Application Note

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Analysis of N-Nitrosodimethylamine and N-Nitrosodiethylamine Using the Agilent 7697A Headspace Sampler, 8890/5977 GC/MSD System

Impurity analysis of sartan drug products following US FDA guidance

Author

Abbey Fausett
Agilent Technologies, Inc.

Abstract

This Application Note describes the use of the Agilent 8890 GC to analyze impurities in sartan products according to a United States Food and Drug Administration (US FDA) method. The workflow also features an Agilent 7697A headspace sampler and a 5977 GC/MSD. Results corresponded to expected detection values.

Introduction

Hypertension, or high blood pressure, affects between 35 and 46% of adults over 25 years of age, according to the World Health Organization.[1] The condition impacts over one billion people worldwide.[2] Valsartan, losartan, and other angiotensin II receptor blockers (ARBs) are a type of pharmaceutical commonly prescribed to treat hypertension, as they are globally available and relatively affordable. The mechanism of ARBs is to inhibit the binding process between angiotensin II and its receptor, preventing blood vessel constriction, and effectively lowering blood pressure.[3]

In July 2018, a worldwide recall was announced on valsartan products due to contamination risk. The initial contaminant was identified as N-nitrosodimethylamine (NDMA). The US FDA released a procedure to guide production facilities in screening valsartan products for these probable carcinogenic impurities before distribution.[4] A second contaminant was identified shortly after the recall and confirmed by the European Medicines Agency.[5] N-nitrosodiethylamine (NDEA), like NDMA, is classified as a probable carcinogen, and is believed to be generated as a secondary product during synthesis of the tetrazole ring common to sartan-class pharmaceuticals. With this new impurity confirmation and link to the tetrazole ring, the initial recall expanded to affect all sartans in production. Following the reports from the European Medicines Agency, the US FDA created a follow-up regulatory procedure to address both contaminants in a single method. The modifications include a change in preparation solvent along with a different GC column chemistry.[6] This document was released in January 2019, and established limit of quantitation (LOQ) values for NDMA and NDEA at 0.10 and 0.05 ppm, respectively, using GC/MS with headspace sampling to analyze both the drug substance and finished product. While the FDA procedure was executed on an Agilent 7890A GC, this Application Note shows that the Agilent 8890 GC system is capable of the same excellent performance as the 7890 system.

Experimental

NDMA and NDEA standards were purchased through MilliporeSigma (Burlington, MA, USA) in neat form. The FDA guidance[6] requires the use of 1-methyl-2-pyrrolidinone (NMP) with a minimum purity of 99.0%. However, use of a higher purity solvent (99.7%, MilliporeSigma) resulted in improved resolution of the target compounds from solvent impurities when following the provided conditions. Because this analysis is applicable to additional global regulatory agencies, there may or may not be flexibility in chromatographic parameters to achieve improved separations when lower purity solvents are implemented in the sample preparation. For the data contained within this evaluation, the FDA protocol was followed without deviation.

Method conditions are summarized in the following tables. Although this work was performed on a fast GC oven (240 V), this option is not required to obtain these results. Another option useful in development of headspace methods is the headspace transfer line accessory, which allows both headspace and liquid injection capability on the same inlet without changing the configuration. Table 1 contains consumables used in the analysis. Calibration solutions were prepared in accordance with the FDA guidance, with an additional point prepared at 1 µg/mL.

Method conditions

Agilent 7697A Headspace Sampler Conditions	
Oven Temperature	130 °C
Loop Temperature	180 °C
Transfer Line Temperature	185 °C
Vial Equilibration	15 minutes
Injection Duration	1 minute
Vial Size (mL)	20 mL
Vial Shaking	Level 5
Fill Mode	Default
Fill Pressure	15 psi
Loop Fill Mode	Default

Agilent 8890 GC Parameters	
Inlet (Split/Splitless)	Helium
Temperature	220 °C
Mode	Split
Split Ratio	5:1
Inlet Pressure (Initial)	7.33 psi
Oven Type	240 V-Fast oven
Equilibration Time	1 minute
Oven Program	40 °C for 0.5 minutes, 20 °C/min to 160 °C, 10 °C/min to 240 °C, Hold 2 minutes Total cycle time: 16.5 minutes
Column	Agilent J&W DB-1701, 30 m × 250 µm, 1.0 µm
Mode	Constant flow
Flow	1 mL/min

Agilent 5977 GC/MSD Conditions	
Source Type	Extractor
Source Temperatre	230 °C
Mass Filter Mode	Selection ion monitoring (SIM)
NDMA *m/z*	74.00
NDEA *m/z*	102
Lens Diameter	6 mm
Quad Temperature	150 °C
NDMA Dwell	150
NDEA Dwell	150

Table 1. Consumables used in analysis.

Description	Part Number
Headspace Vials (20 mL)	5182-0837
Vial Caps	5183-4477
GC Liner	5190-2295
GC Septa	5183-4759
Column DB-1701	122-0733
Extractor Lens (6 mm)	G3870-20448

Results and discussion

Separations were sufficient, and the target peaks are well resolved from solvent and matrix species. Figure 1 shows an example chromatogram.

Retention times agreed with those provided in the FDA regulation. Figures 2A and 2B show overlays of the three lowest calibration points (0.025, 0.05, and 0.1 µg) for NDMA and NDEA.

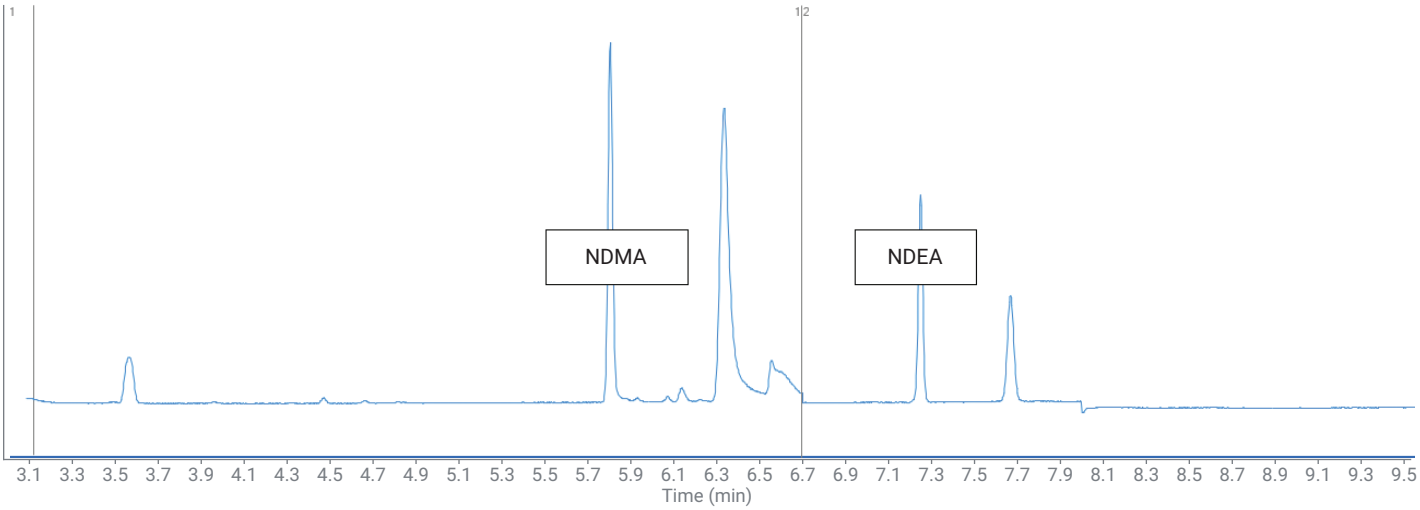

Figure 1. Generated chromatogram in select ion mode of a 1 µg standard mix of NDMA and NDEA in NMP. NDMA retention time is 5.80 minutes, and NDEA is 7.25 minutes.

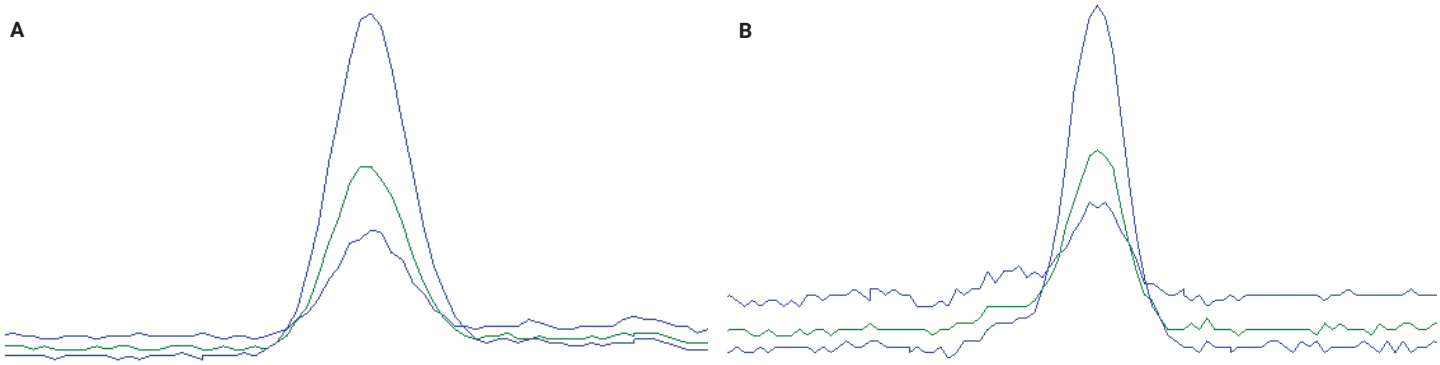

Figure 2. Overlays of NDMA (A) and NDEA (B) at three lowest calibration points.

Two calibration curves were generated using Agilent MassHunter Quantitative Analysis software. As the LOQ and limit of detection (LOD) studies were done at the low end of the curve, the quantitative results were processed against a calibration curve between 0.025 and 0.5 µg standards. The full range curve extends from 0.025 to 100 µg with a linear correlation exceeding 0.995 with inverse weighting. Figures 3A and 3B display the low-level calibration curve information obtained on this instrument. LOD is defined by recording the smallest amount injected on-column that has at least a 3:1 signal-to-noise ratio. In NDMA and NDEA calibrations, the lowest point of 0.025 µg exceeded this setting.

After generating calibration curves for each compound, five replicate standards were analyzed at three levels within the curve. Table 2 summarizes the results of this reproducibility evaluation.

The final evaluation of method performance was a spike and recovery test in matrix. The two lowest calibration standards were spiked into triplicate vials, each containing 500 mg of dissolved valsartan drug tablets, targeting the cited LOQs. A batch sample was analyzed to show nondetects for NDMA and NDEA compounds prior to the spike and recovery test. Table 3 provides the results

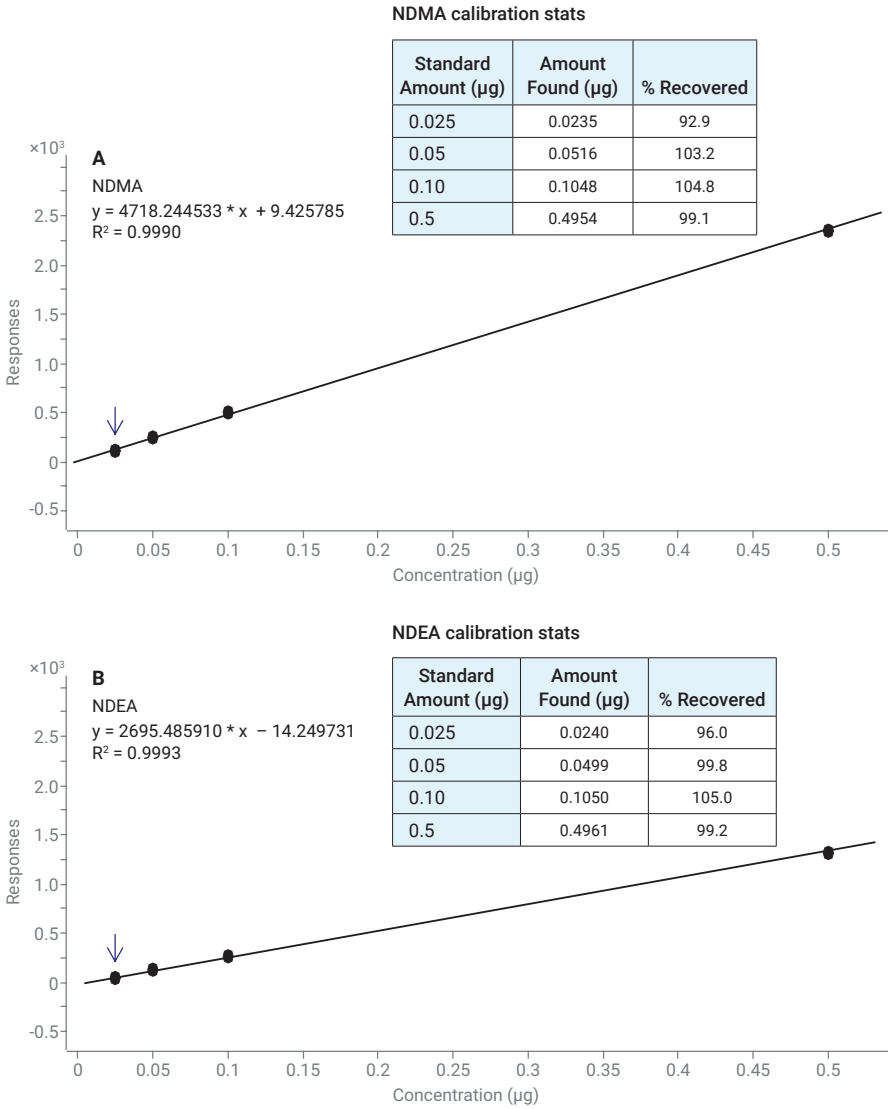

Figure 3. Linear regression determined for the low-level calibration range and accuracy of the calibration standards after processing against the calibration curve.

Table 2. Statistics for reproducibility of standards (n = 5).

	0.025 µg Standard		0.05 µg Standard		0.1 µg Standard	
	NDMA	NDEA	NDMA	NDEA	NDMA	NDEA
Average Found Amount	0.0249	0.0248	0.0518	0.0506	0.1012	0.0988
%RSD	4.95	2.89	6.24	0.7	2.65	4.07

Table 3. LOQ determinations from three replicate samples for NDMA and NDEA target compounds.

	Target LOQ	Average Recovery	Average Recovery
	1 ppm	0.05 ppm	0.10 ppm
NDMA	0.10	0.056	0.11
NDEA	0.05	0.057	0.11

Conclusion

The Agilent 8890 GC extends the legacy of robust and reproducible performance necessary for safe screening of drug products. The 8890 GC paired with the Agilent 7697A headspace sampler and an Agilent 5977 GC/MSD comply with FDA directives and expected detection values for the critical analysis of impurities in sartan products.

References

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