

Overview

Purpose:

Validate four fXa inhibitors in human plasma including protein binding parameters.

Methods:

Protein binding parameters validated utilizing a HTD96b dialysis device. Plasma/buffer was extracted and analyzed by LC/MS/MS.

Results:

All methods validated with Accuracy/Precision better than %15 including determinations of protein binding parameters.

Introduction

fXa inhibitors are a class of anticoagulant drugs widely prescribed to prevent strokes, venous thromboembolism, and deep vein thrombosis. The major concern for patients using this class of drugs is an increased risk of major bleeding events. Andexanet alfa (AnXa) is a modified fXa protein that has a high affinity for fXa inhibitors. When patients treated with fXa inhibitors are exposed to AnXa the unbound plasma concentration of the fXa inhibitors are sequestered and coagulation is restored. In order to establish the effectiveness of AnXa the unbound concentration of the fXa inhibitors must be determined. In this work, we report the validation of four fXa inhibitors in human plasma by HPLC-MS/MS used to support multiple AnXa clinical studies.

Methods

Plasma Extractions:

- Sample volume: 50 μ L
- Add 25 μ L Internal Standard
- Precipitate with acetonitrile or add ethyl acetate
- Evaporate under nitrogen
- Reconstitute with 100 μ L of DMF

LC/MS/MS:

- Shimadzu Binary LC Systems
- Gradient using acetonitrile and water with formic acid
- Flow rate: 700 μ L/minute
- Column: Supelco HS C18 (50 X 2.1 mm, 3 μ m)
- Column temperature: 50°C
- ABSciex 5500/6500 QTRAP operating in MRM mode

- ESI
- Positive ion mode

Protein Binding:

- HTDialysis HTD96b dialysis device
- 12-14 kDA MWCO membrane
- Plasma/PBS volume: 100 μ L
- Incubated at 37°C

Conclusions

- Developed and validated LC/MS/MS methods for four fXa inhibitors including the determination of the protein binding parameters
- Methods are accurate/precise and have been used to support multiple clinical studies.

Table 1: Protein Binding Data

fXa inhibitor	Incubation Recovery (%)	Non-Specific Binding Recovery (%)	Opt Inc Time (hours at 37°C)	%PB Predose Sample	%PB Plasma + AnXa
Apixaban	80.2-91.2	81.8-93.0	4	89	99
Betrixaban	85.6-92.8	86.1-128	4	58	99
Edoxaban	92.6-98.5	82.2-84.3	4	51	97
Rivaroxaban	87.6-97.0	73.4-92.7	4	94	99

* Averaged from Clinical Data

Table 2: Plasma Validation Data

fXa inhibitor	LLOQ (ng/mL)	Intraday A/P (%Bias \pm CV)	Interday A/P (%Bias \pm CV)	Freeze/Thaw (cycles)	BT Stability (hours)	AS Stability (hours)
Apixaban	0.100	12.0 \pm 16.2	3.3 \pm 9.1	4	7.0	39.0
Betrixaban	0.200	6.0 \pm 7.5	2.3 \pm 6.8	4	6.9	32.9
Edoxaban	1.00	3.7 \pm 4.8	7.2 \pm 7.0	4	6.0	31.7
Rivaroxaban	0.100	1.0 \pm 5.8	6.3 \pm 10.6	4	24.8	96.0

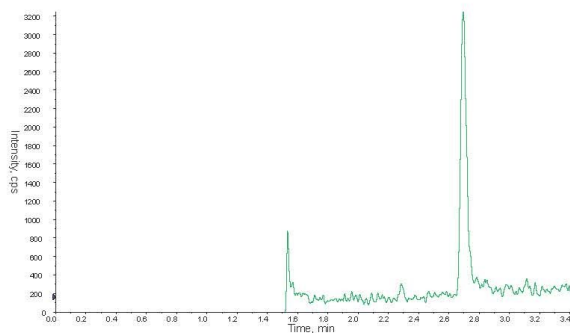


Figure 1: Apixaban Extracted from Human Plasma (0.100 ng/mL)

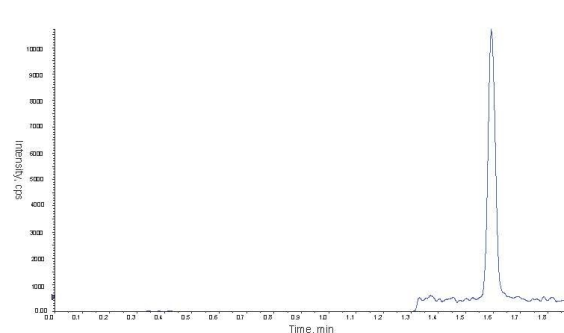


Figure 2: Betrixaban Extracted from Human Plasma (0.200 ng/mL)

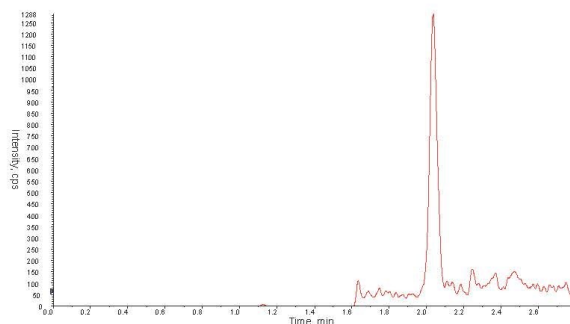


Figure 3: Edoxaban Extracted from Human Plasma (1.00 ng/mL)

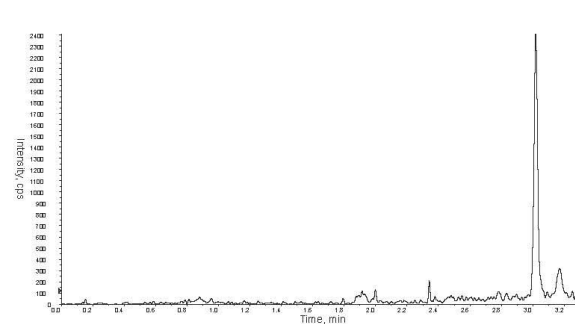


Figure 4: Rivaroxaban Extracted from Human Plasma (0.100 ng/mL)