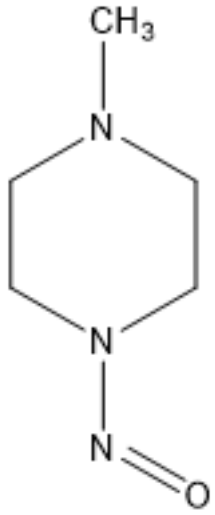
Test for 1-Methyl-4-nitroso piperazine (MeNP) in rifampicin active pharmaceutical ingredient or rifampicin tablets / capsules by HPLC-MS/MS

2025-01

Molecular formula. $C_5H_{11}N_3O$

Relative molecular mass. 129.16.

Graphic formula



1-methyl-4-nitrosopiperazine

Chemical name. 1-Methyl-4-nitrosopiperazine; CAS Reg. No. 16339-07-4.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (15 cm x 4.6 mm) packed with porous particles of silica gel, the surface of which has been modified with chemically-bonded phenyl groups (3 µm).

Use the following conditions for gradient elution:

-**Mobile phase A**: 10 mM ammonium formate solution, pH = 9.0;

-**Mobile phase B**: methanol R.

Prepare the 10 mM ammonium formate solution, pH 9.0, by dissolving 630 mg of ammonium formate for chromatography R in 900 mL of water R, adjust the pH to 9.0 by adding ammonia (~260 g/L) TS and diluting to 1000 mL with water R.

Time (minutes)	**Mobile phase A** (% *V*/*V*)	**Mobile phase B** (% *V*/*V*)	**Comments**
0–3	60	40	Isocratic
3–7	60 to 0	40 to 100	Linear gradient
7–11	0	100	Isocratic
11–11.1	0 to 60	100 to 40	Return to initial composition
11.1–15	60	40	Re-equilibration

Operate with a flow rate of 0.6 mL per minute. Maintain the column at 30 °C. As a detector, use a triple-quadrupole mass spectrometer equipped with an Atmospheric Pressure Chemical Ionization (APCI) source

Set the ion source and scan settings of the MS spectrometer as follows and optimize the settings for ion source temperature and auxiliary gases. Use the additional MRM transition of 130.1→ 58.1 (qualifier ion transition) to identify the MeNP response.

Acquisition mode:	Multiple Reaction Monitoring (MRM)
Polarity:	Positive
MRM transitions (m/z):	130.1→ 100.1(for MeNP)

	134.2→ 104.1(for the Internal Standard MeNP-d4)

Use valve switches to channel rifampicin and excipients to waste, as appropriate, to avoid excessive contamination of MS detector.

Prepare the following solutions freshly. As a diluent, use methanol R. (If 'gelling' occurs during the preparation of sample solutions of finished products, a mixture of 80 volumes of methanol R and 20 volumes of water R can be used as an alternative diluent.). After preparation, keep the solutions protected from light at about 4-8 °C or use an autosampler with cooling (4-8 °C).

For the MeNP stock solution (1), prepare a solution containing 1 µg of 1-nitroso-4-methyl piperazine R per mL.

Forthe Internal Standard (IS) stock solution (1), prepare a solution containing 1 µg of 1-nitroso-4-methyl piperazine-d4 R (MeNP-d4)per mL.

For the Internal Standard (IS) stock solution (2) , dilute 1.0 mL of the Internal Standard (IS) stock solution (1) to 100.0 mL.

Prepare a set of 5 standard solutions (solutions (1) to (5)) and a sensitivity solution (solution (6)) by diluting the MeNP stock solution according to the following table.

Solution	Volume of MeNP stock solution (1) (mL)	Volume of IS stock solution (1) (mL)	Final volume (mL)	MeNP concentration(ng/mL)
1	0.500	1.0	100.0	5
2	1.0	1.0	100.0	10
3	2.0	1.0	100.0	20
4	5.0	1.0	100.0	50
5	10.0	1.0	100.0	100
6	0.100	1.0	100.0	1

Prepare the sample solutions in duplicate (solutions (7) and (8)):

For the analysis of rifampicin active pharmaceutical ingredient, transfer 250.0 mg of the test substance into a 15 mL conical centrifuge tube . Add 5.0 mL of Internal Standard (IS) stock solution (2), mix and sonicate for 10 minutes. Centrifuge the dispersion for 10 minutes at 4,000 rpm, filter the supernatant (a 0.2 µm PVDF membrane syringe filter was found suitable) and use the filtrate.

For the analysis of rifampicin tablets / capsules, weigh and powder 20 tablets / capsules. Transfer a quantity of the powdered tablets or capsule powder, nominally containing 250.0 mg of Rifampicin , into a 15 mL conical centrifuge tube and proceed as described above.

For solution (9), transfer 5.0 mL of Internal Standard (IS) stock solution (2) into a 15 mL conical centrifuge tube, mix and sonicate for 10 minutes. Centrifuge the solution for 10 minutes at 4,000 rpm, filter the supernatant (a 0.2 µm PVDF membrane syringe filter was found suitable) and use the filtrate.

Inject 5 µL each of solutions (1) to (9).

Monitor the 130.1/ 100.1 and the 134.2/ 104.1 (m/z) transitions and measure the signals due to MeNP and MeNP-d4 (retention times of about 5 minutes).

The test is not valid unless, in the chromatogram obtained with solution (6), the signal due to MeNP is detected with a signal-to-noise ratio of at least 10.

Plot the ratios of the signals due to MeNP and to MeNP-d4 versus the concentration of MeNP in each of the standard solutions (1) to (5) (in ng/mL) and determine the regression line using the least-squares method. From the graph so obtained, determine the concentration (C) of MeNP in the sample solutions (7) and (8) (in ng/mL).

The test is not valid when the correlation coefficient of the calibration curve is less than 0.99 nor if the concentrations of MeNP in sample solutions (7) and (8) differ by more than 15%.

Calculate the concentration of MeNP in µg per g rifampicin, R_t

$$R_t = C \times 5 / 1000 / W$$

W = amount of rifampicin taken (in g) or labelled amount of rifampicin in the amount of rifampicin tablets taken (in g).

The result is the mean of the values R_t obtained for the two samples.