Detection: spray with about 7 ml of a mixture of 1 volume of a 25 g/l solution of *vanillin R* in *ethanol (96 per cent) R* and 4 volumes of *sulphuric acid R* and heat at 130 °C for 5-10 min.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Acidity or alkalinity. Mix 5.0 g thoroughly for 1 min with a mixture of 0.1 ml of *bromothymol blue solution R1*, 2 ml of *heptane R* and 10 ml of *water R*. If the aqueous layer is blue, not more than 0.15 ml of *0.01 M hydrochloric acid* is required to change the colour of the indicator to yellow. If the aqueous layer is yellow, add 0.45 ml of *0.01 M sodium hydroxide* and shake vigorously. After standing to ensure complete separation, the aqueous layer is blue.

Optical rotation (*2.2.7*): – 0.10° to + 0.10°.

Dissolve 2.50 g in *ethanol (96 per cent) R* and dilute to 25 ml with the same solvent.

Hydroxyl value (*2.5.3, Method A*): 175 to 190.

Iodine value (*2.5.4, Method A*): maximum 8.0.

Peroxide value (*2.5.5, Method A*): maximum 5.0.

Saponification value (*2.5.6*): maximum 5.0.

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

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 0.5 per cent, determined on 2.00 g.

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

ASSAY

Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 0.4 g of *tetradecane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution. Dissolve 0.100 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution. Dissolve 0.100 g of *octyldodecanol CRS* in the internal standard solution and dilute to 10.0 ml with the same solution.

Column:

– *material*: stainless steel,
– *size*: *l* = 60 m, Ø = 0.25 mm,
– *stationary phase*: *poly(dimethyl)(diphenyl)(divinyl)siloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 0.68 ml/min.

Split ratio: 1:50.

Temperature:

	Time (min)	Temperature (°C)
	0 - 2	180
Column	2 - 22	180 → 280
	22 - 52	280
Injection port		290
Detector		300

Detection: flame ionisation.

Injection: 1 µl.

Calculate the content of $C_{20}H_{42}O$ in the substance to be examined.

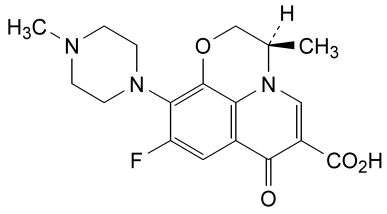
STORAGE

Protected from light.

01/2008:1455
corrected 6.0

OFLOXACIN

Ofloxacinum



and enantiomer

$C_{18}H_{20}FN_3O_4$ M_r 361.4
[82419-36-1]

DEFINITION

(*RS*)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid.

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

CHARACTERS

Appearance: pale yellow or bright yellow, crystalline powder.

Solubility: slightly soluble in water, soluble in glacial acetic acid, slightly soluble or soluble in methylene chloride, slightly soluble in methanol.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *ofloxacin CRS*.

TESTS

Optical rotation (*2.2.7*): – 0.10° to + 0.10°.

Dissolve 0.300 g in a mixture of 10 volumes of *methanol R* and 40 volumes of *methylene chloride R* and dilute to 10 ml with the same mixture of solvents.

Absorbance (*2.2.25*): maximum 0.25 at 440 nm.

Dissolve 0.5 g in *0.1 M hydrochloric acid* and dilute to 100 ml with the same solvent.

Impurity A. Thin-layer chromatography (*2.2.27*).

Solvent mixture: *methanol R, methylene chloride R* (10:40 *V/V*).

Test solution. Dissolve 0.250 g of the substance to be examined in the solvent mixture and dilute to 5.0 ml with the solvent mixture.

Reference solution. Dissolve 10 mg of *ofloxacin impurity A CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Plate: *TLC silica gel GF_{254} plate R* (2-10 µm).

Mobile phase: *glacial acetic acid R, water R, ethyl acetate R* (10:10:20 *V/V/V*).

Application: 10 µl.

Development: over a path of 10 cm.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Limit:

– *impurity A*: any spot due to impurity A is not more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.2 per cent).

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R, water R* (10:60 *V/V*).

Test solution. Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (b). Dissolve 10.0 mg of *ofloxacin impurity E CRS* in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Mix 10.0 ml of the solution with 5.0 ml of the test solution and dilute to 50.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50.0 ml with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

– *temperature*: 45 °C.

Mobile phase: dissolve 4.0 g of *ammonium acetate R* and 7.0 g of *sodium perchlorate R* in 1300 ml of *water R*; adjust to pH 2.2 with *phosphoric acid R* and add 240 ml of *acetonitrile R*.

Flow rate: adjust so that a retention time of about 20 min is obtained for ofloxacin.

Detection: spectrophotometer at 294 nm.

Injection: 10 µl.

Run time: 2.5 times the retention time of ofloxacin.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurity E and ofloxacin.

Limits:

– *impurities B, C, D, E, F*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

– *total*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

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

2.0 g complies with test C. Prepare the reference solution using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 0.2 per cent, determined on 1.000 g by drying at 105 °C for 4 h.

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

ASSAY

Dissolve 0.300 g in 100 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

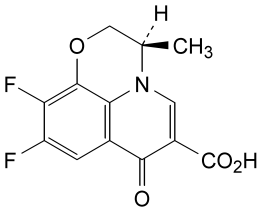
1 ml of *0.1 M perchloric acid* is equivalent to 36.14 mg of $C_{18}H_{20}FN_3O_4$.

STORAGE

In an airtight container, protected from light.

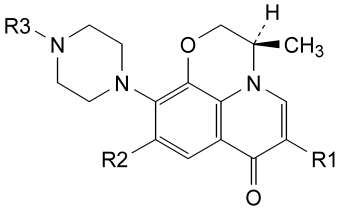
IMPURITIES

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



and enantiomer

A. (*RS*)-9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid (FPA),

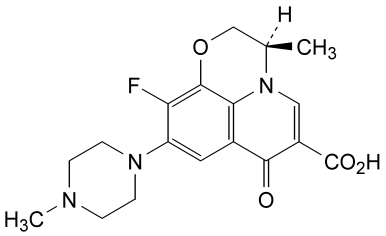


and enantiomer

B. R1 = H, R2 = F, R3 = CH_3: (*RS*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazin-7-one,

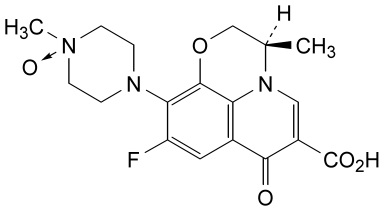
C. R1 = CO_2H, R2 = H, R3 = CH_3: (*RS*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,

E. R1 = CO_2H, R2 = F, R3 = H: (*RS*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



and enantiomer

D. (*RS*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid,



and enantiomer

F. 4-[(*RS*)-6-carboxy-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-10-yl]-1-methylpiperazine 1-oxide.