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Original Article

Simultaneous Estimation of Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz in Bulk and Tablet Dosage Form by Cramer's Rule

Atul Baravkar[1], Gandhi Santosh[2,*], Bawaskar Namrata[2], Vitthal Chopade[3], Makarand Puri[4], Vishnu Neharkar[5], Ramdas Kale[6], Dattatray Kature[7], Rahul Jadhav[8], Manisha Patil[8], Reshma Devkate[9], Alfa Jain[9], Deepali Kadam[10], Amol Khedkar[11], Sachin Vijapure[12], Nilima Wani[13], Ganesh Shinde[14]

[1]Agricultural Development Trust's Shardabai Pawar Institute of Pharmaceutical Sciences and Research, Baramati, Maharashtra, INDIA.
[2]AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune, Maharashtra, INDIA.
[3]Modern College of Pgarmacy, Pune, Maharashtra, INDIA.
[4]School of Pharmacy, Vishwakarma University, Pune, Maharashtra, INDIA.
[5]Rasiklal Dhariwal Institute of Pharmaceutical Education and Research, Pune, Maharashtra, INDIA.
[6]SVPM's College of Pharmacy, Pune, Maharashtra, INDIA.
[7]Delonix Society's Baramati College of Pharmacy, Baramati, Pune, Maharashtra, INDIA.
[8]Shivraj College of Pharmacy, Gadahinglaj, Kolhapur, Maharashtra, INDIA.
[9]Institute of Pharmaceutical Sciences and Research for Girls, Bhigwan, Pune, Maharashtra, INDIA.
[10]K. K. Wagh College of Pharmacy, Nashik, Maharashtra, INDIA.
[11]Saikrupa Institute of Pharmacy, Ghargaon, Ahmednagar, Maharashtra, INDIA.
[12]Sarsam College of Pharmacy, Palashiwadi, Pune, Maharashtra, INDIA.
[13]Pravara Rural College of Pharmacy, Pravaranagar, Loni, Ahmednagar, Maharashtra, INDIA.
[14]Institute of Pharmacy, Loni, Ahmednagar, Maharashtra, INDIA.

ABSTRACT

Background: Cramer's rule is one of the significant techniques applied to settle an arrangement of conditions. In this rule, the upsides of the factors in the framework are to be determined utilizing the determinants of networks. Consequently, Cramer's rule is otherwise called the determinant rule. Few analytical methods for simultaneous estimation of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz available currently are UPLC, RP-HPLC which are quit affordable. UV-Visible method is also available but that are not based on Cramer's rule which affords more accurate results in analytical research protocols. Traditional method needs to separate the LAM, TDF and EVZ before analysis. Proposed method did not need to separate these 3 drugs and only needs to prepare the sample solution directly as per given in the assay procedure and measure the absorbance at 262 nm, 247nm and 272 nm. **Materials and Methods:** Jasco V- 730 double beam UV- vis-spectrophotometer at wavelength range of 200-400 nm was used for research protocol. Trioday tablet containing three Anti-HIV drugs and manufactured by Cipla were used for the study. Methanol and freshly prepared distilled water was used as solvents. UV-visible spectroscopy method is applied for simultaneous estimation of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in their ternary mixture and their tablet dosage form. UV-vis-spectrophotometry is based on the additivity of absorbance of drugs. The drugs show maximum absorbance at 247 nm for Efavirenz, 262 nm for Tenofovir and 272 for Lamivudine in methanol so these wavelengths were selected for further analysis. Matrix was drawn using the standard absorptivity values obtained at all the three wavelengths and the amount of drug in the tablet dosage form was calculated by solving matrix using Cramer's rule. The developed method was validated as per ICH guidelines. **Results:** The maximum wavelength found to be linear in the range of 5-30 µg/mL for Lamivudine and Tenofovir disoproxil fumarate while 10-60 µg/mL for Efavirenz. The precision was carried out at two level viz intra-day and inter-day for which the RSD was found within limit (<%2). Recovery study was carried out on the developed method and the recovery was found to be in the range of 97.5 – 102.5%. **Conclusion:** From analytical data it can be concluded that all the three drugs obey the Beers-Lambert's law at these selected wavelengths of maximum. Method was found to be simple, sensitive, precise and accurate. The developed method can be applied for the routine analysis of the Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in combined dosage form using Cramer's rule.

Keywords: Lamivudine, Tenofovir disoproxil fumarate, Efavirenz, Cramer's rule.

Correspondence:
Gandhi Santosh
AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune-411006, Maharashtra, INDIA.
Email: atul200678@gmail.com

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INTRODUCTION

Tablet containing Lamivudine (LAM), Efavirenz (EFZ), Tenofovir Disoproxil Fumarate (TDF), is prescribed combination used to stop or slow down the progression of HIV infection; also

used to treat chronic hepatitis B virus infection in adults. This combination shows promising results as it helps to increase the lifespan of patient by restricting the HIV growth in the body. Basically, it is an antiretroviral medicine which boost up the immunity to fight against the Acquired Immune Deficiency Syndrome (AIDS).

Lamivudine and Tenofovir are designated as synthetic nucleoside reverse transcriptase inhibitor while Efavirenz belong to class non-nucleoside reverse transcriptase inhibitor. Lamivudine is soluble in methanol and in water. Efavirenz is soluble in methanol, insoluble in water while Tenofovir disoproxil fumarate is soluble in methanol and distilled water.[1-4]

Currently, the literature survey reveals few methods available for the simultaneous estimation of LAM, EFZ and TDF in a combined dosage form like UPLC,[5,6] RP-HPLC[7-12] and UV-vis-derivative spectroscopic methods.[12,13] The aim of present study is to develop a simple, accurate, effective and rapid method for the simultaneous estimation of LAM, EFZ and TDF in a combined dosage form.

MATERIALS AND METHODS

Instrumentation

Jasco V-730 double beam UV-vis-spectrophotometer with 1 cm matched quartz cells was used for study. The spectra in the presented study were recorded at spectral band width of 1.0 nm with the scanning speed 400 nm/min and data pitch 1nm. Range for scanning wavelength was 200-400 nm.

Trioday tablets manufactured in India by Cipla containing Lamivudine (300 mg) Efavirenz (600mg) and Tenofovir Disoproxil Fumarate The methanolwas obtained from multi-speciality hospital pharmacy. Methanol used was of AR grade (LOBA Chemie, India). Freshly prepared double distilled water was used in the experiment.

Experimental

Preparation of standard stock solution

Stock solution of 100 µg/mL for LAM, TDF and EFZ was prepared by weighing accurately 10 mg of standard drugs and dissolving in 100 mL of methanol to get concentration of 100 µg/mL each.

Determination of wavelength of maximum absorbance

The prepared dilution of LAM, EFZ and TDF having concentration of 30 µg/mL was scanned in the range of 200-400 nm. The wavelength of maximum i.e., lambda max for LAM, EFZ and TDF were found to be at 272, 247, 262 nm respectively. Overlain spectra of EVZ, TDF and LAM in methanol is shown in Figure 1.

Preparation of calibration curve

The stock solution of LAM and TDF was diluted appropriately with methanol and six dilutions were prepared in concentration range of 5-30 µg/mL and the dilutions for both drugs were measured for absorbance at 272, 247, 262 nm (Figures 2 and 3).

The stock solution of EFZ was diluted appropriately with methanol and dilutions was prepared in concentration range of 10-60 µg/mL and the absorbance of these six dilutions was measured at 272, 247, 262 nm (Figure 4).

RESULTS

Assay of marketed formulation

Marketed tablets of LAM, TDF and EVZ, Trioday (300 mg, 300 mg, 600 mg) were weighed accurately and finely powdered. Tablet powder equivalent to 10 mg of TDF (10 mg of and LAM; 20 mg of EVZ) was taken and transferred to 10 mL volumetric flask and was diluted with 5 mL of methanol. The prepared solution was sonicated for 10 min, and after sonication volume made up to 10 mL. Whatman filter paper no. 41 was used to filter the sonicated solution. 5 mL of filtrate was taken in 50 mL of volumetric flask and diluted to 50 mL with methanol. The procedure was repeated 6 times for tablet formulation. Absorbance was measured at three selected wavelengths and concentrations were determinedby solving matrix by using a Cramer's rule. The assay results are presented in Table 1.

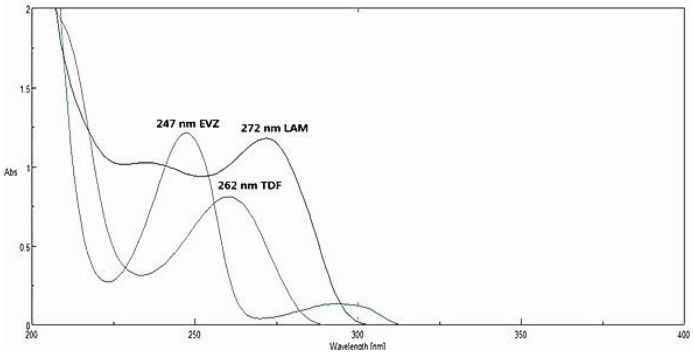

Figure 1: Overlain spectra of EVZ, TDF and LAM in methanol.

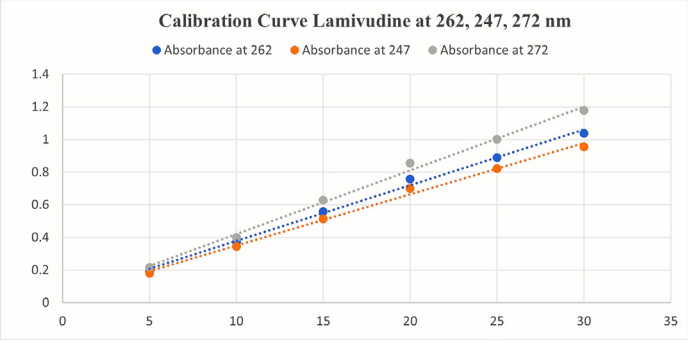

Figure 2: Calibration curve of LAM at 262, 247, 272 nm.

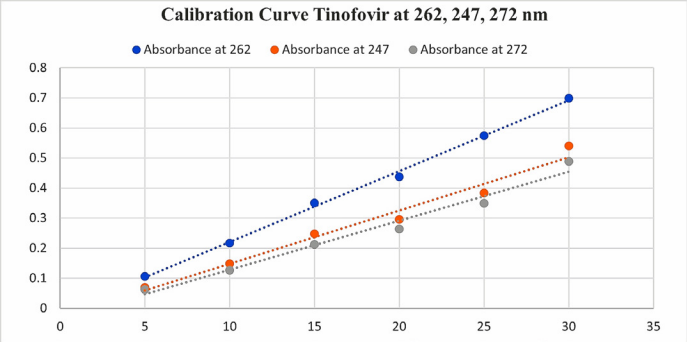

Figure 3: Calibration Curve TDF at 262, 247, 272 nm.

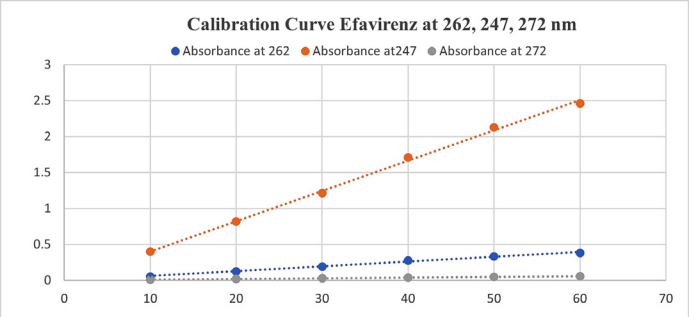

Figure 4: Calibration Curve EFZ at 262, 247, 272 nm.

Table 1: Results of formulation analysis.

Assay Result								
LAM	EVZ	TDF	LAM	EVZ	TDF	LAM	TDF	EVZ
Actual conc. (mcg/mL)			Obtained conc. (mcg/mL)			% Recovery		
10	20	10	9.93	9.98	19.78	99.32	99.87	98.90
10	20	10	9.90	10.14	20.00	99.09	101.40	100.01
10	20	10	9.75	9.77	19.73	97.57	97.78	98.68
10	20	10	9.72	9.89	19.52	97.20	98.94	97.63
10	20	10	10.17	10.14	19.61	101.76	101.49	98.08
10	20	10	9.60	9.98	19.71	96.09	99.84	98.55
					Mean	98.50	99.88	98.64
					SD	1.997	1.426	0.812
					% RSD	2.02	1.42	0.823

Method validation[14]

Precision

The intraday and inter-day precision of the method was carried expressed as relative standard deviation. Intraday precision was determined at three concentration level in triplicate for all three drugs (n = 9). For LAM and TDF at 10, 15, 20 µg/mL and for EVZ at 10, 20, 30µg/mL. Inter-day precision was determined by analysing each drug on the next consecutive day with the same concentration as mentioned above.

Accuracy

The accuracy study was carried out by standard addition method. The standard solution was spiked into the sample solution at three levels 50%, 100% and 150% of assay concentration. The sample concentration used were 10 µg/mL for LAM and TDF and 20 µg/mL for EVZ. The prepared samples were scanned in the range of 200-400 nm. The amount of drugs was calculated by solving the Cramer's matrix. Accuracy data is presented in Tables 2, 3 and 4.

DISCUSSION

To begin with, the overlain spectra of LAM, TDF and EVZ were studied in the different solvents. As a result, the methanol was found to be more suitable solvent for the analysis of these drugs in the UV spectrophotometry as it offered the advantages of ease of solubility over the other solvents. All the three drugs LAM, TDF and EVZ shows absorbance at the wavelength of maximum of each other. So, the absorbance of the mixture at one of the drug's wavelength of maximum is the sum of absorbance all three drug. By applying the Beer- lambert's equation (A= abc, where a = absorptivity, b = path length (1 cm), c= concentration)The absorbance of the mixture at the three wavelength represents as following:

$Am_1 = ax_1cx + ay_1cy + az_1cz$…… at λ_1 (262 nm, λ_{max} of TDF)

$Am_2 = ax_2cx + ay_2cy + az_2cz$…….. at λ_2 (247 nm, λ_{max} of EVZ)

$Am_3 = ax_3cx + ay_3cy + az_3cz$……at λ_3 (272 nm, λ_{max} of LAM)

Here, Am_1, Am_2, Am_3 are the absorbances of the mixture at 262 nm (λ_1), 247 nm (λ_2), 272 nm (λ_3), respectively where cx, cy and

cy are the concentration of LAM, TDF and EVZ. In the above equation, ax1, ax2, and ax3 are the absorptivity's of LAM; ay1, ay2, and ay3 are the absorptivity's of TDF and az1, az2, and az3 are the absorptivity's of EVZ at the three wavelength of maximum 262 nm, 247 nm, 272 nm; respectively.

The above matrix was further solved by calculating the absorptivity's from the calibration curve and the equation was derived as follows.

$Am_1 = 36.218cx + 22.368cy + 6.383cz$……. at λ_1 (262 nm, λ_{max} of TDF)

$Am_2 = 33.691cx + 15.516cy + 41.27cz$……... at λ_2 (247 nm, λ_{max} of EVZ)

$Am_3 = 41.222cx + 13.772cy + 0.9462cz$……at λ_3 (272 nm, λ_{max} of LAM)

So, in the above equation there are three unknown concentrations, to easily solve the above matrix Cramer's rule method was applied. The result was the concentrations of the LAM, TDF, and EVZ in the mixture.

It was observed that the all drugs obey the Beer-Lambert's law in the different concentration range. Precision was carried at intraday and inter-day level and result found that the method was precise, as RSD for proposed method was satisfactory (< 2%) as shown in Table 5.

Recovery study was also carried out on the developed method and result found to be in the range of 97.5-102.5%. The assay was

Table 2: Accuracy result for Lamivudine.

Level %	Sample Conc. µg/mL	Amount Added µg/mL	Total Conc µg/mL	% Recovery	% RSD
50%	10	5	15	100.59	1.168
				99.92	
				98.32	
100%	10	10	20	98.09	1.820
				99.87	
				101.72	
150%	10	15	25	98.17	1.553
				98.03	
				98.03	

Table 3: Accuracy result for Tenofovir disoproxil fumarate.

Level %	Sample Conc. µg/mL	Amount Added µg/mL	Total Conc µg/mL	% Recovery	% RSD
50%	10	5	15	101.88	1.326
				99.58	
				99.57	
100%	10	10	20	97.63	1.466
				97.98	
				100.29	
150%	10	15	25	100.14	1.087
				99.79	
				98.12	

Table 4: Accuracy result for Efavirenz.

Level %	Sample Conc. µg/mL	Amount Added µg/mL	Total Conc µg/mL	% Recovery	% RSD
50%	10	5	15	100.17	0.317
				99.01	
				99.74	
100%	10	10	20	101.28	0.663
				99.99	
				99.99	
150%	10	15	25	100.01	0.566
				99.00	
				99.00	

Table 5: Summary of results.

Parameters		Lamivudine	Tenofovir disoproxil fumarate	Efavirenz
Wavelength maximum		272 nm	262 nm	247 nm
Linearity Range (µg/mL)		5-30 µg/mL	5-30 µg/mL	10-60 µg/mL
Correlation Coefficient(R^2)		0.995	0.997	0.997
Slope		0.998	0.996	0.985
LOD (µg/mL)		1.21	0.56	0.41
LOQ (µg/mL)		3.67	1.70	1.26
% Recovery		98.50	99.88	98.64
Assay (%RSD)		2.027	1.428	0.82
Accuracy(%RSD)	50%	1.168	1.326	0.317
	100%	1.820	1.466	0.663
	150%	1.553	1.087	0.566
Precision (%RSD)	Intra-day	1.315	0.515	0.975
	Inter-day	1.315	1.796	0.540

performed on the marketed tablet Trioday with the label claim 300 mg LAM, 300 mg TDF and 600 mg EVZ. The amount of drug found within pharmacopeial limits. Assay was performed ($n = 6$) and results are given in Table 1.

Traditional method needs to separate the LAM, TDF and EVZ before analysis. Proposed method needs to prepare the sample solution as per given in the assay procedure and measure the absorbance at 262 nm, 247 nm and 272 nm. Matrix was drawn using the absorbances obtained at the entire three wavelengths and then the matrix was solved using Cramer's rule. The final answer was the amount of each drug in the sample solution. The results for the validation parameters are given in Table 5.

CONCLUSION

The proposed UV-vis spectrophotometric method based on Cramer's rule was found to be less time consuming and easy, as it involves very limited steps for analysis. Method was found to be simple, sensitive, precise and accurate. The developed method can be applied for the routine analysis of the LAM, TDF and EVZ.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LAM: Lamivudine; **EFZ:** Efavirenz; **TDF:** Tenofovir Disoproxil Fumarate; **ICH:** International Conference for Hormonization; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation;

AR: Analytical Reagent; **Conc:** Concentration; **LOQ:** Limit of Quantitation; **LOD:** Limit of Detection; **UV-vis:** UV-visible; **UPLC:** Ultra Performance Liquid Chromatography; **RP-HPLC:** Reverse Phase High Performance Liquid Chromatography; **HIV:** Human Immuno Virus; **AIDS:** Acquired Immuno Deficiency Syndrome; **nm:** Nanometer; **cm:** Centimetre; **mg:** Milligram; **min:** Minute; **µg:** Microgram; **mcg:** Microgram; **%:** Percentage.

SUMMARY

Cramer's standard is one of the critical strategies applied to settle a plan of conditions. In this standard, the potential gains of the variables in the structure are not set in stone using the determinants of organizations. Hardly any logical techniques for concurrent assessment of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz accessible as of now are UPLC, RP-HPLC which are stopped reasonable. UV-vis technique is additionally accessible however that are not in light of Cramer's standard which manages the cost of additional exact outcomes in logical exploration conventions. Customary technique needs to isolate the LAM, TDF and EVZ before investigation. Proposed strategy didn't have to isolate these 3 medications and just has to set up the example arrangement straightforwardly according to given in the examine methodology and measure the absorbance at 262 nm, 247nm and 272 nm.

The medications show greatest absorbance at 247 nm for Efavirenz, 262 nm for Tenofovir and 272 for Lamivudine in methanol so these frequencies were chosen for additional examination. Lattice was drawn utilizing the standard absorptivity values got at every one of the three frequencies and how much medication in the tablet measurement structure was determined by addressing network utilizing Cramer's standard. The created technique was approved according to ICH rules. The greatest frequency viewed as direct in the scope of 5-30 µg/mL for Lamivudine and Tenofovir disoproxil fumarate while 10-60 µg/mL for Efavirenz. The accuracy was completed at two level viz intra-day and between day for which the RSD was found inside limit (<%2). Recuperation study was done on the created strategy and the recuperation was viewed as in the scope of 97.5-102.5%. From insightful information it tends to be presumed that every one of the three medications comply with the Brews Lambert's regulation at these chose frequencies of greatest. Strategy was viewed as basic, delicate, exact and precise. The created strategy can be applied for the normal examination of the Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in joined measurements structure utilizing Cramer's standard.

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