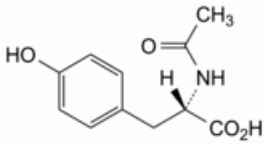
Edition: BP 2025 (Ph. Eur. 11.6 update)

# Acetyltyrosine

General Notices

*(N-Acetyltyrosine, Ph. Eur. monograph 1384)*




$C_{11}H_{13}NO_4$ 223.2 *537-55-3*

Ph Eur

## DEFINITION

(2*S*)-2-(Acetylamino)-3-(4-hydroxyphenyl)propanoic acid.

**Content**

98.5 per cent to 101.0 per cent (dried substance).

## CHARACTERS

**Appearance**

White or almost white, crystalline powder or colourless crystals.

**Solubility**

Freely soluble in water, practically insoluble in cyclohexane.

## IDENTIFICATION

*First identification: A, B.*

*Second identification: A, C, D.*

A. Specific optical rotation (see Tests).
B. Infrared absorption spectrophotometry (2.2.24).

*Comparison* *N-acetyltyrosine CRS*.

C. Thin-layer chromatography (2.2.27).

*Test solution* Dissolve 80 mg of the substance to be examined in a mixture of 3 volumes of *glacial acetic acid R*, 3 volumes of *water R* and 94 volumes of *anhydrous ethanol R*, and dilute to 10 mL with the same mixture of solvents.

*Reference solution* Dissolve 80 mg of *N-acetyltyrosine CRS* in a mixture of 3 volumes of *glacial acetic acid R*, 3 volumes of *water R* and 94 volumes of *anhydrous ethanol R*, and dilute to 10 mL with the same mixture of solvents.

*Plate* *TLC silica gel $F_{254}$ plate R*.

*Mobile phase* *water R*, *glacial acetic acid R*, *ethyl acetate R* (10:15:75 *V/V/V*).

*Application* 5 µL.

*Development* Over 2/3 of the plate.

*Drying* In air.

*Detection* Examine in ultraviolet light at 254 nm.

*Results* 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 the reference solution.

D. Solution S (see Tests) is strongly acid (2.2.4).

## TESTS

### Solution S

Dissolve 2.50 g in *water R* and dilute to 100.0 mL with the same solvent.

### Appearance of solution

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

### Specific optical rotation (2.2.7)

+ 46 to + 49 (dried substance).

Dilute 10.0 mL of solution S to 25.0 mL with *water R*.

### Related substances

Liquid chromatography (2.2.29). *Carry out the test protected from light.*

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

*Reference solution (a)* Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

*Reference solution (b)* Dissolve 20.0 mg of *tyrosine CRS* (impurity A) in 2 mL of a 40 g/L solution of *sodium hydroxide R* and dilute to 20.0 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

*Reference solution (c)* Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase A.

*Reference solution (d)* Dilute 1.0 mL of reference solution (b) to 20.0 mL with the test solution.

*Column*:

— *size*: *l* = 0.15 m, Ø = 3 mm;

— *stationary phase*: spherical *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 40 °C.

*Mobile phase*:

— *mobile phase A*: mix 1.0 mL of *phosphoric acid R* and 1000 mL of *water for chromatography R*;

— *mobile phase B*: *acetonitrile R1*;

| Time (min) | Mobile phase A (per cent *V/V*) | Mobile phase B (per cent *V/V*) |
|---|---|---|
| 0 - 2 | 97 | 3 |
| 2 - 15 | 97 → 62 | 3 → 38 |

*Flow rate* 0.7 mL/min.

*Detection* Spectrophotometer at 219 nm.

*Injection* 2 µL of the test solution and reference solutions (a), (c) and (d).

*Relative retention* With reference to *N*-acetyltyrosine (retention time = about 6 min): impurity A = about 0.5.

*System suitability* Reference solution (d):

— *resolution*: minimum 5.0 between the principal peak and the peak due to impurity A.

*Limits*:

— *impurity A*: not more than 0.8 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.8 per cent);

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *total*: maximum 1.0 per cent;

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

**Chlorides** (*2.4.4*)

Maximum 200 ppm.

Dilute 10 mL of solution S to 15 mL with *water R*.

**Sulfates** (*2.4.13*)

Maximum 200 ppm.

Dissolve 1.0 g in *distilled water R* and dilute to 20 mL with the same solvent.

**Ammonium** (*2.4.1, Method B*)

Maximum 200 ppm, determined on 0.100 g.

Prepare the standard using 0.2 mL of *ammonium standard solution (100 ppm $NH_4$) R*.

**Iron** (*2.4.9*)

Maximum 20 ppm.

In a separating funnel, dissolve 0.5 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. The aqueous layer complies with the test.

**Loss on drying** (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

**Sulfated ash** (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

**Bacterial endotoxins** (*2.6.14*)

Less than 25 IU/g, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

## ASSAY

Dissolve 0.180 g in 50 mL of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (*2.2.20*).

1 mL of *0.1 M sodium hydroxide* is equivalent to 22.32 mg of $C_{11}H_{13}NO_4$.

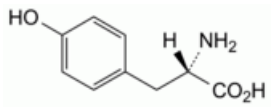
## STORAGE

Protected from light. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

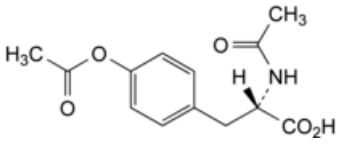
## IMPURITIES

*Specified impurities* A.

*Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use)* B.




A. (2*S*)-2-amino-3-(4-hydroxyphenyl)propanoic acid (tyrosine),




B. (2*S*)-2-(acetylamino)-3-[4-(acetoxy)phenyl]propanoic acid (diacetyltyrosine).