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The impact of Matrix effect in extraction efficiency of N-nitroso-N-methyl-4-aminobutyric acid in Metformin Extended-Release tablets using ESI-LC/HRMS

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Introduction

A sample preparation protocol with great extraction efficiency is extremely important in achieving better analyte detection using liquid chromatography mass spectrometry instruments. The sample extraction method can contribute immensely to the matrix effect and may result in poor analyte extraction efficiency or recovery.

This study explores the effect of pH on the extraction efficiency and recovery of N-nitroso-4-methyl-4-aminobutyric acid (NMBA) contamination in extended release Metformin tablets. NMBA is a nitrosamine impurity found to be carcinogenic and its presence in metformin drug product is under regulatory scrutiny due to various recent recalls.

The liquid chromatography mass spectrometry-based method described in this poster was performed with the Agilent 6546 Quadrupole/ Time of Flight LC/MS (LC/Q-TOF) mass spectrometer.

Instrumentation

1290 Infinity II high-speed pump (G7120A)
1290 Infinity II multisampler (G7167B)
1290 Infinity II multicolumn thermostat (G7116B)
1290 Infinity II Diode Array detector (G7117B)
6546 LC/QTOF (G6546A)

Table 1: Instrumentation detail

Figure 1: 6546 LC/Q-TOF

Experimental

Sample Preparation

The sample preparation procedure was optimized using the following steps.

1.Weigh 280mg (± 2.8mg) equivalent to 250mg Metformin Extended-release crushed tablets.

2. Add 5 mL extraction solvent to make 50 mg/mL and vortex for 2minute.

3.Now put the sample in shaker at 400rpm for 40 minutes.

4. Centrifuge the sample at 8000 rpm for 10 minutes at 15-degree C temperature.

5. Filter the supernatant using 0.2µm PVDF syringe filter into an LCMS vial.

LC Conditions	
Needle wash	Methanol: Water/ 80:20
Sample diluent	Water: methanol (95:5), 0.25 % NH_3
(v/v)Extraction Solvent	Water: methanol (95:5) with 0, 0.25 and 0.5 % NH_4OH
Multisampler temperature	10 °C
Injection volume	40 µL
Analytical column	Agilent InfinityLab Poroshell HPH-C18, 4.6 × 150 mm, 2.7 µm (p/n 693975-702(T))
Column temperature	40 °C
Mobile phase A	0.1 % formic acid in water
Mobile phase B	0.1 % formic acid in Methanol
Flow rate	0.4 mL/min
Gradient	Time (min) %A %B 0.0 95 5 3.0 95 5 12.0 50 50 12.01 10 90 15.0 10 90 15.01 95 5 18.0 95 5
Runtime	18 minutes
UV Wavelength	230 nm

Table 2: LC conditions

Method Optimization

The 6546 LC/Q-TOF was used for detecting the mass conditions for NMBA in Negative Ionization mode where the *m/z* 145.0619 (M-H) ion was used for quantitation. The method was optimized using the Agilent Jet Stream Electrospray ionization (AJS-ESI) source as NMBA gives a better response with this source.

MS Experimental Conditions

Experiment Type	Acquisition Start *m/z*	Acquisition End *m/z*
MS Acquisition	100	200

MS Source Conditions	
Gas Temperature	200 ^{0}C
Gas Flow	6 L/min
Capillary Voltage	4000V
Nebulizer Pressure	55 psi
Sheath Gas Temperature	300 ^{0}C
Sheath gas Flow	12 L/min
Nozzle Voltage	0V
Fragmentor	80V
Skimmer	40V
Oct 1 RF Vpp	300V

Note1: The 6546 LC/QTOF is tuned before starting the project using TOF-Transmission Tune in Negative Ionization Mode. The source parameters are optimized to have best possible response for NMBA.

The chromatographic separation of Metformin drug and NMBA impurity was best achieved using Infinitylab Poroshell HPH-C18 column and diverter valve was programmed such that Metformin peak was diverted to waste and monitored using UV detector.

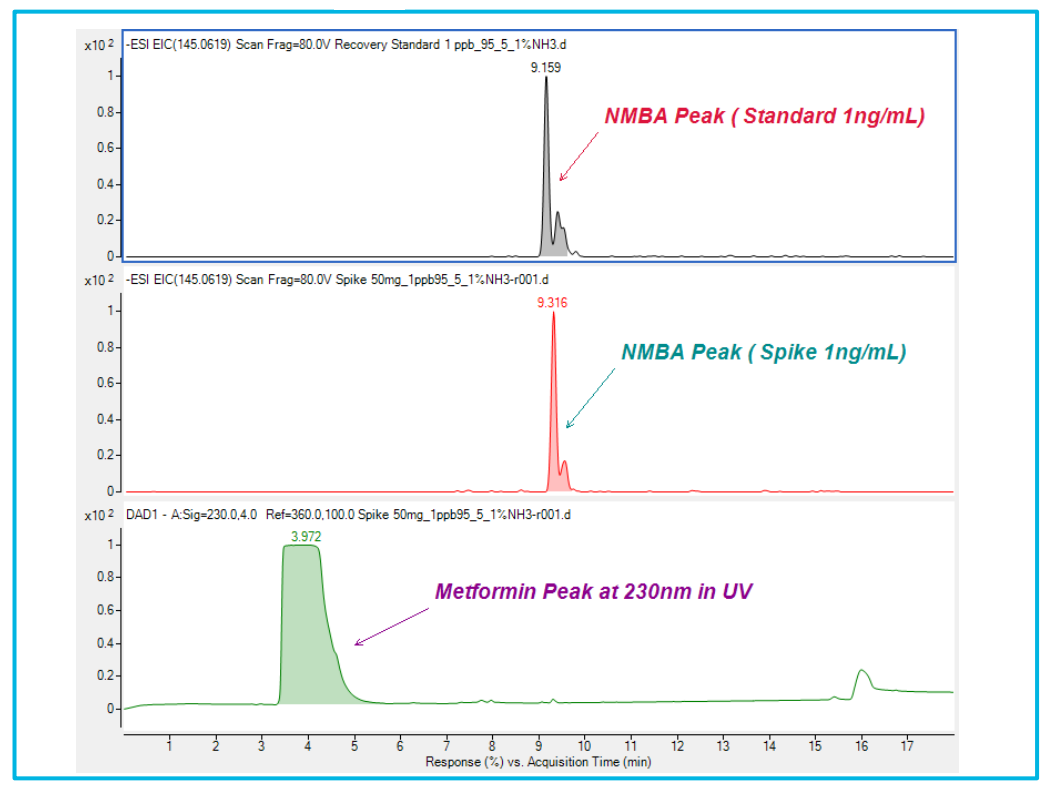

Figure 2: Representative EIC of NMBA at 1 ng/mL conc. Standard and Spike using 50mg/mL of Metformin-ER Drug.

The metformin peak was monitored in the UV detector at 230 nm wavelength and a diverter valve program prevented the high concentration of Metformin from entering the mass spectrometer. The chromatographic separation between the Metformin peak and the NMBA impurity was sufficient to elimination the suppression of ionization of NMBA due to the elution of Metformin peak.

Note2: NMBA is known to exist in the syn and anti conformers due to the restricted rotation of N-N bond and these conformers can be partially separated by chromatographic method. Generally, NMBA appears as a doublet or split peak.

Results and Discussion

Method Performance Characterization

Figure 3 shows the extracted ion chromatograms of NMBA at various concentrations like 0.02, 0.05, 0.1,0.25 and 0.5ng/mL to give some idea about the sensitivity of the method at such low concentrations. In addition, the calibration curve is shown with $r^2 > 0.997$. This shows that method can meet the regulatory requirements with respect to sensitivity for quantitation of NMBA in Metformin-ER tablets.

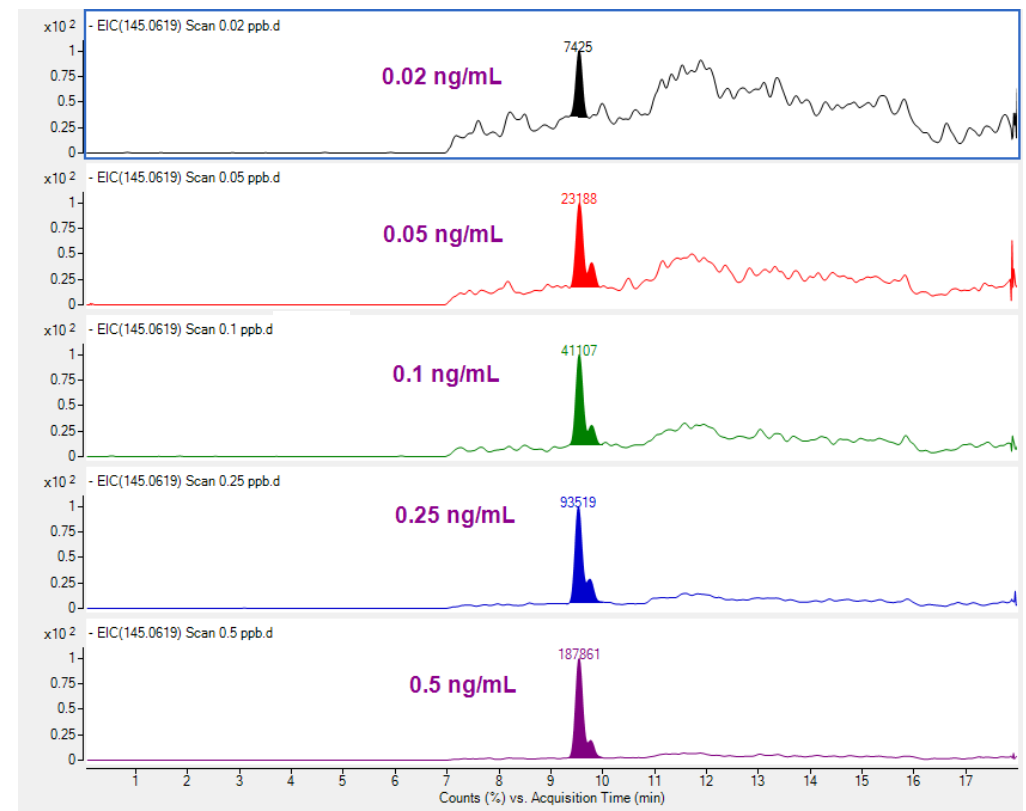

Figure 3: Sensitivity and linearity data for NMBA and the calibration curve with an $r^2 > 0.997$.

Recovery Study and Matrix Effect

The recovery experiment shows excellent recovery of ± 20 % of the spiked concentrations using 0.25 % NH_3.

Spike NMBA Conc. (ppb)	Recovery %		
	No NH_3	0.25 % NH_3	0.5 % NH_3
0.5	58	91	21
1	54	89	62
3	57	95	65

Table 5: The impact of Matrix in Recovery

Conclusions

- The method demonstrates the criticality of extraction solvent pH in achieving the best recovery of NMBA from Metformin-ER tablets using scan mode of High-resolution Mass spectrometer quantitation workflow.
- The method is highly sensitive in terms of achieving the regulatory defined limits for detection of NMBA in Metformin drug.
- As there is nice chromatographic separation between NMBA and High concentration of Metformin which allows the Metformin peak to be easily diverted to waste resulting in a more rugged method.

References

1. FDA guidance document: Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs.
2. FDA guidance document: Liquid Chromatography Electrospray Ionization high resolution mass spectrometry method for determination of nitrosamine impurities in Metformin drug substance and drug products.
3. Simultaneous determination of eight nitrosamine impurities in Metformin Extended-Release tablets using the Agilent 6470 Triple Quadrupole LC/MS. 5994-2533 EN,2020

https://explore.agilent.com/asms

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