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TECHNICAL NOTE

A multivariate approach for the simultaneous determination of losartan potassium and hydrochlorothiazide in a combined pharmaceutical tablet formulation

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Abstract A convenient new method for the simultaneous determination of losartan potassium and hydrochlorothiazide, with minimum sample pretreatment, is described. The procedure, based on the multivariate analysis of spectral data in the 220–274 nm region by the partial least squares algorithm, is linear in the concentration range 1.06–5.70 mg L^{-1} for hydrochlorothiazide and 4.0–22.2 mg L^{-1} for losartan. It is simple, rapid and robust, allowing accurate and precise results, with drug recovery rates of 99.3 and 100.4% and relative standard deviations of 1.7 and 1.0% obtained for hydrochlorothiazide and losartan, respectively. The method was applied to the simultaneous determination of both analytes in tablets, and it provided good results which were in statistical agreement with those provided by independent HPLC analyses of the samples. The method has also been successfully applied for the construction of drug dissolution profiles of a commercial pharmaceutical preparation containing both analytes.

Keywords Chemometric method · Drug content · Dissolution profiles · Simultaneous determination · Losartan potassium · Hydrochlorothiazide

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Introduction

Losartan potassium (LSK) is a nonpeptidic angiotensin II receptor antagonist [1]; the drug is widely used in antihypertensive therapy and in patients with moderate heart failure [2] when administered in a 4:1 (w/w) co-formulation with the known thiazide-type diuretic hydrochlorothiazide (HCT). HCT, but not LSK nor their association, is included in the thirtieth edition of the United States Pharmacopoeia [3] and the fourth edition of the European Pharmacopoeia [4].

Recently, the HCT-LSK combination has been the subject of intense analytical research and several methods have been reported for the simultaneous determination of its components, including HPTLC [5], SFC [6], CE [7], capillary electrochromatography [8], and HPLC [9–14]. In biological fluids, the active principles have been determined simultaneously by LC-MS [15–17]. These methods are not always simple to perform; they are also time-consuming, require careful and often tedious sample preparation due to the different acid-base characteristics of the analytes, and make use of expensive instruments that are not always available in all quality control laboratories.

Spectrophotometric analysis, being simple, economic and fast, constitutes a highly convenient alternative approach for the analysis of drugs in pharmaceutical formulations. However, only a handful of these methods have been reported for the analysis of the HCT-LSK association. These include two-wavelength (206.6/270.6 nm or 236/270 nm) [18, 19], dual-wavelength ($A_{261.4} - A_{206.6}$) [19] and area under the spectral curve [20] determinations, fourth-derivative spectroscopy [21], as well as first-derivative [5], first derivative of spectral ratio and compensation techniques [22], all of which have different and important

drawbacks, including low signal, poor sensitivity, inconvenient signal-to-noise ratio, unsuitable linear range for the construction of dissolution profiles, poor robustness, the need to carefully obtain high-resolution spectra (< 0.1 nm), the use of highly concentrated samples, and susceptibility to the effect of potential interferences from excipients that absorb at short wavelengths.

Chemometric techniques have twice been employed to investigate this association; in one case their use was in conjunction with chromatographic methods [23], while the other reported the application of artificial neural networks to spectrophotometric data; however, the latter method does not appear to be an appropriate choice for the problem in terms of simplicity, mainly due to its linear nature [24].

Prompted by these disadvantages, here we propose an alternative method for the simultaneous determination of HCT and LSK in a combined pharmaceutical formulation, based on the chemometric analysis of the spectra of the analytes in the UV region.

Since analytical methods to control the quality of pharmaceutical dosage forms require validation, data indicative of the accuracy, precision and robustness of the proposed method in the corresponding linear ranges of the analytes are included. In addition, a statistical assessment of the suitability of the proposed method for the determination of HCT and LSK with reference to HPLC as well as its application for the elaboration of the dissolution profiles of both drugs are disclosed.

Experimental

Apparatus and software

The UV measurements were performed in a UV-1601PC (Shimadzu, Kyoto, Japan) double-beam ultraviolet–visible spectrophotometer using 1-cm quartz cells. For HPLC analyses, a Varian (Palo Alto, CA, USA) Prostar liquid chromatograph was employed, which was fitted with a 5 mm analytical C18 column (Erbasil C18, Carlo Erba, Milan, Italy), a manual injector with a 20-mL injection loop, and a variable wavelength UV detector. Chromatograms were acquired and processed with Varian's Galaxie software. The dissolution experiments were carried out with the aid of a Hanson (Chatsworth, CA, USA) SRS 8 Plus dissolution workstation, configured with paddles (apparatus 2 of the USP XXX).

The chemometrics computations [25], including variable selection [26–28] and PLS [29,30] data analysis, were performed in Matlab 5.3 (Mathworks, Inc., Natwick, MA, USA). PLS was run on mean-centered data. Statistical analyses were performed with SPSS 9 (SPSS, Inc., Chicago, IL, USA).

Chemicals and reagents

Pharmaceutical-grade hydrochlorothiazide and losartan potassium (Laboratorios Gador SA, Buenos Aires, Argentina) were used. HPLC-grade acetonitrile was purchased from Fischer Scientific (Pittsburgh, PA, USA). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Samples were prepared with double-distilled water. Commercial tablets containing the HCT-LSK pharmaceutical association were acquired from a local pharmacy. All other chemicals were of analytical grade and were used as received.

Samples

Stock standard and calibration solutions

For UV-PLS: Stock standard solutions of HCT (52.4 mg L^{-1}) and LSK (100.8 mg L^{-1}) were prepared in 1000-mL flasks by dissolving accurately weighed amounts of the drugs in water and completing to the mark with the same solvent. A set of 24 calibration solutions containing the analytes in the ranges 1.06–5.70 mg L^{-1} for HCT and 4.0–22.2 mg L^{-1} for LSK were prepared by appropriate dilution of the corresponding stock standard solutions with water, employing 25-mL volumetric flasks.

For HPLC: The stock standard solution of LSK was prepared in a 10-mL volumetric flask by dissolving an accurately weighed (25.4 mg) amount of the drug in acetonitrile. Analogously, a stock standard solution of HCT was prepared in a 25-mL volumetric flask by dissolving 32.8 mg of accurately weighed HCT in mobile phase. Calibration solutions in the ranges 2.10–5.24 mg L^{-1} for HCT and 10.0–20.1 mg L^{-1} for LSK were prepared by appropriate dilution of the corresponding stock standard solutions with mobile phase.

Validation samples

These synthetic samples, containing the analytes in the ranges 3.60–5.30 mg L^{-1} for HCT and 12.1–20.2 mg L^{-1} for LSK, were prepared in 25-mL volumetric flasks by appropriate dilution of the corresponding, independently prepared, stock standard solutions (50.3 mg L^{-1} for HCT and 100.6 mg L^{-1} for LSK) with water.

Samples for the determination of drug content

The commercial tablets used in the present investigation were declared to contain 12.5 mg HCT and 50 mg LSK per tablet. Twenty tablets were weighed and finely powdered in

a mortar. A quantity of the powder (ca. 260 mg), equivalent to one tablet, was transferred into a 100-mL calibrated flask containing 50 mL water; the flask was shaken mechanically for 30 min, completed to the mark with water, shaken well and the resulting fine suspension was filtered, discarding the first portion of the filtrate. An aliquot of the filtrate (2 mL) was transferred to a 10-mL volumetric flask and diluted quantitatively with water (for UV-PLS) or mobile phase (HPLC). Samples were processed according to the "General analytical procedure."

Samples for the elaboration of dissolution profiles

The experiments were carried out in six vessels filled with dissolution medium (900 mL of water) thermostated at 37 ± 0.1 °C. Stirring speed was 100 rpm. Samples (5 mL) were withdrawn from each vessel at pre-established times and individually filtered through 0.47-mm nylon filters; in each case, the first milliliter was discarded and an aliquot of the filtrate was conveniently diluted with water. Samples were processed according to the "General analytical procedure" for UV-PLS.

General analytical procedure

For UV-PLS: The UV spectrum of the sample was acquired at 1 nm intervals between 220 and 300 nm against a blank of water and saved in ASCII format. Spectra were processed in MATLAB with the PLS algorithm, employing the HCT and LSK calibration models obtained with the aid of the calibration samples, furnishing the predicted concentrations of the corresponding analytes.

For HPLC: The sample was filtered through a 0.47-mm nylon filter before injection. The mobile phase was 35:65 (v/v) MeCN–phosphate buffer (0.1 M, pH 4.0), where the retention times of HCT and LSK were approximately 5.8 and 6.5 min, respectively. The determinations were performed at room temperature with the eluent pumped at 1.0 mL min^{-1}, while detection was performed at 230 nm. The concentrations of the analytes were obtained from their peak areas, with the aid of the corresponding calibration equations; they were concomitantly obtained through the analysis of five calibration solutions, each injected in duplicate.

Results and discussion

The chemical structures of HCT and LSK are shown in Fig. 1, which also displays the electronic absorption spectra of the pure drugs and the spectrum of a mixture of them between 220 and 360 nm. The spectra overlap considerably below 300 nm, which hinders the resolution of the HCT-LSK mixtures through the use of normal univariate spectroscopy. Between 300 and 345 nm, the absorbance of LSK becomes negligible and the absorptivity of HCT is rather low, making it of little use for the analysis of the latter in the required concentration range.

These spectral characteristics explain the difficulties involved in the simultaneous spectroscopic determination of both analytes, which different authors have attempted to circumvent by devising various normal or derivative spectroscopic strategies [14, 18–22], but which have not been exempt from the drawbacks detailed above.

As an efficient and useful alternative for the resolution of the HCT-LSK mixture, we have developed a fast and simple chemometrics-assisted method, which couples the versatile partial least squares (PLS) algorithm with variable selection to the analysis of the UV spectra of the analytes present in mixtures.

PLS is a highly flexible extension of multiple linear regression, which allows the prediction of the responses of the dependent variables from factors underlying the levels of the predictor variables. PLS, the mathematical basis for which has been extensively discussed [29], has been employed for the resolution of complex mixtures in different fields, including pharmaceutical analysis, due to its speed, appealing simplicity and because it takes into account spectral and concentration data for the analytes during the regression stage.

For the PLS calibration stage, 24 training samples were employed, conforming to an asymmetrical design [31]. Their concentrations (Fig. 2A) were selected in order to also allow the determination of the initial points of the dissolution profiles with the required accuracy and precision. The embedded 4^2 full factorial design comprising

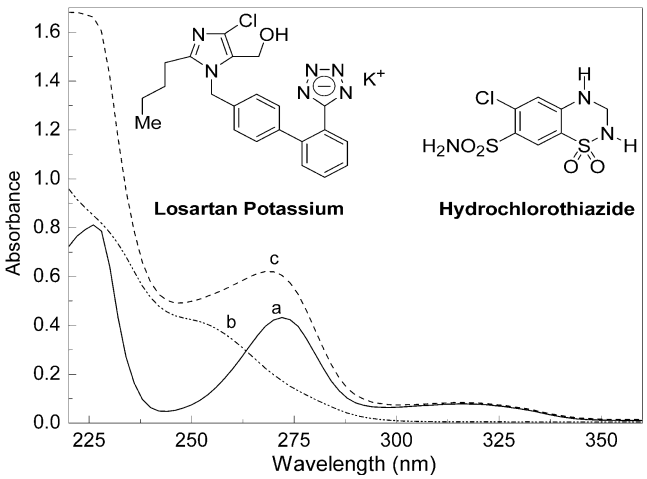

Fig. 1 Electronic excitation spectra, between 220 and 360 nm, of (*a*) HCT (6.35 mg L^{-1}), (*b*) LSK (25.2 mg L^{-1}), and (*c*) a mixture of HCT (6.35 mg L^{-1}) and LSK (25.2 mg L^{-1}), in water

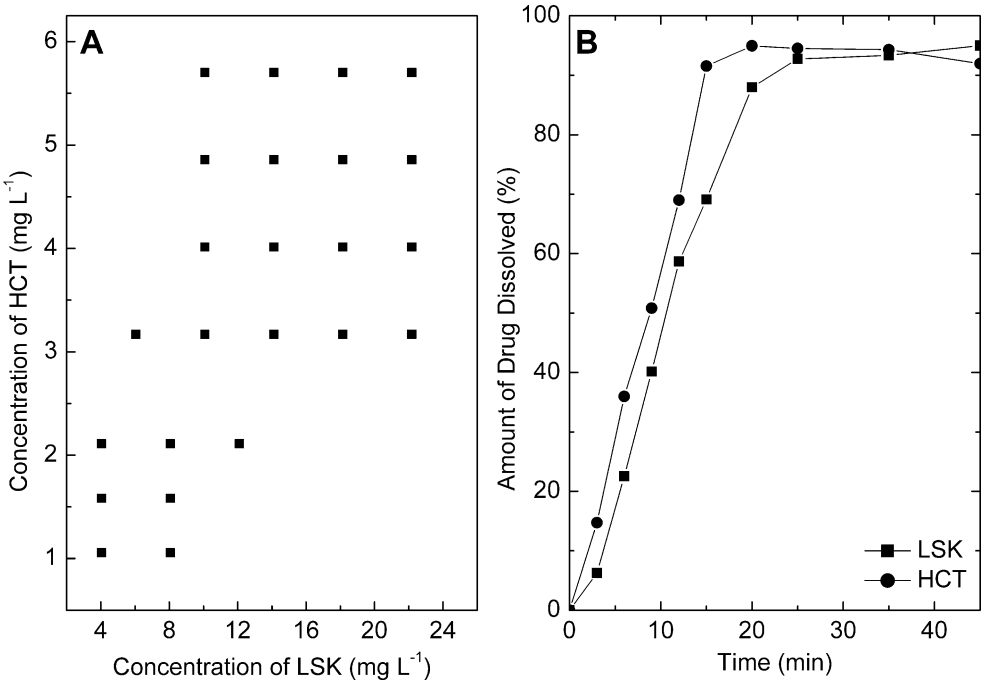

Fig. 2 **A** Concentrations of HCT and LSK in the mixtures comprising the calibration set. **B** Dissolution profiles of HCT and LSK in the combined tablet formulation. The dissolution test was performed with a USP Apparatus II (paddle) at 100 rpm; the dissolution medium was water (900 mL) at 37 °C

analyte mixtures with concentrations of 100±30% of the expected final concentrations is convenient for the determination of the drugs during the later stages of dissolution and during drug content assays. This calibration array is more functionally effective than 5^2 or 6×4 full factorial designs.

The UV spectra of the training samples were mean-centered and submitted to PLS processing with the internal leave-one-out cross-validation scheme and variable selection, employing the moving window of variable size strategy for sensor selection, and assisted by a minimum PRESS (prediction residual error sum of squares) search [32]. The most important details of the resulting calibration models for the analysis of HCT and LSK, reflecting their fitness for the purpose, are collected in Table 1. These include figures of merit such as PRESS, which is a good estimation of the predicting ability of the calibration model; r^2, which approaches unity, revealing that a plot of the predicted versus real data gives a straight line; the root mean square deviation (RMSD), indicative of the average error in the determination of each component; and the relative error of prediction [REP (%)], which provides the value (a percentage) of the RMSD relative to the mean concentration of the training samples.

Once the calibration models were established, the multivariate method was subjected to validation. Under

Table 1 Key statistical parameters for the calibration using the UV-PLS method

Parameter	Analyte	
	HCT	LSK
Number of training samples (*N*)	24	24
Analyte concentration range (mg L^{-1})	1.06–5.70	4.0–22.2
Spectral window (nm)	220–274	220–274
Number of latent variables	2	2
PRESS [(mg L^{-1})^2][a]	0.38	0.25
RMSD (mg L^{-1})	0.13	0.10
REP (%)	1.11	0.77
r^2	0.9994	0.9997
Selectivity [34]	0.52	0.46
Sensitivity [34]	0.23	0.14

[a] $\text{PRESS} = \sum_{i=1}^{N}(y_i - \hat{y}_i)^2$; RMSD=(PRESS/*N*)$^{0.5}$; REP (%)=100×RMSD/$\bar{y}$, and $r^2 = \left[\sum_{i=1}^{N}(y_i - \hat{y}_i)^2\right] \Big/ \left[\sum_{i=1}^{N}(y_i - \bar{y}_i)^2\right]$, where y, $\hat{y}$ $\bar{y}$ represent the true, predicted and mean concentrations of the analyte in the *N* training samples, respectively.

Table 2 Validation of the simultaneous UV-PLS determination of HCT and LSK

Parameters	HCT	LSK
Accuracy		
Number of samples (*N*)	9	9
Concentration range (mg L^{-1})	3.6–5.3	12.1–20.2
$a \pm$ SD (intercept, mg L^{-1} added/mg L^{-1} found)	0.05±0.12	−0.30±0.31
$b \pm$ SD (slope, mg L^{-1} added/mg L^{-1} found)	0.98±0.03	1.02±0.02
R	0.9945	0.9980
Precision		
Number of samples (*N*)	3 × 9	3× 9
Sum of squares (within days)	2.13	0.272
Sum of squares (between days)	41.96	26.18
F ($F_{(0.95,\ 2,\ 24)}$=3.40)	0.61	0.13
Within-day mean recovery (%)	99.2 99.3 98.7	100.4 100.3 100.5
Overall mean recovery (%)	99.3	100.4
Within-day RSD (%)	1.7	1.0
Between-day RSD (%)	1.1	0.14
Robustness[a]		
Wavelengths (nm) [% drug recovery (± RSD)]		
220–269	99.14 (1.28)	100.45 (1.16)
220–274	99.18 (1.23)	100.45 (1.11)
220–279	99.20 (1.21)	100.43 (1.08)
224–269	99.20 (1.19)	100.45 (1.11)
224–279	99.21 (1.19)	100.43 (1.09)
229–269	99.13 (1.21)	100.45 (1.13)
229–274	99.20 (1.20)	100.45 (1.13)
229–279	99.22 (1.21)	100.43 (1.09)
	99.18 (1.22)	100.44 (1.10)
Mean % drug recovery (± RSD)		
Detection and quantitation Limits		
LOD (mg L^{-1})	0.06	0.14
LOQ (mg L^{-1})	0.21	0.49

[a] The ANOVA test resulted in HCT: F_{exp}=0.006; LSK: F_{exp}=0.001; $F_{(0.99,\ 8,\ 72)}$=2.769

the specified optimum wavelength region conditions, the calibration curves for both analytes gave straight lines with very small intercepts and good correlation coefficients in the selected concentration ranges (Table 1). The method's accuracy was assessed by analyzing nine independently prepared validation samples (Table 2) covering the linear range. A plot of percentage of drug recovered against drug added also gave straight lines for both analytes (r=0.9945 and 0.9980 for HCT and LSK, respectively), which passed a joint confidence test for their slopes and intercepts [33]. These results indicated that the method was accurate for both analytes.

Table 3 Simultaneous determination of HCT and LSK in a combined pharmaceutical formulation

Method	Parameter	HCT	LSK
HPLC[a]	Drug recovery (%)	94.4	95.2
	RSD (%)	1.4	1.3
UV-PLS	Drug recovery (%)	95.6	93.5
	RSD (%)	0.6	1.7
	t_{calc} ($t_{(0.05,\ 5)}$=2.23)[b]	1.93	1.95

[a] [HCT, mg L^{-1}] = (−0.02±0.05) + (132.2±1.7) × 10^{-8} AUC; r= 0.9993, N=10
[LSK, mg L^{-1}] = (0.19±0.38) + (141.6±3.6) × 10^{-8} AUC; r=0.9974, N=10

[b] Two-tailed *t*-tests, relative to the HPLC method; six independent samples were analyzed in each case

The precision of the method, including within-day and between-day precisions, was determined with the aid of three independent sets of nine validation samples covering the 100% level as well as lower and higher concentrations (Table 2). The intermediate precision was verified by ANOVA of the three lots. The within-day precision was determined as the RSD of the recoveries of the nine samples contained in the first validation set in a single run, while the between-day precision gave RSD values of less than 2%. This level of precision is considered adequate for the routine quality control analysis of pharmaceuticals.

The evaluation of method robustness was carried out by performing drug recovery analysis of six validation samples after producing deliberate modifications of the initial and final sensors around the selected spectral ranges (Table 2). An ANOVA of the results demonstrated that the method is robust in relation to this variable.

The detection (LOD) and quantitation (LOQ) limits were inferred from the real against the predicted calibration lines using the formulae LOD (or LOQ) = $\kappa SD_a/b$, where SD_a is the standard deviation of the intercept, b is the slope of the calibration line, κ=3.3 for LOD and κ=10 for LOQ. The observed values reflect that the linear working ranges are above the LOQs for both analytes.

Then the validated method was applied to the simultaneous analysis of HCT and LSK in a combined tablet formulation (Table 3). A comparison between the proposed UV-PLS method and HPLC (used as a reference method) revealed that they provide statistically equivalent results. In addition, the observed RSD values are below 2%, indicating that the method is suitable for routine quality control. Analysis of the residuals indicated that the method is selective and that the excipients do not interfere with the determination of the analytes. It is worth noting that the drug recovery results allow us to conclude that the amounts of the drugs contained in the tablets are within the typical specification [3] for each analyte (labeled amount ± 10%).

In another application, the validated method was used to build the dissolution profiles of HCT and LSK in a combined formulation. To do this, the HCT and LSK calibration models were employed for the determination of both drugs in samples taken at pre-established intervals from the vessels of the dissolution workstation (Fig. 2B). The dissolution was carried out in water with a paddle stirring rate of 100 rpm, in agreement with the rate proposed by Suhagia et al. [35] and by Lusina et al. [36]. It is worth noting, however, that while the former team performed a dissolution assay employing 0.1 N HCl as the dissolution medium, the latter team (as in the present case) used water.

A typical USP requirement [3] is that more than 80% of the drug(s) must be dissolved after 30 min. Under the above test conditions, the dissolution profiles constructed with the aid of the UV-PLS method revealed that both drugs are >80% dissolved after 20 min, which means that the tablets comply with the official requirement.

In conclusion, a new and alternative method for the simultaneous determination of LSK and HCT in combined pharmaceutical formulations has been developed. The method, which is based on the PLS analysis of spectral information in the ultraviolet region, was found to be adequately sensitive and robust, and provides reproducible and accurate results without interference from excipients. The method minimizes the time needed for sample preparation as well as the amounts of solvent used and the waste generated. Its suitability for the simultaneous determination of both analytes was also assessed by performing a statistical comparison with the results obtained using HPLC. The method was shown to provide results that were not statistically different from those obtained with the HPLC methodology used as reference. It is of sufficient analytical quality that it can be applied to test the drug contents of finished pharmaceutical products, and its application to the elaboration of drug dissolution profiles was successfully demonstrated. The simplicity and high sample throughput of this method make it suitable for routine quality control testing of the LSK-HCT pharmaceutical association.

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