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ORIGINAL PAPER

Multivariate Versus Classical Univariate Calibration Methods for Spectrofluorimetric Data: Application to Simultaneous Determination of Olmesartan Medoxamil and Amlodipine Besylate in their Combined Dosage Form

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Abstract Olmesartan medoxamil (OLM, an angiotensin II receptor blocker) and amlodipine besylate (AML, a dihydropyridine calcium channel blocker), are co-formulated in a single-dose combination for the treatment of hypertensive patients whose blood pressure is not adequately controlled on either component monotherapy. In this work, four multivariate and two univariate calibration methods were applied for simultaneous spectrofluorimetric determination of OLM and AML in their combined pharmaceutical tablets in all ratios approved by FDA. The four multivariate methods are partial least squares (PLS), genetic algorithm PLS (GA-PLS), principal component ANN (PC-ANN) and GA-ANN. The two proposed univariate calibration methods are, direct spectrofluorimetric method for OLM and isoabsorptive method for determination of total concentration of OLM and AML and hence AML by subtraction. The results showed the superiority of multivariate calibration methods over univariate ones for the analysis of the binary mixture. The optimum assay conditions were established and the proposed multivariate calibration methods were successfully applied for the assay of the two drugs in validation set and combined pharmaceutical tablets with excellent recoveries. No interference was observed from common pharmaceutical additives. The results were favorably compared with those obtained by a reference spectrophotometric method.

Keywords Olmesartan medoxamil · Amlodipine besylate · Spectrofluorimetry · Multivariate calibration methods · Pharmaceutical tablets

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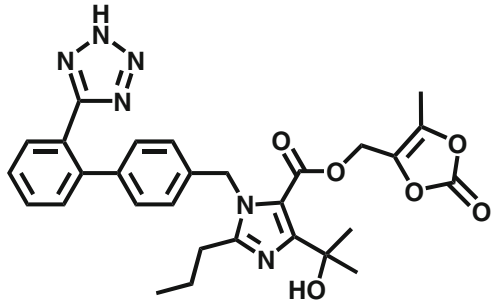
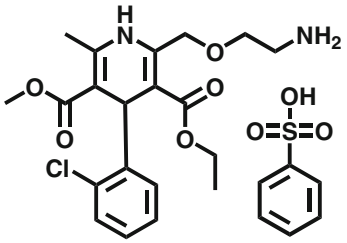
Introduction

Olmesratan medoxamil (OLM, Fig. 1) is chemically known as (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl] phenyl}methylimidazol-5-carboxylate. It is a potent and selective angiotensin AT1 receptor blocker. [1] It has been approved for the treatment of hypertension in the United States, Japan and European countries. The drug contains a medoxomil ester moiety which is cleaved rapidly by an endogenous esterase to release the active olmesartan. [2] There are various methods for analysis of OLM alone or in combination with other drugs. These methods include spectrophotometry [3–9], spectrofluorimetry [8], HPTLC [10], Mass [11], LC–MS–MS [12], CZE [13, 14], and HPLC [15, 16].

Amlodipine besylate (AML, Fig. 1) is chemically known as 3-ethyl-5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate benzene sulphonate. It is a dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris [17]. AML is official in the British Pharmacopoeia (BP) which describes HPLC for its assay in the bulk powder [18]. Several analytical methods have been reported for the determination of AML in pharmaceutical formulations and/or biological fluids. These methods include spectrophotometry [19–21], spectrofluorimetry



Olmesartan medoxomil (OLM)



Amlodipine besylate (AML)

Fig. 1 Chemical structures of olmesartan medoxomil (OLM) and amlodipine besylate (AML)

[22, 23], anodic stripping voltammetry [24, 25], HPLC [26–31], HPTLC [32], capillary electrophoresis [33] and micellar electrokinetic chromatography [34].

Recently, OLM has been marketed in combination with AML in tablet dosage form (Olmesar® tablets). The oral administration of this combination has been proved to be more effective than either of the two drugs in a single-drug therapy for treatment of hypertension [35]. Few methods are available for the simultaneous analysis of OLM and AML combination. These methods include spectrophotometry [35–42], HPLC [36, 43, 44] and TLC [44]. These methods suffered from lower sensitivity and selectivity (e.g. UV-based spectrophotometry), employed intensive instrumentation (e.g. HPLC) or need laborious manipulation (e.g. TLC).

Spectrofluorimetric technique is characterized by its inherent high sensitivity, improved selectivity, practical simplicity, and wide availability of in quality control laboratories. However, to the best of our knowledge, based on extensive literature survey, no attempt has yet been made to employ spectrofluorimetry for the simultaneous determination of OLM and AML. Moreover, the reported techniques analyzed only one ratio for this combination (4:1), while FDA approved other two ratios (2:1 and 8:1). Therefore, the aim of this work was directed to develop simple, sensitive and selective spectrofluorimetric methods for the simultaneous determination of OLM and AML in their combined dosage form in all FDA approved ratios. At first glance, OLM and AML binary mixture appeared to be simple mixture that can be easily resolved by classical univariate methods such as direct and isoabsorpitive methods. However, experimental analysis revealed difficulties in resolving such mixture by these methods. Hence multivariate calibration methods such as PLS and ANN could be methods of choice.

Experimental

Apparatus

Fluorescence measurements were carried out on a RF-3501 version 3.0 spectrofluorimeter (Shimadzu Corporation Kyoto, Japan) equipped with a 150 W xenon lamp and 1 cm quartz cells. The slit widths for both the excitation and emission monochromators were set at 5.0 nm. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (0.01 μg mL^{-1}). Wavelength calibration was performed by measuring λ_{exc} at 275 nm and λ_{em} at 430 nm; no variation in the wavelength was observed. All recorded spectra converted to ASCII format by RFPC software. Hanna pH-Meter (Romania) was used for pH adjustments.

Software

All multivariate calibration methods were implemented in Matlab® 7.1.0.246 (R14). PLS, GA-PLS, GA-ANN and PC-ANN were carried out by using PLS toolbox software version 2.1 in conjunction with Neural Network toolbox. The t test, *F* test and ANOVA test were performed using Microsoft® Excel. All calculations were performed using intel ®core ™ i5-2400, 3.10 GHz, 4.00 GB of RAM under Microsoft Windows 7.

Materials

OLM was obtained from AK Scientific Inc. (CA, USA). AML was obtained from Pfizer Inc. (New York, USA). The purities of OLM and AML were 99.5 %. Olmesar® tablets (Macleods Pharmaceutical Ltd., Mumbai, India) labeled to contain 5 mg of AML and 20 mg of OLM (Batch No: PM00058803). Double distilled water was obtained through WSC-85 water purification system (Hamilton Laboratory Glass Ltd., KY, USA) and used throughout the work. A phosphate buffer solution (pH 5, 0.1 M) was employed for pH adjustment. All solvents and materials used throughout this study were of analytical grade.

Preparation of OLM and AML Standard Solutions

Stock solutions of OLM (200 μg mL^{-1}) and AML (200 μg mL^{-1}) were prepared separately by dissolving 20 mg of each of OLM and AML in 100 mL methanol. Appropriate volumes of these stock solutions were diluted with distilled water to give working solutions of 40 and 10 μg mL^{-1} for OLM and AML, respectively. Stock and working solutions were stable for at least 2 weeks when stored refrigerated at 4 °C.

Preparation of Pharmaceutical Tablets Sample Solutions

Olmesar® tablets were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 40 mg of OLM and 10 mg of AML was extracted into methanol with the aid of shaking and the methanolic extract was filtered. The filtrate was diluted with methanol to obtain final concentrations of 200 and 50 μg mL^{-1} for OLM and AML, respectively. Aliquots of Olmesar® tablet solution were diluted with water to obtain working solution of 40 and 10 μg mL^{-1} for OLM and AML, respectively. To prepare tablet extract containing 40 μg of OLM and 20 μg of AML (2:1), 80 μg of OLM and 10 μg of AML (8:1), standard solutions of AML and OLM were added respectively to Olmesar® tablets. Spectral acquisition and the calculations were performed in the same manner as described in "Calibration Procedures".

Calibration Procedures

– Univariate calibration procedures

Aliquots of standard working solutions equivalent to 0.4–3.2 μg mL^{-1} of OLM and AML were transferred into a series of 5-mL volumetric flasks. 2 mL of 0.1 M phosphate buffer of pH 5.0 was added and the solutions were diluted to the volume with water and mixed well. Fluorescence spectra of the solutions were recorded from 220 to 600 nm using λ_{exc} at 251 nm and stored in the computer. Fluorescence Intensity (FI) of emission spectra was measured at 375 nm and 431 nm (isoabsorpitive point) for determination of OLM and total concentration of OLM and AML respectively (AML can be determined by subtraction). The calibration graphs were constructed by relating the FI at mentioned wavelengths to the corresponding concentrations of OLM and AML (or OLM) respectively. The regression equations for the data were computed.

– Multivariate calibration procedures

Five level, two factor calibration design [45] was used for construction of 25 samples by transferring different volumes of OLM and AML from their standard working solutions into 5-mL volumetric flasks then 2 mL of phosphate buffer (pH5.0, 0.1 M) was added and the solutions were diluted to the volume with water and mixed well (Table 1). The last 15 samples were used to build the multivariate calibration models (training set) while the first 10 samples were used to test the predictive ability of the proposed models (validation set). Concentrations chosen for each compound in the 25 samples were based on; calibration range of each of the two drugs and the ratio of OLM to AML in the pharmaceutical product approved by FDA (8:1, 4:1 and 2:1).

The emission spectra of the 25 samples were scanned from 300 to 600 nm using λ_{exc} at 251 nm and stored in the computer. The 2D Scores plot for the first two PCs of the whole concentration matrix was obtained to confirm the well position of the mixtures in space, orthogonality, symmetry and rotatability [45] as indicated in Fig. 2. Mean centring of the data proved to be the best preprocessing method for getting the optimum results.

Overview for Multivariate Calibration Methods

– Partial least squares regression (PLS)

PLS method involves the decomposition of the experimental data, such as spectrofluorimetric data in our case, into systematic variations (latent variables) that explain the observed variance in data. The purpose of PLS method is to build a calibration model between the concentration of the analytes under study (OLM and AML in our case) and the latent variables of the data matrix. PLS performs the decomposition using both spectrum data matrix and analyte concentration [46].

Including extra latent variables in the model increases the possibility of the known problem of overfitting. Therefore

Table 1 The 5 level 2 factor experimental design of the training and validation set mixtures shown as concentrations of the mixture components in μg mL^{-1}

Mix No.	OLM	AML	Mix No.	OLM	AML
1	3.2	0.4	14	2.4	1
2	3.2	0.6	15	2.4	1.2
3	3.2	0.8	16	2	0.4
4	3.2	1	17	2	0.6
5	3.2	1.2	18	2	0.8
6	2.8	0.4	19	2	1
7	2.8	0.6	20	2	1.2
8	2.8	0.8	21	1.6	0.4
9	2.8	1	22	1.6	0.6
10	2.8	1.2	23	1.6	0.8
11	2.4	0.4	24	1.6	1
12	2.4	0.6	25	1.6	1.2
13	2.4	0.8			

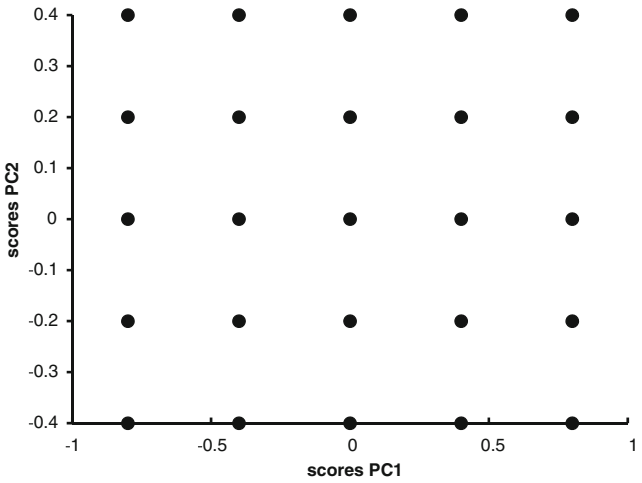


Fig. 2 Scores plot for the mean centred 25 samples concentration matrix of the five level two component experimental design

optimization of number of the latent variables is a critical issue in PLS method

- Optimisation of number of latent variables for PLS model

Cross validation (CV) [46] was applied to predict how many are the optimum number of PLS latent variables. CV involves repeatedly dividing the data into two sets, a training set used to determine a model and a test set to determine how well the model performs so that each sample (or portion of the data) is left out of the training set once only.

Leave one out (LOO) CV is used in our study for optimizing the number of PLS components, by building the model using I-1 samples set (training set consisting of 14 samples) to predict the one sample left (validation sample). The root mean square error of CV (RMSECV) is calculated as

$$\mathrm{RMSECV} = \sqrt{\frac{1}{I}\sum_{i=1}^{I}\left(c_i - \hat{c}_{i\_cv}^{A}\right)^2}$$

where I is the number of objects in the calibration set, c_i is the known concentration for sample i and $\hat{c}_{i\_cv}^{A}$ is the predicted concentration of sample i using A components. Mean centring was performed on the training set each time successive samples were left out.

– Genetic Algorithm

Genetic algorithms (GA) [47–50] have been used to solve difficult problems with objective functions that do not possess 'nice' properties such as continuity, differentiability… etc. These algorithms maintain and manipulate a family, or population, of solutions and implement a 'survival of fittest' strategy in their search for better solutions.

GA searches the solution space of a function through the use of simulated evolution, i.e. the survival of the fittest strategy. GA have been shown to solve the optimization problem by exploring all regions of the potential solutions and exponentially exploiting promising area through mutation, crossover and selection operation applied to individuals in the populations. A complete discussion of genetic algorithms can be found in the literature [50–52].

GA can be used successfully for wavelength selection. GA consists of five steps: A-Initiation: different combinations of wavelengths are generated randomly; each combination represents a possible solution. Each wavelength in the spectrum is assigned randomly a value of 1 or 0, where 1 indicates selection and 0 indicates omitting. B- Evaluation: each different chromosome is used to construct the model and cross validation is used to evaluate the prediction error of each chromosome. C-Exploitation: selection of good chromosomes. D- Exploration: recombination of good genes. E-Mutation: changing chromosomes locally to hopefully form better chromosomes. The new chromosomes produced are tested again for performance and the algorithm continues until a certain number of generations are produced.

- Optimisation of parameters of Genetic Algorithm

A critical issue of successful GA performance is the adjustment of GA parameters. The parameters are: the maximum number of generations, the number of wavelengths in a window, percent genes included at initiation, the mutation rate, breeding cross over rule and percent of population the same at convergence. Other parameters to be chosen by the user are: maximum number of latent variables for the PLS, cross validation type random or contiguous blocks, number of subsets to divide data into for cross validation, number of iterations for cross validation at each generation. The configuration of GA parameters was shown in Table 2.

– Neural networks

Artificial neural network (ANN) is a type of artificial intelligence method that resembles biological nervous system

Table 2 Optimum parameters of the genetic algorithms GA

Parameter	Value
Population size	20
Maximum generations	50
Mutation rate	0.005
The number of variables in a window (window width)	2
Per cent of population the same at convergence	100
% wavelengths used at initiation	50
Crossover type	Single
Maximum number of latent variables	2
Number of subsets to divide data into for cross validation	4
number of iterations for cross validation at each generation	2

in having the ability to find the relationship between inputs and outputs. ANN is composed of elements called artificial neurons that are interconnected by connections called weights. Commonly neural networks are trained, so that a particular input leads to a specific target output. The network is adjusted, based on a comparison of the output and the target, until the network output matches the target. Typically many input/target pairs are used to train a network [53].

ANN has a great advantage over other traditional multivariate methods in modeling linear and non-linear relationship between variables. There are many papers that describe the application of ANN on linear and non-linear data [54, 55].

The type of ANN used in this paper is feed-forward model which was trained with the back propagation of errors learning algorithm. The back-propagation ANN is used in signal processing, data reduction and optimization, interpretation and prediction of spectra and calibration [55]. It is composed of three layers, an input layer in which the input data are introduced (e.g. fluorescence intensities in spectrofluorometry). These inputs are passed to second hidden layer in which inputs are corrected and adjusted by weights and then finally passed to outer most layer (output layer) to give outputs (e.g. concentrations). The connections (weights) between layers are passed forward (from input to output layer), so it is called feed-forward ANN. The outputs (predicted concentrations) are compared with targets (actual concentrations) and the difference between them is called the error which is back propagated (and so called feed-forward ANN with the back propagation of errors learning algorithm) to network once more to be minimized through further adjustment of weights. ANN is iterated several times in such way till the error reaches a minimum value.

In this paper, ANN models preceded by principal component analysis (PC-ANN) and GA (GA-ANN) as input data reduction procedures were applied.

- Optimization of parameters of ANN

For proper training of ANN model, several parameters have to be optimized. There are two transfer functions used in ANN, one between input and output of a node in the hidden layer and the other is applied in output layer. The use of these functions depends on relationship between the inputs and outputs. Tan sigmoid followed by purelin are commonly used for non-linear systems while purelin-purelin transfer functions are used for linear one (as in our case).

Among other ANN parameters, the hidden neurons number (HNN) is related to the converging performance of the output error function during the learning process. The learning coefficient (Lc) controls the degree at which connection weights are modified during the learning phase. The learning coefficient decrease (Lcd) and learning coefficient increase (Lci) control the variation of Lc value. It varies as a function of performance of the ANN (the Lc decreases or increases with the mean square error). For optimization of the ANN parameters, many experiments have to be done through which we can improve the model performance. Optimized ANN parameters are summarized in Table 3.

Results and Discussion

Olmesar® tablets are combined dosage form containing the angiotensin II receptor blocker OLM and the calcium channel blocker AML. It has been used in the treatment of hypertension. The ratio of OLM to AML in Olmesar® tablets is 4:1. FDA approved this combination in three ratios, 2:1, 4:1 and 8:1. So this study was designed to develop simple, robust and accurate multivariate calibration methods for the simultaneous determination of OLM and AML in all ratios approved by FDA. Because of the inherent high sensitivity, improved selectivity, practical simplicity, and wide availability of spectrofluorimetry in quality control laboratories, it was attempted in this study. Another goal of this study was to show the superiority of multivariate calibration methods over univariate calibration ones even for binary mixture that seems to be simple to be resolved as in our case study.

- Spectral characteristics and optimization of assay conditions

Both of OLM and AML exhibited native fluorescence in aqueous phosphate buffer (pH 5, 0.1 M) with $\lambda_{emission}$ at 375 and 455 nm for OLM and AML, respectively, after excitation of both drugs at 251 nm (Fig. 3). The conditions for the emission spectra of both OLM and AML were optimized.

Different experimental parameters affecting the emission spectra were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors included pH, buffer volume, type of the diluting solvent and stability time.

- Effect of pH

The influence of pH on the FI and fluorescence range of the OLM and AML was studied using phosphate buffer covering the pH range from 1.0 to 7.0 only because at basic pH, OLM hydrolysed (as a prodrug) to the olmesartan moiety that did not exhibit fluorescence [8]. pH had no effect on AML emission spectra. On the other hand OLM emission spectra changed versus pH range. Sequentially two different emission wavelengths were observed for OLM over pH ranges from 1.0 to 7.0. Maximum excitation and emission wavelengths were 251 and 417 nm in the pH 1.0, 251 and 375 nm in the pH range 1.5 to 7.0, the fluorescence intensity of OLM did not change from pH 1.0 to 3.0 and decreased markedly from pH 3.0 to 6.0 and ceased totally at pH 7.0. This behavior at neutral and basic pH was attributed

Table 3 Optimized parameters of ANN

Method	GA-ANN		PC-ANN	
Drugs	OLM	AML	OLM	AML
Architecture	67-4-1	18-2-1	2-1-1	2-1-1
Hidden neurons number	4	2	1	1
Transfer Functions	Purelin-purelin	Purelin-purelin	Purelin-purelin	Purelin-purelin
Learning coefficient	0.001	0.001	0.001	0.1
Learning coefficient decrease	0.1	0.1	0.1	0.001
Learning coefficient increase	10	10	10	100

to its hydrolysis (as a prodrug) to the olmesartan moiety that did not exhibit fluorescence [8] (Fig. 4). Therefore, phosphate buffer of pH 5.0 was used throughout the study to allow the incorporation of all ratios of OLM and AML especially 8:1.

- Effect of buffer volume

The effect of phosphate buffer volume on the fluorescence intensity of OLM was studied. It was clear from Fig. 5 that 2 mL of phosphate buffer was sufficient to reach maximum FI for OLM.

- Effect of diluting solvent

Dilution with different solvents including water, methanol, ethanol, and acetonitrile was employed. Among the all tested solvents water gave the highest FI compared with the other solvents (Fig. 6). This may be attributed to change in the medium polarity that may result in physical interaction between these solvents and the excited singlet state of the drug molecules Thus, water was chosen as the diluting solvent throughout the study.

- Effect of time

The effect of time on the stability of the fluorescence intensity of the drugs was also studied. It was found that the FI developed instanteously and remained stable for at least 1 hour (Fig. 7).

– Univariate calibration methods

By examining emission spectra of both OLM and AML, it was clear that OLM can be determined directly by measuring FI at λ_{em} of 375 where AML did not interfere (Fig. 3). For AML, isoabsorptive method was applied for determination of total concentration of OLM and AML by measuring FI at $\lambda_{emission}$ 431 nm (Fig. 8) and by subtraction, AML can be determined solely. The calibration graphs were constructed by relating the FI at mentioned wavelengths to corresponding drugs concentrations and the regression equations were:

$$\text{ForOLM}: FI_{375} = 220.25C_1 + 38.14 (r = 0.9980)$$

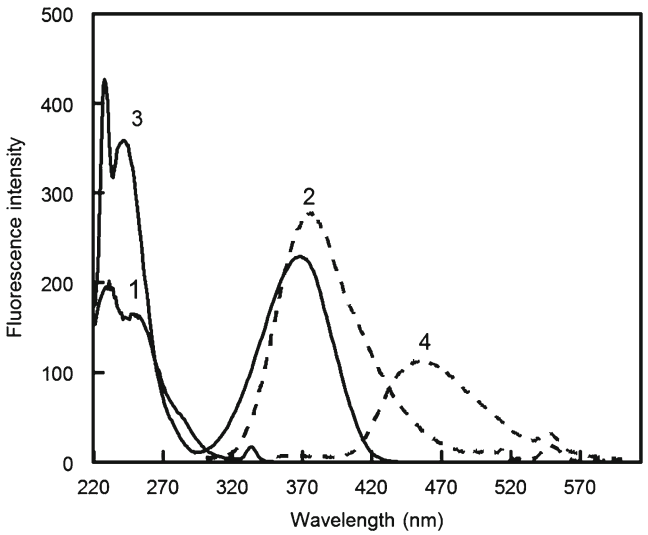


Fig. 3 Excitation (*solid lines*) 1 and emission (*dashed lines*) for OLM (1 and 2) and AML (3 and 4). Concentrations of both OLM and AML were 1 μg mL^{-1} in phosphate buffer solution (pH 5, 0.1 M)

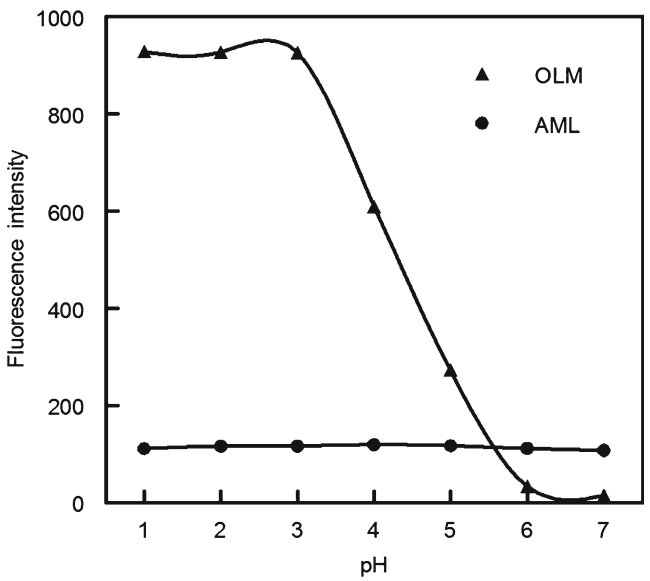


Fig. 4 Effect of pH on FI of OLM (1 μg mL^{-1}) and AML (1 μg mL^{-1})

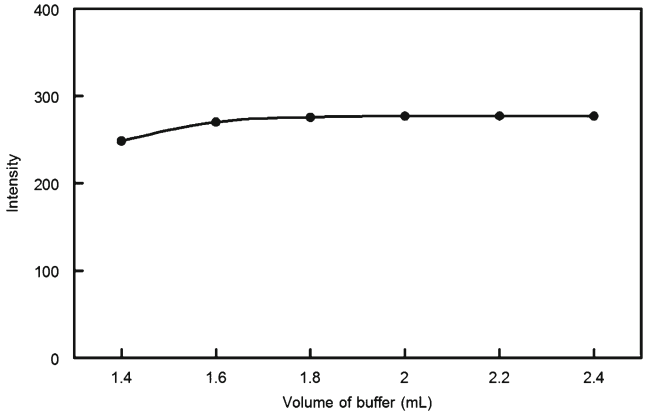


Fig. 5 Effect of volume of phosphate buffer on FI of OLM (1 μg mL^{-1})

$$\text{ForAML}: FI_{431} = 61.04C_2 + 29.40 (r = 0.9995)$$

where FI was fluorescence intensity of the emission spectra, C_1 and C_2 were the concentrations of OLM and AML + OLM in μg mL^{-1}, respectively, and r was the correlation coefficient. To assess the specificity of the proposed univariate calibration methods, OLM and AML were determined in laboratory prepared mixtures containing different ratios of the two drugs. It was obvious that on increasing concentration of AML in the mixtures, recovery % of OLM decreased (Table 5). This may be attributed to the self absorption of emission radiation of mixture at 375 nm by AML because AML had maximum excitation at 360 nm (post inner filter effect). For determination of AML, FI was measured at isoabsorpitive point (431 nm) to determine total concentration of OLM and AML then by subtraction, AML was determined solely. It was noticed that on decreasing total concentration of two drugs, slight shift occurred at

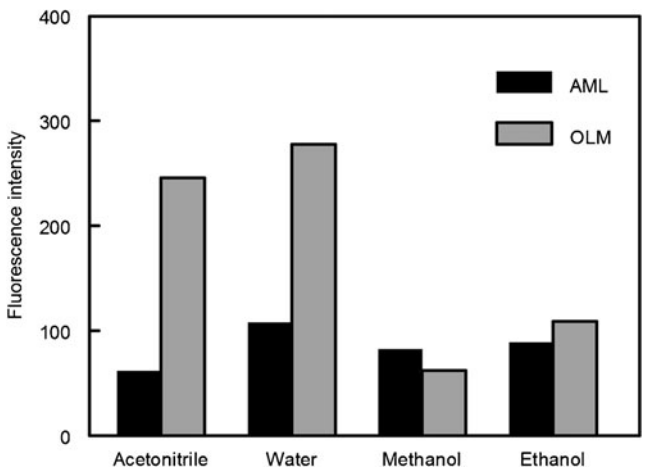


Fig. 6 Effect of the type of diluting solvents on FI of OLM (1 μg mL^{-1}) and AML (1 μg mL^{-1}) in phosphate buffer solution (pH 5, 0.1 M)

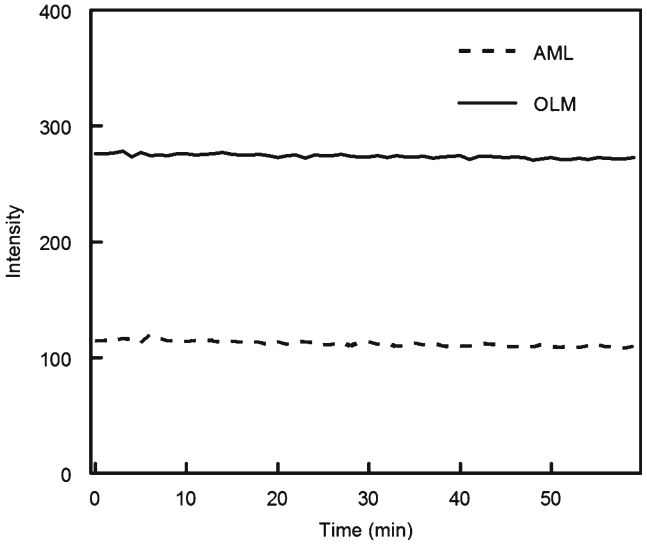


Fig. 7 Effect of time on FI of OLM (1 μg mL^{-1}) and AML (1 μg mL^{-1})

isoabsorpitive point from 431 nm to 432 nm as depicited in Fig. 8. This shift affected the recovery % of total concentration and hence AML so AML recovery % was not good (105.66 % ±2.326) as shown in Table 5. From above discussion, it was clear that univariate methods had limitations that led us to analyze this mixture by multivariate calibration methods.

– Multivariate calibration methods

Four chemometric methods – PLS, GA-PLS, GA-ANN and PC-ANN– were applied for the simultaneous determination of OLM and AML in combined dosage form in all ratios approved by FDA. In general, this study was primarily designed to present the proposed multivariate methods as attractive alternatives for classical univariate calibration methods in handling fluorescence spectral data. Secondary,

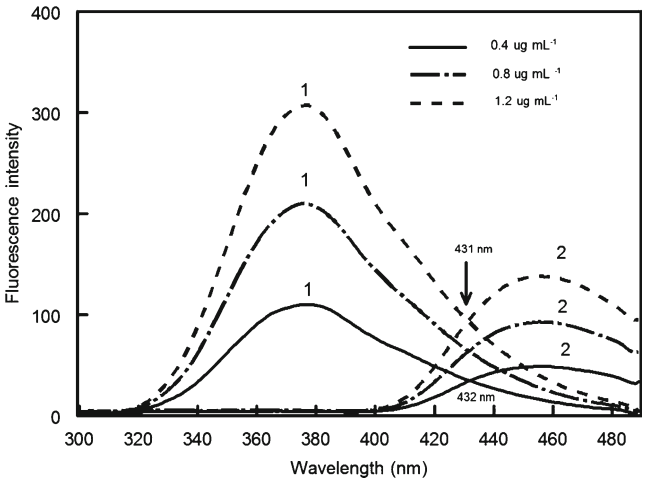


Fig. 8 Emission spectra of 0.4 μg mL^{-1}, 0.8 μg mL^{-1} and 1.2 μg mL^{-1} of AML and OLM showing isoabsorptive points at 432, 431.5 and 431 nm respectively

Table 4 Analysis results for the prediction of the training set by the proposed multivariate calibration methods

Method		PLS				GA-PLS				GA-ANN				PC-ANN			
OLM	AML	OLM		AML		OLM		AML		OLM		AML		OLM		AML	
True (μg ml^{-1})		Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%	Found (μg ml^{-1})	R%
2.4	0.4	2.4	100.00	0.41	102.50	2.4	100.00	0.41	102.50	2.4	100.00	0.4	100.00	2.41	100.42	0.41	102.5
2.4	0.6	2.4	100.00	0.6	100.00	2.39	99.58	0.6	100.00	2.4	100.00	0.59	98.33	2.4	100	0.6	100
2.4	0.8	2.41	100.42	0.8	100.00	2.41	100.42	0.8	100.00	2.41	100.42	0.79	98.75	2.42	100.83	0.8	100
2.4	1	2.4	100.00	1.01	101.00	2.4	100.00	1.01	101.00	2.39	99.58	0.99	99.00	2.4	100.00	1.01	101
2.4	1.2	2.41	100.42	1.19	99.17	2.41	100.42	1.2	100.00	2.4	100.00	1.19	99.17	2.41	100.42	1.19	99.17
2	0.4	1.98	99.00	0.39	97.50	1.98	99.00	0.4	100.00	2.05	102.50	0.4	100.00	1.98	99.00	0.39	97.5
2	0.6	2.02	101.00	0.61	101.67	2.02	101.00	0.6	100.00	2.03	101.50	0.59	98.33	2.02	101.00	0.61	101.67
2	0.8	1.98	99.00	0.78	97.50	1.98	99.00	0.79	98.75	1.98	99.00	0.79	98.75	1.99	99.50	0.78	97.5
2	1	1.99	99.50	0.99	99.00	1.99	99.50	1	100.00	2	100.00	0.99	99.00	1.99	99.50	1	100
2	1.2	1.98	99.00	1.2	100.00	1.98	99.00	1.2	100.00	2.01	100.50	1.18	98.33	1.98	99.00	1.19	99.17
1.6	0.4	1.63	101.88	0.4	100.00	1.63	101.88	0.4	100.00	1.6	100.00	0.4	100.00	1.63	101.88	0.4	100
1.6	0.6	1.58	98.75	0.6	100.00	1.59	99.38	0.6	100.00	1.6	100.00	0.59	98.33	1.58	98.75	0.6	100
1.6	0.8	1.59	99.38	0.79	98.75	1.59	99.38	0.8	100.00	1.61	100.63	0.79	98.75	1.59	99.38	0.79	98.75
1.6	1	1.6	100.00	1.01	101.00	1.6	100.00	1.01	101.00	1.6	100.00	0.99	99.00	1.6	100.00	1.02	102
1.6	1.2	1.62	101.25	1.21	100.83	1.62	101.25	1.2	100.00	1.61	100.63	1.18	98.33	1.62	101.25	1.22	101.67
Mean (%)			99.97		99.93		99.99		100.22		100.32		98.94		100.06		100.06
S.D			0.912		1.399		0.872		0.807		0.819		0.621		0.91		1.52

Table 5 Analysis results for the prediction of the independent validation test set by all the proposed methods

Method		Univariate calibration methods		Multivariate calibration methods							
		Direct	Isoabsorptive	PLS		GA-PLSR		GA-ANN		PC-ANN	
OLM (μg ml^{-1})	AML (μg ml^{-1})	OLM R%	AML R%	OLM R%	AML R%	OLM R%	AML R%	OLM R%	AML R%	OLM R%	AML R%
3.2	0.4	98.36	104.32	99.38	105.00	99.38	105.00	99.06	102.50	99.69	105
3.2	0.6	96.48	104.92	99.06	101.67	99.06	101.67	99.06	103.33	99.38	101.67
3.2	0.8	95.82	107.89	99.06	101.25	99.06	101.25	100.00	100.00	99.38	101.25
3.2	1	95.16	108.24	99.06	102.00	99.06	102.00	99.06	103.00	99.38	101
3.2	1.2	92.4	104.36	96.88	99.17	96.56	98.33	97.19	100.00	96.88	98.33
2.8	0.4	99.1	105.2	100.00	107.50	99.64	105.00	99.29	102.50	100	105
2.8	0.6	100.01	110.7	101.43	103.33	101.43	103.33	102.14	105.00	101.43	103.33
2.8	0.8	97.01	99.88	98.93	98.75	98.93	98.75	98.57	98.75	99.29	98.75
2.8	1	94.35	104.9	98.93	98.00	98.93	98.00	99.64	100.00	98.93	98
2.8	1.2	94.95	105.74	99.29	98.33	99.29	99.17	99.29	100.00	99.64	98.33
Mean (%)		96.36	105.66	99.20	101.50	99.13	101.25	99.33	101.51	99.4	101.07
S.D		2.326	2.888	1.118	3.113	1.170	2.643	1.239	2.011	1.120	2.710

comparing variable selection procedure (GA) versus data compression procedure (PC) and their effect on increasing predictive power of PLS and ANN models. Ultimately, analyzing OLM and AML pharmaceutical preparations in all FDA approved ratios.

- Optimization of parameters of multivariate calibration models

The purpose of multivariate methods is to build a calibration model between the concentration of the analytes under study (OLM and AML in our case) and the experimental data (FI in our case). The first step in model building, involves constructing the calibration matrix for the binary mixture. In this study calibration set was optimized with the aid of the five level two factor design [45] resulting in 25 sample mixtures. Table 1 show the composition of the 25 sample mixtures. Upon designing the calibration set, ratios of OLM to AML in combined dosage form that approved by FDA has been taken into account as in mixture number 1 (8:1), mixtures number 3, 12, and 21 (4:1) and mixtures number 15, 19 and 23 (2:1). These 25 sample mixtures were splitted to 15 training mixtures (for building the models) and 10 validation mixtures (for measuring predictive power of the models).

For PLS model, calibration was done by performing the decomposition of experimental data matrix into latent variables using both FI data matrix and analytes concentration matrix [46].

The emission spectra of these mixtures were collected and examined; the near zero FI in the regions; above 500 nm for both analytes and 300–400 nm for AML accounted for the rejection of these parts from the emission spectra. After manipulation of data matrices, PLS method was run for optimizing the number of latent variables using leave one out (LOO) CV and RMSECV was calculated as mentioned above. The selection of the optimum number of latent

Table 6 Comparison of different error estimates of the four multivariate calibration methods

Method	PLSR		GA-PLSR		GA-ANN		PC-ANN	
Drugs	OLM	AML	OLM	AML	OLM	AML	OLM	AML
RMSEC (μg mL^{-1})	0.0145	0.0094	0.0149	0.054	0.0169	0.0110	0.0159	0.0113
Parameters for RMSEC calculation	Comps=2	Comps=2	Comps=2	Comps=2	Stated in Tables 2 and 3	Stated in Tables 2 and 3	Stated in Tables 2 and 3	Stated in Tables 2 and 3
RMSEP (μg mL^{-1})	0.0411	0.0182	0.0437	0.0167	0.0411	0.0158	0.0378	0.0167
Parameters for RMSEP calculation	Comps=2	Comps=2	Comps=2	Comps=2	Stated in Tables 2 and 3	Stated in Tables 2 and 3	Stated in Tables 2 and 3	Stated in Tables 2 and 3
RMSECV (μg mL^{-1})[a]	0.0186	0.0114	0.0192	0.0065	–	–	–	–
Parameters for RMSECV calculation	Comps=2	Comps=2	Comps=2	Comps=2	–	–	–	–

[a] Each of the two drugs is handled separately in all the models (i.e. a single concentration vector is used at a time)

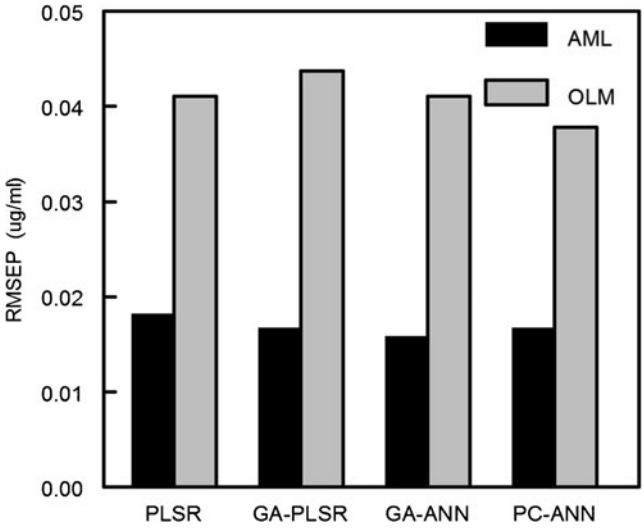


Fig. 9 Bar plots for comparison of the RMSEP values obtained by application of the proposed multivariate calibration methods for the analysis of validation set

variables was a very important pre-construction step: if the number of factors retained was more than required, more noise would be added to the data; if the number retained was too small, meaningful data that could be necessary for the calibration might be discarded. Optimum number of latent variables for PLS model was two (Table 6).

In GA-PLS method, the same procedures were applied for construction of the model but PLS model was preceded by GA procedure as variable selection to choose most correlated wavelengths to the concentrations of the analytes. A critical issue of successful GA performance is the adjustment of GA parameters. Optimum parameters for genetic algorithm were summarized in Table 2. The fitness values were used as response variables for adjustment of these parameters. The GA was run for emission spectra using a PLS with maximum number of latent variables allowed is the optimal number of components determined by cross-validation on the model. FI matrix was reduced to about one third for OLM and one fourth for AML (67 nm for OLM and 18 nm for AML).The chosen wavelengths for OLM are 302–303, 314–319, 326–331, 334–335, 338–341, 350–353, 358–359, 366–367, 390–391, 394–95, 404–405, 410–411, 414–417, 420–421, 424–427, 430–431, 440–443, 452–453, 460–463, 468–471, 474–475, 478–479, 482 (totally, 67 nm) while that for AML are 416–423, 426–427, 436–437, 440–441, 444–445, 474–475 (totally, 18 nm).

The third and fourth methods depended on ANN approach. Since the large number of nodes in the input layer of the network (i.e. the number of wavelength readings for each mixture) increases the CPU time for ANN modeling, the FI matrix was reduced either by genetic algorithm (variable selection procedure) to one third for OLM and one fourth for AML (as in GA-PLS) or principal component analysis (PCA) (variable compression procedure) to two principal components. Thus two ANNs (GA-ANN and PC-ANN)) were applied in our work. The output layer is the concentration matrix of OLM or AML. The hidden layer consists of just single layer which has been considered sufficient to solve similar or more complex problems. Moreover, more hidden layers may cause overfitting [54]. For proper modeling of ANNs, different parameters should be optimized. These parameters are summarized in Table 3. From the most important parameters that should be optimized carefully, transfer function pair. Choosing of transfer function depends on the nature of data you work on. In our case, purelin-purelin transfer function was implemented in our models due to linear correlation between FI and concentrations.

After optimization of parameters and architectures of the two ANNs, the training step is proceeded. ANNs were trained by different training functions and there is no difference in performance (i.e. there is no decrease in root mean square error of prediction (RMSEP)). Levenberg–Marquardt training algorithm (TRAINLM) was thus preferred as it is time saving. To avoid overfitting, the validation set was

Table 7 Analysis results for the prediction of the dosage form by the proposed multivariate calibration methods

Dosge form				PLSR		GA-PLSR		GA-ANN		PC-ANN	
Ratio		True conc. (μg ml^{-1})		OLM	AML	OLM	AML	OLM	AML	OLM	AML
OLM	AML	OLM	AML	R%	R%	R%	R%	R%	R%	R%	R%
4:1		1.6	0.4	101.13	101.02	99.66	100.59	99.50	101.38	101.04	100.75
		2.4	0.6	99.47	98.427	97.64	99.156	99.44	98.98	99.58	99.01
		3.2	0.8	99.25	100.51	98.49	100.31	98.75	100.92	99.38	100.38
2:1[a]		1.6	0.8	101.30	98.84	100.65	102.02	100.75	102.19	100.64	102.13
8:1[a]		3.2	0.4	98.31	99.14	98.60	100.09	99.00	100.09	99.71	99.58
Mean				99.89	99.59	99.01	100.43	99.14	100.37	100.07	100.71
S.D				1.285	1.119	1.165	1.038	0.319	1.195	0.727	1.231

[a] These ratios are synthetically prepared by addition of AML and OLM standards respectively to OLMESAR ® tablet extract

Table 8 Statistical comparison of the results obtained by proposed chemometric methods and the reference method [42] for the analysis of OLMESAR® tablets (Batch No. PM00058803)

Parameters	PLSR		GA-PLSR		GA-ANN		PC-ANN		Reference Method[a]	
	OLM	AML	OLM	AML	OLM	AML	OLM	AML	OLM	AML
R%	101.13	101.02	99.66	100.59	99.50	100.75	101.04	101.38	99.14	98.25
	99.47	98.43	97.64	99.156	99.44	99.01	99.58	98.98	100.35	99.71
	99.25	100.51	98.49	100.31	98.75	100.38	99.38	100.92	100.35	100.78
	101.30	98.84	100.65	102.02	99.01	102.13	100.64	102.19	98.38	100.29
	98.31	99.14	98.60	100.09	99.00	99.58	99.71	100.09	98.70	98.64
Mean (%)	99.89	99.59	99.01	100.43	99.14	100.37	100.07	100.71	99.38	99.53
S.D	1.285	1.119	1.165	1.038	0.319	1.195	0.727	1.231	0.922	1.073
Variance	1.652	1.252	1.357	1.077	0.102	1.429	0.528	1.515	0.850	1.151
Number of samples	5	5	5	5	5	5	5	5	5	5
Student's *t* statistic	0.718	0.077	0.566	1.347	0.193	1.164	1.307	1.613	–	–
F ratio	1.942	1.088	1.596	1.069	1.431	1.242	1.611	1.317		
ANOVA	1.552	0.882	1.552	0.882	1.552	0.882	1.552	0.882		

For $p=0.05$ and 4 ° of freedom the critical values of t and F are 2.306 and 6.388 respectively and F critical for Anova: single factor, 3 ° of freedom is 3.239

[a] Reference method is that published in the literature [42]

involoved in training step and ANNs stops when RMSEP of calibration set decreased and that of independent set increased.

- Prediction ability of the multivariate calibration models

After optimization of parameters and calibration (training) step, all models were applied for analysis of OLM and AML in training set (Table 4) and in validation set (Table 5). RMSEC, RMSEP and RMSCEV were calculated (Table 6). RMSEC and RMSEP were calculated as same manner as RMSECV but for calibration and validation set respectively. RMSEP was used as a measure for performance of the proposed models (Fig. 9) showing that the four methods predicted OLM and AML successively in their binary mixtures. However GA-ANN was the efficient one for AML determination as indicated by decreasing S.D of AML results in validation set (Table 5).

The above mentioned models were applied for analysis of OLM and AML in their pharmaceutical preparations in all ratios that approved by FDA. But because OLMESAR tablets (which contained 4 :1) were only available in our hand, OLM and AML standard solutions were added to tablets extract to prepare the other two ratios (8:1 and 2:1). It was clear from Table 7 that all models were accurate and precise for both OLM and AML determination. The results of the proposed methods were statistically compared to the reference spectroscopic method [42] (Table 8). The t and F values were computed and generally found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision. The t test results indicate no significant difference between the mean of the proposed method and the reference method. The F test reflects no significant difference between the variance of the selected methods and the reference one. Also ANOVA test was computed indicating that there is no significant difference between the four multivariate calibration methods.

Conclusion

Different calibration models have been applied for spectrofluorimetric determination of OLM and AML in their binary mixtures. This paper showed the superiority of the multivariate calibration over univariate calibration in handling such spectrofluorimetric data. Univariate calibration methods applied in this study were direct and isoabsorpitive methods for determination of OLM and AML respectively whereas, the multivariate methods assayed were: PLS, GA-PLS, GA-ANN and PC-ANN methods. These methods are considered powerful alternatives for traditional univariate methods, especially in handling spectrofluorimetric data. GA as a variable selection procedure increased predictive power of ANN particularly in estimation of AML concentration.

The applied methods combine rapidness and simplicity advantages of traditional spectrometric methods together with other important analytical merits, such as sensitivity and specificity. The suggested methods were validated and can be applied for routine quality control analysis of OLM and AML in their combined dosage forms in all FDA approved ratios without prior separation or interference from impurities/excipients.

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References

1. Koga K, Yamagishi S, Takeuchi M, Inagaki Y, Amano S, Okamoto T, Saga T, Makita Z, Yoshizuka M (2002) CS-866, a new angiotensin II type 1 receptor antagonist, ameliorates glomerular anionic site loss and prevents progression of diabetic nephropathy in Otsuka Long-Evans Tokushima fatty rats. Mol Med Camb MA NY 8(10):591–599
2. Schwocho LR, Masonson HN (2001) Pharmacokinetics of CS-866, a new angiotensin II receptor blocker, in healthy subjects. J Clin Pharmacol 41(5):515–527
3. Celebier M, Altinoz S (2007) Determination of olmesartan medoxomil in tablets by UV-Vis spectrophotometry. Pharm Int J Pharm Sci 62 (6):419–422
4. Gowrishankar D, Sharma T, Si S (2010) Difference spectrophotometric method for the determination of olmesartan medoxomil in tablets. J Pharm Res 3(7)
5. Patel J, Kevin G, Patel A, Raval M, Sheth N (2011) Development of the UV spectrophotometric method of Olmesartan medoxomil in bulk drug and pharmaceutical formulation and stress degradation studies. Pharm Methods 2(1):36
6. Rote AR, Bari PD (2009) Ratio spectra derivative and zero-crossing difference spectrophotometric determination of olmesartan medoxomil and hydrochlorothiazide in combined pharmaceutical dosage form. AAPS PharmSciTech 10(4):1200–1205. doi:10.1208/s12249-009-9318-y
7. Rote AR, Bari PD (2010) Spectrophotometric estimation of olmesartan medoxomil and hydrochlorothiazide in tablet. Indian J Pharm Sci 72 (1):111–113. doi:10.4103/0250-474X.62245
8. Farouk M, EL-Aziz OA, Hemdanb A, Shehata M (2011) Simple novel spectrophotometric and spectrofluorimetric methods for determination of some anti-hypertensive drugs. J Am Sci 7(1)
9. Hemke A, Bhure M, Chouhan K, Gupta K, Wadodkar S (2010) UV Spectrophotometric determination of hydrochlorothiazide and olmesartan medoxomil in pharmaceutical formulation. E-J Che 7:1156–1161
10. Shah N, Suhagia B, Shah R, Patel N (2007) Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form. Indian J Pharm Sci 69(6):834
11. Liu D, Hu P, Matsushima N, Li X, Li L, Jiang J (2007) Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. J Chromatogr B 856(1–2):190–197
12. Vaidya VV, Roy SMN, Yetal SM, Joshi SS, Parekh SA (2008) LC–MS–MS determination of olmesartan in human plasma. Chromatographia 67(1):147–150
13. Celebier M, Altinöz S (2007) Development of a CZE method for the determination of olmesartan medoxomil in tablets. Chromatographia 66(11):929–933
14. Çelebier M, Altinöz S (2009) Application of capillary electrophoresis to investigate the degradation kinetic of olmesartan medoxomil. Lat Am J Pharm 28(1):108–111
15. Murakami T, Konno H, Fukutsu N, Onodera M, Kawasaki T, Kusu F (2008) Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques. J Pharm Biomed Anal 47(3):553–559
16. Patel CV, Khandhar AP, Captain AD, Patel KT (2009) Validated absorption factor spectrophotometric and reversed-phase high-performance liquid chromatographic methods for the determination of ramipril and olmesartan medoxomil in pharmaceutical formulations. Eurasian J Anal Chem 2(3):159–171
17. Sweetman SC (2009) Martindale-the complete drug reference, 36th edn. The Pharmaceutical Press, London
18. The British Pharmacopoeia (2010) Her Majesty's Stationery Office:, London
19. Barman RK, Islam MA, Ahmed M, Ibne Wahed MI, Islam R, Khan A, Hossain MB, Rahman BM (2007) Simultaneous high-performance liquid chromatographic determination of atenolol and amlodipine in pharmaceutical-dosage form. Pak J Pharm Sci 20 (4):274–279
20. Muthu AK, Gupta TR, Sharma S, Smith AA, Manavalan R, Kannappan N (2008) Simultaneous estimation of amlodipine and atorvastatin in tablets using orthogonal function ratio spectrometry. Int J Chem Sci 6(4):2233–2241
21. Saxena C, Chturvedi S (2009) Simultaneous estimation of amlodipine besylate and benazepril hydrochloride using derivative spectroscopy. Asian J Chem 21(5):4118–4120
22. Abdel-Wadood HM, Mohamed NA, Mahmoud AM (2008) Validated spectrofluorometric methods for determination of amlodipine besylate in tablets. Spectrochim Acta A Mol Biomol Spectrosc 70(3):564–570. doi:10.1016/j.saa.2007.07.055
23. Shaalan RA, Belal TS (2010) Simultaneous spectrofluorimetric determination of amlodipine besylate and valsartan in their combined tablets. Drug Test Anal 2(10):489–493. doi:10.1002/dta.160
24. Gazy AA (2004) Determination of amlodipine besylate by adsorptive square-wave anodic stripping voltammetry on glassy carbon electrode in tablets and biological fluids. Talanta 62(3):575–582. doi:10.1016/j.talanta.2003.08.025
25. Kazemipour M, Ansari M, Mohammadi A, Beitollahi H, Ahmadi R (2009) Use of adsorptive square-wave anodic stripping voltammetry at carbon paste electrode for the determination of amlodipine besylate in pharmaceutical preparations. J Anal Chem 64(1):65–70
26. Dongre VG, Shah SB, Karmuse PP, Phadke M, Jadhav VK (2008) Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC. J Pharm Biomed Anal 46(3):583–586. doi:10.1016/j.jpba.2007.11.006
27. Joshi SJ, Karbhari PA, Bhoir SI (2009) RP-LC simultaneous determination of nebivolol hydrochloride and amlodipine besilate in bi-layer tablets. Chromatographia 70(3):557–561
28. Bahrami G, Mirzaeei S (2004) Simple and rapid HPLC method for determination of amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies. J Pharm Biomed Anal 36(1):163–168. doi:10.1016/j.jpba.2004.05.016
29. Baranda AB, Jimenez RM, Alonso RM (2004) Simultaneous determination of five 1,4-dihydropyridines in pharmaceutical formulations by high-performance liquid chromatography-amperometric detection. J Chromatogr A 1031(1–2):275–280
30. Massaroti P, Moraes L, Marchioretto M, Cassiano N, Bernasconi G, Calafatti S, Barros F, Meurer E, Pedrazzoli J (2005) Development and validation of a selective and robust LC-MS/MS method for quantifying amlodipine in human plasma. Anal Bioanal Chem 382(4):1049–1054
31. Zou Q, Zhan Y, Ge Z, Wei P, Ouyang P (2009) Liquid chromatography-mass spectrometry method for the determination of amlodipine in human plasma and its application in a bioequivalence study. Arzneim Forsch 59(8):383–391
32. Kakde R, Bawane N (2009) High-performance thin-layer chromatographic method for simultaneous analysis of metoprolol succinate and amlodipine besylate in pharmaceutical preparations. JPC J Planar Chromatogr Mod TLC 22(2):115–119
33. Fakhari AR, Nojavan S, Haghgoo S, Mohammadi A (2008) Development of a stability-indicating CE assay for the determination of amlodipine enantiomers in commercial tablets. Electrophoresis 29 (22):4583–4592. doi:10.1002/elps.200800330
34. Martinez V, Lopez JA, Alonso RM, Jimenez RM (1999) Micellar electrokinetic chromatography as a fast screening method for the

determination of 1,4-dihydropyridine calcium antagonists. J Chromatogr A 836(1):189–199
35. Mehulkumar P, Ramesh V, VinayKumar V, Srinivas R, Diwan P (2009) Simultaneous spectroscopic estimation of amlodipine besylate and olmesartan medoximil in tablet dosage form. Asian J Res Chem 2:127–132
36. Wankhede SB, Wadkar SB, Raka KC, Chitlange SS (2009) Simultaneous estimation of amlodipine besilate and olmesartan medoxomil in pharmaceutical dosage form. Indian J Pharm Sci 71(5):563–567. doi:10.4103/0250-474X.58190
37. Kardile D, Kalyane N, Thakkar T, Patel M, Moradiya R (2010) Simultaneous estimation of amlodipine besylate and olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods. J Pharm Sci Res 2(9):599–614
38. Patil P, Barge V, More H, Piswikar S (2011) Spectrophotometric method for simultaneous determination of olmesartan medoxamil and amlodipine besylate from tablet dosage form. Int J Curr Pharm Res 3(2):74–79
39. Sharma H, Jain N, Jain S (2011) Development of spectrophotometric method for quantitative estimation of amlodipine besylate, olmesartan medoxomil and hydrochlorthiazide in tablet dosage form. Pharm Anal Acta 2(126):2
40. Patil Pournima S, Chivate Niranjan D, Shinde Shubhangi S (2011) Simulteneous determination of Olmesartan medoxomil and Amlodipine besylate from tablet formulation by multiwavelength method. Int J Pharm Tech 3:267–272
41. Patil PS, Kulkarni PD, Burkul MS, More HN, Pishawikar SA (2011) Simultaneous estimation of amlodipine besylate and olmesartan medoxomil by first order derivative spectroscopy from tablet. Int J PharmTech Res 3:668–675
42. Sharma T, Mishra N, Si SC, Shankar DG (2010) Simultaneous estimation of olmesartan medoxomil and amlodipine besylate in solid dosage form by UV spectrophotometry. Pharm Lettre 2:302–307
43. Patil KR, Rane VP, Sangshetti JN, Yeole RD, Shinde DB (2010) Stability indicating LC method for the simultaneous determination of amlodipine and olmesartan in dosage form. J Chromatogr Sci 48 (7):601–606
44. Kamble AY, Mahadik MV, Khatal LD, Dhaneshwar SR (2010) Validated HPLC and HPTLC method for simultaneous quantitation of amlodipine besylate and olmesartan medoxomil in bulk drug and formulation. Anal Lett 43 (2):251–258
45. Brereton RG (1997) Multilevel multifactor designs for multivariatecalibration. Analyst 122(12):1521–1529
46. Kramer R (1998) Chemometric techniques for quantitative analysis. CRC
47. Lucasius CB, Kateman G (1993) Understanding and using genetic algorithms part 1. Concepts, properties and context. Chemom Intell Lab Syst 19(1):1–33
48. Lucasius CB, Kateman G (1994) Understanding and using genetic algorithms part 2. Representation, configuration and hybridization. Chemom Intell Lab Syst 25(2):99–145
49. Hibbert DB (1993) Genetic algorithms in chemistry. Chemom Intell Lab Syst 19(3):277–293
50. Michalewicz Z (1996) Genetic algorithms+data structures. Springer
51. Leardi R (2000) Application of genetic algorithm–PLS for feature selection in spectral data sets. J Chemom 14(5–6):643–655
52. Leardi R, Seasholtz MB, Pell RJ (2002) Variable selection for multivariate calibration using a genetic algorithm: prediction of additive concentrations in polymer films from Fourier transform-infrared spectral data. Anal Chim Acta 461(2):189–200
53. Demuth H, Beale M, Hagan M (2009) MATLAB user's guide Neural Network toolbox™ version 6. MathWorks Inc, Massahusetts
54. Khanchi AR, Mahani MK, Hajihosseini M, Maragheh MG, Chaloosi M, Bani F (2007) Simultaneous spectrophotometric determination of caffeine and theobromine in Iranian tea by artificial neural networks and its comparison with PLS. Food Chem 103(3):1062–1068
55. Abbaspour A, Baramakeh L (2006) Application of principle component analysis–artificial neural network for simultaneous determination of zirconium and hafnium in real samples. Spectrochim Acta A Mol Biomol Spectrosc 64(2):477–482