

**NEAR INFRARED SPECTROSCOPY AS A FIELD ADAPTED
TECHNOLOGY FOR QUALITY ASSESSMENT OF EFAVIRENZ
AND COTRIMOXAZOLE**

Geovin George Mgoylela (B.Pharm)

**M.PHARM (Quality Control and Quality Assurance) Dissertation
Muhimbili University of Health and Allied Sciences
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TECHNOLOGY FOR QUALITY ASSESSMENT OF EFAVIRENZ
AND COTRIMOXAZOLE**

By

Geovin George Mgoyela, (B.Pharm)

**A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree
of Masters of Pharmacy in Quality Control and Quality Assurance of
Muhimbili University of Health and Allied Sciences**

**Muhimbili University of Health and Allied Sciences
July, 2013**

CERTIFICATION

The undersigned certify that they have read and hereby recommend for submission by Muhimbili University of Health and Allied Sciences, a dissertation entitled **near infrared spectroscopy as a field adapted technology for quality assessment of efavirenz and cotrimoxazole** (partial) fulfillment of the requirement for the degree of Master of Pharmacy in Quality Control and Quality Assurance of Muhimbili University of Health and Allied Sciences.

.....
Dr. E.A.Kaale, PhD

(Supervisor)

Date __ __ / __ __ / __ __

.....
Dr. J.Sempombe, PhD

(Supervisor)

Date __ __ / __ __ / __ __

.....
Dr. M.H.S Chambuso, PhD

Date __ __ / __ __ / __ __

DECLARATION AND COPYRIGHT

I, **Geovin George Mgoyela**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature.....

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DEDICATION

To my father George Mgoyela and mother Veronica Mugoyela and my brother Geoffrey and sisters Georgia and Georgina.

Table of Contents

CERTIFICATION	ii
DECLARATION AND COPYRIGHT	iii
ACKNOWLEDGMENTS	iv
DEDICATION	v
LIST OF TABLE	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS/ACRONYMS	x
ABSTRACT	xii
CHAPTER ONE	1
1.0. BACKGROUND INFORMATION	1
1.0.1 Near Infrared Spectroscopy as tool for drugs quality assessment	1
1.0.2 Overview of analytical methods available for Efavirenz and cotrimoxazole ..	3
1.0.3 Efavirenz	4
1.0.4 Cotrimoxazole	5
1.2. PROBLEM STATEMENT	6
1.3. RATIONALE	6
1.4. OBJECTIVES	7
1.4.1. BROAD OBJECTIVE	7
1.4.2. SPECIFIC OBJECTIVES	7
1.5. HYPOTHESES	7
CHAPTER TWO	8
2.0. METHODS	8
2.1. Materials	8
2.1.1. Efavirenz	8
2.2. Equipments	9
2.3. Procedure	9
2.3.1. UV reference procedure	9
2.3.2. NIR spectra scanning for efavirenz	9
2.3.3. NIR spectra scanning for Zentrim [®]	10
2.4. Quantitative analysis	10

2.5. Qualitative Analysis.....	11
CHAPTER THREE	12
3.0. RESULTS AND DISCUSSION	12
3.1. Development and Validation of the Quantitation Method for Efavirenz.....	12
3.1.1. Method development	12
3.2. The Identification Method For Zentrim [®] Tablets.....	17
3.2.1. Method Development and Validation.....	18
CHAPTER FOUR.....	21
4.0. CONCLUSION AND RECOMMENDATION.....	21
4.1. CONCLUSION	21
4.2. RECOMENDATION.....	22
REFERENCES	23

LIST OF TABLE

TABLE 2.1: NOMINAL CONCENTRATION OF EACH COMPONENT IN THE CALIBRATOR TABLETS.	8
TABLE 3.1: STATISTICAL PARAMETERS AND NUMBER OF PRINCIPAL COMPONENTS IN THE PLS METHOD.....	15
TABLE 3.2: PREDICTION REPORT FOR LABORATORY SAMPLE AND SAMPLES OF EFAVIRENZ TABLETS FROM OTHER MANUFACTURER (MATRIX TABLET).	16
TABLE 3.3: THE THRESHOLD SET AT 0.9998648 WITH THE POSITIVE CONTROL PRODUCT (ZENTRIM [®]).....	19
TABLE 3.4: THE TRIAL AND ERROR METHOD AT DIFFERENT SETTING OF THRESHOLDS.....	19
TABLE 3.5: THE THRESHOLD WAS FINALLY SET AT 0.9994648.....	20

LIST OF FIGURES

FIGURE 1.1: DISPLAYS THE ESTIMATION OF PRINCIPAL COMPONENT	2
FIGURE 1.2: DISPLAYS OF THE PLS COMPONENT BY COMPARISON OF BOTH SPECTRA AND TARGET INFORMATION	3
FIGURE 1.3: CHEMICAL STRUCTURE OF EFAVIRENZ.....	4
FIGURE 1.4: CHEMICAL STRUCTURE OF SULFAMETHAXOLE (A) AND CHEMICAL STRUCTURE OF TRIMETHOPRIM (B)	5
FIGURE 3.1: REFLECTANCE SPECTRA OF THE ENTIRE EFAVIRENZ CALIBRATOR TABLETS	13
FIGURE 3.2: REFLECTANCE SPECTRA OF EFAVIRENZ TABLETS AT THREE CONSECUTIVE LEVELS	13
FIGURE 3.3: THE PLOT OF PREDICTED CONCENTRATIONS VERSUS THE ACTUAL CONCENTRATIONS FOR EFAVIRENZ TABLETS	17
FIGURE 3.4: THE GRAPH DISPLAYING THE MATCH VALUES OF REFERENCE LIBRARY.....	18

LIST OF ABBREVIATIONS/ACRONYMS

ANN's	Artificial Neural Network
API	Active Pharmaceutical Ingredient
ASD	Advance System Development
CC	Correlation Coefficient
HIV	Human Immuno-deficiency Virus
HPLC	High Performance Liquid Chromatography
ID	Identification
M-distance	Mahalanobis distance
MSD	Medical Stores Department
MUHAS	Muhimbili University of Health and Allied Sciences
MVDA	Multivariate Data Analysis
NIR	Near Infrared
NIRS	Near Infrared Spectroscopy
NNRTI	Non Nucleoside Reverse Transcriptase Inhibitor
PC	Principal component
PCA	Principal Component Analysis
PCR	Principal Component Regression
PEP	Post Exposure Prophylaxis
PLS	Partial Least Square
R ²	Coefficient of determination
R&D	Research and Development
RSEC	Relative Standard Error Calibration
SEC	Standard Error Calibration
SECV	Standard error of cross validation

SMZ	Sulfamethaxole
TMP	Trimethoprim
USA	United States of America
USAID	United States Agency for International Development
UV	Ultraviolet

ABSTRACT

Background information

Near-infrared-spectroscopy (NIRS) combined with multivariate data analysis (MVA) represents the most recent and efficient technology in analytical chemistry. NIRS is simple, fast and suitable analytical method for quantitative and qualitative analysis. Although the common HPLC technique is accurate and precise with good reproducibility, excellent with respect to selectivity and sensitivity, however, it cannot be used for routine analysis because of its unique requirement which include cost, expertise and time requirement.

Objective

The main objective of this study was to utilize near infrared spectroscopy as a field adapted technology for quality assessment of selected drugs. Which specifically developed and validated a quantitative model for estimating amount of efavirenz in efavirenz tablets using NIR technology and a qualitative model for consistency assessment of cotrimoxazole tablets using hand held microPHAZIR™.

Methods

For efavirenz, the active principle (efavirenz) was quantified with partial least-square algorithm and constructed by cross-validation. Ultra-Violet (UV) spectrophotometric procedure was used as a reference method. Different pre-processing methods were used for development of calibration models. For Zentrim® the development of the method involved trial and error methods which were proposed to reach to the ultimate threshold that would truly identify the product. The identification method used for expression of similarity was Spectral Matching.

Results

For efavirenz, the best calibration model was found when partial least square (PLS) was used as regression algorithm in association with Multiplicative Scattering Correction as pre-processing spectrum method. The model estimators were as follows; coefficient of determination (R^2) was 0.9815, standard error of cross validation (SECV) was 2.0346 and a factor of 5. The chosen model correlated well with the prediction results in accordance with the Mahalabinos distance (M-distance) limits. Samples for Zentrim® were identified

by comparison of their spectra with standard spectra in a reference library. An unknown sample was assumed to be positively identified if its correlation coefficient exceeded the established threshold ($\rho = 0.9994648$).

Conclusion

The developed NIR methods allows for the identification of zentrim[®] and also estimation of amount of efavirenz in tablet form without sample preparation. Thus, NIR-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 the process analytical technology.

CHAPTER ONE

1.0. BACKGROUND INFORMATION

1.0.1 Near Infrared Spectroscopy as tool for drugs quality assessment

Near-infrared spectroscopy (NIRS) has developed into an indispensable tool for academic research and industrial quality control in a wide field of applications. This includes pharmaceutical technology, microbiology, toxicology, counterfeit detection, determination of physicochemical properties and quality control of a drug product^(1,2,3). Pharmaceutical technology needs NIR spectroscopy for implementation of in- and on-line processes control of many phases of manufacturing process.

The near-Infrared (NIR) region of the electromagnetic spectrum extends from the end of visible spectral region of wavelength range of (700nm or 12820cm^{-1}) to the beginning of the fundamental infrared (IR) spectral region (2500nm or 3959cm^{-1}). The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of $-\text{CH}$, $-\text{NH}$, $-\text{OH}$ and $-\text{SH}$ functional groups^(1,4). Furthermore, intermolecular hydrogen bonding and dipole interactions have to be considered, since they alter vibrational energy states, thus shifting existing absorption bands and/or giving rise to new ones.

Near-infrared-spectroscopy (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). Multivariate data analysis 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 objects, such as, concentrations of selected substances or intensities of spectral signals. The fundamental hypothesis for multivariate data interpretations is the existence of relationships between the locations or the distances of points (objects) and relevant properties. The essential concept of multivariate data analysis is based on the use of the principal component⁽⁸⁾. A principal component (PC) is a mathematical function of all features and therefore may contain much more information than all the features individually. Figure 1.1, displays the estimation of principal component graphically⁽⁹⁾.

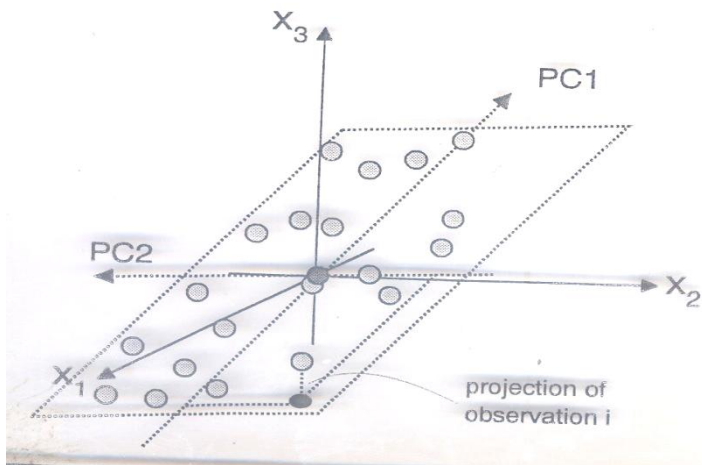


Figure1.1: Displays the estimation of principal component

In other words, 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 (ANN's)⁽¹⁰⁾. By assuming that all the relationships between a component and observed variables are linear, we can use PCA (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). But, if the relationships are thought to be non-linear, then PCA and/or PLS are not suitable since these assume linearity. Alternatively, Artificial Neural Network (ANNs) can be used since it does not assume linearity⁽¹⁰⁾.

The most common multivariate regression methods used in quantitative NIR analysis are principal component regression (PCR) and partial least-squares (PLS) regression⁽¹¹⁾. The 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. Figure 1.2, displays of the PLS component by comparison of both spectra and target information⁽¹²⁾.

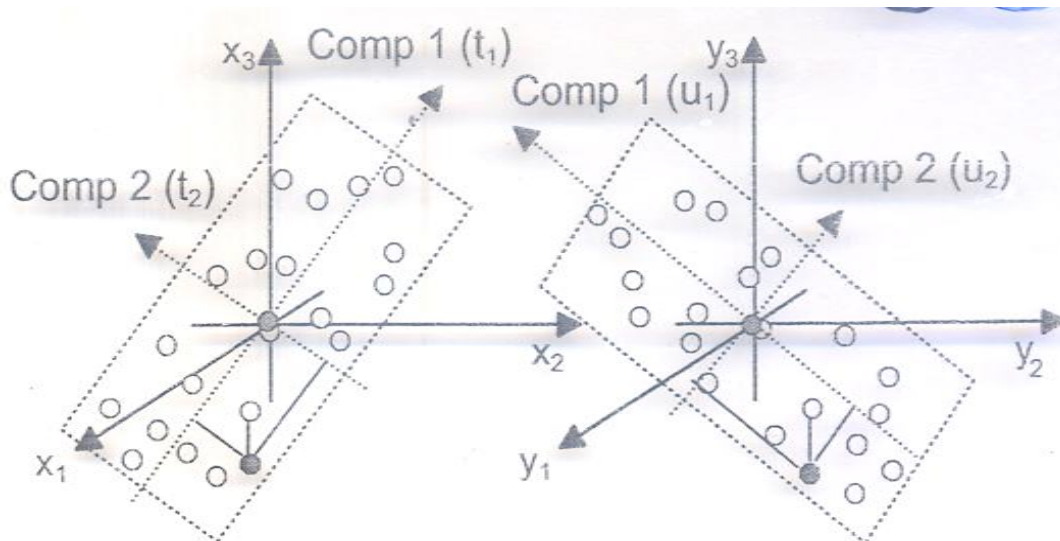


Figure1.2: Displays of the PLS component by comparison of both spectra and target information

The regression model equation can be represented in a mathematical equation both in element and matrix form as follows⁽¹³⁾:

$$(y_{im} \sum_a c_{ma} \sum_k w_{ka} * x_{ik} + f_{im} = \sum_k b_{mk} x_{ik} + f_{im}) \mathbf{Y} = \mathbf{XW} * \mathbf{C}' + \mathbf{F} = \mathbf{XB} + \mathbf{F},$$

Whereby \mathbf{Y} represent matrix of response variables, \mathbf{X} represent matrix of predictor variable, \mathbf{W} represent matrix of transformed PLSR weight, \mathbf{C} represent the Y- weight matrix, \mathbf{F} represent the matrix of Y- residual and \mathbf{B} represent a matrix of regression coefficient of all Y's.

The aim of this work was to develop an analytical method for quantitative and qualitative analysis of efavirenz and cotrimoxazole respectively using NIRS coupled with multivariate methods.

1.0.2 Overview of analytical methods available for Efavirenz and cotrimoxazole

Literature survey reveals that a common analytical method available for determination of efavirenz and cotrimoxazole from biological matrices, bulk drug dosage form is High Performance Liquid Chromatography (HPLC)^(14,15,16). HPLC technique is accurate and precise with good reproducibility. The technique is excellent with respect to selectivity and sensitivity, however, it cannot be used for routine analysis because of its unique requirements i.e. special instrumentation, reagents and expertise⁽¹⁷⁾.

1.0.3 Efavirenz

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used as part of highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV) type 1 infection. It is used in combination with other antiretroviral agents as part of an expanded post exposure prophylaxis (PEP) regimen to prevent HIV transmission for those individuals exposed to materials associated with a high risk for HIV transmission⁽¹⁸⁾.

Efavirenz is named as (S)-6-chloro-4-(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one(Figure 1.3). Its molecular formula is $C_{14}H_9ClF_3NO_2$ with the chemical structure shown in Figure 1.3.

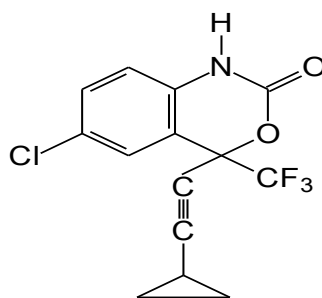
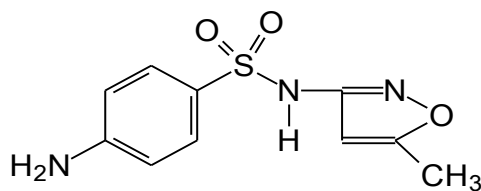


Figure 1.3: Chemical structure of Efavirenz

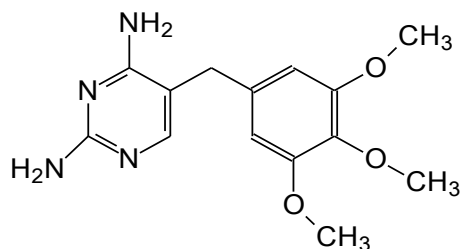
Literature reports development and a successful validation of a HPLC method for simultaneous analysis of lamivudine, tenofovir disoproxil fumarate and efavirenz⁽¹⁹⁾. Thus, the development and validation of the NIR method using a NIR instrument would be an alternative, efficient and faster analytical method for efavirenz.

1.0.4 Cotrimoxazole

Co-trimoxazole (Septrin[®], Bactrim[®]) is a combination of trimethoprim and sulfamethoxazole in the ratio of 1 to 5. It is used in the treatment of a variety of bacterial and protozoal infections⁽¹⁸⁾. Sulfamethoxazole (5-methyl-3-sulfanilamidoisoxazole, SMZ) (Figure 1.4a) is a sulfonamide with broad spectrum of activity that competitively inhibits the bacterial enzyme dihydropteroatesynthetase⁽²⁰⁾, trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine, TMP) (Figure 1.4b) is a dihydrofolate-reductase inhibitor⁽²¹⁾. Both drugs sequentially block folic acid metabolism and produce a synergistic antibacterial activity⁽¹⁶⁾.



(a)



(b)

Figure 1.4: Chemical structure of sulfamethaxole (a) and Chemical structure of trimethoprim (b)

Literature reports on the development and a successful validation of a HPLC with ultraviolet (UV) detection method for simultaneous determination of sulfamethoxazole and trimethoprim in biological fluids for high-throughput analysis⁽¹⁶⁾. The development and validation of the NIR method would be an alternative more efficient and faster analysis of cotrimoxazole.

1.2. PROBLEM STATEMENT

To date, there is hardly any literature that reports on quantitative model determination of efavirenz or qualitative assessment of an essential medicine like cotrimoxazole by Near Infrared Spectroscopy. The most commonly reported method is HPLC which is accurate and precise with good reproducibility, excellent with respect to selectivity and sensitivity. However, this method cannot be used for routine analysis because of its unique requirements which include special instrumentation, reagents and expertise⁽¹⁷⁾.

Hence it is worthwhile to develop a simpler, faster and little or no sample preparation method with no destructive measurement for estimation of drugs for routine and real time analysis. NIRS is one of the analytical methods that fulfill such requirements in which the estimation of the drug can be done with similar effectiveness as in HPLC methods. In addition, it allows on line and in line analysis of pharmaceuticals and monitoring of industrial processes because it uses long fiber optics⁽⁵⁾. NIR spectroscopy can be used to analyze tablets through the plastic blister and glass material since its radiation can penetrate through the packaging material^(5, 17). Also it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environmental pollution.

1.3. RATIONALE

The establishment of the NIR validated analytical technique is of critical importance because it ensures the required API is adequately present in the formulation for therapeutic effect. Nonetheless the method helps regulatory authorities to curb counterfeit drugs that may penetrate the market by early quantification of API before registering the drug or when the drug is already on the market and shows clinical failure. Furthermore the technique enables online monitoring and control of industrial processes since there is no alteration of samples whatsoever, hence will help to reduce the government spending on the substandard drugs. Moreover, the technique prevents environmental pollution because it operates in environmentally friendly conditions where there is no any kind of waste or pollution produced. Thus, it avoids deleterious effect to the ecosystem as well as the environment.

1.4. OBJECTIVES

1.4.1. BROAD OBJECTIVE

To utilize near infrared spectroscopy as a field adapted technology for quality assessment of selected drugs.

1.4.2. SPECIFIC OBJECTIVES

- i. To develop and validate a quantitative model for estimation of amount of efavirenz in efavirenz tablets using NIR technology.

- ii. To develop and validate a qualitative model for consistency assessment Zentrim[®] using a hand held microPHAZIR[™].

1.5. HYPOTHESES

1. NIR technology coupled with chemometric methods is a simple, fast and suitable field adapted analytical technique for quantification of the API in the efavirenz formulation.

2. NIR technology coupled with chemometric methods is a simple, fast and suitable field adapted analytical technique for assessment of consistency of manufacturing of zentrim tablets.

CHAPTER TWO

2.0. METHODS

2.1. Materials

The samples studied included in-house made efavirenz tablets from Muhimbili University of Health and Allied Sciences at Research and Development Laboratory, commercially available cotrimoxazole (Zentrim[®]) from Zenufa Laboratory, Sheltrim[®] from Shelys Pharmaceutical company, Alprim[®] from Elys Pharmaceutical company and Matrix[®] tablets from Matrix Laboratories Limited.

2.1.1. Efavirenz

Calibrator tablets: Efavirenz tablets were made at different strength of 80, 100 and 120% related to the nominal efavirenz concentration (600mg). These were all prepared at R&D MUHAS lab as calibration tablets. This covers the range usually required by the approval authorities ⁽²²⁾ for assay. Table 1 lists the concentration value for each component in the three formulations. All pharmaceuticals have the same active ingredient (efavirenz) but with different nominal levels. The tablets were 2 cm in diameter and 5 mm in thickness, all weighed about 800 mg.

Table 2.1: Nominal concentration of each component 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 for each formulation was 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 respective NIR instrument and analyzed by UV as the reference method.

2.2. Equipments

Near-Infrared spectra for Efavirenz tablets were recorded on a NIR system 5000 spectrophotometer from advanced system development (ASD) Inc (NIR systems, Boulder, Colorado, USA). The instrument is equipped with Grams ALv.9 w/PLS IQ fiber-optic module for quantitative analysis. The system is governed by Indico pro version software suitable for acquisition and processing of spectra.

The UV spectra used in the reference method were recorded on a JENWAY 6405 UV/Vis spectrophotometer from Agilent technologies, Santa Clara California, USA.

The Near-Infrared spectra for cotrimoxazole tablets were recorded on microPHAZIR spectrometer from Polychromix, Wilmington, North Carolina, USA.

2.3. Procedure

2.3.1. UV reference procedure

The following UV spectrophotometric procedure was used as a reference in the estimation of amount of efavirenz.

About 67 mg equivalent weight of finely powdered sample was dissolved in 20 ml of methanol and the mixture was sonicated for 15 minutes and then diluted to 200 ml with the same solvent. The diluted mixture was properly mixed, filtered and a 5 ml of supernant was added to 100 ml volumetric flask and diluted to volume with methanol.

Finally, the spectrum for the resulting solution was recorded at 248 nm against a blank solution consisting of 1M of methanol.

2.3.2. NIR spectra scanning for efavirenz

The spectrum for each tablet was recorded in triplicate over the wavelength range of 1100-2500 nm. Spectra were recorded in the reflectance mode, using fibre optic module. The

spectra of the tablets were recorded in a custom-built holder source Probe MugLite. 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.

2.3.3. NIR spectra scanning for Zentrim®

Zentrim® tablets were taken through the scanning process. Near-infrared spectra were recorded on a NIR Systems microPHAZIR™. Each tablet was scanned in triplicate on flat side over the wavelength range of 1595 – 2396 nm and spectra were recorded.

2.4. Quantitative analysis

The analysis was based on the Partial Least Square (PLS) algorithm and constructed by cross-validation using as many segment 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⁽²³⁾.

The quality of the results was assessed in terms of the standard error cross validation (SECV), coefficient of determination (R²) and Mahalanobis distance.

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

Where n is the number of the observation, 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.

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

Where \bar{Y} is the mean of the dependent variable. Mahalanobis distance (D), 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 provided a way to measure distances that takes into account the scale of the data⁽²⁴⁾.

$$D^2 = F ((n-1)/ (n-k))$$

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

2.5. Qualitative Analysis.

Samples were identified by comparison of their spectra with standard spectra in a reference library. The identification method used for expression of similarity was Spectral Matching. The shape of each sample spectrum collected was compared with the shapes of the spectra in the library and assigned a “degree of match” value ranging from -1 (perfectly anti-matched) to +1 (perfectly matched) using a proprietary algorithm⁽²⁵⁾. The library entries that have the highest match values to the unknown sample were then used to identify the unknown.

CHAPTER THREE

3.0. RESULTS AND DISCUSSION

3.1. Development and Validation of the Quantitation Method for Efavirenz

This part, reports on the development and validation of a NIR-chemometric method suitable for the direct quantification of efavirenz tablets, without any sample preparation. To ensure appropriate quantitative results, a set of calibration samples had to be representative of the changes in the properties of samples that would be found in routine analysis⁽²²⁾. In general, the calibration set would contain an approximately balanced distribution of samples across the concentration domain and a considerable number of representative samples for each level of concentration of the substance of interest. The matrix of the calibration protocol is presented in Table 2.1.

3.1.1. Method development

Spectra investigation: The development of a calibration model consisted of checking different spectral pre-treatments⁽²⁶⁾ as well as their combination 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 3.1.

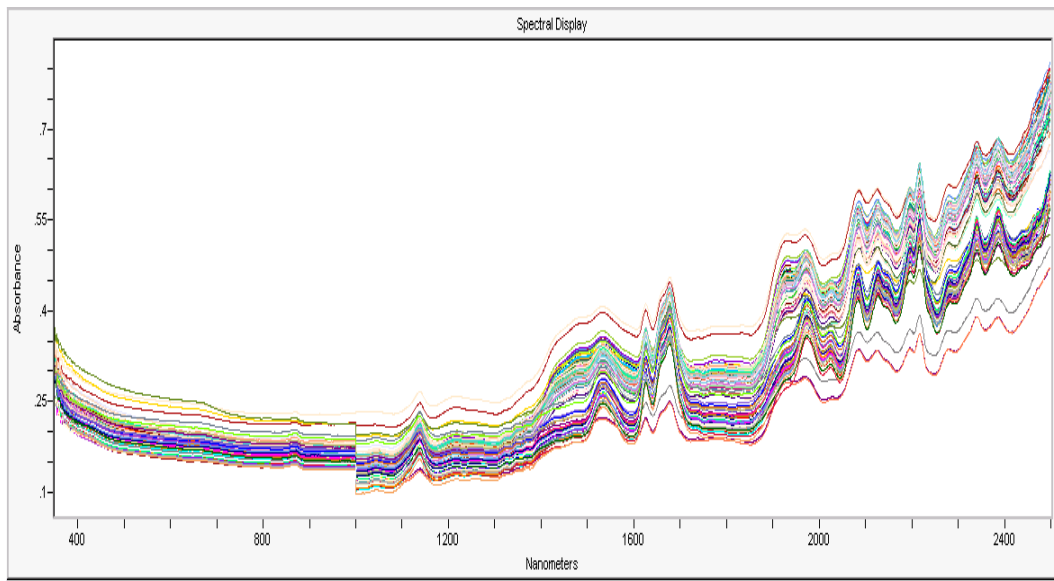


Figure 3.1: Reflectance spectra of the entire efavirenz calibrator tablets

The spectra of sample at three consecutive levels used in the calibration model are shown in Figure 3.2.

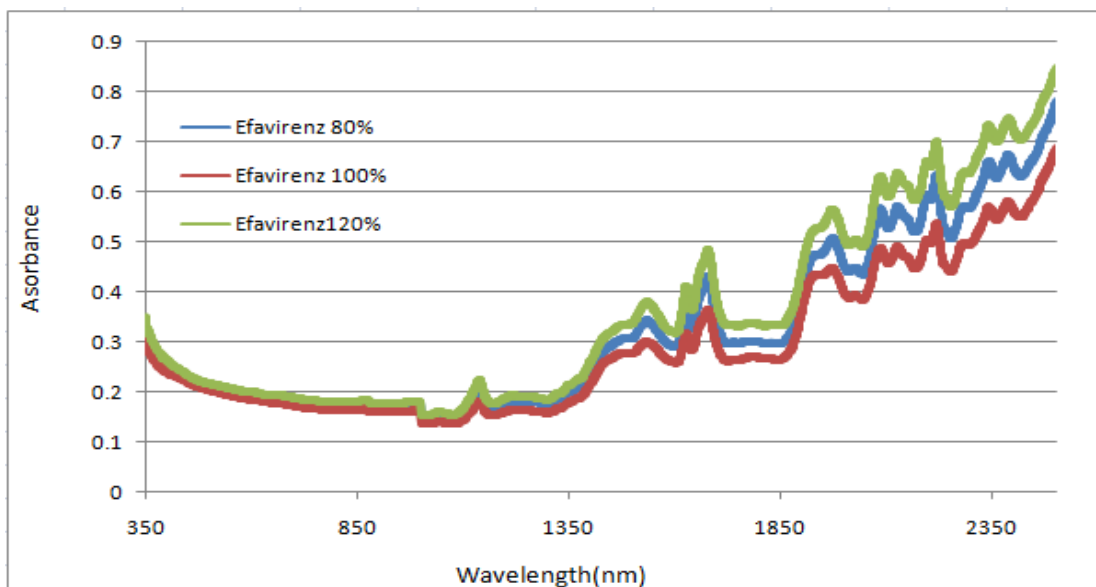


Figure 3.2: Reflectance spectra of efavirenz tablets at three consecutive levels

As shown in Figure 3.1 and figure 3.2, the strong bands for efavirenz molecule are present especially in the 1100-2500 nm range of the spectra because this is Near Infrared region whereby these bands are related to overtone and combination of fundamental vibration of –CH, -NH and -OH in Efavirenz molecule. This region was used for model development. The other portion which is the visible region showed very weak bands due to lack of overtone and fundamental vibration.

To make quantitative analysis using NIR spectroscopy, chemometric methods were used, which extracted the relevant information and reduced the irrelevant one ⁽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 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 the predicted concentrations were compared 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 ⁽²⁷⁾. 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 using the following criteria: the selected model is 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 ⁽²⁶⁾.

The NIR-chemometric model was developed taking into account all the samples of the three series of samples from the calibration matrix. The results obtained during the method development are presented in Table 3.1.

Table 3.1: Statistical parameters and number of principal components in the PLS method.

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

Concerning the results, the R² 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 R² values, the (c) and (g) model could not be chosen as the good model because (c) for instance had larger SECV value of 2.3619 and small R² value of 0.9751 and for (g) model had large SECV of 7.9910 and smaller R². Considering the SECV and R² for those model, together with number of factors, (b) model using Multiplicative Scattering Correction method, was chosen as the best fitted model for efavirenz quantification in tablets form, using the PLS algorithm.

Further tests were done for the model to check its predictability in accordance to the Mahalanobis distance (M-distance). When using this prediction, the M-distance value was the estimation of model performance that was seen with every prediction. I.e. If the M-distance value is less than 3.0 then the sample is represented in the calibration model. But if the M-distance value is greater than 3.0, this indicates that the sample is not well represented by the model^(28, 29). Table 3.2 shows the results of the validation sample as well as sample from other sources.

Table 3.2: Prediction report for laboratory sample and samples of efavirenz tablets from other manufacturer (Matrix tablet).

SAMPLE	NIR 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 on the Tables 3.1 and 3.2, Multiplicative Scattering Correction pre-treatment has been chosen as the best fitted model for Efavirenz tablet quantification, using a PLS algorithm and optimal number of factors as 5.

The plot of predicted concentrations versus actual concentrations for Efavirenz tablets is shown in Figure 3.3. The predicted values were obtained using the Multiplicative Scattering Correction pre-treatment method, PLS algorithm and 5 PLS factors chosen in the calibration model.

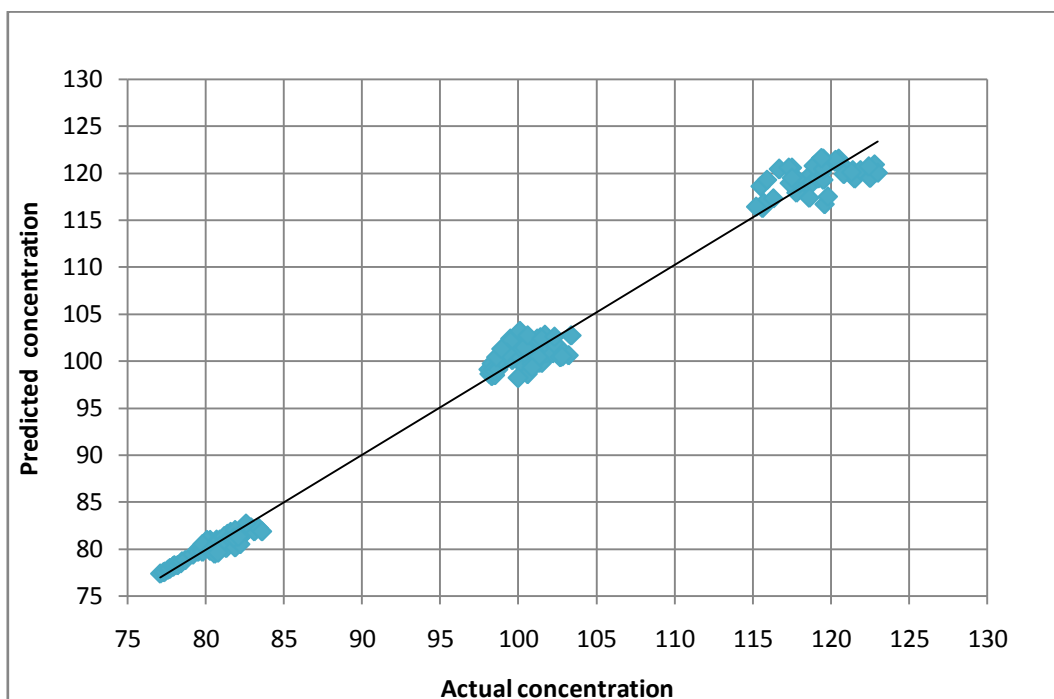


Figure 3.3: The plot of predicted concentrations versus the actual concentrations for Efavirenz tablets

This calibration model allowed for accurate prediction of efavirenz tablets with no adverse effect on the quantification process. The specification limit for acceptance of active principle in the formulation was $\pm 2\%$ of the nominal value, which is clearly larger than the prediction error obtained with the optimum calibration procedure for the production samples. Therefore, the proposed calibration procedure is precise enough for use as a control methodology for this particular pharmaceutical product.

3.2. The Identification Method For Zentrim[®] Tablets

A library consisting of 181 spectra for 60 tablets were compiled using correlation Spectral Matching method. An unknown sample was assumed to be positively identified if its correlation coefficient exceeded the established threshold ($\rho = 0.9994648$). If any sample surpassed such a threshold in the library, it was positively matched to that with the higher coefficient⁽²³⁾. Figure 3.4, the graph displaying the match value of reference library in a semi-logarithmic plot.

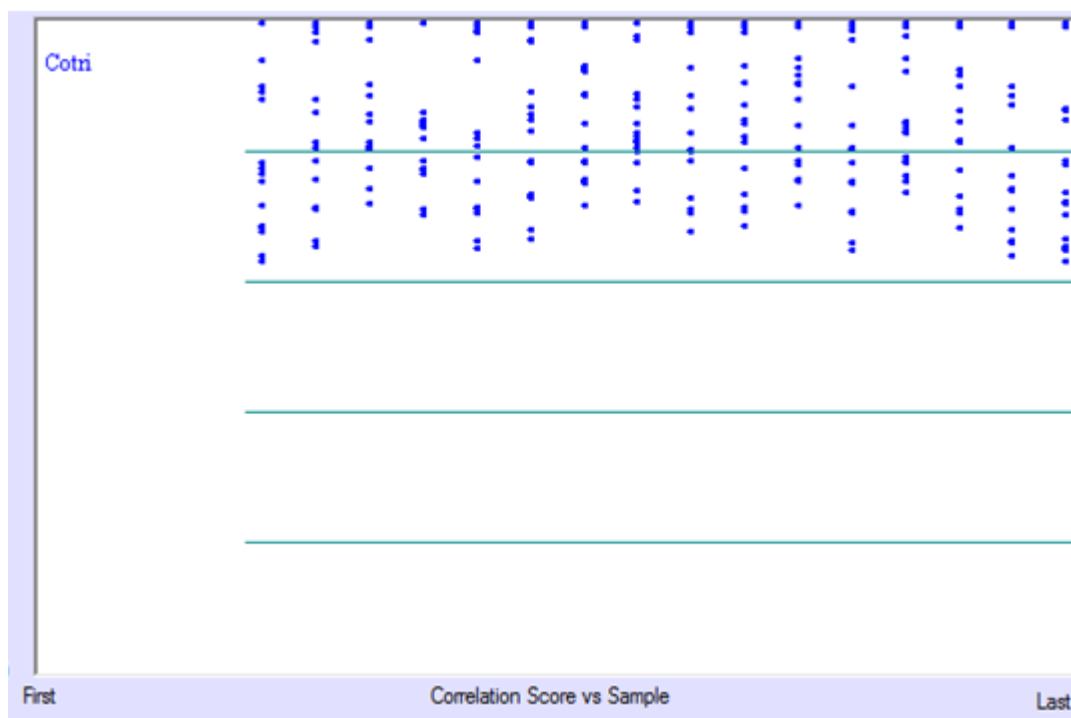


Figure 3.4: The graph displaying the match values of reference library

Each spectrum in the datafile has its own column. The above graph displays the representative spectrum for the entire datafile of the reference library.

3.2.1. Method Development and Validation

The development of the method involved trial and error methods which were proposed to reach to the ultimate threshold that would truly identify the product. The first threshold was set just below the lowest correlation coefficient i.e. 0.9998648 and it resulted into false negatives. The trial and error method continued by lowering the threshold and hence rendering more samples to be correctly identified. Tables 3.3, 3.4 and 3.5 provide the results of the trial and error method for the right threshold.

The trial and error method included both positive and negative control test at varied times, starting from 0 to 10 minutes in 5 minutes interval.

Table 3.3: The threshold set at 0.9998648 with the positive control product (Zentrim®)

PRODUCT	ZENTRIM					
BATCHES	2035		2049		2050	
TIME /min	SCAN #	RESULTS	SCAN #	RESULTS	SCAN #	RESULTS
0	5475	P	5478	P	5481	P
	5476	P	5479	P	5482	P
	5477	P	5480	P	5483	F
5	5484	P	5487	P	5490	F
	5485	P	5488	P	5491	F
	5486	F	5489	F	5492	F
10	5493	P	5496	P	5502	F
	5494	P	5497	P	5503	P
	5495	P	5498	P	5504	F

P- PASS
F- FAIL

The assigned threshold resulted into false negatives as it can be seen above, hence the threshold was lowered as shown in table 3.4 together with negative control i.e. alprim® and sheltrim®.

Table 3.4: The trial and error method at different setting of thresholds

	THRESHOLD	ZENTRIM®		ALPRIM®		SHELTRIM®	
		PASS	FAIL	PASS	FAIL	PASS	FAIL
1	0.9988648	9	0	6	3	0	9
2	0.9989648	9	0	3	6	0	9
3	0.9990648	9	0	1	8	0	9
4	0.9992648	9	0	1	8	0	9

From table 3.4, it showed that at the threshold of 0.9988648 all the positive control i.e. Zentrim® were positively identified but the negative control false positives were seen for Alprim®. At the threshold number 2 i.e. 0.9989648 all positive control were identified but negative control false positives were fewer compared to the threshold 0.9988648. The threshold number 3 and 4 i.e. 0.9990648 and 0.9992648, Zentrim® as positive control was positively identified and they both showed only one false positive on Alprim® as a negative control.

Finally, at the threshold of 0.9994648, all Zentrim® were positively identified and all negative control (Alprim® and Sheltrim®) were identified as false.

Table 3.5: The threshold was finally set at 0.9994648

PRODUCT	ZENTRIM [®]					
BATCHES	2035		2049		2050	
TIME	SCAN #	RESULT	SCAN #	RESULT	SCAN#	RESULT
0	6180	P	6183	P	6186	P
	6181	P	6184	P	6187	P
	6182	P	6185	P	6188	P
5	6195	P	6198	P	6202	P
	6196	P	6199	P	6203	P
	6197	P	6200	P	6205	P
10	6211	P	6214	P	6217	P
	6212	P	6215	P	6218	P
	6213	P	6216	P	6219	P
PRODUCTS	ALPRIM [®]			SHELTRIM [®]		
BATCHES	BN-2H160			BN 110012		
TIME	SCAN #	RESULT	SCAN #	RESULT		
0	6189	F	6192	F		
	6190	F	6193	F		
	6191	F	6194	F		
5	6205	F	6208	F		
	6206	F	6209	F		
	6207	F	6210	F		
10	6220	F	6223	F		
	6221	F	6224	F		
	6222	F	6225	F		
					P-PASS	
					F-FAIL	

The Table 3.5 above displays positive control i.e. zentrim[®] at various batches, all the positive control were positively identified and negative control gave false results. Based on the results presented in Table 3.5, the threshold of 0.9994648 has been chosen as the best fitted model for Zentrim tablet using Spectra Matching algorithm.

CHAPTER FOUR

4.0. CONCLUSION AND RECOMMENDATION

4.1. CONCLUSION

A simple, faster field adapted technology with no sample preparation method involving no destructive measurement for estimation of drugs for routine and real time analysis has been developed.

The quantitative analysis was done using Labspec spectrometer based on PLS algorithm and constructed by cross-validation. The quality of the results were estimated by various chemometric tools such as by comparing number of factors, coefficient of determination (R^2), SECV, Mahalanobis distance and various spectra pretreatment. The best model was selected based on the fact that the selected model is 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.

Hence the best fitted model for efavirenz quantification in tablets form was obtained by using Multiplicative Scattering Correction method which had R^2 of 0.9815, SECV of 2.0346 and a factor of 5 using the PLS algorithm. 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 analytical process involved the identification of the Zentrim[®] tablets and the estimation of amount of Efavirenz tablets using two different spectrometers. Identification process using handheld microPHAZIR relied on correlation Spectral Matching method. An unknown sample was assumed to be positively identified if its correlation coefficient exceeded the established threshold ($\rho = 0.9994648$). If any sample surpassed such a threshold in the library, it was positively matched to that with the higher coefficient. This procedure has been proved to have enough discrimination power to correctly identify Zentrim[®].

Henceforth, NIR method allows for the identification of zentrim[®] and also quantitative determination active substance efavirenz content in pharmaceutical tablet form without sample preparation. Thus, NIR-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 the process analytical technology (PAT), but can not replace all reference assay analyses because these methods are still needed to update and test the calibration model.

4.2. RECOMENDATION

The ICH guidelines were developed mainly for the validation of analytical procedure primarily based on the analyte being in solution but further studies can be implemented using NIR spectroscopy which would require ICH guidelines. Finally the developed method may be used in pharmaceutical preparations and pharmaceutical industries for routine screening, manufacturing and consistency assessment of the particular pharmaceuticals.

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