Pharmaceutical Analysis and Characterization of Nitrosamine Impurities within Angiotensin II Receptor Blocker Drug Products

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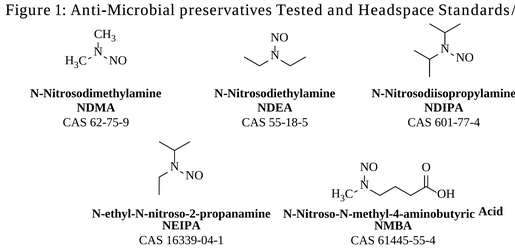
Abstract

Introduction: On July 13, 2018, FDA issued a series of recalls for several lots of valsartan (an ARB) that contained the API originating from Zhejiang Huahai Pharmaceuticals. They were found to contain N-Nitrosodimethylamine (NDMA), a potent carcinogen. **Methods:** Office of Testing & Research scientists were asked by the FDA ARB Taskforce to develop a gas chromatography-mass spectrometer headspace method in August-2018 to analyze Valsartan drug products for an NDMA impurity at sub ppm levels. Three additional nitrosamine impurities were later identified: N-nitrosodiethylamine, N-Nitrosodiisopropylamine and N-ethyl-N-nitroso-2-propanamine. In January 2019, OTR scientists rapidly developed and validated a highly novel GC-MS-HS method to screen and analyze the four nitrosamine impurities and this method was implemented to provide the Agency a comprehensive analytical tool for its on-going Public Health response to the valsartan incident. **Results:** Chromatographic separation was achieved in 24 mins with linearity of 0.05 ppm to 100 ppm and correlation coefficients for standard calibration curves were >0.99. Intra- and inter-day accuracy and precision of all analytes in DMSO were found to be acceptable with RSD% ≤ 10% and accuracy ≥ 85%. Matrix selectivity was tested in two different lots of valsartan API and no interference signal was observed at the retention time of the analyte of interest. **Conclusions:** The regulatory impact of this work was significant and has provided FDA regulators with analytical tools to rapidly monitor ARB drug products as well as other related pharmaceutical drug products. These methods have been shared with regulators throughout the world. As result of the rapid implementation of new characterization methods, the Agency and its worldwide regulatory collaborators can more effectively address the response to the valsartan incidents.

Introduction

Angiotensin II receptor blockers (ARBs) are a class of pharmaceutical drug products used to treat hypertension and congestive heart failure. In July 2018, FDA issued a series of recalls for several lots of valsartan (an ARB) that contained the API originating from Zhejiang Huahai Pharmaceuticals. They were found to contain N-Nitrosodimethylamine (NDMA), a potent carcinogen. OTR scientists were asked by the FDA ARB Taskforce to develop a gas chromatography-mass spectrometer headspace (GC-MS-HS) method in August 2018 to analyze Valsartan drug products for an NDMA impurity at sub ppm levels. Three additional nitrosamine impurities were later identified: N-nitrosodiethylamine (NDEA), N-Nitrosodiisopropylamine (NDIPA) and N-ethyl-N-nitroso-2-propanamine (NEIPA), Figure 1.

Figure 1: Anti-Microbial preservatives Tested and Headspace Standards/Diluent



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From a technical perspective, the structurally related nitrosamine analytes posed a significant analytical challenge and there were no analytical methods available as a starting point for the four analytes in combination: NEIPA, NDIPA, NDBA and NMBA. In January 2019 OTR scientists rapidly developed and validated a third and highly novel GC-MS headspace method to screen and analyze the two additional nitrosamine impurities.

Objectives

Primary Center Of Excellence Goal: Pharmaceutical Analysis and Characterization

FDA Mission Relevance statement: This work supported the characterization of Valsartan API and drug products and provided the FDA ARB task force with timely information to effectively respond to the incident.

Project Objective/s: The objective of this part of the research work is to develop and validate a headspace GC-MS method to quantify four nitrosamine impurities including NDMA, NDEA, NDIPA, and NEIPA in ARP drug products.

Materials and Methods

Experimental:
An Agilent GC 7890A/ MS 5977B interfaced with an Agilent headspace analyzer 7697A system was employed for the method development, validation and implementation phases of the study. The system was equipped with a split/splitless injector (5:1 split ratio, 220 °C). Separation was achieved on a DB-WAX column (30 m × 0.25 mm × 0.5 µm) The drug products or API, were placed in a 20 mL GC vial containing 5.0 mL of dimethyl sulfoxide for analysis.

GC-MS Headspace Materials and Method Validation:
The assay was validated for accuracy, precision, linearity, specificity, and analytical range per the requirements of USP <1225> and ICHQ2R1 for analytical range. Carry-over, and stability studies were conducted. Reference standards N-nitrosodimethylamine (NDMA): 1 mg/mL in MeOH, N-nitrosodiethylamine (NDEA): 1 mg/mL in MeOH were purchased and solid Valsartan API was purchased to test for matrix effects.

Sample Preparation for API: Accurately weigh 500 mg of Valsartan drug substance into a 20 mL headspace vial. Add 4.5 mL of DMSO and 0.5 mL of ISTD mix solution to the vial and immediately cap and crimp the vial. Mix the sample solution using a vortex mixer. Drug substance weight could be increased or decreased, depending on the amount of nitrosamine impurities in the drug substance.

Sample Preparation for Drug Product: Valsartan drug product equivalent to 320 mg Valsartan was placed into a 20 mL headspace vial. Add 4.5 mL of DMSO and 0.5 mL of ISTD mix solution to the vial and immediately cap and crimp the vial. Mix the sample solution using a vortex mixer for 30 secs. Drug product weight could be increased or decreased, based on the amount of nitrosamine impurities in the drug substance.

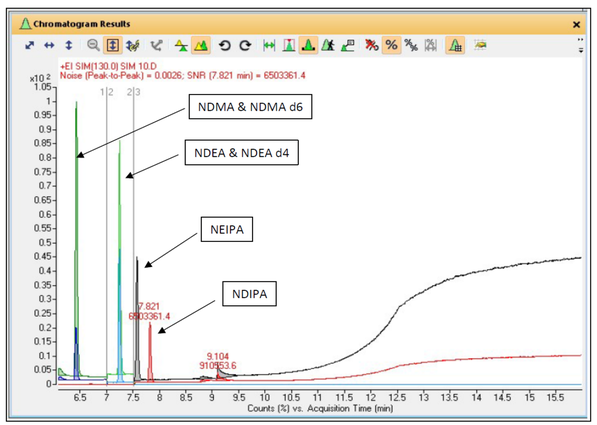

Figure 2: Chromatogram of 0.25 µg NDMA, NDEA, NDIPA, NEIPA Working Standard and ISTD NDMA d6 and NDEA d4

Results and Discussion

System Suitability:
System suitability testing was conducted on each day during the method validation and in-study. Analyte retention time and total area under the curve (AUC) were tested for compliance with USP or method specifications and determined acceptable. The average RT for NDMA was 6.5 min, %RSD 0.03, NDEA was 7.3, %RSD 0.03, NDIPA was 7.9, %RSD 0.0 and NEIPA was 7.6 min, %RSD 0.04.

Linearity:
The correlation coefficient (r2) of each calibration curves was ≥ 0.995 over the analytical range. The S/N ratio of the 0.05 µg working standard was ≥ 10.

Linearity of calibration curves	Day	Equation	R^2
NDMA (0.05-100 ppm)	Validation day 1	Y=2.608038x+0.001476	0.9995
	Validation day 2	Y=2.616180x-0.003536	0.9997
	Validation day 3	Y=2.525428x-0.003593	0.9999
NDEA (0.05-100 ppm)	Validation day 1	Y=0.742240x+0.00045	0.9993
	Validation day 2	Y=0.743481x-0.00019	0.9989
	Validation day 3	Y=0.696714x-0.00036	0.9993
NDIPA (0.05-100 ppm)	Validation day 1	Y=0.390604x+0.00068	0.9997
	Validation day 2	Y=0.210985x-0.00013	0.9985
	Validation day 3	Y=0.198497x-0.00023	0.9983
NEIPA (0.05-100 ppm)	Validation day 1	Y=0.205972x+0.00058	0.9998
	Validation day 2	Y=0.109292x-0.00037	0.9957
	Validation day 3	Y=0.370818x-0.00009	0.9994

Table 1: Linearity details of NDMA, NDEA, NDIPA, and NEIPA

LOD, LOQ, Recovery, Accuracy & Precision:
The limit of detection (LOD) was determined by preparing standards of known concentrations and calculating the signal to noise ratio. The lowest standard concentration with a S/N of ≥ 3 was designated as the method LOD. Limit of Quantitation (LOQ) was determined by spiking a known amount of standard at different concentration levels into replicate samples (n = 3) of Valsartan drug substance. The spiked sample level with recoveries of 80 – 120% and a % RSD of ≤ 10 was designated as the method LOQ. Intra- and inter-day accuracy and precision of all quality control standards in DMSO were found to be acceptable with RSD% ≤ 10% and accuracy ≥ 85%.

Impurity	Drug Substance LOQ, ppm	Drug Substance LOD, ppm	Drug Product LOQ, ppm	Drug Product LOD, ppm
NDMA	0.05	0.01	0.05	0.01
NDEA	0.05	0.01	0.05	0.01
NEIPA	0.05	0.025	0.05	0.025
NDIPA	0.05	0.025	0.05	0.025

Table 2. GC-MS Headspace ARB Method Sensitivity

Analyte	Validation data	QC Concentration (ppm, N=18)			
		0.05	0.15	6.0	80
NDMA	Average (ppm)	**0.050**	**0.153**	**6.05**	**89.91**
	Precision (%RSD)	10.13	2.92	0.79	1.11
	Accuracy (%)	100.16	101.41	100.78	112.38
NDEA	Average (ppm)	**0.052**	**0.149**	**6.04**	**90.45**
	Precision (%RSD)	4.46	3.81	0.93	0.95
	Accuracy (%)	103.57	99.08	100.62	113.06
NDIPA	Average (ppm)	**0.049**	**0.152**	**6.01**	**90.12**
	Precision (%RSD)	4.96	6.03	1.85	1.38
	Accuracy (%)	99.78	101.66	100.15	112.65
NEIPA	Average (ppm)	**0.046**	**0.147**	**5.97**	**89.41**
	Precision (%RSD)	5.00	4.85	1.21	0.99
	Accuracy (%)	92.49	97.75	99.43	111.77

Table 3. Intra-day average concentration for NDMA, NDEA, NDIPA, and NEIPA

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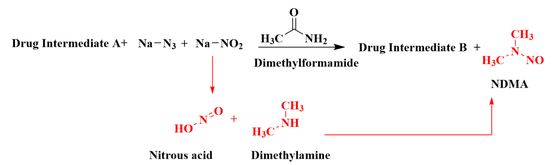
Table 4. Average Inter-day accuracy and precision for NDMA, NDEA, NDIPA, and NEIPA

Drug Master File Review: The process conditions of over 175 drug master files were reviewed by OTR to help OND determine the source for the formation of the nitrosamine impurities. It was necessary to determine if the nitrosamine impurities were a product related substance or related to a residual solvent. In general, the formation of nitrous acid in the presence of N,N-dimethylformamide (in the case of NDMA) can result in solvent decomposition and form an amine which leads to the formation of nitrosamines.



Conclusion

- OTR conducted the review of drug master files for ARB API manufacturers and classified each as high or low risk for NDMA formation.
- The newly developed GC-MS Headspace method for multiple nitrosamine impurities was rapidly implemented to provide the Agency a comprehensive analytical tool for its on-going Public Health response to the valsartan drug substance and drug product incident.
- The regulatory impact of this original analytical method has provided FDA regulators with analytical tools to rapidly monitor ARB drug products and have been shared with regulators throughout the world.
- As a result of the rapid implementation of new impurity characterization methods, the Agency and its worldwide regulatory collaborators can more effectively address the response to the valsartan incident.

Project Contributors:
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