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Stability Indicating Lc–Ms/Ms Method For Lamivudine And Nevirapine

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Abstract

The aim of the stability studies is to perform to meet the quality, safety and efficacy. This type of study is used to know the changes in physical, chemical or microbiological properties of the drug with respect to time. Based on our study, noticed that Lamivudine and Nevirapine were stable to thermal stress. Percentage stability of all the stock solutions met the acceptance criteria. Working Solution percentage Stability values for Lamivudine and Nevirapine at LQC & HQC levels were 104.35 & 99.73% and 100.82 & 99.49%, respectively. Working Solution percentage Stability values for Lamivudine 13C 15N2 and Nevirapine D5 at LQC & HQC levels were 99.66 & 99.85% and 101.41 & 101.93, respectively. The stability studies were determined by calculating the Low Quality Control and High Quality Control samples percentage nominal beside freshly pointed, prepared calibration curve standards and compared with freshly spiked and prepared comparison samples at Low Quality Control and High Quality Control level..

Keywords: Autosampler stability, Bench top stability,Elution,Free Thaw Stability, Wetextract stability

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.

MATERIALS & METHODS

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.

STABILITY

Freeze-Thaw Stability

Lamivudine and Nevirapine six replicates were determined in K3EDTA human plasma at Low Quality Control and High Quality Control concentration after four cycles of freeze thaw (at both -70ºC ± 15ºC and -30ºC ± 10ºC storage temperatures). The stability studies were resolute by calculate the Low Quality Control and High Quality Control samples percentage nominal against freshly spiked, prepared calibration curve standards and compared with freshly pointed and prepared relationship samples at Low Quality Control and High Quality Control level.

Lamivudine:

The average percentage nominal of FT4 (Fourth Freeze Thaw cycle) stability samples calculated against freshly spiked, prepared CC at LQC and HQC concentrations for -70ºC ± 15ºC and -30ºC ± 10ºC were 96.01 & 93.75% and 98.31 & 94.27%, respectively. The average percentage nominal of

stability samples when related with freshly spiked, prepared comparison samples at LQC and HQC levels for - 70ºC ± 15ºC and -30ºC ± 10ºC were 90.53 & 98.93% and 92.71 & 99.48%, respectively four freeze thaw stability cycles (refer Table 1) demonstrating acceptable.

Nevirapine:

The average percentage nominal of FT4 (Fourth Freeze Thaw cycle) stability samples calculated against freshly spiked, prepared CC at LQC and HQC concentrations for -70ºC ± 15ºC and -30ºC ± 10ºC were 95.84 & 95.81% and 95.00 & 94.65%, respectively. The mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels for - 70ºC ± 15ºC and -30ºC ± 10ºC were 94.47 & 99.07% and 93.64 & 97.86%, respectively demonstrating acceptable four freeze thaw stability cycles (refer Table 2)

Bench-Top Stability

Bench top stability of Lamivudine and Nevirapine was estimated at room temperature in K3EDTA human plasma. LQC and HQC Six duplicate samples were processed for about 15.15 hours after keeping the samples on worktop. Bench top stability will be calculated by evaluating the stability of samples against freshly spiked. Should be placed on the bench for 4 to 24 hours and kept on bench during extraction process.

Lamivudine:

Stability studies were calculated by freshly prepared calibaration curve at Low Quality Control and High Quality Control levels were found to be 99.07 and 95.16% . Mean percentage nominal of stability samples at LQC and HQC levels were 93.42 & 100.41%, respectively, representing acceptable bench-top stability at room temperature for at least 15.15 hours (refer Table 3).

Nevirapine:

The mean percentage nominal of bench top stability samples calculated against freshly prepared CC at LQC and HQC levels were 97.59 & 94.37%, respectively and the mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were 96.19 & 97.57%, respectively, representing suitable bench-top stability at room temperature for at least 15.15 hours (refer Table 4).

Auto Sampler Stability for Lamivudine and Nevirapine

Autosampler stability is for to establish to prove the stability of samples in auto sampler at 10°C for 45.10 hours. It is calculated with processed samples by calculating samples percentage nominal against freshly spiked.

Lamivudine:

The average percentage nominal of auto sampler stability samples was calculated against freshly spiked and prepared CC at both Low and High quality control levels after 45.10 hours at 10°C was 102.89 & 114.42% and the mean percentage nominal of stability samples
when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were 104.75 & 105.54% demonstrating acceptable auto sampler stability for at least 45.10 hours at 10°C (refer Table).

Nevirapine:
The average percentage nominal of auto sampler stability samples was calculated against freshly spiked and prepared CC at LQC and HQC levels after 45.10 hours at 10°C was 99.71 & 98.85% and the mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were 97.74 & 100.22% demonstrating acceptable auto sampler stability for at least 45.10 hours at 10°C.

Auto Sampler Stability for Internal Standard
Auto sampler stability for six replicates of both Low Quality Control and High Quality Control samples were processed in auto sampler at 10°C for 45.10 hours. Autosampler stability was done by comparing the internal standard area of freshly sharp and prepared comparison QC samples at LQC and HQC levels against the internal standard area of stability samples.
The percentage of auto sampler stability for Lamivudine 13C 15N2 and Nevirapine D5 were calculated by compared with comparison samples at LQC and HQC levels was 90.84 and 99.32% demonstrating acceptable auto sampler stability for at least 45.10 hours at 10°C.

Wet Extract Stability

Wet Extract Stability in Refrigerator
Wet extract stability for six replicates were processed and transferred into injector vials and stored at 2-8°C for 50.47 hours then transferred into autosampler and finally determined.

Lamivudine:
The average percentage nominal of wet extract stability in refrigerator samples calculated against freshly prepared CC at LQC and HQC after 50.47 hours were 98.55 & 94.94%, respectively. The mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were found to be 92.93 & 100.18%, respectively representing suitable wet extract stability

Nevirapine:
The values of freshly prepared CC at LQC and HQC after 50.47 hours were 97.14 & 95.00%, respectively. The mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were found to be 95.75 & 98.22%, respectively demonstrating acceptable wet extract stability in refrigerator at 50.47 hours.

Wet Extract Stability at Room temperature
Wet extract stability at room temperature for six replicates were processed and transferred into injector vials and stored at room temperature for 06.33 hours then transferred into autosampler and finally comparison at LQC and HQC level.

Lamivudine:
Wet extract stability in room temperature, samples calculated against freshly prepared calibration curve at Low Quality Control and High Quality Control after 06.33 hours were 95.30 & 93.80%. The mean percentage nominal of stability samples when compared with freshly spiked, prepared

comparison samples at LQC and HQC levels were found to be 89.87 & 98.98%, respectively demonstrating acceptable wet extract stability for atleast 06.33 hours at room temperature.

Nevirapine:

Values of freshly prepared Calibration curve at Low Quality Control and High Quality Control after 06.33 hours were 97.49 & 94.95%, respectively. The mean percentage nominal of stability samples when compared with freshly spiked, prepared comparison samples at LQC and HQC levels were found to be 96.10 & 98.17%, respectively demonstrating acceptable wet extract stability for atleast 06.33 hours at room temperature.

Effect of Haemolysis on Lamivudine and Nevirapine

Effect of haemolysis on Lamivudine and Nevirapine in K3EDTA haemolyzed matrix was estimated at 20-250c. LQC and HQC samples were spiked in haemolyzed matrix and six replicates of each LQC and HQC levels were processed as per method SOP along with freshly spiked and prepared calibration curve standards in normal plasma. Prepared calibration curve standards and haemolyzed LQC and HQC samples were analysed.

The mean percentage nominal and %CV of haemolytic effect of Lamivudine at LQC & HQC levels were 107.42 & 103.70% and 4.69 & 1.62% and for Nevirapine, 102.13 & 99.06% and 3.82 & 0.87%, respectively.

Stock Solution Stability Experiments

Working Solution Stability for Lamivudine and Nevirapine

The stock solution 1001.9978 µg/mL and 1011.2941 µg/mL of Lamivudine and Nevirapine, respectively were divided in two portions. Part one is diluted with two stages of low and high concentration should be placed on the top for 40.43 hours at room temperature and other part is in cold place. The stability of the Lamivudine and Nevirapine stock solution should be keep on the top at room temperature for 40.43 hours (low and high) were compared against the freshly prepared stock solutions at LQC and HQC level from the other portion stored in the fridge.The percentage stability of Lamivudine and Nevirapine at LQC & HQC levels were 104.35 & 99.73% and 100.82 & 99.49%, respectively.

Working Solution Stability for Internal Standard

Concentrations of stock solutions 1011.9985 µg/mL and 1001.6699 µg/mL of Lamivudine 13C 15N2 and Nevirapine D5, respectively were divided in two portions. One solution was diluted to internal standard solution keep it on top at room temperature for 40.43 hours and other solution is placed in refrigerator. The percentage stability of Lamivudine 13C 15N2 and Nevirapine D5 at LQC & HQC levels were 99.66 & 99.85% and 101.41 & 101.93, respectively.

Short-Term Stock Solution Stability for Lamivudine and Nevirapine

The stock solution 1001.9978 µg/mL and 1011.2941 µg/mL of Lamivudine and Nevirapine, respectively were divided in two parts. One portion was placed on bench at 20-250c for 43.62 hours and other portion in cold place until analysis. Two of the stock solutions were diluted after intended storage at two levels of low and high (LQC and HQC) concentration. The stability of both the stock

solutions keep at a temperature of 20-250c for a duration of 43.62 hours should compared against the freshly prepared stock solutions at low and high quality control level from other portion in refrigerator.The percentage stability of Lamivudine and Nevirapine at LQC & HQC were 103.83 & 103.93% and 99.91 & 101.76%, respectively (refer table 5, 7 and 9, 11).

Short-Term Stock Solution Stability for Internal Standard

The stock solution 1011.9985 µg/mL and 1001.6699 µg/mL of Lamivudine 13C 15N2 and Nevirapine D5, respectively were divided in two portions. One portion was placed on bench at room temperature for 43.62 hours and other portion in refrigerator until analysis. Both the stock solutions were diluted after intended storage to internal standard concentration and placed on the bench at room temperature for 43.62 hours was compared against the freshly prepared internal standard of intended concentration stored in the refrigerator from other portion. The percentage stability of Lamivudine 13C 15N2 and Nevirapine D5 a LQC & HQC levels were 97.40 & 97.47% and 97.49 & 98.12% (refer table 6, 8 and 10, 12).

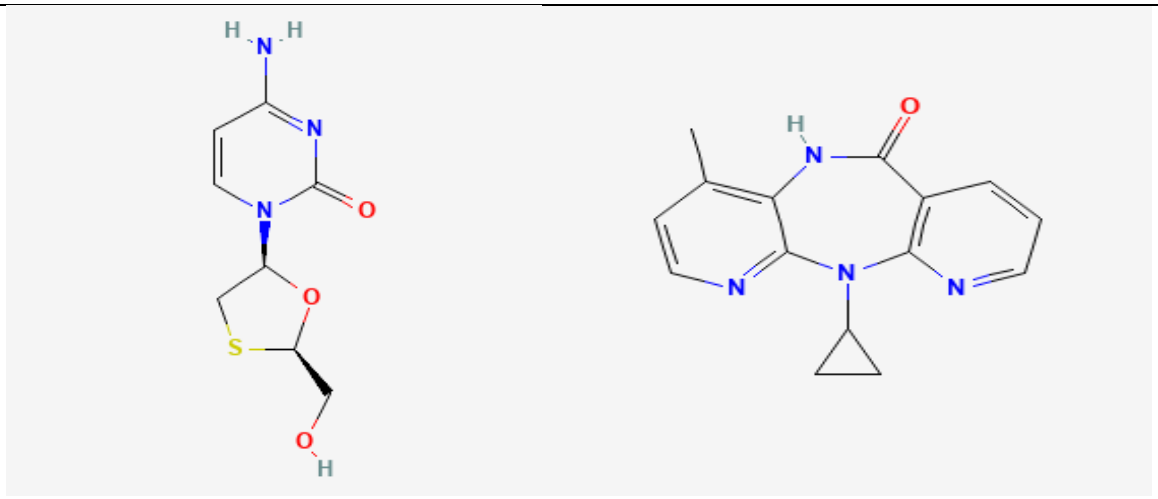

Figure 1: Chemical structures of LamivudineFigure 2: Chemical structures of Nevirapine

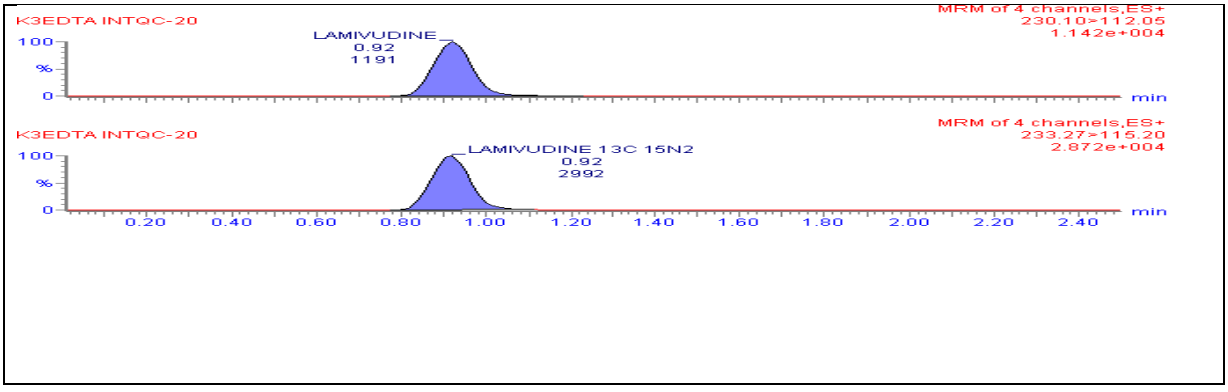

Figure 3: Representative Chromatogram of INTQC Sample for Lamivudine

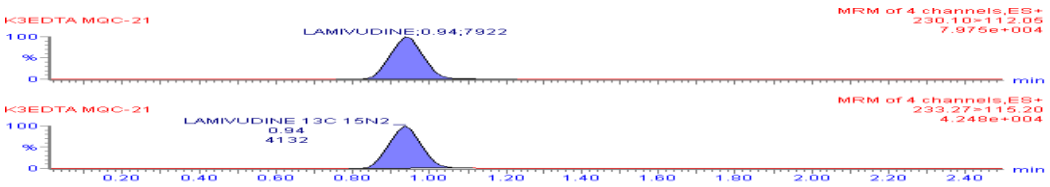

Figure 4: Representative Chromatogram of MQC Sample for Lamivudine

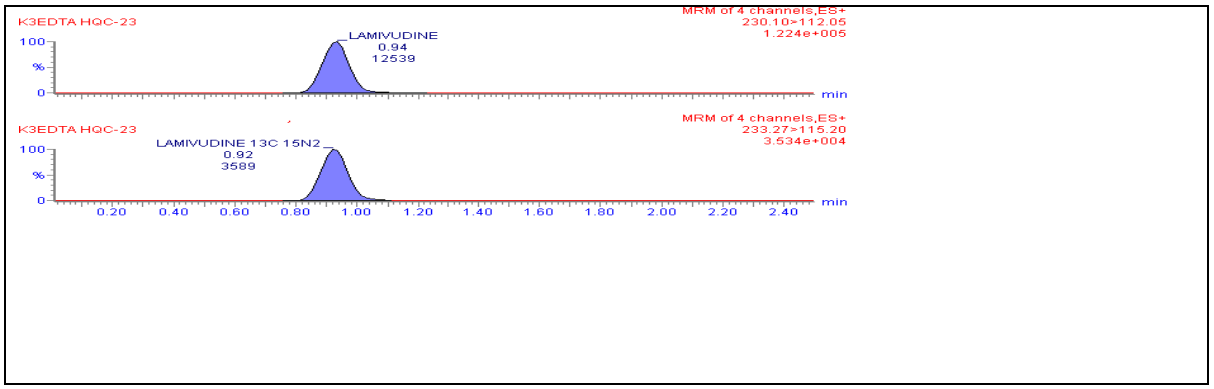

Figure 5: Representative Chromatogram of HQC Sample for Lamivudine

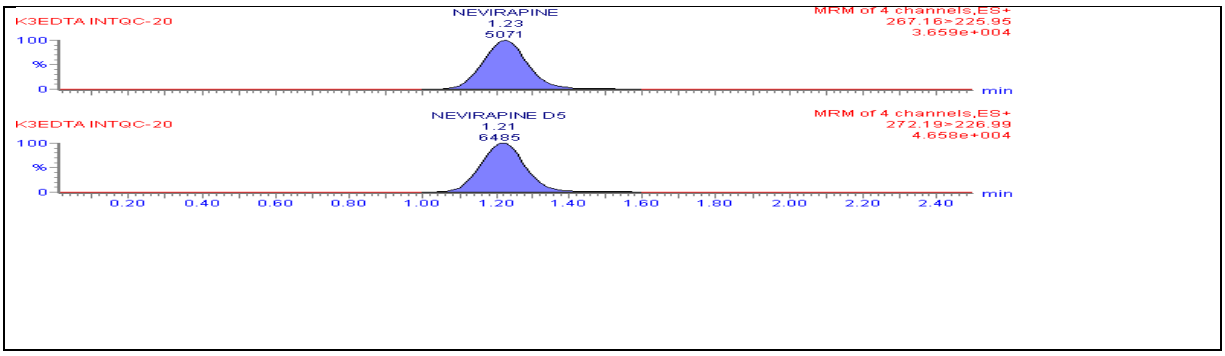

Figure 6: Representative Chromatogram of INTQC Sample for Nevirapine

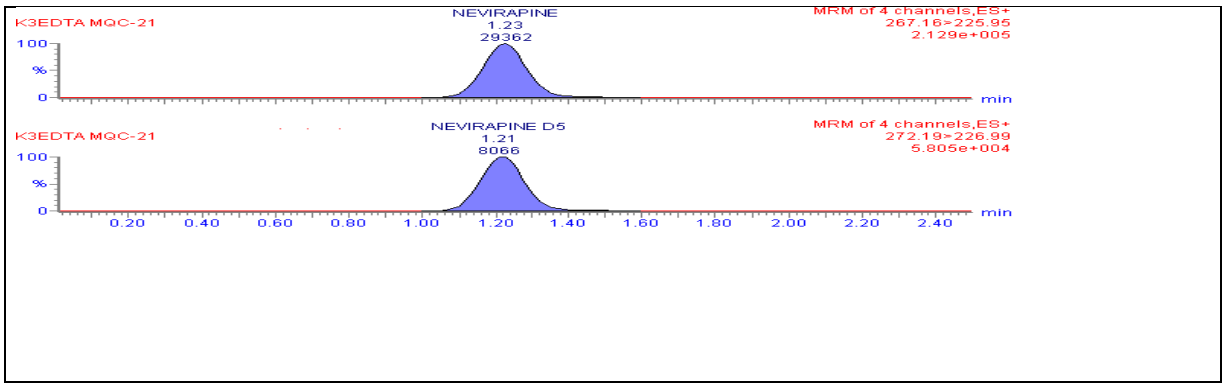

Figure 7: Representative Chromatogram of MQC Sample for Nevirapine

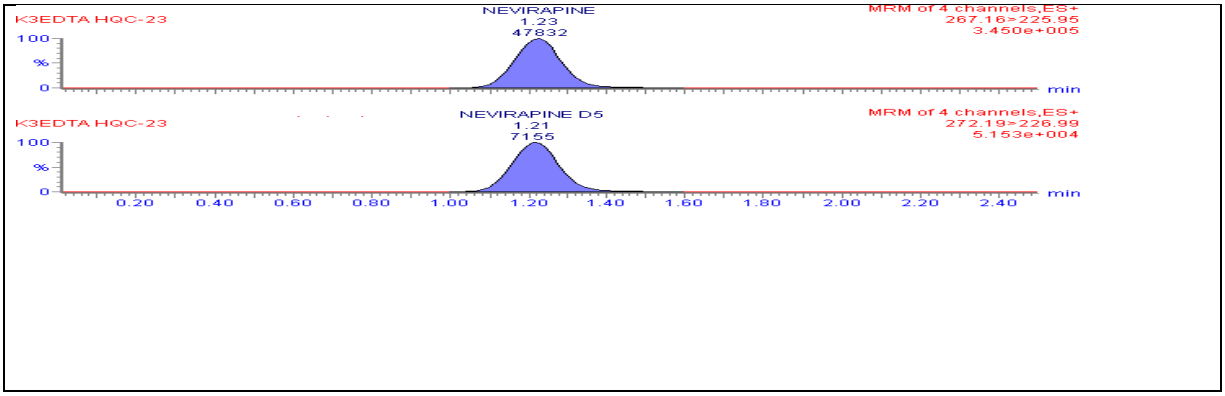

Figure 8: Representative Chromatogram of HQC Sample for Nevirapine

QCID	LQC FreshCS	HQC FreshCS	LQCFT4 (-70ºC±15ºC)	HQCFT4 (-70ºC±15ºC)	LQCFT4 (-30ºC±10ºC)	HQCFT4 (-30ºC±10ºC)
ActualConcentration(ng/mL)	**67.3950**	**2982.0820**	**67.4090**	**2995.9730**	**67.4090**	**2995.9730**
CalculatedConcentrations(ng/mL)	74.4968	2912.8813	60.7298	2939.5403	65.6778	2747.0832
	74.7140	2843.3703	66.3421	2784.5539	65.3056	2838.4918
	67.0256	2831.6301	63.6179	2769.6063	61.9536	2832.3118
	69.2433	2832.1492	68.1663	2804.1336	68.3991	2860.1483
	74.1948	2768.7311	68.8665	2766.1371	71.2025	2844.1340
	69.2385	2846.5700	60.5883	2788.9592	65.0878	2824.0326

Mean	**71.48550**	**2839.22200**	**64.71848**	**2808.82173**	**66.27107**	**2824.36695**
SD	**3.370419**	**45.985916**	**3.630672**	**65.502059**	**3.167721**	**39.765722**
%CV	**4.71**	**1.62**	**5.61**	**2.33**	**4.78**	**1.41**
%Nominal	**106.07**	**95.21**	**96.01**	**93.75**	**98.31**	**94.27**
%NominalagainstCS:			**90.53**	**98.93**	**92.71**	**99.48**

Table 1: Freeze-Thaw Stability for Lamivudine (at -70° C ± 15° C and -30° C ± 10° C)

QCID	**LQC Fresh CS**	**HQC Fresh CS**	**LQCFT4 (-70ºC±15ºC)**	**HQCFT4 (-70ºC±15ºC)**	**LQCFT4 (-30ºC±10ºC)**	**HQCFT4 (-30ºC±10ºC)**
ActualConcentration(ng/mL)	**101.0960**	**4473.2820**	**101.0280**	**4490.1460**	**101.0280**	**4490.1460**
CalculatedConcentrations(ng/mL)	101.4729	4397.2413	95.2255	4208.6814	90.3469	4266.7602
	99.0885	4444.0123	93.1382	4358.8388	98.5105	4318.7163
	106.4337	4249.6694	100.7202	4307.8322	93.9452	4241.2373
	105.3919	4396.3387	99.7147	4336.0935	96.5364	4261.2762
	101.2639	4245.0736	98.1537	4306.7060	95.1733	4238.9506
	101.3236	4324.0063	94.0206	4295.0521	101.3453	4172.5600
Mean	**102.49575**	**4342.72360**	**96.82882**	**4302.20067**	**95.97627**	**4249.91677**

SD	**2.808633**	**83.247947**	**3.140187**	**51.391769**	**3.795322**	**47.593640**
%CV	**2.74**	**1.92**	**3.24**	**1.19**	**3.95**	**1.12**
%Nominal	**101.38**	**97.08**	**95.84**	**95.81**	**95.00**	**94.65**
%NominalagainstCS:			**94.47**	**99.07**	**93.64**	**97.86**

Table 2: Free-Thaw Stability for Nevirapine (at -70° C ± 15° C and -30° C ± 10° C)

StabilityHours	**0 Hour**		**15.15hours**	
QCID	**LQC (FreshCS)**	**HQC (FreshCS)**	**LQC (Stability)**	**HQC (Stability)**
Actual Concentration(ng/mL)	**67.3950**	**2982.0820**	**67.4090**	**2995.9730**
CalculatedConcentrations(ng/mL)	74.4968	2912.8813	72.3088	2838.8830
	74.7140	2843.3703	63.1334	2828.7747
	67.0256	2831.6301	64.8801	2850.0801
	69.2433	2832.1492	64.2735	2812.7889
	74.1948	2768.7311	70.9170	2865.8526
	69.2385	2846.5700	65.1597	2908.7986
Mean	**71.48550**	**2839.22200**	**66.77875**	**2850.86298**
SD	**3.370419**	**45.985916**	**3.833996**	**33.653782**
%CV	**4.71**	**1.62**	**5.74**	**1.18**
%Nominal	**106.07**	**95.21**	**99.07**	**95.16**
%NominalagainstCS:			**93.42**	**100.41**

Table 3: Bench - Top Stability for Lamivudine

StabilityHours	**0 Hour**		**15.15hours**	
QCID	**LQC (FreshCS)**	**HQC (FreshCS)**	**LQC (Stability)**	**HQC (Stability)**
Actual Concentration(ng/mL)	**101.0960**	**4473.2820**	**101.0280**	**4490.1460**
CalculatedConc	101.4729	4397.2413	97.3552	4270.7541
	99.0885	4444.0123	101.5451	4211.0570
	106.4337	4249.6694	98.0574	4262.8366
	105.3919	4396.3387	103.4854	4268.2833

entrations(ng/ mL)	101.2639	4245.0736	96.8297	4220.1621
	101.3236	4324.0063	94.2703	4191.1023
Mean	**102.49575**	**4342.72360**	**98.59052**	**4237.36590**
SD	**2.808633**	**83.247947**	**3.354532**	**34.199017**
%CV	**2.74**	**1.92**	**3.40**	**0.81**
%Nominal	**101.38**	**97.08**	**97.59**	**94.37**
%NominalagainstCS:			**96.19**	**97.57**

S.No.	**Solution1 (43.62 hours)**			**Solution3 (0 Hour)**		
	AnalyteArea	**ISArea**	**AreaRatio**	**Analyte Area**	**ISArea**	**AreaRatio**
1	720	6597	0.1092	713	6791	0.1051
2	687	6625	0.1036	704	6859	0.1026
3	728	7101	0.1026	654	6724	0.0972
4	747	7187	0.1039	622	6384	0.0975
5	753	7334	0.1027	697	6767	0.1030
6	717	6839	0.1048	697	7090	0.0983
		Mean	**0.10447**		**Mean**	**0.10062**

Table 4: Bench - Top Stability for Nevirapine

Table 5: Short-Term Stock Solution Stability for Lamivudine at LQC level

Short-Term Stock Solution Stability for Lamivudine = 103.83%

Solution 1: Analyte (43.62 hours) at Room Temperature + IS (0 Hour) at Refrigerator Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

Table 6: Short-Term Stock Solution Stability of Lamivudine 13C 15N2 at LQC level

S.No.	Solution2 (43.62 hours)			Solution3 (0 Hour)		
	AnalyteArea	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	738	6957	9.4268	713	6791	9.5245
2	751	7270	9.6804	704	6859	9.7429
3	772	7374	9.5518	654	6724	10.2813
4	739	7391	10.0014	622	6384	10.2637
5	705	6936	9.8383	697	6767	9.7088
6	731	7047	9.6402	697	7090	10.1722
		Mean	**9.68982**		**Mean**	**9.94890**

Short-Term Stock Solution Stability for Lamivudine 13C 15N2 = 97.40%
Solution 2: Analyte (0 Hour) at Refrigerator + IS (43.62 hours) at Room Temperature

S.No.	Solution1 (43.62 hours)			Solution3 (0 Hour)		
	AnalyteArea	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	30337	6698	4.5295	27956	6262	4.4647
2	28762	5996	4.7972	28424	6308	4.5058
3	30565	6626	4.6128	28784	6472	4.4476
4	31443	6846	4.5930	27240	5951	4.5774
5	31956	6828	4.6801	26067	5954	4.3782
6	30789	6594	4.6695	27261	6121	4.4540
		Mean	**4.64702**		**Mean**	**4.47128**

Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

Table 7: Short-Term Stock Solution Stability for Lamivudine at HQC
Short-Term Stock Solution Stability for Lamivudine = 103.93% Solution 1: Analyte (43.62 hours) at Room Temperature + IS (0 Hour) at Refrigerator Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

S.No.	Solution2 (43.62 hours)	Solution3 (0 Hour)

	Analyte Area	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	30752	6406	0.2083	27956	6262	0.2240
2	31108	6773	0.2177	28424	6308	0.2219
3	31530	6981	0.2214	28784	6472	0.2248
4	32740	7303	0.2231	27240	5951	0.2185
5	31073	6740	0.2169	26067	5954	0.2284
6	29361	6482	0.2208	27261	6121	0.2245
		Mean	**0.21803**		**Mean**	**0.22370**

Table 8: Short-Term Stock Solution Stability of Lamivudine 13C 15N2 at HQC

Short-Term Stock Solution Stability for Lamivudine 13C 15N2 = 97.47%

Solution 2: Analyte (0 Hour) at Refrigerator + IS (43.62 hours) at Room Temperature Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

S.No.	Solution1 (43.62 hours)			Solution3 (0 Hour)		
	Analyte Area	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	1289	6713	0.1921	1456	7531	0.1933
2	1365	6969	0.1958	1331	7075	0.1881
3	1529	7775	0.1966	1299	6421	0.2024
4	1499	7830	0.1915	1294	6626	0.1953
5	1517	7800	0.1945	1454	7358	0.1976
6	1411	7228	0.1952	1429	7520	0.1900
		Mean	**0.19428**		**Mean**	**0.19445**

Table 9: Short-Term Stock Solution Stability for Nevirapine at LQC level

Short-Term Stock Solution Stability for Nevirapine D5 = 99.91%

Solution 1: Analyte (43.62 hours) at Room Temperature + IS (0 Hour) at Refrigerator Solution 3: Analyte (0 Hour) at
Refrigerator + IS (0 Hour) at Refrigerator

S.No.	Solution2 (43.62 hours)			Solution3 (0 Hour)		
	AnalyteArea	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	1544	7680	4.9741	1456	7531	5.1724
2	1640	8398	5.1207	1331	7075	5.3156
3	1709	8234	4.8180	1299	6421	4.9430
4	1510	7657	5.0709	1294	6626	5.1206
5	1403	7085	5.0499	1454	7358	5.0605
6	1526	7731	5.0662	1429	7520	5.2624
		Mean	**5.01663**		**Mean**	**5.14575**

Table 10: Short-Term Stock Solution Stability of Nevirapine D5 at LQC level
Short-Term Stock Solution Stability for Nevirapine D5 = 97.49%
Solution 2: Analyte (0 Hour) at Refrigerator + IS (43.62 hours) at Room Temperature Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

S.No.	Solution1 (43.62 hours)			Solution3 (0 Hour)		
	AnalyteArea	ISArea	AreaRatio	AnalyteArea	ISArea	AreaRatio
1	58723	6601	8.8958	60014	7010	8.5611
2	56479	6427	8.7875	57281	6636	8.6324
3	62842	7314	8.5925	54311	6301	8.6195
4	64871	7338	8.8403	50589	5825	8.6847
5	64542	7382	8.7431	49522	5795	8.5458
6	6195	704	8.7950	55490	6377	8.7015

	7	5				
		Mean	**8.77570**		**Mean**	**8.62417**

S.No.	Solution2 (43.62 hours)			Solution3 (0 Hour)		
	Analyte Area	**ISArea**	**AreaRatio**	**Analyte Area**	**ISArea**	**AreaRatio**
1	62121	7189	0.1157	60014	7010	0.1168
2	70968	8091	0.1140	57281	6636	0.1158
3	69450	7819	0.1126	54311	6301	0.1160
4	65413	7425	0.1135	50589	5825	0.1151
5	60717	6813	0.1122	49522	5795	0.1170
6	59965	6874	0.1146	55490	6377	0.1149
		Mean	**0.11378**		**Mean**	**0.11596**

Table 11: Short-Term Stock Solution Stability of Nevirapine at HQC
Short-Term Stock Solution Stability for Nevirapine = 10
Solution 1: Analyte (43.62 hours) at Room Temperature + IS (0 Hour) at Refrigerator
Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator
Table 12: Short-Term Stock Solution Stability of Nevirapine D5 at HQC
Short-Term Stock Solution Stability for Nevirapine D5 = 98.12%
Solution 2: Analyte (0 Hour) at Refrigerator + IS (43.62 hours) at Room Temperature
Solution 3: Analyte (0 Hour) at Refrigerator + IS (0 Hour) at Refrigerator

CONCLUSION

A validated stability-indicating LC/MS/MS assay method was established to study the degradation pattern of Lamivudine and Nevirapine under hydrolysis, oxidation, photolysis and thermal stress conditions.

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