

**§ 436.328 High pressure liquid chromatographic assay for sulfisoxazole acetyl content.**

(a) *Equipment.* A suitable high pressure liquid chromatograph, such as a Waters Associates Model 244<sup>1</sup> or equivalent 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;
- (5) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with a suitable reverse phase packing such as: Waters Associates, Micro-Bondapak C18;<sup>1</sup> and
- (6) A suitable integrator.

(b) *Reagents*—(1) *Mobile phase.* Mix acetonitrile (high pressure liquid chromatography grade): water (40:60). Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. De-gas the mobile phase just prior to its introduction into the chromatograph pumping system.

(2) *Internal standard solution.* Dissolve 0.33 milligram of benzanilide per milliliter in acetonitrile (high pressure liquid chromatography grade). Filter the solution through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for ref-

erence standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the baseline.

(d) *Preparation of the working standard and sample solutions*—(1) *Working standard solution.* Prepare a solution containing 1.0 milligram per milliliter of sulfisoxazole acetyl in the internal standard solution.

(2) *Sample solution.* Reconstitute the sample as directed in the labeling. Allow to stand for 1 hour. Shake gently and transfer 5.0 milliliters of the sample to a separatory funnel. Extract the suspension three times with 75-milliliter portions of chloroform. Collect the chloroform layers in a 250-milliliter volumetric flask. Dilute the flask to volume with chloroform and mix. Filter a portion of the solution through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. Transfer a 4.0-milliliter aliquot of the filtrate into a 25-milliliter glass-stoppered flask and evaporate to dryness under a stream of dry air. Dissolve the residue in 10.0 milliliters of the internal standard solution, stopper, and mix.

(e) *Procedure.* Using the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 5 microliters of 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, sulfisoxazole acetyl and benzanilide.

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

$$\text{Milligrams of sulfisoxazole per milliliter of sample} = \frac{A \times \text{Concentration of the standard solution in milligrams per milliliter} \times 125 \times 0.864}{B}$$

where:

A=Area of sample peak (at a retention time equal to that of the standard) di-

vided by the area of the internal standard peak;

B=Area of the standard peak divided by the area of the internal standard peak;

<sup>1</sup>Available from: Waters Associates, Inc., Maple Street, Milford, MA 10757.

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 244<sup>1</sup> 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 Phase<sup>2</sup> 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]

<sup>1</sup>Available from: Waters Associates, Inc., Maple St., Milford, MA 10757.

<sup>2</sup>Available from: The Separations Group, 16640 Spruce St., Hesperia, CA 92345.