Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2025, 17(2): 638-643
(http://www.derpharmachemica.com/archive.html)

Advanced Analytical Methods: LC-MS/MS with Multiple Reaction Monitoring (MRM Mode) For the Quantification of Two Potential Genotoxic Impurities in Emtricitabine Drug Substance

Pantula Nagendra Srinivas[1*], P Shyamala[2], K Rama Srinivas[1], Ch Nagaraju[1]

[1]*Department of Chemistry, Aurobindo Pharma Limited, Andhra Pradesh, India*
[2]*Department of Chemistry, Andhra University, Visakhapatnam, India*
[*]***Corresponding author:*** *Pantula Nagendra Srinivas, Department of Chemistry, Aurobindo Pharma Limited, Andhra Pradesh, India, Tel: 8501094273; E-mail: pnsrinivas70@gmail.com*

Received: 20-December-2023, Manuscript no: DPC-23-123113, **Editor assigned:** 23-December-2023, Pre QC No: DPC-23-123113 (PQ), **Reviewed:** 08-January-2024, QC No: DPC-23-123113, **Revised:** 21-February-2025, Manuscript No: DPC-23-123113 (R), **Published:** 28-February-2025, DOI: 10.4172/0975-413X.17.2.638-643

ABSTRACT

To detect Possible Genotoxic Impurities (PGIs) in Emtricitabine drug substance 2-Methoxy 4-Amino-5-Fluro pyramidine and L-Methyl glyoxalate monohydrate, two sensitive LC-MS/MS techniques were developed and verified. 2-Methoxy 4-Amino-5-Fluro pyramidine and L-menthyl glyoxalate contaminants in the Active Pharmaceutical Ingredient (API) of Emtricitabine. Multiple Reaction Monitoring (MRM) mode was employed with the LC-MS/MS method on acquity UPLC CSH phenyl hexyl, 150x2.1mm, 1.7µm and X-Select CSH C18, 150x4.6 mm, 3.5 µm respectively, using Electrospray Ionization (ESI). The suggested approach was exact, robust, accurate, linear, and specific. Over the concentration range of 0.6 µg/g to 9.5 µg/g and 0.6 µg/ml to 9.4 µg/ml, the calibration curves demonstrated satisfactory linearity; the correlation coefficients were.0.999 and.0.999 in each case. The method's very Low Limit of Quantification (LOQ) and Limit of Detection (LOD) were 0.3 µg/g and 0.6 µg/g, respectively.

Keywords: L-Methyl glyoxalate monohydrate and 2-Methoxy 4-Amino-5-Fluro pyramidine; LC-MS/MS; Emtricitabine; Genotoxic impurities; Trace analysis

INTRODUCTION

A nucleoside reverse transcriptase inhibitor called emtricitabine is used to treat HIV (human immunodeficiency virus) infection in adults. The medication copies HIV RNA (ribonucleic acid) into new virus DNA (deoxyribonucleic acid) by suppressing the reverse transcriptase enzyme. Tenofovir disoproxil fumarate is frequently given in conjunction with emtricitabine; the daily maximum dose of emtricitabine is 0.2 g. Because they are reactive by nature, synthetic starting materials and intermediates could appear as contaminants in the finished API. These substances are frequently carcinogens or mutagens due to the biological reactivity that commonly results from the chemical reactivity [1-4]. It has been repeatedly demonstrated that the significant chemical reactivities of the various alkylating agents prevented their retention within the final API (Figure 1).

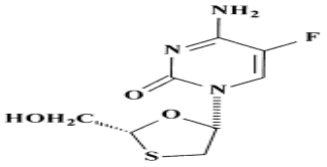

Figure 1: Structure of emtricitabine.

L-methyl glyoxylate monohydrate (LMGH) is a crucial step in the production of lamivudine and emtricitabine 1, two significant antiretroviral medications. These two commonly prescribed medications serve as cytidine reverse transcriptase inhibitors in combination therapy for the treatment of HIV-1, HIV-2, and Hepatitis B. Since Sub-Saharan Africa has a higher rate of AIDS cases than any other region in the world, these generic medications are especially crucial in these nations (Figure 2).

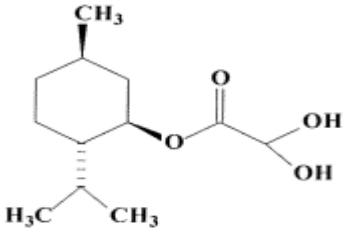

Figure 2: Structure of L-menthyl glyoxylate monohydrate.

2-methoxy 4-amino-5-fluro pyramidine introduction part (Figure 3).

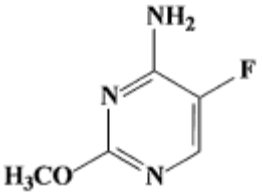

Figure 3: Structure of 2-methoxy 4-amino-5-fluro pyramidine.

Based on the maximum daily dosage of Emtricitabine L -Menthyl glyoxylate monohydrate (LMGH) and 2-Methoxy 4-Amino-5-Fluro pyramidine (MAFP) are required to be controlled at a limit of NMT 6.25 µg/g and NMT 6.25 µg/g respectively in the API.

L-Menthyl glyoxylate monohydrate and 2-Methoxy 4-Amino-5-Fluro pyramidine are used in the synthesis of Emtricitabine.Route of synthesis shown below (Figure 4).

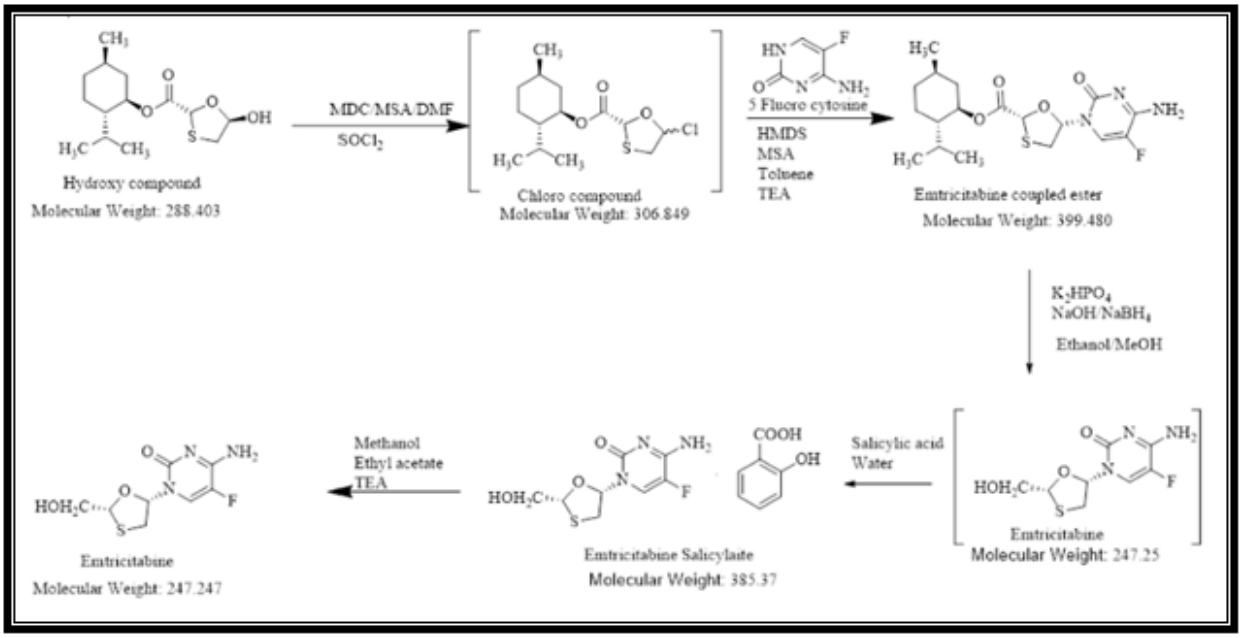

Figure 4: L-Menthyl glyoxylate monohydrate and 2-Methoxy 4-Amino-5-Fluro pyramidine.

MATERIALS AND METHODS

The variety of analytical procedures has expanded and there has been a general revival due to the growing regulatory awareness on the potential risks. For the determination of L-Menthyl glyoxylate monohydrate (LMGH) and 2-Methoxy 4-Amino-5-Fluro pyramidine (MAFP), the suggested LC-MS/MS (liquid chromatography/mass spectrometery/mass spectrometery) approach provides a straightforward, robust, and labor-saving alternative that requires no time-consuming sample preparation steps. When comparing this method to arduous sample preparation techniques, it has numerous advantages over the method documented in the literature in terms of specificity, accuracy, and reproducibility involving direct analysis [5-7]. In order to deal with matrix interference, a diluent that makes the emtricitabine API insoluble and allows for the extraction of analytes from the API matrix is used. The suggested technique uses electrospray ionization in the MRM mode to quantify L-Menthyl glyoxylate monohydrate (LMGH) and 2-Methoxy 4-Amino-5-Fluro pyramidine (MAFP).

Experimental

Chemical and Reagents

List of chemicals, reagents, chemicals, sample, standards and impurities show in Table 1.

Table 1: List of Chemicals and Reagents.

S. No	Name of the materials		Grade
1	LMHG	MAFP	
2	Formic acid	Formic acid	LCMS
3	Methanol	Ammonia	LCMS
4	Sodium hydroxide	Ammonium Formate	AR &LCMS
5	Water	Water	Milli-Q
6	L-Menthyl glyoxylate monohydrate		NA
7	2-Methoxy 4-Amino-5-Fluro pyramidine		NA
8	Salicylic acid		NA
9	Emtricitabine coupled ester		NA
10	Emtricitabine sulfoxide		NA
11	Emtricitabine acid		NA
12	Desfluoro Emtricitabine		NA
13	5-Fluorouracil Analog		NA
14	Emtricitabine		NA

Instrumentation

For L -Menthyl glyoxylate monohydrate (LMHG) and 2-Methoxy 4-Amino-5-Fluro pyramidine (MAFP):
An ultra-performance liquid chromatography, acquity H-class system, with a quaternary solvent manager, gradient mixer assembly, sample manager-F.T.N (Flow through needle) auto injector, with a column oven coupled to Xevo TQ-S Triple Quadrupole LC/MS/MS Mass Spectrometer, (Make Waters).Column was employed in the method was acquity UPLC CSH Phenyl Hexyl 1.7 µm. (150 mm x 2.1 mm) and X-Select CSH C18 150 x 4.6 3.5 µm mm respectively. All the weighing in the experiments was done with Sartorius balance capable of measuring with an accuracy of 0.01 mg.

Chromatographic conditions for LC

Table 2: Chromatographic Conditions for LC.

Chromatographic Conditions for LC			
S. No	**Parameters**	**L-Menthyl glyoxylate monohydrate (Method-A)**	**2-Methoxy 4-Amino-5-Fluro pyramidine (Method-B)**
1	Mobile phase-A	Mix 1.0 mL of formic acid 1000 ml of water	Weigh 126 mg of ammonium formate in 1000 mL of water and add 1.0 ml ammonia to the solution
2	Mobile phase-B	Methanol	Acetonitrile
3	Column	Acquity UPLC CSH phenyl hexyl 1.7 µm. (150 mm x 2.1 mm)	X-select CSH C18 150x4.6 mm,3.5 µm
4	Flow rate	0.25 mL/min	0.3 mL/min
5	Injection volume	5.0 µl	5.0 µl
6	Column oven temperature	40°C	45°C
7	Auto sampler temperature	10°C	10°C
8	Run time	20 min	20 min
9	Pump mode	Gradient	Gradient
Time (min)		Mobile Phase A (%, v/v)	Mobile Phase B (%, v/v)
T_0		90	10
T_0		90	10
$T_{10}.0$		10	90

$T_{13}.0$	10	90
$T_{13}.5$	90	10
$T_{20}.0$	90	10
Time (min)	Mobile Phase A (%, v/v)	Mobile Phase B (%, v/v)
$T_{0.01}$	85	15
$T_{2.0}$	85	15
$T_{5.0}$	65	35
$T_{10.0}$	65	35
$T_{12.0}$	10	90
$T_{14.0}$	10	90
$T_{14.1}$	85	15
$T_{20.0}$	85	15

Mass spectroscopic conditions

Source type: ESI
Mode of ionization: Positive
Source parameters
Capillary: 3.9 kv
Source temperature: 150°C
Desolvation temperature: 500°C
Cone gas flow: 150 L/Hr
Desolvation gas flow: 1000 L/Hr
Nebulizer (Bar): 7.0
Condition for MRM:
Scan type: MRM
Function type: MRM of 1 channel

Table 3: MRM of 1 channel.

Name	Q1 mass (amu)	Q3 mass (amu)	Cone(v)	Collision energy (v)
L-methyl glyoxalate monohydrate	253.2 $[M+Na]^+$	115	26	10
2-methoxy 4-amino-5-fluro pyramidine	143.9	112.8	48	18

Preparation of diluent for L-methyl glyoxalate monohydrate

Prepare a degassed mixture of Methanol, Water and methanolic sodium hydroxide solution in the ratio of 800:200:0.1 v/v/v.

Preparation of diluent for 2-methoxy 4-amino-5-fluro pyramidine

Prepare a degassed mixture of Acetonitrile, Water and Formic acid in the ratio of 500:500:0.1 v/v/v.

Preparation of standard solution for L-methyl glyoxalate monohydrate

Primary standard stock solution

Accurately weigh and transfer about 11.0 mg of L-Menthyl Glyoxalate reference sample into a 50 mL clean, dry volumetric flask, add 30 mL of diluent sonicate to dissolve and make up to volume with diluents (0.22 mg/mL).
Pipette 3.0 mL of Primary standard stock solution into 50 mL of volumetric flask make up to the mark with diluents (528.0 µg/g). Further pipette 3.0 mL of solution into 50 mL of volumetric flask make up to the mark with diluents (31.68 µg/g). Further pipette 2.0 mL of solution into 10 mL of volumetric flask make up to the mark with diluent (6.34 µg/g).

Preparation of standard solution for 2-methoxy 4-amino-5-fluro pyramidine

Primary standard stock solution
Accurately weigh and transfer about 9.8 mg of 2-Methoxy 4-Amino-5-Fluro pyramidine reference sample into a 50 mL clean, dry volumetric flask, add 30 mL of diluent sonicate to dissolve and make up to volume with diluents (0.196 mg/mL).
Pipette 2.0 mL of primary standard stock solution into 50 mL of volumetric flask make up to the mark with diluents (784.0 µg/g). Further pipette 2.0 mL of solution into 50 mL of volumetric flask make up to the mark with diluents (31.36 µg/g). Further pipette 2.0 mL of solution into 10 mL of volumetric flask make up to the mark with diluent (6.27 µg/g).

Preparation of sample solution for L-methyl glyoxalate monohydrate
Accurately weigh and transfer 250 mg of sample into a clean, dry 10 mL volumetric flask/Centrifuge tube, add 5.0 mL of diluent sonicate to dissolve and then add 5.0 mL of diluent and mix well. Total volume is 10.0 mL [8-10].
Preparation of sample solution for 2-methoxy 4-amino-5-fluro pyramidine
Accurately weigh and transfer 100 mg of sample into a clean, dry 10 mL volumetric flask/Centrifuge tube, add 5.0 mL of diluent sonicate to dissolve and then add 5.0 mL of diluent and mix well. Total volume is 10.0 mL [11-13].

RESULTS AND DISCUSION

Validation

System precision: Prepared the standard solution of L-menthyl glyoxalate and 2-methoxy 4-amino 5-fluoropyrimidine as per methodology.

Table 4: L-menthyl glyoxalate and 2-methoxy 4-amino 5-fluoropyrimidine.

Sample preparation ID	Area of L-menthyl glyoxalate	Area of 2-methoxy 4-amino 5-fluoropyrimidine
1	78439	39706
2	80464	37454
3	78524	38826
4	79475	38255
5	76820	36404
6	77051	37046
Mean	78462	37949
SD	1394.68	1216.33
%RSD	1.8	3.2

%RSD for six standard solution injections were observed below 10.0% for both impurities and results are 1.8% for L-menthyl glyoxalate and 3.2% for 2-methoxy 4-amino 5-fluoropyrimidine. System precision met the acceptance criteria.

Identification and specificity

The identification and specificity is defined as the ability to assess and ensure that the impurities and diluent do not affect the sample analyzed.

For L-methyl glyoxalate monohydrate
Inject the blank (as diluent), standard solution, and control sample, spiked sample with all related compounds with and without LMGM and at specification level. Check the interference at the retention time and mass of analyte.
For 2-methoxy 4-amino-5-fluro pyramidine
Inject the blank (as diluent), standard solution, and control sample, spiked sample with all related compounds with and without MAFP and at specification level. Check the interference at the retention time and mass of analyte.

Results table for identification

Table 5: Table for Identification.

Impurity Name	Impurity stock solution (RT in minutes)	Spiked sample solution (RT in minutes)
L-methyl glyoxalate monohydrate	10.94	11.01
2-methoxy 4-amino-5-fluro pyramidine	9.77	9.71

Results table for Specificity

Table 6: Table for Specificity.

Impurity name	Area		MRM trace
	Control sample	Spiked sample	
L-methyl glyoxalate monohydrate	ND	44077	253.2⟶115.0
2-methoxy4-amino-5-fluro pyramidine	ND	36770	143.9 ⟶ 112.8

Spiked sample Retention Times (RT) were comparable to that of reference sample for both impurities and both methods. Did not show any

response at the retention time of the L-methyl glyoxalate monohydrate and 2-methoxy 4-amino-5-fluro pyramidine respective methodologies. Hence methods can be capable for the identification and proved the specificity.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Prediction of LOD and LOQ

Prepared a series of diluted solutions of L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities with respect to analyte concentration were prepared and injected into the LC-MS/MS system until to get the signal to noise (USP S/N) ratio is more than 3 for LOD and more than 10 for LOQ and achieved the target. The Signal to Noise (S/N) ratio of L-menthyl glyoxalate and 2-methoxy 4-Amino-5-Fluro pyramidine peaks were recorded using system data software. The LOQ and LOD results were captured in the below tables.
Results Limit of Quantitation and Limit of detection.

Table 7: Limit of quantitation and limit of detection.

Impurity name	LOQ concentration (µg/g)	USP S/N	LOD concentration (µg/g)	USP S/N
L-menthyl glyoxalate	0.63	222.7	0.32	82.6
2-methoxy 4-amino-5-fluro pyramidine	0.63	298.8	31	171.8

Precision at LOD and LOQ and accuracy at LOQ

Six different solutions were prepared to contain L-menthyl glyoxalate impurity and 2-methoxy 4-amino-5-fluro pyramidine impurity separately as per methodology at proposed LOD and LOQ level and each solution was injected once into the L-MS/MS, area of L-menthyl glyoxalate impurity and 2-methoxy 4-amino-5-fluro pyramidine impurity in each solution was recorded. %RSD for the content of L-menthyl glyoxalate impurity and 2-methoxy 4-amino-5-fluro pyramidine impurity in each preparation was calculated. The % recovery of each solution was calculated.

Table 8: Area of L-menthyl glyoxalate.

Injection ID	Area of L-menthyl glyoxalate		Area of 2-methoxy 4-amino-5-fluro pyramidine	
	LOD	LOQ	LOD	LOQ
1	5308	8329	2818	4750
2	5271	8147	2793	4716
3	5311	8235	2771	4712
4	5239	8245	2713	4701
5	5271	8153	2763	4706
6	5255	8134	2744	4804
Mean	5276	8207	2767	4732
SD	26.67	76.1	36.75	39.5
%RSD	0.5	0.9	1.3	0.63

Table 9: Area of Spiked Sample (L-Menthyl Glyoxalate).

Sample Preparation ID	Area of Spiked Sample (L-Menthyl Glyoxalate)	Area of Neat Standard (L-Menthyl Glyoxalate)	% Recovery
LOQ Accuracy-1	8237	8284	100
LOQ Accuracy-2	8284	8171	100.7
LOQ Accuracy-3	8272	8257	100.4

Table 10: Area of Spiked Sample (2-Methoxy 4-Amino-5-Fluro pyramidine).

Sample Preparation ID	Area of spiked sample (2-methoxy 4-amino-5-fluro pyramidine)	Area of Neat Standard (2-Methoxy 4-Amino-5-Fluro pyramidine)	% Recovery
LOQ accuracy-1	4992	4768	105.3
LOQ accuracy-2	4994	4822	105.3
LOQ accuracy-3	4904	4633	103.4

S/N ratio, %RSD and Accuracy results were met the acceptance criteria.

Linearity

Seven linearity solutions were prepared for L-Menthyl Glyoxalate and 2-Methoxy 4-Amino-5-Fluro pyramidine impurities from LOQ to 150% with respect to test concentration (Table 11 and Figure 5, Table 12 and Figure 6).

Table 11: L-menthyl glyoxalate

L-menthyl glyoxalate		
% Level	**Concentration (µg/g)**	**Area**
LOQ	0.63	8179
25	1.58	18135
50	3.16	34542
75	4.75	50457
100	6.33	67243
125	7.91	82933
150	9.49	101450
Slope	10426	
Intercept	1429.942	
Correlation coefficient	0.9998	

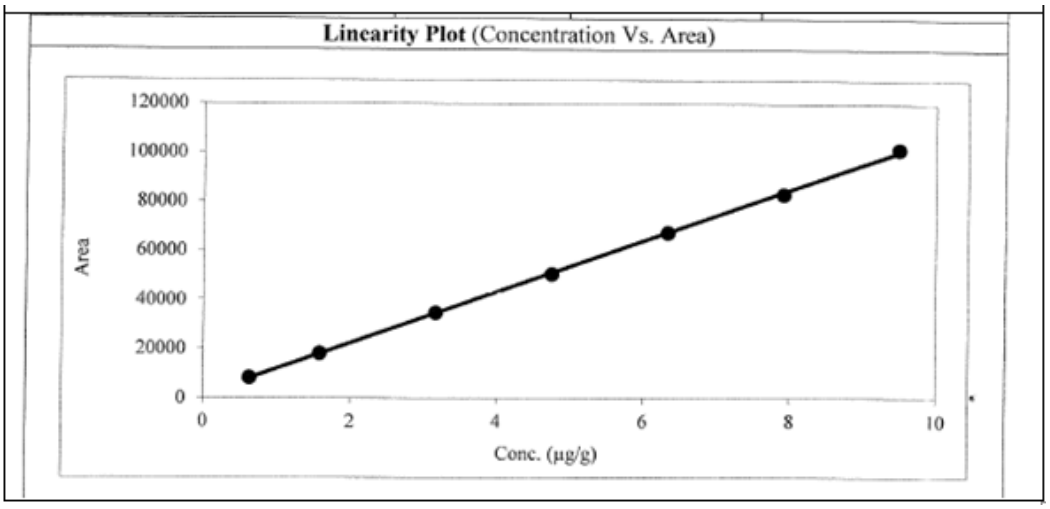

Figure 5: Linearity Plot for L-menthyl glyoxalate.

Table 12: 2-methoxy 4-amino-5-fluro pyramidine.

2-methoxy 4-amino-5-fluro pyramidine		
% Level	**Concentration (µg/g)**	**Area**
LOQ	0.63	4736
25	1.57	11110
50	3,14	21872
75	4.71	33167
100	6.28	43059
125	7.85	53542
150	9.41	65466
Slope	6857	
Intercept	376.77	
Correlation Coefficient	0.9998	

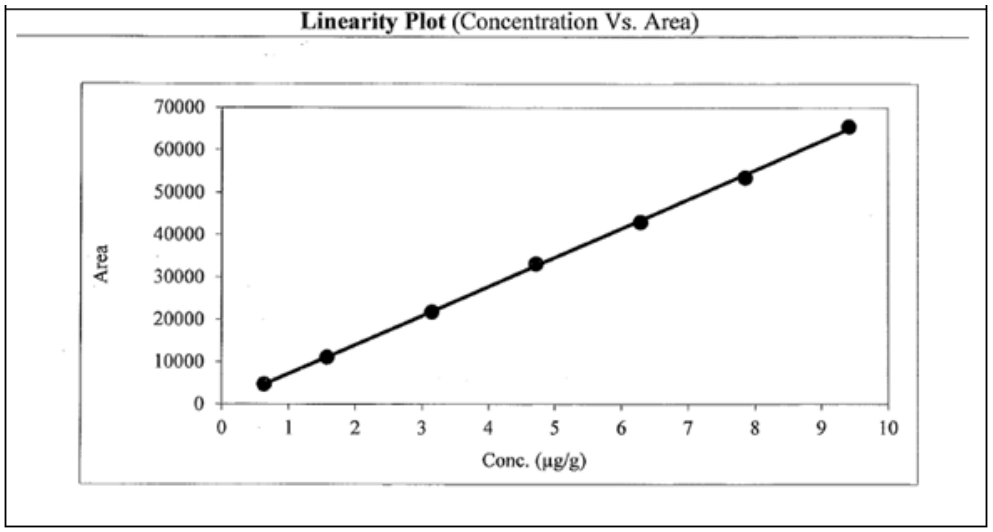

Figure 6: Linearity Plot for 2-Methoxy 4-Amino-5-Fluro pyramidine.

The correlation coefficient is more than 0.99 for L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities in both methodologies. Hence the response of for L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities is linear LOQ to 150% of specification level in both methods.

Method precision

Six sample solutions were prepared individually by spiking the impurity into the Emtricitabine as per test method at specification level and injected into LC-MS/MS as per methodologies (Tables 13 and 14).

Table 13: Sample Preparation.

Sample preparation ID	Area of L-menthyl glyoxalate	Area of 2-methoxy 4-amino-5-fluro pyramidine
1	71543	45275
2	72164	44776
3	71999	45304
4	70869	45001
5	71076	45341
6	71901	45270
Mean	71592	45161
SD	525.3	224.3
%RSD	0.7	0.5

Table 14: Preparation Method precision.

Preparation ID	Added of L-menthyl glyoxalate in µg/g	Found of L-menthyl glyoxalate in µg/g	Added of 2-methoxy 4-amino-5-fluro pyramidine in µg/g	Found of 2-methoxy 4-amino-5-fluro pyramidine in µg/g
1	6.33	5.77	6.28	6.68
2	6.33	5.82	6.28	6.61
3	6.33	5.81	6.28	6.69
4	6.33	5.72	6.28	6.64
5	6.33	5.73	6.28	6.69
6	6.33	5.8	6.28	6.68
	Mean	5.78	Mean	6.67
	SD	0.04	SD	0.03
	%RSD	0.7	%RSD	0.4

%RSD of area and obtained concentration for method precision met the acceptance criteria as per set criteria is not more than 20.0% for both L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities.

Accuracy

Prepared the samples spiked with L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities at 100% level and 150% level in presence of Emtricitabine drug substance (prepared in triplicates) against L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities at 100% level and 150% level in absence of Emtricitabine drug substance and injected into LC-MS/MS as per methodologies (Tables 15-18).

Table 15: 100% of spiked sample (L-menthyl glyoxalate).

Sample Preparation ID	Area of Spiked Sample (L-Menthyl Glyoxalate)	Area of Neat Standard (L-Menthyl Glyoxalate)	% Recovery
100% accuracy-1	69244	69451	100.7
100% accuracy-2	69438	68026	100.9
100% accuracy-3	69809	68883	101.5

Table16: 150% of spiked sample (L-menthyl glyoxalate).

Sample preparation ID	Area of spiked sample (L-menthyl glyoxalate)	Area of neat standard (L-menthyl glyoxalate)	% Recovery
150% accuracy-1	100660	100096	100.2
150% accuracy-2	99903	100545	99.5
150% accuracy-3	102057	100656	101.6

Table 17: 100% of spiked sample (2-methoxy 4-amino-5-fluro pyramidine).

Sample preparation ID	Area of spiked sample (2-methoxy 4-amino-5-fluro pyramidine)	Area of neat standard (2-methoxy 4-amino-5-fluro pyramidine)	% Recovery
100% accuracy-1	44497	42879	104.8
100% accuracy-2	44288	42452	104.8
100% accuracy-3	43803	42029	103.2

Table 18: 150% of Spiked Sample (2-Methoxy 4-Amino-5-Fluro pyramidine).

Sample preparation ID	Area of Spiked Sample (2-Methoxy 4-Amino-5-Fluro pyramidine)	Area of Neat Standard (2-Methoxy 4-Amino-5-Fluro pyramidine)	% Recovery
150% accuracy-1	63279	64281	99.7
150% accuracy-2	64376	62791	101.4
150% accuracy-3	65428	63390	103.1

The individual percentage recovery for each sample at 100% level & 150% specification level are meeting set criteria of between 80.0% to 120.0%.

Range

Range of analytical method can be obtained from linearity and recovery data of L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities in both methodologies. Reported the range in LOQ to 150% with respect to specification level.

Robustness

Prepared standard solution as per test methods for L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine impurities at specification level. Injected to in to LC-MS/MS at deliberately varied conditions to evaluate the system suitability and methods ability to remain unaffected (Tables 19 and 20).

Table 19: L-menthyl glyoxalate.

Parameter	Variation	L-menthyl glyoxalate	
		RT (minutes)	%RSD
Flow rate	-10%	11.49	2.1
	10%	10.76	1.2
Source cleaning	Before	10.96	0.7
	After	10.98	0.9

Table 20: 2-Methoxy 4-amino-5-fluro pyramidine.

Parameter	Variation	2-methoxy 4-amino-5-fluro pyramidine	
		RT (minutes)	%RSD
Flow rate	-10%	10.66	0.9
	10%	8.9	9.6
Source cleaning	Before	9.77	2.9
	After	9.71	3.2

The system suitability results at each of the varied conditions complied the requirements as per the test procedure. Hence it can be concluded that the test method is robust across the extent of changes studied for each of the above parameters.

Specimen chromatograms

The result show in Figures 7-10.

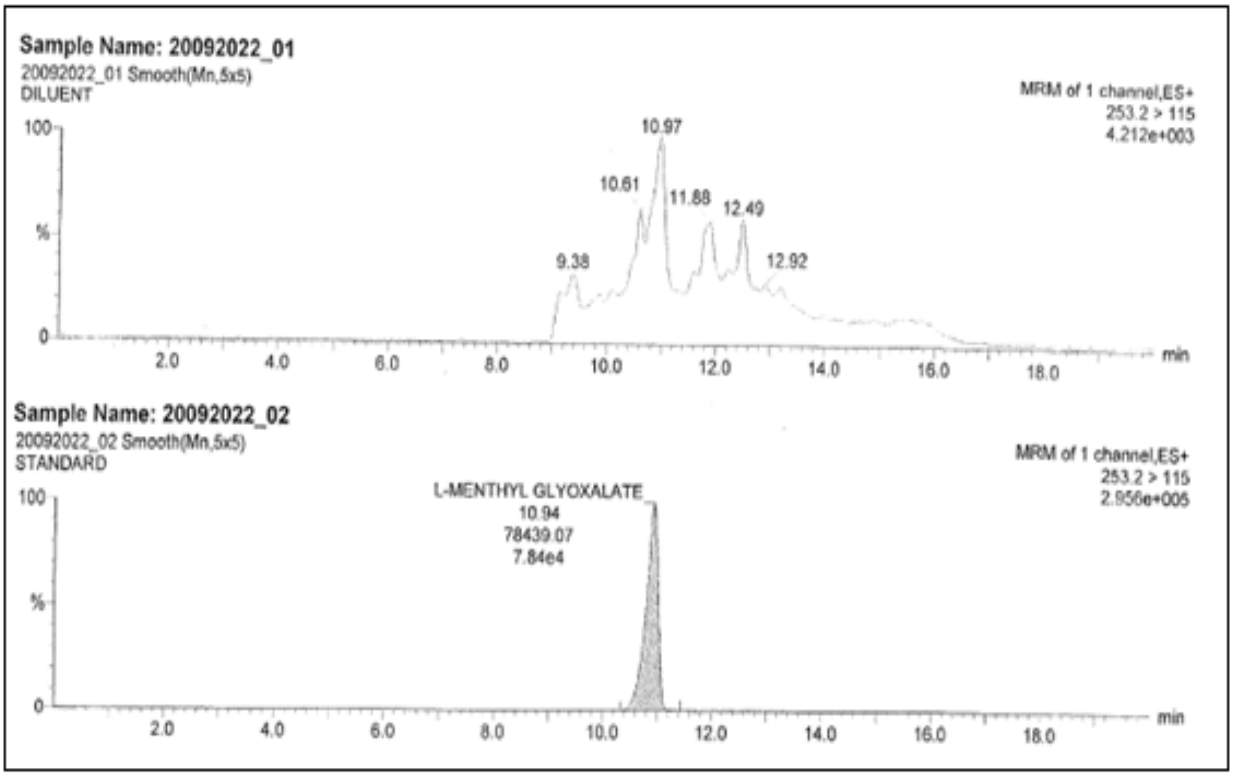

Figure 7: Blank and L-menthyl glyoxalate LCMS chromatograms.

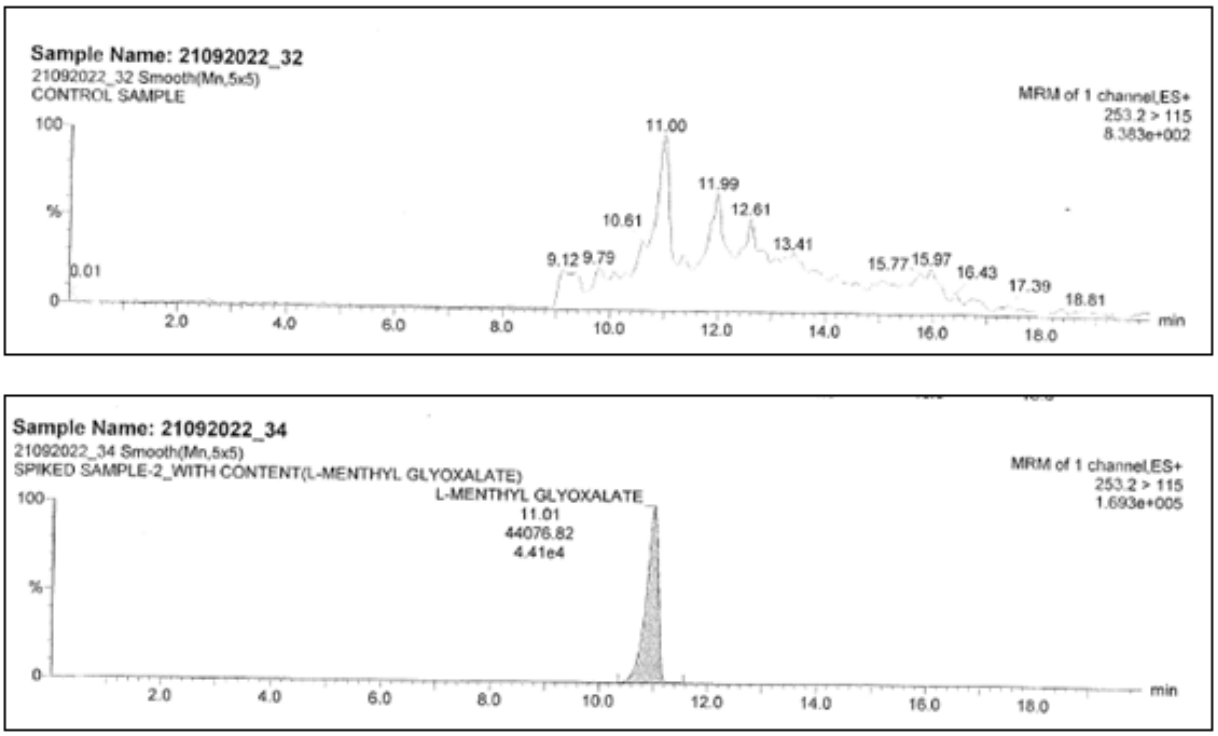

Figure 8: Control sample and spiked sample LCMS chormatograms.

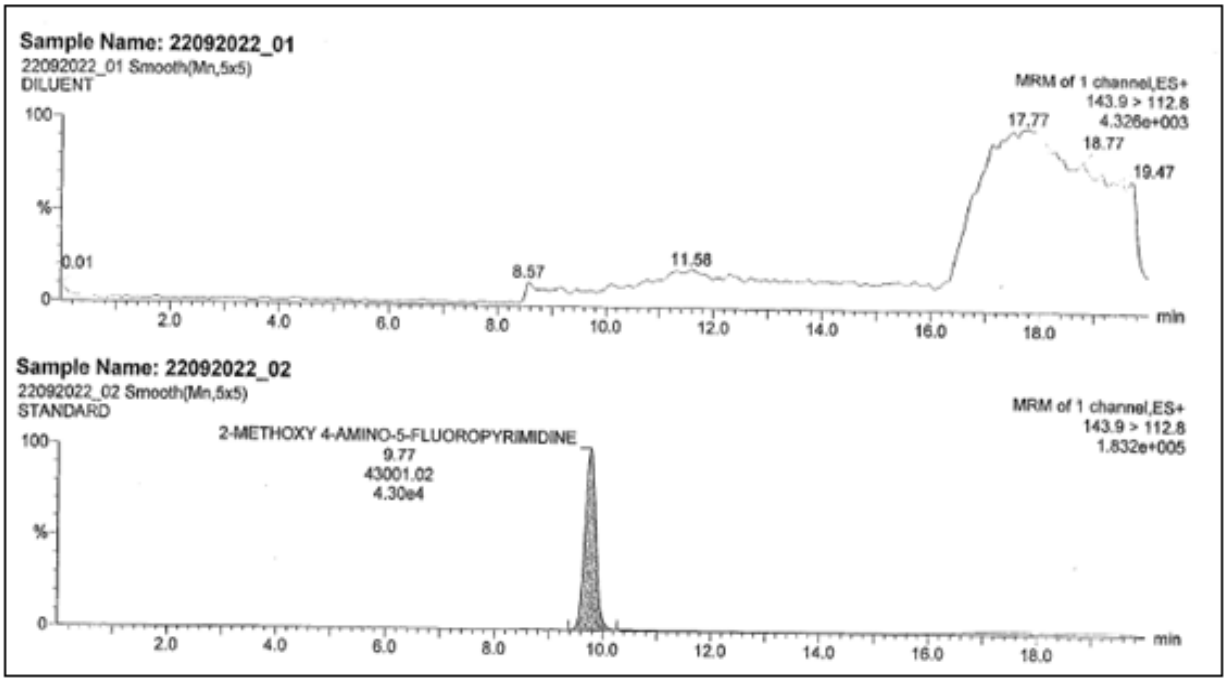

Figure 9: Blank and 2-methoxy-4-amino-5-fluoro pyrimidine LCMS chromatograms.

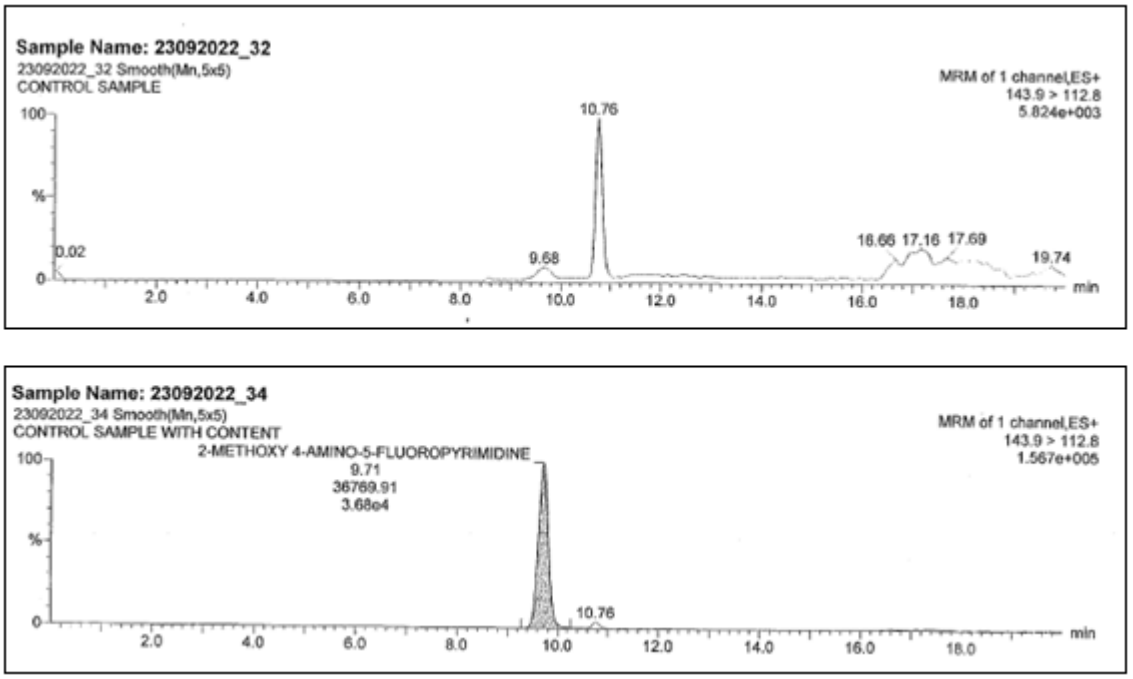

Figure 10: Control sample and spiked sample LCMS Chormatograms.

CONCLUSION

Analytical test method for determination of L-menthyl glyoxalate and 2-methoxy 4-amino-5-fluro pyramidine in emtricitabine by LC-MS/MS was validated for system suitability, identification, specificity, linearity, method precision, accuracy, range (linearity, precision and accuracy) and robustness (flow rate and source cleaning) and meets all the pre-established acceptance criteria.

REFERENCES

[1] E de Clercq. Biophys Acta. **2002**; 1587: p.258–275.
[2] JW Beach. Clin Ther. **1998**; 20: p. 2–25.
[3] JE Gallant. J Clin Virology. **2002**; 25: p.317–333.
[4] Darbyshire J. Trop Med Int Health. **2000**; 5: p. A26–A31.
[5] Clevenbergh P, Mouly S, Sellier P, et al. Curr HIV Res. **2004**; 2(4):309-321.
[6] Boffito M, Acosta E, Burger D, et al. Antivir Ther. 2005; 10(3): p.375-392.
[7] SC Piscitelli, K D Gallicano. N Engl J Med. **2001;** 344: p. 984–996
[8] Aarnoutse RE, Schapiro JM, Boucher CA, et al. Drugs. **2003**; 63: p.741-753.
[9] Saxena D, Damale S, Joshi A, et al. IJLBPR. **2014**; 3(3):196.
[10] NH Supriya, AM Ashis, C Meena. RJPT. **2012**; 5(1): p.133-137.
[11] Delahunty T, Bushman L, Robbins B, et al. J Chromatogr B Analyt Technol Biomed Life Sci. **2009**; 877(20-21): p.1907-1914.
[12] BS Rao, S Nagaraju, BV Kiran. AJRC. **2013**; 6(10): p.936-944.
[13] Nagaraju PT, Channabasavaraj KP, Shantha Kumar PT. Int J Chemtech Res. **2011**; 3(1):23-28.