

# Development and validation of LC-MS/MS assay for the determination of the prodrug dabigatran etexilate and its active metabolites in human plasma

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## Abstract

Dabigatran etexilate (DABE) is a low-molecular-weight prodrug that is converted after oral administration to dabigatran (DAB) a directly acting oral anticoagulant. In this study, an LC-MS/MS assay was developed and validated for the determination of DABE, free DAB and its equipotent O-glucuronide conjugates in plasma. Owing to the susceptibility of DABE and DAB to chemical hydrolysis, cleavage of the O-glucuronide moiety was carried out using  $\beta$ -glucuronidase enzyme. Free and total plasma concentrations of DAB were determined in incurred plasma samples before and after enzymatic cleavage (50 °C and 3 h), respectively. RP-HPLC separation was carried out using acetonitrile: water (30:70, v/v), adjusted to pH 3.0 using formic acid. Tandem mass spectrometric detection at positive electrospray ionization in the MRM mode was then employed for the determination of DABE and DAB. The analysis was carried out within 5.0 min over a linear concentration range of 1.00–600.00 ng/mL for the prodrug and its active metabolite. Validation was carried out according to FDA guidelines for bioanalytical method. The recoveries were higher than 89.48%, the accuracy was within 98.33–110.12% and the RSD was below 10% for the studied compounds in both incurred plasma and quality control samples. Results of incurred sample analysis and incurred sample stability revealed less than 10% variability. This indicated good assay precision and sufficient stability of target analytes in their real matrix at the employed experimental conditions. The applicability of the assay for therapeutic drug monitoring and the determination of the pharmacokinetic parameters were demonstrated.

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