

## Quantitation of 6 Nitrosamines in 5 Sartans by LC-MS/MS system as per the proposed USP General Chapter <1469>

Shailendra Rane<sup>1</sup>, Devika Tupe<sup>1</sup>, Deepti Bhandarkar<sup>1</sup>, S.Saravanan<sup>2</sup> and B.Karthikeyan<sup>2</sup>  
1 Shimadzu Analytical (India) Pvt. Ltd., 2 Spinco Biotech Pvt. Ltd.

### User Benefits

- ◆ An LCMS method for the determination 6 nitrosamines in 5 Sartans as per the proposed USP general chapter <1469>.
- ◆ The LCMS-8045 system easily meets the criteria as per the proposed USP general chapter <1469>.

### Introduction

**Overview :** The Drug Regulatory Authorities first noticed the presence of the nitrosamine (NSA) impurity, N-Nitrosodimethylamine (NDMA) in products containing valsartan in July 2018. Valsartan is an Angiotensin II Receptor Blocker (ARB) and belongs to a family of analogue compounds commonly referred to as the Sartans. Further, few other nitrosamines were subsequently detected in other drug substances belonging to the Sartan family, other Active Pharmaceutical Ingredients (API's), and Finished Pharmaceutical Products (FPP). These included N-nitroso-dimethylamine (NDMA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitroso-diethylamine (NDEA), N-ethyl-N-nitroso-isopropylamine (NEIPA), N-nitroso-diisopropylamine (NDIPA) and N-nitroso-di-n-butylamine (NDBA).

**Occurrence :** Formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines and nitrite salts under acidic reaction conditions. Under these conditions, nitrite salts may form nitrous acid, which can react with an amine to form a nitrosamine. Apart from these there are other routes such as; vendor-sourced starting materials and raw materials; recovered solvents, catalysts and reagents; cross contamination from common manufacturing facility; quenching process using nitrous acid; and packing/storage; which may result in nitrosamines formation or contamination.

**Toxicity/ Regulation/ Methods:** NDMA and NDEA belong to the so-called "cohort of concern", which is a group of highly potent mutagenic carcinogens that have been classified as probable human carcinogens. Hence, United state Food and Drug Administration (USFDA) recommends the following acceptable intake (AI) limits for NDMA, NMBA, NDEA, NEIPA, NMPA and NDIPA (Table 1). These limits are applicable only if a drug product contains a single nitrosamine, and lowest of which is 0.03 ppm for drug substances (DS) with Maximum daily dose (MDD) of 880 mg/day. If more than one nitrosamine impurity is identified in the same DS the limit for total nitrosamines listed in table 1 is still not more than 26.5 ng/day or 0.03 ppm.

Hence, it is imperative to detect above mentioned NSA's with Limit of Quantitation (LOQ) as low as possible to be sure that not just single nitrosamine impurity is below 0.03 ppm, but also total nitrosamine impurities are below 0.03 ppm.

Table 1 . Acceptable Intake (AI) Limits for nitrosamines

Nitrosamine	AI Limit (ng/day)	Limit in ppm for MDD 880 mg/day
NDMA	96.0	0.109
NMBA	96.0	0.109
NDEA	26.5	0.030
NEIPA	26.5	0.030
NMPA	26.5	0.030
NDIPA	26.5	0.030

The United States Pharmacopeia (USP) published the proposed General Chapter <1469> which has been used for the quantification of nitrosamines in 5 different Sartans namely Olmesartan, Telmisartan, Irbesartan, Losartan and Valsartan. In this application note "procedure 3" has been performed on LCMS-8045 system by using Raptor ARC-18 (150mm X 3.0mm x 2.7 µm).

### Experimental

Six nitrosamines namely NDMA, NMBA, NDEA, NEIPA, NDIPA and NDBA were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera™ XS coupled with LCMS™-8045, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Figure 1).

LCMS-8045, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

All six nitrosamines are mid polar compounds. They were easily ionized by Atmospheric Pressure Chemical Ionization (APCI interface).



Fig. 1 Nexera™ XS with LCMS™-8045 system

## Method

The MRM transitions of 6 nitrosamines and 4 internal standards are given in table 2 and analytical conditions in table 3.

Table 2 MRM transitions of nitrosamines

MRM Transitions			
Nitrosamine Impurity	Acquisition Mode	Polarity	MRM (Quantifier, Qualifier)
NDMA	MRM	Positive	75>43,75>44
NDMA-d6	MRM	Positive	81>46,81>64
NMBA	MRM	Positive	147>44,147>117
NMBA-d3	MRM	Positive	150>47,150>120
NDEA	MRM	Positive	103>75,103>47
NDEA-d10	MRM	Positive	113>34,113>49
NEIPA	MRM	Positive	117>75,117>45
NDIPA	MRM	Positive	131>89,131>47
NDBA	MRM	Positive	159>41,159>29
NDBA-d18	MRM	Positive	177>66,177>46

Table 3 Analytical conditions

HPLC System	: Nexera XS
Column	: Raptor ARC-18 (150mm X 3.0mm x 2.7µm)
Column Oven	: 60° C
Mobile Phases	: A-0.1% Formic acid in Water B-0.1% Formic acid in Methanol
Flow Rate	: 0.5mL/min
Gradient program (B%)	: 0-1.5 min → 3(%) ; 1.5-4 min → 3-50(%) ; 4-7 min → 50-75 (%) ; 7-8.1 min → 75-85 (%) ; 8.1-9.2min → 85-95(%) ; 9.2-12 min → 95 (%) ; 12-12.1 min → 95-3 (%) ; 17 min → STOP
Injection Volume	: 20 µL
LCMS System	: LCMS-8045
	: APCI
Temperature	: Interface: 270° C Desolvation Line: 220° C Heater Block: 220° C
Gas Flow	: Nebulizing Gas: 3 L/min Drying Gas: 3 L/min

A representative LCMS chromatogram for nitrosamines with the UV chromatogram of sartans is given in figure 2. A divert valve was employed to direct all API peaks to waste and to protect LCMS from the contaminations.

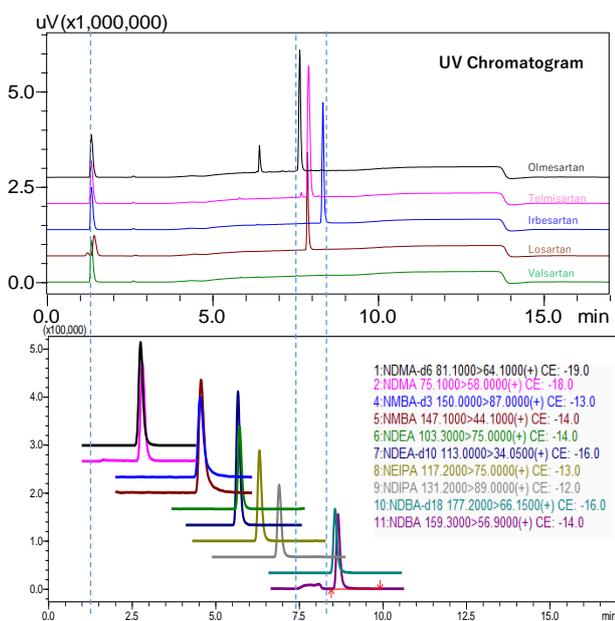


Fig. 2 Representative LCMS chromatogram of 6 NSAs and its overlapping with UV chromatogram

## Linearity of the nitrosamines

Nine-point calibration curves for all 6 NSAs with 4 ISTDs were prepared in 1% formic acid in water and analyzed using the conditions described in Table 3. The figures 3 to 7 depicts the calibration curves, overlay of linearity standards & LOQ solution chromatograms for NDMA, NMBA, NDEA, NEIPA, NDIPA, & NDBA, respectively.

### NDMA

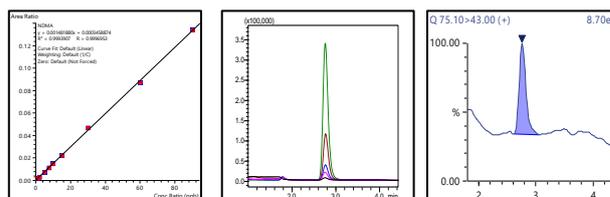


Fig.2 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDMA

### NMBA

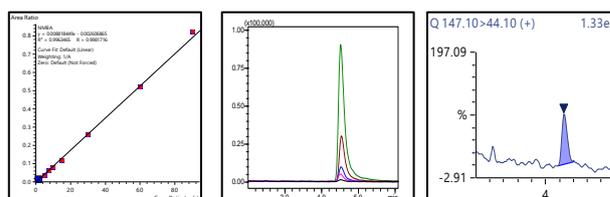


Fig.3 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NMBA

### NDEA

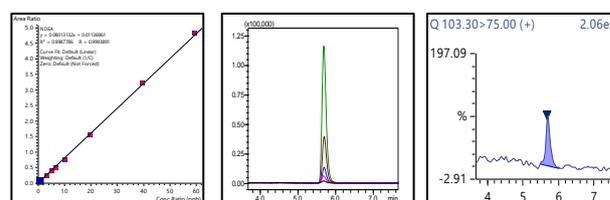


Fig.4 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDEA

### NEIPA

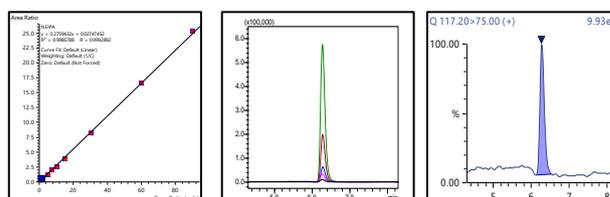


Fig.5 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NEIPA

### NDIPA

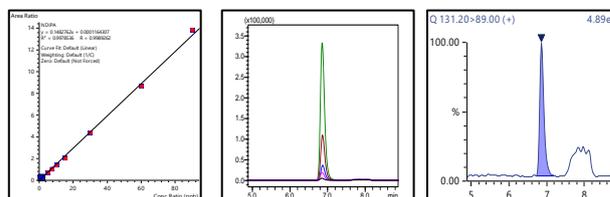


Fig.6 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDIPA

### NDBA

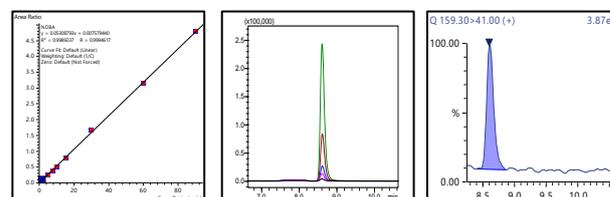


Fig.7 Calibration Curve, Overlay of Linearity Standards & LOQ Solution chromatogram for NDBA

Table 4 Summary of Calibration Curves

Comp.	CC range (ppb)	R2	LOQ		
			Conc. (ppb)	%RSD (n=6)	S/N
NDMA	1.33 to 90	0.999	1.33	2.68	25.34
NMBA		0.998		15.66	15.08
NEIPA		0.999		10.3	40.19
NDIPA		0.998		1.85	90.03
NDBA		0.999		3.66	89.85
NDEA	0.66 to 59.4	0.999	0.66	5.90	29.84

The range for calibration curves, LOQ established from S/N and % RSD at LOQ are shown in table 4.

### ■ Sample Analysis

Weigh 80 mg of sample drug substance in 2 mL centrifuge tube, then add 1188 µL of diluent and 12 µL of internal standard. Vortex at 2500 rpm for 20 min, except for losartan (vortex losartan NMT 5 min). Then centrifuge at 10,000 rpm for 10 minutes. Filter the supernatant through 0.22-µm hydrophilic PTFE syringe filter and inject the sample for LC-MS analysis.

The overall concentration was 66.6 mg/mL. The results of the sample spiked study (recovery study) for the five sartans at LOQ level are given in table 5.

Table 5 The sample spiked study for 5 sartans at LOQ level

% Recovery at 0.03ppm for 5 NSAs and 0.014ppm for NDEA					
Name	Olmesartan	Telmisartan	Irbesartan	Losartan	Valsartan
NDMA	90.7	86.9	114.2	95.7	92.1
NMBA	117.7	114.5	108	112.6	118.7
NEIPA	94.5	93.7	94.4	93.7	93.7
NDIPA	100.7	105.5	98.0	81.4	86.1
NDBA	104.5	110.5	115.2	108.7	111.8
NDEA	71.8	73.2	99.6	77.1	86.8

Note: Criteria for % Recovery as per USP <1469> is 70 to 130%.

### ■ Conclusion

- A single LC-MS/MS quantification method for six nitrosamines in 5 sartans as per proposed USP general chapter <1469> (procedure-3) has been successfully applied into the Shimadzu LCMS-8045 system.
- Nine levels of linearity was performed for all the six nitrosamines by internal standard method.
- Correlation coefficient was greater than 0.99 for all the six nitrosamines.
- The repeatability (n=6) at LOQ level was found to be less than 25% RSD.
- Recovery analysis was performed at LOQ level in all five sartans and it matched to the acceptance criteria between 70 to 130 %.

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