Application of Fourier Function to Double Divisor Ratio Spectra Curves for Analysis of Some Amoeboic 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)amine]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 furoate (DF), BP, [1] and USP, [4] a frequently described antiamoebic (p-methoxy- alphamethylphenethyl)amino]butyl ester, [5]. Metronidazole (MET), BP [1] and USP [4], [5] is nitroimidazole 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 trichomoniasis 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 sulphide, [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], proline 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 benzaldehyde [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.

**Theoretical 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:

\[ A_{i} = \alpha_{i} + \beta_{i}C_{x} + \gamma_{i}C_{y} + \delta_{i}C_{z} \]

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Where $A_{\lambda i}$ is the absorbance of the mixture at $\lambda_i$ and $aX, \lambda_i, bY, \lambda_i$ and $yZ, \lambda_i$ are the absorptivities of $X$, $Y$, and $Z$, respectively. $CX$, $CY$ and $CZ$ represent the concentrations.

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

$$A_{\lambda i}l = aX, \lambda_iCX + bY, \lambda_iCY + yZ, \lambda_iCZ$$  \hspace{1cm} (1)

Where $A_{\lambda i}l$ is the absorbance of the mixture at $\lambda_i$ and $aX, \lambda_i, bY, \lambda_i$ and $yZ, \lambda_i$ are the absorptivities of $X$, $Y$, and $Z$, respectively. $CX$, $CY$ and $CZ$ represent the concentrations.

The concentration $CZ$ is only dependent on the concentration values $CZ$ and $C0X$ but is independent of $C0Y$ and $C0Z$. Also the concentration values $CX$ and $CY$ are proportional to the derivative signals corresponding to a maximum or a minimum in the transformed double divisor ratio spectrum, $Ar (\lambda)$, obtained for the analyte with a suitably selected combination of trigonometric 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 $2\pi\times[2\pi/(n+1)]$, at intervals of $2\pi/(n+1)$:

$$\sum_{j=0}^{n} t'_{rj} = ArT_{i}/ D$$  \hspace{1cm} (7)

Where $T_{i}$ represents the combined Fourier functions whose order $j=1, 2, 3, \ldots n$. $T'_{i}= [\cos jX + \cos j(Y+2\pi/(n+1))$ or $T_{i}'=[\sin jX + \sin j(Y+2\pi/(n+1))]$. 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 $\lambda m$ [where $\lambda m = ([\lambda_{initial} + \lambda_{final}]/2)$ 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 $\lambda m$. The optimum coefficient $t'_{rj}$ is proportional to the concentration of the analyte, so:

$$t'_{rj} = aC$$  \hspace{1cm} (8)

Where $a$ 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 $\lambda m$). 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 (2-25 μg ml⁻¹ each of MEH and DF and 1-24 μg ml⁻¹ of MET).

Procedure

Spectral characteristics

The absorption spectra of 3, 15 and 22.5 μg ml⁻¹ each of MEH, DF and MET, respectively and ternary mixtures containing the three drugs 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 interference from the other two components in the ternary mixture, unfortunately bad results (regarding selectivity) were obtained. The choice of such components is based on their negligible chemical interaction and on their pharmaceutical relevance.

| n | x_i (degree) | λ nm | A_i (1cm) | [Cos x_i +Cos (X+60)] | T_i |
|---|-------------|-----|----------|-----------------------|-----|
| 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'_i = Σ T_i A_i / D

Table 1: Calculation of Fourier functions-ratio spectrum coefficients t'_i, 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.
Double divisor ratio spectra derivative method (DDRDM)

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 μg ml\(^{-1}\) each of MEH and MET) while for determination of MET (normalized, 4, 10, 14 and 20 μg ml\(^{-1}\) each of MEH and DF). Binary mixture of 8 μg ml\(^{-1}\) each of DF and MET was used as a double divisor for MEH, for DF, double divisor consisting of 4 μg ml\(^{-1}\) 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 \(\lambda_m = 313.8-318.6\) nm (minimum to maximum) or at \(\lambda_m = 318.6-320.6\) (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 \(\lambda_m\) and \(\lambda_{m'}\) for determination of MET gave the same results regarding selectivity and sensitivity.

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 \(\Delta\lambda = 8, 2, 4\) and 16 on the derivative of the obtained ratio spectra was also tested, where \(\Delta\lambda = 8, 2, 4\) and 16 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 DDRDM method) were tested, where binary mixture of 20 μg ml\(^{-1}\) each of DF and MET, mixture of 10 μg ml\(^{-1}\) 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 \(\lambda_m = 224\) nm (minimum) while DF at \(\lambda_m = 249\) nm (maximum). MET could be determined at \(\lambda_m = 306\) 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].

![Figure 2: The coincidence spectra of the derivative of the ratio spectra of (a) 10 μg ml\(^{-1}\) MEH (_____ ) and ternary mixture (-----) (10 μg ml\(^{-1}\) MEH, 15 μg ml\(^{-1}\) DF and 20 μg ml\(^{-1}\) MET), (b) 10 μg ml\(^{-1}\) DF (-----) and ternary mixture (-----) (2 μg ml\(^{-1}\) MEH, 10 μg ml\(^{-1}\) DF and 15 μg ml\(^{-1}\) MET), (c) 20 μg ml\(^{-1}\) MET (_____ ) and ternary mixture (-----) (10 μg ml\(^{-1}\) MEH, 15 μg ml\(^{-1}\) DF and 20 μg ml\(^{-1}\) MET).](image-url)
**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.

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

| Parameters | DDRD | HDDR |
|------------|------|------|
| Calibration range | MEH | DF | MET | MEH | DF | MET |
| Slope | 2.24 µg/ml<sup>1</sup> | 4.25 µg/ml<sup>1</sup> | 1.24 µg/ml<sup>1</sup> | 2.25 µg/ml<sup>1</sup> | 2.25 µg/ml<sup>1</sup> | 1.24 µg/ml<sup>1</sup> |
| Intercept | 0.1155 | 0.5161 | 395.23 | 0.0281 | 0.0624 | 162.22 |
| 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 |
| 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 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 | % Recovery<sup>a</sup> | | |
| 3: 15: 22.5<sup>b</sup> | 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 |

<sup>a</sup>The same ratio of Dimetrol® tablets.
<sup>b</sup>Average of three determinations.

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].

| 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<sup>a</sup> | 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 |

<sup>a</sup>Average of 3 determinations.
<sup>b</sup>The values in the parenthesis are the corresponding theoretical values at p= 0.05.

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References

1. “The British Pharmacopoeia”, Her Majesty’s (2007) The Stationary Office, London.
2. Martindale- Extra Pharmacopoeia 34th Ed (2005) “The Complete Drug References” The 334 pharmaceutical Press, London, UK.
3. Budavari, S (2002) “The Merck Index”, An Encyclopedia of Chemicals, Drugs and Biologicals, (13<sup>e</sup> Ed), Merck and Co.Inc. Whitehouse Station, NJ.
4. The United States Pharmacopeia, 30 Ed (2007) National Formulary 25, United States Pharmacopeiaconvention Inc.
5. Al-Ghanam SM, Belal F (2001) Spectrophotometric determination of diloxanide furoate in its dosage forms. Il Farmaco 56: 677-681.
6. Gholivand MB, Torkashvand,M, Malekzadeh G (2012) Fabrication of an electrochemical sensor based on computationally designed molecularly imprinted polymers for determination of cyanazine in food samples. Anal Chim Acta 713: 36-44.
7. Zhang ZL, Zhou GL (20047) J. chromatogr. A 1154: 205-213. 342
8. Haggag RS, Shaalan RA, Belal TS (2010) Validated HPLC determination of the two fixed dose combinations (chlordiazepoxide hydrochloride and mebeverine hydrochloride; carvedilol and hydrochlorothiazide) in their tablets. J AOAC Int 93: 1192-1200.
9. Elmasry MS, Blagbrough IS, Rowan MG, Saleh HM, Kheir AA, et al. (2011) Quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a Venalink monitored dosage system with co-prescribed medicines. J Pharm Biomed Anal 54: 646-652.
10. Naguib IA, Abdelkawy M (2010) Development and validation of stability indicating HPLC and HPTLC methods for determination of sulpiride and mebeverine hydrochloride in combination. Eur J Med Chem 45: 3719-3725.
11. El-Damony AM (2008) Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange. Spectrochim Acta A Mol Biomol Spectrosc 69: 770-775.
12. Ouyang LQ, Wu HL, Liu YJ, Wang JY, YU YJ, et al. (2010) Chem Lett 21: 223-1226.
13. Marina S, Simone S, Eunice K, Valentine P, Cristine S (2009) J Chromatogr Sci 47: 781-784.
14. Fraselle S, Derop V, Degroodt JM, Van Loco J (2007) Validation of a method...
for the detection and confirmation of nitroimidazoles and the corresponding hydroxyl metabolites in pig plasma by high performance liquid chromatography-tandem mass spectrometry. Anal Chim Acta 586: 383-393.

15. Faisal SM, Godwill I, Laxman K P, Jeff M, Paul G, et al. (2010) Anal Bioanal Chem 397: 687-693.

16. Maher HM, Youssef RM, Khalil RH, El-Bahr SM (2008) Simultaneous multi-residue determination of metronidazole and spiramycin in fish muscle using high performance liquid chromatography with UV detection. J Chromatogr B Analys Technol Biomed Life Sci 876: 175-181.

17. Xia X, Li X, Zhang S, Ding S, Jiang H, et al. (2007) Confirmation of four nitroimidazoles in porcine liver by liquid chromatography-tandem mass spectrometry. Anal Chim Acta 586: 394-398.

18. Tavakoli N, Varshosaz J, Dorkoosh F, Zargarzadeh MR (2007) Development and validation of a simple HPLC method for simultaneous in vitro determination of amoxicillin and metronidazole at single wavelength. J Pharm Biomed Anal 43: 325-329.

19. Ping W, Jie L, Hehui Z, Chin (2007) J Chromatogr A 1166: 743-746.

20. Tashtoush BM, Jacobson EL, Jacobson MK (2008) Validation of a simple and rapid HPLC method for determination of metronidazole in dermatological formulations. Drug Dev Ind Pharm 34: 840-844.

21. El-Gindy A, Emara S, Shaaban H (2010) Validation and application of chemometrics-assisted spectrophotometry and liquid chromatography for simultaneous determination of two ternary mixtures containing drotaverine hydrochloride. J AOAC Int 93: 536-548.

22. Adegoke OA, Umoh OE (2009) A new approach to the spectrophotometric determination of metronidazole and tindazole using p-dimethylaminobenzaldehyde. Acta Pharm 59: 407-419.

23. Mishal A, Sober D (2005) Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzolate and diloxanide furoate as bulk drug and in suspension dosage form. J Pharm Biomed Anal 39: 819-823.

24. Al Shaalan, N H (2007) J Appl Sci 4: 66-72.

25. El-Ghobashy MR, Abo-Talib NF (2010) J Adv Res 1: 323-329.

26. Bimalendu G (2003) Journal of the Institution of Chemists (India) 75: 183-185.

27. Dinc E, Baleanu D (2008) Application of Haar and Mexican Hat Wavelets to Double Divisor-Ratio Spectra for the Multicomponent Determination of Ascorbic Acid, Acetylsalicylic Acid and Paracetamol in Effervescent Tablets. J Braz Chem 19: 434-444.

28. Dinc E, Onur F (1998) Application of a new spectrophotometric method for the analysis of a ternary mixture containing metamizol, paracetamol and caffeine in tablets. Analytica Chimica Acta 359: 93-106.

29. Dinc E (1999) 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 48: 1145-1157.

30. Dinc E, Baydan E, Kanbur M, Onur F (2002) 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 58: 579-594.

31. Wahi M, Mahgoub H (2003) Bull Fac Cairo Univ 41: 265-275.

32. Mahgoub H (2003) Bull Fac Cairo Univ 41: 285-298.

33. Youssef RM, Maher HM (2003) A new hybrid double divisor ratio spectra method for the analysis of ternary mixtures. Spectrochim Acta A Mol Biomol Spectrosc 70: 1152-1166.

34. Wahi AM, Abdine H, Korany MA, El-Yazbi FA (1978) Spectrophotometric determination of chlorpheniramine-sulphacetamide in eye drops. Pharmazie 33: 721-722.

35. Korany MA, Elsayed MA, Bedair MM, Mahgoub H, Korany EA (1990) Computer-assisted spectrophotometry: multicomponent analysis with a discrete fourier transform. Talanta 37: 1183-1188.

36. Wahi AM, Abdine H, Korany MA, El-Yazbi FA (1978) Spectrophotometric analysis of binary mixtures of antazoline and naphazoline. J Pharm Sci 67: 140-141.

37. Shibata SM, Furukawa Goto K (1969) Anal Chim Acta 46: 271-279.

38. ICH Q2 (R1) (2005) Validation of Analytical Procedures, Proceedings of the International Conference on Harmonization, Geneva.

39. HPLC manufacturer procedure (EVA PHARMA for Pharmaceuticals and Medical Appliances S.A.E, Egypt) by personal communications.