– elute with a 1.91 g solution of *disodium tetraborate R* in 1000 ml of *water R* as the mobile phase for 15 min; change to 100 per cent of *0.1 M sodium hydroxide* for a period of 0.5 min; elute with this solution for 10 min; return to the initial conditions for a period of 0.5 min; the flow rate is 1.0 ml/min,
– a detector with a sensitivity of 30 µS.

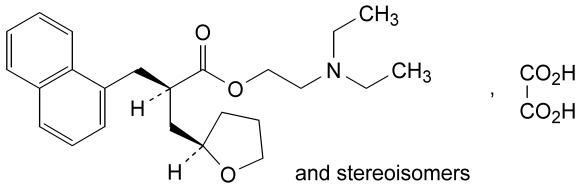
Continuously pump the chemical neutralisation system in counter-flow with a 2.45 g/l solution of *sulphuric acid R*, at a flow rate of 4 ml/min.

Inject 50 µl of each solution. The chromatogram obtained with the reference solution shows a principal peak which corresponds to the sulphate ion (retention time of about 7.5 min). Change the composition of the mobile phase, if necessary, to obtain the prescribed retention time. Calculate the sulphate content of the substance to be examined.

01/2008:1594
corrected 6.0

NAFTIDROFURYL HYDROGEN OXALATE

Naftidrofuryli hydrogenooxalas



$C_{26}H_{35}NO_7$ M_r 473.6
[3200-06-4]

DEFINITION

Mixture of 4 stereoisomers of 2-(diethylamino)ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate hydrogen oxalate.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: freely soluble in water, freely soluble or soluble in alcohol, slightly or sparingly soluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: dissolve 1.0 g in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulphate R*, shake, filter and evaporate the filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Use the residue obtained.

Comparison: *Ph. Eur. reference spectrum of naftidrofuryl.*

B. Dissolve 0.5 g in *water R* and dilute to 10 ml with the same solvent. Add 2.0 ml of *calcium chloride solution R*. A white precipitate is formed. The precipitate dissolves after the addition of 3.0 ml of *hydrochloric acid R*.

TESTS

Absorbance (*2.2.25*): maximum 0.1 at 430 nm.

Dissolve 1.5 g in *water R* and dilute to 10 ml with the same solvent. If necessary use an ultrasonic bath.

Related substances

A. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 80.0 mg of the substance to be examined in the mobile phase and dilute to 20.0 ml with the mobile phase. Treat in an ultrasonic bath for 10 s. A precipitate is formed. Filter through a 0.45 µm membrane filter, discarding the first 5 millilitres. *Use a freshly prepared solution.*

Reference solution (a). Dissolve 5.0 mg of *naftidrofuryl impurity A CRS* in *acetonitrile R* and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *naftidrofuryl impurity B CRS* and 5 mg of the substance to be examined in *acetonitrile R* and dilute to 50 ml with the same solvent. Dilute 1 ml of this solution to 50 ml with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 350 m^2/g, a pore size of 10 nm and a carbon loading of 14 per cent.

Mobile phase: mix 60 ml of *methanol R* with 150 ml of *tetrabutylammonium buffer solution pH 7.0 R* and dilute to 1000 ml with *acetonitrile R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 283 nm.

Injection: 20 µl.

Run time: 2.3 times the retention time of naftidrofuryl.

Relative retention with reference to naftidrofuryl (retention time = about 7 min): impurity A = about 0.5; impurity B = about 0.8; impurity C = about 1.8.

System suitability: reference solution (b):

– *resolution*: minimum 3.0 between the peaks due to naftidrofuryl and impurity B.

Limits:

– *impurities A, B, C*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *any other impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent),
– *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
– *disregard limit*: 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

B. Gas chromatography (*2.2.28*).

Test solution (a). Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 50 ml with the same solvent. Add 2 ml of *concentrated ammonia R* and shake with 3 quantities, each of 10 ml, of *methylene chloride R*. To the combined lower layers, add *anhydrous sodium sulphate R*, shake, filter and evaporate the

filtrate at a temperature not exceeding 30 °C, using a rotary evaporator. Take up the residue with *methylene chloride R* and dilute to 20.0 ml with the same solvent.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *methylene chloride R*.

Reference solution. Dissolve 5 mg of *naftidrofuryl impurity F CRS* in *methylene chloride R* and dilute to 50 ml with the same solvent.

Column:

- *material*: fused silica,
- *size*: *l* = 25 m, Ø = 0.32 mm,
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 0.45 µm).

Carrier gas: *helium for chromatography R*.

Splitter flow rate: 25 ml/min.

Flow rate: 2.9 ml/min.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 4	210
	4 - 8	210 → 230
	8 - 18	230 → 260
	18 - 30	260
Injection port		290
Detector		290

Detection: flame ionisation.

Injection: 1 µl.

Relative retention with reference to the second eluting peak of naftidrofuryl: impurity D = about 0.14; impurity B = about 0.55 (for the second eluting peak); impurity E = about 0.86; impurity F = about 1.04 (for the second eluting peak).

System suitability: test solution (b):

- *resolution*: minimum 1.0 between the 2 peaks due to the diastereoisomers of naftidrofuryl.

Limits: test solution (a):

- *impurity F*: for the sum of the areas of the 2 peaks, maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent),
- *impurity E*: maximum 0.20 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.20 per cent),
- *impurity D*: maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent),
- *any other impurity*: for each impurity, maximum 0.10 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.10 per cent),
- *total*: maximum 0.50 per cent of the sum of the areas of the 2 peaks due to naftidroduryl (0.50 per cent),
- *disregard limit*: 0.02 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl (0.02 per cent); disregard any peaks due to impurity B.

Diastereoisomer ratio. Gas chromatography (*2.2.28*) as described in test B for related substances.

Limits: test solution (b):

- *first eluting naftidrofuryl diastereoisomer*: minimum 30 per cent of the sum of the areas of the 2 peaks due to naftidrofuryl.

Heavy metals (*2.4.8*): maximum 10 ppm.

In a silica crucible, mix thoroughly 1.0 g of the substance to be examined with 0.5 g of *magnesium oxide R1*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. If after 30 min of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800 ± 50 °C for about 1 h. Take up the residue with 2 quantities, each of 5 ml, of a mixture of equal volumes of *hydrochloric acid R1* and *water R*. Add 0.1 ml of *phenolphthalein solution R* and then *concentrated ammonia R* until a pink colour is obtained. Cool, add *glacial acetic acid R* until the solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water R*. The solution complies with limit test E. Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

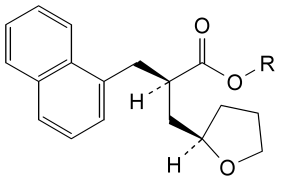
ASSAY

Dissolve 0.350 g in 50 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 47.36 mg of $C_{26}H_{35}NO_7$.

IMPURITIES

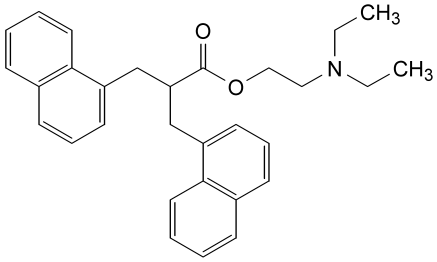
Specified impurities: A, B, C, D, E, F.



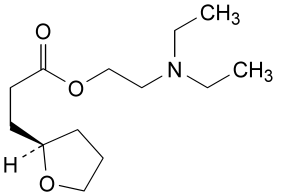
and stereoisomers

A. R = H: 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoic acid,

B. R = C_2H_5: ethyl 2-[(naphthalen-1-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate,

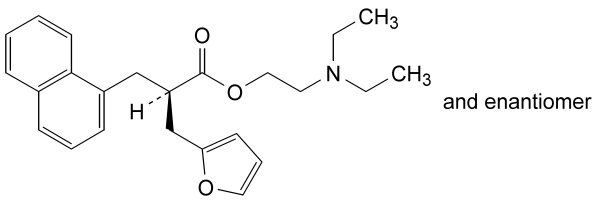


C. 2-(diethylamino)ethyl 3-(naphthalen-1-yl)-2-[(naphthalen-1-yl)methyl]propanoate,

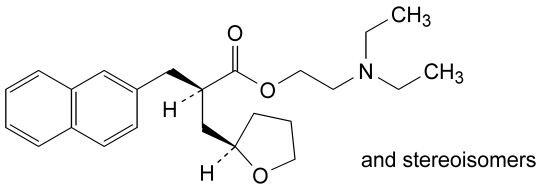


and enantiomer

D. 2-(diethylamino)ethyl 3-[(2*RS*)-tetrahydrofuran-2-yl]propanoate,



E. 2-(diethylamino)ethyl (2*RS*)-2-[(furan-2-yl)methyl]-3-(naphthalen-1-yl)propanoate,

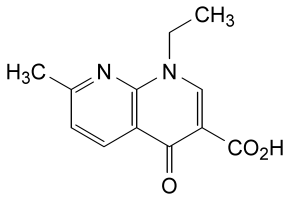


F. 2-(diethylamino)ethyl 2-[(naphthalen-2-yl)methyl]-3-(tetrahydrofuran-2-yl)propanoate.

01/2008:0701
corrected 6.0

NALIDIXIC ACID

Acidum nalidixicum



$C_{12}H_{12}N_2O_3$ M_r 232.2
[389-08-2]

DEFINITION

Nalidixic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, calculated with reference to the dried substance.

CHARACTERS

An almost white or pale yellow, crystalline powder, practically insoluble in water, soluble in methylene chloride, slightly soluble in acetone and in alcohol. It dissolves in dilute solutions of alkali hydroxides.

It melts at about 230 °C.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Dissolve 12.5 mg in *0.1 M sodium hydroxide* and dilute to 50.0 ml with the same solvent. Dilute 2.0 ml of this solution to 100.0 ml with *0.1 M sodium hydroxide*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows two absorption maxima, at 258 nm and 334 nm. The ratio of the absorbance measured at 258 nm to that measured at 334 nm is 2.2 to 2.4.

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *nalidixic acid CRS*. Examine the substances prepared as discs.

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with the test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 2 ml of *hydrochloric acid R*. Add 0.5 ml of a 100 g/l solution of *β-naphthol R* in *alcohol R*. An orange-red colour develops.

TESTS

Absorbance. Dissolve 1.50 g in *methylene chloride R* and dilute to 50.0 ml with the same solvent. The absorbance (*2.2.25*) measured at 420 nm is not greater than 0.10.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution (a). Dissolve 0.20 g of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 20 ml with *methylene chloride R*.

Reference solution (a). Dissolve 20 mg of *nalidixic acid CRS* in *methylene chloride R* and dilute to 20 ml with the same solvent.

Reference solution (b). Dilute 2 ml of test solution (b) to 10 ml with *methylene chloride R*.

Reference solution (c). Dilute 1 ml of reference solution (b) to 10 ml with *methylene chloride R*.

Reference solution (d). Dilute 1 ml of reference solution (b) to 25 ml with *methylene chloride R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 10 volumes of *dilute ammonia R1*, 20 volumes of *methylene chloride R* and 70 volumes of *alcohol R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.1 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (d).

Heavy metals (*2.4.8*). 1.0 g complies with limit test D for heavy metals (20 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 10 ml of *methylene chloride R* and add 30 ml of *2-propanol R* and 10 ml of *carbon dioxide-free water R*. Keep the titration vessel covered and pass *nitrogen R* through the solution throughout the titration. Keep the temperature of the solution between 15 °C and 20 °C. Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*) using a silver-silver chloride comparison electrode with a sleeve diaphragm or a capillary tip, filled with a saturated solution of *lithium chloride R* in *ethanol R*, and a glass electrode as indicator electrode.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 23.22 mg of $C_{12}H_{12}N_2O_3$.

STORAGE

Store in an airtight container, protected from light.