How to Cite:
Lavanya, B., & Shanmugasundaram, P. (2022). Determination of lamivudine and nevirapine in human plasma by Lc-Ms/Ms method. *International Journal of Health Sciences*, *6*(S2), 10655–10669. https://doi.org/10.53730/ijhs.v6nS2.7834

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 µL 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 µm) 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 × 4.6 mm, 5.0 µm) 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 µL of internal standard solution Lamivudine 13C 15N2- 5 µg/ml and Nevirapine D5- 10 µg/ml into all individually labeled vacant Radioimmunoassay(RIA) vials except blank. Pipette 300 µL of plasma samples into respectively labeled RIA vials containing standard solution. Add 200 µL 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.

$$\text{Matrix Factor for Analyte} = \frac{\text{Peak area in the presence of matrix (post-extracted spiked sample of the analyte)}}{\text{Mean peak area in absence of matrix (pure solution of the analyte)}}$$

$$\text{Matrix Factor for Internal Standard} = \frac{\text{Peak area in the presence of matrix (post-extracted spiked sample of the IS)}}{\text{Mean peak area in absence of matrix (pure solution of the IS)}}$$

$$\text{Matrix Factor for IS Normalized} = \frac{\text{Matrix Factor of the Analyte}}{\text{Matrix Factor for Internal Standard}}$$

Lamivudine

The IS normalized matrix factor at LQC and HQC level were found to be between 0.95 to 1.07 and 0.96 to 1.04. The percentage CV of IS normalized matrix factor at LQC and HQC level was found to be 4.17 and 2.54%, respectively evaluated.

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 to107.47 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°C ± 15°C and -30°C ± 10°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

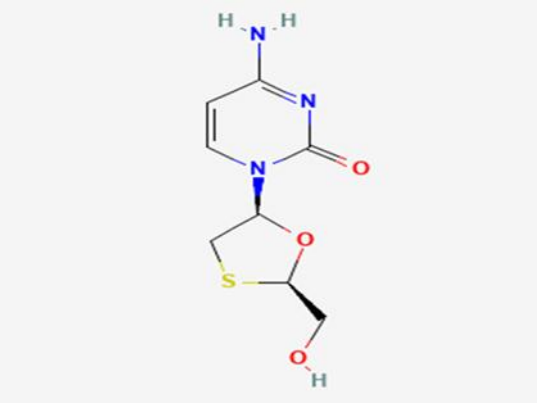

Figure 1: Chemical structures of Lamivudine

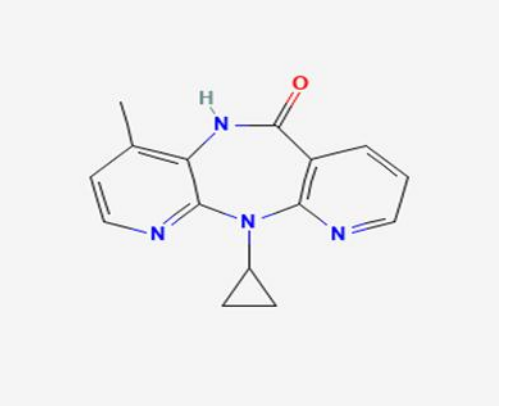

Figure 2: Chemical structures of Nevirapine

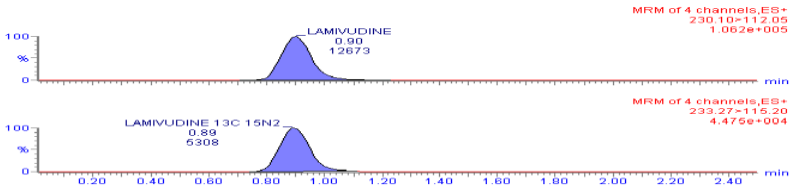

Figure 3: Representative Chromatogram of an Aqueous Standard Solution for Lamivudine

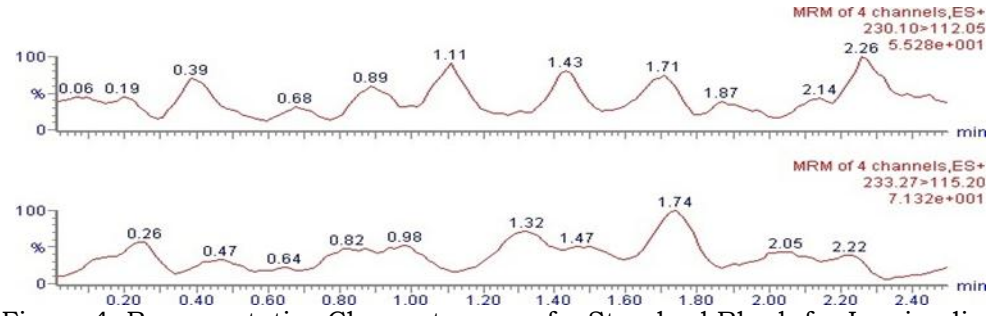

Figure 4: Representative Chromatogram of a Standard Blank for Lamivudine

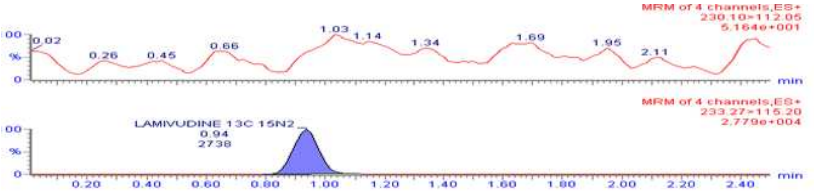

Figure 5: Representative Chromatogram of a Standard Zero for Lamivudine

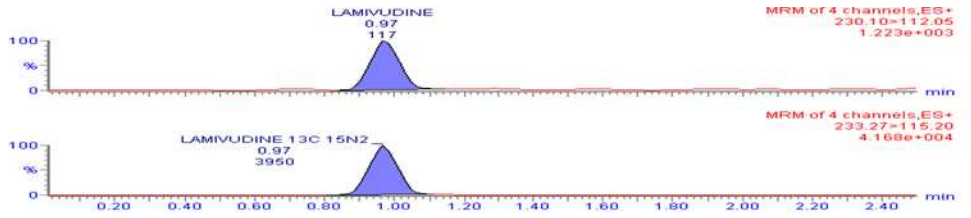

Figure 6: Representative Chromatogram of LOQQC Sample for Lamivudine

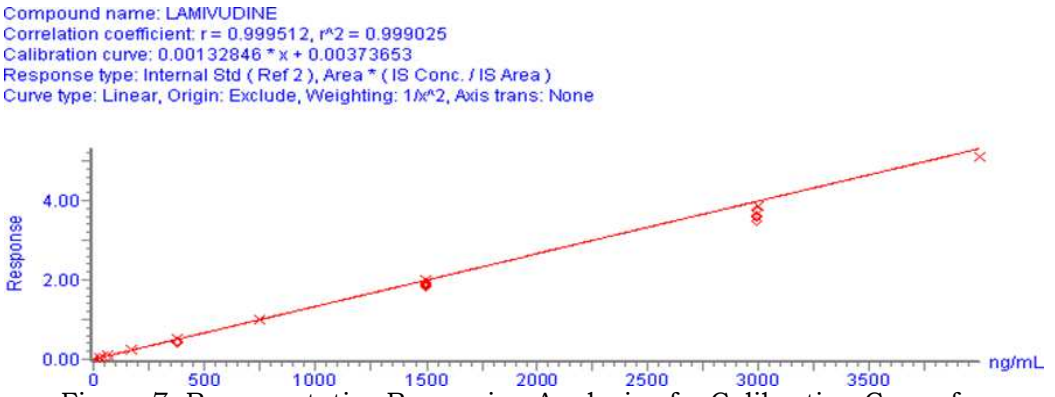

Figure 7: Representative Regression Analysis of a Calibration Curve for Lamivudine

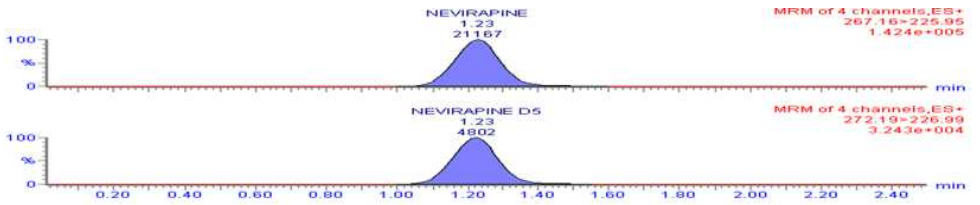

Figure 8: Representative Chromatogram of an Aqueous Standard Solution for Nevirapine

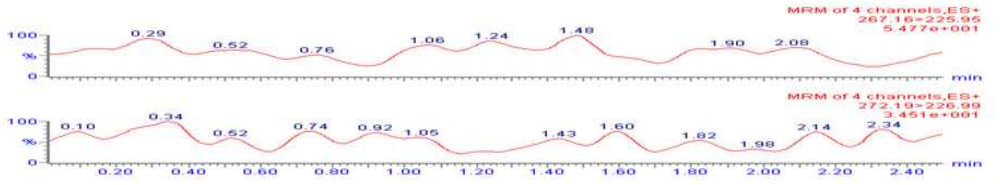

Figure 9: Representative Chromatogram of a Standard Blank for Nevirapine

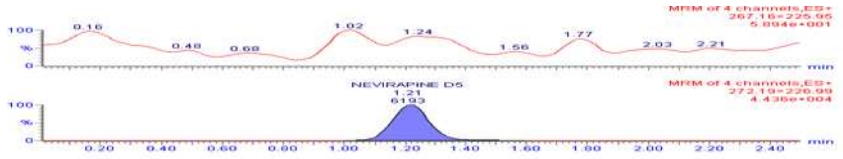

Figure 10: Representative Chromatogram of a Standard Zero for Nevirapine

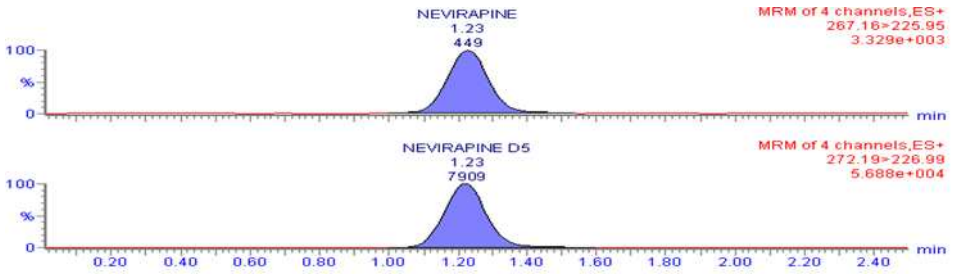

Figure 11: Representative Chromatogram of LOQQC Sample for Nevirapine

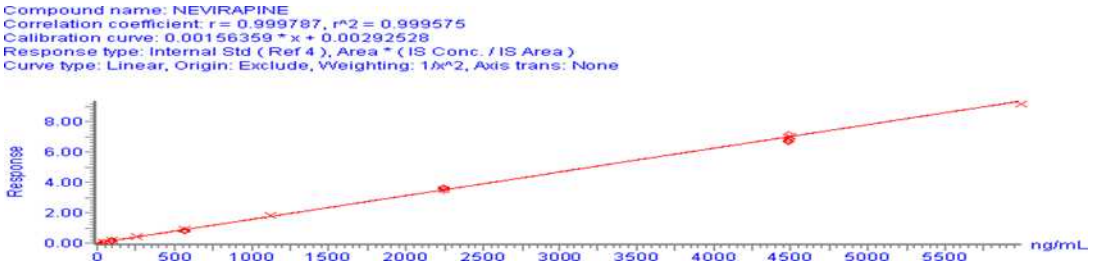

Figure 12: Representative Regression Analysis of a Calibration Curve for Nevirapine

QC ID	LOQQC	LQC	INTQC	MQC	HQC
Actual Concentration(ng/mL)	25.0760	67.4090	374.4970	1497.9870	2995.9730
Calculated concentration (ng/mL) PA – 01	16.4668	70.3348	357.5534	1448.6811	2877.9462
	21.7429	63.8352	362.3295	1463.5894	2832.0383
	20.5109	73.5113	352.0956	1350.7448	2924.4002
	25.4234	59.8411	328.0901	1420.8089	2885.3106
	18.4358	61.6311	355.8411	1407.9936	2911.3789
	20.4158	58.8817	311.6551	1397.2196	2930.2760
Mean	20.49927	64.67253	344.59413	1414.83957	2893.55837
SD	3.043665	5.950008	20.112758	40.057205	36.642758
%CV	14.85	9.20	5.84	2.83	1.27
%Nominal	81.75	95.94	92.02	94.45	96.58
Calculated concentration (ng/mL) PA – 02	20.6493	59.0323	328.4642	1372.4068	2721.6249
	20.4496	58.3954	296.8746	1398.9498	2823.0726
	19.5100	61.6952	322.4722	1440.2016	2689.5978
	17.3999	52.1042	328.6884	1397.4574	2815.6980

	23.0949	60.3845	320.9648	1458.1937	2626.7090
	20.2410	57.9462	326.7702	1413.4944	2826.7659
Mean	20.22412	58.25963	320.70573	1413.45062	2750.57803
SD	1.841709	3.314249	12.097975	31.203244	83.905987
%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 (ng/mL)	25.0760	67.4090	374.4970	1497.9870	2995.9730
Calculated concentration	25.5052	74.5982	399.4359	1515.8712	3183.9551
	25.5961	71.7544	408.8927	1585.1505	3146.6539
(ng/mL)					
PA – 03	27.4842	71.3043	398.1696	1559.6747	3094.2254
	28.2530	70.3642	389.9253	1566.6303	3324.0033
	27.7069	70.3290	384.6938	1562.9046	3153.5980
	26.9612	68.3987	400.4494	1588.4596	3005.9325
Mean	26.91777	71.12480	396.92778	1563.11515	3151.39470
SD	1.137582	2.056043	8.509981	26.015864	105.174920
%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	3022.8816
	25.8789	56.2863	361.5058	1433.0446	3049.4576
(ng/mL)					
PA – 04	24.6361	62.9203	361.8903	1395.5283	2961.3865
	23.2327	64.2931	357.8206	1459.8491	2921.6885
	22.7042	66.3525	352.4636	1458.2072	2948.1999
	21.5232	70.1431	360.5312	1522.2746	3003.8701
Mean	23.79613	63.34030	358.90135	1452.77490	2984.58070
SD	1.595658	4.838258	3.494945	41.476534	48.713477
%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	4164.98802
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
Calculated concentration (ng/mL) PA – 02	36.0293	103.3148	548.6921	2245.4359	4440.1225
	37.5381	106.6095	568.2068	2240.8389	4287.7256
	39.4014	104.4827	545.4490	2145.6407	4376.6774
	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	102.24298	538.99260	2191.90342	4400.39257
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
Calculated concentration (ng/mL) PA – 03	33.4112	98.6530	529.9816	2205.7601	4261.2329
	32.5940	95.0223	498.2373	2284.1236	4312.0988
	34.4187	95.0193	521.3407	2326.1871	4381.1720
	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	4369.22513
SD	0.977739	3.055867	13.045615	50.837580	121.976303
%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
Calculated concentration (ng/mL) PA – 04	36.5674	106.2539	602.9738	2228.6802	4330.3047
	36.8903	104.9024	592.3347	2207.7541	4412.4050
	38.3895	100.6381	589.1570	2208.4599	4393.0096
	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	103.88733	587.53492	2238.55383	4372.18878
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
Calculated concentration	32.8942	101.4940	553.6989	2232.0145	4360.3774
	31.8227	101.2631	553.0814	2181.0282	4404.2260

(ng/mL)					
PA – 05	36.9875	97.4557	543.1689	2209.2881	4475.3401
	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	4468.08900
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

Dilutions	LLOQ	Dilution-01	Dilution-02	Dilution-03	LOD	S/N Ratio of LOD(≥5)	
Analyte Area	301	250	188	116	56	59.507	
	297	231	185	112	59	37.131	
	277	244	203	123	58	28.446	
	311	238	196	119	62	26.440	
	292	249	183	114	57	85.987	
	310	228	181	115	51	53.879	
Mean	298.00000	240.00000	189.33333	116.50000	57.16667	Minimum	26.440
SD	12.649111	9.230385	8.500980	3.937004	3.656045		
%CV	4.24	3.85	4.49	3.38	6.40	Maximum	85.987

Table 3: Limit of Detection for Lamivudine

Dilutions	LLOQ	Dilution-01	Dilution-02	Dilution-03	LOD	S/N Ratio of LOD(≥5)	
Analyte Area	535	402	366	239	125	34.550	
	520	457	379	249	131	54.620	
	566	453	388	260	132	37.345	
	529	461	380	235	125	42.158	
	512	464	345	231	140	55.851	
	497	450	353	279	135	35.505	
Mean	526.50000	447.83333	368.50000	248.83333	131.33333	Minimum	34.550
SD	23.501064	23.025348	16.861198	18.115371	5.819507		
%CV	4.46	5.14	4.58	7.28	4.43	Maximum	55.851

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.

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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.

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