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# Simultaneous determination of amiloride hydrochloride and hydrochlorothiazide in synthetic samples and pharmaceutical formulations by multivariate analysis of spectrophotometric data

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**Abstract**

The use of multivariate spectrophotometric calibration for the simultaneous analysis of synthetic samples and commercial tablet preparations containing hydrochlorothiazide (HCT) and amiloride hydrochloride (AMH) is reported. Partial least squares (PLS-1) analysis of electronic absorption spectral data allowed the rapid and accurate resolution of mixtures in which the analyte ratios were approximately 10:1, without the need of a previous separation step and without interference from other sample constituents. The method, validated by the analysis of synthetic mixtures of both drugs, where accuracy over the linear working range as well as inter- and intra-assay precision were determined, was used in the concentration ranges of 21.7–30.4 mg $l^{-1}$ for HCT and 1.8–3.0 mg $l^{-1}$ for AMH. The proposed method was successfully applied to the evaluation of the stability of the stock solutions of the analytes in MeOH–$H_2O$ and to the elaboration of drug dissolution profiles of commercial tablets, results being concordant with those furnished by the USP technique. The method was also employed for the determination of drug content in two different pharmaceutical formulations, providing results that were in excellent agreement with those obtained by HPLC.



*Keywords:* Hydrochlorothiazide; Amiloride hydrochloride; Multivariate calibration; Simultaneous determination; Spectrophotometric method

## 1. Introduction

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, HCT), an almost white and odorless crystalline

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powder slightly soluble in water and sparingly soluble in methyl alcohol (MeOH), is a thiazide diuretic. It acts reducing the re-absorption of electrolytes from the renal tubules, thereby increasing the excretion of sodium and chloride ions, and consequently of water. The excretion of other ions, such as magnesium and potassium is also increased by HCT, while the loss of calcium is reduced [1].

Like other thiazide diuretics, HCT is used in the treatment of the oedema associated with congestive hearth failure and renal and hepatic disorders. Alone or in combination with other antihypertensive agents, it is also employed for the treatment of hypertension.

Amiloride hydrochloride (*N*-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride, AMH), is a photosensitive yellow or yellowish-green and odorless powder, sparingly soluble in MeOH and slightly soluble in water, imparting acidic character to its solutions. The drug, available as the dihydrate, behaves as a mild diuretic and acts blocking the $Na^+$ channels in the late distal tubules and collecting ducts. By increasing the loss of sodium and chloride ions while reducing the excretion of potassium, AMH adds to the natriuretic effects of other diuretics, while diminishing their kaliuretic effects [1].

HCT and AMH (Fig. 1) are official drugs in the BP 98 [2], the USP 24 [3] and the European Pharmacopoeia [4]. Compounded preparations of both drugs have the British approved name of co-amilozide, when their mass proportion is 10 to 1, respectively [3,5]. Co-amilozide tablets are official in the USP 24 [3].

Large clinical trials demonstrated that the use of the co-amilozide combination provides the best results in terms of reduced cardiovascular morbility and mortality [6]. The conservation of potassium ions by this association diminishes the risk of alkalosis during the prolonged treatments, such as in hypertension management and refractory oedema associated with hepatic cirrhosis and congestive hearth failure, making administration of potassium supplements unnecessary.

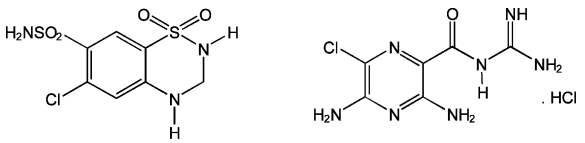


Fig. 1. Chemical structures of HCT and AMH.

The joint use of HCT and AMH for the treatment of nephrogenic diabetes insipidus [7], as well as of oxalate stone formation in patients with an inherited cellular defect in oxalate transport, has been proposed [8]. It was also observed that administration of co-amilozide in nitroglycerin therapy has important antianginal effects, improving exercise capacity of patients with stable angina [9,10].

The widespread use of the co-amilozide combination has aroused great interest in devising analytical methods for the simultaneous determination of both of its ingredients. A literature survey revealed that simultaneous quantification of HCT and AMH has been achieved by UV spectroscopy [11,12], including absorbance ratio spectroscopy [13], derivative spectroscopy [14–16] and multiple linear regression of UV–visible data [15].

In addition, other important methods based on HPLC [11,14,17,18], HPTLC [19], TLC separation followed by fast atom bombardment mass spectrometry [20] and differential pulse polarography with partial least squares (PLS-1) calibration [21] have been described. HCT and AMH have also been simultaneously quantified by HPLC in biological fluids, such as plasma [22] and urine [23,24].

Spectrophotometric techniques provide practical and significant economic advantages over other methods; therefore, they are a frequent choice for pharmaceutical analyses. However, the strong overlap of spectral bands exhibited by mixtures of active principles and some excipients in the UV region, usually constraints the application of such methodologies and these cases often require the use of separation methods prior to spectrophotometric quantification of the analytes of interest.

The need of more complex instrumentation for the simultaneous determination of drugs in pharmaceutical preparations renders methods, being

either more expensive, limited in their applications or rather tedious and time consuming.

These drawbacks, in an era of spectrophotometers able to provide digitized spectra and powerful desktop computers in the analytical laboratory, capable of their rapid analysis, is promoting the development of highly selective numerical methods to facilitate the simultaneous determination of analytes in complex mixtures without the need of previous separations.

Among them, multivariate calibration techniques such as the PLS-1 regression with one dependent variable (PLS-1) is one of the methods of choice for solving spectrally the overlapped mixtures of pharmaceutically relevant analytes [25–28], and an interesting alternative to the more expensive chromatographic techniques.

In this paper we report a simple, precise and accurate spectrophotometric method for the simultaneous determination of HCT and AMH in synthetic mixtures and commercial combined tablet preparations, based on the PLS-1 analysis of their ultraviolet spectral data in selected zones. Comparison with HPLC results is discussed, and the use of the proposed method for solution stability and commercial tablet analysis, including drug content and dissolution profiling, is presented.

## 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 at "normal" speed, over the wavelength range 200–420 nm at intervals of 1 nm (221 data points/spectrum) against a blank of solvent. Samples were analyzed in duplicate in order to provide for better modeling of noise.

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 analysis.

Selection of appropriate wavelength ranges for calibration/prediction by means of a minimum PRESS (prediction residual error sum of squares) search through a variable size moving-window [29] and PLS-1 data evaluation were performed with an in-house set of routines written for MATLAB according to Martens and Naes [30] and Thomas [31]. The software was validated against MULTIVAR [32], results being in full agreement.

HPLC analyses were carried out isocratically at room temperature employing a Gilson liquid chromatograph, equipped with a 307 type pump, a SC10 pump head and a Rheodyne 7725i valve injector fitted with a 20 μl fixed loop. Separation was performed using a 4.6 mm × 25 cm Spherisorb C-18 column with 5 μm particles and the eluate was monitored with a Gilson 112 type UV/visible fixed wavelength detector. A Hanson SRS 8 Plus dissolutor was employed for dissolution analysis of commercial tablets.

### 2.2. Materials

The experiments were carried out with USP-grade HCT and AMH (as the dihydrate) and analytical-grade reagents. Stock solutions of HCT (121.6 mg $l^{-1}$) and AMH (106.4 mg $l^{-1}$) were prepared by dissolving accurately weighed amounts of the drugs in MeOH–$H_2O$ (1:1, v/v). They were conserved at 4 °C, covered with aluminum foil and left to attain room temperature before use. Working solutions of AMH were prepared before use, by 1:10 dilution of the respective stock solution. All the solutions containing AMH and HCT were protected from light throughout the experiments. Pharmaceutical preparations (average weight of 219 and 241 mg/tablet for the different brands) were obtained from a local drugstore. They declared to contain 50 mg of HCT, 5 mg of AMH and excipients (dibasic calcium phosphate, guar gum, magnesium stearate, lactose, sunset yellow FD&C No. 6 and corn starch). MeOH was generously provided by Resinfor Metanol (Puerto General San Martín, Argentina) and used as-received. HPLC-grade solvents were employed for HPLC analyses.

### 2.3. Methods

#### 2.3.1. Calibration system

A training set of 16 mixtures was prepared by convenient dilution of the stock solutions of the drugs in MeOH–$H_2O$ (1:1, v/v) to final concentrations in the range 21.7–30.4 mg $l^{-1}$ for HCT and 1.8–3.0 mg $l^{-1}$ for AMH. The analyte levels were chosen in ratios close to that of co-amilozide, including the range of $100 \pm 10\%$ of the expected amount of the analytes in the unknowns [3,33].

#### 2.3.2. Validation sets

A validation set of 12 synthetic samples, covering the concentration range of interest of both analytes, was prepared for evaluation of accuracy over the working linear range by comparison of the amounts of drugs found and those added to the solutions. A second validation set, consisting of 15 independently prepared synthetic mixtures containing the same concentrations of the analytes, was prepared and analyzed in groups of five at three different times, allowing the evaluation of inter- and intra-assay precision.

#### 2.3.3. Stability of stock solutions

Stability tests were done weekly, over a 7-week period. For analysis, appropriate volumes of the stock solutions were pipetted into 25 ml volumetric flasks in such a way that the final concentrations of both drugs lied within the range of interest, diluted to the mark with MeOH–$H_2O$ (1:1, v/v) and evaluated for percentage of drug recovery.

#### 2.3.4. Resolution of synthetic mixtures—drug content

Binary synthetic mixtures of HCT and AMH were prepared by diluting into 25 ml flasks known amounts of their stock solutions with MeOH–$H_2O$ (1:1, v/v), to obtain final concentrations of 25.0 mg $l^{-1}$ HCT and 2.5 mg $l^{-1}$ AMH.

#### 2.3.5. Analysis of pharmaceutical preparations—content uniformity

Pharmaceutical formulations of the two different brands, Moduretic® and Hidrenox-A®, commercially available in Argentina were evaluated. In each case, groups of five tablets were individually weighed, mixed and finely powdered in a mortar. Portions of the powder (28–30 mg depending upon the brand) equivalent to about 6.25 mg of HCT and 0.625 mg of AMH were accurately weighed and transferred to 50 ml volumetric flasks using 25 ml of MeOH. The flasks were mechanically shaken for 30 min, completed to the mark with distilled $H_2O$ and left for 30 min at room temperature for solids to decant. Then, aliquots of 5 ml were transferred from each flask to 25 ml volumetric flasks and diluted to the mark with MeOH–$H_2O$ (1:1, v/v).

#### 2.3.6. Analysis of pharmaceutical preparations—dissolution profile

The procedure of the USP 24 was followed. In separate experiments, six tablets of Moduretic® and Hidrenox-A® were assayed at 37 °C in 900 ml of dissolution medium, employing USP dissolution method II, at a rotation speed of 50 rpm [3]. Aliquots of 10 ml were periodically withdrawn for evaluation. For UV-PLS analysis, appropriate volumes of filtered samples were pipetted into 10 ml flasks and diluted with MeOH–$H_2O$ (1:1, v/v).

#### 2.3.7. Analysis of pharmaceutical preparations—comparison with an HPLC method

With minor modifications, the procedure of the USP 24 was followed [3]. 20 tablets were accurately weighed and finely powdered; the amount corresponding to one tablet was transferred into a 50 ml flask; then, 15 ml MeOH and 2 ml of 1 N HCl were added, the mixture was mechanically shaken for 10 min and diluted to the mark with $H_2O$. An aliquot was centrifuged and the supernatant used for analysis.

For quantification by HPLC, the injection volume was 20 μl, MeOH–$H_2O$–phosphate buffer (pH 3; 0.1 M) (25:71:4, v/v/v) was employed as mobile phase at a flow rate of 1.0 ml $min^{-1}$ and detection was made at 280 nm. All solvents were filtered through a 0.45 μm millipore filter and degassed before use. Acquisition parameters were: sensitivity = 0.05 AUFS and time constant = 0.5 s; digitized chromatograms were stored and processed on a PC AT 486 DX2 66 MHz computer, employing Gilson's software.

Single-point calibration [3] was carried out with five injections of a standard containing both analytes (concentration of HCT = 1.04 mg $ml^{-1}$, concentration of AMH = 0.09 mg $ml^{-1}$). Triplicate injections were made for each solution of the unknowns. For simultaneous PLS-1 analysis of the samples submitted to HPLC, aliquots of 1.30 ml were transferred to 50 ml flasks and diluted to the mark with MeOH–$H_2O$ (1:1, v/v).

## 3. Results and discussion

The electronic absorption spectra of pure HCT (26.0 mg $l^{-1}$) and AMH (2.61 mg $l^{-1}$), as well as the spectrum of the mixture of the drugs in MeOH–$H_2O$ (1:1, v/v) are shown in Fig. 2. Both drugs, which spectra were recorded over the wavelength range 200–420 nm, exhibit three maxima: $\lambda_{max}$ of HCT are at 225, 270 and 317 nm, while those of AMH lie at 215, 287 and 363 nm. At the selected concentrations, the absorbance of the minor component at $\lambda_{max} = 363$ nm is only 0.17, while HCT, being 10 times more concentrated, exhibits absorbance values as high as 2.80 at $\lambda_{max} = 225$ nm, and 1.70 at $\lambda_{max} = 270$ nm. When plotted against their respective concentrations,

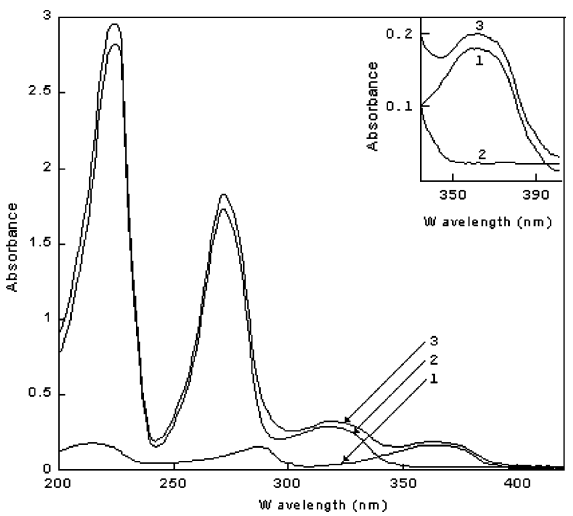


Fig. 2. Ultraviolet spectra of (1) 2.61 mg $l^{-1}$ AMH, (2) 26.0 mg $l^{-1}$ HCT and (3) mixture of HCT (26.0 mg $l^{-1}$) and AMH (2.61 mg $l^{-1}$) in the 200–420 nm region.

absorbance readings of HCT at $\lambda = 225$ nm present a slight deviation from linearity.

In spite that the signal of AMH around $\lambda = 363$ nm is almost free of interference from HCT, due to the poor absorption of the latter in this region, spectra were found to be overlapping, hence complicating direct analysis [12]. In addition, it was anticipated that excipients as well as the high HCT/AMH ratio present in the pharmaceutical preparations could hinder the resolution of the mixture by conventional spectrophotometry.

A few publications reported good results of the use of derivative spectroscopy for the simultaneous quantification of HCT and AMH, when high-resolution (0.1 nm) spectra were employed [13,14]. On the other hand, the interference of one analyte in the determination of the other [16] or results containing high errors [15] have been reported, when hard calibration methods were employed.

We assumed that a soft modeling strategy such as PLS-1 could be a good alternative for the simultaneous quantification of the co-amilozide ingredients. Its election is in principle advantageous, because PLS-1 can handle severe spectral overlap, high concentration ratio of the analytes, small deviations from absorbance-concentration linearity, and knowledge of the spectra of all the absorbing species is not absolutely necessary. The theory and application of PLS-1 and other multivariate calibration methods in analytical chemistry have been thoroughly reported in several books and monographs [30,31,34].

PLS-1 modeling was carried out on the mean-centered ultraviolet spectra of 16 calibration samples of HCT and AMH conforming a four-level full factorial design, recorded in duplicate in the range 200–420 nm. Table 1 summarizes the most relevant information of the calibration system, including its figures of merit. Critical values of the calibration, such as the square of the correlation coefficient ($r^2$), the relative error of prediction during calibration (REC) and the root mean square difference (RMSD), a measure of the average error in the analysis of each component, demonstrated the quality of fit of the calibration data.

Table 1
PLS-1 analysis of HCT and AMH: statistical parameters for the calibration

| Parameter of interest[a] | HCT | | AMH |
|---|---|---|---|
| Spectral range (nm) | 260–277 | 217–330 | 345–395 |
| Concentration range (mg $l^{-1}$) | 21.7–30.4 | 21.7–30.4 | 1.8–3.0 |
| Number of PLS factors | 2 | 2 | 2 |
| PRESS (mg $l^{-1})^{-2}$ | 0.303 | 0.440 | 0.0016 |
| RMSD (mg $l^{-1}$) | 0.097 | 0.141 | 0.0071 |
| REC (%) | 0.373 | 0.451 | 0.309 |
| $r^2$ | 0.9993 | 0.9994 | 0.9995 |
| Selectivity [39] | 0.399 | 0.437 | 0.449 |
| Sensitivity (SEN) | 0.224 | 0.152 | 0.187 |
| Analytical sensitivity, [($\gamma$ ), 1 mg $^{-1}$] | 0.79 | 0.36 | 47.93 |
| Minimum concentration difference, [($\gamma^{-1}$), (mg $l^{-1}$] | 1.26 | 2.75 | 0.021 |

[a]

$$\mathrm{PRESS} = \sum_{1}^{I}(C_{\mathrm{act}} - C_{\mathrm{pred}})^2, \qquad \mathrm{RMSD} = \left[\frac{1}{I}\sum_{1}^{I}(C_{\mathrm{act}} - C_{\mathrm{pred}})^2\right]^{1/2}, \qquad \mathrm{REC\%} = \frac{100}{\bar{C}}\left[\frac{1}{I}\sum_{1}^{I}(C_{\mathrm{act}} - C_{\mathrm{pred}})^2\right]^{1/2},$$

$$r^2 = 1 - \frac{\sum_{1}^{I}(C_{\mathrm{act}} - C_{\mathrm{pred}})^2}{\sum_{1}^{I}(C_{\mathrm{act}} - \bar{C}_{\mathrm{pred}})^2},$$

where $\bar{C}$ is the average component concentration in the $I$ calibration mixtures; sensitivity $= 1/||\mathbf{b}_k||$, where $\mathbf{b}_k$ is the final regression coefficients vector for component $k$, and $\gamma = (\mathrm{SEN}/\sigma_0)$, where $\sigma_0$ is the standard deviation of the blank. Selectivity was approximated as $1/(||\mathbf{b}_k||||A^{\mathrm{T}}C/C^{\mathrm{T}}C||)$, where $A$ and $C$ are the mean-centered absorbance (within the region of interest) and concentration data blocks, respectively.

Several authors have pointed out that in multivariate analysis of spectroscopic data not all wavelengths carry the same quality of information [29,35], and different estimators have been used in order to evaluate the predictive ability of multivariate models [35]. One of them, consisting in a variable-size moving window [29] across the spectra, was used for selection of the appropriate regions of interest guided by a minimum PRESS search as the optimization criterion.

Employing a minimum window of 10 sensors, for each starting wavelength (search over 212 wavelengths), 32 models each with variable number of sensors (10–221) and one and three factors were constructed. The PRESS was computed in each of the 22 578 cases, totaling the evaluation of 2 167 488 different models for each analyte. The spectral ranges affording the minimum PRESS values were considered as having the best predictive abilities. These selections of optimal wavelength range were further confirmed by an additional criterion such as the relative error of prediction.

Both optimum spectral zones included one $\lambda_{\max}$ of their respective analytes. As expected, the AMH range was built around the less interfered absorbing peak with $\lambda_{\max} = 363$ nm, while the HCT preferred region was constructed to enclose the maximum at $\lambda_{\max} = 270$ nm. Interestingly, however, analysis of the tri-dimensional initial sensor-window size-PRESS surface plot revealed the presence of a slightly less than optimal zone for HCT between 217 and 330 nm, covering its three absorption maxima, one of which presents slight deviation from Beer's law (Table 1). Prediction results obtained operating in this sub-optimal zone were similar to those obtained employing the 260–277 nm wavelength range; however, the latter gave

slightly lower variances and therefore it was employed for the rest of the predictions.

On the other hand, the optimum number of factors ($a$), required to avoid overfitting, was chosen for each analyte by application of the $F$-ratio criterion proposed by Haaland and Thomas [36]. For each latent variable ($h$), the minimum PRESS [PRESS($h$*)] within its optimum spectral range was employed in the calculation of the $F$-ratio as shown in the following equation:

$$F(h) = \text{PRESS}(h)/\text{PRESS}(h^*). \quad (1)$$

The optimum number of factors ($a \leq h$*) was considered as that corresponding to a probability of less than 75%. Calibration data were also checked for spectral and leverage-related outliers following the criteria of Ref. [30] and a linear response between actual and predicted values was observed over the examined concentration ranges of both analytes [$\text{HCT}_{\text{unk}} = 0.0018(\pm 0.0048) + 0.9993(\pm 0.0126)\text{HCT}_{\text{act}}$ and $\text{AMH}_{\text{unk}} = 0.0001(\pm 0.0009) + 0.9995(\pm 0.0040)\text{AMH}_{\text{act}}$]. As shown in Table 2, the use of the two factors accounted for more than 99.9% of spectral and concentration variances in the calibration models of both drugs, within their respective optimum spectral ranges.

Validation parameters such as accuracy and precision were studied. Comparison between the amounts of drug obtained in the determination of a set of 12 independent synthetic mixture compositions with those actually added to the solutions allowed the assessment of model accuracy. The results given in Table 3 indicated that essentially quantitative recoveries of the analytes were achieved over the range of concentrations tested, satisfying the acceptance criteria for this study. On the other hand, precision was determined by means of a one-way ANOVA of a second set of 15 samples, analyzed in groups of five at three different times. Mean sample recoveries of this second set were also close to 100%, and data analysis indicated that no statistical difference was found among inter- and intra-assay values for both analytes. Interestingly, in spite of the comparatively low concentration of AMH in the samples and its low absorbance readings within the selected wavelength range, the UV-PLS combination was able to provide results as precise and accurate as those furnished for the most concentrated HCT.

Taking into account that AMH is known to photodegrade, specially in non-acidic solutions

Table 3
Accuracy and precision data for the spectrophotometric PLS-1 simultaneous determination of HCT and AMH

| Parameter | HCT | AMH |
|---|---|---|
| *Accuracy* | | |
| $N$[a] | 24 | 24 |
| Concentration range (mg $l^{-1}$) | 21.7–27.9 | 1.9–2.7 |
| $y_0 \pm$SD | 0.019 ± 0.017 | 0.0018 ± 0.002 |
| Slope ± SD | 0.9996 ± 0.007 | 0.9924 ± 0.0085 |
| $r$ | 0.9995 | 0.9992 |
| *Precision* | | |
| Between-days variation | $7 \times 10^{-2}$ | $1.1 \times 10^{-2}$ |
| Within-days variation | $9 \times 10^{-2}$ | $1.2 \times 10^{-2}$ |
| $F$-ratio[b] | 0.76 | 0.95 |
| Mean recovery (%) | 101.06 | 101.30 |
| Between-days RSD (%) | 0.29 | 0.34 |
| Within-days RSD (%) | 0.27 | 0.31 |

[a] Number of measurements.

Table 2
Spectral and concentration model variances explained by the PLS-1 calibration system

| Number of factors | HCT | | AMH | |
|---|---|---|---|---|
| | Spectral variance (%) | Concentration variance (%) | Spectral variance (%) | Concentration variance (%) |
| 1 | 99.97 | 99.12 | 99.47 | 98.07 |
| 2 | 99.98 | 99.93 | 99.94 | 99.95 |
| 3 | 99.994 | 99.93 | 99.94 | 99.97 |
| 4 | 99.996 | 99.95 | 99.97 | 99.97 |

[37], concomitant with validation, the PLS-1 calibration model was applied to evaluation of the stability of the stock solutions used throughout the study. The outcome of this experiment is shown in Table 4. The ANOVA analysis of the results indicated that both solutions exhibited statistically unaltered drug concentrations during the test period, being stable at 4 °C in MeOH–$H_2O$ over at least 7 weeks.

The validity of the proposed method for the analysis of pharmaceutical preparations and the effect of possible interferences were studied by the simultaneous determination of drug content in synthetic samples and in two different commercial tablet formulations available in Argentine. The results, in terms of percent mean recovery, relative standard deviation and standard error of prediction are consigned in Table 5; they attest the high reliability and reproducibility of the method.

In addition to the high and consistent drug recoveries, low residual spectral errors indicated that the method is free from interference of the excipients. It was observed that drug contents were slightly above their nominal amounts in one of the brands, while the recovery data for AMH were slightly below those declared in the other; nevertheless, all the values were within their respective specifications and in good agreement with their manufacturers' labeled contents.

In order to obtain additional evidence on the accuracy of the proposed method in tablet analysis, two samples each of both pharmaceutical formulations were simultaneously determined by UV-PLS and HPLC, according to the USP 24 [3] with the results collected in Tables 6 and 7. Statistical $t$-test comparison of the means indicated, with a high level of assurance, that recovery data obtained employing the proposed spectroscopic method with multivariate calibration were in concordance with those furnished by HPLC and, as expected, that they were all consistent with the manufacturer's labeled contents. However, the UV-PLS technique demonstrated to be less time-consuming, simpler and more convenient, taking into account that chromatograms required as much as 25 min to develop.

This UV-PLS method was also applied to the evaluation of the dissolution behavior of the commercial tablets. The USP 24 requires that samples taken at a single time of 30 min should contain no less than 80 and 75% of the labeled amounts of AMH and HCT, respectively. In this procedure, concentration of AMH is evaluated at this single time from absorbance readings at its $\lambda_{max} = 363$ nm, while quantification of HCT is based on the difference of absorbances at $\lambda = 270$ and 363 nm, to account for AMH contribution. This procedure is straightforward; however, it is worth noting that the absorbance of AMH at $\lambda =$

Table 4
Stability analysis of stock solutions of HCT and AMH in MeOH–$H_2O$ (1:1, v/v)

| Parameter | HCT | | | | AMH | | | |
|---|---|---|---|---|---|---|---|---|
| Week number | 0 | 1 | 2 | 7 | 0 | 1 | 2 | 7 |
| Mean recovery (%) | 100.8 | 100.2 | 101.6 | 102.1 | 100.6 | 99.8 | 98.8 | 100.2 |
| RSD (%) | 1.0 | 0.4 | 1.3 | 1.2 | 1.5 | 1.0 | 0.5 | 1.1 |
| Number of samples assayed | 4 | 3 | 3 | 4 | 5 | 4 | 4 | 5 |
| *ANOVA of stability tests* | | | | | | | | |
| Mean recovery (%) | 101.2 | | | | 99.9 | | | |
| Between-days variation | 4.10 | | | | 4.14 | | | |
| Within-days variation | 1.15 | | | | 1.25 | | | |
| $F$-ratio | 3.56 | | | | | | 3.31 | |
| $F$-critical[a] | 4.10 | | | | 3.74 | | | |
| Between-days RSD (%) | 1.19 | | | | 1.20 | | | |
| Within-days RSD (%) | 0.91 | | | | 0.99 | | | |

[a] Confidence level of 95%. Degrees of freedom are 2 and 10 for HCT and 2 and 14 for AMH.

Table 5
Spectrophotometric PLS-1 determination of HCT and AMH in synthetic mixtures and pharmaceutical preparations

| Parameter | Synthetic | | Brand 1 | | Brand 2 | |
|---|---|---|---|---|---|---|
| | HCT | AMH | HCT | AMH | HCT | AMH |
| Mean recovery (%)[a] | 100.56 | 100.24 | 103.80 | 103.55 | 99.55 | 94.89 |
| RSD (%) | 0.45 | 0.63 | 1.32 | 1.70 | 0.62 | 1.10 |
| N | 32 | 32 | 24 | 24 | 18 | 18 |
| SEP (mg $l^{-1}$)[b] | 0.11 | 0.014 | 0.50 | 0.045 | 0.16 | 0.028 |

$$\mathrm{SEP} = \left[\frac{1}{N-1}\sum_{1}^{N}(C_{\mathrm{exp}} - C_{\mathrm{pred}}\overline{(C_{\mathrm{exp}} - C_{\mathrm{pred}})})^2\right]^{1/2},$$

where $C_{\mathrm{exp}}$ are the expected sample concentrations based on label claims and $C_{\mathrm{pred}}$ are their predicted concentrations [30,38].

[a] Mean recovery and relative standard deviation relative to nominal content for two determinations.

[b] Label claims are 50 mg HCT/tablet and 5 mg AMH/tablet. Final sample concentrations were approximately 26 mg $l^{-1}$ HCT and 2.6 mg $l^{-1}$ AMH.

363 nm is slightly different from that at $\lambda = 270$ nm potentially affecting the accuracy of the determination of HCT.

Table 8 summarizes the outcome of the quantification of AMH and HCT at different times, in samples of two commercial brands. Results of the UV-PLS method were in excellent agreement with those obtained by applying the USP protocol and showed both brands to comply with the dissolution requirement.

## 4. Conclusions

An accurate, precise and convenient method, based on PLS-1 multivariate calibration analysis of ultraviolet spectral data, was developed for the simultaneous determination of HCT and AMH in synthetic binary mixtures and pharmaceutical dosage forms.

In spite of the unfavorably low concentration of AMH in the samples, this spectrophotometric

Table 6
Spectrophotometric PLS-1 determination of HCT in pharmaceutical preparations

| Method | Parameter | Brand 1 | | Brand 2 | |
|---|---|---|---|---|---|
| UV/PLS-1 | Mean recovery (%)[a] | 104.55 | 106.1 | 99.14 | 99.12 |
| | RSD (%)[b] | 0.35 | 0.28 | 0.25 | 0.05 |
| HPLC[c] | Mean recovery (%) | 104.62 | 104.73 | 98.16 | 100.37 |
| | RSD (%) | 1.42 | 0.99 | 0.41 | 0.39 |
| | $t_{(\mathrm{calc})}$[d] | 0.065 | 1.821 | 2.944 | 4.283 |

Comparison with HPLC.

[a] Mean recovery and relative standard deviation relative to nominal content for two determinations.

[b] Label claims are 50 mg HCT/tablet. Final sample concentrations were approximately 26 mg $l^{-1}$.

[c] Single-point calibration with five samples of standard at a concentration of 1.04 mg $ml^{-1}$. RSD of calibration was 0.73%. Sample concentrations were approximately 1.0 mg $ml^{-1}$. Mean recovery and RSD of three determinations.

[d] $t_{(5,\ 0.01)} = 5.8409$.

Table 7
Spectrophotometric PLS-1 determination of AMH in pharmaceutical preparations

| Method | Parameter | Brand 1 | | Brand 2 | |
|---|---|---|---|---|---|
| UV/PLS-1 | Mean recovery (%)[a] | 102.13 | 104.05 | 95.85 | 95.15 |
| | RSD (%)[b] | 0.24 | 0.49 | 0.52 | 0.23 |
| HPLC[c] | Mean recovery (%) | 102.90 | 103.96 | 96.05 | 95.49 |
| | RSD (%) | 1.92 | 1.43 | 1.67 | 1.04 |
| | $t_{(calc)}$[d] | 0.536 | 0.082 | 0.157 | 0.433 |

Comparison with HPLC.
[a] Mean recovery and relative standard deviation relative to nominal content for two determinations.
[b] Label claims are 5 mg AMH/tablet. Final sample concentrations were approximately 2.6 mg $l^{-1}$.
[c] Single-point calibration with five samples of standard at a concentration of 0.09 mg $ml^{-1}$. RSD of calibration was 0.87%. Sample concentrations were approximately 0.1 mg $ml^{-1}$. Mean recovery and RSD of three determinations.
[d] $t_{(5,\ 0.01)} = 5.8409$.

PLS-1 method was able to allow its quantification and highly reproducible recoveries of this drug were obtained in all cases.

The method is quick and the sample preparation is minimal; it requires simple instrumentation avoiding expensive or time-consuming separations and is capable of providing a high throughput of results; therefore, it seems amenable for routine and quality control analysis of the investigated drugs.

It was successfully employed for the evaluation of stock solutions' stability, drug dissolution profiling and drug quantification in synthetic binary mixtures and pharmaceutical formulations. In addition, the results obtained from the analyses of two commercial brands of tablet preparations were consistent with those furnished by the HPLC method of the USP 24.

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Table 8
Dissolution of co-amilozide tablets, as monitored by the proposed method

| Time (min) | Brand 1 | | | | Brand 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | HCT[a] (%) | | AMH (%) | | HCT (%) | | AMH (%) | |
| | PLS-1 | USP[b] | PLS-1 | USP | PLS-1 | USP | PLS-1 | USP |
| 5 | ND[c] | ND | ND | ND | 49.1 | 48.6 | 82.1 | 86.3 |
| 10 | 67.4 | 64.3 | 84.5 | 85.0 | 69.1 | 68.6 | 92.4 | 89.7 |
| 15 | 82.1 | 78.0 | 88.9 | 91.0 | 80.6 | 82.4 | 97.7 | 97.1 |
| 20 | 90.6 | 88.4 | 96.6 | 99.9 | 89.6 | 88.3 | 99.2 | 99.6 |
| 30 | 95.3 | 95.6 | 98.4 | 99.6 | 94.2 | 93.7 | 99.9 | 99.6 |
| 40 | 97.1 | 97.6 | 98.3 | 99.1 | 97.3 | 96.5 | 100 | 99.8 |

Comparison with drug quantification by the spectrophotometric technique based on USP 24.
[a] Percentage of drug dissolved, mean of six vessels.
[b] The following equations provide AMH and HCT contents in mg $l^{-1}$: $AMH_{unk} = -0.051 + 13.361A_{363}$ ($N = 15$, $r = 0.990$); $HCT_{unk} = -0.229 + 16.89A_{270}$ ($N = 15$, $r = 0.999$). Samples showed complete dissolution of both analytes at time = 60 min. $A_{270}$ values were corrected for the interference of AMH by deduction of the respective $A_{363}$ readings.
[c] Not determined.

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