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Synchronized Spectrophotometric Determination of Tenofovir & Zidovudine in Tablets by Chemometric Approaches

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ABSTRACT

Simultaneous spectrophotometric determination of Tenofovir and Zidovudine was performed by partial least squares (PLS) and principal component regression (PCR) methods do not require any prior graphical treatment of the overlapping spectra of two drugs in the mixture. The absorbance values in the UV- Vis spectra were measured for the wavelength points (from 210 to 330) in the spectral region 200–400 nm considering the intervals of 1 nm. The calibration range was found to be 1-5 µg/ml for Tenofovir, 2-10 µg/ml for Zidovudine with a correlation coefficient of 0.9999 (PLS) , 0.9999 (PCR) for Tenofovir and 0.9999 (PLS), 0.9999 (PCR) for Zidovudine. The validation of the multivariate methods was realized by analyzing the synthetic mixtures of Tenofovir and Zidovudine. The numerical calculations were implemented with 'Unscrambler 10.1 X' software. The Chemometrics analysis methods were satisfactorily applied to the simultaneous determination of Tenofovir and Zidovudine in the pharmaceutical formulation.

Keywords: *Tenofovir, Zidovudine, Chemometrics, spectrophotometry, partial least square, principal component regression.*

I. INTRODUCTION

Chemometrics may be described as the application of mathematical and statistical tools to design and optimize the methods to provide chemical information by analyzing relevant data.

For a successful design the following procedure should be adopted:

- Consider what results are required and what variables (factors) may affect the outcome of the experiment whose results have to be evaluated

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- Select an appropriate design to solve the problem
- Carryout the experimental work
- Examine the data thus obtained, separating the important variables from those of little significance and deciding if other techniques may produce useful information

Designs can be divided into two broad classes

I. Simultaneous designs mean that all experiments are completed before the analysis of the data is carried out

II. Sequential designs mean that the results from the previous experiments determine the conditions for the next experiment

In analytical chemistry, chemometrics techniques have its applications in spectroscopy like UV-Visible spectrophotometry, NMR spectroscopy, fluorescence spectroscopy, chromatography like Liquid Chromatography and also various other aspects of analytical chemistry like flow-injection analysis in pharmaceutical preparations. Chemometrics uses multivariate methods hence it offers many advantages in qualitative as well as quantitative spectroscopic analysis. The name chemometrics can be divided into two terms namely chemo (from chemistry) and metric (meaning measurement). The aim of this paper is to investigate the ability of PLS and PCR Methods to quantify binary mixture of Tenofovir and Zidovudine with overlapping spectrum and to apply the optimized method in pharmaceutical preparations.

II. MATERIAL AND METHODS

Instruments and software:

Digitized UV/VIS absorbency spectra were collected using a UV- visible spectrometer Perkin-Elmer Lambda 25 with 1 cm quartz cells. The data acquisition was made with UV solutions software at a scan rate of 1000 nm min−1 and the slit width of 2 nm. The UV spectra of mixtures were recorded over the wavelength 220–300 nm with one data point per nm. All spectral measurements were performed using a blank solution as a reference. Partial least squares regression and principal component regression were used for chemometric analysis of data. For all calculations, Unscrambler for windows (Version10.1 X) was used.

Pharmaceutical tablet formulations:

A commercial pharmaceutical formulation (TAVIN-L) tablet containing 300 mg of TENO and 150 mg of ZIDA was analyzed by the proposed chemometric methods.

Standard solutions:

Stock solutions of Tenofovir and Zidovudine of 10 mg were prepared in 100 ml volumetric flasks with

methanol. The training set containing 1-5µg/ml Tenofovir and 2-10µg/ml a Zidovudine working standard solutions were prepared by diluting the stock solutions for each drug according to its linear calibration range. Two sets of standard solutions were prepared, the calibration set contained 25 standard solutions and the prediction set contained 9 standard solutions. To a series of 10 ml volumetric flasks, aliquots of Tenofovir and Zidovudine solutions, containing the appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml with methanol. UV spectra of the mixtures were recorded in the wavelength range 220–300 nm versus a solvent blank, and digitized absorbance was sampled at 1 nm intervals. All the solutions were prepared freshly and were protected from light.

Sample preparation:

Twenty tablets were weighed and their average weight was taken. An accurately weighed amount of the powder equivalent was dissolved in methanol in 100 ml calibrated flasks. 20ml of methanol was added and ultra sonicated for 25minutes and the volume was made up to100 ml with methanol and shake well. Then, the solution was filtered through what man filter paper No. 41 and the residue were washed three times with 10 ml of solvent, and then the volume was completed to 100 ml with methanol. The resulting solution was diluted to 1:2 in a 100 ml calibrated flasks. Both techniques were applied to the prepared sample solutions. The volume was made up to 100 ml with methanol. The resulting solution was diluted to 1:2 in a 100 ml calibrated flasks. Both techniques were applied to the prepared sample solutions. Experimental design of sample set calibration and test sets for two-component systems were designed according to factorial principle five-level factorial design was used to produce a calibration set (Training step) of 25 samples. Calibration spectra are shown in Figure 2. A three-level set was derived to produce a prediction set (Validation step) of nine samples. Prediction spectra are shown in Figure 3. The compositions of the used calibration and Validation sets are summarized in Tables 1 & 2 respectively.

Selection of optimum number of factors and the spectral region:

The most commonly employed validation criterion is to divide the dataset into two subsets, a calibration set, and a validation set. The calibration model is calculated using the calibration set. Then, the root means square errors of calibration and validation, RMSEC – root mean square error of calibration and RMSEP – root mean square error of prediction, are calculated using the calibration model under investigation to predict the samples in the calibration set and validation set, respectively.

Market sample analysis: (Assay)

The proposed PLS and PCR methods were applied to the simultaneous determination of TENO and ZIDA in commercial tablets. Determination of six replicates was made. Satisfactory results were obtained for each drug in good agreement with the label claims. The results are presented in Table 4. The method was found to be precise with six sample preparations for the quantification of TENO and ZIDA. The precision and intermediate precision variations were calculated in terms of relative standard deviation and the results were found to be less than 2.0%.To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the pre-analyzed formulation.

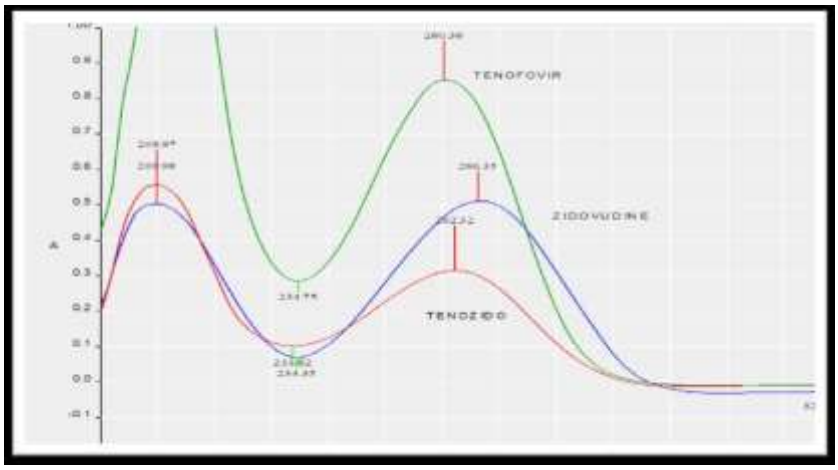
Precision:

The method was found to be precise with six sample preparations for the quantification of TENOFOVIR and ZIDOVUDINE. The precision and intermediate precision variations were calculated in terms of relative standard deviation and the results were found to be less than 2.0% and the results are presented in Table. 5.

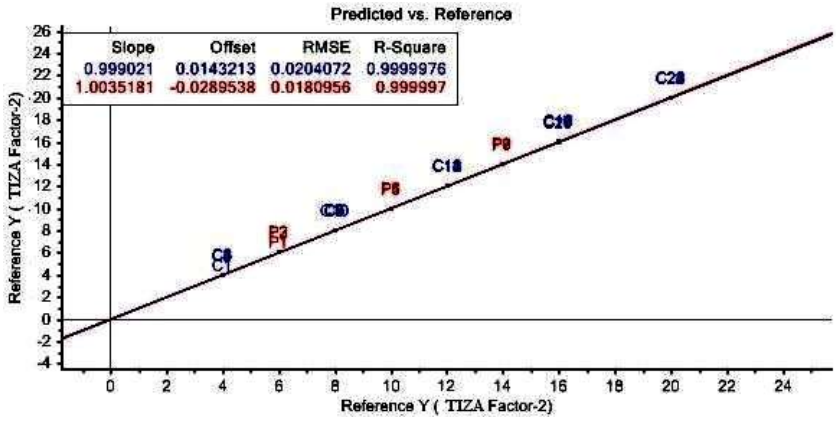
Recovery Studies:

To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the pre-analyzed formulation. The results are presented in Table. 6.

Figure 1: Overlaid spectra of Tenofovir and Zidovudine



Graph 1: Calibration and prediction spectra of Tenofovir



Graph 2: Calibration and prediction spectra of Zidovudine

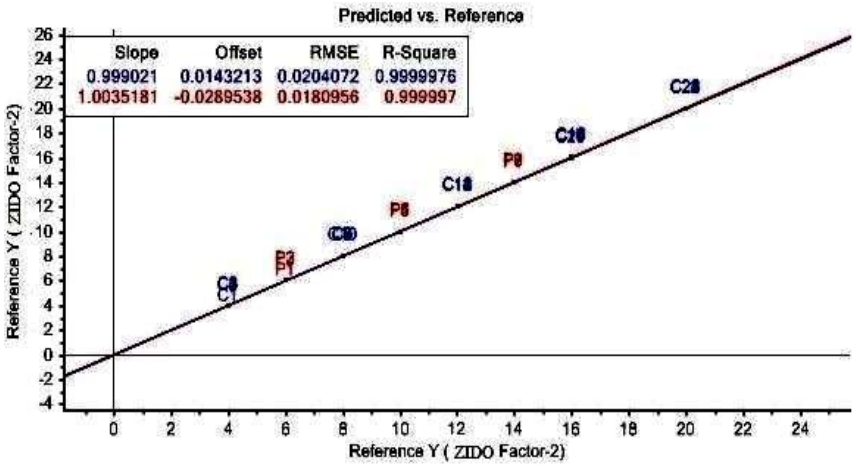


Table 1: Composition of calibration (Training set) for PLS and PCR methods.

Sno	Tenofovir			Zidovudine		
	Reference ug/ml	Predicted ug/ml		Reference ug/ml	Predicted ug/ml	
		PLS	PCR		PLS	PCR
1	4	4.09	4.04	2	2.10	2.05
2	4	4.08	4.04	4	3.98	3.95
3	4	4.07	4.04	6	6.03	6
4	4	4.06	4.04	8	7.97	7.96
5	4	4.05	4.04	10	10.05	10.04
6	8	7.99	7.95	2	2.09	2.05
7	8	7.98	7.95	4	3.97	3.95
8	8	7.97	7.95	6	6.02	6
9	8	7.96	7.95	8	7.96	7.96
10	8	7.95	7.95	10	10.04	10.04

11	12	12.02	11.99	2	2.08	2.05
12	12	12.01	11.99	4	3.96	3.95
13	12	11.99	11.99	6	6.01	6
14	12	11.98	11.99	8	7.95	7.96
15	12	11.97	11.99	10	10.03	10.04
16	16	16.01	15.99	2	2.07	2.05
17	16	16.00	15.99	4	3.95	3.95
18	16	15.99	15.99	6	6	6
19	16	15.98	15.99	8	7.94	7.96
20	16	15.97	15.99	10	10.02	10.04
21	20	20.03	20.02	2	2.06	2.05
22	20	20.02	20.02	4	3.94	3.95
23	20	20.01	20.02	6	5.99	6
24	20	20.00	20.02	8	7.93	7.96
25	20	19.99	20.02	10	10.01	10.04

Table 2: Composition of validation [prediction set] for PLS and PCR methods

Sno	Tenofovir			Zidovudine		
	Reference ug/ml	Predicted ug/ml		Reference ug/ml	Predicted ug/ml	
		PLS	PCR		PLS	PCR

1	10	10.59	10.55	3	2.98	2.94
2	10	10.58	10.55	5	5.06	5.04
3	10	10.57	10.55	7	7.02	7
4	14	12.49	14.46	3	2.97	2.94
5	14	12.48	14.46	5	5.06	5.04
6	14	12.47	14.46	7	7.01	7
7	18	18.48	18.46	3	2.96	2.94
8	18	18.47	18.46	5	5.05	5.04
9	18	18.46	18.46	7	7	7

Table 3: Summary of statistics in PLS and PCR.

RUG	RMSEP		RMSEC		r2		INTERCEPT		SLOPE	
	LS	CR	LS	CR	LS	CR	LS	CR	LS	CR
ENO	.0489	.0425	.02040	.0181	.999	.999	.0143	0.028	.999	.0035
IDA	.0397	.0358	.02040	.0180	.999	.999	.0143	0.028	.999	.0035

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Table 4: Analysis of tablet formulation (Assay).

Formulation	Lab el claim	PLS mg/tab found	PCR mg /tab found
TAVIN-L	Teno 300mg	299.98	298.76
	Zida 150mg	150.27	150.27

Table 5: Precision data.

	SYSTEM PRECISION				METHOD PRECISION			
	Tenofovir (µg/ml)		Zidovudine (µg/ml)		Tenofovir % purity		Zidovudine % purity	
	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR
Mean	1.50	1.50	11.03	11.03	100.31	100.31	100.44	100.44
SD	0.012	0.012	0.018	0.018	0.2265	0.2265	0.1526	0.1532
%RSD	0.76	0.76	0.15	0.15	0.20	0.20	0.1519	0.1526

Table 6: Recovery studies.

% of	TENOFOVIR			ZIDOVUDINE	
TARGET		PLS	PCR	PLS	PCR
	Mean	100.83	100.83	100.01	99.87

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80%	S.D	0.73	0.73	0.44	0.44
	RSD	0.81	0.81	0.22	0.22
100%	Mean	99.77	99.77	99.87	99.83
	S.D	0.37	0.37	0.32	0.25
	RSD	0.37	0.37	0.32	0.25
120%	Mean	99.44	99.44	100.14	100.17
	S.D	0.54	0.54	0.24	0.24
	RSD	0.54	0.54	0.24	0.24

III. CONCLUSION

The developed UV spectrophotometric methods were used for routine spectral analysis for the simultaneous determination of Tenofovir and Zidovudine. This method shows a high percentage of recovery and free from additional signals and suitable for combination drugs having overlapping spectra. Satisfactory results were obtained by these methods. The proposed methods can be used for the analysis of drugs in laboratory and process quality control.

REFERENCES

[1] Oneil MJ, Heckelman PE, Koch CB, In: The Merck Index, 14th ed., Whitehouse station, NJ,Merck and Co Inc., 2006. p. 503.

[2] 2. Toegel, S, Huang, W, Piana, C, Unger,M, Wirth M, Goldring MB, 2007.BMC Molecular Biology, p. 8, 13.

[3] Indian Pharmacopoeia Vol II. Delhi: The Indian Pharmacopoeia commission; Published by the controller of Publication, 2007.p. 681.

[4] Bhure M.V, Hemke A.T, K.R. Gupta. UV-Spectrophotometric Methods for Determination of Tenofovir and Zidovudine in Pharmaceutical Formulation.J. Pharm. Sci. & Res. 2010;Vol.2 (7): 426-432.

[5] Carolin Nimila I, Balan P, Yaswanth Kumar D, Rajasekar S. Simultaneous estimation of Tenofovir and Zidovudine in bulk and pharmaceutical dosage form by UV spectroscopy method. Int J PharmTech Res. 2010;2(4):2313-8.

[6] Ritu V. Kimbahune, Nikunjkumar Patel, Prachi V. Kabra, Bhavin Vaishnani, Vaibhav Deshmukhe, Narendra Sharath Chandra JN. Simultaneous spectrophotometric estimation of Tenofovir and Zidovudine in tablet dosage form. J Pharm Res. 2011;4(6):1757-9.

[7] Sohan S. Chitlange, Ganesh R. Pawbake, Amir I. Mulla, Sagar B. Wankhede. Simultaneous Spectrophotometric Estimation of Tenofovir and Zidovudine in tablet dosage form. Der Pharma Chemica, 2010; 2(1): 335-41.

[8] Gandhi S.P,Dewani M.G, Borole T.C ,Damle M.C Development and validation of stability Indicating HPLC method for determination of Diacerein and aceclofenac as bulk drug and In tablet dosage form. Int J Res Pharm & Chem.2011;1(4):799-806. 39

[9] Sekar V, Jayaseelan S, Udhaya Kumar E, Subash N, Prakash M, Perumal P. Development and validation of RP-HPLC method for the simultaneous estimation of Tenofovir and Zidovudine in dosage form. Int J ChemTech Res. 2010;2(1):168-71.

[10] Gandhi S.P, Dewani M.G, Borole T.C ,Damle M.C. Development and Validation of Stability Indicating HPTLC Method for Determination of Tenofovir and Zidovudine as Bulk Drug and in Tablet Dosage Form E- Journal of Chemistry 2012;9(4):2023-28.

[11] Vigneau E, Devaux M.F, Qannari E.M, Robert P, 1997. Principal component regression, ridge regression and ridge principal component regression in spectroscopy calibration. J Chemom,11, 239-49.

[12] Brereton R, Chemometrics, Data Analysis for the Laboratory and Chemical Plant, Wiley, Chichester:2003.

[13] Chemometrics Steven D. Brown, Thomas B. Blank, Stephen T. Sum, and Lois G. Weyer Anal. Chem., 1994, 66 (12), pp 315–359 2001.

[14] Bhavsar DS, Patel BN, Patel CN. RP-HPLC method for simultaneous estimation of tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharmaceutical methods. 2012 Jul;3(2):73.

[15] Sharma R, Mehta K. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian J Pharm Sci Tech 2010 Jul; 72(4):527.

[16] Srinath A, Sneha B, Alladi A, Ahmed R, Kulkarni RG. Method development and validation for

simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV- spectroscopic method. International Journal of Pharmaceutical Sciences and Research. 2014 Dec 1;5(12):5491.

[17] Ghorpade SA, Sali MS, Kategaonkar AH, Patel DM, Choudhari VP, Kuchekar BS. Simultaneous determination of emtricitabine and tenofovir by area under curve and dual wavelength spectrophotometric method. Journal of the Chilean Chemical Society. 2010;55(1):115-7.

[18] Choudhari VP, Ingale S, Gite SR, Tajane DD, Modak VG, Ambekar A. Spectrophotometric simultaneous determination of Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form by ratio derivative, first order derivative and absorbance corrected methods and its application to dissolution study. Pharmaceutical methods. 2011 Jan;2(1):47.

[19] Ingale KD, Barhate AL, Kale AN, Bobade CD, Choudhari VP, Kuchekar BS. Spectrophotometric estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form by simultaneous equation and absorbance ratio methods. Journal of Pharmaceutical Research. 2010 Jan 1;9(1):11-3.

[20] El Barkil M, Gagnieu MC, Guitton J. Relevance of a combined UV and single mass spectrometry detection for the determination of tenofovir in human plasma by HPLC in therapeutic drug monitoring. Journal of chromatography B. 2007 Jul 1;854(1-2):192-7.

[21] Murugan S, Debnath S, Sikdar P, Babu NM. Simultaneous Estimation of Tenofovir Disoproxil Fumarate, Efavirenz and Lamivudine in Bulk and Tablet Dosage Form by UV Spectrophotometry. Research Journal of Pharmacy and Technology. 2013 Feb 1;6(2):VIII.

[22] AbdelHay MH, Gazy AA, Shaalan RA, Ashour HK. Simple Spectrophotometric Methods for Determination of Tenofovir Fumarate and Emtricitabine in Bulk Powder and in Tablets. Journal of Spectroscopy. 2013 Aug 6;2013.

[23] Bezy V, Morin P, Couerbe P, Leleu G, Agrofoglio L. Simultaneous analysis of several antiretroviral nucleosides in rat-plasma by high-performance liquid chromatography with UV using acetic acid/hydroxylamine buffer: Test of this new volatile medium-pH for HPLC–ESI-MS/MS. Journal of Chromatography B. 2005 Jul 25;821(2):132-43.

[24] Srinath A, Sneha B, Alladi A, Ahmed R, Kulkarni RG. Method development and validation for simultaneous estimation of lamivudine, tenofovir and efavirenz in combined tablet dosage form by RP-HPLC and UV- spectroscopic method. International Journal of Pharmaceutical Sciences and Research. 2014 Dec 1;5(12):5491.

[25] Atul SA, Charushila BH, Sanjay SJ. Application of UV-spectrophotometric methods for estimation of tenofovir disoproxil fumarate in tablets. Pakistan Journal of Pharmaceutical Sciences. 2009 Jan 1;22(1):27-9.