



(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 0.01M phosphoric acid.

(2) *Mobile phase*. Mix acetonitrile (high-pressure liquid chromatography grade):0.01M phosphoric acid (350:650). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions*. Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution*. Place approximately 5 milligrams of the plicamycin working standard, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(2) *Preparation of sample solution*. Prepare the sample solution as described in the individual monograph for the drug being tested.

(e) *Procedure*. Use the equipment, reagents, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test*. Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by two or more replicate injections of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the relative standard deviation as described for system suitability tests in the U.S.P. XX General

Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the minimum performance requirement for the relative standard deviation is not more than 2.0 percent. If the minimum performance requirement is not met, adjustment must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram*. Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations*. Calculate the plicamycin content as described in the individual monograph for the drug being tested.

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**§ 436.342 High-pressure liquid chromatographic assay for cefazolin.**

(a) *Equipment*. A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection; and

(5) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *Buffer solution, pH 3.6*. Transfer 0.9 gram of sodium phosphate, dibasic USP and 1.298 grams of citric acid USP to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(2) *Buffer solution, pH 7.0*. Transfer 5.68 grams of sodium phosphate, dibasic