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A QUANTITATIVE NEAR INFRARED SPECTROSCOPY MODEL FOR THE ASSAY OF EFAVIRENZ IN TABLETS

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Near-infrared-spectroscopy combined with multivariate data analysis represents the most recent and efficient technology in analytical chemistry. The objective of this study was to utilize near infrared spectroscopy as an adapted technology for the quantitative assay of efavirenz. The study developed and validated a quantitative model for estimating the amount of efavirenz in efavirenz uncoated tablets. The quantification was based on the partial least squares algorithm and constructed by cross-validation. A UV spectrophotometric procedure was used as the reference method. Different pre-processing methods were employed in the development of calibration models. The best calibration model was that using partial least squares as the regression algorithm in association with Multiplicative Scattering Correction as the spectrum pre-processing method. The model estimators were: coefficient of determination (R^2) 0.9815, standard error of cross validation 2.0346 and a factor of 5. The chosen model correlated well with the prediction results in accordance with the Mahalabinos distance limits. The developed NIR method allows the estimation of the amount of efavirenz in tablets without sample preparation thus proving to be a simple, fast and suitable method for the quantitative assay of efavirenz in uncoated tablets. Hence, NIR coupled with chemometric methods can be used for on-line, in-line or at-line monitoring of the manufacturing process and are helpful in achieving the goals of Process Analytical Technology.

Keywords: Near Infrared Spectroscopy, chemometrics, multivariate data analysis, efavirenz, Partial Least Squares, cross validation

INTRODUCTION

Near-infrared spectroscopy (NIRS) has developed into an indispensable tool for academic research and industrial quality control in a wide field of applications. These include pharmaceutical technology, microbiology, toxicology, counterfeit detection, determination of physicochemical properties and quality control of a final product [1-3]. Pharmaceutical technology needs NIRS for implementation of in- and on-line process control of many phases of the manufacturing process, better final product certification, and even counterfeit detection for tablets already on the market.

The near infrared (NIR) region of the electromagnetic spectrum extends from the end of the visible spectral region to the beginning of the fundamental IR spectral region (700-2500 nm). The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of –CH, –NH, –OH and –SH functional groups [1,4]. Furthermore, intermolecular hydrogen bonding and dipole interactions have to be considered, since they

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alter vibrational energy states, thus shifting existing absorption bands and/or giving rise to new ones.

NIRS combined with multivariate data analysis (MVDA) represents the most recent and efficient technology in analytical chemistry and particularly in pharmaceutical industry [2-3,5-6]. MVDA is defined as any statistical, mathematical or graphical approach which considers multiple features simultaneously [7]. A feature is a numerical variable that describes an aspect of the object such as concentrations of selected substances or intensities of spectral signals. The fundamental hypothesis for multivariate data interpretation is the existence of relationships between the locations or the distances of points (objects) and relevant properties. The essential concept of MVDA is based on the use of the principal component [8], a mathematical function of all features that may contain much more information than all the features individually.

A principal component is not observed directly, but can be viewed graphically by principal component analysis (PCA), partial least squares (PLS) and artificial neural networks (ANNs) [9]. By assuming that all the relationships between a component and the observed variables are linear, we can use PCA to analyze data matrices, say **X** and/or **Y** (if we assume that only the **X** or the **Y** variables are affected by the principal component) or PLS (assuming that both **X** and **Y** are affected). If the relationships are thought to be non-linear, ANN can be used since it does not assume linearity [9].

The most common multivariate regression methods used in quantitative NIR analysis are principal component regression (PCR) and PLS regression (PLSR) [10]. PCR uses the principal components provided by PCA to perform regression on the sample to be predicted. PLS finds the directions of greatest variability by comparing both spectral and target property information with the new axes, called PLS components or PLS factors.

The regression model equation can be represented in a matrix form as follows [11].

$$Y = XW * C' + F = XB + F$$

Where Y represents the matrix of response variables, X represents the matrix of predictor variables, W represents matrix of transformed PLSR weight, C' represents the Y- weight matrix, F represents the matrix of Y- residual and B represents a matrix of regression coefficient of all Y's.

Literature survey reveals that HPLC is the gold standard analytical method for the assay of efavirenz [11-14]. The development and successful validation of a HPLC method for the simultaneous analysis of lamivudine, tenofovir disoproxil fumarate and efavirenz has been described [15]. However, the method can not achieve the goals of process analytical technology (PAT).Thus, the development and validation of the NIR method would be an alternative, efficient and faster analytical method with no destructive measurement for the estimation of efavirenz both in routine and real time analyses. The aim of this work was to develop an analytical method for the quantitative analysis of efavirenz in efavirenz uncoated tablet using NIRS coupled with MVDA.

MATERIALS AND METHODS

Sample

The samples studied included efavirenz uncoated tablets produced in-house at the Pharmaceutical Research and Development Laboratory of Muhimbili University of Health and Allied Sciences. Tablets were produced at strengths of 80, 100 and 120 % relative to the nominal efavirenz concentration (600 mg) as a calibrator tablet. This covered the range usually required by the approval authorities for assay [16]. Table 1 lists the proportion of each component in the three formulations. The tablets measured 2 cm in diameter and were 5 mm thick with a weight of 800±5 mg.

Table 1: Nominal concentration of components in the calibrator tablets (n=300)

		Efavirenz 80%	Efavirenz 100%	Efavirenz 120%
(a)	Efavirenz	480.0 mg	600.0 mg	720.0 mg
(b)	Sodium CMC CL	75.0 mg	78.0 mg	25.0 mg
(c)	MCC PH 101	225.0 mg	100.0 mg	30.0 mg
(d)	HPMC (Pharmacoat 606)	8.0 mg	10.0mg	10.0 mg
(e)	Sodium Lauryl Sulphate	8.0 mg	8.0 mg	10.0 mg
(f)	Magnesium stearate	4.0 mg	4.0mg	5.0 mg
	Total	**800 mg**	**800 mg**	**800 mg**

A set of 20 tablets from each of the batches were used for calibration. The calibration was checked for each formulation as an independent set of test sample (validation sample) covering the whole calibration range. The intact tablets were scanned with the NIR instrument and the results compared with UV spectroscopic assay as the reference method.

Equipment

NIR spectra were recorded on an NIR system 5000 spectrophotometer from Advanced System Development (ASD) Inc. (NIR Systems, Boulder, CO, USA). The instrument is equipped with Grams Suite ver 9.0 chemometrics application software from Galactic Industries, CA, USA. UV spectra were recorded on a JENWAY 6405 UV/Vis spectrophotometer from Agilent technologies, Santa Clara, CA, USA.

UV reference procedure

To ensure appropriate quantitative results, the UV spectrophotometric procedure for estimation of the amount of efavirenz was used as a reference method [16]. About 67 mg equivalent weight of finely powdered efavirenz uncoated tablet was dissolved in 20 ml of methanol, sonicated for 15 min and diluted to 200 ml with methanol. The mixture was thoroughly shaken and filtered. A 5 ml aliquot of the filtrate was transferred to a 100 ml volumetric flask and diluted to volume with methanol. UV absorbances of the resulting solution were read at 248 nm against a methanol.

NIR spectra scanning

The spectra of the tablets were recorded in triplicate in a custom-built holder source Probe MugLite in the reflectance mode, using fibre optic module over the wavelength range of 350-2500 nm. One tablet was placed on a sampling tray adapter and the probe was brought into direct contact with the sample. After each run the position of the tablet was changed.

Quantitative analysis

The analysis was based on the PLS algorithm and constructed by cross-validation using as many segments as samples in the calibration set. The number of PLS components was taken to be the minimum number for which the prediction error sum of squares (PRESS) was not significantly different from the lowest PRESS value [17]. The quality of the results was assessed in terms of the standard error cross validation (SECV), coefficient of determination (R^2) and Mahalanobis distance given by equations (1), (2) and (3), respectively.

$$SECV = \sqrt{\frac{\sum_{i-1}^{n}(y - \bar{y})^2}{n - m - 1}} \qquad (1)$$

Where n is the number of observations, y is an observation of the dependent variable, $\bar{y}$ is the predicted value of a given observation of the dependent variable and m is the number of independent variables in the model.

$$R^2 = 1 - \frac{\sum_{i-1}^{n}(y - \bar{y})^2}{\sum_{i-1}^{n}(y - \bar{Y})^2} \qquad (2)$$

Where $\bar{Y}$ is the mean of the dependent variable.

Mahalanobis distance (D) is a way of measuring distance that accounts for correlation between variables. It accounts for the variance of each variable and the covariance between variables hence it provides a way to measure distances that takes into account the scale of the data [18].

$$D^2 = F\,\frac{n-1}{n-k} \qquad (3)$$

Where n is the number of scans, k represents the factors for the model and F is the degree of freedom.

RESULTS AND DISCUSSION

Development and validation of the quantitation method

Spectra investigation: The development of a calibration model consisted of checking different spectral pre-treatments [19] as well as their combinations with different spectral ranges. Both the whole spectral range and specific spectral regions containing bands and different spectral pre-treatments were tested with a view of constructing the calibration models. The Near Infrared spectra of the entire efavirenz calibration tablets are shown in Figure 1.

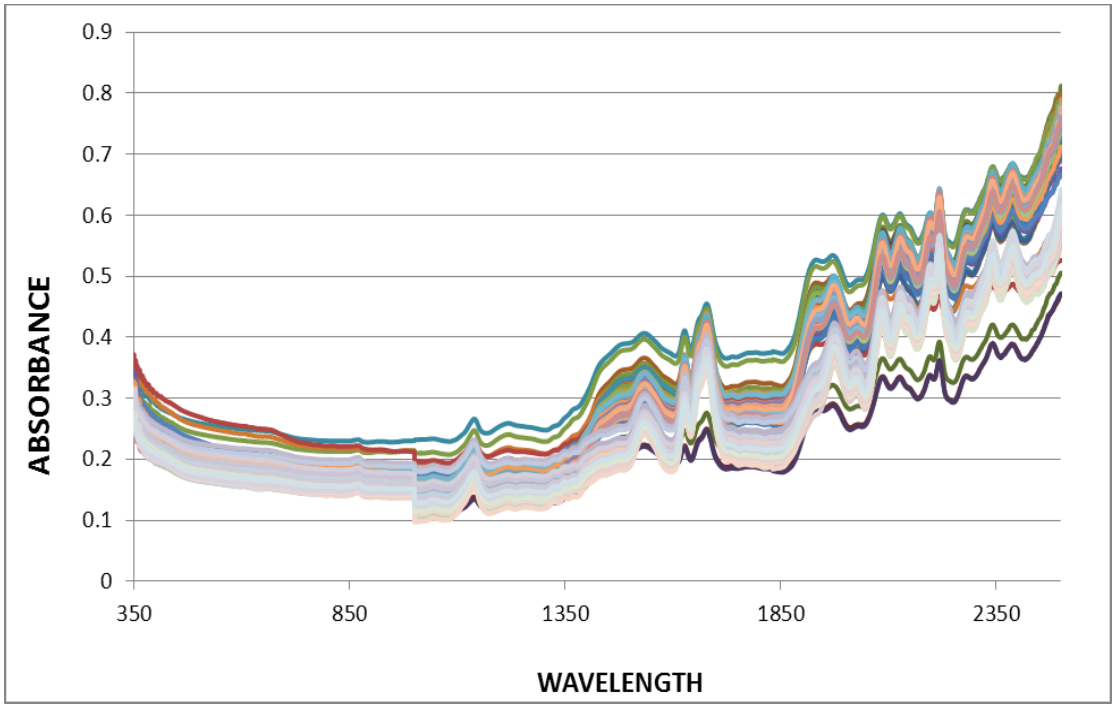

Figure 1: Reflectance spectra of the entire efavirenz calibrator tablets. ***Wavelength in nm***

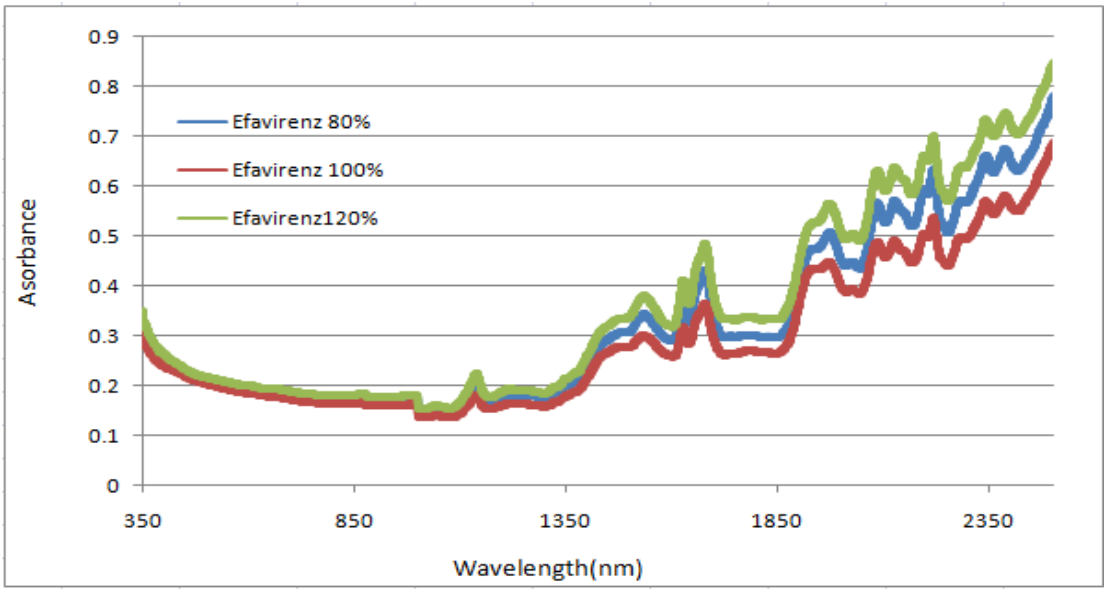

Figure 2: Reflectance spectra of efavirenz tablets at three consecutive levels

As shown in Figure 1 and Figure 2, the strong bands for efavirenz are present in the 1100-2500 nm range of the spectrum. This is the Near Infrared region whereby these bands are related to overtones and combinations of fundamental vibrations of –CH, -NH and -OH in efavirenz. This region was used for model development.

To carry out quantitative analysis using NIR spectroscopy, chemometric methods were used, which extracted the relevant bits of information and minimized irrelevant ones [7]. Spectral interference parameters called for mathematical correction (spectra pre-treatments) in order to reduce, eliminate or standardize their impact on the spectra.

Therefore, the model development consisted of checking different spectra pre-treatments in combination with the specific spectral regions containing strong bands of efavirenz. Multivariate calibration based on PLS regression was then applied. During the design of the calibration model, its predictive ability was tested with the samples used during its development. The validation of the model was done using the cross-validation method, leaving out one sample at a time, and comparing the predicted concentrations with the known concentrations of the compounds in each sample. The standard error of cross validation (SECV) was used as a diagnostic test for examining the errors in the predicted concentrations because it indicates both precision and accuracy of predictions [20]. It was calculated upon addition of each new factor to the PLS models. For each pre-processing method, the coefficient of determination, R^2, between actual known concentration and predicted concentration was computed to evaluate the predictive ability of the model.

The optimal number of factors was selected by ensuring that the selected model was that with the smallest number of factors for which SECV was not significantly greater than SECV for the model with one or more additional factors [19].

The NIR-chemometric model was developed taking into account all samples of the three batches from the calibration matrix. The results obtained during the method development are presented in Table 2. The R^2 values for the proposed models were greater than 0.98, in (b), (e) and (f). The lowest number of PLS factors was 3 and 4 for models (g) and (c), respectively. Considering the SECV together with the R^2 values, models (c) and (g) could not be selected as good models because (c) had the second largest SECV (2.3619) and the second lowest R^2 (0.9751) while (g) had the largest SECV (7.9910) and the lowest R^2 (0.7306). Model (b) was chosen as the best fitted model for efavirenz quantification in tablets, using the PLS algorithm with Multiplicative Scattering Correction (MSC) pretreatment method.

Table 2: Statistical parameters and number of principal components in the PLS method.

	Pretreatment	PC number	SECV	R^2
(a)	None	9	2.2957	0.9765
(b)	Multiplicative Scattering Correction (MSC)	5	2.0346	0.9815
(c)	Normalisation[a]	4	2.3619	0.9751
(d)	Standard Normal Variate alone	5	2.1202	0.9799
(e)	Standard Normal Variate detrend	5	2.0438	0.9813
(f)	Thickness	5	2.101	0.9803
(g)	Multiplicative Scattering Correction second derivate	3	7.991	0.7306

[a]data variables are scaled to unit variance prior to fitting of PCA model.

Further tests were conducted on the model to assess its prediction ability in accordance with the Mahalanobis distance (M-dist.). When using this prediction, the M-dist. value was the estimation of model performance that was seen with every prediction, i.e. if the M-dist.>3.0, the sample is represented in the calibration model whereas if M-dist.<3.0, the sample is not well represented by the model [21-22]. Table 3 shows the results of the validation samples as well as samples from other sources.

Table 3: Prediction report for laboratory samples and samples of efavirenz tablets from a different manufacturer (Matrix).

SAMPLE	NIRS PREDICTED VALUE (%)	M-DISTANCE
(a) Efavirenz 120%	116.61	1.16
(b) Efavirenz 100%	100.30	0.96
(c) Efavirenz 80%	80.78	0.84
(d) Matrix coated	136.84	484.69
(e) Matrix uncoated	64.16	111.71

Based on the results presented in Tables 2 and 3, MSC spectra pre-treatment was chosen as the best fitted model for quantification of efavirenz in uncoated tablets as it gave the lowest SECV and highest R^2 of all the other pre-treatment methods. A further consideration in the choice of the MSC was the principal component. The smaller the number of principal components the better the method, provided the SECV and R^2 are acceptable. Using the M-distance method, the prediction ability of the MSC method was upheld in that the samples were represented in the calibration model. Figure 3 shows the predicted concentrations versus actual concentrations for efavirenz uncoated tablets obtained using the MSC pre-treatment method.

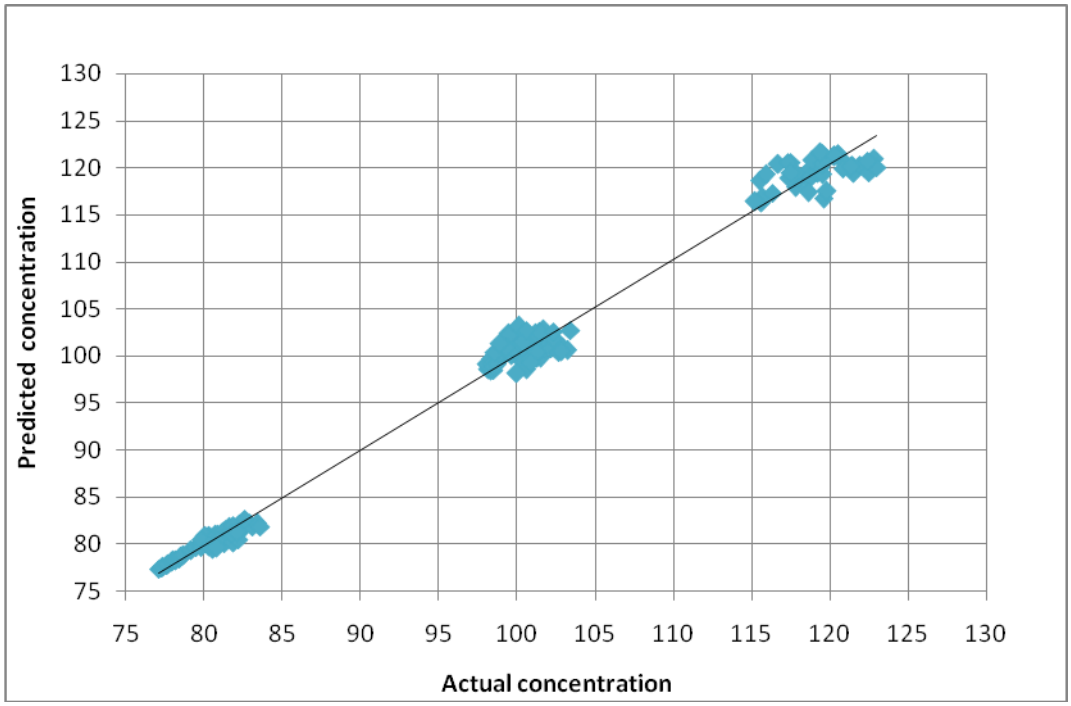

Figure Error! No text of specified style in document.**: The plot of predicted concentrations versus the actual concentrations for Efavirenz tablets**

CONCLUSION

A near infrared method for the quantification of efavirenz in tablets has been developed. Using an NIRS-chemometric technique, different calibration models were evaluated for the quantification of efavirenz in tablets containing 80 to 120% of nominal efavirenz content. The quantitative analysis was based on PLS algorithm and constructed by cross-validation. The best fitted model for efavirenz quantification in tablets was obtained using the MSC method which had R^2 of 0.9815, SECV of 2.0346 and a factor of 5. The chosen model correlated well with the prediction results. The predicted results were all within the model meaning the Mahalanobis distance was not exceeded. The developed NIR method allowed for the determination of efavirenz in uncoated tablets with no need for sample preparation. Such a quick NIRS-chemometric method can be used for on-line, in-line or at-line monitoring of the manufacturing process of efavirenz uncoated tablets and is helpful in achieving the goals of process analytical technology.

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