In the Name of God

Razi University

Faculty of Chemistry
Department of Analytical chemistry

M.Sc.Thesis

Title of the thesis:

Simultaneous determination of Atorvastatin and Amlodipine and Ethinylestradiol and Levonorgestrel binary mixtures in human serum and pharmaceutical formulations by Partial Least-Squares method

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March 2013

Abstract

Part 1

The partial least squares modeling (PLS) as a powerful multivariate statistical tool was applied to the spectrophotometric simultaneous determination of Atorvastatin (AT) Calcium and Amlodipine (AM) Besylate in pharmaceutical formulations. The linear concentration range for AT and AM were 0.3-40.0 mg/L, 0.1-100.0 mg/L, respectively. For simultaneous determination by PLS, 28 calibrations and 12 external test samples containing both AM and AT were prepared. The absorption spectra were recorded from 205 through 310 nm. The root-mean-square errors of predictions (RMSEP) for AM and AT were 0.169, 0.927, respectively. The proposed method was successfully applied for the determination of AM and AT in synthetic and real matrix samples .

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Part 2

Resolution of complex binary mixtures of Ethinylestradiol (ETE) and Levonorgestrel (LEV) was successfully achieved with minimum sample pre-treatment and without analyte separation. The work is based on the radial basis functions-partial least squares (RBF-PLS) analysis of UV spectral data. The calibration graphs were linear in the ranges of 0.3-30 mg/L for ETE and LEV. For calibration and external test sets, binary mixtures were rationally designed. The results for modeling and subsequent prediction in external test set samples resulted in Q^2 values of 94.1 and 99.4% for ETE and LEV, respectively. The RBF-PLS models were then successfully applied to determine the analytes in real samples consist of pharmaceutical preparations. In the analysis of the real samples, we excluded the spectral regions where the unknown interferences absorb and matrix effect exists. The mean recoveries for the real samples were between 90.6 and 106.8%. The estimated precisions of the method in terms of RSD% were in most cases below 4%.

Part 3

Radial Basis Functions-Partial Least Squares (RBF-PLS) calibration model was developed for Simultaneous Detemination of Atorvastatin Calcium and Amlodipine Besylate in pharmaceutical formulations using spectrofluorimetry data . The calibration graphs were linear in the ranges of 0.1-6 mg/L, 0.5-9 mg/L for AM and AT, respectively. The experimental calibration matrix was designed with 17 mixtures of these chemicals.The concentration were varied between calibration graph of drugs. Fluorescence data were taken between 240-600 nm. 18 synthetic sample mixtures were used for validate the proposed method. The root-mean-square errors of predictions (RMSEP) for AM and AT were 0.004, 0.067 respectively. In the analysis of the real samples, we excluded matrix effect exists. The recoveries for the real samples were very near to 100.

Part one

Simultaneous Determination of Atorvastatin Calcium and Amlodipine Besylate in pharmaceutical formulations by Chemometrics Methods

Chapter One

Introduction

1. Chemometrics

The development of the discipline chemometrics is strongly connected with the use of computers in chemistry. As clearly as in the seventies some analytical groups worked with statistical and mathematical methods that are ascribed now a days to chemometric methods. Those early investigations were connected with the use of mainframe computers. The notation chemometrics was introduced in 1972 by the Swedish Svante Wold and the American Bruce R. Kowalski [1, 2]. The foundation of the International Chemometrics Society (ICS) in 1974 led to the first description of this discipline.

Chemometrics is a chemical discipline that uses mathematical and statistical methods, to design or select optimal measurement procedures and experiments and to provide maximum chemical information by analyzing chemical data [3]. According to ICS, chemometrics is a combination of mathematical, statistical, graphical or symbolic methods to improve the understanding of chemical information [4]. Useful at any point in an analysis, from the first conception of an experiment until the data is discarded.

The data flood generated by modern analytical instrumentation is one reason that analytical chemists in particular have developed the applications of chemometrics methods. Chemometrics in analytical chemistry is a discipline that uses mathematical and statistical methods to obtain relevant information on material systems [3].

1.1. Calibration

The calibration of an analytical instrument means the construction of a quantitative relationship between, on the one hand the instrument signal as they are measured on

analytical samples, and on the other hand one or several properties of the samples, usually concentrations of analytes in the samples.

In univariate calibration (UVC) the amplitude of one signal, e.g. the adsorption of light at a certain wavelength is related to the concentration of one analyte. In multivariate calibration (MVC), several signals, for instance from a whole spectrum digitizes at a regularly spaced wavelengths, are used to derive a multivariate model (a generalized standard curve) [5]. This model simultaneously relate the amplitudes of all signals, or a substantial part of them, to the concentration of one or several analytes in the samples. The multivariate model is then used to estimate the analyte concentration in new samples from the multiple signals measured on them. The use of MVC and spectral profiles to measure concentrations or other properties is often referred to as indirect measurement in contrast to the direct measurement of, say, concentration by mean of titration, or precipitation and weighing.

In both UVC and MVC, the deviation between the model and the data are used to derive statistical measure of uncertainty of the model and of the estimated concentration of new samples.

As an example, one may think of the spectroscopic analysis of mixture in order to measure the concentration of one or more of its constituents. The goal of calibration is to replace a measurement of the property of interest by one that is cheaper, or faster, or better accessible, yet sufficiently accurate. Developing the calibration model includes stating the objective of study, designing the experiment, choosing the type of model, estimating its parameters and the final stage of assessing the precision of the predictions.

1.1.1. Multicomponent Analysis

The term multicomponent analysis is used for procedures in which several components in a sample are determined simultaneously. Over the years, we have observed

the inclusion of experiments involving analysis of two-component mixtures in undergraduate instrumental analysis laboratory manuals [6, 7].

In spectroscopic experiments, the concentrations of the components are determined by employing simultaneous equations after obtaining the absorptivity coefficients of the components at two wavelengths. Another approach uses a multiwavelengths linear regression analysis [8], which is above more effective in resolving heavily overlapped signals. Applications of the above techniques become limited when systems with three or more components are involved.

The availability of scanning instruments and spread sheets capable of performing advanced mathematics had led to parallel development in multicomponent analysis techniques, which are collectively called multivariate calibration techniques [9, 10].

With respect to the overlapped signals, chemometrics methods have provided very good results in the resolution of mixture of several components. Where these techniques are successful, they offer an advantage in simplicity over well-established separation techniques such as gas or liquid chromatography.

1.1.2. Univariate Calibration

There is a huge literature on univariate calibration [11, 12]. One of the simplest problems is to determine the concentration of a single compound using the response of a single detector, for example a single spectroscopic wavelength or a chromatographic peak area. Mathematically a series of experiments can be performed to give:

$$\mathbf{x} \approx \mathbf{c} \, . \, s$$

Where, in the simplest case, **x** is a vector consisting of absorbances at one wavelength for a number of samples (or the response), and **c** is of the corresponding concentrations. Both vectors have length *I*, equal to the number of samples. The scalar s relates these parameters and is determined by the experiments.

1.1.3. Multivariate Calibration

Multivariate calibration is the collective term used for the development of a quantitative model for the reliable prediction of properties of interest (y_1, y_2,…, y_q) from a number of predictor variables (x_1, x_2, …, x_p). However, multivariate calibration is a general selectivity and reliability enhancement tool. It is applicable to determination of major constituents as well as microcomponent and other qualities and for a very wide range of instrument types. In MVC, the multivariate information can be used for additional statistical diagnostics such as the similarity between a new sample and the calibration set, the presence of clusters in the data, and the relative information content of the signals (predictor variables).

MVC has six steps, the first five of which comprise the training phase, and the sixth is the prediction steps. These steps are:

1. Specification of the analyte with concentration ranges. Selection of the instrumental method, including the range of wavelengths, reflectance or transmission mode, etc.
2. Selection of a representative set of calibration samples- the training set or calibration set. This calibration set should span the range of analyte concentration and also the concentration ranges of interferents.
3. The multivariate signal (typically the digitzed spectra) are recorded for the calibration samples and stored in an appropriate data base. The analyte concentration is measured by a references method.
4. The data are investigated for the presence of outliers and other anomalies. Thereafter, the data are preprocessed and transformed to a form suitable for the subsequent data analysis.
5. The calibration model is developed and optimized. This includes checking for linearity, and the determination of selectivity, detection limits, precision,

accuracy, and other measure of performance. Statistical measure of uncertainty are calculated and used to construct confidence intervals for predicted values. Also, the model is interpreted chemically , important variables (wavelength regions) looked at, interferences are identified, etc.

6. The model is used to estimate the analyte concentration in new samples (prediction set), including confidence intervals. Diagnostics for dissimilarity (outliers) are checked.

MVC has a much improved precision and selectivity compared to UVC. Also MVC can handle complicated samples with unknown interfering compounds, and it works even when there is no selective wavelength region for the analyte this has made MVC particularly useful with nonselective spectral methods, such as near- infrared spectroscopy (NIR). In slightly generalized sense, MVC can be applied to relate any instrumental profile, including chromatography, kinetic curve, thermogravimetric curves, sound spectra, and any properties of the analyzed sample. The properties can be other the concentrations, for instance viscosity and molecular weight of polymer samples, the energy content of oil, gasoline, coal, or peat, or the taste of cheese, wine, or beer. Hence the MVC methodology provides exciting possibilities for the indirect measurement of complicated samples.

The other advantage of multicomponent analysis using multivariate calibration is the speed of method of determination for the components of interest in a mixture, as a separation step can be avoided.

1.1.4. Partial Least-Squares Regression (PLSR)

PLS is a quantitative spectral decomposition technique that is closely related to principal component regression (PCR). However, in PLS, the decomposition is performed in a slightly different fashion. Instead of first decomposing the spectral matrix into a set of eigenvectors and scores, and regressing them against the concentrations as a

separate step, PLS actually uses the concentration information during the decomposition process. This causes spectra containing higher constituent concentrations to be weighted more heavily than those with low concentrations. Thus ,the eigenvectors and scores calculated using PLS are quite different from those of PCR . The main idea of PLS is to get as much concentration information as possible into the few loading vectors.

In actuality , PLS is simply taking advantage of the correlation relationship that exists between the spectra data and the constituent concentrations . However PLS calibration is one of the best known regression techniques for multivariate data analysis [13, 14]. The two type of PLS calibration are PLS1 and PLS2 algorithms [15, 16].

1.1.4. 1. PLS1 Calibration

The PLS decomposition most often used in calibration is called PLS1 [17] and it performs the decomposition and regression for each compound individually. PLS is performed using nonlinear iterative partial least squares (NIPALS) algorithm [18]. It differs from in that the covariance between the spectral matrix $\mathbf{X}^{\wedge}$ and concentration vector $\mathbf{y}_K$ is used for decomposition given by:

$$\mathbf{X}^{\wedge} = \mathbf{T}_K \mathbf{P}'_K \qquad (1.2.5)$$

$$\mathbf{y}_K = \mathbf{U}_K \mathbf{q}'_k \qquad (1.2.6)$$

where $\mathbf{T}_K$ and $\mathbf{U}_k$ are the scores of matrix $\mathbf{X}$ and the concentration vector $\mathbf{y}_k$ for compound k, and $\mathbf{P}_K$ and $\mathbf{q}_K$ are the corresponding loadings. The dimensions of $\mathbf{U}_K$ and $\mathbf{q}_K$ are $i \times n$ and $1 \times n$, respectively. When both $\mathbf{X}^{\wedge}$ and $\mathbf{y}_K$ are used to estimate components, the components for the $\mathbf{X}^{\wedge}$ and $\mathbf{y}_k$ have the following relationship:

$$\mathbf{U}_{nk} = \mathbf{t}_{nk} \mathbf{b}_{nk} \qquad (1.2.7)$$

Where $\mathbf{U}_{nk}$ and $\mathbf{t}_{nk}$ represent the nth PLS component for compound k and $\mathbf{b}_{nk}$ is a

regression coefficient.

PLS algorithms consist of two steps , calibration and prediction . In contrast to other regression methods, a matrix inversion is performed not in the calibration step but in the prediction step. The important part of regression is predicting the concentration vector $\mathbf{y}_k$ from the **X** matrix. This is done by decompositing the matrix **X** and building up the predict concentration vector $\mathbf{y}_k$. The regression model can be obtained for prediction of compound k as follows:

$$\hat{\mathbf{y}}_K = \mathbf{X}\,\mathbf{b}_k \qquad (1.2.8)$$

Where the coefficient b_k is calculated by:

$$\mathbf{b}_k = \mathbf{W}\,(\mathbf{P}'\mathbf{W})^{-1} q_k' \qquad (1.2.9)$$

1.1.4.2. PLS2 Calibration

The PLS2 algorithm [19, 20] was designed for the case when several concentration vectors $\mathbf{y}_k$ are to fitted using the same measured spectra **X**. The vectors are collected as columns in a matrix **Y**. PLS2 is performed using linear combination of the **Y** variables. In contrast to must PLS1 algorithm, PLS2 must involve an iterative step for each of the components. the decomposition of the **Y** matrix is now given by:

$$\mathbf{Y} = \mathbf{U}\mathbf{Q}' \qquad (1.2.10)$$

Where Q is the loading matrix with dimensions k× n for matrix Y and K is the number of all the concentration vectors simultaneously. The model is given as follows:

$$\mathbf{Y} = \mathbf{X}\mathbf{B} \qquad (1.2.11)$$

the coefficients B are calculated by:

$$\mathbf{B} = \mathbf{W}\,(\mathbf{P}'\mathbf{W})^{-1}\,\mathbf{Q}' \qquad (1.2.12)$$

It should be noted that the same PCs occur in the model for each concentration vector, only the regression coefficient change.

1.1.5. Identification of the Drug

1. 1.5.1. Amlodipine

Name: Amlodipine (AM)
Synonyms : Methyl ethyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl - 1, 4-dihydropyridine-3, 5-dicarboxylate

Molecular Structure of Amlodipine are shown in Fig .1.1.

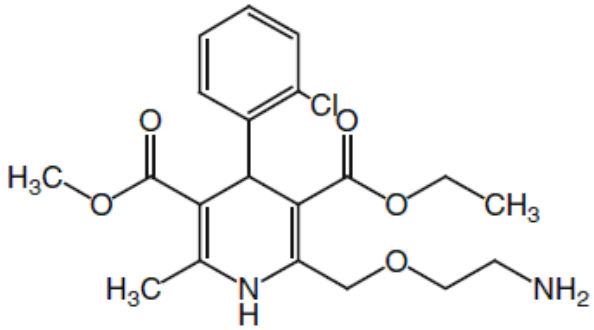

Fig .1.1. Molecular Structure of Amlodipine

Amlodipine is a calcium channel blocker. It relaxes the arterial wall and makes it easier for blood to pass through blood vessels. The drug can be used to treat hypertension, coronary artery disease, angina pectoris (Prinzmetal's angina and chronic stable angina pectoris), heart failure (including decompensated heart failure). It can be used as a monotherapy or in combination with other medicines.

1.1.5.2. Atorvastatin

Name : Atorvastatin

Synonyms : (3R, 5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5 propan 2-ylpyrrol-1-yl]-3, 5-dihydroxyheptanoate

Molecular Structure of Atorvastatin are shown in Fig .1.2.

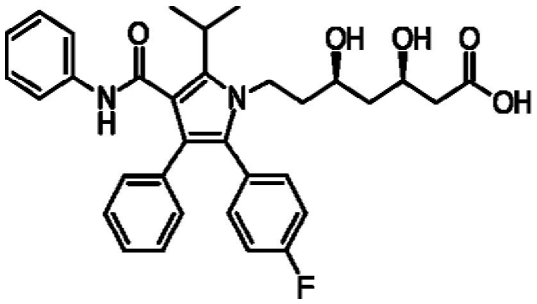

Fig .1.2. Molecular Structure of Atorvastatin

Atorvastatin is in a group of drugs called HMG CoA reductase inhibitors, or "statins." Atorvastatin reduces levels of "bad" cholesterol (low-density lipoprotein, or LDL) and triglycerides in the blood, while increasing levels of "good" cholesterol (high-density lipoprotein, or HDL). Atorvastatin is used to treat high cholesterol, and to lower the risk of stroke , heart attack , or other heart complications in people with type 2 diabetes ,coronary heart disease, or other risk factors.

1.1.5.3. Amostatin

Amostatin tablets combine the calcium channel blocker amlodipine besylate with the lipid-lowering agent atorvastatin calcium. Amostatin tablets are formulated for oral administration in the 20 mg AT/5 mg AM strength combinations.

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Chapter Two

Historical Review

1.2.1. Historical Review on Multivariate Calibration (Principal Component Regression and Partial Least Squares)

In 1966 H.Wold [1,2] showed that the multivariate analysis methods such as partial least squares regression (PLS) and principal component regression (PCR) methods were gaining importance in many fields of chemistry ; analytical , physical ,clinical chemistry and industrial proces control can benefit from use of the methods. This article also represents the basic concept of PLS and PCR regression.

In 1982 S. Wold and H. Martens [4] and in 1983 and 1984 S. Wold et al. [4, 5] pioneered the use of the PLS method for chemical application. In spite of the large amount of literature that emerged from these groups, most articles described PLS gave algorithms and theory that were incomplete and often difficult to understand.

In 1986 P. Geladi and B. R. Kowalski [6] reported the tutorial on the PLS and this article, paragraphs on multiple linear regression (MLR), principal component (PCA) and PCR were included because they are necessary for a good understand of PLS.

In 1987 K. R. Beebe and B.R. Kowalski [7] reported an introduction to the area of multivariate analysis. The multivariate methods that discussed are MLR, PCR and PLS. Their goal was to give the chemist insight into the workings of a collection of a statistical technique and to enable him to judge the appropriatene of the techniques for an application of interest. The examples chosen illustrate how the methods work rather than compare among them.

In 1988 D. M. Haaland and E. D. Thomas [8] studied the relation of PLS methods with other quantitative calibration methods such as classical least squares (CLS), inverse