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An ultra-high-performance liquid chromatography coupled with a tandem mass spectrometry method for the quantification of edoxaban

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Publication date:
2019

[Link to publication](#)

Citation for published version (HARVARD):

Siriez, R, Evrard, J, Laloy, J, Dogne, J-M & Douxfils, J 2019, '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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Background and aim

- Although DOACs do not require regular measurements of their blood concentrations, some clinical situation may require an assessment of their concentration.
- Among the factor Xa inhibitors, edoxaban is the only compound for which some of the **metabolites** (edoxaban-M4, -M6 and -M8 (► **Figure 1**)) are reported to be pharmacologically active.
- Metabolites could potentially interfere with chromogenic assays usually used for the estimation of edoxaban concentration.
- Considering their respective **IC₅₀ towards human factor Xa**, these metabolites would inhibit factor Xa at different degree.
- In this context, we developed a **validated UHPLC-MS/MS method** to quantify simultaneously edoxaban and edoxaban-M4 in **human plasma**.

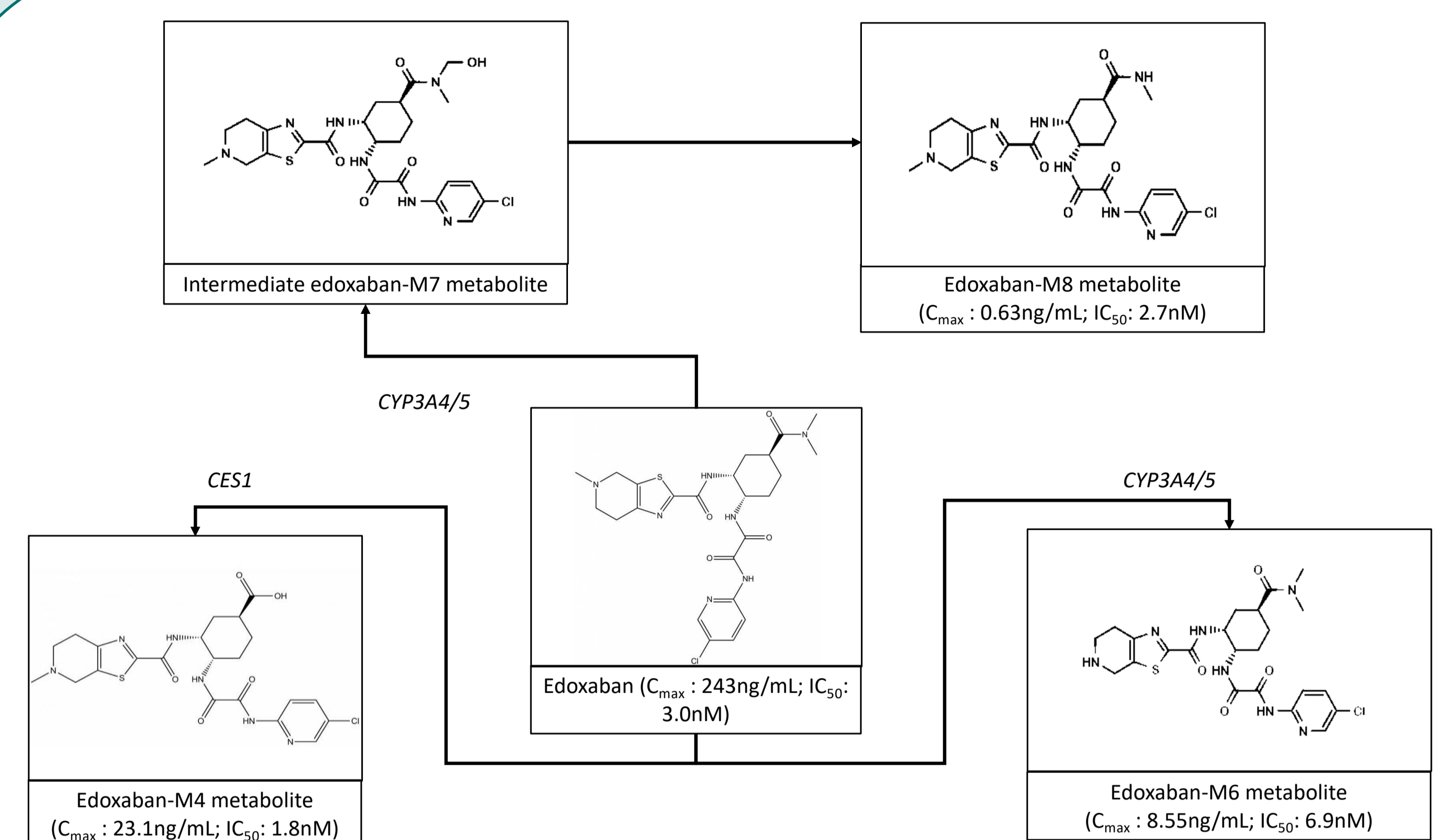


Figure 1: Postulated edoxaban metabolism for active metabolites. CES1: carboxylesterase-1; CYP3A4/5: Cytochrome P450 isoenzyme 3A4/5; IC_{50} : half-maximal inhibitory concentration; C_{max} : maximum observed plasma drug concentration

Table 1: MS/MS parameters for edoxaban, edoxaban-M4 and corresponding internal standard.

ESI+: Electrospray positive ionization mode

Compound	Ion mode	Transition type	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (eV)	Dwell time (s)
Edoxaban	ESI+	Quantification	548.212	152.169	40	32	0.035
	ESI+	Confirmation	548.212	366.19	40	20	0.035
Edoxaban-M4	ESI+	Quantification	521.162	321.176	38	24	0.035
	ESI+	Confirmation	521.162	339.12	38	18	0.035
[² H ₆]-edoxaban	ESI+	Quantification	554.316	158.160	32	30	0.035
	ESI+	Confirmation	554.316	372.27	32	18	0.035

Methods

- Electrospray ionization and chromatographic separation were optimized for the simultaneous dosage of edoxaban (3 to 500ng/mL) and edoxaban-M4 (3 to 150ng/mL) with [²H₆]-edoxaban in plasma (► **Table 1**). Ranges were chosen to cover (supra)-therapeutic ranges.
- The method was validated on a total run time of 6 minutes for calibration curves, precision, accuracy, carry-over, selectivity, matrix effect and short-time stability according to the requirements of regulatory guidelines for bioanalytical method validation provided by the EMA and the FDA.

Results and discussion : Importance of measuring pharmacologically active metabolites of edoxaban

- The method was **validated** according to the **regulatory guidelines** provided by the EMA and the FDA for the simultaneous dosage of **edoxaban (3 to 500ng/mL)** and **edoxaban-M4 (3 to 150ng/mL)** with [²H₆]-edoxaban in plasma (► **Figure 2**).
- A potential interest of synchronously measuring edoxaban and edoxaban-M4 is to obtain complementary information about the **impact of the active metabolite in chronometric or chromogenic assays**. This is especially important since at **low concentration (<30ng/mL)** a **deviation of more than 50% has been observed (anti-Xa vs LC-MS/MS)**, suggesting that **anti-Xa assays are not able to provide reliable results** in these low values.
- **Limitation** : Edoxaban-M6 was not investigated. Regarding its IC_{50} (6.9nM) and C_{max} (8.55ng/mL), the impact on chromogenic assays should be negligible contrary to the impact of the edoxaban-M4 which has a lower IC_{50} (1.8nM) and a higher C_{max} (23.1ng/mL) (► **Figure 1**).
- In addition, this technique could be interesting in case of **drug-drug interactions** which are frequently reported (e.g. co-treatment with *quinidine*, *verapamil*, *ketoconazole*, *rifampin*, *cyclosporine*, *erythromycin*, ...). These interactions disturbed the parent-to-metabolite ratio explaining for ther the imprecision of standard chromogenic methods.

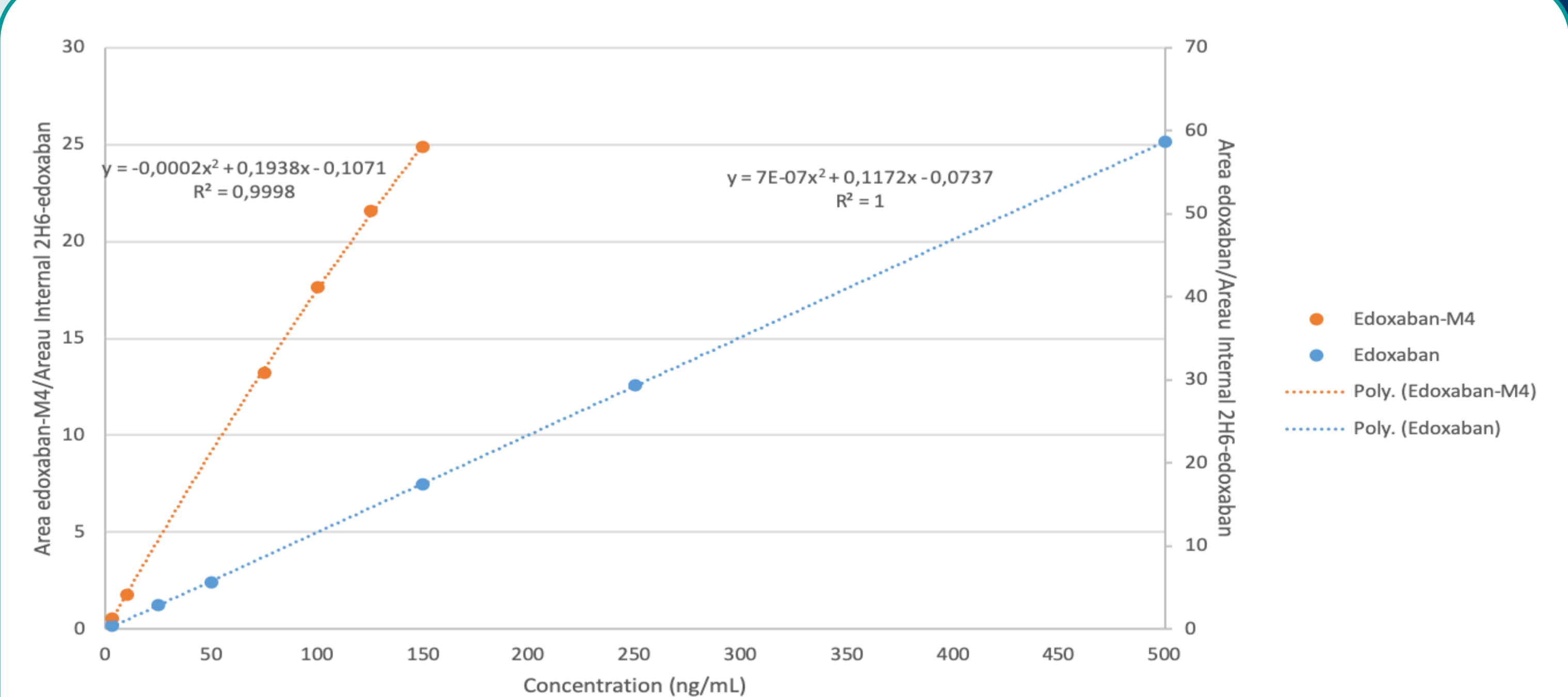


Figure 2: Calibration curves for measurement of edoxaban and edoxaban-M4 in plasma with UHPLC-MS/MS in presence of ²H₆-edoxaban (internal standard). The blue and orange lines represent the calibration lines of the edoxaban (3 to 500ng/mL) and edoxaban-M4 (3 to 150ng/mL), respectively.

Conclusion

- This method permits quantification of **edoxaban and edoxaban-M4** providing complementary information about the inhibitory effect of this active metabolite in chronometric or chromogenic assays.
- Although patients treated with edoxaban exhibits usually low concentrations of active metabolites, the measurement of edoxaban-M4 is interesting; especially in case of **drug interactions**. Indeed, concomitant prescriptions of edoxaban and *carbamazepine* or *rifampicin* is frequent and may lead to disturbance of the estimations of edoxaban concentration by chromogenic anti-Xa assays.
- Therefore, patients are at risk of having **inadequate control of anticoagulation** supporting the need of measuring the most representative edoxaban metabolite concomitantly to the parent compound.