

### 3.3.04

#### AOAC Official Method 951.01

##### Cobalt in Plants

##### Nitrosocresol Method

First Action 1951

Final Action 1965

#### A. Reagents

(Make all distillations in Pyrex stills with standard tapered joints. Store reagents in glass-stoppered Pyrex bottles.)

(a) *Redistilled water*.—Distil twice, or pass through column of ion exchange resin (IR-100A, H-form, or equivalent) to remove heavy metals.

(b) *Hydrofluoric acid*.—48%. Procurement in vinyl plastic bottles is advantageous.

(c) *Perchloric acid*.—60%. No further purification necessary.

(d) *Hydrochloric acid*.—1 + 1. Add equal volume 12 M HCl to distilled water and distil.

(e) *Ammonium hydroxide*.—1 + 1. Distil concentrated  $\text{NH}_4\text{OH}$  into equal volume redistilled water.

(f) *Ammonium hydroxide*.—0.02 M. Add 7 mL  $\text{NH}_4\text{OH}$  (1 + 1), (e), to 2.5 L redistilled water.

(g) *Carbon tetrachloride*.—Distil over CaO, passing distillate through dry, acid-washed filter paper. Used  $\text{CCl}_4$  may be recovered as in 941.03A(a) (see 3.3.18).

(h) *Dithizone*.—Dissolve 0.5 g dithizone in 600–700 mL  $\text{CCl}_4$  (technical grade is satisfactory). Filter into 5 L separator containing 2.5–3.0 L 0.02 M  $\text{NH}_4\text{OH}$ , shake well, and discard  $\text{CCl}_4$  layer. Shake with 50 mL portions redistilled  $\text{CCl}_4$  until  $\text{CCl}_4$  phase as it separates is pure green. Add 1 L redistilled  $\text{CCl}_4$  and acidify slightly with the HCl (1 + 1). Shake the dithizone into  $\text{CCl}_4$  layer and discard aqueous layer. Store in cool, dark place, preferably in refrigerator.

(i) *Ammonium citrate solution*.—40%. Dissolve 800 g citric acid in 600 mL distilled water, and, while stirring, slowly add 900 mL  $\text{NH}_4\text{OH}$ . Reaction is exothermic; take care to prevent spattering. Adjust pH to 8.5, if necessary. Dilute to 2 L and extract with 25 mL portions dithizone solution until aqueous phase stays orange and  $\text{CCl}_4$  remains predominantly green. Then extract solution with  $\text{CCl}_4$  until all orange is removed.

(j) *Hydrochloric acid*.—0.1 M. Dilute 16.6 mL HCl (1 + 1) (6 N) to 1 L with redistilled water.

(k) *Hydrochloric acid*.—0.01 M. Dilute 100 mL of 0.1 M HCl to 1 L with redistilled water.

(l) *Sodium hydroxide solution*.—1 M. Dissolve 40 g NaOH in 1 L redistilled water.

(m) *Borate buffer*.—pH 7.8. Dissolve 20 g  $\text{H}_3\text{BO}_3$  in 1 L redistilled water. Add 50 mL 1 M NaOH and adjust pH, if necessary.

(n) *Borate buffer*.—pH 9.1. To 1 L borate buffer, pH 7.8, add 120 mL 1 M NaOH and adjust pH, if necessary. Equal volumes borate buffer and 0.01 M HCl should give solution of pH 7.9.

(o) *Skellysolve B*.—Essentially *n*-hexane. Purify by adding 20–30 g silica gel/L, let stand several days, and distil.

(p) *Cupric acetate solution*.—Dissolve 10 g  $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  in 1 L redistilled water.

(q) **o*-Nitrosocresol solution*.—Dissolve 8.4 g anhydrous  $\text{CuCl}_2$  and 8.4 g  $\text{NH}_2\text{OH} \cdot \text{HCl}$  in 900 mL  $\text{H}_2\text{O}$ . Add 8 mL *m*-cresol (practical grade) and stir vigorously while slowly adding 24 mL 30%  $\text{H}_2\text{O}_2$ . Stir mechanically 2 h at room temperature. (Standing for longer periods results in excessive decomposition.) Add 25 mL HCl and extract *o*-nitrosocresol with four 150 mL portions Skellysolve B, (o), in large

separator. Then add additional 25 mL HCl and again extract with four 150 mL portions Skellysolve B. Wash combined Skellysolve B extracts twice with 50–100 mL portions 0.1 M HCl and twice with 50–100 mL portions redistilled water. Shake *o*-nitrosocresol solution with successive 50–100 mL portions 1%  $\text{Cu}(\text{CH}_3\text{COO})_2$  solution until aqueous phase is no longer deep blood-red. When light purple is evident, extraction is complete. Discard Skellysolve B phase, acidify aqueous solution of Cu salt with 25 mL HCl, and extract reagent with two 500 mL portions Skellysolve B; wash twice with 150–200 mL portions 0.1 M HCl and several times with 150–200 mL portions redistilled water. Store *o*-nitrosocresol solution in refrigerator at ca 4°C. Reagent is stable 6 months.

(r) *Sodium *o*-nitrosocresol solution*.—Extract 100 mL *o*-nitrosocresol by shaking with two 50 mL portions borate buffer, pH 9.1, in separator. (If this is carried out as 2 extractions, resulting reagent is more concentrated. It is important that total volume *o*-nitrosocresol solution equal total volume buffer.)

(s) *Cobalt standard solutions*.—(1) *Stock solution*.—Dry  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  in oven at 250°–300°C to constant weight (6–8 h). Weigh exactly 0.263 g of the dried  $\text{CoSO}_4$  and dissolve in 50 mL redistilled water and 1 mL  $\text{H}_2\text{SO}_4$ . Dilute to 1 L. (2) *Working solution*.—0.5 g/mL. Transfer 5 mL stock solution to 1 L volumetric flask and dilute to volume with redistilled water.

(t) *Hydroxylamine acetate buffer*.—pH 5.1 0.1. Dissolve 10 g  $\text{NH}_2\text{OH} \cdot \text{HCl}$  and 9.5 g anhydrous  $\text{CH}_3\text{COONa}$  in 500 mL redistilled water.

#### B. Apparatus

(a) *Platinum dishes*.—Approximately 70 mL; for ashing.

(b) *Automatic dispensing burets*.—100 mL; type that can be fitted to ordinary 5 lb (2.27 kg) reagent bottle and filled by means of aspirator bulb is most convenient.

(c) *Wooden separator rack*.—Twelve-unit 125 mL separator size is convenient for dithizone extractions. Rack is fitted across top with removable bar padded with sponge rubber so all 12 separators can be shaken as unit.

(d) *Racks*.—Consisting of 5 5 65 cm (2 2 25 in.) wooden bars with holes drilled at close intervals to take 50 mL centrifuge tubes fitted with No. 13 standard taper glass stoppers. To make these tubes, ream out necks of heavy-wall Pyrex centrifuge tubes (Rockefeller Institute type) with standard taper C rod and grind to take standard taper stopper. Place tubes upright in one section, and place other section (fitted with sponge rubber disks 13 mm thick in bottom of holes) across their tops. Fasten 2 sections at ends with removable rubber connectors made from ordinary tubing of convenient size, so that any number of tubes can be shaken as unit. Use these tubes for reaction of Co with nitrosocresol, extraction of complex into Skellysolve B, and washing of Skellysolve B solution.

(e) *Shaking machine*.—Mechanical shaker giving longitudinal stroke of 5 cm at ca 180 strokes/min; use to make dithizone extractions and to extract Co complex, or shake by hand.

#### C. Cleaning of Glassware

Clean 120 mL Pyrex separators for dithizone extractions by initially soaking 30 min in hot  $\text{HNO}_3$  and rinsing several times with  $\text{H}_2\text{O}$ . As added precaution, shake with several portions dithizone in  $\text{CCl}_4$ . After use, clean by rinsing with  $\text{H}_2\text{O}$ , drain, and stopper to avoid contamination. It is not necessary to clean every time with acid. Repeat  $\text{HNO}_3$  cleaning if blanks are unusually high.

Clean 50 mL glass-stoppered Pyrex centrifuge tubes by soaking 30 min in HNO<sub>3</sub> followed by several rinsings in H<sub>2</sub>O.

Completely submerge pipets in cylinder of chromic acid cleaning solution overnight, rinse several times with H<sub>2</sub>O, and suspend upright in rack to dry.

Wash all other glassware thoroughly in detergent and rinse well with tap water followed by dip in chromic acid cleaning solution. Rinse off cleaning solution with tap water followed by several distilled water rinses.

Clean Pt by scrubbing with sea sand followed by boiling in HCl (1 + 2) 30 min, and rinse several times with H<sub>2</sub>O.

#### D. Preparation of Test Sample

See 922.02(a) (see 3.1.02). Oven-dry all plant material 48 h and prepare for ashing by either of following methods:

(a) Grind material in Wiley mill equipped with stainless steel sieve, mix thoroughly by rolling, and sample by quartering.

(b) Using stainless steel shears, cut material by hand fine enough for convenient sampling.

#### E. Ashing of Test Portions

Weigh 6 g dry plant tissue into clean Pt dish. Cover with Pyrex watch glass and place in cool furnace; heat slowly to 500°C and hold at this temperature overnight. Remove dish and cool. Wet down ash carefully with fine stream redistilled water. From dispensing buret, slowly add 2–5 mL HClO<sub>4</sub>, dropwise at first to prevent spattering. Add ca 5 mL HF, evaporate on steam bath, transfer to sand bath, and keep at medium heat until fuming ceases.

Cover with Pyrex watch glass, return to partially cooled furnace, heat gradually to 600°C, and keep at this temperature 1 h. Remove dish and cool. Add 5 mL HCl (1 + 1) and ca 10 mL redistilled water. Replace cover glass and warm on steam bath to dissolve. (Usually clear solution essentially free of insoluble material is obtained.) Transfer test solution to 50 mL volumetric flask, washing dish several times with redistilled water, dilute to volume with H<sub>2</sub>O, and mix thoroughly. (Pt dishes can ordinarily be used several times between sand and acid cleanings.)

#### F. Dithizone Extraction

Transfer suitable aliquot (2–3 g dry material) to 120 mL separator (use petroleum jelly as stopcock lubricant). Add 5 mL ammonium citrate solution, A(i), and 1 drop phenolphthalein; adjust to pH 8.5 with NH<sub>4</sub>OH (1 + 1). If precipitate forms, add additional ammonium citrate. Add 10 mL dithizone in CCl<sub>4</sub> and shake 5 min. Drain CCl<sub>4</sub> phase into 100 mL beaker. Repeat as many times as necessary, using 5 mL dithizone solution and shaking 5 min each time. Extraction is complete when aqueous phase remains orange and CCl<sub>4</sub> phase remains predominantly green. Then add 10 mL CCl<sub>4</sub>, shake 5 min, and combine with CCl<sub>4</sub> extract. Final 10 mL CCl<sub>4</sub> should be pure green. If not, extraction was incomplete and must be repeated.

Add 2 mL HClO<sub>4</sub> to combined CCl<sub>4</sub> extracts, cover beaker with Pyrex watch glass, and digest on hot plate until colorless. Remove cover glass and evaporate slowly to dryness. (If residue is heated any length of time at high temperature when dry, losses of Co may occur. Heat only enough to evaporate completely to dryness. If free acid remains, it interferes with next step where pH control is important.)

Add 5 mL 0.01M HCl to residue. Heat slightly to assure solution. If Cu is to be determined, transfer with redistilled water to 25 mL volumetric flask, and dilute to volume. Transfer 20 mL aliquot to 50 mL glass-stoppered centrifuge tube or 60 mL separator and reserve remainder for Cu determination, 953.03B (see 3.3.06). If Cu is not to be determined, transfer entire acid solution with redistilled water to centrifuge tube or separator.

#### G. Determination

Add 5 mL borate buffer, pH 7.8, and 2 mL freshly prepared sodium *o*-nitrosocresol solution to test solution. Add exactly 5 mL Skellysolve B and shake 10 min. Remove aqueous phase by moderate suction through finely-drawn glass tube. To Skellysolve B layer add 5 mL Cu(CH<sub>3</sub>COO)<sub>2</sub> solution and shake 1 min to remove excess reagent. Again remove and discard aqueous phase. Wash Skellysolve B by shaking 1 min with 5 mL redistilled water, removing aqueous layer as before; finally shake Skellysolve B 1 min with 5 mL NH<sub>2</sub>OH–NaCH<sub>3</sub>COO buffer to reduce Fe. Transfer Skellysolve B solution of the Co complex to 5 cm cell and read in spectrophotometer as close as possible to point of maximum *A*, 360 nm.

#### H. Blanks and Standards

With each set of determinations include ashing blank and Co standards of 0.0, 0.5, and 1.0 g. Beer's law holds for this range. *A* of 0.0 g point should be <0.05. If above, repurify *o*-nitrosocresol by transferring alternately to aqueous phase as Cu salt and to Skellysolve B phase as free compound after acidifying aqueous phase.

Include reference sample with each set of test samples to detect contamination or unusual losses of Co in method. Commercial buckwheat flour containing 0.05 ppm (g/g) Co has proved satisfactory for this purpose.

#### I. Calculations

Express results in terms of ppm Co, based upon dry weight of test sample.

$$\text{Co, ppm (g/g)} = \left( \frac{\text{g Co/mL dithizone aliquot}}{\text{mL total solution/g dry test portion}} \right)$$

Value for g Co is obtained from curve minus ashing blank.

References: *JAOAC* 34, 710(1951); 36, 405(1953).

CAS-7440-48-4 (cobalt)