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Benzylpenicilloyl Polylysine Concentrate

» Benzylpenicilloyl Polylysine Concentrate has a molar concentration of benzylpenicilloyl moiety ($C_{16}H_{19}N_2O_5S$) of not less than 0.0125 M and not more than 0.020 M. It contains one or more suitable buffers.

Packaging and storage—Preserve in tight containers.

Labeling—The label states that this article is not intended for direct administration to humans or animals.

USP Reference standards ⟨11⟩—
USP L-Lysine Hydrochloride RS

pH ⟨791⟩: between 6.5 and 8.5, the undiluted Concentrate being used.

Limit of penicillenate and penamaldate—Transfer 1 mL of Concentrate to a 50-mL volumetric flask, dilute with *Saline phosphate buffer,* prepared as directed in the *Assay,* to volume, and mix. Using a suitable spectrophotometer and using *Saline phosphate buffer* as a blank, determine the absorbances at the wavelengths of maximum absorption at about 322 nm and 282 nm. Calculate the molar concentration of penicillenate taken by the formula:

$$50A_{322}/26{,}600b$$

in which A_{322} is the absorbance at 322 nm, 26,600 is the molar absorptivity of the penicillenate moiety at pH 7.6, and b is the length of the cell, in cm: not more than 0.00020 M is found. Calculate the molar concentration of penamaldate taken by the formula:

$$50A_{282}/22{,}325b$$

in which A_{282} is the absorbance at 282 nm, 22,325 is the molar absorptivity of the penamaldate moiety at pH 7.6, and b is the length of the cell, in cm: not more than 0.00060 M is found.

Benzylpenicilloyl substitution—

Citrate buffer—Dissolve 19.69 g of sodium citrate dihydrate, 0.1 mL of pentachlorophenol, and 5 mL of 2,2'-thiodiethanol in 900 mL of 0.2 N hydrochloric acid, adjust with hydrochloric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

Ninhydrin reagent—Dissolve 18 g of ninhydrin and 0.7 g of hydrindantin in 675 mL of dimethyl sulfoxide, add 225 mL of 4 M lithium acetate solution previously adjusted with glacial acetic acid to a pH of 5.2, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP L-Lysine Hydrochloride RS in *Citrate buffer* to obtain a solution having a known concentration of about 91 µg per mL (5×10^{-4} M).

Test preparation—Transfer 1.0 mL of Concentrate to a 10-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to an ampul, add 1.5 mL of 6 N hydrochloric acid, and seal the ampul under nitrogen. Heat the ampul at 110° for 22 hours. Transfer the contents of the ampul to a round-bottom, 50-mL flask, and dry by vacuum rotary evaporation. Dissolve the residue three times, using 5-mL portions of water, evaporating to dryness after each dissolution. Dissolve the residue in 10 mL of *Citrate buffer.*

Chromatographic system (see *Chromatography ⟨621⟩*)—The liquid chromatograph is equipped with a 1.75-mm × 50-cm column that contains a packing of 8-µm 8% cross-linked sulfonated divinylbenzene polystyrene cation-exchange resin. The column effluent is mixed continuously with flowing *Ninhydrin reagent,* and the flowing mixture is heated at 130° for 1.5 minutes in a reaction coil. The absorbance of the reaction mixture is measured continuously by a 570-nm detector. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 4.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 57 minutes for L-lysine. Calculate the molar concentration of lysine in the Concentrate taken by the formula:

$$(0.1C/182.65)(r_U/r_S)$$

in which C is the concentration, in µg per mL, of USP L-Lysine Hydrochloride RS in the *Standard preparation;* 182.65 is the molecular weight of anhydrous lysine hydrochloride; and r_U and r_S are the peak responses obtained from the *Test preparation* and the *Standard preparation,* respectively. Calculate the percentage of benzylpenicilloyl substitution taken by the formula:

$$100(B/L)$$

in which B is the molar concentration of benzylpenicilloyl moiety in the Concentrate, as determined in the *Assay;* and L is the molar concentration of lysine in the Concentrate: not less than 50% and not more than 70% is found.

Assay—

Saline phosphate buffer—Dissolve 9 g of sodium chloride and 1.38 g of monobasic sodium phosphate in 900 mL of water, adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 7.6, dilute with water to 1000 mL, and mix.
Mercuric chloride solution—Dissolve 35 mg of mercuric chloride in 500 mL of water, and mix.
Assay preparation—Transfer 1.0 mL of Concentrate to a 500-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.
Procedure—Transfer 3.0 mL of *Assay preparation* to a spectrophotometric cell. Using a suitable spectrophotometer and using *Saline phosphate buffer* as the blank, determine the initial absorbance at the wavelength of maximum absorbance at about 282 nm. Add 0.02 mL of *Mercuric chloride solution* to the *Assay preparation* in the spectrophotometric cell, mix, and determine the absorbance at the same wavelength after 1 and 3 minutes. Repeat the addition of 0.02-mL portions of *Mercuric chloride solution* until a maximum absorbance reading is obtained. Calculate the molar concentration of benzylpenicilloyl moiety in the Concentrate taken by the formula:

$$500\{[A_m(3 + 0.02n)/3] - A_i\}/22{,}325b$$

in which A_m is the highest absorbance observed; A_i is the initial absorbance, n is the number of 0.02-mL portions of *Mercuric chloride solution* added to the *Assay preparation* to obtain the maximum absorbance; 22,325 is the molar absorptivity of the penamaldate formed by the reaction of benzylpenicilloyl with mercuric chloride at pH 7.6; and b is the length of the cell, in cm: between 0.0125 M and 0.020 M is found.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
BENZYLPENICILLOYL POLYLYSINE CONCENTRATE	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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