

TECHNICAL BRODIFACOUM

Full specification: WHO/SRoT/1
Approved 10 December 1999

1. Specification

1.1 Description

The material shall consist of brodifacoum together with related manufacturing impurities and shall be in the form of a white to pale buff powder, free from odour and visible extraneous matter and added modifying agents.

1.2 Chemical and Physical Requirements

The material, sampled from any part of the consignment (see method WHO/M/1.R1), shall comply with the requirements of Section 1.1 and with the following requirements.

1.2.1 *Brodifacoum content (g/kg basis)*

The brodifacoum content shall be declared (not less than 880 g/kg) and when determined by the method described in section 2.1, the mean measured content obtained shall not be lower than the declared content.

1.2.2 *Brodifacoum cis-trans isomer ratio*

The cis-trans isomer ratio shall be declared and shall be in the range of 50: 50 to 80: 20 when determined by the method described in section 2.2.

1.2.3 *Loss on drying at 100°C*

The maximum loss on drying at 100°C when determined by the method described in section 2.3 shall be 5 g/kg.

1.3 Packing and marking of packages

The technical brodifacoum shall be packed in suitable clean containers, as specified in the order.

All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Technical brodifacoum

Actual cis/trans isomer ratio of the batch
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice.

Brodifacoum is an indirect anticoagulant and is hazardous if swallowed. Avoid skin contact; wear protective gloves, clean protective clothing, and suitable respiratory protection when handling the material. Open and handle only in a cabinet with adequate exhaust ventilation. Wash hands and exposed skin thoroughly after using and destroy contaminated clothing.

Keep containers out of the reach of children and well away from foodstuffs and animal feed and their containers.

Brodifacoum is toxic to aquatic wildlife. Avoid accidental contamination of water.

If poisoning occurs, call a physician. Treatment is symptomatic.

2. Methods of determining chemical and physical properties

2.1 Brodifacoum

2.1.1 Summary of method

A weighed sample of technical brodifacoum is dissolved in triphenylbenzene internal standard solution and determined by reverse phase HPLC with UV detection

2.1.2 Apparatus

1. *Liquid chromatograph*, equipped with 10 µL loop injector. UV detector capable of operating at 254 nm and a suitable electronic integrator or laboratory data system.
2. *Chromatographic column*, stainless steel, 250 x 4.6 mm (i.d.) with Zorbax ODS 5 µm, reverse phase column (DuPont Instruments Inc), or equivalent.

2.1.3 Reagents

1,3,5 Triphenylbenzene, internal standard, purity greater than 97%.

Acetic acid, glacial, HPLC grade

Dichloromethane, HPLC grade

Methanol, HPLC grade

Water, HPLC grade

Brodifacoum of known purity

Diluting solvent: dichloromethane-methanol (2+3) v/v

Eluting solvent: methanol-water-acetic acid (94.2 + 5.0 + 0.8) v/v

Mix 942 mL methanol, 50 mL water and 8 mL acetic acid, filter and degas.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh about 100 mg of 1,3,5 triphenylbenzene into a 500 mL volumetric flask, dissolve in dichloromethane (200 mL) and dilute to volume with methanol.

Mix thoroughly. Check for interfering components by injecting 10 μ L into the liquid chromatograph. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

Brodifacoum calibration solution. Weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (s g) into a 100 mL volumetric flask, dissolve in dichloromethane (40 mL) and make up to volume with methanol. Mix thoroughly. Transfer by pipette 10.0 mL into a 50 mL volumetric flask, add by pipette internal standard solution (10.0 mL) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

2.1.5 *Operating conditions*

The conditions given below are typical values and may have to be adjusted to obtain optimum results from the apparatus used.

Eluting solvent flow rate	1 mL min ⁻¹
Temperature	ambient
Injection volume	10 μ L
Wavelength	254 nm
Retention times	Brodifacoum: 6.2 min, internal standard: 11.7 min

2.1.6 *Sample preparation*

Weigh (to the nearest 0.1 mg) into a 100 mL volumetric flask enough sample (w g) to contain about 100 mg brodifacoum. Dissolve in dichloromethane (40 mL) and dilute to volume with methanol. Mix thoroughly. Transfer 10.0 mL into a 50 mL volumetric flask, add by pipette internal standard solution (10.0 mL) and make up to volume with the diluting solvent. Mix thoroughly.

2.1.7 *Equilibration of the system*

Inject two or more 10 μ L aliquots of the calibration solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument. Continue with injections until the peak area ratios of the brodifacoum peaks to the internal standard peaks for successive injections agree within 2%.

2.1.8 *Analysis of sample*

Carry out duplicate injections of calibration and sample solutions (in the order calibration, sample, sample, and calibration). Average the peak area ratios of the calibration injection (R') and of the sample injections. (R).

$$(R) = \frac{\text{area brodifacoum peak}}{\text{area internal standard peak}}$$

2.1.9 Calculation

$$\text{Brodifacoum content} = \frac{R \times \underline{s} \times P}{R' \times \underline{w}} \quad \text{g/kg}$$

- R = average peak area ratio of brodifacoum and internal standard for the sample
 R' = average peak area ratio of brodifacoum and internal standard for the calibration solution
 \underline{w} = mass of sample (g)
 \underline{s} = mass of brodifacoum in the calibration solution (g)
 P = purity of brodifacoum (g/kg).

2.2 Determination of cis/trans isomer ratio

2.2.1 Summary of method

A weighed sample of technical brodifacoum is dissolved in dichloromethane and the isomer ratio determined by HPLC with UV detection

2.2.2 Apparatus

Liquid chromatograph, equipped with 10 μL loop injection, UV detector capable of operating at 254 nm and a suitable recorder, or electronic integrator, or laboratory data system.

Chromatographic column, stainless steel, 250 x 4.6 mm (i.d.) with Spherisorb S5W or Lichrosorb SI60.

2.2.3 Reagents

Acetic acid, glacial, HPLC grade

Dichloromethane, HPLC grade

Hexane, HPLC grade

Brodifacoum of known purity

Eluting solvent: Hexane-dichloromethane-acetic acid (74.8 + 25 + 0.2) v/v

Mix 748 mL hexane, 250 mL dichloromethane and 2 mL acetic acid, filter and degas.

2.2.4 *Operating conditions*

The conditions given below are typical values and may have to be adjusted to obtain optimum results from the apparatus used.

Eluting solvent flow rate	2 mL min ⁻¹
Temperature	ambient
Injection volume	10 µL
Wavelength	254 nm
Retention times	Trans isomer 14 minutes Cis isomer 15.7 minutes

2.2.5 *Sample preparation*

Weigh (to the nearest 0.1 mg) into a 50 mL volumetric flask, enough sample to contain 20 mg brodifacoum. Dissolve in dichloromethane (20 mL) and dilute to volume with dichloromethane. Mix thoroughly.

2.2.6 *Equilibration of the system*

Inject two or more 10 µL aliquots of sample solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument.

2.2.7 *Analysis of sample*

Inject two 10 µL aliquots of sample solution in succession and record the integrated areas for each peak. Average the integrated areas for each of the peaks.

2.2.8 *Calculation*

$$\text{Cis isomer content of sample} = \frac{C}{C + T} \times 100\%$$

$$\text{Trans isomer content of sample} = \frac{T}{C + T} \times 100\%$$

C = mean area of cis isomer peaks

T = mean area of trans isomer peaks

2.3 **Loss on drying at 100°C**

Outline of Method

The material is heated at 100°C for 4h, and the loss of water and volatile materials calculated.

Apparatus

Squat type weighing bottle - 50 mm diameter, 30 mm high

Oven at $100 \pm 2^\circ\text{C}$

Desiccator

Procedure

Heat the weighing bottle at $100 \pm 2^\circ\text{C}$ for 1h, cool in a desiccator and weigh. Place about 2.5 g of the sample in a thin layer in the tared weighing bottle (x g) and weigh (y g). Heat (without lid) to $100 \pm 2^\circ\text{C}$ for 4h, replace lid, cool in the desiccator, and reweigh (z g).

$$\text{Loss on drying} = \frac{100 (y-z)}{(y-x)} \% \text{ w/w}$$

BRODIFACOUM **5 and 25 g/kg CONCENTRATES**

Full specification: WHO/SRoF/1
Approved 10 December 1999

1. Specification

1.1 Description

The material shall consist of a solution of technical brodifacoum, complying with the WHO specification WHO/SRoT/1, as its triethanolamine salt, together with any necessary formulants and a red or blue dye. It shall be free from visible suspended matter and sediment.

1.2 Chemical and physical requirements

The material sampled from any part of the consignment (see method WHO/M/1;R1), shall comply with the requirements of sections 1.1 and with the following requirements.

1.2.1 Brodifacoum content (g/kg basis)

The content of brodifacoum determined by the method described in section 2.1 shall not differ from the declared content by more than the following amounts:

<i>Declared content</i>	<i>Tolerance permitted</i>
Up to 25g/kg	± 15% of the declared content

The average content of all samples taken shall not be lower than the declared content.

1.3 Packing and marking of packages

The brodifacoum concentrate shall be packed in suitable clean containers, as specified in the order. All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Brodifacoum concentrate
Brodifacoummg/kg
Batch or reference number, and date of test
Net weight of contents
Date of manufacture
Instruction for use

and the following minimum cautionary notice:

Brodifacoum is an indirect anticoagulant and is hazardous if swallowed. Avoid skin contact; wear protective gloves and clean protective clothing when handling the material. Wash hands and exposed skin thoroughly after using.

Keep containers out of the reach of children and well away from foodstuffs and animal feed and their containers.

Brodifacoum is toxic to aquatic wildlife. Avoid accidental contamination of water.

If poisoning occurs, call a physician. Treatment is symptomatic.

2. Methods of determining chemical and physical properties

2.1 Brodifacoum content

2.1.1 Summary of method

A weighed sample of brodifacoum concentrate is dissolved in triphenylbenzene internal standard solution and determined by reverse phase HPLC with UV detection.

2.1.2 Apparatus

1. *Liquid chromatograph*, equipped with 10 μ L loop injector. UV detector capable of operating at 254 nm and a suitable electronic integrator, or laboratory data system.
2. *Chromatographic column*, stainless steel, 250x4.6 mm (i.d.) with Zorbax ODS 5 μ m, reverse phase column (DuPont Instruments Inc), or equivalent.

2.1.3 Reagents

1,3,5-Triphenylbenzene internal standard purity greater than 97%.

Acetic acid, glacial, HPLC grade

Dichloromethane, HPLC grade

Methanol, HPLC grade

Water, HPLC grade

Brodifacoum of known purity

Diluting solvent: dichloromethane-methanol (2+3) v/v

Eluting solvent: methanol-water-acetic acid (94.2 + 5.0 + 0.8) v/v

Mix 942 methanol, 50 mL water and 8 mL acetic acid, filter and degas

2.1.4 Preparation of standard solutions

Internal standard solution. Weigh about 100 mg 1,3,5-triphenylbenzene into a 500 mL volumetric flask, dissolve in dichloromethane (200 mL) and dilute to volume with methanol. Mix thoroughly. Check for interfering components by injecting 10 μ L into the liquid chromatograph. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

Brodifacoum calibration solution. Weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (\underline{s} g) into a 100 mL volumetric flask, dissolve in dichloromethane (40 mL) and make up to volume with methanol. Mix thoroughly. Transfer by pipette 10.0 mL into a 50 mL volumetric flask, add by pipette internal standard solution (10.0 mL) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

2.1.5 *Operating conditions*

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Eluting solvent flow rate	1 mL min ⁻¹
Temperature	ambient
Injection volume	10 μ L
Wavelength	254nm
Retention times	Brodifacoum: 6.2 min, internal standard: 11.7 min

2.1.6 *Sample preparation*

Weigh (to the nearest 0.1 mg) into a 100 mL volumetric flask enough sample (\underline{w} g) to contain about 100 mg of brodifacoum. Dissolve in dichloromethane (40 mL) and dilute to volume with methane. Mix thoroughly. Transfer by pipette 10.0 mL into a 50 mL volumetric flask, add by pipette internal standard solution (10.0 mL) and make up to the mark with diluting solvent. Mix thoroughly and filter through a 0.45 μ m filter.

2.1.7 *Equilibration of the system*

Inject two or more 10 μ L aliquots of the calibration solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument. Continue with injections until the peak area ratios of the brodifacoum peaks to the internal standard peaks for successive injections agree within 2%.

2.1.8 *Analysis of sample*

Carry out duplicate injections of calibration and sample solutions (in the order calibration, sample, sample and calibration). Average the peak area ratios of the calibration injections (R') and of the two sample injections (R).

$$(R) = \frac{\text{area brodifacoum peak}}{\text{area internal standard peak}}$$

2.1.9 *Calculation*

$$\text{Brodifacoum content} = \frac{R \times \underline{s} \times P}{R' \times \underline{w}} \text{ g/kg}$$

Where:

- R = average peak area ratio of brodifacoum and internal standard for the sample.
R' = average peak area ratio of brodifacoum and internal standard for the calibration solution
w = mass of sample (g)
s = mass of brodifacoum in the calibration solution (g)
P = purity of brodifacoum (g/kg)