

0.864=The molecular weight of sulfisoxazole divided by the molecular weight of sulfisoxazole acetyl.

[46 FR 2990, Jan. 13, 1981]

§ 436.329 High-pressure liquid chromatographic assay for meclocycline.

(a) *Equipment.* A suitable high-pressure liquid chromatograph, such as a Waters Associates Model 244¹ or equivalent equipped with:

- (1) A low dead volume cell 8 to 20 microliters;
- (2) A light path of 1 centimeter;
- (3) A suitable ultraviolet detection system operating at a wavelength of 340 nanometers;
- (4) A suitable recorder of at least 25.4 centimeter deflection;
- (5) A suitable integrator;
- (6) A column approximately 25 centimeters in length having an inside diameter of approximately 4 millimeters and packed with a suitable reverse-phase packing such as: 10 micrometer silica gel particles bonded to octadecyl silane, Vydac 201 TP Reverse Phase² or equivalent.

(b) *Reagents*—(1) *0.001M Ammonium (ethylenedinitrilo) tetraacetate.* Moisten 293 milligrams of (ethylenedinitrilo) tetraacetic acid with 1 milliliter of methanol and dissolve in 7 milliliters of concentrated ammonium hydroxide. Dilute to 900 milliliters with distilled water, adjust the pH to 6.6 with glacial acetic acid, and dilute to 1,000 milliliters with distilled water.

(2) *Mobile phase.* Mix 150 milliliters of tetrahydrofuran (high-pressure liquid chromatography grade) with 850 milliliters of 0.001M ammonium (ethylenedinitrilo) tetraacetate. 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 0.8 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh an amount of sample or working standard equivalent to approximately 25 milligrams of meclocycline into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methanol and mix. Transfer exactly 3.0 milliliters of this solution to a 25-milliliter volumetric flask, dilute to volume with mobile phase, and mix.

(e) *Procedure.* Using the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the sample or working standard solution prepared as described in paragraph (d) of this section into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. The elution order is void volume, oxytetracycline (if present), demeclocycline (if present), methacycline (if present), and meclocycline.

(f) *Calculations.* Calculate the meclocycline content as follows:

$$\text{Micrograms of meclocycline per milligram} = \frac{A \times \text{Milligrams of working standard} \times \text{Potency of the working standard in micrograms per milligram}}{B \times \text{Milligrams of sample}}$$

where:

A= Area or peak height of the sample peak (at a retention time equal to that observed for the standard);

B= Area or peak height of the standard peak.

[46 FR 3836, Jan. 16, 1981]

¹Available from: Waters Associates, Inc., Maple St., Milford, MA 10757.

²Available from: The Separations Group, 16640 Spruce St., Hesperia, CA 92345.