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HPLC and chemometric methods for the simultaneous determination of cyproheptadine hydrochloride, multivitamins, and sorbic acid

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Abstract

Three methods are presented for the simultaneous determination of cyproheptadine hydrochloride (CP), thiamine hydrochloride (B1), riboflavin-5-phosphate sodium dihydrate (B2), nicotinamide (B3), pyridoxine hydrochloride (B6), and sorbic acid (SO). The chromatographic method depends on a high performance liquid chromatographic (HPLC) separation on a reversed-phase, RP 18 column. Elution was carried out with 0.1% methanolic hexane sulphonic acid sodium salt (solvent A) and 0.01 M phosphate buffer containing 0.1% hexane sulphonic acid sodium salt, adjusted to an apparent pH of 2.7 (solvent B). Gradient HPLC was used with the solvent ratio changed from 20:80 to 70:30 (over 9 min), then to 80:20 (over 11 min) for solvent A:B, respectively. Quantitation was achieved with UV detection at 220 and 288 nm based on peak area. The other two chemometric methods applied were principal component regression (PCR) and partial least squares (PLS). These approaches were successfully applied to quantify each drug in the mixture using the information included in the UV absorption spectra of appropriate solutions in the range 250–290 nm with the intervals $\Delta\lambda = 0.4$ nm at 100 wavelengths. The chemometric methods do not require any separation step. The three methods were successfully applied to a pharmaceutical formulation and the results were compared with each other.

Keywords: Cyproheptadine hydrochloride; Multivitamins; Sorbic acid; HPLC; Chemometrics

1. Introduction

Cyproheptadine hydrochloride (CP) is a sedating antihistamine with antimuscarinic, serotonin-antagonist, and calcium-channel blocking action [1]. It is dispensed with some water-soluble vitamins such as thiamine hydrochloride (B1), riboflavin-5-phosphate sodium dihydrate (B2), nicotinamide (B3), and pyridoxine hydrochloride (B6). They are formulated in the form of syrup containing sorbic acid (SO) as antibacterial and antifungal. The studied drugs show a severe overlap between their absorption spectra. There is, in addition, a strong UV absorption due to

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SO [2]. Hence, their simultaneous determination is hard when conventional, derivative, and derivative ratio spectrophotometric techniques are used. The method used to resolve a complex mixture of such drugs is mainly high performance liquid chromatography (HPLC). No analytical method has been reported for the simultaneous determination of CP, B1, B2, B3, B6, and SO in a multicomponent mixture. The application of multivariate calibration such as partial least squares to the absorbance signals produced by drugs during their simultaneous determination in pharmaceutical preparations is an effective means for quality control of their manufacture [3]. Control analyses on pharmaceutical preparations using multivariate calibration method has been proved to be a valid alternative to HPLC [4]. Partial least squares (PLS) is a multivariate calibration method based on factor analysis. PLS-1 performs the optimization of the number of factors for only one component at a time [5]. The basic concept of PLS regression was originally developed by Wold [6]. A detailed description of the mathematical principles of the PLS algorithms have been reported by Martens and Naes [7].

The principle component regression (PCR) is simply a principle component analysis followed by a regression step [8]. PLS and PCR were found to be specially suited to multicomponent analysis, particularly for mixtures with highly overlapped spectra [9]. The utility of PCR and PLS methods in analysis of multivitamins drug mixtures has been published for the determination of B6 with different drugs such as B1, B2, B3, in composite vitamin B tablets [10], B1 in vitamin tablets [11], metoclopramide [12], diazepam [13], meclozine [12], vitamin B12 with dexamethasone sodium phosphate [14], and B1 with acetylsalicylic acid and caffeine [9].

The aim of this paper is to investigate the ability of PLS and PCR methods for quantifying six components mixture of CP, B1, B2, B3, B6 as relatively weakly absorbing components with overlapping UV spectra, formulated at a lower concentration and SO as major strongly absorbing component, without prior separation, and to apply the optimized models in pharmaceutical preparation. In addition, an HPLC method was developed for the assay of the components of the studied mixture. The proposed methods are simple, accurate, reduced the duration of the analysis, and are suitable for routine determination of the six components of the mixture.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Kyoto, Japan) UV-Vis spectrophotometer, model UV-1601 PC equipped with 1 cm quartz cells and connected to an IBM compatible computer and HP600 inkjet printer was used. The bundled software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}. PLS and PCR analyses were carried out by using PLS-Toolbox software version 2.1-PC [15] for use with MATLAB 5.

The HPLC (Shimadzu) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A degasser, Rheodyne 7725i injector with a 20 μl loop and an SPD-10 AVP UV-Vis detector. Separation and quantitation were made on a 250 mm × 4.6 mm (i.d.) Waters XTerra™ RP 18 column (5 μm particle size). The detector was set at $\lambda = 220$ nm for 14 min, then changed to 288 nm till the end of run. Data acquisition was performed on class-VP software.

2.2. Materials and reagents

Pharmaceutical grade CP (Dipharma, Italy), B1, B2, B3, B6 (all vitamins were supplied by F. Hoffmann-La Roche Ltd., Switzerland) and SO (Merck, Germany) were used and certified by the supplier to contain 99.9, 99.8, 99.7, 99.9, 99.8, and 99.9%, respectively. The methanol used was HPLC grade (BDH, Poole, UK). Potassium dihydrogen phosphate, hexane sulphonic acid sodium salt, hydrochloric, and phosphoric acids were analytical grade.

Phosphate buffer (0.01 M) was prepared by dissolving 1.36 g of KH_2PO_4 in 1 L of distilled water, adjusted to pH 2.7 using 1 M phosphoric acid.

Periavit syrup (Pharaonia Pharmaceuticals, New Borg El-Arab City, Alexandria, A.R.E.) was used. Each 5 ml syrup contains 2 mg CP, 5 mg SO, and 1.2 mg of each of B1, B2, B3, and B6.

2.3. HPLC conditions

The elution was carried out with 0.1% methanolic hexane sulphonic acid sodium salt (solvent A) and 0.01 M phosphate buffer containing 0.1% hexane sulphonic acid sodium salt, adjusted to an apparent pH of 2.7 (solvent B). Gradient HPLC with the ratio changing from 20:80 to 70:30 (over 9 min), then to 80:20 (over 11 min) for solvent A:B, respectively with gradient curve 6 (which is a concentration delivery parameter) was performed. This is an exponential concentration curve. The concentration curve that is implemented is derived according to the following equation:

$$C(t) = C_A + (C_B - C_A)\frac{e^{(6t/T)} - 1}{e^6 - 1}$$

where $C(t)$ is the concentration at time t and T is the total time.

The flow rate was 1 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Standard solutions and calibration

Standard solutions of each of CP, B1, B2, B3, B6, and SO were prepared in 0.1 M hydrochloric acid (for spectrophotometric methods) or solvent A of the mobile phase (for HPLC) within the concentration range of 2.4–5.6 μg ml^{-1} for CP, 1.4–3.4 μg ml^{-1} for each of B1, B2, B3, B6, and 6–14 μg ml^{-1} for SO.

2.4.1. For PLS and PCR methods

A training set of 45 synthetic mixtures with different concentrations of each compound were prepared in 0.1 M hydrochloric acid in range of 2.4–5.6 μg ml^{-1} for CP, 1.4–3.4 μg ml^{-1} for each of B1, B2, B3, B6, and 6–14 μg ml^{-1} for SO. The UV absorption spectra were recorded over the range 250–290 nm. The data points of the spectra were collected at every 0.4 nm. The computation was made in PLS-Toolbox software version 2.1.

PCR and PLS-1 models were applied to the UV absorption spectra of these mixtures using six principal component for PCR and six latent variables for PLS-1 for determination of each compound.

2.4.2. For HPLC method

Triplicate 20 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations. Linear relationships were obtained.

2.5. Sample preparation

A volume of the syrup equivalent to 0.4 mg of CP, 1 mg of SO, and 0.24 mg of each of B1, B2, B3, and B6 was diluted to 100 ml with 0.1 M hydrochloric acid (for spectrophotometric methods) or solvent A of the mobile phase (for HPLC method). The general procedures for PCR, PLS-1, and HPLC methods described under calibration were followed and the concentration of each compound was calculated.

3. Results and discussion

3.1. PCR and PLS-1 methods

Fig. 1 shows the UV absorption spectra of CP, B1, B2, B3, B6, and SO at their nominal concentrations in syrup. As can be seen, CP and each of the vitamins contribute very little to overall absorption of the sample; also, the absorption band of SO is extensively overlapped with CP and the vitamin spectra. The simultaneous determination of CP, B1, B2, B3, B6, and SO in syrup by conventional, derivative, and derivative ratio spectrophotometric methods is thus hindered by strong spectral overlap throughout the wavelength range. PLS or PCR calibration method were necessary for such determination due to the presence of this spectral interference.

The quality of multicomponent analysis is dependent on the wavelength range and spectral mode used [16]. In this work, spectral resolution was assayed with absorbance spectra for PLS-1 and PCR methods, measured at 0.4 nm intervals over the range 250–290 nm. Wavelengths less than 250 nm were rejected due to the differences between the synthetic mixture and pharmaceutical syrup spectra.

Wavelengths more than 290 nm were not used because B1 and B3 do not absorb in this region, so any absorbance values obtained at these wavelengths would have introduced a significant amount of noise

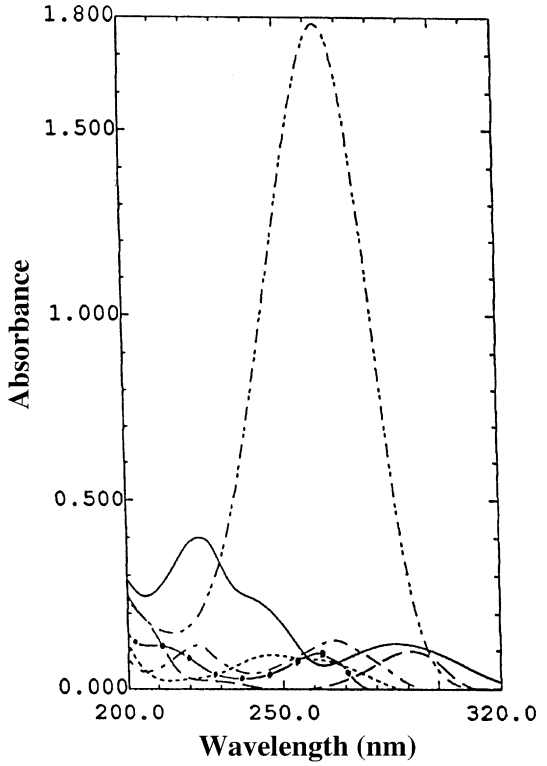

Fig. 1. UV absorption spectra of 4 μg ml^{-1} of CP (—), 2.4 μg ml^{-1} of B1 (. . .), 2.4 μg ml^{-1} of B2 (– - –), 2.4 μg ml^{-1} of B3 (–·–), 2.4 μg ml^{-1} of B6 (– – –), and 10 μg ml^{-1} of SO (– - - –) in 0.1 M hydrochloric acid.

in the calibration matrix, thereby decreasing the precision.

To select the number of factors in the PLS-1 and PCR algorithms, a cross-validation method leaving out one sample at a time [17] was employed using a training (calibration) set of 45 calibration spectra. The predicted concentrations of the components in each sample were compared with the actual concentrations in these training samples and the root mean square error of cross-validation (RMSECV) was calculated for each method. The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It plays the same role as standard deviation in indicating the spread of the concentration errors [18].

Appropriate selection of the number of factors to be used to construct the model is a key to achieving correct quantitation in PLS-1 and PCR calibrations.

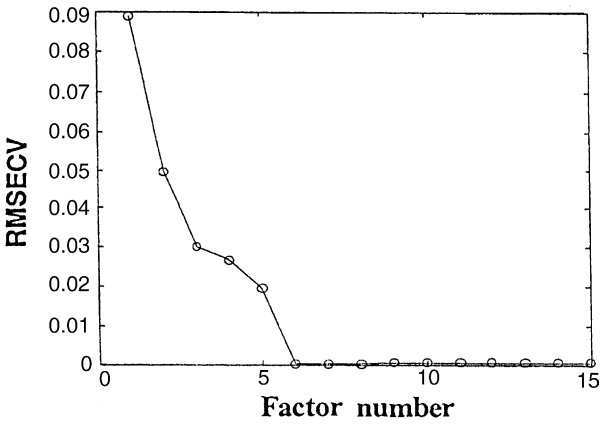

Fig. 2. RMSECV plot of a calibration set prediction using cross-validation of PLS-1 model for CP.

The method developed by Haaland and Thomas [19] was also used for selecting the optimum number of factors. A number of factors of 6 was found to be optimum for each component by the PLS-1 and PCR methods as in Figs. 2 and 3. The selected model is that with the smallest number of factors such that RMSECV for that model is not significantly greater than RMSECV from the model with additional factors.

The evaluation of the predictive abilities of the models was performed by plotting the actual known concentrations against the predicted concentrations [20]. The results are obtained in Table 1. Satisfactory correlation coefficient (r) values were obtained for each compound in the training set by PLS-1 and PCR optimized models indicating good predictive abilities of

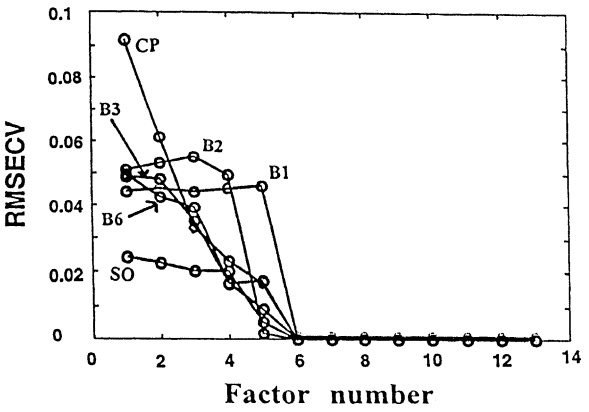

Fig. 3. RMSECV plot of a calibration set prediction using cross-validation of PCR model for CP, B1, B2, B3, B6, and SO.

Table 1
RMSECV and statistical parameter values for simultaneous determination of CP, B1, B2, B3, B6, and SO using PLS-1 and PCR methods

Item	Method	Compound					
		CP	B1	B2	B3	B6	SO
RMSECV	PLS-1	3.10×10^{-4}	9.14×10^{-4}	1.39×10^{-4}	1.87×10^{-4}	3.12×10^{-4}	1.09×10^{-4}
	PCR	3.20×10^{-4}	9.22×10^{-4}	1.40×10^{-4}	1.92×10^{-4}	3.15×10^{-4}	1.13×10^{-4}
Intercept	PLS-1	1.52×10^{-5}	5.53×10^{-5}	6.99×10^{-6}	-4.60×10^{-15}	3.47×10^{-6}	2.67×10^{-5}
	PCR	-3.20×10^{-6}	1.05×10^{-4}	-1.10×10^{-5}	-6.60×10^{-6}	-1.10×10^{-15}	7.18×10^{-6}
Slope	PLS-1	0.99996	0.99997	0.99999	1.00000	0.99998	0.99997
	PCR	1.00000	0.99996	1.00000	1.00000	1.00000	0.99999
Correlation coefficient (r)	PLS-1	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	PCR	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
S.E. of intercept	PLS-1	1.80×10^{-4}	6.90×10^{-4}	9.51×10^{-5}	1.30×10^{-4}	1.99×10^{-4}	6.72×10^{-5}
	PCR	2.20×10^{-4}	7.40×10^{-4}	1.00×10^{-4}	1.50×10^{-4}	2.40×10^{-4}	7.10×10^{-5}
S.E. of slope	PLS-1	4.40×10^{-4}	2.80×10^{-3}	4.00×10^{-4}	5.50×10^{-4}	8.20×10^{-4}	6.60×10^{-5}
	PCR	5.20×10^{-4}	2.90×10^{-3}	4.10×10^{-4}	6.00×10^{-4}	1.00×10^{-3}	7.07×10^{-5}

the models. The RMSECV obtained by optimizing the calibration matrix of the absorption spectra for the PLS-1 and PCR methods are shown in Table 1 indicating good accuracy and precision.

Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. The residuals appear randomly distributed around zero, indicating adequate models as shown in Figs. 4 and 5.

The quantitative prediction abilities of PLS and PCR for spectral analyses are compared. It is difficult to generalize about the superiority of one method over another, because the relative performance of the methods is often dependent on particular data set being analyzed. The major difference between these two methods is that PLS seems to predict better than PCR in the cases when there are random linear base lines or independently varying major spectral components which overlap with the spectral features of the analyte [21].

3.2. HPLC method

The developed HPLC method was applied to the simultaneous determination of CP, B1, B2, B3, B6, and SO. The mobile phase composition and pH were studied and optimized. A satisfactory separation was obtained with gradient elution as described in Section 2. Quantitation based on peak area was achieved with UV detection at 220 nm for 14 min, changing to 288 nm to decrease base line noise during the change in the composition of the mobile phase, and to give suitable response of the separated compounds. The specificity of the HPLC method is illustrated in Fig. 6 where complete separation of the six compounds was noticed. The average retention time±standard deviation for B1, B3, B6, B2, SO, and CP were found to be 2.5 ± 0.02, 6.4 ± 0.03, 10.2 ± 0.05, 12.1 ± 0.04, 16.3 ± 0.06, and 18.5 ± 0.09 min, respectively, for 10 replicates.

3.3. Analysis of pharmaceutical syrup

The proposed PLS-1, PCR, and HPLC methods were applied to the simultaneous determination of CP, B1, B2, B3, B6, and SO in commercial syrup. Seven replicates determination were made. Satisfactory results were obtained for each compound in good agreement with label claims (Table 3). No published method has been reported for simultaneous determination of the components of this mixture. The results of the proposed PLS-1 and PCR methods were also compared with those of the proposed HPLC method. Statistical comparison between the results was performed with regards to accuracy and precision using Student's t-test and F-ratio at 95% confidence level (Table 3). There was no significant difference between the results.

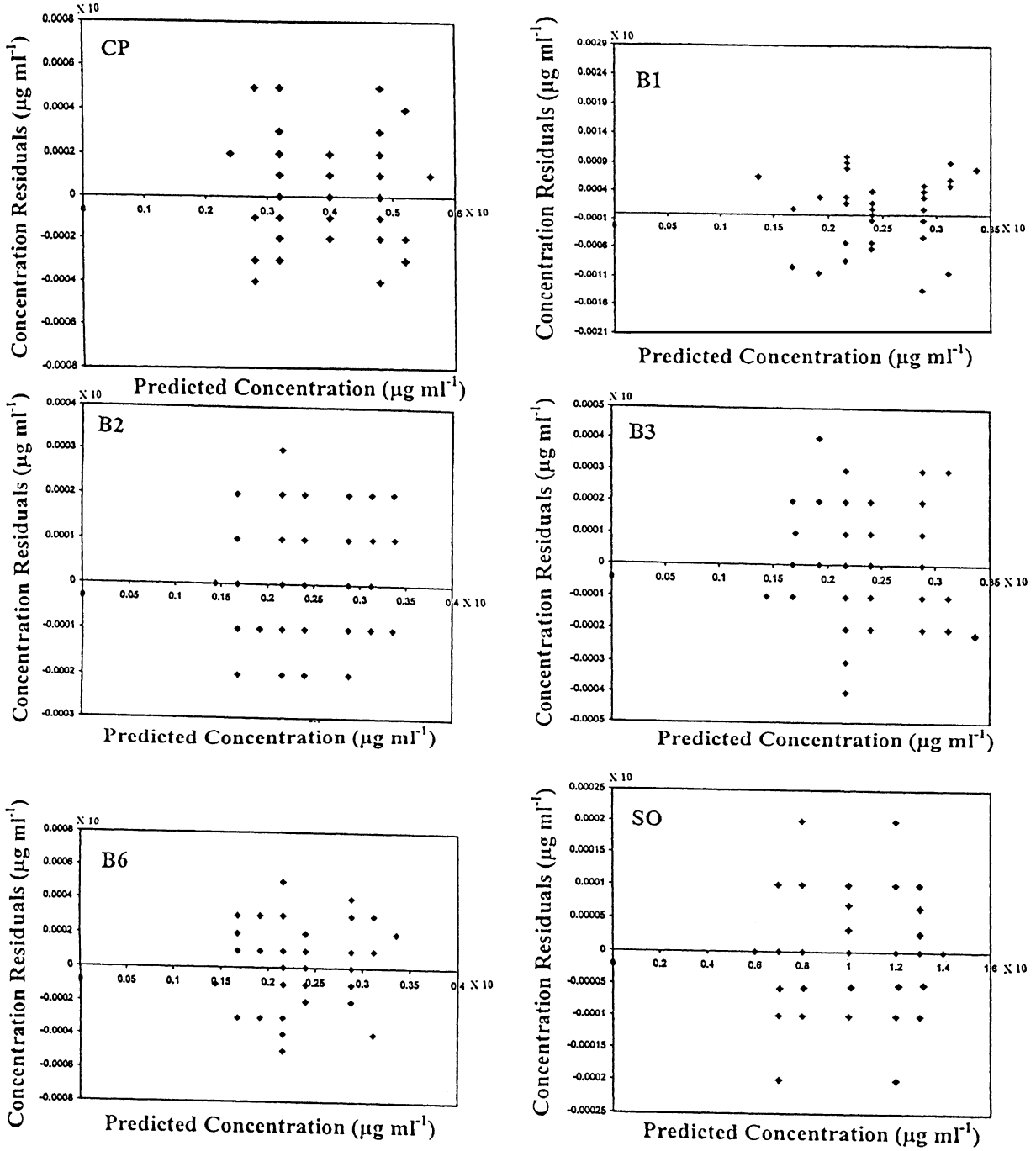

Fig. 4. Concentration residuals vs. predicted concentration of CP, B1, B2, B3, B6, and SO using PLS-1.

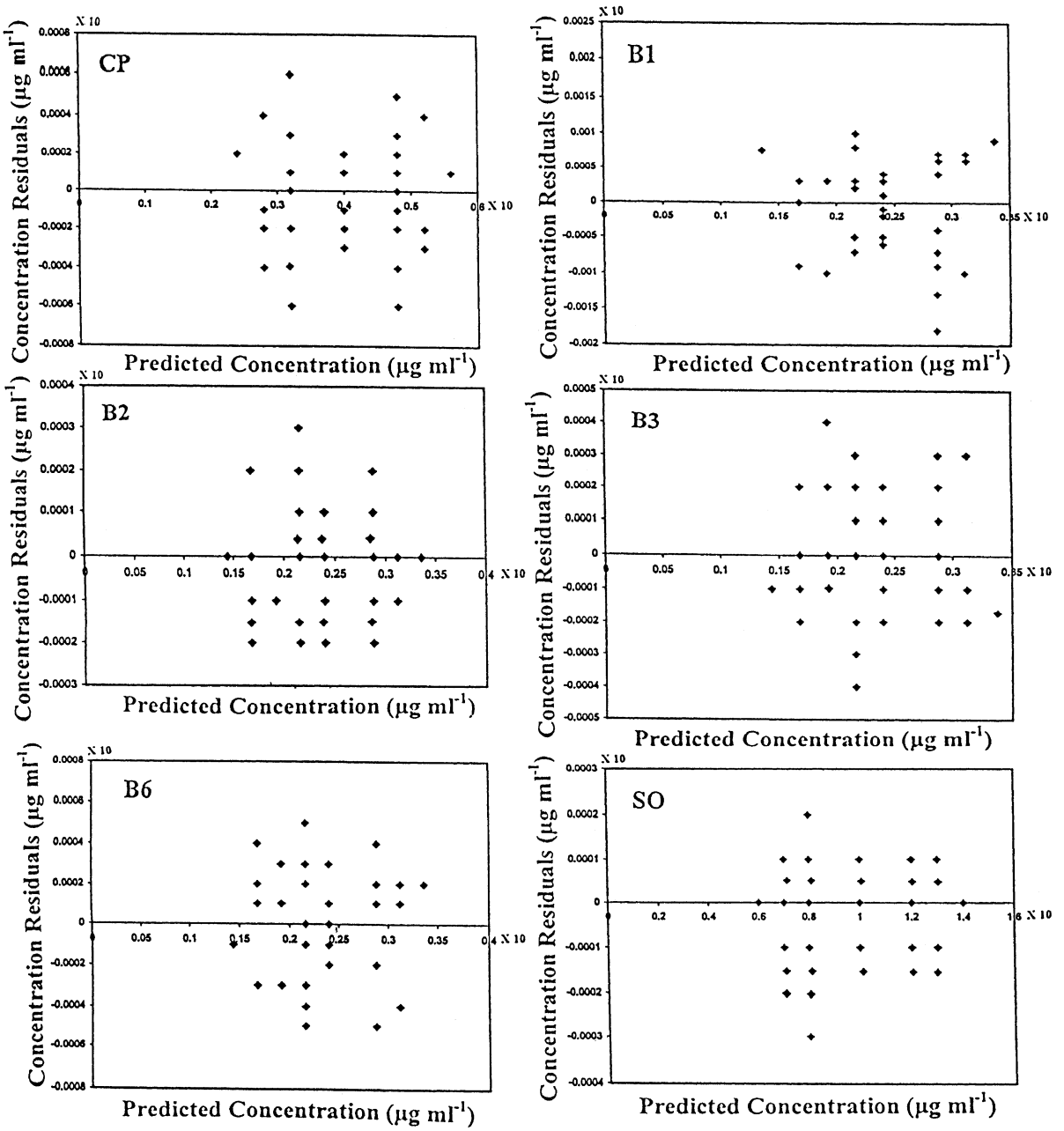

Fig. 5. Concentration residuals vs. predicted concentration of CP, B1, B2, B3, B6, and SO using PCR.

3.4. Validation of the methods

3.4.1. Linearity

The linearity of the HPLC detector response for determination of CP, B1, B2, B3, B6, and SO was evaluated by analyzing a series of different concentrations of each compound. According to the International Conference on Harmonization [22], at least five concentrations must be used. In this study, eight concentrations were chosen, ranging between 2.4 and

Table 2
Characteristic parameters of the calibration equations for the proposed HPLC method for simultaneous determination of CP, B1, B2, B3, B6, and SO

Parameters	CP	B1	B2	B3	B6	SO
Calibration range ($\mu g\,ml^{-1}$)	2.4–5.6	1.4–3.4	1.4–3.4	1.4–3.4	1.4–3.4	6–14
Detection limit ($\mu g\,ml^{-1}$)	0.19	0.06	0.07	0.06	0.08	0.22
Quantitation limit ($\mu g\,ml^{-1}$)	0.63	0.20	0.23	0.20	0.27	0.73
Regression equation (Y)[a]: slope (b)	285.3×10^3	183.0×10^3	357.7×10^3	894.8×10^3	391.4×10^3	241.9×10^3
Standard deviation of the slope (S_b)	3.6×10^3	2.4×10^3	5.0×10^3	11.4×10^3	6.4×10^3	2.6×10^3
Relative standard deviation of the slope (%)	1.26	1.31	1.39	1.27	1.63	1.07
Confidence limit of the slope[b]	282.2×10^3–288.5×10^3	180.9×10^3–185.2×10^3	353.3×10^3–362.0×10^3	884.9×10^3–904.7×10^3	385.8×10^3–379.0×10^3	239.6×10^3–244.2×10^3
Intercept (a)	-3.1×10^3	-2.1×10^3	0.3×10^3	-0.3×10^3	14.3×10^3	-9.6×10^3
Standard deviation of the intercept (S_a)	15.1×10^3	6.1×10^3	12.4×10^3	28.5×10^3	16.0×10^3	24.5×10^3
Confidence limit of the intercept[b]	(-30.8×10^3)–11.6×10^3	(-7.4×10^3)–3.1×10^3	(-10.4×10^3)–11.1×10^3	(-25.0×10^3)–24.3×10^3	0.4×10^3–28.2×10^3	(-30.8×10^3)–11.6×10^3
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9999	0.9998	0.9999
Standard error of estimation	9.65×10^3	2.11×10^3	4.30×10^3	9.83×10^3	5.54×10^3	9.65×10^3

[a] $Y = a + bC$, where C is the concentration of compound in $\mu g\,ml^{-1}$ and Y is the peak area.
[b] 95% confidence limit.

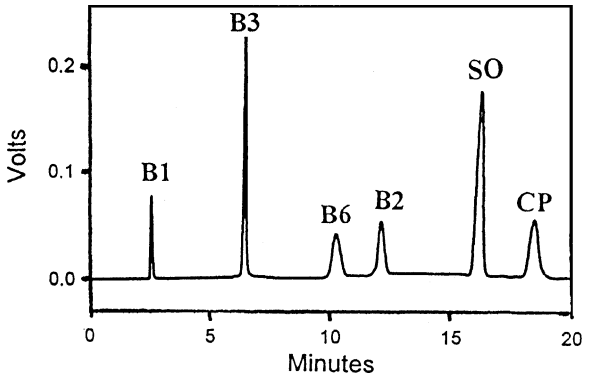

Fig. 6. HPLC chromatogram of 20 μl injection of syrup sample containing 4 μg ml^{-1} of CP, 2.4 μg ml^{-1} of each of B1, B2, B3, B6, and 10 μg ml^{-1} of SO.

5.6 μg ml^{-1} for CP, 1.4 and 3.4 μg ml^{-1} for B1, B2, B3, B6, and 6 and 14 μg ml^{-1} for SO. Each concentration was repeated three times; this approach provided information on the variation in peak area between samples of same concentration. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($P < 0.05$) different from zero. Characteristic parameters for regression equations of the HPLC method obtained by least squares treatment of the results are given in Table 2.

3.4.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each compound. The data for each concentration level were evaluated by one-way ANOVA. An 8 days X 2 replicates design was performed. Statistical comparison of the results was performed using the *P*-value of the *F*-test. Three univariate analyses of variance for each concentration level were made. Since the *P*-value of the *F*-test was always greater than 0.05, there was no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

3.4.3. Range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in pharmaceutical product, to give accurate, precise, and linear results. The calibration range of the proposed HPLC method is given in Table 2.

3.4.4. Detection and quantitation limits

According to ICH recommendations [22], 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 and are given in Table 2.

Table 3
Determination of CP, B1, B2, B3, B6, and SO in synthetic mixtures and commercial syrup using the proposed methods

	Mean found ± S.D.[a]		
	PLS-1	PCR	HPLC
Synthetic mixtures			
For CP	100.0 ± 0.57	100.0 ± 0.67	100.2 ± 0.81
For B1	99.9 ± 0.50	100.0 ± 0.53	100.4 ± 0.64
For B2	99.9 ± 0.35	100.0 ± 0.36	100.1 ± 0.43
For B3	100.0 ± 0.47	100.0 ± 0.38	99.9 ± 0.51
For B6	100.0 ± 0.42	100.0 ± 0.44	99.9 ± 0.69
For SO	100.0 ± 0.35	100.0 ± 0.41	100.0 ± 0.49
Commercial syrup			
For CP	99.7 ± 0.49	99.8 ± 0.40	100.0 ± 0.77
t	0.87	0.61	(2.18)[b]
F	2.47	3.70	(4.28)[b]
For B1	100.1 ± 0.51	100.1 ± 0.54	100.3 ± 0.62
t	0.66	0.64	(2.18)[b]
F	1.48	1.32	(4.28)[b]
For B2	99.7 ± 0.57	99.8 ± 0.50	100.0 ± 0.74
t	0.85	0.59	(2.18)[b]
F	1.69	2.19	(4.28)[b]
For B3	99.9 ± 0.31	100.0 ± 0.26	100.2 ± 0.49
t	1.36	0.95	(2.18)[b]
F	2.49	3.55	(4.28)[b]
For B6	100.0 ± 0.69	100.1 ± 0.59	99.8 ± 0.82
t	0.49	0.78	(2.18)[b]
F	1.41	1.93	(4.28)[b]
For SO	99.9 ± 0.42	100.0 ± 0.52	100.2 ± 0.74
t	0.93	0.59	(2.18)[b]
F	3.10	2.03	(4.28)[b]
Recovery[c]			
For CP	100.0 ± 0.41	100.0 ± 0.43	100.1 ± 0.55
For B1	100.1 ± 0.43	100.0 ± 0.47	99.9 ± 0.50
For B2	100.1 ± 0.54	100.0 ± 0.41	100.2 ± 0.72
For B3	99.9 ± 0.49	100.0 ± 0.52	100.2 ± 0.44
For B6	100.0 ± 0.57	100.2 ± 0.61	99.8 ± 0.77
For SO	100.0 ± 0.61	100.0 ± 0.58	100.1 ± 0.69

[a] Mean and S.D., percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F*.
[c] For standard addition of 50% of the nominal content.

3.4.5. Selectivity

Methods selectivity was achieved by preparing different synthetic mixtures (or validation set) of the studied compounds at various concentrations. The synthetic mixtures were analyzed according to the previous procedures described under the proposed methods. Satisfactory results were obtained (Table 3), indicating the high selectivity of the proposed methods for simultaneous determination of the studied compounds.

3.4.6. Accuracy

This study was performed by addition of known amounts of the studied compounds to a known concentration of the commercial pharmaceutical syrup (standard addition method). The resulting mixtures were analyzed and results obtained were compared with the expected results. The excellent recoveries of standard addition method (Table 3) suggested the good accuracy of the proposed methods.

The influence of the commonly used syrup excipients (glycerin, saccharin sodium, sucrose, ethanol, and mandarin oil) was investigated before the determination of the studied compounds in syrup. No interference could be observed with the proposed methods.

3.4.7. Robustness

Variation of pH of the solvent B of the mobile phase by ± 0.1 did not have significant effects on chromatographic resolution in the HPLC method. Variation of strength of hydrochloric acid by ± 0.02 M did not have significant effect on the UV-based chemometric methods.

3.4.8. Stability

The studied compound solutions in solvent A of the mobile phase or 0.1 M hydrochloric acid exhibited no chromatographic or absorbance changes for 24 h when kept at room temperature, and for 5 days when stored refrigerated at $5\,^{\circ}$C.

4. Conclusion

Two chemometric methods in spectrophotometric analysis, PLS-1 and PCR, are proposed for the simultaneous determination of CP, B1, B2, B3, B6, and SO in their six component mixture. These techniques were applied successfully to a commercial pharmaceutical syrup. The assay results obtained using these chemometric methods were compared with the proposed HPLC method and good coincidence was observed. Although the HPLC method is more specific than the chemometric spectrophotometric methods it needs expensive equipment and materials such as columns and HPLC grade solvents. Chemometric methods are less expensive by comparison and they do not require sophisticated instrumentation and any prior separation step. This can be considered an advantage for these chemometric techniques over HPLC. But they need software for resolution and determination of the components of the mixture. The chemometric methods proposed are very powerful techniques for the simultaneous analysis of multicomponent mixtures in which the spectra of the active compounds overlap with each other and also, by the fact that zero-order spectra is enough for the analysis, there is no need for the spectrophotometer to have any other modes such as derivation and ratio spectra. The proposed three methods, PLS-1, PCR, and HPLC, were found to be suitable for the routine analysis of the component of pharmaceutical syrup containing CP, B1, B2, B3, B6, and SO.

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