FABAD J. Pharm. Sci., 47, 3, 301-316, 2022
Doi: 10.55262/fabadeczacilik.1134580
RESEARCH ARTICLE

Simultaneous Determination of Spiramycin and Metronidazole in Coated Tablets by Derivative and Wavelet Transforms of UV Spectra and Ratio Spectra

Doan Thanh XUAN[*°], Vu Dang HOANG[**°]

Simultaneous Determination of Spiramycin and Metronidazole in Coated Tablets by Derivative and Wavelet Transforms of UV Spectra and Ratio Spectra

SUMMARY

Signal transformation (derivative and wavelet) was applied to UV spectra and ratio spectra to directly quantify spiramycin and metronidazole in binary mixtures. Linear calibration graphs were examined for either drug in the concentration range of 6.25 – 25 mg/L with R^2 > 0.990. First derivative-transformed (i.e., using Savitzky-Golay filter) and wavelet-transformed (i.e., using families such as sym6, haar, db5, bior2.4, rbio2.4, meyr with scaling factor = 256) UV spectrophotometric methods were statistically comparable to the reversed phase-HPLC reference method (p > 0.05) with regard to accuracy and precision when assaying spiramycin and metronidazole in their coated tablets. These analytical methods used only green solvent, and proved to be time-saving and cost-effective.

Key Words: *UV spectrophotometry, derivative transform, wavelet transform, spiramycin, metronidazole, coated tablets, RP-HPLC*

UV Spektrum ve Oran Spektrumlarının Türev ve Dalgacık Dönüşümleri ile Kaplanmış Tabletlerde Spiramycin ve Metronidazolün Eşzamanlı Belirlenmesi

ÖZ

İkili karışımlarda spiramisin ve metronidazolün doğrudan kantitatif analizi için UV spektrumlarına ve oran spektrumlarına sinyal dönüşümü (türev ve dalgacık) uygulandı. Her iki ilaç için de R^2 > 0.990 ile kalibrasyon grafikleri 6.25 – 25 mg/L doğrusal konsantrasyon aralığında çalışıldı. Birinci türevle dönüştürülmüş (yani, Savitzky-Golay filtresi kullanılarak) ve dalgacıkla dönüştürülmüş UV spektrofotometrik yöntemler yani skala faktörü = 256 olan sym6, haar, db5, bior2.4, rbio2.4, meyr gibi aileleri kullanarak, kaplanmış tabletlerinde spiramisin ve metronidazol test edilirken doğruluk ve kesinlik açısından RP-HPLC referans yöntemiyle (p > 0.05) istatistiksel olarak karşılaştırılabilir bulundu. Bu çalışmada analitik yöntemlerde yalnızca yeşil solvent kullanıldı ve yöntemlerin zamandan tasarruf ve düşük maliyetli olduğu kanıtlandı.

Anahtar Kelimeler: *UV spektrofotometrisi, türev dönüşümü, dalgacık dönüşümü, spiramisin, metronidazol, kaplanmış tabletler, RP-HPLC*

Received: 01.02.2022
Revised: 12.05.2022
Accepted: 12.05.2022

[*] ORCID: 0000-0003-2428-3757, Department of Mechatronics, School of Mechanical Engineering, Hanoi University of Science and Technology, No. 1 Dai Co Viet, Hai Ba Trung, Hanoi, Vietnam
[**] ORCID: 0000-0001-9605-3470, Department of Analytical Chemistry and Toxicology, Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Hoan Kiem, Hanoi, Vietnam

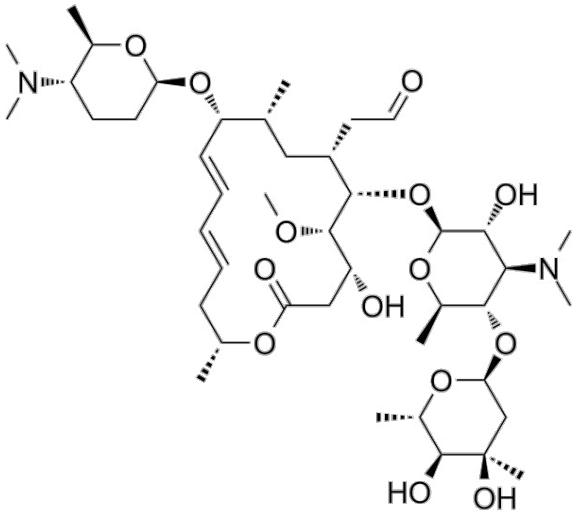
[°] Corresponding authors: Doan Thanh XUAN, Vu Dang HOANG
Tel.: +84 438 254539; fax: +84 438 264464; e-mail: xuan.doanthanh@hust.edu.vn; hoangvd@hup.edu.vn

INTRODUCTION

Spiramycin (SPI) is an antiparasitic and antibiotic, first isolated in 1954 from *Streptomyces ambofaciens*. This macrolide is chemically composed of a 16-member lactone ring with the substitution of two amino sugars (mycaminose and forosamine) and one neutral sugar (mycarose) (Figure 1A). It is a mixture of three major components: I (3-OH) being the most important (accounted for at least 85%) together with II (3-*O*-acetyl) and III (3–*O*-propionyl), showing a greater activity against *Treponema* than other macrolides such as erythromycin or oleandomycin (Kwon, 2017). The conversion factor of International Unit (IU) to milligram (mg) for SPI is 10^{-3} (Drugs.com, 2022).

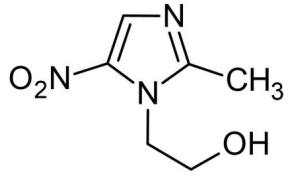
Metronidazole (MET) is a synthetic drug that chemically belonged to the family of nitroimidazole antibiotics. Since its development in 1959 specifically for trichomoniasis treatment, this 5-nitroimidazole medication (Figure 1B) has been still prescribed for anaerobic infections (both parasitic and bacterial) and listed among the 'essential medicines' according to the World Health Organization. This fact is very likely associated with MET's pleiotropic mode of action, separating it from most other antimicrobials i.e., it does target many molecules rather than a few or exactly a single one as it enters the cell and is reduced to its nitro group under low oxygen concentrations (Leitsch, 2017).

As early as the 1980s, the *in vitro* activity of Rodogyl (SPI-MET combined tablet) was reported against putative periodontopathic bacteria (Quee et al., 1983). It was proved that there is synergy between SPI and MET in treating polymicrobial infections (Brook, 1988). The SPI-MET combination (1500000 units/250 mg, three times a day) could mostly eliminate bacterial pathogens in the treatment and follow-up treatment of patients with active periodontitis (except for *Fusobacterium spp*.) (Poulet et al., 2005).



(A)

(4R,5S,6R,7R,9R,10R,11E,13E,16R)-10-{[(2R,5S,6R)-5-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside



(B)

2-(2-methyl-5-nitroimidazol-1-yl) ethanol

Figure 1. Chemical structure and IUPAC name of (A) SPI and (B) MET.

In pharmaceutical analysis, the quantification of either drug alone in their bulk, single and combined dosage forms could be done by various chromatographic methods (Akay et al., 2002; Chepkwony et al., 2001; Horie et al., 1988; Tavakoli et al., 2007), NMR spectroscopic methods (Salem & Mossa, 2012; Salem et al., 2006), Visible light (VIS) and Near Infrared (NIR) spectrophotometric methods (Saffaj et al., 2004; Saffaj et al., 2006; Abdel-Kader & Hashem, 2021; Sakira et al., 2021), and electrochemical methods (Li & Xu, 2014). It was also reported that MET could be co-assayed in multicomponent mixtures by using derivative and chemometrics-assisted UV spectrophotometry (Erk & Altun, 2001; Mahrouse & Elkady, 2011; Elkhoudary et al., 2014; Korany et al., 2015; Attia et al., 2016), direct and ratio-subtraction UV spectrophotometry (El-Ghobashy & Abo-Talib, 2010), kinetic spectrophotometric H-point standard addition method (Issa et al., 2013). To the best of our knowledge, there has not been any pharmacopoeial monograph for SPI-MET combined tablets yet. In the literature, the co-assay of SPI and MET in pharmaceutical mixtures was reliably performed by HPTLC and/or RP-HPLC (both isocratic and gradient) (Maher & Youssef, 2009; Elkhoudary et al., 2016). In 2010, Khattab and co-workers used different UV spectrophotometric techniques to determine SPI and MET in bulk and tablets (Khattab et al., 2010). Their study, nonetheless, has some limitations, such as (i) using methanol as the spectrophotometric solvent that is environmentally unfriendly and (ii) impossibility to determine SPI and MET simultaneously by zero-crossing point technique.

This study was undertaken to develop and validate UV spectrophotometric methods for simultaneous determination of SPI and MET in coated tablets by using both signal transform algorithms: derivative and wavelet. It proves to be better than the above-mentioned study by Khattab and co-workers with regard to (i) the use of a much less toxic solvent (ethanol) (Hansen, 2020) and (ii) the outperformance of wavelet transform over derivative transform in resolving UV spectral overlaps of binary mixtures as pointed out by Dinç and co-workers in reviews (Dinç, 2013, Dinç & Yazan, 2018) and experimental studies (e.g., Dinç et al., 2005, Üstündağ, Ö., & Dinç, E., 2021).

MATERIALS AND METHODS

Apparatus and software

A double-beam UV-1800 spectrophotometer (Shimadzu, Japan) equipped with 1-cm pairs of quartz cuvette was employed. Absorption spectra were registered in the range of 200 - 400 nm with a 0.1-nm fixed slit width, slow scanning speed and 0.1-nm sampling interval. Spectral manipulation (i.e., arithmetic, transformation such as smoothing and derivative) was performed using built-in UV Probe software. For wavelet transform, the spectral processing was done using MATLAB R2020a software (MathWorks, Natick, MA, USA).

RP-HPLC analysis was performed using an Agilent 1200 Series Diode-Array-Detector chromatograph (Agilent Technologies, USA). The chromatographic operating conditions were used exactly as proposed by Elkhoudary et al. (Elkhoudary et al., 2016).

Reagents and standard solutions

Chemical reference standards (HPLC purity): SPI (97%) and MET (99.65%) were provided from LGC Standards – UK. All chemicals and solvents were of analytical grade. Stock solutions of SPI and MET (1250 mg/L) were freshly prepared in ethanol. These solutions were appropriately diluted with ethanol to make a set of standard solutions in 25-mL volumetric flasks.

Sample solutions

Four coated tablet formulations (containing SPI 750000 IU + MET 125 mg) were purchased at local retail pharmacies i.e., Rodogyl (Sanofi Aventis, France), Novogyl (Mekophar, Vietnam), Arme-Rogyl (Armephaco, Vietnam), and Zolgyl (Bidiphar, Vietnam). For each formulation, twenty tablets were carefully peeled off the film coating using a very thin blade, then accurately weighed and finely pulverized in a mortar.

An accurate quantity equivalent to one-half of a tablet was dissolved in about 70 mL of ethanol in a 100-mL volumetric flask by 15-min sonication that was filled subsequently to the calibration mark with the same solvent. Further dilution in 25-mL volumetric flasks was required to obtain the test solutions i.e., SPI 12.5 mg/L + MET 6.25 mg/L (for SPI assay) and MET 12.5 mg/L + SPI 25 mg/L (for MET assay). Unless stated otherwise, only Rodogyl's transformed UV spectra and ratio spectra were displayed as "sample" in all Figures to demonstrate the applicability of our UV spectrophotometric methods.

RESULTS AND DISCUSSION

Figure 2 displays the zero-order UV spectra of SPI, MET and their corresponding mixtures at working concentrations, showing that SPI and MET have broad absorption bands peaked at 226.6 and 308.8 nm, respectively. This observation could be attributable to the electronic transition n → π*, provided that the drugs under study are constituted of the chromophores (i.e., 16-member lactone ring and carbonyl group in SPI, and 5-nitroimidazole structure in MET) and auxochromes (i.e., methyl, hydroxyl, and amino groups). In comparison with the data published by Khattab et al (Khattab et al., 2010) (i.e., SPI and MET peaked at ca. 232 and 311 nm in methanol, respectively), the hypsochromic shift for both drugs was noted under our spectrophotometric conditions. It is probably ascribed to the broadening of n → π* electronic transition in ethanol, which is a hydrogen bond donor stronger than methanol.

It is clear to indicate that (i) the obedience of the law of spectral additivity was reasonably justified in the range 200 – 400 nm, and (ii) the assay of SPI in binary mixtures was hindered since MET absorbed UV radiation markedly in the SPI spectral peak region. Although a direct determination of MET in the binary mixture could be done in the range of 280 – 360 nm as suggested by Khattab et al. (Khattab et al., 2010), it is still advisable to apply signal processing techniques (such as derivative and wavelet transforms) for MET quantification. This is because such signal transformation may effectively eliminate baseline drift as well as unwanted spectral absorption of the sample matrix. Thus, in our study, signal transformation was applied to both UV spectra and ratio spectra for the assay of SPI and MET in binary mixtures.

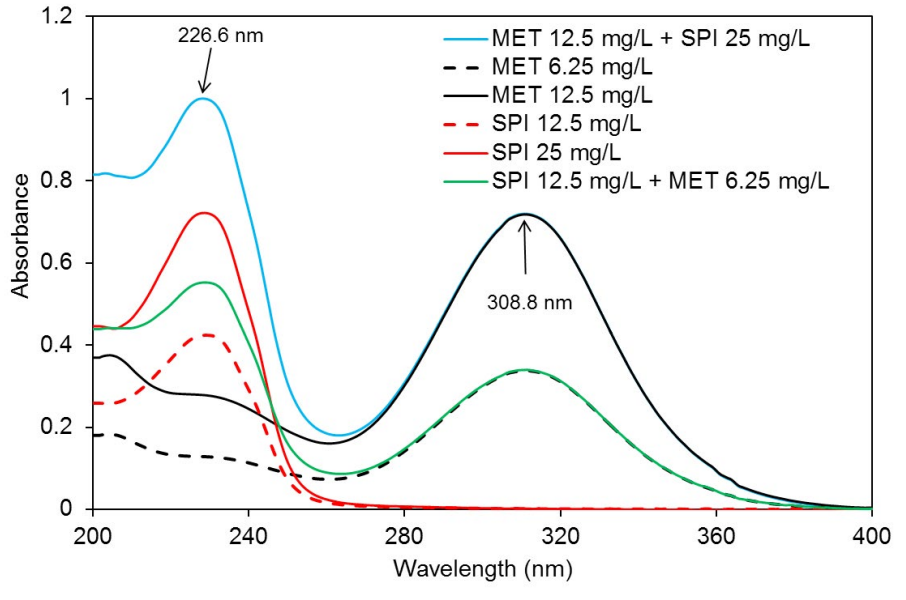


Figure 2. UV absorption spectra of SPI, MET and corresponding SPI-MET mixtures at working concentrations

By using the Savitzky-Golay smoothing and differentiation filter (Savitzky & Golay, 1964), the first-derivative transform is based on the convolution operation exquisitely fitting pieces of 17 data points inclusive of former and later data to a polynomial using least-squares regression. This signal processing was optimally implemented with $\Delta\lambda$ = 4 nm. In Figure 3 A, the first-derivative spectra of SPI and MET exhibited zero-crossing points at 289.8 and 259.8 nm, at which the amplitude of MET and SPI signals was not annulled accordingly. However, the assay of SPI is not feasible herein because a good correlation is not secured for a linear relationship between its derivative response and concentration (Table 1). Figures 3 B and

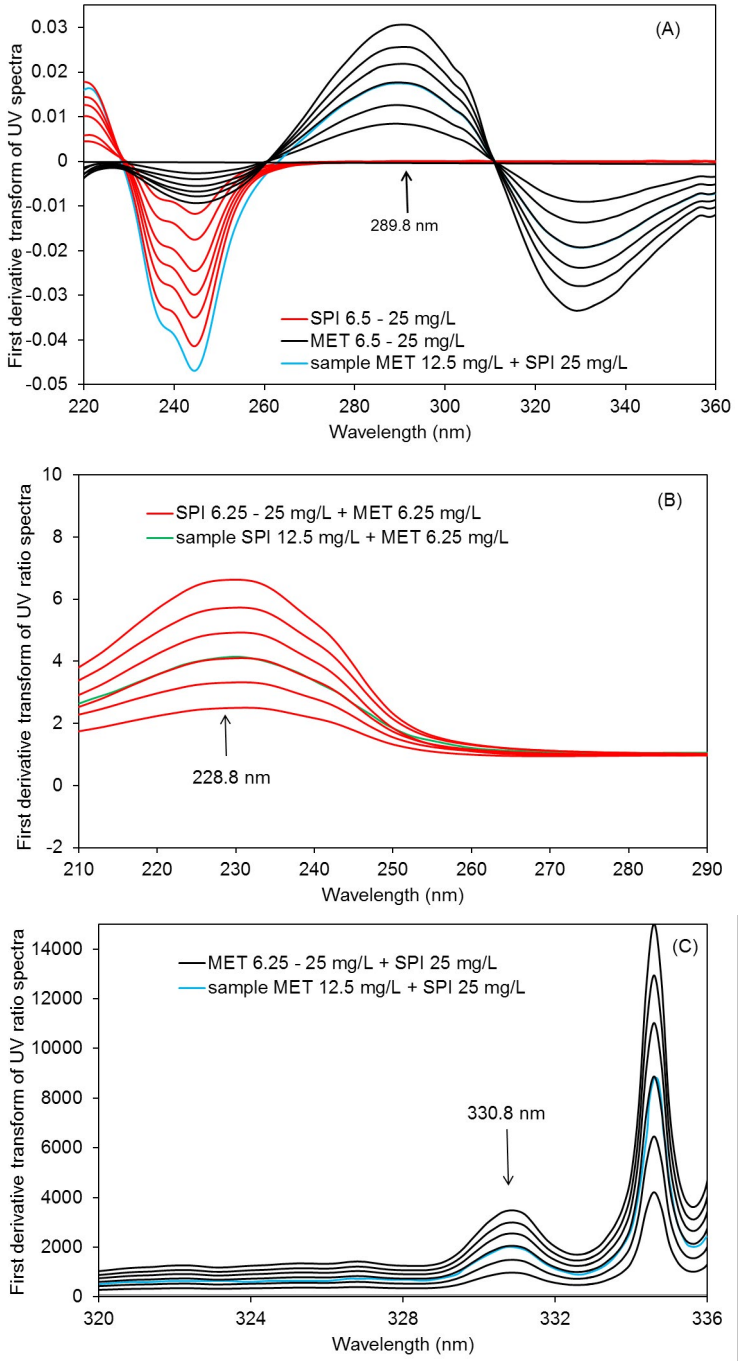
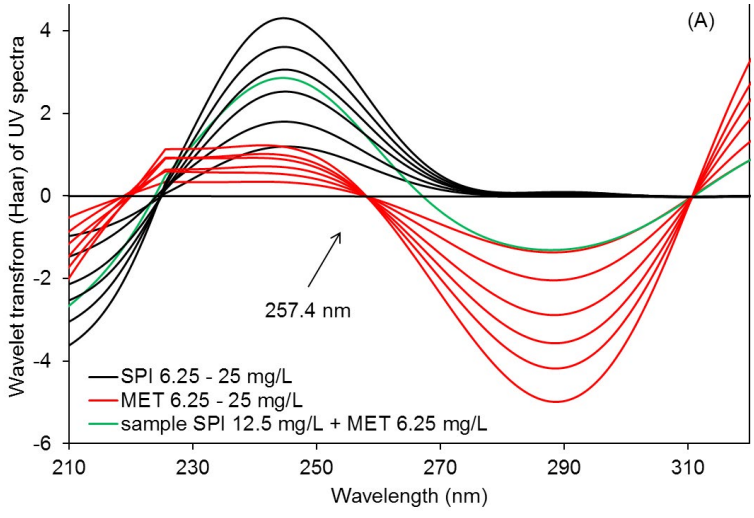


Figure 3: First-order derivatives of UV spectra (A) and UV ratio spectra (B and C)

C present the first-order derivatives of ratio spectra with suitable divisor standard concentrations found to be SPI 25 mg/L (for MET assay) and MET 6.25 mg/L (for SPI assay). The working wavelengths 228.8 and 330.8 nm were subsequently selected to determine SPI and MET, respectively, for their derivative amplitudes were the highest and directly proportional to the concentration ranges under investigation of SPI and MET (6.25 – 25 mg/L).

In the wavelet transform procedure, a signal is decomposed into a set of basic functions (a.k.a. wavelets) by dilating and translating a prototype mother function Ψ(t) (Kaiser, 2011). Spectral data, explicitly speaking, are subjected to mathematical processing for conversion into various coefficients, and each coefficient is then analyzed at a resolution matched to its scale. It means that concerning a wavelet expansion, the representation of spectra could be realized by using coefficients in a linear combination of the wavelet functions. In this study, different wavelet families such as continuous (i.e., Symlets, Coiflets, Mexican hat function, Meyer, Dmeyer, Gaussian, BioSplines, ReverseBio, Morlet) and discrete (Haar, Daubechies) were scrutinized at different dilation parameters (a) to separate overlapping spectral bands of SPI and MET in their binary mixtures. It was shown that the best spectral recovery values were obtained with a = 256. Figures 4 and 5 selectively present the wavelet-transformed UV spectra and ratio spectra, respectively. It is essential to indicate that SPI and MET could be simultaneously determined at 287.6 and 300.4 nm, respectively, by using rbio2.4-based transformed UV spectra. Moreover, the wavelet transform-based amplitudes were higher than the corresponding derivative ones, as expected. This is always true, in particular, for the assay of SPI using signal transformation of UV ratio spectra.

The suitability of our spectrophotometric assay was examined by repeating the absorbance measurement of SPI and MET standard solutions at working concentrations (RSD < 1%, n = 6). The developed spectrophotometric methods were validated in conformity with ICH guidelines for some criteria such as linearity, accuracy, within-run precision (repeatability), and ruggedness (intermediate precision) (ICH Harmonised Tripartite Guideline, 2005). Calibration curves were established for SPI and MET at the working wavelengths with $R^2 > 0.990$ for six standard points in the concentration range of 6.25 - 25 mg/L, suggesting a good relation between two variables: concentration – signal amplitude. Based on the statistical analysis of calibration curve data, LOD and LOQ values (expressed in mg/L) for all the UV spectrophotometric methods proposed were also calculated (Table 1).



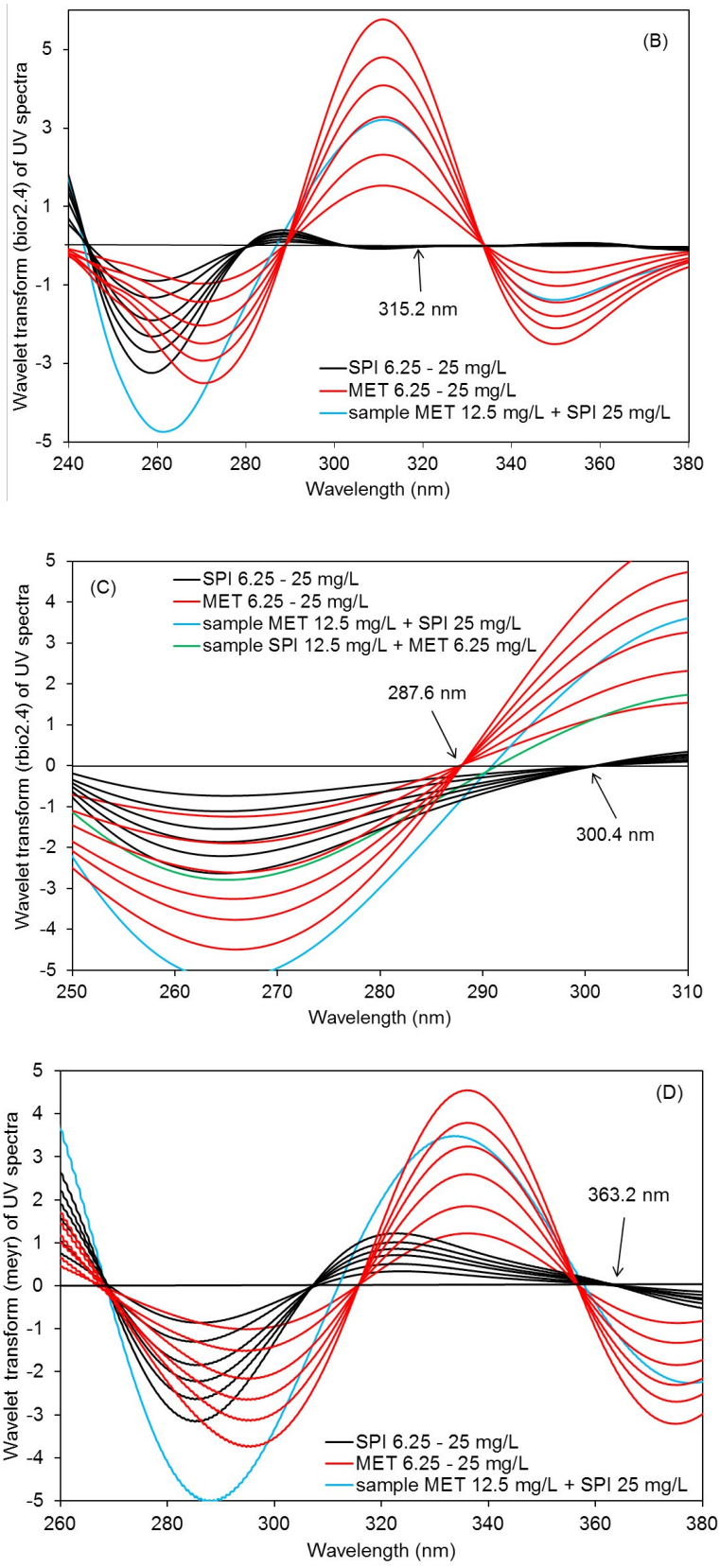
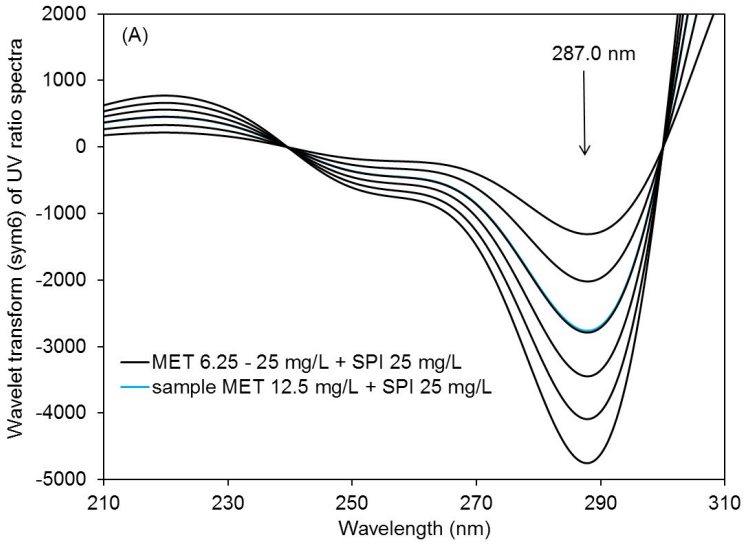
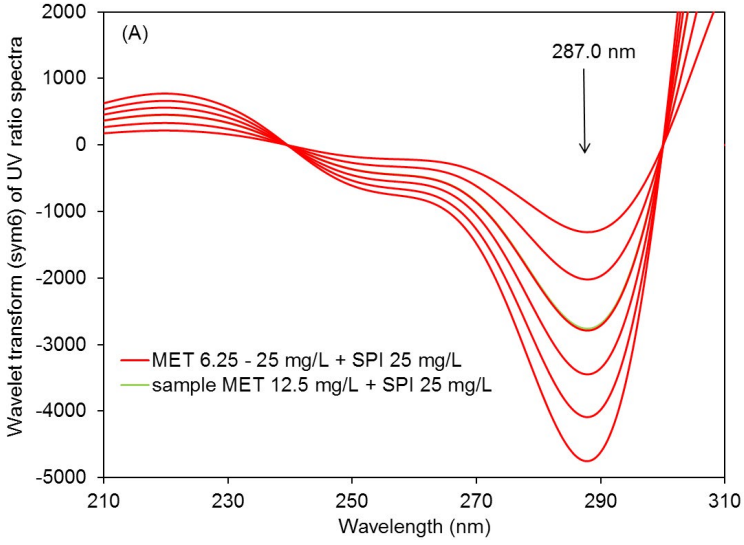
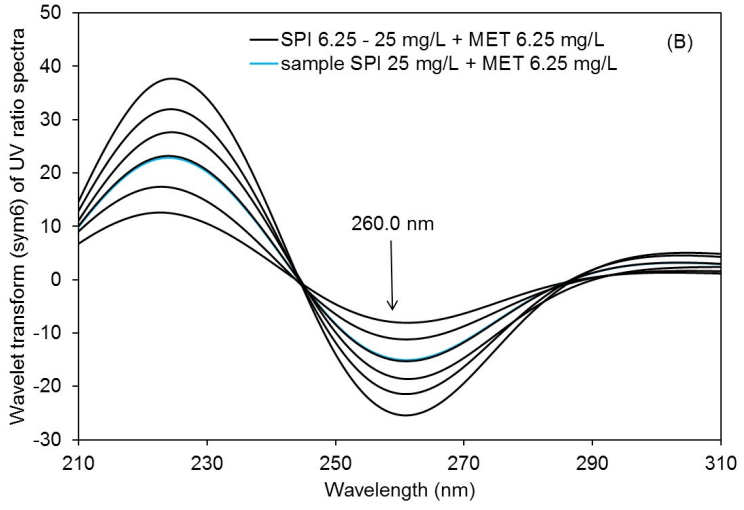
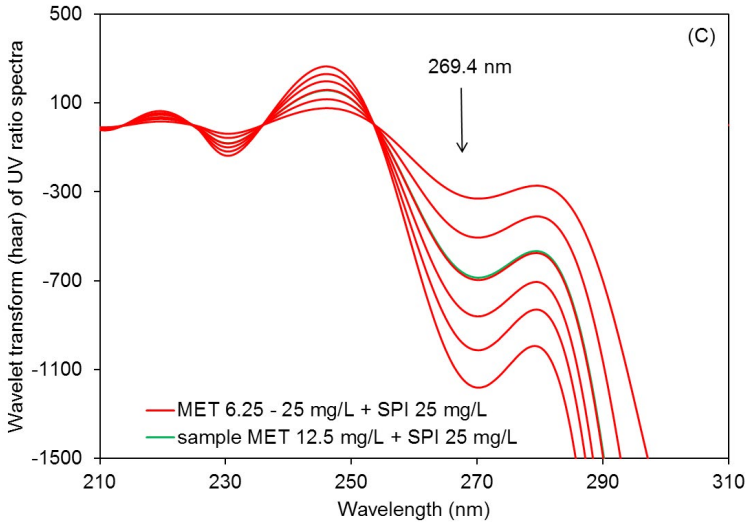


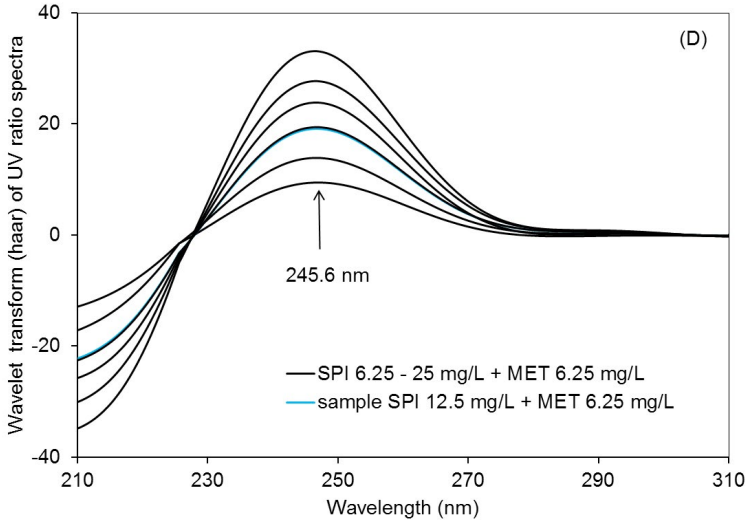
Figure 4. Wavelet transform of UV spectra: (A) haar, (B) bior2.4, (C) rbio2.4, (D) meyr

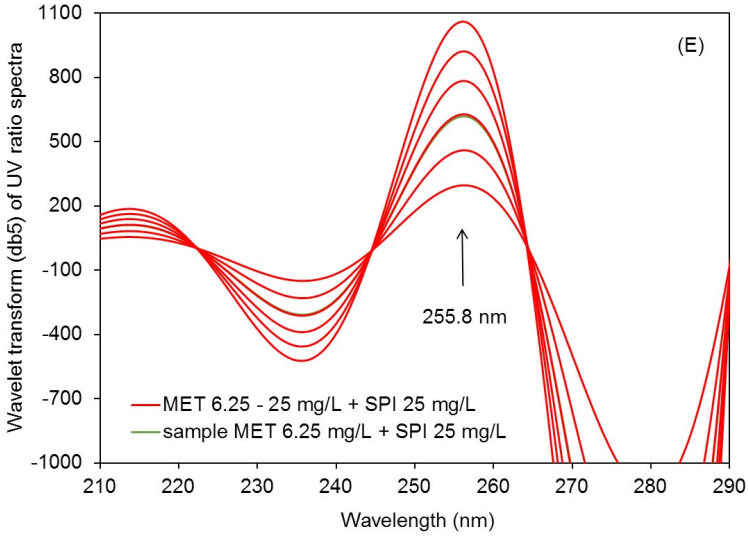

(A)
287.0 nm
MET 6.25 - 25 mg/L + SPI 25 mg/L
sample MET 12.5 mg/L + SPI 25 mg/L
Wavelet transform (sym6) of UV ratio spectra
Wavelength (nm)
2000
1000
0
-1000
-2000
-3000
-4000
-5000
210
230
250
270
290
310


(A)
287.0 nm
MET 6.25 - 25 mg/L + SPI 25 mg/L
sample MET 12.5 mg/L + SPI 25 mg/L
Wavelet transform (sym6) of UV ratio spectra
Wavelength (nm)
2000
1000
0
-1000
-2000
-3000
-4000
-5000
210
230
250
270
290
310


SPI 6.25 - 25 mg/L + MET 6.25 mg/L
sample SPI 25 mg/L + MET 6.25 mg/L
(B)
260.0 nm
Wavelet transform (sym6) of UV ratio spectra
Wavelength (nm)
50
40
30
20
10
0
-10
-20
-30
210
230
250
270
290
310


(C)
269.4 nm
Wavelet transform (haar) of UV ratio spectra
Wavelength (nm)
MET 6.25 - 25 mg/L + SPI 25 mg/L
sample MET 12.5 mg/L + SPI 25 mg/L


(D)
245.6 nm
Wavelet transform (haar) of UV ratio spectra
Wavelength (nm)
SPI 6.25 - 25 mg/L + MET 6.25 mg/L
sample SPI 12.5 mg/L + MET 6.25 mg/L


(E)
255.8 nm
Wavelet transform (db5) of UV ratio spectra
Wavelength (nm)
MET 6.25 - 25 mg/L + SPI 25 mg/L
sample MET 6.25 mg/L + SPI 25 mg/L

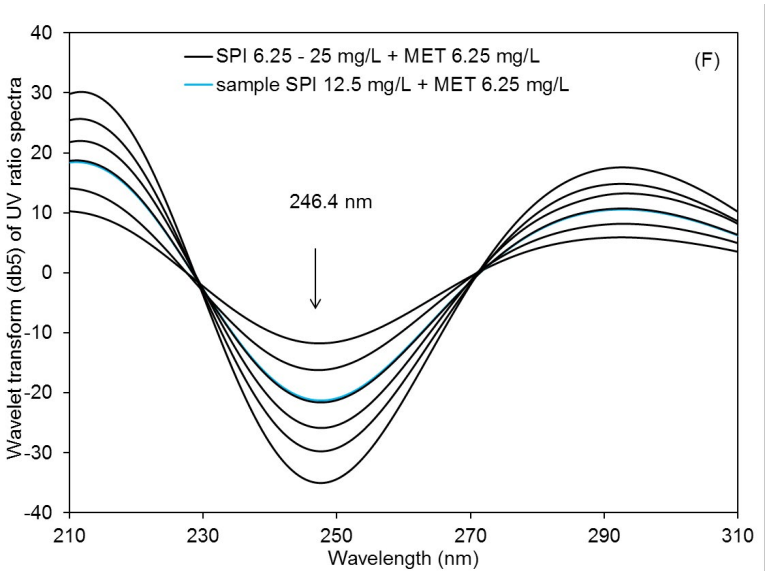


Figure 4. Wavelet transform of UV ratio spectra: (A)-(B) sym6, (C)-(D) haar, (E)-(F) db5

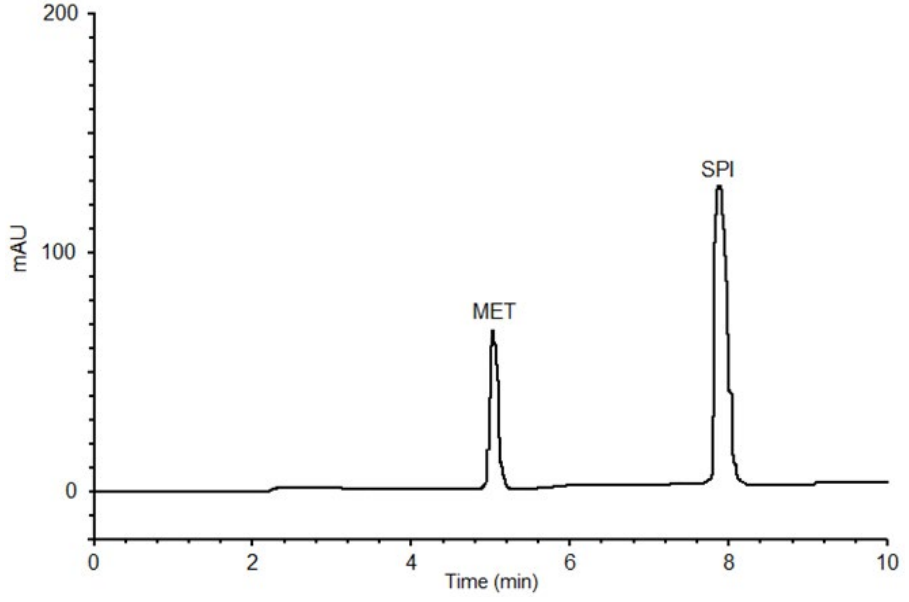


Figure 5. A typical HPLC chromatogram of the mixture of MET 6.25 mg/L + SPI 12.5 mg/L

Table 1. Statistical analysis of 6-point calibration graphs of the proposed spectrophotometric methods (6.25 –25 mg/L)

Method	Compound	Wavelength (nm)	a ($\times10^4$)	b ($\times10^4$)	Sa ($\times10^4$)	Sb ($\times10^4$)	Sy.x ($\times10^4$)	R^2	LOD	LOQ
Derivative transform										
UV spectra	MET	289.8	12.68	1.050	0.189	3.09	2.74	0.9991	0.71	2.16
UV ratio spectra	SPI	228.8	2370	923.2	33.2	542	481	0.9992	0.67	2.03
	MET	330.8	1449336	582381	28240	460039	408274	0.9984	0.93	2.82
Wavelet transform										
UV spectra										
haar	SPI	257.4	1047	747.6	18.3	297	264	0.9987	0.83	2.52
bior2.4	MET	315.2	2304	– 474.5	23.2	377	335	0.9995	0.48	1.45
rbio2.4	SPI	287.6	– 353.3	– 240.4	8.68	141	126	0.9975	1.17	3.57
	MET	300.4	1705	84.55	17.1	278	246	0.9995	0.48	1.44
meyr	MET	363.2	– 772.6	309.6	6.05	98.6	87.5	0.9997	0.37	1.13
UV ratio spectra										
sym6	SPI	260.0	– 9969	– 14764	128	2084	1849	0.9993	0.61	1.85
	MET	287.0	– 1984345	– 441943	36668	597338	530142	0.9986	0.88	2.67
haar	SPI	245.6	13567	5720	164	2670	2370	0.9994	0.57	1.74
	MET	269.4	– 489942	– 189578	8623	140473	124667	0.9987	0.84	2.54
db5	SPI	246.4	– 13261	– 31312	142	2320	2058	0.9995	0.51	1.55
	MET	255.8	442718	173415	10983	178923	158790	0.9975	1.18	3.59

Y = aC + b; where C is concentration in mg/L and Y is signal amplitude in arbitrary unit.
a: slope; b: intercept; Sa: SD of the slope; Sb: SD of the intercept; Sy.x: SD of the residuals; R^2: coefficient of determination; LOD = 3.3×Sy.x/a; LOQ =10×Sy.x/a

Table 2. Assay results for SPI and MET in coated tablets

	% of label claim (mean ± SD, = 6)							
Method	Rodogyl		Novogyl		Arme-Rogyl		Zolgyl	
	SPI	MET	SPI	MET	SPI	MET	SPI	MET
RP-HPLC	100.1 ± 1.2	99.5 ± 1.3	100.4 ± 1.0	100.2 ± 1.1	99.9 ± 0.9	99.3 ± 1.2	100.8 ± 1.4	100.9 ± 1.5
Derivative transform								
UV spectra	-	100.2 ± 1.5	-	99.5 ± 1.7	-	99.0 ± 1.8	-	101.4 ± 2.1
UV ratio spectra	99.8 ± 1.5	100.1 ± 1.7	100.8 ± 1.4	99.8 ± 1.8	100.3 ± 1.5	99.3 ± 1.8	100.1 ± 1.8	101.4 ± 1.9
Wavelet transform								
UV spectra								
haar	100.0 ± 1.4	-	101.0 ± 1.6	-	99.9 ± 1.8	-	100.3 ± 1.8	-
bior2.4	-	99.9 ± 1.7	-	99.9 ± 1.6	-	99.6 ± 1.9	-	101.0 ± 2.1
rbio2.4	100.3 ± 1.3	99.6 ± 1.6	100.7 ± 1.8	100.0 ± 1.8	100.1 ± 1.9	99.9 ± 2.0	100.5 ± 2.0	100.9 ± 1.9
meyr	-	100.2 ± 1.8	-	99.7 ± 1.7	-	99.8 ± 1.7	-	101.5 ± 1.9
UV ratio spectra								
sym6	99.7 ± 1.6	100.3 ± 1.6	100.5 ± 1.7	100.5 ± 1.8	100.3 ± 1.9	99.5 ± 2.1	100.5 ± 1.9	101.0 ± 2.2
haar	99.9 ± 1.4	99.9 ± 1.5	100.6 ± 1.8	100.2 ± 1.9	99.8 ± 1.7	99.7 ± 1.9	100.8 ± 1.7	100.7 ± 1.8
db5	100.2 ± 1.6	99.8 ± 1.5	100.8 ± 1.9	99.9 ± 1.8	99.9 ± 1.8	99.9 ± 1.9	100.1 ± 2.1	101.7 ± 2.0

Table 3. Evaluation of one-way ANOVA and Bartlett tests at the significance level 5% for assay results

One-way ANOVA test					
Source of variation	Compound		Between-groups	Within-groups	Total
Sum of squares	SPI	I	1.680	72.10	73.78
		II	1.491	92.50	93.99
		III	1.526	98.25	99.78
		IV	3.103	116.8	119.8
	MET	I	3.733	112.9	2.976
		II	4.320	130.6	134.9
		III	4.573	150.3	154.8
		IV	5.520	169.9	175.4
Degree of freedom	SPI		6	35	41
	MET		8	45	53
Mean of squares	SPI	I	0.2800	2.060	
		II	0.2486	2.643	
		III	0.2543	2.807	
		IV	0.5171	3.336	
	MET	I	0.4667	2.509	
		II	0.5400	2.902	
		III	0.5717	3.339	
		IV	0.6900	3.776	
Calculated F value	SPI	I	0.1359		
		II	0.0941		
		III	0.0906		
		IV	0.1550		
	MET	I	0.1860		
		II	0.1861		
		III	0.1712		
		IV	0.1828		
Tabulated F value	SPI		2.371		
	MET		2.152		
Bartlett test					
Degree of freedom	SPI		6		
	MET		8		
Calculated χ^2 value	SPI	I	0.6167		
		II	2.295		
		III	2.997		
		IV	0.8994		
	MET	I	0.6654		
		II	1.630		
		III	1.641		
		IV	0.8768		
Tabulated χ^2 value	SPI		12.592		
	MET		15.507		

I: Rodogyl; II: Novogyl; III: Arme-Rogyl; IV: Zolgyl

The accuracy was accessed using the standard addition technique (i.e., spiking a pre-assayed sample with a known amount of standard substances equal to 20% of the nominal content), revealing that the total recovery of standard addition was 98.5 - 101.4% (data not shown). Table 2 displays the analytical results when applying these UV spectrophotometric methods for the assay of SPI and MET in their coated tablets commercially available, clearly demonstrating the good precision of our methods (RSD < 2%) and the actual content of both drugs i.e., 97.7 ÷ 101.0% for SPI and 99.3 ÷ 101.7% for MET as compared to

the label claim. Assay results obtained by two different analysts on two different days (n = 6 for each day) were found to be insignificant different ($p > 0.05$) with RSD values ≤ 1.9%, meaning that our methods could be considered to be rugged enough. In our study, RP-HPLC proposed by Elkhoudary et al. (Elkhoudary et al., 2016) was used as the reference method. Figure 5 displays a typical HPLC of the binary mixture of MET 6.25 mg/L + SPI 12.5 mg/L, obviously showing that MET and SPI were respectively well resolved at ca. 5.0 and 7.9 minutes with tailing factor < 1.5 and acceptable column efficiency (number of theoretical plates > 2000). Statistical interpretation of both RP-HPLC and UV spectrophotometric data shows that they are comparably precise (Bartlett test: calculated χ^2 values smaller than tabulated ones) and accurate (one-way ANOVA test: calculated F values smaller than tabulated ones) at the significance level of 0.05 (Table 3). It also indicates that the excipients used in the pharmaceutical dosage forms under study did not interfere with the specificity of our spectrophotometric assay.

CONCLUSION

The signal transformation was successfully exploited for UV spectrophotometric assay of SPI and MET in their binary mixtures requiring no separation step for either drug. With regard to the assay of SPI, especially, wavelet transform manifested some noticeable advantages over derivative transform, such as the higher signal amplitude in transformed UV ratio spectra and the possibility to determine SPI and MET using transformed UV spectra simultaneously. In comparison with the work previously reported by Khattab and co-workers (Khattab et al., 2010), our spectrophotometric methods used only ethanol (a less toxic solvent than methanol) and offered the possibility of co-assaying SPI and MET in binary mixtures by zero-crossing point technique (i.e., transforming UV spectra by the mother wavelet rbio2.4). They were also statistically comparable with the RP-HPLC reference method (Elkhoudary et al., 2016) in terms of accuracy and precision when assaying SPI and MET in their combined coated tablets. Taking into consideration that they used only green solvent and proved to be time-saving and cost-effective, these analytical methods are strongly suggested for the routine analysis of SPI-MET coated tablets.

CONFLICT OF INTEREST

There is no conflict of interest among the authors of this manuscript.

AUTHOR CONTRIBUTION STATEMENT

The authors equally contributed to this work.

REFERENCES

Abdel-Kader, D. A., & Hashem, E. Y. (2021). Spectrophotometric determination of Metronidazole antibacterial drug via oxidation with alkaline potassium permanganate. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 259*, 119858. doi:https://doi.org/10.1016/j.saa.2021.119858

Akay, C., Özkan, S. A., Şentürk, Z., & Cevheroğlu, Ş. (2002). Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms by RP-HPLC. *Il Farmaco, 57*(11), 953-957. doi:https://doi.org/10.1016/S0014-827X(02)01296-X

Attia, K. A. M., Nassar, M. W., El-Zeiny, M. B., & Serag, A. M. (2016). Zero order and signal processing spectrophotometric techniques applied for resolving interference of metronidazole with ciprofloxacin in their pharmaceutical dosage form. *Spectrochimica acta. Part A: Molecular and Biomolecular Spectroscopy, 154*, 232-236.

Brook, I. (1988). Synergy between spiramycin and metronidazole in the treatment of polymicrobial infections. *Journal of Antimicrobial Chemotherapy, 22* (Suppl B), 77-85.

Chepkwony, H. K., Vermaelen, A., Roets, E., & Hoogmartens, J. (2001). Development and validation of an reversed-phase liquid chromatographic method for analysis of spiramycin and related substances. *Chromatographia, 54*(1), 51-56. doi:10.1007/BF02491832

Dinç, E., Ozdemir, A., & Baleanu D. (2005). An application of derivative and continuous wavelet transforms to the overlapping ratio spectra for the quantitative multiresolution of a ternary mixture of paracetamol, acetylsalicylic acid and caffeine in tablets. *Talanta*, *65*(1), 36-47. doi: 10.1016/j.talanta.2004.05.011

Dinç, E. (2013). Wavelet transforms and applications in drug analysis. *FABAD Journal of Pharmaceutical Sciences*, *38*(3), 159-165, 2013.

Dinç, E., & Yazan, Z. (2018). Wavelet transform-based UV spectroscopy for pharmaceutical analysis. *Frontiers in Chemistry*, *6*, 503. doi:10.3389/fchem.2018.00503

Drugs.com. (2022). Spiramycin. Retrieved from https://www.drugs.com/cons/spiramycin-oral-injection-rectal.html (accessed on 31 January 2022)

El-Ghobashy, M. R., & Abo-Talib, N. F. (2010). Spectrophotometric methods for the simultaneous determination of binary mixture of metronidazole and diloxanide furoate without prior separation. *Journal of Advanced Research, 1*(4), 323-329. doi:https://doi.org/10.1016/j.jare.2010.06.001

Elkhoudary, M. M., Abdel Salam, R. A., & Hadad, G. M. (2014). Comparative artificial neural network and partial least squares models for analysis of Metronidazole, Diloxanide, Spiramycin and Cliquinol in pharmaceutical preparations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 130*, 222-229. doi:10.1016/j.saa.2014.04.002

Elkhoudary, M. M., Abdel Salam, R. A., & Hadad, G. M. (2016). Development and optimization of HPLC analysis of metronidazole, diloxanide, spiramycin and cliquinol in pharmaceutical dosage forms using experimental design. *Journal of Chromatographic Science, 54*(10), 1701-1712. doi:10.1093/chromsci/bmw126

Erk, N., & Altun, M. L. (2001). Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and RP-LC. *Journal of Pharmaceutical and Biomedical Analysis, 25*(1), 115-122.

Hansen, B. (2020). Methanol toxicity. Retrieved from https://toxedfoundation.org/methanol-toxicity/ (accessed on 31 January 2022)

Horie, M., Saito, K., Hoshino, Y., Nose, N., Shida, Y., Nakazawa, H., & Fujita, M. (1988). Determination of spiramycin components in pharmaceutical preparations by HPLC. *Bunseki kagaku, 37*(11), 580-584. doi:10.2116/bunsekikagaku.37.11_580

ICH Harmonised Tripartite Guideline. (2005). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Validation of Analytical Procedures: Text and Methodology Q2(R1)* (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).

Issa, M. M., Nejem, R. A. M., Shanab, A. M. A., & Shaat, N. T. (2013). Kinetic spectrophotometric H-point standard addition method for the simultaneous determination of diloxanide furoate and metronidazole in binary mixtures and biological fluids. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 114*, 592-598. doi: 10.1016/j.saa.2013.05.062

Kaiser, G. (2011). Continuous Wavelet Transforms. In Kaiser G. (Ed.), *A Friendly Guide to Wavelets* (pp. 60-77). Birkhäuser Boston: Modern Birkhäuser Classics.

Khattab, F. I., Ramadan, N. K., Hegazy, M. A., & Ghoniem, N. S. (2010). Simultaneous determination of metronidazole and spiramycin in bulk powder and in tablets using different spectrophotometric techniques. *Drug Testing and Analysis, 2*(1), 37-44.

Korany, M. A., Abdine, H. H., Ragab, M. A. A., & Aboras, S. I. (2015). Application of derivative spectrophotometry under orthogonal polynomial at unequal intervals: Determination of metronidazole and nystatin in their pharmaceutical mixture. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 143*, 281-287. doi: 10.1016/j.saa.2015.01.076

Kwon, J. H. (2017). 141 - Macrolides, Ketolides, Lincosamides and Streptogramins. In J. Cohen, W. G. Powderly, & S. M. Opal (Eds.), *Infectious Diseases (Fourth Edition)* (pp. 1217-1229.e1211): Elsevier.

Leitsch, D. (2017). A review on metronidazole: an old warhorse in antimicrobial chemotherapy. *Parasitology, 146*, 1167 - 1178.

Li, X., & Xu, G. (2014). Simultaneous determination of ranitidine and metronidazole in pharmaceutical formulations at poly(chromotrope 2B) modified activated glassy carbon electrodes. *Journal of Food and Drug Analysis, 22*(3), 345-349. doi:10.1016/j.jfda.2013.09.050

Maher, H. M., & Youssef, R. M. (2009). Development of validated chromatographic methods for the simultaneous determination of metronidazole and spiramycin in tablets. *Chromatographia, 69*, 345-350.

Mahrouse, M. A., & Elkady, E. F. (2011). Validated spectrophotometric methods for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in tablets. *Chemical and Pharmaceutical Bulletin, 59*(12), 1485-1493.

Poulet, P. P., Duffaut, D., Barthet, P., & Brumpt, I. (2005). Concentrations and in vivo antibacterial activity of spiramycin and metronidazole in patients with periodontitis treated with high-dose metronidazole and the spiramycin/metronidazole combination. *Journal of Antimicrobial Chemotherapy, 55* (3), 347-351.

Quee, T. C., Roussou, T., & Chan, E. C. S. (1983). In vitro activity of rodogyl against putative periodontopathic bacteria. *Antimicrobial Agents and Chemotherapy, 24*, 445 - 447.

Saffaj, T., Charrouf, M., Abourriche, A., Abboud, Y., Bennamara, A., & Berrada, M. (2004). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations. *Il Farmaco, 59*(10), 843-846. doi: 10.1016/j.farmac.2004.06.001

Saffaj, T., Charrouf, M., Abourriche, A., Aboud, Y., Bennamara, A., & Berrada, M. (2006). Spectrophotometric determination of metronidazole and secnidazole in pharmaceutical preparations based on the formation of dyes. *Dyes and Pigments, 70*(3), 259-262. doi:10.1016/j.dyepig.2005.01.009

Sakira, A. K., Mees, C., Braekeleer, K. D., Delporte, C., Yameogo, J., Yabre, M., & Kauffmann, J. M. (2021). Determination of the quality of metronidazole formulations by near-infrared spectrophotometric analysis. *Talanta Open, 3*, 100027. doi:10.1016/j.talo.2020.100027

Salem, A. A., & Mossa, H. A. (2012). Method validation and determinations of levofloxacin, metronidazole and sulfamethoxazole in an aqueous pharmaceutical, urine and blood plasma samples using quantitative nuclear magnetic resonance spectrometry. *Talanta, 88*, 104-114. doi:10.1016/j.talanta.2011.10.016

Salem, A. A., Mossa, H. A., & Barsoum, B. N. (2006). Application of nuclear magnetic resonance spectroscopy for quantitative analysis of miconazole, metronidazole and sulfamethoxazole in pharmaceutical and urine samples. *Journal of Pharmaceutical and Biomedical Analysis, 41*(2), 654-661. doi:10.1016/j.jpba.2005.12.009

Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry, 36*(8), 1627-1639.

Tavakoli, N., Varshosaz, J., Dorkoosh, F., & Zargarzadeh, M. R. (2007). Development and validation of a simple HPLC method for simultaneous in vitro determination of amoxicillin and metronidazole at single wavelength. *Journal of Pharmaceutical and Biomedical Analysis, 43*(1), 325-329. doi:10.1016/j.jpba.2006.06.002

Üstündağ, Ö., & Dinç, E. (2021). Continuous wavelet transforms and ultra performance liquid chromatography applied to the simultaneous quantitative determination of candesartan cilexetil and hydrochlorothiazide in tablets. *Monatshefte für Chemie - Chemical Monthly*, *152*, 1097–1106. doi:10.1007/s00706-021-02822-7