LC-MRM-MS Method for the Detection and Quantification of Six Nitrosamine Impurities in Sartan (ARBs) Drugs

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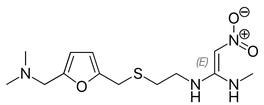
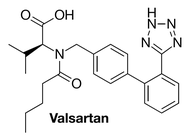
Introduction

Angiotensin II receptor blocker drugs (ARBs) are widely used to lower high blood pressure (Valsartan). It was recently discovered that various ARBs contained genotoxic nitrosamine impurities. Consequently, a recall was issued and regulatory agencies such as the FDA (Food and Drug Administration) became interestedin the detection of such impurities.



Ranitidine



Valsartan

There is still the need for a sensitive method capable of detecting genotoxic nitrosamines at sub-ppm levels in different Sartan drugs. Here, we describe an LC-MRM-MS method for the detection and quantification of sixN-nitrosamine (NDMA, NDEA, NMBA, NEIPA, NDBA, NDPA) impurities at sub-ppm levels in drug substancesusing a SCIEX® Triple Quad™ mass spectrometer and reversed phase liquid chromatography.

Materials and Methods

Table 1.
HPLC Methods

Column: Kinetex® 2.6 µm F5 **Dimension**: 100 x 3 mm	**Column**: Luna® Omega 3 µm Polar C18 **Dimension**: 100 x 4.6 mm	**Column**: Kinetex® 2.6 µm Biphenyl **Dimension**: 100 x 4.6 mm
Part Number: 00D-4723-Y0 **Gradient**:	**Part Number**: 00D-4760-E0 **Gradient**:	**Part Number**: 00D-4622-E0 **Gradient**:

Time (min)	%A	%B	µL/min
0	80	20	400
1.97	45	55	400
5.54	45	55	400
5.57	10	90	400
6.97	10	90	400
7	80	20	400
13	80	20	400

Injection Volume: 4 µL

Time (min)	%A	%B	µL/min
0	97.5	2.5	300
2	97.5	2.5	300
7	50	50	300
7.01	50	50	750
12	2.5	97.5	750
12.9	2.5	97.5	750
13-15	97.5	2.5	750

Injection Volume: 20 µL

Time (min)	%A	%B	µL/min
0	95	20	400
6	95	55	400
6.1	20	55	400
6.5	20	90	400
6.6	20	90	550
13.5	20	20	550
13.6-17	95	20	550

Injection Volume: 30 µL

Table 2.
MS Methods MRM Transitions

Q1 Mass (Da)	Q3 Mass (Da)	ID	DP (volts)	CE (volts)	Source Parameters
147	117	NMBA1	28	10	
147	87	NMBA2	28	17	APCI probe
131	43	NDIPA1	35	22	+ Polarity
131	89	NDIPA2	35	12	Current = 2
75	58	NDMA1	55	17	SourceTemp = 250
75	43	NDMA2	55	23	CUR = 40
159	57	NDBA1	38	21	GS1 = 50
159	103	NDBA2	38	15	GS2 = 30
103	75	NDEA1	50	17	CXP = 10
103	47	NDEA2	50	26	EP = 10
117	75	NEIPA	30	14	

Optimized MRM transitions for the 6 N-nitroso compounds N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), and N-nitrosodibutylamine (NDBA). MS instrument used was a SCIEX API 4000™ Triple Quad.

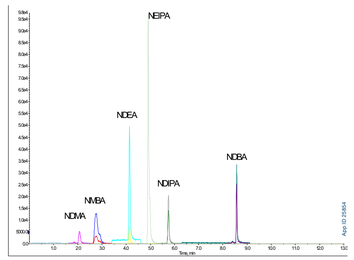
Sample Preparation

Drug substances were spiked with various concentrations obtained by serial dilution of a stock solution containing all six nitrosamines to obtain a suitable linear range of detection for the different drugs. MultiQuant™ software was used for qualitative and quantitative purposes.

Analytical reference standards were purchased from Sigma Aldrich. Drug substance analytical standards were purchased from USP.

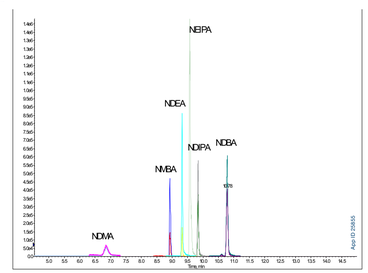
Results

Figure 2.
Representative Chromatogram Using Kinetex 2.6 µm F5 Column



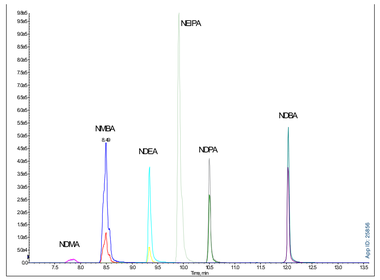
Representative Chromatogram for 20 ng/mL nitrosamine mixture using Kinetex 2.6 µm F5, 100 x 3.0 mm column (P/N: 00D-4723-Y0).

Figure 3.
Representative Chromatogram Using Kinetex 2.6 µm Biphenyl Column



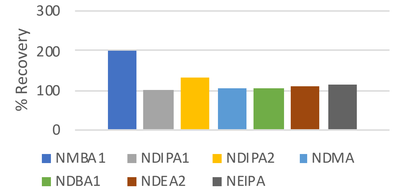
Representative Chromatogram for 20 ng/mL nitrosamine mixture using Kinetex 2.6 µm Biphenyl, 100 x 4.6 mm column (P/N: 00D-4622-E0).

Figure 4.
Representative Chromatogram Using Luna Omega 3 µm Polar C18 Column



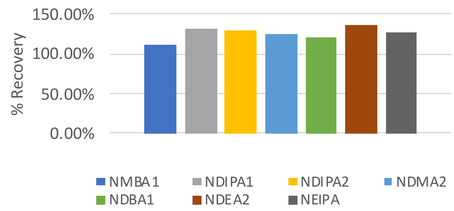
Representative Chromatogram for 20 ng/mL nitrosamine mixture using Kinetex Luna Omega Omega 3 µm Polar C18 100 x 4.6 mm column (P/N: 00D-4760-E0).

Figure 5.
Nitrosamine % Recovery in Valsartan Using Kinetex 2.6 µm F5 Column



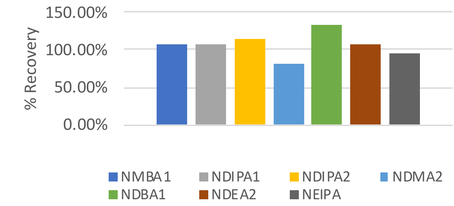
% Recovery was evaluated for the samples spiked in 100 mg/mL of Valsartan. The recovery of all six compounds in the three replicates fell between 202 % and 100 %, with %RSD (n=3) between 1.40 % and 12.5 %.

Figure 6.
Nitrosamine % Recovery in Metformin Using Kinetex Biphenyl Column



% Recovery was evaluated for the samples spiked in 100 mg/mL of Metformin. The recovery of all six compounds in the three replicates fell between 110 % and 135 %, with %RSD (n=3) between 18.5 % and 5.6 %.

Figure 7.
Nitrosamine % Recovery in Valsartan Using Luna Omega Polar C18 Column



% Recovery was evaluated for the samples spiked in 100 mg/mL of Valsartan. The recovery of all six compounds in the three replicates fell between 110 % and 135 %, with %RSD (n=3) between 18.5 % and 5.6 %.

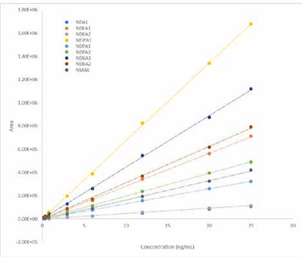
Discussion

Conventional C18 columns provide little interaction for small highly polar compounds. Nitrosamines are compounds of low molecular weight and high polarity, thus making their chromatography separation a challenge. It is of utmost importance for the column to be capable of retaining the polar nitrosamines without generating secondary interactions. Secondary interactions can affect peak shape leading to tailing, reduced peak height, and thus a reduction in sensitivity. When sensitivity is reduced, compound quantitation is negatively affected.

Conclusion

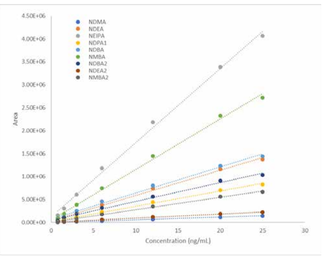
Here, we present LC-MS methods capable of detecting highly polar nitrosamines at 0.007 ppm in the drug product, which is lower than the specification limit for NDMA. Three different stationary phases, a highly inert pentafluoro phenyl, an aqueous stable biphenyl, and a Polar C18 phase capable of providing excellent retention of polar nitrosamines with good peak shape were used for this purpose.

Figure 8.
Linear Dynamic Range Using Kinetex F5



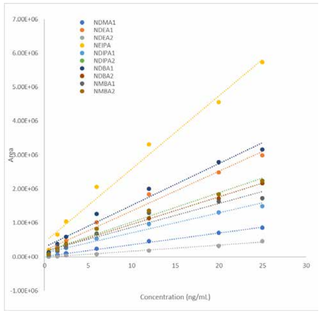
Calibration curves for the six nitrosamine compounds. The response for all eight compounds is linear over the calibration range from 0.5 to 25 ng/mL with correlation coefficient >0.980. LOD was 0.007 ppm.

Figure 9.
Linear Dynamic Range Using Kinetex Biphenyl



Calibration curves for the six nitrosamine compounds. the response for all eight compounds is linear over the calibration range from 0.5 to 25 ng/mL with correlation coefficient >0.998. LOD was 0.005 ppm.

Figure 10.
Linear Dynamic Range Using Luna Omega Polar C18



Calibration curves for the six nitrosamine compounds. The response for all six compounds is linear over the calibration range from 0.5 to 25 ng/mL with correlation coefficient >0.980.. LOD was 0.005 ppm.

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