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Simultaneous Determination of Montelukast and Fexofenadine Using Fourier Transform Convolution Emission Data Under Non- Parametric Linear Regression Method

Marwa A. A. Ragab · Rasha M. Youssef

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Abstract New hybrid chemometric method has been applied to the emission response data. It deals with convolution of emission data using 8-points sin *xi* polynomials (discrete Fourier functions) after the derivative treatment of these emission data. This new application was used for the simultaneous determination of Fexofenadine and Montelukast in bulk and pharmaceutical preparation. It was found beneficial in the resolution of partially overlapping emission spectra of this mixture. The application of this chemometric method was found beneficial in eliminating different types of interferences common in spectrofluorimetry such as overlapping emission spectra and self- quenching. Not only this chemometric approache was applied to the emission data but also the obtained data were subjected to non-parametric linear regression analysis (Theil's method). The presented work compares the application of Theil's method in handling the response data, with the least-squares parametric regression method, which is considered the de facto standard method used for regression. So this work combines the advantages of derivative and convolution using discrete Fourier function together with the reliability and efficacy of the non-parametric analysis of data. Theil's method was found to be superior to the method of least squares as it could effectively circumvent any outlier data points.

Keywords Chemometrics · Spectrofluorimetry · Convoluted derivative curves · Overlapped emission spectra · Self- quenching · Theil's method

M. A. A. Ragab (✉) · R. M. Youssef
Faculty of Pharmacy, Department of Pharmaceutical Analytical Chemistry, University of Alexandria, El-Messalah, Alexandria 21521, Egypt
e-mail: marmed_2001@yahoo.com

Introduction

The reproducibility of fluorescence spectrometry has enabled it to be useful analytical tool. However, when substances whose individual spectral profiles contain broad bands, which often overlap, must be determined simultaneously by spectrofluorimetry, it would be difficult to select a pair of excitation and emission wavelengths which could permit the determination of one of them without any interference from the other. For such a reason, it is worth investigating new methods to solve this problem without restoring too expensive or time consuming techniques.

Several approaches, such as synchronous [1, 2], derivative [3] as well as variable-angle [4] fluorescence spectroscopy have been developed to overcome such problems. All these methods are based on improving the selectivity of co-existing components.

Few chemometric techniques have been found in the literature based on emission data, such as factor analysis [5, 6], three-way resolution [7] and modified PARAFAC algorithm with a Penalty Diagonalization Error (PDE) [8]. They have become more popular in solving problems that are difficult to handle using conventional techniques. These techniques utilized mathematical separation procedures to substitute the traditional chemical separation procedure.

Korany et al. developed a chemometric method based on non-parametric linear regression of derivative/discrete Fourier transform convoluted high performance liquid chromatographic peak responses in non-ideal conditions in the HPTLC [9] and HPLC analysis using external and internal standard methods [10, 11]. It was found that this chemometric treatment was beneficial in eliminating different types of interferences. This was successfully applied to handle some of the most common chromatographic problems and non-ideal

conditions [10, 11]. Several examples dealing with the non-parametric treatment of data were mentioned [9–12].

The survey in the literature reveals that this chemometric hybrid of the two methods presented in this work of derivative and convolution of the resulting derivative curves by discrete Fourier functions was not applied in handling different types of interferences and non- ideal cases in spectrofluorimetry up till now. Also it lacks the application of non-parametric regression methods (Theil's "incomplete" method) as a statistical method of analysis and an important alternative against the parametric regression in the spectrofluorimetric handling of data.

The application of the parametric (least squares) regression method assumes that the data being examined follow normal (Gaussian) distribution. Some support for this assumption is provided by the central limit theorem, which shows that the sampling distribution of the mean may be approximately normal. However, the theorem is not really valid for the very small data sets (often only three or four readings) frequently used in analytical work [13]. This makes it of interest to apply non-parametric regression approaches to fitting a straight line to a set of points, the simplest of the non-parametric regression methods is Theil's "incomplete" method, so called to distinguish it from another more complex procedure developed by the same author (the "complete" Theil's method) [13].

Novel combination of Fexofenadine 'FEX' (anti histaminic drug) and Montelukast 'MTK' (A leukotriene antagonist) is available as tablet dosage form in the ratio of 12:1. It is used to treat seasonal allergies. The literature revealed few methods for simultaneous determination of FEX and MTK, these methods includes; spectrophotometry [14], HPLC [15] and HPTLC [15] but it lacks any spectrofluorimetric method for their simultaneous determination.

Concerning the work presented in this paper, chemometrics were applied in the handling of emission data. Derivative treatment of emission response data was followed by convolution of the resulting derivative curves using 8-points sin *xi* polynomials (discrete Fourier functions). This was found beneficial in eliminating different types of interferences. It was successfully applied to handle some of the most common spectrofluorimetric problems and non-ideal conditions, namely: overlapping emission spectra and self- quenching. The self-quenching is a concentration dependant non-ideal case which distorts the linearity parameters of the regression line. In the presented work, application of the chemometric treatment along with the non-parametric regression method pronouncedly improves the linearity parameters with the possibility of using wider linearity ranges.

The aim of this work was to investigate the application of the derivative technique (D method) alone and the derivative technique followed by convolution using discrete Fourier functions (D/FF method) on the emission data. The application of these chemometric techniques demonstrates the validity of the method to resolve overlapping emission spectra of a binary mixture of FEX and MTK in their pharmaceutical preparation. Also expanding the linearity ranges was achieved by solving the self- quenching in case of MTK and by enhancing the regression equation parameters of both drugs which led to calculating low limits of quantitation after the chemometric treatment. It combines the advantages of derivative and convolution using discrete Fourier functions together with the reliability and efficacy of the non-parametric analysis of data.

Theory

Derivative Technique (D Method)

Application of derivative techniques to spectrophotometric data has become a well-established analytical method [16]. The elimination of interference by the use of derivative techniques depends on the fact that the first derivative of a constant function is zero and that of a linear function is constant. Consequently, a first derivative would eliminate constant interferences and a second derivative would eliminate linear interferences.

The application of this method depends on the fact that the relative fluorescence intensity (F) is a function of wavelength (λ) [10, 11], thus:

$$D1 = dF/d\lambda \tag{1}$$

and

$$D2 = d^2F/d\ \lambda^2 \tag{2}$$

where D1 and D2 are first and second derivative of the analyte, respectively.

Derivative Technique Followed by Convolution Using Fourier Functions (D/FF Method)

The basis of harmonic analysis is that a given function, for example, D1 or D2 curves of emission, $f(\tau)$ can be expanded in terms of the Fourier series [17, 18].

If $(n+1)$ is an even number then:

$$\begin{aligned} f(\tau) = a_0 + a_1\cos x + a_2\cos 2x + \ldots\ldots \\ + a_{(n+1)/2}\ \cos((n+1)/2)x + b_1\sin x \\ + b_2\sin 2x + \ldots.. + b_{(n-1)/2}\ \sin((n-1)/2)x \end{aligned} \tag{3}$$

Calculation of the coefficients a_1, a_2, a_3 …… a_j and b_1, b_2, b_3 ….. b_j is simplified since the trigonometric functions are mutually orthogonal.

Any coefficient t_j , can be calculated from a set of response data measured at equally spaced wavelength intervals, by the following summation, in which x takes values from 0 to 2π-[$2\pi/(n+1)$], at intervals of $2\pi/(n+1)$:

$$(t_j) = \sum f(\tau)_i Tx_i \Big/ \sum (Tx_i)^2 \quad (4)$$

where T represents cosine or sine.

The Fourier function coefficients, (t_j) are proportional to $f(\tau)$. That is:

$$(t_j) = \alpha_j c \quad (5)$$

where α is a constant and c is the concentration of the analyte.

Experimental

Materials and Reagents

FEX was kindly supplied as a gift sample by Sedico Company for Pharmaceuticals, Egypt while MTK, was supplied by SIGMA Pharmaceutical Corp., Egypt. All reagents used were of analytical grade. The pharmaceutical formulation analyzed was MONTAIR FX tablets (label claim: 120 mg FEX and 10 mg MTK per tablet, B. no. ACF1010, Cipla Ltd.).

Apparatus

Fluorescence measurements were carried out using a Shimadzu (Kyoto, Japan) RF-1501 version 3.0 spectrofluorophotometer equipped with a 150 W xenon lamp and 1-cm quartz cells.

Solutions

Stock solutions of 240 mg % FEX and 10 mg % MTK were prepared by dissolving appropriate amounts of each of them in methanol.

Procedures

Construction of Calibration Curves

Aliquots from stock solutions of FEX and MTK were diluted with methanol. The aliquots of FEX were covering the concentration range of 120–2,400 μg mL^{-1} while of MTK, the aliquots were covering the concentration range of 1–10 μg mL^{-1} (Ideal case of linearity) and 1–20 μg mL^{-1} (Non- ideal case of linearity 'self- quenching'). These FEX and MTK solutions were stable for at least 2 h at room temperature. The emission fluorescence spectrum was scanned for each drug under the following operating conditions.

- Emission wavelength, 290–520 nm, at 1.0 nm intervals.
- Excitation wavelength, fixed at 280 nm.

The emission data were processed using Excel software. Derivative technique (D method) was applied, first (D1) and second (D2) derivative data at 1.0 nm interval were calculated. Then convolutions of the two types of derivative data were made using discrete Fourier functions of 8- points sin x_i polynomials (D/FF method) at 1.0 nm interval to get convoluted first derivative curves; $D1$/FF and convoluted second derivative curves; $D2$/FF at 1.0 nm interval as follows:

$$t = \left\{ \begin{matrix} (0)D_0 + (+0.707)D_1 + (+1)D_2 + (+0.707)D_3 \\ +(0)D_4 + (-0.707)D_5 + (-1)D_6 + (-0.707)D_7 \end{matrix} \right\} /4 \quad (6)$$

where D_0 to D_7 stand for eight derivative values; at 1.0 nm interval. The numbers in brackets are values of the selected Fourier function. The derivative values (peak to peak or peak to zero) and the convoluted derivative data (peak to peak or peak to zero) were measured at the corresponding wavelength range for each compound as shown in Table 1 and in Fig. 1.

Preparation of Synthetic Mixtures

Different synthetic mixtures of both drugs were prepared in methanol at the concentration levels of 20:240, 10:120, 10:240 and 20:120 μg mL^{-1} for MTK and FEX, respectively. These synthetic mixtures are examples of the overlapping emission spectra of both drugs in the ideal case of linearity ranges for both drugs and in the non- ideal case of linearity range for MTK (self- quenching). The emission fluorescence spectra of the mixtures were scanned and processed as under the operating conditions discussed in the section of Construction of Calibration Curves.

Assay of Tablets

Ten tablets were accurately weighed and powdered. A sample equivalent to one tablet was weighed and transferred to a 50 ml volumetric flask. Thirty milliliters of methanol were added and the flask was sonicated for 30 min then completed to the volume with methanol; and the solution was filtered. After filtration, two dilutions were made in methanol to give concentration ratios of 10: 120 and 20: 240 for MTK and

Table 1 Selected points (wavelength range in nm) of Fexofenadine (FEX) and Montelukast (MTK) for the derivative and convoluted derivative in the ideal and non- ideal cases of linearity ranges of MTK

	FEX	MTK
Direct measurement		
Fluorescence intensity (F)	309	398
Derivative technique (*D* method)		
First derivative (D1)	327	420
Second derivative (D2)	312–323	391
Derivative under Fourier functions (*D*/FF method)		
First derivative under Fourier functions (D1/FF)	315	381
Second derivative under Fourier functions (D2/FF)	309–315	378

FEX, respectively. The emission fluorescence spectra of the two diluted solutions were scanned and processed as under the operating conditions discussed in the section of Construction of Calibration Curves.

Results and Discussion

Upon dissolving FEX and MTK in methanol, a native fluorescence was observed. Fluorescence spectra of FEX and MTK are considerably overlapped so that the conventional fluorescence does not allow the simultaneous determination of them. Scanning the emission spectra of both drugs showed λ_{em} at 309 nm and 399 nm for FEX and MTK, respectively upon excitation at 280 nm. Different solvents as methanol, water, acetonitrile, acetone and buffer solutions were tried. Methanol was found to be the best solvent that gave high emission intensity for both drugs.

Treatment of Analytical Data

Application of Derivative Technique (D Method) to Emission Response Data

Derivative calculations were applied to emission data of the scanned standard solutions, the overlapped synthetic mixtures and the dosage form final solutions of the previously mentioned two cases; the ideal case of linearity ranges for FEX and MTK and the non- ideal case of linearity range for MTK (self- quenching). Direct measurement of the emission data exhibits some kind of interference. Constant interferences could be eliminated by calculating the first derivative (D1), while second derivative (D2) can eliminate any linear interference [10, 11]. For each case, the D1 and D2 values at the selected points (Table 1) at 1.0 nm interval for each of the two compounds were correlated to the concentration. The points selected for the overlapped emission spectra of both drugs were based on that, maximum response was obtained for each compound at these points with nearly zero contribution of the other. Also in the non- ideal case of self- quenching, the selected points were based on that, at each point MTK was of the most suitable response minimizing the negative deviation of the measured emission. Consequently, minimizing the distortion of the regression line of MTK occurred. This led to expanding the range of linearity without the need of extra experimental work by dilution; Table 2 is a representative example of MTK non ideal case of linearity.

Application of Fourier Functions to Derivative Data (D/FF Method)

For each case, the first and second derivative curves were convoluted using 8-points sin *xi* polynomials at 1.0 nm interval then the optimum convoluted D1/FF, D2/FF, values selected for each of the two compounds were related to concentration. Since convolution using Fourier functions corrects all types of interferences except for linear interference, application of Fourier functions on derivative data would eventually lead to removal of all types of interference producing high degree of purity of the analytical peaks at the selected points [10, 11]. This would be beneficial in case where high incidence of interferences could be found from other mixture components, as in the assay of FEX and MTK in presence of each other. At which the selected points would represent the pure compound and neglect the other interfering compound. Also in the non- ideal case of self- quenching of MTK, the selected points were based on minimizing the errors distorting the MTK regression line. This led to enhancing the linearity parameters consequently enhancing the recovery of MTK in this non ideal case as will be discussed later in details in the section of Precision and Accuracy.

Validation

ICH guidelines [19] for method validation were followed for the developed spectrofluorimetric method. All validation parameters will be discussed below in details.

Parametric Calibration Graphs and Statistical Data

The linearity of the proposed methods was evaluated by analyzing series of different concentrations of each of FEX and MTK. According to ICH, at least five concentrations must be used.

Under the experimental conditions described for each of the two cases, the graphs obtained by plotting relative fluorescence response (F), derivative and convoluted derivative data versus concentration for each of the two compounds, show various degrees of linearity.

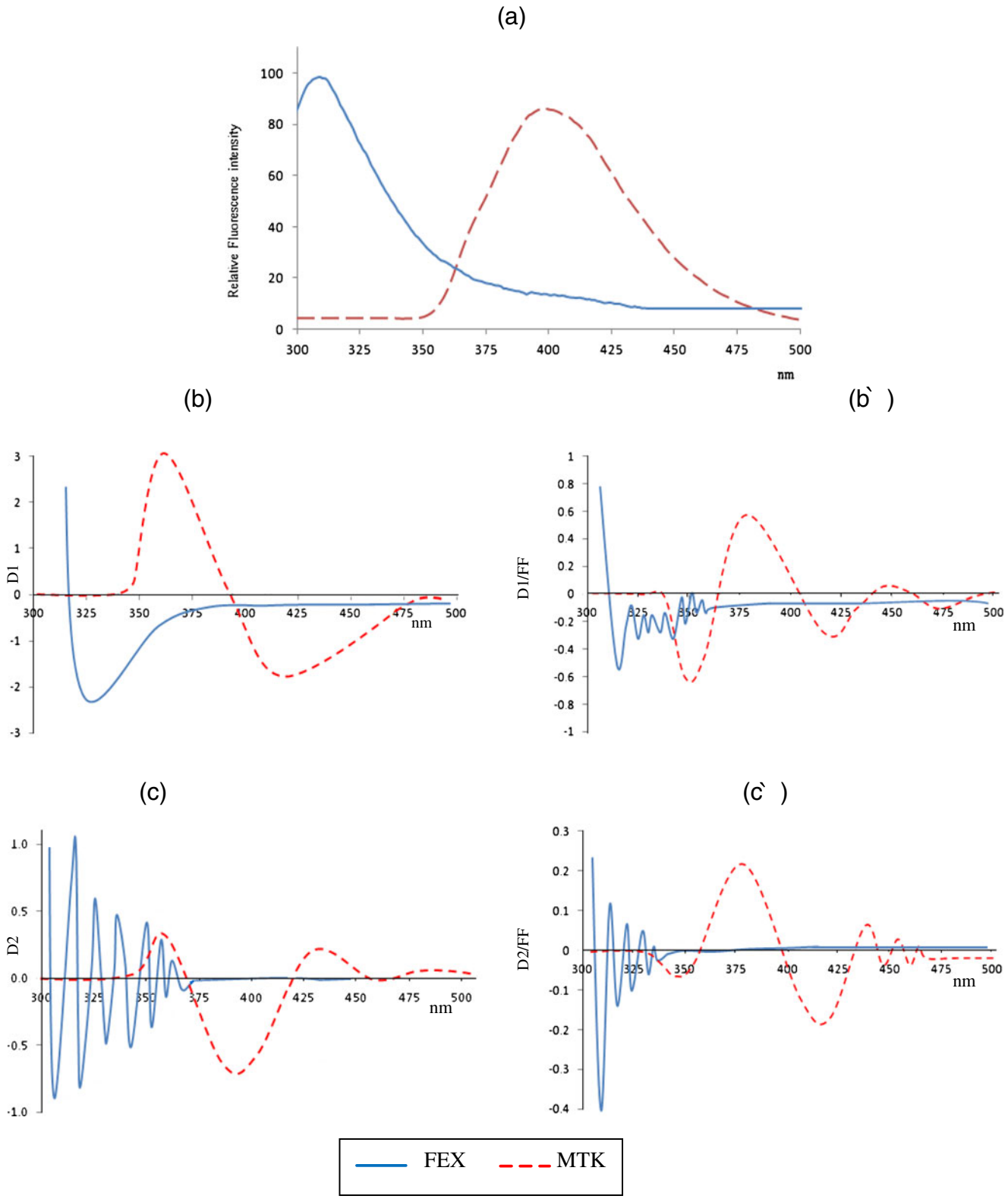

Fig. 1 Emission spectra of 2,400 μg mL^{-1}Fexofenadine (FEX) and 10 μg mL^{-1}Montelukast (MTK) (**a**), their first derivative (**b**), second derivative *curves* (**c**) and the corresponding Fourier functions curves (**b`**) and (**c`**)

Generally, in the ideal case of linearity no great enhancement in the linearity parameters was achieved upon applying D methods then D/FF methods. On the other hand, in non- ideal case of linearity of MTK (self-

quenching) the direct measurement of data showed low values of correlation coefficients with high values of intercepts indicating a bad linearity of the calibration graphs obtained. After treatment of data by applying D methods then D/FF methods, an increase in the correlation coefficient values with decrease in the intercept values were obtained (Table 2). As convolution using Fourier functions corrects all types of interferences except for linear interference. Thus, application of Fourier functions on derivative data especially D2/FF would be beneficial in this case giving the best linearity parameters, (Table 2). Good regression lines show high values for both (r) and (F) values [17].

Application of Non-Parametric Regression Methods

The statistical parameters concerning the parametric method have all assumed that data being examined follow the normal (Gaussian) distribution. Some support for this assumption is provided by the central limit theorem, which shows that the sampling distribution of the mean may be approximately normal. However, the theorem is not really valid for the very small data sets (often only three or four readings) frequently used in analytical work [13] .

There are several non-parametric methods that can be used for fitting a straight line to a set of points. Of the several methods available, perhaps the simplest is Theil's "incomplete" method which was first applied to the data of HPTLC, HPLC and polarography [9–12]. It was also applied for the first time to the emission data in the present study.

When the normal distribution is assumed, the arithmetic mean as the ' measure of central tendency' of a set of results is to be used. In non-parametric statistics, the median is usually used instead as in many cases it is more realistic measure of central tendency than the arithmetic mean [13].

For all of the previously mentioned types of linearity, and for each drug, the emission response data were handled using Theil's method. The best-fit straight line obtained using Theil's method was compared with the least squares best fit line calculated using the parametric regression method. Tables 3 and 4 and Figs. 2 and 3 illustrated that the non-parametric regression model could be considered superior over the parametric one and this was proved by calculating the percentage change in the intercept and slope, in general the intercept decreases and the slope increases. Figures (2 and 3) showed the improvement in the non-parametric regression lines when compared with the parametric ones. This was done using two points which are the intercept and the first point of the linearity range to clearly show the improvement of the regression lines intercepts and slopes values.

In the ideal case of linearity for MTK and FEX (Tables 3 and 4), the change in the intercept and slope is not great but the dramatic change is observed in the non- ideal case of linearity for MTK, Table 4.

Taking Table 4 as an illustrating example, concerning the MTK in the non- ideal case of linearity where high incident of error was encountered, a great enhancement in the intercept and slope is obtained. It can be seen that the percentage change in the intercept when applying the non-parametric relative to the parametric models was from −0.210 to −42.831 % and the intercept decreases almost near the

Table 2 Parametric linear regression and statistical parameters for the determination of Montelukast (MTK) by the proposed Spectrofluorimetric method in the non- ideal case

	r	a	b	$S_{y/x}$	S_a	S_b	F	LOD µg/mL	LOQ µg/mL
Direct measurement									
Fluorescence intensity (F)	0.96667	23.201	5.0808	8.2982	4.2887	0.50858	99.80	4.900	16.332
Derivative technique (*D* method)									
First derivative (D1)	0.95390	0.7979	0.1551	0.3009	0.1555	0.01845	70.719	5.821	19.402
Second derivative (D2)	0.99066	0.1556	0.0498	0.0422	0.0218	0.00259	369.5	2.546	8.487
Derivative under Fourier functions (*D*/FF method)									
First derivative under Fourier functions (D1/FF)	0.97765	0.1393	0.0346	0.0459	0.0237	0.00282	151.39	3.978	13.261
Second derivative under Fourier functions (D2/FF)	0.999	0.0193	0.0097	0.0009	0.0005	0.00018	2769	0.281	0.937

F: Variance ratio, equals the mean of squares due to regression divided by the mean of squares about regression (due to residuals)

r correlation coefficient, *a* intercept, *b* slope

$S_{y/x}$ standard deviation of residuals

S_a standard deviation of intercept

S_b standard deviation of slope

LOD limit of detection

LOQ limit of quantitation

Table 3 Comparison between parametric and non-parametric regression models for the determination of Fexofenadine (FEX) by the proposed Spectrofluorimetric method

	|*a*|		|*b*|			
	Parametric	Non-Parametric	Parametric	Non-Parametric	Percentage change in |*a*|	Percentage change in |*b*|
Direct measurement						
Fluorescence intensity (F)	8.2780	5.6933	0.0382	0.0434	−31.2238	13.5827
Derivative technique (D method)						
First derivative (D1)	0.2070	0.1426	0.000957	0.001087	−31.1111	13.5841
Second derivative (D2)	0.1530	0.10531	0.000707	0.000803	−31.1699	13.5785
Derivative under Fourier functions (*D*/FF method)						
First derivative under Fourier functions (D1/FF)	0.0300	0.020694	0.000139	0.000158	−31.0200	13.6691
Second derivative under Fourier functions (D2/FF)	0.0520	0.036085	0.000242	0.000275	−30.6058	13.6364

|***a***| modulus of intercept

|***b***| modulus of slope

Percentage change in |*a*| means percentage change in |*a*| of NP vs. |*a*| of P=[(|*a*|of NP−|*a*|of P)/|*a*|of P]×100

Percentage change in |*b*| means percentage change in |*b*| of NP vs. |*b*| of P=[(|*b*|of NP−|*b*|of P)/|*b*|of P]×100

origin when applying the non-parametric regression model. The percentage change in slope was from 1.833 to 51.774 % indicating an increase in the slope 'increasing the sensitivity of the method'.

Table 4 Comparison between parametric and non-parametric regression models for the determination of Montelukast (MTK) by the proposed Spectrofluorimetric method in the ideal and non- ideal cases of linearity

	|*a*|		|*b*|			
	Parametric	Non-Parametric	Parametric	Non-Parametric	Percentage change in |*a*|	Percentage change in |*b*|
Ideal case						
Direct measurement						
Fluorescence intensity (F)	12.881	14.02731	7.513652	7.339615	8.899	−2.316
Derivative technique (*D* method)						
First derivative (*D*1)	0.418	0.455725	0.244107	0.238453	9.025	−2.316
Second derivative (*D*2)	0.106	0.114946	0.06157	0.060144	8.440	−2.316
Derivative under Fourier functions (*D*/FF method)						
First derivative under Fourier functions (*D*1/FF)	0.082	0.089651	0.048021	0.046909	9.330	−2.316
Second derivative under Fourier functions (*D*2/FF)	0.017	0.015608	0.01019	0.01112	−8.188	9.127
Non- ideal case						
Direct measurement						
Fluorescence intensity (F)	23.247	14.04218	5.082	7.241011	−39.596	42.483
Derivative technique (D method)						
First derivative (*D*1)	0.798	0.456208	0.155	0.235249	−42.831	51.774
Second derivative (*D*2)	0.156	0.115068	0.050	0.059336	−26.238	18.672
Derivative under Fourier functions (*D*/FF method)						
First derivative under Fourier functions (*D*1/FF)	0.139	0.089746	0.035	0.046279	−35.435	32.226
Second derivative under Fourier functions (*D*2/FF)	0.01904	0.019	0.00982	0.0101	−0. 210	1.833

|***a***| modulus of intercept

|***b***| modulus of slope

Percentage change in |*a*| means percentage change in |*a*| of NP vs. |*a*| of P=[(|*a*|of NP−|*a*|of P)/|*a*|of P]×100

Percentage change in |*b*| means percentage change in |*b*| of NP vs. |*b*| of P=[(|*b*|of NP−|*b*|of P)/|*b*|of P]×100

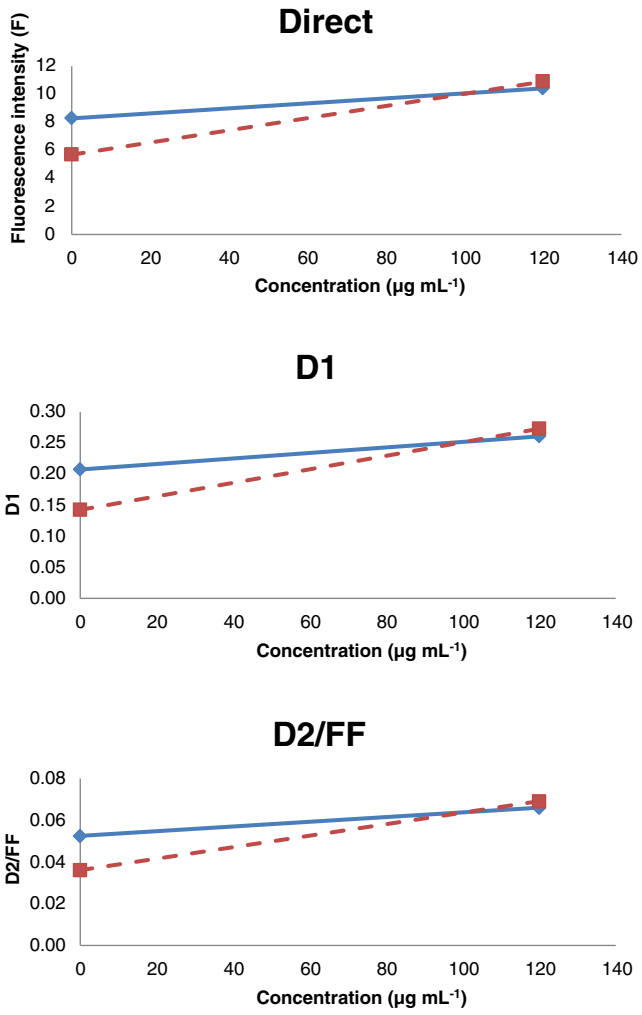

Fig. 2 *Regression lines* calculated by Theil's method, non-parametric (– –), and by the least squares method, parametric (——), for the determination of Fexofenadine (FEX) using direct, D1 and D2/FF as representative examples

From Table 4 in the non- ideal case of MTK linearity, it was also noticed that the change in the intercept and slope upon using Theil's method decreases after the chemometric treatment of the data. For example, using the D2/FF corrects for all types of interferences, so the effect of using Theil's method on the intercept and slope is less pronounced in the D2/FF than in the direct measurement.

Theil's method has three distinct advantages over the least squares method: first, it does not assume that all the errors are in the y-direction; second, it does not assume that either the x- or y- direction errors are normally distributed; and third it is not affected by the presence of outlaying results, Generally, an outlier value does not affect the Theil's calculation at all since it does not affect the median estimate of the slope or intercept. In the least squares calculation, however, the outlying point carries as much weight as the other points. This leads to the fact that, the least squares line passes closer to the outlier than the non-parametric line does.

Detection and Quantitation Limits

Limit of detection (LOD) according to Miller [13] is equal to $y_B+3\ S_B$ where y_B is the value of the calculated intercept and S_B is the $S_{y/x}$ while limit of quantitation LOQ will be equal to $y_B+10\ S_B$. LOD and LOQ for each compound at each case were calculated. The LOD and LOQ were lower than those obtained before the treatment of data especially in the non ideal case of MTK. This indicated that the linearity ranges for the determination of both drugs could be expanded to lower limits of quantitation by enhancing the regression equation parameters after the chemometric treatment of the data, Table 2.

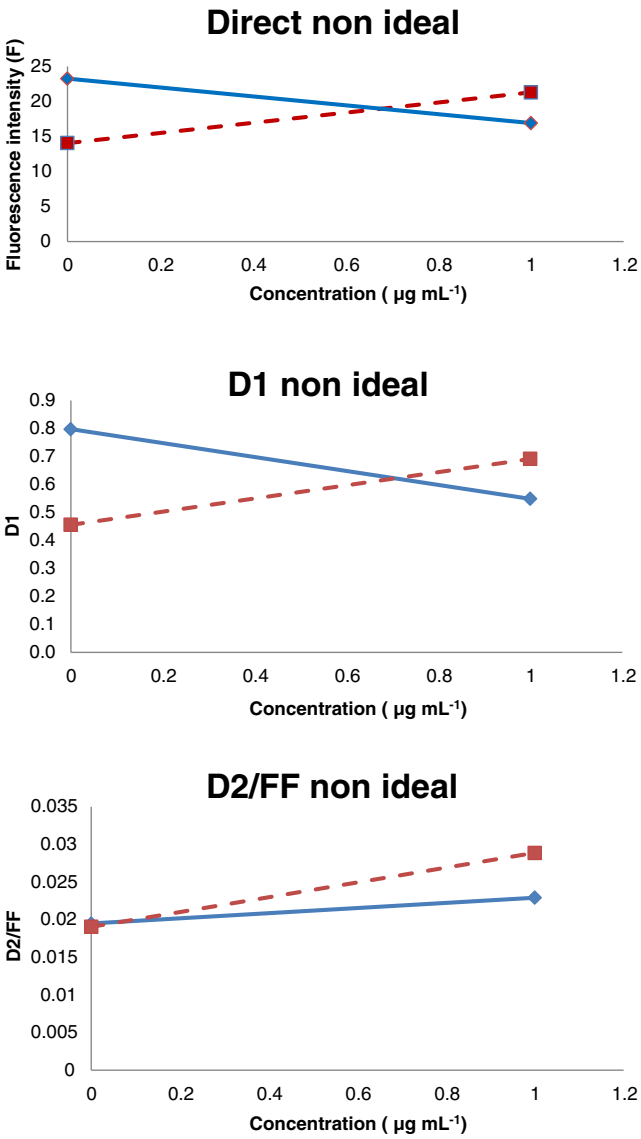

Fig. 3 *Regression lines* calculated by Theil's method, non-parametric (– –), and by the least squares method, parametric (——), for the determination of Montelukast (MTK) using direct, D1 and D2/FF in the non ideal case of linearity as representative examples

Precision and Accuracy

For the parametric regression method, in order to assess the precision, as percentage relative standard deviation (RSD %) and the accuracy, as mean percentage recovery, triplicate determinations were carried out on the synthetic mixtures stated in the section of Preparation of Synthetic Mixtures. The data shown in Table 5 indicate that good accuracy and precision were not obtained for FEX determination in the synthetic mixtures until the application of D1 method due to the overlap with MTK.

For MTK determination in these synthetic mixtures in the ideal case of its linearity range, Table 6 showed bad precision and accuracy due to its overlap with FEX. However when derivative and convoluted derivative were applied, the (RSD %) and mean percentage recovery became in the accepted ranges of each indicating good precision and accuracy.

For MTK determination in its non- ideal case of linearity (self- quenching) with the overlapping with FEX in these synthetic mixtures, good precision and accuracy were obtained at the D2/FF method, Table 7. So application of Fourier functions on derivative data would eventually lead to removal of all types of interference producing high degree of purity of the analytical peaks at the selected points.

For the non-parametric regression method, the same procedures were done except that the (RSD %) and the mean percentage recovery calculations were based on the intercepts and slopes obtained by the non-parametric method. The mean percentage recovery became better and the RSD% became lower indicating good accuracy and precision, this was proved by the calculations of percentage change in Er% and RSD% in the non- parametric method of regression compared with the parametric method, Tables 5, 6 and 7 and Figs. 4 and 5.

Analysis of Pharmaceutical Formulations

For the parametric method, the data shown in Table 8 indicated good accuracy and precision after treatment of data using derivative and convoluted derivative.

For the non-parametric method, the same procedures were done, the (RSD %) and the mean percentage recovery calculations were based on the intercepts and slopes obtained by the non-parametric method. Table 8 showed that by using the non-parametric method, the RSD% became lower than the parametric one and the mean percentage recovery became closer to 100 %, indicating that the non-parametric method was superior over the parametric one.

Table 5 Parametric and Non- parametric evaluation of the precision and accuracy for the determination of Fexofenadine (FEX) in different synthetic mixtures with Montelukast (MTK) by the proposed spectrofluorimetric method

	Recovery %									
	Parametric (P)					Non- Parametric (NP)				
FEX Nominal Conc μg mL^{-1} in MTK:FEX Mixtures	Direct	D1	D1/FF	D2	D2/FF	Direct	D1	D1/FF	D2	D2/FF
1:12(20:240)	128.75	98	98.2	99	100	119.1	99.52	99.5	99.8	100
1:12(10:120)	186.16	99.7	98.9	100	101	140	99.3	100.7	100	100.5
1:24(10:240)	137.97	100	101	101.6	98	125	99.9	99.52	100.7	99.51
10:12(20:120)	165.21	101.7	98	98.1	98	130	101	98.78	98.78	99.9
mean%	154.52	99.85	99.03	99.68	99.25	128.53	99.93	99.63	99.82	99.98
E_r %	54.52	−0.15	−0.97	−0.32	−0.75	28.53	−0.07	−0.38	−0.18	−0.02
SD	26.16	1.52	1.37	1.50	1.50	8.85	0.76	0.80	0.79	0.41
RSD(%)	16.93	1.52	1.39	1.50	1.51	6.89	0.76	0.80	0.79	0.41
%change in E_r %	–	–	–	–	–	−47.67	−53.33	−60.82	−43.75	−97.33
%change in RSD(%)	–	–	–	–	–	−59.32	−50.21	−42.40	−47.17	−73.03

Recovery% is the mean recovery of three determinations at each concentration level

Mean% is the mean of all recoveries of different concentration in the same method.

E_r% is the percentage relative error

SD is the standard deviation of the recoveries of different concentration in the same method.

RSD% is the percentage relative standard deviation.

% change in Er% of NP versus that of P=[(Er% of NP−Er% of P)/Er% of P]∗100

% change in RSD (%) of NP versus that of P=[RSD(%) of NP−RSD (%) of P]/RSD (%) of P]∗100

Table 6 Parametric & Non- parametric evaluation of the precision and accuracy for the determination of Montelukast (MTK) in different synthetic mixtures with Fexofenadine (FEX) by the proposed spectrofluorimetric method in the ideal case of linearity

	Recovery %									
	Parametric (P)					Non- Parametric (NP)				
MTK Nominal Conc μg mL^{-1} in MTK :FEX mixtures	Direct	D1	D1/FF	D2	D2/FF	Direct	D1	D1/FF	D2	D2/FF
1:12 (10:120)	91.18	93.87	96.08	100.59	99.93	93.32	95.39	98.12	99.79	99.18
1:24 (10:240)	92.08	91.31	94.62	101.76	100.76	90.68	93.95	98.29	100.93	99.95
mean%	91.63	92.59	95.35	101.18	100.35	92.00	94.67	98.21	100.36	99.57
E_r %	−8.37	−7.41	−4.65	1.18	0.34	−8.00	−5.33	−1.79	0.36	−0.44
SD	0.64	1.81	1.03	0.83	0.59	1.87	1.02	0.12	0.81	0.54
RSD(%)	0.69	1.96	1.08	0.82	0.58	2.03	1.08	0.12	0.80	0.55
%change in E_r %	–	–	–	–	–	−4.42	−28.07	−61.51	−69.49	−229.4
%change in RSD(%)	–	–	–	–	–	192.2	−44.99	−88.69	−1.77	−6.50

Recovery% is the mean recovery of three determinations at each concentration level

Maen% is the mean of all recoveries of MTK concentration in different synthetic mixtures in the same method

E_r% is the percentage relative error

SD is the standard deviation of the recoveries of different concentration in the same method

RSD% is the percentage relative standard deviation

% change in Er% of NP versus that of P=[(Er% of NP−Er% of P)/Er% of P]∗100

% change in RSD (%) of NP versus that of P=[RSD(%) of NP−RSD (%) of P]/RSD (%) of P]∗100

Table 7 Parametric and Non- parametric evaluation of the precision and accuracy for the determination of Montelukast (MTK) in different synthetic mixtures with Fexofenadine (FEX) by the proposed spectrofluorimetric method in the non- ideal case of linearity

	Recovery %									
	Parametric (P)					Non- Parametric (NP)				
MTK Nominal Conc μg mL^{-1} in MTK :FEX mixtures	Direct	D1	D1/FF	D2	D2/FF	Direct	D1	D1/FF	D2	D2/FF
1:12 (20:240)	71.5	63.9	70.9	77.09	98.2	92.91	85.90	87.52	87.83	99.28
1:12 (10:120)	101.9	97.37	95.13	101.2	99.75	127.15	125.7	112.9	112.60	100.82
1:24 (10:240)	103.1	95.88	92.53	102.11	100.76	128.83	123.4	109.2	113.56	100.99
10:12 (20:120)	70.9	64.6	72.30	79.45	98.24	92.08	86.97	89.35	90.61	99.2
mean%	86.85	80.44	82.72	89.96	99.24	110.24	105.5	99.73	101.15	100.07
E_r %	−13.15	−19.56	−17.29	−10.04	−0.76	10.24	5.48	−0.27	1.15	0.07
SD	18.08	18.70	12.89	13.54	1.25	20.51	22.02	13.15	13.83	0.96
RSD(%)	20.82	23.25	15.58	15.05	1.25	18.60	20.87	13.18	13.67	0.96
%change in E_r %	–	–	–	–	–	−177.9	−128.0	−98.44	−111.5	−109.2
%change in RSD(%)	–	–	–	–	–	−10.63	−10.23	−15.41	−9.17	−23.20

Recovery% is the mean recovery of three determinations at each concentration level

Maen% is the mean of all recoveries of MTK concentration in different synthetic mixtures in the same method

E_r% is the percentage relative error

SD is the standard deviation of the recoveries of different concentration in the same method

RSD% is the percentage relative standard deviation

% change in Er% of NP versus that of P=[(Er% of NP−Er% of P)/Er% of P]∗100

% change in RSD (%) of NP versus that of P=[RSD(%) of NP−RSD (%) of P]/RSD (%) of P]∗100

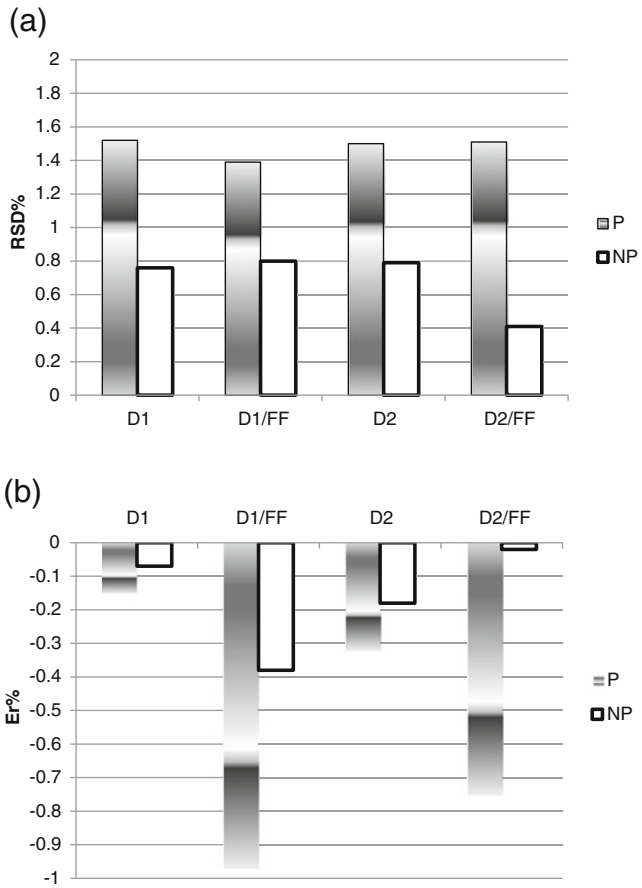

Fig. 4 Comparison between RSD (%), (**a**), and E_r (%), (**b**), calculated for Fexofenadine (FEX), after the chemometric treatment of data for D1, D1/FF, D2 and D2/FF, using the two types of regression models, parametric (P) and non-parametric (NP)

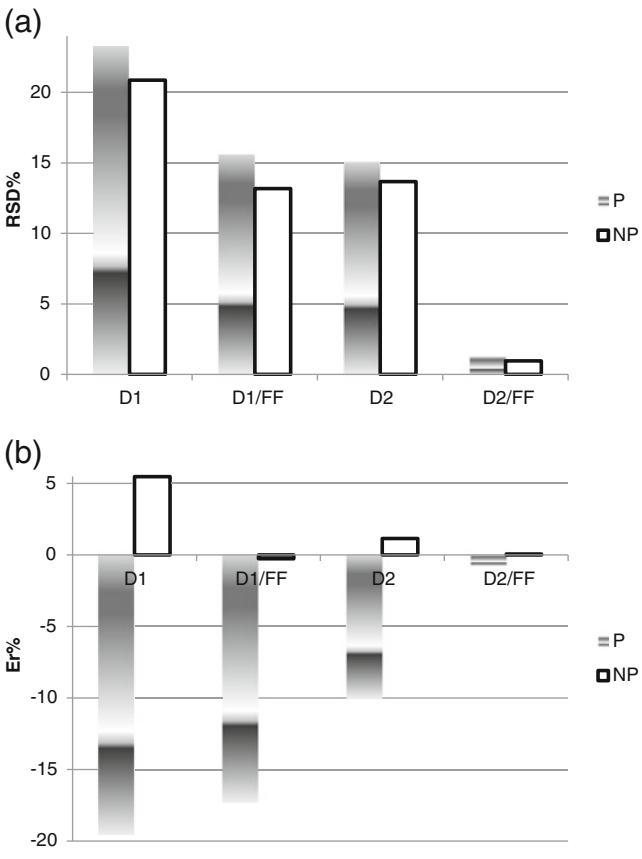

Fig. 5 Comparison between RSD (%), (**a**), and E_r (%), (**b**), calculated for Montelukast (MTK) in the non ideal case of linearity, after the chemometric treatment of data for D1, D1/FF, D2 and D2/FF, using the two types of regression models, parametric (P) and non-parametric (NP)

Conclusion

Derivative treatment of data followed by convolution with discrete Fourier functions has been successfully applied for handling overlapped emission spectra and bad linearity cases in spectrofluorimetry. This is highly needed in cases where sources of interference could dramatically affect the emission response data. This improves the spectrofluorimetric

Table 8 Parametric and non- parametric evaluation of the precision and accuracy for the determination of Fexofenadine (FEX) and Montelukast (MTK) in their pharmaceutical preparation in the ideal case of linearity and Non- ideal case of linearity of MTK

			Parametric					Non- parametric				
Nominal Value μg mL^{-1}			Direct	D1	D1/FF	D2	D2/FF	Direct	D1	D1/FF	D2	D2/FF
FEX 120		recovery	140.1	98.8	99.7	100.5	100.9	110	99.6	100.3	99.7	99.9
		RSD(%)	10.63	1.73	1.56	1.31	1.11	4.11	0.99	0.86	0.66	0.53
FEX 240		recovery	112.7	98.2	97.9	100	101.1	119.1	98.9	100.2	99.7	99.9
		RSD(%)	16.93	1.67	1.50	1.61	1.31	6.55	0.98	0.72	0.66	0.52
MTK 10	Ideal	recovery	93.88	95.44	96.32	98.59	100.93	94.37	97.67	97.77	99.7	100.8
		RSD(%)	1.72	1.64	1.77	1.02	0.65	1.13	1.05	0.99	0.45	0.32
MTK 20	Non- ideal	recovery	75.5	80.5	85.9	90.06	98.5	78.88	87.9	92.10	95.52	99.83
		RSD(%)	12.81	8.78	5.85	3.09	1.5	10.80	7.9	7.12	2.18	0.99

Recovery% is the mean recovery of triplicate determination

RSD% is the percentage relative standard deviation

quantitation of the drugs consequently could expand the linearity range.

Non-parametric regression of the response data using Theil's method is highly advantageous over the usual least squares method. It has effectively circumvented the outlier problem.

In this paper, validation parameters were not only mentioned but also compared using different regression methods, this was done by the analysis of synthetic mixtures and the application of a pharmaceutical formulation. It was found that the non-parametric method was superior over the parametric one in the simultaneous determination of FEX and MTK after the chemometric treatment of their emission spectra.

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