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Spectrophotometric simultaneous determination of triamterene and hydrochlorothiazide in Triamterene-H tablets by multivariate calibration methods

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Abstract

The multivariate calibration methods of partial least-square regression and principal component regression were applied for the simultaneous spectrophotometry determination of triamterene (TRM) and hydrochlorothiazide (HYD) in their mixtures. The parameters of the chemometric procedure were optimized, and the proposed methods were validated with synthetic samples and applied to analyze these drugs in pharmaceutical products with good accuracy and precision. The results were compared with those given by the British Pharmacopoeia (BP) method. The square of the correlation coefficients (R^2) for predicted TRM and HYD with the proposed method in a test sample were 0.9994 and 0.9992, respectively. The relative standard deviation for commercial tablets in the proposed method and BP standard method were 0.405 and 2.142%, respectively.

Keywords: Triamterene; Hydrochlorothiazide; Pharmaceutical analysis; Multivariate calibration

1. Introduction

Triamterene (2,4,7-tri-amino-6-phenylpteridine) (TRM) is used commonly in combination with hydrochlorothiazide (6chloro-3,4-dihydro-2*H*-1, 2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide) (HYD). The mixture of these two drugs is used in treatment for reducing edema and medium hypertension. This combination, Triamterene-H, is commercialized for human treatment. Therefore, the determination of these drugs is a frequent analytical problem in quality control of the pharmaceutical industries. The two drugs studied in this work show a strong overlap between their absorption spectra. Hence, their simultaneous determination is hard when conventional spectrophotometric techniques are used [1–3]. Normally, the method used to resolve a complex mixture of these drugs is mainly high-performance liquid chromatography (HPLC) [4,5].

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In recent years, multivariate calibration methods have been used to resolve mixtures of two or more compounds with similar spectral characteristics. Partial least-squares (PLS) is a multivariate calibration method based on factor analysis, and PLS-1 and PLS-2 types have been described. PLS-2 differs from PLS-1 in the way used to perform the signal decomposition and the regression analysis. The basic concept of PLS regression was originally developed by Wold [6], and the use of the PLS method for chemical analysis was pioneered by Wold et al. [7,8]. A detailed description on the mathematical principles of the PLS algorithms have been reported by Martens and Naes [9] and other workers [10–12]. Principal component regression (PCR) is simply a principal component analysis followed by a regression step [9,13,14]. PLS is related to PCR in that the spectral decomposition is also performed, but this decomposition step is performed differently. In PCR, the information about the concentrations is not used, while PLS use both spectral data and concentration data in the modeling [9–14].

In most cases, multivariate methods plus spectroscopic data have such advantages as simplicity and no expensive. Therefore, these methods have been applied to the determination of drugs [15–21], because HPLC methods and conventional spectroscopic methods were slow, expensive and complex.

The aim of this paper is to investigate the ability of PLS and PCR methods for quantifying binary mixtures of triamterene and hydrochlorothiazide without prior separation and to apply the optimized models in pharmaceutical preparations.

2. Materials and methods

2.1. Materials

Commercial samples of Triamterene-H were bought from pharmacies. Analytical grade TRM and HYD were obtained from Food and Drug Control Laboratories (Tehran, Iran). All other chemical and solvents were of analytical reagent grade.

2.2. Apparatus and software

A Shimadzu UV-2101 scanning spectrophotometer connected to a PC fitted with UV-2101 data software was used for all the measurements and treatment of data.

The Chemometric toolbox and Statistic toolbox of MATLAB 5.2 software was used for the statistical treatment of the data and application of various multivariate methods.

2.3. Procedure

Accurately weighed amounts of finely powdered pure TRM and HYD were placed in two 50 ml volumetric flasks and methanol was added. The volumetric flasks were subjected to ultrasonication for 20 min by a laboratory ultrasonic water-bath. After cooling and further dilution with methanol, the calibration and synthetic mixture mixing of these two solutions with different ratios produced test sets of samples. Compositions of two commercial Triamterene-H tablets, which were purchased from Sobhan and Irandaru companies, are summarized in Table 1. For these tablets, after grinding and homogenizing, an accurately weighed set of ten Triamterene-H tablets, 0.05 g each, was used for analysis. Each weighed sample was mixed with 80 ml methanol and the mixture was subjected to ultrasonication for 20 min. After cooling and further dilution to 100 ml with methanol, a 10 ml portion of the sample was centrifuged at 3000 r.p.m. A 2-ml portion of the supernatant was then diluted to 50 ml with methanol. The absorption spectra between 200 and 600 nm against methanol were recorded for all solutions.

The stability of TRM and HYD and commercial sample solutions were checked for 8 h, and the UV–Vis absorption spectra of all sample solutions were found to be stable for this period of time. It is also to be noted that the simultaneous determination of the aforementioned two drugs with the proposed method can be carried out in less than 1 h.

Commercial Triamterene-H tablets were also analyzed using the British Pharmacopoeia (BP) method [4].

Table 1
Composition of commercial Triamterene-H tablets

Sobhan		Irandaru	
Component	Amount (per tablet)	Component	Amount (per tablet)
Triamterene	50 mg	Triamterene	50 mg
Hydrochlorothiazide	25 mg	Hydrochlorothiazide	25 mg
Lactose	65–85% (w/w)	Lactose	65–85% (w/w)
Corn starch	5–25% (w/w)	Corn starch	5–25% (w/w)
Colloidal silicon dioxide	0.1–0.5% (w/w)	Povidone	0.5–5% (w/w)
Carboxymethyl cellulose sodium	1–6% (w/w)	Crospovidone	2–5% (w/w)
Talc	5–30% (w/w)	Magnesium stearate	0.25–5% (w/w)
Magnesium stearate	0.25–5% (w/w)		

3. Results and discussion

3.1. UV–Vis spectra of TRM and HYD

In Fig. 1, the absorption spectra of standard TRM and HYD solutions recorded between 200 and 600 nm are shown. The two drugs studied show a strong overlap in their absorption spectra, and the univariate analysis method cannot be applied for resolving this mixture.

3.2. Experimental design of sample sets

Calibration and test sets for two component systems were designed according to factorial principles. Solutions containing drug concentrations in the range 0.0–4.0 p.p.m. for HYD and 0.0–8.0 p.p.m. for TRM were produced by dilution of the stock solutions. A five-level factorial design was used to produce a full set of 25 samples. A three-level set was derived from this to produce a calibration set of nine samples, with the remaining 16 samples used for an independent test set [22]. The compositions of the used calibration and test sets are summarized in Tables 2 and 3, respectively.

3.3. Selection of the optimum number of factors and the spectral region

To select the correct number of factors in the PLS and PCR algorithm, a cross-validation method, leaving out one sample at a time, was employed [9].

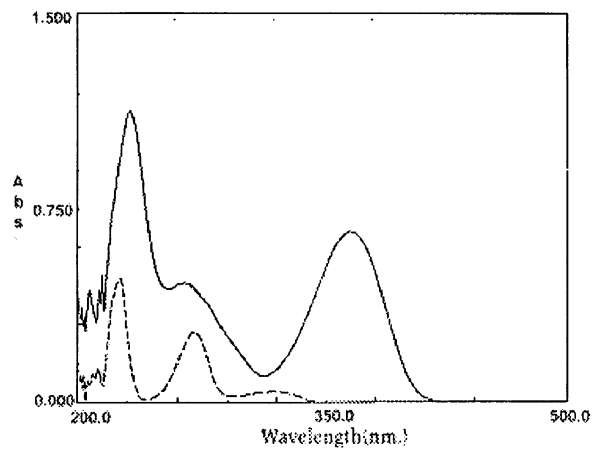

Fig. 1. The absorption spectra of TRM (top) and HYD (bottom) standard solutions.

Table 2
Calibration set composition

Standard	TRM (mg/l)	HYD (mg/l)
P1	0	0
P2	0	2
P3	0	4
P4	4	0
P5	4	2
P6	4	4
P7	8	0
P8	8	2
P9	8	4

Table 3
Test set composition

Sample	TRM (mg/l)	HYD (mg/l)
1	0	1
2	0	3
3	2	0
4	2	1
5	2	2
6	2	3
7	2	4
8	4	1
9	4	3
10	6	0
11	6	1
12	6	2
13	6	3
14	6	4
15	8	1
16	8	3

Table 4
PRESS values obtained for the calibration set

Method	Compound	Number of factors	PRESS
PCR	TRM	4	0.1244
	HYD	4	0.1244
PLS-1	TRM	4	0.0928
	HYD	5	0.0127
PLS-2	TRM	4	0.1011
	HYD	4	0.1011

For the set of nine spectra, PLS-2, PLS-1 and PCR calibration on eight calibration spectra were performed and, using this calibration, the concentration of the samples left out during the calibration process was performed. These processes were repeated a total of nine times until each sample had been left out once. The predicted concentrations ($\hat{X}_{ij}(K)$) of the compounds in each sample, obtained with k factors, were compared with the already known concentrations (X_{ij}) and the prediction error sum of squares (PRESS) was calculated for each of the k factor levels as follows:

$$\mathrm{PRESS}(k) = \sum_{i=1}^{r} \sum_{j=1}^{c} (X_{ij} - \hat{X}_{ij}(K))^2$$

The optional value for k is the level that yields the smallest PRESS(k) value [9]. To check the selected factor number, the external validation method was also used by simply computing the mean squared error of predication (MSEP) for I objects in test set for each of the k factor levels as follows:

$$\mathrm{MSEP}(k) = \sum_{i=1}^{r} \sum_{j=1}^{m} (Z_{ij} - \hat{Z}_{ij}(k))^2 / I^2$$

where Z_{ij} is the known concentration in the test set and is predicted for concentrations with k factors [9]. This showed that the external test set validation and the internal cross-validation indicated about the same number of factors.

To select the spectral region, all of the top steps used repeatedly and the spectral region that lead to the lowest values of MSEP was selected [17]. The spectral region between 246 and 358 nm was selected for analysis and, as a consequence, 113 experimental points per spectrum were used.

The optimal number of factors and PRESS values obtained by PLS-2, PLS-1 and PCR algorithms are summarized in Table 4.

The proposed PLS-2, PLS-1 and PCR calibration models were evaluated by prediction of drug concentrations in their own designed calibration set, obtaining recoveries between 98.4 and 102.8% for TRM, and between 99.4 and 102.3% for HYD.

3.4. Statistical parameters for the optimized models

Using the internal validation in their own designed calibration set, the following statistical parameters have been obtained.

1. The values of root mean square error of estimation (RMSEE), which is an indication of the average error in the analysis for each component.
2. The square of the correlation coefficients (R^2), which is an indication of the quality of the straight line that fits the data.

In Table 5, the results obtained for these parameters by PCR, PLS-1 and PLS-2 are shown. We can see that the R^2 values are in some cases very near to 1 and in some cases equal to 1, which is an indication of similarity between predicted and known values. On the other hand, in general

terms, the statistical parameters obtained by PLS-1 and PLS-2 are better than PCR. Hence, the PLS-1 and PLS-2 methods were selected as more adequate to resolve the binary mixture of drugs.

3.5. Validation of PLS-1 and PLS-2 models

Sixteen synthetic mixtures in the test set were predicted by applying both PLS-1 and PLS-2 methods. The recovery values obtained using the calibration models in the resolution of the test set by PLS-1 and PLS-2 are summarized in Table 6. Satisfactory values are obtained in most of mixtures analyzed by the methods.

The results of PLS models also were compared with the actual values. Table 7 presents linear regression statistics for prediction results. For PLS-1 and PLS-2 models, an intercept significantly equal to zero and a slope significantly equal to unity were achieved (95% confidence level). PLS-2 and PLS-1 models are reliable because these models have small confidence intervals and large R^2 and F-ratio values [23].

The standard deviation and relative standard deviation for three standards of test sample that were randomly selected are given in Table 8.

Limits of detection (LODs) were calculated as three standard error of estimation (SEE) values [24]. SEE values were calculated by using the expression:

Table 5
Statistical parameters of the models optimized

	PCR		PLS-1		PLS-2	
	RMSEE	R^2	RMSEE	R^2	RMSEE	R^2
TRM	0.0485	0.9998	0.0365	1.0000	0.0361	0.9998
HYD	0.0260	0.9998	0.0199	0.9998	0.0258	1.0000

Table 6
The recovery percentage of TRM and HYD from test set solutions

Sample	PLS-1				PLS-2			
	TRM		HYD		TRM		HYD	
	Found (mg/l)	%Rec	Found (mg/l)	%Rec	Found (mg/l)	%Rec	Found (mg/l)	%Rec
1	0.01	–	0.93	92.6	0.01	–	0.94	93.7
2	0.00	–	2.99	99.6	0.00	–	3.01	100.4
3	1.93	96.3	−0.03	–	1.92	96.0	−0.02	–
4	1.95	97.6	0.98	97.5	1.95	97.3	0.99	99.0
5[a]	2.01	100.4	1.95	97.6	2.00	100.0	1.97	98.5
6	2.02	101.0	3.06	101.8	2.01	100.6	3.08	102.7
7	1.98	99.0	3.96	98.9	1.97	98.5	3.98	99.5
8	4.05	101.3	0.97	97.2	4.04	101.0	0.99	99.4
9[a]	4.05	101.3	3.03	100.9	4.05	101.2	3.02	100.7
10	6.04	100.7	−0.01	–	6.04	100.6	0.00	–
11[a]	6.12	102.0	0.98	98.4	6.12	102.0	0.99	99.1
12	5.95	99.1	1.98	99.2	5.94	99.1	1.99	99.3
13	6.05	100.8	3.01	100.4	6.05	100.8	3.02	100.5
14	5.99	99.9	3.97	99.2	5.99	99.8	3.98	99.6
15	8.00	100.0	0.96	96.3	8.00	100.0	0.98	98.2
16	7.82	97.8	2.96	98.6	7.82	97.7	2.96	98.8

[a] Mean of three measurements.

Table 7
Regression statistics for predicted versus actual TRM and HYD values in test set samples

Method	Compound	Intercept ± CI[a]	Slope ± CI	R^{2b}	F-ratio[c]
PLS-1	TRM	0.008 ± 0.062	1.002 ± 0.013	0.9992	24906.64
	HYD	−0.033 ± 0.027	1.008 ± 0.012	0.9992	31875.94
PLS-2	TRM	0.002 ± 0.062	1.002 ± 0.014	0.9994	24404.85
	HYD	−0.022 ± 0.025	1.007 ± 0.011	0.9992	39109.75

[a] Confidence interval (95% confidence level).
[b] The square of correlation coefficients.
[c] Variance modeled by regression to residual variance ratio.

Table 8
Precision for synthetic mixtures in the test set ($n = 3$)

	Sample	TRM		HYD	
		S.D.	%R.S.D.	S.D.	%R.S.D.
PLS-1	5	0.014	0.720	0.001	0.057
	9	0.010	0.230	0.004	0.147
	11	0.019	0.314	0.008	0.808
PLS-2	5	0.014	0.716	0.010	0.055
	9	0.010	0.250	0.004	0.122
	11	0.022	0.354	0.009	0.931

Table 9
Analysis of commercial tablets[a]

	Irandaru		Sobhan	
	TRM ± %R.S.D.[b]	HYD ± %R.S.D.[b]	TRM ± %R.S.D.[b]	HYD ± %R.S.D.[b]
Declared contents	50	25	50	25
PLS-1 results	48.75 ± 0.639	27.19 ± 0.119	49.34 ± 0.239	26.75 ± 0.397
PLS-2 results	48.70 ± 0.690	27.20 ± 0.248	49.28 ± 0.247	26.50 ± 0.509
BP results	48.71 ± 2.224	26.46 ± 1.787	49.27 ± 2.497	25.25 ± 2.416

[a] Results presented as milligrams per tablet.
[b] Relative standard deviation for three measurements.

$$\mathrm{SEE} = \sqrt{\frac{\sum_{i=1}^{n}(C_i - \hat{C}_i)^2}{n - l - 1}}$$

where n is the number of standards in the calibration test, l is the number of independent variables in the calibration equation, C_i is the actual concentration of the analyte in the sample I, and $\hat{C}_i$ represents the predicted concentration of the analyte in the sample i. LODs of 164.1 p.p.b. for TRM, 103.5 p.p.b. for HYD, 162.6 p.p.b. for TRM, and 116.1 p.p.b. for HYD were obtained in PLS-1 and PLS-2 models, respectively.

3.6. Analysis of commercial samples

Two commercial Triamterene-H tablets produced by the Irandaru and Sobhan factories were analyzed using two methods: the proposed spectrophotometric method and the BP standard method [4]. Results are summarized in Table 9. As can be seen, satisfactory results were obtained

in all cases by the proposed methods. PLS-1 and PLS-2 results appear to be same for TRM and higher for HYD than BP results in all cases. The only explanation, which appears to be rational, is the aforementioned higher dissolution of HYD in our proposed method compared with the BP method. The relative standard deviation (R.S.D.) results show that the precision of the proposed method is better than BP method.

Excipients frequently added to dosage forms did not interfere with the proposed method.

4. Conclusion

A comparative study of the use of PLS-1 and PLS-2 for the resolution and simultaneous determination of TRM and HYD in a binary mixture has been accomplished, showing that these methods provide a clear example of the high resolving power of these techniques. In several terms, similar results were obtained for these two drugs in both synthetic and commercial applications by PLS-1 and PLS-2.

The results obtained confirm the suitability of the proposed method for simple, accurate and precise analysis of triamterene and hydrochlorothiazide in pharmaceutical preparations. The proposed methods do not need prior separation of TRM and HYD before analysis. The BP chromatographic standard procedure is rather time consuming and expensive for routine assays. In addition, the proposed methods are suitable for application without interference of the excipients, and can be applied directly to the commercial preparations without previous treatment.

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