Non-parametric linear regression of discrete Fourier transform convoluted densitometric peak responses

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Abstract

The manuscript discusses the application of chemometrics to the handling of TLC response time data. Derivative treatment of chromatographic response data followed by convolution of the resulting derivative curves using 8-points $\sin x_i$ polynomials (discrete Fourier functions) was found to be beneficial in eliminating the interference due to background noise in TLC-densitometric measurements. It also compares the application of Theil's method, a non-parametric regression method, in handling the response data, with the least squares parametric regression method, which is considered the *de facto* standard method used for regression. Theil's method was found to be superior to the method of least squares as it assumes that errors could occur in both *x*- and *y*-directions and they might not be normally distributed. In addition, it could effectively circumvent any outlier data points.
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1. Introduction

Chemometric techniques have been widely applied for exploring complex chromatograms, e.g., application of photodiode array (PDA) detection HPLC of chlorophyll a allomers [1,2]. Deconvolution of chromatograms into orthogonal polynomials has been applied for characterizing the quality of separation in patterns of strongly overlapping peaks, e.g., sulpha drugs analyzed by reversed phase HPLC column [3]. Analysis of enantiomers giving partially overlapping peaks was also made possible by using different chemometric treatments of chromatographic ultraviolet signals, e.g., quantification of pseudoephedrine enantiomers [4]. Deconvolution of partially overlapping tailing peaks in photodiode array HPLC using purity ratios has also been applied [5]. Semi-automatic deconvolution of chromatographic data to give pure spectral and chromatographic profiles has been found beneficial in the quantitative determination of some pharmaceutical mixtures [6].

Exploratory chemometric analysis has been used in the classification of pharmaceutical substances based on chromatographic data [7] and for the purpose of pharmaceutical fingerprinting to distinguish among same-product manufacturers [8].

The non-parametric evaluation of the chromatographic response has been applied for the immunoassay of antigens [9], environmental analysis [10], for data audits to improve the analytical performance [11] and in many aspects of quantitation [12]. Non-parametric regression of data to a straight line has been extended to by-pass any outlier problem with subsequent refitting of the regression line [13,14].

Application of derivative techniques to spectrophotometric data has become a well-established analytical method [15–18]. 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.

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 [19]. This technique was extended to be used in TLC response data by applying the derivative technique by itself and followed by convolution using 8-points $\sin x_i$ polynomials The present study deals with applying the derivative technique by itself and followed by convolution

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using 8-points $\sin x_i$ polynomials (discrete Fourier functions). It was applied on mixtures of bezafibrate (BZ) and its degradation products; bezafibric acid (BZA) and p-chlorobenzoic acid (PCL). The chromatographic response data was treated by applying the derivative technique (D method) alone and the derivative technique followed by convolution using discrete Fourier functions (D/FF method).

The study presents also a comparison between two statistical regression methods for handling data; parametric- and non-parametric-regression methods [20]. The application of the parametric (least squares) regression method assumes that the data being examined follow normal (Gaussian) distribution. However, the non-parametric regression methods can handle data that may not be normally distributed. Since the central limit theorem of the parametric regression is not really valid for the very small data sets frequently used in analytical work, this make 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) [20].

2. Theory

2.1. Derivative technique (D method)

The application of this method depends on the fact that the chromatographic response (R) is a function of distance (d) on the TLC chromatogram and that is:

$$D1 = \frac{dR}{dd} \tag{1}$$

$$D2 = \frac{d^2R}{dd^2} \tag{2}$$

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

2.2. 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 a chromatographic peak, $f(d)$ can be expanded in terms of the Fourier series [21–24].

If $(n+1)$ is an odd number, the expansion is,

$$f(d) = a_0 + a_1 \cos x + a_2 \cos 2x + \cdots + a_{(n/2)} \cos\left(\frac{n}{2}\right)x$$

$$+ b_1 \sin x + b_2 \sin 2x + \cdots + b\left(\frac{n}{2}\right)\sin\left(\frac{n}{2}\right)x \tag{3}$$

If $(n+1)$ is an even number, the expansion is,

$$f(d) = a_0 + a_1 \cos x + a_2 \cos 2x$$

$$+ \cdots + a_{(n+1)/2} \cos\frac{(n+1)}{2x} + b_1 \sin x$$

$$+ b_2 \sin 2x + \ldots + b_{(n-1)/2} \sin\frac{(n-1)}{2x} \tag{4}$$

The calculation of the coefficients a_1, a_2, a_3 ... and b_1, b_2, b_3 ... is simplified since the trigonometric functions are mutually orthogonal.

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

$$t_j = \sum f(d)_i \frac{Tx_i}{\sum (Tx_i)^2} \tag{5}$$

where T represents cosine or sine.

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

$$t_j = \alpha_j C \tag{6}$$

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

3. Experimental

3.1. Instrumentation

The chromatographic system consisted of CAMAG^{TM} Linomat TLC-applicator. A CAMAG^{TM} Linomat syringe (100 µl) was used for application of solutions onto silica gel GF254 precoated TLC plates of particle size of 0.25 mm (FLUKA^{TM}). The plates were developed in CAMAG^{TM} chromatographic tanks (15 cm × 20 cm × 30 cm), and scanned densitometrically using CAMAG^{TM} TLC – Scanner3 version 4.06, supported with UV-lamp short wavelength 254 nm, and interfaced to an IBM computer loaded with CAMAG^{TM}-TLC-SOFTWARE(CATS) and connected to a laser printer. The digital chromatographic response data were transferred to a personal computer for subsequent processing using Microsoft Excel XP (Microsoft Corp. Richmond, VA, USA).

3.2. Materials and reagents

Bezafibrate was kindly supplied by Glaxo Wellcome, Egypt. All solvents were of analytical grade.

3.2.1. Preparation of bezafibric acid (BZA) and p-chlorobenzoic acid (PCL)

Five hundred milligram of bezafibrate were transferred into a 250-ml conical acid were added and the solution was refluxed for 6 h.

The solution was cooled and extracted with chloroform (4 × 25 ml).

The chloroform was distilled under reduced pressure. The residue left was mixed with 5 ml of absolute ethanol and the ethanol was distilled under reduced pressure. The residue (PCL) was collected and dried. The aqueous solution left after extraction with chloroform was warmed (80 °C) till no chloroform odour. The solution was distilled under reduced pressure. The residue left (BZA) was collected and dried.

3.2.2. Preparation of stock and standard solutions

Bezafibrate (0.5 mg ml^{-1} in methanol) was prepared by dissolving 50 mg of BZ in 100 ml of methanol.

Bezafibric acid (0.2 mg ml^{-1} in methanol) was prepared by dissolving 20 mg of the residue left from the aqueous solution (3.2.1) in 100 ml of methanol.

p-Chlorobenzoic acid (0.2 mg ml^{-1} in methanol) was prepared by dissolving 20 mg of the residue left from the chloroformic extract (3.2.1) in 100 ml of methanol.

3.3. Assay of mixtures containing BZ, BZA and PCL

Aliquots of the stock solutions of each of BZ, BZA and PCL were transferred into a set of 10 ml volumetric flasks, mixed well and diluted to volume with methanol to prepare six mixtures containing BZ in the concentration range of 150–400 µg ml^{-1} and each of BZA and PCL in the concentration range of 20–120 µg ml^{-1}. Ten microlitres aliquot of each mixture was applied onto TLC-plate using CAMAG-Linomat TLC Applicator as 5 mm wide bands. The plate was developed using a mobile phase composed of ethyl acetate–methanol–glacial acetic acid (8:1.5:0.5) mixture by ascending technique. The developed plate was dried and the bands were investigated densitometrically at 230 nm under the following conditions:

Mode	Absorbance (reflectance)
Lamp	Deuterium
Bandwidth	10 nm
Slit dimensions	5 mm × 0.45 mm
Scanning speed	0.2 mm s^{-1}

For every concentration of each compound, the peak area and peak height were recorded. For each chromatogram (Figs. 1 and 2) the response readings at 0.2 mm interval (17.6–22 mm for BZA, 42–47 mm for BZ and 54–59 mm for PCL) were recorded. The response data were processed using Microsoft Excel software. Derivative technique (D method) was first applied. For each compound, first (D1) and second (D2) derivative data at one distance interval (0.2 mm) were calculated for each concentration (D11 and D21). The derivative values
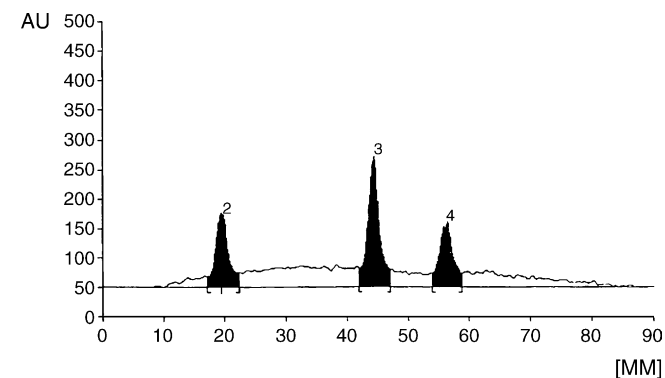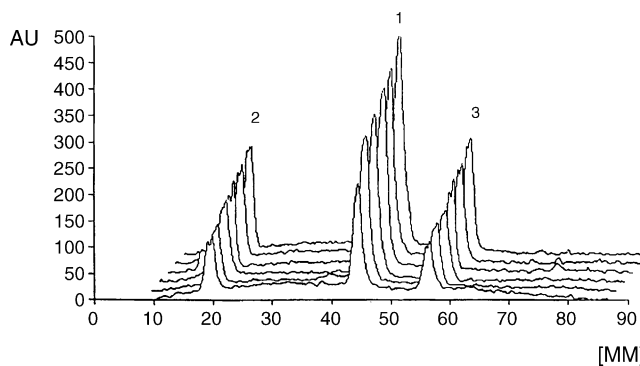

Fig. 2. TLC-chromatogram obtained from serial dilutions of bezafibrate (1) in the concentration range of 150–400 µg ml^{-1} bezafibric acid (2) and *p*-chlorobenzoic acid (3) in the concentration range of 20–120 µg ml^{-1} in their synthetic mixtures using ethyl acetate–methanol–glacial acetic acid (8:1.5:0.5) as mobile phase and UV-scanning at 230 nm.

(peak to peak) were measured at the corresponding distance range for each compound (Figs. 3–5).

By using the previously obtained first and second derivative data, the Fourier function coefficient, *t*, was calculated for each drug using the following equation expressed for eight equally spaced distance intervals as follows:

$$t = \frac{\{(0)D_0 + (+0.707)D_1 + (+1)D_2 + (+0.707)D_3 + (0)D_4 + (-0.707)D_5 + (-1)D_6 + (-0.707)D_7\}}{4}$$

(7)

where D_0 to D_7 stand for eight derivative values; at one distance interval;. The numbers between brackets are values of the selected Fourier function [22]. Thus 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 one (0.2 mm) and two (0.4 mm) distance intervals to get convoluted first derivative curves; D11/FF; and convoluted second derivative curves; D21/FF at one and two distance intervals. The convoluted derivative data (peak to peak) were measured at the corresponding distance range for each compound (Figs. 3–5).

4. Results and discussion

Being an amide, bezafibrate (III) would undergo acid-induced degradation to yield *p*-chlorobenzoic acid (PCL) (IV) and bezafibric acid (BZA) (V) (Scheme 1). The details of structural elucidation and characterization of the separated degradation products has been reported [23]. The development and optimization of the TLC chromatographic conditions for the separation of bezafibrate, bezafibric acid and *p*-chlorobenzoic acid has been discussed [23].

4.1. Application of peak area and peak height

For each of the three compounds, peak areas and peak heights of each concentration in the linearity range were recorded and correlated to the concentration (Tables 1–4).

Fig. 1. TLC-chromatogram of a synthetic mixture of 150 µg ml^{-1} bezafibrate acid (3) and 40 µg ml^{-1} bezafibric acid (2) and 40 µg ml^{-1} *p*-chlorobenzoic acid (4) using ethyl acetate–methanol–glacial acetic acid (8:1.5:0.5) as mobile phase and UV-scanning at 210 nm.
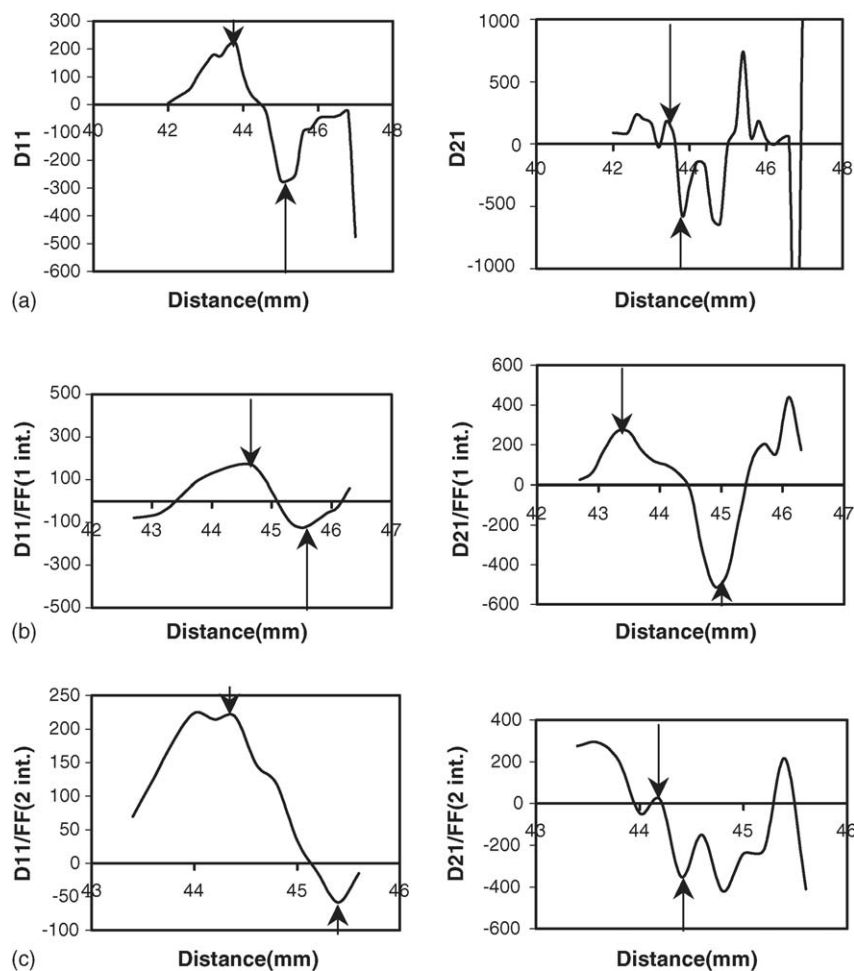
Fig. 3. Derivative curves of 200 μg ml^{-1} bezafibrate (a) and their corresponding convoluted Fourier functions curves at 1 interval (b) and at 2 interval (c).

4.2. Application of derivative technique (D method)

For each of the three compounds, first (D1) and second (D2) derivative of the peak response at one (0.2 mm) interval were calculated for each concentration. The derivative values (peak to peak) were measured at the corresponding distance range for each compound and correlated to the concentration (Tables 1–4).

Table 1
Parametric linear regression and statistical parameters for the determination of bezafibrate, bezafibric acid and *p*-chlorobenzoic acid in their synthetic mixtures by the proposed TLC-densitometric method

Type of linearity	Bezafibrate			Bezafibric acid			*p*-Chlorobenzoic acid		
	r^a	F^b	$S_{y/x}{}^c$	r^a	F^b	$S_{y/x}{}^c$	r^a	F^b	$S_{y/x}{}^c$
(1) Peak area	0.9977	530	222.5	0.9989	1430.6	139.5	0.9980	714.6	95.4
(2) Peak height	0.9903	203.9	12.7	0.9969	643.1	8.6	0.9976	835.2	7.85
(3) First derivative (D11)	0.9981	610.2	3.1	0.9989	1372.6	2	0.9976	818	2.95
(4) Second derivative (D21)	0.9985	665	2.3	0.9947	283.8	4	0.9987	754.2	3.6
(5) First derivative under Fourier functions (D11/FF)									
(a) D11/FF (1 int.)	0.9986	730.7	2.7	0.9878	80.8	18.4	0.9876	158.4	8.2
(b) D11/FF (2 int.)	0.9972	367.2	3.1	0.9995	3680.5	1.2	0.9973	758	5.4
(6) Second derivative under Fourier functions (D21/FF)									
(a) D21/FF (1 int.)	0.9981	549.9	5.8	0.9993	2167.6	9.8	0.9976	860.4	5.6
(b) D21/FF (2) int.)	0.9990	893.4	2.1	0.9998	10627.5	2.5	0.9993	2961.6	6.4

[a] Correlation coefficient.

[b] Variance ratio (*F*) is equal to the mean of squares due to regression divided by the mean of squares about regression (due to residuals).

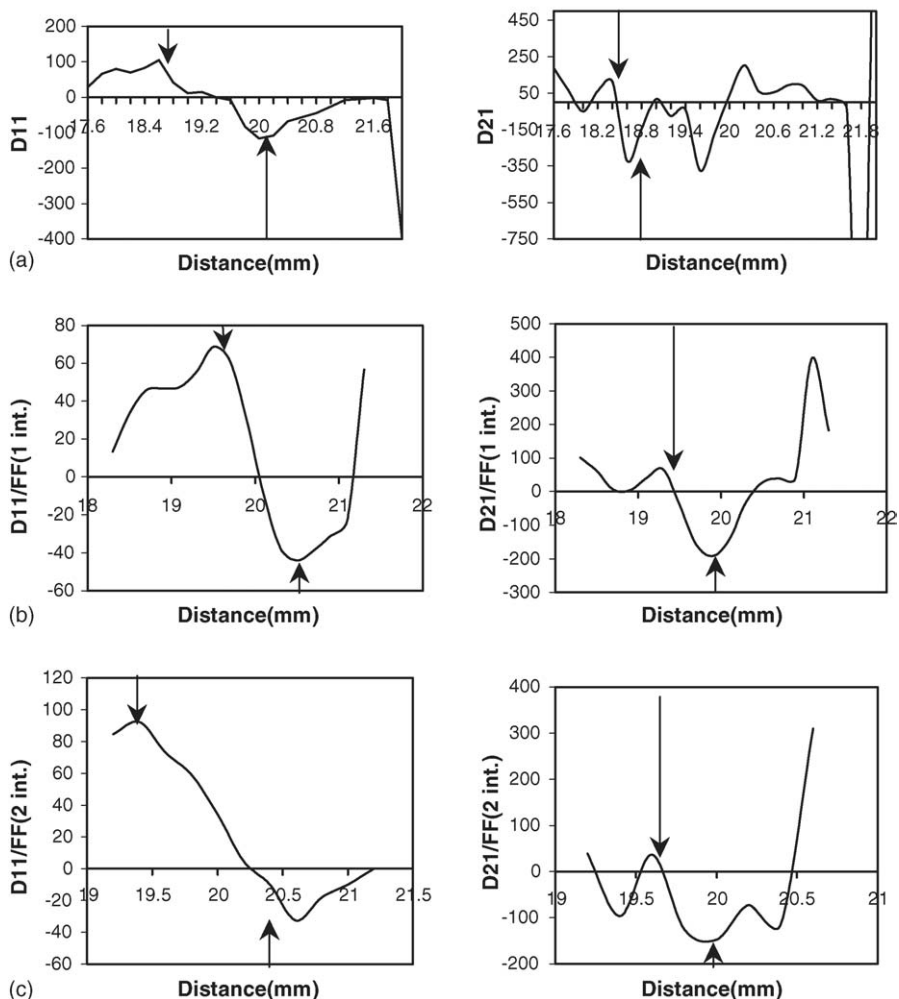[c] Standard error of estimate (standard deviation of residuals).

Fig. 4. Derivative curves of 40 μg ml^{-1} bezafibric acid (a) and their corresponding convoluted Fourier functions curves at 1 interval (b) and at 2 interval (c).

4.3. Application of Fourier functions to derivative data (D/FF) method

For each of the three compounds, the first and second derivative curves were convoluted using 8-points sin x_i polynomials at one and at two distance intervals, then the optimum convoluted D11/FF and D21/FF values selected for each compound were correlated to concentration (Tables 1–4).

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 interferences producing high degree of purity of chromatographic peaks. This would be beneficial in case of interferences arising from background noise as in the TLC-densitometric measurements.

4.4. Methods validation

4.4.1. Calibration graphs and statistical data

Under the previously described conditions, the graphs obtained by plotting derivative and convoluted derivative data versus concentration for each of the three compounds, show var-

ious degrees of linearity and were compared to those obtained using the peak area and peak height as the response signals. Using the method of least squares, regression equations, correlation coefficients (r) and standard errors of estimate ($S_{y/x}$) were calculated. Variance ratios (F-values) were also determined (Tables 1 and 2).

The value of the correlation coefficient (r) indicates the degree of goodness of fitting of the calibration graph. Standard deviation of residuals ($S_{y/x}$) is also called standard error of estimate as it estimates random errors in the y-direction. ($S_{y/x}$) is a measure of the extent of deviation of the found (measured) y-values from the calculated ones. The smaller the ($S_{y/x}$), the closer the points are to the linear regression line. It was noticed that regression lines with high (r) values showed low ($S_{y/x}$) values.

For equal degrees of freedom, increase in the variance ratio (F-values) means increase in the mean of squares due to regression (regression) and decrease in the mean of squares about regression (residuals). The greater the mean of squares due to regression, the more the steepness of the regression line. The smaller the mean of squares about regression, the less the scatter of the experimental points around the regression line. Consequently, regression lines with high F-values (low significance
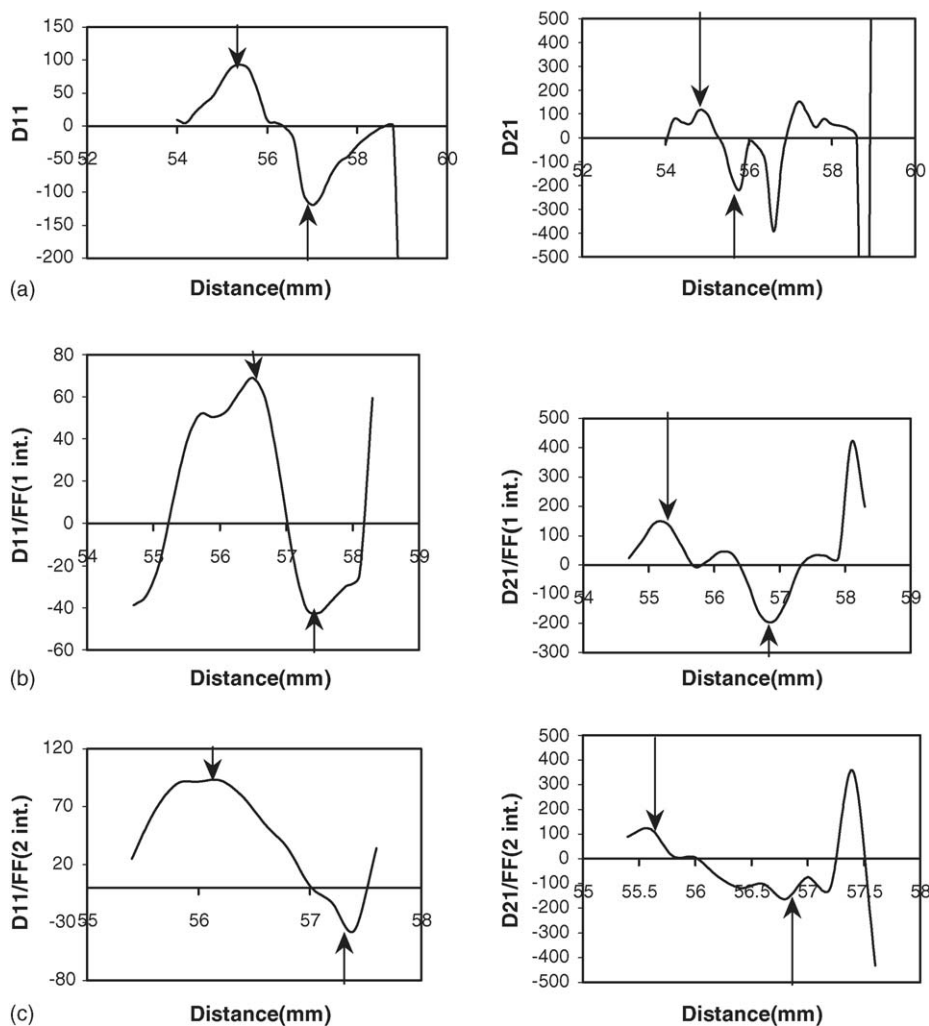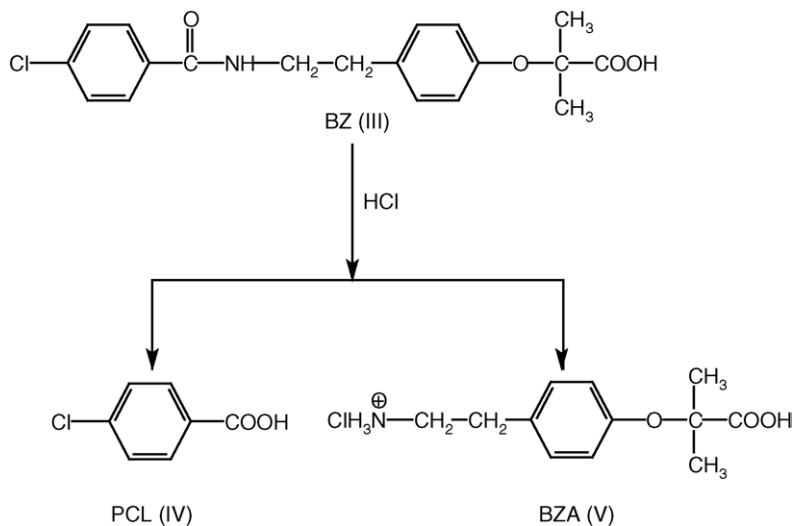
Fig. 5. Derivative curves of 40 μg ml^{-1} p-chlorobenzoic acid (a) and their corresponding convoluted Fourier functions curves at 1 interval (b) and at 2 interval (c).

Scheme 1. Acid-induced degradation of bezafibrate.

Table 2

Comparison between parametric (P) and non-parametric (NP) regression models for the determination of bezafibrate by the proposed TLC-densitometric method

	Bezafibrate				Percentage of change in $|a|$[c]	Percentage of change in $|b|$[d]
	$|a|$[a]		$|b|$[b]			
	P	NP	P	NP		
Direct measurement						
Peak area	462	237	31.5	30.4	−48.7	−3.5
Peak height	27	14	0.87	0.82	−48.1	−5.7
Derivative technique (D method)						
First derivative (D11)						
D11	14.9	14.3	0.34	0.35	−4.02	2.9
Second derivative (D21)						
D21	8.7	4	0.41	0.4	−54.02	−2.4
Derivative under Fourier functions (D/FF method)						
First derivative under Fourier functions (D11/FF)						
D11/FF (1int)	22.6	14	0.89	0.84	−38.08	−5.6
D11/FF (2int)	12.9	11.4	0.54	0.54	−11.62	0
Second derivative under Fourier functions (D21/FF)						
D21/FF (1int)	38.1	30.5	1.23	1.26	−19.9	2.4
D21/FF (2int)	27.4	24	2.5	2.65	−12.4	6

[a] Modulus of intercept.
[b] Modulus of slope.
[c] Percentage of change in $|a|$ means percentage of change in $|a|$ of NP vs. $|a|$ of $P = [(|a|$ of NP $- |a|$ of P$)/|a|$ of P$] \times 100$.
[d] Percentage of change in $|b|$ means percentage of change in $|b|$ of NP vs. $|b|$ of $P = [(|b|$ of NP $- |b|$ of P$)/|b|$ of P$]$.

F) are much better than those with lower ones. Good regression lines show high values for both (r) and (F-values) [25].

Concerning all the previous regression and statistical parameters, regression lines obtained using different types of linearities were compared for the three investigated compounds. Derivative values (D) produced better results than either peak area or peak height. However, convoluted derivative values (D/FF) showed

the best regression lines especially for convoluted derivative values at two intervals, which gave high (r) values up to 0.9998 (Table 1).

4.4.2. Application of non-parametric regression methods

In parametric (P) statistics, the arithmetic mean or average was used as the "measure of central tendency" or "measure of

Table 3

Comparison between parametric (P) and non-parametric (NP) regression models for the determination of bezafibric acid by the proposed TLC-densitometric method

	Bezafibric acid				Percentage of change in $|a|$[c]	Percentage of change in $|b|$[d]
	$|a|$[a]		$b|$[b]			
	P	NP	P	NP		
Direct measurement						
Peak area	80.2	209	103	101	161.25	1.94
Peak height	9	9.6	2.61	2.77	6.6	1.5
Derivative technique (D method)						
First derivative (D11)						
D11	1.2	1.2	1.21	1.21	0	0
Second derivative (D21)						
D21	1.8	0.2	1.07	1.09	−88.8	1.8
Derivative under Fourier functions (D/FF method)						
First derivative under Fourier functions (D11/FF)						
D11/FF (1int)	14.5	4.1	3.7	3.91	−71.7	5.6
D11/FF (2int)	2	1.1	1.16	1.15		
Second derivative under Fourier functions (D21/FF)						
D21/FF (1int)	12	12	7.24	7.24	0	0
D21/FF (2int)	0.5	0	3.12	3.12	−100	0

[a] Modulus of intercept.
[b] Modulus of slope.
[c] Percentage of change in $|a|$ means percentage of change in $|a|$ of NP vs. $|a|$ of $P = [(|a|$ of NP $- |a|$ of P$)/|a|$ of P$] \times 100$.
[d] Percentage of change in $|b|$ means percentage of change in $|b|$ of NP vs. $|b|$ of $P = [(|b|$ of NP $- |b|$ of P$)/|b|$ of P$]$.

Table 4
Comparison between parametric (P) and non-parametric (NP) regression models for the determination of *p*-chlorobenzoic acid by the proposed TLC-densitometric method

	p-Chlorobenzoic acid				Percentage of change in $\|a\|$[c]	Percentage of change in $\|b\|$[d]
	$\|a\|$[a]		$\|b\|$[b]			
	P	NP	P	NP		
Direct measurement						
Peak area	87	139	129	130	59.8	0.78
Peak height	5.5	7.5	2.71	2.74	36.36	1.1
Derivative technique (D method)						
First derivative (D11)						
D11	0.73	0.5	1.01	1.03	−31.5	1.98
Second derivative (D21)						
D21	2.3	0.8	1.24	1.26	−65	1.6
Derivative under Fourier functions (D/FF method)						
First derivative under Fourier functions (D11/FF)						
D11/FF (1int)	42.3	41	1.24	1.27	−3	2.4
D11/FF (2int)	7.7	7.5	1.78	1.78		
Second derivative under Fourier functions (D21/FF)						
D21/FF (1int)	3.44	3.1	1.97	1.97	−9.9	0
D21/FF (2int)	2.4	2	4.17	4.12	−16.6	−1.2

[a] Modulus of intercept.
[b] Modulus of slope.
[c] Percentage of change in $\|a\|$ means percentage of change in $\|a\|$ of NP vs. $\|a\|$ of $P = [(\|a\|$ of NP $- \|a\|$ of P$)/\|a\|$ of P$] \times 100$.
[d] Percentage of change in $\|b\|$ means percentage of change in $\|b\|$ of NP vs. $\|b\|$ of $P = [(\|b\|$ of NP $- \|b\|$ of P$)/\|b\|$ of P$]$.

location". This is logic enough when "symmetrical" normal distribution is assumed, but in non-parametric (NP) statistics, the median is usually used instead [20].

Determining the median of a set of experimental results usually requires little or no calculation. Moreover, in many cases it may be a more realistic measure of central tendency than the arithmetic mean.

Concerning the linear regression methods, the assumption of normally distributed *y*-direction errors was emphasized, and the complexity of some of the calculation methods was apparent. This complexity is overcome by using modern calculators or personal computers, and there are some rapid approximation methods for fitting straight lines to experimental data. There is still an interest to non-parametric approaches to fitting a straight line to a set of points. Of the several methods available, perhaps the simplest is Theil's "incomplete" method that was applied to our data [20].

Theil's method determines the slope of a regression line as the median of the slopes calculated from selected pairs of points, and the intercept of the line is the median of the intercept values calculated from the slopes and the coordinates of the individual points [20].

For all of the previously mentioned types of linearities the 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 2–4).

Theil's method has three distinct advantages over the parametric (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
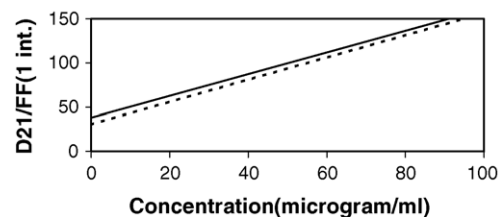

Fig. 6. Calibration graphs calculated by Theil's method (...) and by the least squares method (—) for the determination of bezafibrate using D21/FF (at 1 interval) method.

third, it is not affected by the presence of outlying 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 [16,20] (Figs. 6–8) show examples illustrating these facts. Since regression methods that
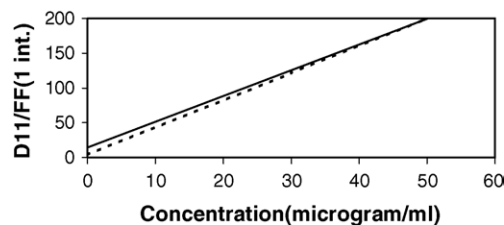

Fig. 7. Calibration graphs calculated by Theil's method (...) and by the least squares method (—) for the determination of bezafibric acid using D11/FF (at 1 interval) method.
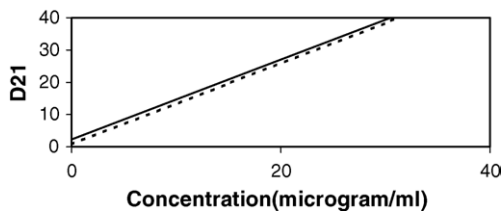
Fig. 8. Calibration graphs calculated by Theil's method (. . .) and by the least squares method (—) for the determination of *p*-chlorobenzoic acid using D21 method.

are relatively unaffected by outlying data are necessary to provide unbiased estimates of the slope for data conforming to the straight-line function, the non-parametric "Theil's" method is highly beneficial in this respect, and is said to be a "robust" method [20].

By calculating the percentage change in intercept and slope values obtained using both the least squares and Theil's method, it was found that the slope remains almost unchanged but in most cases the value of the intercept was less for Theil's method in comparison to that obtained using the least squares method (Tables 2–4).

5. General conclusion

Chemometric techniques could be successfully applied for handling complex chromatograms. This is highly needed in cases where sources of interferences could affect the chromatographic response, e.g., background noise in TLC-densitometric measurements. Derivative treatment of the chromatographic response data followed by application of Fourier functions on the resulting derivative data gives improved quantitation of the chromatographic signals.

Non-parametric (NP) regression of the chromatographic response data using Theil's method is highly advantageous over the usual least squares method. Theil's method could be used in cases where there are both *x*- and *y*-direction errors assuming that the errors are not normally distributed. It also has effectively circumvented the outlier problem.

References

[1] K. Kavianpour, R.G. Brereton, Analyst (London) 123 (1998) 2035–2042.
[2] K.D. Zissis, S. Dunkerley, R.G. Brereton, Analyst (London) 124 (1999) 971–979.
[3] H.J.G. Debets, A.W. Wijnsma, D.A. Doornbos, H.C. Smit, Anal. Chim. Acta 171 (1985) 33–43.
[4] J. Verdu-Andres, R. Herraez-Hernandez, P. Campins-Falco, J. Chromatogr. A. 930 (2001) 95–107.
[5] R.G. Brereton, S. Dunkerley, Analyst (London) 124 (1999) 705–711.
[6] F. Gan, X.N. Li, Y.Z. Liang, Fenxi – Huaxue 28 (2000) 833–836.
[7] A. Detroyer, V. Schoonjans, F. Questier, Y. Vander-Heyden, A.P. Borosy, Q. Guo, D.L. Massart, J. Chromatogr. A. 897 (2000) 23–36.
[8] W.J. Welsh, W. Lin, S.H. Tersigni, E. Collantes, R. Duta, M.S. Carey, W.L. Zielinski, J. Brower, J.A. Spencer, T.P. Layloff, Anal. Chem. 68 (1996) 3473–3482.
[9] Y. Kurtulmus, T. Tanyalcin, G. Bozkaya, O. Gunduz, O. Cerci, F.Z. Kutay, B. Ersoz, Accred. Qual. Assur. 6 (2001) 424–426.
[10] L. Paksy, Mikrochim. Acta 123 (1996) 197–205.
[11] G.E. Rayment, R.O. Miller, E. Sullaeman, Commun. Soil. Sci. Plant. Anal. 31 (2000) 1513–1530.
[12] G. Lippi, C. Brentegani, C. Mazzi, C. Recchi, O. Ruzzenente, G. Guidi, Euro. J. Clin. Chem. Clin. Biochem. 35 (1997) 877–880.
[13] M.O. Moen, K.J. Griffin, A.H. Kalantar, Anal. Chim. Acta 277 (1993) 477–487.
[14] J.S.O. Odonde, J. Autom. Chem. 14 (1992) 25–27.
[15] M.A. Korany, A.M. Wahbi, S. Mandour, IL. Pharmaco. 39 (1984) 243–252.
[16] M.A. Korany, A.M. Wahbi, S. Mandour, M.A. El-Sayed, Anal. Lett. 18 (1985) 21–34.
[17] A. El-Gindy, M.A. Korany, M.M. Bedair, J. Pharm. Biomed. Anal. 113 (1998) 1357–1370.
[18] K. Kovacs-Hadady, I.T. Kiss, M. Kiss, K. Barnakatona, Analyst (London) 113 (1988) 569–572.
[19] M.A. Korany, O.T. Fahmy, H. Mahgoub, H.M. Maher, Talanta 66 (2005) 1073–1087.
[20] J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, fourth ed., Prentice Hall, 2000, pp. 170–172.
[21] A.M. Wahbi, H. Abdine, M.A. Korany, Pharmazie 33 (1978) 278–282.
[22] I.M. Dubrovkin, Izv. Sev-Kavk.Nauchn.Tsentra Vyssh.Shk., Estestv. Nauki 1 (1981) 57.
[23] I.I. Hewala, M.A. Korany, K.M. Abdel-Hai, Anal. Chim. Acta, in press.
[24] M.A. Korany, M.A. El-Sayed, M.M. Bedair, H. Mahgoub, Talanta 37 (1990) 1183–1188.
[25] P. Armitage, G. Berry, Statistical Methods in Medical Research, third ed., Blackwell Scientific Publications, 1994, pp. 283–285.