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Application News

No. AD-0199

Drug Contaminants / GCMS-TQ8050 NX

Determination of Nitrosamine Impurities in Sartan Drug Products by GC-MS/MS Method

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❑ Introduction

Drugs containing sartan APIs (active pharmaceutical ingredients), such as valsartan, candesartan, irbesartan, losartan and olmesartan, are used for treatment of hypertension and heart failure. Recently, US FDA has released findings of probable human carcinogens, N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) in sartan drug products and the affected medicines have been recalled. These nitrosamine impurities can form during the production of sartans that contain a functional group known as a tetrazole ring (Figure 1) under certain conditions. Additionally, the presence of the impurities in some sartans is possibly due to uses of contaminated equipment or reagents in the manufacturing process [1].

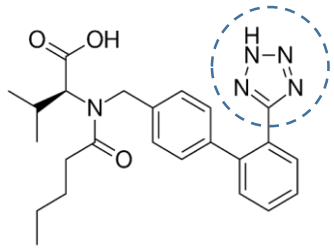

Figure 1. Chemical structure of valsartan with a tetrazole ring

To detect the presence of the nitrosamines impurities, US FDA has released two methods based on headspace GC-MS and direct liquid injection GC-MS/MS [2]. With reference to the direct injection method, a comprehensive GC-MS/MS method was established and evaluated for analysis of seven nitrosamines including the three impurities (NDMA, NDEA and NDBA) listed by FDA and four other nitrosamines (NMEA, NDPA, NPYR and NDIP) which are known to be probable human carcinogens. Four sartan drug products, which contain olmesartan, losartan, irbesartan and valsartan are used as samples and matrices.

❑ Experimental

Instruments Used and Analytical Conditions

Experiments were conducted via GCMS-TQ^{TM}8050 NX, EI (utilizing electron ionization) - MRM (multiple reaction monitoring) acquisition mode. A GC column of SH-Rxi^{TM}-624Sil MS (30 m length, 0.25 mm I.D., 1.40 µm film thickness) was used in this study. Method parameters and sample preparation procedures were referenced to the FDA short form report titled Combined Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS [3]. Necessary modifications in analytical settings on the GC-MS/MS were made to suit this study. Details of the analytical conditions are shown in Table 1.

Table 1. GC-MS/MS analytical conditions

[GC]		
Column Temp.	:	38°C (1 min) → 12°C/min to 160°C → 5°C/min to 200°C (1 min)
Injection Conditions	:	Splitless, sampling time 2 min; High pressure injection (250 kPa, 2 min)
Injection Volume	:	2 µl
Carrier Gas	:	Helium
Carrier Gas Control	:	42.6 cm/sec (Constant linear velocity)
[MS]		
Ion Source Temp.	:	230°C
Interface Temp.	:	280°C
Acquisition Mode	:	MRM (multiple reaction monitoring)
Ionisation Type	:	Electron ionisation (EI)

Preparation of Standards and Drug Products

Seven nitrosamines including three listed by FDA are analyzed in this study (Table 2). A mixed stock standard solution of each nitrosamine in 200 ng/ml was prepared from a 1000 µg/ml commercially available standard solution in dichloromethane (DCM) . From the mixed stock solution, calibration standard solutions of 2.5, 5.0, 10, 25, 50, 80 and 100 ng/ml were prepared. An internal standard, NMDA-D6, was added at a concentration of 50 ng/ml to the calibration standard solutions.

Four sartan drug products (A, B, C and D) were used in this study. The type and content of API per tablet in these drug products are shown in Table 3. One tablet of a drug product was crushed into powder form and weighed into a 15 ml centrifuge tube. Adding 4.5 ml of extraction solvent (DCM) and 0.5 ml of internal

Table 2. Compound information of nitrosamines*

No.	Target Compound	Acronym	CAS No.
1	N-Nitrosodimethylamine	NDMA	62-75-9
2	N-Nitrosodiethylamine	NDEA	55-18-5
3	N-Nitrosodi-n-butylamine	NDBA	924-16-3
4	N-Nitrosomethylethylamine	NMEA	10595-95-6
5	N-Nitrosodi-n-propylamine	NDPA	621-64-7
6	N-Nitrosopyrollidine	NPYR	930-55-2
7	N-Nitrosopiperidine	NPIP	100-75-4

** Nos 1, 2, and 3 are the targeted nitrosamine impurities listed by US FDA.*

Table 3. Drug products and APIs tested for nitrosamines

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

standard (NDMA-D6, 0.5 µg/ml), the mixture was vortexed for 1 min, followed by centrifuging at 4000 rpm for 4.5 min. Using a disposable syringe, approximately 1 ml of the DCM layer was filtered through a 0.45 µm Nylon filter. All samples were stored at 4°C before GC-MS/MS analyses.

❑ Results and Discussion

The elution order of the seven nitrosamines and ISTD is shown in Figure 2. All the analytes were monitored via one quantitative MRM transition and one or two qualitative MRM transitions for confirmation. The details of MRM transitions, CE values and GC-MS/MS method for quantitation of the seven nitrosamines are compiled in Table 4.

Four identification criteria were applied for reporting of positive detection(s) (if any) in drug products tested. The four identification criteria were as follows:

(1) ±0.10 min deviation of absolute retention time
(2) Presence of quantitative MRM transition
(3) Presence of qualitative MRM transition(s)
(4) The relative intensity% of the qualitative MRM transition(s) should fall within ±30% of the set ion ratio.

Table 4. MRM parameters and GC-MS/MS method for quantitative determination of nitrosamines with ISTD

Compd.	MRM transition	CE (V)	Ion Ratio (int)	RT (Min)	Cali Range (ng/ml)	R^2	%RSD (n=6) 5 ng/ml	%RSD (n=6) 10 ng/ml	S/N Ratio 5 ng/ml
NDMA	74.10 > 44.10 74.10 > 42.10	6 18	100 30.1	7.27	2.5 – 100	0.9995	4.37	2.31	323
NDEA	102.10 > 85.10 102.10 > 56.10	6 16	100 29.7	9.88	2.5 – 100	0.9998	3.42	1.77	3511
NDBA	116.10 > 99.10 158.20 > 99.10 158.20 > 116.10	6 8 4	100 20.7 16.7	16.12	2.5 – 100	0.9999	3.96	1.74	2666
NMEA	88.10 > 71.10 88.10 > 43.00 88.10 > 57.10	6 8 10	100 51.2 21.6	8.73	2.5 – 100	0.9997	1.79	3.50	1849
NDPA	130.10 >113.10 130.10 > 88.10	4 4	100 21.2	12.60	2.5 – 100	0.9993	5.61	4.25	2445
NPYR	100.10 > 55.10 100.10 > 68.10	8 8	100 23.3	12.98	2.5 – 100	0.9991	2.68	5.16	141
NPIP	114.10 > 84.10 114.10 > 97.10	8 6	100 67.1	13.32	2.5 – 100	0.9997	5.27	2.67	1262
NDMA-D6 (ISTD)	80.10 > 50.10 80.10 > 46.10	6 18	100 24.0	7.23	50	NA	NA	NA	-

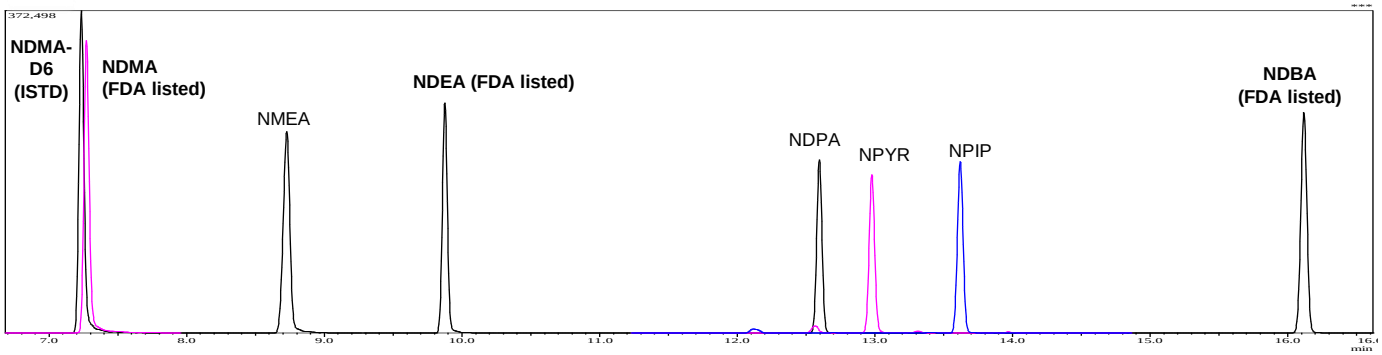

Figure 2. MRM chromatograms of 50 ng/ml nitrosamines and ISTD. Quantitative MRM transition of each analyte is displayed.

Calibration Curves and System Suitability

The ratios of the nitrosamines peak area to that of the ISTD peak area were plotted against standard concentrations, 2.5, 5.0, 10, 25, 50, 80 and 100 ng/ml. All linear calibration curves displayed correlation coefficients, $R^2 \geq 0.999$, which fulfils the FDA requirement of $R^2 \geq 0.998$. Calibration curves of the three nitrosamines listed by US FDA are displayed in Figure 3. The MRM peaks of 5 ng/ml of the three nitrosamines are also shown in Figure 3.

As requirement of system suitability, the signal to noise (S/N) ratio of the 5.0 ng/ml linearity standard should be ≥ 10. As shown in Table 4, the S/N values calculated by the Peak to Peak method are significantly greater than the requirement. The repeatability %RSD (n=6) measured by peak area ratio at 5.0 and 10 ng/ml is between 1.74% and 5.61% (Table 4).

Analyses of Drug Products

Four drug products of different sartan medicines and APIs were tested (Table 3). According to the referenced FDA method, the concentration of detected nitrosamine(s) (NS) in a drug product is calculated following this formula:

$$NS\ (ppm) = [\ (y - b)\ /m\ ] \times EV \times 1\ \mu g/1000\ ng \div wt.$$

where: y = Nitrosamine to ISTD response factor
b = intercept of the linear curve
m = slope of the linear curve
EV = Extraction Volume (5 ml)
wt. = weight of API in drug product (g)

The analysis results with application of the four identification criteria described previously, indicate that all four drug products A, B, C and D are free of the targeted nitrosamines including NDMA, NDEA and NDBA. It is noteworthy that a DCM blank was analysed after each sample run to check for carry-over. As SOP, a QC sample of 10 ng/ml calibration standard was analysed after analyses of the above-mentioned drug products. Results of the QC sample showed that the quantitated values of the nitrosamines were well-within 70 – 130% of the 10 ng/ml calibration standard, except NPIP and NDBA, which displayed higher recovery% of 137% and 133%, respectively.

It was observed that after analyses of the DCM extracts of the drug products, a significant rise of baseline of MRM transition(s) occurred. This can be attributed to the complexity of each sample matrix. Figure 4a shows one example, a high and very noisy baseline was observed with the qualitative MRM of NDPA. In this case, column conditioning was

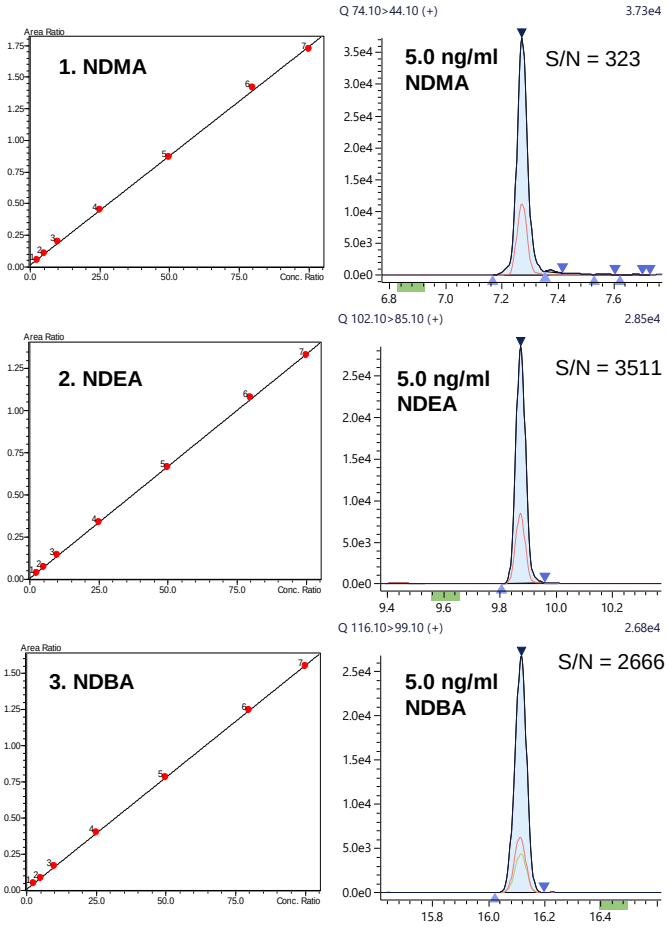

Figure 3. Calibration curves (ISTD method) and MRM chromatogram of 5.0 ng/ml nitrosamines.

carried out to remove contaminants from the column, and hence recover the column performance (Figure 4b).

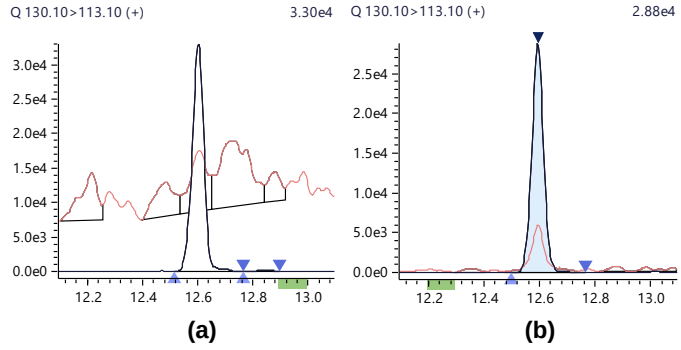

Figure 4. MRM baseline rise of NDPA (2.5 ng/ml) due to column contamination (left) and full recovery after column conditioning (right).

Instrument Recovery and Matrix Effect

Instrument recovery checks were conducted by adding known concentrations of nitrosamines into extracted solutions of Olmesartan drug product A. Standard solutions of 5, 10 and 50 ng/ml were added into 1 ml of DCM extract obtained from drug product A.

Instrument recovery% of nitrosamines was calculated according to formula below:

$$\text{Inst. recovery\%} = \frac{\text{Conc.of post-spiked sample}}{\text{True value}} \times 100$$

where 'Conc. of post-spiked sample' was the quantitated value obtained from neat (DCM) calibration curves

Additionally, matrix effect was also evaluated at concentrations of 5, 10 and 50 ng/ml. Matrix effect was calculated by comparing peak area ratios of post-spiked nitrosamines in the Olmesartan drug matrix and of that in DCM.

$$\text{Matrix effect\%} = \left(\frac{A_{Post}}{A_{DCM}}\right) \times 100$$

where
A_{Post} = Peak area ratio of post-spiked nitrosamine
A_{DCM}= Peak area ratio of nitrosamine in DCM

Overall, the matrix effects were above 100% and there is a good co-relation between the instrument recovery% and matrix effect% (Table 6). Therefore, it can be inferred that matrix enhancement could be the cause of a higher than 100% instrument recovery. These findings also suggest that a matrix-matched calibration could yield more accurate quantitation results.

Table 6. Summary of instrument recovery (Rev%) and matrix effect (ME%)

Compd.	5 ng/ml		10 ng/ml		50 ng/ml	
	Rev%	ME%	Rev%	ME%	Rev%	ME%
NDMA	111	105	121	113	114	116
NMEA	121	117	124	121	122	124
NDBA	144	143	150	145	144	144
NDEA	105	103	116	111	116	117
NDPA	111	106	127	120	126	126
NPYR	139	131	136	124	125	127
NPIP	120	118	135	128	134	134

❑ Conclusions

A sensitive and reliable GC-MS/MS method with direct injection has been developed for analysis of seven nitrosamines in sartan drug products. The seven nitrosamines analysed include the three impurities (i.e., NDMA, NDEA and NDBA) listed by US FDA. Four sartan drug products of olmesartan, losartan, irbesartan and valsartan were used as samples and matrices in method performance evaluation. This reported method is able to achieve the system suitability requirements as stated by FDA. Additionally, the method displayed highly sensitive and repeatable results with high S/N ratios and low %RSD values at 5 ng/ml spiked samples for NDMA, NDEA and NDBA.

❑ References

1. Sartan medicines: companies to review manufacturing processes to avoid presence of nitrosamine impurities EMA/44960/2019
2. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartan-losartan
3. FDA U.S. Food & Drug Administration, Center for Drug Evaluation and Research: Combined Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethyl-amine (NDEA) Impurity Assay by GC/MS

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