**An ultra-high-performance liquid chromatography coupled with a tandem mass spectrometry method for the quantification of edoxaban:
The importance to measure active metabolite**

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**Abstract**

**Background**

Although DOACs do not require regular measurements of their blood concentrations, some clinical situation may require an assessment of their concentration such as the detection of drug accumulation in case of acute renal or hepatic failure, recurrence of a stroke, occurrence of bleedings, planification of urgent invasive procedures, undocumented or multiple drug interactions or in case of multiple interfering factors. Among the factor Xa inhibitors, edoxaban is the only compound for which some of the metabolites (edoxaban-M4, -M6 and -M8 metabolites - sorted by relative occurrence in patients) are reported to be pharmacologically actives. Therefore, the contribution of these metabolites could potentially interfere with chromogenic assays usually used for the estimation of edoxaban concentration. However, considering their respective IC50 towards human factor Xa, these metabolites would inhibit factor Xa at different degree. The scientific literature contains many articles about liquid chromatography measurement of edoxaban, few of them provide and discuss the simultaneous quantification of edoxaban and its M4 metabolite.

**Aims**

To develop a validated UHPLC-MS/MS method to quantify simultaneously edoxaban and edoxaban-M4 metabolite in human plasma.

**Methods/Materials**

Electrospray ionization and chromatographic separation were optimized for the simultaneous dosage of edoxaban and edoxaban-M4 metabolite. The method was validated according to the requirements of regulatory guidelines for bioanalytical method validation provided by the EMA and the FDA.

**Results**

The total run time was 6 minutes. The method was validated for calibration curves, precision, accuracy, carry-over, selectivity, matrix effect and short-time stability.

**Conclusions**

This validated UHPLC-MS/MS method allows the quantification of edoxaban and its active M-4 metabolite. The interest of synchronously measuring edoxaban and edoxaban-M4 metabolite is to obtain complementary information about the inhibitory effect of this active metabolite in chronometric or chromogenic assays. This is of great importance to understand the relevance of pharmacokinetic interaction in treated patients. Although edoxaban has usually low concentrations of active metabolites, which should *de facto* not contribute significantly to the anticoagulant activity, the measurement of the M4-metabolite method is interesting in case of drug interactions (e.g. co-treatment with quinidine, verapamil, ketoconazole, rifampicin, phenytoin, carbamazepine…). Indeed, concomitant prescriptions of edoxaban and carbamazepine or rifampicin, some first-intention treatments, is frequent. As these drugs are inducers of CYP3A4, the metabolite to parent compound ratio is increased (Figure 1). In lights of the differences in terms of inhibitory activity between edoxaban and the M4-metabolite, the estimations of edoxaban concentration by chromogenic anti-Xa assays may be disturbed. Specifically, this estimation relies on the global anti-Xa activity of the plasma sample. The different inhibitory activities of edoxaban and its metabolites may introduce a bias in the estimation of the edoxaban equivalent concentrations given by the anti-Xa assay. Therefore, patients are at risk of having inadequate control of coagulation supporting the need to measure the most representative edoxaban metabolite concomitantly to the parent compound.

**Figure 1: Postulated edoxaban metabolism for active metabolites[[1]](#footnote-1)**

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1. CES1: carboxylesterase-1; CYP3A4/5: Cytochrome P450 isoenzyme 3A4/5 ; IC50: half-maximal inhibitory concentration; Cmax: maximum observed plasma drug concentration [↑](#footnote-ref-1)