MEASUREMENT OF WARFARIN STEREOISOMERS IN CLINICAL SAMPLES

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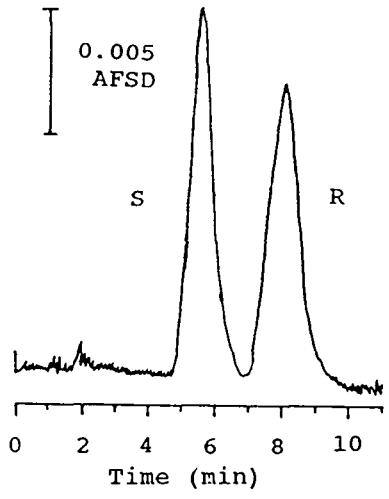
Many drugs, including warfarin, are administered as racemates, the isomers of which often exhibit different efficacy and toxicology profiles. In addition to these pharmacodynamic effects, the warfarin isomers have differing pharmacokinetic parameters (Breckenridge et al 1974) and drug interaction effects (Lewis et al 1974). Taking these into consideration, the S-isomer is approximately 8 times more potent than the R-isomer.

In the past there has been an impetus placed upon developing efficient methods to separate racemates. One method based on the principle of achiral/chiral coupled high-performance liquid chromatography has demonstrated the resolution of warfarin isomers (Chu and Wainer, 1988). Warfarin is separated from serum constituents on the achiral stationary phase and is then transferred onto a chiral stationary phase. Using the same principle, we have developed and optimized an improved method. The total run time is less than 12 minutes at room temperature with a sensitivity of 10mcg/L for each isomer.

Fig. 1. Separation of Racemic Warfarin



Patient serum samples are extracted using solid phase extraction columns and total serum warfarin concentrations are quantified on an achiral 5cm Pinkerton internal surface reversed phase (ISRP) stationary phase (Regis, Illinois) with p-chlorowarfarin as the internal standard and UV detection at 308nm. The warfarin peak is selectively transferred via a switching rheodyne, onto the second column containing alpha$_1$-acid glycoprotein (Chiral-AGP, Chromtech AB, Sweden). For this system fluorescence detection is employed with excitation at 300nm and emission at 370nm. The mobile phase used for both columns is 0.035M phosphate buffer with 15%v/v isopropanol at pH 7.0 and a flow rate of 1.0 and 0.9ml/min on the achiral and chiral systems respectively.

The retention time of racemic warfarin on the ISRP is 1.8 minutes and figure 1 shows that on the chiral-AGP the respective times for the S- and R-isomers are 5.7 and 8.0 minutes. Elution order was ascertained using an optical rotation HPLC detector (ChiraMonitor™, Applied Chromatography Systems Ltd., UK) and the pure R-isomer (a gift from Wainer, 1988). Spiked serum racemic warfarin concentrations of 0.5, 1, 2 and 4mg/L have shown S:R area ratios of 1.035, 1.024, 1.007 and 1.003 respectively, with coefficients of variation of 6.97, 3.12, 1.92 and 2.18%. Preliminary data from patients suggests that the R-isomer is eliminated faster than the S and that when non-compliance is suspected the S:R ratios change. The higher potency of the S-isomer was demonstrated in one patient who had a low serum racemic warfarin concentration of 1.37mg/L yet a high INR of 3.4. This could have been due to the relatively high S-isomer concentration of 0.89mg/L. Several "in use" clinical studies are in progress and the pharmacodynamics and pharmacokinetics of each isomer is under full investigation.

Breckenridge, M. et al (1974) J. Clin. Pharmacol. Ther. 15: 424-30
Lewis, R.J. et al (1974) J. Clin. Invest. 53: 1607-17
Chu, Y.Q. and Wainer, I.W. (1988) Pharm. Res. 5: 680-3