Chemometric Determination of Naproxen Sodium and Pseudoephedrine Hydrochloride in Tablets by HPLC

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A new chemometric determination by high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection was implemented for the simultaneous determination of naproxen sodium and pseudoephedrine hydrochloride in tablets. Three chemometric calibration techniques, classical least squares (CLS), principle component regression (PCR) and partial least squares (PLS) were applied to the peak area at multiwavelength PDA detector responses. The combinations of HPLC with chemometric calibration techniques were called HPLC-CLS, HPLC-PCR and HPLC-PLS. For comparison purposes the HPLC method called the classic HPLC method was used to confirm the results obtained from combined HPLC-chemometric calibration techniques. A good chromatographic separation between two drugs with losartan potassium as an internal standard was achieved using a Waters Symmetry® C18 Column 5 *μ*m 4.6±250 mm and a mobile phase containing 0.2 M acetate buffer and acetonitrile (v/v, 40 : 60). The multiwavelength PDA detection was measured at five different wavelengths. The chromatograms were recorded as a training set in the mobile phase. Three HPLC-chemometric calibrations and the classic-HPLC method were used to test the synthetic mixtures of naproxen sodium and pseudoephedrine hydrochloride in the presence of the internal standard. The HPLC-chemometric approaches were applied to real samples containing drugs of interest. The experimental results obtained from HPLC-chemometric calibrations were compared with those obtained by a classic HPLC method.

Key words   HPLC-chemometric calibration; quantitative determination; naproxen sodium; pseudoephedrine hydrochloride; pharmaceutical preparation

The combination of naproxen sodium (NAP) and pseudoephedrine hydrochloride (PSE) has an effect as a pain reliever, fever reducer and nasal decongestant in pharmaceutical tablet formulations. Particularly, the above combination temporarily relieves cold, sinus and flu symptoms such as sinus pressure, minor body aches and pains, headache, nasal and sinus congestion (promotes sinus drainage and restores freer breathing through the nose) and fever.

In the literature, NAP in single component samples was investigated by voltametry[1] and spectrofluorometry.[2] HPLC was applied to the simultaneous quantitation of NAP and PSE impurities.[3] NAP with other compounds in the samples was determined by using HPLC,[4] chemometric methods[5,6] and capillary electrophoresis.[7] Determination of PSE in human plasma was carried out by HPLC.[8] PSE with other compounds in the sample was carried out by using HPTLC,[9] capillary electrophoresis.[10] HPLC,[11] spectrophotometry,[12,13] derivative and ratio spectra derivative spectrophotometry[14] and ratio spectra derivative spectrophotometry, and Vierord's method.[15]

Although HPLC, as a comparison method, is the current method of choice for the analysis of multicomponent pharmaceutical formulations, it requires a separation treatment and several injections during analysis. In chromatographic analysis, the main problems of this method involve the optimization of experimental conditions such as selection of column type, temperature of column, variety and composition of mobile phase, selection of one specific wavelength and cheap instrumentation. In spite of the fact that this method undoubtedly provides more sensitive determination than the spectrophotometric methods, the calculations at one specific wavelength produce some errors in the process of construction of linear regression equations. Namely, in the case of a single wavelength detector response classic HPLC gives some chromatographic area errors coming from injection and instrumental fluctuations, as well as the other sources. Therefore, the above mentioned facts affect the result of analysis. For these reasons, the simultaneous use of chromatograms to obtain a multiwavelength PDA detector response will eliminate the errors of single regression equations based on single wavelength. The injection (times of) frequency until achievement of the final result will decrease by the simultaneous use of HPLC and chemometric calibration techniques. This procedure reduces the time of analysis and consumption of reactants.

In recent years, chemometric calibrations such as classical least-squares (CLS), inverse least squares (ILS), principle component regression (PCR) and partial least squares (PLS) have been applied to the analysis of analytical data obtained from many instruments.[16—20] Chemometric calibration techniques have been subjected to the resolution of overlapping spectra for the determination of active compounds in samples containing two or more compounds.[21—24]

In our study, CLS, PCR and PLS calibration techniques were applied to the HPLC data set at multiwavelengths using PDA detectors for the binary mixture analysis. These combined numerical methods with HPLC were denoted as HPLC-CLS, HPLC-PCR and HPLC-PLS. The HPLC method based on multiwavelength with chemometric calibration techniques eliminated the disadvantages of the single re-

gression equation and its chromatographic conditions. At the same time, HPLC-chemometric approaches did not require any use of a specific wavelength in chromatographic analysis. In this study, three HPLC-chemometric approaches were applied to the simultaneous determination of NAP and PSE in synthetic mixtures and tablets. As an alternative method classic HPLC was used to analyze the same samples. For a statistical comparison, t-test, F-test and ANOVA test were applied to the obtained results. The proposed methods gave us successful results.

Theoretical Outline In this chemometric HPLC study, CLS, PCR and PLS calibrations were used to analyze the ratio of the peak areas of analyzed drugs to IS at the five wavelengths using a PDA detector. The chromatograms of analyzed drugs were plotted and stored in a computer, and the detector responses were evaluated as a function of peak area. The application procedure of the combined HPLC-chemometrics calibrations was developed and is discussed in the following section for each chemometric calibration.

HPLC-CLS Approach This approach is based on the application of multi linear regression (MLR) to the ratio of the peak area of individual drugs. Let us consider the responses as ratio values of the peak area at five wavelengths (R) and 6 standard series (concentration set (C)) of the analyzed drug. The corresponding matrix equation would thus be:

$$
\begin{bmatrix}
R_{11} & R_{12} & R_{13} & R_{14} & R_{15} & R_{16} \\
R_{21} & R_{22} & R_{23} & R_{24} & R_{25} & R_{26} \\
R_{31} & R_{32} & R_{33} & R_{34} & R_{35} & R_{36} \\
R_{41} & R_{42} & R_{43} & R_{44} & R_{45} & R_{46} \\
R_{51} & R_{52} & R_{53} & R_{54} & R_{55} & R_{56}
\end{bmatrix}
$$

$$
=
\begin{bmatrix}
K_{11} \\
K_{21} \\
K_{31} \\
K_{41} \\
K_{51}
\end{bmatrix}
\times
\begin{bmatrix} C_{11} & C_{12} & C_{13} & C_{14} & C_{15} & C_{16} \end{bmatrix}
\tag{1}
$$

where $R_{5\times6}$ represents the matrix of the peak area responses (ratio of the peak area of the analyte to the peak area of the internal standard), $K_{5\times1}$ is the matrix of the calibration coefficients and $C_{6\times1}$ is the concentration set of the analyzed compound.

We may write the Eq. 1 in a compact form as follows:

$$
R_{5\times6}=K_{5\times1}C_{1\times6}
\tag{2}
$$

Using Eq. 2, and after doing some matrix calculation, the values of the matrix $K_{5\times1}$ are obtained as

$$
K_{5\times1}=R_{5\times6}C_{6\times1}^{\mathrm{T}}[C_{1\times6}C_{6\times1}^{\mathrm{T}}]^{-1}
\tag{3}
$$

where, $C_{6\times1}^{\mathrm{T}}$ is the transpose of $C_{1\times6}$ and $[C_{1\times6}C_{6\times1}^{\mathrm{T}}]^{-1}$ is the inverse of $C_{1\times6}C_{6\times1}^{\mathrm{T}}$.

The mathematical computation using Matlab 6.5 software is carried out by the following algorithm:

$$
\mathrm{Ka}_{1\times5}=\frac{1}{[K_{1\times5}^{\mathrm{T}}K_{5\times1}]}\times K_{1\times5}^{\mathrm{T}}
\tag{4}
$$

Finally, the obtained $\mathrm{Ka}_{1\times5}$ is introduced into the following equation

$$
C_{\mathrm{prediction}_{1\times n}}=\mathrm{Ka}_{1\times5}\times R_{\mathrm{sample}_{5\times n}}
\tag{5}
$$

As can be seen from Eq. 5, we observe that the concentration of the content of analyte in the mixture is obtained by multiplying $\mathrm{Ka}_{1\times5}$ and $R_{\mathrm{sample}_{5\times n}}$.

HPLC-PCR Approach In the application of HPLC-PCR, the ratio (R) of the peak area of the individual drug and the drug concentration set were reprocessed by mean-centering as R_{o} and C_{o}, respectively. The covariance dispersion matrix of the centered matrix R_{o} was investigated.

Using the square covariance matrix, normalized eigenvalues and eigenvectors were obtained.

The numbers of optimal principal components (eigenvectors (P)) were chosen by considering only the highest values of the eigenvalues. Therefore, the remaining eigenvalues and their corresponding eigenvectors were ignored.

For these reasons coefficient b, defined as $b=P\times q$ was determined, where P is the matrix of eigenvectors and q is the C-loadings given by $q=D\times T^{\mathrm{T}}\times R_{\mathrm{o}}$. T^{T} represents the transpose of the score matrix T, and D is a diagonal matrix having the components inverse to the selected eigenvalues. The drug content in samples was evaluated by using the $C_{\mathrm{prediction}}=b\times R_{\mathrm{sample}}$. PLS toolbox 3.5 in Matlab 7.0 software was used for the data treatment.

HPLC-PLS Approach PLS calibration using the orthogonalized PLS algorithm involves, simultaneously, independent and dependent variables on the data compression and decomposition operations.

In the HPLC data analysis, HPLC-PLS calibration was obtained by decomposition of both concentration and the ratio of peak area matrix into latent variables, $R=T\times P^{\mathrm{T}}+E$ and $C=U\times Q^{\mathrm{T}}+F$. The linear regression equation $C_{\mathrm{prediction}}=b\times R_{\mathrm{sample}}$, was used for the estimation of the drugs in the samples. Here, vector b was given as $b=W\times(P^{\mathrm{T}}\times W)^{-1}\times Q$, where W represents a weight matrix.

Application of this method was done using PLS toolbox 3.5 in Matlab 7.0 software.

Experimental

Instrumentation and Chromatography Chromatography was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., California, U.S.A.) provided with a quaternary pump, a thermostatted autosampler, a thermostatted column compartment, and a multiwavelength diode array detector (DAD). Data were acquired and processed using HP Chem Station for LC (Rev. A0.01 [403]) software from Heawlet-Packard. The column used was a Waters Symmetry® C18 Column 5 μm 4.6×250 mm. The flow rate was maintained at 1.7 ml/min and the injection volume was 30 μl. The mobile phase was prepared daily, filtered through a 0.45 μm membrane filter.

Commercial Tablet Formulation The commercial pharmaceutical formulation, ALEVE® COLD&SINUS (produced by Bayer Corp., U.S.A., Batch no. 257082N) containing 220 mg NAP and 120 mg PSE, were analyzed using the proposed methods. The active compounds were kindly obtained from National Drug Industry Firms.

Standard Solutions Stock solutions of 100 mg/100 ml NAP, PSE and losartan potassium (IS) were prepared in a mixture of 0.2 M acetate buffer and acetonitrile (v/v, 40 : 60) (pH=5.0). A standard series (training set) of solutions containing 20—320 μg/ml NAP and 100—1000 μg/ml PSE was obtained from the stock solutions. A validation set consisting of 12 synthetic mixture solutions in the concentration range of 20—320 μg/ml NAP and 100—1000 μg/ml PSE was prepared. For the standard addition technique, six solutions using the stock solutions and tablet solutions were prepared. Throughout chromatographic study, 10 μg/ml IS was added to each solution. All the solutions were prepared freshly and protected from light.

Tablet Analysis Twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet was dissolved in the mobile

phase in a 100 ml calibrated flask. The solution was filtered into a 100 ml calibrated flask with a 0.45 μm membrane filter. Tablet solutions were diluted to the concentration range of 20 μg/ml for NAP and 12.5 μg/ml for PSE in a 25 ml-calibrated flask.

Results and Discussion

Method Development and Optimization First, several mobile phase systems with other chromatographic conditions were tested for experimental optimization. The following chromatographic parameters were found to be suitable for separation and determination of NAP and PSE in samples.

Chromatographic separation was carried out at ambient temperature on a column with a mobile phase consisting of acetate buffer (0.2 M) and acetonitrile (v/v, 40 : 60). The flow rate was set 1.7 ml/min with 30 μl as the injection volume. The IS as an internal standard was found to be appropriate in our study as seen in Fig. 1. The retention times at a flow rate of 1.7 ml/min were obtained as 5.870 for NAP, 1.345 for PSE and 2.91 for IS (Fig. 1).

Chromatograms corresponding to the training set in the range of 20—320 μg/ml NAP and 100—1000 μg/ml PSE with 10 μg/ml IS were plotted by diode array detectors in a five-wavelength set, 245 nm (A), 250 nm (B), 255 nm (C), 260 nm (D) and 265 nm (E), as shown in Fig. 1.

Introduction of multiwavelength PDA detectors to the HPLC systems make possible, simultaneous multi-detection of samples at multiwavelengths. The obtained multiwavelength detections produce different peak area information about qualitative and quantitative properties of the analyzed compounds. Simultaneous data collection at multiwavelengths provides the application of multivariate calibration techniques, to these HPLC data for quantitative studies. The application of multivariate methods, including CLS, one specific, and PLS, to the chromatographic data is a new approach for the simultaneous quantitative analysis of NAP and PSE in samples.

As in single wavelength HPLC, HPLC-chemometric data sets were obtained by using the peak-area ratio of each compound to IS *versus* its concentration. Afterwards, these peak area ratios as HPLC data sets were used to construct the multivariate calibrations as HPLC-CLS, HPLC-PCR and HPLC-PLS.

For a comparison of these HPLC-chemometric calibrations, a mixture of the subjected drugs was analyzed by the classic HPLC method based on a single wavelength detection response. The experimental results of the HPLC-chemometric calibration methods were compared with each other, as well as with those obtained by classic-HPLC method.

In the present study, the first aim of multivariate calibration techniques based on the multivariate HPLC data is the elimination of errors resulting from sample injection and an experimental environment that may affect the peak area. In addition, the second aim of this an application is to avoid the repetition of injection. Therefore, HPLC-chemometric calibration permits the removal of errors and residuals of calibration of classic HPLC based on a single wavelength. Thus, the sensitivity, accuracy and precision of the HPLC-chemometric calibrations are bigger that provided by the classic-HPLC method.

Implementation of the multivariate calibration algorithms presented in the theoretical section is applied in the following section.

Processing of HPLC Data A training set consisting of the mixture solution in the concentration range of 20—320 μg/ml NAP and 100—1000 μg/ml PSE with 10 μg/ml IS was prepared. The peak area for the training set was obtained at a five-wavelength set (245, 250, 255, 260, 265 nm) and at the retention time of 5.87 for NAP, 1.345 for PSE and 2.91 for IS. Chromatograms of the training set in the concentration range for both drugs were presented in Fig. 1. The HPLC data set corresponding to the training set was given in Table 1. The chemometric calibration techniques, CLS, PCR, and PLS, were applied to the prepared training set and its measured HPLC data set. The amount of NAP and PSE in samples was determined by the HPLC-chemometric calibrations.

HPLC-CLS Approach The algorithm of HPLC-CLS was briefly explained in the theoretical section. The coefficient vector matrix (K) was calculated using the linear equation system based on the relationship between the peak area data and training set (Table 1). This HPLC data set corresponds to the chromatograms shown in Fig. 1. By replacing the coefficient matrix (K) into the linear equation system, HPLC-CLS calibration was obtained. The prediction of an unknown concentration of NAP and PSE in samples was carried out by the HPLC-CLS calibration. The calibration and data treatment were done by CLS algorithm written in Matlab 7.0 software.

HPLC-PCR Approach The HPLC-PCR calibration was constructed using the PCR algorithm as it was explained in the above theoretical section. In this case, the square matrix of peak area data was obtained by decomposition of peak area values. Linear correlation between the training set and decomposed peak area values was used to obtain the HPLC-PCR calibration. This procedure was applied individually for NAP and PSE, respectively. The obtained HPLC-PCR calibration was subjected to the determination of the above drugs in the synthetic mixtures and tablets. The data given in Table 1 (corresponding to Fig. 1) were used for HPLC-PCR calibration. Previously, the PLS toolbox 3.5 in Matlab 7.0 was used for both the calculation of calibration and data treatment.

HPLC-PLS Approach As in the theoretical outline, PLS calibration algorithm was applied to HPLC data summarized in Table 1, which corresponds to Fig. 1. In this calibration model, both peak area data and concentration set were decomposed. HPLC-PLS calibration was obtained using the relationship between the decomposed peak area data and concentration set. The amount of NAP and PSE in samples was determined using the HPLC-PLS calibration. The mathematical treatments have been performed by means of the PLS toolbox 3.5 in Matlab 7.0 software.

Classical HPLC In the classic HPLC technique, the ratio of peak area of analyte to IS was plotted against the concentration of NAP and PSE. At wavelengths of, 245 nm (A), 250 nm (B), 255 nm (C), 260 nm (D) and 265 nm (E), five linear regression equations for each drug were obtained from the HPLC data given in Table 1. At a specific wavelength of 250 nm (B), a linear equation giving successful results for each drug was selected from Table 2. Satisfactory results were not obtained for the determination of NAP and PSE in samples when the other four wavelengths (245 nm
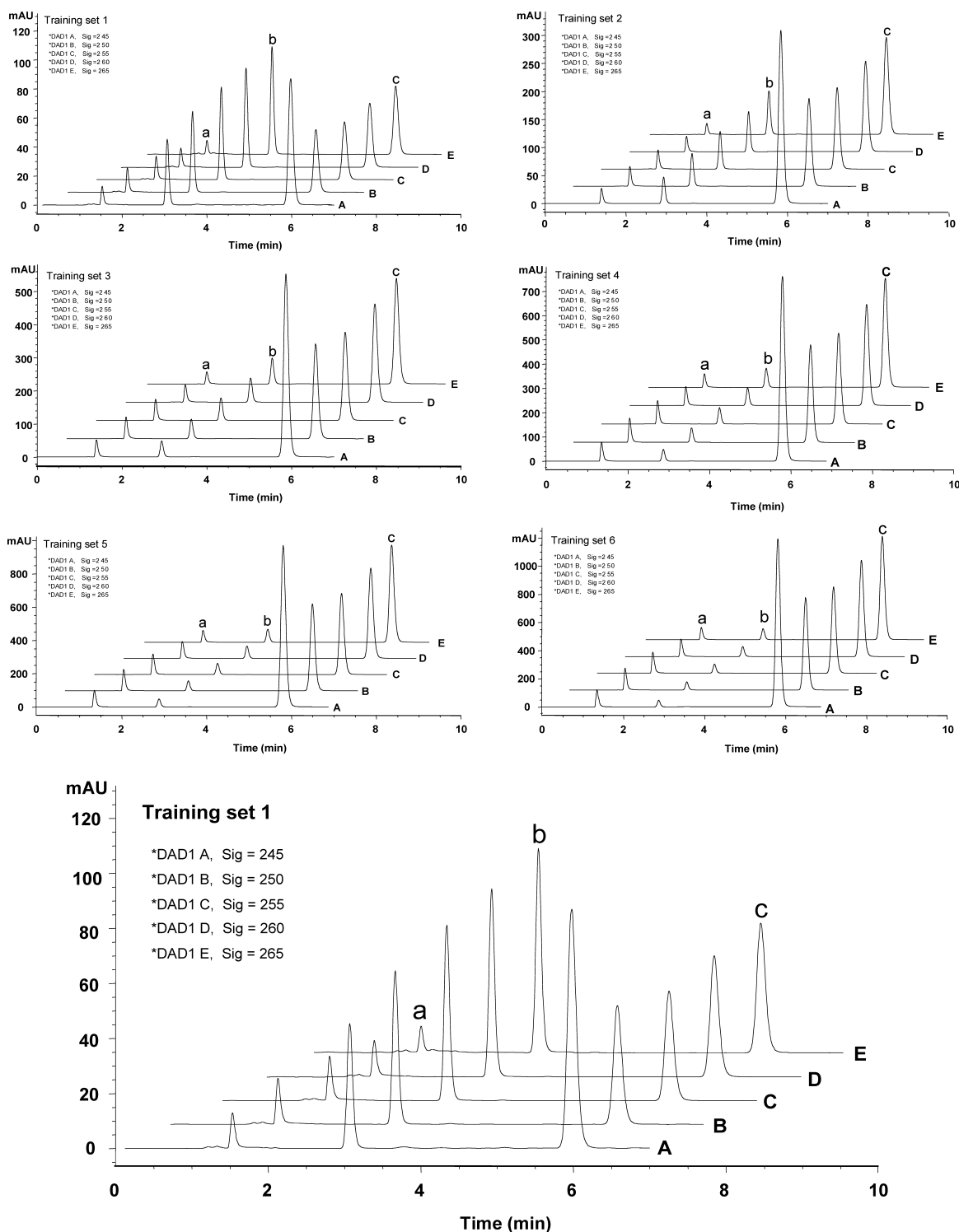
Fig. 1.   HPLC 3-Dimensional Chromatograms of Concentration Set 1 Containing 160 μg/ml PSE (a), 300 μg/ml NAP (c) and IS (b) at Five Different Wavelengths

The small chromatograms correspond to training sets 1—6.

(A), 255 nm (C), 260 nm (D) and 265 nm (E)) were used as individual wavelengths.

All linear regression equations and their statistical parameters are presented in Table 2. The correlation coefficients of regression equations were found to be higher than 0.999. At the subject wavelength point, the calibration equations gave us good linearity for NAP and PSE.

The validity of the HPLC method was assessed by applying a standard addition technique for five replicates. The results are presented in Table 3. In this table, the results indicate that there is no interference from the excipients used in the formulation of the tablets. Microsoft Excel was used for the classic HPLC calculations.

Ability Parameters in Chemometric Calibration   In the HPLC-chemometric calibrations, the predictive ability of a regression model can be defined in several ways. The most

Table 1. The HPLC Data Set Corresponding to the Training Set

No.	Training set			The ratio of peak areas (PSE/IS)					The ratio of peak areas (NAP/IS)				
	PSE	NAP	IS	245	250	255	260	265	245	250	255	260	265
1	100	20	10	0.09566	0.14645	0.19725	0.23437	0.22091	1.71199	1.31275	0.91351	1.14944	1.51481
2	200	80	10	0.18474	0.29583	0.40693	0.48099	0.45632	6.34510	4.88977	3.43443	4.33886	5.72733
3	400	140	10	0.39164	0.59869	0.80575	0.95481	0.90504	11.20485	8.68928	6.17372	7.81704	10.33583
4	600	200	10	0.58823	0.91085	1.23347	1.46324	1.38537	15.91245	12.41119	8.90994	11.28929	14.93404
5	800	260	10	0.77687	1.19558	1.61429	1.91356	1.81722	20.30054	15.92463	11.54873	14.63778	19.38296
6	1000	320	10	0.99315	1.52759	2.06203	2.43820	2.31615	25.08047	19.77797	14.47547	18.35962	24.32076

Table 2. The Calculated Straight Lines and Their Statistical Parameters

Drug	λ (nm)	Regression equation	R	SE(m)	SE(n)	SE(r)
PSE	245	$A=0.0778C+0.1970$	0.9999	9.40×10^{-6}	8.87×10^{-3}	7.33×10^{-3}
	250	$A=0.0615C+0.0457$	0.9999	1.30×10^{-5}	9.37×10^{-3}	1.01×10^{-2}
	255	$A=0.0452C-0.1055$	0.9998	1.87×10^{-5}	9.86×10^{-3}	1.46×10^{-2}
	260	$A=0.0573C-0.1496$	0.9998	2.05×10^{-5}	1.05×10^{-2}	1.60×10^{-2}
	265	$A=0.0760C-0.2170$	0.9998	1.89×10^{-5}	1.21×10^{-2}	1.47×10^{-2}
NAP	245	$A=0.0010C-0.0089$	0.9998	4.90×10^{-4}	1.97×10^{-1}	1.23×10^{-1}
	250	$A=0.0015C-0.0094$	0.9999	3.31×10^{-4}	4.57×10^{-2}	8.31×10^{-2}
	255	$A=0.0020C-0.0099$	0.9998	4.23×10^{-4}	1.06×10^{-1}	1.06×10^{-1}
	260	$A=0.0024C-0.0105$	0.9999	5.56×10^{-4}	1.50×10^{-1}	1.40×10^{-1}
	265	$A=0.0023C-0.0121$	0.9999	7.62×10^{-4}	2.17×10^{-1}	1.91×10^{-1}

SE(m): standard error of slope, SE(n): standard error of intercept, SE(r): standard error of regression constant, C: concentration (μg/ml), A: peak area, r: regression coefficient.

Table 3. Statistical Results for Standard Addition Method

	Standard addition							
	Classical HPLC		HPLC-PCR		HPLC-PLS		HPLC-CLS	
	PSE	NAP	PSE	NAP	PSE	NAP	PSE	NAP
Mean	301.7	164.6	299.5	160.2	299.2	159.6	301.2	159.5
SD	5.45	3.30	1.98	2.28	1.98	3.32	1.94	1.59
RSD	1.81	2.00	0.66	1.42	0.66	2.09	0.65	1.03
SE	2.73	1.65	0.99	1.14	0.99	1.66	0.97	0.79
CL ($P=0.05$)	5.34	3.23	1.94	2.24	1.94	3.26	1.90	1.56

SD: standard deviation, RSD: relative standard deviation, SE: standard error.

Table 4. Statistical Calculations of HPLC-Chemometric Calibrations

Parameter	Classical HPLC		HPLC-PCR		HPLC-PLS		HPLC-CLS	
	PSE	NAP	PSE	NAP	PSE	NAP	PSE	NAP
SEP	2.0481	1.6672	1.1963	1.2972	0.4922	0.8430	1.3383	2.2037
SEC	0.9094	1.3203	0.6494	0.7819	0.5563	0.8390	0.7830	0.6126
r	0.9999	0.9995	0.9999	0.9997	0.9999	0.9996	0.9999	0.9996
n	2.1077	2.9789	2.5145	1.8715	2.4845	2.8005	-1.3501	2.7608
m	0.9781	0.9796	0.9862	0.9864	0.9832	0.9815	0.9952	0.9833

SEP: standard error of prediction, SEC: standard error of calibration, r: regression, n: intercept, m: slope.

general expression is the standard error of calibrations (SEC). In our case, six chromatograms corresponding to the concentration set with IS were used in calibration steps for both drugs. The SEC values of NAP and PSE were calculated by the data obtained from the difference between the added and predicted concentrations in the calibration steps. Linear regression analysis and other statistical results based on the relationship between added and predicted concentra-

tions were obtained. Their statistical results with SEC values are presented in Table 4. The first two factors for HPLC-PCR and HPLC-PLS were found to be reliable for the prediction of both drugs. The above SEC values and other statistical values, correlation coefficient (r), slope (m) and intercept (n), were calculated by HPLC-PCR and HPLC-PLS calibrations using the first two factors.

The standard error of prediction (SEP) values and their

Table 5. The Recovery Results Obtained by Applying the Proposed Methods to Synthetic Binary Mixtures

No.	Added (μg/ml)		Recovery (%)							
			Classic HPLC		HPLC-PCR		HPLC-PLS		HPLC-CLS	
	PSE	NAP	PSE	NAP	PSE	NAP	PSE	NAP	PSE	NAP
1	100	300	99.2	95.3	100.6	98.9	100.6	98.9	98.7	99.2
2	200	300	95.8	100.6	99.1	99.7	99.3	100	99.7	101.4
3	400	300	98.4	100.3	99.2	98.8	99.5	100.1	100.1	100.1
4	600	300	99	98.7	100.2	101.1	99.6	100.1	100.7	99.9
5	800	300	95.7	101.2	99.4	99.1	99.8	100.0	100.6	101.4
6	1000	300	97.4	103.3	98.2	98	99.7	100.0	100.9	95.3
7	160	20	99.4	94.2	100.5	101.5	99.8	98.8	98.1	102.5
8	160	80	96.6	97.6	100.8	101.3	99.4	102.1	98.9	99.3
9	160	140	103	95.7	102.2	102	100	99.9	97.2	99.4
10	160	200	94.5	100.4	99.2	98.5	99.6	99.9	98	95.8
11	160	260	96.3	100	99.2	99.9	99.3	100.0	99.3	96.9
12	160	320	99.4	102.8	97.9	98.8	100.6	98.7	100.6	100.3
	Mean		97.9	99.2	99.7	99.8	99.8	99.9	99.4	99.3
	RSD		2.26	2.83	1.15	1.29	0.42	0.85	1.18	2.16

RSD=relative standard deviation.

Table 6. The Experimental Results of Tablets Prepared by Proposed Methods and Their Statistical Test Results

	Tablet							
	Classic HPLC		PCR		PLS		CLS	
	PSE	NAP	PSE	NAP	PSE	NAP	PSE	NAP
Mean	306.75	164.53	304.42	162.18	303.43	161.91	305.34	163.83
SD	2.24	2.11	1.86	1.74	1.55	1.36	2.07	2.08
RSD	0.73	1.28	0.61	1.07	0.51	0.84	0.68	1.27
SE	1.00	0.94	0.83	0.78	0.69	0.61	0.92	0.93
CL ($P=0.05$)	1.96	1.85	1.63	1.53	1.36	1.19	1.81	1.83
ANOVA test	2.62	2.36	2.62	2.36	2.63	2.36	2.63	2.36
$F_{theoretical}$	3.24	3.24	3.24	3.24	3.24	3.24	3.24	3.24
$F_{calculated}$			1.45	1.46	2.10	2.39	1.18	1.02
$F_{theoretical}$			6.39	6.39	6.39	6.39	6.39	6.39
$t_{calculated}$			0.056	0.0185	0.053	0.088	0.474	0.603
$t_{theoretical}$			2.78	2.78	2.78	2.78	2.78	2.78

Label claim: 300 mg PSE and 160 mg NAP per tablet.

statistical values were calculated according to the difference between added and predicted concentrations in the synthetic mixtures. The obtained results, SEP, correlation coefficient (r), slope (m) and intercept (n), are shown in Table 4.

All the statistical data indicate that the minimum values of SEC and SEP gave us acceptable results under optimized conditions in the calibration and prediction steps.

Method Validation The validation of HPLC-CLS, HPLC-PCR, HPLC-PLS and classic-HPLC was measured by their ability to obtain reliable results of analysis. For this, twelve different synthetic mixtures in the concentration range of 20—320 μg/ml NAP and 100—1000 μg/ml PSE with 10 μg/ml IS were analyzed by the proposed calibration method. The mean recoveries and relative standard deviations of our proposed methods were computed and presented in Table 5. Their numerical values were found satisfactory for the validity of HPLC-CLS, HPLC-PCR, HPLC-PLS and classic-HPLC. The reliable accuracy and higher precision in application of these methods were observed for the analysis of both drugs. During the analysis procedure, interference and systematic errors were not observed.

As another validation procedure, a standard addition technique was used. In this technique, the standard of two pure drugs, equal to the content of tablet formulation, was added to the tablet solutions in the working concentration range. The results and their standard deviations were calculated and are presented in Table 3. The recovery results were obtained from the average of five replicates for each drug. Good coincidence was observed for the standard addition assay results by application of our methods.

Tablet Analysis HPLC-CLS, HPLC-PCR, HPLC-PLS and classic-HPLC techniques were applied to the quantitative analysis of NAP and PSE in tablets. The experimental results related to a pharmaceutical dosage form are presented in Table 6. The results of all the methods were very close to each other, as well as to the labeled value of the commercial pharmaceutical dosage form. Good agreement was reported for all the above applied methods.

Statistical Analysis Three statistical tests, t-test, F-test and ANOVA tests, were applied to the tablet assay results to determine the significance of difference between methods. In the t-test, F-test and ANOVA test, HPLC-CLS, HPLC-PCR and HPLC-PLS were compared with classic-HPLC. Their theoretical and calculated results are shown in Table 6. The calculated statistical test values did not exceed the theoretical statistical values, so there was no significant difference

among the methods. The numerical values of all statistical tests indicate that the investigated techniques are appropriate for the determination of both drugs in the pharmaceutical dosage form.

In analytical study, the HPLC method is frequently used as a comparison method for the analysis of samples. In this study, the HPLC method was called as a classic HPLC method. For good separation and determination, the period of analysis time may get longer in order to find a specific wavelength for measurement of the responses of a detector in some cases. For this reason, the HPLC-chemometric calibration technique plays an important role in the evaluation of chromatograms at the multiwavelength points in presence of PDA responses.

Alternative combined calibration approaches, HPLC-CLS, HPLC-PCR and HPLC-PLS calibration models, were proposed for the simultaneous prediction of a drug amount in samples. This study contains the evaluation of HPLC data for chemometric calibrations. Thus, HPLC-chemometric approaches have many advantages, *e.g.*, robust and reliable results, a decreased the injection size due to the use of a multichromotographic data procedure, and noise elimination due to mainly the mechanisms of the PLS and PCR approaches. We observed that the HPLC-chemometrics approaches give better results than classic HPLC as seen in Tables 4 and 5.

In a practical way good chromatographic separation and determination were satisfactorily performed using classic HPLC and HPLC-chemometric methods under the above mentioned chromatographic conditions. We believe that use of the HPLC method in combination with chemometric calibrations is a viable new alternative determination method that eliminates or reduces inconvenient errors in the chromatographic analysis.

HPLC-chemometric calibration techniques can be successfully applied to the routine and quality control analysis of subject drugs and other drugs in samples.

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