

5.1.17

AOAC Official Method 977.35 Carbadox in Feeds

Spectrophotometric Method First Action 1977 Final Action 1981

(Applicable to levels 0.0055%. Carbadox solutions are light sensitive. Extracts must be protected from direct sunlight or artificial light.)

A. Apparatus

(a) *Filter aid*.—Celite 545 (Celite Corp.) or Millipore prefilter pad (No. AP2504700, Millipore Corp., Ashby Rd, Bedford, MA 01730, USA), or equivalent.

(b) *Spectrophotometer*.—For use at 520 nm.

B. Reagents

(a) *Carbadox standard solutions*.—(1) *Stock solution*.—1.10 mg/mL. Weigh 110.0 mg Carbadox Reference Standard (available from Phibro Animal Health, 65 Challenger Rd, Third Floor, Ridgefield Park, NJ 07660, USA, Tel: +1-888-403-0074, Fax: +1-201-329-7070; www.philbroah.com) into 100 mL volumetric flask, dissolve in CHCl_3 -methanol (3 + 1), and dilute to volume with same solvent. Ultrasonic bath speeds dissolution. Prepare fresh daily. (2) *Working solution*.—0.110 mg/mL. Pipet 10 mL stock solution into 100 mL volumetric flask, dilute to volume with CHCl_3 -methanol (3 + 1), and mix well. Prepare fresh daily.

(b) *Methanolic hydrochloric acid solution*.—1M. Dilute 85 mL HCl to 1 L with methanol.

(c) *Methanolic sodium hydroxide solution*.—0.05M. Dissolve 2.0 g NaOH in methanol and dilute to 1 L with methanol. Prepare fresh weekly or sooner if precipitate forms.

(d) *Potassium phosphate solution*.—1M. Dissolve 136 g KH_2PO_4 in H_2O and dilute to 1 L.

(e) *Sodium hydroxide-sodium chloride solution*.—Dissolve 100 g NaCl in 0.1M NaOH and dilute to 1 L with 0.1M NaOH.

(f) *Stannous chloride solution*.—Prepare immediately before use. Add 8.0 g $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ to 100 mL methanolic 1M HCl. Place in 55 –60 C water bath and swirl intermittently until solution is clear (ca 20 min). Stopper and cool to room temperature. Use within 2 h.

C. Extraction of Analyte

Weigh duplicate test portions of ground feed into 250 mL Erlenmeyers: 2.000 g for 0.0330–0.0606% carbadox; 5.000 g, 0.0110–0.0330%; and 20.00 g, 0.0055–0.0110%. Wet each portion with 10 mL H_2O , let stand 5 min, and add 140 mL CHCl_3 -methanol (3 + 1). Add 15.0 mL working standard solution to 1 portion. Stopper both flasks loosely or with polyethylene stopper with pinhole, and boil gently 1 h. Cool to room temperature.

Using three 25 mL portions CHCl_3 -methanol (3 + 1), quantitatively transfer mixture to Büchner precoated with Celite or containing prefilter pad, collecting filtrate under vacuum in 250 mL volumetric flask. Dilute to volume with CHCl_3 -methanol (3 + 1),

and mix well. Pipet 100 mL aliquot into 250 mL separator containing 50 mL NaOH–NaCl solution. Shake 10 s and discard lower CHCl_3 layer. Add 50 mL CHCl_3 , shake 10 s, and discard CHCl_3 layer. Add 10 mL KH_2PO_4 solution, and extract with three 50 mL portions CHCl_3 , combining extracts in round-bottom flask. *Do not let any solids at interface drain into flask*. Evaporate to dryness, using rotary evaporator and 60 C water bath.

Conduct reagent blank of H_2O and CHCl_3 -methanol (3 + 1) through boiling, filtration, extractions, and evaporation, omitting addition of feed and carbadox.

Alternatively, weigh ground feed as above and extract as follows: Wet each portion with 10 mL H_2O , let stand 5 min, and add 140 mL CHCl_3 -methanol (3 + 1) to one flask. Prepare spiked sample by adding 130 mL CHCl_3 -methanol (3 + 1) and 10 mL carbadox working standard solution to other flask. Break up any clumps with spatula. Stopper tightly and let stand overnight in dark at room temperature. Using three 25 mL portions of CHCl_3 -methanol (3 + 1) quantitatively transfer mixture to Büchner precoated with Celite or containing prefilter pad, collecting filtrate under vacuum in 250 mL volumetric flask for unspiked sample and in 500 mL volumetric flask for spiked sample. Proceed as above, beginning “Dilute to volume with CHCl_3 -methanol ”.

D. Determination

Dissolve residue in flask from test portion, test portion plus standard, and blank in 5.00 mL 0.05M methanolic NaOH. Add 20.0 mL SnCl_2 solution, swirl gently, and let stand 10 min for complete color development. If necessary, clarify solution by filtration through small glass wool plug. If alternative overnight leach was used, clarify solution by centrifuging 10 min at 100 rpm. Within 15 min after completion of color development, determine *A* of clear solutions at 520 nm against methanol as reference solvent. Subtract *A* of blank from *A* of test portion and *A* of test portion plus standard.

$$\text{Carbadox, \%} = \frac{(A/\text{g test portion}) [1(A - A)]}{(\text{mg carbadox/mL working standard solution}) (1 \text{ g}/1000 \text{ mg}) 15 \text{ mL aliquot } 100}$$

$$\text{Carbadox, mg/kg} = \frac{(A/\text{g test portion}) [1(A - A)]}{(\text{mg carbadox/mL working standard solution}) 15 \text{ mL aliquot}}$$

When alternative overnight leach was used, change calculation to:

$$\text{Carbadox, \%} = \frac{(A/\text{g test portion}) [1(2A - A)]}{(\text{mg carbadox/mL working standard solution}) (1 \text{ g}/1000 \text{ mg}) 10 \text{ mL aliquot } 100}$$

$$\text{Carbadox, mg/kg} = \frac{(A/\text{g test portion}) [1(2A - A)]}{(\text{mg carbadox/mL working standard solution}) 10 \text{ mL aliquot}}$$

References: *JAOAC* **60**, 1059(1977); **62**, 982(1979).

CAS-6804-07-5 (carbadox)