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Application News

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Drug Contaminants/ HS-20 GCMS-QP2020 NX

Determination of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in Drug Products by Headspace GC-MS

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❑ Introduction

The detection of probable human carcinogens, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in valsartan manufactured by a pharmaceutical company in China has recently led to an EU-wide review of all valsartan medicines [1]. The presence of trace amounts of nitrosamines in foods has been known to be produced from chemical reactions between secondary amines and nitrites [2]. Valsartan is used for treatment of hypertension, congestive heart failure, and to increase the chances of living longer after a heart attack. In the following months, the review was subsequently extended to other 'sartan' medicines when low levels of NDEA were found in losartan and irbesartan made by pharmaceutical companies in India. Examples of other 'sartan' medicines include candesartan and olmesartan. To detect the presence of impurities NDEA and/or NDMA in valsartan drug products, FDA released two headspace GC-MS methods [3, 4], both of which should be validated by the user if the resulting data are used to support a required quality assessment of the API or drug product, or if the results are used in a regulatory submission. With reference to the methods released by FDA, a headspace (HS) GC-MS method using HS-20 and GCMS-QP2020 NX was developed with some modifications.

❑ Experimental

Instruments Used and Analytical Conditions

Experiments were conducted using HS-20 and GCMS-QP2020 NX, utilizing electron ionization via FASST (Fast Automated Scan/SIM Type) mode, which enables simultaneous Scan/SIM acquisitions. In this way, both scan and SIM chromatograms are obtained in a single analysis. A GC column of SH-Stabilwax™ (30 m length, 0.25 mm I.D., 0.5 µm film thickness) was used in this study. Method parameters were referenced from the headspace (HS) GC-MS method for combined NDMA and NDEA determination, published by FDA, with some modifications to suit the study [4]. The details of analytical conditions are shown in Table 1.

Figure 1. GCMS-QP2020 NX and HS-20

Table 1. Headspace and GC-MS analytical conditions

[HS-20 Loop Mode]	
Pressurizing Gas Pressure	: 116.0 kPa
Oven Temp.	: 160°C
Sample Line Temp	: 170°C
Shaking Level	: 2
Equilibrating Time	: 45 min
Pressurizing Time	: 0.50 min
Load Time	: 0.50 min
Injection Time	: 1.00 min
Loop Volume	: 1 ml
[GC]	
Column Temp.	: 70°C (4 min) → 20°C/min to 240°C (3.5 min)
Injection Mode	: Split mode (split ratio 5)
Carrier Gas	: Helium
Carrier Gas Control	: 63.5 cm/sec (Constant linear velocity)
[MS]	
Ion Source Temp.	: 230°C
Interface Temp.	: 240°C
Acquisition Mode	: FASST (Fast Automated Scan/SIM Type)
Event time	: 0.10 sec (scan), 0.10 sec (SIM)

Methods and Chemicals

A 100 µg/ml mixed stock standard solution of NDMA and NDEA in dimethyl sulfoxide (DMSO) was prepared from commercially available NDMA and NDEA standard solutions. From the 100 µg/ml stock solution, standard solutions of 0.025, 0.050, 0.10, 0.50, 5.0, 10 and 100 µg/ml were prepared. Calibration working standards were prepared by transferring 1 ml of the standard solutions into individual 20 ml headspace vials containing 4 ml of DMSO. Headspace vials were capped and crimped immediately. This would produce 0.025, 0.050, 0.10, 0.50, 5.0, 10 and 100 µg calibration working standards.

For sample preparation of drug product, one tablet of each drug product was crushed into powder form and weighed into a 20 ml headspace vial. 5.0 ml of DMSO was added into the vial and crimped immediately. For recovery checks, one crushed tablet was weighed into a 20 ml headspace vial. 1 ml of working standard solution and 4 ml of DMSO was transferred into the respective vial. Headspace vial was capped and crimped immediately.

❑ Results and Discussion

The elution order of NDMA and NDEA is shown in Figure 2. The monitoring ions of NDMA and NDEA are m/z 74 and m/z 102, respectively. To allow further confirmation of the targeted nitrosamines, analyses were performed in FASST mode. In this way, the highly sensitive and selective SIM data was acquired for calibration and quantitation, whereas the scan data was used for identification confirmation via mass spectra search. Therefore, any positive detection(s) of NDMA and NDEA (in drug products) could be verified via retention time, presence of targeted ion(s) and similarity to standard mass spectra.

Calibration

The NDMA and NDEA peak areas were plotted against working standard concentrations, 0.025, 0.050, 0.10, 0.50, 5.0, 10.0 and 100 µg. Two calibration curves were plotted for each nitrosamine and all linear calibration curves displayed correlation coefficients, $R^2 \geq 0.999$, which fulfils the FDA requirement of $R^2 \geq 0.995$. The low-range calibration curve was plotted from 0.025 – 0.50 µg and the high-range calibration curve was plotted from 0.025 – 100 µg (Figure 3).

This is in accordance to FDA recommendations, which state that NDMA and NDEA peak areas ≤ 0.50 µg working standard peak area should be quantitated using the 0.025 – 0.50 µg calibration curve, and peak areas larger than that of 0.50 µg working standard should be quantitated using the 0.025 – 100 µg calibration curve.

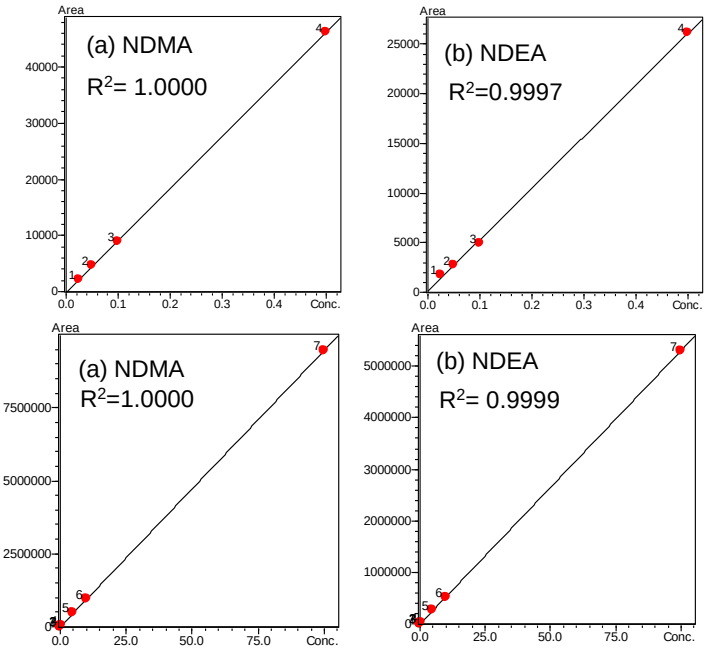

Figure 3. Calibration curves for low range of 0.025~0.50 µg (top) and high range for 0.025~100 µg (bottom)

Repeatability

Peak area repeatability of NDMA and NDEA (n=6) at 0.025 and 0.050 µg, were assessed (Table 2). The low %RSD values showed that the HS GC-MS method was repeatable at the two lowest calibration levels.

Table 2. %RSD (n=6) of peak area at 0.025 and 0.050 µg calibration levels

	NDMA		NDEA	
Working standard (µg)	0.025	0.050	0.025	0.050
%RSD (n=6)	2.55%	2.81%	5.09%	3.66%

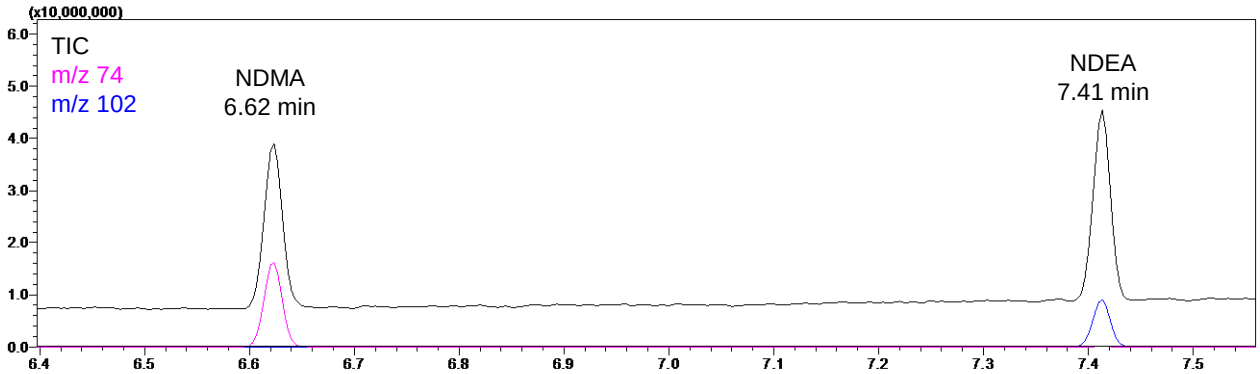

Figure 2. Chromatogram of 50 ug/ml NDMA and NDEA standard solution

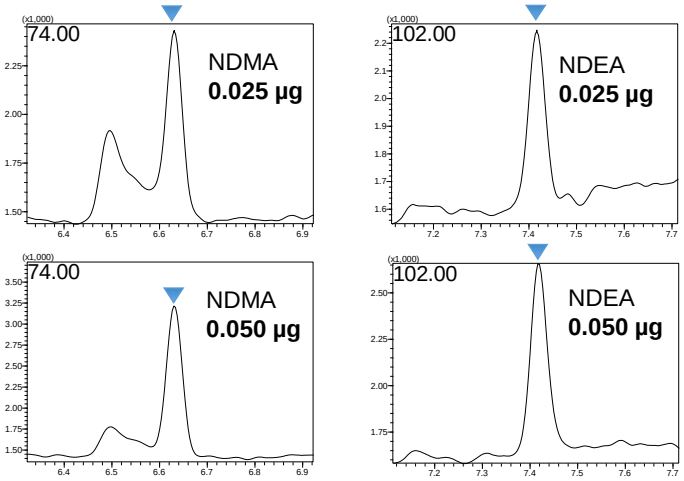

Figure 4. SIM mass chromatograms of working standard

Analyses of Drug Products

Four drug products (A to D) of different 'sartan' medicines and API (Active Pharmaceutical Ingredient) weights were tested (Table 3). The concentration of NDMA or NDEA in the API is calculated using following formula:

NDMA or NDEA (ppm) = [(y–b) /m] ÷ wt.

where: y = NDMA or NDEA peak area
b = intercept of the linear curve
m = slope of the linear curve
wt. = weight of API (g)

According to FDA, the NDMA or NDEA must be reported when it is detected above the LOQ (NDMA LOQ = 0.10 ppm ; NDEA LOQ = 0.05 ppm).

All four drug products showed negative detection of the targeted nitrosamines. Hence, they were used as blank matrices for recovery checks. Details of drug products are shown in Table 3.

Table 3. Drug products and APIs tested

Drug Product	API	Weight of API per tablet (mg)
A	Olmesartan	40
B	Losartan	100
C	Irbesartan	150
D	Valsartan	160

Recovery Checks

To assess the efficiency of the headspace pre-treatment of drug products, recovery checks were conducted. 0.025, 0.050, 10 and 50 µg of NDMA and NDEA were spiked into the mixtures of DMSO with one crushed tablet of each drug product. DMSO blanks were analysed after sampling of each drug product. 0.50, 10 and 20 µg working standards were prepared as QC samples, and were analysed after every eight sample runs, including blanks. QC samples were analysed for continuing calibration check (CCC) purposes. As the CCCs were well within 70-130% of the true values, the same calibration curves were applied for all recovery check results. Recovery% of the spiked samples were derived from quantitation results using low-range or high-range calibration curves. Calibrated standard concentrations (µg) after HS GC-MS analyses were compared with spiked concentrations (µg). Recoveries of spiked and QC samples were no less than 70%. The results of recovery check is shown in Table 4.

Table 4. Summary of recovery check results in drug products and QC samples

Spiked Conc. in Drug Product (µg)	QC Std Conc. in DMSO ((µg)	% Recovery									
		NDMA					NDEA				
		A	B	C	D	QC	A	B	C	D	QC
0.025		76.7	70.6	72.7	70.4		79.8	84.5	78.0	79.9	
	0.05					89.9					87.9
0.05		79.6	110.4	73.0	85.4		105.7	87.4	80.3	73.2	
	0.5					92.3					85.4
	10					87.4					88.4
10		90.0	91.3	91.5	93.4		98.8	93.9	93.0	95.8	
	20					83.0					85.7
50		99.3	95.0	93.7	95.3		104.6	97.0	96.9	98.6	

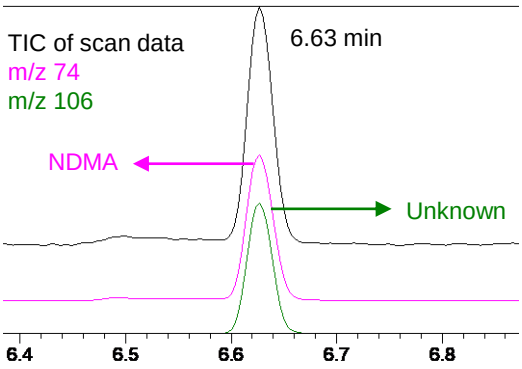

Figure 6. NDMA peak of 10 µg spiked drug product B

Identification of Interferences by Deconvolution

In this study, NDMA and NDEA were quantitated using the SIM data, i.e. peak areas of the monitoring ions m/z 74 and m/z 102, respectively. In addition, the mass spectra of scan data at the retention times of NDMA and NDEA were also matched against NIST library for confirmation. However, for the spiked drug analyses, it was noticed that there is a matrix interference of the scan data (m/z 106) at the retention time of NDMA (Figure 6). As a result, spectrum match of NDMA was low in the spiked drug samples at 6.63 min (Figure 7).

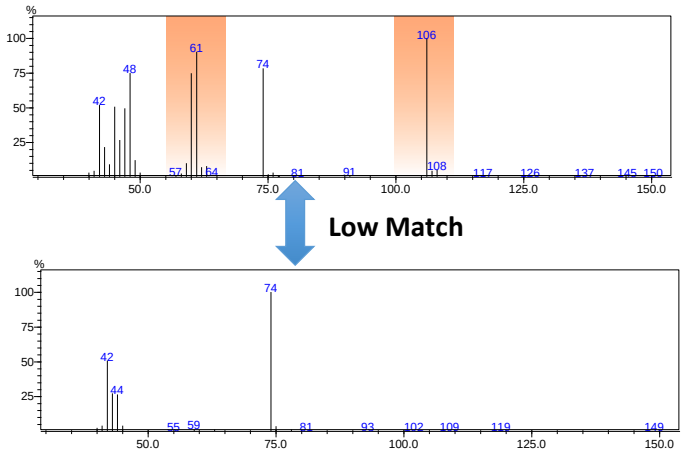

Figure 7. Mass spectrum of scan data at the retention time of NDMA in spiked drug product (top) and standard mass spectrum of NDMA (bottom). Orange: Interferences

Peak deconvolution of a scan mass spectrum can be useful for differentiation and identification of targeted analytes from interference(s). Figure 8 shows the peak deconvolution result performed using SmartDalton™ software [5]. Mass spectra of Est. #1 was obtained after deconvolution (Figure 9). A library match of the deconvoluted mass spectrum gave a good and conclusive match to NDMA. The R.Match (reverse match) value was 791 out of 1000 (to NDMA).

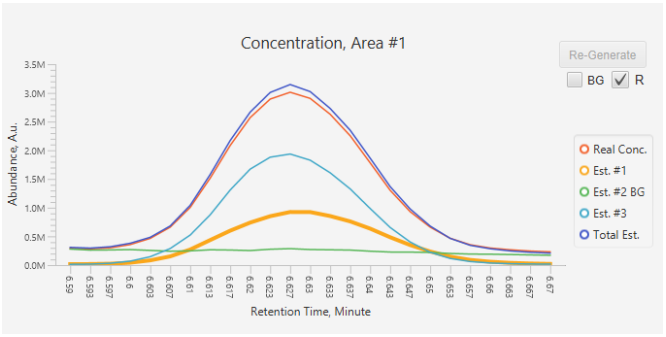

Figure 8. Results of peak deconvolution. Est #1 and #3 correspond to NDMA and unknown, respectively.

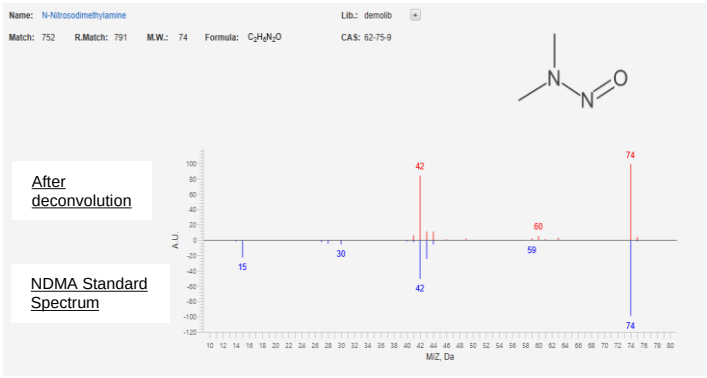

Figure 9. NIST matching of mass spectrum Est. #1 after deconvolution to NDMA standard mass spectrum

Conclusions

A sensitive and reliable HS GC-MS method for pre-treatment and analysis of NDMA and NDEA impurities in 'sartan' drug products was developed and its performance was evaluated with spiked samples of drug products. The method exhibits excellent repeatability and good recoveries at LOQ level. Peak deconvolution using SmartDalton software was applied for accurate library matching in confirmation of NDMA in the presence of significant interference.

References

1. European Medicines Agency, Science Medicines Health: EU authorities take further action in ongoing review of sartans: Zheijiang Huahai placed under increased supervision; Aurobindo Pharma stopped from supplying irbesartan to the EU (Press release 15/10/2018)
2. RONALD, S. KIRK and RONALD SAWYER. Pearson's composition and analysis of foods. 9th ed. United Kingdom: Pearson Education, 1991.
3. FDA U.S. Food & Drug Administration, Center for Drug Evaluation and Research: GC/MS Headspace Method for Detection of NDMA in Valsartan Drug Substance (2018)
4. FDA U.S. Food & Drug Administration, Center for Drug Evaluation and Research: Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS-Headspace
5. http://sg.chemopower.com/smartdalton/

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