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N-Nitrosamines in active substances

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NOTE ON THE GENERAL CHAPTER

This chapter has been elaborated with the objective of developing validated limit tests for N-nitrosamine impurities in "sartan" active substances with a tetrazole ring, following the EC's Implementing Decision concerning, in the framework of Article 31 of Directive 2001/83/EC of the European Parliament and of the Council, the marketing authorisations of medicinal products for human use which contain these active substances (EMA/248364/2019 Rev 1). During publication in Pharmeuropa 32.2, an updated CHMP opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 referral for nitrosamine impurities in human medicinal products has been published, making a quantitative determination of N-nitrosamine impurities necessary in certain cases. On November 13, 2020, the EMA published a news announcing that "EMA's human medicines committee (CHMP) has aligned recommendations for limiting nitrosamine impurities in sartan medicines with recent recommendations it issued for other classes of medicines."

In order to provide further support to users and given that procedure C (GC-MS/MS) had initially been developed and validated as a quantitative test, conditions and calculations for a quantitative use of the procedure, have been added to the chapter.

As a further consequence, the section of the European Pharmacopoeia where the proposed general chapter will be listed needs to be changed from "2.4 Limit tests" to "2.5 Assays", and the general chapter will bear a new reference 2.5.42 (previously 2.4.36).

01/2022:20542

2.5.42. *N*-NITROSAMINES IN ACTIVE SUBSTANCES

This chapter describes analytical procedures for the detection of various *N*-nitrosamines in particular active substances. Procedures A and B have been validated as limit tests (30 ppb) and procedure C has been validated as a quantitative test. The scope of each procedure is defined in Table 2.5.42.-1. With these three procedures, it is possible to analyse the following *N*-nitrosamines: *N*-nitroso-dimethylamine (NDMA); *N*-nitroso-diethylamine (NDEA); *N*-nitroso-dibutylamine (NDBA); *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA); *N*-nitroso-diisopropylamine (NDiPA); *N*-nitroso-ethyl-isopropylamine (NEiPA) and *N*-nitroso-dipropylamine (NDPA).

Procedure A uses deuterated *N*-nitroso-diethylamine (NDEA-d_{10}) as internal standard. Procedures B and C use *N*-nitroso-ethylmethylamine (NEMA) as internal standard.

When a procedure is applied to substances outside of the scope covered by the initial validation (see Table 2.5.42.-1) or to medicinal products or if procedure A or B is used quantitatively, then it must be validated.

Table 2.5.42.-1. – *Scope of the validation*

Active substance (monograph number)	NDMA	NDEA	NDBA	NMBA	NDiPA	NEiPA	NDPA
Candesartan cilexetil (2573)	A*BC	ABC	C	A	AC	AC	C
Irbesartan (2465)	A*BC	ABC	C	A	AC	AC	C
Losartan potassium (2232)	A*BC	ABC	C	A	AC	AC	C
Olmesartan medoxomil (2600)	A*BC	ABC	C	A	AC	AC	C
Valsartan (2423)	A*BC	ABC	C	A	AC	AC	C

* In procedure A, the presence of dimethylformamide (DMF) in the substance to be examined may interfere with the detection of NDMA.

PROCEDURE A (LC-MS/MS)

Liquid chromatography (*2.2.29*) coupled with mass spectrometry (*2.2.43*).

Internal standard solution. 9.0 ng/mL solution of *deuterated N-nitroso-diethylamine R* (NDEA-d_{10}) in *methanol R3*.

N-Nitrosamines spiking solution. For each *N*-nitrosamine concerned, use the corresponding CRS (*N-nitroso-dimethylamine CRS*, *N-nitroso-diethylamine CRS*, *N-nitroso-N-methyl-4-aminobutyric acid CRS*, *N-nitroso-diisopropylamine CRS* and *N-nitroso-ethyl-isopropylamine CRS*). In a single volumetric flask, dilute 300 µL of each of these CRS to 50.0 mL with *methanol R3*. Dilute 300 µL of this solution to 100.0 mL with *methanol R3*.

Test solution. Suspend 150.0 mg of the substance to be examined in 0.5 mL of *methanol R3*. Add 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter the supernatant through a membrane filter (nominal pore size 0.20 µm)(1). Use the filtrate.

Spiked solution. Suspend 150.0 mg of the substance to be examined in 0.5 mL of the *N*-nitrosamines spiking solution. Add 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter the supernatant through a membrane filter (nominal pore size 0.20 µm)(1). Use the filtrate.

Reference solution. Dilute 0.5 mL of the *N*-nitrosamines spiking solution with 0.5 mL of the internal standard solution. Mix thoroughly for 5 min and sonicate for 15 min. Add 4.0 mL of *water for chromatography R*. Mix thoroughly for 5 min and sonicate for 15 min. Centrifuge at about 3000 *g* for 5 min. Filter through a membrane filter (nominal pore size 0.20 µm)(1). Use the filtrate.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm)(2);
- *temperature*: 40 °C.

Mobile phase:

- *mobile phase A*: 0.1 per cent *V/V* solution of *formic acid R* in *water for chromatography R*;

(1) Membrane used for the development of the procedure: GHP (hydrophilic polypropylene).
(2) Gemini C18, 110 Å is suitable.

– *mobile phase B*: *methanol R3*;

Time[3] (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 1	80	20
1 - 6	80 → 55	20 → 45
6 - 14	55	45
14 - 16	55 → 5	45 → 95
16 - 35	5	95

Flow rate: 0.5 mL/min.

Detection[4]: triple-quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode. The following settings have been found to be suitable and are given as examples; the settings may be adjusted so as to comply with the system suitability criteria:

– *ionisation mode*: APCI-positive;
– *heater temperature*: 350 °C;
– *nebuliser pressure*: 310 kPa;
– *gas temperature*: 300 °C;
– *drying gas flow*: 5 L/min;
– *corona current*: 6 µA;
– *dwell time*: 200 ms;
– *capillary voltage (Vcap)*: 1.5 kV;
– *cell acceleration voltage*: 3 V;
– *MRM mode parameters*:

Substance	MRM transitions (*m/z*) principal (*qualifier*)	Collision energy (V)	Fragmentor voltage (V)
NDMA	75 → 58	11	20
	(*75 → 43*)	15	15
NMBA	147 → 117	2	41
	(*147 → 87*)	6	46
NDEA	103 → 75	9	25
	(*103 → 47*)	17	20
NEiPA	117 → 75	7	54
	(*117 → 47*)	15	59
NDiPA	131 → 89	2	20
	(*131 → 47*)	10	25
NDEA-d_{10}	113 → 81	6	76
	(*113 → 34*)	14	81

NOTE: acquisition can be started at 3.0 min and stopped at 15.5 min before elution of the active substances; during non-acquisition the eluent is directed to waste. Time segments can be defined accordingly.

Autosampler: set at 5 °C.

Injection: 20 µL of the spiked solution, the test solution and the reference solution.

Relative retention with reference to NDEA-d_{10} (retention time = about 8.6 min): NDMA = about 0.6; NMBA* = about 0.7; NDEA = about 1.0; NEiPA* = about 1.3; NDiPA = about 1.6.

**Exist as conformers that can be separated under the specified chromatographic conditions. Integrate both peaks or include the tailing, as appropriate.*

System suitability: for each *N*-nitrosamine:

– *repeatability*: use the principal MRM transitions, maximum relative standard deviation of 20 per cent for the ratio between the area of the peak due to the *N*-nitrosamine and the area of the peak due to the internal standard, determined on 6 injections of the reference solution;
– *signal-to-noise ratio 1*: for the spiked solution, minimum 5 for the peak corresponding to the principal transition of NDMA and minimum 10 for the peak corresponding to the principal transition of other *N*-nitrosamines;
– *signal-to-noise ratio 2*: for the spiked solution, minimum 3 for the peak corresponding to the qualifier transition.

Limit: use the principal MRM transitions.

For the test solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine detected and the area of the peak due to the internal standard (R_s).

For the spiked solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine and the area of the peak due to the internal standard (R_r).

For each *N*-nitrosamine detected, the ratio of R_s to R_r must be smaller than 0.50.

The test is not valid unless the ratio between the area of the peak corresponding to the principal transition and the area of the peak corresponding to the qualifier transition for the test solution is within 20 per cent of the same ratio calculated for the spiked solution.

PROCEDURE B (GC-MS)

Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Sample preparation 1 (valsartan, losartan potassium and olmesartan medoxomil).

Internal standard solution (1). Dissolve 5 mg of *N-nitroso-ethylmethylamine R* (NEMA) in *methanol R3* and dilute to 10.0 mL with the same solvent. Dilute 500 µL of the solution to 10.0 mL with *water for chromatography R*.

Extraction mixture. Dissolve 40.0 g of *sodium hydroxide R* in about 800 mL of *water for chromatography R*. Add 100 µL of the internal standard solution (1) and dilute to 1000 mL with *water for chromatography R*.

N-Nitrosamines spiking solution (1). For each *N*-nitrosamine concerned, use the corresponding CRS (*N-nitroso-dimethylamine CRS* and *N-nitroso-diethylamine CRS*). In a single volumetric flask, dilute 200 µL of each of these CRS to 20.0 mL with *water for chromatography R*. Dilute 300 µL of this solution to 20.0 mL with *water for chromatography R*.

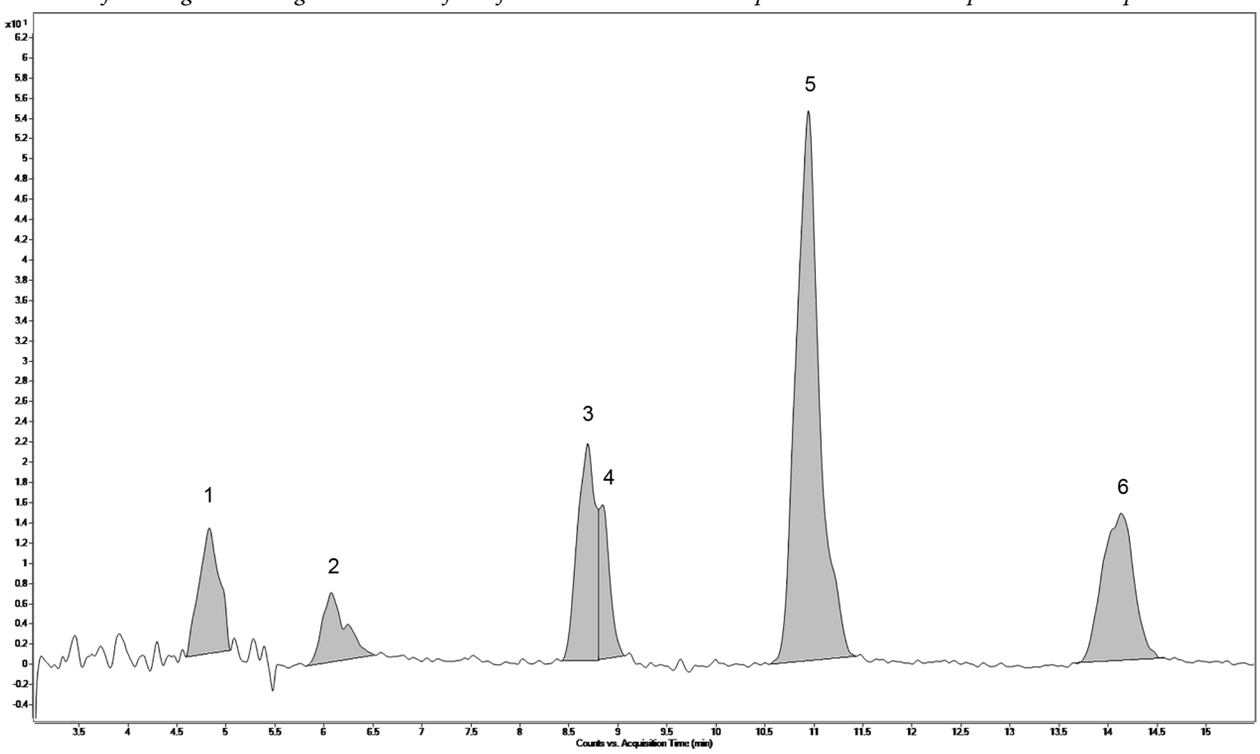
Test solution (1). Suspend 250.0 mg of the substance to be examined in 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min then centrifuge at about 4000 *g* for 5 min. Collect the lower layer (organic). If necessary, centrifuge to obtain a clear solution. Use the clear solution.

Spiked solution (1). Suspend 250.0 mg of the substance to be examined in 10.0 mL of the extraction mixture. Add 100 µL of the *N*-nitrosamines spiking solution (1). Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min then centrifuge at about 4000 *g* for 5 min. Collect the lower layer (organic). If necessary, centrifuge to obtain a clear solution. Use the clear solution.

Reference solution (1). Add 100 µL of the *N*-nitrosamines spiking solution (1) to 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min then centrifuge at about 4000 *g* for 5 min. Use the lower layer (organic).

(3) D_0 (dwell volume used for development of the procedure) = 0.2 mL.
(4) Instrument used for the development of the procedure: Agilent 1290 fast LC / Agilent 6460 Triple Quad.

The following chromatogram is shown for information but will not be published in the European Pharmacopoeia.



1. NDMA
2. NMBA
3. NDEA-d_{10}
4. NDEA
5. NEiPA
6. NDiPA

Figure 2.5.42.-1. – *Chromatogram of N-nitrosamine analysis in active substances by procedure A: spiked solution*

Sample preparation 2. (candesartan cilexetil and irbesartan).

Internal standard solution (2). Dissolve 5 mg of *N-nitroso-ethylmethylamine R* (NEMA) in *methanol R3* and dilute to 10.0 mL with the same solvent. Dilute 100 µL of the solution to 10.0 mL with *methanol R3*.

Extraction solution. Dilute 100 µL of internal standard solution (2) to 100.0 mL with *methylene chloride R1*.

N-Nitrosamines spiking solution (2). For each *N*-nitrosamine concerned, use the corresponding CRS (*N-nitroso-dimethylamine CRS* and *N-nitroso-diethylamine CRS*). In a single volumetric flask, dilute 200 µL of each of these CRS to 10.0 mL with *methanol R3*. Dilute 300 µL of this solution to 20.0 mL with *methanol R3*.

Test solution (2). Suspend 500.0 mg of the substance to be examined in 5.0 mL of the extraction solution. Add 100 µL of *methanol R3*. Shake well for at least 5 min, then centrifuge at about 4000 *g* for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (nominal pore size 0.45 µm)(5) to obtain a clear solution. Use the clear solution.

Spiked solution (2). Suspend 500.0 mg of the substance to be examined in 5.0 mL of the extraction solution. Add 100 µL of the *N*-nitrosamines spiking solution (2). Shake well for at least 5 min, then centrifuge at about 4000 *g* for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (nominal pore size 0.45 µm)(5) to obtain a clear solution. Use the clear solution.

Reference solution (2). Add 100 µL of the *N*-nitrosamines spiking solution (2) to 5.0 mL of the extraction solution. Shake well for at least 5 min, then centrifuge at about 4000 *g* for 5 min. Collect the supernatant solution. If necessary, filter the supernatant through a membrane filter (nominal pore size 0.45 µm)(5) to obtain a clear solution. Use the clear solution.

Column:
- *material*: fused silica;
- *size*: *l* = 30 m, Ø = 0.25 mm;
- *stationary phase*: *cyanopropylphenylene(6)me thyl(94)polysiloxane R* (film thickness 1.4 µm)(6).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.0 mL/min

Injection mode: *pulsed splitless*: 172 kPa, 0.5 min.

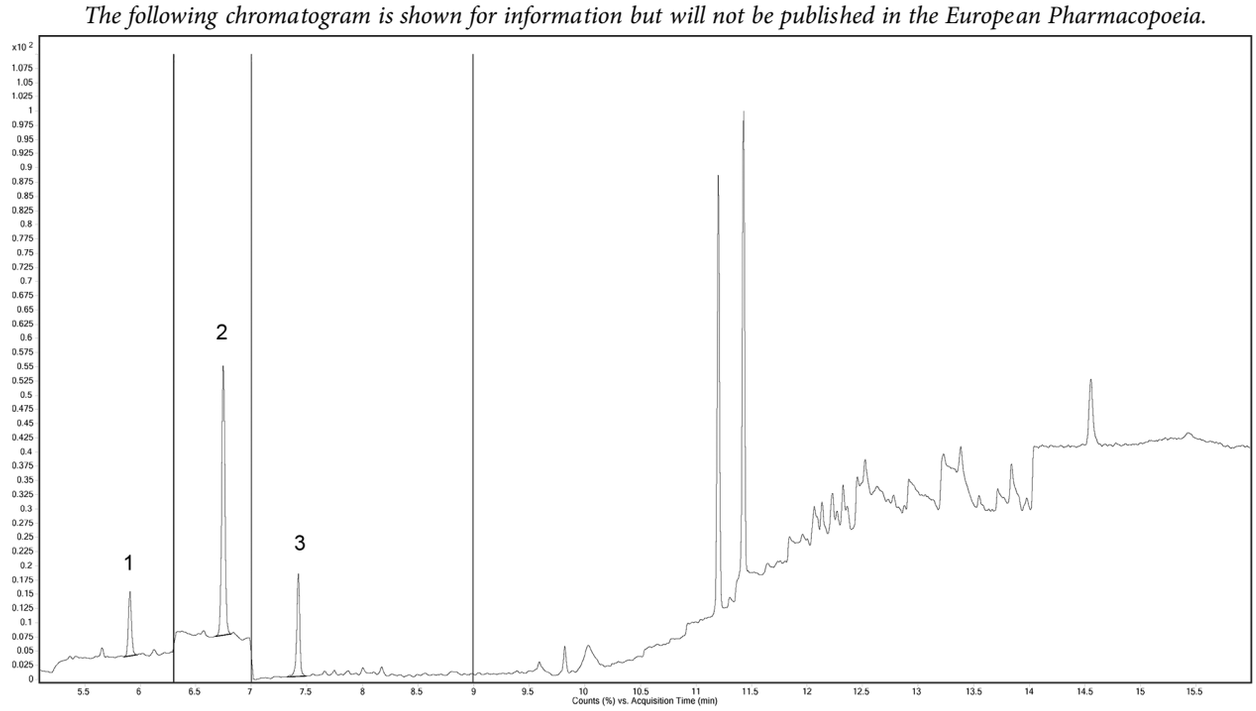
Temperature:

	Time (min)	Temperature (°C)
Column	0 - 0.5	40
	0.5 - 7.0	40 → 170
	7.0 - 10.7	170 → 280
	10.7 - 17.0	280
Injection port		250
Transfer line		240

Detection(7): single quadrupole mass spectrometer in single ion monitoring (SIM) mode. The following settings have been found to be suitable and are given as examples; the settings may be adjusted so as to comply with the system suitability criteria:
- *electron impact ionisation mode*: 70 eV;
- *ion source temperature*: 230 °C;
- *analyser temperature*: 150 °C;
- *dwell time*: 300 ms;
- *gain factor*: 5;
- *SIM mode parameters*:

(5) Membrane used for the development of the procedure: regenerated cellulose (Chromafil Xtra RC).
(6) Agilent VF-624ms is suitable.
(7) Instrument used for the development of the procedure: GC Agilent 7890A coupled to MS detector 5975C.

The following chromatogram is shown for information but will not be published in the European Pharmacopoeia.



1. NDMA 2. NEMA 3. NDEA

Figure 2.5.42.-2. – *Chromatogram for N-nitrosamine analysis in active substances by procedure B: spiked solution*

Substance	*m/z*	Start of monitoring (min)
NDMA	74	5.0
NEMA	88	6.3
NDEA	102	7.0

NOTE: acquisition can be started at 5 min; during non-acquisition the eluent is directed to waste. Time segments can be defined accordingly.

Injection: 2.5 µL of the spiked solution, the test solution and reference solution, (1) or (2) as appropriate.

Relative retention with reference to the internal standard (NEMA) (retention time = about 6.7 min): NDMA = about 0.9; NDEA = about 1.1.

System suitability:

- *repeatability*: maximum relative standard deviation of 20 per cent for the ratio between the area of the peak due to each *N*-nitrosamine and the area of the peak due to the internal standard, determined on 6 injections of the reference solution.
- *signal-to-noise ratio*: for the spiked solution, minimum 10 for the peak due to each *N*-nitrosamine.

Limit: use the stated *m/z*.

For the test solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine detected and the area of the peak due to the internal standard (R_s).

For the spiked solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine and the area of the peak due to the internal standard (R_r).

For each *N*-nitrosamine detected, the ratio of R_s to R_r must be smaller than 0.50.

PROCEDURE C (GC-MS/MS)

Gas chromatography (*2.2.28*) coupled with mass spectrometry (*2.2.43*).

Internal standard solution. Dissolve 5 mg of *N-nitroso-ethylmethylamine R* (NEMA) in *methanol R3* and dilute to 10.0 mL with the same solvent. Dilute 500 µL of the solution to 10.0 mL with *water for chromatography R*.

Extraction mixture. Dissolve 40.0 g of *sodium hydroxide R* in 500 mL of *water for chromatography R*. Add 100 µL of the internal standard solution, then 50 mL of *acetonitrile R1*, and dilute to 1000 mL with *water for chromatography R*.

N-Nitrosamines spiking solution. For each *N*-nitrosamine concerned, use the corresponding CRS (*N-nitroso-dimethylamine CRS*, *N-nitroso-diethylamine CRS*, *N-nitroso-dibutylamine CRS*, *N-nitroso-diisopropylamine CRS*, *N-nitroso-ethyl-isopropylamine CRS* and *N-nitroso-dipropylamine CRS*). In a single volumetric flask, dilute 100 µL of each of these CRS to 10.0 mL with *water for chromatography R*. Dilute 300 µL of this solution to 20.0 mL with *water for chromatography R*.

Test solution. Suspend 250.0 mg of the substance to be examined in 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min, then centrifuge at about 10 000 *g* for 5 min. Use the lower layer (organic).

Spiked solution. Suspend 250.0 mg of the substance to be examined in 10.0 mL of the extraction mixture. Add 100 µL of the *N*-nitrosamines spiking solution. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min, then centrifuge at about 10 000 *g* for 5 min. Use the lower layer (organic).

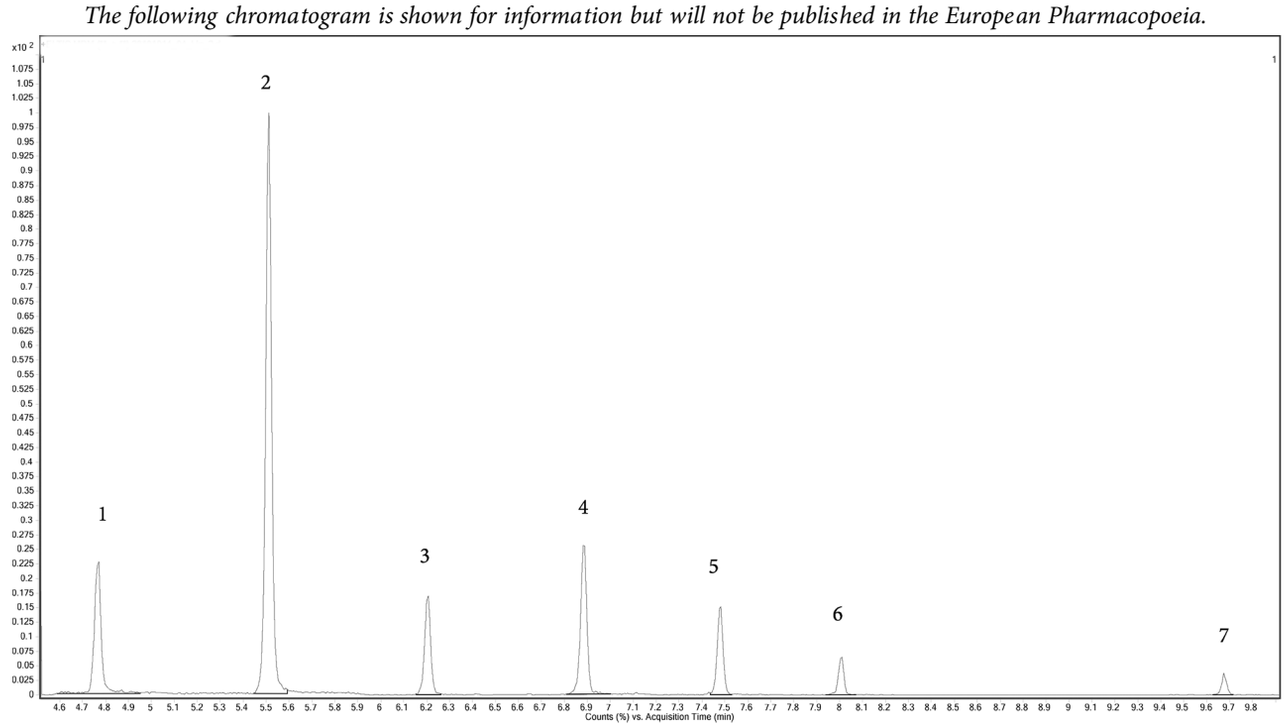
Reference solution (a). Add 50 µL of the *N*-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min, then centrifuge at about 10 000 *g* for 5 min. Use the lower layer (organic).

Reference solution (b). Add 100 µL of the *N*-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min, then centrifuge at about 10 000 *g* for 5 min. Use the lower layer (organic).

Reference solution (c). Add 200 µL of the *N*-nitrosamines spiking solution to 10.0 mL of the extraction mixture. Vortex and shake well for 5 min. Extract with 2.0 mL of *methylene chloride R1*. Shake well for at least 5 min, then centrifuge at about 10 000 *g* for 5 min. Use the lower layer (organic).

Column:

The following chromatogram is shown for information but will not be published in the European Pharmacopoeia.



1. NDMA
2. NEMA
3. NDEA
4. NEiPA
5. NDiPA
6. NDPA
7. NDBA

Figure 2.5.42.-3. – *Chromatogram for N-nitrosamine analysis in active substances by procedure C: spiked solution*

– *material*: fused silica;
– *size*: *l* = 30 m, Ø = 0.25 mm;
– *stationary phase*: *cyanopropylphenylene(6)me thyl(94)polysiloxane R* (film thickness 1.4 µm)[8].

Carrier gas: *helium for chromatography R.*

Flow rate: 1.3 mL/min.

Injection mode: pulsed splitless: 276 kPa, 0.5 min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 0.5	40
	0.5 - 2.2	40 → 140
	2.2 - 4.2	140
	4.2 - 6.2	140 → 180
	6.2 - 6.7	180
	6.7 - 8.7	180 → 240
	8.7 - 10.5	240
	10.5 - 11.5	240 → 280
	11.5 - 14.0	280
Injection port		250
Transfer line		240

Detection[9]: triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode. The following settings have been found to be suitable and are given as examples; the settings may be adjusted so as to comply with the system suitability criteria:

– *electron impact ionisation mode*: 40 eV;
– *ion source temperature*: 230 °C;
– *analyser temperature*: 150 °C (both);
– *dwell time*: 200 ms;
– *gain factor*: 15;
– *collision gas*: *nitrogen for chromatography R* at 1.5 mL/min;
– *MRM mode parameters*:

Substance	MRM transitions (*m/z*) principal (*qualifier*)	Collision energy (V)
NDMA	74 → 44	5
	(*74 → 42*)	22
NEMA	88 → 71	5
	(*88 → 42*)	22
NDEA	102 → 85	3
	(*102 → 56*)	19
NEiPA	116 → 99	5
	(*116 → 44*)	14
NDiPA	130 → 88	5
	(*130 → 71*)	14
NDPA	130 → 113	1
	(*130 → 88*)	1
NDBA	158 → 141	1
	(*158 → 99*)	7

NOTE: acquisition can be started at 4.5 min; during non-acquisition the eluent is directed to waste. Time segments can be defined accordingly.

Injection: 3 µL of the spiked solution, the test solution and reference solution (b).

(8) Agilent VF-624ms is suitable.
(9) Instrument used for the development of the procedure: GC Agilent 7890B coupled to MS detector 7000D.

Relative retention with reference to the internal standard (NEMA) (retention time = about 5.5 min): NDMA = about 0.9; NDEA = about 1.1; NEiPA = about 1.3; NDiPA = about 1.4; NDPA = about 1.5; NDBA = about 1.8.

System suitability: for each *N*-nitrosamine:

- *repeatability*: use the principal MRM transitions, maximum relative standard deviation of 20 per cent for the ratio between the area of the peak due to the *N*-nitrosamine and the area of the peak due to the internal standard, determined on 6 injections of reference solution (b);
- *signal-to-noise ratio 1*: for the spiked solution, minimum 10 for the peak corresponding to the principal transition;
- *signal-to-noise ratio 2*: for the spiked solution, minimum 3 for the peak corresponding to the qualifier transition.

Limit: use the principal MRM transitions.

For the test solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine detected and the area of the peak due to the internal standard (R_s).

For the spiked solution, calculate the ratio between the area of the peak due to each *N*-nitrosamine and the area of the peak due to the internal standard (R_r).

For each *N*-nitrosamine detected, the ratio of R_s to R_r must be smaller than 0.50.

The test is not valid unless the ratio between the area of the peak corresponding to the principal transition and the area of the peak corresponding to the qualifier transition for the test solution is within 20 per cent of the same ratio calculated for the spiked solution.

When procedure C is used as a quantitative test. Inject 3 µL of the spiked solution, the test solution and reference solutions (a), (b) and (c).

System suitability: for each *N*-nitrosamine:

- *repeatability*: use the principal MRM transitions, maximum relative standard deviation of 20 per cent for the ratio between the area of the peak corresponding to the *N*-nitrosamine and the area of the peak due to the internal standard, determined on 6 injections of reference solution (b);
- *signal-to-noise ratio 1*: for reference solution (a), minimum 10 for the peak corresponding to the principal transition;
- *signal-to-noise ratio 2*: for reference solution (a), minimum 3 for the peak corresponding to the qualifier transition.

Calculation: use the principal MRM transitions.

Use reference solutions (a), (b) and (c) to establish a calibration curve using the ratio between the area of the peak due to each *N*-nitrosamine detected and the area of the peak due to the internal standard versus the concentration of the *N*-nitrosamines. The concentration of any *N*-nitrosamines in the test solution is interpolated from the calibration curve.

The test is not valid unless:

- the ratio between the area of the peak corresponding to the principal transition and the area of the peak corresponding to the qualifier transition for the test solution is within 20 per cent of the same ratio calculated for the spiked solution;
- the calculated recovery in all spiked solutions is between 70-130 per cent.

Reagents

Formic acid, anhydrous. *1039300.* [64-18-6].
See *formic acid R*.

Formic acid. CH_2O_2. (M_r 46.03). *1039300.* [64-18-6].

Content: minimum 98.0 per cent *m/m*.

Colourless liquid, corrosive, miscible with water and with ethanol (96 per cent).

d_{20}^{20}: about 1.22.

Assay. Weigh accurately a conical flask containing 10 mL of *water R*, quickly add about 1 mL of the acid and weigh again. Add 50 mL of *water R* and titrate with *1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*) or using 0.5 mL of *phenolphthalein solution R* as indicator.

1 mL of *1 M sodium hydroxide* is equivalent to 46.03 mg of CH_2O_2.

When used for mass spectrometry detection, a special grade may be needed.

Methanol R3. *XXXXXXX.*

Content: minimum 99.9 per cent.

When used for mass spectrometry detection, a special grade may be needed.

Methylene chloride R1. *XXXXXXX.*
Content (*2.2.28*): minimum 99.8 per cent.

Deuterated *N*-nitroso-diethylamine. $C_4{}^2H_{10}N_2O$. (M_r 112.2). *XXXXXXX.* [1219794-54-3]. *N*,*N*-Bis[($^2H_5$)ethyl]nitrous amide. NDEA-d_{10}.

Degree of deuteration: minimum 98 per cent.

***N*-Nitroso-ethylmethylamine.** $C_3H_8N_2O$. (M_r 88.1). *XXXXXXX.* [10595-95-6]. *N*-Ethyl-*N*-methylnitrous amide. NEMA.
Yellow liquid.
bp: about 170 °C.

Cyanopropylphenylene(6)methyl(94)polysiloxane. *XXXXXXX.*
Polysiloxane substituted with 6 per cent of cyanopropyl and phenylene groups and 94 per cent of methyl groups.

