Acetylcysteine Injection

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Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

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Acetylcysteine Injection

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

Acetylcysteine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of acetylcysteine, $C_5H_9NO_3S$.

Usual strength. 200 mg per ml.

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. 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. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acetylcysteine IPRS* or with the reference spectrum of acetylcysteine.

Tests

pH (2.4.24). 6.5 to 7.5.

Hydrogen sulphide

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 m1 of *5 M 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* decolorized with *activated charcoal* before use, if necessary, and 5 ml of a 5 per cent 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, at 665 nm run 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 per cent w/v solution of *sodium sulphide*. Standardise this solution in the following manner. To 25 ml of *0.05M iodine* add 8 ml of *hydrochloric acid* and 25 ml of the *sodium sulphate solution*. Titrate with *0.1 M sodium thiosulfate solution* using starch solution, added towards the end point, as indicator. Repeat the operation without the *sodium sulphide solution*. The concentration of the sodium sulphide solution expressed in parts per million of hydrogen sulphide 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 sulphide solution* in place of the injection being examined. The absorbance of the solution obtained from the injection is not greater than the absorbance obtained from the reference solution (l00 ppm with reference to the content of acetylcysteine).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use, except reference solution (b).

Test solution. Dilute a volume of the injection with the mobile phase to obtain a solution containing the equivalent of 0.2 per cent w/v of Acetylcysteine.

Reference solution (a). A 0.2 per cent w/v solution of *N-acetyl-L-cysteine* in the mobile phase.

Reference solution (b). A 0.2 per cent w/v solution of *N-acetyl-L-cysteine* in the mobile phase and store at room temperature for at least 2 hours before use.

Reference solution (c). Weigh and transfer 20 mg each of, *L-cysteine* and *L-cystine* to a 100-ml volumetric flask, add 10 ml of *1 M hydrochloric acid*, add 40 mg of *N-acetyl-L-cysteine* and immediately dilute to volume with the mobile phase. Dilute 10.0 ml of the solution to 200.0 ml with the mobile phase.

Reference solution (d). Dilute 1.0 ml of reference solution (c) to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless-steel column 25 cm × 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Lichrosorb RP 18),
- mobile phase: a mixture of 90 volumes of a 0.5 per cent w/v solution of *ammonium sulphate* containing *0.02 M sodium pentanesulfonate* and 10 volumes of *methanol*, adjusted to pH 2.0 with *2 M hydrochloric acid*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume: 20 µl.

Name	Relative retention time
Cystine	0.60
Cysteine	0.66
Acetylcysteine	1.0

Inject reference solution (a), (b) and (d). The test is not valid unless the height of the trough separating the peaks due to cysteine and cystine is less than one quarter of the height of the peak due to cysteine in the chromatogram obtained with reference solution (d), the peak due to *N,N'*-diacetylcystine appears (retention time: about 13 minutes) in the chromatogram obtained with reference solution (b) and the area of this peak is more than the area of any corresponding peak in the chromatogram obtained with reference solution (a).

Inject reference solution (c), (d) and the test solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to *N,N'*-diacetylcystine is not more than the area of the acetylcysteine peak in the chromatogram obtained with reference solution (d) (1.0 per cent), the area of any peak corresponding to cystine and cysteine, each of, is not more than the area of corresponding peak in the chromatogram obtained with reference solution (d) (0.5 per cent) and the sum of areas of all the secondary peaks, other than *N,N'*-diacetylcystine, cystine and cysteine is not more than the area of the acetylcysteine peak in the chromatogram obtained with reference solution (d) (0.3 per cent). Ignore any peak with an area less than the area of the acetylcysteine peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.3 Endotoxin Unit per ml of a 1 per cent w/v solution in water for injections.

Assay. Add 20 ml of *glacial acetic acid* to volume of the injection containing equivalent of 0.4 g of Acetylcysteine. Titrate with *0.05 M iodine* until a permanent pale yellow colour is obtained. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 0.01623 g of $C_5H_9NO_3S$.

Storage. Store protected from light, at a temperature not exceeding 30°.

4.2 General Reagents

N-Acetyl-L-cysteine
$C_5H_9NO_3S$ = 163.2
$[\alpha]_D^{20}$: about +4.6.

mp, about 110°
General reagent grade of commerce.