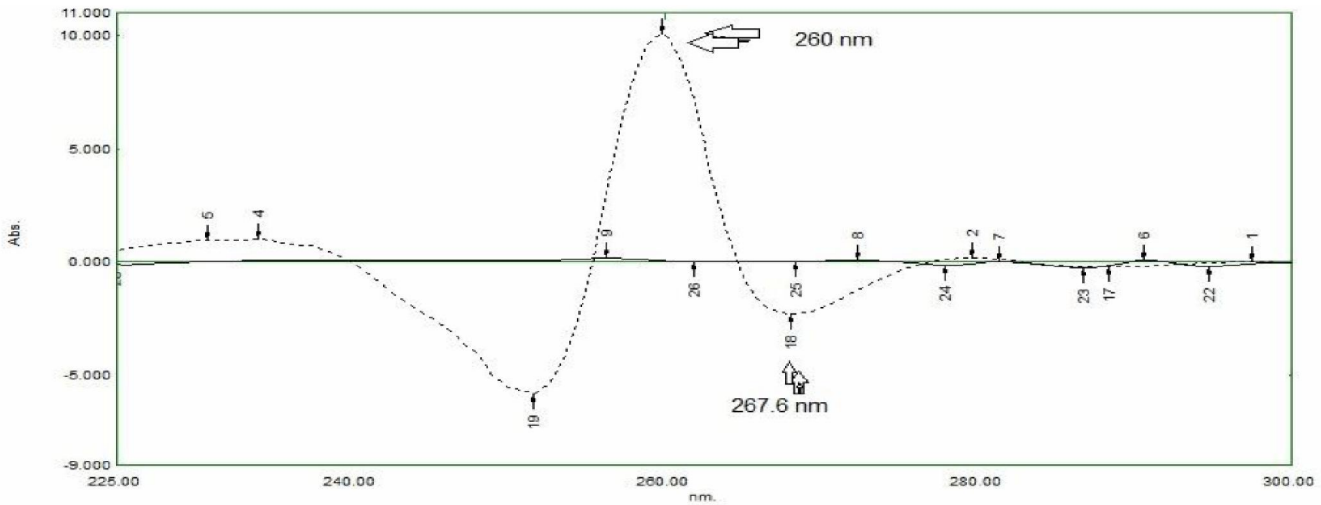


Figure 5 : Third derivative of DIQ12 µg mL-1 (-----) and of MTR 12 µg mL-1(———) where DIQ could be determined at 260 nm and 267.6 nm when MTR has no contribution

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noise ratio. Linear calibration graphs were obtained for MTR in concentration range of 2-24 μg mL^{-1} by recording the peak amplitude at 286nm and 328.5nm using 5μg mL^{-1} of DIQ as a divisor. The regression equations were computed and found to be:

(^{1}DD) = 0.3036 C - 0.0944 (r=0.9998), at 286 nm

(^{1}DD) = 0.1769 C+ 0.0173 (r=0.9999), at 328.5 nm

Where $^1DD_1$ is the peak amplitude of the first-derivative curve for (MTR/DIQ), C is the concentration of MTR (μg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples

The mean percentage recoveries were found to be 99.878 at 286 nm and 99.974 at 328.5 nm

Derivative ratio spectrophotometric method was used to determine DIQ in presence of MTR. The zero-order of the derivative ratio spectra of DIQ and the first-order of the derivative ratio spectra were presented in Figure 8 & Figure 9, respectively. The concentration of the divisor was studied, it was found that upon dividing by 12μg mL^{-1} of MTR product led to the best results in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graphs were obtained for DIQ in concentration range of 1-12μg mL^{-1} by recording the peak

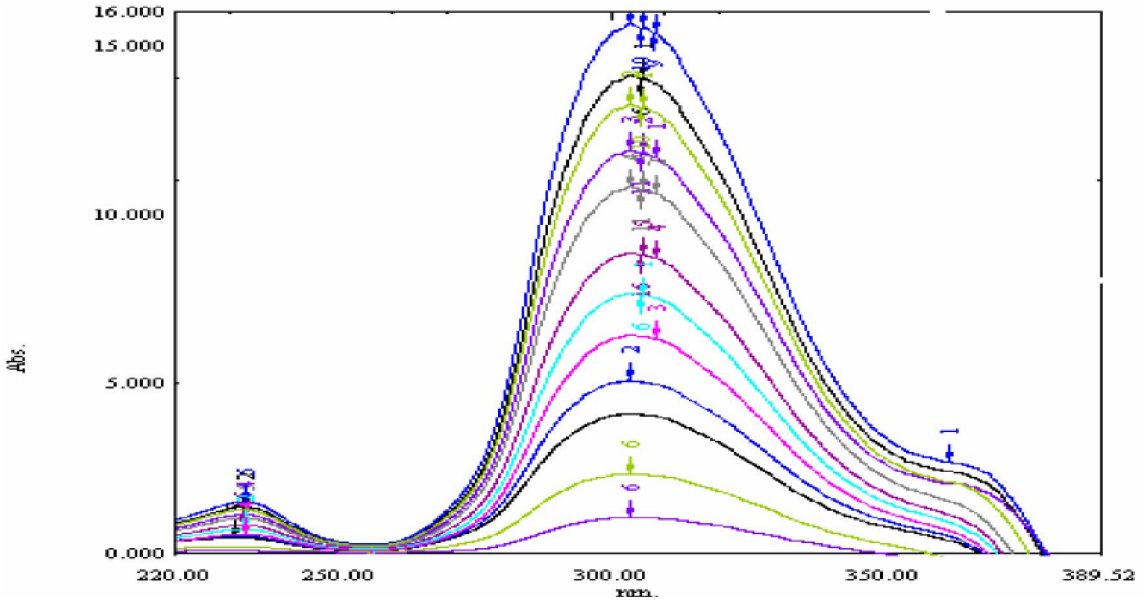

Figure 6 : Divisionof MTR (2-24)μg mL^{-1} using 5ug mL^{-1} of DIQ as divisor

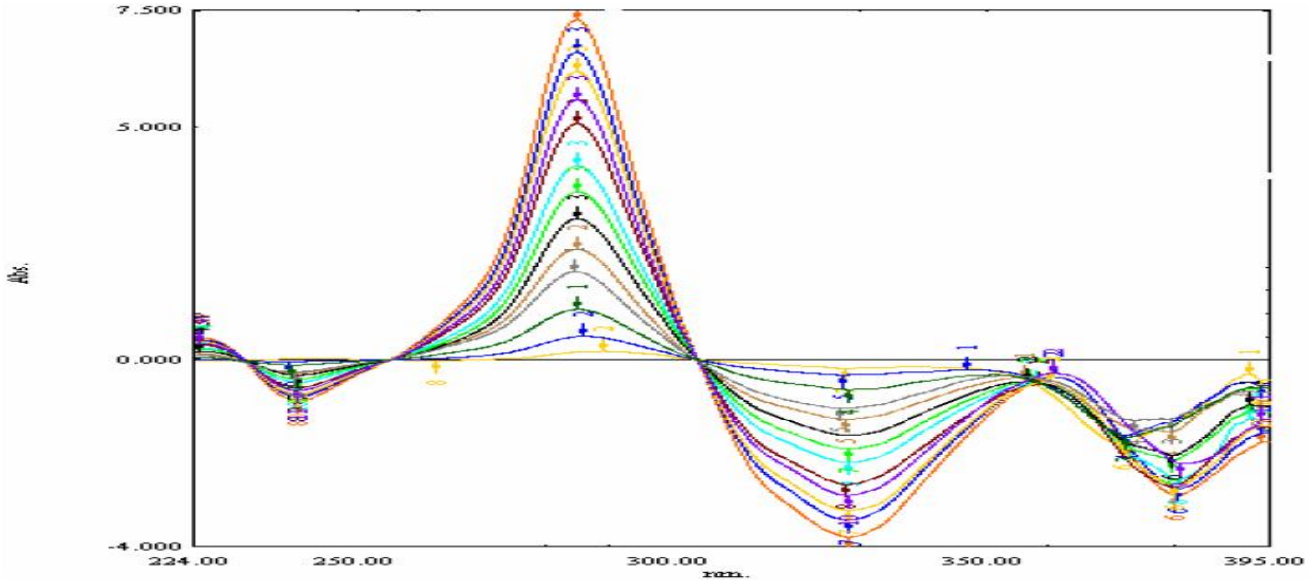

Figure 7 : First derivative of ratio spectra MTR (2-24) μg mL^{-1} using 5μg mL^{-1} DIQ as divisor

amplitude at 250nm and 260.7 using 12µg mL^{-1} of MTR as a divisor. The regression equations were computed and found to be:

(^{1}DD) = 12.9746 C –3.0204 (r=0.9999), at 250 nm

(^{1}DD)= 18.3730 C –8.3705 (r=0.9999), at 260.7 nm

Where ^{1}DDis the peak amplitude of the first-derivative curve for (DIQ/MTR), C is the concentration of DIQ (µg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples.

The mean percentage recoveries were found to be 99.949 at 250 nm and 99.998 at 260.7 nm.

Isosbestic point

In this method DIQ is determined by derivative ratio technique at 260.7 nm where MTR don·t interfere while total is measured at the isosbestic point at 280 nm then concentration of MTR is determined by subtraction. To ensure the validity of the chosen isosbestic point Figure 3 shows the isosbestic point of MTR, DIQ and their mixture in which each sample either single or in mixture contains 8 ug/mL.

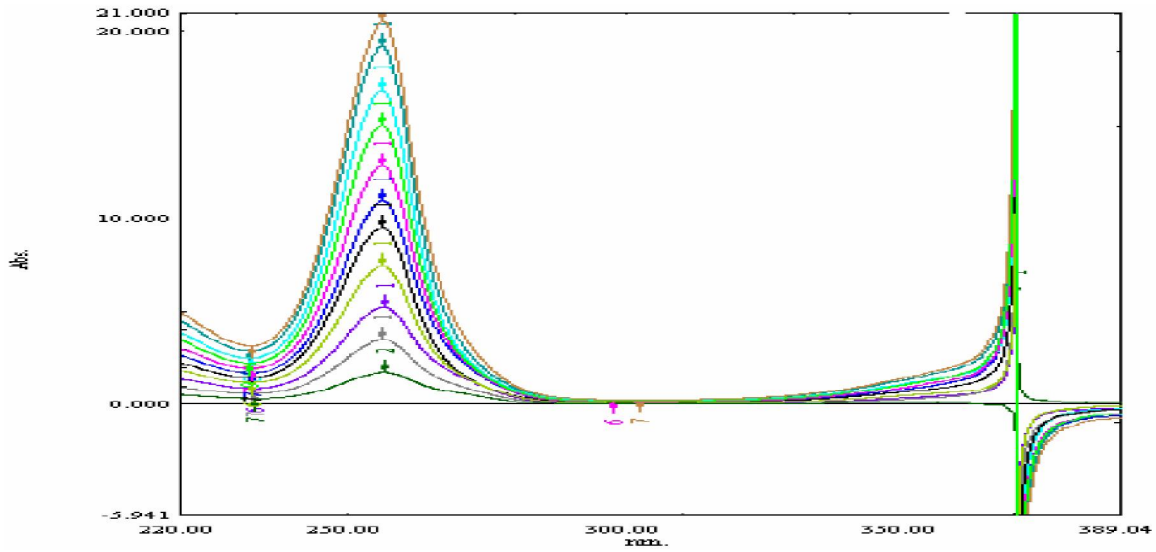

Figure 8 : Division of DIQ (1-12) µg mL^{-1} using 12 ug mL^{-1} of MTR as divisor

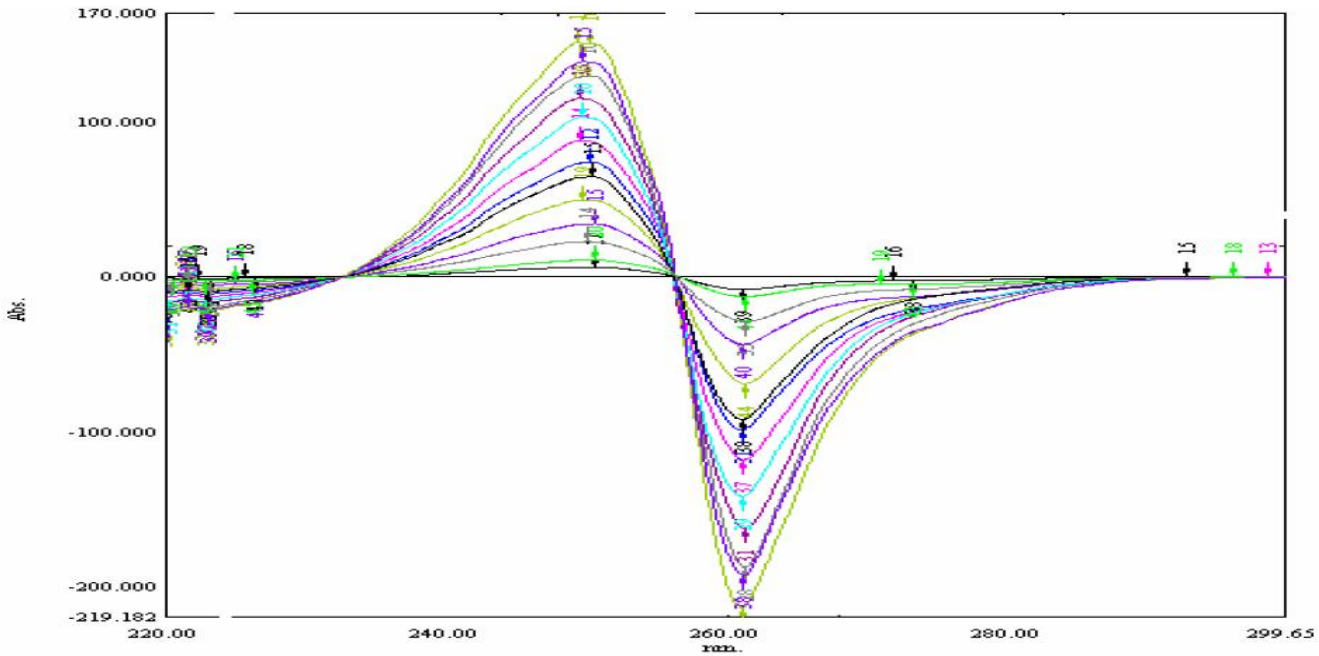

Figure 9 : First derivative of ratio spectra DIQ (1-12) µg mL^{-1} using 12 µg mL^{-1} MTR as divisor

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Statistical analysis

The suggested methods were successfully applied for the determination of MTR and DIQ in their laboratory prepared mixtures with good precision as shown in TABLE 3 & 4. The proposed methods were also used for estimating the concentration of both drugs in

TABLE 1a : Assay parameters and validation sheet for determination of metronidazole (MTR) and diiodohydroxyquine (DIQ) by second derivative and Isosbestic point techniques

Parameter	2D-method			Isosbestic point method
Range	MTR at	DIQ at		MTR at
	311 nm	238.5 nm	255. 3 nm	280 nm
	2-24 µg mL^{-1}	1-12 µg mL^{-1}	1-12 µg mL^{-1}	1-12 µg mL^{-1}
Slope	0.012	0.097	0.302	0.02
Intercept	0	-0.022	-0.091	-0.007
Mean	100.347	100.053	99.929	100.433
±S.D.	0.847	0.463	0.939	0.603
Variance	0.717	0.214	0.882	0.364
Coefficient of variation	0.844	0.463	0.939	0.600
Correlation coefficient (r)	0.9999	1	0.9998	0.9998
R.S.D. (%)	0.844	0.463	0.939	0.600

TABLE 1b : Assay parameters and validation sheet for determination of metronidazole (MTR) and diiodohydroxyquine (DIQ) by third derivative and first derivative of ratio spectra techniques

Parameter	3D-method		1DD-order method			
Range	DIQ at		MTR		DIQ at	
	260 nm	267.6 nm	286 nm	328.5 nm	250 nm	260.7 nm
	1-12 µg mL^{-1}	1-12 µg mL^{-1}	2-24 µg mL^{-1}	2-24 µg ml^{-1}	1-12 µg mL^{-1}	1-12 µg mL^{-1}
Slope	0.841	0.193	0.304	0.158	12.975	18.373
Intercept	-0.233	0.147	-0.094	0.017	-3.020	-8.371
Mean	99.978	100.041	99.878	99.974	99.949	99.998
±S.D.	0.995	0.701	0.821	0.433	0.586	0.641
Variance	0.990	0.491	0.674	0.187	0.343	0.411
Coefficient of variation	0.995	0.701	0.822	0.433	0.586	0.641
Correlation coefficient (r)	0.9998	0.9998	0.9998	0.9999	0.9999	0.9999
R.S.D. (%)	0.995	0.701	0.822	0.433	0.586	0.641

TABLE 2a : Statistical comparison for the results obtained by the proposed methods and the official method for analysis of MTR and official method for analysis of DIQ

Parameters	2D-method			Isosbestic point	official method	official method
	MTR	DIQ		MTR	MTR	DIQ
	311 nm	238.5 nm	255. 3 nm	280 nm		
Mean	100.347	100.053	99.929	100.433	100.07	100.18
±S.D	0.845	0.463	0.939	0.603	0.507	0.641
Variance	0.714	0.214	0.882	0.364	0.257	0.411
N	12	12	12	8	6	6
F-test	2.78 (4.74)a	1.92 (4.74)a	2.15 (4.74)a	1.42 (4.88)a		
Student's t-test	0.866 (2.120)a	0.432 (2.120)a	0.667 (2.120)a	1.22 (2.179)a		

a The values in the parenthesis are corresponding theoretical t- and F-values at P = 0.05[16]

TABLE 2b : Statistical comparison for the results obtained by the proposed methods and theofficial method for analysis of MTR and official method for analysis of DIQ

Parameters	^{3}D-method		1DD-method				official method	official method
	DIQ		MTR		DIQ		MTR	DIQ
	260 nm	267.6 nm	286 nm	328.5 nm	250 nm	260.7 nm		
Mean	99.978	100.041	99.878	99.974	99.949	99.998	100.07	100.18
±S.D	0.995	0.701	0.821	0.433	0.586	0.641	0.507	0.641
Variance	0.990	0.491	0.674	0.187	0.343	0.411	0.257	0.411
N	12	12	12	12	12	12	6	6
F-test	2.41 (4.74)a	1.19 (4.74)a	2.62 (4.74)a	1.37 (4.74)a	1.198 (4.74)a	1 (4.74)a		
Student's t-test	0.519 (2.120)a	0.420 (2.120)a	0.609 (2.120)a	0.395 (2.120)a	0.741 (2.120)a	0.568 (2.120)a		

a The values in the parenthesis are corresponding theoretical t- and F-values at P = 0.05[16]

TABLE 3 : Determination of metronidazole and diiodohydroxyquine in laboratory prepared mixtures by the proposed methods

Method	^{2}D-method					
	MTR		DIQ			
	311 nm		238.5 nm		255.3 nm	
(Mean ± SD)	100.107±0.471		99.920±0.358		100.337±0.311	
Method	^{3}D-method		^{1}DD-order method			
	DIQ		MTR		DIQ	
	260	267.6	286	328.5	250	260.7
	nm		nm		nm	
(Mean ± SD)	100.929±0.789	100.197±1.005	100.307±0.570	100.243±0.258	99.856±0.487	99.84±0.425

TABLE 4 : Determination of metronidazole (MTR) in lab prepared mixtures by proposed isosbestic point method

	Total		MTR		
Mixture ratio DIQ: MTR	Taken (µg mL-1)	Found (µg mL-1)	Taken (µg mL-1)	Found (µg mL-1)	R%
1:7	8	8.05	7	7.05	100.71
2:6	8	8.05	6	6.05	100.83
1:12	13	12.95	12	11.95	99.58
4:4	8	8.05	4	4.03	100.75
1:10	11	10.95	10	9.95	99.50
3:5	8	8.05	5	5.06	101.20
5:3	8	8.05	3	3.01	100.33
1:9	10	10.05	9	9.05	100.56
				Mean	100.433
				SD	0.603

TABLE 5 : Determination of MTR and DIQ in Paramibe compound tablet by proposed methods

Method	^{2}D-method					
	MTR		DIQ			
	311 nm		238.5 nm		255.3 nm	
(Mean ± SD)	100.098 ± 0.388		99.973±0.275		99.878±0.179	
Method	^{3}D-method		^{1}DD-order method			
	DIQ		MTR		DIQ	
	260	267.6	286	328.5	250	260.7
	nm		nm		nm	
(Mean ± SD)	100.535±0.448	100.190±0.563	99.753±0.394	99.66±0.415	99.535±0.450	100.253±0.510

their pharmaceutical formulations. The results are shown in TABLE 5. Assay parameters and a validation sheet for determination of the studied drugs are shown in TABLE 1. Statistical comparison for the results obtained by the proposed methods and the reference ones for the studied drugs are shown in TABLE 2. The calculated t- and F-values were found to be less than the tabulated ones confirming good accuracy and excellent precision.

CONCLUSION

Unlike the mostly recommended HPLC procedure, the proposed spectrophotometric methods are simple and not expensive. The reagents used in the proposed methods are cheap and available. The procedures applied in each method do not involve any critical reactions or tedious sample preparations. This aspect of spectrophotometric analysis is of major interest in ana-

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lytical pharmacy since it offers distinct possibility of assaying the studied drugs in their mixtures and in their pharmaceutical formulation without interference from the excipients. The suggested methods are found to be accurate and selective with no significant difference of the precision compared with the reference methods of analysis. The proposed methods could be applied successfully, for routine analysis of MTR and DIQ singly, in their mixtures or in their pharmaceutical formulations

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