Agence nationale de sécurité du médicament et des produits de santé

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Determination of NDMA and NDEA in SARTAN drug substances by HPLC/UV

Method reference : 19A0416-01

1. Principle

The method is intended to the detection and the quantitative determination by HPLC-UV of N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) in Sartan drug substances (*valsartan*, *losartan*, *irbesartan*, *candesartan* and *olmesartan*). The analytical protocole involves the use of a 2 standard solutions (containing NDMA and NDEA), 2 sample solutions (raw material) and one spiked solution (raw material containing known content of NDMA/NDEA). Solutions are prepared in order to promote the maximal solubility of the active substance. Spike amounts of NDMA and NDEA are not greater than the proposed current limits, and are prepared in order to not too much load the analytical column with interfering substances.

The sensitivity of the method (determined with spiked solutions) is compared to the current toxicological limits issued by EMA in table 1.

	NDMA			NDEA		
	LOD	**LOQ**	**EMA**	**LOD**	**LOQ**	**EMA**
VALSARTAN	0.02	0.04	0.30	0.04	0.08	0.08
LOSARTAN	0.025	0.05	0.96	0.05	0.10	0.27
IRBESARTAN	0.02	0.04	0.32	0.04	0.09	0.09
CANDESARTAN	0.10	0.25	3.00	0.10	0.40	0.83
OLMESARTAN	0.10	0.25	2.40	0.10	0.50	0.66

Table 1: Limits of the HPLC-UV method (ppm)

Caution: Nitrosamines present carcinogenic properties and therefore require appropriate safety conditions for their manipulation.

2. Reagents and reference substance

- Methanol HPLC grade
- Purified water: milli-Q
- NDMA solution 1000 µg/mL in methanol (RESTEK, reference: 31427)
- NDEA solution 100 µg/mL in methanol (LGC – D_R Ehrenstorfer, reference: XA15603500ME)

3. Chromatographic system

- Liquid chromatograph equipped with UV-VIS Detector
- Analytical column: Inertsil ODS-3, 4.6 x 250 mm, 5 µm
- Column temperature: 30 °C
- Detection: 228 nm
- Flow rate: 1.0 mL/min
- Injection volume : 50 µL
- Mobile Phase A: methanol, water (35:65 *V/V*)
- Mobile Phase B: water, methanol (25:75 *V/V*)
- Run Time: 35 min (NDMA RT = 3.8 min, NDEA RT = 8.0 min)
- Gradient elution :

Time (min)	Mobile Phase A (% V/V)	Mobile Phase B (% V/V)
0 – 5	100	0
5 – 10	100 → 0	0 → 100
10 – 25	0	100
25 – 27	0 → 100	100 → 0
27 – 35	100	0

The gradient elution is run in order to flush the column with a high ratio of methanol therefore avoiding the presence of unexpected peaks on following chromatograms.

4. Preparation of solutions

Solutions are prepared in low-actinic glassware.

- **Dilution solvent:** methanol, water (35:65 *V/V*)

- **Standard solutions:**

Stock solution NDMA (2.5 µg/mL):

Accurately transfer 250 µL of the commercial NDMA solution (1000 µg/mL) into a 100 mL volumetric flask and make up to volume with methanol. The solution is stored at 2-8°C.

Stock solution NDEA (2.5 µg/mL):

Accurately transfer 250 µL of the commercial NDEA solution (100 µg/mL) into a 10 mL volumetric flask and make up to volume with methanol. The solution is stored at 2-8°C.

Suitability solution (NDMA/NDEA = 5 ng/mL):

Accurately transfer 0.25 mL of stock solution NDMA and 0.25 ml of stock solution NDEA into a 125 mL volumetric flask and make up to volume with the dilution solvent.

Standard solution 1 for assay (NDMA/NDEA = 10 ng/mL):

Accurately transfer 0.5 mL of stock solution NDMA and 0.5 ml of stock solution NDEA into a 125 mL volumetric flask and make up to volume with the dilution solvent.

Standard solution 2 for the preparation of spiked solution (NDMA/NDEA = 25 ng/mL):

Accurately transfer 0.25 mL of stock solution NDMA and 0.25 ml of stock solution NDEA into a 25 mL volumetric flask and make up to volume with the dilution solvent.

Other concentrations of standard solution may be obtained from stock solutions.

- **Test solutions for Valsartan drug substance (spike concentration NDMA/NDEA = 0.08 ppm)**:

Accurately weigh 320 mg of sample into a 15 mL centrifuge tube.

Add 1 mL of methanol, shake vigorously during 5 minutes and sonicate for 5 minutes more.

Add 4 mL of purified water and shake vigorously during 5 minutes.

Centrifuge the solution at 4000 rpm during 5 minutes. Transfer the supernatant solution into a 2 mL Eppendorf tube and centrifuge at 12000 rpm during another 5 minutes. Filter an aliquot of the latter supernatant solution through a 0.45 µm GHP membrane and transfer into an injection vial.

The *spiked solution* is prepared using the same previous protocol substituting 1 mL of methanol by 1 mL of standard solution 2.

- **Test solutions for Losartan drug substance (spike concentration NDMA/NDEA = 0.10 ppm)**:

Accurately weigh 250 mg of sample into a 15 mL centrifuge tube and add 5 mL of dilution solvent in order to prepare the *test solution*. Shake vigorously during 5 minutes and sonicate for 5 minutes more.

Filter the solution through a 0.45 µm GHP membrane and transfer into an injection vial.

The *spiked solution* is prepared using the same previous protocol replacing 5 mL of dilution solvent by 1 mL of standard solution 2 + 4 ml of dilution solvent.

- **Test solutions for Irbesartan drug substance (spike concentration NDMA/NDEA = 0.08 ppm)**:

Accurately weigh 300 mg of sample into a 15 mL centrifuge tube and add 5 mL of dilution solvent in order to prepare the *test solution*. Shake vigorously during 5 minutes and sonicate for 5 minutes more.

Centrifuge the solution at 4000 rpm during 5 minutes. Filter an aliquot of the supernatant solution through a 0.45 µm GHP membrane and transfer into an injection vial.

The *spiked solution* is prepared using the same previous protocol replacing 5 mL of dilution solvent by 1 mL of standard solution 2 + 4 ml of dilution solvent.

- **Test solutions for Candesartan drug substance (spike concentration NDMA/NDEA = 0.40 ppm)**:

Accurately weigh 62 mg of sample into a 15 mL centrifuge tube and add 5 mL of dilution solvent in order to prepare the *test solution*. Shake vigorously during 5 minutes and sonicate for 5 minutes more.

Centrifuge the solution at 4000 rpm during 5 minutes. Filter an aliquot of the supernatant solution through a 0.45 µm GHP membrane and transfer into an injection vial.

The *spiked solution* is prepared using the same previous protocol replacing 5 mL of dilution solvent by 1 mL of standard solution 2 + 4 ml of dilution solvent.

- **Test solutions for Olmesartan drug substance (spike concentration NDMA/NDEA = 0.60 ppm)**:

Accurately weigh 83 mg of sample into a 15 mL centrifuge tube and add 5 mL of dilution solvent in order to prepare the *test solution*. Shake vigorously during 5 minutes and sonicate for 5 minutes more.

Centrifuge the solution at 4000 rpm during 5 minutes. Filter an aliquot of the supernatant solution through a 0.45 µm GHP membrane and transfer into an injection vial.

The *spiked solution* is prepared using the same previous protocol replacing 5 mL of dilution solvent by 2 mL of standard solution 2 + 3 ml of dilution solvent.

Other concentrations of spiked solutions may be obtained adjusting the preparation of solutions.

	VALSARTAN		LOSARTAN		IRBESARTAN		CANDESARTAN		OLMESARTAN	
	TEST	*SPIKE 0.08 ppm*	*TEST*	*SPIKE 0.10 ppm*	*TEST*	*SPIKE 0.08 ppm*	*TEST*	*SPIKE 0.40 ppm*	*TEST*	*SPIKE 0.60 ppm*
Weigh	320 mg	320 mg	250 mg	250 mg	300 mg	300 mg	62 mg	62 mg	83 mg	83 mg
MeOH	1.0 mL	-	-	-	-	-	-	-	-	-
Water	4.0 mL	4.0 mL	-	-	-	-	-	-	-	-
STANDARD 2 25 ng/mL	-	1.0 mL	-	1.0 mL	-	1.0 mL	-	1.0 mL	-	2.0 mL
DILUENT MeOH, water (35:65 *V/V*)	-	-	5.0 mL	4.0 mL	5.0 mL	4.0 mL	5.0 mL	4.0 mL	5.0 mL	3.0 mL

Table 2: Summary table for the preparation of test solutions

5. Procedure

Before starting a sequence of injections, the chromatographic system will be tested injecting the *Suitability solution*. Both peaks originating from NDMA and NDEA present signal-to-noise ratio greater than 10.

Standard solutions are prepared in duplicate from 2 different stock solutions. Test solutions are prepared in duplicate. The NDMA/NDEA quantitation will be valid if the recovery of the 2 standard solutions is found between 90.0% and 110.0%.

For contaminated samples, the spiked solution may be used in order to confirm the identification and the quantification of NDMA/NDEA.

A typical sequence of injections is:

- Blank (initialization of the system),
- Standard solutions 1 (x2),
- Blank solution,
- Test solutions (x2),
- Blank solution,
- Spiked solution,
- Blank solution.

6. Calculations

The NDMA/NDEA content in the sample is calculated in ppm related to the *sartan* drug substance based on the following formula:

$$C_{NDMA/NDEA}\ (ppm) = \frac{A_{TEST} \cdot Vol_{TEST} \cdot C_{STD} \cdot P_{STD}}{A_{STD} \cdot Vol_{STD} \cdot W_{TEST}} \cdot 10$$

A_{TEST}: Area of NDMA/NDEA peak in the test solution
A_{STD}: Average area of NDMA/NDEA peak in standard solutions 1
Vol_{TEST}: Dilution volume of test solution (mL)
Vol_{STD}: Dilution volume of standard solution 1 (mL)
W_{TEST}: Weigh of SARTAN drug substance (mg)
C_{STD}: NDMA/NDEA concentration in the commercial standard solution (µg/mL)
P_{STD}: NDMA/NDEA purity in the commercial standard solution (%)