Development and validation of LCïMSMS assay for the determination of the prodrug dabigatran etexilate and its active metabolites inhuman plasma

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Abstract

tDabigatran etexilate (DABE) is a low-molecular-weight prodrug that is converted after oral adminis-tration to dabigatran (DAB) ð a directly acting oral anticoagulant. In this study, an LCïMSMS assay wasdeveloped and validated for the determination of DABE, free DAB and its equipotent O-glucuronide con-jugates in plasma. Owing to the susceptibility of DABE and DAB to chemical hydrolysis, cleavage of theO-glucuronide moiety was carried out using -glucuronidase enzyme. Free and total plasma concentra-tions of DAB were determined in incurred plasma samples before and after enzymatic cleavage (50ŮCand 3 h), respectively. RP-HPLC separation was carried out using acetonitrile: water (30:70, v/v), adjustedto pH 3.0 using formic acid. Tandem mass spectrometric detection at positive electrospray ionization inthe MRM mode was then employed for the determination of DABE and DAB. The analysis was carriedout within 5.0 min over a linear concentration range of 1.00ï600.00 ng/mL for the prodrug and its activemetabolite. Validation was carried out according to FDA guidelines for bioanalytical method. The recov-eries were higher than 89.48%, the accuracy was within 98.33ï110.12% and the RSD was below 10% forthe studied compounds in both incurred plasma and quality control samples. Results of incurred samplere-analysis and incurred sample stability revealed less than 10% variability. This indicated good assayprecision and sufficient stability of target analytes in their real matrix at the employed experimentalconditions. The applicability of the assay for therapeutic drug monitoring and the determination of thepharmacokinetic parameters were demonstrated

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