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

Clenbuterol Injection

General Notices

Action and use

Beta$_2$-adrenoceptor agonist; bronchodilator.

DEFINITION

Clenbuterol Injection is a sterile solution of Clenbuterol Hydrochloride in Water for Injections. It is supplied as a ready-to-use solution. Multi-dose containers may contain a suitable antimicrobial preservative.

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

Content of clenbuterol hydrochloride, $C_{12}H_{18}Cl_2N_2O,HCl$

95.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.

Solution A 0.06% w/v of *sodium chloride*, adjusted to pH 4.0 with 1M *hydrochloric acid*.

(1) Dilute the injection with *methanol*, if necessary, to produce a solution containing 0.003% w/v of Clenbuterol Hydrochloride.
(2) Dilute 1 volume of a 0.06% w/v solution of *clenbuterol hydrochloride BPCRS* in *methanol* to 20 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

(a) Use a *silica gel F$_{254}$* pre-coated plate (Merck silica gel 60 F$_{254}$ HPTLC plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 5 µL of each solution.
(d) Develop the plate to 6 cm.
(e) After removal of the plate, allow it to dry in air and examine under *ultraviolet light (254 nm)*.

MOBILE PHASE

0.15 volume of *concentrated ammonia*, 5 volumes of *toluene*, and 10 volumes of *ethanol*.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) is similar in position and size to the principal spot in the chromatogram obtained with solution (2).

B. In the Assay, the principal peak in the chromatogram obtained with solution (1) has the same retention time as that in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 5.0, Appendix V L.

Clarity and colour of solution

The injection is not more opalescent than *reference suspension I*, Appendix IV A, and is not more intensely coloured than *reference solution* Y_7, Appendix IV B, Method I.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. *Solution A* 0.06% w/v of *sodium chloride*, adjusted to pH 4.0 with 1M *hydrochloric acid*.

(1) Dilute the injection with *methanol*, if necessary, to produce a solution containing 0.003% w/v of clenbuterol hydrochloride.
(2) Dilute 1 volume of solution (1) to 100 volumes with *methanol*.
(3) Dilute 1 volume of a solution containing 0.06% w/v of *clenbuterol hydrochloride BPCRS* and 0.006% w/v of *clenbuterol hydrochloride impurity B EPCRS* in *methanol* to 20 volumes with solution A.
(4) Dilute 1 volume of solution (2) to 10 volumes with *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (12 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable) fitted with a pre-column (0.5 cm × 4.0 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Nucleosil C18 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 245 nm.
(f) Inject 20 µL of each solution.
(g) For solution (1) allow the chromatography to proceed for twice the retention time of the peak due to clenbuterol.

MOBILE PHASE

167 volumes of *acetonitrile*, 333 volumes of *methanol* and 500 volumes of 0.25% w/v *citric acid monohydrate*, previously adjusted to pH 4.5 with *dilute ammonia solution*. Dissolve *sodium decanesulfonate* in the resulting solution to give a concentration of 0.2% w/v.

When the chromatograms are recorded under the prescribed conditions, the relative retentions with reference to clenbuterol (retention time of about 7.4 minutes) are: impurity A, about 0.5 and impurity B, about 0.8.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and clenbuterol is at least 2.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%);

the sum of the areas of all the *secondary peaks* is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than 3 times the area of the principal peak in the chromatogram obtained with solution (4) (0.3%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

Solution A 0.06% w/v of *sodium chloride*, adjusted to pH 4.0 with 1M *hydrochloric acid*.

(1) Dilute the injection with *methanol*, if necessary, to produce a solution containing 0.003% w/v of clenbuterol hydrochloride.

(2) 0.06% w/v of *clenbuterol hydrochloride BPCRS* in *methanol*. Dilute 1 volume of this solution to 20 volumes with solution A.

(3) Dilute 1 volume of a solution containing 0.06% w/v of *clenbuterol hydrochloride BPCRS* and 0.006% w/v of *clenbuterol hydrochloride impurity B EPCRS* in *methanol* to 20 volumes with solution A.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related Substances may be used with an injection volume of 10 µL.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to impurity B and clenbuterol is at least 2.0.

DETERMINATION OF CONTENT

Calculate the content of $C_{12}H_{18}Cl_2N_2O$,HCl in the injection from the chromatograms obtained using the declared content of $C_{12}H_{18}Cl_2N_2O$,HCl in *clenbuterol hydrochloride BPCRS*

STORAGE

Clenbuterol Injection should be protected from light and stored at a temperature not exceeding 25°.

IMPURITIES

The impurities limited by the requirements of this monograph include impurities A and B listed under Clenbuterol Hydrochloride.