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QUANTITATIVE ANALYSIS OF MEXILETINE IN HUMAN PLASMA BY UPLC MASS SPECTROMETRY

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ABSTRACT

To determine mexiletine reliability and also reproducibility. To choose proper IS & analyte. And collect literature review. Then dvelop method and to quantify the method. Mass spectrometry parameters, fragmentation pattern, and mode of ionization are the main task in mass spectrometry tuning to obtain respective fragmented ions and response for both MX and MXD6. ESI-LC-MS/MS is a very powerful technique for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. MRM technique was chosen for the assay development. The MRM parameters were optimized to maximize the response for the analyte. The pre method validation is done with six PA batches, out of which three are non-PA batches (except LLOQQC). Along with the PA batches a recovery was performed. The recovery is determined with neat standards and expressed as a percentage. These PA batches are followed with matrix effects and matrix factor. This matrix effect was determined by injecting 6 different lots of blank plasma which are infused with neat standards. Therefore, full-method validation that include 24 parameters performed for the method development for mexilitine within acceptance criteria.

Keywords: Mexiletine, Anti-arrhythmic agent, Liquid chromatography-Mass Spectrometry/ Mass Spectrometry, Liquid phase extraction (LPE)

INTRODUCTION

Mexiletine is most widely used for treating as local anesthetic, it is an orally active class I anti-arrythmic agent, it is also structurally similar to lidocaine **[1, 2]**. Chemical named is 1-methyl-2-(2,6-xylyloxy)-ethylamine **[3] (Figure 1)**. It is powder form and its colour is white, solubility in water is freely soluble and **[4]** mix in ethanol, partially **[5]** soluble with ACN (Acetonitrile) **[6]**. And also well, good method development and full method validation is done in below article. PA is done in interday and intraday with respect to ICH guidelines **[7]**. This is a cost efficient and low time consuming method for bio-analytical. The method is developed with proper documentation and standard method procedure is establishing a new method for mexiletine in development and validation. UPLC is used for evaluation of drug. And to evaluate pharmacokinetic and dynamic studies with respect guidelines **[8]**. Half-life for mexilitine drug is 10-12 hours. Route of elimination is 10% excreted by kidney at elimination.

Toxicity when dug is overdosed:

- Av heart block,
- Nausea,
- Paresthesia,
- Cardiovascular collapse and Seizures.

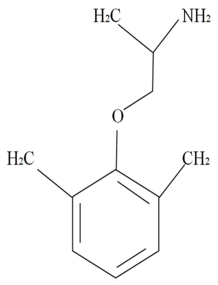


Figure 1: Mexiletine Strructure

MATERIALS AND METHODS

HPLC/LCMS grade Acetonitrile and methanol were purchased, Formic acid were purchased from Merck, Ammonium formate were purchased from qualagenic, Mexiletine Hydrochloride (MX) is an analyte and Mexiletine D6 Hydrochloride (MXIS) is used as an internal standard. Blank human plasma (K2 EDTA, anticoagulant) was obtained from clinical lab. All other chemicals and solvents were of analytical grade and water was Milli-Q grade (Novitium Pharma Limited, India).

Stock Solutions

a) MEXILETINE (MX)

Dissolve an accurately weighed quantity of Mexiletine Hydrochloride (Corrected for free base) in 10 mL volumetric flask with methanol and made up to volume with methanol to obtain an equivalent concentration of 500 µg/mL.

b) MEXILETINE D_6 (MXIS)

Dissolve an accurately weighed quantity of Mexiletine D_6

Hydrochloride (Corrected for free base) in a 20 mL volumetric flask with methanol and made up to volume with methanol to obtain an equivalent concentration of 100 µg/mL.

Further, dilute this solution with 50% acetonitrile as follows:

MXIS-MS x 0.200 mL / 20 mL = MXIS-I (Appx 1000 ng/mL)

CHROMATOGRAPHIC CONDITIONS:

Analytical column: Zorbax SB C_{18} 4.6 x 75 mm, 3.5 µm and the temperature is 40° C ± 5°C. Mobile phase: Acetonitrile and 10mM Ammonium Formate in the ratio 60:40, v/v. Flow rate is 0.500ml/min and the Injection volume is 5L.

PROCEDURE

SAMPLE PREPARATION:

Calibration Standards, QC Samples and Subject Samples (Except standard blank)

Add 50 µL of MXIS-I (Appx. 1000 ng/mL) into labelled polypropylene tubes/ria vials. Pipette 100 µL of plasma sample to respectively labelled polypropylene tubes/ ria vials and vortex briefly. Add 2.5 mL of Methyl-Tert Butyl Ether, cap and vortex for 10 min at 1500-2000 rpm. Centrifuge at 4000 rpm for 5 min, at 4°C ± 2°C temperature. Flash freeze and transfer the supernatant into labelled polypropylene tubes/ria vials. Evaporate to dryness in LV evaporator at 40°C± 1°C. Reconstitute the dried residue samples with 400 µL of reconstitution solution and vortex briefly. Transfer sample into auto sampler vials / polypropylene shell vials and cap for injection.

Calibration Standard Blank Sample

Pipette 50µL of 50% acetonitrile into labelled polypropylene tubes/ ria vials. Add 100 µL of blank plasma into each tube and vortex.

Recovery Standards (as required)

Pipette 50µL of 50% acetonitrile into labelled polypropylene tubes/ ria vials. Add 100 µL of blank plasma into each tube and vortex. Reconstitute the dried residue samples with 400 µL of appropriate neat standard solutions and vortex briefly. Transfer sample into auto sampler vials / polypropylene shell vials and cap for injection.

Matrix Factor (as Required)

Pipette 50 µL of 50% acetonitrile into labelled polypropylene tubes/ ria vials. Add 100 µL of blank plasma into each tube and vortex briefly. Reconstitute the dried residue samples with 400 µL of appropriate neat standard solutions and vortex briefly. Transfer sample into auto sampler vials and cap / polypropylene shell vials and caps for injection.

RESULTS AND DISCUSSION

Mass spectrometry fragmented ions MX and MXD6 which were shown in **Figure 2a, 2b, 3a, 3b**. ESI-LC-MS/MS.

The instrumental parameters for mass spectroscopy were optimized. The ionisation mode was Positive ion electrospray with the source temperature 150°C. The capillary voltage, cone gas flow, Desolvation gas flow, probe flow rate are 2.0 kV, 50 L/hr, 800L/hr, 500µL/min respectively. The dwell time was 0.100 seconds for both MX and MXD6. Whereas the collision energy, cone voltage is 28.0, 12.0 and 28.0, 14.0 respectively for MX and MXD6.

The collisionally associated dissociation (CAD) mass spectrum of MX shows formation of characteristic product ions at m/z 41.00, 57.90, 77.05 and 104.98. The major product ion at m/z 57.90 for MX. The CAD mass spectrum of MXD6 shows formation of characteristic product ions at m/z 46.01, 63.95, 77.05 and 105.10. The major product ion at m/z 63.95. The inherent selectivity of MS-MS detection was also expected to be beneficial in developing a selective and sensitive method.

The Calibration curve was found to be linear and the slope was found to be 0.00220336 and the intercept was 0.00196563 and the determination coefficients (r2) was 0.999393. These results indicate adequate reliability and reproducibility of this method within the analytical range of 10.00 to 1000.00 ng/mL **(Table 1)**.

Acceptance Criteria:

Mean accuracy (% Nominal) must be within ± 15.0 % of nominal value for calibration standards except LLOQ standard (i.e. STD 1) for which it must be within ± 20.0 % of nominal value.

Precision (% CV) ≤ 15.0 % for each calibration standards except LLOQ standard (i.e. STD 1) for which it must be ≤ 20.0 % **(Figure 4)**.

Acceptance Criteria:

QCs Intra-Run (Within Run):

Accuracy should be within ± 20.0 % for LLOQQC and within ± 15.0 % for other QCs of nominal value, all QCs at each concentration must be within specifications. Mean Accuracy should be within ± 20.0 % for LLOQQC and within ± 15.0 % for other QCs.

Precision ≤ 20.0 % for LLOQQC and ≤ 15.0 % for other QCs.

QCs Inter-Run (from acceptable runs only) (Between runs):

Mean accuracy must be within ± 20.0 % for LLOQQC and within ± 15.0 % of nominal value for other QCs.

Precision ≤ 20.0 % for LLOQQC and ≤ 15.0 % for other QCs.

Limit of Quantification (LOQ):

The LOQ signal-to-noise (S/N) value found for 6 injections of MX. The mean of it is 162.40which is in the acceptance criteria of mean S/N ≥ 5:1 **(Table 3)**.

Acceptance Criteria: % CV should be ≤ 15.0.

Auto sampler Carry-Over Test (ASCOT)

No significant carryover has observed when Auto sampler carryover test was analyzed.

Stability Studies

There was no significant change observed in concentration of SM in matrix, when tested for freeze thaw stability over 3 cycles, bench top stability for 25.0 hrs, processed sample stability of Mexiletine for 28.0 hrs at RT, 90.0 hrs at Auto sampler and Long term stability for 59 days. Stability of Mexiletine in Whole blood for room temperature and ice bath determines at 2.0 hrs **(Table 5)**.

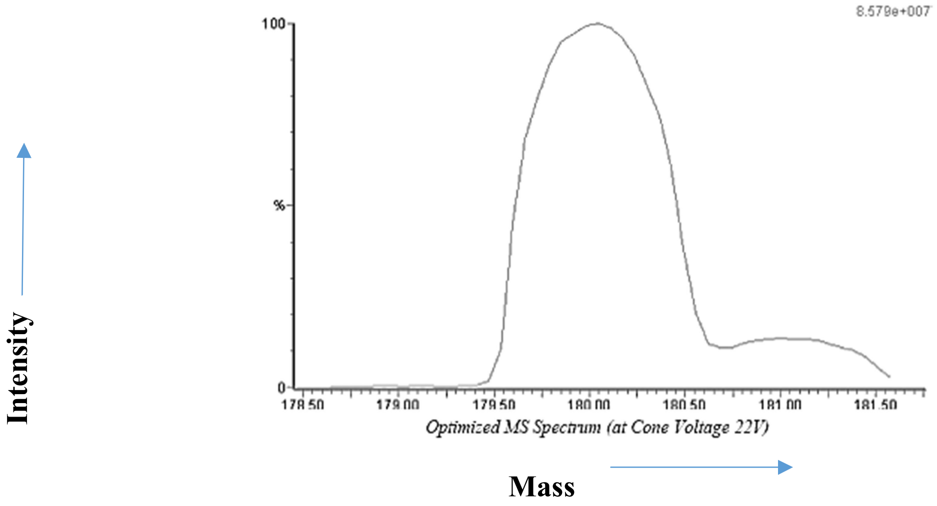


Figure 2 (a): Mass spectrum of Mexiletine

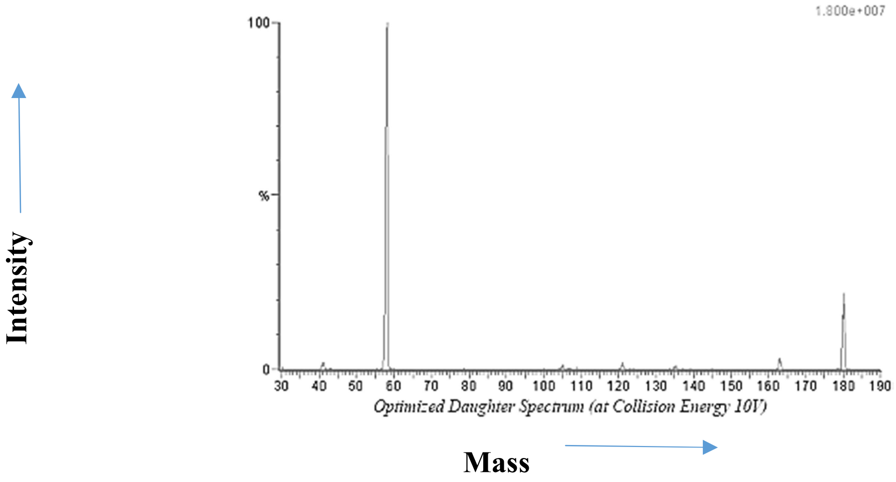


Figure 2 (b): Mass spectrum Mexiletine daughter ions

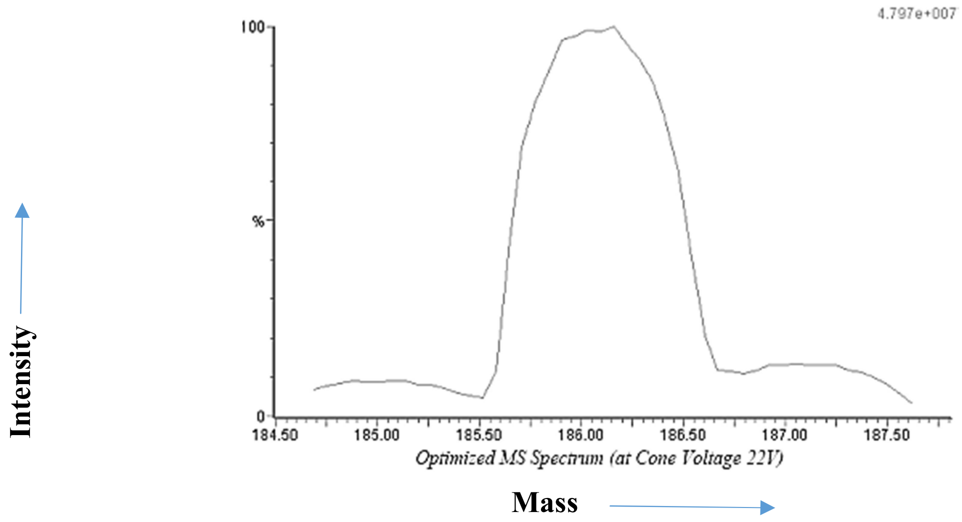


Figure 3 (a): Mass spectrum of Mexiletine D6

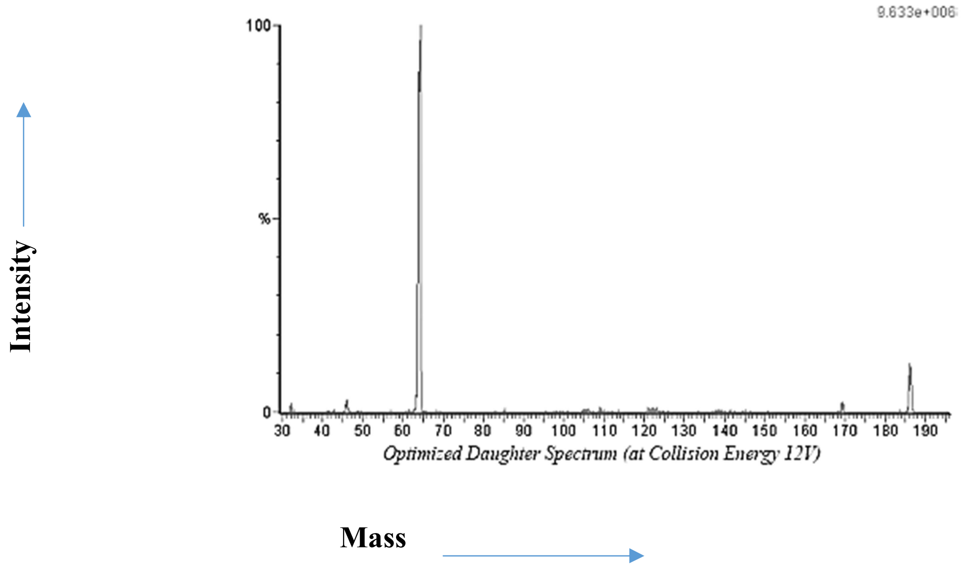
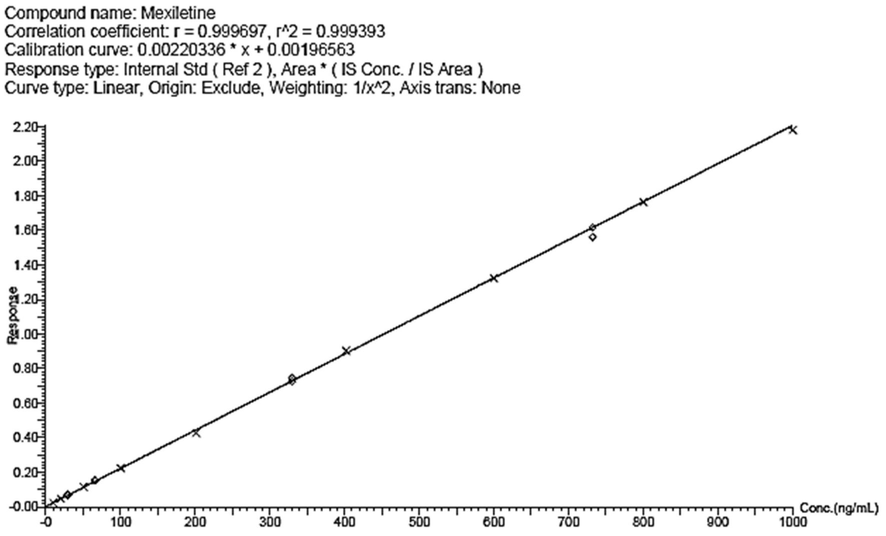


Figure 3 (b): Mass spectrum of Mexiletine D4

Table 1: Back Calculated Concentration of Calibration Curve

Concentration of CC	Mean	S.D	%CV	% Nominal	% Deviation
STD 1 10.00 (ng/mL)	9.98	0.13	1.26	99.77	-0.23
STD 2 20.00 (ng/mL)	20.47	0.28	1.39	102.33	2.33
STD 3 50.00 (ng/mL)	50.16	1.39	2.78	100.31	0.31
STD 4 100.00 (ng/mL)	100.97	1.24	1.23	100.97	0.97
STD 5 200.00 (ng/mL)	200.61	6.31	3.15	100.31	0.31
STD 6 400.00 (ng/mL)	391.71	13.47	3.44	97.93	-2.07
STD 7 600.00 (ng/mL)	596.85	17.51	2.93	99.47	-0.53
STD 8 800.00 (ng/mL)	794.61	16.32	2.05	99.33	-0.67
STD 9 1000.00 (ng/mL)	1032.08	26.13	2.53	103.21	3.21



Compound name: Mexiletine
Correlation coefficient: r = 0.999697, r^2 = 0.999393
Calibration curve: 0.00220336 * x + 0.00196563
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x^2, Axis trans: None

Figure 4: Calibration curve for Mexiletine

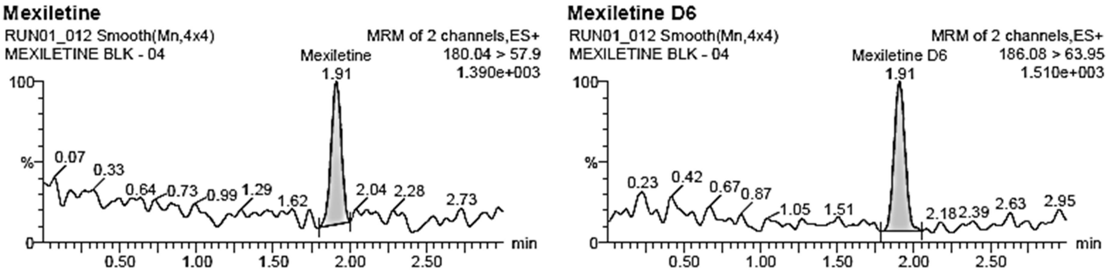


Figure 5: Representative chromatogram of blank human plasma

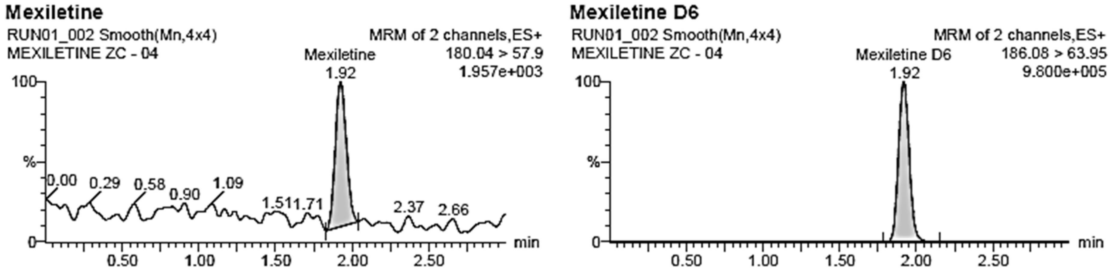


Figure 6: Representative chromatogram of blank human plasma spiked with internal standard

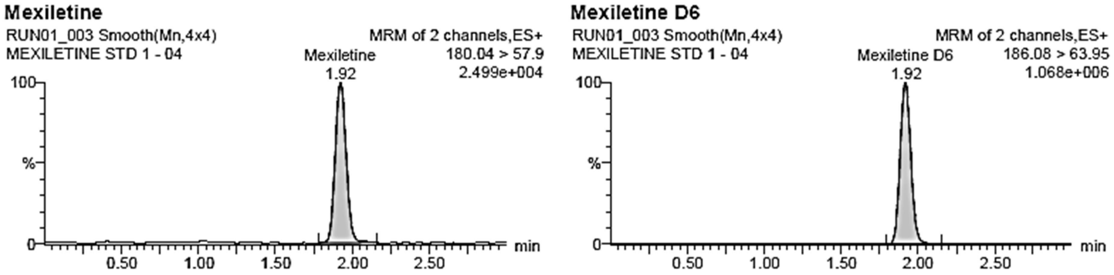


Figure 7: Representative chromatogram of LLOQ level

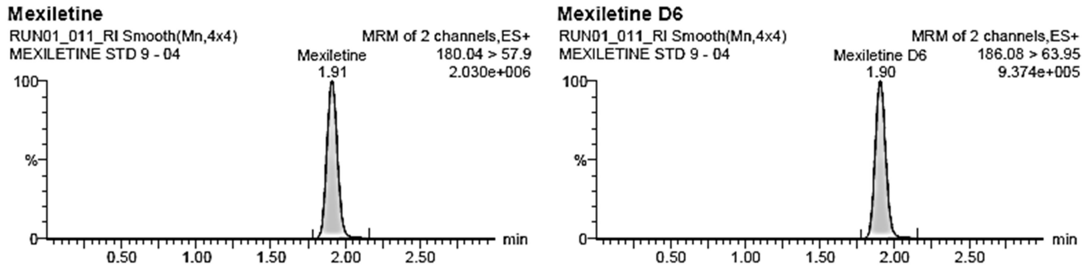


Figure 8: Representative chromatogram of ULOQ level

Table 2: Intra-and Inter- assay Precision and Accuracy of QC samples

Type of assay	Parameters	LLOQQC 10 (ng/mL)	LQC 30 (ng/mL)	MQC 330 (ng/mL)	HQC 730 (ng/mL)
Intra-assay P&A (n=6)	Intra run mean	10.33	30.54	345.31	770.04
	Intra run SD	0.16	0.69	8.04	12.54
	Intra run CV%	1.59	2.27	2.33	1.63
	Intra run % Nominal	103.28	101.80	104.64	105.48
	Intra run % deviation	3.28	1.80	4.64	5.48
Inter-assay P&A (n=18)	Inter run mean	10.49	30.13	346.90	780.13
	Inter run SD	0.35	0.75	8.60	14.91
	Inter run CV%	3.33	2.50	2.48	1.91
	Inter run % Nominal	104.89	100.42	105.12	106.87
	Inter run % deviation	4.89	0.42	5.12	6.87

Table 3: Recovery for Mexiletine

Sample level	Recovery	Mean	SD	%CV
HQC	61.09	60.58	0.84	1.39
MQC	61.05			
LQC	59.61			

Acceptance Criteria: % CV should be ≤ 15.0

Table 4: Recovery Mexiletine D6 (IS)

Sample level	Recovery	Mean	SD	%CV
HQC	64.33	64.02	0.61	0.96
MQC	64.42			
LQC	63.31			

Table 5: Stability of Mexiletine

S. No.	QC concentration	Mean	SD	%CV	%Nominal	%Deviation
Freeze/ thaw stability	FT-LOW (30.00 ng/mL)	28.94	0.61	2.10	96.47	-3.53
	FT-HIGH (730.00 ng/mL)	735.63	13.99	1.90	100.77	0.77
Bench Top Stabilit	BTS-LOW	29.15	0.61	2.08	97.17	-2.83
	BTS-HIGH	738.69	12.42	1.68	101.19	1.19
Processed sample Stability	PS-LOW-RT	28.88	0.94	3.25	96.27	-3.73
	PS-HIGH-RT	746.04	33.99	4.56	102.20	2.20
Long term Stability	LT-LOW	29.98	0.77	2.58	99.92	-0.08
	LT-HIGH	736.53	0.13	0.02	100.89	0.89
Dry extract Stability	LT-LOW	29.20	0.39	1.35	97.34	-2.66
	LT-HIGH	742.92	1.14	0.15	101.77	1.77

Acceptance criteria:

Accuracy = % Nominal for QC at each concentration should be within 85 -115 (± 15.0 %), at least 4 out of 6 individual concentrations must be within specification.

Precision= % Deviation must be ≤ 15% for each concentration tested

Ruggedness:

Ruggedness was achieved by different lot/batch in analytical column and instruments and it was found to be unaffected by variations in using different of column and different instruments.

CONCLUSION

The method validation supporting data with precision and accuracy batches, recovery, matrix effects and few stabilities are required to determine Mexiletine reliability and also reproducibility. The mean recovery and % CV of MX and MXIS are 65.15%, 64.21% and 2.84%, 1.16% respectively. These PA batches are followed with matrix effects and matrix factor. This matrix effect was determined by injecting 6 different lots of blank plasma which are infused with neat standards. There were no suppression or enhancement observed at room temperature of analyte and IS, whereas matrix factor was to analyze variability of matrix effects in each subjects. The %CV for these was found to be 2.89 % (low QC) and 1.56 % (high QC).The Low and high QC samples (n=6) were retrieved from the deep freezer; samples were processed for three freeze/thaw cycles. Parameters are run respective to FDA guidelines.

ACKNOWLEDGMENT

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