*Asian Journal of Chemistry* Vol. 19, No. 4 (2007), 2642-2646

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

A. SHANTA KUMARI*, K. PRAKASH, K.E.V. NAGOJI† and M.E.B. RAO†
*Department of Analytical Chemistry, St. Peter's Institute of Pharmaceutical Sciences*
*Hanamkonda, Warangal-506 001, India*
*E-mail: pkatakam9@rediffmail.com*

A new reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the estimation of lamivudine (3TC) in bulk and pharmaceutical dosage forms using RP-$C_{18}$ column using an isocratic HPLC system. The mobile phase consisted of acetonitrile and 0.05 M potassium dihydrogen phosphate (pH 4.2) in the ratio of 50 : 50 at a flow rate of 1 mL/min. The run time was 15 min. Nelfinavir mesylate (NEM) (50 µg/mL) was used as internal standard. The detection was carried out at 271 nm and the linearity was found to be in the range of 0.5-160 µg/mL. The retention times for drug (3TC) and internal standard (NEM) were 3.342 and 12.833 min, respectively. Recovery studies shown that about 100.07 % of 3TC could be recovered from the pre-analyzed samples indicating high accuracy of proposed method. There was no intra-day and inter-day variation found in the method of analysis. The mean drug content in branded 3TC tablet dosage forms was quantified and found to be between 99.79 and 100.86 %.

**Key Words: RP-HPLC, Lamivudine, Nelfinavir mesylate.**

## INTRODUCTION

Lamivudine (3TC) is a purine nucleoside analog used against HIV-1 and HIV-2 in the treatment of AIDS. It is (-)-4-amino-1-[(2R,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*[2]. It is active against hepatitis-B virus in HIV-infected patients[3]. Some analytical methods for the estimation of lamivudine were reported such as HPTLC[4], HPLC[5-7], LC-MS[8] and radioimmuno assay[9]. The present study is aimed at developing a simple, reproducible and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of 3TC in bulk and pharmaceutical dosage forms using nelfinavir mesylate (NEM) as an internal standard (IS).

†Roland Institute of Pharmaceutical Sciences, Berhampur-760 010, India.

## EXPERIMENTAL

An isocratic HPLC system (Shimadzu®) consisting of LC-10 AT liquid pump, SPD-10A UV-visible detector, a ODS-18 RP column (4.6 mm I.D. X 25 cm length), 25 μL Hamilton® injecting syringe and MS Windows based Single channel software (Class VP®). Afcoset® electronic balance was used for weighing the materials. Pure samples of lamivudine and nelfinavir mesylate were obtained from Matrix Laboratories, Hyderabad, India. Acetonitrile of HPLC grade and potassium dihydrogen phosphate of AR grade were purchased from E. Merck (India) Ltd., Mumbai. Water used was triple distilled prepared by all glass distillation apparatus.

Chromatographic conditions: The optimized chromatographic conditions were as follows:

| | |
|---|---|
| Chromatograph | Schimadzu HPLC system |
| Mobile phase | Acetonitrile : 0.05 M potassium dihydrogen phosphate (pH 4.2) (50 : 50) |
| Column | ODS $C_{18}$ RP column (4.6 mm I.D. × 25 cm length) |
| Flow rate | 1 mL/min |
| Detection | UV set at 271 nm |
| Injection volume | 20 μL |
| Temperature | Ambient |
| Retention time | |
| of Drug | 3.342 min |
| of IS | 12.833 min |
| Run time | 15 min |

Stock solutions of 3TC and NEM were prepared by dissolving accurately weighed 25 mg of 3TC and NEM in 25 mL of acetonitrile : 0.05 M potassium dihydrogen phosphate (50 : 50) to obtain 1 mg/mL solutions. From these solutions 2.5 mL was pipetted out into 25 mL volumetric flask and diluted with the same solvent system to obtain 100 μg/mL solutions. Working standard solutions of 3TC each containing internal standard (NEM) solution in the concentration of 50 μg/mL were prepared by taking required aliquots of 3TC solutions and then diluted with the same solvent system. The standard solutions prepared above were injected five times into the column at a flow rate of 1mL/min. The ratios of AUC of drug to I.S. were calculated for each of the drug concentrations. The regression equation of drug concentration over the ratio of drug peak is to that of IS was obtained. The regression equation was used to estimate the amount of 3TC in pharmaceutical tablet dosage forms.

The proposed HPLC method was tested for intra-day and inter-day variations. The recovery studies were carried out by adding known amounts of (10 and 30 μg) of the 3TC to the pre-analyzed samples and subjecting them to the proposed HPLC method.

**Estimation of lamivudine in its commercial tablet formulations:** Contents of ten tablets containing 3TC were pooled and powdered. The powder equivalent to 25 mg of 3TC was extracted into acetonitrile and the volume was adjusted to 25 mL, mixed and filtered through a 0.45 μ filter. From the filtrate 0.1 mL was pipetted into a 10 mL graduated test tube and spiked with the required aliquot of I.S. solution and then the volume was adjusted to 10 mL with the mobile phase such that the concentration of IS in each sample was 50 μg/mL and was injected 5 times into HPLC column. The mean concentration of 3TC corresponding to the ratio of AUC of 3TC to that of IS was calculated from the standard graph. The same procedure was followed for remaining branded tablets.

## RESULTS AND DISCUSSION

The present study was carried out to develop a specific sensitive, precise and accurate HPLC method for the analysis of lamivudine in pharmaceutical tablet dosage forms. The column pressure varied from 210-220 $kgf/cm^2$. The retention times for 3TC and IS (NEM) were 3.342 and 12.833 min, respectively. Each of the samples was injected five times and almost the same retention times were observed in all the cases.

The ratio of peak area of 3TC to peak area of I.S. for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1.

TABLE-1
CALIBRATION OF HPLC METHOD FOR ESTIMATION OF LAMIVUDINE

| Concentration of Lamivudine (μg/mL) | Mean ratio of AUC of drug to IS (n = 5) | CV (%) |
|---|---|---|
| 0.5 | 0.1542 | 2.54 |
| 1.0 | 0.2077 | 2.11 |
| 2.0 | 0.4151 | 1.37 |
| 5.0 | 0.9663 | 2.15 |
| 10.0 | 1.8252 | 1.84 |
| 20.0 | 3.0065 | 1.57 |
| 40.0 | 5.5025 | 1.81 |
| 80.0 | 10.8071 | 2.16 |
| 100.0 | 13.4240 | 3.07 |
| 160.0 | 22.6740 | 2.86 |

CV= coefficient variation, regression equation (from 0.5 to 160 μg/mL)

The peak areas of both drug and internal standard were reproducible as indicated by the low coefficient of variation (< 3.07 %). A good linear relationship (r = 0.9992) was observed between the concentration of drug and the respective ratio of peak areas. The calibration graph was found to

be y = 0.1378x + 0.1287 (where y is the ratio of peak area of drug to that of internal standard and x is the concentration of drug in the range of 0.5 to 160 μg/mL). When 3TC solutions containing 10 and 30 μg/mL were analyzed by the proposed HPLC method for finding out intra-day and inter-day variation, a low coefficient of variation was observed (< 2.01 %) showing that the method is highly precise (Table-2). About 100.07 % of 3TC could be recovered from the preanalyzed samples indicating high accuracy of proposed method as shown in Table-3.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

| Lamivudine concentration (μg/mL) | Concentration of lamivudine (μg/mL) found on | | | |
|---|---|---|---|---|
| | Intra-day | | Inter-day | |
| | Mean (n = 5) | % CV | Mean (n = 5) | % CV |
| 10 | 10.16 | 1.72 | 10.21 | 1.94 |
| 30 | 30.22 | 1.48 | 30.27 | 2.01 |

TABLE-3
RECOVERY STUDIES OF LAMIVUDINE

| Amount of drug added (μg) | Mean (± S.D.) amount (μg) found (n = 5) | Mean % recovery |
|---|---|---|
| 10 | 10.071 (± 0.014) | 100.07 |
| 30 | 30.025 (± 0.070) | 100.08 |

The 3TC content in branded tablet formulations was quantified using the proposed analytical method and details are shown in Table-4. The absence of additional peaks indicated no interference of the excipients used in the tablets. The tablets were found to contain 99.79 to 100.86 % of the labeled amount. The low percent of CV (< 2.04 %) indicates the reproducibility of the assay of 3TC in the tablet dosage forms. The proposed method was found to be simple, precise, accurate, specific and economical. Hence this method can be employed to estimate 3TC in bulk and tablet dosage forms effectively.

TABLE-4
ASSAY OF DIFFERENT BRANDS OF LAMIVUDINE TABLETS

| Brand | Labeled amount of drug (mg) | Mean % of labeled amount (n = 5) | CV (%) |
|---|---|---|---|
| A | 100 | 99.79 | 1.73 |
| B | 100 | 100.18 | 1.91 |
| C | 100 | 100.86 | 2.04 |

## ACKNOWLEDGEMENTS

The authors are thankful to Matrix Laboratories, Hyderabad, India for providing gift samples of lamivudine and nelfinavir mesylate. The authors are also grateful to Roland Institute of Pharmaceutical Sciences, Berhampur for providing necessary facilities to carry out the research work.

## REFERENCES

1. J.E.F. Reynolds, in: Martindale, The Extra Pharmacopoeia, Lamivudine, Antiviral Agents, Royal Pharmaceutical Society, London, edn. 31, p. 659 (1996).
2. J.A. Coates, N. Cammack, H.J. Jenkinson, A.J. Jowett, B.A. Pearson, C.R. Penn, P.L. Rouse, K.C. Viner and J.M. Cameron, *Antimicrob. Agents Chemother.*, **36**, 733 (1992).
3. I.W. Fong, *Lancet*, **344**, 1702 (1994).
4. S. Anbazhagan, N. Indumathy, P. Shanmugapandiyan and S.K. Sridhar, *J. Pharm. Biomed. Anal.*, **39**, 801 (2005).
5. E.K. Kano, C.H. Dos Reis Serra, E.E. Koono, S.S. Andrade and V. Porta, *Int. J. Pharm.*, **297**, 73 (2005).
6. K. Henry, R. Brundage, D. Weller, O. Akinsete and A. Shet, *J. Acquir. Immun. Defic. Syndr.*, **35**, 537 (2004).
7. B. Fan, M.G. Bartlett and J.T. Stewart, *Biomed. Chromatogr.*, **16**, 383 (2002).
8. C. Estrela Rde, M.C. Salvadori and G. Suarez-Kurtz, *Rapid Commun. Mass Spectrom.*, **18**, 1147 (2004).
9. S.A. Wring, R.M. O'Neill, J.L. Williams, W.N. Jenner, M.J. Daniel, M.R. Gray, J.J. Newmann, G.N. Wells and D.R. Sutherland, *J. Pharm. Biomed. Anal.*, **12**, 1573 (1994).

(*Received*: 2 February 2006; *Accepted*: 27 December 2006) AJC-5300