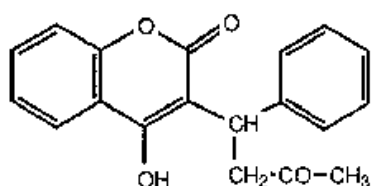


Simple routine method for warfarin enantiomers in plasma on CHIRAL-AGP

A method for the separation of warfarin enantiomers in human blood plasma has been published by I. Locatelli et al in *J. Chromatogr., B*, 818 (2005) 191-198. The article also describes the separation of warfarin and five hydroxylated metabolites by reversed phase non-chiral chromatography.

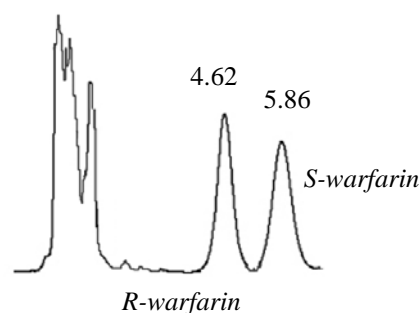


Warfarin is used as oral anticoagulant drug in the treatment and prevention of thromboembolism. Careful monitoring is necessary to tailor the treatment of individual patients, mainly due to complex pharmacokinetics. The two enantiomers, R- and S-warfarin, show distinct pharmacological properties. After administration of the racemate, the concentrations of the enantiomers in plasma differ due to stereoselective metabolism. S-warfarin is 2-5 times more potent than R-warfarin and the plasma clearance is also twice as fast.

Anticoagulation with warfarin is increasingly indicated and individual dosing can decrease the risk of adverse effects. These are reasons for continuing interest in improved methodology for the determination of warfarin enantiomers and metabolites in biological matrices.

The aim of the authors was to develop simple methods for routine use at hospitals for the separation of warfarin and hydroxylated metabolites as well as the enantiomers of warfarin. The assay has been successfully applied in a study of influence of CYP2C9 polymorphism, demographic factors and concomitant drug therapy on warfarin metabolism and maintenance dose in a group of 204 patients.

Plasma samples were extracted to diethylether, evaporated and reconstituted. The samples were injected on an HPLC system equipped with a UV photodiode-array detector. First a sample was injected onto a reversed phase column for the separation of racemic warfarin and hydroxylated metabolites. Then a sample was injected onto the CHIRAL-AGP column. A CHIRAL-AGP 100x3.0 mm column was used with a 10x3 mm CHIRAL-AGP guard column. The mobile phase was 15% acetonitrile in 50 mM phosphate buffer pH 7.0. The warfarin enantiomers were detected at 310 nm. Flow rate was set at 0.9 ml/min (which is higher than recommended 0.5 ml/min for 3 mm ID columns). Retention time for R-warfarin is 4.6 min and for S-warfarin 5.8 min.



The method has been validated. Accuracy and repeatability were calculated using calibration lines derived from peak areas versus analyte concentration as no internal standard was used. Values at 500 ng/ml are shown in the table below.

	Repeatability		Accuracy
	Intraday % (n=3)	Interday R.S.D.% (n=6)	% (n=3)
R-warfarin	1.3-4.0	5.1	99.1
S-warfarin	0.5-3.3	4.6	99.2

The limit of detection was 25 ng/ml and the linear range 75-2500 ng/ml for both enantiomers.

No significant matrix effect was observed when examining blank plasma samples from six independent sources.

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