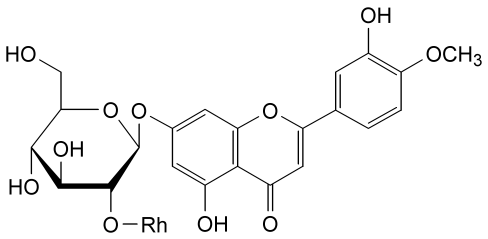
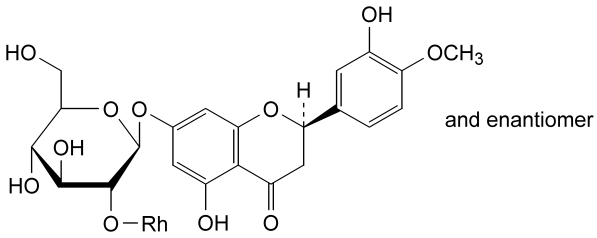
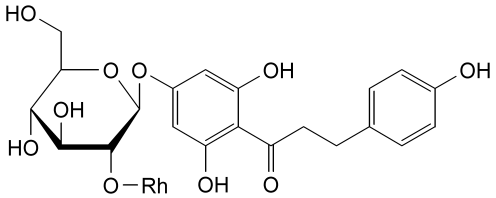


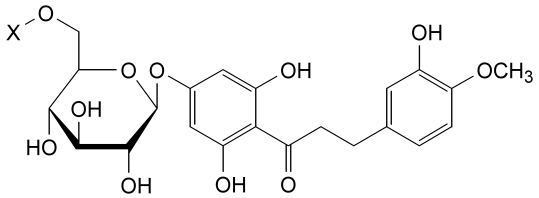
B. 7-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (neodiosmin),



C. (2*RS*)-7-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (neohesperidin),

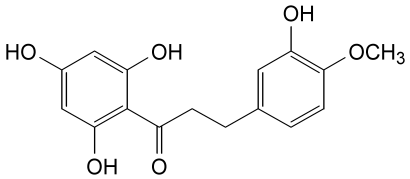


D. 1-[4-[[2-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)propan-1-one (naringin-dihydrochalcone),



E. X = Rh: 1-[4-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperidin-dihydrochalcone),

F. X = H: 1-[4-(β-D-glucopyranosyloxy)-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperetin-dihydrochalcone 7′-glucoside),

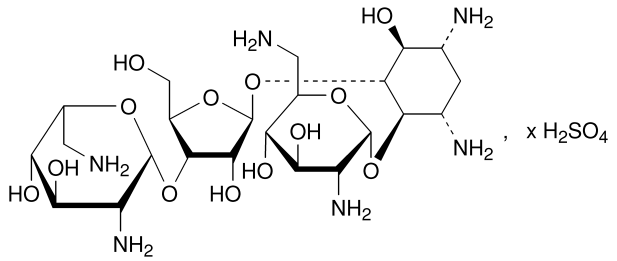


G. 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one (hesperetin-dihydrochalcone).

**01/2008:0197**

# NEOMYCIN SULPHATE

## Neomycini sulfas



$C_{23}H_{46}N_6O_{13},xH_2SO_4$ $M_r$ 615 (base)

DEFINITION

Mixture of sulphates of substances produced by the growth of certain selected strains of *Streptomyces fradiae*, the main component being the sulphate of 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B).

*Content*: minimum of 680 IU/mg (dried substance).

CHARACTERS

*Appearance*: white or yellowish-white powder, hygroscopic.

*Solubility*: very soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

A. Examine the chromatograms obtained in the test for related substances.

*Results*:

- the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (e),
- it complies with the limits given for impurity C.

B. It gives reaction (a) of sulphates (*2.3.1*).

TESTS

**pH** (*2.2.3*): 5.0 to 7.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

**Specific optical rotation** (*2.2.7*): + 53.5 to + 59.0 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 ml with the same solvent.

**Related substances**. Liquid chromatography (*2.2.29*).

*Test solution.* Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

*Reference solution (a).* Dissolve 25.0 mg of *framycetin sulphate CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

*Reference solution (b).* Dilute 5.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

*Reference solution (c).* Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

*Reference solution (d).* Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 50.0 ml with the mobile phase.

*Reference solution (e).* Dissolve 10 mg of *neomycin sulphate CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase.

*Column*:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,

– *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (5 µm),

– *temperature*: 25 °C.

*Mobile phase*: mix 20.0 ml of *trifluoroacetic acid R*, 6.0 ml of *carbonate-free sodium hydroxide solution R* and 500 ml of *water R*, allow to equilibrate, dilute to 1000 ml with *water R* and degas.

*Flow rate*: 0.7 ml/min.

*Post-column solution*: *carbonate-free sodium hydroxide solution R* diluted 1 in 25 previously degassed, which is added pulse-less to the column effluent using a 375 µl polymeric mixing coil.

*Flow rate*: 0.5 ml/min.

*Detection*: pulsed amperometric detector with a gold indicator electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively 0.00 V detection, + 0.80 V oxidation and – 0.60 V reduction potentials, with pulse durations according to the instrument used.

*Injection*: 10 µl; inject the test solution and the reference solutions (b), (c), (d) and (e).

*Run time*: 1.5 times the retention time of neomycin B.

*Relative retention* with reference to neomycin B (retention time = about 10 min): impurity A = about 0.65; impurity C = about 0.9; impurity G = about 1.1.

*System suitability*:

– *resolution*: minimum of 2.0 between the peaks due to impurity C and to neomycin B in the chromatogram obtained with reference solution (e); if necessary, adjust the volume of the carbonate-free sodium hydroxide solution in the mobile phase,

– *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with reference solution (c).

*Limits*:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent),

– *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent) and not less than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent),

– *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent),

– *total of other impurities*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (15.0 per cent),

– *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (1.0 per cent).

**Sulphate**: 27.0 per cent to 31.0 per cent (dried substance).

Dissolve 0.250 g in 100 ml of *water R* and adjust the solution to pH 11 using *concentrated ammonia R*. Add 10.0 ml of *0.1 M barium chloride* and about 0.5 mg of *phthalein purple R*. Titrate with *0.1 M sodium edetate* adding 50 ml of *alcohol R* when the colour of the solution begins to change, continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 9.606 mg of $SO_4$.

**Loss on drying** (*2.2.32*): maximum 8.0 per cent, determined on 1.000 g by drying at 60 °C over *diphosphorus pentoxide R* at a pressure not exceeding 0.7 kPa for 3 h.

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

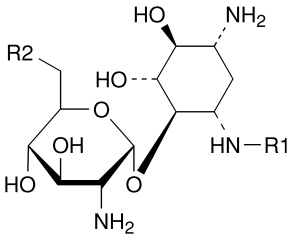
## ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *neomycin sulphate for microbiological assay CRS* as the reference substance.

## STORAGE

In an airtight container, protected from light.

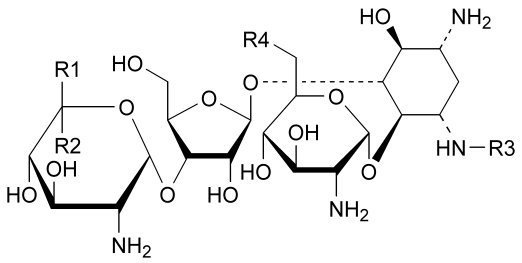
## IMPURITIES




A. R1 = H, R2 = $NH_2$: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (neamine or neomycin A-LP),

B. R1 = $CO\text{-}CH_3$, R2 = $NH_2$: 3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-D-streptamine (3-acetylneamine),

D. R1 = H, R2 = OH: 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-D-streptamine (paromamine or neomycin D),



C. R1 = $CH_2$-$NH_2$, R2 = R3 = H, R4 = $NH_2$:
2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin C),

E. R1 = R3 = H, R2 = $CH_2$-$NH_2$, R4 = OH:
4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin I or neomycin E),

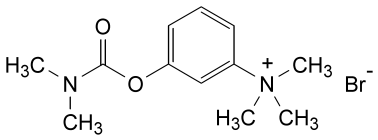
F. R1 = $CH_2$-$NH_2$, R2 = R3 = H, R4 = OH:
4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxy-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-β-D-ribofuranosyl]-D-streptamine (paromomycin II or neomycin F),

G. R1 = H, R2 = $CH_2$-$NH_2$, R3 = CO-$CH_3$, R4 = $NH_2$:
3-*N*-acetyl-2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine (neomycin B-LP).

**01/2008:0046**
**corrected 6.0**

# NEOSTIGMINE BROMIDE

## Neostigmini bromidum



$C_{12}H_{19}BrN_2O_2$ $M_r$ 303.2
[114-80-7]

DEFINITION

3-[(Dimethylcarbamoyl)oxy]-*N*,*N*,*N*-trimethylanilinium bromide.

*Content*: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

*Appearance*: white or almost white, crystalline powder or colourless crystals, hygroscopic.

*Solubility*: very soluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

*First identification: B, D.*

*Second identification: A, C, D.*

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

*Test solution*. Dissolve 20 mg in *0.5 M sulphuric acid* and dilute to 100 ml with the same acid.

*Spectral range*: 230-350 nm.

*Absorption maxima*: at 260 nm and 266 nm.

*Specific absorbances at the absorption maxima*:

– at 260 nm: about 16;

– at 266 nm: about 14.

B. Infrared absorption spectrophotometry (*2.2.24*).

*Comparison*: *neostigmine bromide CRS*.

C. Heat about 50 mg with a mixture of 0.4 g of *potassium hydroxide R* and 2 ml of *ethanol (96 per cent) R* on a water-bath for 3 min, replacing the evaporated ethanol (96 per cent). Cool and add 2 ml of *water R* and 2 ml of *diazobenzenesulphonic acid solution R1*. An orange-red colour develops.

D. It gives the reactions of bromides (*2.3.1*).

TESTS

**Solution S**. Dissolve 2.5 g in *distilled water R* and dilute to 50 ml with the same solvent.

**Appearance of solution**. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

**Impurity A**: maximum 0.33 per cent.

Dissolve 50 mg in a mixture of 1 ml of *sodium carbonate solution R* and 9 ml of *water R*. The absorbance (*2.2.25*) measured immediately at 294 nm is not greater than 0.25.

**Sulphates** (*2.4.13*): maximum 200 ppm, determined on solution S.

**Loss on drying** (*2.2.32*): maximum 1.0 per cent, determined on 1.00 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 0.225 g in 2 ml of *anhydrous formic acid R*. Add 50 ml of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

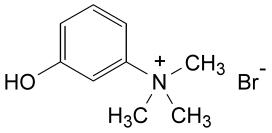
1 ml of *0.1 M perchloric acid* is equivalent to 30.32 mg of $C_{12}H_{19}BrN_2O_2$.

STORAGE

Protected from light.

IMPURITIES

*Specified impurities: A.*



A. 3-hydroxy-*N*,*N*,*N*-trimethylanilinium bromide.