U.S. FOOD & DRUG ADMINISTRATION

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs

Background: Angiotensin II receptor blocker (ARB) drug products are commonly used to treat high blood pressure and heart failure. In July 2018, it was found that some ARB drug products contained carcinogenic nitrosamine impurities. As this incident continues to evolve, it has resulted in numerous recalls and ARB drug shortages in the US. As a member of the FDA's working group to address this continually-evolving incident, CDER/OPQ/OTR is responsible for testing for nitrosamine impurities in ARB drug products and drug substances of interest. OTR has successfully developed and implemented GC/MS methods to quantitate N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) at trace levels. However, these GC/MS methods cannot yet directly detect N-nitroso-N-methyl-4-aminobutyric acid (NMBA), another nitrosamine impurity that was found in certain ARB drug products by some firms. In addition, it is speculated that three other nitrosamine impurities may also be present in ARB drugs from reviews of manufacturing processes and published literature sources, namely N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), and N-nitrosodibutylamine (NDBA). Thus, a single method was developed that was capable of detecting and quantifying all of the six aforementioned impurities simultaneously. Herein, we report an LC-HRMS method that has been validated for the simultaneous determination of the six nitrosamine impurities in losartan drug substance and drug product at sub-ppm levels. The method may also be capable of testing for these six impurities in other ARB drug substances and drug products pending verification and/or validation.

Conclusions:

An LC-HRMS method was developed and validated following ICH Q2(R1) for the detection and quantitation of six nitrosamine impurities in losartan drug substance and drug product, including N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA). The limit of detection (LOD), limit of quantitation (LOQ) and range of the method are summarized below:

	NDMA	**NDEA**	**NEIPA**	**NDIPA**	**NDBA**	**NMBA**
LOD (ng/mL)	0.10	0.32	0.05	0.15	0.10	0.20
(ppm)	0.005	0.016	0.003	0.008	0.005	0.010
LOQ (ng/mL)	1.0	1.0	1.0	1.0	1.0	1.0
(ppm)	0.05	0.05	0.05	0.05	0.05	0.05
Range (ng/mL)	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 200
(ppm)	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 10.0

LC-HRMS Method for the Determination of Six Nitrosamine Impurities in Losartan Drug Substance or Drug Product

Purpose

This method is to quantitate the following six nitrosamine impurities in losartan drug substance or drug product: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA)and N-nitroso-N-methyl-4-aminobutyric acid (NMBA).

Principle

The six nitrosamine impurities (NDMA, NDEA, NEIPA, NDIPA, NDBA, and NMBA) are separated from each other and from losartan by reverse phase chromatography and are detected by a high-resolution and high-mass accuracy (HRAM) mass spectrometer. A high sensitivity of detection is achieved by monitoring the accurate *m/z* values of the protonated or deprotonated impurity ions or their fragments. Quantitation is performed by comparing the peak area of an impurity in extracted ion chromatograms of samples to its standard in an external calibration standard solution containing the reference standards for the six impurities.

Reagent

- Reference standards for NDMA, NDEA, NEIPA, NDIPA, NDBA, and NMBA
- Formic acid, LC/MS grade (Fisher A117-50 or equivalent)
- Methanol, LC/MS grade (Fisher A456-4 or equivalent)
- Water, LC/MS grade or equivalent
- 2-propanol, LC grade

Equipment/Instrument

- HPLC or UHPLC system equipped with temperature-controlled autosampler and column compartment
- Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer or Q Exactive™ HF-X hybrid quadrupole-orbitrap mass spectrometer (ThermoFisher Scientific)
- HPLC column: Kinetex® 2.6 µm F5 100 Å, 100 x 4.6 mm (Phenomenex, Part No. 00D-4723-E0)
- Analytical Balance
- Vortex Mixer
- 15 mL glass centrifuge tubes
- Wrist action shaker
- 0.22 µm PVDF syringe filters
- Centrifuge
- HPLC vials

Mobile phase preparation

- Mobile phase A (0.1% formic acid in water): mix formic acid and water at a volume ratio of 1:1000
- Mobile phase B (0.1% formic acid in methanol): mix formic acid and methanol at a

volume ratio of 1:1000

Note: Avoid using commercially available pre-made 0.1% formic acid in water and 0.1% formic acid in methanol which may interfere with the detection of the analytes.

Diluent and Blank: Methanol

Mixed Stock Standard preparation
Prepare a mixed stock standard solution in methanol with the following concentrations.

Nitrosamine	Conc. (ng/mL)
NDMA	100
NDEA	100
NEIPA	100
NDIPA	100
NDBA	100
NMBA	200

Standard Preparation (2.0/4.0 ng/mL)
Transfer a 1.0 mL aliquot volume of the mixed stock standard into a 50 mL volumetric flask and dilute to volume with methanol. Prepare fresh daily.

Drug substance sample preparation
Accurately weigh 100 mg of drug substance into a 15 mL glass centrifuge tube. Add 5.0 mL of methanol and mix the solution using a vortex mixer until dissolved.

Drug product sample preparation
Crush the appropriate number of tablet(s) to obtain a target concentration of 20 mg/mL of API in 5.0 mL of methanol, and transfer into a 15 mL glass centrifuge tube. Add 5.0 mL of methanol and mix for about a minute using a vortex mixer. Shake the sample for 40 minutes using a mechanical wrist action shaker.

After extraction, centrifuge the sample for 15 minutes at 4500 rpm. Filter the supernate using a 0.22 µm PVDF syringe filter, discard the first 1 mL and transfer the filtered sample into an hplc vial for LC/MS analysis.

Chromatographic Conditions

HPLC Column	Kinetex® 2.6 µm F5 100 Å, 100 x 4.6 mm (Phenomenex, Part No. 00D-4723-E0)		
Column Temp.	40 °C		
Flow Rate	0.6 mL/min		
Mobile Phase A	0.1% formic acid in water		
Mobile Phase B	0.1% formic acid in methanol		
Gradient	Time (min)	A%	B%
	0	90	10
	1.5	90	10
	7.0	45	55
	17.0	45	55
	17.1	10	90
	21.0	10	90
	21.1	90	10
	25.0	90	10
Injection Volume	3 µL		
Autosampler Temp.	4 - 8 °C		
Needle Wash	80:20, Methanol:Water with 0.1% Formic Acid		

Mass spectrometer conditions

- Instrument
  Q Exactive™ mass spectrometer (ThermoFisher) or Q Exactive™ HF-X mass spectrometer (ThermoFisher)

- Ion Source Settings (apply to both negative and positive modes)

  Note: Ion source parameters can be adjusted to achieve the desired sensitivity.

Sheath Gas Flow Rate	55 arbitrary units
Aux Gas Flow Rate	15 arbitrary units
Sweep Gas Flow Rate	0 units
Spray Voltage	3.5 kV
Capillary Temp.	400 °C
S-Lens	55 (applied to Q Exactive™)
Funnel RF Level	25 (applied to Q Exactive™ HF-X)
Aux Gas Heater Temp.	350 °C

- Scan Settings
  Note: 1) The scan start-end time should be adjusted for the user's HPLC system since the

retention times of the impurities may vary between different HPLC systems, 2) The divert valve can be used to divert the eluent to waste when a scan is not performed.

Impurity	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
Scan Type	PRM	SIM	PRM	PRM	SIM	PRM
Polarity	Positive	Negative	Positive	Positive	Positive	Positive
Scan Start -End (min)	0 - 3.5	3.5 - 5.5	5.5 - 7.3	7.3 - 8.5	8.5 - 9.5	14 - 16
m/z Isolated for PRM	75.0553	N/A	103.0866	117.1022	N/A	159.1492
NCE	80	N/A	30	10	N/A	30
Isolation Window	1.5 m/z	N/A	1.5 m/z	1.5 m/z	N/A	1.5 m/z
Scan Range	N/A	m/z 144.3 - 145.8	N/A	N/A	m/z 130.4 - 131.9	N/A
Microscans	3	3	3	3	3	3
Resolution	35,000 or 45,000	70,000 or 60,000	35,000 or 45,000	35,000 or 45,000	70,000 or 60,000	35,000 or 45,000
AGC target	2e5	1e6	2e5	2e5	1e6	2e5
Maximum IT	100 ms	100 ms	100 ms	100 ms	100 ms	100 ms

Injection Order

- Inject Blank (use diluent) at least once at the beginning of a sequence
- Inject Standard solution for six consecutive times before the injection of the first sample
- Inject Standard solution once every six injections of samples and at the end of a sequence.
- Example:

Order	Solution	No. of Injections
1	Blank	2
2	Standard	6
3	Blank	1
4	Sample 1	1
5	Sample 2	1
6	Sample 3	1
7	Sample 4	1
8	Sample 5	1
9	Sample 6	1
10	Standard	1
…	…	…

System Suitability

- The area of an interference peak for NDBA in the blank injection, if present, should be no more than 5% of the peak area of NDBA in the standard solution.

- The % RSD of the peak area for each nitrosamine impurity for the first six injections of standard solution should be no more than 10%.
- The cumulative % RSD of the peak area for each nitrosamine impurity should be no more than 15%. (cumulative % RSD of the peak area is calculated by combining the initial six replicate injections of the standard solution and each subsequent bracketing standard)

Data Processing

- Peak areas in the extracted ion chromatograms (EIC) with a *m/z* tolerance of 15 ppm are used for quantitation. The *m/z* values to be extracted are listed below:

Impurity	**NDMA**	**NMBA**	**NDEA**	**NEIPA**	**NDIPA**	**NDBA**
m/z to be extracted	75.0553	145.0619	75.0553, 103.0866	75.0553	131.1179	57.0704, 103.0872, 159.1492

- NMBA and NEIPA exist as *syn* and *anti* conformers due to the restricted rotation of N-N bond (reference 1, 2), and these conformers can be partially separated by the method's chromatographic conditions.
    - The NMBA peak is observed as a doublet at a ratio of approximately 3:1. Integrate both peaks and use the combined peak area for NMBA.
    - Depending on the column and the concentration of the sample, the NEIPA peak may appear as doublet or a single peak with a tailing shoulder. Include the resolved second peak or the tailing of the main peak when integrating the NEIPA peak(s).
- The retention time difference of any impurity in the analyzed samples should not be more than 2% of the retention time of the corresponding standard in the standard solution.

Calculation

Drug Substance:

Nitrosamine impurity (ppm) = $\frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times 10^6\ ng} \times \frac{V}{W} \times 10^6$

Where: Nitrosamine impurity refers to NDMA, NDEA, NEIPA, NDIPA, NDBA or NMBA
A_{spl} = Area of the nitrosamine impurity peak in the sample solution
As = Average area (n = 6) of the nitrosamine impurity peak from the first six consecutive injections of the standard solution
C_s = Concentration of the nitrosamine impurity in the standard solution (ng/mL)
W = Weight of drug substance (mg)
V = Volume of the diluent in the sample solution (mL)

Drug Product:

Nitrosamine impurity (ppm) = $\frac{A_{spl}}{As} \times C_s \times \frac{1\ mg}{1\times 10^6\ ng} \times \frac{1}{20\ mg/mL} \times 10^6$

Where: Nitrosamine impurity refers to NDMA, NDEA, NEIPA, NDIPA, NDBA or NMBA
A_{spl} = Area of the nitrosamine impurity peak in the sample solution
As = Average area (n = 6) of the nitrosamine impurity peak from the first six consecutive injections of the standard solution
C_s = Concentration of the nitrosamine impurity in the standard solution (ng/mL)

Report

- Report the nitrosamine impurity content in ppm with three significant figures if the value is ≥ LOD
- Report 'not detected' if no nitrosamine impurity is detected or the value is < LOD

References

1. U.P. Senthilkumar and R. Jeyaraman, J. Org. Chem., 1992, 57 (22), 6006-6014
2. C.F. Cheng and C.W. Tsang, J. Chromatogr. A., 1999, 849, 389 - 402

Example Chromatograms

Standard

Extracted ion chromatograms (EIC) of each nitrosamine impurity at a mass accuracy of 15 ppm

Combined

NDMA (EIC m/z 75.0553)

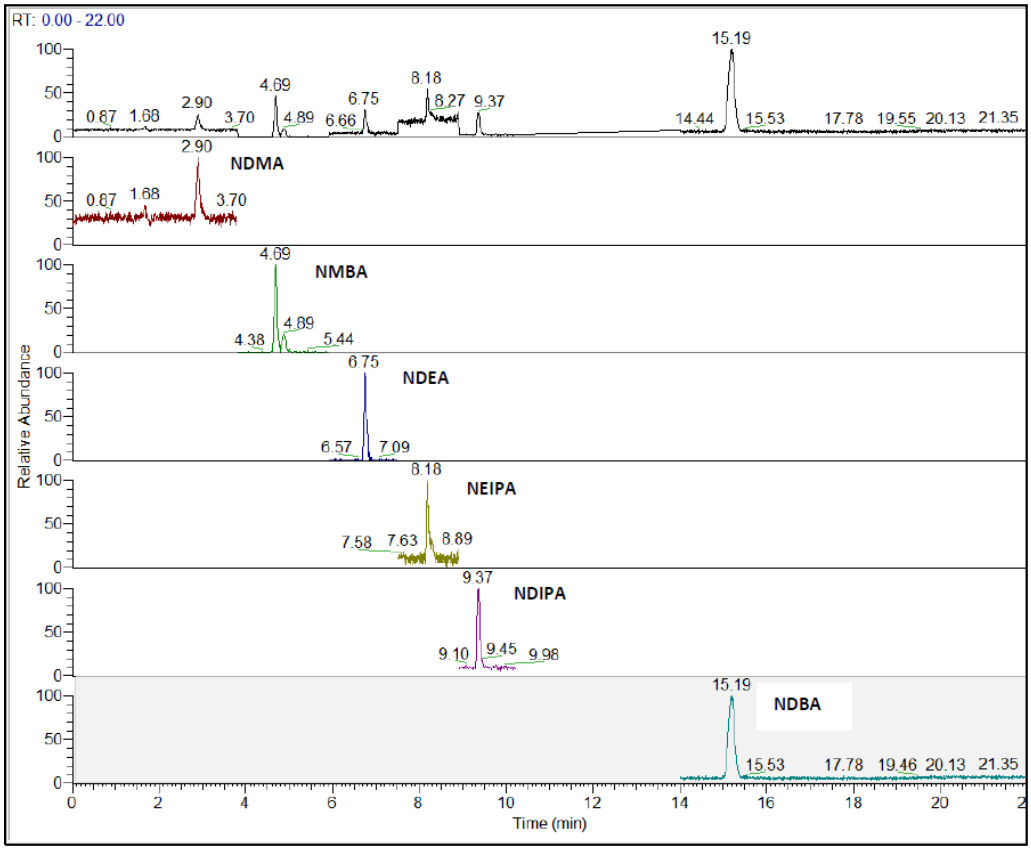
NMBA (EIC m/z 145.0619)

NDEA (EIC of m/z 75.0553, 103.0866)

NEIPA (EIC m/z 75.0553)

NDIPA (EIC m/z 131.1179)

NDBA (EIC m/z 57.0704, 103.0872, 159.1492)



Methanol Blank

Extracted ion chromatograms of each nitrosamine impurity at a mass accuracy of 15 ppm

Combined

NDMA (EIC m/z 75.0553)

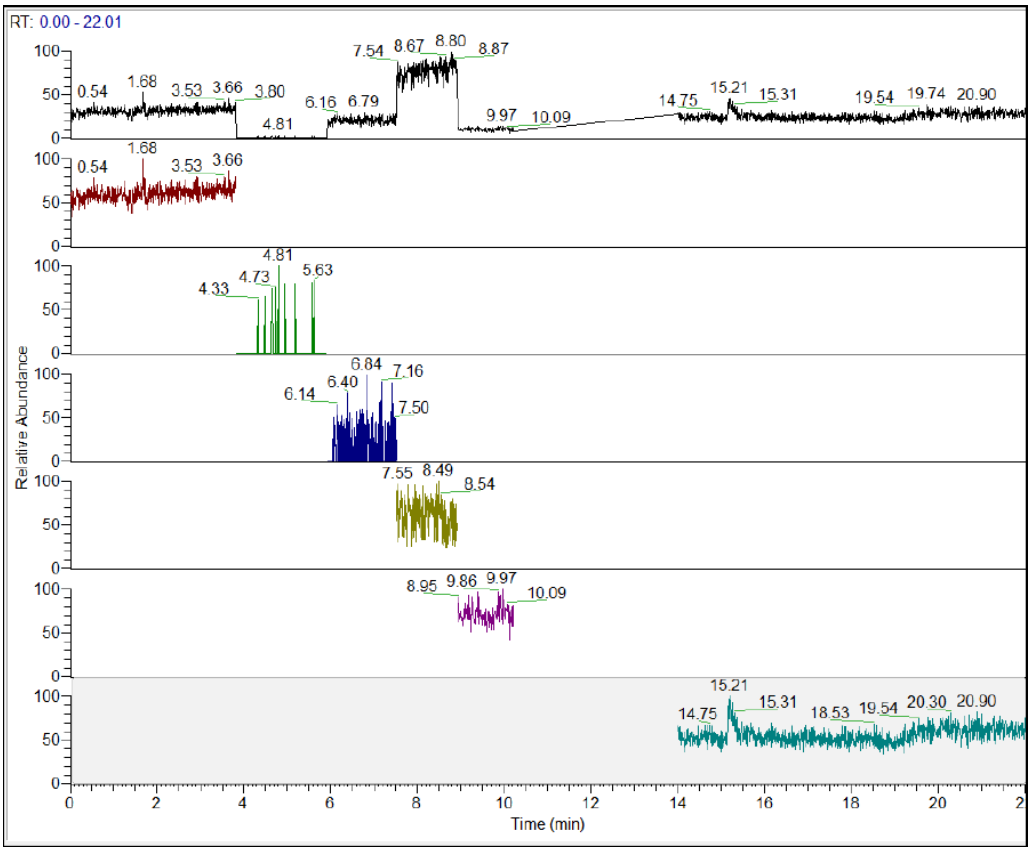
NMBA (EIC m/z 145.0619)

NDEA (EIC of m/z 75.0553, 103.0866)

NEIPA (EIC m/z 75.0553)

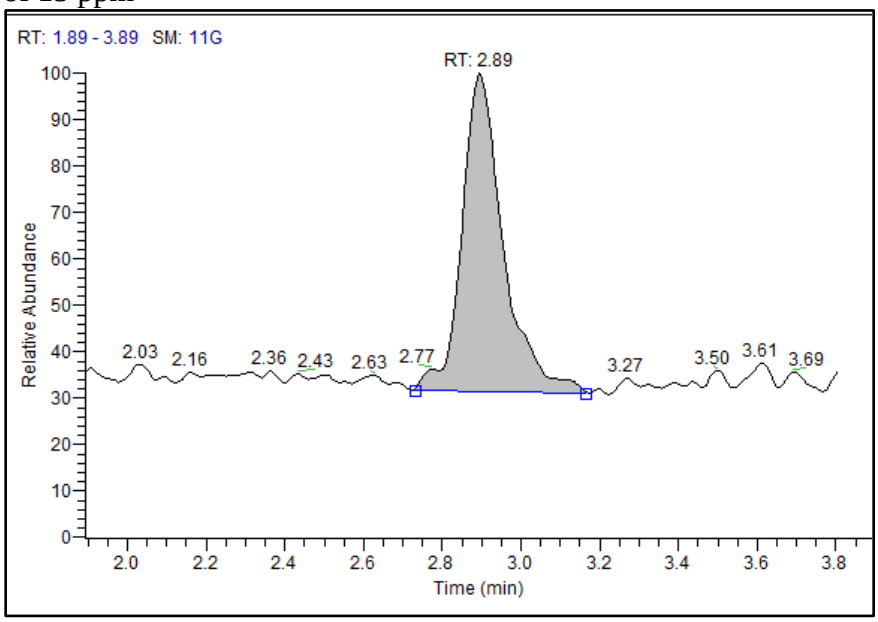
NDIPA (EIC m/z 131.1179)

NDBA (EIC m/z 57.0704, 103.0872, 159.1492)



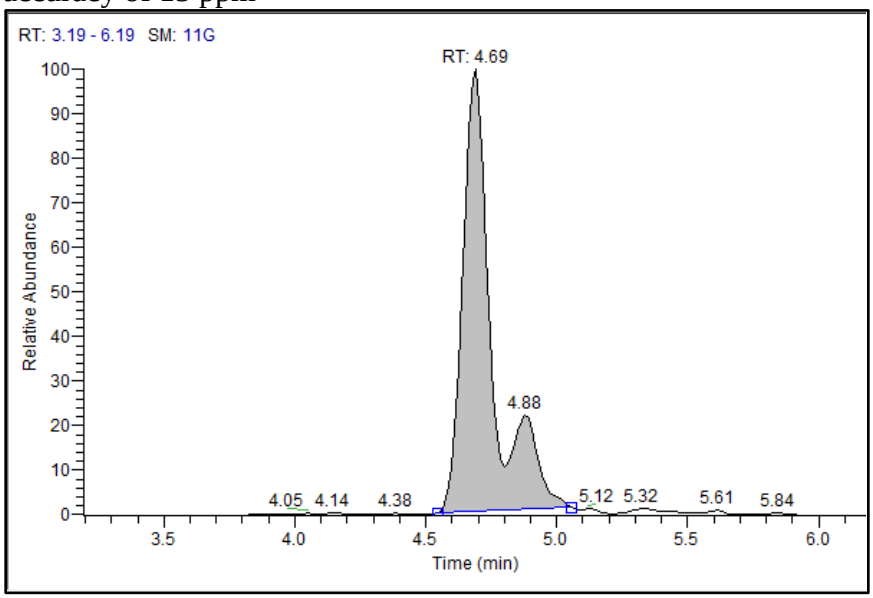
NDMA (2.0 ng/mL Standard)

Extracted ion chromatogram of m/z 75.0553 from PRM scan of m/z 75.0553 at a mass accuracy of 15 ppm



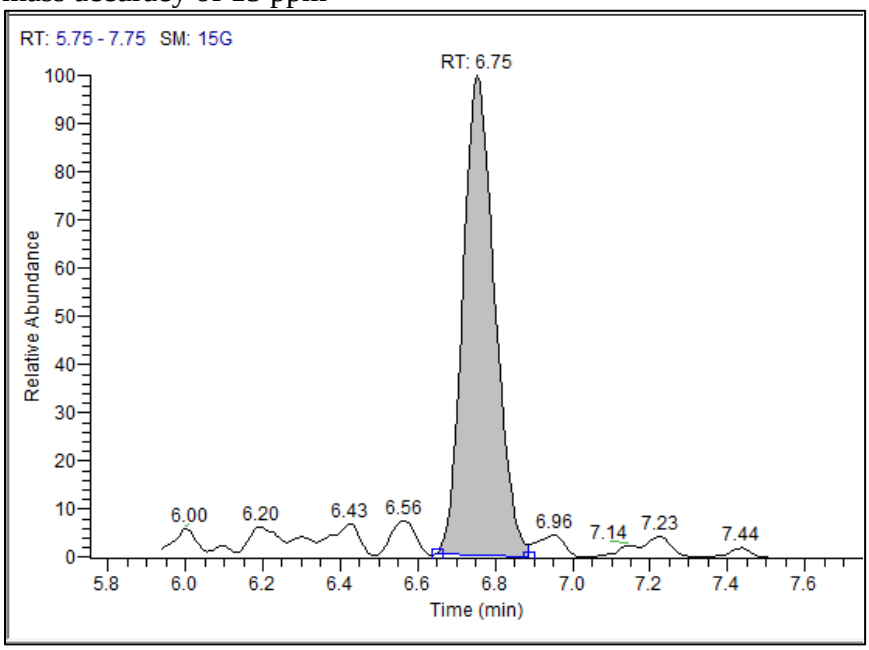
NMBA (4.0 ng/mL Standard)

Extracted ion chromatogram of m/z 145.0619 from SIM scan of m/z 144.3 to 145.8 at a mass accuracy of 15 ppm



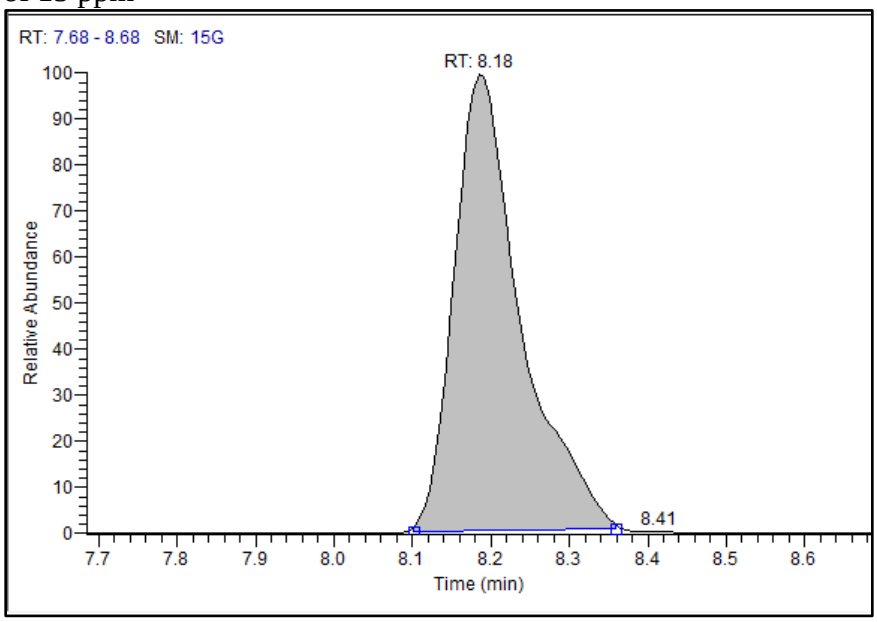
NDEA (2.0 ng/mL Standard)

Extracted ion chromatogram of m/z 75.0553 and 103.0866 from PRM scan of m/z 103.0866 at a mass accuracy of 15 ppm



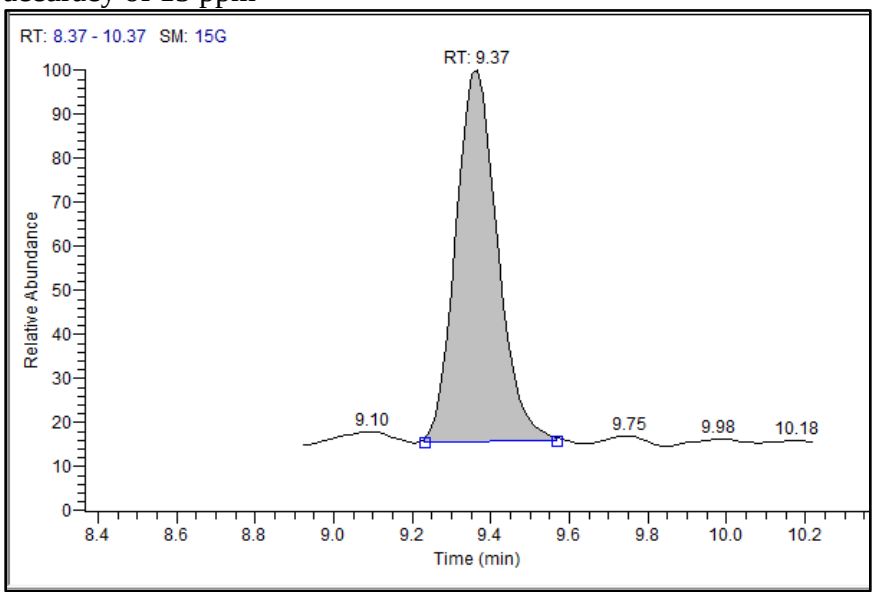
NEIPA (2.0 ng/mL Standard)

Extracted ion chromatogram of m/z 75.0553 from PRM scan of m/z 117.1022 at a mass accuracy of 15 ppm



NDIPA (2.0 ng/mL Standard)

Extracted ion chromatogram of m/z 131.1179 from SIM scan of m/z 130.4 - 131.9 at a mass accuracy of 15 ppm



NDBA (2.0 ng/mL Standard)

Extracted ion chromatogram of m/z 57.0704, 103.0872 and 159.1492 from PRM scan of m/z 159.1492 at a mass accuracy of 15 ppm