DETERMINATION OF *N*-NITROSO-*N*-METHYLAMINO BUTYRIC ACID (NMBA) IN SARTAN MEDICINES BY LIQUID CHROMATOGRAPHY HYBRID TANDEM MASS SPECTROMETRY

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1 Scope

This document outlines the testing method for the determination of *N*-Nitroso-*N*-methylamino butyric acid (NMBA) in sartan medicines by Liquid Chromatography Hybrid Tandem Mass Spectrometry.

2 Determination of NMBA by QTRAP LC-MS/MS

2.1 Reagents and Chemicals

N-Nitroso-*N*-methylamino butyric acid (NMBA)
N-Nitroso-*N*-methylamino butyric acid-D3 (NMBA-D3)
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 5500 MS/MS coupled with Agilent 1260 Infinity LC)
Ultrasonic bath
Volumetric flask (Class A, 10 mL)
Membrane filter (Nylon 0.2 µm)
Micropipette
2 mL vials
1.5 mL Eppendorf tube
Conical centrifuge tube, Polypropylene (PP)

2.3 LC-MS/MS Method

HPLC

Column:	Dionex Bonded Silica Acclaim™ Trinity™ P1 column (3.0 mm x 100 mm, 3 µm), 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:	0.3 mL/min		
Gradient:	Time (min)	Mobile phase A (%)	Mobile phase B (%)
	0	90	10
	1	90	10
	4	5	95
	7	5	95
	7.1	90	10
	10	90	10

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

MS/MS

MS:	QTRAP 5500					
Polarity:	Positive					
Ionization mode:	ESI (Ion Electrospray Ionization)					
MS parameter:	CUR: 20 psi; CAD: Medium; TEP: 500 °C; GSI: 40 psi; CXP: 13					
MRM:	ID	Q1	Q3	DP	EP	CE
	NMBA 1	147.0	117.0	35	8	9
	NMBA 2	147.0	87.0	35	8	17
	NMBA 3	147.0	102.0	35	8	13
	NMBA 4	147.0	98.0	35	8	22
	NMBA IS	150.0	120.0	35	8	9

[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* (100 mg/L): Weigh 1 ± 0.1 mg NMBA standard and transfer to a 10 mL volumetric flask, top up to volume with MeOH.
2. *Stock Internal Standard Solution* (20 mg/L): Prepared from commercially available NMBA-D3 standard and dilute with MeOH.
3. *Intermediate Stock Standard Solution* (1 mg/L): accurately transfer 100 µL of NMBA *Stock Standard Solution* to a 10 mL volumetric flask and top up to volume with *Diluent*.
4. *Intermediate Stock Internal Standard Solution* (1 mg/L): Accurately transfer 500 µL of NMBA-D3 *Stock Internal Standard Solution* to a 10 mL Volumetric flask and top up to volume with MeOH.
5. *Stock Spiking Standard Solution* (0.45 mg/L): accurately transfer 45 µL of NMBA *Stock Standard Solution* to a 10 mL Volumetric flask and top up to volume with MeOH.
6. *Working Standard Solutions* (with 10 µg/L IS; prepared in 10 mL Volumetric flask individually):

Working Standard Solution	Standards Conc.	Vol of *Intermediate Stock Standard solution* (1 mg/L)	Vol of *Intermediate Stock IS Solution* (1 mg/L)	
1	0	0	100 µL	Top up to volume (10 mL) with *Diluent*
2	5 µg/L	50 µL	100 µL	
3	10 µg/L	100 µL	100 µL	
4	20 µg/L	200 µL	100 µL	
5	50 µg/L	500 µL	100 µL	
6	100 µg/L	1000 µL	100 µL	
7	200 µg/L	2000 µL	100 µL	

[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 an amount of powdered sample, corresponding to 500 mg of the sartan API into a 15 mL PP conical centrifuge tube.
3. Add 100 µL *Intermediate Stock IS Solution* (1 mg/L) 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 a 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

Spike Sample Solution [LOD of NMBA: 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 centrifuge tube.
2. Transfer 100 µL *Stock Spiking Standard Solution* into the tube.
3. Repeat step 3-5 as in Section 2.4.2 to obtain *Spike Sample Solution* (NMBA 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: *NMBA_MRM_ESI*
2. Inject solvent blank (*Diluent*).
3. Inject *Standard Solutions 1-7*.
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.*
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 NMBA are 0.09 µg/g and 0.30 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 NMBA in the chromatogram of all the ion pairs from the *Sample Solution* and *Spiked Sample Solutions* have close retention time (±0.5 min) to the peaks from the *Standard Solutions* chromatogram;
   ii. The deviation of the ion ratios of NMBA obtained from the *Standard Solutions* and *Sample Solution* for the MRM transitions are not more than 20%;

3. The quantification is performed using the peak area ratios from ion pair of [NMBA 1 (147.0/117.0) / IS] through linearity plot from *Standard Solutions 1-7*. The quantification result is valid only if:
   i. The deviation of the peak area ratios for NMBA 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 0.3 µg/g' if the analysis result of NMBA is above 0.09 µg/g but less than 0.3 µg/g.

4. For negative identification, the result is valid only if:
   i. No peaks corresponding to NMBA was observed in the chromatogram obtained from the *Sample Solution.* Positive results are observed in *Spiked Sample Solution*;
   ii. Report as 'Not Detected' and indicate the LOD of NMBA as 0.09 µg/g.

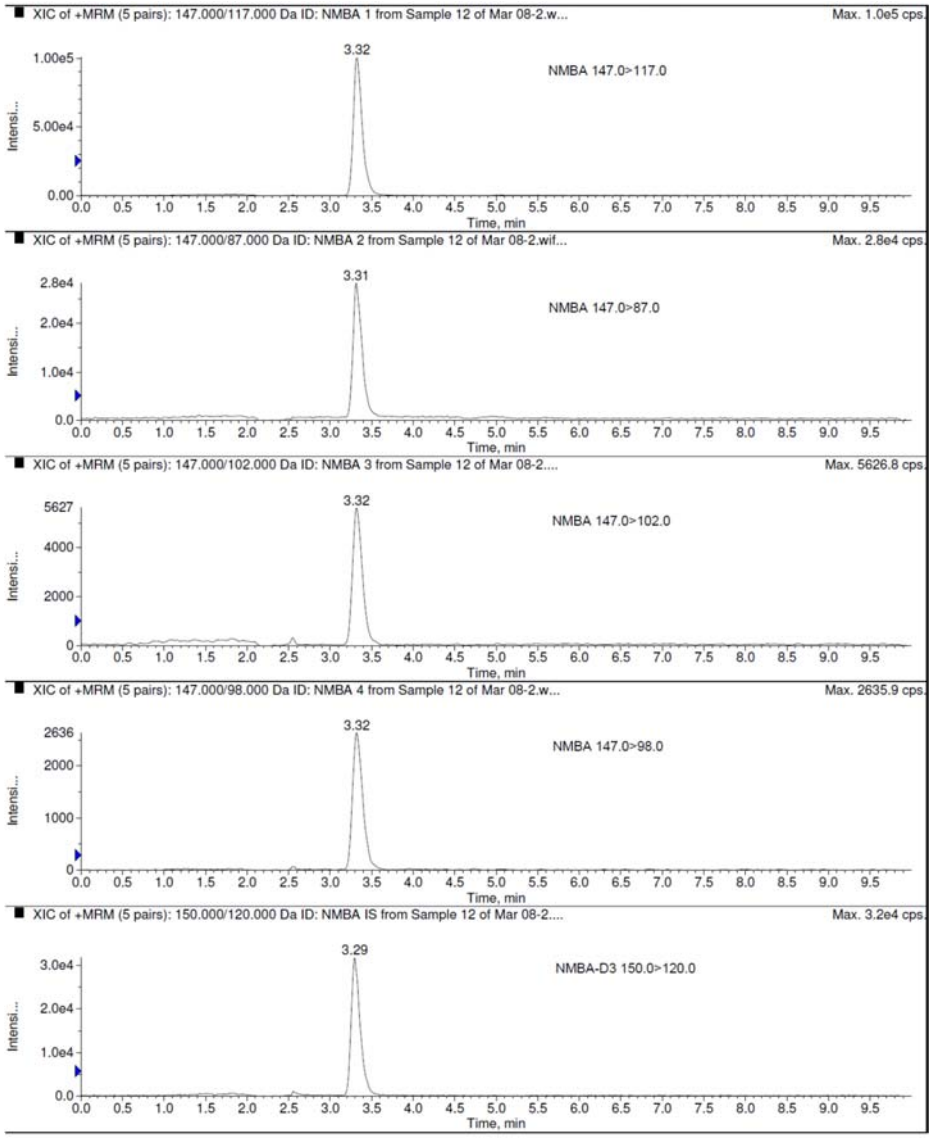

Fig1. The MRM chromatograms of NMBA and NMBA-D3