

PLS and first derivative of ratio spectra methods for determination of hydrochlorothiazide and propranolol hydrochloride in tablets

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Abstract Two new analytical methods have been developed as convenient and useful alternatives for simultaneous determination of hydrochlorothiazide (HCT) and propranolol hydrochloride (PRO) in pharmaceutical formulations. The methods are based on the first derivative of ratio spectra (DRS) and on partial least squares (PLS) analysis of the ultraviolet absorption spectra of the samples in the 250–350-nm region. The methods were calibrated between 8.7 and 16.0 mg L⁻¹ for HCT and between 14.0 and 51.5 mg L⁻¹ for PRO. An asymmetric full-factorial design and wavelength selection (277–294 nm for HCT and 297–319 for PRO) were used for the PLS method and signal intensities at 276 and 322 nm were used in the DRS method for HCT and PRO, respectively. Performance characteristics of the analytical methods were evaluated by use of validation samples and both methods showed to be accurate and precise, furnishing near quantitative analyte recoveries (100.4 and 99.3% for HCT and PRO by use of PLS) and relative standard deviations below 2%. For PLS the lower limits of quantification were 0.37 and 0.66 mg L⁻¹ for HCT and PRO, respectively, whereas for DRS they were 1.15 and 3.05 mg

L⁻¹ for HCT and PRO, respectively. The methods were used for quantification of HCT and PRO in synthetic mixtures and in two commercial tablet preparations containing different proportions of the analytes. The results of the drug content assay and the tablet dissolution test were in statistical agreement ($p < 0.05$) with those furnished by the official procedures of the USP 29. Preparation of dissolution profiles of the combined tablet formulations was also performed with the aid of the proposed methods. The methods are easy to apply, use relatively simple equipment, require minimum sample pre-treatment, enable high sample throughput, and generate less solvent waste than other procedures.

Keywords Hydrochlorothiazide · Propranolol hydrochloride · First derivative of ratio spectra · PLS · Dissolution test · Drug content assay

Introduction

Combination of hydrochlorothiazide (HCT), a thiazide-type diuretic, with fixed doses of the non-selective β -adrenergic blocker propranolol hydrochloride (PRO) is a pharmacological combination [1] currently used in anti-hypertensive therapy [2].

Analytical methods reported for simultaneous quantification of this officially recognized combination [3] in pharmaceutical dosage forms include TLC–densitometry [4], two-wavelength spectroscopy [5], derivative spectroscopy [6], and HPLC [7, 8]. The procedure of USP 29 for drug content involves HPLC determination, and tablet dissolution is assayed by classical spectrophotometry after a selective extraction with an organic solvent [3].

Multivariate calibration is a useful chemometric tool for analysis of complex mixtures, because it enables rapid and

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simultaneous determination of each component in the mixture, with minimum sample preparation and without the need for lengthy separations [9, 10]. Partial least-squares (PLS) is a factor-based multivariate calibration method which decomposes data into spectral loadings and scores and, assuming compliance with Beer's Law, builds the corresponding calibration models from these new variables. The background of PLS has been extensively discussed [11–14].

Because of their potential ability to increase minor spectral features, derivative techniques are also used in conjunction with spectrophotometric methods, especially when improved selectivity is required [15, 16]. In recent years, these methods have emerged as attractive strategies for rapid determination of several analytes in mixtures.

Herein we report the development of two methods for the simultaneous determination of HCT and PRO, based on PLS and first-derivative of ratio spectra (DRS) analysis of the ultraviolet spectra of HCT–PRO mixtures. The convenient use of these methods for accurate and precise determination of drug content and tablet dissolution in pharmaceutical formulations containing both drugs is also discussed.

Results and discussion

The electronic absorption spectra of HCT, PRO, and a mixture of both drugs in 0.01 mol L⁻¹ HCl are shown in Fig. 1a. They overlap severely and their first derivative spectra (Fig. 1b) have no convenient zero-crossing points. In our hands this feature resulted in unreliable application of previously reported procedures based on conventional absorbance readings and derivative techniques [5, 6], leading to unsatisfactory results. We therefore decided to develop PLS and DRS methods, with the objective of providing new, rapid, and advantageous alternatives for determination of these analytes in their combined dosage forms.

The pharmaceutical combination HCT–PRO is available in two different proportions (25 mg HCT mixed with 40 mg or 80 mg PRO). A training set of 24 samples (in the form of

a 4×6 full asymmetric factorial design) in 0.01 mol L⁻¹ HCl was therefore designed for the PLS method, with the objective of testing both formulations with a single set of calibration standards. Calculations were performed on mean-centered data. Variables were selected by searching the minimum PRESS (prediction residual error sum of squares, a measure of the predictive ability of the model) with the changeable size moving-window strategy [17–19] and the leave-one-out internal cross-validation scheme, for one to five latent variables. The appropriate number of model dimensions, which was found to be two for each analyte, was found applying the Haaland and Thomas [20] statistical criterion ($\alpha=0.75$).

Relevant information related to the PLS calibration models for HCT and PRO using full spectra (250–350 nm) and in their optimum spectral regions is collected in Table 1. As expected, improved calibrations were obtained when selected spectral regions were used instead of full spectra. Low values were obtained for both RMSD (root mean square deviation) and REC (the percentage relative error in calibration), which measure the average error in the analysis and evaluate the goodness of fit of the calibration data to the models developed during calibration. Both of these, and highly satisfactory results for LLOQ (lower limit of quantification), r^2 (a measurement of the goodness of fit of the predicted concentrations to their actual values), and figures of merit, are evidence of the quality of the models and the suitability of the method for the proposed determinations.

The calibration models were validated for accuracy and precision with three external sets of nine samples each, prepared by following a full factorial design, which were analyzed on three separate occasions. These yielded almost quantitative analyte recoveries (100.4 and 99.3% for HCT and PRO, respectively) and relative standard deviations below 2%. Repeatability was demonstrated by an ANOVA test conducted on the three validation sets (F -ratios 1.90 and 0.18 for HCT and PRO, respectively; $F_{(0.95,2,24)}=3.40$).

For binary mixtures, first derivative of ratio spectra spectrophotometry is performed by taking the first deriva-

Fig. 1 (a) Electronic absorption spectra obtained from 16.0 mg L⁻¹ HCT (1), 25.8 mg L⁻¹ PRO (2), and a mixture containing 16.0 mg L⁻¹ HCT and 25.8 mg L⁻¹ PRO (3) in 0.01 mol L⁻¹ HCl. (b) First derivative of the electronic absorption spectra of 16.0 mg L⁻¹ HCT (1), 25.8 mg L⁻¹ PRO (2), and a mixture containing 16.0 mg L⁻¹ HCT and 25.8 mg L⁻¹ PRO (3)

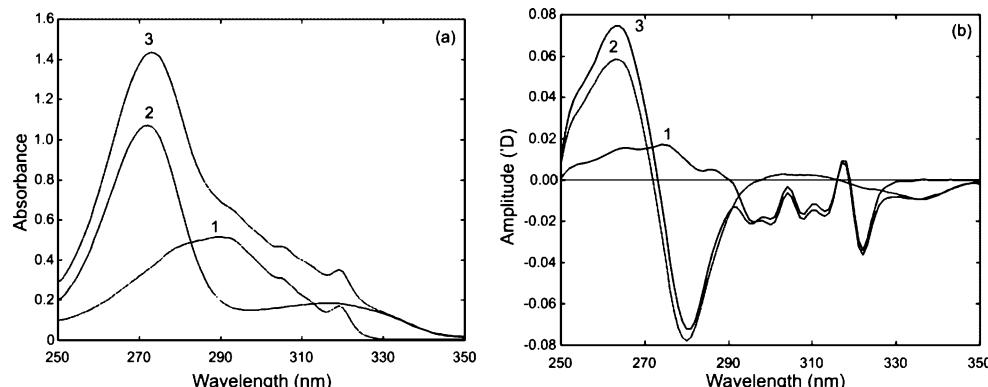


Table 1 Results from PLS analysis of HCT and PRO. Statistical data for the calibration models

Property ^a	HCT	PRO		
Spectral range (nm)	250–350	277–294	250–350	297–319
Linear range (mg L ⁻¹)	8.7–16.0	8.7–16.0	14.0–51.5	14.0–51.5
Concentrations of the calibration samples (mg L ⁻¹) ^b	8.7, 10.4, 13.9, 16.0		14.0, 17.9, 25.8, 28.0, 36.4, 51.5	
Concentrations of the validation samples (mg L ⁻¹)	9.6, 12.2, 14.8		15.1, 32.5, 43.7	
Number of factors (latent variables)	2	2	2	2
PRESS ((mg L ⁻¹) ²)	0.10	0.044	0.60	0.43
Root mean square deviation, RMSD, (mg L ⁻¹)	0.07	0.04	0.16	0.13
Relative error in calibration, REC (%)	0.52	0.34	0.53	0.45
<i>r</i> ²	0.9996	0.9998	0.9999	0.9999
Selectivity	0.32	0.57	0.68	0.51
Sensitivity (SEN)	0.19	0.07	0.07	0.021
Analytical sensitivity, γ (L mg ⁻¹)	12.0	15.4	8.7	6.3
Minimum concentration difference, γ^{-1} (mg L ⁻¹)	0.08	0.07	0.11	0.16
Lower limit of detection (LLOD, mg L ⁻¹)	0.17	0.11	0.23	0.20
Lower limit of quantification (LLOQ, mg L ⁻¹)	0.56	0.37	0.78	0.66

^aREC(%)=(100/C_a)×RMSD, where C_a is the average component concentration in the calibration mixtures. Sensitivity=1/||b||, where b is the final regression coefficients vector for the analyte, and γ =(SEN/ σ_o), where σ_o is the standard deviation of the blank. Selectivity=1/(||b|| ||AC^T C||), where A and C are the mean centered absorbance (within the region of interest) and concentration data blocks, respectively

^bA full asymmetric factorial design of 24 samples was used

tive of the curves resulting from the division, amplitude by amplitude, of normal spectra of the analyte by an appropriate spectrum of the other component of the mixture. The corresponding calibration graph is constructed by plotting the amplitude of the derivative spectra at a maximum or a minimum, against the corresponding concentrations of the analyte [21]. This strategy has been particularly recommended when traditional derivative spectrophotometry gives poor results [22, 23].

To develop the DRS method for HCT and PRO the number of smoothing points, the wavelength increment ($\Delta\lambda$) to obtain the first derivative [24, 25], and the concentration of the divisor spectrum were optimized; the best results are shown in Table 2 and Fig. 2. Straight lines passing through the origin confirmed each derivative signal was independent of the presence of the other analyte. PRO could be quantified by measuring the spectral ratio amplitude at 288, 307, 312, or 322 nm, the determination being most sensitive at 322 nm.

The accuracy and precision of the DRS method were determined by assay of nine mixtures containing different amounts of both analytes within their linear ranges. Plots of the predicted concentrations against known values furnished straight lines, with correlation coefficients of 0.9981 and 0.9999 for HCT and PRO, respectively. In addition, the concentration residuals appeared randomly distributed around zero when plotted against the actual concentrations of the prepared mixtures.

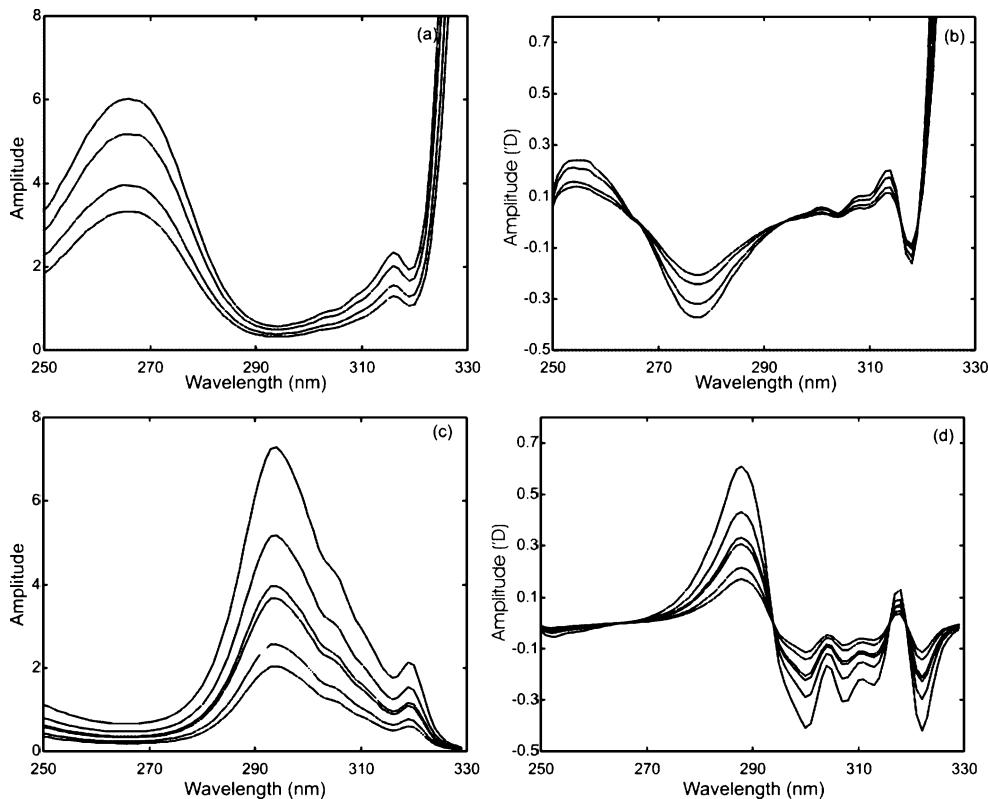
Essentially quantitative recoveries and good standard deviations for both analytes were recorded when the PLS and DRS methods were performed on six synthetic mixtures of HCT and PRO (Table 3).

PLS and DRS predictions of the amounts of the active principles in tablet preparations containing two different combinations of HCT and PRO were also in statistical agreement and results were not significantly different from those obtained by use of the HPLC-based USP method. The performance of PLS and DRS was also better than that of a recently reported derivative method (Table 3) [6]. Taking into account the tolerance levels established by the USP for pharmaceutical tablet formulations [3], both commercial preparations proved to comply with the declared amounts of their active ingredients.

Table 2 First derivative of ratio spectra analysis of HCT and PRO. Statistical data for the calibration models

Property	HCT	PRO
Working wavelength (nm)	276	322
Linear range (mg L ⁻¹)	8.7–16.0	14.0–51.5
Concentrations of the calibration samples (mg L ⁻¹)	8.7, 10.4, 13.9, 16.0	14.0, 17.9, 25.8, 28.0, 36.0, 51.5
Concentration of the divisor spectrum (mg L ⁻¹)	14.0 (PRO)	13.9 (HCT)
Number of smoothing points	9	9
Wavelength increment for differentiation ($\Delta\delta$, nm)	9	9
Intercept ($\pm SD$) of the calibration line	-1.8 (± 5.9) × 10 ⁻³	4.2 (± 8.6) × 10 ⁻⁴
Slope ($\pm SD$) of the calibration line	-22.6 (± 0.5) × 10 ⁻³	8.21 (± 0.03) × 10 ⁻³
<i>r</i> ² of the calibration line	0.9996	0.9999
Lower limit of detection (LLOD, mg L ⁻¹)	0.35	0.92
Lower limit of quantification (LLOQ, mg L ⁻¹)	1.15	3.05

Fig. 2 Ratio spectra (**a**) and first derivative of ratio spectra (**b**) of HCT (8.7, 10.4, 13.9 and 16.0 mg L⁻¹; divisor: PRO, 14.0 mg L⁻¹); and ratio spectra (**c**) and first derivative of ratio spectra (**d**) of PRO (14.0, 17.9, 25.8, 28.0, 36.0 and 51.5 mg L⁻¹; divisor: HCT, 13.9 mg L⁻¹). Solvent: 0.01 mol L⁻¹ HCl



The dissolution test is a convenient in-vitro approach to measurement of drug bioavailability; it is one of the key tests used for determination of the quality of tablets, capsules, and other solid pharmaceutical dosage forms, and of drug-release behavior. The methods were used as analytical tools to evaluate the dissolution of commercial samples. The dissolution test was performed in accordance with USP 29,

with the dissolution test system configured as apparatus 1 (basket), at a rotation speed of 100 rpm; 0.01 mol L⁻¹ HCl (900 mL), thermostatted at 37.0±0.1 °C, was used as dissolution medium. Six tablets were analyzed per run in six separate flasks and the amounts of the dissolved drugs were determined in filtered samples (20-μm filters), withdrawn from the dissolution medium after a dissolution

Table 3 Determination of HCT and PRO in synthetic mixtures and in tablets (drug content and dissolution) by PLS and DRS. Comparison with the USP procedure

Sample (analytical purpose)	Analyte	Drug recovery (%) ^a			
		Literature method ^d	PLS	DRS	USP ^e
Synthetic (drug content)	HCT	102.8±2.3	100.1±0.7	100.1±1.4	
	PRO	94.5±1.8	99.3±0.8	100.3±1.0	
Tablets ^b 25 mg HCT/40 mg PRO (drug content)	HCT	96.5±1.3	94.7±1.5	94.2±0.9	95.8±1.6 ^f
	PRO	90.9±1.7	95.0±0.7	94.0±0.8	94.3±1.8 ^f
Tablets ^b 25 mg HCT/80 mg PRO (drug content)	HCT	99.8±1.3	98.5±0.7	97.4±0.9	
	PRO	96.1±0.6	101.3±0.4	100.4±0.4	
Tablets ^c 25 mg HCT/40 mg PRO (dissolution test)	HCT	96.0±1.4	92.7±1.3	94.7±1.8	94.5±1.5 ^f
	PRO	91.4±2.0	95.3±1.4	96.9±1.9	97.6±2.5 ^f
Tablets ^c 25 mg HCT/80 mg PRO (dissolution test)	HCT	97.6±4.3	98.2±2.5	98.8±3.0	98.8±2.6 ^f
	PRO	93.3±3.4	101.2±3.7	101.2±3.8	102.9±3.4 ^f

^a Mean and standard deviation for six determinations

^b Percentage recovery from the label-claimed amount. Samples taken from 20 powdered tablets

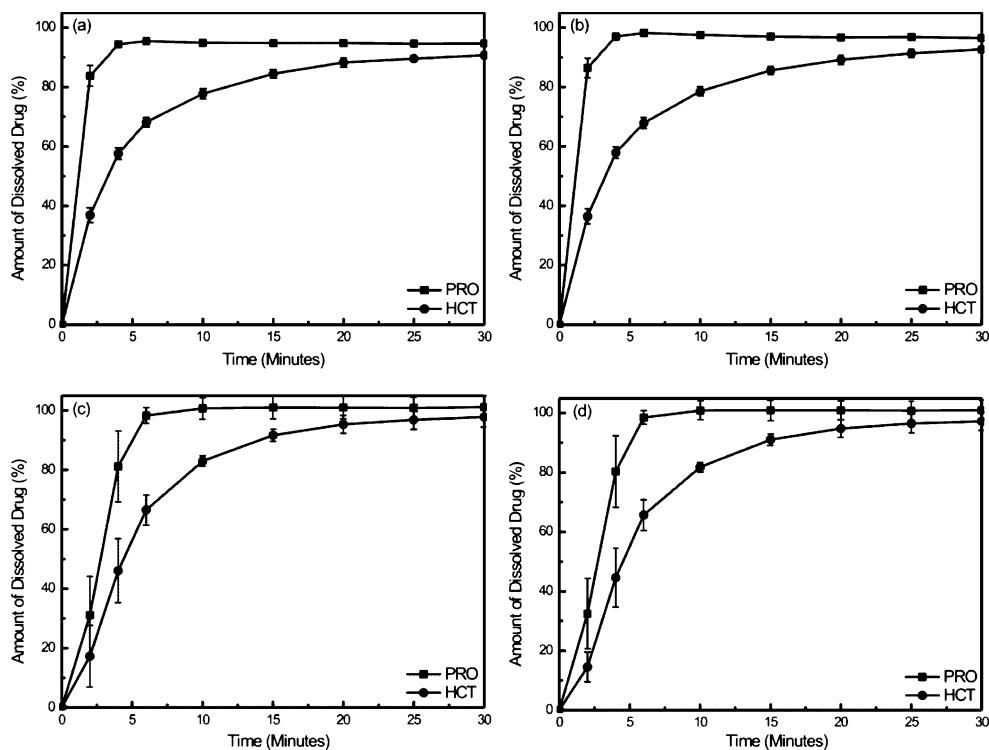
^c Percentage recovery from the label-claimed amount for six individual tablets

^d Derivative spectroscopic method reported in Ref. [6]

^e Determinations were performed in accordance with USP 29

^f Drug recoveries for the PLS, DRS, and USP procedures are statistically equivalent ($p<0.05$)

Fig. 3 Dissolution profiles of HCT–PRO tablets containing 25 mg HCT and 40 mg PRO, as determined by PLS (a) and DRS (b), and tablets containing 25 mg HCT and 80 mg PRO, as determined by PLS (c) and DRS (d)



time of 30 min [3]. Results were compared with those obtained by use of the USP procedure based on a selective extraction with hexane as solvent. In this test it was observed that the results obtained by PLS and DRS for the amounts of HCT and PRO dissolved in the 30 min period were statistically concordant with those predicted by the USP official method ($p < 0.05$) for both formulations (Table 3). The advantages of HCT and PRO, however, were that they were less time-consuming and produced less organic solvent waste.

The proposed methods were also used to construct dissolution profiles of tablets containing 25 mg HCT combined with 40 or 80 mg PRO. This was achieved by quantifying the amount of dissolved HCT and PRO at different times, under the USP 29 conditions for the dissolution test [3]. As shown in Fig. 3, both methods furnished similar dissolution profiles for each drug combination.

In conclusion, we have developed two useful and effective analytical methods for determination of HCT and PRO in drug content and dissolution tests on tablets containing this pharmacological combination. The proposed methods (PLS and DRS) were rapid, accurate, and reproducible, enabled high sample-throughput and required minimum sample pretreatment.

They gave results statistically similar to those furnished by compendial procedures, with the advantages of being more rapid, less expensive, and producing less solvent waste. The DRS method is simpler and less time-consuming than PLS and can be advantageously applied in routine analysis because it requires only a few solutions for calibration.

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Electronic Supplementary Material

**PLS and first derivative of ratio spectra methods
for the determination of hydrochlorothiazide and
propranolol hydrochloride in tablets**

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Experimental

Equipment

Spectrophotometric measurements were carried out between 250 and 350 nm, at 1-nm intervals (101 data points/spectrum) against a blank of solvent, in 1-cm quartz cells, with a Shimadzu UV-1601 PC spectrophotometer. Variable selection [1-4], data transformation, ratio spectra [5-7], smoothing and derivation [8, 9], and PLS [10-13] data analysis were performed in Matlab 5.3 (Mathworks, Inc.). PLS was run on mean-centered data.

A Hanson SRS 8 Plus dissolutor configured as USP Apparatus 1 (basket) was used for the dissolution tests. Statistical treatment of the data was performed with the SPSS 9 (SPSS, Inc.) application software.

Reagents

The experiments were carried out employing USP-grade drugs, analytical-grade solvents and double-distilled water. Stock solutions of HCT (870 mg L^{-1}) and PRO (2800 mg L^{-1}) were prepared in MeOH. Working solutions of HCT (43.5 mg L^{-1}) and PRO (140 mg L^{-1}) were made before use, by dilution of the corresponding stock solutions in 0.01 M HCl. Tablets were obtained from Wyeth Laboratories, Inc. Argentina. Their label claim was 40 mg PRO and 25 mg HCT, and 80 mg PRO and 25 mg HCT.

Samples

Training Set for PLS

A set of 24 mixtures was prepared in 25 mL flasks, by diluting the working solutions in 0.01 M HCl to obtain final concentrations in the ranges $8.7 - 16.0 \text{ mg L}^{-1}$ for HCT and $14.0 - 51.5 \text{ mg L}^{-1}$ for PRO. The analyte levels were chosen in ratios close to those of the commercial tablet preparations, covering the range of $100 \pm 30\%$ of their expected concentrations in the unknowns.

Calibration Solutions for the Ratio Spectra Derivative Method

Two sets, containing four solutions of HCT ($8.7 - 16.0 \text{ mg L}^{-1}$) and six solutions of PRO ($14.0 - 51.5 \text{ mg L}^{-1}$), were prepared in 25 mL flasks by dilution of the corresponding working solutions in 0.01 M HCl. For building the calibration plots, their spectra were divided by spectra of PRO (14.0 mg L^{-1}) and HCT (13.9 mg L^{-1}), respectively.

External Validation Set for PLS and Ratio Spectra Derivative Methods

Three groups of nine samples each covering the concentration ranges $9.6 - 14.8 \text{ mg L}^{-1}$ for HCT and $15.1 - 43.7 \text{ mg L}^{-1}$ for PRO were prepared in 25 mL flasks, by dilution of appropriate volumes of the working solutions with 0.01 M HCl. Spectra of the mixtures were acquired at three different times, recorded and analyzed by PLS and ratio spectra derivative methods.

Synthetic Samples for PLS and Ratio Spectra Derivative Methods

A set of six mixtures covering the concentration ranges $9.6 - 14.8 \text{ mg L}^{-1}$ for HCT and $15.1 - 43.7 \text{ mg L}^{-1}$ for PRO was prepared in 25 mL flasks, by dilution of appropriate volumes of the working solutions with 0.01 M HCl. Spectra of the mixtures were acquired, recorded and processed by both methods.

Determination of Drug Content in Pharmaceutical Dosage Forms

Groups of 20 tablets were weighed, ground and finely powdered in a mortar. Portions of the powders were accurately weighed, transferred to 25 mL volumetric flasks and suspended in 12.5 mL of 0.01 M HCl. The flasks were mechanically shaken for 30 min, completed to the mark with 0.01 M HCl, mixed, and left for 30 min at room temperature to fully decant the solids. Then, 2mL aliquots were transferred from each flask to 25 mL volumetric flasks and individually diluted to their marks with 0.01 M HCl. Finally, spectra of the mixtures were acquired, recorded and analized by both methods.

Dissolution Test of Hydrochlorothiazide-Propranolol Tablets

The test was carried out according to the method of the USP (apparatus 1; basket rotation speed: 100 rpm; medium: 900 mL of 0.01 M HCl; dissolution time: 30 min; bath temperature: $37.0 \pm 0.1^\circ\text{C}$; number of flasks: 6) [14]. The samples were filtered through 20 μm filters, their spectra were acquired, recorded and analized by both methods.

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