

PT18 LC-MS/MS analysis of S- and R-warfarin in human plasma and ultrafiltrate

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A Novel LC-MS/MS assay for S-/R-warfarin in plasma and UF was developed.

Background: Warfarin is a coumarin anticoagulant drug administered as a racemic mixture. The anticoagulant activity primarily resides in the S-enantiomer and the metabolic clearance of the S-enantiomer is almost twice that of the R-enantiomer. When relating warfarin concentrations to clinical results, it is therefore important to measure S- and R-warfarin separately. As part of a study to investigate the free clearance of warfarin in young and elderly, an assay was needed to measure the free concentrations of S- and R-warfarin in plasma and ultrafiltrate (UF). Warfarin is highly protein bound (~99%), meaning that the free (unbound) concentrations in ultrafiltrate will be ~100 fold lower than total concentrations in plasma. A sensitive technology such as LC-MS/MS was therefore required.

Aim: To develop and validate a sensitive and reliable LC-MS/MS assay for measuring S- and R-warfarin in human plasma and ultrafiltrate.

Methods: Warfarin was extracted from plasma and ultrafiltrate samples by liquid-liquid extraction. The enantiomers were separated by chiral chromatography and detected by MS/MS (m/z 307→161) using d₆-warfarin as internal standard.

Results & Conclusion: An LC-MS/MS assay for measuring S- and R-warfarin in human plasma and ultrafiltrate has been developed. Preliminary validation data and application of the assay on selected clinical samples will be presented.