Partial Least Squares and Continuous Wavelet Transformation in Simultaneous Spectrophotometric Determination of Amlodipin and Atorvastatine

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Abstract

A simple spectrophotometric method for simultaneous determination of amlodipin and atorvastatine in pharmaceutical preparation is proposed based on the partial least squares and continuous wavelet transformation. The resulted partial least squares models were employed for prediction of the two drugs in two brands of combined pharmaceutical preparations which resulted in percent recoveries of 100.7 and 101.4 for amlodipin and 98.6 and 100.1 for atorvastatine. In the best conditions, the results of percent recoveries by continuous wavelet transformation zero-crossing were: 110.3 and 109.0 for amlodipin and 99.7 and 99.7 for atorvastatine for brand 1 and 2, respectively.

Keywords: Partial least squares; Continuous wavelet transformation; Recovery; Atorvastatine; Amlodipin

Introduction

Amlodipin (AM), 2[(2-aminoethoxy) methyl]-4-(2-chloro-phenyl)-1, 4-dihydro-6-methyl-3, 5-pyridine carboxylic acid, 3-ethyl, 5-methylester (Figure 1) [1] is a dihydropyridine derivative with calcium antagonist activity. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina [2]. AM inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle [3]. Atorvastatine (AT) is chemically described as [R-(R*,R*)]-2-(4-fluorophenyl),β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid (Figure 1) [1]. AT is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of the sterols, including cholesterol. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides and to increase HDL-cholesterol in the treatment of hyper-lipidaemias [3].

There are several combined commercial product of these two drugs [4]. Many methods have been reported for the determination of either AM [5-11] or AT [12-17] alone or in combination with other drugs in pharmaceutical dosage forms or individually in biological fluids.

Only two papers have reported simultaneous determination of AM and AT which are based on HPLC [4,18]. However, none of the reported analytical methods describe simultaneous determination of AM and AT using spectrophotometrics methods. To our knowledge, this is the first report for the simultaneous determination of both AM and AT in pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate spectrophotometric method using partial least squares (PLS) as a multivariate calibration method for the simultaneous determination of AT and AM in the pharmaceutical preparations.

PLS is a useful chemometrics tool for analysis of complex mixtures, because it enables rapid and simultaneous determination of each component in the mixture, with minimum sample preparation and without the need for lengthy separations [19,20].
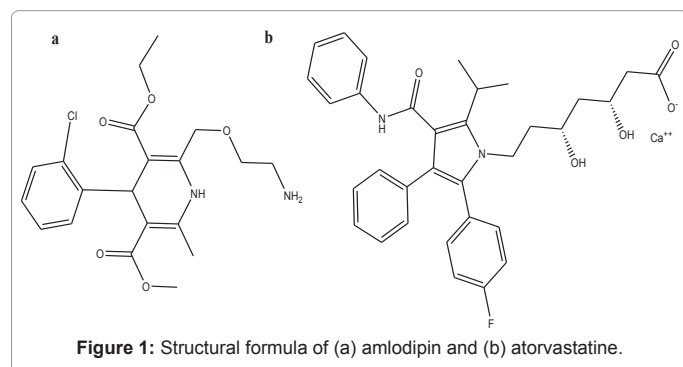
Continuous wavelet transformation (CWT) with zero-crossing has been applied for multi-component determination [21-23]. The importance of CWT comes from the transformation of the original signal to the other forms.

Theory

Partial least squares (PLS)

PLS is a predictive two-block regression method based on estimated latent variables (LVs) and is applied to the simultaneous analysis of two datasets (e.g. spectra and concentration) on the same objects [19,24].

The purpose of the PLS is to build a linear model enabling the prediction of desired characteristics e.g. concentration (involved in $\mathbf{y}$) from measured spectra (involved in X). There is a linear model $\mathbf{y}=\mathbf{X}\mathbf{b}$ in matrix notation where $\mathbf{b}$ contains the regression coefficients that are

Figure 1: Structural formula of (a) amlodipin and (b) atorvastatine.

determined during the calibration step, and X is the matrix of collected spectra. PLS was firstly applied to evaluate near infrared (NIR) spectra by Martens and Jensen [24] and is now used routinely in academic institutions and industry to correlate instrumental measurements with related chemical/physical data.

Interestingly, multivariate method PLS1 is advantageous and appealing for the determination of analytes in complex matrices, because calibration does not require knowledge of the concentrations of all other components except the analyte of interest. However, interferences should be present in the calibration set in order to be modeled and taken into account during the prediction step.

Continuous wavelet transformation (CWT)

A wavelet is a waveform of effectively limited duration that has an average value of zero. Wavelet analysis is the breaking up of a signal into shifted and scaled versions of the original (or mother) wavelet [25,26].

The continuous wavelet transform (CWT) is defined as the sum over all wavelength (λ) of the signal ($f(\lambda)$) multiplied by scaled, shifted versions of the mother wavelet function Ψ:

$$C(scale, position) = \int_{-\infty}^{+\infty} f(\lambda)\, \psi(scale, position, \lambda)\, d\lambda = \frac{1}{\sqrt{s}} \int_{-\infty}^{+\infty} f(\lambda)\, \psi\left(\frac{\lambda - \tau}{s}\right) d\lambda \quad (1)$$

The result of the CWT is many wavelet coefficients C, which are a function of scale (s) and position (τ). Multiplying each coefficient by the appropriately scaled and shifted wavelet yields the constituent wavelets of the original signal. We have already seen that wavelet analysis produces a wavelength-scale view of the signal. Now, we introduce scaling and shifting wavelets. Scaling a wavelet simply means stretching (or compressing) it. This is shown by scale factors. The scale factor works exactly the same with wavelets. Shifting is shown by τ.

The wavelet is placed at the beginning of the signal at the point which corresponds to the first wavelength. The wavelet function at scale "1" is multiplied by the signal and then integrated over all wavelengths. The final result is the value of the transformation, i.e., the value of the continuous wavelet transform at first wavelength and scale $s=1$. In other words, it is the value that corresponds to the point $\tau=0$, $s=1$ in the wavelength-scale plane. The wavelet at scale $s=1$ is then shifted towards the right by τ amount to the location $\lambda=\tau$, and the above equation is computed to get the transform value at $\lambda=\tau$, $s=1$. This procedure is repeated until the wavelet reaches the end of the signal. One row of points on the wavelength-scale plane for the scale $s=1$ is now completed. Then, s is increased by a small value. Note that, this is a continuous transform, and therefore, both τ and s must be incremented continuously. When the process is completed for all desired values of s, the CWT of the signal has been calculated.

At every location, it is multiplied by the signal. Obviously, the product is nonzero only where the signal falls in the region of support of the wavelet, and it is zero elsewhere (zero-crosses points). If the signal has a spectral component that corresponds to the current value of s (which is 1 in this case), the product of the wavelet with the signal at the location where this spectral component exists gives a relatively large value. If the spectral component that corresponds to the current value of s is not present in the signal, the product value will be relatively small, or zero.

For quantitation by CWT in binary mixtures, calibration of each analyte in the mixture can be performed by modelling CWT-signal against concentration at zero-crosses of the second component.

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Statistical measures and figures of merits of the PLS model

Quantitatively, all model performances of PLS are expressed in terms of root mean square error of cross-validation (*RMSECV*), root mean square error of prediction (*RMSEP*), and cross-validated correlation coefficient (Q^2) [27]:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m}} \quad (2)$$

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(c_i - \hat{c}_i)^2}{n}} \quad (3)$$

$$Q^2 = 1 - \frac{\sum_{i=1}^{n}(c_i - \hat{c}_i)^2}{\sum_{i=1}^{n}(c_i - c_{mean})^2} \quad (4)$$

In all of these expressions, c_i, $\hat{c}_i$, and c_{mean} are the real and predicted concentration of the component, and mean of the real concentrations, respectively. It must be mentioned that c_i and $\hat{c}_i$ in Eq. (2) are those in calibration set and c_i, $\hat{c}_i$, and c_{mean} in Eqs. (3) and (4) are those in validation set. m and n are the number of the samples in the calibration and validation sets, respectively. *RMSECV* was calculated in a leave-one-out approach. The F statistic was used to make the significance determination of the number of LVs and to avoid over-fitting [28].

Selectivity, sensitivity, and limit of determination can be calculated and used for method comparison or to study the quality of a given analytical technique.

One method for calculation of the figures of merits of the multivariate calibration methods relies on net analyte signal (NAS) calculations. The method was extended to inverse multivariate methods like PLS by Lorber [29]. In this definition, LOD is defined as

$$LOD = \Delta(\alpha, \beta)\delta r \quad (5)$$

where $\Delta(\alpha,\beta)$ is the noncentrality parameter of a non-central t-distribution, which can be calculated numerically or taken from statistical tables [30] and δr is an estimate for the standard deviation of the measurement errors.

NAS calculations also allow estimation of other figures of merit such as the selectivity for k^{th} component [31]:

$$SEL = \frac{\left\| s_k^* \right\|}{\left\| s_k \right\|} \quad (6)$$

and its sensitivity

$$SEN = \frac{1}{\|b\|} \qquad (7)$$

where b is the vector of final regression coefficients, which can be obtained by any multivariate method. s_k is the pure spectrum of the analyte (kth component) in unite concentration and s_k^* is the NAS of the analyte.

Another useful parameter is analytical sensitivity γ. It may be defined, in analogy to univariate calibration, as the quotient:

$$\gamma = \frac{SEN}{\delta r} \qquad (8)$$

Experimental

Apparatus and software

An Agilent 8453 UV-Vis spectrophotometer with diode array detector connected to a computer equipped with ChemStation software was used for recording of the absorption spectra of the compounds and their samples in the spectral range of 200-320 nm. The treatment of the absorption spectral data was performed by using MATLAB 6.5 software (MATLAB 6.5, The Mathworks Inc., Natick). PLS multivariate calibrations were performed using a series of m-files written in MATLAB environment.

Standard solutions

All solutions were prepared with analytical-reagent grade chemicals. Stock solutions of the pure compounds (AM and AT) were separately prepared by dissolving 20 mg of AM and AT in 100 ml mixture of methanol-water (20:80, v/v) in a calibrated flask. Standard calibration solutions in the studied concentration ranges were obtained by appropriate dilution of the above standard solutions.

Analysis of tablet formulations

Ten tablets of AM-AT co-formulation (two different brands) were accurately weighed and grounded to fine powder and a sample of the powder equivalent to the amount of a tablet was accurately weighed and transferred into a 100 mL volumetric flask. 20 mL of methanol was added and the mixture was stirred for 30 min at room temperature. Then, it was diluted to volume with doubly distilled water and mixed. This solution was filtered through a Whatman paper. A 10 mL aliquot of filtrate was transformed in a 100 mL volumetric flask and completed to the mark with methanol-water mixture (20:80, v/v) and mixed. This sample was then submitted for the analysis.

Results and Discussion

Figure 2 displays the UV absorption spectra for AM and AT in the standard solutions recorded between 205 and 310 nm. As can be seen they overlap severely. In order to find the linear dynamic range for each compound, possible concentration levels of the related compounds were tested (0.1–100.0 mg L^{-1} for AM and 0.3–100.0 mg L^{-1} for AT). Individual calibration curves were constructed with more than 25 points as absorbance versus drugs concentrations in the dynamic linear ranges and were evaluated by linear regression [32]. Statistical characteristics of the regression equations of individual calibrations using absorption UV spectra are shown in table 1.

Determination of amlodipin and atorvastatine in synthetic binary mixtures using PLS multivariate calibration

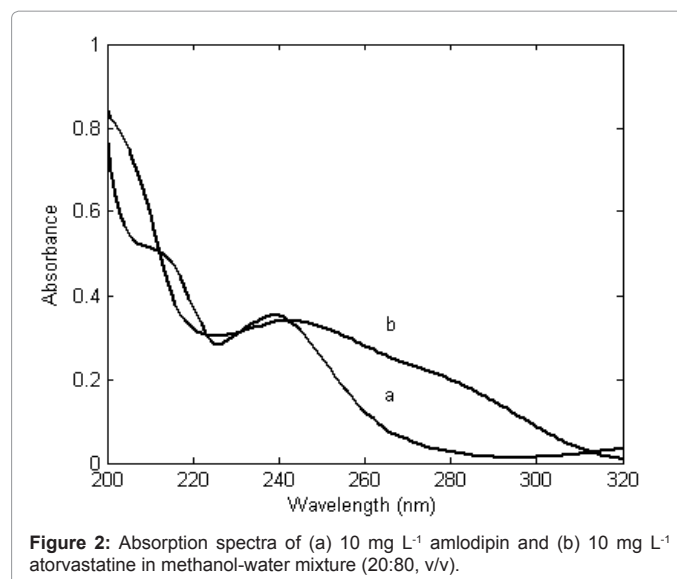AM shows a maximum absorbance at 246 nm and that of AT has

Figure 2: Absorption spectra of (a) 10 mg L^{-1} amlodipin and (b) 10 mg L^{-1} atorvastatine in methanol-water mixture (20:80, v/v).

Parameter	AM	AT
Sample number	28	36
Linear range (mgL^{-1})	0.3-40.0	0.1-100.0
Intercept of calibration curve	5.2×10^{-3}	2.3×10^{-2}
Slope of calibration curve	3.7 ×10^{-2}	2.8×10^{-2}
Standard error of intercept	2.1×10^{-3}	2.9×10^{-3}
Standard error of slope	1.3×10^{-4}	8.5×10^{-3}
Correlation coefficient	0.997	0.997
Limit of detection (mgL^{-1})	1.1×10^{-2}	6.2×10^{-2}
Limit of quantitation (mgL^{-1})	3.7×10^{-2}	0.2

Table 1: Parameters resulted from univariate calibration for AM and AT.

been located at 240 nm. For the both drugs, there exists an increasing trend of absorbance toward lower wavelengths (Figure 2). It is evident that spectra strongly overlap, making difficult the simultaneous determination of both drugs by classical methodology due to their severe mutual interference. Therefore, we expected that the use of multivariate calibration could be a better resource to circumvent spectral overlapping and mutual interference problems.

For simultaneous determination by PLS, 28 calibrations and 12 external test samples containing both AM and AT were prepared. Dividing the samples into calibration and validation sets was carried out by Kennard-Stone algorithm [33]. The concentration of AM and AT lye in their known linear absorbance-concentration ranges. In table 2, the concentrations of standard calibration and external test samples have been represented. In order to evaluate the performance of the PLS models, 12 external test samples were considered in the validation set. The spectrum of each standard mixture was recorded in the wavelength range of 205 to 310 nm with 1 nm intervals. Therefore, the **X** matrix for calibration is of 28×106 and the matrix for external test set is of 12×106 dimensions.

Models were internally validated employing the well-known leave-

one-out procedure, and the number of latent variables for prediction was chosen in agreement with the Haaland-Thomas criterion [28]. The statistical parameters and figure of merits found upon application of the PLS to calibration and external test sets have been given in table 3. High Q^2 and R^2 values and low *RMSECV* and *RMSEP* values indicate the validity of the multivariate calibration and the analytical method.

Determination of amlodipin and atorvastatine in synthetic binary mixtures using CWT zero-crossing

Two wavelet families of Mexican hat (Mexh) and Morlet (Morl) were tested to find the optimal signal processing function for obtaining reliable results in determination of the investigated analytes. Mexh was

No.	AM (mgL^{-1})	AT(mgL^{-1})
	Calibration set	
1	5.9	19.3
2	3.3	10.8
3	9.7	26.6
4	9.8	12.7
5	5.2	25.8
6	8.3	16.5
7	3.8	12.4
8	6.9	21.8
9	9.7	20.4
10	3.5	17.6
11	9.2	17.1
12	2.4	21.2
13	8.9	10.2
14	7.7	26.3
15	5.3	26.9
16	4.6	10.7
17	4.7	18.3
18	9.4	19.3
19	6.4	25.8
20	4.1	22.8
21	6.1	18.1
22	6.3	20.8
23	4.9	12.7
24	7.8	28.6
25	8.2	18.8
26	5.9	11.1
27	7.2	26.6
28	6.9	19.6
	External test set	
1	6.3	27.9
2	8	10.9
3	8.5	27.7
4	5.8	27.8
5	9.3	16.5
6	5.3	11.6
7	6	24.5
8	7.5	20.8
9	7.9	18.1
10	9.1	17.5
11	7.4	17.7
12	6.9	24.5

Table 2: Composition of the 40 binary mixtures of AM and AT used for PLS calibration and external test set.
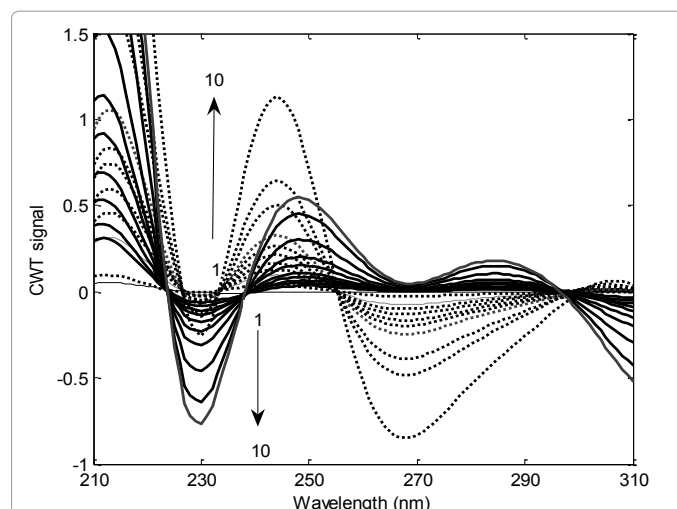
Figure 3: CWT signals of AM (dotted lines) and AT (solid lines) with concentrations of (1) 1.0, (2) 3.0, (3) 4.0, (4) 5.2, (5) 6.8, (6) 8.0, (7) 10.0, (8) 16.0, (9) 20.0 and (10) 35.0 mg L^{-1}.

selected as optimal mother wavelet for transformation of absorption spectra.

The absorption spectra of the standard solutions of the AM and AT with different concentrations recorded in the wavelength range 205–310 nm were transferred to wavelet domain in MATLAB 7.1.

The optimum value of scaling factor for determination of AM and AT in their binary mixture was found as a=10. Figure 3 shows the graphs of CWT for calibration sets of AM and AT obtained by Mexh (a=10) on the absorption spectra. Calibration graphs of AM were obtained by measuring the CWT signals at 238, 268, 269, and 270 nm where the CWT signals of AT zero crosses at 235, 256, 309, and 310 nm. Linear regression analyses and their statistical results have been reported in table 4. Application of these calibration models to synthetic binary mixtures in table 1 (the external test set) gave the results (Table 5).

Assay of pharmaceutical formulations

The methods developed were applied for the estimation of AM and AT in pharmaceutical preparations.

The calibration models were validated for accuracy and precision with five times determination of tablets contents. The precision is expressed as the RSD%. The percentage recoveries and other statistics have been presented in tables 6 and 7 for PLS and CWT zero-crossing methods, respectively. As the data in table 6 show, the amounts of drugs were found to be within the range of 98.6-101.4% relative to the declared content by PLS method. None of the tablet excipients were found to interfere with the analyte determination. The analysis of tablets in most cases yielded RSD% values below 2. The results, contained in table 6, show that the proposed method is accurate and precise for both analytes.

Comparison between PLS and CWT zero-crossing methods

By comparing the results contained in tables 3 and 5, it is clear that the statistical parameters of PLS (Q^2 and *RMSEP*) are significantly better than those of CWT analysis. The better performances of PLS can be attributed to its multivariate character. CWT is only a preprocessing

Analyte	NuNumber of LVs	RMSECV	RMSEP	R^2_{test}	Q^2_{test}	SEL	SEN	LOD	γ
AM	4	0.087	0.169	0.987	0.981	0.354	0.300	0.496	68.804
AT	3	0.267	0.927	0.980	0.974	0.348	0.956	0.156	219.010

Table 3: Performance characteristics of PLS upon application to the calibration and external test set in Table 2.

Analyte	λ (nm)	Dynamic linear range (mg L^{-1})	Regression equation	R^2	LOD (mg L^{-1})
AM					
	238	1.7-20	S=0.019C$_{AM}$-0.0125	0.981	0.074
	268	1.7-20	S=-0.0229C$_{AM}$+0.0187	0.967	0.011
	269	1.7-20	S=-0.0228C$_{AM}$+0.0183	0.968	0.010
	270	1.7-20	S=-0.0224C$_{AM}$+0.02	0.965	0.010
AT					
	235	3.6-35	S=-0.019C$_{AT}$-0.0283	0.991	0.079
	256	2.2-35	S=0.012C$_{AT}$-0.0184	0.986	0.037
	309	2.2-35	S=-0.0167C$_{AT}$+0.0098	0.991	0.013
	310	2.2-35	S=-0.018C$_{AT}$+0.008	0.993	0.010

Table 4: Statistical results of calibration curves at zero-cross points for AM and AT.

method and the analyses are eventually performed on the transformed data as zero order data. Results of PLS are better than those of CWT even at its best circumstances (λ=238 and 256 nm for AM and AT, respectively).

If we consider the results of CWT zero-crossing in the two mentioned wavelengths in application to real pharmaceutical samples,

	λ (nm)	RMSEP	R^2_{test}	Q^2_{test}
AM				
	238	0.281	0.960	0.953
	268	0.506	0.914	0.849
	269	0.498	0.917	0.854
	270	0.524	0.909	0.838
AT				
	235	1.107	0.968	0.966
	256	0.968	0.975	0.974
	309	1.075	0.972	0.968
	310	1.027	0.974	0.971

Table 5: Statistical performances of CWT zero-crossing in external test set prediction.

Brand 1		Declared content (mg)	Found^a (mg)	Recovery%	RSD%
	AM	5	5.04 ± 0.05	100.7	0.81
	AT	20	19.72 ± 0.61	98.6	3.07
Brand 2	AM	5	5.07 ± 0.04	101.4	0.80
	AT	20	20.02 ± 0.38	100.1	1.88

^{a}Mean of five determinations

Table 6: Results of application of PLS models on the spectra of the pharmaceutical preparations.

	λ (nm)	Declared content (mg)	Found^a (mg)	Recovery%	RSD%
AM					
Brand 1					
	238	5	4.34 ± 0.06	86.9	1.41
	268	5	5.54 ± 0.02	110.8	0.38
	269	5	5.52 ± 0.02	110.4	0.37
	270	5	5.51 ± 0.02	110.3	0.37
Brand 2					
	238	5	4.28 ± 0.11	85.6	2.68
	268	5	5.45 ± 0.10	109.0	1.75
	269	5	5.45 ± 0.08	109.1	1.41
	270	5	5.47 ± 0.06	109.4	1.08
AT					
Brand 1					
	235	20	20.88 ± 0.03	104.4	0.15
	256	20	19.52 ± 0.03	97.6	0.16
	309	20	19.93 ± 0.01	99.7	0.04
	310	20	19.79 ± 0.01	98.9	0.07
Brand 2					
	235	20	20.88 ± 0.03	104.4	0.13
	256	20	19.51 ± 0.02	97.5	0.12
	309	20	19.93 ± 0.01	99.7	0.04
	310	20	19.78 ± 0.01	98.9	0.06

^{a} Mean of five determinations.

Table 7: Results of application of CWT zero-crossing method on the spectra of the pharmaceutical preparations.

the results of PLS are yet better. This statement is especially true in determination of AM. Also, in all of the other wavelengths, PLS performances are better for AM. However, for AT determination in pharmaceutical preparations, recoveries of CWT zero-crossing in the other wavelengths are comparables with those of PLS.

For a more precise statistical comparison of the two methods t values were calculated [32]. For AM, t values of 512.51 and 243.25 and

for AT, 1.78 and 1.97 were obtained in analyzing Brand 1 and Brand 2, respectively. It must be mentioned for this comparison the best results of CWT zero-crossing have been used. Considering 5 determinations in each case, the degree of freedom will be 8 with the tabulated t value of 2.36 (p=0.05). Therefore, the accuracy of the PLS method is significantly better than that of CWT zero-crossing in AM determination but there is no significant difference in determination of AT.

Due to its multivariate character, PLS is not largely affected by fluctuations raised from random errors (noise) from one determination to another. Therefore, it is more accurate than CWT zero-crossing method which uses signal at only single wavelengths.

RSD% values obtained with two methods in AM determinations are comparable but in pharmaceutical analysis for AT, CWT zero-crossing method seems to be more precise than PLS.

Conclusions

In the rare reports published, the methods for the simultaneous determination of amlodipin and atorvastatine recommend analysis by HPLC. Nonetheless, these methods are time consuming and require the use of large volumes of HPLC-grade solvents. In this paper, a novel analytical method for the simultaneous determination of amlodipin and atorvastatine in pharmaceutical preparations by using partial least squares was demonstrated. Results of application to pharmaceutical preparations indicate that this method can be easily and conveniently used for the routine quality control of the drugs in pharmaceutical dosage forms. Also, the method can be used instead of the conventional HPLC in order to minimize cost of analysis and the toxic wastes produced during the analysis.

PLS results were compared with those of CWT zero-crossing method. The results indicate the superiority of PLS method.

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