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Research Article

METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF NEVIRAPINE & LAMIVUDINE BY RP-HPLC IN COMBINATION TABLET DOSAGE FORM

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ABSTRACT

A new method was established for simultaneous estimation of Nevirapine and Lamivudine by RP-HPLC method. The chromatographic conditions were successfully developed for these parathion of Nevirapine and Lamivudine by using Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm column, flowrate was1ml/min, mobile phase ratio was Potassium dihydrogen phosphate buffer (0.02 M, pH 2.5): Acetonitrile (57:43) (pH was adjusted with orthophosphoric acid), detection wavelength was 273 nm. The instrument used was Hitachi HPLC Auto Sampler, Separation module1575. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)).The linearity study for Nevirapine and Lamivudine was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient(r2)was foundtobe 0.999and 0.999,%mean recovery was found to be100% and 100.5%,%RSD for repeatability was 0.2 and 0.4,%RSD for intermediate precision was 0.5 and 0.1 respectively.

Keywords: Nevirapine, Lamivudine, RP-HPLC, Phosphate buffer and acetonitrile.

I. INTRODUCTION

Lamivudine

Lamivudine ($C_8H_{11}N_3O_3S$) is a synthetic nucleoside analog and its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP) as an intracellular phosphorylated. This nucleoside is made up of analgesic viral DNA by HIV reverse transcripts and HBV polymerase, resulting in removal of DNA chain

IUPAC Name

4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one

Nevirapine

Nevirapine ($C_{15}H_{14}N_4O$**)** interacts with antiretroviral in the drug is NNRTI class. Both NNRTI and RTIs interfere with the same goal, the reverse transcriptase enzyme, the main viral enzyme transcribing the viral RNA into the DNA. Unlike Nucleoside RTIs, on the active site of the enzyme, NNRTIs bind a different area away from the active site NNRTI Pocket**.**

IUPAC Name

11-cyclopropyl-4-methyl-5, 11-dihydro-6H-dipyrido [3,2-b:2′,3′-e][1,4]diazepin-6-one.

Literature survey revealed that is have been projected concurrently in combination with other drugs using RP-UPLC, LC-MS, and LC-MS/MShave be projected individually or in combination with other drugs using UV, Capillary Electrophoresis, HPLC and HPTLC.Since, No spectrophotometric and RP-HPLC technique have been reported yet for instantaneous assessment of lamivudine and Nevirapine.

The current plan describes the development of an easy, precise, exact and reproducible spectrophotometric and RP-HPLC technique for the instantaneous assessment of Lamivudine and Nevirapine in Pharmaceutical forms.

MATERIALS

All experiments will be carried out in the Analytical R & D of Comprime Labs Pvt. Ltd. Hydernagar, Hyderabad. Pure samples of **Lamivudine and Nevirapine** will be procured from industries involved in bulk manufacture of this drug.Dosage formulation will be procured from local market.The methods will be developed and validated in Analytical R & D of Comprime Labs Pvt. Ltd. Hydernagar, Hyderabad. The methods will be first developed, then Validated as per ICH guidelines, then the method will be applied to the formulations.

Preparation of mobile phase

Mobile phase was prepared by taking Potassium dihydrogen phosphate buffer (0.02 M, pH 2.5): acetonitrile (57:43) Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Preparation of standard and sample solutions of Nevirapine and Lamivudine

Preparation of Standard Stock Solution

10 mg of Nevirapine and Lamivudine were weighed accurately and transferred into 100 ml volumetric flask. About 10 ml mobile phase was added and keep under Sonicator to dissolve. The volume was made up to the mark with same solvent. The final sample contained about 150 µg/ml of Nevirapine and Lamivudine

Standard solution

The 100 percent mixed standard solution of Nevirapine andLamivudine was prepared by transferring 0.15 ml of Nevirapine and0.75 ml Lamivudine to the 10 ml volumetric flasks and made up to the mark with dilute up to the mark with diluents.

Preparation of Sample Stock Solution

20 Tablet contents were weighed and triturate to fine powders. An accurately weighed 10 mg equivalent weight of Nevirapine and Lamivudine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent.

Sample solution

From this stock solution pipette 0.15 ml of Nevirapine and 0.75 ml Lamivudine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHODOLOGY

The selected and optimized mobile phase was Potassium dihydrogen phosphate buffer (0.02 M, pH 2.5): acetonitrile (57:43)and conditions optimized were: flow rate (1.0 ml/minute), wavelength (273 nm), Run time was 10 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry.

RESULTS AND DISCUSSION

The developed method of analysis was validated as per the ICH for the parameters like, linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation(LOQ).

Linearity

The linearity line was found to be 0-600 µg / ml for Nevirapineand 0-300 µg / ml for Lamivudine. Correlation modules have been identified as 0.999 & 0.996, the slopes are labeled as 12421 & 8127, and 80625 & 70773 for Nevirapine andLamivudine and the regression equations were calculated is shown in Fig.4&5 and results were presented in Table 4.

Precision

To check the intra-day and inter-day variation of the method, standard concentration was subjected to the proposed HPLC method of analysis. The precision of the proposed method i.e. the intra and inter-day variations in the peak area of the drug solutions was calculated in terms of percent RSD. A statistical evaluation revealed that the relative standard deviation of drugs at differentconcentration levels for 6 injections was less than 2.0. The results for intra-day and inter-day precision were presented in Table 5

Accuracy

The recovery studies were carried out for the accuracy parameter. Accuracy at different concentrations (80%, 100%, and 120%) wereprepared and the % recovery was calculated. The percentage recovery was found to be within the limit i.e. 98-102%). The results obtained for recovery at 80%, 100%, 120% are within the limits. Hence method is accurate.The results were presented in Table 6

Limit of Detection and Limit of Quantification
LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations, **LOD = 3.3 × σ / s** and **LOQ=10×σ/S**., The results were presented in Table 7.

CONCLUSION
A sensitive & selective stability indicting RP-HPLC technique have been developed & validated for the analysis of Lamivudine and Nevirapine. Depending on the on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Lamivudine and Nevirapine indicated that the developed method is specific for the estimation of Lamivudine and Nevirapine.Further the proposed RP-HPLC technique has excellent sensitivity, precision and reproducibility.

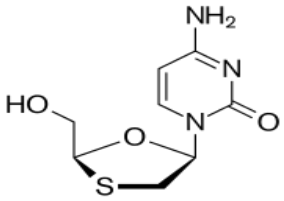

Fig. 1: Chemical Structure of Lamivudine

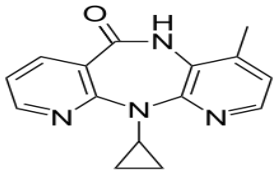

Fig. 2: Chemical Structure of Nevirapine

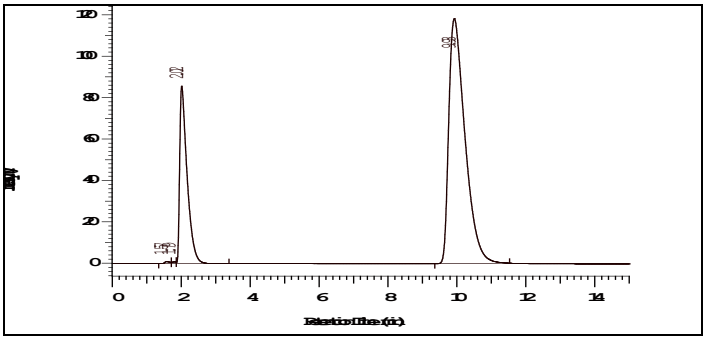

Fig. 3: Optimized chromatogram of Lamivudine (RT 2.02 min)& Nevirapine (RT= 9.93 min)

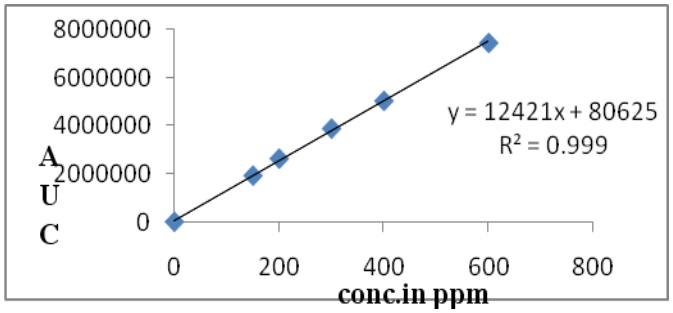

Fig. 4: Calibration curve of Nevirapine

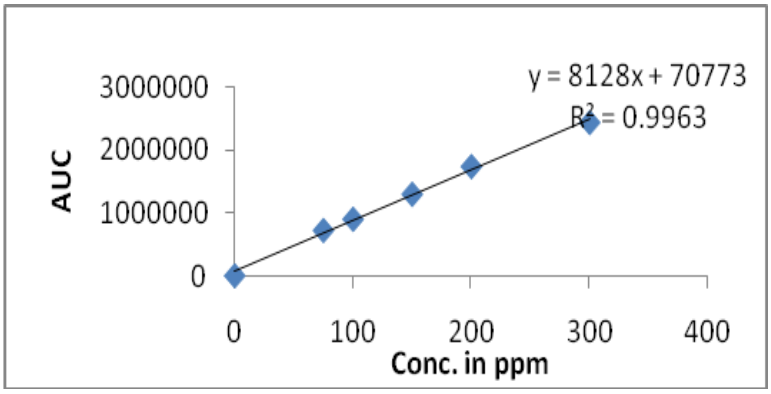

Fig. 5: Calibration curve of Lamivudine

Table1: List of various equipments used

S. no.	Name of Instrument	Instrument Model	Name of manufacturer
1	UV-Visible double beam spectrophotometer	UV 1800	Shimadzu, corp. Japan.
2	HPLC	1575	Hitachi
3	Ultra Sonicator	--------	Entrech electronics limited

Table 2: List of various materials used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	----	----	In house laboratory.
2.	Methanol	99.9%	HPLC	LobaChem; Mumbai.
3.	Sodium Hydroxide	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	LobaChem; Mumbai.
5.	Ortho phosphoric acid	96%	L.R.	Sd fine-Chem ltd; Mumbai

Table 3: Optimized Chromatographic Conditions

Mobile phase	Potassium dihydrogen phosphate buffer(0.02 M, pH 2.5): acetonitrile (57:43)
Wavelength	273nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Column	Develosil ODS HG-5 RP C_{18}, 5µm, 15cmx4.6mm i.d.

Table 4: Data of linearity

S.No	Nevirapine		Lamivudine	
	Working conc. (µg/ ml)	Peak area	Working conc. (µg/ ml)	Peak area
1	150	1928747	75	724838
2	200	2638131	100	904737
3	300	3892572	150	1302869
4	400	5049436	200	1746831
5	600	7469310	300	2450813
Correlation Coefficient (r)		0.9993	0.9963	
Slope (m)		12421	8128	
Intercept (c)		80625	70773	

Table 5: Precision data for Nevirapine and Lamivudine

Injection No.	Nevirapine		Lamivudine	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	9.93	3983572	2.02	1302869
2	9.93	3985214	2.02	1302586
3	9.93	3990228	2.02	1318521
4	9.92	3985261	2.01	1302569
5	9.92	3996512	2.02	1302896
Mean		3988157		1305888
SD		5295.407		7063.605
%RSD		0.7694		0.8498

Table 6: Accuracy data for Nevirapine and Lamivudine

%Concentration (at specification Level)	Nevirapine			Lamivudine		
	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
80	240	300	99.38	120	100	99.48
100	300	300	99.86	150	100	99.25
120	360	300	99.34	180	100	99.68
Mean % Recovery **99.52%**				**99.47%**		

Table 7: Data table of LOD & LOQ for Nevirapine and Lamivudine

Drug	LOD (µg/ml)	LOQ (µg/ml
Neverapine	0.1µg/ml	0.3µg/ml
Lamivudine	0.08µg/ml	0.24µg/ml

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