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A Simple and Rapid RP-HPLC Method for the Estimation of Nevirapine in Bulk and Pharmaceutical Dosage Forms

PALANIAPPAN MOHANRAJ, DEB KUMAR SARKAR, TIRTHANKAR CHOUDHURY and KARUNAKARAN GAUTHAMAN*

Department of Pharmaceutical Sciences,
Himalayan Pharmacy Institute,
E.Sikkim-737136, India.
gauthamank@gmail.com; Fax: +91-3592-246462

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Abstract: A reverse phase HPLC method is described for the determination of nevirapine in tablet dosage forms. Chromatography was carried on an ODS column using a mixture of methanol and water (89:11 v/v) as the mobile phase at a flow rate of 1 mL/min with detection at 284 nm. The retention time of the drug was 3.22 min. The detector response was linear in the concentration of 25-200 mcg/mL. The limit of detection and limit of quantification was 0.004 and 0.0121 mcg/mL respectively. The percentage assay of nevirapine was 99.52%. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, economical, fast, accurate and precise and hence can be applied for routine quality control of nevirapine in bulk and tablet dosage forms.

Keywords: Nevirapine, RP-HPLC, Estimation, Tablet dosage forms.

Introduction

Nevirapine, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-b:2',3'-e][1,4] diazepin 6-one[1] is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). Treatment with nevirapine mono-therapy is notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI binding site[2]. However, in association with two other antiretroviral products, nucleoside reverse transcriptase inhibitors (NRTIs) and/or protease inhibitors (PIs), nevirapine significantly reduces the viral load and increases CD4 cell count, particularly in treatment-naive patients[3].

Nevirapine is metabolized by cytochrome P450 (CYP3A4) and is a relatively potent inductor of the enzyme; consequently, it has the ability to reduce plasma concentrations of other drugs that are also biotransformed by CYP3A4 as PIs[4]. Therapeutic drug monitoring of nevirapine may be warranted to prevent or delay the occurrence of viral resistance, and to ensure optimal therapy for HIV-infected patients[5]. Literature survey reveals that chromatographic methods for the determination of nevirapine in tablet dosage forms by simultaneous quantification with other drugs HPTLC[6], several HPLC[7-13] methods in combination with other antiretrovirals by plasma levels. Marzolini C[14] *et al.* describe method in transplacental passage at delivery in pregnant woman, but due to ethical concern, pregnant women are generally excluded from clinical trials[15] and LC/MS/MS[16] method in human plasma with other antiretroviral drugs, Few individual HPLC methods developed for nevirapine like Hollanders R M F[17] *et al.* developed a method in phosphate buffer and acetonitrile with flow rate 1.5 mL/min and retention time was 6.5 min, Joesph W P[18] *et al.* developed method LC-8 analytical column with mobile phase phosphate buffer,1-butane sulfonic acid as anion pair reagent, methanol and acetonitrile and peak area was detected with retention time 10 min at a flow rate of 1.0 mL/min. Lopaz R M[19] *et al.* showed chromatogram with phosphate buffer and acetonitrile as mobile phase at a flow rate of 1 mL/min with retention time was 2 min. Our aim is to report an isocratic RP-HPLC method for the estimation of nevirapine with mobile phase methanol and water used as mobile phase in the ratio of (89:11), and flow rate 1 mL/min, retention time was 3.22 min. This paper reports simple, economical, accurate, reliable and reproducible analytical method for the estimation of nevirapine in formulation. This procedure is applied successfully for the analysis of the commercial tablets, purchased from market and the results are compared statistically.

Experimental

Nevirapine was obtained as a gift sample from Cipla Ltd, Methanol HPLC grade (S.d. fine-Chem Ltd.), Water HPLC grade (Universal Laboratories Pvt. Ltd.), were used.

Instrument

RP-HPLC was performed in Shimadzu (Japan) HPLC VP Series, Pump LC 20AT & UV Detector model SPD–20A with Rheodyne injector and 20 µL loop. The column used was C18-ODS-Hypersil column (250 mm×4.6 mm, 5 µ). Spinchrome (Mumbai, India) computer based data station was used.

Chromatographic conditions

Chromatographic separations were achieved using a Luna 5u C18 Hypersil column (250×4.6 mm, 5 µ). The mobile phase consisting of methanol and water (89:11 v/v) was passed through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 1 mL /min and the measurements were made at 284 nm. The column and the RP-HPLC system were kept in ambient temperature.

Preparation of standard stock solution

Accurately weighed 100 mg of nevirapine standard was taken in 100 mL volumetric flask. This was dissolved in 70 mL of methanol and sonicated for 30 min and then diluted to 30 mL with the mobile phase to get 1 mg/mL standard stock solution.

Preparation of sample solution

Each twenty tablets (Nevimune, Nevivir, Cipla Ltd.) were weighed accurately and finely powdered. The powder equivalent to 100 mg was taken in 100 mL volumetric flask.

This was dissolved in 70 mL methanol and sonicated for 30 min with internal shaking. Then the volume was finally made to 100 mL with mobile phase to get a clear solution of 1 mg/mL. The above solution was centrifuged at 3000 rpm for 5 min.

Linearity

Several aliquots of standard stock solutions 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 mL of nevirapine were taken in different 10 mL volumetric flask and diluted up to the mark with methanol. Evaluation was performed with Ultra-Violet Dual alpha absorbance detector at 284 nm. Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area *versus* concentration of nevirapine (Figure 1). The plot of peak area of each sample against respective concentration of nevirapine was found to be linear in the range of 25.0-200.0 µg/mL, using regression analysis the linear equation Y= 15.71x - 2.116 with correlation coefficient of r^2= 0.9999. Linear regression least square fit data obtained from the measurements are given in Table 1.

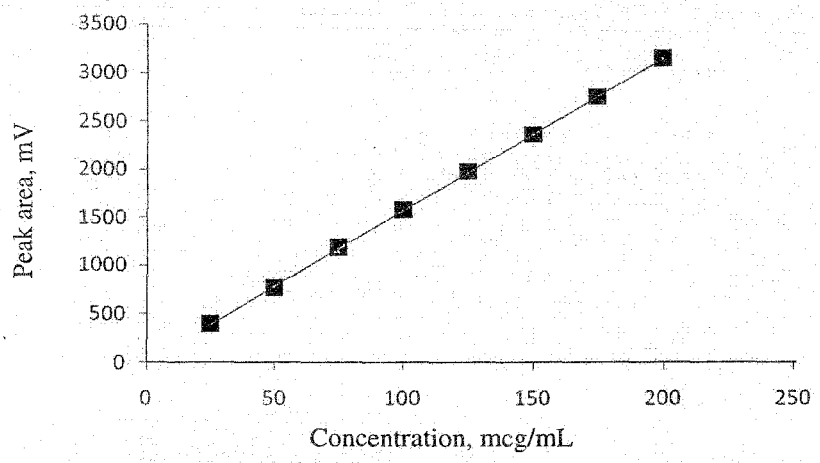

Figure 1. Calibration curve of nevirapine by RP- HPLC

Table 1. Calibration curve points of the proposed method

Concentration of nevirapine µg/mL	Peak area* mV.s
25	396.208
50	770.874
75	1179.528
100	1574.426
125	1969.221
150	2351.261
175	2745.28
200	3142.885

**Mean of five determinations.*

Results and Discussion

The system suitability tests were carried out on freshly prepared standard stock solution of nevirapine. Parameters that were studied to evaluate the suitability of the system are given in Table 2.

Table 2. System suitability test parameters

Parameters	Values
HETP, mm	0.009
Tailing factor	1.113
Theoretical plates/m	110099.73
Resolution	2.38
LOD, µg/mL	0.004
LOQ, µg/mL	0.0121

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for nevirapine were found to be 0.004 and 0.0121 µg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of nevirapine as shown in Figure 2, it was found that the retention time was 3.22 min. A mixture of methanol and water ratio of 89:11 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 25.0-200.0 mcg/mL. The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as CV (%) of a series of measurement Table 3. The result obtained shows CV (%) less than 1% indicating good intra-day precision.

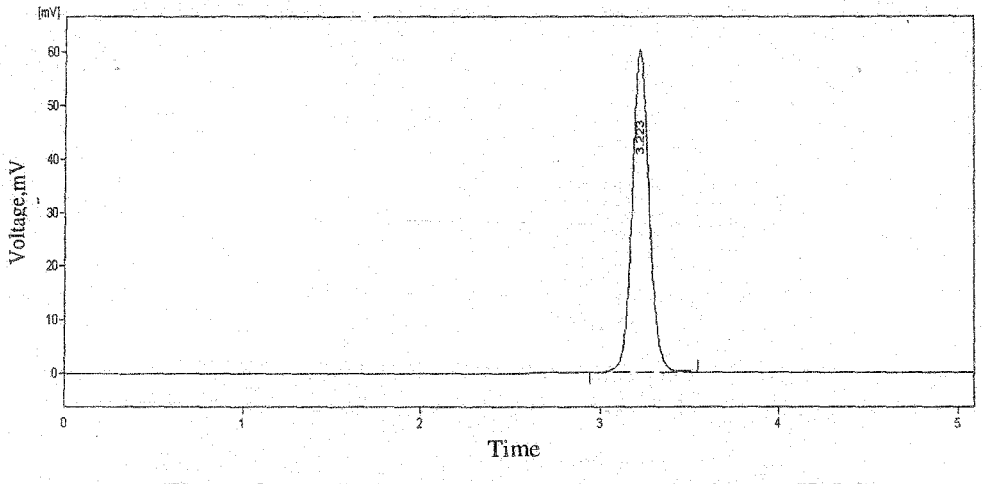

Figure 2. A typical chromatogram of nevirapine by RP- HPLC

Table 3. Intra and inter - day precision for nevirapine assay

Conc. of nevirapine µg/mL	Concentration of nevirapine found			
	Intra-day		Inter-day	
	Mean , n= 5	CV, %	Mean, n=5	CV, %
50	50.05	0.61	50.51	0.99
100	99.63	1.21	99.47	0.28
200	200.14	0.93	200.16	1.03

The assay of nevirapine tablets was found to be 99.52%. The results are given in Table 4. From the recovery studies it was found that about 99.56% of nevirapine was recovered which indicates high accuracy of the method. The results are given in Table 5. The absence of additional peaks in the chromatogram indicates non interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage form of nevirapine within a short analysis time.

Table 4. Results of assay of formulation by the proposed method

Sample	Amount claim mg/tab	Amount found mg/tab	% Recovery
1	200	199.44	99.72
2	200	199.79	99.33

**Mean of five determinations.*

Table 5. The recovery studies for nevirapine by proposed method

Labeled Claim (200 mg)	Amount of drug added, mg	Mean amount found, mg	Mean % Recovery*
1	10	10.24	100.39
2	20	19.07	98.96
3	30	29.88	99.33

**Mean of five determinations.*

Assay

20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 3.22 min. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 4.

Recovery studies

Accuracy was determined by recovery studies of nevirapine, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table 5. The study was done at three different concentration levels.

Conclusion

Developed high performance liquid chromatographic method is simple, reliable, reproducible and economical for the analysis of nevirapine in pharmaceutical formulations. The reported method can be used successfully for the effective qualitative and quantitative analysis of nevirapine in Tablet or other pharmaceutical formulations.

Acknowledgement

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Reference

1. The Merck Index, 14th Ed., Merck Research Laboratories, (Monograph No:6490), 2006, p 1123.
2. De Clercq E, *Il Farmaco*, 1999, **54**, 26-45.

3. Barry M, Mulcahy F, Merry C, Gibbons S and Back D, *Clin Pharmacokinet.*,1999, **36**, 289.
4. Malaty L I and Kupper J J, *Drug Saf.* 1999, **20**, 147.
5. Jajaray A, Alexander J, Price C, Daly D, Pav J, Hatoxx S and Keirns J, *Pharm. Res.* 1995, **9**, S334.
6. Anbazhagan S, Indumathy N, Shanmugapandiyan P and Sridhar S K, *J. Pharm Biomed, Anal.*, 2005, **39**, 801.
7. D' Avolio A, Siccardi M, Sciandra M, Lorena B, Bonora S, Trentini L and Perri G D, *J.Chromatogr.B, 2007,* **859,** 234.
8. Ramachandran G, Hemanthkumar A K, Kumaraswami V and Swaminathan S, *J.Chromatogr.B,* 2006, **843**, 339.
9. Fan B and Stewart J T, *J. Pharm. Biomed. Anal.*, 2002, **28**, 903.
10. Dailly E, Raffi F and Jolliet P, *J. Chromatogr. B,* 2004, **813**, 335.
11. Rezk N L, Richard R, Tidwell and Kashuba A D M, *J.Chromatogr.B,* 2002, **774**, 79.
12. Rezk N L, Richard R, Tidwell and Kashuba A D M, *J.Chromatogr.B* ,2004, **805**, 241.
13. Aymard G, Legrand M, Trichereau N and DiquetB, *J.Chromatogr.B,* 2000, **774**, 227.
14. Marzolini C, Beguin A, Telenti A, Schreyer A, Buclin T, Biollaz J and Decostered L A, *J.Chromatogr.B* , 2002, **774**, 127.
15. Little B B, Bawdon R E, Christmas J T, Sobhi S, Gilstrap L and Gynecol A J O, *J.Chromatogr. B,*1989, **732**, 161.
16. Mistri H N, Jangid A G, Pudage A, Gomes N, Sanyal M and Shrivastav P, *J.Chromatogr.B* , 2007, **853**, 320.
17. Hollanders R M F, Kolmer E B, Burger D M, Wuis E W, Koopmans P P and Hekster Y A, *J.Chromatogr.B,* 2000, **744**, 65.
18. Pav J W, Rowland L S and Korpalski D J, *J. Pharm Biomed. Anal.*, 1999, **20**, 91.
19. Lopez R M, Pou L, Gomez M R, Ruiz I and Monterde J, *J. Chromatogr. B,* 2001, **751**, 371.

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UV and RP-HPLC Method for the Estimation of Lamivudine in Bulk and Pharmaceutical Dosage Forms

P. MOHANRAJ and K. GAUTHAMAN*
Department of Pharmaceutical Sciences
Himalayan Pharmacy Institute, East Sikkim-737 136, India
Fax: (91)(3592)246247; E-mail: pmohanraj1972@yahoo.com

Two assay procedures based on UV-spectrophotometry and reversed phase high performance liquid chromatography (RP-HPLC) have been developed for the determination of lamivudine in bulk drug and pharmaceutical formulations. UV spectrophotometry involves the determination of lamivudine by dissolving it in methanol followed by measuring absorbance at 272 nm. The HPLC determination carried out on a Luna 5u C-18 column using a mobile phase consisting of methanol:water (89:11) at a flow rate of 1.0 mL/min with UV detection at 272. UV spectroscopic method is applicable over 2.5-20 μg/mL range of lamivudine with a molar absorptivity 1.155×10^3 L mol^{-1} cm^{-1} and a Sandell's sensitivity of 0.02265 μg/cm^2. In HPLC method a rectilinear relationship was observed between 5-100 μg/mL and analysis time was 10 min. The retention time was found to be 2.7 min. The methods when apply to the determination of lamivudine in dosage form gave satisfactory results. The developed methods were found to be precise and accurate from the statistical validation of the analysis data.

Key Words: Lamivudine, UV spectrophotometry, RP-HPLC.

INTRODUCTION

Lamivudine is a purine nucleoside analog used against HIV-1 and HIV-2 in the treatment of AIDS. It is (-)-4-amino-1-(2RS,5S)-2-(hydroxymethyl)-1,3- oxathiolan-5-yl]pyrimidin-2(1*H*)-one classified under nucleoside reverse transcriptase inhibitors category of antiretroviral drugs[1]. It is a potent and highly selective inhibitor of human immuno deficiency virus type-1 and type-2 replication *in vitro*. It is active against hepatitis-B virus in HIV- infected patients[2]. The literature survey indicates that lamivudine has been determined simultaneously with some other drugs by HPTLC[3] HPLC[4-9] and spectrophotometric[10] methods in biological fluids and buffer solutions. Nevertheless, no combined HPLC and UV Spectrophotometric method have been reported for the determination of lamivudine in individual formulations. The aim of this study was to develop and validate a simple and fast LC and UV method, through evaluation of the parameter of linearity, precision and accuracy, detection and quantitation limits, robustness and specificity to determine lamivudine in pharmaceutical formulations.

This paper reports two simple, economical, accurate, reliable and reproducible analytical methods for the estimation of lamivudine in formulation. The procedure is applied successfully for the analysis of the commercial tablets, purchased from market and the results are compared statistically.

EXPERIMENTAL

A Shimadzu UV/visible double beam spectrophotometer (model 1700 pharmaspec) with matched quartz cells were used for all spectral measurements.

Shimadzu LC-20 AT liquid pump, SPD-20A UV-Visible detector, a Luna 5u C-18 RP-HPLC column (250 mm × 4.60 mm 5 μm, ID), 25mL Hamiltonâ injecting syringe and spinchrom software were used. Sartorius (CP225D) electronic balance was used for weighing the materials. Pure sample of lamivudine and HPLC grade methanol (Thomas baker), HPLC grade qualigens water was used for preparing the mobile phase.

UV-Spectrophotometric method (method A): An accurately weighed 100 mg lamivudine transferred into 100 mL calibrated flask and added with 30 mL of methanol dissolved well and make up to the volume with distilled water. Aliquots of standard drug solution ranging from 2.5-20 μg/mL the contents were mixed well and absorbance maximum is found at 272 nm. The obtained absorbance values when plotted against the concentration of lamivudine give the calibration graph. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from Beer's law data.

Preparation of sample solution: An accurately weighed tablet powder of lamivudine equivalent to 100 mg of drug transferred into 100 mL calibrated flask and added with 30 mL of methanol dissolved well and make up to the volume with distilled water and filtered. Different aliquots (0.25-1 mL) of 100 μg/mL of lamivudine were accurately transferred into a series of 10 mL calibrated flasks and made up to the volume with water. The contents were mixed well and absorbance maximum was found at 272 nm. The obtained absorbance values when plotted against the concentration of lamivudine gives the calibration graph. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from beer's law data.

Calibration graph: (Method B): About 100 mg of pure sample of lamivudine was weighed accurately and transferred to a 100 mL calibrated flask and dissolved in 70 mL of methanol. The solution was sonicated for 20 min and then the volume made up with a further quantity of mobile phase to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 5-100 μg/mL were made in 10 mL calibrated flasks. The solutions prepared as above were filtered through 0.45 μm membrane filter and then 20 μL of filtrate was injected each time in to the column flow rate of 1 mL/min. Each concentration was injected six times into the column and corresponding chromatograms were obtained. Detection of the drug was performed at 272 nm. From the chromatograms, the retention time and mean peak area ratio

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Calibration graph: (Method B): About 100 mg of pure sample of lamivudine was weighed accurately and transferred to a 100 mL calibrated flask and dissolved in 70 mL of methanol. The solution was sonicated for 20 min and then the volume made up with a further quantity of mobile phase to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 5-100 μg/mL were made in 10 mL calibrated flasks. The solutions prepared as above were filtered through 0.45 μm membrane filter and then 20 μL of filtrate was injected each time in to the column flow rate of 1 mL/min. Each concentration was injected six times into the column and corresponding chromatograms were obtained. Detection of the drug was performed at 272 nm. From the chromatograms, the retention time and mean peak area ratio

were recorded for all concentrations. A calibration curve of peak area ratio *vs.* the respective concentration was plotted. From this, the regression of drug concentration over the peak area plot was computed using least squares method of analysis. This regression equation was used to estimate the amount of lamivudine in pharmaceutical formulations.

Analysis of formulations: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate lamivudine in tablet dosage forms. For this, 20 tablets were weighed and powdered. Accurately weighed portion of the tablet equivalent to 100 mg was taken in 100 mL calibrated flask and 70 mL of methanol was added, shaken well and allowed to stand for 20 min with intermittent sonication to ensure complete solubility of the drug. The mixture was thoroughly mixed and made up to the mark with mobile phase and filtered through a 0.45 μm membrane filter. From the filtrate, different aliquots were taken in separate 10 mL calibrated flasks. The contents of the flask were made up to the volume with the methanol and mixed well. Each of these solutions was then injected into the column. All the determinations were conducted 5 times from the peak area ratios. The drug content in the tablets was quantified using the regression equation obtained from the pure sample.

Optimized chromatographic conditions: The optimized chromatographic conditions are given in Table-1.

TABLE-1

Parameters	Method
Stationary phase (column)	Shimadzu LC-20AT liquid pump, SPD-20A UV-Visible detector, a Luna 5u C-18 RP-HPLC column (250 × 4.60 mm 5 μm, ID),
Mobile phase	Methanol: Water (89:11)
Flow rate (mL/min)	1
Column back pressure (1500 psi)	1500
Run time (min)	1
Column temperature (°C)	Ambient
Volume of injection loop (μL)	20
Detection wavelength (nm)	272
Retention time (min)	2.7

TABLE-2
SYSTEM SUITABILITY TEST PARAMETERS

Parameters	Value	Parameters	Value
HETP (mm)	0.0222	Resolution	1.2500
Tailing factor	1.1200	LOD (μg/mL)	0.0033
Theoretical plates (m)	43327	LOQ (μg/mL)	0.0100

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity, Sandell's sensitivity and the regression analysis using the method of least squares was made for the slope (b), intercept (a), correlation(r^2) obtained from different concentration and results are summarized in Table-3.

TABLE-3
OPTICAL CHARACTERISTICS FOR METHOD-A

λ_{max} (nm)	272
Beer's law limits (μg/mL) (C)	2.5-20
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.155×10^3
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.02265
Regression equation (y*)	
Slope (b)	0.04320
Intercept (a)	0.00262
Correlation coefficient (r^2)	0.99960
Range of errors**	
Confidence limits with 0.05 level	0.78630
Confidence limit with 0.01 level	0.86990

*y = a + bc where c is the concentration of lamivudine in μg/mL and y is the absorbance at the respective λ_{max}; **For average of six measurements.

The graphs showed negligible intercept as described by the regression equation:

$$Y = a + bc$$

(where y is the absorbance unit and C is concentration in mg/mL) obtained by the method of least squares. The per cent range of error (0.05 and 0.01 confidence limit) calculated from the six measurements, 3/4th of the amount of upper Beer's law limits in each method are summarized in Table-3. The result showed that these methods have reasonable precision. The other active ingredient and excipients usually present in the pharmaceutical dosage form or not present in this formulation, so they doesn't interfere in the estimation when formulation was analyzed by this method. The optimum concentration for the estimation of lamivudine was established by varying drug concentration. To evaluate the accuracy of the method a known amount of pure drug was added to the previously analyzed pharmaceutical preparation and the mixture was analyzed by the proposed method. The per cent recovery is given in Table-6. Inter-day and Intra-day precision of the methods were determined by repeat analysis (n = 5) of the standard solution containing lamivudine at three different levels. The results of method are presented in Table-4. The % of RSD value for the peak area based and retention time based value did not exceed 0.3 %, which shows the method is more precise. The method reported here is simple, sensitive, accurate, precise, reproducible and economical and can be used in the determination of lamivudine in bulk drug and its pharmaceutical dosage forms in a routine manner.

TABLE-4
INTRA-AND INTER-DAY PRECISION FOR LAMIVUDINE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED UV METHOD

Conc. of lamivudine (μg/mL)	Concentration of lamivudine found on			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
5	5.35	0.414	4.66	1.190
10	9.97	0.359	9.53	0.456
15	14.67	0.208	4.57	0.172

The objective of present study was to develop a rapid and sensitive HPLC method for the analysis of lamivudine in bulk drugs and its formulation using the most commonly employed RP-HPLC C-18 column with UV detection at 272 nm. Mobile phase consisting of methanol:water (89:11) had given immaculate results when compared to other mobile phases at a flow rate of 1.0 mL/min, the retention time for lamivudine was 2.7 min. The run time was set as 10 min/mL tried for each sample 6 times and the retention times were same. The peak area of the drug was reproducible as indicated by low coefficient of variance (0.74 %).The calibration curve points of the proposed method were shown in Table-7. Under the described experimental conditions the analyte peak was well defined. Lamivudine was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r^2) over the concentration range 5-100 mg/mL. Using regression analysis the linear equation,

$$Y = 24.572x + 8.187$$

(where Y is the mean peak area and X is the concentration in mg/mL). LOD and LOQ were calculated on the basis of signal to noise ratio. Experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the method. The limit of detection and limit of quantification were found 0.0033 and 0.010 μg/mL, respectively (Table-2). The regression equation was used to estimate the amount of lamivudine either in formulation or in validation studies (precision and accuracy). To evaluate the accuracy of the method, a known amount of pure drug was added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The accuracy of the method was determined and the mean recovery was found to be 99.57 % and the values are given in Table-6. It is more sensitive method as signal/concentration of drug value varies more from one concentration to other.

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as CV (%) of a series of measurement. The result obtained shows CV (%) of 1.21 % indicating good intra-day precision. Inter-day variability was calculated from assay on 3rd day and shows a mean CV (%) of 0.892 %. The experimental values for the estimation of lamivudine in samples are presented in Table-5.

TABLE-5
INTRA-AND INTER-DAY PRECISION FOR LAMIVUDINE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Conc. of lamivudine (μg/mL)	Concentration of lamivudine found on			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
20	20.19	1.73	19.96	1.27
30	30.45	0.61	29.51	0.99
40	39.84	1.21	39.46	0.28
80	79.47	0.93	79.68	1.03

TABLE-6
EXPERIMENTAL VALUES OBTAINED IN THE RECOVERY STUDIES FOR LAMIVUDINE BY PROPOSED METHODS

Method	Amount of drug added (mg)	Mean amount found (mg)	Mean % recovery*
HPLC	10	9.93	99.10
	20	19.60	100.46
	30	29.61	99.16
UV	10	10.09	99.11
	20	19.27	99.68
	30	29.36	98.82

*Mean of five determinations.

TABLE-7
CALIBRATION CURVE POINTS OF THE PROPOSED METHOD (HPLC) FOR ESTIMATION OF LAMIVUDINE

Concentration of lamivudine (μg/mL)	Peak area *(mV s)	CV (%)
5	124.772	2.13
10	269.741	1.09
15	374.011	2.79
20	503.710	0.74
30	749.583	0.57
40	983.959	0.99
50	1226.043	1.49
80	2001.963	1.49
100	2448.166	1.51

*Mean of six determinations.

TABLE-8
RESULTS OF ASSAY OF FORMULATION BY THE PROPOSSED METHODS

Pharmaceutical formulation	Labelled claim (mg)	Found (% recovery of nominal amount ± SD*)	
		Method A	Method B
Brand 1	150	99.68 ± 0.64	99.99 ± 0.48
Brand 2	100	99.72 ± 0.45	99.87 ± 0.21

*Mean of five determinations.

Conclusion

Developed high performance liquid chromatographic and ultraviolet spectroscopic methods are simple, reliable, reproducible and economical for the analysis of lamivudine in pharmaceutical formulations. The reported method can be used successfully for the effective qualitative and quantitative analysis of lamivudine in tablet or other pharmaceutical formulations. However, The UV spectroscopic method is simpler and more economical than the HPLC method.

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REFERENCES

1. The Merck Intex, edn. 14, p. 927 (2006).
2. J.E.F. Reynolds, in Martindale, The Extra Pharmacopoeia, Lamivudine, Antiviral Agents, Royal Pharmaceutical Society, London, edn. 31, p. 659 (1996).
3. S. Anbazhagan, N. Indumathy, P. Shanmugapandian and S.K. Sridhar, *J. Pharm. Biomed. Anal.*, **39**, 801 (2001).
4. A.S. Kumari, K. Prakash, K.E.V. Nagoji and M.E.B. Ravi, *Asian J. Chem.*, **19**, 2642 (2007).
5. K.K. Nerurkar, U.J. Dhorda, S.I. Bhoir and M. Sunderasan, *Indian J. Pharm. Sci.*, **65**, 412 (2003).
6. M.S. Palled, P.M.N. Rajesh, M. Chatter and A.R. Bhat, *Indian J. Pharm. Sci.*, **67**, 110 (2005).
7. B. Fan and J.T. Setwart, *J. Pharm. Biomed. Anal.*, **28**, 903 (2002).
8. C.P.W.G.M. Verweij-van Wissen, R.E. Aarnoutse and D.M. Burger, *J. Chromatogr. B*, **816**, 121 (2005).
9. G. Aymard, M. Legrant, N. Trichereau and B. Diquet, *J. Chromatogr. B*, **744**, 227 (2000).
10. D.G. Sankar, M.V.V.N. Reddy, J.M.R. Kumar and T.K. Murthy, *Indian J. Pharm. Sci.*, **64**, 504 (2002).

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