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Liquid Chromatograph Mass Spectrometer LCMS™-8045

Application News

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

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User Benefits

- An LC-MS/MS method for the determination of 6 nitrosamines in Losartan API 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 States 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 Losartan API. 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).

■ Experiment

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 (Fig. 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 is given in Fig. 2.

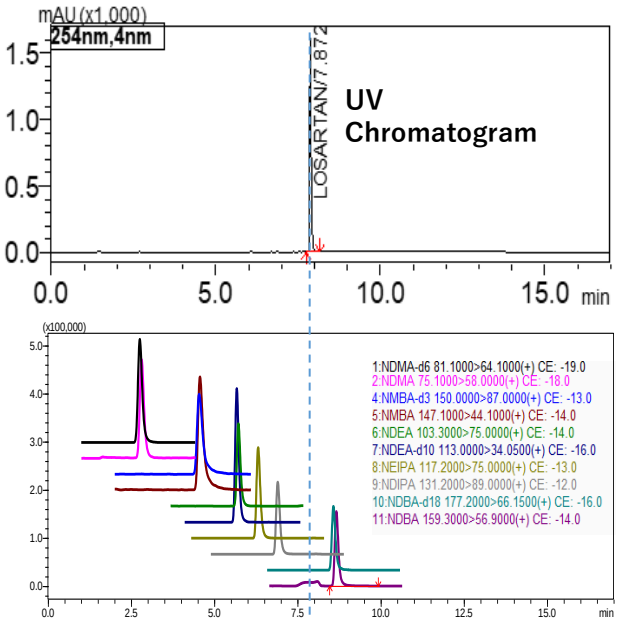

Fig. 2 Representative LCMS chromatogram of 6 NSAs and its overlapping with UV chromatogram

■ Linearity of the nitrosamines

Nine-points 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. Divert valve was employed in order to avoid Losartan API peak into the MS. The Fig. 3 to 8 depicts the calibration curves, overlay of linearity standards and LOQ solution chromatograms for NDMA, NMBA, NDEA, NEIPA, NDIPA, and NDBA, respectively.

NDMA

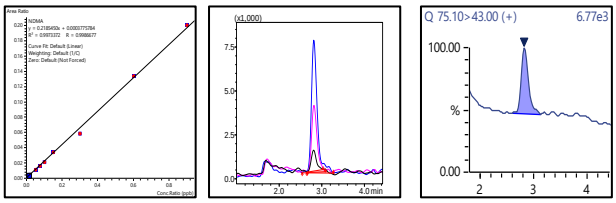

Fig.3 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NDMA

NMBA

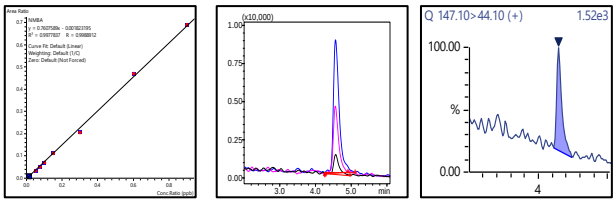

Fig.4 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NMBA

NDEA

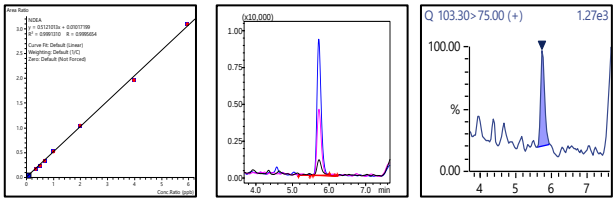

Fig.5 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NDEA

NEIPA

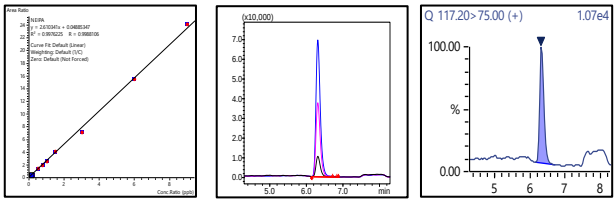

Fig.6 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NEIPA

NDIPA

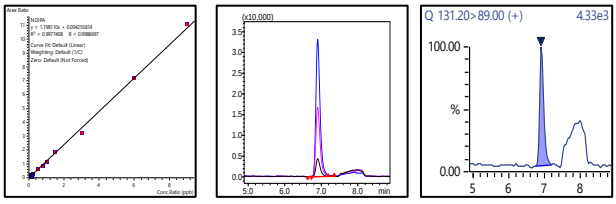

Fig.7 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NDIPA

NDBA

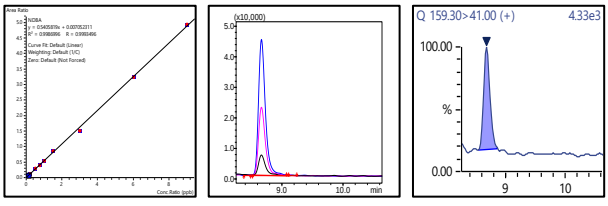

Fig.8 Calibration Curve, Overlay of Linearity Standards and LOQ Solution chromatogram for NDBA

Table 4 Summary of Calibration Curves

Comp.	CC range (ppb)	R^2	LOQ		
			Conc. (ppb)	%RSD (n=6)	S/N
NDMA	1.33 to 90	0.997	1.33	2.68	18.34
NMBA		0.997		15.66	17.54
NEIPA		0.997		10.3	37.10
NDIPA		0.997		1.85	46.80
NDBA		0.998		3.66	32-50
NDEA	0.66 to 59.4	0.999	0.66	5.90	22.10

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 5 minutes. 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 Losartan API at LOQ level is given in Table 5.

Table 5 The sample spiked study for Losartan API at LOQ level
(Results expressed are relative to sample)

Losartan API			
Name	Sample Amt. (ppb)	Found Amt. (ppb)	% Recovery
NDMA	Below LOQ	1.33	85.7
NMBA	Below LOQ	1.54	112.9
NEIPA	Below LOQ	1.19	92.5
NDIPA	Below LOQ	1.07	82.7
NDBA	Below LOQ	1.57	106.4
NDEA	Below LOQ	1.21	70.7

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

■ Conclusion

- An LC-MS/MS quantification method for six nitrosamines in Losartan API as per proposed USP general chapter <1469> (procedure-3) has been successfully applied to the Shimadzu LCMS-8045 system.
- Linearity with nine levels 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, and it matched the acceptance criteria between 70 to 130 %.

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