

PRODUCT INFORMATION
METHODS OF ANALYSIS

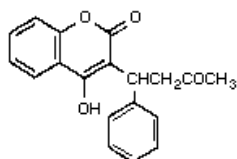
PRODUCT: WARFARIN TECH (ISO, BSI)

REVISE DATE: 10,MAY 2007

VERSION: 03

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CHEMICAL NAME : 4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one



STRUCTURAL FORMULA :

REGISTRATION NO. :

CAS NUMBER : [81-81-2] (unstated stereochemistry), 5543-58-8 (R)-(+)-isomer

CHEMICAL FORMULA : C₁₉H₁₆O₄

MOLECULAR WEIGHT : 308.3

ASSAY WARFARIN TECH

1. OUTLINE:

This sample is dissolved by mobile phase, the Warfarin 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;

Water: the redistilled water

Brodifacoum standard: known purity ≥99.0%

3. APPARATUS:

HPLC: Ultraviolet detector with adjustable wavelength;

Chromatography data treater;

Chromatograph Column: 150mm x 4.6 mm(i.d) mm Lichrospher C18 stainless steel column,

Filter: Filter film hole diameter 0.45 um;

Micro-Syringe:100 μl;

Quantitative-sampler: 10 μl;

Ultrasonic Cleaners.

4. THE OPERATION CONDITION OF CHROMATOGRAPHY

Flow(mobile) phase: (methanol + water + Glacial acetic acid) = 90+10+5(v/v/v)

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

Wavelength: 265 nm;

Flow rate: 1.0ml/min

Admission valve:10 ul

Retention time: Warfarin 2.29min (Picture 1 & 2)

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

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5. DETERMINATION PROCEDURE

Preparation of calibration solution:

Weigh Warfarin standard 0.01g (accurate to 0.0002g) into 100ml volume flask. Dissolve with mobile phase, make up to the mark.

Preparation of sample solution:

Weigh Warfarin sample 0.01g (accurate to 0.0002g) into 100ml volume flask. Dissolve with mobile phase, make up to the mark.

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 1.2%, determine by the injection order below: standard solution, sample solution, sample solution, standard solution

Calculation

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

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

Where :

A1—average ratio of peak area of Warfarin in the standard solution

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

m1—mass of Warfarin standard, g

m2—mass of Warfarin sample, g

P—mass percent of Warfarin in standard, %

Allowable deviation

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