

**PRODUCT INFORMATION**  
**METHODS OF ANALYSIS**

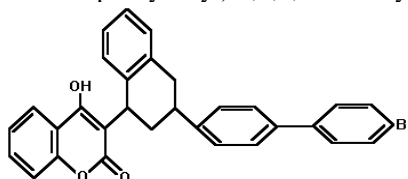
**PRODUCT:** BRODIFACOUM TECH (ISO, BSI)

REVISE DATE: 30,NOV. 2007

VERSION: 04

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**CHEMICAL NAME** : 3-(3-(4'-bromobiphenyl-4-yl)-1,2,3,4- tetrahydro-1-naphthyl)-4-hydroxycoumarin



**STRUCTURAL FORMULA** :

**REGISTRATION NO.** : PD20070323 (98% Tech) ; LS981283 (0.5% Liquid/Powder) ; LS981284 (0.005% RB)

**CAS NUMBER** : [56073-10-0]

**CHEMICAL FORMULA** :  $C_{31}H_{23}BrO_3$

**MOLECULAR WEIGHT** : 523.4

**ASSAY BRODIFACOUM TECH**

**1. OUTLINE:**

This sample is dissolved by methanol, dichloromethane. With methanol, water as mobile phase, the Brodifacoum in sample is separated and determined by HPLC in stainless steel column with Eclipse XDB-C8 and 5um as filling material on wavelength Ultraviolet detector

**2. REAGENTS AND SOLUTIONS**

Methanol: reagent (GR)

Phosphoric acid;

Dichloromethane: reagent (GR)

Water: the redistilled water

Mix-solvent: methanol + dichloromethane: 3+2 (V/V)

Brodifacoum standard: known purity  $\geq 99.0\%$

**3. APPARATUS:**

HPLC: Ultraviolet detector with adjustable wavelength;

Chromatography data treater;

Chromatograph Column: 150cm x 4.6 mm(i.d) mm stainless steel column,

Filling: Eclipse XDB- C8 and 5um

Filter: Filter film hole diameter 0.45 um;

Micro-Syringe:100  $\mu$ l;

Quantitative-sampler: 10  $\mu$ l;

Ultrasonic Cleaners.

**4. THE OPERATION CONDITION OF CHROMATOGRAPHY**

Flow(mobile) phase: methanol + water (dilute to PH=3 with Phosphoric acid )=80:20 (v/v)

Column temperature: room temperature (changes no more than 2 degree)

Wavelength: 254 nm;

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Flow rate: 1.0ml/min;

Admission valve:10 ul;

Retention time: Brodifacoum A almost 17 min, Brodifacoum B almost 18.4 min,

The above chromatograph condition is for the typical operation. To get optimum results, the parameters could be adjusted according to different apparatus.

The typical operation HPLC, the chart 1 & chart 2.

## 5. DETERMINATION PROCEDURE

Preparation of calibration solution:

Weigh Brodifacoum standard 0.05g (accurate to 0.002g) into 100ml volume flask. Dissolve with methanol and dichloromethane, make up to the mark and mix thoroughly.

Preparation of sample solution:

Weigh the sample 0.05g (accurate to 0.002g) into 100ml volume flask. Dissolve with methanol and dichloromethane, make up to the mark and mix thoroughly.

Determination

Under the above operation condition, after stabilized the zero line of apparatus, inject standard solution a couple of time until the variation of the response ratio of the two injection is less than 1.5%, determine by the injection order below: standard solution, sample solution, sample solution, standard solution

Calculation

Sample of Brodifacoum mass percentage X1(%) calculate as formula (1) :

$$X1 = \frac{A2 \times m1 \times P}{A1 \times m2} \quad (1)$$

Where :

A1—average ratio of peak area of Brodifacoum A & B in the standard solution

A2---average ratio of peak area of Brodifacoum A & B in the sample solution

m1—mass of Brodifacoum standard, g

m2—mass of Brodifacoum sample, g

P—mass percent of Brodifacoum in standard, %

Sample of Bromadiolone  $\alpha$  (A/B) calculate as formula (2) :

$$\alpha (A/B) = \frac{AA}{AB} \quad (2)$$

Where :

AA : average ratio of A peak area of Brodifacoum in 2 injection Brodifacoum sample solution

AB : average ratio of B peak area of Brodifacoum in 2 injection Brodifacoum sample solution

Allowable deviation

The deviation of result of parallel determination two times: Brodifacoum is no more than 1.2%