

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

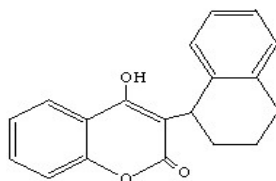
**PRODUCT:** COUMATETRALYL TECH (ISO, BSD)

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VERSION: 03

PAGE: 1 OF 3

**CHEMICAL NAME** : 4-Hydroxy-3-(1,2,3,4-tetrahydro-1- naphthalenyl)-2H-1-benzopyran-2-one



**STRUCTURAL FORMULA** :

**REGISTRATION NO.** :

**CAS NUMBER** : [5836-29-3]

**CHEMICAL FORMULA** : C<sub>19</sub>H<sub>16</sub>O<sub>3</sub>

**MOLECULAR WEIGHT** : 292.35

**ASSAY COUMATETRALYL TECH**

**1. OUTLINE:**

The coumatetralyl in sample is separated and determined by HPLC in stainless steel column with Lichrospher C18 as filling material on wavelength Ultraviolet detector

**2. REGENTS AND SOLUTIONS**

Methanol: reagent (GR)

Glacial acetic acid: reagent (GR)

Water: the redistilled water

Coumatetralyl standard: known purity ≥99.0%

**3. APPARATUS:**

HPLC: Ultraviolet detector with adjustable wavelength ;

Chromatography data treater ;

Chromatograph Column: 150mm x 4.6 mm Lichrospher C18;

Filter: Filter film hole diameter 0.45 um;

Micro-Syringe: 100 µl;

Quantitative-sampler: 5 µl;

Ultrasonic Cleaners.

**4. THE OPERATION CONDITION OF CHROMATOGRAPHY**

Flow phase: methanol + water + glacial acetic acid: 90 + 10 + 5 (v/v/v)

Column temperature: room temperature (changes less than 2 Degree)

Wavelength: 265 nm

Flow rate: 1.0ml/min

Admission valve: 10 ul

Retention time: 2.773 min (Chart 1 & 2)

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

## 5. DETERMINATION PROCEDURE

### Preparation of calibration solution:

Weigh Coumatetralyl standard 0.01g (accurate to 0.0002g) into 100ml volume flask. Dilute with Flow Phase, make up to the mark and mix thoroughly.

### Preparation of sample solution:

Weigh the sample 0.01g (accurate to 0.0002g) into 100ml volume flask. Dilute with Flow Phase, 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 1.0%, determine by the injection order below: standard solution, sample solution, sample solution, standard solution

### Calculation

Sample of Coumatetralyl 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 Coumatetralyl in the standard solution

A2---average ratio of peak area of Coumatetralyl in the sample solution

m1—mass of Coumatetralyl standard, g

m2—mass of Coumatetralyl sample, g

P—mass percent of Coumatetralyl in standard, %

### Allowable deviation

The deviation of result of parallel determination two times: Coumatetralyl is less than 1.2%