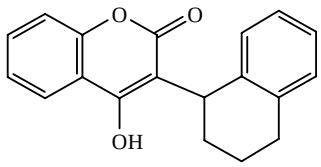
COUMATETRALYL

189



ISO common name	Coumatetralyl
Chemical name	4-Hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)-coumarin (IUPAC); 4-hydroxy-3-(1,2,3,4-tetra-hydro-1-naphthalenyl)-2*H*-1-benzopyran-2-one (CA; *5836-29-3*)
Empirical formula	$C_{19}H_{16}O_3$
RMM	292.6
m.p.	174.5 °C
v.p.	1×10^{-6} Pa at 20 °C
Solubility	In water: 4 mg/l; *n*-hexane: 0.2 g/l; dichloromethane: 52 g/l; 2-propanol: 25 g/l; toluene: 4.1 g/l; all at 20 °C
Description	Colourless crystals
Stability	Half life, considerably more than 1 year at pH 4 to 9 and 22 °C
Formulations	Tracking powders, baits (ready for use), grain baits, bait concentrates

COUMATETRALYL TECHNICAL
*__189__/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 Infrared. Prepare potassium bromide discs from the sample and from coumatetralyl standard using 0.7 mg of material and 200 mg of potassium bromide. Scan the discs from 4000 to 400 cm^{-1} (2.5 to 25 µm). The spectrum produced from the sample disc should not differ significantly from that from the standard.

2.2 HPLC. Use the HPLC method below. The retention time of coumatetralyl for the sample solution should not deviate by more than 2 % from that of the calibration solution.

2.3 TLC. Carry out a thin-layer chromatographic identity test by comparing the sample with the standard using the following conditions:

TLC plate	Coated with silica gel 60 F_{254}, 0.25 mm (e.g. Merck Darmstadt, FRG, Art. No 5715)
Eluting solvent	Toluene-methanol-acetic acid, 95 + 5 + 5 (v/v)
Sample solution	Dissolve an amount of sample to contain about 50 mg of coumatetralyl in methanol (about 5 ml) in a volumetric flask (10 ml) and make up to volume with methanol.
Reference solution I	Dissolve coumatetralyl standard (50 mg) in methanol (about 5 ml) in a volumetric flask (10 ml) and make up to volume with methanol.
Reference solution II	Mix 5 ml of the sample solution with 5 ml of reference solution I.
Loading	10 µl
Travelling distance	10 cm
Visualization	UV at 254 nm
R_F *value*	coumatetralyl: approximately 0.5

* CIPAC method 1994. Prepared by the German Committee (DAPA). Chairman: Dr W Dobrat. Based on a method supplied by Bayer AG, Germany.

Apply the reference solutions I and II, and the sample solution. The major spot from the sample solution should have the same R_F value as that from the reference solutions.

3 Coumatetralyl

OUTLINE OF METHOD Coumatetralyl is dissolved in, or extracted with acetonitrile, and determined by reversed phase HPLC on a C18 column with 0.5% aqueous acetic acid- acetonitrile 50+50 as eluent, UV detection at 310 nm and external standardisation.

REAGENTS

Coumatetralyl standard of known purity
Acetonitrile HPLC grade
Methanol HPLC grade
Tetrahydrofuran HPLC grade
Water HPLC grade
Acetic acid glacial
Acetic acid aqueous solution 0.5% (v/v)
Solvent mixture I methanol - water, 50 + 50 (v/v)
Solvent mixture II methanol - tetrahydrofuran, 50 + 50 (v/v)
Eluent 0.5% acetic acid - acetonitrile, 50 + 50 (v/v)
Calibration solution. Prepare two calibration solutions of which the coumatetralyl contents differ about 10 %. Weigh (to the nearest 0.1 mg) into two volumetric flasks (100 ml) about 80 mg (*s* mg) of coumatetralyl standard. Dissolve in methanol (about 50 ml) using an ultrasonic bath. Allow to cool to room temperature and make up to volume with acetonitrile (Stock solutions A and B). Transfer by pipette 5.0 ml of the stock solution I to volumetric flasks (100 ml) and make up to volume with solvent mixture I (Calibration solutions A and B respectively).

APPARATUS

High performance liquid chromatograph equipped with a loop injection valve (Rheodyne 7010 or equivalent), a column oven set at 50 °C and a UV spectrophotometric detector set at 310 nm
Column stainless steel, 250 × 4 (i.d.) mm, packed with LiChrosorb RP-18 (5 µm)

Disposable filter (Millex HV- 0.45 µm, Millipore SLHV 025 NB or equivalent)
Centrifuge
Ultrasonic bath

PROCEDURE

(a) Operating conditions (typical):

Eluent flow rate	2 ml/min
Temperature	50 °C
Injection volume	20 µl
Detector wavelength	310 nm
Retention time	coumatetralyl: about 5 min
Run time	8 min

(b) Preparation of sample. Weigh (to the nearest 0.1 mg) enough sample to contain about 80 mg (*w* mg) of coumatetralyl into a volumetric flask (100 ml). Dissolve the coumatetralyl in or, extract the sample with, acetonitrile using an ultrasonic bath for about 5 min. Allow to cool to room temperature and make up to volume with acetonitrile. Pipette 5.0 ml of this solution into a volumetric flask (100 ml). Make up to volume with solvent mixture I and mix well. If necessary, filter or centrifuge an aliquot of the suspension to get a clear solution.

(c) Determination. Inject 20 µl portions of calibration solutions A and B and measure the coumatetralyl peak area. Inject each calibration solution twice and calculate the average calibration factor. The individual values must not deviate from the mean by more than 0.8 %, otherwise repeat the calibration. Then inject in duplicate 20 µl portions of each sample solution. After a series of more than 4 sample injections repeat the injection of the calibration solution at the end of the series. Measure the relevant peak areas. Use the average calibration factors of the calibration solutions preceding and following the series of the sample solution injections to calculate the coumatetralyl content.

(c) Calculation

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

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

where:

s = mass of coumatetralyl in the calibration solution (mg)
P = purity of coumatetralyl standard (g/kg)
H_s = peak area of coumatetralyl for the calibration solution
H_w = peak area of coumatetralyl for the sample solution
f = calibration factor
w = mass of sample taken (mg)

Repeatability r = 12.1 g/kg at 997 g/kg active ingredient content
Reproducibility R = 12.3 g/kg at 997 g/kg active ingredient content

COUMATETRALYL TECHNICAL CONCENTRATES
*__189__/TK/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC. As for coumatetralyl technical **189**/TC/M/2.2.

2.2 TLC. As for coumatetralyl technical **189**/TC/M/2.3.

3 Coumatetralyl. As for coumatetralyl technical **189**/TC/M/3.

Repeatability r = 1.9 g/kg at 79 g/kg active ingredient content
Reproducibility R = 2.8 g/kg at 79 g/kg active ingredient content

* CIPAC method 1994. Prepared by the German Committee (DAPA). Chairman: Dr W Dobrat. Based on a method supplied by Bayer AG, Germany

COUMATETRALYL TRACKING POWDERS
*189/TP/M/-

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for coumatetralyl technical **189**/TC/M/2.2.
2.2 TLC. As for coumatetralyl technical **189**/TC/M/2.3.

3 Coumatetralyl
OUTLINE OF METHOD Coumatetralyl is extracted with a water-methanol mixture and determined by reversed phase HPLC.

REAGENTS, APPARATUS and PROCEDURE As for coumatetralyl technical **189**/TC/M/3, except:
(b) Preparation of sample. Weigh (to the nearest 0.5 mg) enough of the well homogenised sample to contain about 4 mg (*w* mg) of coumatetralyl into a ground-glass-stoppered Erlenmeyer flask (300 ml). Pipette 100.0 ml of solvent mixture I into the flask. Extract the coumatetralyl using an ultrasonic bath for about 15 min. Allow to cool to room temperature and filter or centrifuge an aliquot of the supernatant solution. Use the clear solution for injection without further dilution.

(d) Calculation

$$\text{Content of coumatetralyl} = \frac{H_w \times f}{w \times 20} \text{ g/kg}$$

where:
20 = dilution factor

Repeatability r = 0.14 g/kg at 8 g/kg active ingredient content
Reproducibility R = 0.37 g/kg at 8 g/kg active ingredient content

* CIPAC method 1994. Prepared by the German Committee (DAPA). Chairman: W Dobrat. Based on a method supplied by Bayer AG, Germany

COUMATETRALYL GRAIN BAITS
[*] **189**/AB/M/-

1 Sampling. Take at least 1 kg.

2 Identity tests
2.1 HPLC. As for coumatetralyl technical **189**/TC/M/2.2.
2.2 TLC. As for coumatetralyl technical **189**/TC/M/2.3.

3 Coumatetralyl
OUTLINE OF METHOD Coumatetralyl is extracted with methanol and determined by reversed phase HPLC.

REAGENTS, APPARATUS and PROCEDURE As for coumatetralyl tracking powder **189**/TP/M/3, except:
(b) Preparation of sample. Weigh (to the nearest 0.5 mg) enough of the well homogenised sample to contain about 4 mg (*w* mg) of coumatetralyl into a ground-glass-stoppered Erlenmeyer flask (300 ml). Pipette methanol into the flask (100 ml). Extract the coumatetralyl using an ultrasonic bath for about 30 min. Allow to cool to room temperature and filter or centrifuge an aliquot of the supernatant solution. Use the clear solution for injection without further dilution.

Repeatability r = 0.06 g/kg at 0.3 g/kg active ingredient content
Reproducibility R = 0.08 g/kg at 0.3 g/kg active ingredient content

COUMATETRALYL BLOCK BAITS
[*] **189**/BB/M/-

1 Sampling. Take at least 1 kg.

2 Identity tests
2.1 HPLC. As for coumatetralyl technical **189**/TC/M/2.2.
2.2 TLC. As for coumatetralyl technical **189**/TC/M/2.3.

* CIPAC method 1994. Prepared by the German Committee (DAPA). Chairman: Dr W Dobrat. Based on a method supplied by Bayer AG, Germany.

3 Coumatetralyl

OUTLINE OF METHOD Coumatetralyl is extracted with a methanol-tetrahydrofuran mixture and determined by reversed phase HPLC.

REAGENTS, APPARATUS and PROCEDURE As for coumatetralyl tracking powder **189**/TP/M/3, except:

(b) Preparation of sample. Crumble an aliquot of the sample to produce a fine grained material. Weigh (to the nearest 0.5 mg) enough of the well homogenised sample to contain about 4 mg (*w* mg) of coumatetralyl into a ground-glass-stoppered Erlenmeyer flask (300 ml). Pipette into the flask solvent mixture II (100.0 ml). Extract the coumatetralyl using an ultrasonic bath for about 30 min. Allow to cool to room temperature and filter or centrifuge an aliquot of the supernatant solution. Use the clear solution for injection without further dilution.

Repeatability r = 0.06 g/kg at 0.4 g/kg active ingredient content
Reproducibility R = 0.16 g/kg at 0.4 g/kg active ingredient content