Simultaneous spectrophotometric determination of losartan potassium, amlodipine besilate and hydrochlorothiazide in pharmaceuticals by chemometric methods

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In the present work, four different spectrophotometric methods for simultaneous estimation of losartan potassium, amlodipine besilate and hydrochlorothiazide in raw materials and in formulations are described. Overlapped data was quantitatively resolved by using chemometric methods, classical least squares (CLS), multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS). Calibrations were constructed using the absorption data matrix corresponding to the concentration data matrix, with measurements in the range of 230.5–350.4 nm ($\Delta\lambda = 0.1$ nm) in their zero order spectra. The linearity range was found to be 8–40, 1–5 and 3–15 μg mL^{-1} for losartan potassium, amlodipine besilate and hydrochlorothiazide, respectively. The validity of the proposed methods was successfully assessed for analyses of drugs in the various prepared physical mixtures and in tablet formulations.

Keywords: losartan potassium, amlodipine besilate, hydrochlorothiazide, spectrophotometry, chemometry

Losartan potassium (LOS), amlodipine besilate (AML) and hydrochlorothiazide (HYD) are drugs widely used for the treatment of hypertension and cardiovascular diseases in combined pharmaceutical preparations. Losartan potassium and its principal active metabolites block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to angiotensin II receptor type 1 (AT$_1$) receptor found in many tissues (vascular smooth muscle, adrenal gland). Amlodipine besilate inhibits the movement of calcium ions across the cell membrane into vascular smooth muscles and myocytes. Action is stronger in arterial resistance vessels causing peripheral vasodilatation and reduction in afterload. Hydrochlorothiazide inhibits the reabsorption of sodium and chloride at the beginning of the distal convo-

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luted tubule. It causes natriuretic effect mainly by decreasing sodium and chloride reabsorption in the cortical segment of the ascending limb of the loop of Henley by inhibition of a specific Na^+, Cl^- co-transporter (1).

A few estimations in body fluids, in bulk in combination with other drugs and in single dosage forms have been reported for losartan potassium, amlodipine besilate and hydrochlorothiazide such as HPLC (2–7), spectrophotometry (8), multivariate approach (9), multi-syringe chromatography (MSC) (10) and HPTLC (11, 12), ultra performance liquid chromatography (UPLC-MS) (13). All these drugs are available in combined tablet dosage forms, as antihypertensive agents. An extensive literature survey revealed that a number of methods are reported for the individual drugs but there is no report on simultaneous estimation of such a combination in physical mixtures or in pharmaceutical formulations by chemometric methods. The present article discusses the attempts made to develop simple, sensitive and reproducible methods for simultaneous estimation of these drugs in dosage forms.

Chemometric calibration techniques in spectral analysis are widely used in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra where separation procedures are not required in drug determination. We have also used these techniques for simultaneous analyses of mixtures (14–19).

In this study, four chemometric methods for spectral data processing are proposed for simultaneous determination of LOS, AML and HYD in their ternary mixtures and in tablets.

EXPERIMENTAL

Instrument and software

A Shimadzu (Japan) 2550-double beam spectrophotometer was used for all spectrophotometric measurements. Absorption spectra of the reference and test solutions were taken in 1-cm matched quartz cells over the range of 200–400 nm. Chemometric calculations on the resulting data were carried out with the PLS toolbox (Demover5.0) in MATLAB 7 (Math works).

Samples and solvents

Losartan potassium, amlodipine besilate and hydrochlorothiazide were kindly supplied by ATOZ India Ltd., India, and were certified to be 99.8, 99.6 and 99.9 % pure, respectively. The drugs were used without further purification. All the solvents used in spectrophotometric analysis were of analytical reagent grade. Trilopace tablets, batch number BF 70002 (Akums Drugs & Pharmaceuticals Ltd., India), which were claimed to contain 50 mg of losartan potassium USP, 5 mg of amlodipine besilate BP and 12.5 mg of hydrochlorothiazide IP, were used.

Standard solutions and mixtures

Stock solutions of LOS (1 mg mL^{-1}), AML (1 mg mL^{-1}) and HYD (2 mg mL^{-1}) in methanol were diluted with water to prepare the working solutions (0.16 mg mL^{-1}, 0.02 mg mL^{-1} and 0.06 mg mL^{-1}, respectively). The calibration set contained 25 and the prediction set 9 mixtures of calibration samples, so that the concentration of each drug in the resulting solutions was in its own linear dynamic range, as shown in Table 1. Furthermore, we demonstrated that in formulations LOS, AML and HYD range 1: 2.5: 10.

Tablet analysis

Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 50 mg of AML, 125 mg of HYD and 500 mg of LOS was dissolved in 50 mL of methanol. The solution was ultrasonicated for 10 minutes. Then, the solution was filtered through Whatman filter paper No. 41. The filtrate (3 mL) was transferred into a 100 mL volumetric flask and made up to volume with Millipore water. Aliquots of these solutions were used in such a way that the concentration of each drug was within the range of the calibration matrix. The diluted solutions were analyzed six times. All the proposed chemometric methods were applied.

Chemometric methods

Classical least squares (CLS). – This method assumes Beer's law model with the absorbance at each frequency being proportional to the component concentration. In

Table I. Composition of the calibration set

Concentration (µg mL^{-1})				Concentration (µg mL^{-1})			
Mixture	LOS	AML	HYD	Mixture	LOS	AML	HYD
1	24	3	9	14	24	5	15
2	24	1	3	15	40	5	3
3	8	1	15	16	40	1	12
4	8	5	6	17	8	4	3
5	40	2	15	18	32	1	9
6	16	5	9	19	8	3	12
7	40	3	6	20	24	4	12
8	24	2	6	21	32	4	6
9	16	2	12	22	32	2	3
10	16	4	15	23	16	1	6
11	32	5	12	24	8	2	9
12	40	4	9	25	16	3	3
13	32	3	15				

LOS, AML, HYD – losartan potassium, amlodipine besilate, hydrochlorothiazide, respectively.

matrix notation, Beer's law model for *m* calibration standards containing *l* chemical components with the spectra of *n* digitized absorbances is given by:

$$A = C \times K + E_A \tag{1}$$

where *A* is the $m \times n$ matrix of calibration spectra, *C* is the $m \times l$ matrix of component concentration, *K* is the $l \times n$ matrix of absorptivity-path length products, and E_A is the $m \times n$ matrix of spectral errors. *K* then represents the matrix of pure component spectra at unit path length. The classical least squares solution according to Eq. (1) during calibration is:

$$\hat{K} = (C^T C)^{-1} C^T \times A \tag{2}$$

where $\hat{K}$ indicates the least-squares estimation of *K*.

Analysis based on spectrum a, of unknown component concentration (sample):

$$C_0 = (\hat{K}\hat{K}^T)^{-1}\hat{K} \times a \tag{3}$$

where C_0 is vector of predicted concentration and $\hat{K}^T$ is the transpose of matrix $\hat{K}$.

Multiple linear regressions (MLR). – If absorbance measurements for several solutions containing mixtures of the analytes are made in numbers exceeding the number of mixture components, then the system composed of the absorbance and concentration matrices will be overdimensioned and take the following matrix form:

$$A = KC \tag{4}$$

where *A* is the data absorbance calibration matrix, *K* is the matrix from which the proportionality constants are calculated from spectra for standard solutions of pure analytes and/or their mixtures, and *C* is the concentration matrix. The *C* prediction concentration matrix can be calculated from the following equation:

$$C = (K^T K)^{-1} K^T \tag{5}$$

where *K′* is the transpose of *K* and *A* is the absorbance matrix of unknown samples. Matrix *K* can be obtained in various ways. We calculated *K* values by *MLR* of the data for mixtures of analytes of known composition (20).

Principle component regression (PCR). – In the spectral work, the following steps can explain the fundamental concept of PCR. The original data obtained in absorbances (*A*) and concentrations (*C*) of analytes were reprocessed by mean-centring as A_0 and C_0, respectively. Using the ordinary linear regression:

$$C = a + b \times A \tag{6}$$

The coefficient b is: $b = P \times q$, where P is the matrix of eigenvectors and q is the C-loadings given by $q = D \times T^T \times A_0$. Here, T^T is the transpose of the score matrix T. D is a diagonal matrix having on components the inverse of the selected eigenvalues. Knowing b one can easily find a by using the formula $a = C_{mean} \times A^T_{mean} \times b$, where A^T_{mean} represents the transpose of the matrix having the entries of the mean absorbance values, and C_{mean} is the mean concentration of the calibration set.

Partial least squares (PLS). – In the UV-Vis spectra, the absorbance data (A) and concentration data (C) are mean centered to give the data matrix A_0 and vector C_0. The orthogonalized PLS algorithm has the following steps. The loading weight vector W has the following expression:

$$W = \frac{A_0^T C_0}{C_0^T C_0} \tag{7}$$

The scores and loadings are given by:

$$t_1 = \frac{A_0 W}{A_0^T t_1} \tag{8}$$

$$P_1 = t_1^T t_1 \tag{9}$$

$$q_1 = \frac{C_0^T t_1}{t_1^T t_1} \tag{10}$$

The matrix and vector of the residuals in A_0 and C_0 are:

$$A_1 = A_0 - t_1 P_1^T \tag{11}$$

$$C_1 = C_0 - t_1 q_1^T \tag{12}$$

From the general linear equation, the regression coefficients were calculated by:

$$b = W(P^T W)^{-1} q \tag{13}$$

$$a = C_{mean} - A^T_{meanb} \tag{14}$$

The built calibration equation is used for the estimation of the compounds in the samples (21).

RESULTS AND DISCUSSION

A calibration set was randomly prepared as mixtures of LOS, AML and HYD in their possible compositions by applying a multilevel multifactor design (22) (Table I). The UV absorbance data were obtained by measuring the absorbances in the region of 200–400 nm (Fig. 1). From this 230.5–350.4 nm wavelength was selected for construction
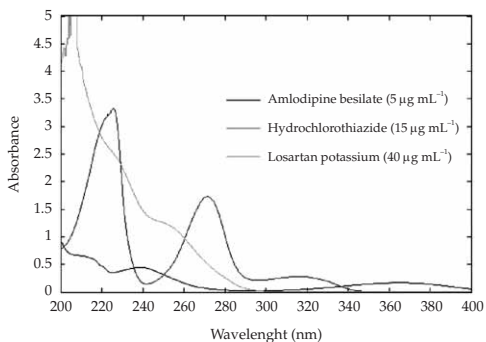

Fig. 1. Overlapping spectrum of LOS, AML and HYD.

Table II. Statistical parameters of chemometric methods in the calibration set

Method	Parameter	LOS	AML	HYD
	RMSECV	0.1647	0.1183	0.1823
CLS	RMSEP	0.1264	0.0908	0.1110
	R	1	0.9940	0.9990
	RMSECV	0.2258	0.2047	0.2583
MLR	RMSEP	0.2062	0.0908	0.2328
	R	1	0.9920	0.9980
	RMSEC	0.1647	0.1183	0.1823
PCR	RMSECV	0.1987	0.1424	0.2134
	RMSEP	0.1250	0.0856	0.1120
	R	1	0.9940	0.9990
	RMSEC	0.1647	0.1183	0.1823
PLS	RMSECV	0.1987	0.1424	0.2134
	RMSEP	0.1250	0.0856	0.1120
	R	1	0.9940	0.9990

CLS – classical least squares, MLR – multiple linear regression, PCR – principal component regression. PLS – partial least squares, RMSEP – root mean square error of prediction, RMSECV – root mean square error of cross validation, RMSEC – root mean square error of calibration. For other acronyms see Table I.

of the calibration model. The fit model was constructed by using the absorption data matrix corresponding to the concentration data matrix in CLS, MLR, PCR and PLS.

Before constructing the model, pre-processing (23) was carried out to reduce the effect of noise, improve the predictive ability of the model and simplify the model by making the data more normally distributed and the wavelength selection based on the best outcome for reduced error of spectral data. In Table II, *R* is defined as the correlation coefficient between constituent concentrations and shows the absorbance effects relating to the constituent of interest. Values obtained in the methods close to 1 mean no interference was coming from other constituents in the respective set of calibration mixtures.

The most commonly employed validation criterion is to divide the dataset into two subsets, a calibration set and a validation set. The calibration model is calculated using the calibration set. Then, the root mean square errors of calibration and validation, RMSEC – root mean square error of calibration and RMSECV – root mean square error of cross validation, are calculated by using the calibration model under investigation to predict the samples in the calibration set and validation set, respectively:

$$RMSEC = \sqrt{\frac{(y - y_{pred})^i (y - y_{pred})}{m - 1}}$$

Selection of the optimum number of factors for PCR and PLS. – For PCR and PLS methods, 25 calibration spectra were used for the selection of the optimum number of factors by using the cross-validation with the leave-out-one technique. This allows modelling of the system with the optimum amount of information and avoidance of over-fitting or under-fitting. The cross-validation procedure consisted of systematically removing one of a group of training samples in turn and using only the remaining ones for the construction of latent variable factors and applied regression. The predicted concentrations were then compared with the actual ones for each of the calibration samples and the root mean square error of prediction (RMSEP) was calculated. The RMSEP was computed in the same manner each time, and then a new factor was added to the PCR and PLS model. The selected model was that with the smallest number of factors such that its
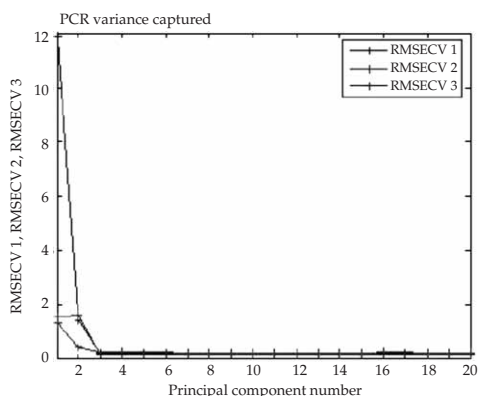

Fig. 2. Representation of RMSECV values generated from calibration by PCR: LOS, AML and HYD.
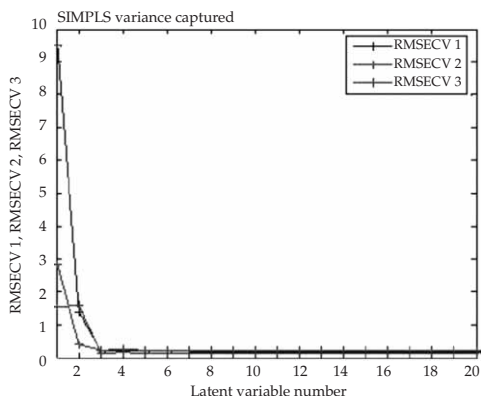
Fig. 3. Representation of RMSECV values generated from calibration by PLS: LOS, AML and HYD.

RMSECV values were not significantly greater than that for the model, which yielded the minimum RMSECV. A plot of RMSECV values against the number of components (Figs. 2 and 3) indicates that the latent variable factor 3 was optimum for PCR and PLS selected based on the RMSEC and RMSECV, respectively, for the estimation of the titled drugs. At the selected principal component of PCR and PLS, the concentrations of each sample was then predicted and compared with the known concentration and the RMSEP was calculated:

$$\text{RMSEC} = \sqrt{\sum_{i=y}^{N} \frac{(y_{\text{ipred}} - y_{\text{iref}})^2}{N}} \qquad\qquad \text{RMSEC} = \sqrt{\frac{(y - y_{pred})^i (y - y_{pred})}{m - 1}}$$

Table III. Composition of synthetic mixtures (formulation) and recovery set

Synthetic formulation (µg mL⁻¹)[a]			Formulation (µg mL⁻¹)[b]			Recovery (µg mL⁻¹)[c]		
LOS	AML	HYD	LOS	AML	HYD	LOS	AML	HYD
24.8	3.6	10.8	30	3	7.5	3	0.45	0.75
24.8	1.2	3.6	30	3	7.5	3	0.45	0.75
9.6	1.2	18.0	30	3	7.5	3	0.45	0.75
9.6	6.0	7.2	30	3	7.5	6	0.90	1.50
48.0	2.4	18.0	30	3	7.5	6	0.90	1.50
19.2	6.0	10.8	30	3	7.5	6	0.90	1.50
48.0	3.6	7.2	30	3	7.5	9	1.35	2.25
28.8	2.4	7.2	30	3	7.5	9	1.35	2.25
19.2	2.4	14.4	30	3	7.5	9	1.35	2.25

[a] Synthetic mixture of LOS, AML and HYD (standards).
[b] Pharmaceutical formulation, tablet.
[c] Recovery – standard addition to pharmaceutical formulation..
For other acronyms see Table I.

Table IV. Prediction results for losartan, amlodipine and hydrochlorothiazide from the synthetic validation samples by different chemometric methods

Component	CLS		MLR		PCR		PLS	
	Mean ± SD[a]		Mean ± SD[a]		Mean ± SD[a]		Mean ± SD[a]	
LOS	100.6	0.9	100.4	1.2	100.6	0.9	100.6	0.9
AML	100.0	1.6	100.6	0.7	100.0	0.5	100.0	0.5
HYD	100.9	0.6	99.7	0.9	100.9	0.7	100.9	0.7

[a] Nine determinations.
For acronyms see Table I and II.

In order to test the proposed techniques, the validation set of synthetic mixtures (from standards) containing the three drugs (Table III) in variable ratios was carried out; the results are given in Table IV. The maximum values of the mean percent errors corresponding to CLS, MLR, and PCR and PLS for the same mixtures were completely acceptable because of their very small numerical values (below 0.2). Results of tablet analyses are shown in Table V.

Recovery and precision studies

To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the preanalyzed formulation. Results of recovery studies were found to be from 99.3 ± 0.3 to 101.3 ± 0.6 % (Table VI). Good precision of the method was indicated by RSD ranging 0.3–1.4 %.

Table V. Prediction results for losartan, amlodipine and hydrochlorothiazide in formulation samples by different chemometric methods

Method	Parameter[a]	LOS	AML	HYD
CLS	Mean (%)	100.02	100.39	101.28
	RSD (%)	0.034	0.720	0.131
MLR	Mean (%)	100.27	98.92	101.52
	RSD (%)	0.078	1.683	1.192
PCR	Mean (%)	100.01	100.36	101.32
	RSD (%)	0.033	0.705	0.128
PLS	Mean (%)	100.01	100.36	101.32
	RSD (%)	0.033	0.705	0.128

[a] Nine determinations.

Table VI. Recovery studies of LOS, AML and HYD in pharmaceutical formulations[a]

Method	LOS Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)[b]	AML Added (μg mL^{-1})	Found (μg mL^{-1})	*Recovery (%)[b]	HYD Added (μg mL^{-1})	Found (μg mL^{-1})	*Recovery (%)[b]
	3	33.1	100.4	0.5	3.4	99.8	0.8	8.4	101.6
CLS	6	36.0	100.0	0.9	3.9	99.2	1.5	9.1	100.6
	9	39.0	100.1	1.4	4.3	99.4	2.3	9.9	101.6
Mean			100.1			99.5			101.3
S.D			± 0.2			± 0.3			± 0.6
	3	33.0	100.1	0.5	3.5	101.3	0.8	8.4	102.2
MLR	6	35.7	99.3	0.9	3.9	99.9	1.5	9.1	100.6
	9	39.1	100.2	1.4	4.4	100.5	2.3	9.8	100.3
Mean			99.9			100.5			101.0
S.D			± 0.7			± 1.0			± 1.4
	3	33.1	100.3	0.5	3.4	99.8	0.8	8.4	101.7
PCR	6	36.0	100.0	0.9	3.9	99.0	1.5	9.1	100.6
	9	39.0	100.0	1.4	4.3	99.2	2.3	9.9	101.6
Mean			100.1			99.3			101.3
S.D			± 0.2			± 0.4			± 0.6
	3	33.1	100.3	0.5	3.4	99.7	0.8	8.4	101.7
PLS	6	36.0	100.0	0.9	3.9	99.0	1.5	9.1	100.6
	9	39.0	100.0	1.4	4.3	99.2	2.3	9.9	101.6
Mean			100.1			99.3			101.3
S.D			± 0.2			± 0.4			± 0.6

[a] Additions to 30 μg mL^{-1} LOS, 3 μg mL^{-1} AML and 7.5 μg mL^{-1} HYD in the formulation.
[b] Nine determinations.

CONCLUSIONS

Based on the results obtained in this work, the UV spectrophotometric method for simultaneous determination of losartan potassium, amlodipine besilate and hydrochlorothiazide in mixtures by multivariate calibration of synthetic and pharmaceutical samples is applicable. PLS and PCR using a calibration matrix constructed with absorption spectra were successfully applied to simultaneous analysis of these drugs in synthetic and pharmaceutical mixtures.

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S A Ž E T A K

Istodobno spektrofotometrijsko određivanje losartan kalija, amlodipin besilata i hidroklorotiazida u farmaceutskim pripravcima kemometrijskom metodom

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U radu su opisane četiri spektrofotometrijske metode za istodobno određivanje losartan kalija, amlodipin besilata i hidroklorotiazida u sirovinama i farmaceutskim pripravcima. Podaci koji su se preklapali kvantitativno su razlučeni kemometrijskim metodama, klasičnom metodom najmanjih kvadrata (CLS), multiplom linearnom regresijom (MLR), regresijom glavnih komponenata (PCR) te metodom parcijalnih najmanjih kvadrata (PLS). Kalibracije su provedene koristeći podatke o ovisnosti apsorpcije o koncentracijama, mjereći spektre nultog reda u rasponu 230,5–350,4 nm ($\Delta\lambda$ = 0,1 nm). Linearnost za losartan kalij bila je 8–40, za amlodipin besilat 1–5, a za hidroklorotiazid 3–15 µg mL^{-1}. Valjanost predloženih metoda uspješno je potvrđena analizom navedenih lijekova u različitim pripremljenim smjesama i tabletama.

Ključne riječi: losartan, amlodipin besilat, hidroklorotiazid, spektrofotometrija, kemometrija

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