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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE BY USING RP-HPLC METHOD

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Abstract:

High Performance Liquid Chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Zidovudine and Lamivudine was done by RP-HPLC. The Phosphate buffer of pH 3.0 and the methanol as mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C_{18} (4.6 x 150mm, 5µm) and equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 265 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. The linearity range of Baclofen and Tramadol were found to be from 100-500 µg/ml of Baclofen and 5-25 µg/ml of Tramadol. Linear regression coefficient was found to be less than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Zidovudine and Lamivudine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Key words: *Zidovudine, Lamivudine, Validation, Linearity.*

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INTRODUCTION:

Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter. Lamivudine (LAMI) is chemically 1-[(2R,5S)-2-(hydroxy methyl)-1-3 oxathiolan-5-yl] cytosine and used as an antiretroviral activity. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B.

It needs to be phosphorylated to its triphosphate form before it is active. 3TC-triphosphate also inhibits cellular DNA polymerase. Zidovudine (ZIDO) is chemically 1- [(2R,4S,5S)-4azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H-dione) and used as an antiretroviral activity.

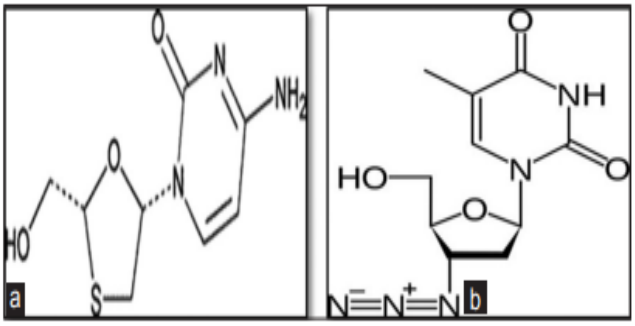

Figure 1: Structure of a) lamivudine and b) zidovudine

The main objective is to develop a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination of lamivudine and zidovudine in pharmaceutical formulation.

MATERIALS AND METHODS:

Experimental work:

All the chemicals and reagents were LR grade.

Preparation of phosphate buffer (pH-4.6): Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of citric acid mono hydrate in sufficient water to produce 1000ml. Adjust the pH 6 by using ortho phosphoric acid.

Preparation of mobile phase: Accurately measured 650 ml (65%) of Buffer and 250 ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent preparation: The Mobile phase was used as the diluent.

Table 1: Optimized chromatographic conditions

Instrument used	Waters HPLC with auto sampler and PDA detector 996 model
Temperature	Ambient
Column	Altima C_{18} (4.6×150mm, 5.0 µm)
Buffer	Phosphate buffer (pH-4.6)-Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of citric acid monohydrate in sufficient water to produce 1000mL. Adjust the pH 4.6 by using ortho phosphoric acid.
pH	4.6
Mobile phase	Buffer: Methanol: ACN (65:25:10v/v)
Flow rate	1 ml/min
Wavelength	265 nm
Injection volume	10 µl
Run time	14 min

Method development and validation:

Assay:

Weigh 20 lamivudine and zidovudine combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 49.26 mg LAMI and ZIDO into 10 ml volumetric flask. Add about 10 ml CAN (acetonitrile) of diluents and sonicate to dissolve it completely and make the volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further, pipette 0.1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10 µg/ml).

The simple chromatogram of test LAMI and ZIDO was shown in Figure 5. The amounts of LAMI and ZIDO per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Analysis of marketed formulation were also % label claim was found to be 99-101% satisfactory were concluded.

Table 2: Assay

Drug	Label claim	Amount found	% Label claim	SD	% RSD
LAMI	20 mg	19.91 mg	99.61	0.07	0.28
ZIDO	40 mg	39.62 mg	99.02	0.55	1.35

Precision:

Accurately weigh and transfer 10 mg of lamivudine and 10mg of zidovudine working standard into a 10 mL and 10 ml of clean dry volumetric flasks add about 7mL and 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml and 3 ml of the above Lamivudine, Zidovudine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents

Linearity:

Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 10 mg of Lamivudine, Zidovudine (marketed formulation-dose of Lamivudine is 150 mg, Dose of Zidovudine is 300 mg in combination tablet) sample into a 10 mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution 100 ppm).

RESULTS AND DISCUSSION:

System suitability:

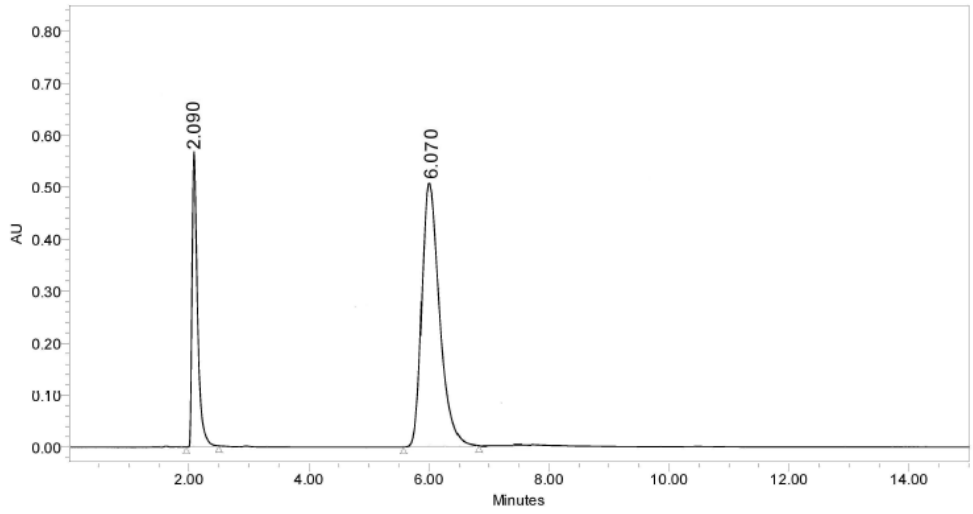

Figure 2: System suitability

Table 3: System suitability parameters for lamivudine and zidovudine

Name	Retention time (min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
Lamivudine	2.090	3468547	567933		1.0	2565.5
Zidovudine	6.070	16289441	517733	2.5	1.1	2355.2

Table 4: Analytical performance parameters of lamivudine and zidovudine

Parameters	Lamivudine	Zidovudine
Slope (m)	19725	68375
Intercept (c)	35332	56388
Correlation coefficient (R^2)	0.999	0.999

LOD & LOQ

Table 5: Results of LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Lamivudine	51	155	3.05
Zidovudine	52	156	3.01

Table 6: Results of LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Lamivudine	51	530	10.4
Zidovudine	52	517	9.96

Linearity

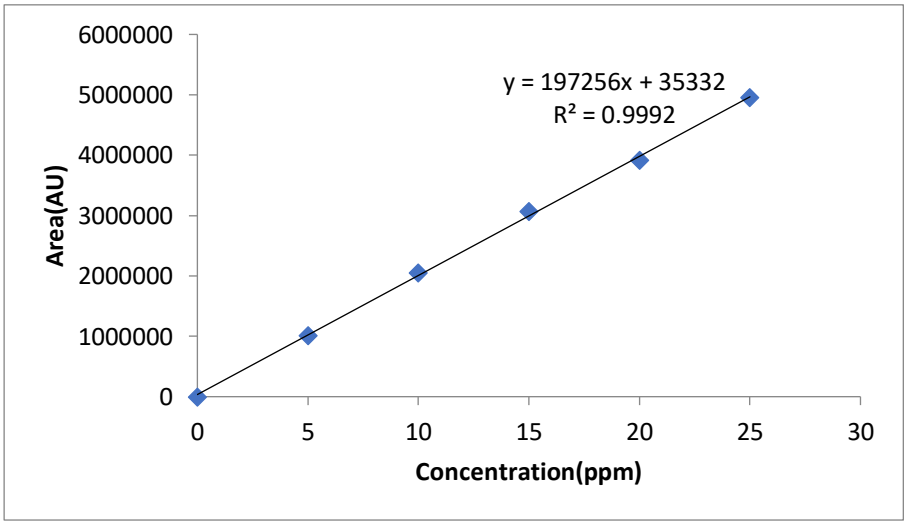

Figure 3: Calibration graph for LAMI

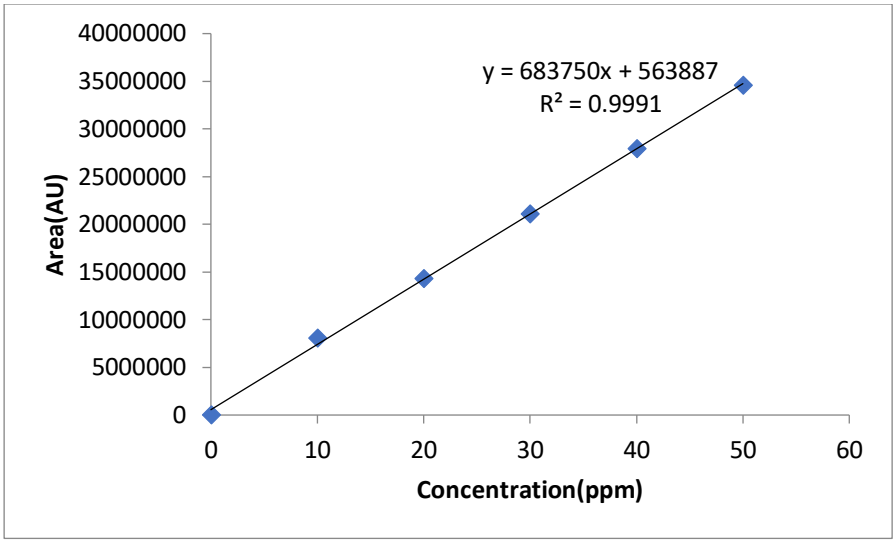

Figure 4: Calibration graph for ZIDO

The proposed methods for simultaneous estimation of LAMI and ZIDO in tablet dosage forms were found to be simple, accurate, economical, and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for LAMI and ZIDO were linear with correlation coefficients (r^2) values The values of %RSD are within the prescribed limit of 2%, showing the high precision of methods, and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of LAMI and ZIDO in formulations.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of lamivudine and zidovudine was done by RP-HPLC. The mobile phase was optimized with consists of Buffer: Methanol: Acetonitrile in the ratio of (65:25:10v/v). An Altima C_{18} column (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1 ml/min. the linearity range of lamivudine and zidovudine were found to be from 5-25ppm, 10-50 µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery of Lamivudine is 99.8 and Zidovudine is 99.9%. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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