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A VALIDATED RP-HPLC DETERMINATION OF LAMIVUDINE IN VARIOUS DOSAGE FORMS AS PER ICH GUIDELINES

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ABSTRACT

A simple, accurate and reproducible RP-HPLC method has been developed for the determination of Lamivudine in tablet dosage form and in liquid dosage form. Chromatography was carried out on a Thermo Hypersil ODS-C18 column using a mobile phase consisting of and water acetonitrile (3:7 v/v) at a flow rate of 0.8 ml/min. The detection was made at 270 nm. The retention times for Lamivudine, was found to be 1.3 min. The calibration curves were linear over the range 10-120 µg/ml for Lamivudine. The proposed method was validated as per ICH guidelines and it was found suitable for the routine quality control analysis of the drugs in tablet as well as dosage forms.

Keywords: Lamivudine, RP-HPLC, Tablet, Liquid form.

INTRODUCTION

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1) and hepatitis B (HBV). Lamivudine is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of lamivudine unfortunately leads to emergence of a resistant hepatitis B virus (YMDD) mutant. Despite this, lamivudine is still used widely as it is well tolerated [1-3].

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 is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth is terminated. Lamivudine is administered orally, and it is rapidly absorbed with a bio-availability of over 80%. Some research suggests that lamivudine can cross the blood-brain barrier. Lamivudine is often given in combination with zidovudine, with which it is highly synergistic. Lamivudine treatment has been shown to restore zidovudine sensitivity of previously resistant HIV. Several mutagenicity tests show that lamivudine should not show mutagenic activity in therapeutic doses [4-6].

The UV spectrophotometic analysis of lamivudine has been done on the Systronics 2210 model of UV double beam spectrophotometer. Absorption is taken on 270nm wavelength for the quality calculation of the data [6].

EXPERIMENT

Apparatus

Chromatographical measurements were carried out on a computerized Lacrom Merch Hitachi Interface D-7000.

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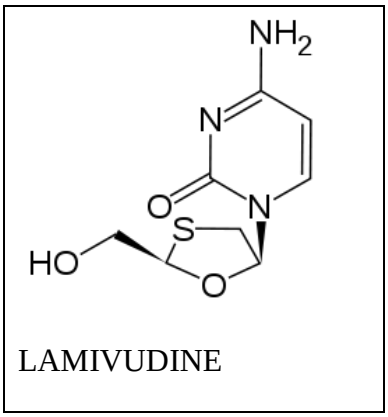
The chromatograms of test and reference solutions were recorded over the 270nm. The subsequent statistical manipulation was performed by transferring the Chromatographical data to Microsoft excel 2003 program and processing them with the standard curve fit package and matrix calculations [7].



LAMIVUDINE

Chemicals:

Pharmaceutical grade lamivudine (Aurbindo Lab. Pvt. Ltd. Hyderabad, Andhra Pradesh India) was used as working standards after confirming their purity and compliance with pharmaceutical requirements. All other reagents used were analytical grade.

Pharmaceutical preparation:

The following pharmaceutical preparation was purchased from the local market and subjected to analysis by the proposed procedures; Lamivir Lamivudine Oral Solution, Mfd. by Cipla Ltd. Verna Indl. Estate, Goa, with label claim 50mg per 5ml Lamivir-150 Lamivudine Film Coated Tablet, Mfd. by Cipla Ltd. Verna Indl. Estate, Goa, with label claim 150mg [8].

PROCEDURES

Preparation of standards:

Into 100-ml volumetric flask an accurately weighed amount (1 mg) of the studies drugs is dissolved in about 100 ml of Distilled Water /acitonitrile (3:7). The resulting solution is diluted quantitatively with Distilled Water to obtain the appropriate dilutions for drug according to its linear calibration range or as specified under the analysis of the laboratory prepared mixtures [9].

Sample preparation:

For tablet

Twenty tablets are weighed and finely powdered. An accurately weighed amount of the powder equivalent to the averaged weight of one tablet is transferred to 100 ml volumetric flask and diluted to about 100 ml with Distilled Water/acitonitrile (3:7). The mixture is shacked for about 10 minutes and then sonicated for additional 15 minutes, and filtered. The first portion of filtrate is discarded. The obtained clear solution is used as stock sample solution. The stock solution is diluted quantitatively with Distilled Water to obtain the suitable working sample solutions for the chromatographic measurements [10].

For liquid

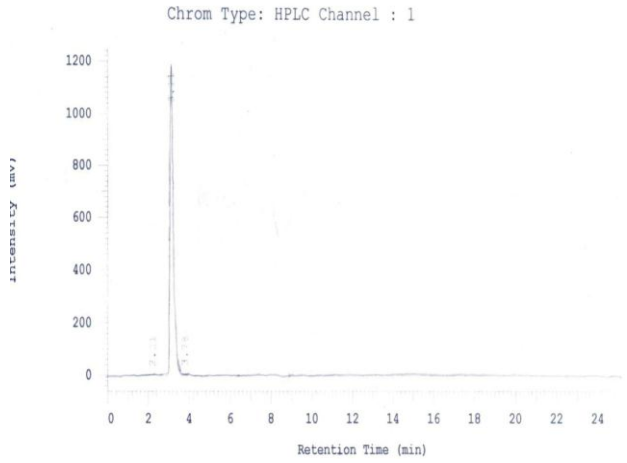
An accurately measured amount equivalent to the average weight is transferred to 100ml volumetric flask and diluted to about 100ml with Distilled water/ acitonitrile (3:7). The mixture is shacked for about 10 minutes and then sonicated for additional 15 minutes, and filtered. The first portion of filtrate is discarded. The obtained clear solution is used as stock sample solution. The stock solution is diluted quantitatively with Distilled Water to obtain the suitable working sample solutions for the chromatographic measurements [11,12].

RESULTS AND DISCUSSION

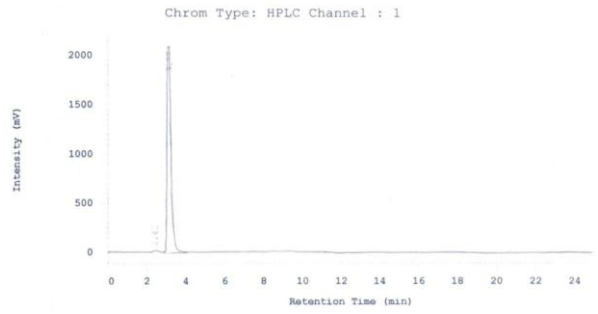
Full chromatogram methods usually provide significant improvement in precision over methods restricted to a single wavelength. It should certainly be preferred when the selection of variables is simple. In such cases, the regression coefficients for different selected collinear wavelengths may have relatively little meaning for interpretation purposes, but the model performs well, both in the calibration and prediction stages, provided that the model having linearity between responses and concentrations and the prediction is performed within the calibration domain. In addition, the baseline effects and noise are probably non-significant or of very low significance [13-16].

The method development and validation procedure is performed according to the ICH guidelines which gives the significant readings for the method performance in the laboratory.

RP-HPLC CROMATOGRAM OF LAMIVUDINE LIQUID



RP-HPLC CROMATOGRM OF LAMIVUDINE TABLET



RP-HPLC CHROMATOGRAM OF LAMIVUDINE STANDARD

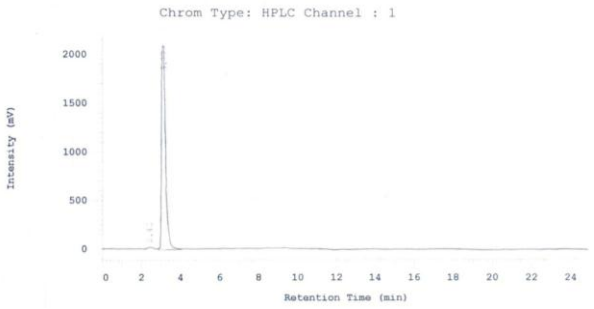
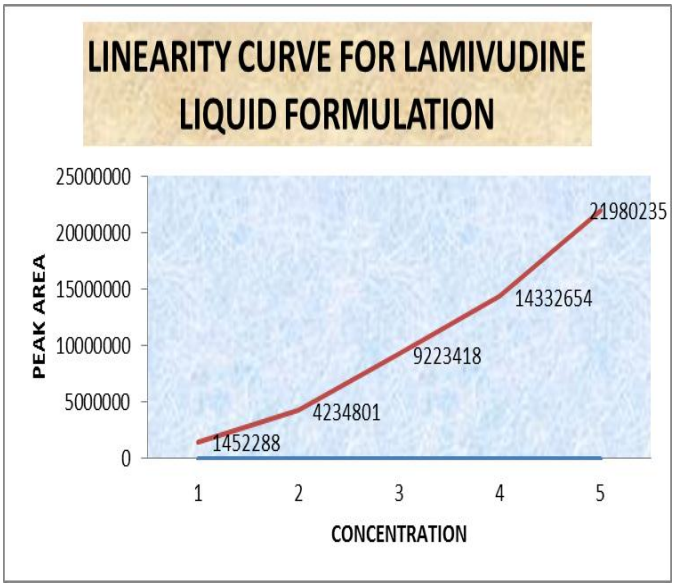


Table 1. Different formulation details

S. No.	Sample type	Concentration µg/ml	Mobile phase	P.A	R.T.	%FIT
1	Standard	150	Water: acetonitrile (3:7)	29587068	3.23	
2	Tablet			30197536	3.17	102.5
3	liquid			31234182	3.11	105.5

Table 2. Linearity of Lamivudine

SN	Con µg/ml	Liquid 5mg/ml						Tablet 150mg					
		P.A.	MPA	%FIT	St.Dv.	RSD	CC	P.A.	M.P.A.	%FIT	SD	RSD	CC
1	10	1452288		98.9				120432		98.5			
2	30	4234801		99.8				346353		99.5			
3	60	9223418	9164679	100.6	1.98	1.9	.99	612402	613326	100.7	1.63	1.6	.99
4	90	14332654		102.2				1064026		101.8			
5	120	21980235		103.9				1323418		102.9			



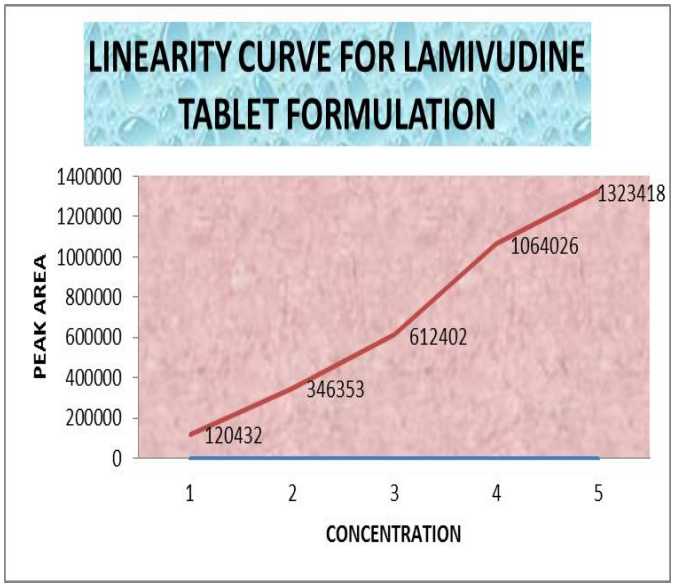


Table 3. Accuracy of Lamivudine

SN	Con µg/ml	Liquid 5mg/ml						Tablet 150mg					
		P.A.	MPA	%FIT	S.D.	RSD	CC	P.A.	M.P.A.	% FIT	SD	RSD	CC
1	10	1452288		98.9				120432		98.5			
2	30	4234801		99.8				346353		99.5			
3	60	9223418	9164679	100.6	1.98	1.9	.99	612402	613326	100.7	1.63	1.6	.99
4	90	14332654		102.2				1064026		101.8			
5	120	21980235		103.9				1323418		102.9			

Table 4. Ruggedness of Lamivudine

SN	ANALYST	Liquid 5mg/ml			Tablet 150mg		
		DATE	P.A.	%FIT	DATE	P.A.	%FIT
1	Analyst 1	25/7/2011	9223418	100.6	20/6/2011	612402	99.7
2	Analyst 2	25/7/2011	9233516	100.6	20/6/2011	612389	99.7

Table 5. LOD & LOQ of Lamivudine

Limit of Detection (LOD)					Limit of Quantification (LOQ)				
Conc.	Tablet 150mg		Liquid 5mg/ml		Conc.	Table 150mg		Conc.	Liquid 5mg\ml	
10µg/ml	RT. (min.)	P.A	RT (min.)	P.A	10µg/ml	RT (min)	P.A	30µg/ml	RT (min)	P.A
	3.27	120432	3.5	1452288		3.27	120432		3.53	4234801

CONCLUSION

The validation parameters were studied at the prescribed wavelength for the method. Linearity and Accuracy were determined by absorbance shown on various concentrations by calculating the recovery that was close to 100%. Precision was calculated as repeatability (SD and CV). Proposed method is simple, precise, accurate, and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis of the various dosage forms of lamivudine.

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