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Application of Fourier Function to Double Divisor Ratio Spectra Curves for Analysis of Some Amoebicide Drugs in Their Ternary Mixtures

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Abstract

This work concerns with development of spectrophotometric methods for analysis of Metronidazole, Diloxanide Furoate and Mebeverine HCl. Method (I) is double divisor ratio spectra derivative method (DDRD) which depends on using derivative signals of the ratio spectra using double divisor. Method (II) is hybrid double divisor ratio spectra (HDDR) which depends on convolution of the double divisor ratio spectra using trigonometric Fourier functions. The developed HDDR method showed better selectivity than DDRD due to its higher resolution power. The methods have been applied for analysis of the studied drugs in their commercial tablets and the results obtained from the analysis of the market sample by the proposed methods were statistically compared with those obtained by manufacturer RP-HPLC method.

Keywords: Fourier function; Hybrid; Double divisor; Ratio spectra; Spectrophotometry

Introduction

Mebeverine Hydrochloride (MEH), BP, [1] is an antispasmodic drug with direct action on the smooth muscle of gastrointestinal tract and is used in conditions such as irritable bowel syndrome [2]. It is chemically designated as 3, 4-dimethoxybenzoic acid 4-[ethyl (p-methoxy- alphamethylphenethyl)amino]butyl ester, [3]. Diloxanide Furoate (DF), BP, [1] and USP, [4] a frequently described antiamoebic drug, [5]. Chemically it is N-dichloroacet-4-hydroxy-N-methyl anilide 3. Metronidazole (MET), BP, [1] and USP, [4] is nitroimidazole derivative which has been widely used for treatment of protozoal diseases including trichromoniasis and giardiasis, [6]. It is chemically described as 1-(2-hydroxyethyl)-2-methyl-5-nitro imidazole, [3]. Dimetrol® is commercial product that contains a combination of amoebicide and antispasmodic drugs.

The literature survey reveals several methods for determination of each of the proposed drugs either alone or in combination with other drugs. BP, [1] reported non aqueous titration methods for determination of each of the studied drugs in its dosage form. Also USP, [4] determined each of DF and MET using the same non aqueous technique. Later MEH was determined by HPLC-MS with various components in water, [7] RP-HPLC in its combination with different drugs in different dosage forms different, [8,9]. Also RP-HPLC and HPTLC techniques were used as stability indicating assay methods for determination of MEH in its combination with sulpiride, [10], MEH with different drugs were also determined by formation of ion pair complexes with methyl orange [11]. On the other hand MET was determined in plasma, [12-14], blood, [15], fish muscles, [16], procine liver, [17] and in different pharmaceutical preparations using different HPLC techniques, [18-21]. MET has been also determined by chemometric assisted spectrophotometric method, [21] by formation of colored product with P-dimethyl amino benzaldhyde [22] and by voltametric method, [6]. Both DF and MET have been determined in their combined dosage form by different methods including RP- HPLC, [23,24] and derivative and ratio derivative spectra spectrophotometric methods [24-26].

From the literature survey, no analytical method has been developed for determination of the proposed drugs in their ternary mixtures. Moreover, analytical studies related to the quality control and routine analysis of a commercial products in the research or industry laboratories use spectrophotometric methods, which are found to be preferable, instead of hyphenated analytical instrumentations or techniques such as LC-MS and GC-MS due to the fast quantitative resolution of samples containing two or more substances without needing any chemical pretreatment [27]. In addition, the above mentioned hyphenated techniques require a prior step such as derivatization, extraction and other tedious analytical process (e.g. smoothing process) during analysis. Taking into account all the above arguments, the quantitative spectrophotometric resolution of the ternary mixture of the studied drugs having overlapped spectra is an interesting issue. In this work DDRD and HDDR methods have been developed for determination of the proposed ternary mixture, besides the suggested methods were found to be easy to apply, rapid, sensitive and economic.

Theoritical Background

Double divisor ratio spectra derivative method (DDRD)

Dinc et al. [28-30] has developed the double divisor ratio spectra derivative (DDRD) method which depended on using the coincident spectra of the derivative of the ratio spectra obtained by using a "double divisor" (sum of two spectra) and measuring at either the maximum or minimum peaks.

If a ternary mixture of analytes X, Y and Z is considered, if Beer's law is obeyed for the three compounds over the whole wavelength range used, the absorption curve of the mixture at λi can be defined as follow:

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$$Am\lambda 1 = \alpha X, \lambda iCX + \beta Y, \lambda iCY + \gamma Z, \lambda iCZ \quad (1)$$

Where Amλ1 is the absorbance of the mixture at λi and αX, λi, βY,λi and γZ,λi are the absorpitivities of X, Y and Z, respectively. CX, CY and CZ represent the concentrations.

If eq. (1) is divided by the spectrum of the sum of two compounds in the ternary mixture, eq. (1) becomes:

$$Am\lambda 1/ (\alpha X, \lambda iC0X + \beta Y, \lambda iC0Y) = (\alpha X, \lambda iCX + \beta Y, \lambda iCY)/ (\alpha X, \lambda iC0X + \beta Y, \lambda iC0Y) + \gamma Z, \lambda iCZ/ (\alpha X, \lambda iC0X + \beta Y, \lambda iC0Y) \quad (2)$$

-The ratio of the sum of (αX, λiCX+ βY, λiCY) to the sum of (αX, λiC0X+ βY,λiC0Y) is equal to constant (K). If this constant is replaced in eq. (2), we obtain eq. (3):

$$Am\lambda 1/ \alpha X, \lambda iC0X + \beta Y, \lambda iC0Y = K + \gamma Z, \lambda iCZ/ (\alpha X, \lambda iC0X + \beta Y, \lambda i C0Y) \quad (3)$$

If the standard concentrations of C0X and C0Y (used as double divisor) are equal or very close to each other (C0X= C0Y) we can write eq. (3) as follow:

$$Am\lambda 1/ (\alpha X, \lambda i + \beta Y, \lambda i) C0X = K + \gamma Z, \lambda iCZ/ [(\alpha X, \lambda i + \beta Y, \lambda) C0X] \quad (4)$$

If the first derivative of eq. (4) is taken and since the derivative of a constant is zero, we obtain eq. (5):

$$d/d\lambda [Am\lambda 1/ (\alpha X,\lambda i + \beta Y,\lambda i)C0X] = d/d\lambda[\gamma Z,\lambda i/ (\alpha X,\lambda i + \beta Y,\lambda)] \times (CZ/ C0X) \quad (5)$$

Eq. (5) is the mathematical foundation of multi-component analysis which permits the determination of each component in the mixture without interference from the other components in the ternary mixture as the derivative of the obtained ratio spectra is dependant only on the concentration values CZ and C0X but is independent of the concentration values CX and CY. Also the concentration CZ is proportional to the derivative signals corresponding to a maximum or minimum point.

Hybrid double divisor ratio spectra method (HDDR)

Wahbi et al. [31,32] has introduced the Fourier function ratio spectrum method for determination of binary mixtures. Lately, Rasha and Hadir, [33] extended the method for determination of ternary mixtures using double divisor ratio spectra.

The constant term (K) appears in eq. (3) can be eliminated by applying the trigonometric Fourier function method [31, 32]. According to the basic principle for the use of the trigonometric Fourier functions to process absorbance ratio spectrum, an absorbance ratio spectrum can be expanded in terms of the Fourier series as follow:

$$Ar(\lambda) = a0 + a1 \cos X + a2 \cos 2X + a3 \cos 3X + \ldots\ldots\ldots\ldots\ldots\ldots + B1 \sin X + b2 \sin 2X + b3 \sin 3X + \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Where Ar (λ) denotes the absorbance ratio obtained by using a double divisor at a set of (n+1) equally spaced wavelengths, aj are the coefficients of Cos jX and bj are the coefficients of Sin jX (j= 1, 2, ……..n). a_0 is the constant component, a1 Cos X is the quadratic component, etc of the absorbance ratio gross curve. A linear component is not present in the above expansion; however a combination of two trigonometric functions has been proved to correct this, [34].

- In view of the orthogonality property of the Fourier functions, contribution from any components other than the specified compound can be automatically eliminated by convoluting the double divisor ratio spectrum, Ar (λ), obtained for the analyte with a suitably selected combined trigonometric Fourier functions simply for any number of points greater than 2. (6, 8 or 10) points are usually selected, [34, 35].

- The suitable function is selected according to the shape of the double divisor ratio spectra.

- The optimum number of points and wavelength interval are selected for a given component at which the calculated Fourier coefficient should afford precise and accurate estimate of the analyte.

- Any coefficient of the combined trigonometric Fourier function –double divisor ratio spectrum, t'rj, can be calculated from a set of (n+1) absorbance ratios measured at equally spaced wavelengths by the following summation, in which X takes values from 0 to 2π-[2π/(n+1)], at intervals of 2π/(n+1):

$$\sum_{i=0}^{n} t'rj = AriT'ji / D \quad (7)$$

Where T'i represents the combined Fourier functions whose order j= 1, 2, 3,……..n. T'i= [Cos jX + Cos j (X+2π/ (n+1))] or T'i= [Sin jX – Sin j (X+2π/ (n+1))]. The denominator (D) has a numerical value of 3 or 4 for six or eight points for the combined Fourier functions [36].

- The optimum coefficient is selected at the optimum mean wavelength λm [where λm = [(λinitial + λfinal)/2] which corresponds to a maximum or a minimum in the transformed double divisor ratio spectrum. Such spectrum is obtained by plotting the t'rj coefficients versus λm. The optimum coefficient t'rj is proportional to the concentration of the analyte, so:

$$t'rj = \alpha C \quad (8)$$

Where α is a constant and C is the concentration of the analyte.

-In practice, a calibration graph for component Z is obtained by recording the spectra of different concentrations of pure Z and dividing each by the double divisor (spectrum of sum of X and Y). Then the ratio spectrum- Fourier function coefficients are calculated from a set of absorbance ratios at specified optimum parameters (including function order, number of points, wavelength interval and λ_m). The coefficients are calculated using eq. (7) as exemplified in Table 1. The calibration graph is then obtained by plotting t'rj against CZ. By analogy, components X and Y can be determined.

Experimental

Instruments

A double beam UV-Visible spectrophotometer (SHIMADZU, Japan), model UV-1601 PC with 1 cm path length quartz cell is used and it is connected to IBM compatible computer. The software was UVPC personal spectroscopy software version 3.7. The absorbance data were processed using excels software.

Chemicals and reagents

Pure samples: Standards MEH, DF and MET with claimed purity of 98.9, 100.5 and 100.4 % according to manufacturer certificate were kindly donated by EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt).

Market sample: Dimetrol® film coated tablets batch No. 909537, were labeled to contain 375 mg MET, 250 mg DF and 50 mg MEH per

tablet were manufactured by EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt.

Methanol: HPLC grade (Sigma-Aldrich® Chemie GmbH, Germany).

Solutions

Solutions and synthetic mixtures: -Stock standard solutions each of *MEH, DF and MET* were prepared in methanol in the concentration of 1 mg ml^{-1}.

-Working standard solutions each of *MEH, DF and MET* were prepared in methanol in the concentration of 0.1 mg ml^{-1}.

Sample solution: The content of twenty coated tablets of Dimetrol® was separately weighed. An accurately weighted portion equivalent to 150 mg MET, 100 mg DF and 20 mg MEH were separately transferred into 100-ml calibrated measuring flask and then 75 ml methanol was added. The prepared solution was sonicated for 30 minutes, the volume was completed with the same solvent and the solution was then filtered. Appropriate dilution of the prepared solution was made to prepare the working solution (0.1 mg ml^{-1} DF and the corresponding amount each of MET and MEH).

Synthetic mixtures: Accurate volumes each of MEH, DF and MET stock standard solutions were transferred into 10-ml volumetric flasks and diluted to the volume with methanol to prepare five synthetic mixtures within the concentration range of each drug (2-25µgml^{-1} each of MEH and DF and 1-24 µgml^{-1} of MET).

Procedure

Spectral characteristics

The absorption spectra of 3, 15 and 22.5 µgml^{-1} each of MEH, DF and MET, respectively and ternary mixtures containing the three drugs in the same ratio were recorded over the range of 200 - 350 nm against methanol Figure 1.

Application of analytical methods

Double divisor ratio spectra derivative method (DDRD): For determination of MEH: The absorption spectra of the solutions prepared at different concentrations of pure MEH and the ternary mixture were recorded and divided by the sum of the absorption spectra of DF and MET (8 µgml^{-1} each as a double divisor). First derivative of these ratio spectra were obtained at Δλ=8 and then the peak amplitudes values at 229.2 nm were plotted against the corresponding concentrations of MEH. Concentration of MEH in the ternary mixture was calculated from the computed regression equation. *In the same way*, the recorded spectra of the solutions at the increasing concentrations of DF were

n (i)	xi (degree)	λ nm	A_r (1cm)	T_{xi} [Cos xi +Cos (Xi+60)]
0	0	214	1.0854	1.5
1	60	218	0.5618	0
2	120	222	1.5076	-1.5
3	180	226	1.2772	-1.5
4	240	230	0.2961	0
5	300	234	0.3079	1.5
			D = 3	
Calculate t'r= Σ T_{xi} A_{ri} / D				

Table 1: Calculation of Fourier functions-ratio spectrum coefficients t'_{rj} for Mebeverine HCl determination.

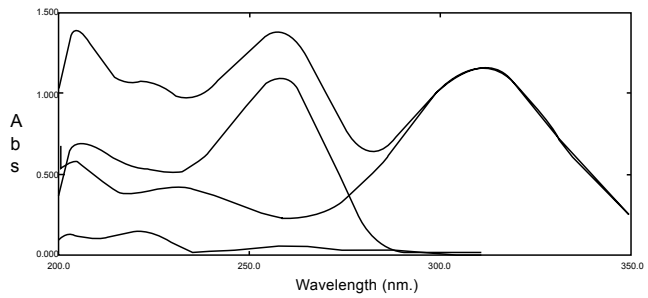

Figure 1: Zero order absorption spectra of 3 µgml^{-1} MEH ( ), 15 µgml-1 DF (-----), 22 µgml^{-1} of MET (.....) and ternary mixture (-.-.-.-.-) (3 µgml^{-1} MEH, 15 µgml^{-1} DF and 22 µgml^{-1} MET) in methanol.

divided by the double divisor (the sum of the absorption spectra of DF and MET, 4 µgml^{-1} each), the obtained ratio spectra were smoothed at Δλ=4 and second derivative of the obtained ratio spectra were traced with intervals of Δλ=2. The amplitudes at 272.8-283.2 nm (peak to peak) were proportional to the concentration of DF, concentration of DF in the ternary mixture was then determined from the obtained calibration graph. *On the other hand, to determine* MET, the spectra of its different concentrations and that of the ternary mixture were recorded and divided by the sum of the spectra of MEH and DF (normalized spectrum of each) to get the ratio spectra. The amplitudes of the first derivative of (Δλ= 4) these ratio spectra at 313.8-318.6 and 318.6-320.6 nm (peak to peak) were measured and plotted against the corresponding concentrations of MET to construct the calibration curve from which the drug concentration in the prepared ternary mixture was obtained.

Hybrid Double divisor ratio spectra method (HDDR): The recorded spectra of the solutions at the increasing concentrations of pure MEB, DF and MET were divided by the spectrum of the double divisor (I), (20 µgml^{-1} each of DF and MET), divisor (II) (10 µgml^{-1} each of MET and MEB) and divisor (III) (normalized spectra of MEH and DF), respectively to obtain their respective ratio spectra. Using six points, T' = [Cos X+ Cos(X+60)] combined Fourier function, [34], the different coefficients, t'rj, were calculated from the respective absorbance ratio values each 4, 6 and 4 nm interval for MEH, DF and MET, respectively (as exemplified for MEH in Table 1). The concentrations of standard MEH were proportional to t'jr values at λ_m = 224 nm in the spectral region selected (206-250 nm), while concentrations of standard DF were proportional to t'jr values at λ_m = 249 nm in the spectral region selected (204-276 nm). On the other hand concentrations of MET was linear with t'jr values at λ_m = 306 nm in the spectral region selected (272-316 nm).

Results and Discussion

As can be seen in Figure 1, MEH, DF and MET cannot be determined using the traditional spectrophotometric methods because of almost complete overlap of their signals in the region 200- 300 nm. On the other hand, MET can be selectively determined at 311 nm without interference from the other two components in the ternary mixture, unfortunately bad results (regarding selectivity) were obtained. The power of DDRD and HDDR methods was demonstrated to resolve the ternary mixtures consisting of MEH, DF and MET. The choice of such components is based on their neglicable chemical interaction and on their pharmaceutical relevance.

Double divisor ratio spectra derivative method (DDRD)

Method optimization: The main instrumental parameter conditions were optimized for a reliable determination of the subject matter components. The selected double divisor was obtained either by the sum of the absorption spectrum of the same concentrations of two components in the same ternary mixture, as is carried out in this work, or it was obtained by preparing the mixed solution of two components of the same concentrations [37]. Some binary mixtures were tested as double divisor (normalized, 4, 8, 14 and 20 μgml^{-1} each of DF and MET) for determination of MEH, for DF (normalized, 4, 10 and 20 μgml^{-1} each of MEH and MET) while for determination of MET (normalized, 4, 10, 14 and 20 μgml^{-1} each of MEH and DF). Binary mixture of 8 μgml^{-1} each of DF and MET was used as a double divisor for MEH, for DF, double divisor consisting of 4 μgml^{-1} each of MEH and MET was selected, while binary mixture of the sum of normalized spectra of MEH and DF was selected for determination of MET.

The order of the derivative of the obtained ratio spectra (first and second) was tested in order to obtain a wavelength corresponding to a maximum or a minimum point at which the pure drug and its ternary mixture coincide to be used as the working wavelength for this drug. MEH was determined using first derivative of double divisor ratio spectra (1DD) at 229.2 nm (minimum), Figure 2a, while DF was measured in the second order (2DD) of the obtained double divisor ratio spectra at 272.8-283.2 (minimum to maximum), Figure 2b**.** On the other hand MET could be measured at λ_1 = 313.8-318.6 nm (maximum to minimum) or at λ_2 = 318.6-320.6 (minimum to maximum) in the first derivative double divisor ratio spectra (1DD), Figure 2c. In case of DF and MET, measuring the amplitudes from peak to peak enhanced the method selectivity (especially for DF), on the other hand using the amplitudes at λ_1 and λ_2 for determination of MET gave the same results regarding selectivity and sensitivity.

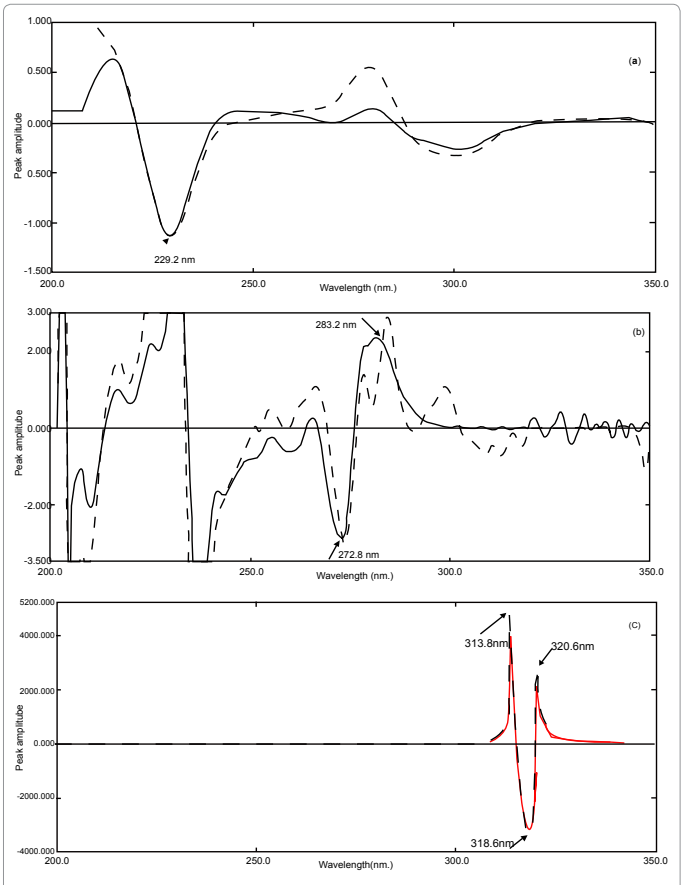

Figure 2: The coincidence spectra of the derivative of the ratio spectra of (a) 10 μgml^{-1} MEH (____) and ternary mixture (-----) (10 μgml^{-1} MEH, 15 μgml^{-1} DF and 20 μgml^{-1} MET). (b) 10 μgml^{-1} DF (------) and ternary mixture (-----) (2 μgml^{-1} MEH, 10 μgml^{-1} DF and 15 μgml^{-1} MET). (c) 20 μgml^{-1} MET (____) and ternary mixture (-----) (10 μgml^{-1} MEH, 15 μgml^{-1} DF and 20 μgml^{-1} MET).

Smoothing function for the obtained double divisor ratio spectra was also tested, where it was found that it had a significant effect only on DF ratio spectra, where smoothing DF ratio spectra with $\Delta\lambda$=4 gave the best results regarding selectivity. Effect of $\Delta\lambda$ (2, 4, 8 and 16) on the derivative of the obtained ratio spectra was also tested, where $\Delta\lambda$ = 8, 2 and 4 were found to be suitable for determination of MEH, DF and MET, respectively.

Hybrid double divisor ratio spectra method (HDDR)

Since convolution using combined trigonometric Fourier functions corrects all types of interferences, application of these functions to double divisor absorbance ratio spectra data would lead to removal of interference from other mixture components as found in pharmaceutical preparations and remove background noise in case of minor concentrations or where sample matrix contribution is significant as in biological fluids, [33].

Method optimization: Different binary mixtures (previously mentioned in optimization of DDRD method) were tested, where binary mixture of 20 μgml^{-1} each of DF and MET, mixture of 10 μgml^{-1} each of MEH and MET and mixture of normalized spectra each of MEH and DF were the most suitable double divisors for MEH, DF and MET, respectively.

Depending on the general rules for the use of Fourier function in processing ratio spectra absorption curves [31,32] different parameters associated with the calculation of the Fourier coefficient were optimized. The quadratic functions T' = [Cos X + Cos (X+60)] and T' =[Cos X+ Cos (X+45)] were tested, where the first function has been chosen as it contributed greatly to the absorption ratio spectra obtained for each of the studied drugs over the wavelength range selected for each. The combined trigonometric Fourier function ratio spectra coefficients, t'rj, were calculated from the absorbance ratio data using different number of points (6 and 8) and at different wavelength intervals (2, 4 and 6 nm). Using six points, T' = [Cos X + Cos (X+60)] combined trigonometric Fourier function and convoluting the obtained ratio spectra at 4 nm interval (for MEH and MET) and at 6 nm interval (for DF) gave the best results.

Mebeverine was determined at λ_m = 224 nm (minimum) while DF at λ_m= 249 nm (maximum). MET could be determined at λ_m= 306 nm (maximum) where the convoluted ratio spectra of each of them coincided with its ternary mixture in the spectral region selected as shown in Figure 3. Moreover, MET showed a minimum point at 314 nm but incorrect results were produced when using this wavelength for measuring MET in the prepared ternary mixtures. Convoluted ratio spectra of different concentration of standard MEH, DF and MET under the selected conditions are shown in Figure 4.

Methods validation

Validation of the suggested spectrophotometric methods has been carried out according to USP requirements 4 and ICH guidelines [38].

Linearity and range: Under the above described experimental conditions, the graphs obtained by plotting the derivative double divisor absorbance ratio (DDRD method) and the combined trigonometric Fourier function coefficients (HDDR method) versus the concentrations of MEH, DF and MET showed good linear relationships. Linearity ranges and regression equations parameters are found in Table 2.

Accuracy (recovery study) and precision: Accuracy was calculated as the percentage recoveries of blind pure drugs. It was further assured by application of standard addition technique at different levels (80, 100 and 120%). Precision was studied with respect to both repeatability and intermediate precision. Repeatability was calculated by the analysis of three different concentrations of pure drugs (5, 10 and 15 μg ml^{-1}, each) in triplicates on the same day in the same equipment. The experiment was repeated on the same concentration seven times on four consecutive days to determine the intermediate precision.

Good percentage recoveries and acceptable RSD%, Table 2, were obtained indicating that the proposed methods could be considered as accurate and precise.

Specificity: It was detected by analyzing mixtures containing different ratios of MEH, DF and MET within their linearity ranges and according to the above stated procedures. From the results shown in Table 3, it was noticed that better percentage recoveries and lower RSD% values were obtained when using HDDR method due to its higher power in eliminating the interference and enhancing signal to noise ratio than DDRD method, indicating its higher selectivity especially when measuring DF.

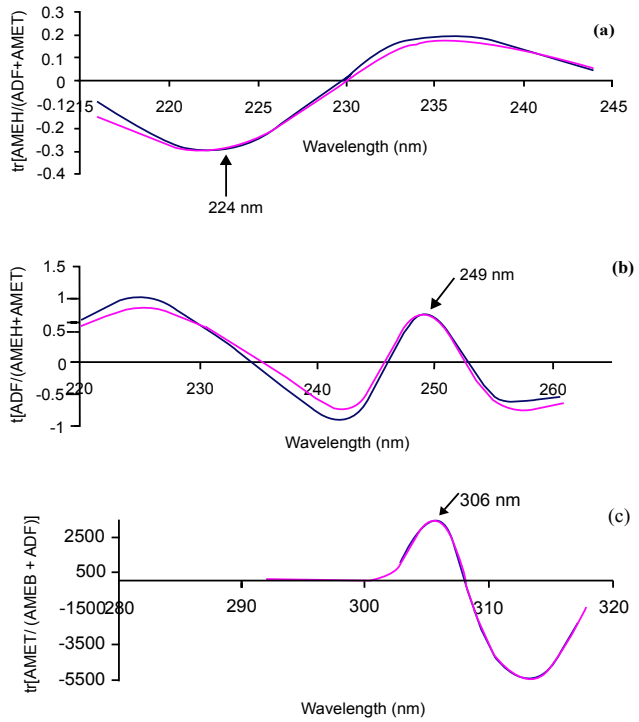

Figure 3: The coincident spectra of the convoluted ratio spectra using combined trigonometric Fourier functions of: (a)) MEH (10 μgml^{-1}) and ternary mixture (10 μgml^{-1} MEH , 15 μgml^{-1} DF and 20 μgml^{-1} MET), (b) DF (12 μgml^{-1}) and ternary mixture (μgml^{-1} MEH , 12 μgml^{-1} DF and 8 μgml^{-1} MET), (c) MET (20 μgml^{-1}) and ternary mixture (10 μgml^{-1} MEH , 15 μgml^{-1} DF and 20 μgml^{-1} MET).

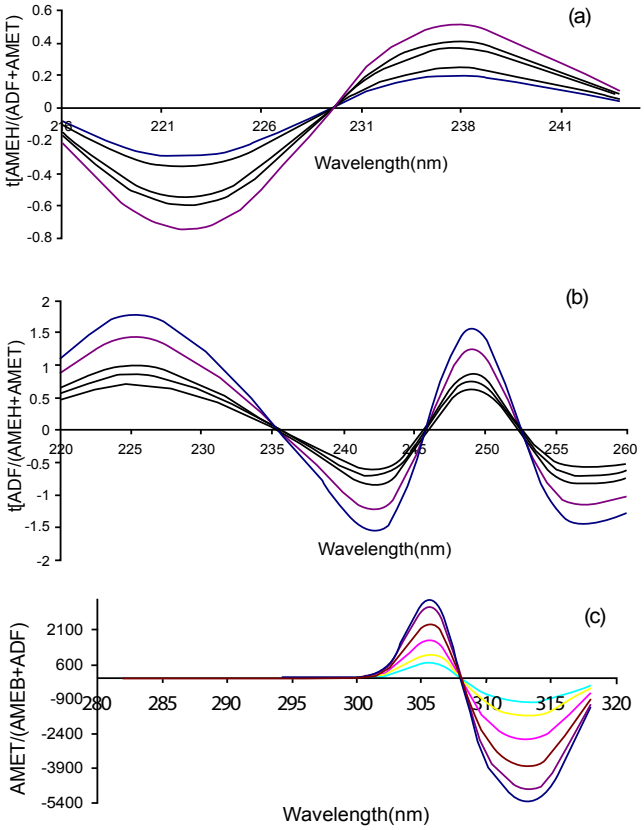

Figure 4: Convoluted ratio spectra of different concentrations of (a) MEH, (b) DF and (c) MET using six-points combined trigonometric Fourier functions in methanol.

Application to market sample

The developed methods have been successfully applied for determination of the studied drugs in Dimetrol® tablets. Good results were obtained indicating that tablet additives did not interfere Table 4.

The results obtained from analysis of Dimetrol® tablets using the developed DDRD and HDDR methods were statistically compared with those obtained from using the manufacturer RP-HPLC one, [39] using F and student's t- test. No significant difference was found regarding both accuracy and precision. The developed methods have advantages over the commercial RP-HPLC method of being simpler, omit the need for expensive instrument and so can be used as alternative methods to LC methods in quality control laboratories.

Conclusion

From the previous discussion, the proposed methods can be applied for rapid determination of MEH, DF and MET combination. The developed HDDR can be considered superior to DDRD method since application of the combined trigonometric Fourier functions to double divisor absorbance ratio spectra eliminates most of interference giving high degree of purity of the analytical signals thus improving the performance of the method without calculation of the derivatives. The developed methods have a great promise for routine analysis of the commercial formulations and quality control of such mixture; they are also suitable and valid for application in laboratories lacking LC instruments.

Parameters	DDRD			HDDR		
	MEH	DF	MET	MEH	DF	MET
Calibration range	2-24 μgml^{-1}	4-25 μgml^{-1}	1-24 μgml^{-1}	2-25 μgml^{-1}	2-25 μgml^{-1}	1-24 μgml^{-1}
Slope	0.1155	0.5161	395.23	0.0281	0.0624	162.22
Intercept	-.0212	0.1515	-2.0476	-0.0043	0.0039	-2.1817
Correlation coefficient	0.9999	0.9997	0.9999	0.9999	0.9999	0.9999
Accuracy	99.96	99.45	99.98	100.02	100.17	100.00
Precision						
Repeatability	0.689	1.231	1.058	0.897	1.210	1.223
Intermediate precision	0.987	1.241	1.230	0.909	1.290	1.112

Table 2: Analytical parameters of the proposed methods for determination of Mebeverine HCl, Diloxanide Furoate and Metronidazole.

Parameters	DDRD			HDDR		
MEH: DF: MET	% Recovery[b]					
	MEH	DF	MET	MEH	DF	MET
3: 15: 22.5[a]	97.00	99.98	102.58	99.16	102.73	102.56
15: 15: 15	96.47	105.50	102.00	100.14	98.47	101.86
10: 15: 20	98.70	106.66	102.10	99.97	100.29	102.04
4: 8: 9	103.00	104.41	99.78	99.37	97.80	99.66
4: 12: 8	103.00	102.08	102.75	97.15	101.34	102.67
Mean ± RSD%.	99.63 ± 3.193	103.73 ± 2.594	101.84 ± 1.173	99.16 ± 1.203	100.13 ± 2.024	101.76 ± 1.199

[a]The same ratio of Dimetrol® tablets.
[b]Average of three determinations.

Table 3: Results of determination of Mebeverine HCl, Diloxanide Furoate and Metronidazole in synthetic mixtures using the proposed spectrophotometric methods.

Parameters	DDRD			HDDR		
	MEH	DF	MET	MEH	DF	MET
Dimetrol® tablets (B.No.909537)	100.39 ± 1.285	99.24 ±1.883	98.88 ± 0.775	101.08 ±1.534	101.16 ±0.653	98.99 ± 0.539
Standard addition[a]	99.46 ±0.962	98.94 ±1.859	99.79 ± 1.206	98.66 ±1.781	102.61 ±1.259	99.50 ± 1.330
Degree of freedom F-test	10 (5.050) b 1.683	10 (5.050) b 4.937	10 (5.050) b 1.014	9 (5.192) b 2.432	9 (6.256) b 0.603	10 (5.050) b 4.205
Degree of freedom Student's –t test	10 (2.228) b 0.819	10 (2.228) b 1.954	10 (2.228) b 2.174	9 (2.262) b 1.522	9 (2.262) b 1.522	10 (2.228) b 0.439

a: Average of 3 determinations.
b: The values in the parenthesis are the corresponding theoretical values at p= 0.05.

Table 4: Application of the proposed spectrophotometric methods for the determination of the studied drugs in tablets and statistical comparison with the manufacturer RP-HPLC method [39].

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