

French National Agency for Medicines and Health Products Safety
Laboratory Controls Division

Determination of NDMA in *valsartan* active substances and finished products by HPLC/UV

Method reference : 18A0399-02

1. Principle

The method is dedicated to the detection and the quantitative determination of N-nitrosodimethylamine (NDMA) in *valsartan* active pharmaceutical ingredients and finished products (powdered tablets) by HPLC-UV. Nitrosamines present carcinogenic properties and therefore require appropriate safety conditions for their manipulation.

The procedure involves the use of a 2 standard solutions (containing NDMA), 2 sample solutions (raw material or finished product) and one spiked solution (NDMA + raw material or finished product). The preparation of 3 spiked solutions corresponding to 0.3 ppm, 1.6 ppm and 7.8 ppm is described. Depending of the NDMA content in sample solutions, the operator has to choose the appropriate spiked solution. Other spiked preparations may be considered.

The linearity of the method has been checked between 0.01 µg/mL and 0.25 µg/mL. For samples found with high NDMA content, an additional dilution of the test solution may be necessary in order to enter the linear range of the method.

Based on the signal-to-noise ratio of the NDMA peak in the standard solution, the detection limit is defined at 0.1 ppm and the reporting value at 0.3 ppm. Results obtained between 0.1 ppm and 0.3 ppm will be provided for information purpose only.

The method was found suitable for the analysis of 6 different formulations. In case of doubt concerning specificity, excipients should be injected in the chromatographic system, or another independent method should be carried out.

2. Reagents and reference substance

- Methanol HPLC grade
- Purified water: milli-Q
- NDMA solution 1000 µg/ml in methanol (Restek, reference: 31427)

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 : 20 µL
- Mobile Phase A: water, methanol (80:20 V/V)
- Mobile Phase B: water, methanol (25:75 V/V)
- Run Time: 40 min (NDMA RT = 4.5 min)
- Gradient elution :

Temps (min)	Phase mobile A (% V/V)	Phase mobile B (% V/V)
0 - 14	100	0
14 - 15	100 → 0	0 → 100
15 - 27	0	100
27 - 28	0 → 100	100 → 0
28 - 40	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 (blank solution):** mobile phase A

- **Standard solutions:**

Stock solution (NDMA = 2.5 µg/mL corresponding to 78 ppm):

Accurately transfer 250 µL of 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.

Standard solution 1 (NDMA = 0.25 µg/mL corresponding to 7.8 ppm):

Accurately transfer 1 mL of stock solution into a 10 mL volumetric flask and make up to volume with dilution solvent.

Standard solution 2 (NDMA = 0.05 µg/mL corresponding to 1.6 ppm):

Accurately transfer 1 mL of stock solution into a 50 mL volumetric flask and make up to volume with dilution solvent.

Standard solution 3 (NDMA = 0.01 µg/mL corresponding to 0.3 ppm):

Accurately transfer 1 mL of stock solution into a 250 mL volumetric flask and make up to volume with dilution solvent.

- **Spiking solutions:**

Spiking solution 1 (NDMA = 1.25 µg/mL):

Accurately transfer 5 mL of stock solution into a 10 mL volumetric flask and make up to volume with methanol.

Spiking solution 2 (NDMA = 0.25 µg/mL):

Accurately transfer 1 mL of stock solution into a 10 mL volumetric flask and make up to volume with methanol.

Spiking solution 3 (NDMA = 0.05 µg/mL):

Accurately transfer 1 mL of stock solution into a 50 mL volumetric flask and make up to volume with methanol.

- **Test solution for active substance** (valsartan = 32 000 µg/mL):

Accurately weigh 160 mg into a 15 mL centrifuge tube.

Add 1 mL of methanol (or 1 mL of spiking solution 1, 2 or 3 in order to obtain respectively a 7.8 ppm, 1.6 ppm or 0.3 ppm spiked solution). 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 PVDF membrane and transfer into an injection vial.

- **Test solution for finished product** (valsartan = 32 000 µg/mL):

Weigh 10 tablets together and calculate the average mass of one tablet. Finely grind the 10 tablets and accurately weigh the equivalent of 320 mg valsartan (for example: 2 tablets for Valsartan 160 mg/tablet or 4 tablets for Valsartan 80 mg/tablet). Transfer the powder into a 15 ml centrifuge tube.

Add 2 mL of methanol (or 2 mL of spiking solution 1, 2 or 3 in order to obtain respectively a 7.8 ppm, 1.6 ppm or 0.3 ppm spiked solution). Shake vigorously during 5 minutes and sonicate for 5 minutes more.

Add 8 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 PVDF membrane and transfer into an injection vial.

5. Procedure

Test solutions are prepared in duplicate. Standard solution are prepared in duplicate from 2 different stock solutions. For samples where no NDMA is detected, the standard solution 3 (corresponding to 0.3 ppm) is injected. For samples where NDMA is detected, the standard solution 2 (corresponding to 1.6 ppm) or 1 (corresponding to 7.8 ppm) is injected.

The NDMA quantitation will be valid if the recovery of the 2 standard solutions is found between 90.0% and 110.0%. Moreover the NDMA contents calculated in the test solutions and the spiked solution deviate not more than 10.0%.

A typical sequence of injections is:

- 1 blank (initialization of the system),
- 2 standard solutions,
- 1 blank solution,
- 2 test solutions,
- 1 spiked solution.

6. Calculations

The NDMA content in the sample is calculated in ppm related to *valsartan*, based on the following formula:

$$C_{\text{NDMA}}(\text{ppm}) = \frac{\text{Concentration NDMA (in TEST solution)}}{\text{Concentration VALSARTAN (in TEST solution)}} \cdot 1\,000\,000$$

- NDMA content in the active substance:

$$C_{\text{NDMA}}(\text{ppm}) = \frac{A_{\text{TEST}} \cdot \text{Vol}_{\text{TEST}} \cdot C_{\text{STD}} \cdot P_{\text{STD}}}{A_{\text{STD}} \cdot \text{Vol}_{\text{STD}} \cdot W_{\text{TEST}}} \cdot 1\,000$$

A_{TEST} : Area of NDMA peak in the test solution

A_{STD} : Average area of NDMA peak in standard solutions

Vol_{TEST} : Dilution volume of test solution (mL)

Vol_{STD} : Dilution volume of standard solution (mL)

W_{TEST} : Weigh of Valsartan active substance (mg)

C_{STD} : NDMA concentration in the commercial standard solution ($\mu\text{g}/\text{mL}$)

P_{STD} : NDMA purity in the commercial standard solution (%)

- NDMA content in the finished product:

$$C_{\text{NDMA}}(\text{ppm}) = \frac{A_{\text{TEST}} \cdot \text{Vol}_{\text{TEST}} \cdot \text{AM}_{\text{TEST}} \cdot C_{\text{STD}} \cdot P_{\text{STD}}}{A_{\text{STD}} \cdot \text{Vol}_{\text{STD}} \cdot W_{\text{TEST}} \cdot C_{\text{API}}} \cdot 1\,000$$

A_{TEST} : Area of NDMA peak in the test solution

A_{STD} : Average area of NDMA peak in standard solutions

Vol_{TEST} : Dilution volume of test solution (mL)

Vol_{STD} : Dilution volume of standard solution (mL)

W_{TEST} : Weigh of tablet powder (mg)

AM_{TEST} : Average mass of tablet (mg)

C_{STD} : NDMA concentration in the commercial standard solution ($\mu\text{g}/\text{mL}$)

P_{STD} : NDMA purity in the commercial standard solution (%)

C_{API} : Valsartan label content of the test sample (80 mg/tab or 160 mg/tab)

7. Example of chromatograms

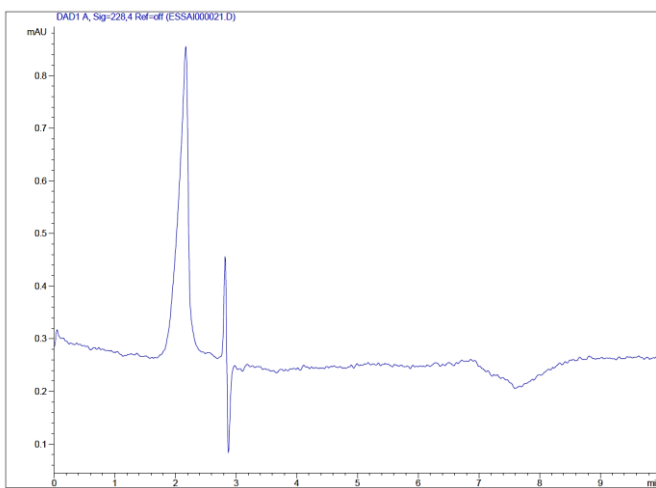


Fig.1: blank solution

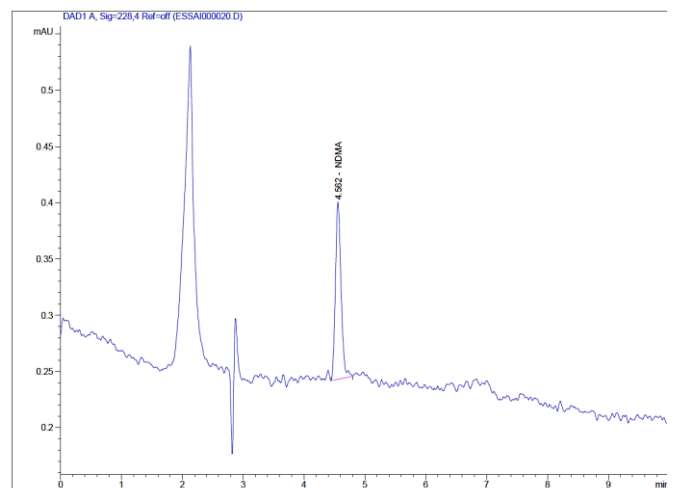


Fig.2: 0.3 ppm standard solution

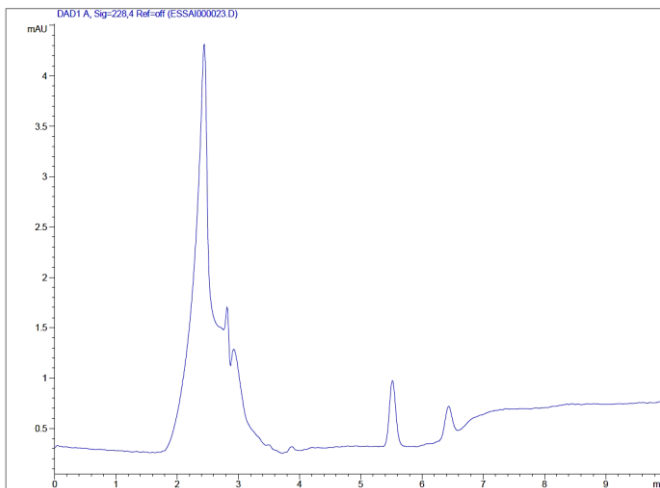


Fig.3: Test solution (no NDMA detected in the sample)

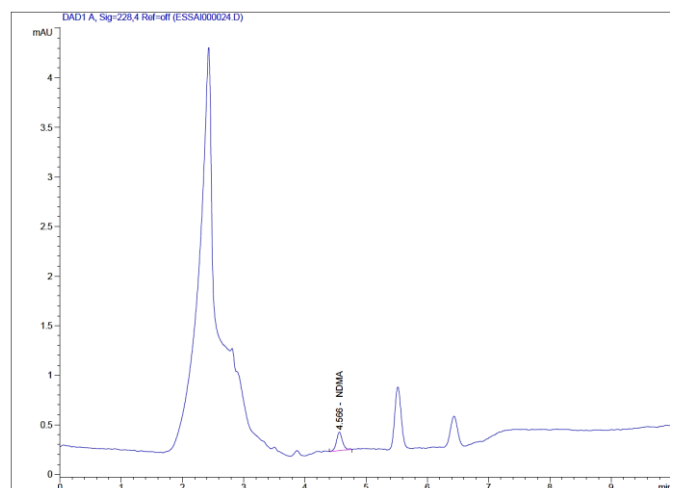


Fig.4: Spiked solution (with 0.3 ppm NDMA)

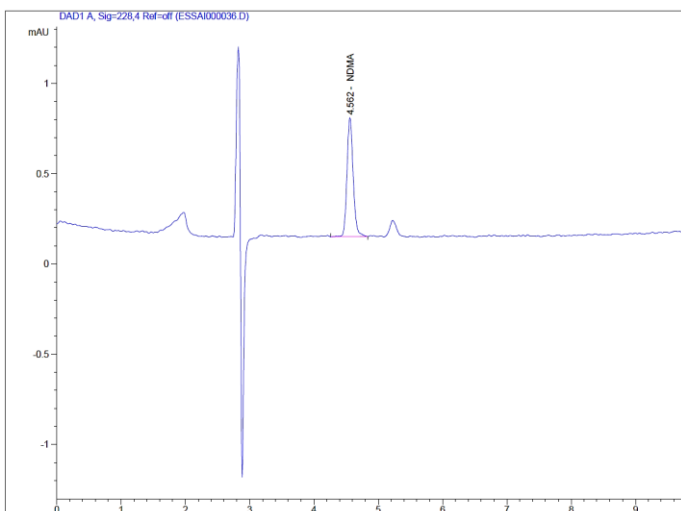


Fig.5: Test solution (containing 1.2 ppm NDMA)

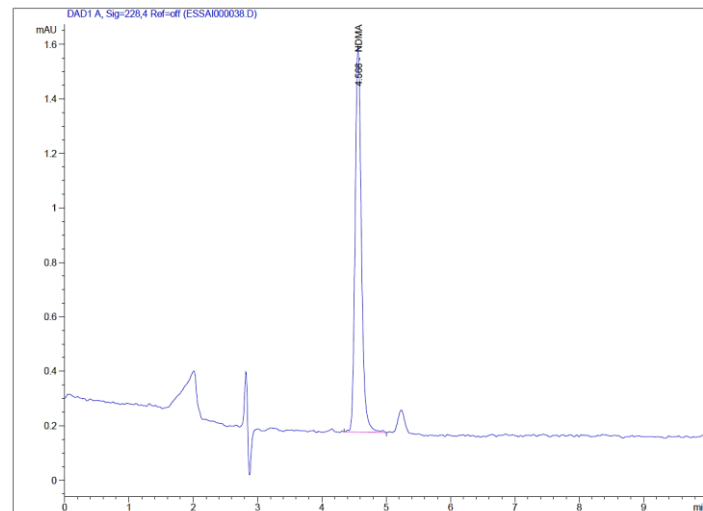


Fig.6: Spiked solution (containing 1.2 ppm and 1.6 ppm spiked NDMA)