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A new technique for spectrophotometric determination of Pseudoephedrine and Guaifenesin in syrup and synthetic mixture

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The accuracy in predicting different chemometric methods was compared when applied on ordinary UV spectra and first order derivative spectra. Principal component regression (PCR) and partial least squares with one dependent variable (PLS1) and two dependent variables (PLS2) were applied on spectral data of pharmaceutical formula containing Pseudoephedrine (PDP) and Guaifenesin (GFN). The ability to derivitize in resolved overlapping spectra chloropheniramine maleate was evaluated when multivariate methods are adopted for analysis of two component mixtures without using any chemical pretreatment. The chemometrics models were tested on an external validation dataset and finally applied to the analysis of pharmaceuticals. Significant advantages were found in analysis of the real samples when the calibration models from derivative spectra were used. It should also be mentioned that the proposed method is a simple and rapid way requiring no preliminary separation steps and can be used easily for the analysis of these compounds, especially in quality control laboratories.

Keywords: derivative; partial least squares (PLS); Pseudoephedrine and Guaifenesin; chemometrics; spectrophotometer; principal component regression (PCR)

Introduction

Cough and cold pharmaceutical preparations are one of the most extended formulations in the world and have many pharmaceutical forms: syrup, suspension, sachets, capsules, and tablets.[1] These preparations represent complex formulae containing several active ingredients and a broad spectrum of excipients, such as flavouring agents, saccharose or aspartame, acidulants, natural or artificial colouring and flavouring agents, dyes, sweeteners, and preservatives.[2,3] The majority of these ingredients are present as a mixture of basic amino compounds and their separation in pharmaceutical forms is quite complicated due to similarities of their physical and chemical properties.[4] The combination of antihistamine such as pyrilamine maleate (PA) and chlorpheniramine maleate (CLP) is used to overcome the allergic effects and reduce or relieve cold symptoms.[5]

The assay of the drug product in the stability test sample needs to be determined using the stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines[6] and USP-26.[7] Although the stability indicating methods have been reported for the assay of various drugs, most of them describe assay procedures for drug products containing only one active drug substance. Only a few stability indicating methods are reported for the assay of combined drug products, containing two or more active drug substances. The objective of this work, for the first time, was to develop a simple, precise, and rapid analytical procedure, which would serve as a stability indicating assay for the combined drug product of Pseudoephedrine (PDP) and Guaifenesin (GFN) in syrup.

PDP and GFN hydrochlorides are widely used in combination with other drugs for the clinical treatment of common cold, sinusitis, bronchitis, and respiratory allergies.[8] Various methods have been used for the determination of PDP or GFN in cough and cold pharmaceuticals.[9–16]

Different methods were developed to determine of drugs in pharmaceutical products.[17–22] Despite the success in the field of pharmaceutical analysis, the methods based on ordinary spectrophotometry have proven to be often unsatisfactory for the analysis of multicomponent mixtures, owing to their low spectral resolution.[23,24] In contrast, derivative spectrophotometry has been successful in resolving complex mixtures because of its ability in extracting high analytical information from spectra composed of overlapped bands. This technique provides

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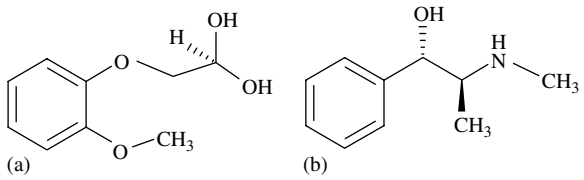

Figure 1. Structural formulae for (A) Guaifenesin and (B) Pseudoephedrine.

a better resolution of the spectral overlap by magnifying small differences between the spectral curves.[25–29] Moreover, minimization of the nonspecific matrix interference is reached by elimination of baseline drifts.[30–32] A very recent review describes derivative spectrophotometry as a powerful tool in pharmaceutical analysis.[33]

In addition, several multicomponent determinations based on the application of these chemometrics to spectrophotometric data have been reported.[34–37] These techniques cannot provide a resolution of the spectral overlap special for real samples, because they have complex matrix.

The combined use of derivative spectrophotometry and chemometric techniques has been demonstrated to be a highly convenient choice in the determination of multicomponent matrices presenting serious spectra overlapping, thanks to their common potential ability to exploit minor spectral features.[38–41]

This work describes a new method for the simultaneous PDP and GFN (Figure 1) determination in syrup in the presence of the overlapping spectra with the PCR, PLS1, and PLS2.

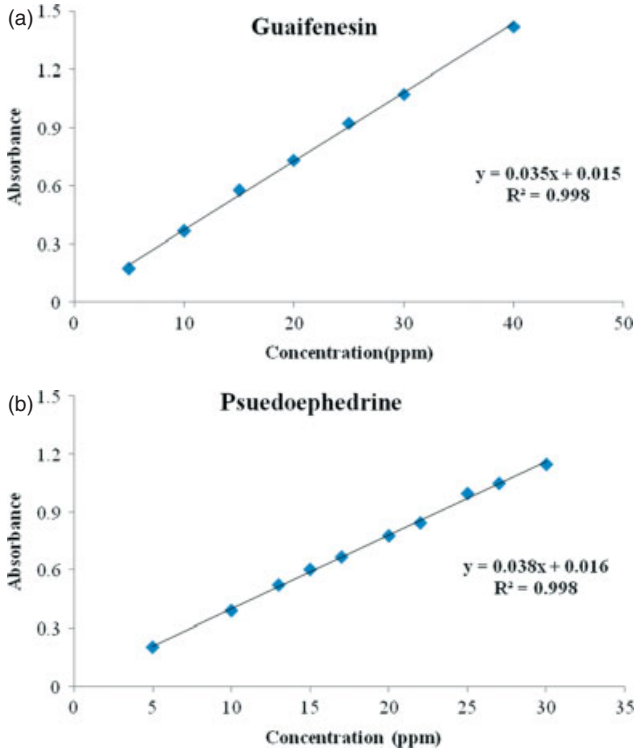

Figure 2. Analytical curve for the univariate determination of (A) Guaifenesin (λ_{max} = 222 nm, A=0.035C_{GFN} + 0.015 and R^2 = 0.998); and (B) Pseudoephedrine (λ_{max} = 207 nm, A=0.038C_{PDP} + 0.016 and R^2 = 0.998).

Experimental

Reagents and chemicals

All reagents were of analytical grade and all the solutions were prepared with distilled water. Phosphate salts (Merck) and phosphoric acid (Merck) were used to prepare buffer solutions (PH=5-8). Expectorant syrup (containing PDP and GFN) was manufactured by Behsa pharmaceutical Co. (Arak, Iran) for Zahravi pharma. Co. with a label of containing 30 mg and 100 mg of the PDP and GFN, respectively.

Apparatus and software

The electronic absorption measurements were carried out on a Perkin Elmer Lambda 800 dual beam spectrophotometer (slit width of 2 nm and scan rate of 400 nm/min) using 1.00 cm quartz cells. The absorption spectra were recorded over the wavelength range of 200–350 nm against a blank (phosphate buffer with PH=6). The PLS1, PLS2, PCR and derivative spectrophotometry (DS) calculations were performed with the software package 'The Unscrambler 9. 8' (Camo Software As, Oslo, Norway).[42]

Stock and standard solutions

Stock solutions of PDP and GFN, containing 100 µg mL^{-1} were prepared in 100-ml volumetric flasks, by dissolving 10 mg of each compound in some drops methanol and distillated water. Working standard solutions were prepared daily by diluting the stock solutions for each drug according to its linear calibration range, 5–30 and 5–40 µg mL^{-1} for PDP and GFN, respectively (Figures 2A and 2B). Two sets of standard solutions were prepared, the calibration set and prediction set which contained 30 and 5 standard solutions, respectively. To a series of 10-ml volumetric flasks, aliquots of PDP and GFN solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml. UV spectra of the mixture were recorded in the wavelength range 200–350 nm versus the blank, while the digitized absorbance was sampled at 1.0 nm intervals. All the solutions were freshly prepared.

Procedure to determine Pseudoephedrine and Guaifenesin in pharmaceutical formulations

A volume of the syrup equivalent to 5 mg guaifenesin, 1.5 mg pseudoephedrine was diluted to 100 mL with distilled water. Further dilution was carried out with the buffer to reach the calibration range of each compound.

Chemometrics Techniques

Experimental Design (ED)

Prediction ability of a regression model is largely affected by the experimental design in building the calibration set. A careful selection of the reference solutions increases the chances of improving quality of information from the calibration set. Statistical ED is a powerful technique for choosing the component concentrations so that to minimize the total number of samples in covering the defined experimental region.

The chemometrics model calibration was optimized with the aid of the orthogonal design method. A set of standard samples was prepared according to a five-level orthogonal array design (OAD) which led to 30 samples, so that the concentration of each

Table 1. Concentration data of the different mixtures used in the calibration set for the determination of Guaifenesin (GFN) and Pseudoephedrine (PDP) concentration values are expressed as $\mu g ml^{-1}$

Sample	GFN	PDP
1	5.00	5.00
2	5.00	10.00
3	5.00	15.00
4	5.00	20.00
5	5.00	25.00
6	5.00	30.00
7	12.00	5.00
8	12.00	10.00
9	12.00	15.00
10	12.00	20.00
11	12.00	25.00
12	12.00	30.00
13	19.00	5.00
14	19.00	10.00
15	19.00	15.00
16	19.00	20.00
17	19.00	25.00
18	19.00	30.00
19	26.00	5.00
20	26.00	10.00
21	26.00	15.00
22	26.00	20.00
23	26.00	25.00
24	26.00	30.00
25	33.00	5.00
26	33.00	10.00
27	33.00	15.00
28	33.00	20.00
29	33.00	25.00
30	33.00	30.00

Table 2. Concentration data of the different mixtures used in the calibration set for the determination of GFN and PDP concentration values are expressed as $\mu g ml^{-1}$

Sample	GFN	PDP
1	6.00	9.50
2	7.50	23.00
3	17.50	13.00
4	22.50	8.00
5	37.50	28.00

drug in the resulting solutions was in its own linear dynamic range (Table 1). For the prediction set, five mixtures were prepared, not being included in the calibration set, and were employed as an independent test (Table 2).

Multivariate analysis

PCR and PLS are factor analysis methods, based on a two-stage procedure: a calibration step, in which a mathematical model is built by using component concentrations and spectral data from a set of references, followed by a prediction step in which the model is used to calculate the concentrations of an unknown sample from its spectrum.

Table 3. Statistical parameters calculated from application of PCR and PLS methods to full spectra of calibration samples

		PCR		PLS1		PLS2	
		^0D	^1D	^0D	^1D	^0D	^1D
GFN	RMSEP	0.1065	0.0840	0.1062	0.1059	0.1113	0.0845
	R^2	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999
	PC	3	3	3	2	3	3
PDP	RMSEP	0.1630	0.1076	0.1530	0.1066	0.1533	0.1089
	R^2	0.9996	0.9998	0.9997	0.9998	0.9997	0.9998
	PC	6	5	5	5	5	4

Both algorithms transform the original variables into a smaller number of orthogonal variables called principal components (PCs), which are linear combinations of the original variables. When multivariate calibration approaches are applied in spectrophotometric multicomponent analysis, a relationship between spectral and concentration data from reference samples, representing the variables of the system, is established. A new matrix constituted by the new variables PCs and scores is built. The calculation of this new matrix is planned by an algorithm specific to the regression method adopted. PCs represent the absorptivity values of the samples at the various concentration and wavelength values, whereas the scores represent the numerical coefficients. Their combination allows to build the mathematical model representing the reference spectra and able to predict the component concentrations of new samples.

Determination of PC number

The optimal number of principal factors is essential in building multivariate models. The prediction error decreases with the number of PCs used to reach an optimal value. Most information is usually contained in the first PCs but it is not assured that the useful information is exclusively reserved to these factors. Full cross validation is the most used validation method, in which one reference at a time is removed from the calibration set; after that the same sample is predicted by using the calibration built with the other references. Several tests have been proposed to select the number of PCs. The Root Mean Square Error of Prediction (RMSEP) was chosen to express the prediction error when PCR and PLS procedures were applied. This parameter represents an estimate of the error when other samples are predicted with that model. The best prediction ability of the models is reached when the prediction error is at its lowest value.

$$\mathrm{RMSEP} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_i - \hat{x}_i)^2\right]^{0.5} \quad (1)$$

where x_i is the true concentration of the analyte in the sample i, $\hat{x}_i$ represents the estimated analyte concentration in the sample i, $\overline{x}$ is the mean of the true concentration in the prediction set and n is the total number of samples used in the prediction set. The values of RMSEP for the PDP and GFN mixtures are summarized in Table 3.

Another important statistical parameter in evaluating the model quality is R^2. It represents an index of quality in fitting all data to a

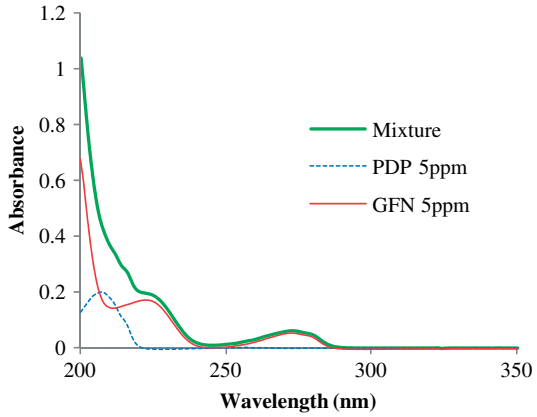

Figure 3. Absorbance spectra of the Guaifenesin (5 μg mL^{-1}, λ max = 222 nm), Pseudoephedrine (5 μg mL^{-1}, λ max = 207 nm) and their mixture.

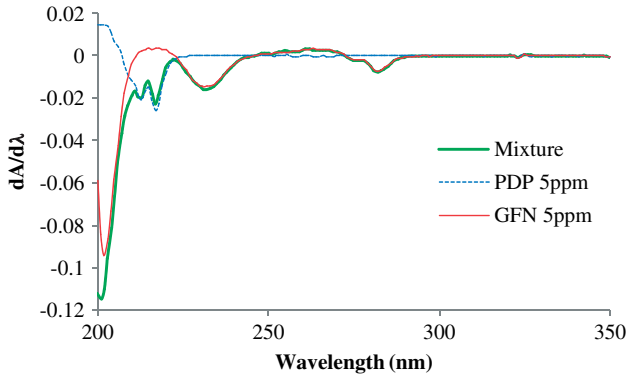

Figure 4. First-order derivative spectra of the Guaifenesin (5 μg mL^{-1}), Pseudoephedrine (5 μg mL^{-1}) and their mixture.

straight line and represents the fraction of total variance explained by the model. It is computed as:

$$R^2 = \sum_{i=1}^{n}(\hat{x}_i - x_i)^2 / \sum_{i=1}^{n}(x_i - \bar{x}_i)^2 \quad (2)$$

where $\bar{x}$ represents the mean of the true concentrations in the prediction set.

Results and Discussion

Data processing and model building

The absorption UV spectra of pure PDP, GFN and their mixture are shown in Figure 3. The wavelength range 200–300 nm was considered throughout because the absence of absorbance after 300 nm for all the drugs. Quantitative determination by ordinary spectrophotometry, which uses measurements at discrete wavelengths, was proven to be inaccurate because of the extensive overlap of the spectral curves. With the aim to extract the most significant analytical information from the spectral data, the mathematical derivatives of the absorbance values against wavelengths (dA/dλ) were calculated. Figure 4 shows the first order derivative spectra of the two drugs.

PCR, PLS1, and PLS2 calibration models were first developed on data from the full-range spectra of ordinary and derivative and a comparative study of the three chemometric approaches was undertaken. The models were validated by full cross-validation and the statistical parameters RMSEP and R^2 were recalculated when a new factor was added to the models. Table 3 lists the optimal PCs and the corresponding statistical parameters. RMSEP values were satisfactory for all the models. PCs resulted ranging between 3 and 6, showing the higher values in correspondence of PDP determination. This table represents by applying chemometrics methods on derivative relative just chemometrics methods not only reduce RMSEP but also reduce PC numbers.

Analogously, the values of RMSE of cross-validation and test for calibration and prediction sets showed that use of derivative spectra precision of determination significantly can be improved (Table 4).

Table 4. Comparison of statistical parameters of cross validation and test set

			Calibration		Prediction	
			$RMSE_{cr.val}$	$RMSE_{test}$	$RMSE_{cr.val}$	$RMSE_{test}$
GFN	PLS1	^{0}D	0.1182	0.0931	0.1331	0.2237
		^{1}D	0.0880	0.0751	0.0978	0.1567
	PLS2	^{0}D	0.1235	0.0981	0.1388	0.2310
		^{1}D	0.0859	0.0749	0.0955	0.1497
	PCR	^{0}D	0.1193	0.0938	0.1341	0.2249
		^{1}D	0.0886	0.0749	0.0978	0.1548
PDP	PLS1	^{0}D	0.2966	0.2836	0.3807	0.5511
		^{1}D	0.1697	0.1588	0.2138	0.3106
	PLS2	^{0}D	0.1138	0.1090	0.1425	0.1491
		^{1}D	0.0840	0.0830	0.1028	0.1120
	PCR	^{0}D	0.3049	0.3073	0.4393	0.6173
		^{1}D	0.0939	0.0833	0.1181	0.1738

Recovery study

In order to test the prediction ability of the constructed models, a series of synthetic mixtures PDP and GFN was analyzed. A prediction set of five samples, containing the drugs in the same concentration ranges used for the calibration set, was randomly prepared. The above defined PCR and PLSs calibrations were applied to the prediction set and the models were tested by comparing accuracy, in terms of percentage recovery reported in Table 5.

In order to assess the applicability of the proposed method to the analysis of real samples, determination of the drugs was conducted in different mixtures of their pharmaceutical formulations. Thus, different mixtures of the commercially available PDP and GFN syrups were prepared and analyzed with the PLS-1, PLS-2 and PCR methods. Each measurement was repeated three times and the obtained mean recovery values are presented in Table 6. It can be observed that in all mixtures the calculated values are in satisfactory agreement with the declared values in the derivative and chemometrics methods. For instance, recovery of PDP in PLS1 method without derivative is 68.93, but with applying derivative to will be 101.5. Furthermore, applying chemometrics methods on derivative reduce PC numbers. For example, in GFN, PC numbers in PCR reduce from 13 to 5. According these results we conclude that applying chemometrics method on derivative spectra largely improved determination of compound with high overlapping in

Table 5. The predicted concentrations obtained by the application of the PLS method and the first-order derivative spectra on the absorption UV spectra for the simultaneous determination of Guaifenesin (μg mL^{-1}) and Psuedoephedrine (μg mL^{-1}) in binary mixtures

PLS1:

	GFN					PDP			
	Found		Recovery			Found		Recovery	
Experimental	^{0}D	^{1}D	^{0}D	^{1}D	Experimental	^{0}D	^{1}D	^{0}D	^{1}D
6.00	5.76	5.90	96.0	98.3	9.50	9.49	9.63	99.9	101.4
7.50	7.54	7.57	100.5	100.9	23.00	23.12	23.20	100.5	100.9
17.50	17.56	17.68	100.3	101.0	13.00	13.27	13.24	102.1	101.8
22.50	22.81	22.77	101.4	101.2	8.00	8.03	8.10	100.4	101.2
37.50	37.66	37.49	100.4	100.0	28.00	27.90	27.77	99.6	99.2

PLS2:

	GFN					PDP			
	Found		Recovery			Found		Recovery	
Experimental	^{0}D	^{1}D	^{0}D	^{1}D	Experimental	^{0}D	^{1}D	^{0}D	^{1}D
6.00	5.76	5.84	96.0	97.3	9.50	9.57	9.64	100.7	101.5
7.50	7.55	7.55	100.7	100.7	23.00	23.14	23.12	100.6	100.5
17.50	17.77	17.65	100.5	100.8	13.00	12.77	12.86	98.2	98.9
22.50	22.81	22.73	101.4	101.0	8.00	7.95	7.80	99.4	104.0
37.50	37.69	37.41	100.5	99.8	28.00	25.29	26.79	90.3	95.7

PCR:

	GFN					PDP			
	Found		Recovery			Found		Recovery	
Experimental	^{0}D	^{1}D	^{0}D	^{1}D	Experimental	^{0}D	^{1}D	^{0}D	^{1}D
6.00	5.76	5.88	96.0	98.0	9.50	9.46	9.56	99.6	100.6
7.50	7.54	7.56	100.5	100.8	23.00	23.10	23.15	100.4	100.6
17.50	17.76	17.68	101.5	101.0	13.00	13.28	13.24	102.2	101.8
22.50	22.81	22.76	101.4	101.2	8.00	8.05	8.08	100.6	101.0
37.50	37.66	37.50	100.4	100.0	28.00	27.80	27.76	99.3	99.1

Table 6. Recovery and PC studies of GFN and PDP in real sample using PCR and PLS methods

			PLS1		PLS2		PCR	
		Actual	^{0}D	^{1}D	^{0}D	^{1}D	^{0}D	^{1}D
GFN	Concentration	25.00	25.10	24.93	25.29	24.76	27.06	25.07
	PC		9	3	10	6	13	5
	Recovery		100.4	99.7	101.2	99.0	108.2	100.3
PDP	Concentration	7.50	5.17	7.61	6.40	7.72	7.56	7.52
	PC		8	5	8	6	9	5
	Recovery		68.93	101.5	85.3	102.9	100.8	100.3
			PLS1		PLS2		PCR	
		Actual	^{0}D	^{1}D	^{0}D	^{1}D	^{0}D	^{1}D
GFN	Concentration	25.00	31.94	24.93	32.04	24.76	31.92	25.07
	PC		3	3	6	6	5	5
	Recovery		127.8	99.7	128.2	99.0	127.7	100.3
PDP	Concentration	7.50	12.43	7.61	12.48	7.72	12.96	7.52
	PC		5	5	6	6	5	5
	Recovery		165.7	101.5	166.4	102.9	172.8	100.3

spectra special for determination in real samples with complex matrix.

Conclusions

UV–visible spectral data can be transformed in increasing derivative orders so to obtain more useful information. Application of chemometric methods to derivative UV spectra can magnify the prediction ability of this spectrophotometric technique. A comparative study on the application of the multivariate calibration methods PCR, PLS1, and PLS2 on first order derivative spectra of two mixtures of PDP and GFN has been accomplished.

According to the results obtained in this work, application of the UV spectrophotometric method is an effective and accurate way for the simultaneous determination of PDP and GFN in binary mixtures by multivariate calibration of synthetic and pharmaceutical samples. In addition, the application of this method is simple, fast, precise and affordable, requiring no complex pretreatment or chromatographic the sample separations containing analytes.

According to these studies, multivariate calibration methods (PCR, PLS1, PLS2) coupled with derivative spectral data can be recommended as a very suitable choice to resolve severe overlapped absorption spectra of drug mixtures. This approach is simple in application, inexpensive, requires an easy treatment of the samples, and provides reliable analytical results.

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