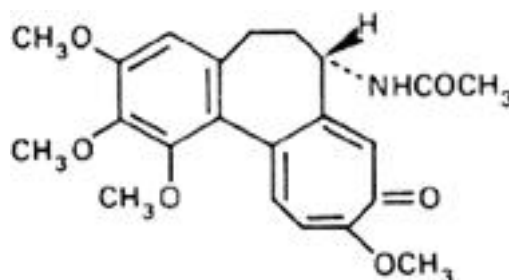


Colchicine (Colchicinum)**Molecular formula.** $C_{22}H_{25}NO_6$ **Relative molecular mass.** 399.4**Graphic formula.****Chemical name.** (S)-N-(5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)acetamide; CAS Reg. No. 64-86-8.**Description.** Pale yellow to pale greenish yellow crystals, amorphous scales or a powder; odourless or almost odourless.**Solubility.** Soluble in water; freely soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.**Category.** Anticancer drug.**Storage.** Colchicine should be kept in a tightly closed container, protected from light.**Additional information.** Colchicine is an alkaloid obtained from *Colchicum autumnale* L. (Fam. Liliaceae). It darkens on exposure to light. CAUTION: Colchicine is extremely poisonous and must be handled with care.**Requirements****Definition.** Colchicine contains not less than 97.0% and not more than 103.0% of $C_{22}H_{25}NO_6$, calculated with reference to the anhydrous and solvent-free substance.**Identity tests**

- Either test A or tests B, C and D may be applied.

A. Carry out the examination as described under [1.7 Spectrophotometry in the infrared region](#). The infrared absorption spectrum is concordant with the spectrum obtained from colchicine RS or with the *reference spectrum* of colchicine.

B. The absorption spectrum of a 10 µg/mL solution in ethanol (~750 g/l) TS, when observed between 230 nm and 400 nm, exhibits 2 maxima at about 243 nm and 350 nm.

C. Dissolve 30 mg in 1 mL of ethanol (~750 g/l) TS and add 1 drop of ferric chloride (25 g/l) TS; a red colour is immediately produced.

D. Mix 1 mg with about 0.2 mL of sulfuric acid (~1760 g/l) TS; a lemon-yellow colour is produced. Add about 0.1 mL of nitric acid (~1000 g/l) TS; the colour changes to greenish blue, rapidly becoming reddish and finally yellow or almost colourless. Then add a few drops of sodium hydroxide (~80 g/l) TS; the colour changes to red.

Specific optical rotation. Use a 10 mg/mL solution and calculate with reference to the dried and solvent-free substance;

$$[\alpha]_D^{20} = -425^\circ \text{ to } -460^\circ.$$

Sulfated ash. Not more than 1.0 mg/g.

Content of solvent and water. Dry at 130 °C for 4 hours, using about 0.5 g of the substance, and determine the loss of weight. Weigh 0.3 g of the dried material and determine the water content as described under [2.8 Determination of water by the Karl Fischer method](#), Method A, using pyridine R as the solvent; the sum of the loss of weight and of the water content, both expressed in mg/g, is not less than 115 mg/g and not more than 145 mg/g.

Colchicine. Dissolve 0.050 g in 4 mL of water, add 0.2 mL of ferric chloride (25 g/l) TS, dilute to 6 mL with water, and mix; the colour produced in the test solution is not more intense than that of the standard colour solution produced by mixing 2 mL of iron colour TS, 1 mL of cobalt colour TS, 2 mL of copper colour TS and 0.70 mL of hydrochloric acid (~70 g/l) TS, when compared as described under [1.11 Colour of liquids](#).

Related substances. Carry out the test as described under [1.14.1 Thin-layer chromatography](#), using a suitable aluminium oxide

containing a substance that fluoresces at about 254 nm as the coating substance and a mixture of 25 volumes of chloroform R, 20 volumes of acetone R, and 0.4 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 2 µl of each of 2 solutions in ethanol (~750 g/l) TS containing (A) 50 mg of the test substance per mL and (B) 2.5 mg of the test substance per mL. After removing the plate from the chromatographic chamber, allow it to dry in air, and examine the chromatogram in ultraviolet light (254 nm). Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B.

Assay. Dissolve about 0.05 g, accurately weighed, in a mixture of 10 mL of acetic anhydride R and 20 mL of toluene R, and titrate with perchloric acid (0.02 mol/l) VS, determining the end-point potentiometrically as described under [2.6 Non-aqueous titration](#), Method A. Each mL of perchloric acid (0.02 mol/l) VS is equivalent to 7.988 mg of $C_{22}H_{25}NO_6$.