SHIMADZU
Excellence in Science

Liquid Chromatograph Mass Spectrometer LCMS-8045

Application News

Quantitation of 8 Nitrosamines in 12 different solvents by LC-MS/MS system

Devika Tupe, Nitish R. Suryawanshi , Jitendra Kelkar and Pratap Rasam
Shimadzu Analytical (India) Pvt. Ltd.

User Benefits

- A single LC-MS/MS method for the determination of 8 nitrosamines in 12 solvents.
- It easily meets the current regulatory requirement i.e. 0.03 ppm for the nitrosamines [EMA(European Medical Agency)].
- The same method can be extended for the other solvents which are not mentioned in the application note.

■ Introduction

Overview : In June 2018, the American Food and Drug Administration (FDA) was informed of the presence of an impurity identified as N-nitrosodimethylamine (NDMA) in the ARB valsartan. Through investigation, the Agency determined that numerous valsartan and a few other ARB drug products from different manufacturers contained unacceptable levels of nitrosamines. The drug product manufacturers voluntarily recalled the affected batches of these drug products, which led to a drug shortage in some of the affected products.

In addition, the FDA evaluated processes that use common amines in API synthesis and learned that common synthetic pathways could also introduce other types of nitrosamine impurities besides NDMA.

General Root Causes for the Presence of Nitrosamine Impurities in APIs:

1) Sources of secondary, tertiary and quaternary amines that can form nitrosamines.
2) Contamination of raw material used for the manufacturing of APIs leads to the addition of nitrosamines.
3) Recovery solvents, catalysts and reagents as sources of contaminations

A manufacturing site may produce the same API by more than one synthetic process that uses common solvents. If any of those synthetic processes produce nitrosamines or contains precursor amines, the solvents sent for recovery are at risk. The use of recovered solvents that are comingled from different processes or across manufacturing lines without control and monitoring can introduce nitrosamine impurities. If a recovered solvent is contaminated in this way and then used to manufacture an API, the API will be contaminated even if the synthetic route is not normally susceptible to nitrosamine formation.

Traditionally the GCMS is a preferred and simple technique to determine the nitrosamines in solvents. However, NMBA can only be detected by LCMS technique. In this application note an LCMS method has been developed for the simultaneous determination of 8 nitrosamines in 12 different solvents.

■ Methods

An LC-MS/MS method was developed for the detection and quantitation of eight nitrosamine impurities, including N-nitroso-dimethylamine (NDMA), N-nitroso-diethylamine (NDEA), N-ethyl-N-nitroso-isopropylamine (NEIPA), N-nitroso-diisopropylamine (NDIPA), N-nitroso-di-n-propylamine (NDPA), N-nitroso-methylphenylamine (NMPA), N-nitroso-di-n-butylamine (NDBA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA) in different solvents by using 4 internal standards referred in Table 2.

Based on the chemical properties of the solvents, three different sample preparation methods were employed.

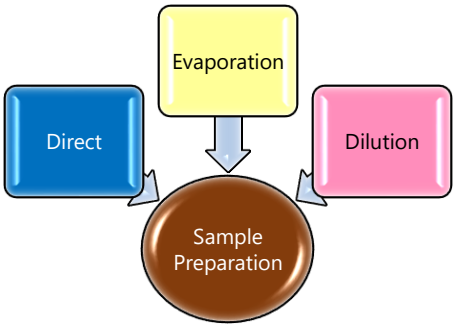

Fig. 1 Different sample preparation methods

1) **Direct method** : For water, methanol, ethanol, acetonitrile, isopropyl alcohol, and dimethyl sulfoxide (DMSO).

Linearity Standards: Prepare linearity standards of Blank, Blank + IS, and 8 NSA Mix of concentrations 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard mix; in water. For DMSO, prepare separate linearity standards in DMSO. Injection volume is 4 µL.

2) **Evaporation method** : For dichloromethane (DCM), acetone, chloroform, and ethyl acetate.

Linearity Standards: Prepare linearity standards of Blank, Blank + IS, and 8 NSA Mix of concentrations 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard mix; in water. Glycerol and methanol was added 20 µL and 50 µL respectively. Further, evaporate the samples at 40° C for 40 minutes. Reconstitute the samples in 1 mL of water. Injection volume is 10 µL.

3) **Dilution method** : For toluene and dimethyl formamide (DMF).

Linearity Standards: Prepare linearity standards of Blank, Blank + IS, and 8 NSA Mix of concentrations 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard mix; in methanol. In 1 mL of sample, add 2 mL methanol. Injection volume is 4 µL.

■ Experiment

Eight nitrosamines 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. 2).

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

Fig. 2 Nexera™ XS with LCMS-8045 system

Table 1 Analytical conditions

HPLC System	: Nexera XS
Column	: Shim-pack™ GIST C18-AQ (100 mm x 4.6 mm, 3 µm) (P/N :227-30724-05)
Column Temperature	: 40°C
Mobile Phases	: A-0.1% Formic acid in Water B-0.1% Formic acid in Methanol
Flow Rate	: 0.7 mL/min
Gradient program (B%)	: 0-2 min →10(%); 2-4 min → 10-50(%); 4-7.5 min →50-85 (%);7.5-9.5 min→85 (%);9.5-9.6 min →85-10(%);.13 min → STOP
Injection Volume	: 4 µL (Direct & Dilution) & 10µL (Evaporation)
LCMS System	: LCMS-8045
Temperature	: APCI : Interface: 350°C Desolvation Line: 200°C Heater Block: 200°C
Gas Flow	: Nebulizing Gas: 3 L/min Drying Gas: 5 L/min

Table 2 Nitrosamines with their MRM transitions

Sr.No.	Abbreviations	cas no	Type	ISTD group	MRM (quantifier)	MRM (qualifier)
1	NDMA	62-75-9	Target	1	75 > 43	75> 58
2	NMBA	61445-55-4	Target	2	147>117	147>44
3	NDEA	55-18-5	Target	3	103>29	103>45
4	NEIPA	16339-04-1	Target	2	117>75	117>27
5	NDPA	621-64-7	Target	4	131>43	131>89
6	NDIPA	601-77-4	Target	4	131>43	131>89
7	NMPA	614-00-6	Target	4	137>66	137>107
8	NDBA	924-16-3	Target	4	159>29	159>41
9*	NDMA-D6	17829-05-9	ISTD	1	81>46	-------
10*	NMBA-D3	1219794-54-3	ISTD	2	150>120	150>47
11*	NDEA-D10	1184996-41-5	ISTD	3	113>34	113>81
12*	NDBA-D18	1219798-82-9	ISTD	4	177>66	177>46

* Internal Standard (ISTD)

■ Linearity of the nitrosamines

The calibration curves for 8 NSAs were prepared from 0.001 ppm to 0.100 ppm. The recovery of 8 NSAs in different solvents were checked at three different levels i.e., 0.005 ppm, 0.010 ppm and 0.030 ppm and analyzed using the conditions described in Table 1. Representative chromatogram of 8 nitrosamines with 4 ISTD is given in Fig. 3.

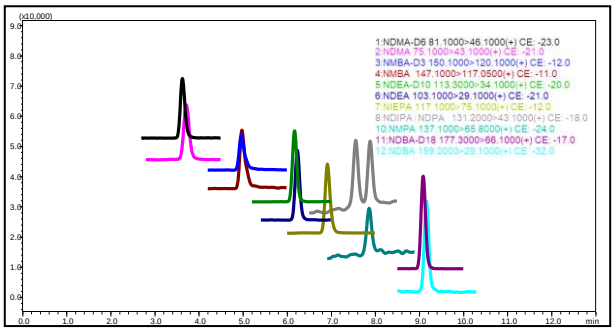

Fig. 3 Representative LCMS chromatogram of nitrosamines

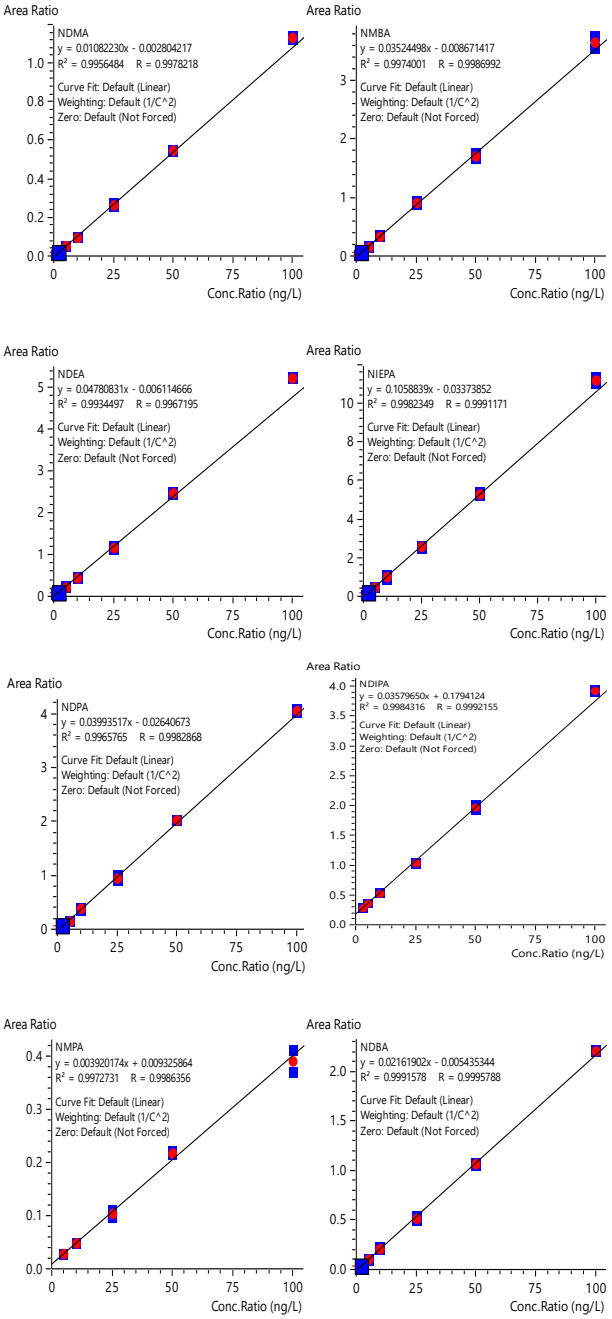

Fig. 4 Calibration curves of 8 Nitrosamines

LOQs for 8 nitrosamines at different solvents are given in Table 3 and % recoveries at these LOQ levels are given in Table 4 by using three different sample preparation techniques.

Table 3 LOQs of nitrosamines in different solvents (in ppm)

Sr. No.	Solvents\ NSA	NDMA	NMBA	NDEA	NEIPA	NDPA	NDIPA	NMPA	NDBA
1	Water	0.001	0.001	0.001	0.001	0.002	0.003	0.010	0.002
2	Methanol	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
3	Acetonitrile	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
4	IPA	0.005	0.010	0.005	0.005	0.005	0.005	0.010	0.005
5	Ethanol	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
6	DMSO	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
7	DCM	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
8	Acetone	0.005	0.005	0.005	0.005	0.010	NA #	0.010	0.005
9	Chloroform	0.005	0.005	0.005	0.005	0.005	0.010	0.010	0.005
10	Ethyl Acetate	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.005
11	Toluene	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
12	DMF	NA	0.010	0.010	0.010	0.010	0.010	0.010	0.010

NSA was found to be present, but can not be calculated.

Table 4 %recoveries of nitrosamines in different solvents at LOQ

Sr.No	Solvents\ NSA	NDMA	NMBA	NDEA	NEIPA	NDPA	NDIPA	NMPA	NDBA
1	Water	101.0	102.0	101.4	101.0	99.5	100.4	100.7	100.7
2	Methanol	101.5	98.6	100.4	98.4	103.8	110.0	106.6	94.2
3	Acetonitrile	93.0	110.8	110.5	111.6	112.1	105.6	57.4	93.4
4	IPA	115.8	92.7	116.2	107.3	98.0	80.8	99.4	95.8
5	Ethanol	118.3	108.2	112.0	106.7	97.8	91.9	101.8	101.3
6	DMSO	99.7	96.0	108.8	90.5	94.9	99.7	66.0	93.5
7	DCM	88.3	96.9	100.0	108.4	105.6	94.1	46.2	95.5
8	Acetone	88.4	91.4	95.8	99.9	94.4	NA	50.9	95.4
9	Chloroform	67.8	87.4	93.3	95.8	93.5	118.3	35.1	107.4
10	Ethyl Acetate	76.3	91.3	96.4	106.8	64.5	65.1	40.1	95.5
11	Toluene	106.4	112.4	101.3	112.9	98.8	162.5	233.9	103.0
12	DMF	NA	104.7	111.0	101.4	80.0	205.9	285.3	103.1

: Direct injection : Evaporation : Dilution

■ Discussion and Conclusion

- A single LC-MS/MS method has been developed for the determination of 8 NSA in different solvents by using the LCMS-8045 system.
- Different solvents have different chemical properties and boiling points; based on these properties three sample pretreatment methods have been developed.
- Apart from the direct method, evaporation method can also be used for solvents like methanol, acetonitrile, IPA and ethanol to enhance the sensitivity.
- For DMSO, response for a few nitrosamines has been observed to be enhanced. Hence, it is necessary to prepare the calibration standards in DMSO only.
- DMF and toluene do not give the proper peak shapes for few nitrosamines even after co-injection. Hence, the dilution method has been employed for these solvents.
- The response of DMF highly interferes with the response of NDMA. Hence, HS GCMS would be the preferred option for NDMA in DMF.
- Except NDIPA & NMPA in toluene and DMF, the rest of the nitrosamines can easily be determined by the LC-MS/MS system.
- All the solvents showed good sensitivity as well as recovery for most of the nitrosamines at the concentration <0.03 ppm. This fulfills the current regulatory requirements very easily.

06-SAIP-081-LC-40-EN First Edition: May. 2021
Revision A: Aug. 2023

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

Shimadzu Analytical (India) Pvt.Ltd.
www.shimadzu.in