ejpmr, 2017,4(6), 570-575

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

APPLICATION OF MULTIVARIATE CALIBRATION METHODS FOR SIMULTANEOUS DETERMINATION OF ANTIRETROVIRAL DRUGS IN FIXED DOSE COMBINATION

Santosh V. Gandhi* and Amit S. Mutha

AISSMS College of Pharmacy, Kennedy Road, Pune- 411001, Maharashtra, India.

***Corresponding Author: Santosh V. Gandhi**

AISSMS College of Pharmacy, Kennedy Road, Pune- 411001, Maharashtra, India.

Article Received on 17/04/2017 **Article Revised on 07/05/2017** **Article Accepted on 27/05/2017**

ABSTRACT

This presented work is based on application of two multivariate calibration methods for simultaneous UV-Vis spectrophotometric determination of active substances in combined pharmaceutical formulation composed of Lamivudine (LAM) and Tenofovir Disoproxil Fumarate (TDF). The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of LAM and TDF were recorded at concentrations within their linear range 5.0-30.0 µg/ml for both drugs. 28 set of mixtures were used for calibration and 12 set of mixtures were used for validation in the wavelength range of 240 to 280 nm with the wavelength intervals λ= 0.5 nm in methanol. The methods were validated as per International Conference on HarmonizationQ2 (R1) (ICH) guidelines. These methods were successfully applied for determination of drugs in pharmaceutical formulation (tablet) with no interference of the excipients as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control as well as in process control of drugs and formulation.

KEYWORDS: Lamivudine, Tenofovir Disoproxil Fumarate, PLS, PCR, Validation.

INTRODUCTION

Lamivudine is [4-amino-1-[(2R,5S)- 2(hydroxymethyl)-1,3-oxathiolan-5-yl] 1,2 dihydro -pyrimidin-2-one] [Fig. 1(a)] is a potent nucleoside analogue reverse transcriptase inhibitor. It can inhibit both types (I and II) of HIV reverse transcriptase of Hepatitis B [1].It is official in Indian Pharmacopeia.[2] Tenofovir disoproxil fumarate is fumaric acid salt of the bis isopropoxy carbonyl oxy methyl ester derivative of Tenofovir. Chemically it is 9-[(R)2[[(isopropoxcarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl] adenine fumarate [Fig. 1(b)] and belongs to a class of antiretroviral drugs. Tenofovir is a nucleotide analogue reverse transcriptase inhibitor, which block reverse transcriptase, an enzyme useful in viral production.[3] It is official in Indian Pharmacopeia.[4] Lamivudine and Tenofovir are effective for the treatment of HIV. Several methods are reported for quantitative determination of LAM and TDF in single and in combination such as UV and RP-HPLC.[5-11]

Chemometric is the science of extracting information from chemical systems. Multivariate calibration method (e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data are the important chemometric approach for determination of mixtures including drugs combination.[12] As there are no reports on chemometric analysis of these drugs, this work was undertaken for which presents simple, accurate and reproducible multivariate spectrophotometric methods for simultaneous determination of LAM and TDF in tablet dosage form.

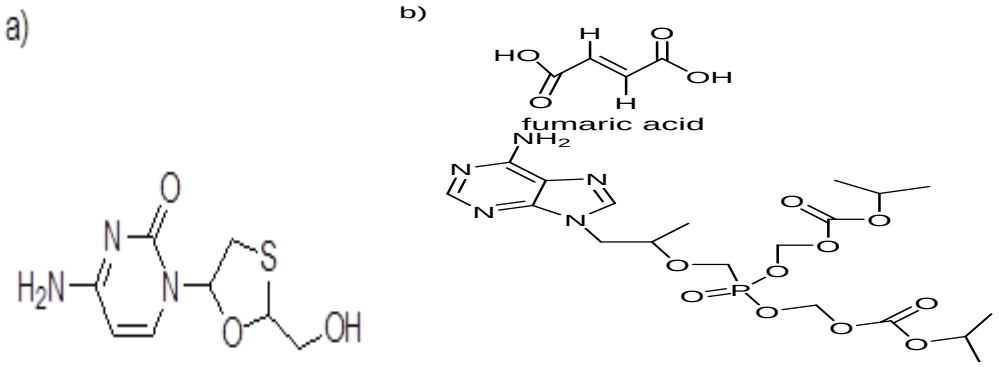

Fig.1. Structure of a) Lamivudine (LAM) and b) Tenofovir Disoproxil Fumarate (TDF)

MATERIALS AND METHODS

Instrumentation

Double beam UV- Vis spectrophotometer (Jasco V-730) with matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at spectral band width of 2.0 nm, scanning speed 100 nm/min and data pitch 0.5 nm.Unscrambler X (10.3) (64-bit) trial version and Microsoft Excel 2013 were used for model generation and application of chemometric.

Material and Reagents

Reference standard of LAM and TDF were obtained from Cipla Ltd, Mumbai Central as gift samples and methanol (AR grade) purchased from LOBA Chemie, India. Tenvir-L tablets manufactured by Cipla Ltd. containing Lamivudine IP 300 mg and Tenofovir Disoproxxil Fumarate IP 300 mg were procured from local pharmacy shop.

One component calibration

To find linear concentration of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 5.0-30.0 µg/ml for both LAM and TDF. Absorbance values wererecorded at λ_{max} of each drug (274 nm for LAM and 260 nm for TDF) against methanol as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance. Fig. 2 represents overlain spectra of LAM and TDF and their mixture.

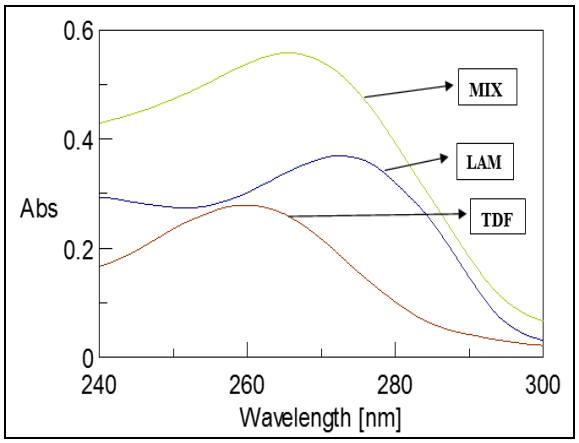

Fig. 2: Overlay spectra of LAM, TDF and mixture.

Preparation of standard stock solution

Stock solution of LAM and TDF were prepared by dissolving accurately weighed 10 mg of standard drug in 10 ml of methanol, separately. The concentration of LAM and TDF were 1000 µg/ml from which further 5 ml was pipetted and diluted to 50 ml to achieve final concentration of 100 µg/ml of LAM and TDF, separately.

Preparation of working stock solution

Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with methanol to obtain final concentration of 5, 10, 15, 20, 25 and 30 µg/ml for both LAM and TDF.

Construction of calibration and validation set

A total set of 40 mixtures were prepared by combining working standard of LAM and TDF in their linear concentration range of 5.0-30.0 µg/ml. (Table I). From these 28 mixtures were used for calibration set and 12 mixtures were used for validation set by random selection. The absorbance spectra were recorded in range of 240- 280 nm with 0.5 nm interval. The spectra were saved as ASCII (.txt) format which were further extracted in MS-Excel as required by Unscrambler software for model generation. The PCR and PLS models were developed utilizing absorption data using Unscrambler software. Selection of proper number of latent variables for development of model was necessary to obtain good prediction. Leave-one-out (LOO) cross validation method was used to obtain necessary number of latent variables (LVs), as shown in Fig. 3 and calculated using formula[13],

$$\text{RMSECV} = \sqrt{\sum \frac{(Cact - Cpre)^2}{Ic}}$$

Where,
RMSECV= Root mean square error of cross validation
Cact= actual concentration of calibration set
Cpre= predicted concentration of validation set
Ic= Total number of samples in calibration set

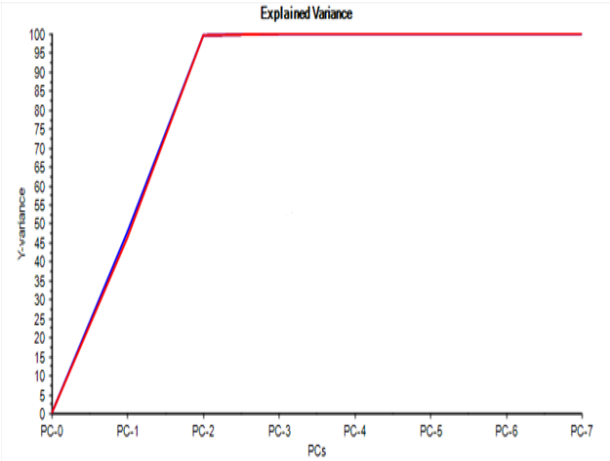

Fig.3: Explained Variance describing number of optimum PCs (Principle Components)

After the PCR and PLS models have been constructed, it was found that the optimum number of LVs were two factors for both PCR and PLS. For validation of generated models, concentration in validation set was predicted by using proposed PCR and PLS models (Table II). The validation of developed methods was performed as per ICH Q2 (R1).[14]

Table I: Composition of Calibration and Validation sets.

MIX. NO	*LAM* (µg/ml)	*TDF* (µg/ml)	*MIX. NO*	*LAM* (µg/ml)	*TDF* (µg/ml)
1	5	5	21	25	20
2	5	15	22	25	25
3	5	25	23	25	30
4	5	30	24	30	5
5	10	5	25	30	10
6	10	10	26	30	15
7	10	15	27	30	20
8	10	20	28	30	25
9	10	25	29	5	10
10	10	30	30	5	20
11	15	5	31	15	10
12	15	15	32	15	25
13	15	20	33	20	10
14	15	30	34	20	15
15	20	5	35	20	25
16	20	15	36	25	15
17	20	20	37	25	5
18	20	30	38	25	30
19	25	10	39	30	5
20	25	15	40	30	30

*Calibration set: - Mix no. 1-28
*Validation set: - Mix no. 29-40

Table II: Predicted results for validation set by PCR and PLS method.

METHOD		**PCR**				**PLS**			
LAM	***TDF***	***LAM***		***TDF***		***LAM***		***TDF***	
Actual (µg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
5	10	5.015	100.3	9.999	100.0	5.006	100.1	10.011	100.1
5	20	4.918	98.4	20.055	100.3	4.905	98.1	20.150	100.8
15	10	14.686	97.9	9.975	99.8	14.668	97.8	9.777	97.8
15	25	15.086	100.6	24.820	99.3	15.015	100.1	24.834	99.3
20	10	19.792	99.0	9.989	99.9	19.755	98.8	9.969	99.7
20	15	20.098	100.5	15.195	101.3	19.786	98.9	15.062	100.4
20	25	20.162	100.8	25.289	101.2	20.000	100.0	25.145	100.6
25	5	24.970	99.9	5.022	100.4	24.965	99.9	5.061	101.2
25	15	25.039	100.2	15.361	102.4	25.548	102.2	15.174	101.2
25	30	25.371	101.5	30.629	102.1	25.170	100.7	30.605	102.0
30	5	30.640	102.1	5.036	100.7	30.413	101.4	5.044	100.9
30	30	30.095	100.3	30.234	100.8	30.063	100.2	30.229	100.8

* % R - % Recovery.

Assay of marketed preparation

20 tablets of Tenvir-L were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of LAM (10 mg of TDF) was taken and transferred to 10 ml volumetric flask and was diluted to 10 ml with methanol. The solution was sonicated for 10 minutes. This solution was then filtered with help of whatman filter paper no. 41. 1 ml of filtrate solution was diluted to 10 ml with methanol. Further 1 ml of this solution was diluted to 10 ml with methanol to get final concentration 10 µg/ml of LAM and TDF each. The procedure was repeated 6 times for tablet formulation. The assay results are presented in Table III.

Table III: Assay result for LAM and TDF in tablet (Tenvir-L) by proposed methods

METHOD		PCR				PLS			
LAM	***TDF***	***LAM***		***TDF***		***LAM***		***TDF***	
Actual (µg/ml)		Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R
10	10	9.972	99.7	10.144	101.4	9.987	99.8	10.040	100.4
10	10	10.253	102.5	10.042	100.4	10.255	102.6	10.048	100.5
10	10	9.996	100.0	10.104	101.0	10.006	100.1	10.100	101.0
10	10	9.978	99.8	10.191	101.9	9.987	99.9	10.187	101.9
10	10	10.037	100.4	10.038	100.4	10.046	100.5	10.080	100.8
10	10	10.096	101.0	10.133	101.3	10.105	101.1	10.127	101.3
MEAN		10.055	100.6	10.109	101.1	10.063	100.6	10.097	101.0
SD		0.107	1.074	0.060	0.600	0.104	1.046	0.054	0.500

* % R - % Recovery.

Accuracy study

The accuracy study was carried out at three levels 50%, 100% and 150% of assay concentration. Calculated amount of LAM and TDF from standard solutions were spiked into sample solution and scanned in range of 240-280 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table IV and Table V.

Table IV: Accuracy data of LAM by PCR and PLS models.

Level %	Sample Conc. µg/ml	Amount added µg/ml	Total Conc. µg/ml	Predicted Conc. µg/ml		% Recovery		% RSD	
				PCR	PLS	PCR	PLS	PCR	PLS
50%	10	5	15	14.760 14.963 14.763	14.760 14.964 14.764	98.4 99.8 98.4	98.4 99.8 98.4	0.786	0.787
100%	10	10	20	19.840 20.399 20.249	19.841 20.400 20.250	99.2 102.0 101.2	99.2 102.0 101.2	1.434	1.435
150%	10	15	25	25.483 25.487 25.467	25.485 25.489 25.469	101.9 101.9 101.9	101.9 102.0 101.9	0.041	0.042

Table V: Accuracy data of TDF by PCR and PLS models.

LEVEL %	Sample Conc. µg/ml	Amount added µg/ml	Total Conc. µg/ml	PREDICTED CONC. µg/ml		% Recovery		% RSD	
				PCR	PLS	PCR	PLS	PCR	PLS
50%	10	5	15	14.942 14.809 15.281	14.943 14.811 15.282	99.6 98.7 101.8	99.6 98.7 101.9	1.620	1.619
100%	10	10	20	20.105 20.377 20.008	20.106 20.379 20.009	100.5 101.8 100.0	100.5 101.9 100.0	0.949	0.951
150%	10	15	25	25.247 25.495 25.495	25.248 25.496 25.496	100.9 101.9 101.9	101.0 102.0 102.0	0.563	0.564

Precision

Precision was carried at three concentration levels (10, 15, 20 µg/ml for both LAM and TDF) in three replicates at each level. The results of intraday and interday precision studies which are presented in Table VI and Table VII.

Table VI: Precision results obtained using developed PCR and PLS models (Intraday Precision)

Amount Taken µg/ml		Predicted Conc. µg/ml				% Recovery				% RSD			
		PCR		PLS		PCR		PLS		PCR		PLS	
LAM	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*
10	10	9.94	9.98	9.94	9.98	99.4	99.8	99.4	99.8				
10	10	9.95	9.93	9.95	9.93	99.5	99.3	99.5	99.3	0.344	0.939	0.342	0.936
10	10	10.00	10.11	10.00	10.11	100.0	101.1	100.0	101.1				
15	15	15.03	15.35	15.03	15.35	100.2	102.3	100.2	102.3				
15	15	14.87	15.09	14.87	15.10	99.1	100.6	99.1	100.6	1.249	1.269	1.248	1.267
15	15	15.24	15.48	15.24	15.48	101.6	103.2	101.6	103.2				
20	20	20.05	19.64	20.05	19.64	100.2	98.2	100.2	98.2				
20	20	19.94	20.17	19.94	20.17	99.7	100.8	99.7	100.8	0.655	1.482	0.653	1.480
20	20	20.20	19.67	20.20	19.67	101.0	98.3	101.0	98.3				

Table VII: Precision results obtained using developed PCR and PLS models (Interday Precision)

Amount Taken µg/ml		Predicted Conc. µg/ml				% Recovery				% RSD			
		PCR		PLS		PCR		PLS		PCR		PLS	
LAM	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*	*LAM*	*TDF*
10	10	9.94	9.99	9.95	9.99	99.4	99.9	99.5	99.9				
10	10	9.96	9.93	9.96	9.99	99.6	99.3	99.6	99.9	0.315	0.931	0.313	0.930
10	10	10.01	10.12	10.01	10.12	100.1	101.2	100.1	101.2				
15	15	15.04	15.35	15.04	15.36	100.3	102.3	100.2	102.4				
15	15	14.90	15.10	14.91	15.12	99.3	100.6	99.4	100.8	1.128	1.290	1.127	1.288
15	15	15.29	15.49	15.24	15.49	101.9	103.2	101.0	103.3				
20	20	20.11	19.69	20.06	19.68	100.5	98.4	100.3	98.4				
20	20	19.94	20.17	19.94	20.17	99.7	100.8	99.7	100.8	0.646	1.423	0.644	1.421
20	20	20.20	19.68	20.20	19.69	101.0	98.4	101.0	98.4				

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

RESULTS

Out of 40 mixtures, 28 set of mixtures were used for calibration and 12 set of mixtures were used for validation. The models were tried to develop with varying Δ λ. The best results were obtained with the wavelengths intervals λ= 0.5 nm in methanol. The developed method found to be accurate as results are close to 100% and precise with % RSD less than 2. Summary of results is presented in Table VIII.

Table VIII: Summary of results

Parameters	Lamivuduine (LAM)		Tenofovir Disoproxil Fumarate(TDF)	
	PCR	PLS	PCR	PLS
Range (µg/ml)	5.0-30.0	5.0-30.0	5.0-30.0	5.0-30.0
Wavelength (nm)	240- 280	240- 280	240- 280	240- 280
Data interval (Δλ)	0.5	0.5	0.5	0.5
Factors / PC's	2	2	2	2
% Recovery	100.6	100.6	101.1	101.0
LOD	0.56	0.56	0.22	0.22
LOQ	1.73	1.73	0.63	0.63
Correlation Coefficient (r^2)	0.9971	0.9971	0.9963	0.9962
Intercept	0.0495	0.0496	0.0670	0.0670
Slope	0.9971	0.9971	0.9962	0.9962
RMSECV	0.4436	0.4560	0.5250	0.5234
RMSEP	0.4555	0.4560	0.5234	0.5234

CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Lamivudine (LAM) and Tenofovir Disoproxil Fumarate (TDF)in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of LAM and TDF in pharmaceutical preparations. The proposed methods do not need separation of LAM and TDF before analysis. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well as for in process quality control.

ACKNOWLEDGMENT

Authors are thankful to the Principal, AISSMS college of Pharmacy for providing necessary facilities to carry out the experiment. Authors are also thankful to Cipla Ltd, Mumbai Central for providing a reference standard of Lamivudine and Tenofovir Disoproxil Fumarate.

REFERENCES

1. Database of Lamivudine for DB00709, https://www.drugbank.ca/drugs/DB00709 (accessed on 23.01.17).
2. The Indian Pharmacopoeia. Vol. 2014; I: 2054-2057.
3. Database of Tenofovir disoproxil fumarate for DB00300, https://www.drugbank.ca/salts/DBSALT000172 (accessed on 23.01.17).
4. The Indian Pharmacopoeia. 2014; I: 2833-2837.
5. Bojja S, Thimmaraju MK, Nerella R. Simultaneous Determination of Tenofovir disoproxil fumarate and Lamivudine by UV Spectrophotometric Method. International Journal of Pharmacy and Pharmaceutical Science Research, 2012; 2(1): 9-15.
6. Mohammed IA, Mirza SB. Determination of Lamivudine and Tenofovir in Pharmaceutical Dosage Form by Simultaneous and Q-analysis Uv-Spectrophotometric Method. World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(3): 688-696.
7. Sharma R, Mehta K. Simultaneous Spectrophotometric Estimation of Tenofovir Disoproxil Fumarate and Lamivudine in Three Component Tablet Formulation Containing Efavirenz. Indian Journal of Pharmaceutical Sciences, 2010; 557-565.
8. Karunakarana A, Kamarajanb K, Thangarasua V. A Validated RP - HPLC method for simulataneous estimation of Lamivudine and Tenofovir disoproxil fumarate in pure and in tablet dosage form. Eurasian Journal Anal Chem, 2012; 7(2): 56-66.
9. Jayadev S, Manikanta K, Syama Sundar B, Jayathirtha Rao V. Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate by RP-HPLC in the Pharmaceutical Dosage Form. Asian Journal of Chemistry, 2011; 23(4): 1683-1686.
10. Sonawane PH, Panzade PS, Kale MA. Simultaneous Estimation of Lamivudine and Tenofovir disoproxil fumarate in Bulk and Combined Pharmaceutical Dosage Form by HPLC method. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013; 3(16): 27-30.
11. Gorja A, Bandla J. Method Development and Validation for the Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate in Pharmaceutical Dosage Forms by RP-HPLC. International Journal of Advanced Research in Pharmaceutical & Bio Science, 2013; 2(1): 22-32.
12. Gandhi SV, Mutha AS. Chemometric – Assisted UV Spectrophotometric Method for Determination of Antihyperlipidemic Agents in Pharmaceutical Formulation. Journal of Applied Pharmaceutical Research, 2017; 5(2): 27 – 33.
13. Gandhi SV, Pahade AR, Tapale SR. Chemometrics - Assisted UV Spectrophotometric Methods for Determination of Metoprolol Succinate and Hydrochlorothiazide in Oral Tablets. Pharm Analysis & Quality Assurance, 2016; 4: 177-181.
14. International Conference on Harmonization. ICH harmonized tripartite guideline validation of analytical procedures text and methodology Q2 (R1) ICH. Geneva, 2005; 1-17.