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Method Development and Validation for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine through Stability indicating RP-HPLC Method

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Abstract

A simple, economic, specific, accurate and precise validated reverse phase liquid chromatographic method has been developed for the estimation of Efavirenz, Lamivudine and Zidovudine in Tablet dosage forms. Here in present method, chromatography was carried out using the instrument Waters HPLC 2695 mode with empower software on a Xterra C18(150mm×4.6mm, 5µ) column with mobile phase of 70 volumes of Water (pH was adjusted to 2.1 with o-phosphoric acid) and 30 volumes of Methanol in isocratic mode. The flow rate was 1ml/min, with injection volume 10µl. Detection was done by using PDA detector at 275nm. The retention time was found to be 1.91, 2.90 and 7.52 min The method was validated in terms of linearity, precision, accuracy, LOD, LOQ and robustness in accordance with ICH guidelines. The linearity was found to be in the range of 300-900 µg/ml, 75-225 µg/ml, 150-450 µg/ml for Efavirenz, Lamivudine and Zidovudine with correlation coefficient 0.999. The LOD values were 1.8196, 0.796, 3.166 µg/ml.The LOQ values were 6.065, 2.654 and 10.55 µg/ml respectively. The percentage assay was 99.89, 99.22 and 99.64% for Efavirenz, Lamivudine and Zidovudine. No chromatographic interference from tablet excipients was found. The developed method with good separation could be successfully applied for the determination of Efavirenz, Lamivudine and Zidovudine in its Tablet dosage form.

Keywords: Efavirenz, lamivudine, zidovudine, waters HPLC, tablets, estimation, validation.

Introduction

Efavirenz[1-3] is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one with the molecular formula $C_{14}H_9ClFNO_2$ and with a molecular weight of 315.7g/mol−1.Efavirenz is insoluble in water, soluble in lower alcohol. Lamivudine is chemically (2R,cis)-4- amino-l-(2-hydroxymethyl-l,3-oxathiolan-5-yl)-(lH)-pyrimidin-2-one with the molecular formula $C_8H_{11}N_3O_3S$ and a molecular weight of 229.256gmol−1. Lamivudine[2,4,5] is soluble in water, sparingly soluble in methanol, slightly soluble in ethanol. Zidovudine[6,7] is chemically 1- (3-azide-2, 3-di deoxy-β-D-ribofuranosyl)-5-methyl Pyrimidin-2,4(1H, 3H) –dione with the molecular formula $C_{10}H_{13}N_5O_4S$ and with a molecular weight of 267.25g/mol−1.Zidovudine is soluble in water, alcohol, acetone, ethanol and sparingly soluble in denatured alcohol.

Very few methods are reported in literature for the assay of Efavirenz, Lamivudine and Zidovudine in Tablet dosage forms using RP-HPLC method. The reported HPLC methods were having disadvantages like high flow rate, high retention time, more organic phase and use of costly solvents. The proposed RP-HPLC method utilizes economical solvent system and having advantages like better retention time, less flow rate, very sharp and symmetrical peak shapes. The aim of the study was to develop a simple, precise, economic and accurate RP-HPLC method for the estimation of Efavirenz, Lamivudine and Zidovudine in Tablet dosage forms.

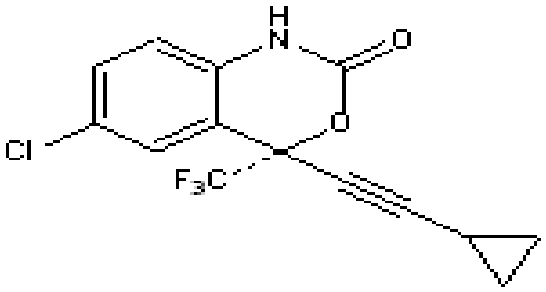

Figure-1
Structure of Efavirenz

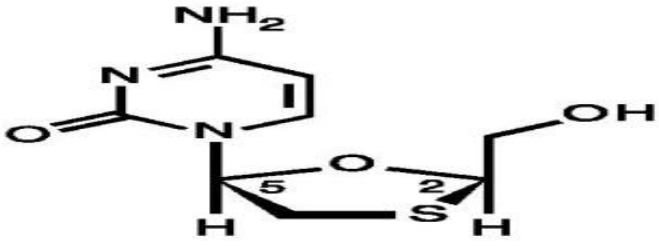

Figure-2
Structure of Lamivudine

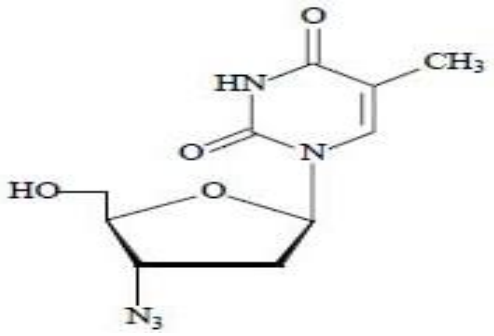

Figure-3
Structure of Zidovudine

Material and Methods

Efavirenz, Lamivudine, Zidovudine were obtained from Mylan Laboatories, Hyderabad, India. Acetonitrile (HPLC grade), Water (HPLC grade), Orthophosphoric Acid (AR grade) were purchased from S D chemicals, Mumbai.

Methodology[8]: Preparation of standard solution for assay: Standard solution of Efavirenz, Lamivudine, Zidovudine (600µg/ml, 150 µg/ml,300µg/ml) was prepared by dissolving 60mg of Efavirenz, 15mg of Lamivudine and 30mg of Zidovudine working standard in 50ml of diluent (mixture of water and Methanol in the ratio 50:50) with sonication and made up to 100ml with the same diluent.

Preparation of sample solution for assay: Five tablets were weighed and finely powdered and a powder quantity equivalent to 150mg of Efavirenz, 300mg of Lamivudine and 600mg of Zidovudine were accurately weighed and transferred to a 100ml volumetric flask and 50ml of diluent (mixture of water and Methanol in the ratio 50:50) was added to the same. The flask was sonicated for 30 min and volume was made up to the mark with diluent. Transferred 5ml of solution into a 50ml volumetric flask and dilute up to the mark with diluent so as to obtain a concentration of 150,300,600 µg/ml. Mixed well and filtered through 0.45µm Nylon disposable syringe filter. The amount present in each tablet was calculated by comparing the area of standard Efavirenz, Lamivudine, Zidovudine and tablet sample.

Validation[9-12] of Analytical Method: Validation of a method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical Parameters.

Accuracy: Accuracy of the method was determined by recovery experiments. Recovery studies were carried out by adding known amount of standard drug solution to the sample solution. The % recovery studies were carried out at three different levels of 50%, 100% and 150% of target assay concentration in triplicate.

Precision: The Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

System precision: System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for five replicate injections of the drug Efavirenz, Lamivudine and Zidovudine at assay concentration (600,150,300µg/ml). The results were within acceptable limits.

Method precision: The precision of the test method was performed by injecting 6 replicates standard preparation and the % RSD was calculated.

Linearity: The linearity of the method was demonstrated over the level of 50 – 150% of the target concentration. Aliquots of Efavirenz 300,450,600,750 and 900µg /ml, Lamivudine 75,112.5,150, 187.5 and 225 µg/ml and Zidovudine 150,225,300,375 and 450 µg /ml were prepared from standard solution. A Calibration curve was produced by analyzing different concentrations of the pure drug. The correlation coefficient for the peak area at each level versus concentration of analyte was calculated.

Observation: i. The correlation coefficient was found to be 0.999. ii. From the above study it was established that the linearity of test method is from 50 % to 150% of the target concentration.

Robustness: The Robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. Analyze the sample separately by deliberate changes in the analytical method as given below:

Effect of variation in flow rate: A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared as per the testing method and was injected into the HPLC system by keeping flow rates 0.8ml/min and1.2ml/min. Evaluate the system suitability parameters for 0.8 ml/min and 1.2ml/min flow.

Acceptance criteria: i. The Tailing Factor of Efavirenz, Lamivudine and Zidovudine standards should be ≤ 2.0 for Variation in flow.

Effect of variation in Temperature: A study was conducted to determine the effect of variation in Temperature. Standard solution was prepared as per the testing method and was injected into the HPLC system by keeping temperatures 40˚C and 50˚C. Evaluate the system suitability parameters for 40˚C and 50˚C

Limits of detection and limits of quantification: The limit of detection is the estimation of sensitivity based on how much low concentration can be detected by the method which was found to be 1.8196, 0.796, 3.166µg/ml (S/N > 3) for Efavirenz, Lamivudine and Zidovudine. In addition the limit of quantification was 6.065,2.654,10.55µg/ml (S/N > 10) for Efavirenz, Lamivudine and Zidovudine.

Forced degradation studies[13]: Degradation by Hydrochloric Acid (Acid Treated Sample): Randomly select 20 tablets from a batch and make it powder, and weigh accurately 2140 mg of powder (equivalent to 600mg Efavirenz, 150mg Lamivudine, and 300mg Zidovudine) and transfer to 100ml volumetric flask. Then add 10ml of acid(0.1N HCL), shake the flask on a rotator shaker for 30 min and sonicate for 1hr with intermediate shaking then add 10ml base(0.1NaOH) and make up the volume with water.

Pipette out 2.5 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with water. Inject 10µl of test solution into HPLC system.

Degradation by Sodium Hydroxide (Base Treated Sample): Randomly select 20 tablets from a batch and make it powder, and weigh accurately 2140 mg of powder (equivalent to 600mg Efavirenz, 150mg Lamivudine, and 300mg Zidovudine) and transfer to 100ml volumetric flask. Then add 10ml of base (0.1NaOH), shake the flask on a rotator shaker for 30 min and sonicate for 1hr with intermediate shaking then add 10ml acid (0.1N HCL), and make up the volume with water.

Pipette out 2.5 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with water. Inject 10µl of test solution into HPLC system.

Degradation by Hydrogen Peroxide (Peroxide Treated Sample): Randomly select 20 tablets from a batch and make it powder, and weigh accurately 2140 mg of powder (equivalent to 600mg Efavirenz, 150mg Lamivudine, and 300mg Zidovudine)) and transfer to 100ml volumetric flask. Then add 1% peroxide and shake the flask on a rotator shaker for 30 min at 60˚C and sonicate for 1hr with intermediate shaking and make up the volume with water.

Pipette out 2.5 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with water. Inject 10µl of test solution into HPLC system.

Degradation by Photo Light: Randomly select 20 tablets from a batch and make it powder, and weigh accurately 2140 mg of powder (equivalent to 600mg Efavirenz, 150mg Lamivudine, and 300mg Zidovudine) and transfer to 100ml volumetric flask and keep the flask in sunlight for 55hrs. Then shake the flask on a rotator shaker for 30 min and sonicate for 1hr with intermediate shaking and make up the volume with water.

Pipette out 2.5 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with water.Inject 10µl of test solution into HPLC system.

Degradation by Water: Randomly select 20 tablets from a batch and make it powder, and weigh accurately 2140 mg of powder (equivalent to 600mg Efavirenz, 150mg Lamivudine, and 300mg Zidovudine) and transfer to 100ml volumetric flask. Then shake the flask on a rotator shaker for 30 min and sonicate for 1hr with intermediate shaking and make up the volume with water.

Pipette out 2.5 ml of above clear solution and transfer it to 25 ml volumetric flask and make up the volume with water. Inject 10µl of test solution into HPLC system.

Results and Discussion

In the present investigation trials were made to develop a simple, precise, accurate, economic method using RP-HPLC.

Trials were carried out with solvents like methanol, water in different compositions. The observed chromatograms was lacking peak shape, Resolution and high RT.

Finally with Water (pH was adjusted to 2.1 with o-phosphoric acid): Methanol in the ratio 70:30 composition with a flow rate of 1ml/min peaks were eluted at 2.90,1.91 and 7.52min and peak shape and symmetry was maintained with no interference. The wavelength was set at 275nm. The column used was Xterra C18 (150×4.6,5µm). The injection volume was 10µl. A sample chromatogram was shown in figure 4. A Standard chromatogram was shown in figure 5.

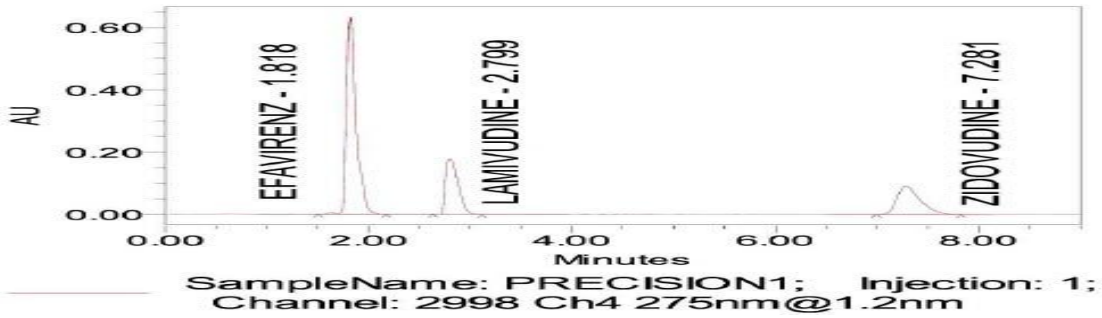

Figure-4
Sample Chromatogram

The developed method was validated as per ICH guidelines. The linearity was studied for Efavirenz, Lamivudine and Zidovudine. The aliquot portions of standard solution were taken and diluted appropriately with diluents to get series of concentration ranges from 300-900µg/ml,75-225 µg/ml,150-450(50-150% level of test concentration) the method was found to be linear in the studied range. The correlation coefficient (r^2) was found to be 0.999 and shows good linearity. The data of linearity was given in table 1.

Precision studies were carried out in terms of repeatability (system precision) and Method precision. Five deter minations of target assay concentration (600,150,300µg/ml) was evaluated for repeatability and data was given in table 4.

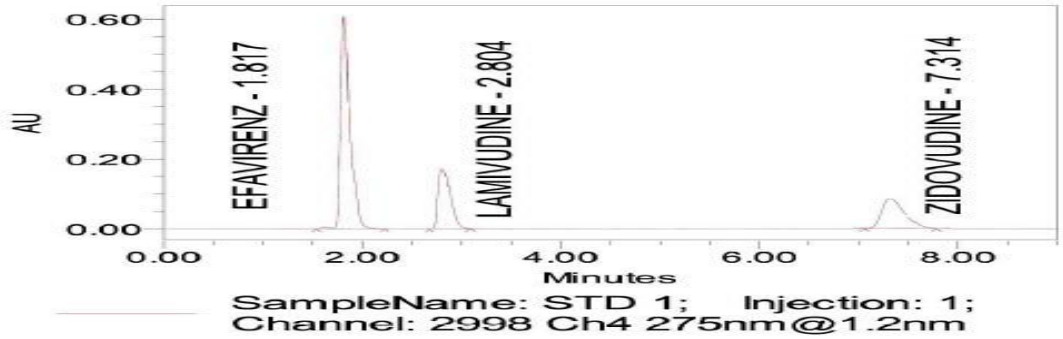

Figure-5
Standard Chromatogram

Table-1
Linearity data for Efavirenz

S No	Concentration (µg/ml)	Peak Area	
1	300	1962758	
2	450	2940680	
3	600	3936292	
4	750	4915675	
5	900	5890842	
		Slope	6551
		Intercept	1148
		r^2	0.999

Table-2
Linearity data for Lamivudine:

S No	Concentration (µg/ml)	Peak Area	
1	75	749659	
2	112.5	1124969	
3	150	1487641	
4	187.5	1862939	
5	225	2234179	
		Slope	9921
		Intercept	2999
		r^2	0.999

Table-3
Linearity data for Zidovudine

S No	Concentration (µg/ml)	Peak Area	
1	150	749390	
2	225	1112840	
3	300	1493110	
4	375	1875266	
5	450	2229699	
		Slope	4982
		Intercept	4010
		r^2	0.999

For accuracy determination, the % recovery studies were carried out at three different levels of 50%, 100% and 150% of target assay concentration (600,150,300µg/ml) for Efavirenz, Lamivudine and Zidovudine. The results of accuracy is good in agreement with limit given in guidelines for accuracy and was given in table 7.

Robustness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as flow rate,Temperature. Evaluated the system suitability parameters for 0.8 and 1.2ml/min flow and the data was given in table 9. Evaluated the system suitability parameters for 40˚C temperature and 50˚C temperature and the data was given in table 10.

Table-4
System precision data

S .No	Injection	Peak Area of Efavirenz	Peak Area of Lamivudine	Peak Area of Zidovudine
1	Injection – 1	3938248	1496399	1419855
2	Injection – 2	3936755	1497949	1410038
3	Injection – 3	3931014	1494020	1419111
4	Injection – 4	3936640	1490661	1419704
5	Injection – 5	3934192	1492222	1418646
Mean		3939370	1494250	1417471
% RSD		0.1	0.1	0.1

Table-5
System Suitability Data

Parameters	Efavirenz	Lamivudine	Zidovudine
Theoretical plates	5217	6630	7767
Tailing Factor	1.725	1.746	1.526
Retention time	2.90	1.91	7.52

Table-6
Method Precision data

S.No	Injection	Peak area of Efavirenz	Peak area of Lamivudine	Peak area of Zidovudine
1	Injection – 1	3939072	1498054	1417566
2	Injection – 2	3932697	1498331	1412369
3	Injection – 3	3937488	1494546	1413563
4	Injection – 4	3933983	1499974	1413144
5	Injection – 5	3938602	1491382	1411585
6	Injection – 6	3948212	1498393	1419728
% RSD		0.2	0.1	0.3

Table-7
Accuracy data for Efavirenz

Level	Peak Area	% Recovery	Mean Recovery
50%	635817	99.83	99.81
	635573	99.82	
	635713	99.83	
	634301	99.80	
	631768	99.85	
	633114	99.73	
100%	1215723	99.89	99.89
	1213239	99.86	
	1201789	99.92	
150%	1805308	99.91	99.91
	1801424	99.93	
	1804708	99.94	
	1802991	99.90	
	1806128	99.91	
	1805487	99.90	

Table-8
Accuracy data for Lamivudine

Level	Peak Area	% Recovery	Mean Recovery
50%	455902	98.98	98.85
	454959	98.75	
	454837	98.66	
	452399	98.80	
	454221	98.98	
	450444	98.93	
100%	869250	99.27	99.22
	869775	99.17	
	869132	99.22	
150%	1235389	99.70	99.69
	1231746	99.68	
	1237708	99.65	
	1236704	99.71	
	1233520	99.74	
	1232127	99.66	

Table-9
Accuracy data for Zidovudine

Level	Peak Area	% Recovery	Mean Recovery
50%	7232462	99.72	99.61
	7340668	99.50	
	7360124	99.68	
	7189978	99.54	
	7144372	99.58	
	7276293	99.69	
100%	14823951	99.67	99.64
	14789567	99.73	
	14790196	99.53	
150%	22153271	99.80	99.80
	22048548	99.79	
	22063175	99.77	
	22058838	99.83	
	22092426	99.81	
	22063658	99.84	

Table-10
Variation in flow rate and temperature for Efavirenz

System Suitability Parameter	Efavirenz			
	Flow (ml/min)		Temperature (°c)	
	Low (0.8)	High (1.2)	Low (40˚C)	High (50˚C)
Retention Time	3.652	1.465	1.824	1.818
Tailing	1.640	1.791	1.687	1.748
Plate Count	6513	6365	6325	6431

Table-11
Variation in flow rate and temperature for Lamivudine

System Suitability Parameter	Lamivudine			
	Flow (ml/min)		Temperature(°c)	
	Low (0.8)	High (1.2)	Low (40°c)	High (50°c)
Retention Time	2.421	2.292	2.807	2.801
Tailing	1.735	1.711	1.730	1.658
Plate Count	5344	5478	5709	5557

Table-12
Variation in flow rate and temperature for Zidovudine

System Suitability Parameter	Zidovudine			
	Flow (ml/min)		Temp(°c)	
	Low (0.8)	High (1.2)	Low (40°c)	High (50°c)
Retention Time	9.657	5.935	7.059	7.050
Tailing	1.480	1.515	1.560	1.479
Plate Count	6669	6196	6317	6754

LOD and LOQ were determined from standard deviation and slope method as per ICH guideline for Efavirenz, Lamivudine and Zidovudine LOD values were found to be 1.8196,0.796 and 3.166µg/ml and LOQ values were found to be 6.065,2.654,10.55µg/ml.

Table-13
Showing LOD and LOQ results

Sample	LOD µg/ml	LOQ µg/ml
Efavirenz	1.8196	6.065
Lamivudine	0.796	2.654
Zidovudine	3.166	10.55

Forced degradation studies were done to Efavirenz, Lamivudine and Zidovudine by using Acid, Base, Peroxide, Light and Water and the results were shown in the following tables.

Table-14
Showing Degradation Results of Efavirenz

TEST	Area of Efavirenz	%Degradation of Efavirenz	% Assay of Efavirenz
ACID	3037621	15.86	84.14
BASE	3200844	16.69	83.31
PEROXIDE	3005006	15.67	84.33
LIGHT	3045409	15.88	84.12
WATER	2993232	15.60	84.40
Average	3084423	15.94	84.06
% RSD	5.4		

Table-15
Showing Degradation Results of Lamivudine

TEST	Areaof Lamivudine	%Degradation of Lamivudine	% Assay of Lamivudine
ACID	1263888	17.91	82.09
BASE	1141502	16.18	83.82
PEROXIDE	1135652	16.10	83.90
LIGHT	1002445	14.21	85.79
WATER	1264497	17.92	82.08
Average	1137597	16.46	83.53
% RSD	2.4		

Table-16
Showing Degradation Results of Zidovudine

TEST	Area of Zidovudine	% Degradation of Zidovudine	% Assay of Zidovudine
ACID	1304626	19.49	80.51
BASE	1268328	18.95	81.05
PEROXIDE	1100506	16.44	83.56
LIGHT	1329703	19.87	80.13
WATER	1380049	20.62	79.38
Average	1088643	19.07	80.92
% RSD	5.9		

Conclusion

The proposed reverse phase liquid chromatographic method was validated over the parameters like linearity, accuracy, precision, robustness, limit of detection and limit of quantification proved to be convenient and effective for the determination of Efavirenz, Lamivudine and Zidovudine in tablet dosage forms. A specific, accurate, precise, economic and sensitive validated reverse phase liquid chromatographic method has been developed for the determination of Efavirenz, Lamivudine and Zidovudine in tablet dosage forms. Moreover, the lower organic solvent consumption along with short analytical run time of 8min, better retention time leads to cost effective chromatographic method.

Acknowledgement

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References:

1. Martindale the complete drug reference, thirty fifth edition, **1**, 921–922 **(2006)**
2. www.rxlist.com/epivir-drug.htm **(2013)**
3. Adkins J.C. and Noble S., Efavirenz Drugs, **56**, 1055-1064 **(1998)**
4. http://www.fda.gov/oia/pepfar.htm **(2013)**
5. www.enwikipedia.org/wiki/Lamivudine **(2013)**
6. www.enwikipedia.org/wiki/zidovudine **(2013)**
7. www.who.int/medicenes/publications/pharmacopoeia/QAS_077Rev3_Zidovudine **(2013)**
8. Mandloi D.K., Tyagi P.K., Rai V.K., Dey S., Ashada R.K., Method Development and validation of RP- HPLC in the application of invitro dissolution study of Lamivudine in bulk drug and tablet formulation, *Journal of Chemical and Pharmaceutical Research*, **1(1)**, 286-296 **(2009)**

9. ICH, validation of analytical procedures: Methodology, international conference on harmonization, IFPMA, Geneva **(1996)**

10. Edwardson P.A., Bhaskar G. and John E., Method validation in pharmaceutical analysis, *Journal of Pharmaceutical Analysis*, **990**, 929-933 **(1990)**

11. Edwardson P.A., Bhaskar G. and John E., Method validation in pharmaceutical analysis, *J Pharm Biomed Anal*, **8**, 929-933 **(1990)**

12. FDA, Guidance for industry, Analytical procedures and methods validation, chemistry, manufacturing and controls documentation **(2000)**

13. www.mongabay.com/health/full/Lamivudine_Zidovudine.htm/ **(2013)**

14. Lloyd R. Snyder, practical HPLC method development, 2nd edition, 687-711 **(1997)**

15. International conference on Harmonisation of technical requirements for the registration of pharmaceutics for Human use. Validation of Analytical procedures: Methodology.ICHQ2B Geneva, (CPMP/ICH/281/95) **(1996)**

16. USP 28, NF 23,The United State Pharmacopoeial Convention, Asian Edition, **(2005)**

17. USP 28 ,NF 23,The United State Pharmacopoeial Convention,Asian Edition, **(2008)**

18. Sethi P.D., Qualitative analysis of drugs and formulations, 4th edition, 1-19 **(1996)**

19. Sharma B.K., Instrumental method of chemical analysis, 18th edition 39-139 **(1999)**

20. Silver stein R.M., Clayton Bassler G.,Terence C., Morill, Spectrophotometric identification of organic compounds, 5th edition; 289 **(1991)**

21. www.ikev.org/haber/stabilite/kitap/35%201.7%20Stability%20Workshop%20ICH%20Q2A%20C%20.pdf **(2013)**

22. Sethi P.D., Quantitative Analysis of Drugs in Pharmaceutical Formulations, 3rd ed. Delhi: CBS Publishers and Distributors; 7-27 **(2005)**

23. Eunice Kazue Kano, Cristina Helena dos Reis Serra, Eunice Emiko Mori Koono, Simone Schramm Andrade,Valentina Porta, Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies: A Review, *International Journal of Pharmaceutics*, **297(1–2)**, 73–79 **(2005)**

24. Jayaseelan S., Suresh S., Sathishkumar G., Sekar V. and Perumal P., Bioanalytical Method Development And Validation of Lamivudine By RP-HPLC Method: A Review, *International Journal of Chemtech Research Coden(USA), Ijcrgg*, **2(1)**, 163-167 **(2010)**

25. Akhilesh Vikram Singh, Lila K. Nath and Nihar R. Pani, Development And Validation of Analytical Method For The Estimation of Lamivudine In Rabbit Plasma: A Review, *Journal of Pharmaceutical Analysis*, **1(4)**, 251–257 **(2011)**

26. Joanna J. Zheng, Steven T. Wu, Thomas A Emm, High-Performance Liquid Chromatographic Assay For The Determination of 2′-Deoxy-3′-Thiacytidine (Lamivudine) In Human Plasma: A Review, *Journal Of Chromatography B: Biomedical Sciences And Applications*, **761(2)**, 195–201 **(2001)**

27. Patro S.K., Swain S.R., Patro V.J. and Choudhury N.S.K., Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation: A Review, *E-Journal of Chemistry*, **7(1)**, 117-122 **(2010)**

28. Deepali G. and Elvis M., UV Spectrophotometric Method for Assay of the Anti-Retroviral Agent Lamivudine in Active Pharmaceutical Ingredient and in its Tablet Formulation: *J Young Pharm*, 417–419 **(2010)**

29. Shalini S., Shanooja V.P., Abdul Jameela S., Basima , K. K. Harilala, Harish Rajakb and V. Ravichandran, Application of UV-Spectrophotometric methods for estimation of Lamivudine in tablets: A Review, *Digest Journal of Nanomaterials and Biostructures*, **4(2)**, 357–360 **(2009)**

30. Y. Anand kumar and N. Rama Rao, Development of Rapid UV Spectrophotometric Method for the Estimation of Efavirenz in Formulations: A Review, *E-Journal of Chemistry*, **7(3),** 856-860 **(2010)**

31. J. Sudhakar reddy, MD. S.Maqsood ahmed I. E. Chakravarthy and K. Prabhavathi, Spectrophotometric Determination of Zidovudine in Pharmaceutical Dosage Forms: A Review, *E-Journal of Chemistry* , **9(1)**, 89-92 **(2012)**

32. Agaiah Goud, Rajineekar Reddy N., Quantitative estimation of Zidovudine by UV Spectrophotometry: A Review, *International Journal of Pharmacy & Technology IJPT*, **2(4)**, 1328 **(2010)**

33. Chidambaram Saravanan, Manni Venkatachari Kumudhavalli Ramalingam kumar, Vijaya kumar Latha, Balasundaram Jayakar, Spectrophotometric Determination of Zidovudine in Pharmaceutical Dosage Forms: A Review", *IRJP*, **1(1)**, 314-323 **(2010)**

34. Sharada C.H., Channabasavaraj K.P. and Tamizh Mani T., Development of a Spectrophotometric Method for the Quantitative Estimation of Zidovudine Concentration in Bulk and Pharmaceutical Dosage Forms: A Review, *KMITL Sci. Tech. J*, **10(1)**, 1-8 **(2010)**

35. Anand Kumar Y. and Rama rao N., Development of rapid UV Spectrophotometric method for the estimation of

Efavirenz in Formulations: A Review, *E-Journal of Chemistry*, **9(2),** 569-575 **(2012)**

36. Brajendra Singh Rajpoot: Method development and validation of RP-HPLC method in the detection of Efavirenz in Bulk drug and Tablet Formulation: A Review, *IRJP*, **3(7)**, 297-300 **(2012)**

37. Geetha Ramachandran, A.K. Hemanth Kumar, Soumya Swaminathan, P.Venkatesan, V. Kumaraswami, David J. Greenblatt, Simple and rapid liquid chromatography method for determination of efavirenz in plasma: A Review, *Journal of Chromatography B*, **835(1–2)**, 131–135 **(2006)**

38. Peter Langmann, Diana Schirmer, Thomas Vath, Michael Zilly, Hartwig Klinker, High-performance liquid chromatographic method for the determination of HIV-1 non-nucleoside reverse transcriptase inhibitor efavirenz in plasma of patients during highly active antiretroviral therapy: A Review, *Journal of Chromatography B: Biomedical Sciences and Applications***, 755(1–2)**, 151–156 **(2001)**

39. Ashok Peepliwal, Sagar D. Vyawahare and Chandrakant G. Bonde, A quantitative analysis of Zidovudine containing formulation by FT-IR and UV spectroscopy: A Review, *Anal. Methods*, **11**, 1756-1763 **(2010)**

40. Venkatesh P., Lydia Smiley and Mahesh, Simultaneous estimation of Zidovudine and Lamivudine tablets by RP-HPLC method: A Review, *International Journal of ChemTech Research*, **3(1)**, 376-380 **(2011)**

41. Jacqueline De Souza, Eunice Kazue Kano, Eunice Emiko Mori Koono, Simone Grigoleto Schramm,Valentina Porta, Sílvia Storpirtis, LC–UV Methodology for Simultaneous Determination of Lamivudine and Zidovudine in Plasma by Liquid–Liquid Extraction : A Review, *Chromatographia*, **69(2)** 231-235 **(2009)**

42. Severino Grangeiro Jr, Miracy M. Albuquerque, Davi P. Santana Maria Fernanda Pimentel , Reniere H. da Silva, Simone S. Simoes, Simultaneous spectrophotometric determination of lamivudine and zidovudine in fixed dose combinations using multivariate calibration: A Review, *Quím. Nova*, **34(5)**, 376-380 **(2011)**

43. Bengi Uslu and Sibel A. Ozkan, Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performanceliquid chromatography–UV methods: A Review, *Analytica Chimica Acta*, **466**, 175–185 **(2002)**

44. Balamuralikrishna K., Mahendra K. and B. Syama Sundar, Development and validation of analytical procedure for the simultaneous estimation of efavirenz, lamivudine and zidovudine through new RP-HPLC method: A Review, *Journal of Pharmacy Research*, **4(10)**, 3766-3768 **(2011)**