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Application News

NO. GC-23-ADI-062

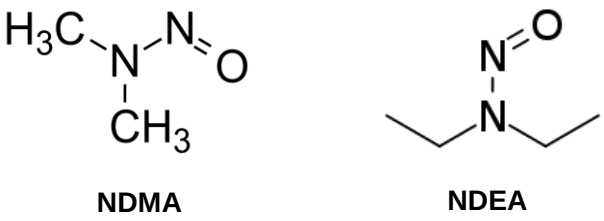
GCMS-TQ8040 NX, HS-20

Analysis of N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) in pharmaceutical substance by HSGCMS/MS

Introduction

N-Nitrosodimethylamine (NDMA) & N-Nitrosodiethylamine (NDEA) are simplest of Dialkylnitrosamines. They are continued to be released as a by-product and contaminant from various industries and from municipal wastewater treatment plants. Major releases of NDMA, NDEA have been from the manufacture of pesticides, rubber tires, alkyl amines, and dyes. Similarly, these compounds are produced as a byproduct in the manufacturing of Active Pharmaceutical Ingredients (API's). These compounds are classified as a Group 2A carcinogen (probable human carcinogen) by the World Health Organization.

Recently some drug products were discovered to have been contaminated with NDMA & NDEA. It is believed to have been introduced into the finished products as a result of the manufacturing process of the drug substance. This contamination, was far exceeding the regulatory exposure limits specific to drug products. Consequently, medical agencies across Europe as well as the US Food and Drug Administration (USFDA) withdrew all affected drug products from the market. Hence it is very essential to have a sensitive, specific, accurate, reliable & robust method by using suitable analytical technique. In this experiment the In this experiment the pharmaceutical API's namely Valsartan, Losartan & Olmesartan-Medoximil prone to contamination with NDMA and NDEA are analyzed by using developed HSGCMS/MS method. Refer figure 1 for structure of NDMA & NDEA.



NDMA **NDEA**

Figure 1: Structure of NDMA & NDEA

Experimental:

This study was conducted using a Shimadzu GCMS-TQ8040 NX with HS-20 Headspace autosampler. The instrument was operated in constant linear velocity mode, equipped with a Stabilwax DA capillary column providing the best chromatographic resolution, symmetrical peak shapes, and enhanced sensitivity for target analytes.

A standard mixture of NDMA & NDEA was used to develop MRM method which was further used to analyze samples. The standard mixture was used to prepare calibration curves ranging from 1.0 – 50 µg/L. The standards were prepared in N,N- Dimethyl sulfoxide. The instrument operating conditions are shown in Table 1.

Table 1: GCMS-TQ8040 NX Operating Conditions

Instrument Details	Shimadzu GCMS-TQ8040 NX with HS-20		
GC Parameters			
Column Details	Stabilwax DA, 30 m, 0.32 mm ID, 1.0 µm df		
Injection Mode	Split		
Flow Control Mode	Linear Velocity		
Detector	Mass spectrometer		
Carrier Gas	Helium		
Column Flow	2.50 mL/min		
Linear Velocity	57.0 cm/sec		
Temp. Program	Ramp Rate (ºC/min)	Temp. (ºC)	Hold Time (min)
	-	40.0	0.0
	8	140.0	0.0
	25	230.0	3.9
Diluent	N, N-Dimethyl sulfoxide		
HS Parameters			
Mode	Loop (1 mL)		
Oven Temp.	160 ºC		
Sample Line Temp.	165 ºC		
Transfer Line Temp.	175 ºC		
Shaking Level	5		
Pressurizing Gas Pressure	80 kPa		
Equilibrating Time	25.0 min		
GC Cycle Time	30.0 min		
MS Parameters			
Ion Source Temperature	200 ºC		
Ionization Mode	EI		
Mode	MRM		
Detector Voltage	Set relative to tune + 0.8 kV		
Electron Voltage	70 eV		

MRM method development:

A mixture of NDMA & NDEA standards were procured from Restek®. For MRM optimization, about 5 ppm individual standard mixtures were analyzed separately using scan mode. For individual components, precursor ions were selected. Using selected precursor ions, product ion scan was performed with different Collision Energies (CE)
For each component, MRM transitions with appropriate CE's were determined. All the above steps were simplified with the help of Shimadzu's ''MRM optimization tool''. These MRM transitions were registered to Smart Database and the final MRM method with optimum segments was generated & used for analysis. Refer Table 2 for optimized MRM's with CE.

Table 2: Optimized MRM's with optimized CE's.

Compound Name	MRM Transitions	CE's
NDMA	74.00>73.00	29
	74.00>59.00	35
	74.00>69.00	37
NDEA	102.00>85.10	5
	102.00>56.10	21
	102.00>87.00	9

Linearity of the Calibration Curve:

A six-point calibration curve from 1.0 to 50 µg/L was analyzed using the conditions described in Table 1. The retention times, correlation coefficient & LOQ established from S/N and % RSD are shown in table 3. The calibration curves established for both components with (r^2) greater than 0.999 for calibration levels 1.0, 2.5, 5.0, 10.0, 20.0 & 50 ppb shown in Figure 2. Figure 3, 4 & 5 depicts chromatographic overlay of all the linearity levels, 6 replicates of LOQ solution (1.0 ppb) & Working solution (10.0 ppb) respectively.

Table 3: LOQ summary

Component	R.T. (min)	LOQ (1.0 ppb)		r^2
		S/N	% RSD	
NDMA	10.57	30	8.9	0.999
NDEA	11.89	238	8.2	0.999

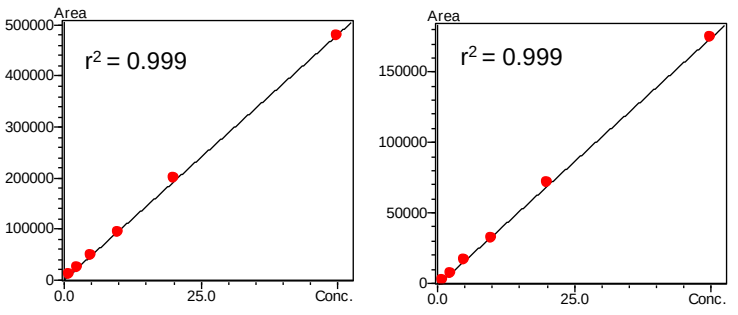


Figure 2: Calibration Curves from 1.0 ppb to 50 ppb

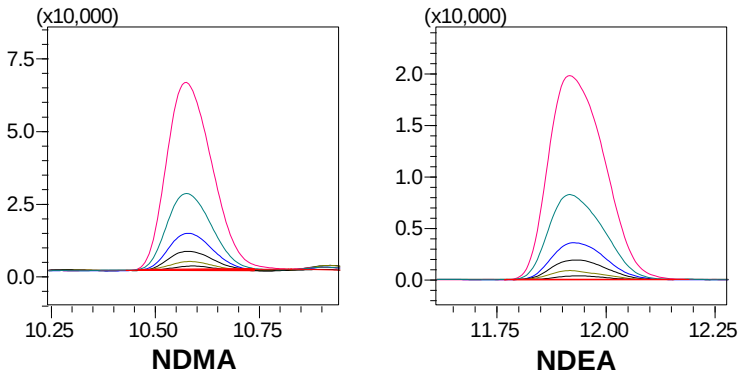


Figure 3: Chromatographic overlay from 1.0 ppb to 50 ppb

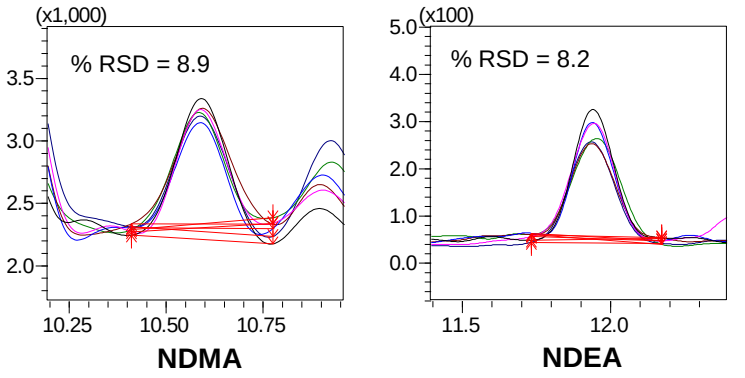


Figure 4: Chromatographic overlay of 6 replicates (LOQ 1 ppb)

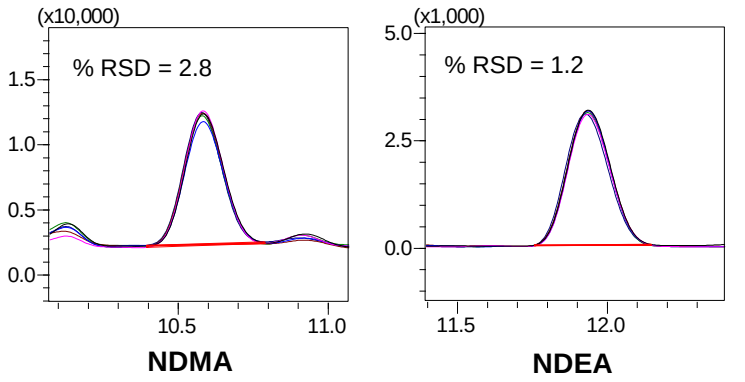


Figure 5: Chromatographic overlay of 6 replicates (10.0 ppb)

Sample Analysis:

Three API samples namely Valsartan, Losartan & Olmesartan-Medoximil were individually weighed in a 20 ml headspace vial to which added 2 ml of DMSO as diluent to make 2 % w/v solution.

Spiked Recovery Test:

Samples of individual API's were weighed in a 20 ml headspace vial which were spiked with 2 ml 1.0 ppb, 10.0 ppb & 50.0 ppb NDMA & NDEA standard solutions respectively and were subjected for HSGCMS/MS analysis.

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Table 3, 4 & 5 shows results of the sample analysis and accuracy study for 3 API's

Valsartan API				
Conc.	Sample Amount (ppb)		% Recovery	
	NDMA	NDEA	NDMA	NDEA
1.0 ppb	ND	117	109.5	85.8
10.0 ppb			97.4	100.5
50.0 ppb			103.7	105.9

Losartan API				
Conc.	Sample Amount (ppb)		% Recovery	
	NDMA	NDEA	NDMA	NDEA
1.0 ppb	ND	73.5	101.2	83.0
10.0 ppb			101.9	99.1
50.0 ppb			106.4	110.4

Olmesartan-Medoximil API				
Conc.	Sample Amount (ppb)		% Recovery	
	NDMA	NDEA	NDMA	NDEA
1.0 ppb	ND	ND	113.3	119.1
10.0 ppb			114.2	96.1
50.0 ppb			119.4	100.9

Conclusion:

- A highly sensitive method was developed for quantitation of genotoxic impurities namely NDMA & NDEA in pharmaceutical API's by using Shimadzu GCMS-TQ8040 NX with HS-20 headspace autosampler.
- The MRM method developed can be used for screening of NDMA & NDEA in various Pharmaceutical products.
- Ultra Fast scanning, UFsweeper® and ASSP™ features enabled sensitive, selective, fast, reproducible, and linear method of analysis.

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