Advances in Crop Science and Technology

ISSN: 2329-8863

Hinge MA, et al., ACST 2021, 9:9

Research Article Open Access

Chemometric assisted Spectrophotometric Method for Simultaneous estimation of Amlodipine Besylate and Candesartan Cilexetil

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Received date: September 06, 2021; **Accepted date:** September 20, 2021; **Published date:** September 27, 2021

Citation: Hinge MA et al. (2021) Chemometric assisted Spectrophotometric Method for Simultaneous estimation of Amlodipine Besylate and Candesartan Cilexetil. Adv Crop Sci Tech 9: 483

Abstract

In the present work, two different spectrophotometric methods for simultaneous estimation of Amlodipine Besylate and Candesartan Cilexetil in bulk and in formulation are described. Overlapped data(spectra) was quantitatively resolved by using two chemometric methods, Inverse least squares (ILS) and Classical least square method (CLS). The Calibration curves were plotted using the absorbance and concentration of the mixed solutions of two drugs. The drugs; Amlodipine Besylate and Candesartan Cilexetil were found to be linear in 5–15 and 8–24 µg/ml range. The data matrix of absorbance was generated by measuring the absorbance in the wavelength range from 300 to 360 nm. A Calibration set composition of the concentration of different mixture of Amlodipine Besylate and Candesartan Cilexetil was assembled in statistical way to optimize the particulate content from the spectra in way to get minimal errors in multivariate calibrations. The algorithms of CLS and ILS were applied to the spectra of the mixture of two drugs in calibration set and a suitable matrix was acquired. The model from CLS and ILS was selected by studying values of RMSEP. Then this algorithms was applied to the prediction set of different mixture of two drugs and marketed formulation. The results of recovery study of the marketed formulation were determined with great sensitivity in terms of limit of detection and limit of quantification. These CLS and ILS methods are validated and proposed methods were successfully employed for analyses of drugs in the mixtures of drugs and formulation.

Keywords: Chemometrics; Spectrophotometry; Amlodipine Besylate; Candesartan Cilexetil

Introduction

The development of chemometric methods of multi-component analysis has allowed the resolution of the complex spectra of mixtures of analytes. The chemometric quantitative analytical techniques have many applications and advantages such as the mixtures can be analyzed without any separation procedures for drug determination; the techniques are very easy to apply, very sensitive, useful and yet very inexpensive as compared to other analytical techniques for simultaneous determination of compounds in multicomponent mixtures. These methods provide additional advantages where calibration can be performed by ignoring the concentration of all other components except the analyte of interest and also the speed in the determination of components in a mixture. Amlodipine Besylate (AML), Benzenesulfonic acid; 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Fig. 1). Amlodipine is showing effect for long time and it affects 1, 4-dihydropyridine calcium channel by blocking it. It acts on smooth muscle cells of vascular system by sustaining voltage-gated L-type calcium channels. It inhibits the entry of calcium in smooth muscle cells and prevents myocyte contraction and vasoconstriction which depends on calcium concentration. Amlodipine Besylate is used in the treatment of Angina pectoris and Hypertension. Amlodipine Besylate is soluble in methanol and slightly soluble in water. Candesartan Cilexitil (CAN), is chemically 1-cyclohexyloxycarbonyloxyethyl, 2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] benzimidazole-4-carboxylate.

Candesartan Cilexitil exhibit antihypertensive property by selectively blocking the binding of angiotensin II to AT1 in many tissues like vascular smooth muscle and the adrenal glands.

It impedes the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and thereby lowers the overall blood pressure.

This drug is indicated for treatment of uncomplicated hypertension and congestive heart failure, myocardial infraction and coronary artery diseases.

Candesartan is Soluble in methanol and Practically insoluble in water. Amlodipine is official in IP 2014 and BP 2003 and estimated by TLC and HPLC. Candesartan Cilexitil is official in JP 2016 and analysed by Liquid chromatography. Literature survey reveals that differents analytical methods have been reported for the determination of Amlodipine Besylate and Candesartan Cilexitil as alone and in combined dosage forms with other drugs. The different methods reported are UV method, HPLC, stability indicating RP-HPLC, HPTLCand UPLC method. Both the drugs Amlodipine Besylate and Candesartan Cilexitil were simultaneously estimated by UV spectrophotmetric and HPLC method [1].

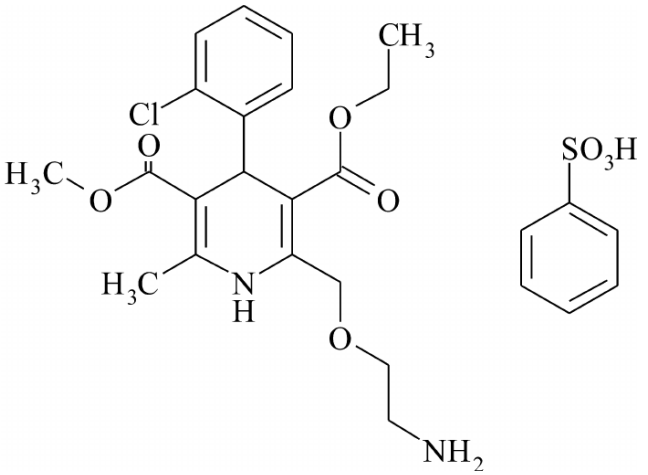

Figure1: Chemical structure of Amlodipine Besylate.

Materials and Methods

Candesartan Cilexetil was procured as gift sample from Alembic Pharmaceutical Ltd., Vadodara and Amlodipine Besylate from Ciron Drugs and Pharmaceutical Pvt. Ltd., Maharashtra. Methanol used for analysis was of AR Grade and Distilled Water. For spectrophotometric analysis, a Shimadzu UV-Vis double beam spectrophotometer equipped with 1 cm quartz cells and connected to personal computer loaded UV Probe Ver.2.10 software was used. CLS and ILS analyses were carried out using the Chemometrics Toolbox 3.02 software for use with MATLAB R2015a Software and Excel.

Preparation of standard solutions and calibration

For spectrophotometric measurement, stock solution (1000 □g/ml) of AML and CAN were prepared separately by dissolving 10 mg of each drug in 10 ml methanol. The zero order spectra were recorded over the wavelength range 200-400 nm against the solvent blank. The dilutions were made in methanol to obtain concentrations ranging from 5-15 µg/ml for AML and 8-24 µg/ml for CAN and their different synthetic mixtures by using the stock solutions [2].

Preparation of binary mixtures of AML and CAN

Appropriate and accurate volume aliquots of the above stock solutions were transferred to the two sets of 10 ml calibrated flasks. The calibration set of 15 and validation set of 10 standard mixture solutions which contain the concentrations with different ratio of AML and CAN was randomly prepared within the linearity range of two drugs. The absorbance data matrix was obtained by measuring the absorbance at 31 wavelength points (300 to 360 nm) with the interval of 2 nm in spectral region between 300 to 360 nm. A calibration set of 15 mixtures was prepared in methanol, applying a multilevel multifactor design in which two levels of concentrations of AML and CAN within the stated range were introduced as shown in Table 1. A validation set of 10 mixtures was prepared in methanol, applying a multilevel multifactor design in which two levels of concentrations of AML and CAN within the stated range were introduced as shown in Table 2.

Mix. No.	AML (µg/ml)	CAN (µg/ml)
1	5	16
2	5	20
3	5	24
4	7.5	8
5	7.5	12
6	7.5	24
7	10	12
8	10	16
9	10	20
10	12.5	8
11	12.5	20
12	12.5	24
13	15	8

Table 1: Composition of Calibration set for two constituents used in CLS and ILS techniques.

Mix. No.	AML (µg/ml)	CAN (µg/ml)
1	5	8
2	5	12
3	7.5	16
4	7.5	20
5	10	24
6	10	8
7	12.5	12
8	12.5	16
9	15	20
10	15	24

Table 2: Composition of Validation set for all two constituents used in CLS and ILS techniques.

Preparation of sample solutions

In spectrophotometric methods, twenty tablets of brand (UNISIA) were weighed separately and powdered in mortar. An amount of the powder equivalent to 80 mg of CAN was taken in a 25 ml calibrated volumetric flask and dissolved in methanol.

After sonication, the solution was filtered through Whatman filter paper number 41. The volume of the solution was made 25 ml mark by using methanol.

Further dilutions of the solution were made with methanol to reach the concentration of calibration range. All the proposed chemometric methods were applied to the solutions [3].

Classical least squares (CLS)

CLS is a simple method which is based on linear relation between the absorbance and the concentrations of components in mixture at each wavelength. By studying the matrix, it was found that the mixture follows Beer's law with m calibration standards containing l chemical components and n as absorbance and it is expressed by equation,

$$A=C\times K+EA \quad \text{--------- (1)}$$

where A is the m x n matrix obtained from calibration spectra, C is the m x l matrix of concentrations of component, K is the l x n matrix of relation between absorbance and concentration proportionality constants, and EA is in m x n matrix used to define spectral errors or residuals which are not fitting in model.

Inverse least squares (ILS)

This method uses concentration as a function of absorbance. The inverse of Beer's law model for m calibration standards with spectra of n digitised absorbance is given by [1,2],

$$C=A\times P+Ec \quad \text{--------- (2)}$$

where A is the m x n matrix obtained from calibration spectra, C is the m x l matrix of concentrations of component, P is the n × l matrix of unknown calibration co-efficient related to the l component concentrations of the spectral intensities, and Ec is the m × l vector of errors. Since in ILS the number of wavelengths should be less than the total number of calibration mixtures. Therefore multiple linear regressions have been used for the selection of wavelengths.

Results and Discussion

Figure 2 shows the zero-order overlay spectra of AML and CAN as well as their corresponding binary mixture in methanol. As shown in the Fig. 3 the spectra of AML and CAN are overlapped in the region of their absorption maxima. The chemometric method seemed to offer great potential. For this reason to solve overlapped spectra, chemometric calibrations using the zero-order spectra have been applied [4].

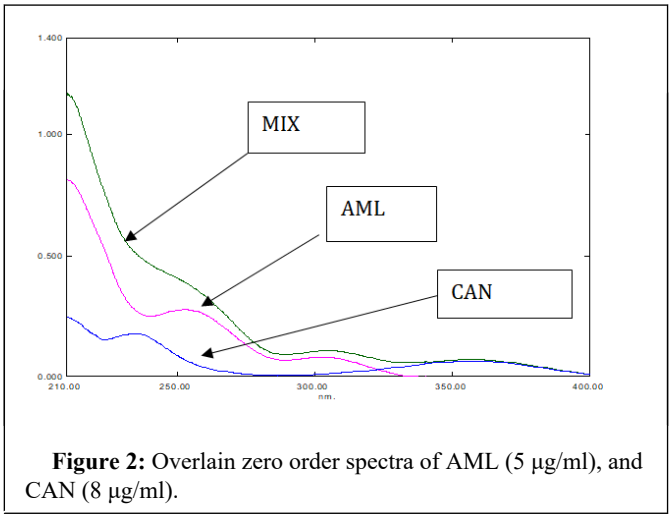

Figure 2: Overlain zero order spectra of AML (5 µg/ml), and CAN (8 µg/ml).

Multivariate calibration

The calibration set of 15 standard mixture solutions which contain the concentrations with different ratio of AML and CAN was randomly prepared within the linearity range of two drugs. The UV absorbance data was obtained by measuring the absorbance in the region of 300-360 nm. By using the correlation between calibration concentrations and its absorbance data, the chemometric calibrations were calibrated within the CLS and ILS algorithms. The quality of multi-component analysis is depends on the parameters like, range of wavelengths, spectral mode, concentrations of solutions of calibration set and calibration range. All the information present in the sample target should be present in the calibration data set. It has been one of the main drawbacks in development studies of multivariate method. The CLS, technique is designated as full spectrum computational procedures, thus wavelength selection is seemingly unnecessary and so all available wavelengths are often used. Stepwise multiple linear regressions have been used for the selection of frequencies in ILS.

CLS Method: In CLS method, the coefficient matrix (K) was calculated by using the equation of straight line for calibration curve plotted between the absorbance and concentration of solutions of calibration set. Replacing the coefficient matrix (K) into the linear equation system, the calibration of CLS can be written as:

ILS method: In ILS method, the coefficient matrix (P) was obtained from the linear equation using the absorbance data and the concentrations of solutions of calibration set. Introducing (P) into the linear equation system we obtain the calibration for ILS as:

Statistical parameter

The model of CLS and ILS shows predictive applicability of a regression and is described in different ways. The expression for the standard error of prediction (SEP) and standard error of calibration denoted by SEC and it is given in the following C is the predicted concentration of drugs and n is the total number of the synthetic mixtures.

The SEP and SEC results and other statistical evaluations obtained by applying CLS and ILS to the above mentioned validation set of the synthetic mixtures are quoted in Table 3.

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing 10 samples of various concentrations of AML and CAN were carried out. The maximum values of the mean percent errors corresponding to CLS and ILS for the same mixtures were completely acceptable because of their very smallest values. The mean recoveries and the relative standard deviations of our proposed methods were computed and indicated in Table 4 and 5. Their numerical values were completely acceptable because of their smallest values and hence found satisfactory for the validity of all calibration methods. The linearity of the proposed chemometric method for determination of AML and CAN was evaluated by analysing a series of different concentrations of standard drug. The linearity was found to be ranging between 5-15 µg/ml for AML and 8-24 µg/ml for CAN. Each concentration was repeated three times. The accuracy study was performed by increasing standard addition of known amounts of studied drugs to an unknown concentration (constant volume) of the commercial pharmaceutical formulations. A constant volume of the unknown solution was added to the volumetric flasks. Then the solution of working standard was added at three different levels.

Finally, each flask was made up to the mark with methanol and mixed well. The resulting mixtures were analyzed and chemometric recoveries were determined. The results obtained were compared with expected results. The good mean recoveries and standard deviation suggested good accuracy of the proposed methods and no interference from formulations excipients. The selectivity of the proposed method was also assessed by the analysis of synthetic mixtures, where satisfactory results were obtained over the stated calibration range [5].

The predicted concentrations of the components in each sample were compared with the actual concentrations of the components in each validation samples and the root mean square error of prediction (RMSEP) was calculated for each method. The RMSEP was used for examining the error in the predicted concentrations. The model is a key to achieving correct quantitation in CLS and ILS calibrations. The resulted models were also validated by prediction of the concentration of analytes in separate validation set which was not used in the model development. The RMSEP values are represented in Table 3. The predictive abilities of the models were evaluated by plotting the actual known concentrations against the predicted concentrations which are shown in Figure 3. Figure indicates that there was good agreement between the predicted (calculated) concentration and actual concentration of drugs. The means recoveries and the relative standard deviation of our proposed methods were computed and indicated in Table 4 for AML and CAN, respectively. Another diagnostic test was carried out by plotting the concentration residuals against the predicted concentrations. Fig. 5 has shown the residuals appear randomly distributed around zero, indicating adequate models building. Satisfactory correlation coefficient (r2) and slope values were obtained for each compound in the validation set by CLS and ILS optimized models indicating good predictive abilities of the models. The assay results are given. Summary of CLS and ILS methods given in Table 4.

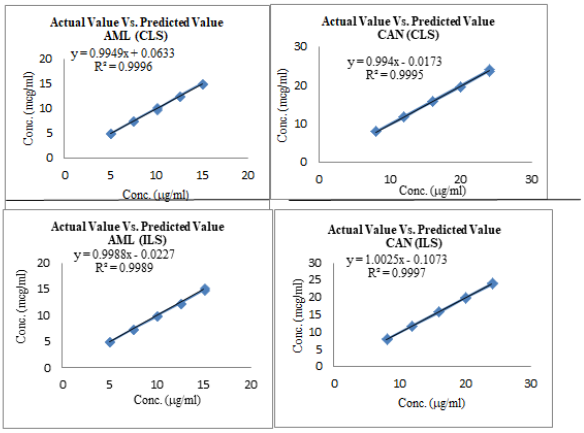

Figure 3: CLS – Expected vs. Predicted Concentration of AML and CAN, ILS – Expected Vs. Predicted Concentration of AML and CAN.

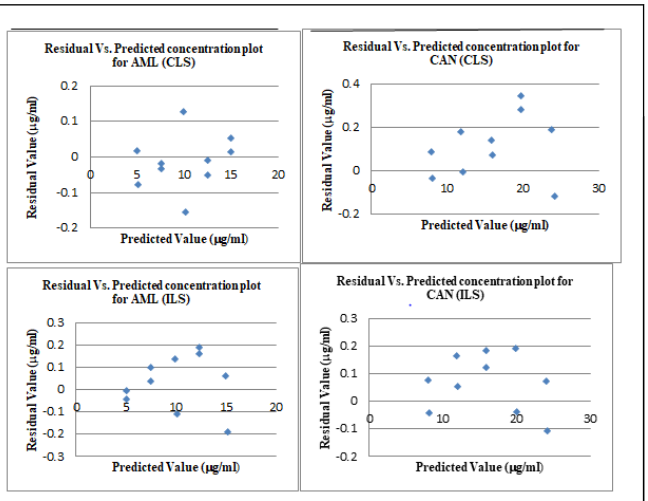

Figure 4: CLS – Expected vs. Residual Concentration of AML and CAN, ILS – Expected Vs. Residual Concentration of AML and CAN.

COMPONENT	RMSEP (CLS)	RMSEP (ILS)
Amlodipine Besylate	0.06069	0.05097
Candesartan Cilexetil	0.70126	0.58312

Table 3: Statistical parameters of chemometric methods in calibration step of Zero-order spectra.

Parameters	**AML**		**CAN**	
	CLS	ILS	CLS	ILS
Linearity (µg/ml)	42125	42125	45505	45505
Wavelength (nm)	300-360	300-360	300-360	300-360
Δλ (nm)	2	2	2	2
% Recovery	100.21	99.68	99.31	99.52
SD	0.855	1.085	0.783	0.678
RSD	0.31	0.406	0.399	0.38
Correlation coefficient (r2)	0.9996	0.9989	0.9995	0.9997
Intercept	0.0633	0.0227	0.0173	0.1073
Slope	0.9949	0.9988	0.994	1.0025
RMSEP	0.06069	0.05097	0.7012	0.5831
LOD (µg/ml)	0.1048	0.1198	0.1398	0.0866
LOQ (µg/ml)	0.3177	0.3632	0.4239	0.2625

Table 4: Summary parameters of Chemometric methods

Assay of Marketed Formulation

Twenty tablets were accurately weighed and finely powdered. Tablets powder equivalent to about 50 mg of AML and 80 mg of CAN accurately weighed and transferred to into 25 mL volumetric flask and 10 mL of methanol was added. The mixture was sonicated for 20 min and diluted up to the mark with methanol and filtered through a whatman filter paper no.41. From this solution, dilutions were made to get the solution containing 5 µg/ml of AML and 8 µg/ml of CAN. The analysis procedure was repeated three times for tablet formulation. The result was shown in Table 6.

Conclusion

Many drugs have come up in combinations in order to improvise the therapy of various ailments. These combinations have forged a

challenge to use a simple method to estimate the individual drugs in combination with respect of time and complexity. Simultaneous determination of AML and CAN in tablet is not reported in the literature as yet. However, the chemometric methods are economical than other methods like chromatography and do not require sophisticated instrumentation and any prior separation of components. The proposed chemometric-assisted spectrophotometric methods are applicable, prompt, and specific for the simultaneous determination of AML and CAN in their synthetic mixtures and commercial pharmaceutical tablets. We attempted to develop two chemometric methods i.e. CLS and ILS. These methods were found to be simple, precise, accurate, rapid and economical methods for their simultaneous determination. The methods were successfully validated and found suitable for quality control laboratories.

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