PRODUCT INFO

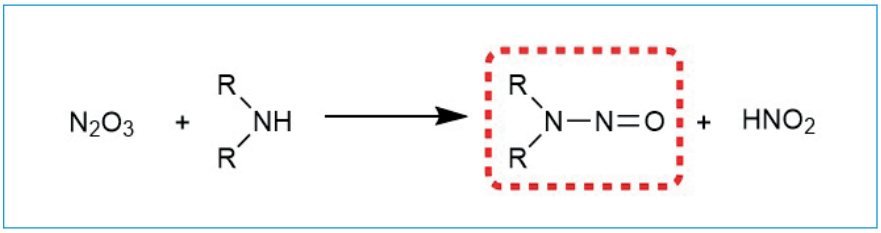
Nitrosamine Analysis

The analysis of nitrosamines in medicines has recently become a key point in which international regulatory agencies such as the FDA (Food and Drug Administration) and the EMA (European Medicines Agency) have focused, due to some impurities detected in the medicines from the group of sartans such as Valsartan, Losartan, Candesartan, Olmesartan and Irbesartan.

90% of nitrosamines are considered carcinogenic substances, which requires a strict control of their presence, as well as their quantification. Gastric and colon cancer are the two most common tumors deriving from these compounds.

What are nitrosamines?

Nitrosamines are organic compounds that contain a nitroso group, whose chemical structure is R1N(-R2)-N=O. They originate from the reaction of a secondary amine with nitrites or nitrogen oxide in a highly acidic environment. Its formation is favored at high temperatures. Proteins contain secondary amines, so nitrosamines can be formed if they react with nitrites or nitrogen oxide under acidic conditions, such as the gastric environment.



In July 2018, health authorities became aware for the first time of the presence of N-nitrosodimethylamine (NDMA), a nitrosamine impurity, in products containing Valsartan.

These compounds are commonly found or generated, in very small amounts, in foods such as meat or oils, preservatives, latex products, drinking and waste water, tobacco smoke, pesticides and, to a greater extent, in some medicines, originating from some impurities present in the solvents, starting materials and reagents used in the production of the drug itself.

Nitrosamine Analysis

Detection methods

The most reliable detection methods are gas or liquid chromatography, coupled with a mass detector. The American and European agencies, FDA and EMA respectively, have issued guidelines on the permitted limits of these impurities in pharmacological products.

Scharlau solvents

Scharlab offers two solvents for this analysis: Dichloromethane for GC-MS (CL0346) and Methanol for LC-MS (ME0326).

In the case of Scharlau's dichloromethane for GC-MS, in addition to ensuring the absence of peaks greater than 3 ppt (expressed as n-tetradecane) from n-undecane to n-tetracontane, the absence of nitrosamines has been ensured following the guidelines set out in the EPA 8270 regulation.

Regarding Scharlau's methanol for LC-MS, in addition to being suitable for LC-MS by means of the reserpine test, the absence of nitrosamines has also been ensured, following the EPA 8270 guidance.

Nitrosamine specifications

The following tables show the 9 nitrosamines analysed in our solvents, specified in the EPA 8270 guideline, as well as the maximum limits of each nitrosamine, according to the solvent and the analysis technique. The maximum limits have been determined based on the detection limit of the equipment and the uneven fragmentation pattern of the different nitrosamines.

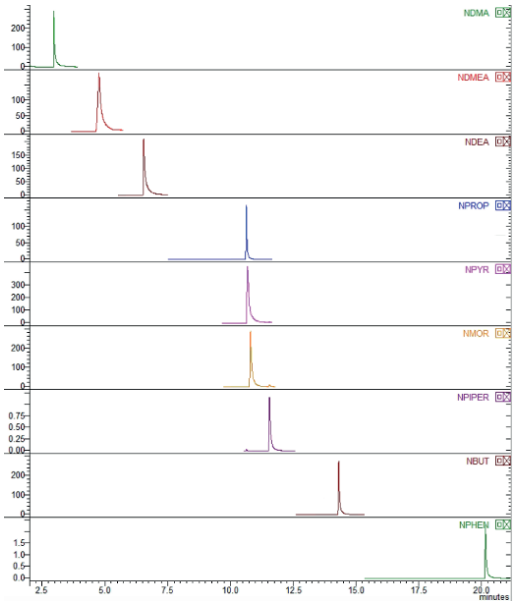
Dichloromethane, by GC/MS/MS

Compound	ppt (ng/l)
N-nitrosodimethylamine	10
N-nitrosomethylethylamine	100
N-nitrosodiethylamine	50
N-nitrosodipropylamine	50
N-nitrosopyrrolidine	20
N-nitrosomorpholine	20
N-nitrosopiperidine	10
N-nitrosodibutylamine	10
N-nitrosodiphenylamine	1

Methanol, by LC/MS/MS

Compound	ppt (ng/l)
N-nitrosodimethylamine	30
N-nitrosomethylethylamine	7
N-nitrosodiethylamine	50
N-nitrosodipropylamine	50
N-nitrosopyrrolidine	5
N-nitrosomorpholine	100
N-nitrosopiperidine	3
N-nitrosodibutylamine	2
N-nitrosodiphenylamine	4

GC/MS/MS conditions:



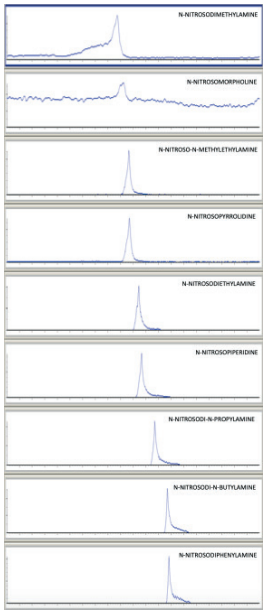
- **Equipment:** Bruker SCION TQ GC/MS/MS System

- **Injector:** Split/Splitless
  - Temperature: 220 °C
  - Constant flow: 1.0 ml/min
  - Mode: Splitless
  - Splitless time: 1.0 min
  - Injection volume: 2 µl

- **Column:** SC-5MS, 30 m, 0.25 mm, 0.25 µm
  - Initial temperature: 40 °C
  - Initial time: 3.0 min
  - Ramp 1: 8 °C/min
  - Final temperature 1: 180 °C
  - Ramp 2: 40 °C/min
  - Final temperature 2: 300 °C
  - Final time: 10 min

- **Mass Spectrometer (MS):**
  - Transfer line temperature: 280 °C
  - Source temperature: 280 °C
  - Acquisition time: 21.15 min
  - Delay: 2 min
  - Ionisation type: Electronic Ionisation (EI)
  - Ionisation energy: 70 eV
  - Collision gas: Ar

LC/MS/MS conditions:



- **HPLC equipment:** Agilent 1200 SL Series
  - Mobile phase A: H_2O, 0.1% Formic Acid Scharlau, LC-MS quality
  - Mobile phase B: MeOH Scharlau, LC-MS quality
  - Column: KromaPhase C18, 150 x 4.6 mm, 5 µm
  - Temperature: 40 °C
  - Constant flow: 1.0 ml/min
  - Injection volume: 20 µl
  - Gradient:

Time	Flow (µl/min)	% A	% B
0.00	1000	95.0	5.0
1.00	1000	95.0	5.0
1.30	1000	60.0	40.0
5.00	1000	0.0	100.0
7.00	1000	0.0	100.0
7.01	1000	95.0	5.0
10.00	1000	95.0	5.0

- **Mass Spectrometer (MS):** ABSCIEX 3200 QTRAP
  - Interphase: APCI+
  - Collision gas: N_2
  - Turbo temperature: 400 °C
  - Curtain gas: 20 psi
  - Nebulisation gas: 50 psi
  - Turbo gas: 50 psi
  - Corona current: 5 µA

Nitrosamine Analysis

Ordering information:

Solvents

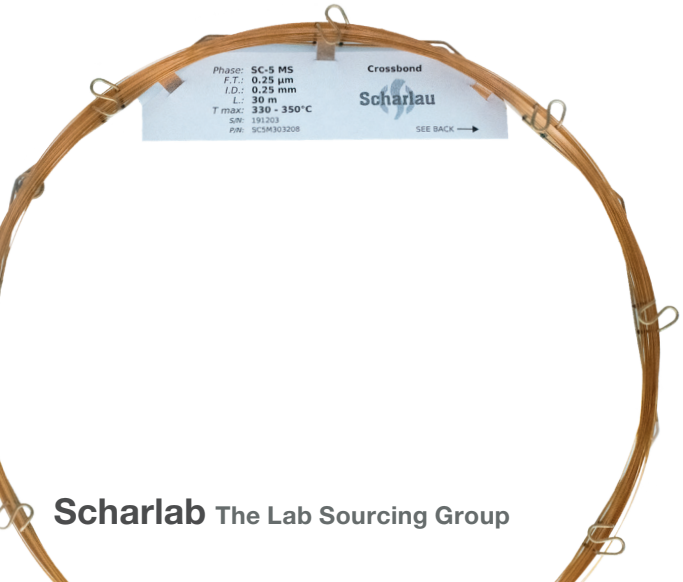
Description	Packaging	Art. No.
Dichloromethane, GC-MS, suitable for nitrosamine analysis	1 l	CL03461000
	2.5 l	CL03462500
Methanol, LC-MS, suitable for nitrosamine analysis	1 l	ME03261000
	2.5 l	ME03262500

Standards

Description	Packaging	Art. No.
Nitrosamines mixture - 9 components (N-Nitrosodibutylamine, N-Nitrosodiethylamine, N-Nitrosodimethylamine, N-Nitrosomethylethylamine, N-Nitrosodiphenylamine, N-Nitrosodipropylamine, N-Nitrosomorpholine N-Nitrosopiperidine, N-Nitrosopyrrolidine), 100 µg/ml in dichloromethane	1 ml	PS53450001
Nitrosamines mixture - 3 components (N-Nitrosodimethylamine, N-Nitrosodiphenylamine, N-Nitrosodipropylamine), 2000 µg/ml in methanol	1 ml	PS71530001
N-nitrosodiethylamine [55-18-5]	100 ml	NS12220100
N-nitrosodimethylamine [62-75-9], 10 µg/ml in methanol	10 ml	PS21880010
N-nitrosodimethylamine [62-75-9], 100 µg/ml in methanol	1 ml	PS21890001
N-nitrosomethylethylamine [10595-95-6]	100 mg	NS23870100
N-nitrosodiphenylamine [86-30-6]	100 mg	NS21650100
N-nitrosodiphenylamine [86-30-6], 10 µg/ml in methanol	10 ml	PS21900010
N-nitrosodiphenylamine [86-30-6], 100 µg/ml in methanol	1 ml	PS21910001
N-nitrosodipropylamine [621-64-7]	100 ml	NS21640100
N-nitrosodipropylamine [621-64-7], 10 µg/ml in methanol	10 ml	PS21920010
N-nitrosodipropylamine [621-64-7], 100 µg/ml in methanol	1 ml	PS21930001
N-nitrosopiperidine [100-75-4]	100 ml	NS12230100

Columns

Description	Packaging	Art. No.
Column GC SC-5MS, 30 m, 0.25 mm, 0.25 µm	1 u	SCMS500050
Column KromaPhase C18, 150 x 4.6 mm, 5 µm	1 u	066-B2Y803



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