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Original research paper

Simultaneous spectrophotometric determination of valsartan and hydrochlorothiazide by H-point standard addition method and partial least squares regression

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Simultaneous determination of valsartan and hydrochlorothiazide by the H-point standard additions method (HPSAM) and partial least squares (PLS) calibration is described. Absorbances at a pair of wavelengths, 216 and 228 nm, were monitored with the addition of standard solutions of valsartan. Results of applying HPSAM showed that valsartan and hydrochlorothiazide can be determined simultaneously at concentration ratios varying from 20:1 to 1:15 in a mixed sample. The proposed PLS method does not require chemical separation and spectral graphical procedures for quantitative resolution of mixtures containing the titled compounds. The calibration model was based on absorption spectra in the 200–350 nm range for 25 different mixtures of valsartan and hydrochlorothiazide. Calibration matrices contained 0.5–3 μg mL^{-1} of both valsartan and hydrochlorothiazide. The standard error of prediction (SEP) for valsartan and hydrochlorothiazide was 0.020 and 0.038 μg mL^{-1}, respectively. Both proposed methods were successfully applied to the determination of valsartan and hydrochlorothiazide in several synthetic and real matrix samples.

Keywords: valsartan, hydrochlorothiazide, H-point standard addition method, partial least squares regression, multicomponent analysis

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High blood pressure is the largest single risk factor for premature death. As it is known, some patients with hypertension require two or more antihypertensive drugs with complementary mechanisms of action to lower their blood pressure. The angiotensin II type 1-receptor antagonist valsartan and the diuretic hydrochlorothiazide (Fig. 1) are two antihypertensive agents with well recognised clinical efficacy. Oral administration of valsartan with hydrochlorothiazide has been found to be more effective than either drug alone in the treatment of hypertension in patients whose blood pressure is not

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adequately controlled by monotherapy. This fixed dose combination should be used as a second-line therapy (1).

Only few methods appear in the literature for determination of valsartan individually based on HPLC (2, 3) and GC-MS (4), spectrophotometry, LC (5) and LC-MS, when in combination with hydrochlorothiazide (6). There have been several reports on the determination of hydrochlorothiazide individually or in its combination with other drugs, including the use of liquid chromatography (7–11) and spectrophotometry (12–16).

Spectrophotometry is a relatively easy method for simultaneous determination of drugs. One of the main drawbacks of spectrophotometric methods for simultaneous determination of valsartan and hydrochlorothiazide is the high degree of spectral overlapping of these drugs (Fig. 2). Nowadays, quantitative spectrophotometry has been greatly improved by the use of a variety of multivariate statistical methods, particularly the partial least squares regression (17–19).

The H-point standard addition method (HPSAM) permits both proportional and constant errors produced by the matrix of the sample to be corrected. This method is

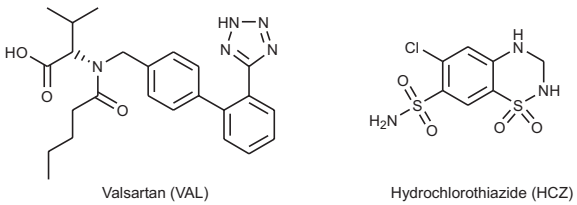

Fig. 1. Chemical structures of the investigated drugs.

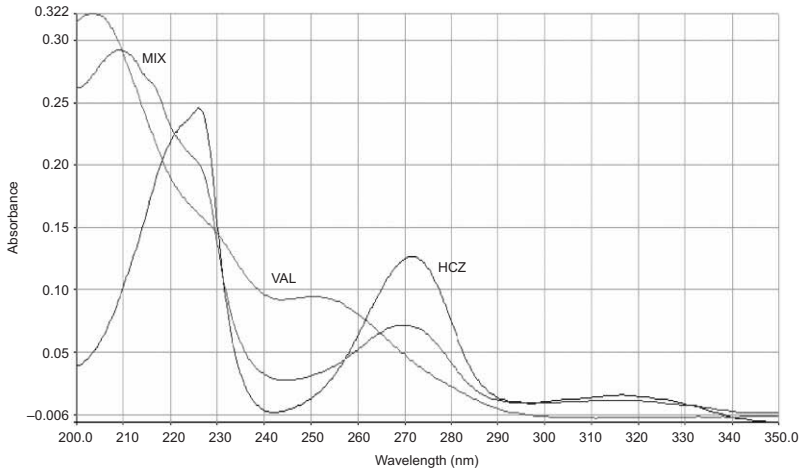

Fig. 2. Absorption spectra of valsartan and hydrochlorothiazide and their mixture (VAL: 2 μg mL^{-1}, HCZ: 2 μg mL^{-1}, MIX: 2 μg mL^{-1} VAL + 2 μg mL^{-1} HCZ).

based on the principles of dual wavelength spectrophotometry and the standard addition method. The greatest advantage of HPSAM is that it can remove the errors resulting from other components in the system. The requirement for the application of the method is that of the two wavelengths, the analytical signal due to one of the species is constant and that of the other as different as possible. By plotting the analytical signal against the added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H), where $-C_H$ is the unknown analyte concentration and A_H the analytical signal due to the interfering species (20).

This paper reports the simultaneous spectrophotometric determination of valsartan and hydrochlorothiazide. The H-point standard addition method and partial least squares were employed for the resolution of binary mixtures of valsartan and hydrochlorothiazide. These methods are based on a solid mathematical and statistical background. The fundamental advantages of our investigated methods are a simultaneous analysis of binary mixture components without any chemical pre-treatment and lasting a short period of time, as well as no high costs and no complex instruments.

EXPERIMENTAL

Apparatus and software

A Perkin Elmer (Lambda 25, USA), spectrophotometer controlled by a computer and equipped with a 1-cm pathlength quartz cell was used for UV-VIS spectra acquisition. The computations were made with Microsoft Excel and Matlab 7.2 Software (Mathworks Limited). PLS calculations were carried out using the PLS Toolbox 5.0 demo version (Eigen Vector Technologies).

Commercial products

The commercial pharmaceutical product Valent-H (Lupin Ltd, India) containing 80 mg valsartan and 12.5 mg hydrochlorothiazide per tablet was analyzed using the proposed methods.

Standard and sample solutions

Standard solutions. – Standard stock solutions (1000 μg mL^{-1}) of valsartan (VAL) and hydrochlorothiazide (HCZ) were prepared separately in 0.05 mol L^{-1} NaOH. The solutions were kept at room temperature. Various aliquots of standard solutions were taken, and then diluted to 10.0 mL with water to give the desired final analyte concentration.

Sample preparation. – For preparation of commercial samples, 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet containing VAL and HCZ was dissolved in a 100 mL calibration flask in 0.05 mol L^{-1} NaOH. The content of the flask was mechanically shaken for 30 min and filtered through a 0.45-μm membrane filter. The obtained solution was diluted to the working concentration range. Appropiate volumes of standard stock solutions of VAL and HCZ at three different con-

centration levels (1.0, 2.0 and 3.0 μg mL^{-1} VAL and 0.5, 1.5 and 2.5 μg mL^{-1} HCZ) were added to the analyzed tablet solutions, respectively, for recovery studies. This procedure was repeated six times for each concentration level.

Individual calibrations. – To verify Beer's law, calibration graphs were prepared for the determination of valsartan and hydrochlorothiazide (Table I). The correlation coefficients obtained indicate that the interaction between the two binary systems does not affect the linear correlation prevailing between the absorbance and concentration of each drug. The limit of detection (*LOD*) was determined using the formula: $LOD = kSD_a/b$ where k = 3.3, SD_a is the standard deviation of the intercept, and b is the slope.

Table I. Calibration graphs for the determination of valsartan and hydrochlorothiazide by the proposed method

Analyte	Slope	Intercept	Correlation coefficient	Linear range (μg mL^{-1})	Limit of detection (μg mL^{-1})
Valsartan	0.043	–0.0071	0.9981	2–10	0.51
Hydrochlorothiazide	0.0707	0.0235	0.9978	2–10	0.62

H-point standard addition method

Synthetic samples containing different concentration ratios of VAL and HCZ were prepared and standard additions of VAL were made; then absorbances were measured at 216 and 228 nm. Concentrations of VAL and HCZ were obtained by construction of H-point graphs (Fig. 3). The concentration of VAL was obtained from C_H. HCZ concentration was evaluated from the calibration curve constructed by plotting A_H (analytical signal due to interferent) *vs.* HCZ concentration.

PLS calibration

Calibration and prediction sets were designed with 25 and 10 binary mixtures of the cited drugs, respectively. Concentrations of VAL and HCZ in calibration and prediction solutions were in the range of 0.5–3.0 μg mL^{-1}. Each calibration or prediction mixture was prepared by diluting the appropriate aliquot of stock solution with distilled water. The spectrum of each solution was recorded against the blank in the range of 200–350 nm with a wavelength interval of 1 nm as shown in Fig. 4.

RESULTS AND DISCUSSION

Requirements for applying HPSAM

Consider an unknown sample containing an analyte X and an interferent Y. Determination of the concentration of X by HPSAM under these conditions requires selection of two wavelengths, λ_1 and λ_2, at which the interfering species, Y, should have the same

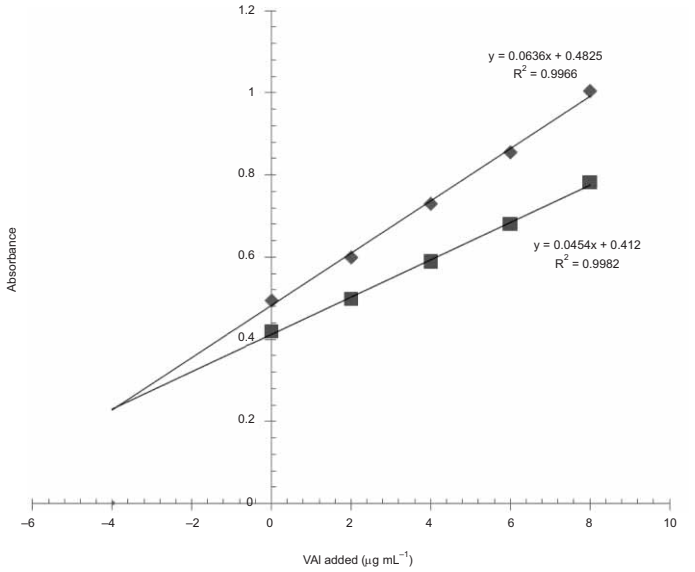

Fig. 3. Plots of the H-point standard addition method for valsartan (4 μg mL^{-1}) and hydrochlorothiazide (0.8 μg mL^{-1}).

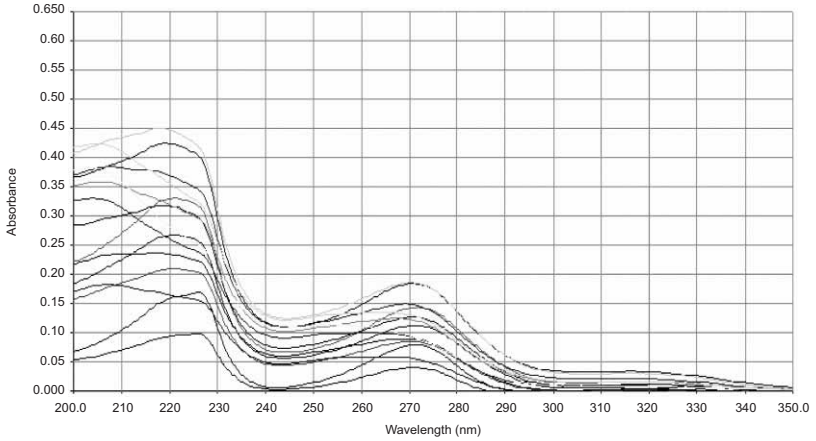

Fig. 4. Absorption spectra of the calibration set consisting of 25 synthetic mixtures of VAL and HCZ in the linear concentration range of 0.5–3.0 μg mL^{-1} within the full spectral range of 200–350 nm.

absorbance. Then, known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two wavelengths and expressed by *Eqs.* (*1*) and (2), where $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$ are the analytical signals measured at λ_1 and λ_2, respectively, b_0 and A_0 ($b_0 \neq A_0$) are the original analytical signals of X at λ_1 and λ_2 respectively, b and A' are the analytical signals of Y at λ_1 and λ_2, respectively, $M_{\lambda 1}$ and $M_{\lambda 2}$ are the

slopes of the standard addition calibration lines at λ_1 and λ_2, respectively, C_i is the added concentration of analyte X. The two straight lines obtained intersect at the so-called H point [$-C_H$, A_H, (Fig. 3, *Eqs.* (*1*) and (*2*)].

$$A_{(\lambda 1)} = b_0 + b + M_{\lambda 1}\, C_i \tag{1}$$

$$A_{(\lambda 2)} = A_0 + A' + M_{\lambda 2}\, C_i \tag{2}$$

At the H-point ($C_i = -C_H$), *Eqs.* (*3*) and (*4*) follow from *Eqs.* (*1*) and (*2*), since $A_{(\lambda 1)} = A_{(\lambda 2)}$.

$$b_0 + b + M_{\lambda 1}\,(-C_H) = A_0 + A' + M_{\lambda 2}\,(-C_H) \tag{3}$$

$$-C_H = [(A_0 - b_0) + (A' - b)] \;/\; (M_{\lambda 1} - M_{\lambda 2}) \tag{4}$$

From *Eq.* (*4*), the following conclusions can be drawn: (*i*) if component Y is the known interferent and the analytical signal corresponding to Y, b (at λ_1) and A' (at λ_2) do not change with the addition of analyte X, that is, $b = A'$ = constant, and then see *Eqs.* (*5*) – (*8*):

$$-C_x = (A_0 - b_0) \;/\; (M_{\lambda 1} - M_{\lambda 2}) = b_0 \;/\; M_{\lambda 1} = A_0 \;/\; M_{\lambda 2} \tag{5}$$

if $C_H = -C_x$ then,

$$-C_H = (A_0 - b_0) \;/\; (M_{\lambda 1} - M_{\lambda 2}) = b_0 \;/\; M_{\lambda 1} = A_0 \;/\; M_{\lambda 2} \tag{6}$$

if the value of $-C_H$ is included in *Eq.* (1), then

$$A_H = b_0 + b + M_{\lambda 1}\,(-C_H) \tag{7}$$

$b_0 = -M_{\lambda 1}\, C_H$ [*Eq.* (4)], then

$$A_H = b \tag{8}$$

and similarly $A_H = A'$.

Hence, the A_H value is only related to the signal of the interfering species Y at the two selected wavelengths and C_H is independent of the concentration of interfering species. Fig. 3 shows the effect of change in valsartan concentration at H-point. According to the above discussion, at H-point C_H is independent of the concentration of interferent and so A_H is also independent of the analyte concentration.

For selection of appropriate wavelengths for applying HPSAM, the following principles were followed. At the two selected wavelengths, the analyte signals must be linear with the concentration, and the analyte signal obtained from a mixture containing

the analyte and the interferent signal should be equal to the sum of individual signals of the two species. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths, λ_1 and λ_2, must be as large as possible in order to get good accuracy and sensitivity (20). Simultaneous determination of Co(II) and Pd(II) were carried out by the complex formation with 2-pyridylazoresorcinol in the presence of sodium dodecyl sulphate. The two wavelengths were selected as 597 (λ_1) and 650 (λ_2), respectively.

In the current system, the analyte is valsartan and hydrochlorothiazide is the interferent. Several wavelength pairs were examined and the wavelength pair of 216 and 228 nm was selected. Under optimal conditions, determination of valsartan and hydrochlorothiazide was carried out using HPSAM. The concentration of interferent was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H-point (A_H). Several synthetic mixtures with different concentration ratios of valsartan and hydrochlorothiazide were analyzed by the proposed method. The results are given in Table II.

Table II. Analysis of valsartan hydrochlorothiazide mixtures at different concentration ratios by HPSAM

A-*C* equation	*R*	Present in sample (μg mL^{-1})		Amount found (μg mL^{-1})	
		VAL	HCZ	VAL	HCZ
$A_{216.32} = 0.0748C + 0.2278$	0.9982	2.00	0.80	2.01	0.78
$A_{228.41} = 0.0536C + 0.1898$	0.9987				
$A_{216.32} = 0.0707C + 0.3094$	0.9990	2.00	1.60	2.02	1.58
$A_{228.41} = 0.0505C + 0.2742$	0.9984				
$A_{216.32} = 0.0620C + 0.5748$	0.9985	2.00	4.00	2.01	3.94
$A_{228.41} = 0.0502C + 0.5530$	0.9991				
$A_{216.32} = 0.0758C + 0.4111$	0.9980	4.00	0.80	4.02	0.79
$A_{228.41} = 0.0508C + 0.3148$	0.9980				
$A_{216.32} = 0.0685C + 0.4565$	0.9988	4.00	1.60	4.01	1.58
$A_{228.41} = 0.0593C + 0.3737$	0.9987				
$A_{216.32} = 0.0636C + 0.4824$	0.9983	4.00	2.00	4.01	1.93
$A_{228.41} = 0.0454C + 0.4120$	0.9990				
$A_{216.32} = 0.0787C + 0.7718$	0.9965	4.00	4.00	4.01	4.03
$A_{228.41} = 0.0569C + 0.6848$	0.9955				
$A_{216.32} = 0.0682C + 0.5328$	0.9961	6.00	0.80	6.01	0.79
$A_{228.41} = 0.0463C + 0.3966$	0.9962				
$A_{216.32} = 0.0695C + 0.4748$	0.9976	1.00	5.00	1.01	4.76
$A_{228.41} = 0.0477C + 0.4969$	0.9956				
$A_{216.32} = 0.0703C + 0.2666$	0.9980	1.00	2.00	1.01	1.83
$A_{228.41} = 0.0492C + 0.2525$	0.9990				

A – absorbance of the mixture at the selected wavelengths
C – concentration of the interferent in μg mL^{-1}
R – correlation coefficient

Repeatability of the HPSAM

To check the repeatability of the method, six replicate experiments of valsartan and hydrochlorothiazide were done (Table III). Then, the concentration of interferent was calculated in each test solution by the calibration method using standard solutions and the ordinate value of the H-point (A_H). The relative standard deviations for six replicate measurements of the mixture of 4.0 and 0.8 μg mL^{-1} of valsartan and hydrochlorothiazide were 1.5 and 2.2 %, respectively.

PLS method

The PLS calibration method is performed by composing both concentration and absorbance matrices into latent variables, $A = TP^T + E$ and $C = UQ^T + F$. Vector b is given as $b = W(P^TW)^{-1}Q$, where W represents a weight matrix. The next step is to use the linear regression $C = a + bA$, where constant a has the form $a = C_{mean} - A^T_{mean} \times b$.

The ability of the partial least squares calibration of resolving overlapped spectra was examined by selecting calibration and prediction sets. Twenty-five binary mixtures were selected as the calibration set for model construction. For evaluation of the constructed model, a prediction set with 10 samples was selected randomly. Composition of

Table III. Replicate for the analyses of valsartan and hydrochlorothiazide mixtures by HPSAM

A-*C* equation	*R*	Present in sample (mg mL^{-1})		Amount found (mg mL^{-1})	
		VAL	HCZ	VAL	HCZ
$A_{216.32} = 0.0706C + 0.3738$	0.9990	4.00	0.80	4.01	0.79
$A_{228.41} = 0.0484C + 0.2895$	0.9986				
$A_{216.32} = 0.0751C + 0.3615$	0.9997	4.00	0.80	3.90	0.79
$A_{228.41} = 0.0514C + 0.2806$	0.9997				
$A_{216.32} = 0.0738C + 0.3495$	0.9987	4.00	0.80	3.90	0.71
$A_{228.41} = 0.0512C + 0.2703$	0.9987				
$A_{216.32} = 0.0732C + 0.3498$	0.9999	4.00	0.80	3.80	0.79
$A_{228.41} = 0.0504C + 0.2715$	0.9999				
$A_{216.32} = 0.0741C + 0.3609$	0.9992	4.00	0.80	3.90	0.79
$A_{228.41} = 0.0510C + 0.2800$	0.9993				
Mean				3.90	0.77
RSD (%) (n = 5)				1.81	4.66

A – absorbance of mixture at the selected wavelength
C – concentration of the interferent in μg mL^{-1}
R – correlation coefficient

Table IV. Sets of calibration and prediction solutions

Calibration set		Prediction set	
Valsartan ($\mu g\ mL^{-1}$)	Hydrochlorothiazide ($\mu g\ mL^{-1}$)	Valsartan ($\mu g\ mL^{-1}$)	Hydrochlorothiazide ($\mu g\ mL^{-1}$)
0.5	0.5	0.5	2.0
0.5	1.0	1.0	2.0
0.5	1.5	1.5	2.5
0.5	2.0	2.0	2.5
1.0	0.5	1.0	0.5
1.0	1.0	1.0	1.0
1.0	1.5	0.5	2.5
1.0	2.0	1.0	2.5
1.5	0.5	1.5	2.5
1.5	1.0	3.0	3.0
1.5	1.5	–	–
1.5	2.0	–	–
2.0	0.5	–	–
2.0	1.0	–	–
2.0	1.5	–	–
2.0	2.0	–	–
2.5	0.5	–	–
2.5	1.0	–	–
2.5	1.5	–	–
2.5	2.0	–	–
3.0	0.5	–	–
3.0	1.0	–	–
3.0	1.5	–	–
3.0	2.0	–	–
3.0	2.5	–	–

calibration and prediction standards is summarized in Table IV. A total of 151 data points were recorded between 200 and 350 nm. The number of latent variables (factors) was determined by the cross-validation method. The prediction error was calculated in the prediction set. This error was expressed as the prediction residual error sum of squares (PRESS). PRESS was calculated for the first latent variable, which was built by the PLS modeling in the calibration set. Then another factor was added and PRESS was calculated again. Calculations were repeated and the corresponding PRESS values were estimated. The optimum number of latent variables was 9 since it gave the minimum PRESS value for both drugs.

Validation of the method

In case of chemometric calibrations, the ability of the calibration model is defined in various ways. The most general expression is the standard error of prediction (SEP) and standard error of calibration (SEC) given as:

$$\text{SEC or SEP} = \sqrt{\frac{\sum_{i=1}^{n} (\hat{C} - C_i^2)}{n}} \qquad (9)$$

where $\hat{C}$ denotes the added drug concentration, C_i is the predicted drug concentration and n represents the total number of synthetic mixtures. SEC and SEP values were calculated and are represented in Table V.

Table V. Statistical parameters for the PLS method

Step	Parameter	VAL	HCZ
Calibration	SEC	0.0067	0.0091
	Slope	0.9984	0.9942
	Intercept	0.0023	0.0076
	R	0.9984	0.9949
Prediction	SEP	0.0204	0.0386
	Slope	1.0165	0.9527
	Intercept	0.0136	0.1053
	R	0.9947	0.9729

SEC – standard error of calibration
SEP – standard error of prediction
R – correlation coefficient

Recovery and precision studies

Recovery results of the prediction set are given in Table VI. Accuracy and precision for the analysis of VAL and HCZ in the proposed synthetic mixtures at three different concentrations (1.0, 2.0, 3.0 μg mL^{-1} for VAL and 0.5, 1.5, 2.5 μg mL^{-1} for HCZ) were tested in intra-day (n = 6) and inter-day (n = 6) experiments. Good accuracy and precision were observed for the results obtained by PLS calibration.

The standard addition method was used to observe the selectivity of the proposed PLS method. Appropriate volumes of the standard stock solutions of VAL and HCZ at three different concentrations were added to the analyzed tablet solutions and re-analyzed by the proposed method. This procedure was repeated six times for each concentration level. The recovery results, standard deviations and relative standard deviations were calculated and are given in Table VII.

Analysis of commercial tablets

Results obtained by the application of HPSAM and PLS calibration to the analysis of VAL and HCZ in Valent H tablet formulation is summarized in Table VIII.

Table VI. Prediction sets by the PLS model

Prediction set (actual)		Prediction set (found)		Recovery (%)	
VAL (μg mL^{-1})	HCZ (μg mL^{-1})	VAL (μg mL^{-1})	HCZ (μg mL^{-1})	VAL (μg mL^{-1})	HCZ (μg mL^{-1})
0.5	2.0	0.507	2.018	101.5	100.9
1.0	2.0	1.053	2.181	105.3	109.1
1.5	2.5	1.508	2.376	100.5	95.1
2.0	2.5	1.923	2.314	96.2	92.6
1.0	0.5	0.996	0.515	99.6	103.1
1.0	1.0	1.052	1.038	105.2	103.8
0.5	2.5	0.510	2.563	102.0	102.5
1.0	2.5	1.081	2.610	108.2	104.4
1.5	2.5	1.608	2.623	107.2	104.9
3.0	3.0	3.108	2.818	103.6	93.9
Mean recovery				103.0	101.0

Table VII. Accuracy and precision for valsartan and hydrochlorothiazide in synthetic mixtures using the PLS method

Intra-day by using PLS							
Added (μg mL^{-1})		Valsartan[a]			Hydrochlorothiazide[a]		
VAL	HCZ	Found[a] (μg mL^{-1})	Recovery[a] (%)	RSD (%)	Found[a] (μg mL^{-1})	Recovery[a] (%)	RSD (%)
0.50	2.00	0.50	100.2	1.0	2.03	101.5	1.0
1.01	1.01	1.05	105.0	0.0	1.03	103.0	1.0
1.51	2.50	1.52	101.3	2.0	2.53	101.2	1.6
Inter-day							
Added (μg mL^{-1})		Valsartan			Hydrochlorothiazide		
VAL	HCZ	Found[a] (μg mL^{-1})	Recovery[a] (%)	RSD (%)	Found[a] (μg mL^{-1})	Recovery[a] (%)	RSD (%)
0.50	2.01	0.51	102.0	1.2	2.12	106.0	1.4
1.01	1.01	1.06	106.0	0.5	1.06	106.0	1.9
1.50	2.50	1.55	103.3	0.5	2.57	102.8	2.3

[a] Six determinations

Table VIII. Simultaneous determination of valsartan and hydrochlorothiazide in commercial tablets by HPSAM and PLS methods

Sample	Label claim (mg)		HPSAM (mg)[a]		PLS (mg)[a]	
	VAL	HCZ	VAL	HCZ	VAL	HCZ
Valent-H	80	12.5	79.0 ± 0.8	12.4 ± 1.2	79.0 ± 2.5	12.4 ± 3.9

[a] Mean ± SD, $n = 3$.

CONCLUSIONS

The above results show that HPSAM and PLS regression allow rapid, accurate and simple resolution of valsartan and hydrochlorothiazide mixtures. HPSAM can be used in complex samples with matrix effects because the standard addition method has the capability of removing these effects. However, partial least squares regression cannot be used in these cases. On the other hand, the PLS method was more rapid than HPSAM. Therefore HPSAM is preferred in mixtures with matrix effects but PLS is better than HPSAM in mixtures without these effects.

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REFERENCES

1. H. Siragy, Angiotensin II receptor blockers: review of the binding characteristics, *Am. J. Cardiol.* **84** (1999) 3S-8S; DOI: 10.1016/2FS0002-9149/2899/2900727-4.
2. E. Francotte, A. Davatz and P. Richert, Development and validation of chiral HPLC methods for the quantitation of valsartan and of the tosylate of valine benzyl ester, *J. Chromatogr. B. Biomed. Appl.* **686** (1996) 77–83; DOI: 10.1016/2FS0378-4347/2896/2900242-3.
3. A. Sioufi, F. Morfil and J. Godbillon, Automated determination of an angiotensin II receptor antagonist in plasma by high performance liquid chromatography, *J. Liq. Chromatogr.* **17** (1994) 2179–2186; DOI: 10.1080/2F10826079408013539.
4. H. H. Maurer, T. Kraemer and J. W. Arlt, Screening for the detection of angiotensin converting enzyme inhibitors, their metabolites and AT II receptor antagonists, *Ther. Drug Monit*. **20** (1998) 706–713; DOI: 10.1097/2F00007691-199812000-00022.
5. E. Satana, S. Altinay, N. G. Goger, S. A. Ozkan and Z. Senturk, Simultaneous determination of valsartan and hydrochlorothiazide by first derivative ultraviolet spectrophotometry and LC, *J. Pharm. Biomed. Anal.* **25** (2001) 1009–1013; DOI: 10.1016/2FS0731-7085/2801/2900394-6.
6. H. Li, Y. Wang, Y. Jiang, Y. Tang, J. Wang, L. Zhao and J. Gu, A LC/MS method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma, *J. Chromatogr. B* **852** (2007) 436–442; DOI: 10.1016/2Fj.jchromb.2007.02.014.
7. I. E. Panderi and P. M. Parissi, Simultaneous determination of benazepril hydrochloride and hydrochlorothiazide by microbore liquid chromatography, *J. Pharm. Biomed. Anal*. **21** (1999) 1017–1024; DOI: 10.1016/2FS0731-7085/2899/2900195-8.

8. D. Farthing, I. Fakhry, E. B. Ripley and D. Sica, Simple method for determination of hydrochlorothiazide in human urine by HPLC utilizing narrow bore chromatography, *J. Pharm. Biomed. Anal*. **17** (1998) 1455–1459; DOI: 10.1016/2FS0731-7085/2898/2900021-1.
9. K. Richter, R. Oerter and W. Kirch, New sensitive method for the determination of hydrochlorothiazide in human serum by high performance liquid chromatography with electrochemical detection, *J. Chromatogr. A* **729** (1996) 293–296; DOI: 10.1016/2F0021-9673/2895/2900900-0.
10. D. L. Hertzog, J. F. McCafferty, X. Fang, R. J. Tyrrell and R. A. Reed, Development and validation of a stability indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide and their degradation products, *J. Pharm. Biomed. Anal*. **30** (2002) 747–760; DOI: 10.1016/2FS0731-7085/2802/2900385-0.
11. M. Kartal and N. Erk, Simultaneous determination of hydrochlorothiazide and amiloride hydrochloride by ratio spectra derivative spectrophotometry and high performance liquid chromatography, *J. Pharm. Biomed. Anal*. **19** (1999) 477–485; DOI: 10.1016/2FS0731-7085/2898/2900241-6.
12. F. A. El-Yazbi, H. H. Abdine and R. A. Shaalan, Simultaneous determination of amiloride HCl, hydrochlorothiazide and atenolol in combined formulations by derivative spectroscopy, *J. Pharm. Biomed. Anal*. **17** (1998) 877–884; DOI: 10.1016/2FS0731-7085/2897/2900241-0.
13. I. E. Panderi, Simultaneous determination of benezapril hydrochloride and hydrochlorothiazide in tablets by second order derivative spectrophotometry, *J. Pharm. Biomed. Anal*. **21** (1999) 257–265; DOI: 10.1016/2FS0731-7085/2899/2900134-X.
14. A. Fattah, M. El Walily, S. F. Belal, E. A. Heaba and A. El-Kersh, Simultaneous determination of enelapril maleate and hydrochlorothiazide by first derivative ultraviolet spectrophotometry and high performance liquid chromatography, *J. Pharm. Biomed. Anal.* **13** (1995) 851–856; DOI: 10.1016/2F0731-7085/2895/2901346-M.
15. K. Beliz, E. Dinc and D. Baleanu, Chemometric methods for the simultaneous spectrophotometric determination of telmisartan and hydrochlorothiazide in the commercial pharmaceuticals, *Rev. Chim*. **60** (2009) 544–560.
16. A. El-Gindy, S. Emara and H. Shaaban, Development and validation of chemometrics assisted spectrophotometric and liquid chromatographic methods for the simultaneous determination of two multicomponent mixtures containing bronchodilator drugs, *J. Pharm. Biomed. Anal.* **43** (2007) 973–982; DOI: 10.1016/2Fj.jpba.2006.09.020.
17. M. D. Luca, F. Oliverio, G. Ioele and G. Ragno, Multivariate calibration techniques applied to derivative spectroscopy data for the analysis of pharmaceutical mixtures, *Chemom. Intell. Lab. Syst*. **96** (2009) 14–21; DOI: 10.1016/2Fj.chemolab.2008.10.009.
18. J. M. L. Gallego and J. P. Arroyo, Spectrophotometric resolution of ternary mixtures of dexamethasone, polymyxin B and trimethoprim in synthetic and pharmaceutical formulations, *Anal. Chim. Acta* **437** (2001) 247–257; DOI: 10.1016/2FS0003-2670/2801/2901008-X.
19. M. Blanco, J. Coello, F. Gonzalez, H. Iturriaga, S. Marpoch and X. Tomas, Spectrophotometric determination of pharmaceutical dosages by partial least squares calibration, *J. Pharm. Biomed. Anal*. **12** (1994) 509–514; DOI: 10.1016/2F0731-7085/2893/29E0004-7.
20. G. Bagherian, M. Arab Chamjangali and H. Eskandari, Simultaneous determination of cobalt and palladium in micellar medium using H-point standard addition method and partial least square regression, *Spectrochim. Acta A* **67** (2007) 378–384; DOI: 10.1016/2Fj.saa.2006.07.026.

S A Ž E T A K

Simultano spektrofotometrijsko određivanje valsartana i hidroklorotiazida metodom H-točke standardne adicije i djelomičnom regresijom najmanjih kvadrata

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U radu je opisano simultano određivanje valsartana i hidroklorotiazida metodom H-točke standardne adicije (HPSAM) i djelomičnom kalibracijom najmanjih kvadrata (PLS). Praćene su apsorbancije na dvije valne duljine, 216 i 228 nm, nakon dodatka standardne otopine valsartana. Rezultati primjene HPSAM pokazali su da se valsartan i hidroklorotiazid dadu odrediti simultano ako je omjer njihovih koncentracija u smjesi od 20:1 do 1:15. Za potpunu rezoluciju smjesa navedenih ljekovitih tvari preporučena PLS metoda ne treba niti kemijsko odjeljivanje niti grafičku obradu. Kalibracija se temelji na apsorpciji pri valnim duljinama 200–350 nm provedenoj na 25 različitih smjesa valsartana i hidroklorotiazida. Koncentracije valsartana i hidroklorotiazida u kalibracijskim matricama bile su 0.5–3 μg mL^{-1}. Očekivane standardne greške (SEP) za valsartan i hidroklorotiazid iznosile su 0,020, odnosno 0,038 μg mL^{-1}. Obje predložene metode uspješno su primijenjene za određivanje valsartana i hidroklorotiazida u nekoliko sintetskih i realnih uzoraka.

Ključne riječi: valsartan, hidroklorotiazid, metoda H-točke standardne adicije, djelomična regresija najmanjih kvadrata, višekomponentna analiza

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