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SIMULTANEOUS EVALUATION OF AMLODIPINE BESYLATE AND CANDESARTAN CILEXETIL BY APPLYING CHEMOMETRIC ASSISTED SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Two distinct spectrophotometric methods are provided in this paper for simultaneous measurement of Amlodipine Besylate and Candesartan Cilexetil in bulk and formulation. Two chemometric approaches were used to quantitatively resolve overlapping spectra, Inverse least squares (ILS) and the Classical least square method (CLS). Calibration curves were plotted using the absorbance and concentration of mixed solutions of two drugs. The drugs; Amlodipine Besylate and Candesartan Cilexetil were found to be linear in the 5–15 and 8–24 µg/ml range. The data matrix of absorbance was generated by determining absorbance in a wavelength range from 300 to 360 nm. A calibration set composition of concentration of a different mixture of Amlodipine Besylate and Candesartan Cilexetil was assembled statistically to optimize the particulate content from the spectra in a way to get minimal errors in multivariate calibrations. The algorithms of CLS and ILS were applied to spectra of the mixed solution of two drugs in a calibration set and a suitable matrix was acquired. The model from CLS and ILS was selected by studying the values of RMSEP. Then this algorithm was applied to the prediction set of different mixtures of two drugs and marketed formulation. The results of the 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 employed for the quantification of drugs in mixtures and formulation.

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Introduction

The introduction of multicomponent quantification by chemometric approaches has enabled the resolution of complicated spectra of analytes or drug mixtures [1]. Apart from the different analytical methods used for the quantification of drugs, chemometric quantitative analytical techniques have more advantages and applications, such as analysing mixtures excluding any form of prior drug separation methods; these approaches are uncomplicated in the application, sensitive, useful, and yet cost-effective. These approaches have additional advantages, such as the ability to do calibration while disregarding the concentrations of all other constituents except analyte and rapidity with which components in a combination can be determined [1]. Amlodipine Besylate (AML) [2-5], Benzenesulfonic acid; 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate **(Figure 1)**. Amlodipine Besylate is showing an effect for a long time and it affects 1, 4-dihydropyridine calcium channel by blocking it. It acts by keeping voltage-gated L-type calcium channels open in vascular smooth muscle cells. It suppresses myocyte contraction and vasoconstriction, both of which are dependent on calcium concentration, by inhibiting calcium entrance into smooth muscle cells. Angina pectoris and hypertension are treated with amlodipine besylate. Amlodipine Besylate is slightly soluble in water and soluble in methanol. 1-cyclohexyloxycarbonyloxyethyl, 2-ethoxy-3-[[4-[2-(2H-tetrazole-5-yl) phenyl] phenyl] methyl] benzimidazole-4-

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carboxylate **(Figure 2)** can also be referred to as Candesartan Cilexitil (CAN) [2, 3, 6, 7]. Candesartan Cilexetil acts as an antihypertensive by preventing angiotensin II binding to angiotensin1 in different tissues such as vascular smooth muscle and adrenal glands. It blocks angiotensin II's angiotensin1-mediated vasoconstrictive and aldosterone-secreting actions, lowering total blood pressure. This drug is used to treat uncomplicated hypertension, heart failure, myocardial infarction, and coronary artery disease. Now a days, Hypertension becomes a major impact disease affecting life in many ways leading to death also and its management and treatment needs a new and better development in drug and formulation. Different studies are performed using different antihypertensive drugs (both synthetic and herbal) and development of formulation were examined considering bioavalability of drugs, design and therapy [8-12]. In methanol, Candesartan Cilexetil is soluble but practically insoluble in water.

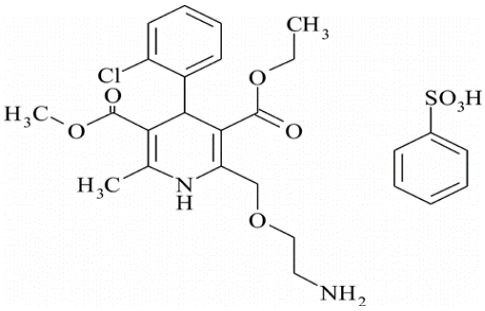

Figure 1. Chemical structure of Amlodipine Besylate

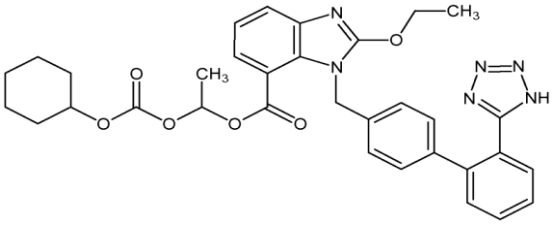

Figure 2. Chemical structure of Candesartan Cilexetil

Amlodipine Besylate is official in IP 2018 [13], BP 2020 [14] and USP 2015 [15] and estimated by TLC and HPLC. Candesartan Cilexitil is official in JP 2016 [16], USP 2015 [17] and BP 2020 [18] and analysed by Liquid chromatography.
A review of the literature reveals that several analytical procedures have been published for the quantification of Amlodipine Besylate including different methods viz; spectrophotometric [19, 20], HPLC [21-25], stability-indicating RP-HPLC [26], in human plasma by HPLC-MS [27] and HPTLC method [28, 29] as alone and in combined dosage forms with other drugs. For Candesartan Cilexetil, the different methods reported are UV method [30-33], HPLC [34-36], stability-indicating RP-HPLC [37], HPTLC [38] and UPLC method [39]. Both the drugs Amlodipine Besylate and Candesartan Cilexitil were simultaneously estimated by UV spectrophotmetric [40, 41] and HPLC method [42-47].

Materials and Methods

Candesartan Cilexetil was procured as a 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. Shimadzu UV-Vis (double beam) spectrophotometer was used for spectrophotometric analysis. It was connected to a personal computer having UV Probe Ver.2.10 software and provided with 1 cm quartz cells. For chemometric calculation, chemometrics toolbox (3.02) software associated with MATLAB R2015a Software and Excel was used to conduct CLS and ILS evaluations.

Preparation of Solutions of Standard and Calibration Set

Stock solutions (1000 µg/ml) of Amlodipine Besylate and Candesartan Cilexetil were produced separately for spectrophotometric measurement by solubilising 10 mg of both constituents in 10 ml methanol. The zero-order spectra were obtained against a solvent blank throughout the wavelength range 200-400 nm. By diluting the stock solutions in methanol, concentrations of 5-15 µg/ml for Amlodipine Besylate and 8-24 µg/ml for Candesartan Cilexetil and their various synthetic combinations were obtained.

Preparation of Binary Mixtures of Amlodipine Besylate and Candesartan Cilexetil

Appropriate volumes of the aforementioned stock solutions were taken in two sets of 10 ml volumetric flasks in an appropriate and precise manner. Within the linearity range of two drugs, a calibration set of 15 and a validation set of 10 standard

combination solutions containing concentrations with varying ratios of Amlodipine Besylate and Candesartan Cilexetil were created at random. By measuring absorbance at 31 wavelength points (300 to 360 nm) in the spectral region between 300 and 360 nm with a 2 nm interval, the absorbance data matrix was made. In methanol, a calibration set of 15 mixtures was created using a multilevel multifactor design with two levels of Amlodipine Besylate and Candesartan Cilexetil concentrations within the 5 - 15 and 8 – 24 5 - 15 and 8 – 24 µg/ml range, as mixture of 5 and 8 µg/ml, 5 and 12 µg/ml and so on for Amlodipine Besylate and Candesartan Cilexetil respectively. Using a multilevel multifactor design,10 mixtures validation set was created in methanol in which two amounts of Amlodipine Besylate and Candesartan Cilexetil concentrations within a 5 - 15 and 8 – 24 µg/ml range were introduced,

Preparation of Sample Solutions

Twenty tablets (UNISIA) were weighed separately and crushed in a mortar. In a 25 ml calibrated volumetric flask, the quantity of tablet powder equivalent to 80 mg of Candesartan Cilexetil was dissolved in methanol. Then, using Whatman filter paper number 41, the solution was filtered after sonication. Using methanol, the volume of the solution was made to 25 ml. The solution was diluted further with methanol to attain the calibration range concentration. The solutions were subjected to all of the recommended chemometric approaches.

Classical Least Squares Method

CLS method was studied on basis of the linear relationship between absorbance and concentration of drugs in the mixture at each wavelength. By studying the matrix, it was found that using m calibration standards with l chemical components and n as absorbance, the mixture follows Beer's law, and below is the expressed equation [1],

$$A=C\times K+EA \quad (1)$$

derived from calibration spectra, A is m x n matrix, C is the concentration of drug in m x l matrix, containing the relationship between absorbance and concentration proportionality constants, K is l x n matrix, and containing spectral errors or residuals that do not fit in the model, EA is a m x n matrix.

Inverse Least-Squares Method

The function of absorbance in this method is calculated by concentration. The inverse of Beer's law model form calibration standards having spectra of n digitised absorbance is seen as the equation below [1],

$$C=A\times P+Ec \quad (2)$$

where derived from calibration spectra, A is m x n matrix, the component concentrations of m x l matrix are C, P is n l matrix of unknown calibration coefficients associated to l component concentrations of spectral intensities and Ec is ml vector of errors. Because the total number of calibration mixtures in ILS should be smaller than the number of wavelengths, as a result, multiple linear regressions were performed to choose the wavelengths.

Results and Discussion

The zero-order overlay spectra of Amlodipine Besylate and Candesartan Cilexetil and their binary combination in methanol is depicted in **Figure 3**. The spectra of Amlodipine Besylate and Candesartan Cilexetil overlap in the region of their absorption maxima, as illustrated in **Figure 3**. The chemometric technique appeared to have a lot of promise. As a result, chemometric calibrations based on zero-order spectra have been used to solve overlapped spectra.

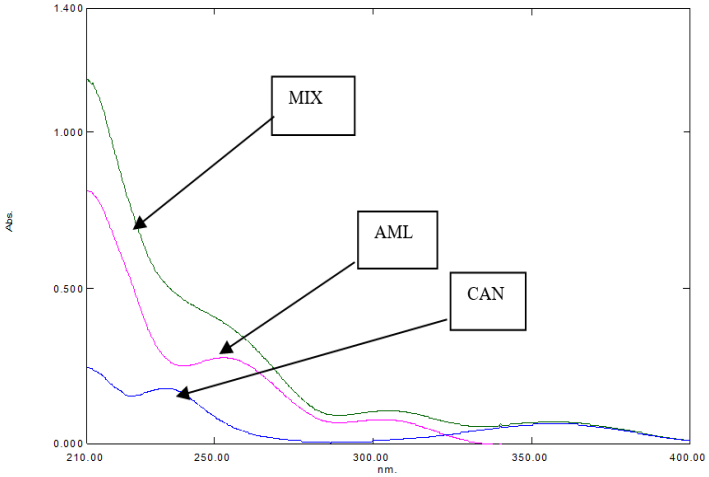

Figure 3. Overlay spectra of Amlodipine Besylate (5 µg/mL) & Candesartan Cilexetil (8 µg/mL).

Multivariate Calibration

Within the linearity range of two drugs, a calibration set of 15 standard combination solutions which consists of concentrations with a varied ratio of Amlodipine Besylate and Candesartan Cilexetil was randomly made. By measuring absorbance in a region of 300-360 nm, UV absorbance data was obtained. By using the correlation between calibration concentrations and absorbance data, chemometric calibrations were calibrated within CLS and ILS algorithms. Wavelength range, spectral mode, calibration set concentrations, and calibration range are some characteristics that determine the quality of multi-component analysis. The calibration data set should contain all of the information from the sample target. It has been one of the major disadvantages in multivariate method development studies. Because the CLS technique is classified as an entire spectrum computational procedure, selection of wavelength appears to be unnecessary and all accessible wavelengths are frequently utilised. The frequency selection in ILS was done using stepwise multiple linear regressions.

CLS Method

Coefficient matrix (K) was generated using the CLS method by applying the equation of a straight line for calibration curve plotted between the absorbance and concentration of solutions of the calibration set. Calibration of CLS Candesartan Cilexetil can be stated as follows by substituting coefficient matrix (K) into linear equation system:

$$\begin{bmatrix} C_{AML} \\ C_{CAN} \end{bmatrix} = \begin{bmatrix} 0.0021 & 0.0024 & 0.0026 & 0.0030 & 0.0033 & 0.0037 & 0.0042 & 0.0046 & 0.0051 & 0.0056 \\ 0.0061 & 0.0067 & 0.0073 & 0.0078 & 0.0108 & 0.0107 & 0.0103 & 0.0098 & 0.0092 & 0.0085 \\ 0.0077 & 0.0068 & 0.0059 & 0.0050 & 0.0041 & 0.0033 & 0.0026 & 0.0020 & 0.0084 & 0.0090 \\ 0.0096 & 0.0101 & 0.0106 & 0.0111 & 0.0118 & 0.0121 & 0.0125 & 0.0128 & 0.0130 & 0.0132 \\ 0.0133 & 0.0133 & 0.0133 & 0.0131 & 0.0129 & -0.0001 & -0.0001 & -0.0001 & & \\ 0.0015 & 0.0010 & 0.0007 & 0.0005 & 0.0003 & 0.0002 & 0.0002 & -0.0000 & -0.0000 & -0.0001 \\ -0.0001 & -0.0001 & -0.0001 & -0.0001 & & & & & & \end{bmatrix} \begin{bmatrix} A1 & A16 \\ A2 & A17 \\ A3 & A18 \\ A4 & A19 \\ A5 & A20 \\ A6 & A21 \\ A7 & A22 \\ A8 & A23 \\ A9 & A24 \\ A10 & A25 \\ A11 & A26 \\ A12 & A27 \\ A13 & A28 \\ A14 & A29 \\ A15 & A30 \\ & A31 \end{bmatrix}$$

ILS Method

The coefficient matrix (P) was calculated using absorbance data and concentrations of calibration set solutions in the ILS method. When we add (P) to the linear equation system, we get the following calibration for ILS:

$$\begin{bmatrix} C_{AML} \\ C_{CAN} \end{bmatrix} = \begin{bmatrix} 0.0020 & -0.0602 & 0.0576 & 0.0005 & 0.008 & -0.0426 & 0.0211 & 0.2374 & 0.2100 & 0.2560 \\ 0.0411 & 0.0101 & -0.0771 & -0.2588 & -0.3332 & 0.0125 & 0.0187 & 0.2287 & 0.0999 & -0.1974 \\ -0.3806 & -0.3409 & -0.1796 & -0.0762 & 0.0264 & -0.1308 & -0.0230 & -0.0788 & 0.8128 & 0.5793 \\ 0.4389 & 0.4171 & -0.3156 & -0.3991 & -0.0834 & -0.0607 & -0.4211 & -0.0560 & -0.2311 & 0.2778 \\ -0.0574 & 0.0061 & -0.1692 & -0.2709 & 0.2105 & 0.0464 & -0.2432 & -0.2121 & 0.2973 & 0.0343 \\ -0.1111 & 0.0683 & -0.1291 & -0.2196 & 0.3575 & 0.3350 & -0.1303 & 0.0244 & 0.1273 & -0.0344 \\ 0.2019 & -0.0256 & & & & & & & & \end{bmatrix} \begin{bmatrix} A1 & A16 \\ A2 & A17 \\ A3 & A18 \\ A4 & A19 \\ A5 & A20 \\ A6 & A21 \\ A7 & A22 \\ A8 & A23 \\ A9 & A24 \\ A10 & A25 \\ A11 & A26 \\ A12 & A27 \\ A13 & A28 \\ A14 & A29 \\ A15 & A30 \\ & A31 \end{bmatrix}$$

Statistical Parameter

CLS and ILS models demonstrate the predictive usefulness of regression and are described in a variety of ways. The following formula is used to calculate standard error of prediction (SEP) and standard error of calibration (SEC);

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{N}(C_i^{added} - C_i^{found})^2}{n}} \quad (3)$$

The estimated drug concentration is C and the total number of synthetic mixtures is n.

The RMSEP values for Amlodipine Besylate and Candesartan Cilexetil was found to be 0.06069 and 0.70126 by CLS method and 0.05097 and 0.58312 by ILS method.

Simultaneous quantitation of the prediction set of 10 samples of varying concentrations of Amlodipine Besylate and Candesartan Cilexetil was carried out to assess the validity (predictive capacity) of calibration models. Maximum values of mean per cent errors corresponding to CLS and ILS for the same combinations were acceptable because of their relatively modest values. Our proposed approaches' mean recoveries and relative standard deviations were calculated and shown in **Tables 1 and 2**. Because of their smallest values, their numerical values were fully acceptable, and all calibration procedures were confirmed to be legitimate. The linearity of the proposed chemometric approach for the measurement of Amlodipine Besylate and Candesartan Cilexetil was determined by analysing a series of varied concentrations of standard drugs. The linearity of Amlodipine Besylate was determined to be between 5-15 µg/ml and 8-24 µg/ml for Candesartan Cilexetil. Three times each concentration was done. Standard drugs of known amounts were added to an unknown concentration of pharmaceutical formulations was used to conduct an accuracy investigation. The volumetric flasks were filled with a consistent volume of the unknown solution. The working standard solution was then added at three separate levels.

Finally, each flask was filled to the capacity with methanol and thoroughly mixed. The chemometric recoveries of resultant mixtures were discovered. The expected outcomes were compared to the acquired results. The proposed procedures were accurate and there was no interference from the formulation excipients as indicated by the good mean recoveries and standard deviation **(Tables 1 and 2)**. The suggested method's selectivity was also tested by analysing synthetic mixes, which yielded satisfactory findings over the given calibration range.

The actual component concentrations in each validation sample were compared to the predicted component concentrations in each sample, and the calculation of the root mean square error of prediction (RMSEP) for each technique was carried out. The RMSEP was utilised to look into the projected concentrations' error. In CLS and ILS calibrations, the model is critical for accurate quantification. The models were additionally validated by predicting analyte concentrations in a separate validation set that had not been included in the model development. The low values of RMSEP shows the acceptability of Model. The models' predictive abilities were assessed by graphing actual known concentrations against expected concentrations, as shown in **Figure 4**. The figure shows that the predicted (calculated) drug concentration and the actual drug concentration were in good agreement. **Tables 1 and 2** show the mean recoveries and relative standard deviations of our suggested approaches for Amlodipine Besylate and Candesartan Cilexetil, respectively. Plotting the concentration residuals against the expected concentrations was used as another diagnostic test. The residuals in **Figure 5** appear to be randomly distributed around zero, indicating that suitable models have been built. CLS and ILS optimised models obtained satisfactory correlation coefficient (r^2) and slope values for each compound in the validation set, showing that the models had strong prediction ability [48, 49].

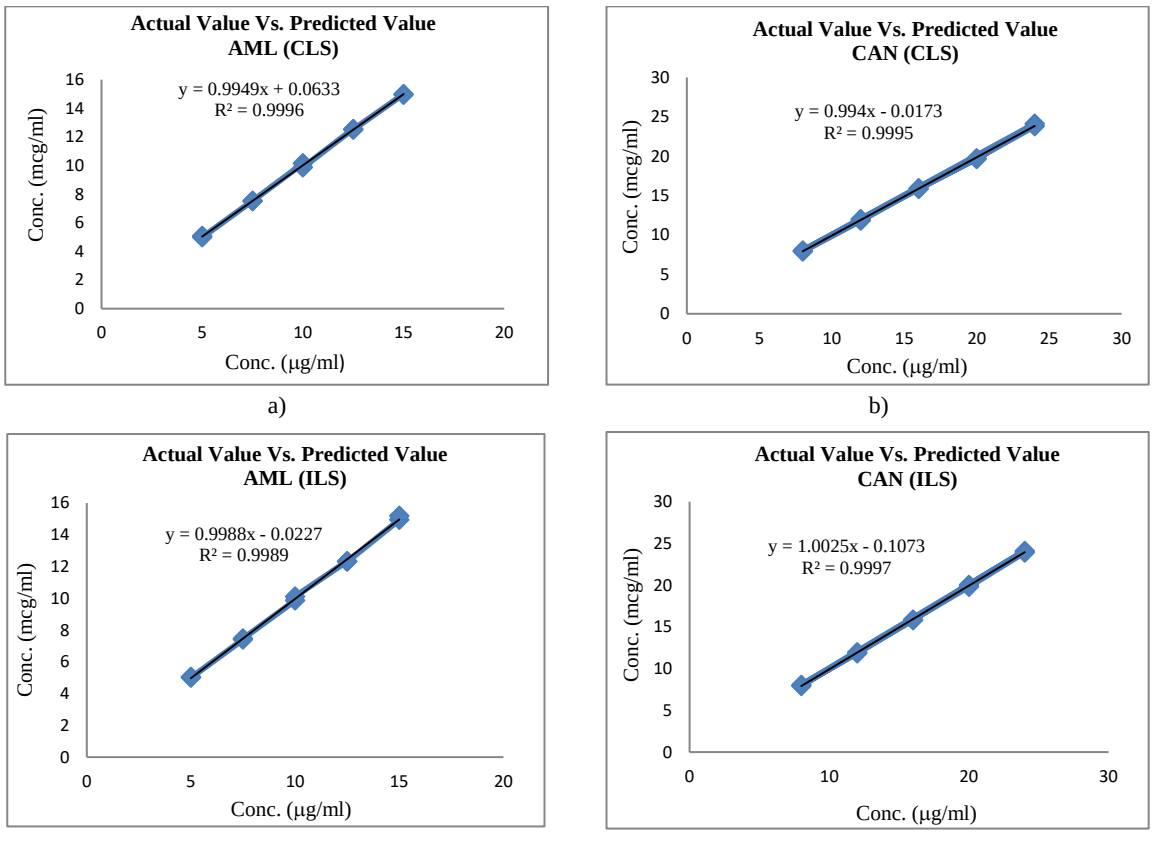

Figure 4. CLS – Expected vs. Predicted Concentration of Amlodipine Besylate and Candesartan Cilexetil, ILS – Expected Vs. Predicted Concentration of Amlodipine Besylate and Candesartan Cilexetil

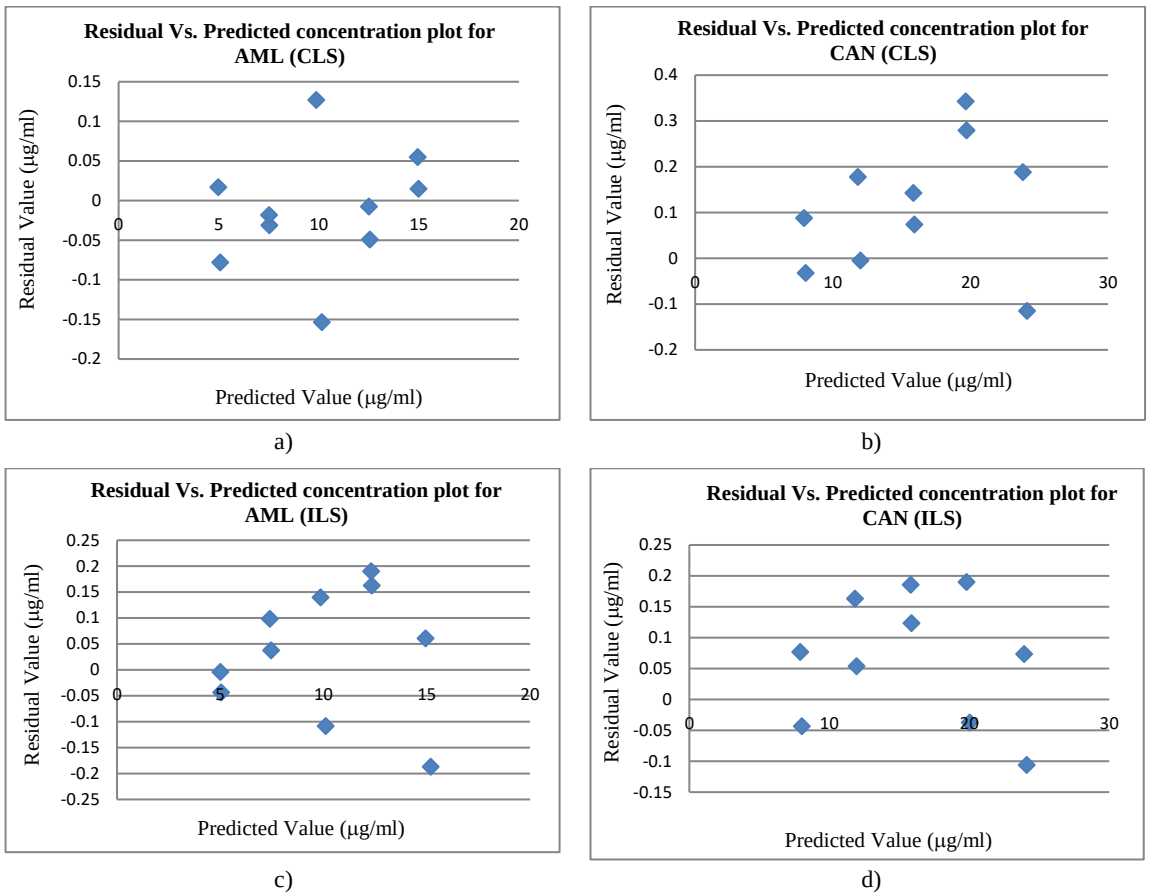

Figure 5. CLS – Expected vs. Residual Concentration of Amlodipine Besylate and Candesartan Cilexetil, ILS – Expected Vs. Residual Concentration of Amlodipine Besylate and Candesartan Cilexetil.

Table 1. Analysis of validation set by CLS method

Expected Conc. (µg/ml)		Predicted Conc. (µg/ml)		% Recovery		Residual (Expected-Predicted) Conc. (µg/ml)		(Expected-Predicted)^2 Conc. (µg/ml)	
AML	**CAN**	**AML**	**CAN**	**AML**	**CAN**	**AML**	**CAN**	**AML**	**CAN**
5	8	5.078	7.912	101.56	98.90	-0.078	0.088	0.00600	0.00774
5	12	4.983	11.822	99.66	98.52	0.017	0.1776	0.00028	0.03154
7.5	16	7.531	15.926	100.42	99.54	-0.0312	0.0738	0.00097	0.00544
7.5	20	7.518	19.657	100.24	98.29	-0.0183	0.3426	0.00033	0.11737
10	24	10.153	24.114	101.53	100.48	-0.1533	-0.1148	0.02350	0.01317
10	8	9.873	8.031	98.73	100.40	0.127	-0.0319	0.01612	0.00101
12.5	12	12.549	12.004	100.39	100.04	-0.0492	-0.0049	0.00242	0.00002
12.5	16	12.507	15.857	100.06	99.11	-0.0076	0.1426	0.00005	0.02033
15	20	14.985	19.720	99.90	98.60	0.0149	0.2794	0.00022	0.07806
15	24	14.945	23.811	99.63	99.22	0.055	0.1881	0.00302	0.03538
Mean%								100.21	99.31
SD[a]								0.8559	0.7834
RSD[b]								0.3107	0.3998

a=Standard Deviation, b=Relative Standard Deviation

Table 2. Analysis of validation set by ILS method

Expected Conc. (µg/ml)		Predicted Conc. (µg/ml)		% Recovery		Residual (Expected-Predicted) Conc. (µg/ml)		(Expected-Predicted)^2 Conc. (µg/ml)	
AML	**CAN**	**AML**	**CAN**	**AML**	**CAN**	**AML**	**CAN**	**AML**	**CAN**
5	8	5.043	7.923	100.87	99.04	-0.0436	0.0769	0.00190	0.00591

5	12	5.004	11.946	100.08	99.55	-0.004	0.0538	0.00002	0.00289
7.5	16	7.401	15.814	98.69	98.84	0.0983	0.1857	0.00966	0.03448
7.5	20	7.462	20.037	99.50	100.19	0.0376	-0.0374	0.00141	0.00139
10	24	9.860	24.106	98.60	100.44	0.1397	-0.1061	0.01951	0.01125
10	8	10.108	8.043	101.08	100.54	-0.1084	-0.0433	0.01175	0.00187
12.5	12	12.337	11.836	98.70	98.64	0.1627	0.1632	0.02647	0.02663
12.5	16	12.309	15.876	98.48	99.23	0.1901	0.1235	0.03613	0.01525
15	20	14.939	19.809	99.59	99.05	0.0608	0.1901	0.00369	0.03613
15	24	15.187	23.926	101.25	99.69	-0.1871	0.0735	0.03500	0.00540
Mean %								99.68	99.52
SD[a]								1.085	0.6786
RSD[b]								0.4063	0.3802

a=Standard Deviation, b=Relative Standard Deviation

Assay of Marketed Formulation

Twenty tablets were finely powdered after being precisely weighed. Tablet powder weighing about 50 mg Amlodipine Besylate and 80 mg Candesartan Cilexetil was precisely weighed and put to a 25 mL volumetric flask with 10 mL methanol. The mixture was sonicated for 20 minutes before being diluted with methanol to the desired concentration and filtered through Whatman filter paper no.41. Dilutions were prepared from this solution to produce a solution containing 5 µg/ml Amlodipine Besylate and 8 µg/ml Candesartan Cilexetil. For tablet formulation, the analysis technique was repeated three times.

The assay results of formulation in percentage for Amlodipine Besylate and Candesartan Cilexetil was found to be 100.28 ± 0.0559 and 100.88 ± 0.0761 by CLS method and 99.32 ± 0.5029 and 99.68 ± 0.0559 by ILS method.

Conclusion

Many drugs have been developed in combination to improve the treatment of various disorders. The assessment of the individual drugs in combination in terms of time and complexity using a simple method have been made very difficult by these combinations. Simultaneous determination of Amlodipine Besylate and Candesartan Cilexetil in tablet has yet to be published in the literature. Chemometric procedures, on the other hand, are less expensive than other methods such as chromatography since they do not require complex instrumentation or any prior component separation. For the simultaneous determination of Amlodipine Besylate and Candesartan Cilexetil in their synthetic mixtures and commercial pharmaceutical tablets, the suggested chemometric-assisted spectrophotometric methods are adaptable, fast, and specific. CLS and ILS are two chemometric approaches that we attempted to create. These two methods were found to be linear with regression coefficient closer to 0.9999. A mathematical model is accepted as the value of RMSEP units was found to be less than three. The sensitivity of the methods were determined by LOD and LOQ. LOD for Amlodipine Besylate and Candesartan Cilexetil by CLS and ILS method was obtained as 0.1048 and 0.1198 µg/ml and 0.1398 and 0.0866 µg/ml, respectively. LOQ for Amlodipine Besylate and Candesartan Cilexetil by CLS and ILS method was obtained as 0.3177 and 0.3632 µg/ml and 0.4239 and 0.2625 µg/ml, respectively. For their simultaneous determination, these approaches were found to be simple, precise, accurate, quick, and cost-effective. The procedures were validated and confirmed to be appropriate for quality control laboratories.

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