Waters™

Application Note

Determination of Nitrosamine Impurities in Losartan Potassium Drug Substance and Drug Product using the Xevo TQ-S micro and Atlantis Premier BEH C_{18} AX Column

Jun Xiang Lee, Zhen Han Mong, Wenlin Zhang

Waters Corporation

Abstract

This application note demonstrates a sensitive and robust UPLC-MS/MS quantification method for three nitrosamine impurities (NDMA, NDEA, NMBA) in Losartan Potassium drug substance and drug product using Xevo TQ-S micro Triple Quadrupole Mass Spectrometry with APCI. The developed instrument method exhibited excellent linearity from 0.5–100 ng/mL for NDMA and NDEA and 1–100 ng/mL for NMBA with an R^2 >0.997. The limit of quantitation (LOQ) of 0.5 ng/mL (0.005 ppm) was achieved for all compounds, which complies with the US FDA's nitrosamine impurities acceptable limits. Mean extraction efficiency was over 70% for all impurities in Losartan Potassium DS and DP. The results in this study also demonstrated that the Atlantis Premier BEH C_{18} AX Column can overcome poor retentivity issues for small polar compounds, which makes it ideal for analysis of polar nitrosamines such as NDMA.

Benefits

- A robust, repeatable, highly selective, and sensitive method was developed to accurately quantify 1 ng/mL NDMA, NDEA, and NMBA in Losartan Potassium drug substance and drug product (0.001 ppm) using the ACQUITY UPLC I-Class coupled with the Xevo TQ-S micro Triple Quadrupole Mass Spectrometry

- The developed method was able to achieve an LOQ of 0.5 ng/mL for NDMA, NDEA, and NMBA, which is below the most recent acceptable limits defined by the United States Food and Drug Administration (FDA) in February 2021[1]
- Superior retention capability for small polar compounds such as NDMA was demonstrated by the Atlantis Premier BEH C_{18} AX Column
- ARB drug substances and drug products were tested for the presence of nitrosamine impurities, and pre-spiked tests determined the extraction efficiency to be over 70% for all 3 nitrosamine impurities

Introduction

Since early 2018, the US FDA has reported on several pharmaceutical drug products used to treat high blood pressure and heart attack that contain unacceptable thresholds of nitrosamine impurities. The consumption of nitrosamine impurities above the acceptable threshold over a long period of time may increase the risk of cancer. This phenomenon led to a withdrawal of several drug products worldwide, causing a shortage of such drugs for a period.

The possible sources of nitrosamine impurities could be from the active pharmaceutical ingredient (API), also known as drug substance, or solvents that were used in the process of synthesizing the drug product. In addition, inappropriate storage conditions could also increase the concentration of nitrosamine impurities over time to an unacceptable threshold.

However, with a good control of the concentration of nitrosamine impurities at-or-below the acceptable threshold, it is not expected that there will be an increased risk of cancer. Hence, it is important for manufacturers to test for the presence of nitrosamine impurities at different stages of drug synthesis. Based on the latest FDA regulations, manufacturers of drug substances and drug products should use an instrument method with LOQs at or below 0.03 ppm to accurately quantitate nitrosamine impurities at not more than an acceptable intake of 26.5 ng/day.[1]

In this application note, a robust and sensitive method meeting the FDA's guidance was developed using the ACQUITY UPLC I-Class coupled with a Xevo TQ-S micro Triple Quadrupole Mass Spectrometry for analysis of three nitrosamine impurities: N-Nitroso-dimethylamine (NDMA), N-Nitroso-diethylamine (NDEA), and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA). Like other studies on nitrosamines impurities,[2] we observed better sensitivity with atmospheric pressure chemical ionization (APCI) as compared to electrospray ionization (ESI), as

such APCI was used in the experiment. Linearity, limit of quantification (LOQ), accuracy, precision, and extraction efficiency are reported here. The Atlantis Premier BEH C_{18} AX Column featured in this study shows excellence performance in retaining highly polar and small molecules such as NDMA.

Experimental

Working Standard Preparation

A standard stock mixture (NDMA, NDEA, and NMBA) and an internal standard stock mixture (NDMA-d6, NDEA-d4, and NMBA-d3) were prepared in methanol at 50 µg/mL and 0.5 µg/mL, respectively.

A calibration series of 0.5, 1, 2, 5, 10, 20, and 100 ng/mL of mix standards spiked with 5 ng/mL internal standards were prepared in methanol:water (5:95).

Drug Substance Sample Preparation

100 mg of Losartan Potassium API was accurately weighed into a centrifuge tube and dissolved in 10 mL of methanol:water (5:95). 50 ng of the internal standard was added into the mixture to achieve a concentration of 5 ng/mL spiked internal standard. Subsequently, the sample was vortexed, sonicated and centrifuged for 15 minutes at 9,000 rpm. The supernatant was collected and filtered through a 0.22 µm nylon filter before injecting into the LC-MS analysis.

Drug Product Sample Preparation

Losartan drug tablet (100 mg) was crushed into a fine powder. Subsequently, the sample preparation from dissolution to sample analysis was the same as that for drug substance sample preparation.

Extraction Efficiency Sample Preparation

2.5 ng/mL of nitrosamine impurities (NDMA, NDEA, and NMBA) were pre-spiked into Losartan Potassium DS and DP, before performing the above DS and DP sample preparation. The supernatant was collected and filtered through a 0.22 µm nylon filter before injecting into the LC-MS analysis.

Chromatographic Conditions

LC system:	ACQUITY UPLC I-Class
Column(s):	Atlantis Premier BEH C_{18} AX, 1.7 2.1 x 100 mm (P/N: 186009368)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.45 mL/min
Mobile phase A:	5 mM Ammonium formate with (formic acid in water
Mobile phase B:	5 mM Ammonium formate with (formic acid in methanol

Gradient

Time (min)	%A	%B	Curve
Initial	95	5	Initial
0.5	95	5	6
3.5	25	75	6
4.0	5	95	6
6.0	5	95	6
8.5	95	5	6

Mass Spectrometer Conditions

MS system:	Xevo TQ-S micro
Ionization mode:	APCI +

APCI probe temperature:	300 °C
Corona current:	2 µA (system/pin specific)
Cone gas flow (L/Hr)	100
Desolvation gas flow (L/Hr):	1200
Data management	Chromatographic software: MassLynx v4.2 Quantitation software: TargetLynx

MRM Transition

Data was collected using the MRM transition mode, which contains two transitions for each compound and the associated internal standard. The associated internal standard for NDMA, NDEA, and NMBA is NDMA-d6, NDEA-d4, and NMBA-d3, respectively. Transitions marked with * are the quantifiers in Table 1.

Compound name	Cone voltage (V)	MRM transition	Collision energy (V)	Internal standard	Cone voltage (V)	MRM transition	Collision energy (V)
NDMA	25	75.00>58.00*	10	NDMA-d6	25	81.00>64.00*	10
	60	75.00>43.00	12		60	81.00>46.00	12
NDEA	16	103.00>75.00*	10	NDEA-d4	16	107.00>77.00*	10
	16	103.00>47.00	16		16	107.00>47.00	16
NMBA	20	147.00>117.00*	7	NMBA-d3	20	150.00>47.00*	13
	20	147.00>44.00	13		20	150.00>87.00	13

Table 1. Transitions of NDMA, NDEA, and NMBA.

Results and Discussion

Retention Capability of the Atlantis Premier BEH C18 AX Column

NDMA is a common nitrosamine that poses some retention issues due to its polar nature. Hence, without the

proper column selection with suitable stationary phase, NDMA might be eluted out along with the polar matrix interference, which will reduce sensitivity and accuracy.

In this study, two different columns of similar dimension, the ACQUITY UPLC CSH Fluorophenyl Column and the Atlantis Premier BEH C_{18} AX Column, were evaluated for the retention of NDMA. From Figure 1, it can be seen that the Atlantis Premier BEH C_{18} AX Column is able to retain the most polar nitrosamine, NDMA, better as compared to CSH Fluorophenyl Column. This allows the polar matrix interferences to be better separated from the analytes, which eventually leads to improved sensitivity and accuracy as reported in this analysis.

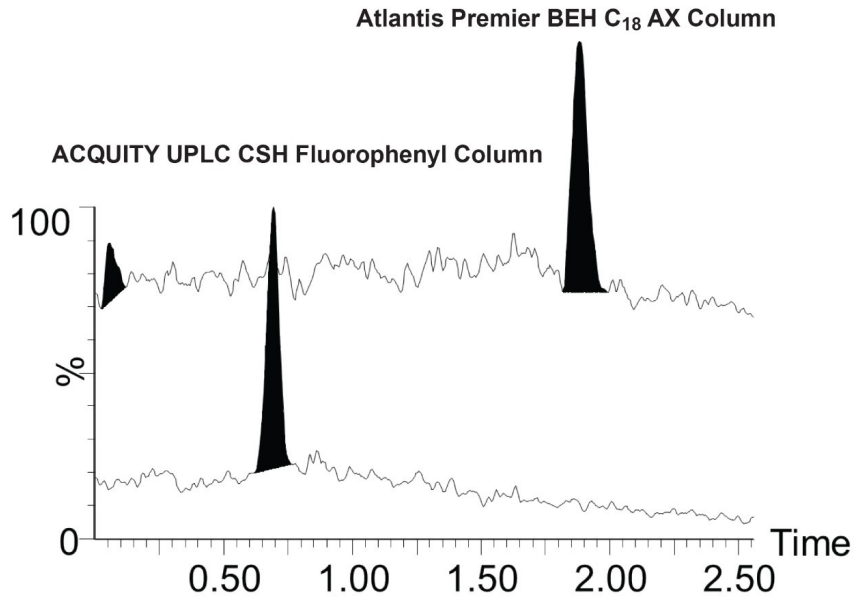

Figure 1. Comparison of 10 ng/mL retention capability of NDMA with Atlantis Premier BEH C_{18} AX Column and ACQUITY UPLC CSH Fluorophenyl Column.

Instrument Linearity and Accuracy

The internal standard calibration curves described in Figure 2 were obtained by averaging the calibration points of two individual curves. The linear dynamic range for NDMA and NDEA ranges from 0.5–100 ng/mL, while NMBA ranges from 1–100 ng/mL. The linear regression, R^2 in Figure 2, achieved for all analytes was greater than 0.997, which demonstrated good linearity between the obtained peak area and prepared concentrations. The % residual difference across all concentrations was <14.6%.

Compound	RT (min)	Range	IS conc	R^2
NDMA	1.13	0.5~100 ng/mL	5 ng/mL	0.9987
NDEA	2.79	0.5~100 ng/mL		0.9975
NMBA	2.23	1.0~100 ng/mL		0.9978

Table 2. Details of retention time and calibration curve for the nitrosamines.

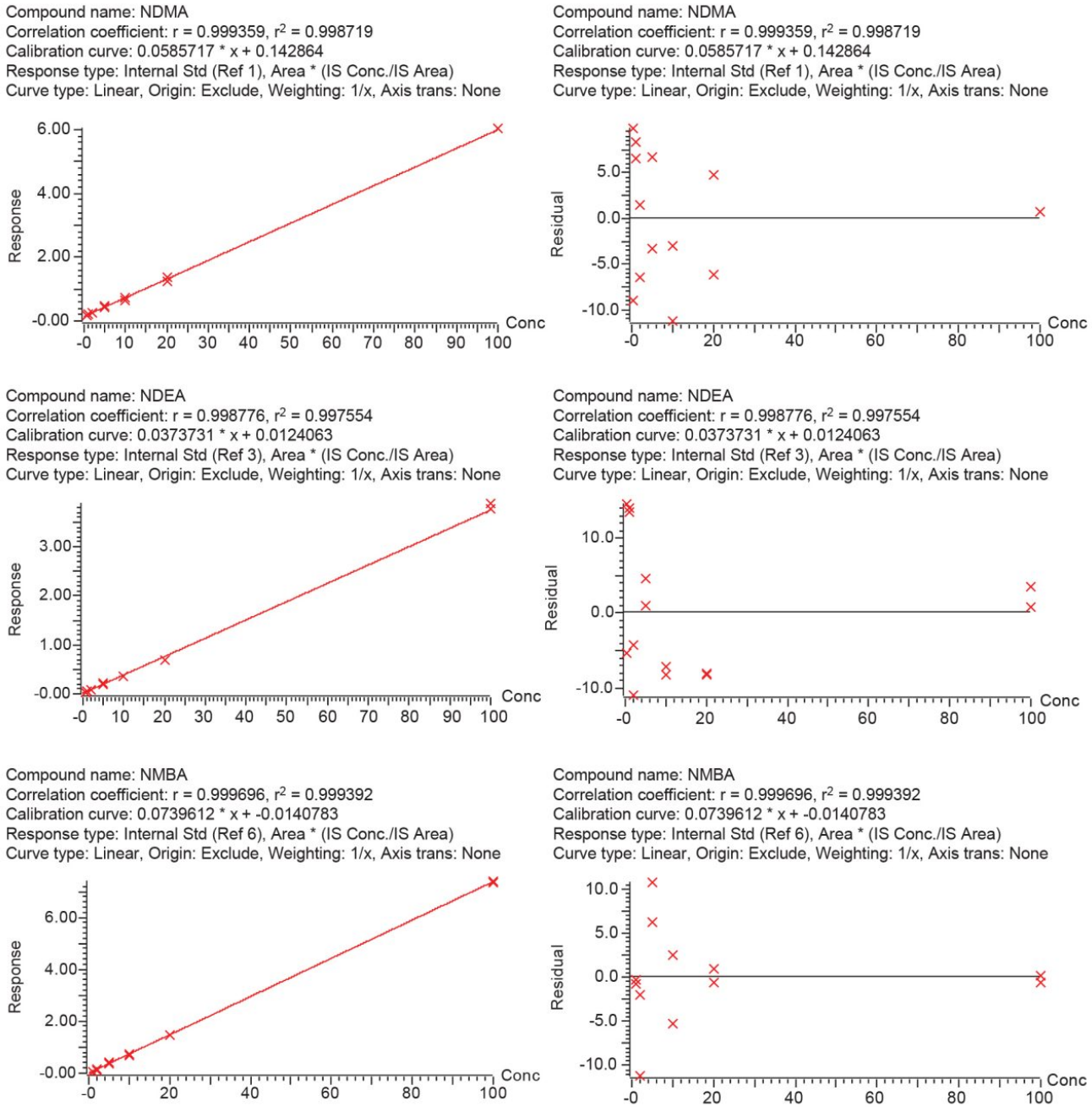

Figure 2. Calibration Curves of NDMA, NDEA, and NMBA.

Instrument Precision

A repeatability study was carried out by injecting ten consecutive runs of standard mixture at 1.0 ng/mL. The concentration %RSD obtained for all three nitrosamines was <12%. The trending plot of calculated concentration for ten repeated injections in Figure 3 demonstrates the capability of the Xevo TQ-S micro Triple Quadrupole Mass Spectrometry to produce repeatable results even at low analyte concentrations.

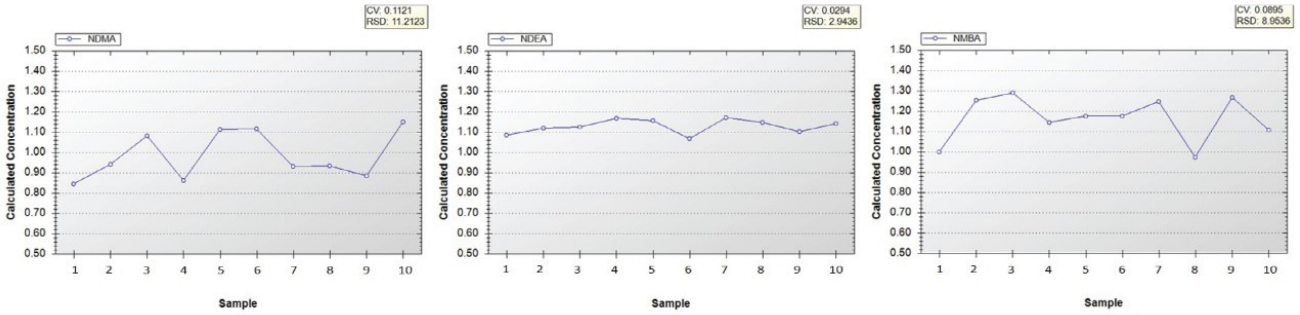

Figure 3. Trending plot of NDMA, NDEA, NMBA calculated concentration over ten repeated injections.

Limit of Quantitation (LOQ)

The LOQ level is the lowest concentration level of analyte that can be accurately identified for quantification. In this study, the LOQ was established by the lowest concentration of the compound that can achieve a signal-to-noise ratio greater than 10.

Figure 4 shows the concentration of NDMA, NDEA, and NMBA at 0.5 ng/mL, which is the lowest concentration that can produce a signal 10 times the baseline. Hence, the LOQ of all three compounds was established at 0.5 ng/mL.

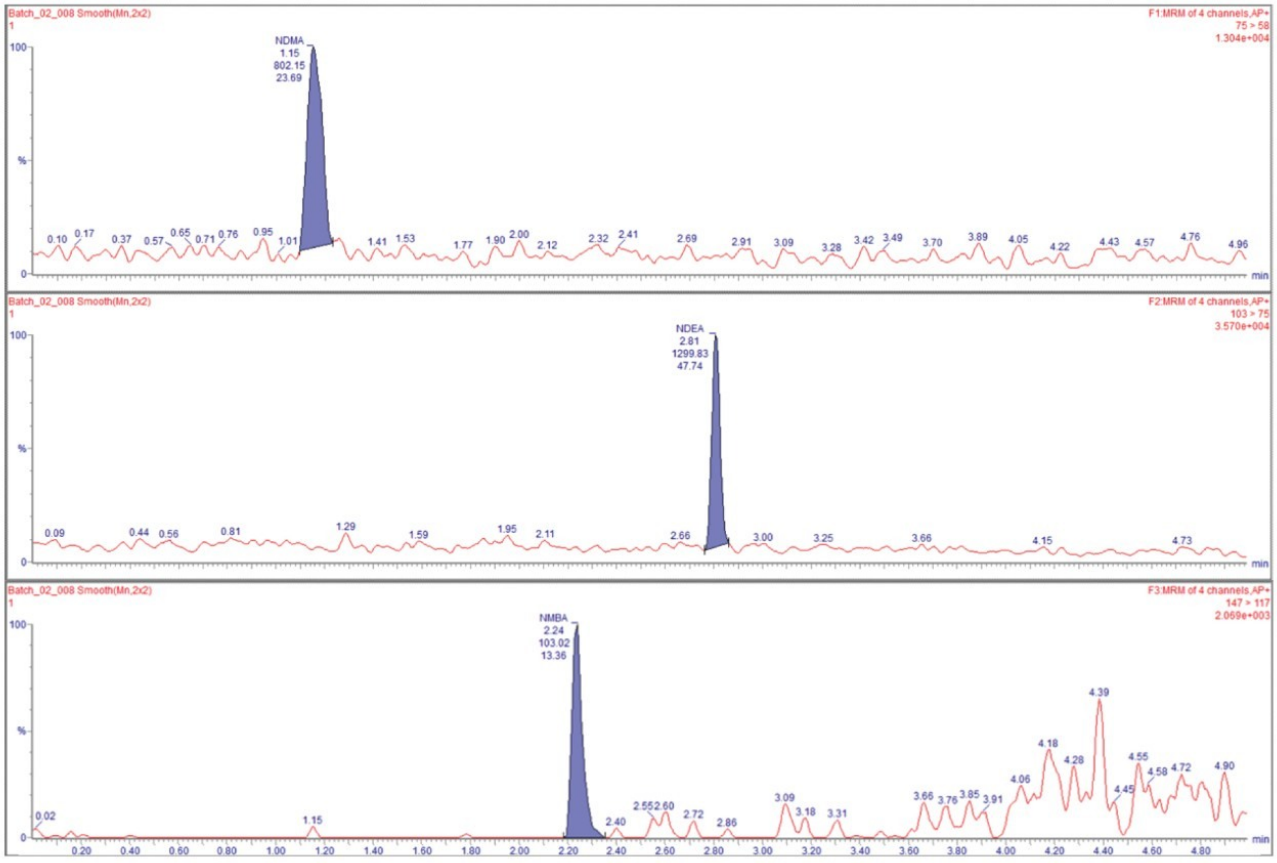

Figure 4. Chromatogram of NDMA (top), NDEA (middle), and NMBA (bottom) at LOQ.

Analysis of Losartan Potassium DS and DP and The Evaluation of Nitrosamine Extraction Efficiency

Losartan Potassium drug substance (DS) and drug product (DP) were screened for nitrosamine impurities using the developed method and all nitrosamines were found to be below the LOQ, thus reported as not detected. To study nitrosamine extraction efficiency in Losartan DS and DP, a final concentration of 2.5 ng/mL (0.025 ppm) of nitrosamine impurities were pre-spiked into DS and DP respectively, and subjected to the sample preparation method stated in the experimental section. Two DS and four DP samples were used for the evaluation.

The % extraction efficiency is calculated by the following formula. As seen in Table 3, the recovery of NDMA, NDEA, and NMBA ranges from 70.7%–88%, 85.3%–105.3% and 89.3%–116.0%, respectively.

% extraction efficiency = [Impurities concentration determined in spiked Losartan potassium DS and DP/2.50 ng/mL] x 100%

		NDMA	NDEA	NMBA
Drug substance 1	Blank sample	ND	ND	ND
	Spiked sample	1.77	2.47	2.50
	Extraction efficiency (%)	**70.7**	**98.7**	**100.0**
Drug substance 2	Blank	ND	0.50	ND
	Spiked	1.93	2.87	2.53
	Extraction efficiency	**77.3**	**94.7**	**101.3**
Drug product 1	Blank sample	ND	ND	ND
	Spiked sample	1.97	2.63	2.90
	Extraction efficiency	**78.7**	**105.3**	**116.0**
Drug product 2	Blank sample	ND	ND	ND
	Spiked sample	2.10	2.30	2.43
	Extraction efficiency	**84.0**	**92.0**	**97.3**
Drug product 3	Blank sample	ND	0.83	ND
	Spiked sample	2.20	3.43	3.00
	Extraction efficiency	**88.0**	**104.0**	**120.0**
Drug product 4	Blank sample	ND	ND	ND
	Spiked sample	1.90	2.13	2.23
	Extraction efficiency	**76.0**	**85.3**	**89.3**

ND: not detected

Table 3. Extraction efficiency based on 0.025 ppm pre-spiked nitrosamines in Losartan Potassium DS and DP.

Instrument Quality Control Checks

Known concentrations of standards at 1 ng/mL and 10 ng/mL were used for Quality Control (QC) checks to evaluate the system performance throughout the analysis. A set of the mentioned QCs (n=7) were analyzed throughout the sample list, by injecting once after every six sample runs.

The precision for all compounds of the quality checks at 1 ng/mL and 10 ng/mL were <10%, which displays good repeatability. The accuracy for the compounds of the quality checks at 1 ng/mL and 10 ng/mL were <21% and <11% respectively, which demonstrates reliable results throughout the whole analysis.

Conclusion

The MRM transitions-based method to rapidly screen and quantify for nitrosamine impurities (NDMA, NDEA, and NMBA) was successfully developed using the ACQUITY UPLC I-Class System - Xevo TQ-S micro Triple Quadrupole Mass Spectrometry with Atmospheric Pressure Chemical Ionization.

The results obtained demonstrate good precision, repeatability, and sensitivity of the instrument. The instrument has also proven the capability to achieve an LOQ of 0.005 ppm for all nitrosamine impurities, which is lower than the acceptable limits established by the US FDA of 0.03 ppm for intake of nitrosamines impurities not more than 26.5 ng/day. Pre-spiked evaluation of Losartan DS and DP also indicated good extraction efficiency for all nitrosamine impurities. Furthermore, the Atlantis Premier BEH AX Column has the capability to retain small polar compounds, even in DS and DP, when compared to other commercial columns.

References

1. Center for Drug Evaluation and Research. Control of nitrosamine impurities in human drugs. *U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER)*. Revision 1, February 2021.
2. Lee, J.-H., Lee, S-U. and Oh, J-E. Analysis of nine nitrosamines in water by combining automated solid-phase extraction with high-performance liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. *International Journal of Environmental Analytical Chemistry*, 93:12, 1261–1273.

Featured Products

- ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>
- Xevo TQ-S micro Triple Quadrupole Mass Spectrometry <https://www.waters.com/134798856>
- MassLynx MS Software <https://www.waters.com/513662>
- TargetLynx <https://www.waters.com/513791>

720007393, October 2021