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Estimation of Stavudine, Lamivudine and Nevirapine by Chemometric UV Spectroscopic Method

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ABSTRACT

A chemometric UV- spectrophotometric method of analysis was successfully developed for the simultaneous determination of stavudine, lamivudine and nevirapine in pharmaceutical formulation. The application of multivariate method to the obtained spectrophotometric data is a new idea for the simultaneous quantitative analysis of stavudine, lamivudine and nevirapine in samples. Multivariate calibration models were built from the raw PLS(Partial least square) method. Verification of the calibration carried out with the aid of prediction set of mixtures of these three drugs produced satisfactory result. The results also showed that for practical purposes, PLS model constructed was effective in their performance prediction. The precision study showed that % RSD was within the range of acceptable limits, the mean recovery (mean ± SD) was found to be 100.24 ± 1.23, 100.52 ± 1.52, 99.72 ± 1.57 and 100.23 ± 1.55, 99.66 ± 1.03, 99.15 ±1.39 for stavudine, lamivudine and nevirapine for two different batches of the formulation respectively. The good recoveries obtained in this case proved that, the proposed procedures could be applied efficiently for determination of studied drug simultaneously in their mixture as well as in the commercial dosage form with satisfactory precision and accuracy.

Keywords: Antiretrovirals, chemometric, UV spectroscopy, stavudine, lamivudine, nevirapine

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) was first recognized by the medical community as a distinct clinical entity in 1981.The most effective means for accomplishing durable suppression of HIV replication is simultaneous initiation of combinations of effective anti-HIV drugs. Current recommendations for initial treatment of HIV infection advocate a minimum of three active antiretroviral agents: two Nucleoside Reverse Transcriptase Inhibitors and either a Ritonavir-boosted Protease Inhibitor or Non-Nucleoside Reverse Transcriptase Inhibitors. Evolution of triple-therapy regimens showed superior virologic efficacy.[1,2]

The multi-component formulations have gained a lot of importance recently. One of the most exploited quantitative procedures for analysis of multi-component formulations is the simultaneous spectrophotometric method, through other hard modeling techniques are also being used for the assay of substance/s in multicomponent samples by spectrophotometry.[3-5]

All these methods fail to use the entire information obtained in the spectrophotometric method of analysis. Only the method that stores the whole spectral data of standard substance and uses the algorithm matching the absorption spectrum of the sample with the spectrum obtained mathematically by adding up the individual spectra of components makes a full use of the information load of the spectrophotometric method. This is also the operating principle of advanced design UV-Vis spectrophotometers equipped with multi- component analysis program.[3-4]

The application of chemometrics which are essentially a self modeling technique allows the interpretation of multivariate data to predict the concentration of each drugs in a mixture. The well known multivariate analysis methods are statistical tools involving factor analysis and have many of the full spectrum advantages for the analysis which can provide significant improvements in precision over methods that are restricted to a small number of wavelengths, since there is signal averaging effect when many or all the spectral intensities are included in the analysis. It concentrated on researching a simple analytical UV- spectroscopic procedure, the results of which could be interpreted with the aid of readily accessible chemometrics methods namely;[6]

- Classical least squares (CLS),
- Principal component regression (PCR) and
- Partial least squares (PLS)

Chemometric Analysis for Spectroscopy [7-12]

Multivariate analysis is a chemometric technique to improve the understanding of chemical information and to correlate quality parameters or physical properties to analytical instrument data.

Most chemical measurements are multivariate. This means

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that more than one measurement can be made with a single sample. An example is spectroscopy where light absorption at hundreds of wavelengths using a single sample can be measured, which leads to very complex spectra containing a lot of information.

In order to extract the information hidden in the complex spectra, we need a computational rather than an analytical approach, when more than one light absorbing components are present in a mixture. This means that we cannot directly compare absorption at a certain wavelength with a certain material property. This is because the composition of the material leads to co- absorption at many different wavelengths in the spectrum, which consists of a large number of overlapping bands. Therefore, we compare the absorption at many different wavelengths (actually, we usually take a whole spectrum) with the property of interest, concentration in the present case.

Both PCR and PLS are factor based methods and involve spectral decomposition. PLS has proven to be a popular and effective approach to predict concentration of different chemicals in a mixture from spectra of unknown samples.

The main advantages of the use of multivariate calibration techniques are fast, cheap and non-destructive analytical measurements can be used to estimate sample concentration which would otherwise require time-consuming, expensive or destructive techniques such as HPLC.

Materials and methods

Stavudine was generously gifted by Matrix Laboratories, Hyderabad, Lamivudine and Nevirapine were gifted by Hetero Drugs Ltd, Hyderabad. All the chemicals and reagents were of analytical grade.

Preparation of standard solutions for linear calibration models

Stavudine/Lamivudine/Nevirapine standard stock solutions

About 25.0 mg of each stavudine, lamivudine and nevirapine working standards were accurately weighed, transferred to three separate 25 ml volumetric flask and dissolved in methanol. The volume was made up to the mark to obtain the final concentration of 1000.0 µg/ml of each stavudine, lamivudine and nevirapine.

The above standard stock solutions stavudine; lamivudine and nevirapine were used to prepare the linearity solutions of desired concentration of 2.0 - 8.0 µg/ml, 12.0 - 18.0 µg/ml, 14.0 - 26.0 µg/ml for stavudine, lamivudine and nevirapine respectively by suitable dilution with methanol. A calibration curve was prepared by plotting absorbance vs concentration at their respective $\lambda_{max.}$

Preparation of standard solutions for multivariate analysis

To develop the Chemometric multivariate calibration, 192 preparations (calibration samples) of standard solutions containing mixture of stavudine, lamivudine and nevirapine were prepared in separate 10 ml flasks by adding appropriate volumes of suitably diluted standard stock solutions in methanol to final concentration in the ranges between 0.0 - 8.0 µg/ml for stavudine, 0.0 - 18.0 µg/ml for lamivudine and 0.0 - 26.0 µg/ml for nevirapine respectively, given in table no 1.

Multivariate calibration models for the analysis of drugs

A multivariate calibration model for the analysis of individual drugs was carried out using UV- Visible spectrophotometer model (UV-1800 Shimadzu Corporation, Kyoto, Japan). Absorption spectrum was recorded for each solution between 240 and 292 nm at 2 nm intervals with methanol as blank. The optimum number of factors used in the PLS algorithm is an important parameter towards obtaining the best prediction performance. This means modeling the system with the optimum amount of information and avoiding over fitting or under fitting. (Overfitting and under fitting means that the defined model must neither overestimate nor underestimate values, and is made by fitting constants). Cross-validation procedures were applied to calculate the number of factor which gives as much as possible information from the absorbance data to measure the property of interest (concentration). Thus the range between 240 - 292 nm was selected as all the three drugs shows closely overlapping bands and optimum number of factors was extracted.

The absorbance data matrices were constructed from the raw spectra containing absorbance values at wavelengths ranges between 240 and 292 nm at 2 nm intervals. The spectrum of the single sample consists of the individual absorbance value for each wavelength at which the sample was measured. Generally MLR techniques (PLS and PCR) employ data that is organized as matrices either as row vector or column vector.

Data organization for PLS:

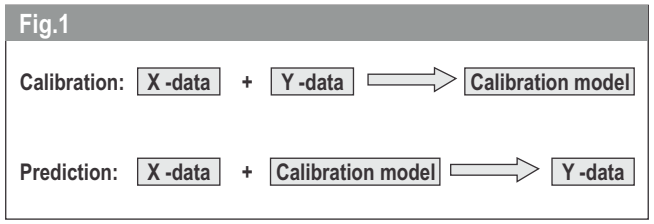
In order to find a useful relation between the two matrices it is necessary e.g. to determine the regression relationship between the two data sets. The process requires the two data sets containing 'known data', also referred to as the training set, to make a model of the relationship. This process is called calibration. Multivariate calibration techniques are then used to construct a mathematical model (calibration model) that relates the multivariate response (spectrum) to the concentration of the analyte of interest, and such a model can be used to efficiently predict 'unknown' Y-values from the measurements of new X-variables. Both calibration and prediction is schematized as follows:

Table 1: Composition of the calibration samples for PLS

Samples	Concentration (µg/ml)																							
	Set A			Set B			Set C			Set D			Set E			Set F			Set G			Set H		
	ST	LM	NV	ST	LM	NV	ST	LM	NV	ST	LM	NV	ST	LM	NV	ST	LM	NV	ST	LM	NV	ST	LM	NV
1	0	0	0	2	0	0	3	0	0	4	0	0	5	0	0	6	0	0	7	0	0	8	0	0
2	0	12	14	2	12	14	3	12	14	4	12	14	5	12	14	6	12	14	7	12	14	8	12	14
3	0	13	16	2	13	16	3	13	16	4	13	16	5	13	16	6	13	16	7	13	16	8	13	16
4	0	14	18	2	14	18	3	14	18	4	14	18	5	14	18	6	14	18	7	14	18	8	14	18
5	0	15	20	2	15	20	3	15	20	4	15	20	5	15	20	6	15	20	7	15	20	8	15	20
6	0	16	22	2	16	22	3	16	2	24	16	22	5	16	22	6	16	22	7	16	22	8	16	22
7	0	17	24	2	17	24	3	17	24	4	17	24	5	17	24	6	17	24	7	17	24	8	17	24
8	0	18	26	2	18	26	3	18	26	4	18	26	5	18	26	6	18	26	7	18	26	8	18	26
9	0	0	0	0	12	0	0	13	0	0	14	0	0	15	0	0	16	0	0	17	0	0	18	0
10	2	0	14	2	12	14	2	13	14	2	14	14	2	15	14	2	16	14	2	17	14	2	18	14
11	3	0	16	3	12	16	3	13	16	3	14	16	3	15	16	3	16	16	3	17	16	3	18	16
12	4	0	18	4	12	18	4	13	18	4	14	18	4	15	18	4	16	18	4	17	18	4	18	18
13	5	0	20	5	12	20	5	13	20	5	14	20	5	15	20	5	16	20	5	17	20	5	18	20
14	6	0	22	6	12	22	6	13	22	6	14	22	6	15	22	6	16	22	6	17	22	6	18	22
15	7	0	24	7	12	24	7	13	24	7	14	24	7	15	24	7	16	24	7	17	24	7	18	24
16	8	0	26	8	12	26	8	13	26	8	14	26	8	15	26	8	16	26	8	17	26	8	18	26
17	0	0	0	0	0	14	0	0	16	0	0	18	0	0	20	0	0	22	0	0	24	0	0	26
18	2	12	0	2	12	14	2	12	16	2	12	18	2	12	20	2	12	22	2	12	24	2	12	26
19	3	13	0	3	13	14	3	13	16	3	13	18	3	13	20	3	13	22	3	13	24	3	13	26
20	4	14	0	4	14	14	4	14	16	4	14	18	4	14	20	4	14	22	4	14	24	4	14	26
21	5	15	0	5	15	14	5	15	16	5	15	18	5	15	20	5	15	22	5	15	24	5	15	26
22	6	16	0	6	16	14	6	16	16	6	16	18	6	16	20	6	16	22	6	16	24	6	16	26
23	7	17	0	7	17	14	7	17	16	7	17	18	7	17	20	7	17	22	7	17	24	7	17	26
24	8	18	0	8	18	14	8	18	16	8	18	18	8	18	20	8	18	22	8	18	24	8	18	26

ST-Stavudine LM-Lamivudine NV-Nevirapine

Fig.1



The prior aim to calibrate is to determine a function f ( ) that allows quantitative predictions of Y (e.g. one or more concentrations) from X (e.g. measured absorbances):

$Y\hat{} = f(X)$

where $Y\hat{}$ is a matrix with the predicted Y-values and X are the predictor variables.

Fig. 2. Strategic approach in Chemometric Multivariate Analysis

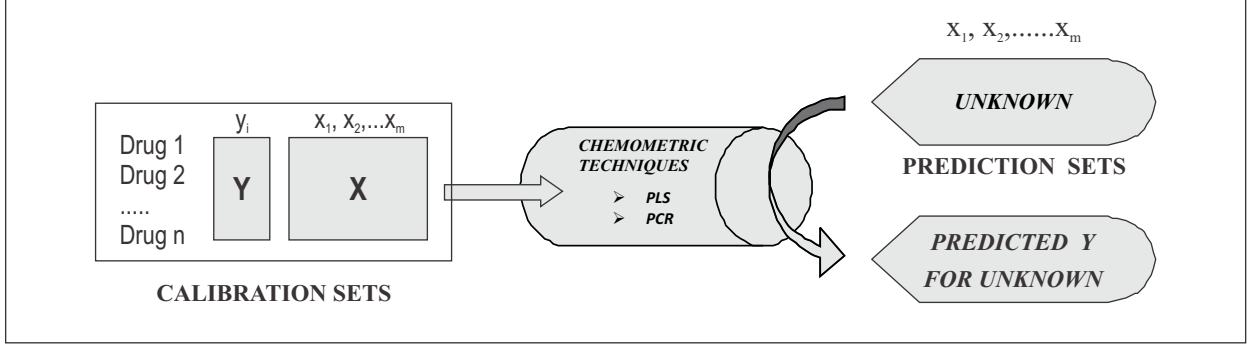
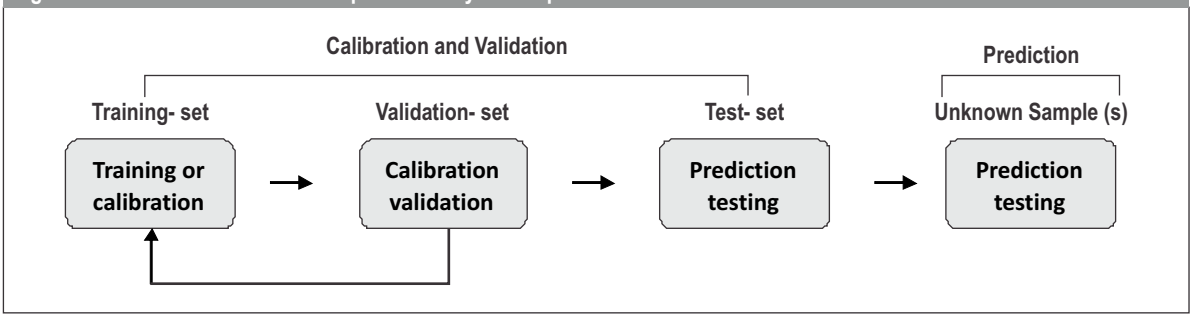


Fig. 3: Calibration and validation steps necessary before prediction.



Absorbance matrix:

Each spectrum is placed into absorbance matrix as a row vector. This is illustrated in data set X shown below, containing absorbance data of a drug samples. The data set consists of a number of objects (drug samples). Each object is a set of values such as absorbance, measured at different variables (wavelengths). In this data set each line represents one object and each column represents one variable.

Data set X containing spectroscopic data (Absorbances)	
Samples	**wavelengths**
	λ_1 λ_2 λ_3 λ_4.....
1	x_{11} x_{12} x_{13} x_{14}
2	x_{21} x_{22} x_{23} x_{24}
3	x_{31}....
.....	

For example the data set X (absorbance matrix, $X_{n\times p}$) in the matrix form would look like:

$$X = \begin{pmatrix} X_{11} & X_{12} & X_{13} & X_{14} & ....X_{1p} \\ X_{21} & X_{22} & X_{23} & X_{24} & \\ X_{31} & .... & & & \\ X_{n1} & X_{n2} & X_{n3} & X_{n4\ .....} & X_{ny} \end{pmatrix} \quad n \times p$$

Where

p = number of wavelengths.

x = absorbances measured at p wavelengths.

n = standard solution mixtures.

m = number of analytes.

$$C = \begin{pmatrix} C_{11} & C_{12} & C_{13} & C_{14} & ....C_{1m} \\ C_{21} & C_{22} & C_{23} & C_{24} & \\ C_{31} & .... & & & \\ C_{n1} & C_{n2} & C_{n3} & C_{n4\ .....} & C_{nm} \end{pmatrix} \quad n \times m$$

Taken together, the concentration matrix and absorbance matrix comprises a data matrix. It is essential that the columns of the absorbance and the concentration matrices correspond to the same mixtures.

In the proposed study 192 samples were measured at 27 different wavelengths, each spectra would hold in a row vector containing absorbance values. These 192 rows assembled into an absorbance matrix which would be 192 × 27 in size (192 rows, 15 columns).

Preparation of Quality Control (QC) samples

Standard stock solutions were prepared by dissolving 10.0 mg of each of stavudine, lamivudine and nevirapine separately in 100 ml of methanol to obtain the final concentration of 100.0 µg/ml for each of the three drugs. From this stock, different dilutions were made with methanol to obtain the final concentrations of 3.0, 4.0 or 5.0 µg/ml for stavudine, 12.0, 15.0 or 18.0 µg/ml for lamivudine and 16.0, 20.0 or 24.0 µg/ml for nevirapine. QC samples at three different levels of HQC [high quality control (5.0, 18.0 24.0 µg/ml)], MQC [medium quality control (4.0, 15.0, 20.0 µg/ml)], LQC (low quality control, 3.0, 12.0, 16.0 µg/ml)] for stavudine, lamivudine and nevirapine respectively, were selected to perform different validation parameters.

Precision and Reproducibility of method

The intra-day, inter-day, analyst to analyst variation, reproducibility of the method and accuracy were determined by assaying three QC samples (LQC, MQC and HQC) in replicates (n = 6) for each drug. Precision is reported as percent relative standard deviation (%RSD).

Intermediate precision expresses within laboratory variations: different days, different analysts and different equipments. The procedure followed for method precision was repeated on two different days, by two different analysts, using two different systems. Six sets of standard solution each containing stavudine, lamivudine and nevirapine for evaluation of intermediate precision were prepared using concentration at three levels (LQC, MQC and HQC) and each concentration level was analyzed six times.

Validation / prediction sets (Percent recovery study)

It is highly desirable to assemble an additional data set containing independent measurements on samples that were independent from the samples used to create the training set. Samples held in reserve this way are called as validation samples. The pair of absorbance and concentration matrices holding these data is known as validation set. We treat the validation samples as if they are unknown. The calibration developed with the training set was used to predict the concentrations of the validation samples. Then the predicted concentrations were compared with the actual concentrations to assess the expected performance of the calibration on actual unknowns.

A validation set of 21 mixtures were prepared at three different levels 80%, 100%, and 120% of assay concentration [100% level in stavudine 4.0µg/ml , lamivudine 15.0µg/ml and nevirapine 20.0µg/ml] from stock standard solution in methanol. Percent recovery was calculated for each of the mixture.

The validation was performed on prediction sets containing 21 different ternary mixtures of stavudine, lamivudine and nevirapine. The recovery percentage was also calculated for each component. For statistical comparison, t test was applied to the obtained results for the significance of difference between known concentrations of all tested samples in prediction sets with concentrations predicted in constructed models.

A validation set of 21 mixtures were prepared at three different levels 80 %, 100 %, and 120 % of assay concentration [stavudine (4.0 µg/ml), lamivudine (15.0 µg/ml) and nevirapine (20.0 µg/ml)] by transferring appropriate volumes of the working solutions into 10 ml flask and made up the final volume with methanol. Percent recovery was calculated for each of the mixture.

Analysis of pharmaceutical preparation

Two different batches of formulation were analyzed using the validated method. Twenty tablets were weighed and average weight was calculated. The tablets were ground and finely powdered in a mortar. An amount equivalent to 40.0 mg of stavudine, 150.0 mg lamivudine and 200.0 mg of nevirapine; was dissolved in methanol in 100 ml volumetric flasks. The solutions were sonicated for 20 min, made to the mark with methanol, and filtered. The first portion of filtrate was discarded. The obtained clear solution was used as stock sample solution. The stock solution was further diluted quantitatively with methanol to obtain the working concentration range of 4.0 µg/ml stavudine, 15.0 µg/ml lamivudine and 20.0 µg/ml nevirapine in a 10 ml volumetric flasks and volume was made up with methanol.

Stability

The stability of the drugs was evaluated under the conditions likely to be encountered during actual sample handling and analysis. The QC samples were kept to evaluate bench top stability and long term storage stability at refrigerated condition (2 - 8ºC). For each concentration and storage condition three replicates were analyzed. The concentration of stavudine, lamivudine and nevirapine after each storage period was related to the initial concentration as determined for the samples that were freshly prepared and processed immediately. The result of % change in the concentration of the samples after each storage period with respect to initial concentration was calculated.

RESULTS AND DISCUSSION

The present study was aimed at developing and validating a simple chemometric based analytical method for stavudine, lamivudine and nevirapine in tablet dosage form.

Standard calibration samples with different concentrations for each compound were prepared and the absorbances were measured at 265 nm, 272 nm and 282 nm for the drugs i.e. stavudine, lamivudine and nevirapine respectively. Parameters of the linear equations are summarized in Table 2, and for all the compounds, there was a high correlation between measured absorbance and concentration.

The PLS technique was typical full-spectrum method, more powerful than the ones based on the measurement at only one wavelength, such as direct spectrophotometry because the simultaneous inclusion of spectral intensities at multiple wavelengths can greatly improve the precision and applicability of quantitative spectral analysis of mixtures. The first step in simultaneous determination of stavudine, lamivudine and nevirapine in mixtures by multivariate methods involved constructing the calibration matrix. Here calibration was performed with the absorption spectra. The

Table 2: Parameters of linear regression equations for Stavudine, Lamivudine and Nevirapine

Parameters	Stavudine	Lamivudine	Nevirapine
Sample number (n)	7	7	7
Wavelength used (nm)	265	272	282
Linear range (µg/ml)	2.0 – 8.0	12.0 – 18.0	14.0 – 26.0
Correlation of coefficient	0.9992	0.9998	0.9997

absorbance data matrix for the training set was obtained by recording the absorbance within the wavelength range 240-292 nm at 2 nm intervals. In order to select the mixtures that provide more information from calibration set, their composition was randomly designed.

Eight sets of standard solutions (calibration sets) contained 192 standard solutions for determination were prepared (Table-1), so that the concentration of each drug in resulting solutions was in its own linear dynamic range.

Data processing and model building

The digitized absorbance of calibration mixtures was gathered in a 192 × 27 data matrix(Y) and absorbances of prediction mixtures were collected in a 18 × 27 data matrix (Y_{unk}). PLS method was run on the calibration data of absorption UV spectra and concentrations in prediction sets were calculated at the optimum number of factors with the help of "SAS 9.1" software. The multivariate calibrations were computed with the PLS algorithms using the correlation for the absorbance data matrix and the corresponding concentration data matrix of calibration (training set). In order to validate proposed method 21 synthetic mixture containing stavudine, lamivudine and nevirapine, were prepared and analyzed with the proposed method. Predicted concentrations obtained by application of PLS method for simultaneous determination of stavudine, lamivudine and nevirapine in mixtures are given in Table 3. The agreement between experimental and prediction values by the method is good. In this model, known concentrations of all tested samples in predicted sets were compared with the predicted concentrations by constructed models and equations; very good agreement(as p value at 99 % & 95 % CI was found to be insignificant) between actual and predicted values for all components are represented. The percentage recoveries were also calculated for each component by application of PLS UV absorption spectra in mixtures; then mean recovery was calculated. According to the t-test, all the calculated t-values were smaller than the theoretical t-values at the 95% & 99% CI. This indicates that there is no significant difference between the performances of the used technique with regards to the mean values. These results testify the high potential of PLS method for multi-component determinations even in the presence of strong spectral overlap between the analytes; and, allows the effective resolution of mixture of stavudine, lamivudine and nevirapine. However, PLS calibration can implicitly model some interference, whenever the calibration solutions and samples have similar compositions and interferences are included in variable concentrations in the calibration sets. This allows the multi-component determination without prior separation and makes the method more robust

Table 3. Predicted concentrations in synthetic mixture obtained by application of PLS method

Set No.	Composition prediction set (µg/ml)			Predicted concentration obtained by application of PLS method (µg/ml)			% Recovery		
	ST	LM	NV	ST	LM	NV	ST	LM	NV
1	2.94	12.0	16.0	2.96	11.97	16.0	100.68	99.75	100.0
2	3.92	15.0	20.0	4.02	14.87	20.06	102.5	99.13	100.3
3	4.9	18.0	24.0	4.99	17.92	23.97	101.8	99.55	99.87
4	3.99	14.98	19.98	3.94	14.80	20.64	98.74	98.79	103.3
5	6.93	26.98	35.98	6.9	26.77	36.64	99.56	99.22	101.8
6	7.91	29.98	39.98	7.96	29.67	40.7	100.63	98.96	101.8
7	8.89	32.98	43.98	8.93	32.72	44.61	100.44	99.21	101.4
8	2.99	11.97	15.94	2.94	11.90	15.97	98.32	99.41	100.18
9	3.99	14.97	19.92	4.0	14.98	19.97	100.25	100.06	100.25
10	4.99	17.96	23.90	4.96	17.89	23.94	99.39	99.61	100.16
11	3.98	14.94	19.92	3.92	14.84	20.19	98.4	99.33	101.35
12	6.97	26.91	35.8	66.86	26.74	36.16	98.4	99.36	100.8
13	7.97	29.91	39.8	47.92	29.82	40.16	99.37	99.96	100.8
14	8.97	32.9	43.8	28.88	32.73	44.13	98.99	99.48	100.7
15	3.01	11.95	15.9	63.0	11.82	16.0	99.66	98.91	100.25
16	4.01	14.93	19.9	63.94	14.87	19.94	98.25	99.59	99.89
17	5.01	17.92	23.95	4.993	17.97	24.01	99.60	100.27	100.25
18	3.99	14.99	19.9	73.92	14.87	20.17	98.24	99.19	101.0
19	7	26.94	35.9	36.92	26.69	36.17	98.85	99.07	100.66
20	8	29.92	39.9	37.86	29.74	40.11	98.25	99.39	100.45
21	9	32.91	43.92	8.91	32.84	44.18	99.0	99.78	100.59
	Mean						**99.37**	**99.34**	**100.67**

ST – Stavudine, LM – Lamivudine, NV – Nevirapine

Method precision

The method precision of the method is expressed as %RSD and it is found to be 0.44 for stavudine, 0.21 for lamivudine and 0.57 for nevirapine respectively, results are tabulated in the Table 4.

Intermediate precision (Ruggedness)

The intermediate precision of the method is expressed as %RSD and it is found to be 0.75 and 1.02 for stavudine, 0.83 and 0.93 for lamivudine and 0.37 and 0.92 for nevirapine respectively in case of inter-day and analyst to analyst variation respectively.

Reproducibility

Reproducibility of the method is expressed as %RSD and it is found to be 1.28 for stavudine, 1.54 for lamivudine and 1.71 for nevirapine respectively.

Accuracy

Accuracy was carried out at 3 different concentrations. Accuracy was expressed by percentage recovery. By means of addition of stavudine, lamivudine and nevirapine reference

Table 4: Results of method precision by chemometrics

Set No.	Stavudine % Recovery	Lamivudine % Recovery	Nevirapine % Recovery
1	99.96	100.30	98.94
2	99.31	100.49	99.94
3	100.40	100.06	100.37
4	100.49	100.11	98.81
5	100.34	99.96	99.98
6	100.24	100.50	99.64
Mean	**100.12**	**100.24**	**99.61**
% RSD	**0.44**	**0.21**	**0.57**

standards to the sample solution, mean percentage recoveries were calculated. To a preanalysed sample mixture, 3 µg/ml, 4 µg/ml and 5 µg/ml of stavudine, 12 µg/ml, 14 µg/ml and 18 µg/ml of lamivudine and 16 µg/ml, 20 µg/ml and 24 µg/ml of nevirapine were added and recovery was calculated by the proposed method, results are shown in table 5-7.

Analysis of formulations

The developed chemometric method was applied to the determination of stavudine, lamivudine and nevirapine in pharmaceutical preparation Triomune Tablets. Satisfactory

Table 5: Accuracy of the assay method for Stavudine (Batch A and B)

	Amount added (µg/ml)		Amount found (µg/ml)		% Recovery		Mean% Recovery± SD	
	A	B	A	B	A	B	A	B
1	2.96	3.0	2.94	2.96	99.65	98.72	100.65 ± 0.89	100.08 ± 1.34
2	2.94	3.0	2.96	3.0	100.92	100.11		
3	3.00	3.0	3.04	3.04	101.39	101.40		
4	4.02	3.97	3.97	3.91	98.55	98.53	98.96 ± 1.02	98.73 ± 0.50
5	4.00	3.94	3.93	3.91	98.21	99.30		
6	3.94	3.99	4.06	3.92	100.10	98.35		
7	4.99	5.01	5.03	5.10	100.69	101.92	101.12 ± 0.59	101.91 ± 0.24
8	4.96	4.97	5.05	5.07	101.80	102.15		
9	4.99	4.93	5.04	5.01	100.88	101.66		
Across all levels							**100.24 ± 1.23**	**100.23 ± 1.55**

Table 6: Accuracy of the assay method for Lamivudine (Batch A and B)

	Amount added (µg/ml)		Amount found (µg/ml)		% Recovery		Mean% Recovery± SD	
	A	B	A	B	A	B	A	B
1	11.97	11.97	12.13	11.84	101.38	98.95	101.82 ± 0.43	99.56 ± 0.85
2	11.90	11.97	12.16	12.03	102.26	100.54		
3	11.82	11.90	12.03	11.88	101.81	99.90		
4	14.87	14.8	14.95	14.60	100.54	98.71	99.15 ± 1.27	99.90 ± 1.27
5	14.98	14.87	14.81	14.83	98.83	99.76		
6	14.87	14.97	14.58	15.15	98.08	101.24		
7	17.92	17.90	17.74	17.69	99.03	98.84	100.62 ± 1.49	99.29 ± 1.29
8	17.89	17.93	18.04	17.62	100.84	98.29		
9	17.97	18.01	18.33	18.14	102.01	100.73		
Across all levels							**100.53 ± 1.52**	**99.66 ± 1.03**

Table 7: Accuracy of the assay method for Nevirapine (Batch A and B)

	Amount added (µg/ml)		Amount found (µg/ml)		% Recovery		Mean% Recovery± SD	
	A	B	A	B	A	B	A	B
1	16.0	15.93	15.98	15.81	99.90	99.27	101.14 ± 1.11	100.50 ± 1.06
2	15.97	16.01	16.19	16.17	101.44	101.00		
3	16.0	15.90	16.33	16.09	102.09	101.22		
4	20.06	20.01	19.91	19.99	99.21	99.95	99.63 ± 1.48	98.72 ± 1.38
5	19.97	19.87	19.65	19.65	98.41	98.94		
6	19.94	19.93	20.19	19.38	101.27	97.26		
7	23.97	23.90	23.79	23.44	99.28	98.11	98.38 ± 0.81	98.24 ± 0.67
8	23.94	23.85	23.38	23.60	97.68	98.94		
9	24.01	24.01	23.57	23.44	98.19	97.65		
Across all levels							**99.72 ± 1.57**	**99.15 ± 1.39**

Batch A and B are the different pre analyzed commercial tablets.
Amount added is the concentration of reference sample solution added to the pre analyzed solution.

Table 8: Analysis of formulations by chemometric method

	Label Claim (mg)	Chemometric Method % Label claim (Mean ± S.D.)	% RSD
Batch - A			
Stavudine	40	98.96±1.01	1.02
Lamivudine	150	99.15±1.26	1.27
Nevirapine	200	99.63±1.48	1.47
Batch - B			
Stavudine	40	98.73±1.50	1.52
Lamivudine	150	99.90±1.27	1.27
Nevirapine	200	98.72±1.38	1.36

† Average of six determinations

results were obtained in good agreement with the label claim. The calculated statistical test value did not exceed the theoretical statistical value at 95 % and 99 % confidence level, indicating that there was no significant difference among the methods with regard to the mean value and their SD (table 8).

Stability:

Three replicates of standard preparation were analyzed initially and then after at regular interval up to 48 h at bench top and at refrigerated condition (2 - 8ºC). The % difference in the standard preparation was compared with the initial concentration, and there was no much difference in the initial concentration and concentration after 48 h. (table 9).

CONCLUSION

Conventional multi component UV spectroscopic methods are not suitable for combination drugs having narrow difference in λmax. In such cases, chemometry serves as an alternative to other sophisticated methods like HPLC. Once the calibration matrix is built and stored in the data computation device, the samples can simply be prepared, diluted and absorbance measured and concentration of the sample read from the stored matrix. This will reduce the time consumed for routine analysis in tablets like content uniformity, dissolution testing, in process quality control testing etc. As the total absorbance of the combination is read, this method is also more accurate and precise than conventional UV methods.

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Table 9: Results of Stability analytical standard solution

	Stavudine		Lamivudine		Nevirapine	
Time(H)	**Conc. (µg/ml)**	**% Difference**	**Conc. (µg/ml)**	**% Difference**	**Conc. (µg/ml)**	**% Difference**
Bench Top Stability						
Initial	4.04	-	14.88	-	20.07	-
3	4.05	0.25	14.97	0.60	20.02	-0.24
6	3.99	-1.24	15.04	1.08	20.00	-0.35
18	4.01	-0.74	15.07	1.30	20.11	0.19
24	4.05	0.17	14.72	-1.07	20.28	1.05
42	4.05	0.24	14.78	- 0.67	20.42	1.74
48	4.06	0.49	14.85	- 0.20	20.20	0.65
Long Term Stability(2-8ºC)						
Initial	4.04	-	14.88	-	20.07	-
12	4.07	0.74	14.95	0.47	20.1	0.15
24	4.0	-0.9	14.77	-0.74	19.97	-0.5
48	4.08	0.99	14.74	-0.94	19.85	-1.09
After 12 days	4.11	1.73	14.83	-0.33	19.79	-1.4

http://www.camo.com/.../Chemometric%20Analysis%20for%20Spectroscopy.pdf

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Concentration matrix:

Similarly concentration matrix holds the concentration data. The concentration of the component for each sample was placed into the concentration matrix as a column vector.