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

Apramycin Veterinary Oral Powder

General Notices

Action and use

Aminoglycoside antibacterial.

DEFINITION

Apramycin Veterinary Oral Powder contains Apramycin Sulfate.

The veterinary oral powder complies with the requirements stated under Veterinary Oral Powders and with the following requirements.

CHARACTERISTICS

Light brown, granular powder.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Dissolve a quantity of the veterinary oral powder containing the equivalent of 60 mg of apramycin in *water* and dilute to 100 mL.
(2) 0.06% w/v of *apramycin BPCRS* in *water*.
(3) 0.06% w/v of each of *apramycin BPCRS* and *tobramycin BPCRS* in *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a silica gel F_{254} precoated plate (Merck silica gel 60 F_{254} plates are suitable).
(b) Use the mobile phase as described below.
(c) Apply 5 µL of each solution.
(d) Develop the plate to 10 cm.
(e) After removal of the plate, allow it to dry in air for 10 minutes, heat at 100° for 10 minutes, spray with *sodium hypochlorite solution* whilst hot and cool for 5 minutes. Spray with *absolute ethanol*, heat at 100° for 5 to 10 minutes, or until an area of the plate below the line of application gives at most a faint blue colour with one drop of a 1% w/v solution of *potassium iodide* containing 1% w/v *soluble starch*, and spray with the potassium iodide-starch solution.

MOBILE PHASE

20 volumes of *chloroform*, 40 volumes of 13.5M *ammonia* and 60 volumes of *methanol*, equilibrated for 1 hour before use.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated principal spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

B. In the test for Related substances, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).
C. Yields reaction A characteristic of *sulfates*, Appendix VI.

TESTS

Related substances

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

(1) Dissolve a quantity of the oral powder containing the equivalent of 0.3 g of apramycin and dilute to 100 mL.
(2) 0.30% w/v of *apramycin BPCRS*.
(3) Dilute 1 volume of solution (1) to 20 volumes.
(4) Dilute 1 volume of solution (3) to 50 volumes.

CHROMATOGRAPHIC CONDITIONS

(a) Use a column (25 cm × 4 mm) packed with fast cation-exchange polymeric beads (13 µm) with sulfonic acid functional groups (Dionex Fast Cation-1^{R} is suitable) and a stainless steel post-column reaction coil (380 cm × 0.4 mm) with internal baffles. Use in the reaction coil *ninhydrin reagent I* at a flow rate approximately the same as that for the mobile phase.
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 0.8 mL per minute.
(d) Use a column temperature of 130°. Maintain the post-column reaction coil at the same temperature.
(e) Use a detection wavelength of 568 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

Mobile phase A A solution containing 1.961% w/v of *sodium citrate*, 0.08% v/v of *liquefied phenol* and 0.5% v/v of *thiodiglycol*, adjusted to pH 4.25 using *hydrochloric acid*.

Mobile phase B A solution containing 4.09% w/v of *sodium chloride* and 3.922% w/v of *sodium citrate* with 0.08% v/v of *liquefied phenol*, adjusted to pH 7.4 with *hydrochloric acid*.

Equilibrate the column using a mixture containing 75% of mobile phase A and 25% of mobile phase B. After each injection elute for 3 minutes using the same mixture and then carry out a linear gradient elution for 6 minutes to 100% of mobile phase B. Elute for a further 21 minutes using 100% of mobile phase B, then step-wise re-equilibrate to a mixture of 75% of mobile phase A and 25% of mobile phase B and elute for at least 10 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution factor* between compound A and 3-hydroxyapramycin, identified as indicated in the reference chromatogram supplied with *apramycin BPCRS*, is at least 0.8.

LIMITS

Multiply the areas of all the *secondary peaks* by 0.5. [*NOTE*: This is to ensure that the impurities are calculated relative to Apramycin Sulfate (which contains about 50% w/w of apramycin).]

In the chromatogram obtained with solution (1):

the areas of any peaks corresponding to 3-hydroxyapramycin, lividamine/2-deoxystreptamine (combined), compound A and compound B (identified as indicated in the reference chromatogram supplied with *apramycin BPCRS*) are not greater than 1.4, 1.0, 0.4 and 0.4 times respectively the area of the principal peak in the chromatogram obtained with solution (3) (7%, 5%, 2% and 2% respectively);

the area of any other *secondary peak* is not greater than 0.4 times the area of the principal peak in the chromatogram obtained with solution (3) (2%);

the sum of the areas of all the *secondary peaks* is not greater than 3 times the area of the principal peak in the chromatogram obtained with solution (3) (15%).

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

ASSAY

Carry out the *microbiological assay of antibiotics*, Appendix XIV A, Method B. The precision of the assay is such that the fiducial limits of error are not less than 95% and not more than 105% of the estimated potency.

Calculate the content of apramycin in the veterinary oral powder taking each 1000 Units found to be equivalent to 1 mg of apramycin. The upper fiducial limit of error is not less than 97.0% and the lower fiducial limit of error is not more than 110.0% of the stated content.

LABELLING

The quantity of active ingredient is stated in terms of the equivalent amount of apramycin.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Apramycin Sulfate.