DETERMINATION OF *N*-NITROSODIMETHYLAMINE (NDMA) AND *N*-NITROSODIETHYLAMINE (NDEA) IN SARTAN MEDICINES BY LC-MS/MS

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1 Scope

This testing method is for the determination of *N*-Nitrosodimethylamine (NDMA) and *N*-Nitrosodiethylamine (NDEA) in sartan medicines by Liquid Chromatography Hybrid Tandem Mass Spectrometry (LC-MS/MS).

2 Determination of NDMA and NDEA by QTRAP LC-MS/MS

2.1 Reagents and Chemicals

N-Nitrosodimethylamine (NDMA)
N-Nitrosodiethylamine (NDEA)
N-Nitrosodimethylamine-D6 (NDMA-D6)
Methanol, HPLC grade (MeOH)
Formic acid, MS grade
Deionized water (DI water)
Diluent: MeOH / DI water (20/80)

2.2 Instruments and Apparatus

Liquid Chromatography Tandem Mass Spectrometry (QTRAP 6500+ MS/MS coupled with Agilent 1290 Infinity LC)
Ultrasonic bath
Volumetric flask (Class A, 10 mL)
Membrane syringe filter (Nylon 0.2 µm)
Micropipette
2 mL vials
1.5 mL Eppendorf tube
Conical bottom centrifuge tube, Polypropylene (PP)

2.3 LC-MS/MS parameters

HPLC

Column:	Phenomenex® Gemini C18 (4.6 x 100 mm, 3 µm), or equivalent, or equivalent		
Column oven Temp:	40 °C		
Injection volume:	10 µL		
Mobile phase A:	0.1% Formic acid in DI water		
Mobile phase B:	Methanol		
Flow rate:	0.6 mL/min		
Gradient:	Time (min)	Mobile phase A (%)	Mobile phase B (%)
	0	95	5
	1	95	5
	5	5	95
	7	5	95
	7.1	95	5
	10	95	5

[Note: The flow rate or run time may be varied to obtain optimum separation.]

MS/MS

MS:	QTRAP 6500+					
Polarity:	Positive					
Ionization mode:	APCI (Atmospheric Pressure Chemical Ionization)					
MS parameter:	CUR: 20 psi; CAD: Medium; TEP: 550 °C; GSI: 40 psi; CXP: 11					
MRM:	ID	Q1	Q3	DP	EP	CE
	NDMA 1	75.0	43.0	68	7	21
	NDMA 2	75.0	58.0	68	7	16
	NDEA 1	103.1	75.1	45	10	14
	NDEA 2	103.1	47.1	45	10	21
	NDMA IS	81.0	46.0	68	7	21

[Note: To avoid excessive contamination of MS detector from API and excipients, valve switches were set to MS detector only at time window: RT_{NMBA} ±~0.5 min]

2.4 Standard, Sample, Sample Blank and Spiked Sample Preparation

2.4.1 Standard Preparation

1. *Stock Standard Solution* (20 mg/L): Diluted from commercially available NDMA and NDEA standard solution with MeOH individually.
2. *Stock Internal Standard Solution* (1 mg/L): Diluted from commercially available NDMA-D6 standard solution with MeOH.
3. *Mixed Stock Standard Solution* (1 mg/L): accurately transfer 500 µL of each *Stock Standard Solution* respectively to a 10 mL volumetric flask and top up to volume with MeOH.
4. *Working Standard Solutions* (with 5 µg/L IS):

NDEA *Working Standard Solutions*

Working Standard Solution	Standards Conc.	Vol of *Mixed Stock Standard Solution* (1 mg/L)	Vol of *Stock IS Solution* (1 mg/L)	Final Vol
1	0	0	50 µL	10 mL
2	1 µg/L	10 µL	50 µL	10 mL
3	2 µg/L	20 µL	50 µL	10 mL
4	5 µg/L	50 µL	50 µL	10 mL
5	10 µg/L	100 µL	50 µL	10 mL
6	20 µg/L	200 µL	50 µL	10 mL

NDMA *Working Standard Solutions*

Working Standard Solution	Standards Conc.	Vol of *Mixed Stock Standard Solution* (1 mg/L)	Vol of *Stock IS Solution* (1 mg/L)	Final Vol
1	0	0	50 µL	10 mL
4	5 µg/L	50 µL	50 µL	10 mL
5	10 µg/L	100 µL	50 µL	10 mL
6	20 µg/L	200 µL	50 µL	10 mL
7	50 µg/L	500 µL	50 µL	10 mL
8	100 µg/L	1000 µL	50 µL	10 mL

Note: Protect all *Standard Solutions* from light.]

2.4.2 Sample Preparation

1. Weigh 10 tablets together and calculate the average mass of one tablet.
2. Accurately weigh certain amount of finely powdered sample, corresponding to 500 mg of the sartan API, into a 15 mL PP conical bottom centrifuge tube.
3. Add 50 µL *Stock Internal Standard Solution* and 2 mL of methanol, vortex to mix well and sonicate for 5 min. Add 8 mL of DI Water, mix well and sonicate for 5 min. [Note: Scale down the sample amount if necessary].
4. Transfer about 1 mL mixture to a 1.5 mL Eppendorf tube, centrifuge the mixture at 15000 rpm for 5 min at room temperature.
5. Filter the supernatant into a HPLC vial through 0.2 µm Nylon Membrane filter. [Note: Protect sample solutions from light. In the situation when the amount of powder is too much for effective sample extraction, please reduce the powder amount or increase the extraction solvent volume. In this case, the LOD of the method will be affected and needed to recalculated accordingly.]

2.4.3 Spiked Sample Preparation

Spiked Sample Solution 1 [LOD of NDEA: 0.02 µg/g]

1. Accurately weigh an amount of powdered sample, corresponding to 500 mg of sartan API into a 15 mL PP Conical bottom centrifuge tube.
2. Add 10 µL *Mixed Stock Standard Solution* into the tube.
3. Repeat step 3-5 as in Section 2.4.2 to obtain *Spiked Sample Solution 1* (NDEA concentration: 1 µg/L).

Spiked Sample Solution 2 [LOD of NDMA: 0.09 µg/g]

1. Accurately weigh an amount of powdered sample, corresponding to 500 mg of sartan API into a 15 mL PP Conical bottom centrifuge tube.
2. Add 45 µL *Mixed Stock Standard Solution* into the tube.
3. Repeat step 3-5 as in Section 2.4.2 to obtain *Spiked Sample Solution 2* (NDMA concentration: 4.5 µg/L).

2.4.4 Sample Blank Preparation

Sample Blank Solution: Prepare the Sample Blank as described for the Sample Preparation in Section 2.4.2 but without the sample addition.

2.5 Test Procedure

1. Select method: *Nitrosamins_MRM*;
2. Inject solvent blank (*Diluent*).

3. For quantification of NDEA, inject *Standard Solutions 1-6*; for quantification of NDMA inject *Standard Solutions 1, 7-10*.
4. Inject solvent blank.
5. Inject *Sample Blank*.
6. Inject *Sample Solution* (Duplicate) [Note: dilute sample with diluent accordingly when the concentration of the *Sample Solution* exceeds the calibration range.].
7. Inject *Spiked Sample Solution 1* and *2*.
8. Inject solvent blank.
9. Flush LC-MS/MS system immediately after the analysis.

2.6 Interpretation of Results

1. The LOD and LOQ for NDMA are 0.09 µg/g and 0.30 µg/g; LOD and LOQ for NDEA are 0.02 µg/g and 0.05 µg/g.
   [Note: The LOD and LOQ calculations were based on the 500 mg of sartan API used in the drug substance].
2. For positive identification, the result is valid only if:
   i. The peaks corresponding to NDMA and NDEA in the chromatogram of all the ion pairs from the *Sample Solution* have close retention time (±0.5 min) to the peaks from the *Standard Solutions* chromatogram;
   ii. The deviation of the ion ratios of NDMA and NDEA obtained from the *Standard Solutions* and *Sample Solution* for the two MRM transitions are not more than 20%;
3. The quantification is performed using the peak area ratios of [NDEA 2 (103.1/47.1) / IS] through linearity plot obtained from *Standard Solutions 1 to 6* (for NDEA) and peak area ratios from ion pair of [NDMA 2 (75.0/58.0) / IS] through linearity plot from *Standard Solutions 1, 4-8*. The quantification results are valid only if:
   i. The deviation of the peak area ratios for NDMA and NDEA obtained from duplicated sample solution are not more than 20%;
   ii. The linearity coefficient of the calibration plot is greater than 0.99;
   iii. Report as 'Less than 0.30 µg/g' if the analysis result of NDMA is above 0.09 µg/g but less than 0.30 µg/g;
   iv. Report as 'Less than 0.05 µg/g' if the analysis result of NDEA is above 0.02 µg/g but less than 0.05 µg/g.
4. For negative identification, the result is valid only if:
   i. No peaks corresponding to NDMA and NDEA was observed in the chromatogram obtained from the *Sample Solution*. Positive results are observed in *Spiked Sample Solution 1* for NDEA and *Spiked Sample Solution 2* for NDMA;
   ii. Report as 'Not Detected' and indicate the LOD of NDMA as 0.09 µg/g;

NDEA as 0.02 µg/g.

3 References

1. Determination of *N*-nitrosodimethylamine in Valsatan Active Pharmaceutical Ingredient and the Related Medicinal Products, Taiwan Food and Drug Administration (TFDA), OMCL TW_TFDA-B
2. Determination of NDMA by LC/UV in Valsartan Active Substances and Finished Products, French National Agency for Medicines and Health Products Safety Laboratory Controls Division *Ref. 18A0399-01*

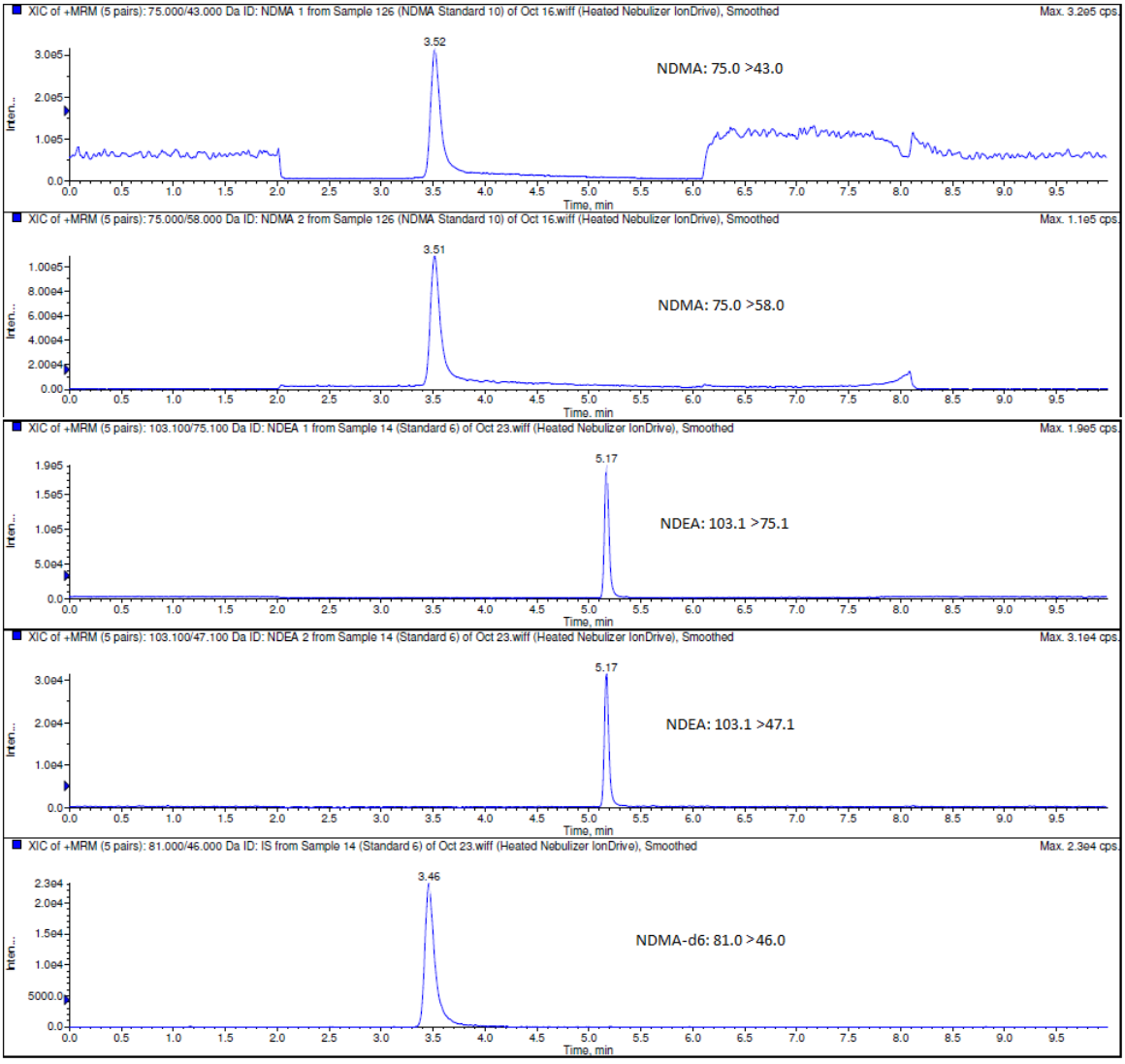

Fig1. The MRM chromatograms of NDMA, NDEA, and NDMA-D6