

§ 556.225

with a 10-milliliter pipette to deliver a reproducible volume.

ii. Increase the flow rate to 2 to 4 milliliters per minute by applying air pressure to the column. A glycerol manostat adjusted to 30 inches and attached between an air supply and column provides adequate pressure.

b. Wash the resin with 10 milliliters of eluting reagent A. Discard eluate.

2. Pass eluate A from procedure step V-D4 through the column. Collect in a 250-milliliter beaker.

3. Pass 50 milliliters of specially denatured alcohol 3A through the column. Combine with the eluate of procedure step V-E2.

F. Reduction. 1. Place the eluate A fraction from procedure step V-E3 on a hot water bath (90 °C.) and evaporate with a stream of air until 5 to 10 milliliters remain. Do not overheat the sample or allow the sample to go to dryness.

2. Transfer to centrifuge tube and rinse beaker three times with 3 milliliters of specially denatured alcohol 3A.

3. Evaporate on a hot water bath (90 °C.) under a stream of air until alcohol has evaporated. Do not overheat the sample or allow the sample to go to dryness.

4. Remove the tube from the water bath and immediately add 5.0 milliliters of water.

5. While mixing, add 2 drops of titanium chloride and 4 drops of 10N sodium hydroxide. Continue mixing until greyish color disappears.

a. Mix on Vortex Jr. mixer, or equivalent, regulated with autotransformer.

b. Precipitate of insoluble tissue substances and white titanium salts is present after reduction is complete.

6. Dilute to 50 milliliters with specially denatured alcohol 3A and mix.

7. Centrifuge for 5 minutes at 2,000 r.p.m.

G. Cation-exchange chromatography—No. 2. 1. Prepare resin column by procedure step V-E.

2. Pass the centrifugate of procedure step V-F7 through column. Use three rinses of specially denatured alcohol 3A, each 5 milliliters, to aid in transferring of sample.

3. Pass 50 milliliters of specially denatured alcohol 3A through the column.

4. Pass 50 milliliters of deionized water through the column.

5. Elute arylamine residue from the resin with 40 to 43 milliliters of 4N HCl into a 50-milliliter volumetric flask (actinic ware) for 3,5-DNBA analysis. Avoid direct sunlight. The arylamine has been found to be photosensitive.

H. Color development and measurement. 1. Cool to 0 °C.–5 °C. by placing in a freezer or ice bath.

2. Perform the Bratton-Marshall reaction in subdued light as follows:

a. Add 1 milliliter of sodium nitrite reagent, mix, and allow to stand for 1 minute.

21 CFR Ch. I (4–1–07 Edition)

b. Add 1 milliliter of ammonium sulfamate reagent, mix, and allow to stand for 1 minute.

c. Add 1 milliliter of coupling reagent, mix, and allow to stand for 10 minutes.

d. Dilute to volume with 4N HCl.

3. Perform colorimetric measurement at 530 millimicrons as follows:

a. Fill two matched 100-millimeter cells with 4N HCl and place into instrument.

b. Adjust dark current.

c. Adjust to zero absorbance.

d. Replace acid in cell of sample side of compartment with sample to be measured.

e. Record absorbance observed.

I. Calculations. Determine parts per billion (observed) from the standard curve.

§ 556.225 Doramectin.

(a) *Acceptable daily intake (ADI)*. The ADI for total residues of doramectin is 0.75 microgram per kilogram of body weight per day.

(b) *Tolerances*—(1) *Cattle*. A tolerance of 100 parts per billion is established for parent doramectin (marker residue) in liver (target tissue) and of 30 parts per billion for parent doramectin in muscle.

(2) *Swine*. A tolerance is established for parent doramectin (marker residue) in liver (target tissue) of 160 parts per billion.

[63 FR 68184, Dec. 10, 1998]

§ 556.227 Eprinomectin.

(a) *Acceptable daily intake (ADI)*. The ADI for total residues of eprinomectin is 10 micrograms per kilogram of body weight per day.

(b) *Tolerances*—(1) *Cattle*. Tolerances are established for residues of eprinomectin B1a (marker residue) in milk of 12 parts per billion, in liver (target tissue) of 4.8 parts per million, and in muscle of 100 parts per billion.

(2) [Reserved]

[63 FR 59715, Nov. 5, 1998]

§ 556.228 Enrofloxacin.

The acceptable daily intake for enrofloxacin is 3 micrograms per kilogram of body weight per day.

(a) *Cattle*. A tolerance of 0.1 part per million for desethylene ciprofloxacin (marker residue) has been established in liver (target tissue) of cattle.