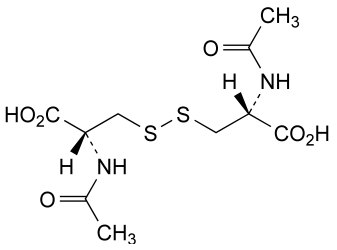
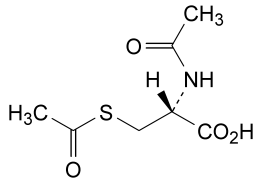


C. *N,N′*-diacetyl-L-cystine,

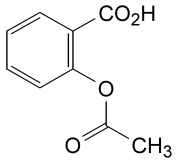


D. *N,S*-diacetyl-L-cysteine.

01/2005:0309

ACETYLSALICYLIC ACID

Acidum acetylsalicylicum



$C_9H_8O_4$ M_r 180.2

DEFINITION

Acetylsalicylic acid contains not less than 99.5 per cent and not more than the equivalent of 101.0 per cent of 2-(acetyloxy)benzoic acid, calculated with reference to the dried substance.

CHARACTERS

A white, crystalline powder or colourless crystals, slightly soluble in water, freely soluble in alcohol.

It melts at about 143 °C (instantaneous method).

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *acetylsalicylic acid CRS*.

B. To 0.2 g add 4 ml of *dilute sodium hydroxide solution R* and boil for 3 min. Cool and add 5 ml of *dilute sulphuric acid R*. A crystalline precipitate is formed. Filter, wash the precipitate and dry at 100 °C to 105 °C. The melting point (*2.2.14*) is 156 °C to 161 °C.

C. In a test tube mix 0.1 g with 0.5 g of *calcium hydroxide R*. Heat the mixture and expose to the fumes produced a piece of filter paper impregnated with 0.05 ml of *nitrobenzaldehyde solution R*. A greenish-blue or greenish-yellow colour develops on the paper. Moisten the paper with *dilute hydrochloric acid R*. The colour becomes blue.

D. Dissolve with heating about 20 mg of the precipitate obtained in identification test B in 10 ml of *water R* and cool. The solution gives reaction (a) of salicylates (*2.3.1*).

TESTS

Appearance of solution. Dissolve 1.0 g in 9 ml of *alcohol R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Related substances. Examine by liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 0.10 g of the substance to be examined in *acetonitrile for chromatography R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 50.0 mg of *salicylic acid R* in the mobile phase and dilute to 50.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10.0 mg of *salicylic acid R* in the mobile phase and dilute to 10.0 ml with the mobile phase. To 1.0 ml of this solution add 0.2 ml of the test solution and dilute to 100.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

– a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm),

– as mobile phase at a flow rate of 1 ml/min a mixture of 2 volumes of *phosphoric acid R*, 400 volumes of *acetonitrile for chromatography R* and 600 volumes of *water R*,

– as detector a spectrophotometer set at 237 nm.

Inject 10 µl of each solution. Continue the chromatography of the test solution for seven times the retention time of acetylsalicylic acid. The test is not valid unless in the chromatogram obtained with reference solution (b), the resolution between the two principal peaks is at least 6.0.

In the chromatogram obtained with the test solution the area of any peak, apart from the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); the sum of the areas of all the peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent). Disregard any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals (*2.4.8*). Dissolve 1.0 g in 12 ml of *acetone R* and dilute to 20 ml with *water R*. 12 ml of this solution complies with limit test B for heavy metals (20 ppm). Prepare the standard using lead standard solution (1 ppm Pb) obtained by diluting *lead standard solution (100 ppm Pb) R* with a mixture of 6 volumes of *water R* and 9 volumes of *acetone R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying *in vacuo*.

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

ASSAY

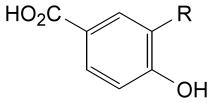
In a flask with a ground-glass stopper, dissolve 1.000 g in 10 ml of *alcohol R*. Add 50.0 ml of *0.5 M sodium hydroxide*. Close the flask and allow to stand for 1 h. Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.5 M hydrochloric acid*. Carry out a blank titration.

1 ml of *0.5 M sodium hydroxide* is equivalent to 45.04 mg of $C_9H_8O_4$.

STORAGE

Store in an airtight container.

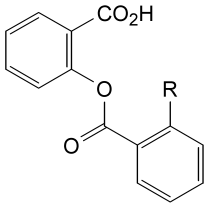
IMPURITIES



A. R = H: 4-hydroxybenzoic acid,

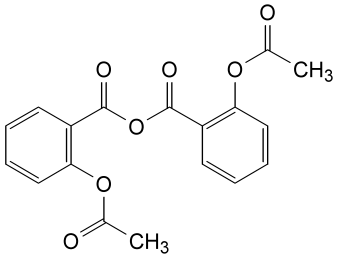
B. R = CO_2H: 4-hydroxybenzene-1,3-dicarboxylic acid (4-hydroxyisophthalic acid),

C. salicylic acid,



D. R = O-CO-CH_3: 2-[[2-(acetyloxy)benzoyl]oxy]benzoic acid (acetylsalicylsalicylic acid),

E. R = OH: 2-[(2-hydroxybenzoyl)oxy]benzoic acid (salicylsalicylic acid),

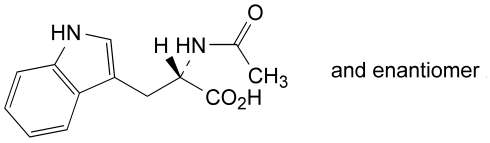


F. 2-(acetyloxy)benzoic anhydride (acetylsalicylic anhydride).

01/2005:1383

N-ACETYLTRYPTOPHAN

N-Acetyltryptophanum



and enantiomer

$C_{13}H_{14}N_2O_3$ M_r 246.3

DEFINITION

N-Acetyltryptophan contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*RS*)-2-acetylamino-3-(1*H*-indol-3-yl)propanoic acid, calculated with reference to the dried substance.

PRODUCTION

Tryptophan used for the production of *N*-acetyltryptophan complies with the test for 1,1′-ethylidenebistryptophan and other related substances in the monograph on *Tryptophan (1272)*.

CHARACTERS

A white or almost white, crystalline powder, or colourless crystals, slightly soluble in water, very soluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

It melts at about 205 °C.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D, E.

A. It complies with the test for optical rotation (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *N-acetyltryptophan CRS*.

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

Test solution. Dissolve 50 mg of the substance to be examined in 0.2 ml of *concentrated ammonia R* and dilute to 10 ml with *water R*.

Reference solution (a). Dissolve 50 mg of *N-acetyltryptophan CRS* in 0.2 ml of *concentrated ammonia R* and dilute to 10 ml with *water R*.

Reference solution (b). Dissolve 10 mg of *tryptophan R* in the test solution and dilute to 2 ml with the same solution.

Apply to the plate 2 µl of each solution. Develop over a path of 10 cm using a mixture of 25 volumes of *glacial acetic acid R*, 25 volumes of *water R* and 50 volumes of *butanol R*. Dry the plate in an oven at 100-105 °C for 15 min and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

D. Dissolve about 2 mg in 2 ml of *water R*. Add 2 ml of *dimethylaminobenzaldehyde solution R6*. Heat on a water-bath. A blue or greenish-blue colour develops.

E. It gives the reaction of acetyl (*2.3.1*). Proceed as described for substances hydrolysable only with difficulty.

TESTS

Appearance of solution. Dissolve 1.0 g in a 40 g/l solution of *sodium hydroxide R* and dilute to 100 ml with the same alkaline solution. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 or GY_7 (*2.2.2, Method II*).

Optical rotation (*2.2.7*). Dissolve 2.50 g in a 40 g/l solution of *sodium hydroxide R* and dilute to 25.0 ml with the same alkaline solution. The angle of optical rotation is −0.1° to + 0.1°.

Related substances. Examine by liquid chromatography (*2.2.29*).

Buffer solution pH 2.3. Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 ml of *water R*. Add about 700 ml of a 2.9 g/l solution of *phosphoric acid R* and adjust the pH to 2.3 with the same acidic solution.

Prepare the solutions immediately before use.

Test solution. Dissolve 0.10 g of the substance to be examined in a mixture of 50 volumes of *acetonitrile R* and 50 volumes of *water R* and dilute to 20.0 ml with the same mixture of solvents.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with a mixture of 10 volumes of *acetonitrile R* and 90 volumes of *water R*.

Reference solution (b). Dissolve 1.0 mg of *1,1′-ethylidenebis(tryptophan) CRS* in a mixture of 10 volumes of *acetonitrile R* and 90 volumes of *water R* and dilute to 100.0 ml with the same mixture of solvents.

Reference solution (c). To 4.0 ml of reference solution (a), add 20.0 ml of reference solution (b) and dilute to 100.0 ml with a mixture of 10 volumes of *acetonitrile R* and 90 volumes of *water R*.