

Stability Indicating Double Divisor Spectrophotometric Method for determination of Diloxanide Furoate and Metronidazole in Their Binary Mixture

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Abstract

Diloxanide furoate (DXF) and Metronidazole (MTZ) are commonly combined as a binary mixture for the treatment of a variety of diseases brought on by bacteria and parasites including amoebiasis and giardiasis. Different analytical methods were established for determination of both DXF and MTZ, However, only one chemometric spectrophotometric method was established for their stability indicating determination which involves problematical steps and specific software program. Herein, double divisor ratio spectra derivative (DDRSD) method is exploited for quantification of DXF and MTZ in presence of DXF-degradation products (FUR and DEG). The recommended DDRSD method is linear over the range of 2–25 and 1–25 µg/mL for DXF and MTZ, correspondingly. Additionally, the suggested method was effectively employed to their pharmaceutical formulations with good findings. Besides, the recommended DDRSD method was validated conforming to ICH guidelines. The introduced DDRSD method is simple, selective, and economic and could be implemented for quality control samples of the studied drugs.

Keywords: Diloxanide furoate; Metronidazole; Double Divisor spectrophotometry, Method validation.

Introduction

Metronidazole (MTZ) is an antiprotozoal nitroimidazole derivative primarily employed to treat parasitic infections by protozoa and anaerobic bacteria [1]. Whereas Diloxanide furoate (DXF) is a derivative of dichloroacetamide that primarily acts as a luminal amebicide in the intestine to treat intestinal amoebiasis. Patients with invasive amoebiasis receive a combination of an amebicide that works in the tissues, such as metronidazole, along with DXF for the treatment of asymptomatic cyst [2]. The recommended treatment for diarrhea caused by *Clostridium difficile*, *Entamoeba histolytica*, and *Giardia lamblia* is a combination of DXF and MTZ for controlling amoebiasis and giardiasis. Additionally, This combination helps treat bacterial infections in the vaginal area and other body parts. [3].

Diloxanide furoate (DXF) and Metronidazole (MTZ) are co-formulated together for their antiprotozoal activity in the treatment of amoebiasis and their combination is presented in different pharmaceutical formulations. Different methods were established for determination of both DXF and MTZ including spectrophotometry [4]–[11], HPLC [12]–[18], and TLC [9], [16], [19] methods. By reviewing the literature there is only stability indicating spectrophotometric chemometric method was exploited for determination of both DXF and MTZ [9], meanwhile this chemometric approach required special software for data manipulation with sophisticated steps.

In the field of pharmaceuticals, spectrophotometry is a commonly manipulated technique. However, it can be affected by co-formulated medications, excipients, and degradation products, which may interfere with direct UV absorption magnitudes.

This means that the absorption observed may not solely be from the analyte, but also from other components, which is referred to as extraneous response. Especially when handling a complex mixture of binary, ternary and quaternary components, their resolution is so difficult. However, some spectrophotometric procedures help in achieving the required resolution between interfering spectra with good selectivity. Among these procedures used to reduce or eliminate such interference or irrelevant absorption is the double divisor ratio spectra derivative (DDRSD) method [20]. Since the development of double divisor ratio spectra derivative (DDRSD) method by E. Dinç [20]. It has been shown that by eliminating interference from degradation products, co-formulated compounds, and extraneous absorbance from the formulation matrix, the detection of pharmaceutical compounds is improved. Additionally, DDRSD method was widely adopted for determination of complex mixture of pharmaceutical compounds with overlapped spectra [21]–[25]. Few stability indicating methodologies have been reported for the assay of the DXF and MTZ, as a combination drug therapy. For the determination of DXF and MTZ in the presence of DXF degradation products namely, 2-furanoic acid (FUR) and 4-hydroxy-N-methyl aniline (DEG). This work attempts to describe a new, selective, accurate and stability indicating method for simultaneous determination of DXF and MTZ in presence of DXF degradation products. Therefore, in this work double divisor ratio spectra derivative (DDRSD) method is attempted for determination of MTZ and DXF in the presence of DXF degradation products (FUR & DEG).

2. Experimental

2.1. Instruments

- A double-beam, IBM-compatible computer-connected UV-Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC, with quartz cell with 1 cm pathlength. Version 3.7 of the UV-PC Personal Spectroscopy Software was applied. The wavelength-scanning speed was 2800 nm min⁻¹, and the spectral bandwidth was 2 nm.
- Sonix TV ss-series ultrasonicator (New York, USA)
- Digital balance (sartorius, Germany)

2.2. Samples

(a) Pure standard

- Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt graciously provided the drugs diloxanide furoate and metronidazole. According to the published procedure, the purity of both was verified to be 100.11% and 99.87%, respectively individually.
- 2-Furanoic acid (C₅H₄O₃), its purity is 99.4% (Winlab, India).

(b) Pharmaceutical formulations

- Flagimide® tablets (Batch No. 0563930) is stated to comprise 250 mg DXF and 250 mg of MTZ, produced by Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt.
- Furazole® tablets (Batch No. 324185) is defined to have 250 mg DXF and 200 mg of MTZ, manufactured by Egyptian Int. Pharmaceutical Industries Co. (EIPICo.), 10th of Ramadan City, Egypt.

(c) Degraded sample

The degradation products of Diloxanide furoate was prepared and identified as described by *Abbas et al.* [9].

2.3. Chemicals and Reagents

All chemicals used were of pure analytical grade and solvents were of spectroscopic grade. Methanol (Merck, Germany) is used as a solvent for all samples organization.

2.4. Standard solutions

1. Stock standard solutions (1 mg/mL)

MTZ, DXF, FUR, and DEG were properly measured and delivered into four separate 100-mL volumetric flasks, where they were dissolved and dispersed to the appropriate volume with methanol.

2. Working standard (100 µg/mL)

To generate 100 µg/mL working standard solutions, 10 mL of the MTZ, DXF, FUR, and DEG stock solutions were precisely placed into four different 100 mL volumetric flasks. Then, the volume was filled with methanol to the appropriate dilution.

2.5. Laboratory prepared mixtures

Accurate aliquots of DXF from its working solution (100 µg/mL) was transferred into a series of 10-mL volumetric flasks, and different aliquots of MTZ working solution (100 µg/mL) were added. Then, different aliquots of FUR and DEG from their respective working solutions were inserted, in the ratio of 10–90% of DXF concentration. These laboratory-prepared mixtures enable the examination of various DXF and MTZ ratios while keeping the

market product ratio into account.

2.6. Procedure

2.6.1. Spectral characteristics of DXF, MTZ, FUR, and DEG

Aliquots equivalent to 10 µg from each of MTZ, DXF, FUR and DEG working standard solutions (100 µg/mL in methanol), were properly and independently placed into 10-mL volumetric flasks. Methanol was used to fill the volume, and absorption spectra were measured for each solution from 200–400 nm, with methanol as the control. The absorbing spectrum of DXF, MTZ, FUR and DEG were segmented by the spectra of different concentrations of MTZ, FUR, DEG and the spectra of mixtures of equal concentrations of MTZ/DEG, MTZ/FUR and FUR/DEG. Then, the first derivative of ratio spectra with scaling factor = 10 and $\Delta\lambda = 4$ nm were accomplished.

3. Method validation

3.1. Linearity

Aliquots equivalent to 10–250 µg of DXF and 20–250 µg of MTZ were accurately transferred into two separate sets of 10-mL volumetric flasks from their respective working standard solutions (100 µg/mL) then completed the volume with methanol. The calibration curve was constructed relating absorbance of MTZ solutions at λ_{\max} 310.8 nm to the corresponding concentration and then the regression equation was calculated. The absorption spectra of DXF were divided by the absorption spectrum of a mixture of equal concentration of MTZ/DEG (20 µg/mL of each) used as a divisor, then the first derivative of the ratio spectra was achieved using $\Delta\lambda = 4$ and scaling factor = 10. To depict the correlation between the peak amplitudes at 250 nm and the relevant concentrations of DXF, a calibration curve was established, and the regression equation was then calculated.

3.2. Accuracy

The previously mentioned procedure under linearity was followed for different concentrations of standard DXF and MTZ. Besides, the concentrations of the studied drugs were measured from their corresponding regression equations then estimated the mean percentage recoveries and standard deviations.

3.3. Precision

Intraday precision (Repeatability)

The described approach was used to investigate three independent intra-day concentrations of DXF (5, 10 and 15 µg/mL) and MTZ (5, 10 and 15 µg/mL) under linearity for each drug. Using the specified double divisor spectrophotometric methodology, the percentage recoveries and standard deviations for every compound were calculated.

Intermediate precision

Using the method described under linearity, the previously revealed concentrations of DXF and MTZ were tested on three consecutive days (inter-day). Afterward, the percentage recoveries and relative standard deviations (%RSD) for the studied drugs were explored operating the suggested DDRSD method.

3.4. Analysis of laboratory prepared mixtures

Into 10-mL volumetric flasks, accurate aliquots of DXF were transported from its working standard solution (100 µg/mL) equivalent to 90–225 µg, aliquots of MTZ working solution (100 µg/mL) equivalent to 100–250 µg were inserted. Then, different aliquots equivalent to 12–100 µg of FUR and 13–110 µg of DEG were combined. Subsequently, the volume was fulfilled with methanol. The absorption spectrum for each prepared solution was scanned using methanol as a blank. The absorbance of the obtained spectra was measured at 310.8 nm to determine MTZ concentration by applying in its corresponding regression equation. The absorption spectrum of a mixture of equal amounts of MTZ/DEG (20 µg/mL of each) was used to segment the attained spectra, then the first derivative of the ratio spectra of the mixtures using $\Delta\lambda = 4$ nm and scaling factor = 10 was obtained. The concentration of DXF was quantified by substituting in its corresponding regression equation.

4. Determination of MTZ and DXF in pharmaceutical formulations

Ten tablets of Flagimide® and Furazole® each were weighed, thoroughly combined, and finely grounded. The crushed tablets were properly weighed and delivered into a 250-mL beaker in an amount that was equal to 250 mg of DXF and 250 mg (or 200 mg) of MTZ. In a subsequent step, 50 mL of methanol was added, and sonicated for 30 minutes, followed by filtering over a filter paper into a 100-mL volumetric flask. The remaining powder was once more treated and filtered before being finished to volume with methanol. Using methanol as the solvent, appropriate dilution was carried out to produce a working solution containing 100 µg/mL. Following that, the suggested spectrophotometric approach was carried out, and using the relevant regression equations for MTZ and DXF, the concentration of each was estimated. Finally, the standard addition technique was adopted.

5. Results and discussion

Stability-indicating assay methods are critical in industries such as pharmaceuticals, where the stability of a product is vital. These methods are essential to ensure the quality, safety, and effectiveness of medications and other chemical substances throughout their shelf life [26], [27]. The purpose of stability-indicating assays is to detect and measure any degradation products that may form during the use or storage of a product. These byproducts can be caused by various factors such as chemical reactions, temperature, humidity, and light. Stability-indicating tests help to determine the extent of product degradation and assess its impact on product quality by identifying and measuring these degradation products [28], [29]. Spectrophotometry is a powerful analytical technique that uses the amount of light absorbed or transmitted by a sample to determine the concentration of a substance or analyze its properties. One of the major advantages of spectrophotometry is its ability to distinguish between interfering spectra, which can occur when there are multiple absorbing species in a sample that may overlap and make analysis more difficult [30]–[32]. To treat amoebiasis and giardiasis, diloxanide furoate and metronidazole are routinely combined in different formulations [3]. DXF as an ester-containing drug is susceptible to hydrolytic degradation. As described by Abbas et al. [9], DXF was degraded and the degradation products were separated and identified as revealed by the authors to obtain DXF-degradation products namely (2-furanoic acid and 4-hydroxy-N-methyl). The researched components' chemical structures are displayed in **Figure 1**.

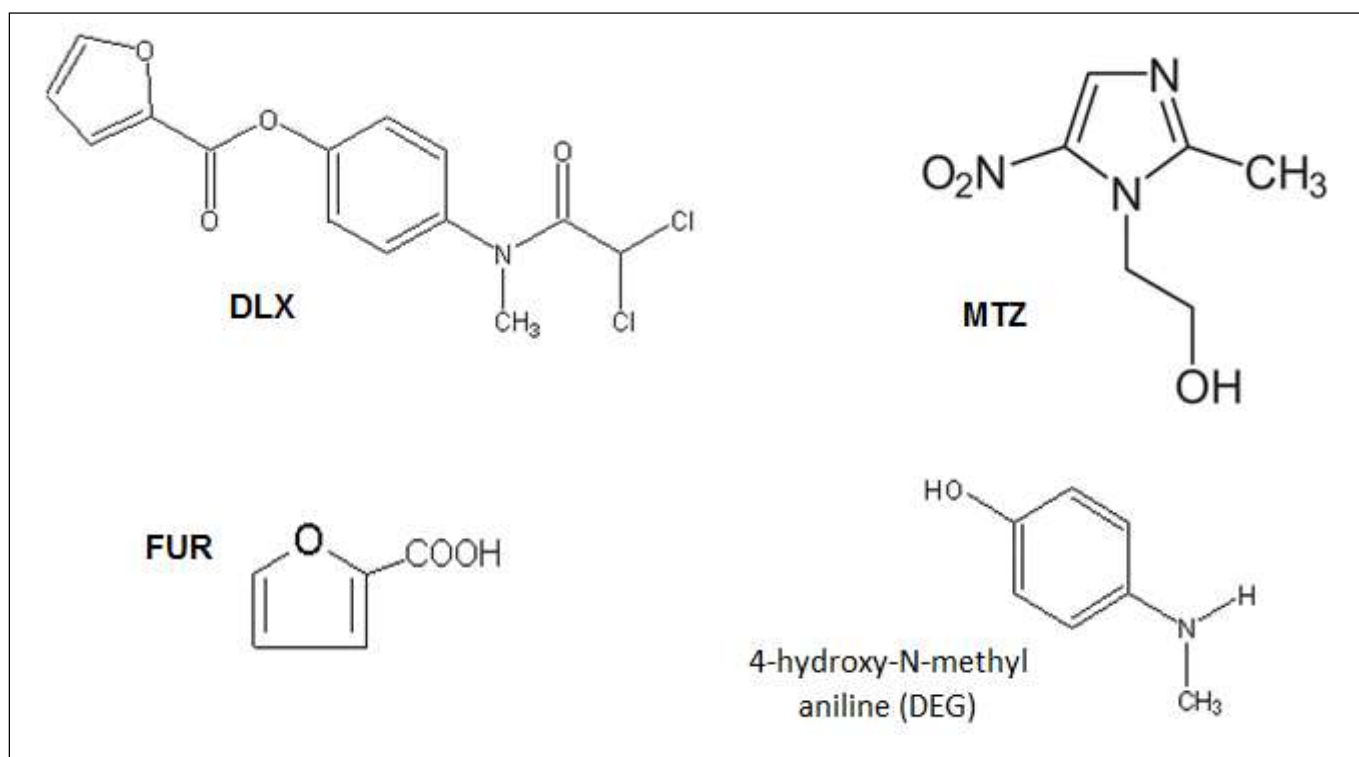


Figure 1: Chemical structure of MTZ, DXF FUR, and Deg.

Figure 2 shows the UV-absorption spectra of DXF and MTZ at their nominal concentrations in the tablets and the spectra of FUR and DEG (degradation products of DXF). A significant overlapping in absorption bands was noticed. Substantial spectral overlapping across the entire wavelength range prevents simultaneous measurement of DXF, MTZ, FUR, and DEG by conventional, derivative, and derivative ratio spectrophotometric approaches. Hence, the UV spectrum of the MTZ is not significantly affected by the presence of other components, it is possible to determine the MTZ using the standard zero-order method even in the presence of DXF, FUR, and DEG.

A spectrophotometric approach for the simultaneous determination of ternary mixtures has been presented by Dinc et al. [20], [23]. This technique is known as the "double divisor ratio spectra derivative method (DDRSD)". It involves using coinciding spectra of the derivative of the ratio spectra by utilizing a "double divisor" (the combination of two spectra) and detecting at the maximum or minimum wavelength. The zero order absorption spectra of MTZ, DXF, FUR and DEG show relative overlapping, which allows only the direct determination of MTZ at its λ_{\max} 310.8 nm but prevents the use of direct absorbance measurements for assaying DXF as shown in **Figure 3**. The issue of overlapping spectra for the selective determination of DXF was addressed using DDRSD technique, which relies on the derivative ratio principle. Various concentrations of MTZ, FUR, and DEG were explored as divisors since the choice of the divisor and its concentration are crucial. Binary mixes of MTZ/FUR, MTZ/DEG, and FUR/DEG at equal

and varied concentrations were examined as well as divisors. The use of a mixture of equal concentrations of MTZ/DEG (20 $\mu\text{g/mL}$ of each) as a divisor for determining DXF achieved the most favorable results in terms of signal-to-noise ratio, sensitivity, and selectivity.

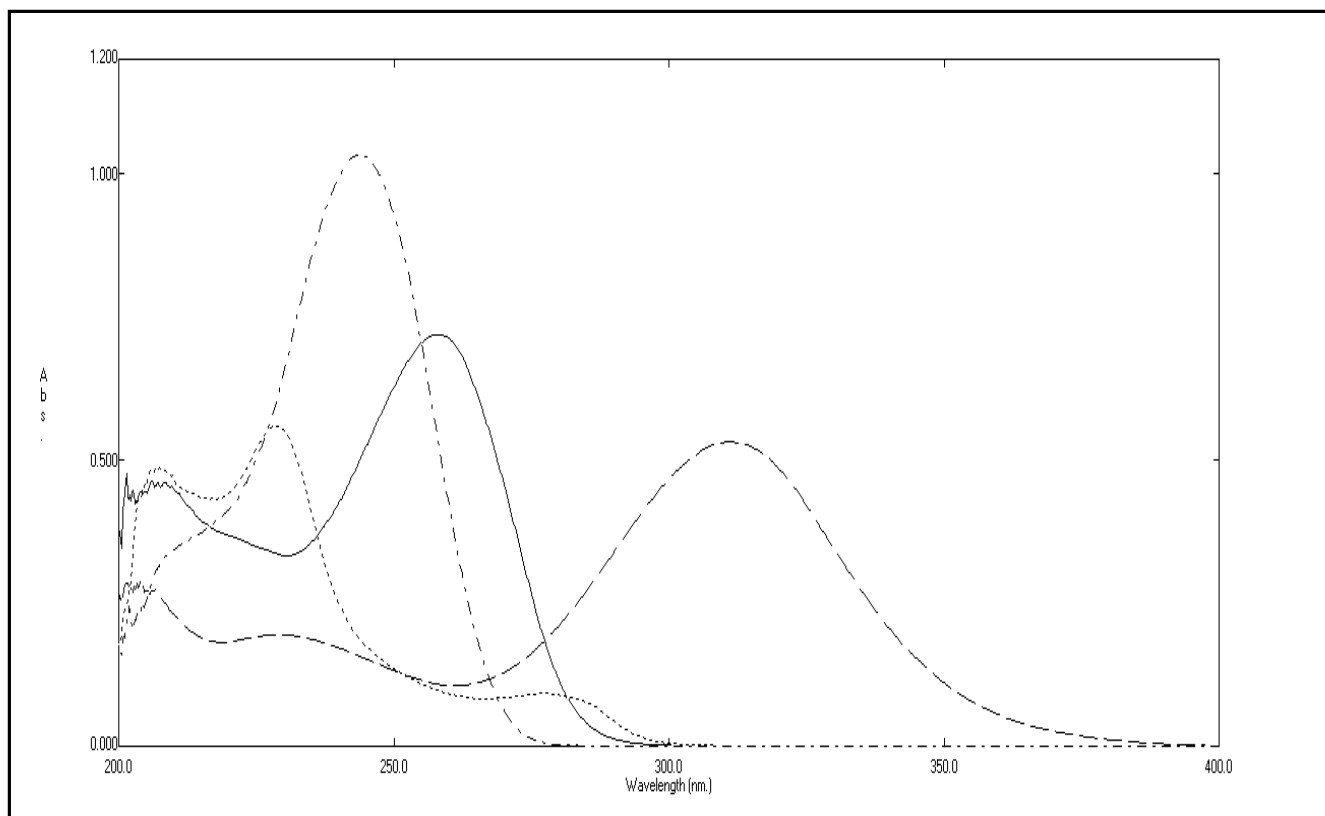


Figure 2: Zero order absorption spectra of 10 $\mu\text{g/mL}$ of DXF (—), 10 $\mu\text{g/mL}$ of MTZ (-----), 10 $\mu\text{g/mL}$ of FUR (-.-.-.-) and 10 $\mu\text{g/mL}$ of DEG (.....) using methanol as a solvent.

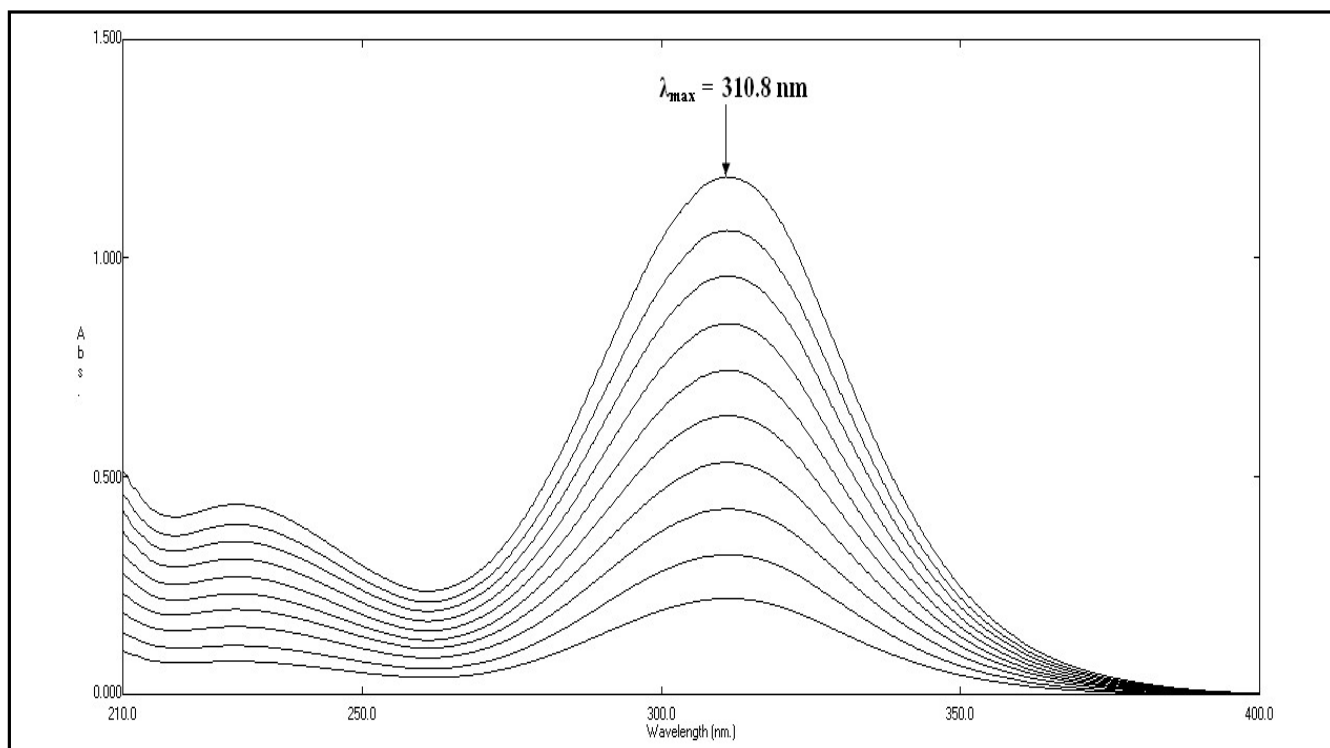


Figure 3: Zero order absorption spectra of Metronidazole (2–25 $\mu\text{g/mL}$) using methanol as a blank.

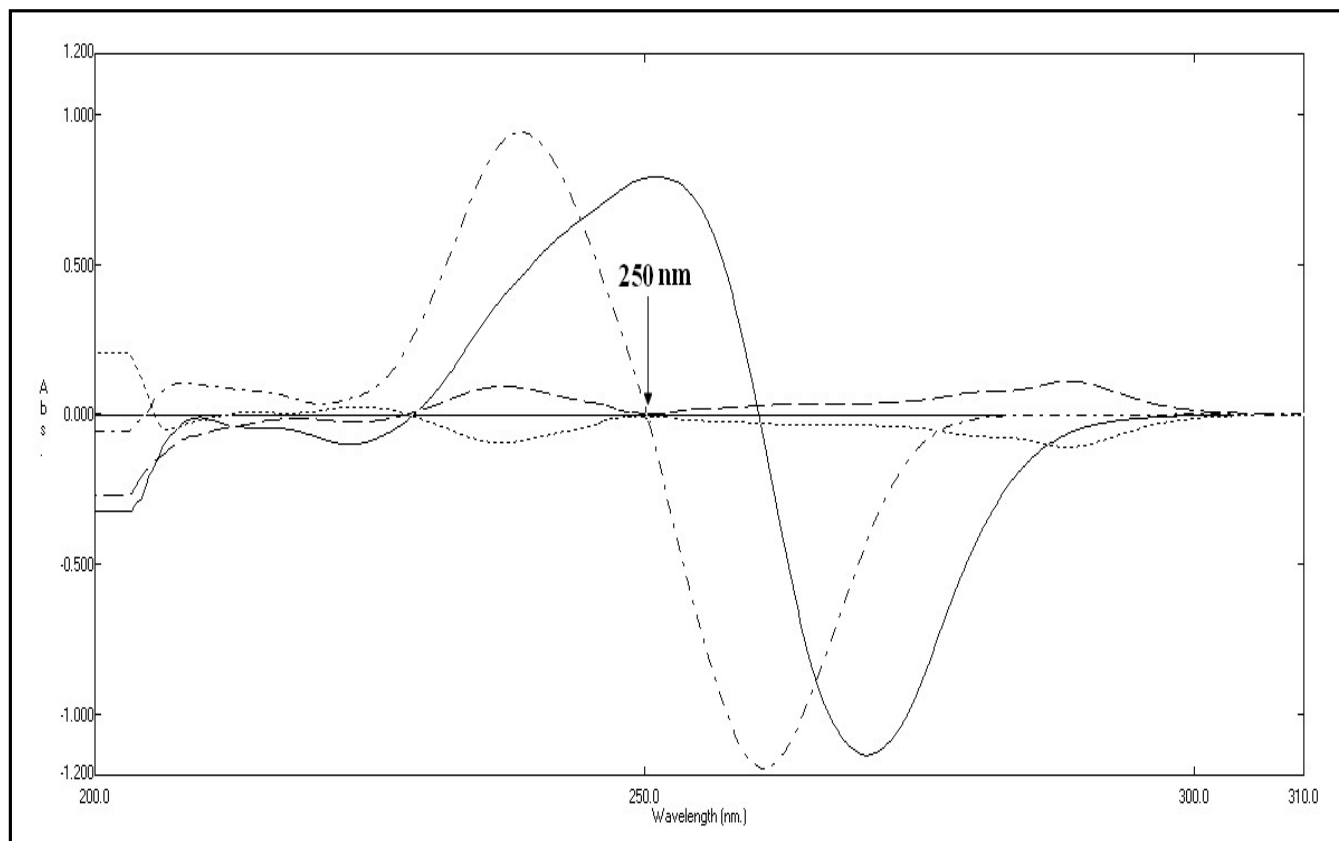


Figure 4: First derivative of ratio spectra of 10 µg/mL of each of DXF (—), MTZ (-----), FUR (-.-.-) and DEG (.....) using mixture of 20 µg/mL of each of MTZ and DEG as a divisor and methanol as a solvent.

By using a divisor consisting of a mixture of MTZ and DEG (each at a concentration of 20 µg/mL), it was determined that DD¹ spectra of DXF obtained and could be detected at 250 nm, which corresponds to the zero crossing of FUR and zero contribution of MTZ and DEG. This was achieved by dividing the absorption spectra of MTZ, DXF, FUR, and DEG by the divisor spectrum, as shown in **Figure 4**. Peak amplitude at 250 nm for DD¹ spectra of DXF in the concentration range of 1-25 µg/mL and absorbance of MTZ at its maximum 310.8 nm in the concentration range of 2–25 µg/mL were derived as linear relationships, from which the linear regression formulas were constructed and demonstrated to be:

$$A = 0.0536 C_{\text{MTZ}} - 0.0002$$

$$PA = 0.0800 C_{\text{DXF}} - 0.0056$$

$$r = 0.9999$$

$$r = 0.9999$$

$$\text{at } 310.8 \text{ nm for MTZ}$$

$$\text{at } 250 \text{ nm for DXF}$$

Where A is the absorbance of MTZ, PA is the peak amplitude of DXF, C₁ and C₂ are the concentrations of MTZ and DXF in µg/mL, respectively, and r is the correlation coefficient.

Table 1 displays the accuracy results of the suggested approach for determining pure MTZ at 310.8 nm and pure DXF at 250 nm in bulk powder. Additionally, the suggested method's selectivity was verified by using it on laboratory-prepared combinations with various ratios of MTZ, DXF, FUR, and DEG. Results exhibited in **Table 2** demonstrates that the suggested approach is valid for determining intact MTZ and DXF in the presence of 10% to 70% of DXF-degradation products. **Table 3** demonstrates the successful implementation of the proposed method to measure the concentrations of MTZ and DXF in the mentioned pharmaceutical formulations. No impact from excipients was observed when the standard addition method was utilized, **Table 4** presents the corresponding results. To ensure accuracy, repeatability, and intermediate precision, the suggested approach was validated in accordance with ICH [33] recommendations. The results of this validation are displayed in **Table 5**, which also shows other parameters of a regression formula. Based on the correlation coefficient, it can be concluded that the method has a strong linear relationship. The descriptive data from the regression analysis showed low standard errors for the intercept and slope, indicating high accuracy with minimal deviation and dispersion of the calibrated values. Statistical comparing of the results acquired for the analysis of MTZ and DXF in the pure form by the suggested DDRSD spectrophotometric method with those obtained by applying the published spectrophotometric method [4] (by measuring the absorption at the their iso-absorptive point at 277.2 nm for DXF and direct determination of MTZ

at λ_{\max} 314 nm) was conducted and there was no meaningful difference between the results was found. **Table 6** reveals that the calculated *t*- and *F*- values were less than the theoretical ones, confirming accuracy and precision at 95% confidence level. On the other hand, the published spectrophotometric method fails to determine DXF in presence of its degradation products due to severe overlapping, while the suggested DDRSD spectrophotometric method is more selective and can be considered as stability indicating one.

Table 1: Results of accuracy for determination of Diloxanide furoate and Metronidazole in pure form by the proposed spectrophotometric method.

DXF at 250 nm			MTZ at 310.8 nm		
Taken ($\mu\text{g/mL}$)	Found* ($\mu\text{g/mL}$)	Recovery %	Taken ($\mu\text{g/mL}$)	Found* ($\mu\text{g/mL}$)	Recovery %
3.00	3.04	101.33	5.00	5.07	101.40
7.00			9.00	8.94	99.33
11.00			13.00	13.06	100.46
15.00			17.00	17.04	100.24
19.00			21.00	20.96	99.81
23.00			25.00	24.60	98.40
	18.84	99.16			
	23.06	100.26			
Mean \pm SD		99.78 \pm 1.04	Mean \pm SD		99.94 \pm 1.02

* Average of 3 determinations.

Table 2: Determination of Diloxanide furoate and Metronidazole in laboratory prepared mixtures by the proposed spectrophotometric method.

Mixture ratio	% Degradation products	Concentration ($\mu\text{g/mL}$)	Recovery % *	
		MTZ: DXF: FUR: DEG	MTZ	DXF
1 : 1**	10	25 : 22.5 : 1.2 : 1.3	100.36	99.33
1 : 1.25**	20	10 : 10 : 1.2 : 1.3	98.50	98.40
1 : 2	30	10 : 14 : 3.1 : 2.9	99.30	100.71
1 : 1.5	40	10 : 9 : 2.9 : 3.1	100.80	100.89
1.25 : 1	50	25 : 10 : 4.8 : 5.2	100.44	98.00
1 : 2	60	15 : 12 : 8.6 : 9.4	99.13	101.08
1 : 3	70	10 : 9 : 10 : 11	98.80	101.11
Mean \pm SD			99.62 \pm 0.90	99.93 \pm 1.33

* Average of 3 determinations.

** The ratio of pharmaceutical formulations.

- Percent of the degradation products were calculated according to their molecular weights.

Table 3: Determination of Diloxanide furoate and Metronidazole in pharmaceutical formulations by the proposed double divisor spectrophotometric method.

Drug	Pharmaceutical formulation (Found %* \pm SD)	
	Flagimide® tablets	Furazole® tablets
DXF	100.12 \pm 1.05	101.12 \pm 1.21
MTZ	101.27 \pm 1.11	98.68 \pm 0.92

* Average of 6 determinations.

Table 4: Application of standard addition technique for the determination of Diloxanide furoate and Metronidazole by the proposed double divisor spectrophotometric method.

Pharmaceutical formulation		Taken (µg/mL)	Standard addition technique		
			Pure added (µg/mL)	Pure found* (µg/mL)	Recovery %
Flagimide® tablets claimed to contain 250 mg MTZ and 250 mg of DXF (Batch No. 0563930)	DXF	10.00	8.00	7.82	97.75
			10.00	9.93	99.30
			12.00	12.15	101.25
			14.00	14.04	100.29
	Mean ± SD				99.65 ± 1.49
	MTZ	10.00	8.00	8.06	100.75
			10.00	9.86	98.60
			12.00	12.10	100.83
			14.00	14.10	100.71
	Mean ± SD				100.22 ± 1.08

* Average of 3 determinations.

Table 5: Results of assay validation parameters of the proposed double divisor spectrophotometric method for the determination of Diloxanide furoate and Metronidazole.

Parameters	The proposed spectrophotometric method	
	DXF	MTZ
Range ($\mu\text{g/mL}$)	1 – 25	2 – 25
<u>Linearity</u>		
Slope	0.0800	0.0536
Intercept	– 0.0056	– 0.0002
Correlation coefficient (r)	0.9999	0.9999
Standard error of the slope	0.00027	0.00025
Confidence limit of the slope	0.07930-0.08049	0.05301-0.05412
Standard error of the intercept	0.00385	0.00398
Confidence limit of the intercept	(-0.01409)-(-0.00287)	(-0.00895)-(-0.00855)
Accuracy (Mean \pm SD)	99.64 \pm 1.06	99.94 \pm 1.02
Specificity and Selectivity	99.93 \pm 1.33	99.62 \pm 0.90
<u>Precision (%RSD)</u>		
Repeatability*	0.78	0.791
Intermediate precision*	0.98	0.92
LOD ($\mu\text{g/mL}$)	0.45	0.88

* The intra-day and inter-day relative standard deviations of the average of concentrations 5, 10 and 15 $\mu\text{g/mL}$ of DXF and 5, 10 and 15 $\mu\text{g/mL}$ of MTZ.

Table 6: Statistical comparison of the results obtained by the proposed double divisor spectrophotometric method and the reported method for the determination of pure Diloxanide furoate and Metronidazole.

Items	Spectrophotometric method		Reported method [4]	
	DXF	MTZ	DXF	MTZ
Mean	99.64	99.94	100.02	100.04
SD	1.06	1.02	1.15	1.15
% RSD	1.06	1.02	1.15	1.15
n	6	6	8	8
Variance	1.12	1.04	1.32	1.32
Student's <i>t</i> -test (2.18)	0.57	0.15		
<i>F</i> -value (4.88)	1.18	1.27		

* by measuring the absorbance at the isosbestic point at 277.2 nm for DXF and direct determination of MTZ at λ_{\max} 314.0 nm.

- Figures between parenthesis represent the corresponding tabulated values of *t* and *F* at *P* = 0.05.

Conclusion

The drugs DXF and MTZ, which are frequently combined as a binary mixture, are known as antibiotics, and are utilized for the treatment of a variety of diseases brought on by bacteria and parasites. According to a survey of the literature, the only stability-indicating spectrophotometric approach for the measurement of DXF and MTZ is the chemometric method, the use of which necessitates complicated and specific software. Consequently, in the study that is being described, a new spectrophotometric technique for selectively determining DXF and MTZ in the presence of DXF-degradation products is introduced adopting DDRSD technique. The offered technique is easy, selective, and precise, and enables stability indicating assay of the investigated mixture both on its own and in their pharmaceutical formulations.

References

- [1] F. P. Tally and C. E. Sullivan, "Metronidazole: in vitro activity, pharmacology and efficacy in anaerobic bacterial infections," *Pharmacother. J. Hum. Pharmacol. Drug Ther.*, vol. 1, no. 1, pp. 28–38, 1981.
- [2] A. A. Al-Majed, F. Belal, and A. A. Al-Badr, "Diloxamide furoate," in *Analytical profiles of drug substances and excipients*, vol. 26, Elsevier, 1999, pp. 247–282.
- [3] H. Qureshi, A. Ali, R. Baqai, and W. Ahmed, "Efficacy of a Combined Diloxanide Furoate—Metronidazole Preparation in the Treatment of Amoebiasis and Giardiasis," *J. Int. Med. Res.*, vol. 25, no. 3, pp. 167–170, 1997.
- [4] M. R. El-Ghobashy and N. F. Abo-Talib, "Spectrophotometric methods for the simultaneous determination of binary mixture of metronidazole and diloxanide furoate without prior separation," *J. Adv. Res.*, vol. 1, no. 4, pp. 323–329, 2010.
- [5] M. M. Issa, A. M. A. Shanab, and N. T. Shaat, "Kinetic spectrophotometric H-point standard addition method for the simultaneous determination of diloxanide furoate and metronidazole in binary mixtures and biological fluids," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 114, pp. 592–598, 2013.
- [6] N. H. Al Shaalan, "Determination of diloxanide furoate and metronidazole in binary mixture using first derivative of the ratio-spectra and highperformance liquid chromatography-UV methods," *J Appl Sci*, vol. 4, pp. 66–72, 2007.
- [7] M. M. Elkhoudary, R. A. A. Salam, and G. M. Hadad, "Comparative artificial neural network and partial least squares models for analysis of Metronidazole, Diloxanide, Spiramycin and Clotrimazole in pharmaceutical preparations," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 130, pp. 222–229, 2014.
- [8] S. M. Galal, M. M. Bedair, and M. A. El-Sayed, "Derivative spectrophotometric determination of antiprotozoal drugs in two-component tablet preparation," *J. Pharm. Belg.*, vol. 46, no. 5, pp. 315–319, 1991.
- [9] S. S. Abbas, N. E. Wagieh, M. Abdelkawy, and M. M. Abdelrahman, "Simultaneous determination of diloxanide furoate and metronidazole in presence of diloxanide furoate degradation products," *J. AOAC Int.*,

- vol. 94, no. 5, pp. 1427–1439, 2011, doi: 10.5740/jaoacint.10-286.
- [10] E. A. Abdelaleem and N. S. Abdelwahab, "Validated chromatographic and spectrophotometric methods for analysis of some amoebicide drugs in their combined pharmaceutical preparation.," *Pak. J. Pharm. Sci.*, vol. 26, no. 1, 2013.
- [11] S. K. Talwar, S. C. Sharma, and S. Das, "Simultaneous spectrophotometric determination of diloxanide furoate and metronidazole in dosage forms," *J. Pharm. Biomed. Anal.*, vol. 4, no. 4, pp. 511–515, 1986.
- [12] M. M. Elkhoudary, R. A. Abdel Salam, and G. M. Hadad, "Development and optimization of HPLC analysis of metronidazole, diloxanide, spiramycin and cliquinol in pharmaceutical dosage forms using experimental design," *J. Chromatogr. Sci.*, vol. 54, no. 10, pp. 1701–1712, 2016.
- [13] K. R. Danao, S. M. Hiradere, R. S. Moon, A. V. Kasture, and P. G. Yeole, "RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination," *Int J pharm life Sci*, vol. 1, pp. 82–85, 2010.
- [14] S. M. El-Gizawy, "HPLC analysis of metronidazole and diloxanide furoate in its dosage forms," *Anal. Lett.*, vol. 28, no. 1, pp. 83–92, 1995.
- [15] A. Mishal and D. Sober, "Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzoate and diloxanide furoate as bulk drug and in suspension dosage form," *J. Pharm. Biomed. Anal.*, vol. 39, no. 3–4, pp. 819–823, 2005.
- [16] M. M. Morcoss, N. S. Abdelwahab, N. W. Ali, and M. T. Elsaady, "Different chromatographic methods for simultaneous determination of diloxanide furoate, metronidazole and its toxic impurity," *J. Iran. Chem. Soc.*, vol. 13, pp. 1643–1651, 2016.
- [17] M. Mabrouk, H. El-Fatratry, I. Hewala, and E. Emam, "Development and application of a novel, dual-mode gradient, stability-indicating HPLC-DAD method for the simultaneous determination and purity assessment of mebeverine hydrochloride, diloxanide furoate and their corresponding major degradation products in," *J. Pharm. Biomed. Anal.*, vol. 83, pp. 249–259, 2013.
- [18] E. A. Bahgat, M. S. Elmasry, and R. A. Sayed, "The application of experimental design and green analytical chemistry in HPLC strategy for the determination of five drugs used in antimicrobial combination therapy," *Microchem. J.*, p. 108915, 2023.
- [19] E. A. Abdelaleem and N. S. Abdelwahab, "Simultaneous determination of some antiprotozoal drugs in their binary and ternary mixtures with mebeverine hydrochloride in different dosage forms," *J. Liq. Chromatogr. Relat. Technol.*, vol. 36, no. 11, pp. 1528–1539, 2013.
- [20] E. Dinç, "The spectrophotometric multicomponent analysis of a ternary mixture of ascorbic acid, acetylsalicylic acid and paracetamol by the double divisor-ratio spectra derivative and ratio spectra-zero crossing methods," *Talanta*, vol. 48, no. 5, pp. 1145–1157, 1999.
- [21] S. Pandey, N. K. Karmakar, R. K. Pandey, and S. S. Shukla, "A double divisor ratio-spectra derivative method for the ternary mixture of aspirin, clopidogrel bisulphate and rosuvastatin calcium," *Anal. Chem. Lett.*, vol. 11, no. 5, pp. 696–707, 2021.
- [22] S. H. Elagamy, "Resolving Problems Encountered in Spectrophotometric Analysis of a Ternary Mixture of Linagliptin, Empagliflozin and Metformin Hydrochloride," *Anal. Chem. Lett.*, vol. 12, no. 3, pp. 349–357, 2022.
- [23] E. Dinç, E. Baydan, M. Kanbur, and F. Onur, "Spectrophotometric multicomponent determination of sunset yellow, tartrazine and allura red in soft drink powder by double divisor-ratio spectra derivative, inverse least-squares and principal component regression methods," *Talanta*, vol. 58, no. 3, pp. 579–594, 2002.
- [24] R. Hajian and A. Soltaninezhad, "The spectrophotometric multicomponent analysis of a ternary mixture of paracetamol, aspirin, and caffeine by the double divisor-ratio spectra derivative method," *J. Spectrosc.*, vol. 2013, 2013.
- [25] H. Salem, H. M. Lotfy, N. Y. Hassan, M. B. El-Zeiny, and S. S. Saleh, "A comparative study of different aspects of manipulating ratio spectra applied for ternary mixtures: derivative spectrophotometry versus wavelet transform," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 135, pp. 1002–1010, 2015.
- [26] M. Bakshi and S. Singh, "Development of validated stability-indicating assay methods—critical review," *J. Pharm. Biomed. Anal.*, vol. 28, no. 6, pp. 1011–1040, 2002.
- [27] S. S. Abbas, H. E. Zaazaa, M. Abdelkawy, and M. M. Abdelrahman, "Spectrophotometric determination of isopropamide iodide and trifluoperazine hydrochloride in presence of trifluoperazine oxidative degradate," *Drug Test. Anal.*, vol. 2, no. 4, pp. 168–181, 2010.
- [28] R. M. Maggio, S. E. Vignaduzzo, and T. S. Kaufman, "Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations," *TrAC Trends Anal. Chem.*, vol. 49, pp. 57–70, 2013.
- [29] H. E. Zaazaa, M. M. Abdelrahman, N. W. Ali, M. A. Magdy, and M. Abdelkawy, "Kinetic study and mechanism of Niclosamide degradation," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 132, pp. 655–662, 2014.
- [30] A. Parmar and S. Sharma, "Derivative UV-vis absorption spectra as an invigorated spectrophotometric method for spectral resolution and quantitative analysis: Theoretical aspects and analytical applications: A

- review," *TrAC Trends Anal. Chem.*, vol. 77, pp. 44–53, 2016.
- [31] A.-A. Y. El-Sayed and N. A. El-Salem, "Recent developments of derivative spectrophotometry and their analytical applications," *Anal. Sci.*, vol. 21, no. 6, pp. 595–614, 2005.
- [32] M. M. Abdelrahman, I. A. Naguib, M. R. El Ghobashy, and N. A. Ali, "Quantitative determination of zopiclone and its impurity by four different spectrophotometric methods," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 137, pp. 617–624, 2015.
- [33] ICH, "Validation of an analytical Procedures: text and methodology Q2(R1)," *Guidance*, vol. 1994, no. November 1996, p. 17, 2005.