



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

Acetylcysteine Injection

[General Notices](#)

Action and use

Sulphydryl donor; antidote to paracetamol poisoning; mucolytic.

DEFINITION

Acetylcysteine Injection is a sterile solution in Water for Injections of acetylcysteine sodium, prepared by the interaction of Acetylcysteine with Sodium Hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of acetylcysteine, C₅H₉NO₃S

95.0 to 105.0% of the stated amount.

IDENTIFICATION

To a volume containing the equivalent of 0.8 g of Acetylcysteine add [3M hydrochloric acid](#) until the pH of the solution is 2.0. Add, while stirring continuously, two 200 -mg portions of finely powdered [sodium chloride](#) followed, if necessary, by further 25-mg portions of [sodium chloride](#) until a precipitate begins to appear. Allow to stand for 15 minutes, filter and dry the residue at 70° at a pressure not exceeding 0.7 kPa for 2 hours. The [infrared absorption spectrum](#) of the residue, [Appendix II A](#), is concordant with the *reference spectrum* of acetylcysteine ([RS 003](#)). Examine as discs prepared using [potassium bromide](#).

TESTS

Acidity or alkalinity

pH, 6.5 to 7.5, [Appendix V L](#).

Related substances

Carry out the method for [liquid chromatography](#), [Appendix III D](#), using the following solutions. With the exception of solution (3), the solutions should be prepared immediately before use.

- (1) Dilute the injection with the mobile phase to produce a solution containing the equivalent of 0.2% w/v of Acetylcysteine.
- (2) Dissolve 20 mg of [L-cysteine](#) (impurity B) and 20 mg of [L-cystine](#) (impurity A) in 10 mL of 1M [hydrochloric acid](#), add 40 mg of [acetylcysteine BPCRS](#) and immediately dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.
- (3) 0.2% w/v solution of [acetylcysteine BPCRS](#) in the mobile phase and store at room temperature for at least 2 hours before use (generation of *N,N'*-diacetylcystine).

(4) Dilute 1 volume of solution (2) to 10 volumes with mobile phase.

CHROMATOGRAPHIC CONDITIONS

- Use a stainless steel column (25 cm × 5 mm) packed with [octadecylsilyl silica gel for chromatography](#) (5 μm) (LiChrosorb RP18 is suitable).
- Use isocratic elution and the mobile phase described below.
- Use a flow rate of 1 mL per minute.
- Use an ambient column temperature.
- Use a detection wavelength of 205 nm.
- Inject 20 μL of each solution.
- Allow the chromatography to proceed for three times the retention time of acetylcysteine.

MOBILE PHASE

10 volumes of [methanol](#) and 90 volumes of a 0.5% w/v solution of [ammonium sulfate](#) containing 0.02M [sodium pentanesulfonate](#). Adjust the pH of the mixture, if necessary, to pH 2.0 using [2M hydrochloric acid](#).

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to acetylcysteine (retention time about 6 minutes) are: cystine, about 0.6; cysteine, about 0.7; *N,N'*-diacetylcysteine, about 2.2.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the [peak-to-valley ratio](#) is at least 2.5, where *H_p* is the height above the baseline of the peak due to cysteine and *H_v* is the height above the baseline of the lowest point of the curve separating this peak from the peak due to cystine.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to *N,N'*-diacetyl-L-cystine (impurity C) is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to L-cysteine (impurity B) is not greater than twice the area of the corresponding peak in the chromatogram obtained with solution (2) (1%);

the area of any peak corresponding to L-cystine (impurity A) is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of any other [secondary peaks](#) is not greater than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (2) (1%).

Disregard any peak with an area less than the area of the peak due to acetylcysteine in the chromatogram obtained with solution (4) (0.1%).

[Hydrogen sulfide](#)

Place a quantity of the injection containing the equivalent of 0.4 g of Acetylcysteine in a round-bottomed, three-necked flask containing 40 mL of [water](#). The flask is fitted with a gas inlet tube which reaches nearly to the bottom of the flask, a dropping funnel containing [hydrochloric acid](#) and an outlet tube leading to a 100 mL graduated flask containing a mixture of 1 mL of 5M [sodium hydroxide](#) and 50 mL of [water](#). Pass through the flask a steady current of nitrogen and add 10 mL of [hydrochloric acid](#) from the dropping funnel. Maintain the current of nitrogen for 30 minutes and then disconnect the absorption flask. Add to the flask 10 mL of a solution prepared by dissolving 0.1 g of [N,N-dimethyl-p-phenylenediamine dihydrochloride](#) in a mixture of 45 mL of [hydrochloric acid](#) and 55 mL of [water](#) decolourised with [activated charcoal](#) before use, if necessary, and 5 mL of a 5% w/v solution of [iron\(III\) chloride hexahydrate](#) in 1M [hydrochloric acid](#) and allow to stand for 20 minutes protected from light. Add sufficient [water](#) to produce 100 mL and measure the [absorbance](#) of the solution, [Appendix II B](#), at 665 nm using a 4 cm pathlength and using in the reference cell a solution prepared in the same manner but without the injection being examined.

Prepare a 0.4% w/v solution of [sodium sulfide](#). Standardise this solution in the following manner. To 25 mL of [0.05M iodine VS](#) add 8 mL of [hydrochloric acid](#) and 25 mL of the sodium sulfide solution. Titrate with 0.1M [sodium thiosulfate solution VS](#) using [starch solution](#), added towards the end point, as indicator. Repeat the operation without the sodium sulfide solution. The concentration of the sodium sulfide solution expressed in parts per million of hydrogen sulfide is the difference between the titrations multiplied by 68.16. Prepare a solution containing the equivalent of 20 ppm of hydrogen sulfide by appropriate dilution of the sodium sulfide solution with [water](#).

Repeat the procedure carried out on the injection using 2 mL of the 20 ppm hydrogen sulfide solution in place of the injection being examined. The [absorbance](#) of the solution obtained from the injection is not greater than the absorbance of the solution obtained from the standard (100 ppm with reference to the content of acetylcysteine).

Bacterial endotoxins

Carry out the [test for bacterial endotoxins, Appendix XIV C](#). If necessary, dilute the injection with [water BET](#) to give a solution containing 10 mg per mL (solution A). The endotoxin limit concentration of solution A is not more than 0.3 IU per mL.

ASSAY

Carry out the method for [liquid chromatography, Appendix III D](#), using the following solutions.

- (1) Dilute the injection with the mobile phase to produce a solution containing the equivalent of 0.2% w/v of Acetylcysteine.
- (2) 0.2% w/v solution of [acetylcysteine BPCRS](#) in the mobile phase.
- (3) Dissolve 20 mg of [L-cystine](#) (impurity A) and 20 mg of [L-cysteine](#) (impurity B) in 10 mL of 1M [hydrochloric acid](#) and dilute to 100 mL with the mobile phase. Dilute 1 volume of the resulting solution to 20 volumes with the mobile phase.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related substances may be used.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the [peak-to-valley ratio](#) is at least 2.5, where H_p is the height above the baseline of the peak due to cysteine and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to cysteine.

DETERMINATION OF CONTENT

Calculate the content of $C_5H_9NO_3S$ in the injection using the declared content of $C_5H_9NO_3S$ in [acetylcysteine BPCRS](#).

STORAGE

Acetylcysteine Injection should be protected from light.

LABELLING

The strength is stated in terms of the equivalent amount of acetylcysteine in a suitable dose-volume.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A, B and C listed under [Acetylcysteine](#).