

Chiral analysis of Warfarin using a Circular Dichroism Detector

Introduction

Warfarin is an anticoagulant used for the treatment and prevention of thromboembolism such as myocardial infarction. In Japan, warfarin potassium is also used as a medicine. Both the S enantiomer and the R enantiomer of warfarin show different pharmacokinetics and physiological activity.

The Circular Dichroism detector is well known for its high accuracy and selectivity for chiral analysis of compounds that have chromophores close to the chiral center. Both CD and UV signals are measured simultaneously and spectral scanning can be used to optimize the wavelength of interest, which is important in the measurement of chiral compounds. Not only can the CD detector help differentiate between two enantiomers, but it can also distinguish between achiral impurities and enantiomers of interest.

Keyword: Warfarin, enantiomer, CD detector, CHIRALCEL OD-RH

Experimental

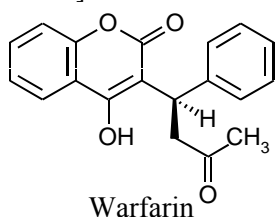
[Equipment]

Pump: PU-4185
 Pump option: Degasser unit
 Autosampler: AS-4050
 Column oven: CO-4060
 Detector: CD-4095

[Conditions]

Column: CHIRALCEL OD-RH (4.6 mm I.D. x 150 mm L, 5 μ m)
 Eluent A: Phosphoric acid aqueous solution (pH 2.0)
 Eluent B: Acetonitrile
 Composition: Eluent A/B (50/50)
 Flow rate: 0.5 mL/min
 Column temp.: 25°C
 Wave length: 270 nm
 Response: 1 sec
 Scan speed: 10 nm/sec
 Injection volume: 10 μ L
 Standard sample: 200 μ g/mL racemic warfarin in eluent A/B (50/50)

[Structure]



Results

Fig. 1 Chromatograms of the racemic warfarin standard. Fig. 2 shows the CD and UV spectra (measured using stopped-flow).

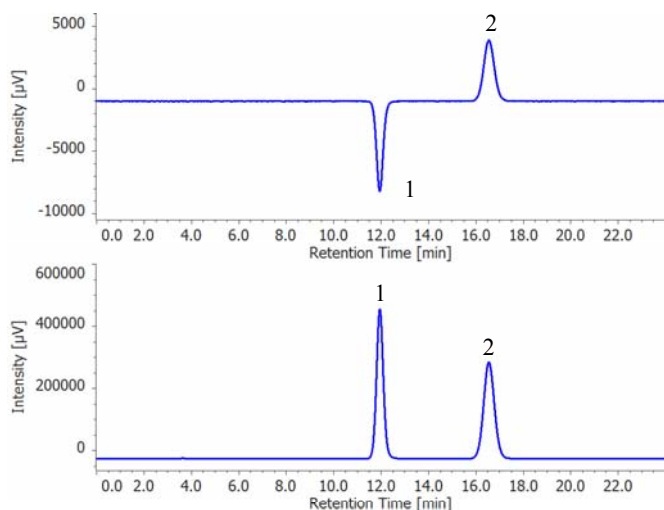


Fig. 1 Chromatogram of Warfarin standard
 Top: CD detection, Bottom: UV detection
 1: R-(+)-Warfarin, 2: S-(-)-Warfarin

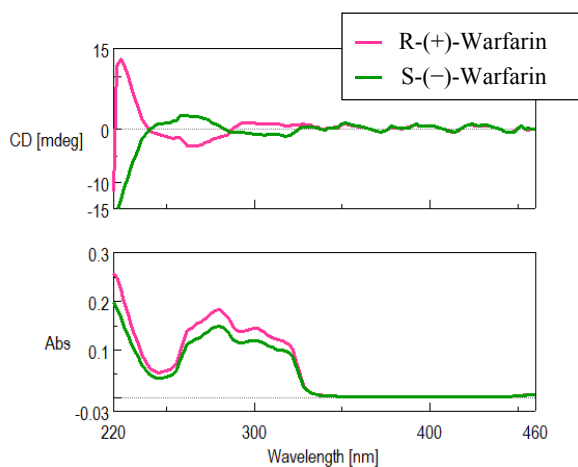


Fig. 2 Spectral measurement
 Top: CD, Bottom: UV

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Application Note

101018H

P/N	Description
7003-J014A	PU-4185 RHPLC Semi-micro Pump (Base Unit)
7006-H003A	DG-4000-04 4-channel Degasser Unit, for Analytical
7017-J002A	AS-4050 HPLC Autosampler
7021-J002A	CO-4060 Column Oven
7029-J002A	CD-4095 Circular Dichroism Chiral Detector
7058-J011A	BS-4000-1 Bottle stand
6688-H564A	LC-Net CG cable
7059-J012A	ChromNAV Ver.2 Chromatography Data System
7001-H401A	HPLC Start Up Kit for LC-4000
7001-H405A	Maintenance tool kit
	19724 CHIRALPAK AD-RH manufactured by Daicel