



SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF FEBUXOSTAT AND DICLOFENAC IN PHARMACEUTICAL DOSAGE USING CHEMOMETRIC METHODS

Ibrahim H.I. Habib¹, Mohamed Rizk², Maha Mahmoud Abou El-Alamin²,
Ghada Sayed Imam^{3*}

¹Microanalytical Chemistry Laboratory, Applied Organic Chemistry Department, National Research Centre, 33 El-Bohouth Str., Dokki, Giza, Egypt, P.O. 12622.

²Analytical Chemistry Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

³National Nutrition Institute, 16 Kasr Al Aini St., Cairo, Egypt.

* Corresponding Author: Dr. Ghada Sayed Imam

National Nutrition Institute, 16 Kasr Al Aini St., Cairo, Egypt.

Article Received on 15/04/2016

Article Revised on 05/05/2016

Article Accepted on 25/05/2016

ABSTRACT

Simultaneous spectrophotometric determination of Febuxostat (FEB) and Diclofenac potassium (DIC) in pharmaceutical dosage using four different chemometric techniques, Iterative Target Transformation Factor Analysis (ITTFA), Classical Least Squares (CLS), Principal Component Regression (PCR) and Partial Least-Squares (PLS-1). In these techniques, the concentration data matrix was prepared according to the full factorial experimental design of three levels and two components in mixtures and using 2.5 mM NaOH solution as solvent. The corresponding absorbance data matrix was measured over the wavelength range of 220 - 400 nm with the intervals of $\Delta\lambda = 1$ nm, then regression was obtained by using the concentration data matrix and absorbance data matrix for the prediction of the unknown concentrations of FEB and DIC in their mixture. The procedure did not require any chemical separation step or prior graphical treatment of the overlapped spectra. The calibration range was found linear over 2-10 and 2.5-25 $\mu\text{g/mL}$ for FEB and DIC, respectively and the multivariate methods were validated on both authentic mixtures and pharmaceutical preparations. The precision and accuracy of the methods were assessed and compared with each other.

KEYWORDS: Febuxostat, Diclofenac potassium, Iterative Target Transformation Factor Analysis, Classical Least Squares, Principal Component Regression, Partial Least-Squares.

1. INTRODUCTION

Febuxostat (FEB), [2-[3-cyano-4-(2-methylpropoxy)phenyl]-4-methylthiazole-5-carboxylic acid] (Figure 1a) is a non-purine xanthine oxidase inhibitor used as a potential alternative to allopurinol in the treatment of hyperuricaemia with chronic gout with molecular weight of 316.38.^[1, 2] It is a non-hygroscopic, white crystalline powder that is soluble in dimethylformamide and dimethylsulfoxide; slightly soluble in acetonitrile and methanol; sparingly soluble in ethanol and practically insoluble in water.^[3, 4] Recent methods for the determination of FEB in single formulation include spectrophotometric^[5, 6] and chromatographic^[7-9] techniques.

Diclofenac potassium (DIC), Potassium [2-[(2,6-dichlorophenyl)amino]phenyl]acetate (Figure 1b) is a nonsteroidal anti-inflammatory drug (NSAID) applied or taken to reduce inflammation and as an analgesic for reducing pain in certain conditions. It is white or

slightly yellowish, slightly hygroscopic and crystalline powder that is sparingly soluble in water; freely soluble in methanol; soluble in ethanol (96%) and slightly soluble in acetone.^[10, 11] Numerous analytical methods include the spectrophotometric^[12-14], spectrofluorimetric^[15], potentiometric^[16, 17], voltammetric^[18, 19] and chromatographic^[20-22] techniques could be found in the literature.

Febuxostat is usually accompanied with diclofenac potassium in pharmaceutical dosage form. Literature review revealed that the two cited components were simultaneously determined in mixture either by HPLC^[23-27] and spectrophotometric methods using simultaneous equation, absorbance ratio^[28-30], dual wavelength^[31] and first derivative methods.^[29, 32] To our knowledge, the simultaneous determination of both drugs using chemometric techniques has not been reported. Main advantages of these techniques are the simultaneous analysis of the mixture components with strongly

overlapping spectra without graphical pre-treatment such as derivative and ratio spectra derivative. They also require shorter time, less costs and the errors of calibration model are minimized by measuring the absorbance values at many points in the wavelength range of the zero-order spectra.

On the other hand, Pandey et al.^[4] demonstrated FEB is slightly soluble in methanol and acetonitrile and because of methanol being expensive and volatile causing spectral drift and consequently high bias in accuracy, diluted NaOH solution is used instead as solvent which gave more accurate, low cost and reduced the solubility of other possible inactive ingredients such as magnesium stearate, talc, avcyl pH 101 and hydroxyl propyl methyl cellulose.

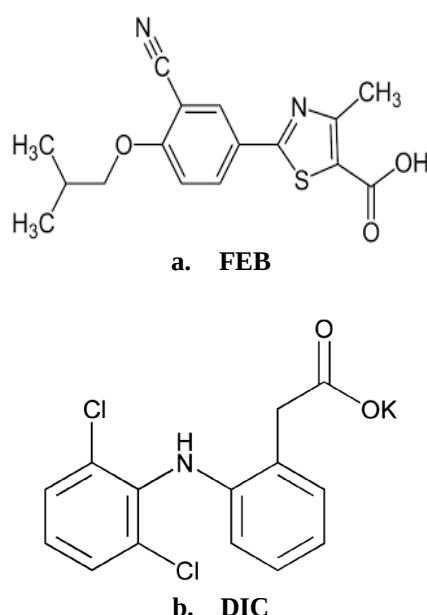


Fig 1: Chemical structures of febuxostat (FEB) and diclofenac potassium (DIC).

2. EXPERIMENTAL

2.1 Apparatus

A JASCO V-570 double beam UV-VIS spectrophotometer with a quartz cuvette, 10-mm path length at scan speed of 1200 nm/min and fixed slit width of 2 nm was used for measuring the light absorption in ultra violet region (200-400 nm) and connected to a computer loaded with Spectra Manager Program (JASCO) used for the spectral acquisition and elaboration of the data obtained in ASCII format to subsequent manipulation by Multivariate Analysis Program Add-in Microsoft Excel written in Macros according to algorithms described by Haaland, Martens and Geladi^[33-35] for CLS, PCR, PLS methods and Gemperline^[36] for ITTFA method.

2.2 Materials and reagents

Pharmaceutical standards of Febuxostat "FEB" (EVA PHARMA, Egypt) and diclofenac potassium "DIC" (Adwia Pharmaceuticals, 10th Ramadan, Egypt), which

were certified to contain 98.3 and 99.8% (w/w), respectively. The combined dosage form was prepared resembling to the formulation sold in India under trade name of XANFEB DSR tablet (Indoco Remedies Ltd., India) containing 40 mg FEB and 100 mg DIC. Sodium hydroxide (Merck, Darmstadt, Germany) and double distilled water were used.

2.3 Standard solutions and Calibration curves

Stock solutions were prepared by dissolving 25 mg of FEB and 20 mg of DIC in 5 ml 0.05 M NaOH and then completed to 100 ml with distilled water to obtain finally a concentration of 250 µg/mL and 200 µg/mL, respectively. To a series of 10-ml volumetric flasks, aliquots of FEB and DIC solutions were added and then diluted to 10 ml with 2.5 mM NaOH. Four sets of standard solutions were prepared, two series of individual solutions containing FEB 2-10 µg/ml and DIC 2.5-25 µg/ml and two series of 9 mixtures. The composition of the 9 mixtures were prepared twice according to the full factorial experimental design (L^n) of three levels of concentrations and two components as summarized in Table 1. The first one of mixture data set will be used for calibration and another for validation method. UV spectra of the solutions were then recorded in the range of 200-400 nm against a blank of 2.5 mM NaOH solution.

2.4 Pharmaceutical sample preparation

Owing to unavailability of tablets containing this combined formula in local market, powders of 40 mg of FEB, 100 mg of DIC, 1.5 mg magnesium stearate, 1.5 mg talc, 67 mg avcyl pH 101 and 90 mg hydroxyl propyl methyl cellulose (equivalent to that formulated in XANFEB DSR tablets, India), were weighted and transferred to a 100 ml beaker, then 5 ml of 0.05 M NaOH solution was added and the solution was shaken vigorously for 15 minutes and decanted into a 100 ml volumetric flask and completed to the mark with washings of distilled water. The solution was then filtered through the Whatman filter paper no.41. Three different aliquots of the filtrate as 50, 100, 200 µL were transferred into three 10-ml volumetric flasks and then diluted to the mark with 2.5 mM NaOH. UV spectra of the solutions were then recorded in the range of 200-400 nm against a blank of 2.5 mM NaOH solution. The reproducibility of the method was studied by performing the assay on the same day (intra-day precision) and on three different days (inter day precision).

3. RESULTS AND DISCUSSION

As shown in Figure 2, the absorption spectra of FEB and DIC in aqueous 2.5 mM NaOH solutions were severely overlapped obstructing the resolution of this binary mixture to direct absorbance measurements. Such a determination could be theoretically facilitated by the use of chemometric methods. The spectra of FEB and DIC scanned over the wavelength range 220–400 nm with 1 nm intervals were selected for chemometric analysis. Wavelengths below 220 nm were rejected due to the

noise appeared on replicating the same sample. It was noted experimentally that using 2.5 mM sodium hydroxide solution as solvent for FEB and DIC gave better reproducible and stable spectra than traditional solvent of methanol.

With the aim of improving the recovery of these compounds, four different chemometric approaches were evaluated, namely classical least squares (CLS), partial least squares regression (PLS), principle component regression (PCR) and iterative target transformation factor analysis (ITTFA). Haaland and Thomas^[33] made a comparison of the different multivariate calibration methods for quantitative spectral analysis. They concluded that it was very difficult to generalize about the superiority of one method over each other, because their relative performance is often dependent on the particular data set to analyze. In the calibration step, two types of calibration data sets were tested which is either individually or in mixture as demonstrated in Table 1. The results were found significant difference between these two data sets and between chemometric methods as demonstrated later. The theory and application of the chemometrics in spectroscopy have been discussed by several workers^[33-36] and here we describe them briefly.

Table 1 Composition of FEB and DIC in mixtures and individual solutions

#	$\mu\text{g/ml}$			
	Mixture solutions		Individual solutions	
	DIC	FEB	DIC	FEB
1	5	2	2.5	2
2	12.5	2	5	3
3	20	2	7.5	4
4	5	5	10	5
5	12.5	5	12.5	6
6	20	5	15	7
7	5	8	17.5	8
8	12.5	8	20	9
9	20	8	22.5	10
10	-	-	25	-

Classical Least Squares (CLS)

This method assumed Beer's law model with the absorbance at each wavelength being proportional to the component concentration.

$$A = KC + E$$

The training set (calibration set) of absorptivity was used for constructing CLS model or (K) matrix with dimension 2 components at 180 different wavelengths. The CLS method requires that all the components in the calibration samples must be known. The absorbance matrix A of the calibration samples of mixtures (9x180) and their corresponding concentration matrix C (9x2) were used to find the absorptivity matrix (K-matrix) by the following equation:

$$K = (C^t \cdot C)^{-1} \cdot C^t \cdot A$$

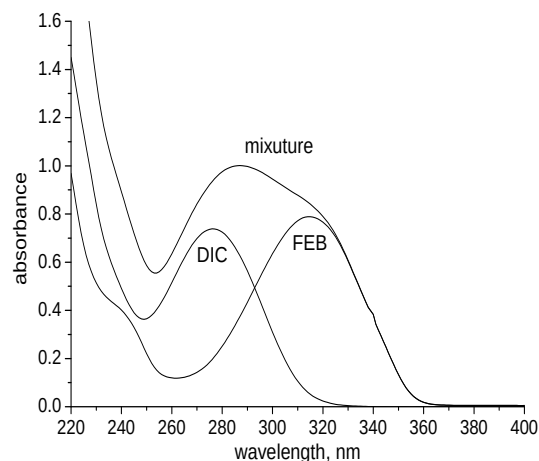


Fig 2. Overlapped Absorption spectra of 10µg Febuxostat and 25µg Diclofenac

Then, the obtained K-matrix was further used for the prediction of unknown concentration C_u of the two components in both the validation and pharmaceutical formulation samples:

$$C_u = (K \cdot K^t)^{-1} \cdot K \cdot A_u$$

Where A_u is spectra matrix of the unknown samples.

Principal Component Regression (PCR) and Partial Least Squares (PLS)

PCR and PLS methods could be used to determine the components under investigation even in the presence of unknown components (interfering substance) which gave these two methods an advantage over CLS.^[37] Unlike CLS method based on direct regression of the concentrations onto the spectroscopic responses, the PCR method consists of two steps; in the first step, it does decomposition of spectral matrix A either by principal component analysis PCA or by singular value decomposition SVD methods into a minimum number of linear independent products of two smaller matrices of what is so called principal component scores (eigenvalues) and loadings (eigenvectors) and then regress the obtained scores against the concentrations as a calibration step^[35] PLS resembles PCR, but the PLS method decomposes simultaneously the spectroscopic data matrix A and the chemical data matrix C into a minimum number of eigenvectors and their eigenvalues containing the most relevant information in A and C.^[34, 35]

The main advantage arise from this decomposition is that each eigenvalue represents the relative importance of the associated eigenvector. A large eigenvalue indicates a major factor and contains meaningful information, whereas a very small eigenvalue indicates an unimportant factor containing mainly noise and can be deleted. This minimum number of eigenvectors/eigenvalues was called factor number. The remaining eigenvectors were the result of experimental

error. If the factor number was greater or smaller than the true one, i.e. either data was deficiently or excessively introduced, under fitting or over fitting concentration data was risked. The regression coefficients K was obtained by regression the optimal number of chemical eigenvectors on the spectral eigenvectors and then used in the prediction step to estimate unknown concentrations of a mixture.

In order to evaluate the factor number without under/over fitting the concentration data, the raw data of the calibration samples were mean centered^[38] as a preprocessing step (the centering makes the following computations numerically well conditioning, i.e. no collinearity, low noise and no constant background) and then the cross validation method^[33], leaving out one sample at a time and the prediction error sum of squares (PRESS) of concentrations was calculated which was a measure of the efficiency for a calibration fit model and the optimum number of factors would be the number that yielded the minimum PRESS.

$$\text{PRESS} = \sum_{i=1}^m (\hat{c}_i - c_i)^2$$

Where $\hat{c}_i$ is the calculated concentration and c_i is the actual concentration for the i^{th} sample left out of the calibration during cross validation. Based on spectral data and their corresponding concentration data of 9 mixture samples, 2 factors were obtained as optima values for determining FEB and DIC components as shown in Figure 3.

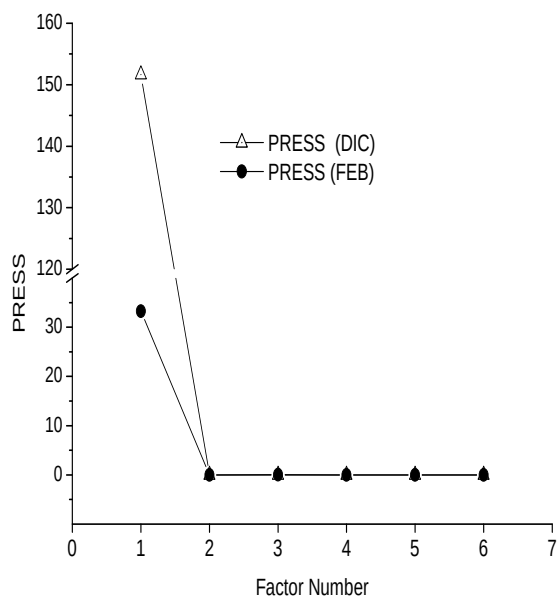


Fig3. PRESS plot of the cross validation results of the calibration set as a function of the number of factors used to construct the PLS or PCR models.

Iterative Target Transformation Factor Analysis (ITTFA)

The spectral data matrix A was first decomposed by principal component analysis PCA or by singular value decomposition SVD into minimum number of two independent matrices of scores T and P loadings. Target transformation matrix R is computed from projection of T matrix onto concentrations of unknowns which were initialized with best guesses and these were then refined by iterative calculations of the target transform.

$$R = (T \cdot T^t)^{-1} \cdot T \cdot c^o$$

$$c' = T \cdot R$$

The iteration was repeated until convergence $\|c' - c^o\|$ was reached. Different from classical factor analysis technique^[39], in ITTFA the absorbance spectrum data of the unknown as well as those of the reference samples were used for calibration. Moreover, the iterative techniques were employed for prediction and better results can generally be obtained.^[36]

3.1 Validation of training set

Two data sets for the calibration either of individual or mixture solutions were applied to identify which the set will give more accurate and precise on determining the two components in their mixtures. As depicted in Table 2, using CLS method, DIC gave higher accurate and precise results by mixture data set than individual data set while no significant difference was observed between the two data sets on determining FEB. This was assessed by the comparative statistics of t - and F -tests to compare between two unpaired groups (independent) with unequal variances.^[40] It was clear that calculated t value for unpaired two-tailed data distribution of DIC higher than the tabulated t value ($t_{cal} = 2.929 > t_{tab} = 2.201$). This suggested that at 95% confidence level differences in accuracy between the recoveries obtained by the two data sets were statistically significant and it was preferably to use mixture data set as training set. This was confirmed by the F -test which revealed that there was significant difference between the precision of the two data sets. The calculated value of F was higher than the critical value ($F_{cal} = 5.282 > F_{8,8} = 3.483$).

The confidence interval CI revealed that at 95% confidence, the source of error was likely systematic on determining DIC and FEB by individual data set and it was random by mixture data set. Thus, the proposed mixture training set can be confidentially applied to real samples and using other chemometric techniques as given in table 3.

3.2 Validation of chemometric methods

After optimization of parameters and calibration (training) step, all models of CLS, ITTFA, PCR and PLS were applied for analysis of FEB and DIC in individual samples (Table 3) and in mixture samples (Table 4). The accuracy expressed by bias (relative error RE, %),

recoveries, and precision expressed by relative standard deviation (RSD, %) values were summarized.

It was clear from Tables 3 and 4 that PCR and PLS had superiority over CLS and ITTFA in the analysis of individuals and mixtures as indicated by best precision

and accuracy. The proposed method PLS1 was then applied for the simultaneous determination of the two components in formulation samples as given in Table 5.

Table 2 Results obtained by CLS for determining DIC and FEB using either individual or mixture data sets as training set.

Taken, µg/ml		Found, %			
		Individual data set		Mixture data set	
DIC	FEB	DIC	FEB	DIC	FEB
5	2	120.75	106.88	105.55	106.72
12.5	2	106.03	106.29	101.55	104.16
20	2	104.44	102.84	100.06	98.58
5	5	108.53	101.70	103.44	102.54
12.5	5	105.37	102.77	100.76	102.79
20	5	104.78	100.00	100.30	99.12
5	8	108.14	98.26	102.72	99.29
12.5	8	103.35	99.09	98.69	99.61
20	8	102.36	98.77	97.89	98.77
Accuracy (bias), RE %		7.08	1.84	1.22	1.29
Precision RSD, %		5.51	3.17	2.40	2.89
Confidence interval CI at $\alpha=0.05$ and $n=9$		2.48	1.42	1.28	1.30
t -test = 2.201 at $\alpha=0.05$ and $df=11$		2.929	0.391		
F -test = 3.438 at $\alpha=0.05$ and $df=8$		5.282	1.203		

Table 3 Recoveries of DIC and FEB individually obtained by different chemometric methods

		taken	found, %			
		ug/ml	CLS	ITTFA	PCR	PLS
DIC		2.5	93.91	93.93	97.13	101.03
		5	98.21	98.22	99.56	97.26
		7.5	98.73	98.74	100.51	98.18
		10	103.11	103.12	102.27	100.03
		12.5	100.52	100.51	98.29	99.14
		15	96.05	96.05	95.81	97.20
		17.5	98.57	98.57	98.99	97.61
		20	94.71	94.71	96.97	100.41
		22.5	94.25	94.25	95.86	101.12
		25	93.88	93.88	99.39	99.81
Accuracy (bias), %			-2.81	-2.80	-1.52	-0.82
Precision (RSD), %			3.150576	3.148226	2.079985	1.521781
Confidence interval at $\alpha=0.05$, $n=10$			1.95	1.95	1.29	0.94
FEB		2	110.12	110.13	102.46	99.37
		3	100.92	100.92	101.01	98.09
		4	103.98	103.98	102.54	99.83
		5	102.04	102.03	102.13	98.99
		6	100.12	100.11	100.30	98.02
		7	100.65	100.64	101.09	99.19
		8	101.43	101.43	101.33	100.57
		9	102.36	102.36	101.83	101.98
		10	100.62	100.62	100.36	100.40
Accuracy (bias), %			2.47	2.47	1.45	-0.40
Precision (RSD), %			3.097	3.101	0.842	1.259
Confidence interval at $\alpha=0.05$, $n=9$			2.02	2.03	0.55	0.82

Table 4 Recoveries of DIC and FEB in mixtures obtained by different chemometric techniques

Taken, µg/ml		Found, %							
		CLS		ITTFA		PCR		PLS1	
DIC	FEB	DIC	FEB	DIC	FEB	DIC	FEB	DIC	FEB
5	2	105.55	106.72	105.55	106.74	101.96	99.11	99.76	99.87
12.5	2	101.55	104.16	101.54	104.17	99.63	101.52	99.82	99.50
20	2	100.06	98.58	100.06	98.58	99.93	98.37	100.12	97.04
5	5	103.44	102.54	103.42	102.55	96.85	101.04	99.37	100.65
12.5	5	100.76	102.79	100.76	102.78	100.61	102.69	100.41	102.30
20	5	100.30	99.12	100.30	99.12	99.92	98.79	100.01	100.10
5	8	102.72	99.29	102.71	99.29	100.55	98.95	100.85	98.94
12.5	8	98.69	99.61	98.69	99.61	100.14	100.11	99.80	100.07
20	8	97.89	98.77	97.89	98.76	100.04	99.97	99.86	99.99
Accuracy (bias), %		1.22	1.29	1.22	1.29	-0.04	0.06	-0.01	-0.17
Precision (RSD), %		2.397	2.889	2.392	2.896	1.349	1.441	0.424	1.398
Confidence interval at $\alpha=0.05$, n=9		1.078	1.299	1.076	1.302	0.607	0.648	0.191	0.629

The repeatability of the PLS method was determined by performing the assay 3 replicates of three different concentrations on the same day (intra-day precision) while the intermediate precision was determined the same but over three different days (inter day precision). Both intra- and inter-day RSD range from 0.45-1.35%

for all analytes, confirming good precision (Table 5). It is clear also from the table 5 that the bias is more than the confidence interval "CI" at 95% confidence, indicating that the source of error is likely systematic, i.e. the content assay was deviated from the true value by about $\pm 1\%$ on determining both DIC and FEB.

Table 5 Recoveries of DIC and FEB in dosage form obtained by PLS on the same day and on the three successive days

Taken, µg/ml		found, Mean $\pm$ SD, µg/ml			
		Intra-days ¹		Inter-days ²	
DIC	FEB	DIC	FEB	DIC	FEB
5	2	5.08 $\pm$ 0.13	2.03 $\pm$ 0.04	5.05 $\pm$ 0.14	2.03 $\pm$ 0.05
10	4	10.10 $\pm$ 0.02	4.01 $\pm$ 0.03	10.10 $\pm$ 0.10	3.94 $\pm$ 0.07
20	8	20.17 $\pm$ 0.26	8.07 $\pm$ 0.06	20.25 $\pm$ 0.38	7.94 $\pm$ 0.20
Accuracy, bias, %		1.17	0.87	1.29	-1.11
Precision, RSD		0.45	0.66	1.35	0.96
CI at $\alpha=0.05$		0.49	0.71	0.85	0.60
1 mean of three determinations					
2 mean of nine determinations over three days					

3.3 Application to dosage form

The proposed method was applied for the determination of DIC and FEB in tablets containing 100 and 40 mg respectively per tablet and the results show 100.15 and 100.90% recovery, respectively (Table 6), which

indicates that the method is selective for the assay of DIC and FEB without interference from the excipients used in these tablets and is more accurate than that obtained by uv-spectrophotometric method.^[26]

Table 6 application of the proposed method for determining FEB and DIC in pharmaceutical product and comparison with first derivative spectrophotometric method as reference method

Dosage form, mg		Proposed Method*		Reference Method ^[29]	
		Found $\pm$ RSD, %		Found $\pm$ RSD, %	
DIC	FEB	DIC	FEB	DIC	FEB
100	40	100.15	100.9	102	93.5
*Mean of three determinations					

4. CONCLUSION

Four different chemometric techniques; CLS, ITTFA, PCR and PLS were developed for the determination of DIC and FEB. The proposed multivariate calibration methods PCR and PLS showed the best results regarding accuracy and precision in the analysis of individuals and

mixtures. PCR and PLS were simple, rapid, sensitive and precise and could be easily applied successfully for the simultaneous determination of DIC and FEB in dosage form without any preliminary separation step.

5. ACKNOWLEDGEMENT

The co-author, Miss Ghada Sayed Imam, thanks sincerely to National Research Centre, Dokki, for providing the laboratory facilities.

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