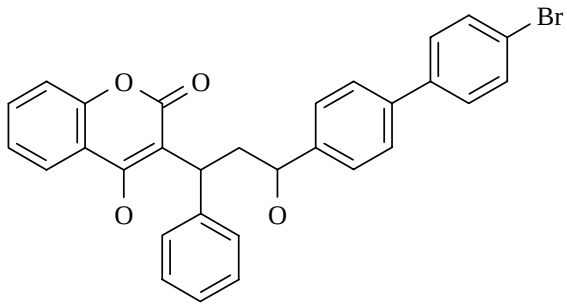
BROMADIOLONE
371



ISO common name	Bromadiolone
Chemical name	3-[3-(4'-Bromobiphenyl-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-coumarin (IUPAC); 3-{3-[4'-bromo(1,1'-biphenyl)-4-yl]-3-hydroxy-1-phenylpropyl}-4-hydroxy-2*H*-1-benzopyran-2-one (CA; *28772-56-7*)
Empirical formula	$C_{30}H_{23}BrO_4$
RMM	527.4
m.p.	200 -210 °C
v.p.	2×10^{-6} Pa at 25 °C
Solubility	In water: 16 mg/l at 20 °C; dimethylformamide: 730 g/l, ethanol: 8.2 g/l, ethylacetate 25 g/l, acetone: 22 g/l
Description	Yellow-white, odourless powder
Stability	Stable at normal storage conditions
Formulations	Bait and concentrate

Note: bromadiolone consists of a mixture of two diastereoisomeric pairs.

BROMADIOLONE TECHNICAL
*371/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity test.
HPLC Use the HPLC method below. The retention times of the bromadiolone diastereoisomers from the sample solution should not differ from those from the calibration solution by more than 2%.

3 Bromadiolone
OUTLINE OF METHOD Bromadiolone is determined by reversed phase high performance liquid chromatography using internal standardization.

REAGENTS
Methanol HPLC grade
Phosphoric acid solution c (1/3 H_3PO_4) = 0.0025 mol/l
Mobile phase. Mix methanol (750 ml) and phosphoric acid solution (250 ml). Filter through a 0.45 µm filter and degas by placing the mixture in an ultrasonic bath.
Bromadiolone standard of know purity
Bromadiolone solution. Weigh (to the nearest 0.1 mg) with a weighing device 50 mg pure bromadiolone (*s* mg) into a volumetric flask (100 ml). Dissolve in methanol by sonification during 2 min. Let cool to room temperature and fill to the mark with methanol. Mix well and store in the dark to avoid decomposition.
Tioclomarol [3-(4-chlorophenyl)-3-hydroxy-1-(5-chloro-2-thienyl)]-4-hydroxy-coumarin, internal standard.
Internal standard solution. Weigh (to the nearest 0.1 mg) with a weighing device 50 mg tioclomarol (*r* mg) into a volumetric flask (100 ml). Dissolve in methanol by sonification during 2 min. Let cool to room temperature and fill to the mark with methanol. Mix well and store in the dark to avoid decomposition.
Calibration solution. Transfer by a pipette 10 ml of the bromadiolone and 10 ml of the internal standard solution to a volumetric flask (100 ml). Dilute to the mark with mobile phase and mix well. Store in the dark to avoid decomposition. In natural light a loss of 5% per hour may occur. In the dark the solution is stable for 24 h.

* CIPAC method 1991. Prepared by PAC-France; Chairman: B Declercq. Based on a method supplied by Lipha SA, France.

APPARATUS

Liquid chromatograph equipped with a constant flow pump, a UV spectrophotometer, a loop injector and an electronic integrator
Column stainless steel, 200 × 4.6 (i.d.) mm, packed with Nucleosil C18, 5 µm
Filtration device equipped with a PFTE membrane, 0.45 µm (Millipore xx 1004700 or equivalent)
Ultrasonic bath
Magnetic stirrer

PROCEDURE

(a) Operating conditions (typical):

Flow rate of mobile phase	1ml/min
Column temperature	20 to 25 °C
Injection volume	20 µl
Detector wavelength setting	254 nm
Run time	18 min
Retention times	bromadiolone main diastereoisomer: 12 min bromadiolone minor diastereoisomer: 11 min tioclomarol: 7 min

(b) Preparation of sample solution. Weigh (to the nearest 0.1 mg) with a weighing device enough sample to contain about 50 mg (*w* mg) pure bromadiolone into a volumetric flask (100 ml). Dissolve in methanol by sonification for 2 min. Let cool to room temperature and fill the mark with methanol. Mix well. Transfer by pipette 10 ml of this solution and 10 ml of internal standard solution to a volumetric flask (100 ml). Dilute to the mark with mobile phase and mix well. Store in the dark to avoid decomposition.
(c) Determination. Inject 20 µl portions of the calibration solution until the response ratio of the bromadiolone peak relative to the internal standard peak agrees within 1% for successive injections. Then inject in duplicate portions of the sample solution followed by another calibration solution injection. Measure the areas of the relevant peaks. Calculate the response factors *(f)* from the calibration solution injection preceding and following the injections of the sample solution. Average the values obtained.
(*d) Calculation*

$$f = \frac{I_r \times s \times P}{H_s}$$

$$\text{Content of bromadiolone} = \frac{H_w \times r \times f}{I_q \times w} \text{ g/kg}$$

where:

H_s = sum of the areas of the bromadiolone peaks in the calibration solution
I_r = area of the internal standard peak in the calibration solution
H_w = sum of the areas of the bromadiolone peaks in the sample solution
I_q = area of the internal standard peak in the sample solution
s = mass of bromadiolone in the calibration solution
r = mass of internal standard in the calibration and sample solutions (mg)
w = mass of sample taken (mg)
f = response factor
P = purity of the bromadiolone standard (g/kg)

Repeatability r = 27 g/kg at 990 g/kg active ingredient content

Reproducibility R = 56 g/kg at 990 g/kg active ingredient content

BROMADIOLONE SOLUBLE CONCENTRATES
*__371__/SL/M/-

1 Sampling. Take at least 500 ml.

2 Identity test. As for bromadiolone technical 371/TC/M/2.

3 Bromadiolone. As for bromadiolone technical **371**/TC/M/3 except:
(b) Preparation of sample solution. Weigh (to the nearest 0.1 mg) with a weighing device enough sample to contain about 5 mg (w mg) pure bromadiolone into a volumetric flask (100 ml). Add by pipette methanol (10 ml) and internal standard solution (10 ml). Dilute to the mark with mobile phase and mix well. Store in the dark to avoid decomposition.
and:
(d) Calculation

$$\text{Content of bromadiolone} = \frac{H_w \times r \times f}{I_q \times w \times 10} \text{ g/kg}$$

Repeatability r = 0.49 g/kg at 10.5 g/kg active ingredient content and
0.11 g/kg at 2.5 g/kg active ingredient content
Reproducibility R = 1.0 g/kg at 10.5 g/kg active ingredient content and
0.23 g/kg at 2.5 g/kg active ingredient content

* CIPAC method 1991. Prepared by PAC-France; Chairman: B Declercq. Based on a method supplied by Lipha SA, France.

BROMADIOLONE SOLID CONCENTRATES
*371/DP/M/-

1 Sampling. Take at least 1 kg.

2 Identity test. As for bromadiolone technical **371**/TC/M/2.

3 Bromadiolone. As for bromadiolone technical **371**/TC/M/3, except:
(b) Preparation of sample solution. Weigh (to the nearest 0.1 mg) with a weighing device enough sample to contain about 3.5 to 4.5 mg (*w* mg) pure bromadiolone into a conical flask (100 ml). Add methanol (75 ml) and stir on a magnetic stirrer for 30 min. Filter with a filtration device into a volumetric flask (100 ml). Rinse the conical flask and the filtration device with methanol (10 ml). Add by pipette (10 ml) internal standard solution, dilute to the mark with mobile phase, and mix well. Store in the dark to avoid decomposition and:
(d) Calculation

$$\text{Content of bromadiolone} = \frac{H_w \times r \times f}{I_q \times w \times 10} \text{ g/kg}$$

Repeatability r = 0.74 g/kg at 9.91 g/kg active ingredient content

Reproducibility R = 1.35 g/kg at 9.91 g/kg active ingredient content

* CIPAC method 1991. Prepared by PAC-France; Chairman: B Declercq. Based on a method supplied by Lipha SA, France.