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A New Analytical Method Development and Validation for the Simultaneous Estimation of Lamivudine and Stavudine in Tablet Dosage Form by Rp-Hplc Method

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Abstract: A high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of two antiretroviral drugs viz. Lamivudine and Stavudine that constitute one of the first line regimens in antiretroviral therapy. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. Chromatography was carried out by isocratic technique on a reversed-phase C-18 SYMMETRY column with mobile phase of methanol and water (80:20 v/v) with UV detection at 266nm. The linearity of the calibration curves for each analyte in the desired concentration range is good ($r2 > 0.999$) by RP-HPLC method. The method was accurate and precise with recoveries in the range of 97 and 103% for all the two drugs and relative standard deviation (R.S.D.) <1%. The proposed methods were highly sensitive, precise and accurate and hence it can be successfully applied for the simultaneous estimation of Lamivudine and Stavudine in tablet dosage form.
Keywords: Lamivudine, Stavudine, simultaneous estimation and RP-HPLC.

Introduction

One of the deadliest and unmanageable chronic health catastrophes is HIV/AIDS. It requires lifelong treatment with potent life saving essential drugs that include nucleoside reverse transcriptase inhibitors, non nucleoside reverse transcriptase inhibitors and protease inhibitors[1]. Amongst these Lamivudine (3TC: 2, 3-dideoxy-3-thiacytidine) and Stavudine (d4T: 2, 3-didehydro-3-deoxythymidine) constitute first line therapy[2]. Combination of these two drugs into fixed dose combinations (FDCs) has been an essential constituent of the Highly Active Anti-retroviral (HAART) therapy[3]. Lamivudine is a nucleoside analog having potent in vitro and in vivo inhibitory activity against HIV reverse transcriptase[4]. Lamivudine specifically refers to the (−) enantiomer of the cis racemate and is marketed as tablets in different strengths. Stavudine is chemically a thymidine nucleoside analogue. It has a complete and less variable oral absorption as compared to other nucleoside analogs[5]. It is a known agent for the treatment of infection by HIV-1 (human immunodeficiency virus, type 1), which acts through non-competitive inhibition of HIV-1 reverse transcriptase [6]. The two antiretroviral drugs are official only in Indian Pharmacopoeia (IP, Addendum 2002).

Materials and methods

Working standards of Lamivudine and Stavudine were kindly provided by Micro Labs Pvt Ltd., Bangalore. HPLC grade water, methanol and Acetonitrile were supplied by Loba chemicals Ltd., India, Qualigens Fine Chemicals Ltd., Mumbai, S.D. Fine chemicals Ltd.,India..

Preparation of standard solutions:

Lamivudine and Stavudine stock Preparation:

An accurately weighed quantity of 750mg of Lamivudine and 150mg of Stavudine working standard were taken in a 100ml volumetric flask and dissolved in 50ml mobile phase by sonicating for 15minutes and made up to the volume with mobile phase. The solution was filtered through Whatmann filter paper no: 40 and 5 ml of the filtrate was diluted to 50ml with mobile phase. The solution was filtered through 0.2µ Nylon membrane syringe filter and 20µl of this solution was injected for HPLC analysis.

Preparation of sample solutions:

Twenty tablets were weighed and crushed to fine powder. A quantity of powder equivalent to 2449.3 mg of marketed formulation was transferred to 100ml standard flask. The powder was dissolved in 50 ml mobile phase by sonicating for 15 mins and made up to the volume with mobile phase. The solution was filtered through Whatmann filter paper no: 40 and 5ml of the filtrate was diluted to 50ml with mobile phase. The solution was filtered through 0.2µ Nylon membrane syringe filter and 20µl of this solution was injected for HPLC analysis.

Analysis of formulation:

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed Standard solution was injected and the chromatogram was recorded. The retention time Lamivudine and Stavudine was 4.288and 7.488.min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factors (peakarea ratio of standard peak area and sample peak area) of the standard solution and sample solution were calculated. A representative chromatogram has been given in the figure-1.The result of analysis reported in (table-1).The stability of the sample in mobile was analyzed after 24 hrs; it was found no change in analytical parameter.

Recovery studies:

To study accuracy of the method recovery studies were carried out by adding a known quantity of the standard to the preanalyzed sample and recovery study was done. The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we conclude that the method was accurate [Table- 2].

Table1: Analysis of formulation:

Drug	**Lamivudine**		**Stavudine**	
Amount of drug(mg/tab)	Labeled amount	Estimated amount	Labeled amount	Estimated amount
	150mg	149.40 mg	30mg	30.38mg
%RSD	0.25%		0.68%	

Figure-1 Chromatogram for formulation

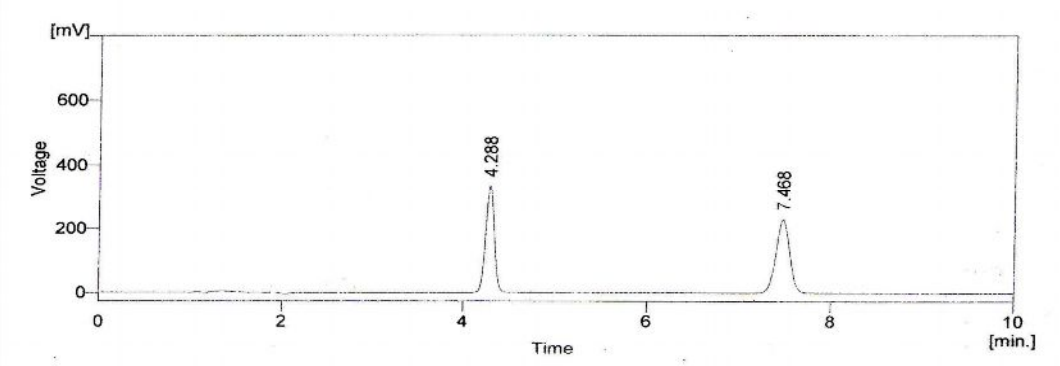

Table 2: Recovery Studies for Lamivudine and Stavudine

S. No	Inj. Sample	Spike level	Amount present	Amount recovered	% recovered
1	LAMIVUDINE	80 %	601.09mg	594.30mg	99.88%
2		100 %	749.80mg	749.50mg	99.97%
3		120 %	898.70mg	890.90mg	99.14%
4.	STAVUDINE	80 %	119.82mg	119.09mg	99.41%
5		100 %	150.40mg	149.80mg	99.60%
6		120 %	179.52mg	179.12mg	99.74%

Solution stability:

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for bothsolutions, the retention time and peak area of Lamivudine and Stavudine remained almost unchanged (% R.S.D.less than 1.0) and no significant degradation within the indicated period, thus indicated that bothsolutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

Result and Discussion

A simple, precision and accuracy RP-HPLC method was developed the simultaneous estimation of Lamivudine and Stavudine in tablet dosage form, consisting of methanol: water system (80: 20 % v/v). The chromatographic condition was set at a flow rate of 1.5 ml/min with the UV detector at 266 nm. The above method was optimized with a view to develop an assay method for Lamivudine and Stavudine.

Several mobile phase compositions were tried to resolve the peaks of Lamivudine and Stavudine. The optimum mobile phase containing methanol and water (80: 20 % v/v) was selected because it was found ideal to resolve the analyte peaks of both the drugs. Quantification was achieved with UV detections at 266 nm based on peak area and absorbance. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions are Lamivudine and Stavudine. Various parameters obtained with 20 µl of injection volume are summarized in the table-3 which is given below.

Table3: Validation and system suitability parameters

S.No	Parameters	Lamivudine	Stavudine
1	System suitability (%RSD of tailing factor)	1.056	1.000
2	Specificity	Specific	Specific
3	Precision: A)System Precision B)Method Precision	 0.38 0.16	 0.33 0.46
4	Linearity	0.999	0.999
5	Accuracy	99.97%	99.60%
6	Robustness	Robust	Robust
7	LOD	0.9µg	0.8 µg
8	LOQ	2.8 µg	2.5 µg
9	Stability studies	stable	Stable

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