

Subchapter 8
MISCELLANEOUS PESTICIDES

7.8.01

AOAC Official Method 983.11
Brodifacoum (Technical)
and Pesticide Formulations

Liquid Chromatographic Method
First Action 1983
Final Action 1989

(Not applicable to wax bait formulations.)

A. Principle

Weighed sample of technical brodifacoum, brodifacoum concentrate, or bait extract is dissolved in triphenylbenzene internal standard solution and determined by reverse phase liquid chromatography and UV detection.

B. Apparatus

- (a) *Liquid chromatograph.* - Flow rate 1 mL/min; loop injection 10 μ L; mobile solvent methanol-H₂O-CH₃COOH (94.2 + 5.0 + 0.8), filtered and degassed; UV detection at 254 nm with range to give peak heights ca 60-80% full scale. Retention times (min) – brodifacoum 6.2, internal standard 11.7.
- (b) *Column.* – 250 x 4.6 mm Zorbax ODS 5 μ m reverse phase column (Agilent Technologies).
- (c) *Centrifuge.* – Equipped with 15 mL capped tubes.
- (d) *Macerator.* – With 400 mL stainless steel cup/h impeller assembly, such as Sorvall Omnimixer homogenizer.
- (e) *Rotary evaporator.* – Fitted with vacuum and cold water supplies.
- (f) *Filter.* – Spread 10 g Celite 545 on 9 cm No. 5 filter paper wetted with methanol in Büchner. Press filter and prewash with 30 mL methanol.

C. Reagents

- (a) *1,3,5-Triphenylbenzene internal standard solution.* – Accurately weigh ca 100 mg pure 1,3,5-triphenylbenzene into 500 mL volumetric flask, dissolve in 200 mL CH₂Cl₂, and dilute to volume with methanol.
- (b) *Brodifacoum standard solution.* – Accurately weigh ca 100 mg brodifacoum standard of known purity (Zeneca Ag Products, www.zenecaagproducts.com) into 100 mL volumetric flask. Dissolve in 40 mL CH₂Cl₂. Dilute to volume with methanol. Transfer 10.0 mL each of brodifacoum standard solution and internal standard solution to 50 mL volumetric flask and dilute to volume with diluting solution.
- (c) *Diluting solution.* – CH₂Cl₂-methanol (2+3).
- (d) *Extracting solution.* – CH₂Cl₂-formic acid (50+1).

Store reagents in tightly capped dark bottles to avoid evaporation and decomposition. Check internal standard solution for interfering components by injecting 10 µL into liquid chromatograph.

D. Determination

- (a) *Technical material.* – Accurately weigh ca 100 mg test portion into 100 mL volumetric flask. Dissolve in 40 mL CH₂Cl₂. Dilute to volume with methanol. Transfer 10.0 mL each of test solution and internal standard solution to 50 mL volumetric flask. Dilute to volume with diluting solution.
- (b) *Powder concentrate.* – Accurately weigh test portion containing ca 5 mg brodifacoum into 250 mL capped conical flask. Add 100 mL extracting solution and shake 1 min. Filter through Celite, using two 30 mL washes of extracting solution. Evaporate filtrate at 60°C under vacuum. Dissolve residue in 20.0 mL diluting solution and 5.0 mL internal standard solution.
- (c) *Liquid concentrate.* – Accurately weigh test portion containing ca 5 mg brodifacoum into 25 mL volumetric flask. Add 5.0 mL internal standard solution, and dilute to volume with diluting solution.
- (d) *Pelleted bait.* – Grind test portion containing ca 2 mg brodifacoum in analytical mill. Transfer to tared macerator cup and accurately weigh. Add 250 mL extracting solution and homogenize 10 min. Filter through Celite using two 50 mL washings of extracting solution. Rotary-evaporate filtrate at 60° under vacuum. Dissolve residue in 8.0 mL diluting solution and 2.0 mL internal standard solution. Centrifuge sample to remove remaining solids.

Inject 2 or more aliquots of standard solution into liquid chromatograph to set integration parameters and stabilize instruments. Monitor response factor until results agree within 2%. Inject 4 aliquots of standard solution and 2 aliquots of test solution in succession. Calculate response factor, R, for each:

$$R = \text{area brodifacoum peak} / \text{area internal standard peak}$$

Peak heights can be used in place of peak areas for technical material and bait formulations, but not for liquid formulations.

$$\text{Brodifacoum, \%} = (R/R') \times (W'/W) \times P \times F$$

where R and R' = average response factor for sample and standard solutions, respectively; W and W' = mg test portion and standard, respectively; P = purity (%) of standard; F = scaling factor = 1 for technical material, 1/20 for powder and liquid concentrates, 1/50 for pelleted bait.

Reference: JAOAC **66**, 993 (1983).

CAS – 56073 – 10 – 0 (brodifacoum)