SHIMADZU

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Quantitation of NDMA, NMBA, NDEA, NEIPA, NDPA, NDIPA, NMPA and NDBA in 12 different solvents by LC-MS/MS system

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1. Overview

Nitrosamines, refer to any molecule containing the nitroso functional group. These molecules are of concern because nitrosamine impurities are probable human carcinogens. Although they are also present in some foods and drinking water supplies, their presence in medicines is nonetheless considered unacceptable.

Recovered materials such as solvents, reagents, and catalysts may pose a risk of nitrosamine impurities due to the presence of residual amines (such as trimethylamine or diisopropylethylamine). If the recovery process involves a quenching step (i.e., nitrous acid used to decompose residual azide), nitrosamines could form during solvent recovery. These nitrosamines may be entrained if they have boiling points or solubility properties similar to the recovered materials, depending on how recovery and subsequent purification takes place (e.g., aqueous washes or distillation). This further increases the risk of contamination in material recovery. For these reasons, some drug products using APIs manufactured by certain “low” risk processes were found to be contaminated[1] .

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

2. Introduction

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, 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.

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 by using 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 coming from different processes or across manufacturing lines are uncontrolled and unmonitored. This can introduce nitrosamine impurities. If a recovered solvent is contaminated as mentioned above and then used for manufacturing an API, then API will be contaminated even if the synthetic route is not normally susceptible to nitrosamine formation.

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 (refer Figure 1).

3. Methods

3-1. Sample preparation Methods

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

Linearity Standards: Prepare Blank, Blank + IS, and linearity mix standards of 8 nitrosamines at concentration levels 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard 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 Blank, Blank + IS, and linearity mix standards of 8 nitrosamines at concentration levels 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard in water. Glycerol and methanol was added 20 µL and 50 µL respectively. Further, evaporate the samples at 40 ^{0}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 Blank, Blank + IS, and linearity mix standards of 8 nitrosamines at concentration levels 1, 2, 3, 5, 10, 25, 50, and 100 ppb; each containing 30 ppb of internal standard in methanol.. In 1 mL of sample, add 2 mL methanol. Injection volume is 4 µL.

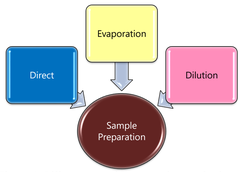

Figure 1. Different sample preparation methods

3-2. LC-MS/MS analysis

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 (Figure 3) in positive mode.

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Figure 2. Nexera XS with LCMS-8045 triple quadrupole mass spectrometer

Figure 3. APCI Probe

Table 1. Instrument parameters

UHPLC condition (Nexera XS system)	
Column	Shim-pack GIST C18-AQ (100 mm x 4.6 mm, 3 micron) (P/N :227-30724-05)
Mobile phase	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 vol.	4 µL (Direct & Dilution) & 10µL (Evaporation)
Column temperature	40 ^{0}C
MS Parameters (LCMS-8045)	
MS interface	APCI
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 5 L/min
MS temperatures	Desolvation line- 200 ^{0}C; Heating block- 200 ^{0}C; Interface- 350 ^{0}C

Table 2 Nitrosamines with their MRM transitions

Sr.No.	Abbreviations	CAS Number	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

4. Results

4.1 Linearity of the nitrosamines

The calibration curves for 8 nitrosamines were prepared from 0.001 ppm to 0.100 ppm. The recovery of 8 nitrosamines 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 ISTDs is given in Figure 4.

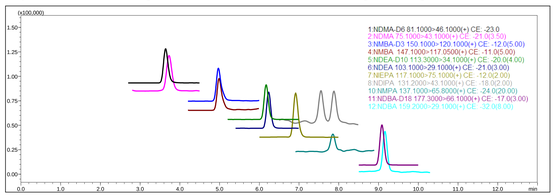

Figure 4. Representative MRM chromatogram of nitrosamines

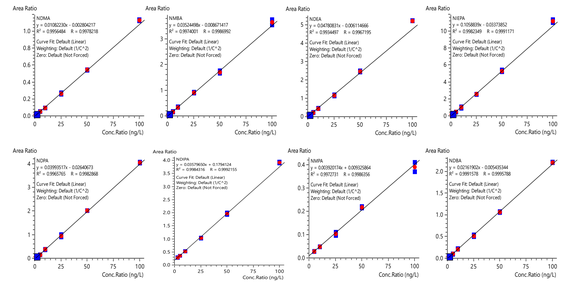

Figure 5. Calibration curves of 8 Nitrosamines

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 in different solvents (in ppm)

Sr.No	Solvents	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	0.010	0.005	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	0.010	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

Table 4 % recoveries in different solvents at LOQ (in ppm)

Sr.No	Solvents	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

5. Discussion and Conclusion

- A single LC-MS/MS method has been developed for the determination of 8 nitrosamines in 12 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, the evaporation method can also be used for the solvents like methanol, acetonitrile, IPA, and ethanol to enhance the sensitivity.
- For DMSO, response for the few nitrosamines have 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 DMF shows very high interferes to NDMA. Hence, HS GCMS would be the preferred option for the determination of NDMA in DMF.
- Except for 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.

6. References

[1] Quantitation of 8 Nitrosamines in 12 different solvents by LC-MS/MS system, 06-SAIP-081-LC-040-EN , Mar,2021.