

5.1.57

**AOAC Official Method 961.26
Zoalene in Feeds**

**Spectrophotometric Method
First Action 1961
Final Action 1962**

(Not applicable in presence of furazolidone, nitrofurazone, and nihydrazone.)

A. Principle

Zoalene is extracted from feeds, premixes, and concentrates containing 40–250 000 mg/kg with 85% CH₃CN. For mixes containing <10 000 mg/kg, alumina is added. After filtration and dilution, zoalene is determined colorimetrically after reaction with ethylenediamine.

B. Reagents

(a) *Acetone*.—95%. Add 5 mL H₂O to 95 mL acetone.

(b) *Acetonitrile*.—85%. Add 850 mL practical grade CH₃CN to 150 mL H₂O (deionized or distilled).

(c) *Activated alumina*.—Alcoa grade F 20, 80–200 mesh. (Available from Fisher Scientific Co. “Alumina, Adsorption, Fisher.”)

(d) *Dimethylformamide (DMF)*.—95%. Add 5 mL H₂O to 95 mL technical DMF. Prepare fresh daily, since old solutions may cause cloudiness.

(e) *Ethylenediamine*.—98–100%. (No. EX0510, EM Science). Reagent must be practically colorless.

(f) *Zoalene standard solution*.—40 g/mL. Weigh 40.0 mg Zoalene Reference Standard (available from Alpharma, Inc.) into 1 L volumetric flask, dilute to volume with 85% CH₃CN, and mix.

C. Determination

Weigh 10.0 g ground test portion into 250 mL Erlenmeyer and add 65 mL 85% CH₃CN. Warm on steam bath to 50 ± 5 C, swirling occasionally. Let cool to room temperature (ca 30 min). Add 20 g alumina and swirl occasionally ca 3 min. (Addition of alumina is unnecessary for concentrates containing 10 000 mg/kg zoalene.) Filter with suction on medium or fine porosity 40 mm diameter fritted glass funnel, transferring as much solids as possible. Transfer remaining solids with minimum volume 85% CH₃CN, and suck dry. Suspend cake in funnel with minimum volume 85% CH₃CN and slight stirring but without suction. Then filter with suction and repeat suspension and filtering, keeping total volume <100 mL. Transfer combined filtrates to 100 mL volumetric flask (or volumetric flask may be used to collect filtrates directly), dilute to volume with 85% CH₃CN, and mix.

Table 961.26. Dilution of test sample for determination

mg/kg Zoalene in test sample	Additional dilution	Aliquot size, mL	Multiplication factor <i>M</i>
40–120	None	4	1
120–250	None	2	2
250–500	10 to 100	10	4
500–1000	10 to 100	5	8
1000–2500	10 to 100	2	20
2500–5000	1 to 100	10	40
5000–10000	1 to 100	5	80
10000–25000	1 to 100	2	200
25000–50000	1 to 1000	10	400
50000–100000	1 to 1000	5	800
100000–250000	1 to 1000	2	2000

Based on zoalene concentration, make additional dilutions with 95% acetone and use aliquots indicated in Table 961.26.

Pipet indicated aliquots into three 50 mL beakers, X, Y, and Z, for concentrations <0.25% (<2500 mg/kg); omit X for products >0.25% (>2500 mg/kg). Pipet 1 mL standard solution into beaker Z and evaporate all solutions to dryness with air current. (Heat may be used but temperature must not exceed 60 C.) Pipet 10 mL 95% DMF into X and 2 mL each into Y and Z. Swirl intermittently during 5 min to dissolve zoalene. Pipet 8 mL ethylenediamine into Y and Z and mix. If turbidity persists after 2 min, filter through small Reeve Angel No. 804, or equivalent, paper. Read *A* of solutions at 560 nm in stoppered 1 cm cells against 95% DMF 5 min after addition of ethylenediamine. Keep cell compartment of spectrophotometer at <30°C to avoid rapid fading of color. If *A* is >1, reanalyze, using greater dilution or smaller aliquot.

$$\text{Zoalene, \%} = (A_Y - A_X) \quad M/100 (A_Z - A_Y)$$

$$\text{Zoalene, mg/kg} = (A_Y - A_X) \quad M \quad 100 (A_Z - A_Y)$$

(*Caution:* CH₃CN and ethylenediamine are toxic. Handle in hood and avoid contact with skin.)

References: *JAOAC* **44**, 18(1961); **45**, 294(1962); **51**, 501(1968).

CAS-148-01-6 (zoalene)