Application Note
CD Spectroscopy

Measurement of warfarin using circular dichroism coupled with HPLC

Introduction

High-pressure liquid chromatography (HPLC) is the most effective method for the quantitative estimation of trace levels of compounds and for mixture separation and purification. HPLC is also widely used for the analysis of food and medicinal products. In these fields, samples often exhibit optical activity, allowing for the analysis of enantiomeric excess and the separation and purification of these enantiomers.

JASCO has made both CD detectors (PMT and InGaAs) compatible for use with HPLC (CD-4095), as well as an HPLC flow cell for use with the J-1500 and J-1700 CD spectrometers. Both models provide high sensitivity, resolution, and a wide dynamic range, with high accuracy detection down to 200 nm.

In this application note, the CD measurement of warfarin was obtained using a J-1500 spectropolarimeter in static mode and coupled to an HPLC system (CD-4095) for in-line CD detection of the HPLC eluent.

JASCO J-1500-PAL high-throughput system
View product information at www.jascoinc.com

Keywords

J-1500, circular dichroism, J-1700, HPLC, CD-4095, medicinal products, chiral analysis, pharmaceutical

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Experimental

CD measurement conditions	
Data acquisition interval	0.1 nm
Response time	2 sec
Spectral bandwidth	1 nm
Scan speed	100 nm/min
Accumulations	1 time
Path length	1 mm
Wavelength range	210-400 nm
Sample	200 µg/mL warfarin

CD chromatogram measurement conditions	
Data interval	1 sec
Response time	1 sec
Spectral bandwidth	1 nm
Path length	10 mm
Sample concentration	200 µg/mL
Injection volume	10 µL
Wavelength range	220, 263 nm
`Flow rate	0.5 mL/min
Mobile phase	pH 2.0 aqueous phosphoric acid/acetonitrile (40/60)
Column	CHIRALCEL OD-RH (4.6 mm I.D. x 150 mm L, 5 µm)

Results

Figure 1 shows the CD and absorption spectra of R-(+)-warfarin and S-(-)-warfarin. CD peaks are observed at 220, 263, and 306 nm which can be used for the measurement of the CD chromatogram.

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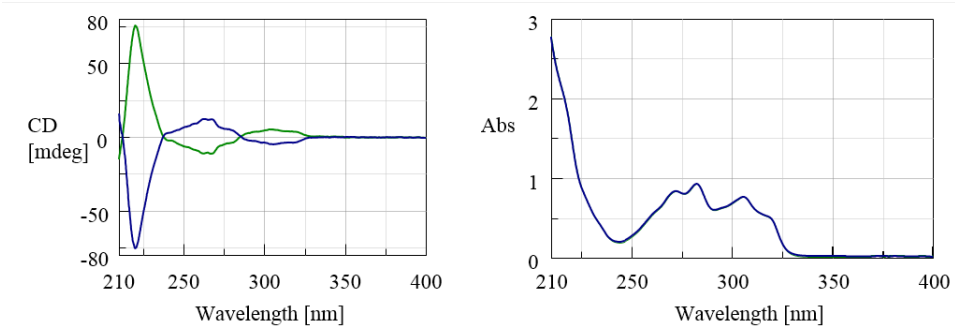

Figure 1. CD (left) and absorption (right) spectra of R-(+)-warfarin (green) and S-(-)-warfarin (blue).

Figure 2 shows the CD and absorption chromatograms of R-(+)-warfarin and S-(-)-warfarin at 220 and 263 nm. The measurement was performed using the Time Course Measurement mode and was initiated by the trigger signal from the manual injector or autosampler. The peak retention for R-(+)-warfarin is observed at 7.61 minutes and the peak retention for S-(-)-warfarin is 9.78 minutes. The CD and absorption signals are generally greater at shorter wavelengths which provides higher sensitivity. The CD peak area detected at 220 nm is six times larger than the peak at 263 nm and the area of the peak detected at 220 nm is double that at 263 nm. The area ratio of R-(+)-warfarin and S-(-)-warfarin is 1:1.

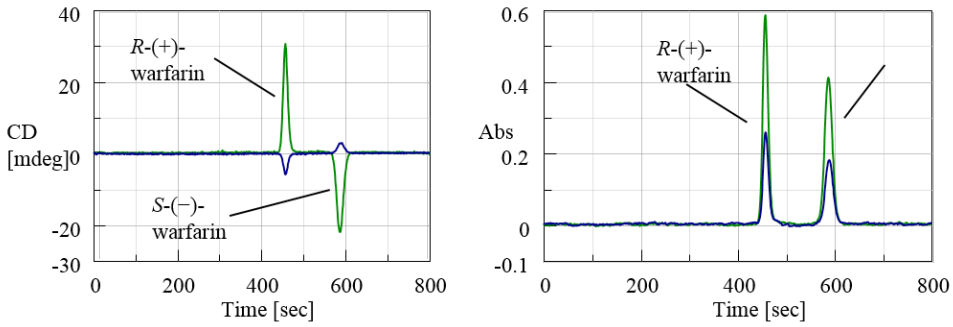

Figure 2. The CD and absorption chromatogram of warfarin detected at 220 nm (green) and 263 nm (blue).

Conclusion

This application note demonstrates that the J-1500 and J-1700 CD spectrometers can be used in conjunction with the JASCO HPLC system (CD-4095) in order to separate and obtain CD chromatograms on chiral substances.

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