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Determination of Lamivudine and Nevirapine in Human Plasma by Lc-Ms/Ms Method

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Abstract

A sensitive and selective LC-MS/MS method to quantify Lamivudine and Nevirapine in K3EDTA human plasma over the concentration range of 25.0240 to 3997.1740 ng/mL and 37.5080 to 5991.0460 ng/mL, respectively were developed and validated. Lamivudine and Nevirapine and its internal standard (Lamivudine 13C 15N2 and Nevirapine D5) were selectively extracted from 300 μ L plasma by direct elution using solid phase extraction technique. Elution was achieved by reverse phase liquid chromatography on Hypurity C18 (100 mm × 4.6 mm, 5.0 μ m) column was done. LLOQ of 25.0240 ng/mL and 37.5080 ng/mL were resolute for this technique for Lamivudine and Nevirapine respectively. Accuracy and precision values for Lamivudine were 99.55 & 1.50 and for Nevirapine the accuracy and precision values are 99.33 & 1.03%, respectively.

Keywords: Bioanalytical method, Antiretroviral Drugs, Biological sample, Solid phase extraction, Validation parameters

Introduction

Antiretroviral drugs are used to treat infections by retro viruses, primarily the human immunodeficiency virus (HIV). The aim of antiretroviral treatment is to maintain Human Immuno Virus at a low level in the body. Since single drug therapy rapidly becomes ineffective due to the development of HIV resistant strains, the new paradigm is to combine two to three anti retro viral drugs. The synergistic action of different classes of antiretroviral drugs prolongs the survival of HIV patients such that combination therapy is now considered first-line treatment. Current treatment guidelines state that a combination antiretroviral regimen should contain at least one nucleoside analog reverse transcriptase inhibitor (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) in a fixed dose combination. Lamivudine(20-deoxy-30-thiacy-tidine) is NRTI whereas Nevirapine (11-cyclopropyl-5,11-dihydro-4- methyl-6H- dipyrido[3,2-b:20, 30-e]1,4 diazepin-6-one) is a highly potent noncompetitive NNRTI. The validated method was applied to a clinical pharmacokinetics study involving formulations of Lamivudine and Nevirapine

Methodology

Reagents and chemicals

HPLC grade acetonitrile and potassium dihydrogen ortho phosphate buffer analytical grade were procured from Clearsynth Lab Limited, Mumbai, India. Analytes Lamivudine (99.92%), Nevirapine (99.95%) and co analyte Zidovudine (99.90%) were obtained from Clearsynth Lab Limited, Mumbai, India. pure standards of Lamivudine 13C 15N2 (99.43%) and Nevirapine D5 (99.53%)were obtained from Clearsynth Lab Limited, Mumbai, India. Blank K3EDTA human plasma lots were used for screening were obtained from Micro therapeutics Lab.

Instrumentation and Chromatographic Conditions

Chromatographic separation was carried out on a Waters HPLC with a Hypurity C18 (100 mm \times 4.6 mm, 5.0 μ m) column and a mobile phase consisting of Acetonitrile: buffer (75:25v/v) delivered at a flow rate of 1 mL/min. The injection volume was 5 ml. Quantitation was achieved in a run time of 2.5min by MS/MSdetection in the positive ion mode using an Quattro Micro Mass, Waters equipped with a Turbo ion spray TM interface at 600 1C and ion spray voltages et at 5500V. Source parameters viz. nebulizer gas(GS1), auxiliarygas(GS2), curtain gas(CUR) and collision gas(CAD) were set at 35, 35, 20 and 6psi,respectively.vCompoundvparameters viz. declustering potential(DP), collisionenergy(CE), entrance potential (EP) and collision cellex it potential(CXP) were respectively 36,16,10 and 6V for lamivudine,70,44,10 and 6V for nevirapine. Detection was supported out by selective reaction monitoring (SRM) of the transitions (precursor ion to product ion) at m/z 230.10-112.05 for lamivudine, m/z 267.16-225.95 for nevirapine and m/z 233.27-115.20 for Lamivudine 13C 15N2, m/z 272.19-226.99 for Nevirapine D5. Quadrupoles Q1and Q3were set on unitre solution. Data Acquisition – Mass Lynx version 4.1 SCN627 supplied by Waters India Ltd.

Sample Preparation

Add 50 μ L of internal standard solution Lamivudine 13C 15N2- 5 μ g/ml and Nevirapine D5- 10 μ g/ml into all individually labeled vacant Radioimmunoassay(RIA) vials except blank. Pipette 300 μ L of plasma samples into respectively labeled RIA vials containing standard solution. Add 200 μ L of Buffer into all the samples. Load the samples into catridges.

VALIDATION AND CHARACTERISTICS OF METHOD

Chromatography

Typical chromatograms of aqueous standard solution (analytes of internal standards), standard blank, standard zero, LOQQC, LQC, INTQC, MQC, HQC and calibration curve of Lamivudine and Nevirapine were shown in Figure 3 to Figure 12, respectively.

Specificity and Selectivity

Selectivity was calculated by determining a total of nine batches (six batches of blank K3EDTA human normal plasma, a batch of haemolyzed plasma, a batch of heparin plasma and a batch of lipemic plasma) for both Lamivudine and Nevirapine, found from non-dependent source

No significant interferences were observed at the retention times of analyte and internal standard in nine out of nine batches evaluated for both Lamivudine and Nevirapine, demonstrating acceptance criteria were met.

Signal-to-Noise (S/N) Ratio

The signal-to-noise ratio was done for both Lamivudine and Nevirapine at LLOQ concentrations in nine different lots of K3EDTA human normal plasma including a batch of haemolyzed plasma, a batch of heparin plasma and a batch of lipemic plasma.

Signal-to-Noise ratios ranged from 46.432 to 148.407 and from 43.070 to 285.999 for both Lamivudine and Nevirapine, respectively across the matrix lots evaluated, representing suitable Signal to noise ratio).

Carry Over Test

Carry over was calculated as the percentage peak area observed in a processed blank plasma injected in duplicate immediately after a processed ULOQ calibration standard which were used from PA-03 batch sample from quality control laboratory. There is no specific carry over detected for both Lamivudine and Nevirapine and its internal standards, respectively.

Matrix Factor

The potential for co-extracted matrix component to influence the detector response of analyte and internal standard was evaluated in nine independent lots of blank K3EDTA human normal plasma including one lot of haemolyzed plasma, one lot of heparin plasma and one lot of lipemic plasma. Aqueous standard equivalent to LQC and HQC level concentration along with intended concentration of internal standard were spiked to the post extracted blank matrix respectively for both LQC and HQC samples.

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| Matrix Factor for Analyte = Peak area in the presence of matrix (post-extracted spiked sample of the analyte) | | | | | | |
|---|--|--|--|--|--|--|
| Mean peak area in absence of matrix (pure solution of the analyte) | | | | | | |
| Matrix Factor for Internal Standard = Peak area in the presence of matrix (post-extracted spiked sample of the IS | | | | | | |
| Mean peak area in absence of matrix (pure solution of the IS) | | | | | | |
| Matrix Factor for IS Normalized = Matrix Factor of the Analyte | | | | | | |
| Matrix Factor for Internal Standard | | | | | | |

Nevirapine:

The IS normalized matrix factor at LQC and HQC level were found to be between

0.96 to 1.01 and 0.98 to 1.02. The percentage CV of IS normalized matrix factor at LQC and HQC level was found to be 1.69 and 1.39%, respectively evaluated.

Matrix Effect:

In each matrix lot, spiked LQC and HQC were prepared and three replicate samples from each matrix lot at both LQC and HQC were processed along with freshly spiked and prepared calibration curve standards in plasma as per method SOP procedure. Processed matrix effect quality control samples were analyzed against freshly spiked and prepared calibration curve standards.

Lamivudine:

The % nominal for matrix effect at LQC and HQC level were 99.77, 100.25 & 102.88% and 97.56, 95.73 & 95.45%, respectively. The % CV for Matrix effect at LQC and HQC level were 6.64, 8.01 & 6.86% and 4.42, 4.68 & 2.69%, respectively.

Nevirapine:

The % nominal for matrix effect at LQC and HQC level were 104.38, 102.75 & 102.71% and 90.66, 90.77 & 90.88%, respectively. The % CV for Matrix effect at LQC and HQC level were 5.08, 5.03 & 5.05% and 3.12, 2.26 & 3.11%, respectively.

Linearity

Linearity values for Lamivudine and Nevirapine were 25.0240 to 3997.1740 ng/mL and 37.5080 to 5991.0460 ng/mL using internal standards as Lamivudine 13C 15N2 and Nevirapine D5.

We were analysed the acceptable precision and accuracy batches in this range for Lamivudine and Nevirapine respectively. Calibration curves were done by least square regression analysis. Calibration curve was found to be linear as shown in figure 7 and figure 12.

Correlation coefficients were found to be more than 0.99 for both Lamivudine and Nevirapine.

Weighting Scheme

One calibration curves from accepted PA batch during pre-method validation was evaluated by least square linear regression analysis with 1/X and 1/X2 weighting factors.

1/X2 weighting factor was found to be least sum value than 1/X value, so selected the 1/X2 value for the method validation.

Sensitivity

The LLOQ for Lamivudine and Nevirapine in K3EDTA human plasma were determined based on the analysis of LLOQ in the precision and accuracy validation batches. LLOQ of 25.0240 ng/mL and 37.5080 ng/mL were determined for this method for Lamivudine and Nevirapine, respectively. The accuracy and precision from the five PA batches for Lamivudine were 99.55 & 1.50 and for Nevirapine the accuracy and precision were 99.33 & 1.03%, respectively.

Calibration Curve Precision and Accuracy

Inter-batch calibration standard accuracy for Lamivudine and Nevirapine went from 98.01 to 102.67% and 98.35 to 101.84% with inter-day precision value of 1.50 to 5.68% and 1.03 to 2.31d%, respectively during the progress of method validation representing suitable assay linearity. Correlation coefficient (r2) were consistently greater than 0.99. A representative calibration curve for Lamivudine and Nevirapine in K3EDTA human plasma is shown in Figure 9 and Figure 18, respectively.

Precision and Accuracy

Assay precision and accuracy (inter batch and intra batch) values were determined through five precision and accuracy batches by analysing six repeats each of LOQQC, LQC, INTQC, MQC and HQC samples in each set. Consecutive numbering of the QC samples may not be recite in the tables as some of the samples have been randomly used to perform various validation parameters.

Accuracy

The accuracy of the assay is defined as absolute value of the ratio of calculated mean values of the quality control samples to their respective nominal values expressed as percentage.

Intra Batch Accuracy

Lamivudine:

The intra batch accuracy at LOQQC ranged from 80.65 to 107.34%. For LQC, INTQC, MQC and HQC it ranged from 76.46 to 105.99% from five PA batches (refer Table 1).

Nevirapine:

The intra batch accuracy at LOQQC ranged from 89.53 to 102.18%. For LQC, INTQC, MQC and HQC it ranged from 89.21 to 104.68% from five PA batches (refer Table 2).

Inter Batch or Total Accuracy

Lamivudine:

The inter batch accuracy at LOQQC were found to be 91.93%. For LQC, INTQC, MQC and HQC, it ranged from 92.62 to 94.62% from five PA batches.

Nevirapine:

The inter batch accuracy at LOQQC were found to be 96.51%. For LQC, INTQC, MQC and HQC, it ranged from 95.99 to 98.66% from five PA batches.

Precision

The precision of the assay were measured by the percentage co-efficient of variation over the concentration range of LOQQC, LQC, INTQC, MQC and HQC sample of Lamivudine and Nevirapine during the course of method validation.

Intra Batch Precision

Lamivudine:

The within batch precision at LOQQC ranged from 4.23 to 14.85%. For LQC, INTQC, MQC and HQC it ranged from 0.97 to 9.20% from acceptable five PA batches .

Nevirapine:

The within batch precision at LOQQC ranged from 2.22 to 9.28%. For LQC, INTQC, MQC and HQC it ranged from 0.65 to 6.80% from acceptable five PA batches .

Inter Batch or Total Precision Lamivudine:

The total precision at LOQQC was found to be 13.37%. For LQC, INTQC, MQC and HQC it ranged from 8.29 to 11.19% from acceptable five PA batches.

Nevirapine:

The total precision at LOQQC was found to be 7.88%. For LQC, INTQC, MQC and HQC it ranged from 2.99 to 6.16% from acceptable five PA batches.

Extended Precision and Accuracy Batch

Forty sets of LQC, MQC and HQC samples were processed and analyzed against a single calibration curve for Lamivudine and Nevirapine, respectively. The % nominal for calibration curve standards for Lamivudine ranged from 95.66 to 107.47 and for Nevirapine ranged from 94.04 to 110.20, respectively.

Lamivudine:

The percentage nominal and %CV at LQC, MQC and HQC levels were found to be 100.68, 95.51 & 96.22% and 5.80, 3.44 & 3.66%, respectively.

Nevirapine:

The percentage nominal and %CV at LQC, MQC and HQC levels were found to be 101.99, 97.05 & 95.52% and 2.92, 1.59 & 2.61%, respectively.

RECOVERY

Recovery of Lamivudine and Nevirapine

The recovery of Lamivudine and Nevirapine were detected by comparing the detector reaction of Lamivudine and Nevirapine at three different stages of extracted low, medium and high quality control samples from PA-04 samples with detector response got from un-extracted aqueous quality control samples. The average recovery of Lamivudine and Nevirapine was 69.18 and 76.10%, respectively. The percentage CV for Lamivudine and Nevirapine was 3.53 and 9.09 at three different QC level, respectively

Recovery of Internal Standard

Internal standard recovery was calculated by taking the internal standards in all types of quality control samples and analyzing the detector response (average). Recovery values of internal standard was 67.71 and 74.89 for Lamivudine 13C 15N2 and Nevirapine D5 respectively

STABILITY

Freeze-Thaw Stability

Six replicates of Lamivudine and Nevirapine samples at LQC and HQC concentration in K3EDTA human plasma were analysed after four freeze-thaw (FT4) cycles (at both $-70^{\circ}C \pm 15^{\circ}C$ and $-30^{\circ}C \pm 10^{\circ}C$ storage temperatures). The stability was determined by calculating the percentage nominal of LQC and HQC samples against freshly spiked, prepared calibration curve standards and compared with freshly spiked and prepared samples at LQC and HQC level.

LIMIT OF DETECTION

Lamivudine: From LLOQ sample (25.0440 ng/mL), four different lower concentrations (20.0352, 15.0264, 10.0176 & 5.0088 ng/mL) including five times the lower concentration (LOD dilution) were prepared and six replicates of these samples were analyzed.

The mean peak area, standard deviations were calculated and % CV for five different concentrations was 4.24, 3.85, 4.49, 3.38 & 6.40%. Signal to noise for five times lower concentration (LOD) prepared from LLOQ ranged from 26.440 to 85.987. This proves that LOD concentration which is 20% of LLOQ concentration has signal to noise ratio more than 5. So, the selected LLOQ (approximately 25.0240 ng/mL) was more suitable to quantify Lamivudine in plasma using LC- MS/MS (refer Table 3).

Nevirapine:

From LLOQ sample (38.3920 ng/mL), four different lower concentrations (30.7136, 23.0352, 15.3568 & 7.6784 ng/mL) including five times the lower concentration (LOD dilution) were prepared and six replicates of these samples were analyzed.

The mean peak area, standard deviations were calculated and % CV for five different concentrations was 4.46, 5.14, 4.58, 7.28 & 4.43%. Signal to noise for five times lower concentration (LOD) prepared from LLOQ ranged from 34.550 to 55.851. This proves that LOD concentration which is 20% of LLOQ concentration has signal to noise ratio more than 5. So, the selected LLOQ (approximately 37.5080 ng/mL) was more suitable to quantify Nevirapine in plasma using LC- MS/MS(refer Table 4).

REINJECTION REPRODUCIBILITY

CC standards, LQC and HQC samples of PA-04 were re-injected after 10.18 hours storage in auto sampler.

The percentage nominal for LQC & HQC levels for Lamivudine and Nevirapine were 113.53 & 112.10% and 104.62 & 96.23% and the percentage CV for LQC & HQC for Lamivudine and Nevirapine were 3.81 & 2.32% and 3.13 & 2.64% and the ratios of means while comparing the mean of back-calculated values against the mean of values obtained from an original injection for LQC and HQC for Lamivudine and Nevirapine were 1.08 & 1.07 and 1.02 & 0.99%, respectively

Lamivudine and Nevirapine samples were prepared in K3EDTA human plasma at around 2 times concentrations of higher quality control samples and diluted 2 times and 4 times with pooled K3EDTA human blank plasma. The percentage nominal of Lamivudine and Nevirapine for 2 times & 4 times dilutions were 95.69 & 90.04% and 95.36 & 92.63%, respectively.

RUGGEDNESS

One Precision & Accuracy batch (PA-05) samples were prepared by different analyst and analysed using different column to ensure the ruggedness of the bioanalytical method







Figure 3: Representative Chromatogram of an Aqueous Standard Solution for Lamivudine

Figure 5: Representative Chromatogram of a Standard Zero for Lamivudine

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Figure 7: Representative Regression Analysis of a Calibration Curve for Lamivudine





Figure 9: Representative Chromatogram of a Standard Blank for Nevirapine



Figure 10: Representative Chromatogram of a Standard Zero for Nevirapine



Figure 11: Representative Chromatogram of LOQQC Sample for Nevirapine



Figure 12: Representative Regression Analysis of a Calibration Curve for Nevirapine

| QC ID | LOQQC | LQC | INTQC | MQC | HQC |
|-------------------------------------|----------|----------|-----------|------------|---------|
| Actual Concentration (ng/mL) | 25.0760 | 67.4090 | 374.4970 | 1497.9870 | 2995.97 |
| | 16.4668 | 70.3348 | 357.5534 | 1448.6811 | 2877.94 |
| Calculated concentration (ng/mL) | 21.7429 | 63.8352 | 362.3295 | 1463.5894 | 2832.03 |
| | 20.5109 | 73.5113 | 352.0956 | 1350.7448 | 2924.40 |
| PA – 01 | 25.4234 | 59.8411 | 328.0901 | 1420.8089 | 2885.31 |
| | 18.4358 | 61.6311 | 355.8411 | 1407.9936 | 2911.37 |
| | 20.4158 | 58.8817 | 311.6551 | 1397.2196 | 2930.27 |
| Mean | 20.49927 | 64.67253 | 344.59413 | 1414.83957 | 2893.55 |
| SD | 3.043665 | 5.950008 | 20.112758 | 40.057205 | 36.6427 |
| %CV | 14.85 | 9.20 | 5.84 | 2.83 | 1.27 |
| %Nominal | 81.75 | 95.94 | 92.02 | 94.45 | 96.58 |

| | 20.6493 | 59.0323 | 328.4642 | 1372.4068 | 2721.62 |
|--------------------------|----------|----------|-----------|------------|---------|
| Calculated concentration | 20.4496 | 58.3954 | 296.8746 | 1398.9498 | 2823.07 |
| (lig/lill) | 19.5100 | 61.6952 | 322.4722 | 1440.2016 | 2689.59 |
| PA - 02 | 17.3999 | 52.1042 | 328.6884 | 1397.4574 | 2815.69 |
| | 23.0949 | 60.3845 | 320.9648 | 1458.1937 | 2626.70 |
| | 20.2410 | 57.9462 | 326.7702 | 1413.4944 | 2826.76 |
| Mean | 20.22412 | 58.25963 | 320.70573 | 1413.45062 | 2750.57 |
| SD | 1.841709 | 3.314249 | 12.097975 | 31.203244 | 83.9059 |
| %CV | 9.11 | 5.69 | 3.77 | 2.21 | 3.05 |
| %Nominal | 80.65 | 86.43 | 85.64 | 94.36 | 91.81 |
| QC ID | LOQQC | LQC | INTQC | MQC | HQC |
| Actual Concentration | 25.0760 | 67.4090 | 374.4970 | 1497.9870 | 2995.97 |
| Calculated concentration | 25.5052 | 74.5982 | 399.4359 | 1515.8712 | 3183.95 |
| | 25.5961 | 71.7544 | 408.8927 | 1585.1505 | 3146.65 |
| (ng/mL) | | | | | |
| PA - 03 | 27.4842 | 71.3043 | 398.1696 | 1559.6747 | 3094.22 |
| | 28.2530 | 70.3642 | 389.9253 | 1566.6303 | 3324.00 |
| | 27.7069 | 70.3290 | 384.6938 | 1562.9046 | 3153.59 |
| | 26.9612 | 68.3987 | 400.4494 | 1588.4596 | 3005.93 |
| Mean | 26.91777 | 71.12480 | 396.92778 | 1563.11515 | 3151.39 |
| SD | 1.137582 | 2.056043 | 8.509981 | 26.015864 | 105.174 |
| %CV | 4.23 | 2.89 | 2.14 | 1.66 | 3.34 |
| %Nominal | 107.34 | 105.51 | 105.99 | 104.35 | 105.19 |
| Calculated concentration | 24.8017 | 60.0465 | 359.1966 | 1447.7456 | 3022.88 |
| | 25.8789 | 56.2863 | 361.5058 | 1433.0446 | 3049.45 |
| (ng/mL) | | | | | |
| PA - 04 | 24.6361 | 62.9203 | 361.8903 | 1395.5283 | 2961.38 |
| | 23.2327 | 64.2931 | 357.8206 | 1459.8491 | 2921.68 |
| | 22.7042 | 66.3525 | 352.4636 | 1458.2072 | 2948.19 |
| | 21.5232 | 70.1431 | 360.5312 | 1522.2746 | 3003.87 |
| Mean | 23.79613 | 63.34030 | 358.90135 | 1452.77490 | 2984.58 |
| SD | 1.595658 | 4.838258 | 3.494945 | 41.476534 | 48.7134 |
| %CV | 6.71 | 7.64 | 0.97 | 2.85 | 1.63 |
| %Nominal | 94.90 | 93.96 | 95.84 | 96.98 | 99.62 |

Table 1 : Intra Batch Precision and Accuracy of Lamivudine

| QC ID | LOQQC | LQC | INTQC | MQC | HQC |
|------------------------------|---------|----------|----------|-----------|-----------|
| Actual Concentration (ng/mL) | 37.5820 | 101.0280 | 561.2680 | 2245.0730 | 4490.1460 |
| Calculated concentration | 35.2874 | 95.7983 | 507.6657 | 2009.0139 | 4125.6673 |
| (ng/mL) | 35.9166 | 103.4165 | 491.6250 | 1989.9680 | 4080.7599 |
| PA - 01 | 41.7850 | 91.2465 | 494.9008 | 1937.4115 | 4100.5334 |

| | 34.3612 | 104.1669 | 505.3689 | 2051.5266 | 4172.2668 |
|----------------------------------|----------|----------|-----------|------------|-----------|
| | 41.8976 | 90.9390 | 503.4032 | 2040.6151 | 4265.8018 |
| | 41.1507 | 105.6730 | 501.1795 | 2042.8512 | 4244.8989 |
| Mean | 38.39975 | 98.54003 | 500.69052 | 2011.89772 | 4164.9880 |
| SD | 3.561608 | 6.705359 | 6.226438 | 43.337466 | 76.677299 |
| %CV | 9.28 | 6.80 | 1.24 | 2.15 | 1.84 |
| %Nominal | 102.18 | 97.54 | 89.21 | 89.61 | 92.76 |
| | 36.0293 | 103.3148 | 548.6921 | 2245.4359 | 4440.1225 |
| Calculated concentration (ng/mL) | 37.5381 | 106.6095 | 568.2068 | 2240.8389 | 4287.7256 |
| | 39.4014 | 104.4827 | 545.4490 | 2145.6407 | 4376.6774 |
| PA – 02 | 40.4061 | 98.5173 | 528.1340 | 2218.8357 | 4318.4012 |
| | 37.7325 | 100.1088 | 543.6999 | 2156.2279 | 4475.0186 |
| | 35.6258 | 100.4248 | 499.7738 | 2144.4414 | 4504.4101 |
| Mean | 37.78887 | 102.2429 | 538.99260 | 2191.90342 | 4400.3925 |
| SD | 1.859633 | 3.065056 | 23.097260 | 48.272934 | 87.116013 |
| %CV | 4.92 | 3.00 | 4.29 | 2.20 | 1.98 |
| %Nominal | 100.55 | 101.20 | 96.03 | 97.63 | 98.00 |
| | 33.4112 | 98.6530 | 529.9816 | 2205.7601 | 4261.2329 |
| Calculated concentration | 32.5940 | 95.0223 | 498.2373 | 2284.1236 | 4312.0988 |
| (ng/mL) | 34.4187 | 95.0193 | 521.3407 | 2326.1871 | 4381.1720 |
| PA - 03 | 32.6215 | 91.0231 | 509.2748 | 2211.1579 | 4395.7007 |
| | 35.0409 | 98.0410 | 503.3016 | 2272.8077 | 4273.5202 |
| | 33.8062 | 98.9819 | 526.6581 | 2205.5860 | 4591.6262 |
| Mean | 33.64875 | 96.12343 | 514.79902 | 2250.93707 | 4369.2251 |
| SD | 0.977739 | 3.055867 | 13.045615 | 50.837580 | 121.97630 |
| %CV | 2.91 | 3.18 | 2.53 | 2.26 | 2.79 |
| %Nominal | 89.53 | 95.15 | 91.72 | 100.26 | 97.31 |
| QC ID | LOQQC | LQC | INTQC | MQC | HQC |
| Actual Concentration (ng/mL) | 37.5820 | 101.0280 | 561.2680 | 2245.0730 | 4490.1460 |
| | 36.5674 | 106.2539 | 602.9738 | 2228.6802 | 4330.3047 |
| Calculated concentration (ng/mL) | 36.8903 | 104.9024 | 592.3347 | 2207.7541 | 4412.4050 |
| | 38.3895 | 100.6381 | 589.1570 | 2208.4599 | 4393.0096 |
| PA – 04 | 36.8320 | 106.2092 | 584.3603 | 2252.0837 | 4373.8524 |
| | 38.1040 | 103.2379 | 586.9229 | 2267.7831 | 4366.3336 |
| | 36.4532 | 102.0825 | 569.4608 | 2266.5620 | 4357.2274 |
| Mean | 37.20607 | 103.8873 | 587.53492 | 2238.55383 | 4372.1887 |
| SD | 0.827180 | 2.291670 | 10.964986 | 27.472247 | 28.516734 |
| %CV | 2.22 | 2.21 | 1.87 | 1.23 | 0.65 |
| %Nominal | 99.00 | 102.83 | 104.68 | 99.71 | 97.37 |

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| | 32.8942 | 101.4940 | 553.6989 | 2232.0145 | 4360.3774 |
|----------------------------------|----------|----------|-----------|------------|-----------|
| Calculated concentration (ng/mL) | 31.8227 | 101.2631 | 553.0814 | 2181.0282 | 4404.2260 |
| | 36.9875 | 97.4557 | 543.1689 | 2209.2881 | 4475.3401 |
| PA – 05 | 32.7583 | 91.8803 | 544.7416 | 2197.9383 | 4566.0511 |
| | 38.4635 | 94.0458 | 559.4828 | 2192.8584 | 4437.8835 |
| | 32.9087 | 99.4336 | 556.0314 | 2194.8913 | 4564.6559 |
| Mean | 34.30582 | 97.59542 | 551.70083 | 2201.33647 | 4468.0890 |
| SD | 2.719670 | 3.932216 | 6.425510 | 17.559961 | 84.340929 |
| %CV | 7.93 | 4.03 | 1.16 | 0.80 | 1.89 |
| %Nominal | 91.28 | 96.60 | 98.30 | 98.05 | 99.51 |

Table 2: Intra Batch Precision and Accuracy of Nevirapine

| Dilution s | LLOQ | Dilution-01 | Dilu tion -02 | Dilution-03 | LOD | S/N Ratio | o of LOD 5) |
|---------------|--------|-------------|---------------------|-------------|------|-----------|----------------|
| | 301 | 25 0 | 188 | 116 | 56 | 59.5 7 | 50 |
| Analyte | 297 | 23 1 | 185 | 112 | 59 | 37.1 1 | 13 |
| Alea | 277 | 24 4 | 203 | 123 | 58 | 28.4 6 | 14 |
| | 311 | 23 8 | 196 | 119 | 62 | 26.4 0 | 14 |
| | 292 | 24 9 | 183 | 114 | 57 | 85.9 7 | 98 |
| | 310 | 22 8 | 181 | 115 | 51 | 53.8 9 | 37 |
| М | 298.00 | 240.00000 | 189. | 116.50000 | 57.1 | Minimum | 26.440 |
| ea | 000 | | 333 | | 6667 | winning | 20.440 |
| n | | | 33 | | | | |
| S | 12.649 | 9.230385 | 8.50 | 3.937004 | 3.65 | | |
| D | 111 | | 098 | | 6045 | | |
| | | | 0 | | | | |
| % | 4.24 | 3.8 | 4.49 | 3.3 | 6.40 | Maximum | 85.987 |
| C | | 5 | | 8 | | | |
| V | | | | | | | |

Table 3: Limit of Detection for Lamivudine

| D i | LLOQ | Dilution-01 | Dilutio n-02 | Dilution-03 | LOD | S/N Ratio of LOD (≥5) |
|--------|------|-------------|-----------------|-------------|-----|--------------------------|
| 1 | | | | | | |
| u | | | | | | |
| t | | | | | | |
| i | | | | | | |
| 0 | | | | | | |

| n | | | | | | | |
|-------|--------|-----------|--------|-----------|--------|---------|--------|
| s | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | 535 | 40 | 366 | 23 | 125 | 34. | 550 |
| | | 2 | | 9 | - | | |
| | 520 | 45 | 379 | 24 | 131 | 54.0 | 520 |
| Analy | | 7 | | 9 | | | |
| Area | 566 | 45 | 388 | 26 | 132 | 37.3 | 345 |
| Theu | | 3 | | 0 | | | |
| | 529 | 46 | 380 | 23 | 125 | 42. | 158 |
| | | 1 | | 5 | | | |
| | 512 | 46 | 345 | 23 | 140 | 55.8 | 851 |
| | | 4 | | 1 | | | |
| | 497 | 45 | 353 | 27 | 135 | 35. | 505 |
| | | 0 | | 9 | | | |
| М | 526.50 | 447.83333 | 368.50 | 248.83333 | 131.33 | Minimum | 34 550 |
| e | 000 | | 000 | | 333 | Willing | 51.550 |
| а | | | | | | | |
| n | | | | | | | |
| S | 23.501 | 23.025348 | 16.861 | 18.115371 | 5.8195 | | |
| D | 064 | | 198 | | 07 | | |
| % | 4.46 | 5.1 | 4.58 | 7. | 4.43 | Maximum | 55.851 |
| С | | 4 | | 28 | | | |
| v | | | | | | | |

Table 4: Limit of Detection for Nevirapine

CONCLUSION

A selective and sensitive hyphenated method i.e. Lc-Ms/Ms is used to quantitate Lamivudine and Nevirapine in K3EDTA human plasma over the concentration range 25.0240 3997.1740 ng/mL and 37.5080 to 5991.0460 ng/mL, respectively were exactly validated. This technique is suitable for analysis of Lamivudine and Nevirapine to support bio-equivalence/bioavailability.

References

- Kaynaklar APA 6.0 formatına göre verilmeli ve alfabetik sıraya konulmalıdır. Makale metni içinde kullanılan tüm kaynaklar burada listelenmeli, makale içinde kullanılmayan kaynaklar burada listelenmemelidir. İki yazarlı çalışmalarda yazar isimleri "&" ile ayrılmalıdır. Çok yazarlı kaynaklarda son yazarın adının kısaltmasından sonra "virgül" ve "&" işareti kullanılmalıdır. Kaynaklar arasında boşluk bırakılmamalıdır. Örnek olarak bazı kaynak yazımları aşağıda verilmiştir.
- 2. Validation of a sensitive LC/MS/MS method for the determination of zidovudine and lamivudine in human plasma
- 3. Joseph E. Rower, Brandon Klein, Lane R. Bushman, & Peter L. Anderson. 2012 Jan; 26(1): 12–20 *CA:Biomed Chromatogr.*

- 4. Development and Validation of a Bioanalytical Method for the Simultaneous Determination of 14 Antiretroviral Drugs using Liquid Chromatography-Tandem Mass Spectrometry.
- 5. Alper Daskapan, Kai van Hateren, Ymkje Stienstra, Jos Kosterink, Tjip van der Werf, Daan Touw & Jan-Willem Alffenaar. Vol.4. No.2. pages 37-50 (2018) *Journal of Applied Bioanalysis*.
- 6. Bioanalytical method development and validation for a large peptide HIV fusion inhibitor (Enfuvirtide, T-20) and its metabolite in human plasma using LC-MS/MS.
- 7. D Chang 1, S J Kolis, K H Linderholm, T F Julian, R Nachi, A M Dzerk, P P Lin, J W Lee, & S K Bansal. 2005 Jul 1;38(3):487-96, *J Pharm Biomed Anal*.
- 8. Development and Validation of a Selective and Rapid LC–MS–MS Method for the Quantification of Abacavir in Human Plasma.
- 9. Manish Yadav, Ajay Gupta, Puran Singhal, & Pranav S. Shrivastav. Vol. 48, September 2010, *Journal of Chromatographic Science*.
- 10. A LC–MS/MS method with column coupling technique for simultaneous estimation of lamivudine, zidovudine and nevirapine in human plasma.
- 11. Srinivasa Reddy, Licto Thomas, K. S. Santoshkumar, Nirmala Nayak, Arindam Mukhopadhyay & Saral Thangam, (2016) 7:17, *Journal of Analytical Science and Technology*.
- 12. LC–MS/MS method for simultaneous quantification of lamivudine, stavudine and nevirapine in human plasma
- 13. Mistri HN, Jangid AG, Pudage A, Gomes N, Sanyal M, Shrivastav & P. Highthroughput, 2007;853:320–32, J Chromatogr B Analyt Technol Biomed Life Sci.
- 14. Simultaneous determination of lamivudine, stavudine and nevirapine in human plasma by LC–MS/MS and its application to pharmacokinetic study in clinic
- 15. Zhou L, Cungang D, Qinghua G, Zhen Z, Xiaojin Z, & Xiaofen L, 2010;24:926–34, Biomed Chroma.