

DISSOLUTION METHOD DEVELOPMENT OF LAMIVUDINE, NEVIRAPINE AND STAVUDINE IN TABLETS BY UPLC

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ABSTRACT

A sensitive, selective and rapid RP-UPLC method was developed and validated for the simultaneous estimation of lamivudine, nevirapine and stavudine in their tablet dosage form. The chromatographic separation was achieved on acuity X-Bridge column (50 x 4.6 mm, 3.5 μm) using phosphate buffer of pH 2.5 and methanol with gradient elution at a flow rate of 1.0 mL min⁻¹. The UV detection was performed at 266 nm, column oven temperature was 30°C and total run time was 5 minutes within which all three compounds were separated. The method was validated for accuracy, repeatability, reproducibility and robustness. Linearity was established for lamivudine, nevirapine and stavudine in the range of 3.2–19.2, 4.4–26.4 and 8.8–52.8 μg min⁻¹ respectively. The percentage recoveries of lamivudine, nevirapine and stavudine were found to be 100 ± 3.2, 100 ± 2.2 and 100 ± 5.0% respectively.

Keywords: UPLC, Lamivudine, Nevirapine and Stavudine Tablets, Dissolution development, Validation.

INTRODUCTION

Lamivudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV. The chemical name of lamivudine is 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Fig. 1). Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine and also been referred to as (-) 2',3'-dideoxy,3'-thiacytidine. Lamivudine is a nucleoside reverse transcriptase (NRT) inhibitor of human immunodeficiency virus type 1 (HIV-1) and Hepatitis B¹. It is a chain terminator, and all three phosphorylation events to give the triphosphate are carried out by cellular enzymes. It is listed in Indian pharmacopoeia and indexed in another sources^{2,3}.

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type

1 (HIV-1). Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. The chemical name of nevirapine is 11-cyclopropyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one⁴ (Fig. 1). Treatment with Nevirapine mono-therapy is notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI binding site⁵. Nevirapine in association with two other antiretroviral products, nucleoside reverse transcriptase inhibitors (NRTIs) and/or protease inhibitors (PIs) significantly reduces the viral load and increases CD4 cell count, particularly in treatment-naïve patients⁶. Nevirapine is metabolized by cytochrome P450 (CYP3A4) and is a relatively potent inducer of the enzyme; consequently, it has the ability to reduce plasma concentrations of other drugs that are also biotransformed by CYP3A4 as PIs⁷.

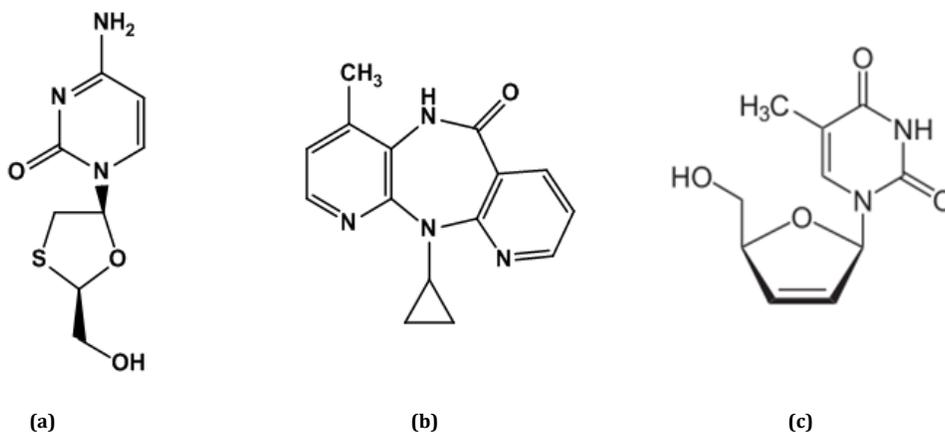


Fig. 1: Structures of Lamivudine (a), Nevirapine (b), Stavudine (c)

Stavudine is a nucleoside analog reverse transcriptase inhibitor (NARTI) active against HIV. It is chemically named as 1-((2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (Fig. 1). The drug is officially listed in monograph of USP⁸. Stavudine is phosphorylated by cellular kinases to the active metabolite Stavudine triphosphate. Stavudine triphosphate inhibits the activity of HIV reverse transcriptase both by competing with the natural substrate deoxythymidine triphosphate

and by its incorporation into viral DNA causing a termination of DNA chain elongation because stavudine lacks the essential 3'-OH group. Stavudine triphosphate inhibits cellular DNA polymerase beta and gamma, and markedly reduces the synthesis of mitochondrial DNA.

There were a number of analytical methods have been evoked for the estimation of lamivudine, nevirapine and stavudine either individually or combination with other anti-viral agents in biological

fluids and stability indicating assays. Rao et. al⁹ published a method for the estimation of stavudine in tablets and serum. Panditi et. al¹⁰ reported a first order derivative spectroscopy method for the estimation of stavudine in bulk and pharmaceutical dosage forms. Saini et. al¹¹ estimated lamivudine and stavudine in tablet dosage forms. Prasada Rao et. al¹² developed a method for the determination of nevirapine in bulk drug and tablets. Kapoor et. al¹³ published a method for the simultaneous determination of lamivudine, stavudine and nevirapine using HPLC and in their method run time was 15 min. Literature survey reveals that various HPLC methods were published^{14,15,16,17,18} for the estimation of lamivudine, nevirapine and stavudine but there is no UPLC method to estimate lamivudine, nevirapine and stavudine. The present research work describes a sensitive, selective and accurate RP-UPLC method for the simultaneous determination of lamivudine, nevirapine and stavudine in the tablet dosage forms.

MATERIALS AND METHODS

Materials and methods

Reference standards of lamivudine, nevirapine and stavudine were received from Aurobindo Pharma (Hyderabad, India) along with their purity confirmation certificates. Tablet samples were received from Dr. Reddy's Laboratories (Hyderabad, India). Potassium dihydrogen phosphate (KH₂PO₄) and orthophosphoric acid were procured from Merck (Mumbai, India). HPLC grade methanol (MeOH) and acetonitrile (ACN) were obtained from Rankem (Delhi, India). Analysis was performed on Waters Aquity UPLC system (Milford, USA). Dissolution study was performed on Distek (New Jersey, USA). An X-Bridge column, 50 x 4.6 mm, 3.5 µm was received from Waters, USA. Water was prepared by using Millipore Milli-Q system.

Chromatographic conditions

Buffer preparation

2.72 gm of KH₂PO₄ was added to 1000 mL of Milli-Q water and to this about 1 mL of triethylamine was added and mixed well. The pH of this solution adjusted to 2.5 with ortho-phosphoric acid and was filtered through 0.22 µm filter using vacuum filtration. Diluent was prepared by mixing phosphate buffer of pH 2.5 and MeOH in the ratio of 50:50. UPLC flow rate was 1.0 mL min⁻¹ and detection was set at 266 nm. UPLC gradient programme was shown in following Table 1.

Table 1: UPLC gradient programme

Time (min)	Flow rate (mL min ⁻¹)	Buffer (%)	Methanol (%)
0.00	1.00	95	5
3.00	1.00	40	60
3.50	1.00	95	5
5.00	1.00	95	5

Dissolution conditions and sample preparation

Dissolution analysis was performed using 0.1 N hydrochloric acid (prepared by dissolving 8.5 mL of concentrated hydrochloric acid in 1 L of water) dissolution medium and the volume of medium was 900 mL. Temperature of the medium was set at 37.0 ± 0.5°C and rotational speed at 75 RPM. Samples were collected at 30 min. Withdrawn a 20 mL of the sample and filtered through 0.22 µm nylon filter. Injected a portion of the sample to estimate stavudine, and diluted 10 mL of this solution to 100 mL for the determination of lamivudine and nevirapine.

Standard solution preparation

Accurately weighed and transferred 16.8, 22.4 and 44 mg of lamivudine, nevirapine and stavudine, respectively, into a 100 mL volumetric flask, dissolved and diluted with the diluent. A 10 mL of this solution was diluted into 100 mL with dissolution media.

RESULTS AND DISCUSSION

UPLC method development

Now-a-days in pharmaceutical industries many HPLC methods are performing on UPLC because of its less time consumption and high sensitivity. The other factors to consider for developing UPLC methods are reduction of total run time, lower cost per assay, dissolution and promote instrument uptime. The advantage of UPLC is the chromatogram generated from UPLC method had very narrow peaks, and the excessive resolution which indicated an opportunity for method improvement, less sample consumption and withstand to high backpressure.

An initial UPLC trial was taken on Aquity BEH 50 x 2.1 mm, 1.7 µm columns. Using this column all peaks were eluted within 2.2 minutes but peak shapes are found to be not good (Fig. 2a).

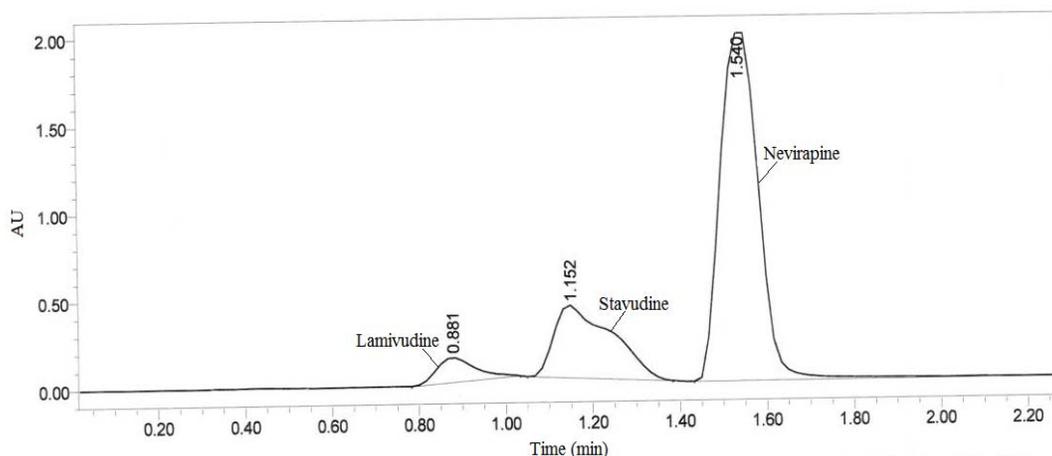


Fig. 2a: UPLC chromatogram of LNS on Aquity BEH column

After that injected the same sample solution on Zorbax, 50 x 4.6 mm, 3 µm column which gives good peak shape for all three analyzing compounds but the resolution between stavudine and nevirapine was less (Fig. 2b).

To increase the resolution, the sample solution was injected on X-bridge column (50 x 4.6 mm, 3 µm) which has higher carbon content and ethylene bridged linkage. Using this column, all the three peaks were eluted within 5 minutes with good shape and well resolution (Fig. 2c).

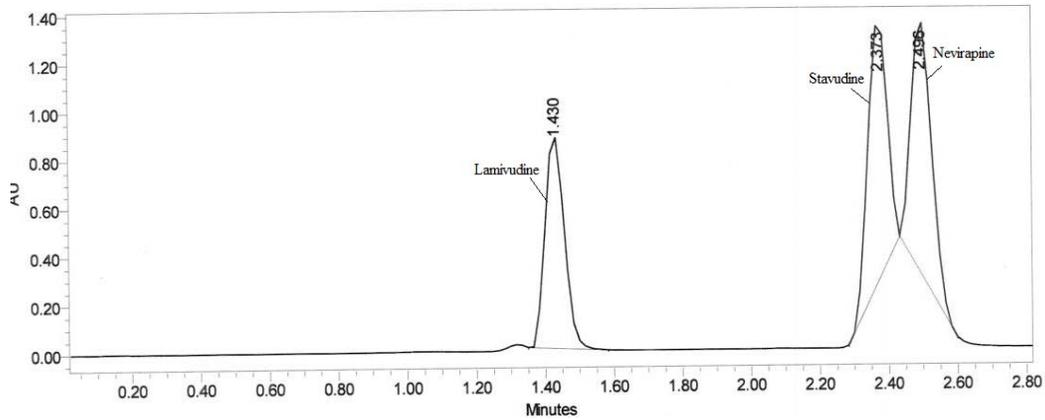


Fig. 2b: UPLC chromatogram of LNS on Zorbax column

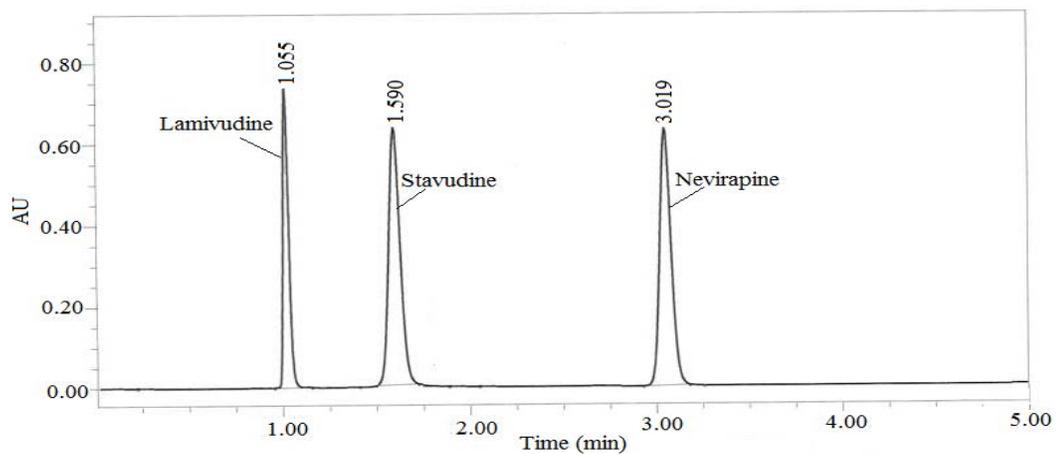


Fig. 2c: UPLC chromatogram of LNS on X-Bridge column

Analytical parameters and validation

After satisfactory development of the method, validation was carried out according to ICH guidelines. The method was validated to demonstrate its suitability for intended purpose using the standard procedure and validation characteristics including specificity, accuracy, precision, robustness, linearity and stability have been evaluated.

Specificity

The amount of placebo used in the preparation was calculated and the solution was prepared as per the test method and injected. No peaks have interfered at the retention time of the lamivudine, nevirapine and stavudine (Fig. 3).

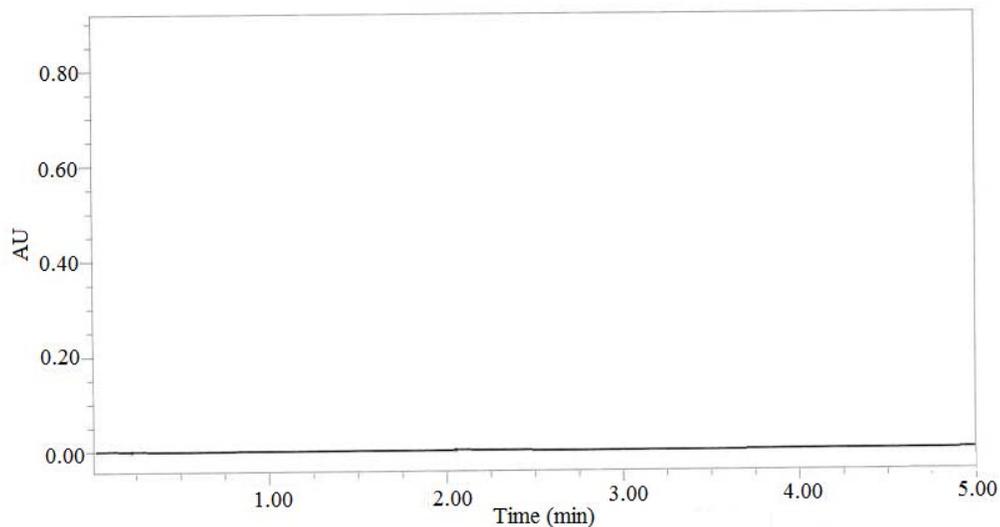


Fig. 3: Blank Chromatogram

Precision**System precision**

System precision was calculated by injecting six replicate injections of standard solution. The % RSD for six injections found to be within the limits (Table 2).

Method precision

Method precision was calculated by injecting six replicate injections of standard and six injections of sample solution from six dissolution vessels. The % dissolution values were found to be within the limit (Table 2).

Intermediate precision

Intermediate precision was calculated by injecting six injections of standard and six injections of sample solution from six dissolution

vessels. The % RSD for six replicate injections of standard and sample was found to be within the limit. This was performed by different analyst on a different instrument and with a different column in different day.

Linearity

The standard solutions were prepared in the concentration ranging from 20% to 120% and the linearity results were found to be within the limits i.e. the correlation coefficient was found to be not less than 0.999 (Table 2).

Accuracy

The placebos were spiked with different ratios of API ranging from 20% to 120% in triplicate. The values which were obtained were found to be within the limits. i.e. the % recovered drug was found to be within 95 % to 105% (Table 3).

Table 2: Results for precision and linearity

Parameter	Lamivudine	Nevirapine	Stavudine
Regression statistics			
Slope	26898	25832	10156
Intercept	8130.6	6868.9	16347
Correlation coefficient (r ²)	0.9994	0.9996	0.9992
Repeatability (% RSD)	1.12	0.94	1.25
Inter-day precision (% RSD)	0.96	0.76	0.85

Table 3: Accuracy results

Drug	Accuracy levels (Average % Recovery) ^a				
	20%	60%	80%	100%	120%
Lamivudine	98.4	100.4	96.8	102.6	103.2
Nevirapine ^a	99.4	97.8	100.4	102.2	101.3
Stavudine ^a	98.5	96.8	95.0	102.3	105.0

a. Average of three determinations

Filter validation

Injected the centrifuged solutions of standard, sample, and compared the peak area of standard and samples which were collected from different filters viz. 0.22 µm nylon, 0.22 µm PVDF and 0.22 µm PTFE filters. It was found that nylon filters were showing good correlation of 99.99% between standard and sample solution when compared to centrifuge. So, finally selected 0.22 µm nylon filters to filter the samples.

Robustness**a. Variation in the flow rate:**

Injected the standard solution six times at different flow rates including, 0.9, 1.0 and 1.1 mL min⁻¹ and calculated % RSD for six injections. In all the flow rates the % RSD for all six injections was found to be less than 1.0% that indicates the method was stable.

b. Change in column temperature

Injected the standard solution six times at various column temperatures (at 25, 30 and 35°C) and calculated the % RSD for six injections. In all the temperature conditions the % RSD for all six injections found to be less than 0.8% which indicates the method was robust.

C. Change in UPLC buffer pH

Injected the standard solution six times at different buffer pH's (pH 2.0, 2.2 and 2.4) and calculated % RSD for six injections. The % RSD for all six replicate injections was 0.9% which shows that the method is suitable even there is slight change in buffer pH.

CONCLUSIONS

A new RP-UPLC method was developed for the estimation of lamivudine, nevirapine and stavudine in their pharmaceutical dosage forms. The total runtime of the developed method was 5

minutes. This method can be used for the estimation of lamivudine, nevirapine and stavudine in their single dosage form also.

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