RTC INTERNATIONAL INC

SIYANG RODENTICIDE FACTORY

RODEN CHEMICAL INTERNATIONAL 洛登克化工有限公司

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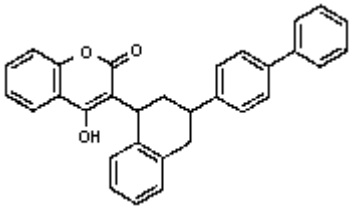
PRODUCT INFORMATION
METHODS OF ANALYSIS

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

REVISE DATE: 10,MAY 2007 VERSION: 03

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

STRUCTURAL FORMULA :



REGISTRATION NO. :

CAS NUMBER : [56073-07-5]

CHEMICAL FORMULA : C19H16O3

MOLECULAR WEIGHT : 292.35

ASSAY DIFENACOUM TECH

1. PRINCIPLE:
Difenacoum has an absorption maximum in ultraviolet region which wavelength is 256nm, the relationship between it's absorption intensity and thickness accord with Beer-Lamberlaw.

2. REGENT
Methyl alcohol (GB 683): Chromatoraphically pure
Glacial acetic acid: analytically pure
Water (redistillation)
Glacial acetic acid aqueous solution (v/v): 10ml Glacial acetic acid dissolve in 90ml redistillation water.
Acetone: analytically pure
Standard Difenacoum product: Content of effective constituent be 99%

3. INSTRUMENTS:
High voltage liquid chromatograph: Beckman 344m highciency liquid chromatograph, 165 variable wavelength ultrabiolet detector.
427 integraph
Chromatographic column: length 150mm, inside diameter
Inner filling agent: AUEX ODS C185 u
Sample injection amulus: 20 ul
Measuring flask: 50ml
Volume pipet: 10ml, 5ml, 2ml

4. CHROMATOGRAM OPERATING CONDITIONS:
Column temperature: room temperature
Detector: wavelength 25 mm
Mobile phase: methyl alcohol + Glacial acetic acid aqueous solution = 90 + 10 (V/V)

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Velocity of flow: 1 ml/min
Recorder paper speed: 0.1 mm/min
Sample size: 20 ul

5. MEASURING STEP:

Get exact 1g (exact abour 0.0002 g) 5% Difenacoum matemal power using decrement method, put them in a 50ml measming flask, add in 40ml methyl alcohol and dip in for 30min then shake up emphatically for 10min, filtrate.

a) Preparation of Difenacoum standard solution:

Get exactly 0.10g (exact about 0.0002g) standard Difenacoum, put them in a 50ml measuring flask, dissolve by 40ml acetonte and dilute to the scale, shake up ,get 10ml such solution by volume pipet, put them in a 50ml measuring flask and dilute by methyl alcohol to the scale.

b) Preparation of sample solution:

Get exactly 0.10g (exact about 0.0002%) Difenacoum sample, put them in a 50ml measming flask, dissolve by 40ml acetone and dilute to the scale, shake up, get 10ml such solution by volume pipet, put them in a 50ml measming flask and dilute by methyl alcohol to the scale.

c) Measuring:

Under above mentioned Chromatogram oprating conditions, analyze according to following procedures only after there is stable working stams of the instruments:
a, Difenacoum standard solution
b, Difenacoum standard solution
c, Difenacoum standard solution
d, Difenacoum standard solution

5.4. Calculation
Difenacoum content % (xl) calculare as per (1) formula under below

$$X1 = \frac{As \times MR \times P}{AR \times MS} \quad (1)$$

Thereunto:
AR--- a d = average peak area value of injected Difenacoum standard solution.
As--- b c = average peak area value of injected Difenacoum sample solution
MR--- quality of standard Difenacoum product (g)
Ms--- quality of Difenacoum sample (g)
P--- content of standard Difenacoum product (%)

Measuring result: average value of the difference between two calculation under this
Method should be no more than 1%