SIMULTANEOUS DETERMINATION OF THE TWO NON-STEROIDAL ANTI-INFLAMMATORY DRUGS; DIFLUNISAL AND NAPROXEN IN THEIR TABLETS BY CHEMOMETRIC SPECTROPHOTOMETRY AND HPLC

ABDEL-AZIZ M WAHBI, MOKHTAR M MABROUK*, MARWA S MONEEB AND AMIRA H KAMAL*

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
[*]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Tanta University, Tanta, Egypt

ABSTRACT

Chemometric spectrophotometry and HPLC were applied to the simultaneous determination of the two non-steroidal anti-inflammatory drugs; diflunisal (I) and naproxen (II). The applied chemometric techniques are multivariate methods including classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS); and the second derivative of the ratio spectra (^2D_r) method. To develop the multivariate methods, the UV absorption spectra of the standard solutions of the training and validation sets in methanol were recorded in the range of 242-274 nm at 2 nm intervals. The specificity of the studied multivariate methods has been tested. In the ^2D_r method, analytical signals at 235 and 259 nm were selected for the determination of (I) and (II), respectively. The HPLC method depends on reversed-phase separation using C18 column. The mobile phase consists of a mixture of acetonitrile - acetate buffer (pH 4.2; 50 mM) (60:40, v/v). The UV detector was set at 255 nm. The developed methods were validated and successfully applied to the simultaneous determination of (I) and (II) in their tablets. The assay results obtained using the chemometric methods were statistically compared to those of the HPLC method and good agreement was observed.

Keywords: Diflunisal, naproxen, chemometrics, reversed-phase HPLC.

INTRODUCTION

Diflunisal (I), 2`,4`-difluoro-4-hydroxy-3-biphenyl carboxylic acid and naproxen (II), (+)-(S)-6-methoxy-α-methyl-2-naphthalene acetic acid, are two non-steroidal anti-inflammatory drugs (NSAIDs) (Martindale, 2005) formulated together in a tablet form. Each tablet is labeled to contain 200 mg (I) and 200 mg (II). Both drugs are official in the USP 28 (2005) and BP 2005 but their combination is not yet official.

Literature survey revealed few methods for the simultaneous determination of (I) and (II). These methods included: a quantitative thin layer chromatography densitometric method for their determination in dosage forms (Bebawy and El-Kousy, 1999), synchronous spectrofluorimetric methods for their determination in serum (Perez-Ruiz *et al*., 1998) and a Liquid chromatographic method with amperometric and UV detection for the determination of (I) and (II) in their combination with other non-steroidal anti-inflammatory drugs in plasma (Kazemifard and Moore, 1990). Neither HPLC nor chemometric spectrophotometric methods were reported yet for the simultaneous determination of (I) and (II) in their pharmaceutical formulations.

In this study, chemometric spectrophotometric and HPLC methods have been developed for the simultaneous determination of (I) and (II) in their synthetic mixtures and their tablets. The studied chemometric spectrophotometric methods are multivariate methods including classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS); and the second derivative of the ratio spectra (^2D_r) method.

In the last few years, the chemometric calibration techniques, such as classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS), have widely been applied to the spectrophotometric resolution of mixtures containing two or more compounds without a preliminary separation (Kramer, 1998; Dinç and Üstündağ, 2002; El-Gindy *et al*., 2004). Classical least squares (CLS), sometimes known as K-matrix calibration, can be applied to simple systems where the concentration values of all the components present in the training samples are provided (Kramer, 1998; Kenneth *et al*., 1998). Principal component regression (PCR) is sometimes described as performing a least squares regression of the projections of the data onto the basis vectors of a factor space using inverse least squares (Kramer, 1998; Kenneth *et al*., 1998). Partial least squares (PLS) method is a multivariate calibration based on factor analysis. The basic concept of PLS regression

Corresponding author: Tel: +20-3-4871-317; Fax: +20-3-4873-273, e-mail: abdel_azizwahbi@yahoo.com

was originally developed by Wold (1966). A detailed description of the mathematical principles of the PLS algorithm was reported by Martens and Naes (1992). PLS method involves simultaneously the independent and the dependent variables in the data compression and decomposition operations (Kramer, 1998; Kenneth *et al*., 1998; Matthias Otto, 1999).

The use of absorbance ratio spectra has been the basis of some analytical procedures (Zayed, 2005; Dinç *et al*., 2002; Dinç *et al*., 2000). Blanco *et al*. (1987) developed a method based on the use of each component in turn as a reference standard and provided an over-dimentioned system that can only be solved graphically. Salinas *et al*. (1990) modified the original equations of Blanco *et al*. (1987) and reported the derivative ratio spectrum and the derivative ratio zero-crossing methods for resolution of binary (Salinas *et al*., 1990) and ternary mixtures (Berzas-Nevado *et al*., 1992).

EXPERIMENTAL

Apparatus and software

For chemometric spectrophotometry

A Jasco (V-530) UV-Vis double beam spectrophotometer equipped with 1 cm quartz cells and connected to a personal computer loaded with [Jasco]-[spectra manager] software was used. CLS, PCR, and PLS analyses were carried out using the Chemometrics Toolbox 3.02 software (Kramer, 1995) for use with MATLAB 6. The visual BASIC program of Wahbi *et al*. (2005) was used for the differentiation of ratio data.

For HPLC

The HPLC (waters) instrument was equipped with a model 600 E pump, 600 controller, U6K injector with a 20 µl loop and 486 UV-Vis detector. Separation and quantitation were carried out on a 250 mm x 4.6 mm (i.d.) Thermo BDS Hypersil™ C18 column (5µm particle size) and the detector was set at λ=255 nm. Hanna HI 8314 pH-Meter was used to adjust the pH of the buffer used in the mobile phase. Data acquisition was performed on the powerful Millennium software (version 2.1) of waters.

Materials

Diflunisal (100.1±0.51%) and naproxen (99.8±0.32%) were kindly supplied by Rameda for pharmaceutical industries and diagnostic reagents, 6th of October City, Egypt.

Pharmaceutical preparation

Commercial Maxipan® film coated tablets batch No.04613 product of Rameda for pharmaceutical industries and diagnostic reagents, 6th of October City, Egypt. Each film coated tablet is labeled to contain 200 mg (I) and 200 mg (II).

Reagents

For chemometric spectrophotometry

Methanol was of analytical grade (Sigma).

For HPLC

Acetonitrile was HPLC grade. Sodium acetate anhydrous and glacial acetic acid were analytical grade. Acetate buffer (50 mM) was prepared using sodium acetate anhydrous and distilled water, adjusted to pH 4.2 using glacial acetic acid then filtered through a membrane filter 0.45µm and degassed using sonication.

Standard solutions and calibrations

Stock standard solution (250 mg/100 ml) of each drug was prepared in methanol (for spectrophotometric methods) and in acetonitrile (for HPLC). Suitable dilutions were made using the specified solvent.

For chemometric spectrophotometry

Multivariate methods

A training set of ten synthetic binary mixture solutions in the possible combinations containing 2-14 µg/ml (I) and 2-16 µg/ml (II) was used to develop the chemometric calibrations. A validation set containing ten synthetic binary mixtures in the range of 2-14 and 4-14 µg/ml for (I) and (II), respectively, was prepared using the above stock solutions.

^2D_r method

Calibration graphs were established for standard solutions containing 2-16 µg/ml of (I) and for standard solutions containing 4-16 µg/ml of (II).

For HPLC

Solutions used for the calibration ranged from 4 to 16 µg/ml for each drug. Triplicate 20 µl injections were made for each concentration and chromatographed under the specified conditions. Peak area versus concentration was plotted.

Conditions

For chemometric spectrophotometry

Multivariate methods

The UV absorbances were recorded within the wave-length range 242-274 nm at 2 nm intervals.

^2D_r method

The UV absorbances were recorded within the wave-length range 220-350 nm at 2 nm intervals.

For HPLC

The elution was carried out with acetonitrile - acetate buffer (pH4.2; 50 mM) (60:40, v/v). The flow rate was 0.7 ml/min. The injection volume was 20 µl. Quantitation based on peak area was achieved using UV detection at 255 nm. All determinations were performed at ambient temperature.

Sample preparation

For chemometric spectrophotometry

Ten Maxipan® film coated tablets were accurately weighed and finely powdered in a mortar. An amount of the tablet mass equivalent to one tablet content [200 mg of (I) and 200 mg of (II)] was dissolved in 60 ml of methanol. After 30 min of mechanical shaking, the solution was filtered in a 100 ml volumetric flask. The residue was washed thrice, each with 10 ml of the solvent. Then the volume was completed to 100 ml and suitable dilutions were made using the same solvent.

For HPLC

The same procedure was followed using acetonitrile as solvent. The sample solutions were filtered through a membrane filter 0.45µm and degassed via sonication.

Results and discussion

Chemometric spectrophotometry

Multivariate methods

Fig. 1 shows the zero-order absorption spectra of diflunisal (I) and naproxen (II) in methanol. It is clear that the spectra of the two drugs display considerable overlap.

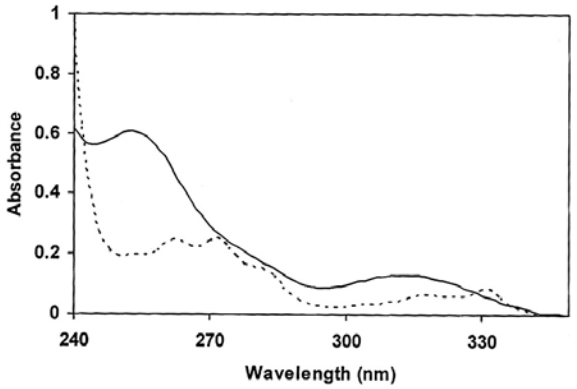

Fig. 1: UV zero-order absorption spectra of 10 µg/ml diflunisal (—) and 10 µg/ml naproxen (- - - -) in methanol.

The composition of the training set was orthogonally designed in order to obtain maximum information on each drug from the calibration procedure. The number of calibration mixtures in the training set was selected according to the rule of five. This rule states that using five times the number of samples as there are components provides enough samples to reasonably represent all possible combinations of different concentration values (Kramer, 1998). A training set was prepared as shown in table 1. The absorbance data matrix for this training set was obtained by recording the absorbances within the wavelength range 242-274 nm at 2 nm intervals. The multivariate calibrations were computed with the CLS, PCR and PLS algorithms using the correlation for the absorbance data matrix and the corresponding concentration data matrix of the training set.

Table 1: Composition of the training set

Mixture No.	Diflunisal (µg/ml)	Naproxen (µg/ml)
1	2	16
2	3	14
3	4	12
4	5	10
5	6	8
6	7	7
7	8	5
8	10	4
9	12	3
10	14	2

In order to validate the developed calibrations, an independent set of validation synthetic mixtures containing (I) and (II) in the different compositions given in table 2, was prepared and analyzed. The mean percentage recoveries, standard deviations (S.D.) and relative standard deviations (R.S.D.) are indicated in table 2. The results contributed to the high accuracy and precision of the developed multivariate methods.

Specificity

The specificity of the studied multivariate methods has been tested. These methods have been applied to the absorbance ratio data, using either diflunisal or naproxen as divisor. The results obtained for the analysis of Maxipan® tablets using the absorbance ratio data were found to be closely similar to those obtained using absorbance data. The results obtained were statistically analyzed and compared using t- test between the smallest mean percentage found and the highest one and using F-test between the smallest variance and the highest one. At 95% confidence level, the difference in the mean percentage found (t-test) or in variance (F-test) was not statistically significant. Therefore there were no significant differences between the results with regard to accuracy (t-test) and precision (F-test). This confirms specificity of the proposed methods for the two components present in the investigated binary mixture. The presence of any absorbing foreign substance in the test solution would lead to different results when using absorbance and absorbance ratio data.

STATISTICAL ANALYSIS

Determining how many factors to be used in the calibration is a key step in factor based techniques (PCR and PLS). Only those factors that contain analytical information must be kept .The discarded factors should contain only noise (Kramer, 1998; Kenneth *et al.,* 1998). The Chemometrics Toolbox 3.02 Software offers several indicators which could be used for determining the optimum number of factors (rank). The Cross validation

Table 2: Assay results of diflunisal (I) and naproxen (II) combinations in synthetic mixtures (validation mixtures) by the proposed chemometric methods

Validation mixtures		Recovery (%)							
		Multivariate methods						Second derivative ratio method (^2D_r)	
Added (μg/ml)		CLS		PCR		PLS			
Diflunisal (I)	Naproxen (II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
2	14	99.76	99.72	99.74	99.71	99.74	99.71	98.70	99.22
3	13	101.32	100.88	101.31	100.88	101.31	100.87	99.85	100.05
4	12	101.21	100.85	101.19	100.84	101.20	100.83	100.25	100.02
5	11	100.78	99.68	100.76	99.66	100.75	99.66	100.80	100.45
6	10	100.41	99.87	100.40	99.86	100.41	99.85	99.79	99.30
7	9	100.54	99.17	100.53	99.18	100.54	99.16	100.68	99.22
8	8	100.73	100.46	100.72	100.45	100.71	100.44	99.82	99.65
9	7	101.27	100.01	101.24	99.99	101.22	99.98	99.20	100.32
12	6	100.63	99.96	100.66	99.94	100.65	99.93	100.46	99.16
14	4	100.72	99.37	100.71	99.37	100.72	99.37	100.18	100.64
Mean		100.74	100.00	100.73	99.99	100.73	99.98	99.97	99.80
± S.D.		0.47	0.58	0.46	0.57	0.46	0.57	0.65	0.56
R.S.D.		0.46	0.58	0.46	0.57	0.46	0.57	0.65	0.57

S.D. standard deviation; R.S.D. relative standard deviation.

procedure leaving out one sample at a time, was used for this purpose (Kramer, 1998; Espinosa-Mansilla *et al.*, 1995) and the predicted residual error sum-of-squares, (PRESS) was calculated.

$PRESS = \sum_{i=1}^{n} (C_i^{True} - C_i^{Predicted})^2$

where C_i^{True} represents the true concentration, $C_i^{Predicted}$ denotes the predicted concentration and n is the total number of validation samples.

A better way for selecting the optimum number of factors involves the generation of a calibration for every possible rank. Each calibration was used to predict the concentrations for a set of independently measured, independent validation samples. Then the PRESS was calculated (Kramer, 1998). Another way to determine the optimum number of factors is the two-way F-test on reduced eigenvalues (REV) according to the method of Malinowski (Kramer, 1998). A rank of two factors was found to be the optimum system rank for both the PCR and PLS models according to the three studied criteria. Fig. 2 shows the obtained (PRESS) values with an independent validation set, those with cross validation technique (CROSS) and the reduced eigenvalues (REV) for the PCR and PLS methods using the different number of factors.

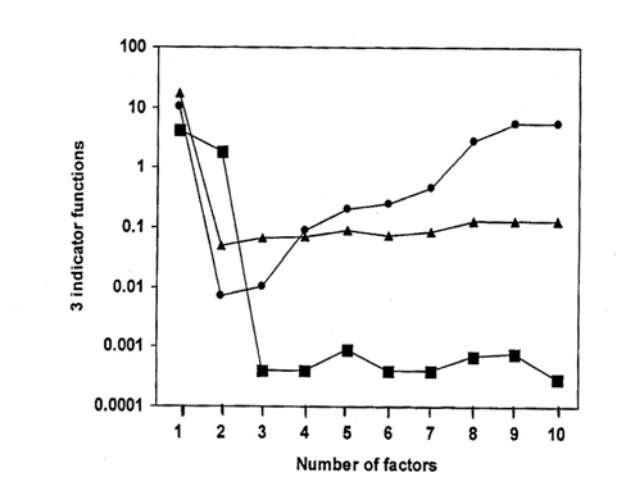

Fig. 2: PRESS (-▲-); CROSS (-●-) and REV (- ■ -) vs. number of factors for the PCR and PLS models of diflunisal and naproxen.

The predictive ability of a model could be defined using several validation diagnostics. These include the standard error of prediction (SEP), the mean squared error of prediction (MSEP), the root mean standard error of prediction (RMSEP) and the variance of prediction (s^2)

Table 3: Statistical parameters of the validation synthetic mixtures using the proposed chemometric methods

Method	SEP ($x10^2$)	MSEP ($x10^3$)	RMSEP ($x10^2$)	s^2 ($x10^2$)	a ($x10^2$)	Lower [a] 95%	Upper[a] 95%	b	Lower[b] 95%	Upper [b] 95%	r
Diflunisal											
CLS	6.65	3.98	6.31	1.77	0.17	-0.034	0.037	1.007	0.999	1.012	0.99998
PCR	6.59	3.92	6.26	1.74	0.08	-0.033	0.035	1.007	0.999	1.012	0.99998
PLS	6.57	3.88	6.23	1.72	0.06	-0.033	0.034	1.007	0.999	1.012	0.99998
^2D_r	3.94	1.38	3.71	0.61	-1.32	-0.076	0.049	1.003	0.995	1.011	0.99995
Naproxen											
CLS	6.15	3.40	5.83	1.51	-4.99	-0.196	0.096	1.006	0.991	1.021	0.99984
PCR	6.13	3.39	5.82	1.50	-5.02	-0.196	0.096	1.006	0.991	1.021	0.99984
PLS	6.11	3.36	5.79	1.49	-5.06	-0.196	0.095	1.006	0.991	1.021	0.99984
^2D_r	1.79	2.87	5.63	1.28	1.77	-0.107	0.142	0.996	0.983	1.008	0.99988

SEP, standard error of prediction; MSEP, mean squared error of prediction; RMSEP, root mean standard error of prediction; s^2, variance of prediction.
a, intercept; b, slope; r, correlation coefficient.
[a] Lower and upper confidence limits for the intercept at the 95% confidence level.
[b] Lower and upper confidence limits for the slope at the 95% confidence level.

(Kramer, 1998; Kenneth *et al.*, 1998). The MSEP and RMSEP characterize both the accuracy and the precision of prediction (Kenneth *et al.*, 1998).

$$SEP = [\sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / n\text{-}1]^{½}$$
$$MSEP = \sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / n$$
$$RMSEP = [\sum^{n}_{i=1} (C_i^{True} - C_i^{Predicted})^2 / n]^{½}$$
$$s^2 = \sum^{n}_{i=1} (C_i^{Predicted} - C_i^{True} - bias)^2 / n\text{-}1$$

where C_i^{True} is the true concentration, $C_i^{Predicted}$ is the predicted concentration and n is the total number of validation samples. The numerical values of SEP, MSEP, RMSEP and s^2 are indicated in table 3. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed methods.

Another way to validate the models and to examine the results is the predicted versus true concentration plot. In this plot, points are expected to fall on a straight line with a slope of one and a zero intercept (Kenneth *et al*, 1998). The correlation coefficient (r) is calculated for each calibration to indicate the quality of fit of all data to a straight line. The regression analysis for these linear relationships was carried out and the results are shown in table 3. The absence of bias was proved by determining the confidence limits for the intercept, a, and the slope, b, at the 95% significance level (Miller and Miller, 2000). The upper and lower confidence limits are shown in table 3. For (I) and (II), using the three developed multivariate models, the 95% confidence interval of the intercept included the ideal value of zero and that of the slope included the ideal value of one. This gave indication of good fitness and absence of bias which confirmed the trueness of the developed methods. Furthermore, no sample(s) appeared to be unusually far from the line than the rest of the data.

^2D_r method

In this study, the second derivative of the ratio spectra method, ^2D_r, was used in analysis rather than the first derivative, ^1D_r, as the statistical parameters provided by the ^2D_r method showed better linearity, fitness, mean recoveries and lower relative standard deviation (R.S.D.) than those obtained using the ^1D_r method.

Fig. 3(a) shows the ratio curves obtained for the UV spectra of (I) serial standard solutions of increasing concentrations in methanol, divided by the spectrum of 12 µg/ml (II) standard solution in the same solvent. The computed second derivative curves of these ratio spectra are shown in fig. 3(b). There exist four maxima (at 235, 252, 258 and 274 nm) and two minima (at 242 and 263 nm). The maximum at 235 nm was selected as the optimum for the determination of (I) in its synthetic mixtures with (II) and in tablets, due to its low standard deviation (S.D.) and suitable mean recovery.

Fig. 3(c) shows the ratio curves obtained for the UV spectra of (II) standard solutions of increasing concentrations in methanol, divided by the spectrum of 4 µg/ml (I) standard solution in the same solvent. The computed second derivative curves of these ratio spectra are shown in fig. 3(d). There exist three maxima (at 247, 271 and 278 nm) and a minimum (at 259 nm). The minimum at 259 nm was selected as the optimum for the determination of (II) in its synthetic mixtures with (I) and in tablets, due to its low standard deviation (S.D.) and suitable mean recovery.

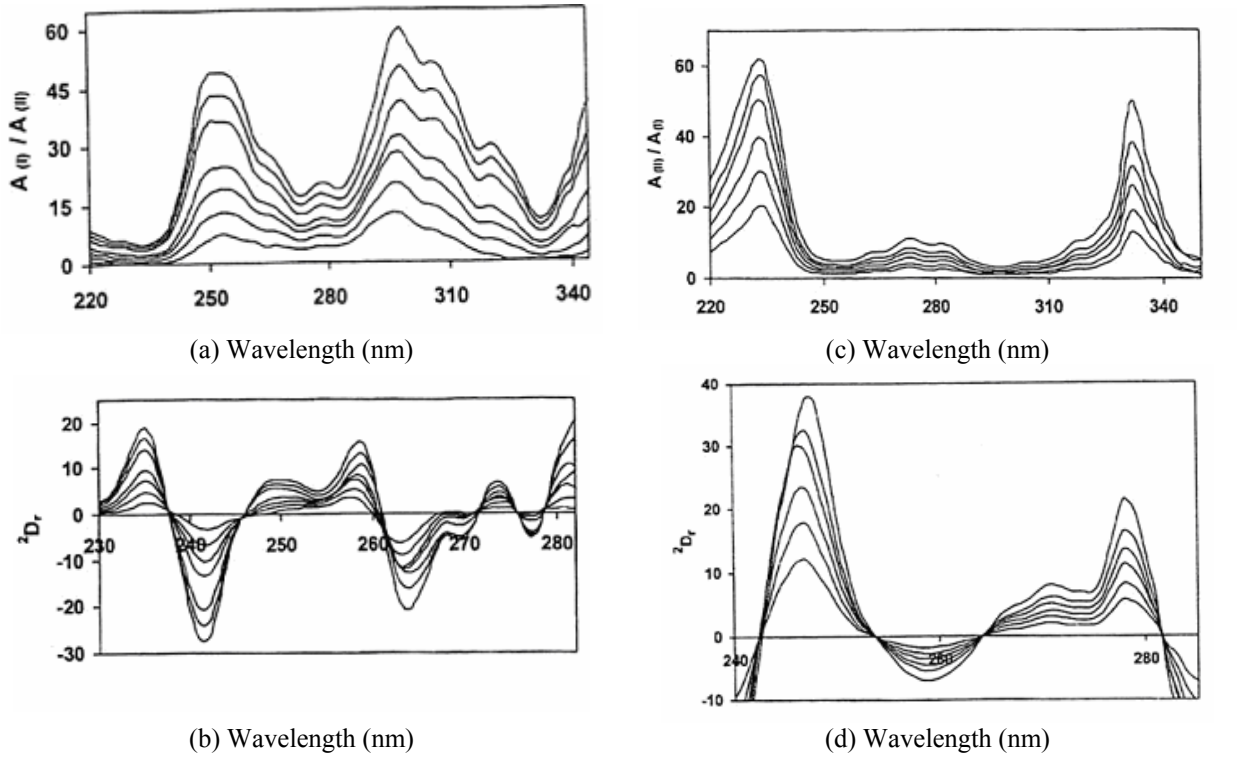

Fig. 3: Ratio spectra of different diflunisal (I) standard solutions at increasing concentrations (2, 4, 6, 8, 12, 14 and 16 µg/ml) in methanol using 12 µg/ml standard solution of naproxen (II) in methanol as divisor (a); their second derivative curves (b); ratio spectra of different naproxen (II) standard solutions at increasing concentrations (4 , 6, 8, 10, 12, and 16 µg/ml) in methanol using 4 µg/ml standard solution of diflunisal (I) in methanol as divisor (c); their second derivative curves (d).

In order to determine the divisor concentration, different concentrations over the range 2-16 µg/ml (I) and 4-16 µg/ml (II) were tested. Then 4 µg/ml (I) and 12 µg/ml (II) were chosen as optimum values. The visual BASIC program of Wahbi *et al*. (2005) was used for the differentiation of ratio data using 10-points orthogonal polynomials at 2 and 4 nm intervals for (I) and (II), respectively.

In order to demonstrate the validity of the developed derivative ratio methods for the determination of (I) and (II) in combination, the 10 binary synthetic mixtures of the validation set were analyzed for (I) and (II). The UV spectra were recorded within the wavelength range 220-350 nm at 2 nm intervals. The ratio curves were developed and their second derivative curves were computed (Wahbi *et al.*, 2005). The mean percentage recoveries, standard deviations (S.D.) and relative standard deviations (R.S.D.) are given in table 2.

Assay validation

Linearity and range

Calibration graphs were established for standard solutions containing 2-16 µg/ml (I) at 235 nm and for standard solutions containing 4-16 µg/ml (II) at 259 nm. Regression analysis has been carried out with correlation coefficient, r, (0.99994 and 0.99990), intercept, $a \pm S_a$, ($6.87 \times 10^{-2} \pm 5.40 \times 10^{-2}$ and $0.47 \times 10^{-2} \pm 3.33 \times 10^{-2}$), slope, b $\pm S_b$, ($1.18 \pm 5.35 \times 10^{-3}$ and $0.44 \pm 3.06 \times 10^{-3}$) and residual standard deviation, $S_{y/x}$, (8.85×10^{-2} and 2.63×10^{-2}) for (I) and (II), respectively. The good linearity of the calibration graphs and negligible scatter of the experimental points were clearly evident by the values of the correlation coefficients and the standard deviation around the slopes and intercepts.

Accuracy

The developed ^2D_r method was applied to six synthetic mixtures of the tablet excipients to which known quantities of (I) and (II) had been added. No interferences were observed. The mean percentage recoveries were found to be 100.5±0.79 % and 99.7±0.58% for (I) and (II), respectively.

Specificity

According to ICH document for specificity (1996), the method is specific when the results are unaffected by the presence of the dosage form excipients, so the above results demonstrated the specificity of the developed method. Furthermore, the specificity was confirmed by

comparing the second derivative ratio curves (using both divisors) of standard and test solutions with regard to general shape and position of the peaks.

Precision

Precision was estimated by the determination of the repeatability of the method. Repeatability was assessed using three determinations at each of three different test concentrations, covering the specified range of the method. The relative standard deviations were found to be 0.75 and 0.58 for (I) and (II), respectively.

The accuracy and precision were furthermore confirmed by comparing the results obtained for the assay of Maxipan® tablets using the ^2D_r method to those of the studied HPLC method. The results obtained were statistically analyzed and compared using t-test and F-test. At 95% confidence level, the difference in the mean percentage recovery (t-test) or in variance (F-test) was not statistically significant (table 5). Therefore, there were no significant differences between the two methods with regard to accuracy (t-test) and precision (F-test).

STATISTICAL ANALYSIS

The predictive ability of the ^2D_r method can be defined using the same validation diagnostics previously discussed with the multivariate methods.

The numerical values of SEP, MSEP, RMSEP, s^2 and the regression data for the predicted versus known concentration plot were calculated and indicated in table 3. The small values of the calculated validation diagnostics indicate the negligible error of prediction and the high predictive ability of the proposed method.

HPLC

Method development

The HPLC method depends on reversed-phase separation using a 250 mm x 4.6 mm (i.d.) Thermo BDS Hypersil™ C18 column (5µm particle size). The detector was set at λ= 255 nm. The method has been optimized after studying the effect of different parameters on the separation. The mobile phase was chosen after several trials with acetonitrile and buffer solutions in various proportions and at different pH. A mobile phase consisting of acetonitrile - acetate buffer (pH 4.2; 50 mM) (60:40, v/v) at a flow rate of 0.7ml/min was found to give optimum separation.

Fig. 4 shows a typical separation chromatogram of 20 µl injection of synthetic mixture containing 6 µg /ml (I) and 10 µg /ml (II) in acetonitrile, where the average retention times (± S.D.) for (I) and (II) were found to be 4.97 ± 0.06 and 7.60 ± 0.04 min, respectively for seven replicates. Run time was found to be less than 10 min.

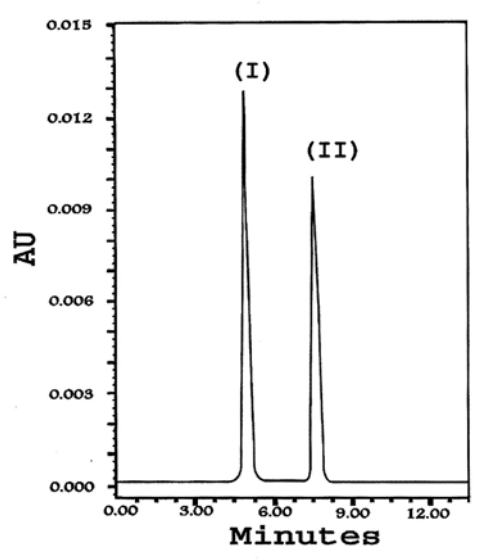

Fig. 4: HPLC chromatogram of 20 µl injection of synthetic mixture containing 6 µg /ml diflunisal (I) and 10 µg /ml naproxen (II) in acetonitrile.

The developed HPLC method was applied to the simultaneous determination of (I) and (II) in their synthetic binary mixtures. The assay results are shown in table 4.

Table 4: Assay results of diflunisal (I) and naproxen (II) combinations in synthetic mixtures by the proposed HPLC method.

Mixtures Added (µg/ml)		Recovery (%)	
(I)	(II)	(I)	(II)
2	14	99.07	101.38
4	12	101.54	98.90
6	10	100.81	99.37
8	8	101.49	98.98
10	7	98.78	101.53
12	5	99.20	99.67
14	2	100.32	100.22
Mean		100.17	100.01
± S.D.		1.16	1.08
R.S.D.		1.16	1.08

S.D. standard deviation; R.S.D. relative standard deviation.

Assay validation

System suitability

The suitability of the chromatographic system was monitored by calculating the capacity factor, K', the resolution, R_s, the selectivity, α, and the asymmetry factor, A_s. These parameters were found satisfactory for both drugs. K' was found to be 3.10 and 5.43 for (I) and

(II), respectively. R_s and α were found to be not less than 3.21 and 1.75, respectively. A_s was found to be 1.04 and 1.02 for (I) and (II), respectively.

Linearity and range

Linearity of HPLC detector response for determination of (I) and (II) was evaluated by analyzing a series of standard solutions of different concentrations of each compound. Calibration graphs were established for standards containing 1.5-16 µg/ml for both drugs. Regression analysis has been carried out with correlation coefficient, r, (0.99992 and 0.99994), intercept, a ± S_a, ($0.90x10^3 \pm 4.45x10^3$ and $0.33x10^3 \pm 1.15\ x10^3$), slope, b ± S_b, ($85.2\ x10^3 \pm 0.44x\ 10^3$ and $25.7\ x10^3 \pm 0.12x10^3$) and residual standard deviation, $S_{y/x}$, ($8.31\ x10^3$ and $2.80\ x10^3$) for (I) and (II), respectively. The good linearity of the calibration graphs and negligible scatter of the experimental points is clearly evident by the values of the correlation coefficients and the standard deviation around the slopes and intercepts.

Accuracy

The developed HPLC method has been applied to six synthetic mixtures of the tablet excipients to which known quantities of (I) and (II) have been added. No interferences were observed. The mean percentage recoveries were found to be 100.2 ± 1.10 % and 99.9 ± 1.35 % for (I) and (II), respectively.

Specificity

According to ICH document for specificity (1996), the method is specific when the results are unaffected by the presence of the dosage form excipients, so the above results demonstrated the specificity of the method. Furthermore, the specificity of the proposed HPLC method was confirmed by comparing the chromatograms of standards and test solutions. The average retention times ± standard deviation for (I) and (II) in the tablets were found to be 5.03 ± 0.05 [4.97 ± 0.06 min for standard (I)], and 7.59 ± 0.05 [7.60±0.04 min for standard (II)] min for (I) and (II), respectively.

Precision

Precision was estimated by the determination of the repeatability of the method. Repeatability was assessed using three determinations at each of three different test concentrations, covering the specified range of the method. The relative standard deviations were found to be 1.25 and 1.08 for (I) and (II), respectively.

The accuracy and precision were furthermore confirmed by comparing the results obtained for the assay of Maxipan® tablets using the developed HPLC method to those of the studied multivariate and ^2D_r methods. The results obtained were statistically analyzed and compared using t-test and F-test. At 95% confidence level, the difference in the mean percentage recovery (t-test) or in variance (F-test) was not statistically significant (table 5). Therefore, there were no significant differences between the methods with regard to accuracy (t-test) and precision (F-test).

Detection and quantitation limits

According to ICH recommendations (1996), the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically. The detection limits were found to be 0.12 and 0.40 µg/ml for (I) and (II), respectively. The quantitation limits were found to be 0.37 and 1.22 µg/ml for (I) and (II), respectively.

Robustness

Variation of pH of the buffer used in the mobile phase by ± 0.2 pH unit as well as variation of acetonitrile % in the mobile phase by ± 5% did not give significant effect on the chromatographic separation.

Stability

The synthetic binary mixtures of (I) and (II); and test solutions in acetonitrile were found to be stable for 24 hours when kept at room temperature and 4 days when stored refrigerated at 5 ^oC based upon 98% recovery limit.

Analysis of tablets

The proposed CLS, PCR, PLS, ^2D_r and HPLC methods were applied to the simultaneous determination of (I) and (II) in commercial Maxipan® tablets. Satisfactory results were obtained for each compound in good agreement with label claim (table 5). The assay results of the proposed CLS, PCR, PLS and ^2D_r methods were compared to those of the proposed HPLC method. Statistical comparison between the results was performed with regards to accuracy and precision using t-test and F-test at 95% confidence level (table 5). The calculated values did not exceed the theoretical ones, indicating that there was no significant difference between the methods compared.

CONCLUSION

Chemometric spectrophotometric and HPLC methods have been developed in this study for the simultaneous determination of diflunisal and naproxen in their synthetic binary mixtures and in their tablets. The assay results obtained using chemometric methods (CLS, PCR, PLS and ^2D_r) were compared to those of the proposed HPLC method and good coincidence was observed as there was no significant difference between the methods compared. The HPLC method is superior with regard to identification and specificity. However, the chemometric methods are less expensive by comparison and do not require sophisticated instrumentation nor any prior separation step. The multivariate methods have higher superiority over the derivative ratio methods for being fast, easy, simple and do not require prerequisite for

Table 5: Determination of diflunisal (I) and naproxen (II) in Maxipan® tablets[a] using the proposed chemometric and HPLC methods

	CLS	PCR	PLS	^2D_r	HPLC
Diflunisal (I)					
Mean [b] ± S.D.	100.48 0.77	100.47 0.77	100.47 0.77	99.25 0.99	99.67 1.29
R.S.D.	0.77	0.77	0.77	1.00	1.29
t	1.20	1.19	1.19	0.58	(1.86) [c]
F	2.79	2.79	2.78	1.70	(6.39) [c]
Naproxen (II)					
Mean [b] ± S.D.	101.26 0.88	101.25 0.88	101.24 0.88	100.43 0.96	100.23 1.28
R.S.D.	0.87	0.87	0.87	0.96	1.28
t	1.49	1.47	1.46	0.28	(1.86) [c]
F	2.12	2.13	2.12	1.77	(6.39) [c]

[a] Maxipan® tablets labeled to contain 200 mg (I) and 200 mg (II) per tablet.
[b] Mean percentage found of five different concentration levels.
[c] Theoretical values for t (0.05) and F (0.05).

successful application. The developed methods are suggested to be used in routine analysis of diflunisal and naproxen in their synthetic binary mixtures and in their tablets.

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