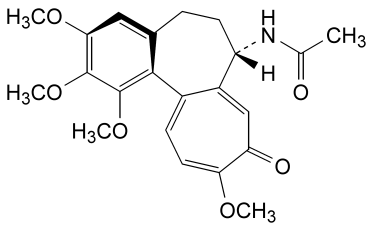
01/2008:0758

COLCHICINE

Colchicinum



$C_{22}H_{25}NO_6$ M_r 399.4
[64-86-8]

DEFINITION

(-)-*N*-[(7*S*,12a*S*)-1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellowish-white, amorphous or crystalline powder.

Solubility: very soluble in water, rapidly recrystallising from concentrated solutions as the sesquihydrate, freely soluble in alcohol, practically insoluble in cyclohexane.

IDENTIFICATION

First identification: B.

Second identification: A, C, D.

A. Dissolve 5 mg in *alcohol R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 25.0 ml with *alcohol R*. Examined between 230 nm and 400 nm (*2.2.25*), the solution shows 2 absorption maxima, at 243 nm and 350 nm. The ratio of the absorbance measured at 243 nm to that measured at 350 nm is 1.7 to 1.9.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs of *potassium bromide R*.

Comparison: *colchicine CRS*.

C. To 0.5 ml of solution S (see Tests) add 0.5 ml of *dilute hydrochloric acid R* and 0.15 ml of *ferric chloride solution R1*. The solution is yellow and becomes dark green on boiling for 30 s. Cool, add 2 ml of *methylene chloride R* and shake. The organic layer is greenish-yellow.

D. Dissolve about 30 mg in 1 ml of *alcohol R* and add 0.15 ml of *ferric chloride solution R1*. A brownish-red colour develops.

TESTS

Solution S. Dissolve 0.10 g in *water R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution GY_3 (*2.2.2, Method II*).

Acidity or alkalinity. To 10 ml of solution S add 0.1 ml of *bromothymol blue solution R1*. Either the solution does not change colour or it becomes green. Not more than 0.1 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to blue.

Specific optical rotation (*2.2.7*): – 235 to – 250 (anhydrous substance).

Dissolve 50.0 mg in *alcohol R* and dilute to 10.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20.0 mg of the substance to be examined in a mixture of equal volumes of *methanol R* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (a). Dissolve 20.0 mg of *colchicine for system suitability CRS* in a mixture of equal volumes of *methanol R* and *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Reference solution (c). Dilute 1 ml of reference solution (b) to 20.0 ml with a mixture of equal volumes of *methanol R* and *water R*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octylsilyl silica gel for chromatography R1* (5 µm).

Mobile phase: mix 450 volumes of a 6.8 g/l solution of *potassium dihydrogen phosphate R* and 530 volumes of *methanol R*. After cooling to room temperature, adjust the volume to 1000 ml with *methanol R*. Adjust the apparent pH to 5.5 with *dilute phosphoric acid R*.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: 3 times the retention time of colchicine.

Relative retention with reference to colchicine (retention time = about 7 min): impurity D = about 0.4; impurity E = about 0.7; impurity B = about 0.8; impurity A = about 0.94; impurity C = about 1.2.

System suitability: reference solution (a):

Peak-to-valley ratio: minimum 2, where H_P = height above the baseline of the peak due to impurity A and H_V = height above the baseline of the lowest point of the curve separating this peak from the peak due to colchicine.

Limits:

– *impurity A*: not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent),
– *any other impurity*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent),
– *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Colchiceine: maximum 0.2 per cent.

Dissolve 50 mg in *water R* and dilute to 5 ml with the same solvent. Add 0.1 ml of *ferric chloride solution R1*. The solution is not more intensely coloured than a mixture of 1 ml of red primary solution, 2 ml of yellow primary solution and 2 ml of blue primary solution (*2.2.2, Method II*).

Chloroform (*2.4.24*): maximum 500 ppm.

Ethyl acetate (*2.4.24*): maximum 6.0 per cent *m/m*.

Water (*2.5.12*): maximum 2.0 per cent, determined on 0.500 g.

Sulphated ash (*2.4.14*): maximum 0.1 per cent, determined on 0.5 g.

ASSAY

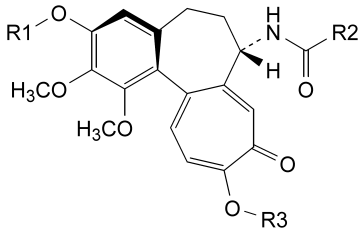
Dissolve 0.250 g with gentle heating in a mixture of 10 ml of *acetic anhydride R* and 20 ml of *toluene R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 39.94 mg of $C_{22}H_{25}NO_6$.

STORAGE

Protected from light.

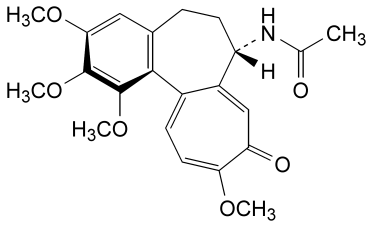
IMPURITIES



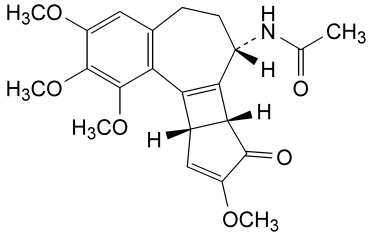
A. R1 = R3 = CH_3, R2 = H: *N*-[(7*S*,12a*S*)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]formamide (*N*-deacetyl-*N*-formylcolchicine),

E. R1 = H, R2 = R3 = CH_3: *N*-[(7*S*,12a*S*)-3-hydroxy-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (3-*O*-demethylcolchicine),

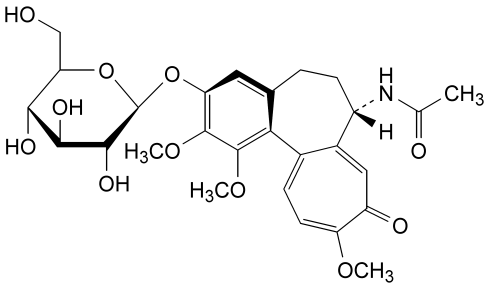
F. R1 = R2 = CH_3, R3 = H: *N*-[(7*S*,12a*S*)-10-hydroxy-1,2,3-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchiceine),



B. (-)-*N*-[(7*S*,12a*R*)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (conformationnal isomer),



C. *N*-[(7*S*,7b*R*,10a*S*)-1,2,3,9-tetramethoxy-8-oxo-5,6,7,7b,8,10a-hexahydrobenzo[*a*]cyclopenta[3,4]-cyclobuta[1,2-*c*]cycloheptan-7-yl]acetamide (β-lumicolchicine),



D. *N*-[(7*S*,12a*S*)-3-(β-D-glucopyranosyloxy)-1,2,10-trimethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide (colchicoside).

01/2008:1775

COLESTYRAMINE

Colestyraminum

[11041-12-6]

DEFINITION

Strongly basic anion-exchange resin in chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium groups.

Nominal exchange capacity: 1.8 g to 2.2 g of sodium glycocholate per gram (dried substance).

CHARACTERS

Appearance: white or almost white, fine powder, hygroscopic.

Solubility: insoluble in water, in methylene chloride and in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: colestyramine CRS.

B. Chloride (see Tests).

TESTS

pH (*2.2.3*): 4.0 to 6.0.

Suspend 0.100 g in 10 ml of *water R* and allow to stand for 10 min.

Dialysable quaternary amines: maximum 500 ppm, expressed as benzyltrimethylammonium chloride.

Test solution. Place a 25 cm piece of cellulose dialysis tubing having a molecular weight cut-off of 12 000-14 000 and an inflated diameter of 3-6 cm (flat width of 5-9 cm) in *water R* to hydrate until pliable, appropriately sealing one end. Introduce 2.0 g of the substance to be examined into the tube and add 10 ml of *water R*. Seal the tube and completely immerse it in 100 ml of *water R* in a suitable vessel and stir the liquid for 16 h to effect dialysis. Use the dialysate as test solution.

Reference solution. Prepare the reference solution in a similar manner but using 10 ml of a freshly prepared 0.1 g/l solution of *benzyltrimethylammonium chloride R* instead of the substance to be examined.

Transfer 5.0 ml of the test solution to a separating funnel and add 5 ml of a 3.8 g/l solution of *disodium tetraborate R*, 1 ml of a solution containing 1.5 g/l of *bromothymol blue R* and 4.05 g/l of *sodium carbonate R* and 10 ml of *chloroform R*. Shake the mixture vigourously for 1 min, allow the phases to separate and transfer the clear organic layer to a 25 ml