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SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND STAVUDINE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

Lamivudine is a potent ‘‘reverse transcriptase inhibitor ’’ belonging to the class of nucleoside analog reverse transcriptase inhibitor (NARTI). Stavudine is an anti-HIV treatment drug in the class of drugs called nucleoside reverse transcriptase inhibitors (NRTIs). Both are excellent anti-retroviral drugs, widely used clinically in the treatment of infections which are related to immuno deficiency virus. The present combination of Lamivudine and Stavudine was marketed as one tablet dosage form formulation with a dose of Lamivudine 150mg/tab and Stavudine 40mg/tab. Although various methods have been developed for the estimation of Lamivudine and Stavudine individually and in combination with other drugs, no official method has been published with the combination of these drugs. The fixed dose combination of Lamivudine and Stavudine was subjected to simultaneous estimation by Reverse phase HPLC method.

Key words: Lamivudine, Stavudine, Retroviral, HPLC, Nucleoside analog, Immuno virus.

1. INTRODUCTION

Lamivudine, ([2R-cis]-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]2(1H) pyrimidinone) (The United states Pharmacopoeia. 2003). It is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against HIV-1, a causative agent of acquired immune deficiency syndrome (AIDS) and Hepatitis B viruses. Stavudine,1-((2R,5S)-5 (hydroxymethyl)-2,5-dihydrofuran-2-yl)-5-ethylpyrimidine - 2,4(1H,3H) –dione (Indian Pharmacopoeia. 2010; Martindale.2005; The Merck Index. 2006). Stavudine, an analog of thymidine, is phosphorylated by cellular kinases into active triphosphate. It is a synthetic thymidine analogue with potent inhibitory activity against HIV-1 in vitro. Since therapy with NRTIs for treatment against HIV-1 results in rapid development of HIC strains, co-administration of other antiretroviral therapies is necessary (Parthiban et al. 2012). Stavudine triphosphate inhibits the HIV reverse transcriptase by competing with natural substrate, thymidine triphosphate. It also causes termination of DNA synthesis by incorporating into it (Umesh M Patel and R Nageswara Rao, 2011). Multi-drug therapy has become the standard treatment for acquired immunodeficiency syndrome (AIDS) (Srivani Mallepelli et al. 2011). The US Department of Health and Human services’ current guideline for treatment of established HIV infection strongly recommends lamivudine in combination with Stavudine and other NRTIs (US Department of Health and Human Services. 2011). 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 (British Pharmacopoeia. 2008; S Jayaseelan et al. 2010). From the Literature Survey it was seen that analytical methods are available for the determination of these drugs individually and in combination with other drugs. Therefore the present study has been undertaken to develop a new, simple, rapid, efficient and reproducible method for the simultaneous determination of Lamivudine and Stavudine combination. An attempt was made in this project to derive simple, cost effective, rugged, accurate, sophisticated and precise HPLC method for this combination of drugs (Namita Kapoor. 2006).

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Lamivudine and Stavudine working standards were procured as gift samples from Hetero Drugs Ltd. Hyderabad, India. Hypersil ODS C_{18}, 150×4.6mm, 5µm is used as stationary phase. Acetonitrile (HPLC grade, Ranbaxy) was used as a solvent for mobile phase preparation. Ammonium acetate, Glacial acetic acid (AR grade, merck) was used for 0.02 M ammonium acetate pH 6.5 buffer preparation.

2.2. HPLC Instrumentation and Chromatographic Conditions

HPLC was performed with an LC-10A VP quaternary pump, a programmable variable wavelength PDA dedector, an SPD-10 AVP column oven and SCL 10 AVP system controller , all from schimadzu and a rheodyne injector fitted with a 10 µl loop (injection volume). Data were recorded and evaluated by use of class – VP 5.032 software. Chromatographic separation was achieved on a 150 × 4.6 mm, i.d, 5µm particle size, Hypersil ODS C_{18} reverse phase column. The mobile phase was 94 : 6 (v/v) 0.02M ammonium acetate buffer pH 6.5 – acetonitrile, at a flow rate of 1mL per min and detection was performed at 250nm.

2.3. Preparation of standard solutions and samples

2.3.1. Preparation of mobile phase

Preparation of 1% v/v glacial acetic acid solution

1.0 ml of glacial acetic acid was diluted to 100 ml with water in 100 ml volumetric flask.

2.3.2. Buffer preparation

1.54 gm of ammonium acetate was dissolved in 1000 ml of water in 1000 ml volumetric flask. The pH of the solution is adjusted to 6.5 with 1%v/v glacial acetic acid and filtered through 0.45µ membrane filter.

2.3.3. Mobile phase composition

A filtered and degassed mixture of buffer and acetonitrile in the ratio of 94: 6 v/v was used as a mobile phase.

2.3.4. Diluent for sample preparation

Mobile phase is used as diluent for sample preparation.

2.3.5. Preparation of combined standard

Lamivudine (150mg) and Stavudine (40mg) were weighed accurately and transferred into 100ml volumetric flask, 60ml of diluent (mobile phase) was added and sonicated to dissolve, and then diluted upto the mark with diluent. The aliquot (5ml) was further diluted to 50ml with the same diluent. The final solution contained 150 ppm of Lamivudine and 40 ppm of Stavudine.

2.3.6. Preparation of pharmaceutical formulation

Twenty tablets were accurately weighed and finely powdered. The powder equivalent to 150mg of lamivudine was accurately weighed, mixed with 60ml of diluent and sonicated for 30 min with occasional shaking and diluted upto volume with diluent. The solution was then filtered through 0.45µ membrane filter. The filtrate (5ml) was further diluted to 50ml with the same diluent and mixed well.

2.4. Assay of marketed formulation

Fixed volumes 10µl of standard and sample preparation were injected into chromatographic system and the chromatograms were recorded and the peak responses were measured. The amount of lamivudine and Stavudine present was calculated

comparing the peak area values of sample with that of standard. All determinations were performed in duplicate. The results obtained by estimating the marketed tablet formulation containing Lamivudine and Stavudine by proposed HPLC method were given in table.

2.5. Method validation

The analytical method was validated for linearity, accuracy, precision, specificity, robustness and ruggedness (ICH guidelines. 2005).

2.5.1. Linearity

Linearity was studied by preparing standard solutions at different concentrations 25%, 50%, 100%, 125%, 150% are prepared by diluting the Lamivudine and Stavudine using diluents, plotting a graph of concentration against peak area, and determining the linearity by Least-squares regression.

2.5.2. Accuracy, as recovery

To confirm the accuracy of the proposed method, recovery experiments were performed by standard addition technique. In this method a known quantity of standard was added at three different concentration of active ingredient and calculating the recovery of Lamivudine and Stavudine RSD (%) and standard error (SE) for each concentration.

2.5.3. Precision

Precision was studied by measuring intra-day (repeatability) and inter-day (by injecting of samples over three consequtive days) variation of the method for three different concentrations of Lamivudine and Stavudine.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

3.1.1. Column and mobile phase

The photo diode Array detector was selected for quantification purpose, since it provides a higher sensitivity.

Several columns and Solvents have been used with RP-HPLC, but hypersil ODS C_{18} 150×4.6 mm i.d, 5µm size was fixed as it gave better resolution when compared with C18 columns. The mobile phase was selected in different proportions (50: 50; 90: 10; 70: 30; 94: 6 v/v), while better results achieved using 94: 6 v/v (buffer: acetonitride), allowing good separation in a short period of time. The retention times of Lamivudine and Stavudine were 3.275 min and 4.475 min respectively and the total time of analysis was than 10 min.

3.1.2. Detector Settings

The detection wavelength used was 270mm.

3.1.3. Linearity

The plot of peak area versus the respective concentration of Lamivudine and Stavudine were found to be linear in the concentration range of 37.5 to 225 ppm and 10 to 60 ppm, respectively as shown in the figures. The corresponding peak areas were given in the table. The linearity obtained between peak areas and concentrations with both Lamivudine and Stavudine were excellent. The R^2 of Lamivudine and Stavudine were found to be 0.9999 and 0.9999 respectively.

Table 1: ***The corresponding peak area of Lamivudine and Stavudine***

S.No.	*Concentration of Lamivudine ppm*	*Lamivudine Peak area*	*Concentration of Stavudine ppm*	*Stavudine Peak area*
1.	37.5	973527	10	262771
2.	75	1900089	20	512603
3.	150	3788081	40	1017378
4.	187.5	4717794	50	1286767
5.	225	5683221	60	1545067

3.1.4. Precision

In order to evaluate the instrumental precision was injected the same standard for six times. The chromatographic method proved to be precise. The accuracy of the method was evaluated by the standard addition procedure (% of recovery) with three addition levels (50, 100 and 150% of the expected values, each one in triplicate). The standard mixture was added to the sample. The results are shown in the table. The results demonstrate good recovery for the compounds under study.

Table 2: ***The % of recovery of Lamivudine and Stavudine***

Drug	***Theoretical concentration (ppm)***	***Intraday measured concentration****		***Interday measured concentration****	
		Mean (ppm)	***RSD(%)***	***Mean (ppm)***	***RSD(%)***
Lamivudine	100	100.0	0.01	99.37	0.49
	150	150.36	0.01	149.57	0.68
	200	200.11	0.01	199.84	0.72
Stavudine	20	20.79	0.05	20.20	2.30
	40	40.62	0.03	39.86	2.22
	60	60.33	0.02	59.44	1.23

The summary of validation data for estimation of Lamivudine and Stavudine in the tablet dosage form are given in the table.

Table 3: ***The of validation data for estimation Lamivudine and Stavudine***

Parameter	***Result***	
	Lamivudine	***Stavudine***
Linearity range	37.5 to 225	10 to 60
Correlation coefficient	0.9999	0.9999
Accuracy	100.1	99.87
Precision Intra-day(n=5) Inter-day (n=5)	0.01-0.01 0.49-0.72	0.02-0.05 1.23-2.30
Specificity	Specific	Specific

3.2. Analysis of pharmaceutical preparation

The peaks with R_f 3.275 min for Lamivudine and 4.475 min for Stavudine were observed in the chromatogram of the samples extracted from tablets. Experimental results of the amount of Lamivudine and Stavudine tablets expressed as % of label claim were in good agreement with label claims, which suggests that there is no interference from any excipients, which are normally present in the tablets. The % RSD for drug content was found to be 0.41 and 0.56 for Lamivudine and Stavudine respectively. The low values of relative standard deviation indicate the high precision of the method. The results are shown in the table.

Table 4: ***The analysis of pharmaceutical preparation of Lamivudine and Stavudine***

Drug	*Label claim (mg/tab)*	*Amount estimated (mg/tab)**	*% Amount estimated**	*R.S.D (%)*
Lamivudine	150	149.99	99.99	0.41
Stavudine	40	40.06	100.06	0.56

4. CONCLUSION

A simple, rapid, accurate, and precise HPLC analytical method has been developed for the determination of Lamivudine and Stavudine in combined tablet dosage forms. The method was validated in accordance with ICH guidelines. The retention time 3.275 min for Lamivudine and 4.475 min for Stavudine enables rapid determination of the drug, which is important for routine analysis. The method seems to be suitable for the quality control in the pharmaceutical industry because of its sensitivity, simplicity and selectivity.

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