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Chemometric determination of amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate in synthetic mixtures and pharmaceutical formulations

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Abstract

Different chemometric methods such as classical least squares (CLS), principal components regression (PCR) and partial least squares with one dependent variable (PLS-1) applied on UV spectral data (^0D) and on their first derivatives (^1D) were evaluated for the simultaneous quantification of samples containing mixtures of amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate. Their performances were compared by means of ANOVA tests, which evidenced that ^0D-PCR, ^0D-PLS-1, ^1D-PCR and ^1D-PLS-1 were reproducible and gave statistically similar results, while ^0D-CLS and ^1D-CLS displayed higher variances than the former and failed to comply with the Levene's variance homogeneity test at different stages of the method comparison and validation process. The four statistically equivalent procedures were successfully applied to the analysis of synthetic samples with two to four analytes and to commercial tablet preparations containing amiloride hydrochloride and hydrochlorothiazide alone or in association with atenolol or timolol maleate.

Keywords: Amiloride hydrochloride; Atenolol; Hydrochlorothiazide; Timolol maleate; Multivariate methods; Chemometric determination

1. Introduction

The resolution of multicomponent preparations is often a complex analytical problem since combined substances may have different chemical structures but similar properties, like chromatographic behavior and UV spectra. Drugs currently employed in antihypertensive therapy, such as amiloride hydrochloride (AMH), atenolol (ATE), hydrochlorothiazide (HCT) and timolol maleate (TIM) constitute an example of this situation, presenting overlapping UV spectra [1].

Atenolol is a β_1-selective adrenergic blocking agent, which is used concurrently with diuretics because of their additive effects; it reduces heart rate and the force of the heart muscle contraction and lowers blood pressure by blocking the action of the nervous system on the heart. Timolol maleate is a non-selective β-adrenergic blocker, which unlike atenolol inhibits both β_1- and β_2-adrenegic receptors, reducing cardiac activity by diminishing or preventing

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sympathetic β-adrenoceptor stimulation. On the other hand, hydrochlorothiazide is a thiazide-type diuretic, which reduces reabsorption of electrolytes from the renal tubules thereby increasing the excretion of sodium, chloride, and consequently of water, being potassium ions also lost as an undesirable side effect. Finally, amiloride hydrochloride is a potassium sparing diuretic that acts on the distal renal tubule of the nephron inhibiting sodium–potassium ion exchange. These pharmacological properties make AMH useful for the prevention of hypokalemia induced by thiazide diuretics in patients with hypertension or congestive heart failure [2].

In the stepped-care approach to antihypertensive drug therapy, diuretics and β-adrenergic blocking agents are the initial drugs of choice. However, very often combination therapy is required and preparations containing hydrochlorothiazide in fixed proportion with β-adrenergic blocking agents are usually employed, after an initial dosage adjustment period. Amiloride hydrochloride is then added to these binary combinations to produce ternary mixtures in which this drug helps reducing the loss of potassium ions, specially during prolonged treatment [3]. Therefore, the HCT–AMH combination and its association with ATE and TIM are widely prescribed.

Multivariate calibration is a useful tool in analysis of multicomponent mixtures because it allows the rapid and simultaneous determination of each component in the mixture, with minimum sample preparation, reasonable accuracy and precision and without the need of lengthy separations. With the aid of modern instrumentation to acquire and digitize spectral information and powerful computers to process large amounts of data, chemometric methods such as classical least squares (CLS), principal component regression (PCR) and partial least squares with one dependent variable (PLS-1), the basis of which have been thoroughly reported in the literature [4–7] are finding increasing use in quantitative analysis of complex mixtures [8–11], offering sometimes an interesting alternative to chromatographic techniques.

All these methods comprise two separate stages. In the first step, termed calibration, an empirical model is built, representing the relationship between the data generated from a set of reference samples and the respective concentrations of their component(s) of interest. This is followed by a second step called prediction, in which the calibration model is used to determine the concentration of the components in the unknowns from their spectral data.

CLS is one of the simplest methods, being a multivariate least-squares procedure based directly on Beer's law, which model accounts for errors in the spectral measurements. It has several disadvantages, among them the fact that it generates a rigid model, which requires full knowledge of all of the components of the mixtures and their respective concentrations and that it is not applicable in case of interactions among the components or with the matrix.

On the other hand, PCR and PLS-1 are factor-based methods, which perform data decomposition into spectral loadings and scores prior to model building with the aid of these new variables. In PCR, the data decomposition is done using only spectral information, while PLS-1 employs spectral and concentration data. All of these methods assume analyte compliance with Beer's Law.

Derivative techniques are widely used in conjunction with spectrophotometric methods, specially in cases where improvements in selectivity are required, due to their potential ability to increase minor spectral features and correct baseline drift [12,13]. However, one of the disadvantages of these data transformation procedures is that some loss of signal occurs during the transformation. Nevertheless, the combined use of derivative methodologies and chemometric techniques has demonstrated to be a highly convenient choice in certain circumstances [14–16].

In spite of their wide use and the availability of many analytical methods for their individual quantification, there are only a few scattered reports on the simultaneous resolution of mixtures containing thiazide diuretics, β-adrenergic blocking agents and AMH; for example, the analysis of AMH, HCT and ATE by derivative spectroscopy [17] and HPLC [18,19] and the simultaneous quantification of AMH, HCT and TIM employing a high-performance flow injection system [20] have been described. However, none of these methods entails the use of multivariate techniques, in spite of their recognized high-resolution potential that has been used advantageously for the elimination of interferences and the resolution of overlapped signals.

In this paper, we report our results on the rapid and simultaneous resolution of samples containing AMH,

ATE, HCT and TIM employing multivariate calibration methodologies such as CLS, PCR and PLS-1 on electronic absorption spectra (^0D) and on their first derivatives (^1D). We also report the successful application of some of these procedures to the quantification of the analytes in synthetic mixtures and pharmaceutical preparations containing AMH and HCT alone or in ternary mixtures with ATE or TIM.

2. Experimental

2.1. Apparatus, hardware and software

Spectrophotometric measurements were carried out with an Unicam Helios β spectrophotometer, employing a 10 mm quartz cell. Spectra were acquired over the wavelength range 200–420 nm at intervals of 1 nm (221 data points/spectrum) against a blank of solvent. Spectra were saved in CSV (comma separated values) format, transferred to a PC Pentium II 466 MHz computer, and then transformed into Matlab 5.3 (Mathworks Inc.) readable files, for their subsequent storage, manipulation and analysis. Variable selection [21], data transformation [22,23] as well as CLS, PCR and PLS-1 data analysis [4,5] were performed with a home-made set of routines based on the equations contained in refs. [4–7], written for Matlab 5.3. Statistical treatment of data was performed with the SPSS 9 application software.

2.2. Materials

All the experiments were carried out with USP-grade drugs and analytical-grade solvents. Stock solutions of AMH (108.2 mg l^{-1}), ATE (1199.6 mg l^{-1}), HCT (600.4 mg l^{-1}) and TIM (240.0 mg l^{-1}) were prepared by dissolving accurately weighed amounts of the drugs in a mixture containing 30% MeOH and 70% of H_2O made 0.05N in HCl. Working solutions of the analytes were prepared before use, by appropriate dilution of the respective stock solutions. All the solutions were protected from light throughout the experiments.

Pharmaceutical preparations were obtained from local drugstores. The AMH–HCT tablets declared to contain 5 mg AMH and 50 mg HCT; the AMH–ATE–HCT tablets indicated 2.5 mg AMH, 50 mg ATE and 25 mg HCT, while the AMH–HCT–TIM tablets reported 2.5 mg AMH, 25 mg HCT and 10 mg TIM. They also contained dibasic calcium phosphate, guar gum, magnesium stearate, lactose, microcrystalline cellulose and pregelatinized starch as declared excipients.

2.3. Methods

2.3.1. Calibration system

A training set of 27 mixtures displaying a central composite design, was prepared in separate 25 ml flasks by adding appropriate volumes of the working solutions of the drugs in MeOH–0.05N HCl (3:7, v/v) to final concentrations in the ranges of 1.04–1.95 mg l^{-1} for AMH, 21.11–38.87 mg l^{-1} for ATE, 10.56–19.44 mg l^{-1} for HCT and 4.22–7.78 mg l^{-1} for TIM. The analyte levels were chosen in ratios close to those of the commercial tablet preparations, covering the range of 100 ± 30% of the expected amount of the analytes in the unknowns [24].

2.3.2. Validation sets

A validation set of 15 synthetic quaternary mixtures was prepared by dispensing appropriate volumes of the working solutions into 25 ml flasks and completing to the mark with MeOH–0.05N HCl (3:7, v/v). Binary, ternary and quaternary synthetic mixtures for evaluation of inter- and intra-day variations were prepared similarly. Readings of six replicates were made each time.

2.3.3. Analysis of synthetic mixtures and pharmaceutical preparations

Pharmaceutical formulations commercially available in Argentina were evaluated. In each case, groups of 20 tablets were weighed, ground and finely powdered in a mortar. Portions of the powder were accurately weighed and transferred to 50 ml volumetric flasks using 15 ml of MeOH. The flasks were mechanically shaken for 30 min, completed to the mark with 0.05N HCl, mixed and left for 30 min at room temperature to decant the solids. Then, aliquots of 2 ml were transferred from each flask to 25 ml volumetric flasks and diluted to the mark with MeOH–0.05N HCl (3:7, v/v). Synthetic mixtures containing two to four components were prepared in MeOH–0.05N HCl (3:7, v/v) by appropriate dilutions of the working solutions in 25 ml flasks.

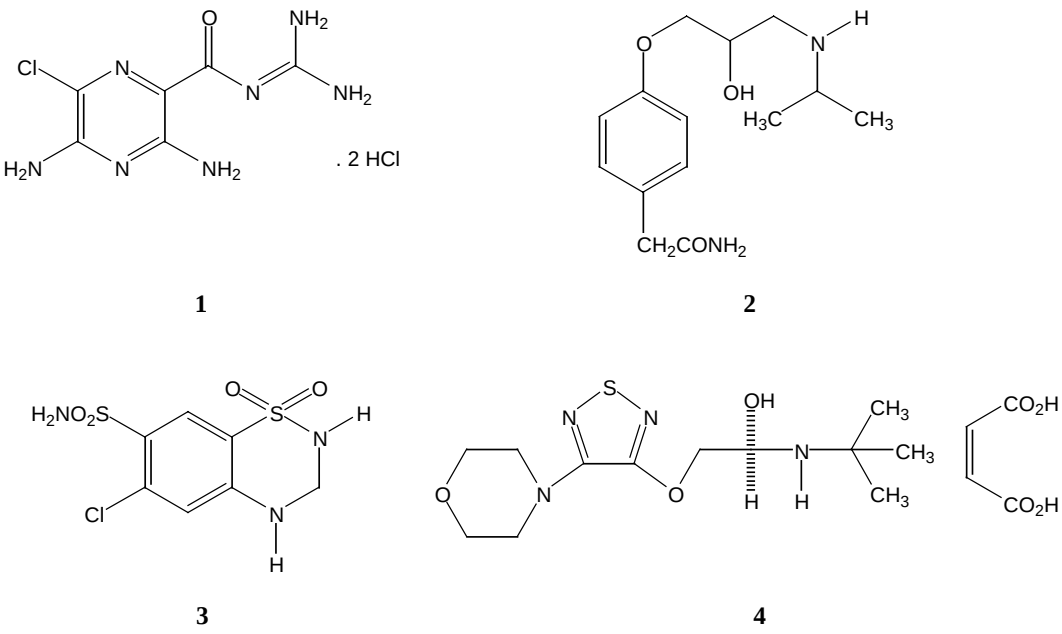

Fig. 1. Structural formulae of amiloride hydrochloride (1), atenolol (2), hydrochlorothiazide (3) and timolol maleate (4).

3. Results and discussion

Fig. 1 contains the formulae of the four analytes evidencing their structural diversity, while the electronic absorption spectra in the 200–420 nm region of pure AMH (1.51 mg l^{-1}), ATE (30.23 mg l^{-1}), HCT (14.88 mg l^{-1}) and TIM (5.95 mg l^{-1}) in MeOH–0.05N HCl (3:7, v/v), are shown in Fig. 2 and the first derivatives of the absorption spectra of the analytes at the same concentrations are displayed in Fig. 3. The analytes exhibit several maxima in their electronic absorption spectra: λ_{max} of AMH lie at 215, 287 and 363 nm, λ_{max} of ATE are at 226 and 261 nm, λ_{max} of HCT are at 225, 270 and 317 nm, while that of TIM lies at 295 nm. The strong signal overlapping of the absorption spectra and their first derivatives points to the difficulties that can be found in this multicomponent determination employing classical univariate techniques. For instance, the overlap of the first derivative spectra does not permit this quaternary mixture to be solved by the zero-crossing strategy.

For the simultaneous determination of mixtures of analytes employing chemometric methods, appropriate calibration designs are required. A literature survey showed that in certain cases, the composition of the calibration set is restricted to satisfy the resolution of only one analyte combination [25], being the calibration matrix of rather limited usefulness; on the contrary, other authors have designed multipurpose calibration sets suitable for evaluation of different analyte combinations [26,27]. In spite of the fact that quaternary mixtures of AMH, ATE, HCT and TIM are not commercially available, our choice was to elaborate a four-component calibration design with the aim of serving as a multipurpose tool, allowing the determination of as many as possible commercial drug combinations as binary and ternary mixtures, with a single and reduced set of calibration standards.

Thus, a training set of 27 samples with a central composite design was prepared, by appropriate dilution of the working solutions. A validation set of 15 quaternary samples, the composition of which is shown in Table 1, was concomitantly prepared and the electronic spectra of both sets were recorded between 200 and 420 nm. The first derivatives of both sets of spectra were then obtained employing the Savitzky–Golay smoothing and differentiation algorithm with a nine-datapoints derivation window [22,23].

Since not all wavelengths in the spectra carry the same quality of information and in order to select

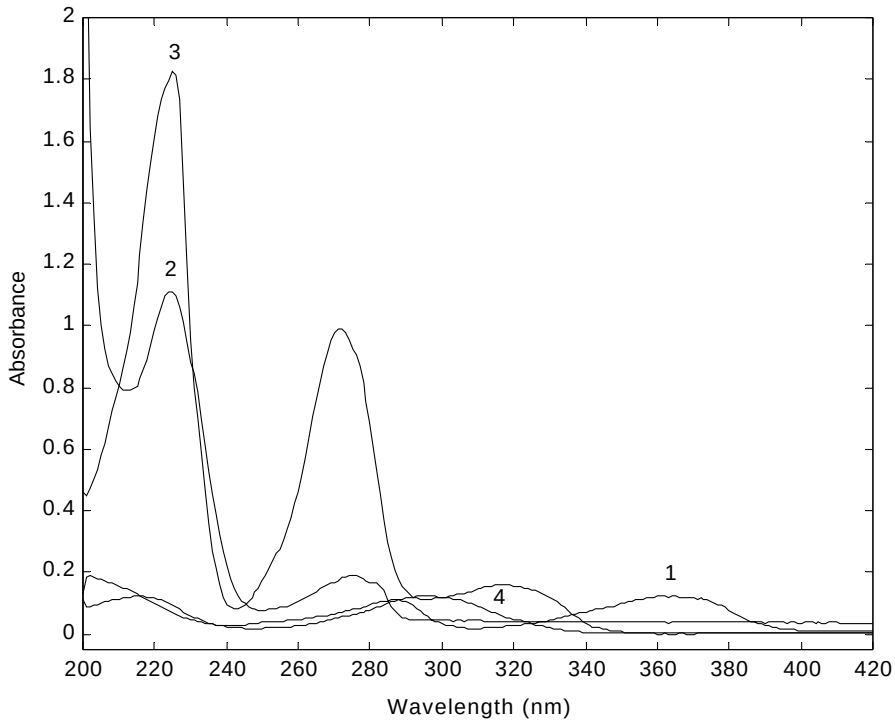

Fig. 2. Electronic absorption spectra of (1) amiloride hydrochloride (1.51 mg l^{-1}), (2) atenolol (30.23 mg l^{-1}), (3) hydrochlorothiazide (14.88 mg l^{-1}), and (4) timolol maleate (5.95 mg l^{-1}), in MeOH–0.05N HCl (3:7, v/v).

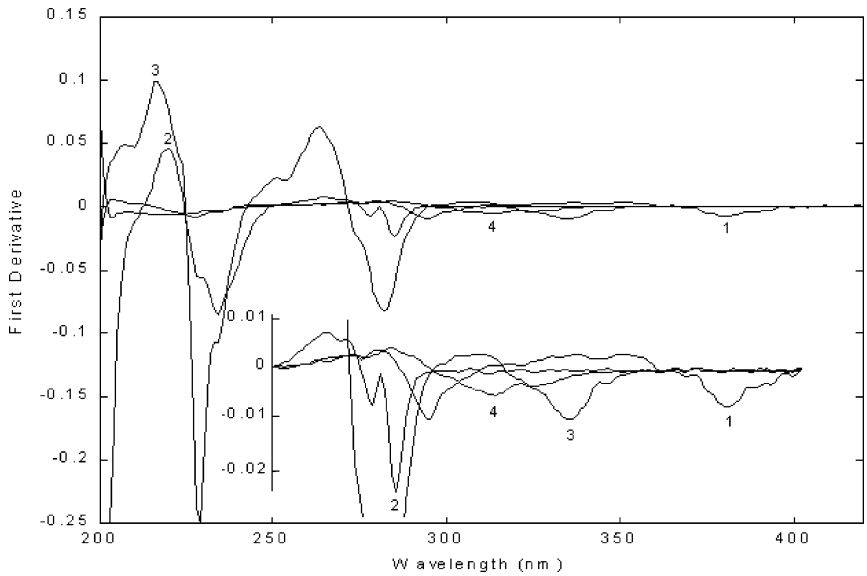

Fig. 3. First derivative of the electronic absorption spectra of (1) amiloride hydrochloride (1.51 mg l^{-1}), (2) atenolol (30.23 mg l^{-1}), (3) hydrochlorothiazide (14.88 mg l^{-1}), and (4) timolol maleate (5.95 mg l^{-1}), in MeOH–0.05N HCl (3:7, v/v). Lower right: magnification of the 250–400 nm range.

Table 1
Composition of the samples of the validation set

Sample	Concentration ($mg\,l^{-1}$)			
	Amiloride hydrochloride	Atenolol	Hydrochlorothiazide	Timolol maleate
1	1.30	25.43	12.72	5.09
2	1.30	25.43	12.72	6.91
3	1.30	25.43	17.28	5.09
4	1.30	34.55	12.72	5.09
5	1.30	34.55	12.72	6.91
6	1.51	21.11	14.88	5.95
7	1.51	30.23	14.88	5.95
8	1.51	30.23	17.28	5.95
9	1.51	30.23	19.44	5.95
10	1.73	25.43	12.72	5.09
11	1.73	25.43	17.28	6.91
12	1.73	25.43	17.28	5.09
13	1.73	34.55	12.72	5.09
14	1.73	34.55	12.72	6.91
15	1.73	34.55	17.28	5.09

each analyte's most appropriate spectral working region and the number of factors to be used in the PCR and PLS-1 methods, a minimum PRESS search guided by a moving window of variable size was employed [21]. Using mean-centered data (spectra or their first derivatives), windows with different initial and final wavelengths were built. For each wavelength interval, models with 1–10 factors were constructed and cross-validated employing the leave-one-out strategy, in which one sample was left out at a time until each sample had been left out once, and its concentration was calculated with the aid of a model made with the remaining samples.

The prediction errors of the samples were each calculated with reference to the known concentrations of the analytes, squared and added all together resulting in the prediction residual error sum of squares (PRESS). This parameter, which is a measure of the predictive ability of the model, was calculated for each window and each factor and then, the window of wavelengths displaying the minimum PRESS for each factor was obtained.

The Haaland and Thomas criterion to avoid data overfitting [7] was now applied in the form of *F*-ratios between the minimum PRESS values of the models constructed with h factors and the first minimum PRESS of the whole 10-factor series, corresponding to a model with h^* factors ($h \leq h^* \leq 10$). The selected optimum model was that with the first PRESS value having an *F*-ratio probability falling below 0.75.

In the case of ${}^0D$-CLS and ${}^1D$-CLS, the spectral regions were chosen by trial and error, attempting to optimize the rmsd (root mean square deviation) values of the analytes' recoveries. They included most of the absorbing maxima of the analytes.

Critical calibration information is shown in Table 2, which summarizes the data of the optimum spectral regions, the number of factors required for the different methods and analytes and their associated statistical parameters r^2 (square of the correlation coefficient) and rmsd, indicative of the methods' performance. rmsd is a measure of the average error in the analysis, while r^2 evaluates the goodness of fit of the predicted concentrations to their actual values. Noteworthy, r^2 values better than 0.995 were obtained in most of the cases, indicating excellent linear relationships between predicted and actual concentration values, over the ranges of interest for most of the procedures and analytes.

Interestingly, optimum wavelength regions found for normal spectra were different than those recorded for their derivatives, reflecting the different degrees of interference in the determination of a given analyte, produced by the others. For the same reason, not all of the selected regions contained the most representative absorption maxima. The most striking case was that of

Table 2
Characteristics of the calibration models

Procedure	Analyte													
	Amiloride hydrochloride				Atenolol				Hydrochlorothiazide				Timolol maleate			
	Range (nm)	r^2	n^a	rmsd	Range (nm)	r^2	n^a	rmsd	Range (nm)	r^2	n^a	rmsd	Range (nm)	r^2	n^a	rmsd
^0D-CLS[b]	234–364	0.9319	–	0.0697	234–364	0.9962	–	0.2749	234–364	0.9967	–	0.1326	234–364	0.9727	–	0.1496
^1D-CLS[b]	241–387	0.9966	–	0.0156	241–387	0.9937	–	0.3610	241–387	0.9971	–	0.1215	241–387	0.9941	–	0.0667
^0D-PCR	355–386	0.9977	2	0.0135	204–219	0.9987	5	0.1884	267–285	0.9980	4	0.1123	286–342	0.9988	4	0.0344
^1D-PCR	355–386	0.9968	1	0.0155	227–313	0.9982	5	0.2240	268–283	0.9981	3	0.1034	287–328	0.9990	4	0.0315
^0D-PLS-1	370–393	0.9977	2	0.0134	237–271	0.9988	4	0.1941	267–285	0.9982	4	0.1136	287–388	0.9987	4	0.0339
^1D-PLS-1	355–386	0.9968	1	0.0154	237–287	0.9991	4	0.1962	268–283	0.9981	3	0.1039	307–377	0.9993	4	0.0310

[a] n is the number of latent variables employed.
[b] Ranges were selected by trial and error, minimizing the overall prediction error.

ATE, for which the 204–219 nm interval was found to be the most appropriate for running the ^0D-PCR procedure, while the best predictive ability of the ^1D-PCR procedure was observed when the 227–313 nm interval was employed.

Results of the CLS algorithm applied to absorption data evidenced some difficulties of this model to resolve AMH and TIM, the less absorbing species. Interestingly, however, when derivative data were processed (^1D-CLS) great improvement in model performance was observed, the results being more consistent with those obtained with the factor-based methods. On the other hand, it was detected that application of the latter on the first derivative of the absorption spectra (^1D-PCR and ^1D-PLS-1) resulted in models of the same or less complexity than those generated with the absorption data.

The validity of the calibration models was tested evaluating their predictive abilities on samples not included in the training set. Thus, the different models were employed to predict the concentration of the four analytes in the 15 samples of the validation set, with the results collected in Table 3, in terms of overall means of the recovery values, their standard errors and 95% confidence intervals (IC_{95}) arranged for each method and component. Mean recoveries were near quantitative and indicative of the methods' accuracy, while the observed standard errors signaled the good repeatability of the measurements.

In order to select the most appropriate procedure(s) for this multiple determination, their performances were evaluated and a method comparison was carried out by means of a two-factor (procedure and component) ANOVA test applied to the percentage recoveries of the analytes of the validation set.

Before running the ANOVA test, verification of variance homogeneity was performed, employing Levene's criterion [28]. Initial results indicated non-compliance with the test; however, a more in-depth analysis including multiple pair-wise comparisons clearly evidenced that CLS applied to absorption spectra (^0D-CLS) was solely responsible for this outcome.

Therefore, the ANOVA test which results are shown in Table 4 was carried out excluding this data set. Comparison of the between analytes mean squares with the residual mean squares gave $F = 2.174$. Being the critical value $F_{3,292,0.05} = 2.6355$, this result indicates

Table 3
Mean recoveries, standard errors and confidence intervals for synthetic samples of the validation set

Factor	Mean recovery (%)	S.E. (%) (internal)	IC_{95} (%)
Procedure			
^0D-CLS[a]	99.71	3.02	93.67–105.75
^1D-CLS	99.99	1.21	97.58–102.42
^0D-PCR	99.97	0.96	98.05–101.89
^1D-PCR	99.96	1.14	97.68–102.24
^0D-PLS-1	100.01	1.08	97.84–102.16
^1D-PLS-1	99.94	1.04	97.86–102.02
Analyte[b]			
Amiloride hydrochloride	100.10	1.14	97.83–102.37
Atenolol	99.78	1.06	97.67–101.89
Hydrochlorothiazide	100.16	1.10	97.97–102.35
Timolol maleate	99.85	1.00	97.86–101.84

[a] ^0D-CLS data are consigned for the sake of comparison.
[b] ^0D-CLS results were excluded from calculation.

that there is not a statistically significant difference between the mean recoveries of the different analytes at a 95% confidence level. Likewise, the *F*-ratio value of 0.032 obtained by comparison of the between procedures mean squares with the residual mean squares proved to be below the critical value $F_{4,292,0.05} = 2.4026$, revealing that there is no statistically significant difference between the mean recoveries given by the five different procedures tested.

Next, intra-day and inter-day variations were examined. Sets of six synthetic samples, each containing two (AMH and HCT), three (AMH, ATE and HCT or AMH, HCT and TIM combinations) and all four analytes, with analyte levels and relationships similar to those of the commercial products were prepared. Synthetic mixtures were measured at different times and the analyte recoveries obtained by application of the five different procedures for each sample and analyte were recorded and statistically analyzed.

When subjected to Levene's test of variance homogeneity, it was observed that the whole data set passed the test only after exclusion of the results provided by the ^1D-CLS model. A more in-depth analysis also evidenced that recoveries furnished by the latter model satisfied Levene's criterion only in the case of four-analyte mixtures. Presumably, this outcome is a consequence of both, the characteristic poor flexibility of the CLS model and the structure of the training set, devoid of samples containing just two or three analytes.

The ANOVA test carried out on the ^0D-PCR, ^0D-PLS-1, ^1D-PCR and ^1D-PLS-1 results for two to four analyte mixtures indicated no statistical difference among these procedures. Therefore, it was concluded that they were suitable for the quantification purposes.

The four chemometric procedures, which proved to have statistically similar performances, were then applied to the analysis of synthetic samples and commercial tablet preparations containing different combinations of the analytes. As shown in Table 5, excellent recovery results were obtained in all cases.

Table 4
Results of the two-factor ANOVA

Source of variation	Sum of squares	Degrees of freedom	Mean square	*F*-ratio
Between procedures	0.150	4	0.038	0.032
Between analytes	7.686	3	2.562	2.174
Residual	344.131	292	1.179	
Total	351.967	299		

Recoveries for the different procedures and analytes. $F_{(4,292,0.95)} = 2.4026$ and $F_{(3,292,0.95)} = 2.6355$.

Table 5
Multivariate determination of synthetic mixtures and commercial tablets with the different chemometric procedures

Samples	Components	% Drug recovery by different procedures (mean ± S.D.)[a]			
		^0D-PCR	^1D-PCR	^0D-PLS	^1D-PLS
Brand 1	AMH	100.70 ± 2.75	99.03 ± 3.00	100.70 ± 2.76	98.73 ± 3.00
	ATE	99.75 ± 0.40	100.44 ± 0.58	101.37 ± 0.52	100.38 ± 0.62
	HCT	102.51 ± 0.64	101.73 ± 0.76	101.93 ± 0.98	101.72 ± 0.75
Brand 2	AMH	98.68 ± 1.54	97.65 ± 1.46	98.66 ± 1.52	97.62 ± 1.45
	TIM	100.93 ± 0.36	100.64 ± 0.35	100.84 ± 0.38	100.53 ± 0.86
	HCT	101.59 ± 0.75	100.18 ± 0.80	101.16 ± 0.77	100.12 ± 0.80
Brand 3	AMH	104.65 ± 1.10	103.88 ± 1.08	104.64 ± 1.10	103.87 ± 1.08
	HCT	104.10 ± 0.92	103.32 ± 1.01	104.12 ± 0.96	103.25 ± 1.02
Brand 4	AMH	98.15 ± 2.42	98.18 ± 2.54	98.16 ± 2.41	98.17 ± 2.53
	HCT	101.20 ± 0.86	100.15 ± 0.89	100.76 ± 0.86	100.09 ± 0.89
Synthetic 1	AMH	100.25 ± 0.30	99.92 ± 0.29	100.25 ± 0.30	99.91 ± 0.29
	HCT	100.48 ± 0.15	99.29 ± 0.14	100.06 ± 0.31	99.21 ± 0.14
Synthetic 2	AMH	100.12 ± 0.30	98.73 ± 0.40	100.11 ± 0.30	98.74 ± 0.40
	HCT	100.11 ± 0.47	100.86 ± 0.48	100.51 ± 0.47	100.95 ± 0.47
	TIM	100.63 ± 0.56	101.62 ± 0.62	99.76 ± 0.57	98.77 ± 0.76
Synthetic 3	AMH	100.29 ± 0.50	98.92 ± 0.51	100.29 ± 0.51	98.93 ± 0.51
	ATE	102.64 ± 0.50	102.97 ± 0.54	100.17 ± 0.38	102.30 ± 0.36
	HCT	100.13 ± 0.42	100.85 ± 0.50	100.61 ± 0.45	100.86 ± 0.50
Synthetic 4	AMH	98.87 ± 0.20	100.05 ± 0.11	98.97 ± 0.20	100.05 ± 0.11
	ATE	98.80 ± 1.48	99.39 ± 0.59	100.16 ± 0.57	100.76 ± 0.88
	HCT	99.52 ± 0.64	99.84 ± 0.60	99.48 ± 0.66	99.85 ± 0.59
	TIM	99.27 ± 0.89	101.14 ± 1.25	99.31 ± 0.93	98.95 ± 1.66

[a] Percentage recovery from the label claimed amount. Mean and standard deviation for six determinations.

In comparison to the tolerance levels established in the USP for pharmaceutical tablet preparations [29] and in spite that the prediction errors were slightly higher than those obtained with the validation set, it can be observed that the concentrations of the active principles were predicted with highly acceptable errors and that all of the commercial preparations proved to comply with the manufacturers declared amounts of their active ingredients. Interference due to the excipients was not observed.

In conclusion, we have applied chemometric methods such as CLS, PCR and PLS-1 to mean-centered UV absorption spectra and their first derivatives for the simultaneous evaluation of mixtures containing amiloride hydrochloride, atenolol, hydrochlorothiazide and timolol maleate. Comparison of the different procedures indicated that ^0D-PCR, ^1D-PCR, ^0D-PLS-1 and ^1D-PLS-1 satisfied Levene's test for variance homogeneity, were reproducible and proved to be not statistically different in their ability to evaluate the four analytes. Noteworthy, however, from a practical point of view experimental setups which do not require data pretreatment, such as ^0D-PCR and ^0D-PLS-1 should be preferred due to their time saving characteristics which makes them comparatively more efficient. The four statistically equivalent multipurpose calibration models were successfully applied to the quantitative analysis of synthetic samples and commercial tablet preparations containing mixtures of the analytes, providing a new and alternative tool for the rapid and convenient determination of these multicomponent mixtures with minimal sample preparation.

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