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Application News

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

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User Benefits

- A single LC-M/MS method for the determination 8 nitrosamines in 12 solvents
- It easily meets the current regulatory requirement for the nitrosamines (i.e. 0.03ppm).
- 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 lots of 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, 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 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 produces 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 preferred and simple technique to determine the nitrosamines in solvents. However, NMBA can only be detected by LCMS technique. In this application note a LCMS method has been developed for the simultaneous determination 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 table2.

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

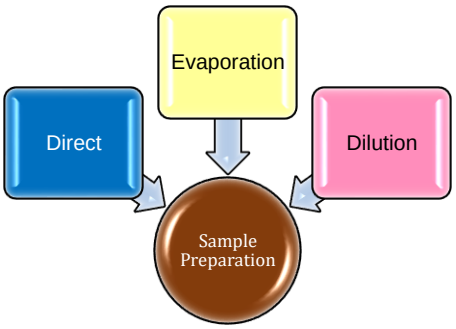

Fig. 1 Different sample preparation methods

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 4uL.

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 and evaporate the samples at 40° C for 40 minutes. Reconstitute the samples in 1 ml of water. Injection Volume is 10uL.

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

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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. Add 2 ml methanol to the samples. Injection Volume is 4 uL.

■ Experimental

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 (Figure 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 of 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 micron) (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	: 0.7mL/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) & 10uL (Evaporation)
LCMS System	: LCMS-8045
	: APCI
Température	: 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	115>69	115>41
8	NDBA	924-16-3	Target	4	137>66	137>107
9*	NDMA-D6	17829-05-9	ISTD	1	81>46	-------
10*	NDEA-D10	1219794-54-3	ISTD	2	150>120	150>47
11*	NMBA-D3	1184996-41-5	ISTD	3	113>34	113>81
12*	NDBA-D18	1219798-82-9	ISTD	4	177>66	177>46-

■ Linearity of the nitrosamines

The calibration curves for 8 NSA's were prepared from 0.001ppm to 0.100 ppm. The recovery of 8 NSA's 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 Figure 3.

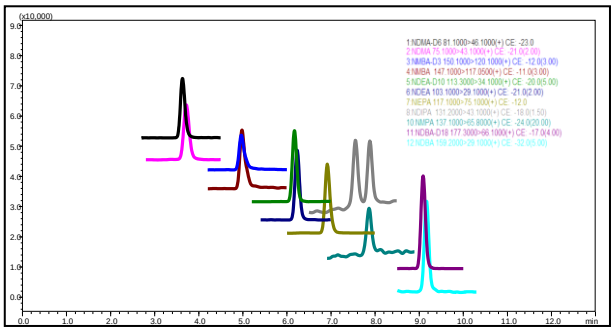

Fig. 3 Representative LCMS chromatogram of nitrosamines

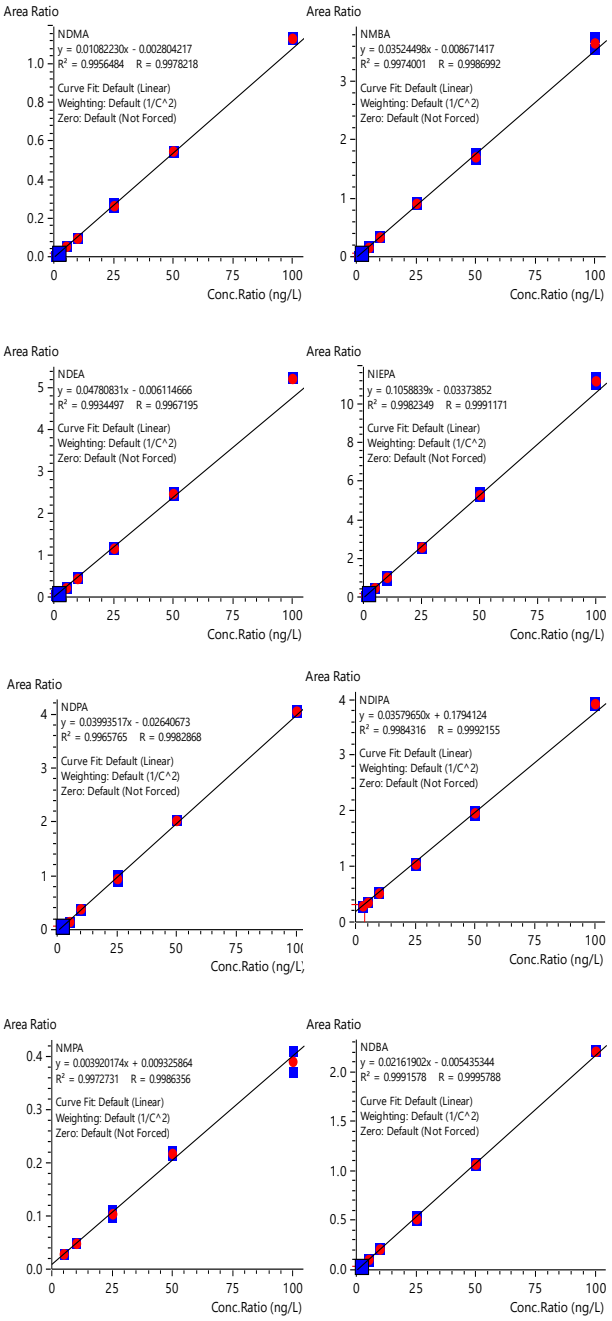

Fig. 4 Calibration curves of 8 Nitrosamines

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

Sr. No.	Solvents\ NSA	NDMA	NMBA	NDEA	NIEPA	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, hence can not be calculated.

Table 4 %recoveries of nitrosamines in different solvents at LOQ

Sr.No	Solvents\ NSA	NDMA	NMBA	NDEA	NIEPA	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 having different chemical properties and boiling points; based on these properties three sample pretreatment methods have been developed.
- Apart from the direct method, for solvents like methanol, acetonitrile, IPA and ethanol can also be determined by the evaporation method to enhance the sensitivity.
- For DMSO, it has been observed the enhanced response for few nitrosamines, hence calibration standards also necessary to be prepared 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.
- DMF gives the very high interference to NDMA hence, HS GCMS would be the preferred option for NDMA in DMF, however, rest of 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 <0.03ppm. So, it fulfills the current regulatory requirements very easily.

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