

Free MMAE toxin quantitation by triple quadrupole in Antibody Drug Conjugate analysis

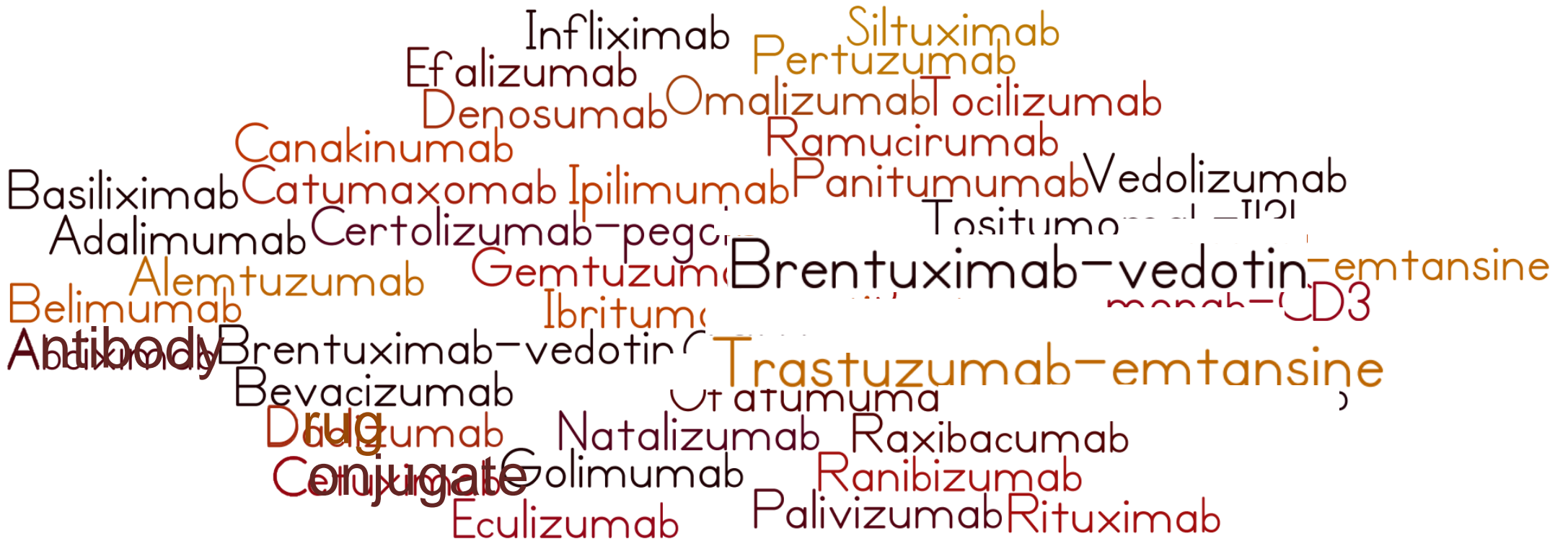
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PIT2
Shimadzu*

**Sega NDIAYE
EBF Barcelona 2013**

Overview

- Introduction
- Antibody Drug Conjugate
 - General
 - ADC Brentuximab-Vedotin
 - Monomethyl Auristatin E
- Equipment
- Methods
- Results
- Conclusion

Monoclonal antibody



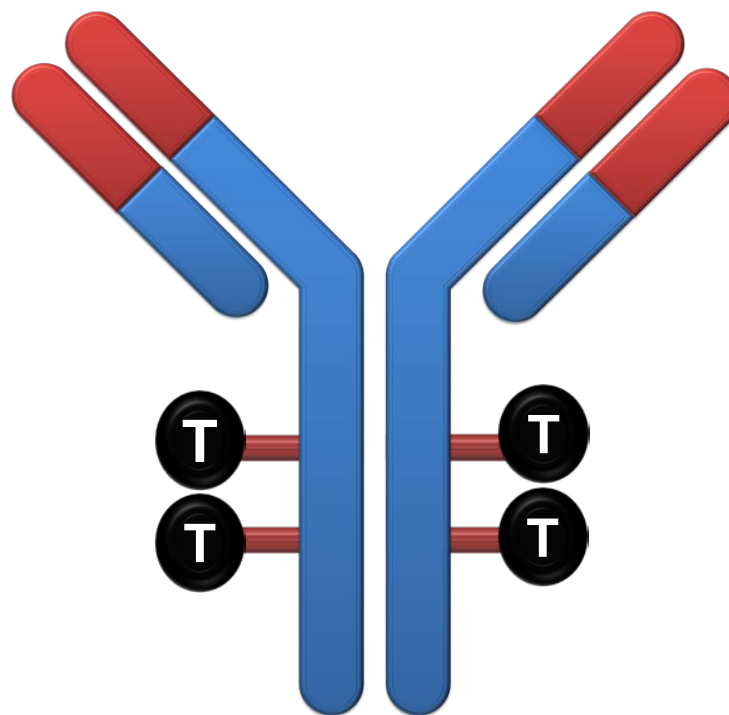
A word cloud of monoclonal antibodies, with the most prominent words being **Trastuzumab-emtansine** and **Brentuximab-vedotin-CD3**. Other visible antibodies include: **Infliximab**, **Siltuximab**, **Pertuzumab**, **Efalizumab**, **Denosumab**, **Omalizumab**, **tocilizumab**, **Canakinumab**, **Ramucirumab**, **Basiliximab**, **Catumaxomab**, **Ipilimumab**, **Panitumumab**, **Vedolizumab**, **Adalimumab**, **Certolizumab-pegol**, **Tositumomab**, **Alemtuzumab**, **Gemtuzumab**, **Brentuximab-vedotin-CD3**, **Belimumab**, **Ibritumomab**, **Trastuzumab-emtansine**, **Brentuximab-vedotin**, **Bevacizumab**, **Ustatumumab**, **Daliumab**, **Natalizumab**, **Raxibacumab**, **Conjugate**, **Golimumab**, **Ranibizumab**, **Eculizumab**, **Palivizumab**, and **Rituximab**.

Anatomy of an ADC

Antibody

Drug

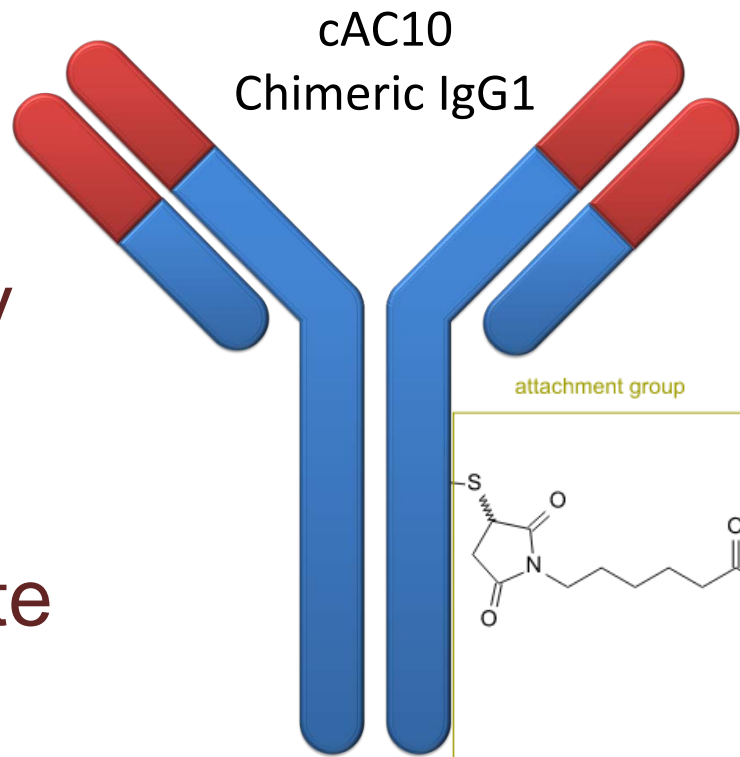
Conjugate



Linker

Toxin

Brentuximab-Vedotin

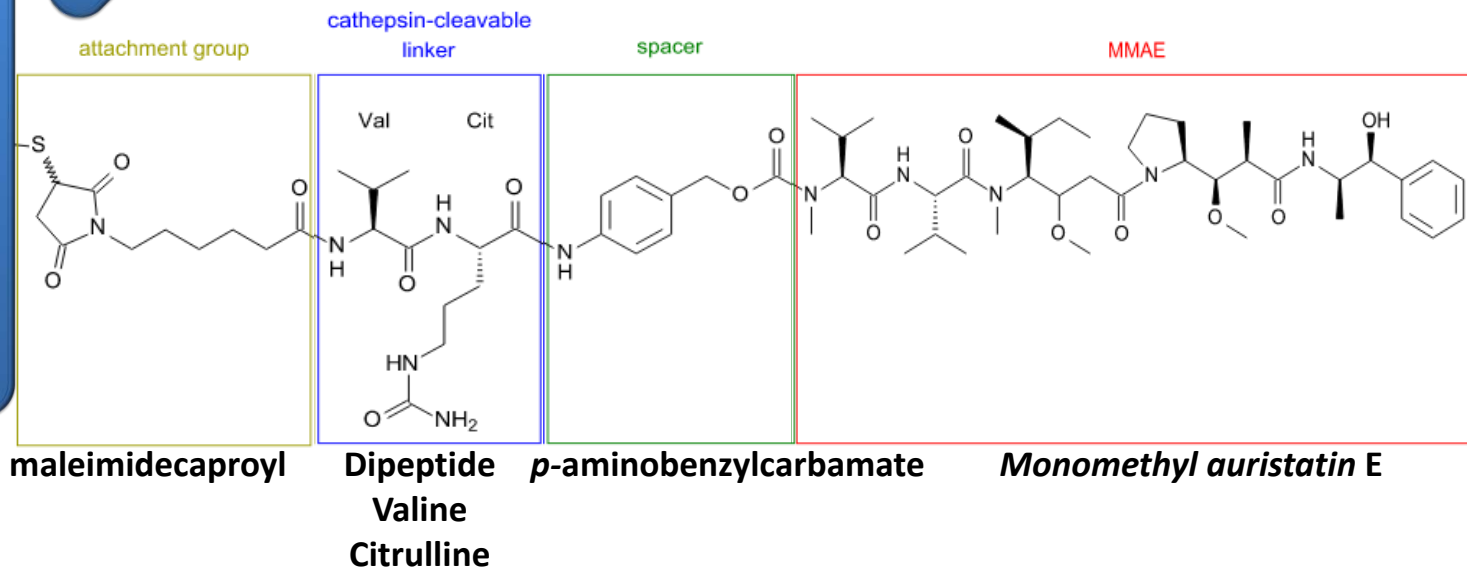


ADCETRIS

Antibody

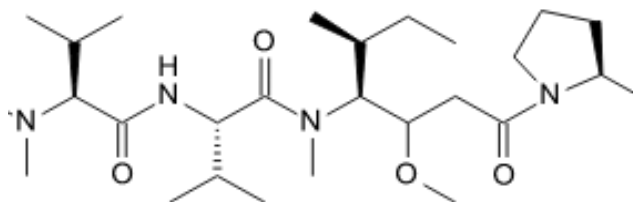
Drug

Conjugate



Monomethyl auristatin E

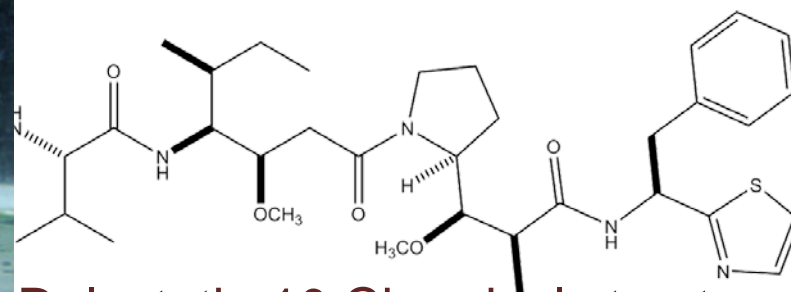
- The **auristatin E** synthetic analog of the natural product **dolastatin 10**
- Dolastatin 10 was originally isolated from the Indian Ocean sea hare, *Dolabella auricularia*



MMAE Chemical structure



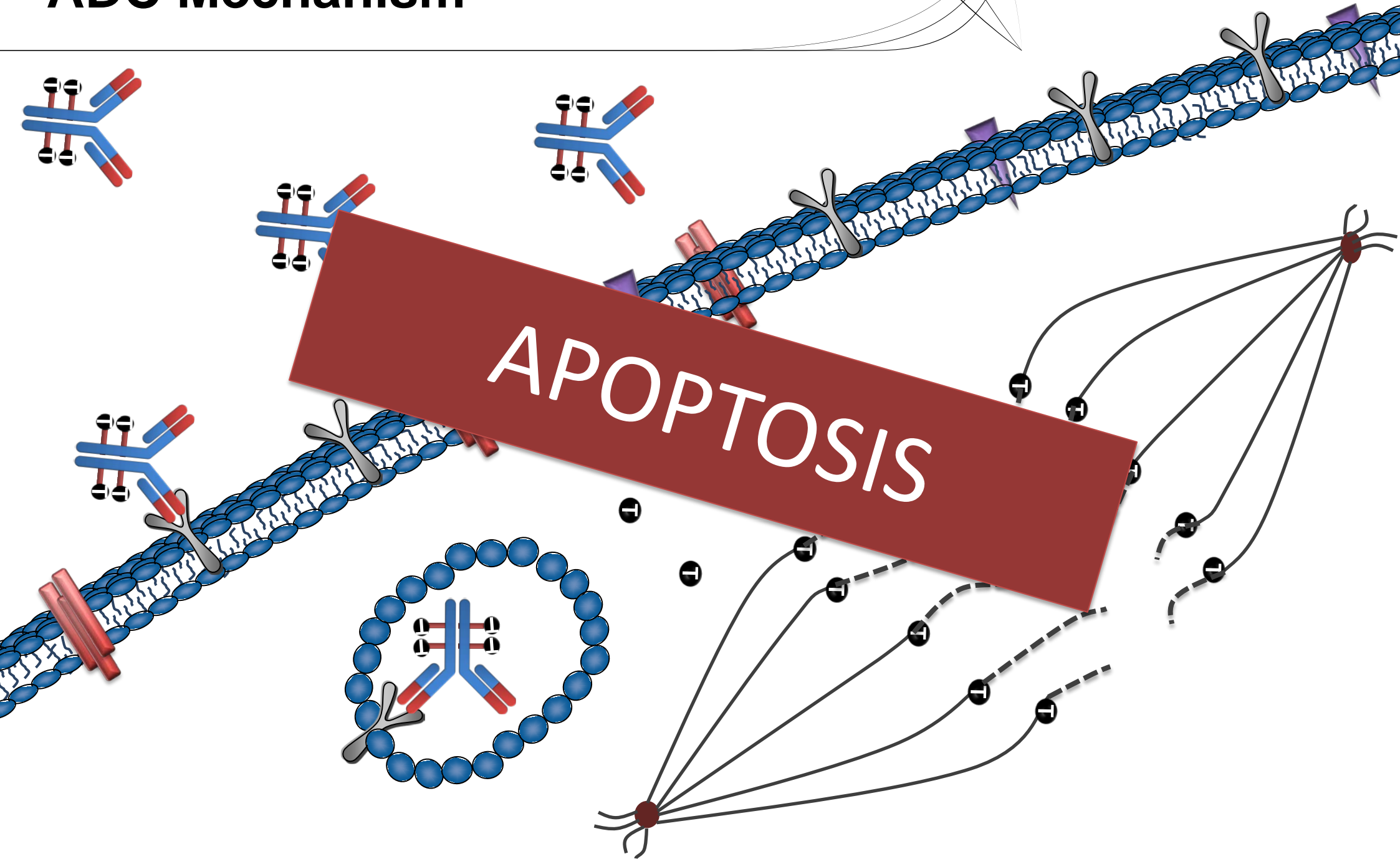
Wedge sea hare



Dolastatin 10 Chemical structure

- High toxicity
- Blocks tubulin polymerization

ADC Mechanism

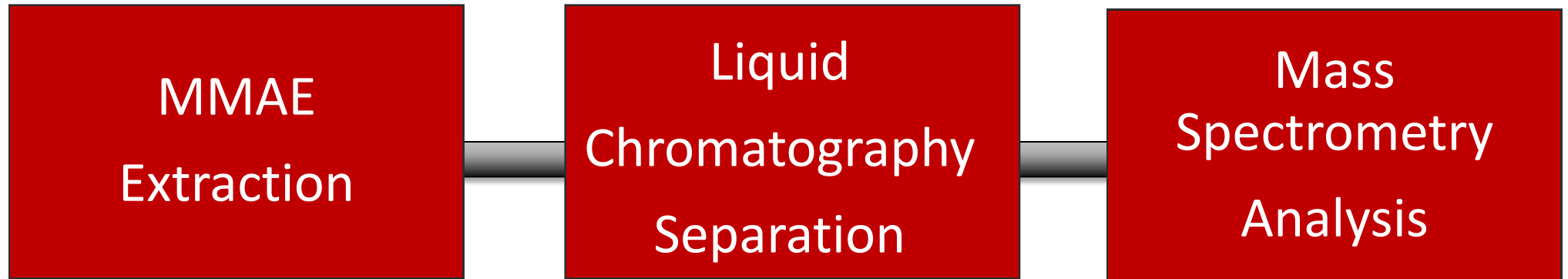


LC-MS/MS system

- SHIMADZU 8040 LC-MS/MS
 - Liquid Chromatography UHPLC Nexera SHIMADZU
 - Electrospray interface
 - Triple quadrupole
 - Speed : up to 555 MRM/sec
 - Sensitivity : ≈ 1 fmol
(Réserpine M=608.7 S/N 10000)

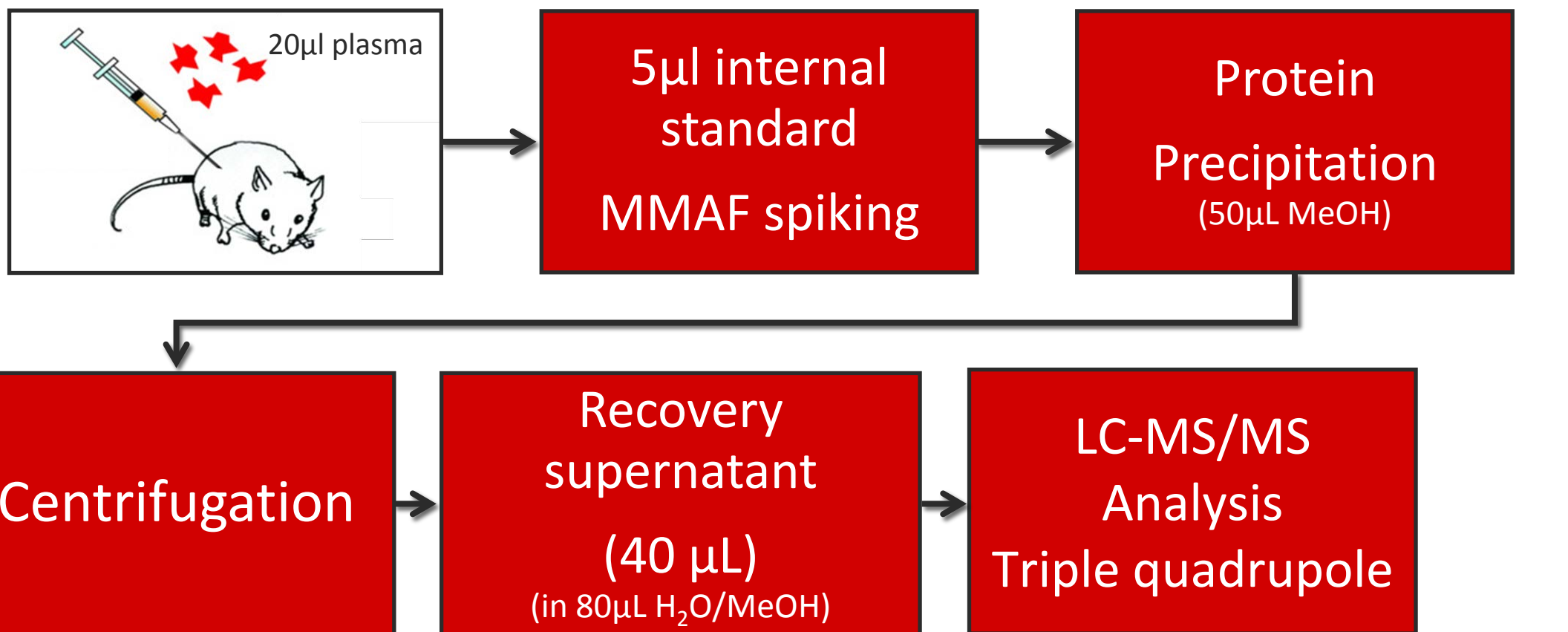


Methods



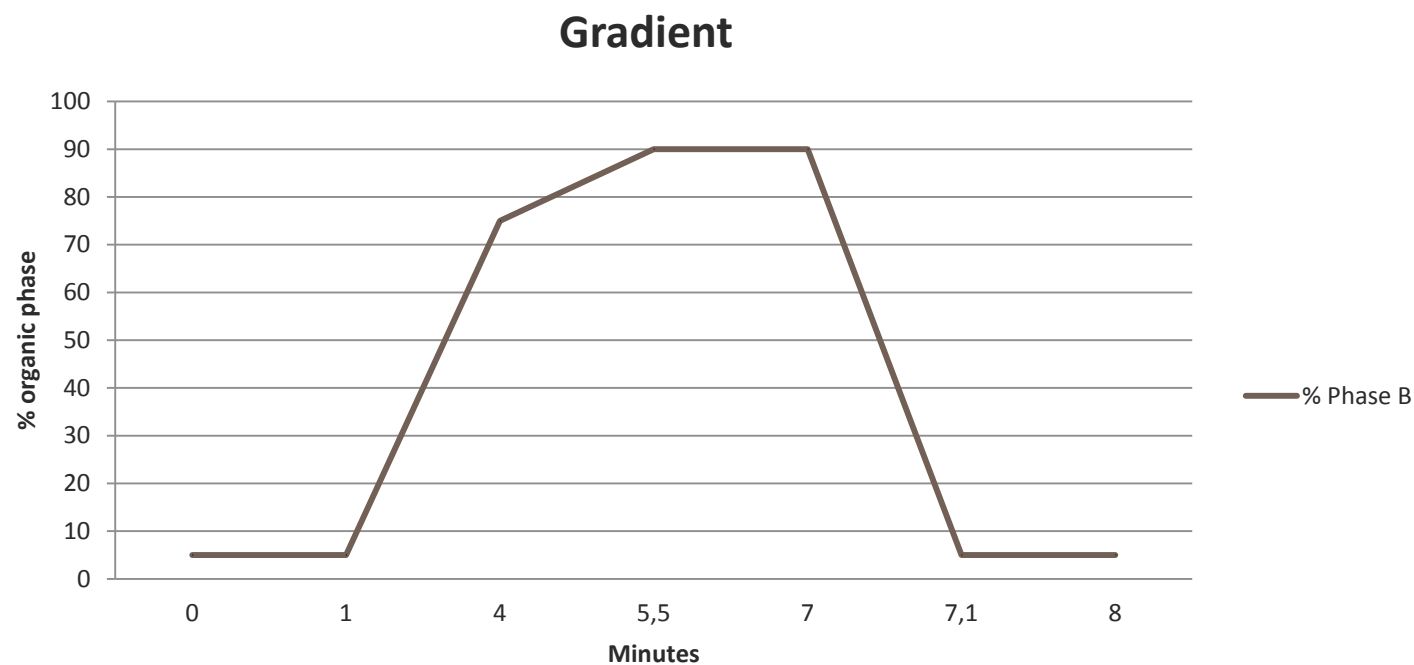
Methods

- MMAE protocol extraction



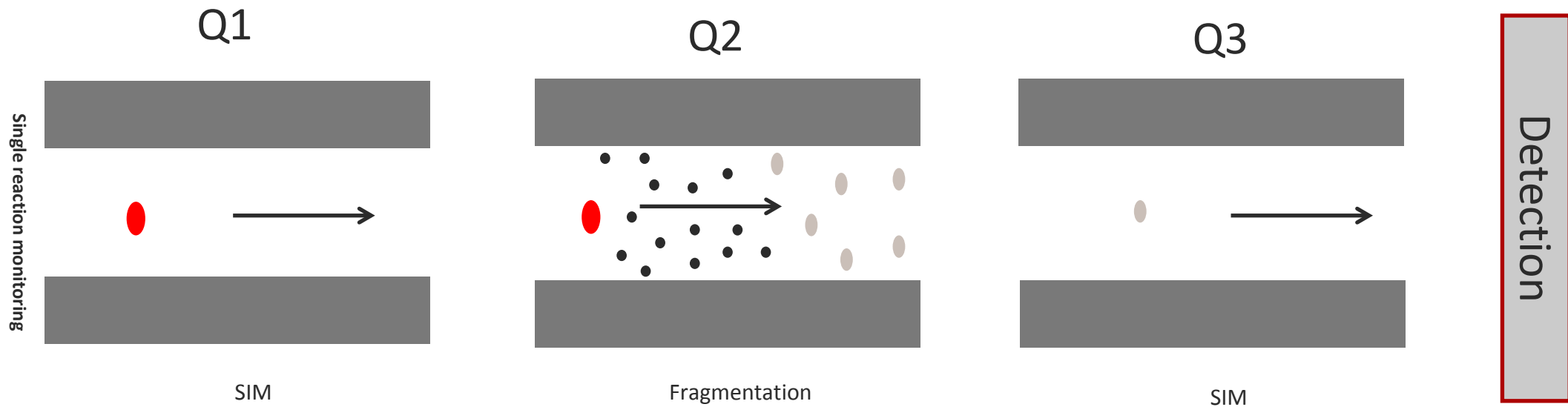
Methods

- Chromatography
 - Kinetex XB-C18 Phenomenex UHPLC Column
 - Eluent : H₂O = A et MeOH = B Flow rate : 0.7 mL/min T : 40°C
 - Total duration of chromatographic run : 8 min
 - Assay with internal standard: MMAF



Methods

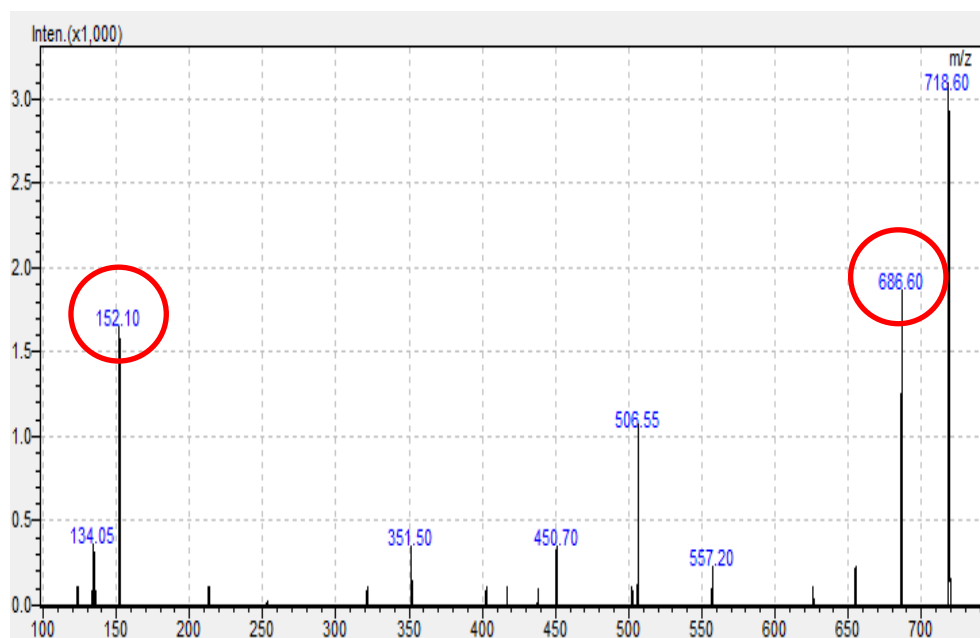
- Mass spectrometry triple quadrupole
- Triple quadrupole using SRM (Single Reaction Monitoring)



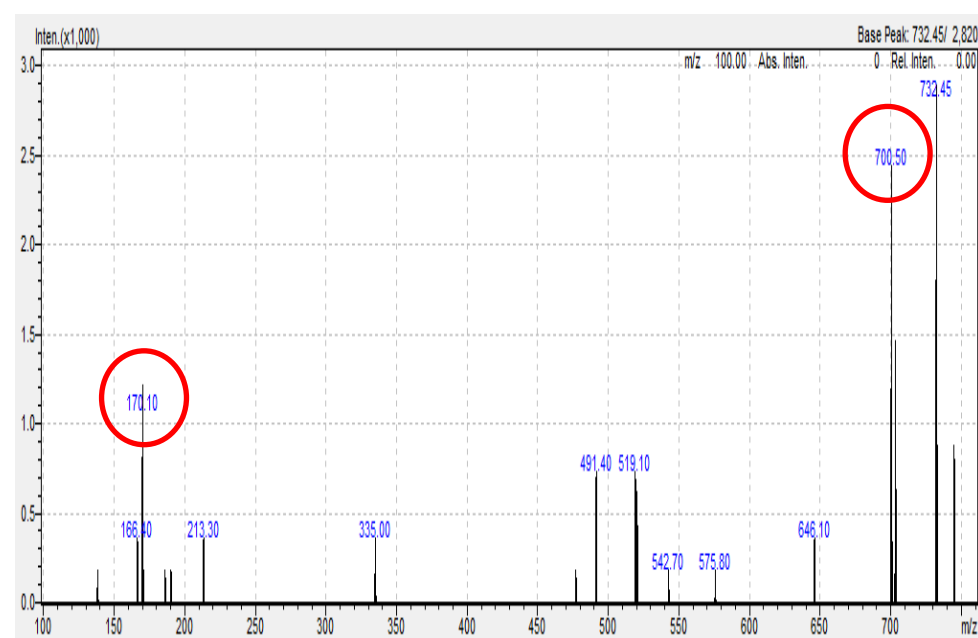
- Increase the signal to noise ratio

Methods

- Mass spectrometry
- 2 SRM transition by compound : MRM Mode
 - MMAE : 718.50 → 686.50 ; 718.50 → 152.10
 - MMAF : 732.50 → 700.30 ; 732.50 → 170.30
 - Fragments verified by Product Ion Scan and literature search



Product Ion Scan MMAE



Product Ion Scan MMAF

MMAE

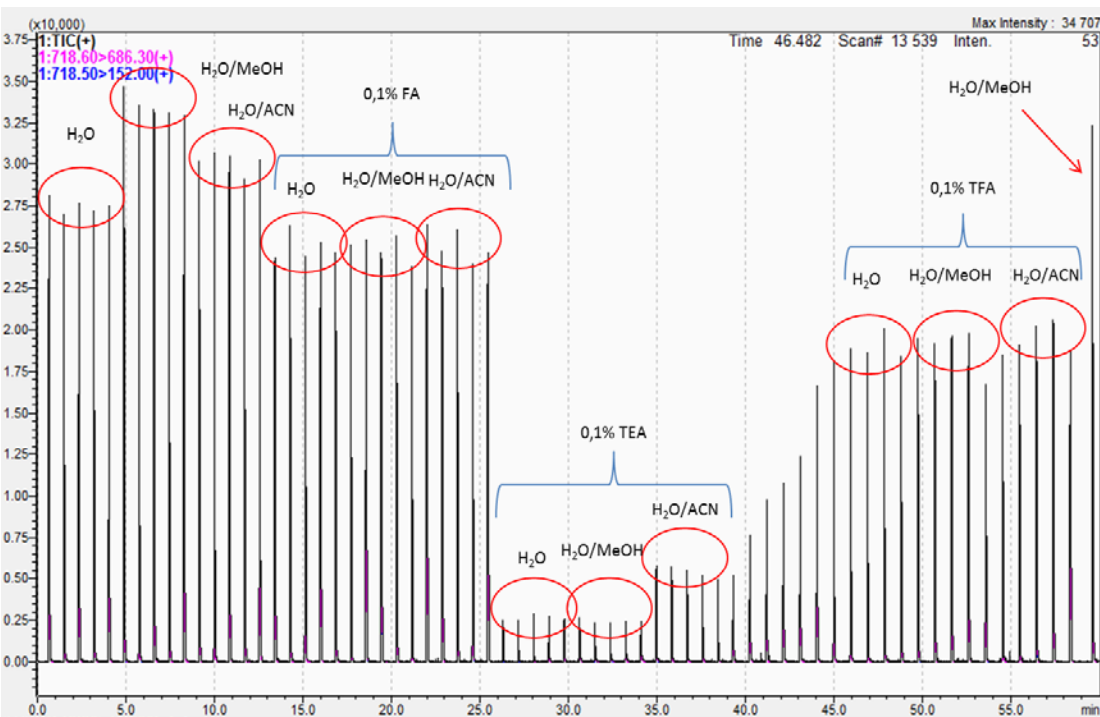
Transition	Dwell time (ms)	Q1 PreBias (V)	Collision Energy (V)	Q3 PreBias (V)
718,5->686,3	100	-28	-31	-34
718,5->152,0	100	-28	-36	-29

MMAF

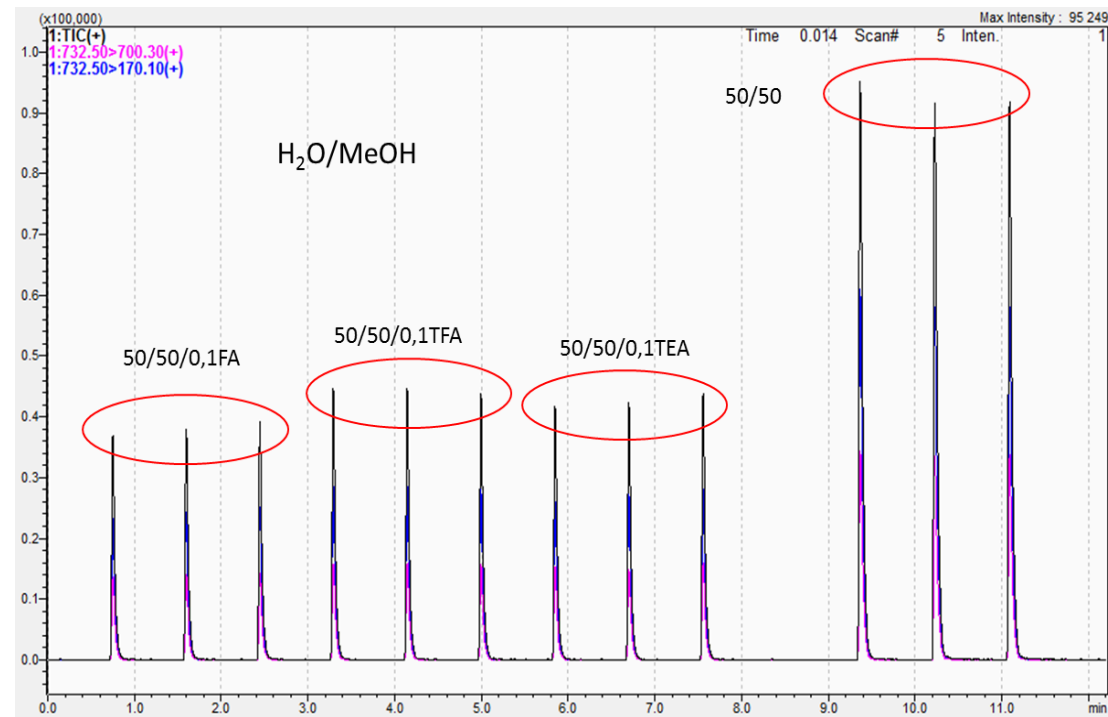
Transition	Dwell time (ms)	Q1 PreBias (V)	Collision Energy (V)	Q3 PreBias (V)
732,5->700,3	100	-28	-29	-26
732,5->170,1	100	-28	-46	-30

Optimization

Mobile phase optimization



MMAE signal response optimization

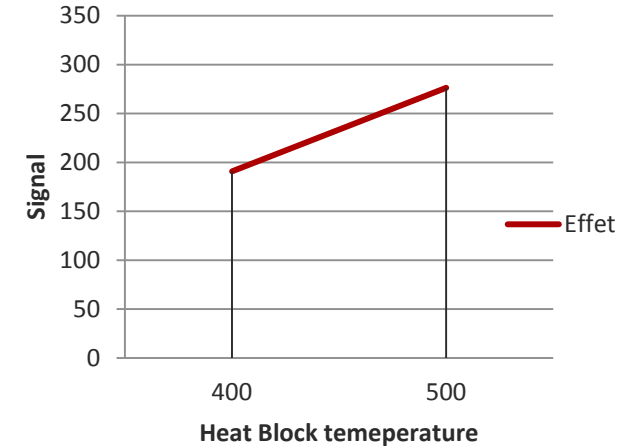
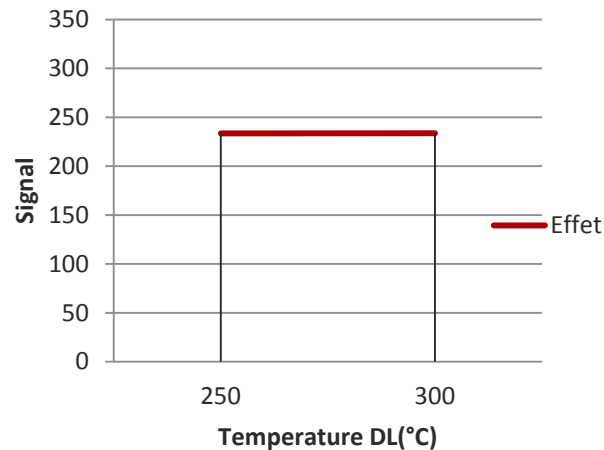
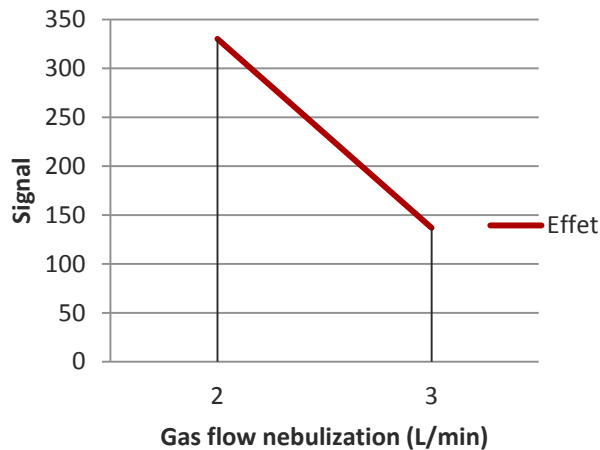


MMAF signal response optimization

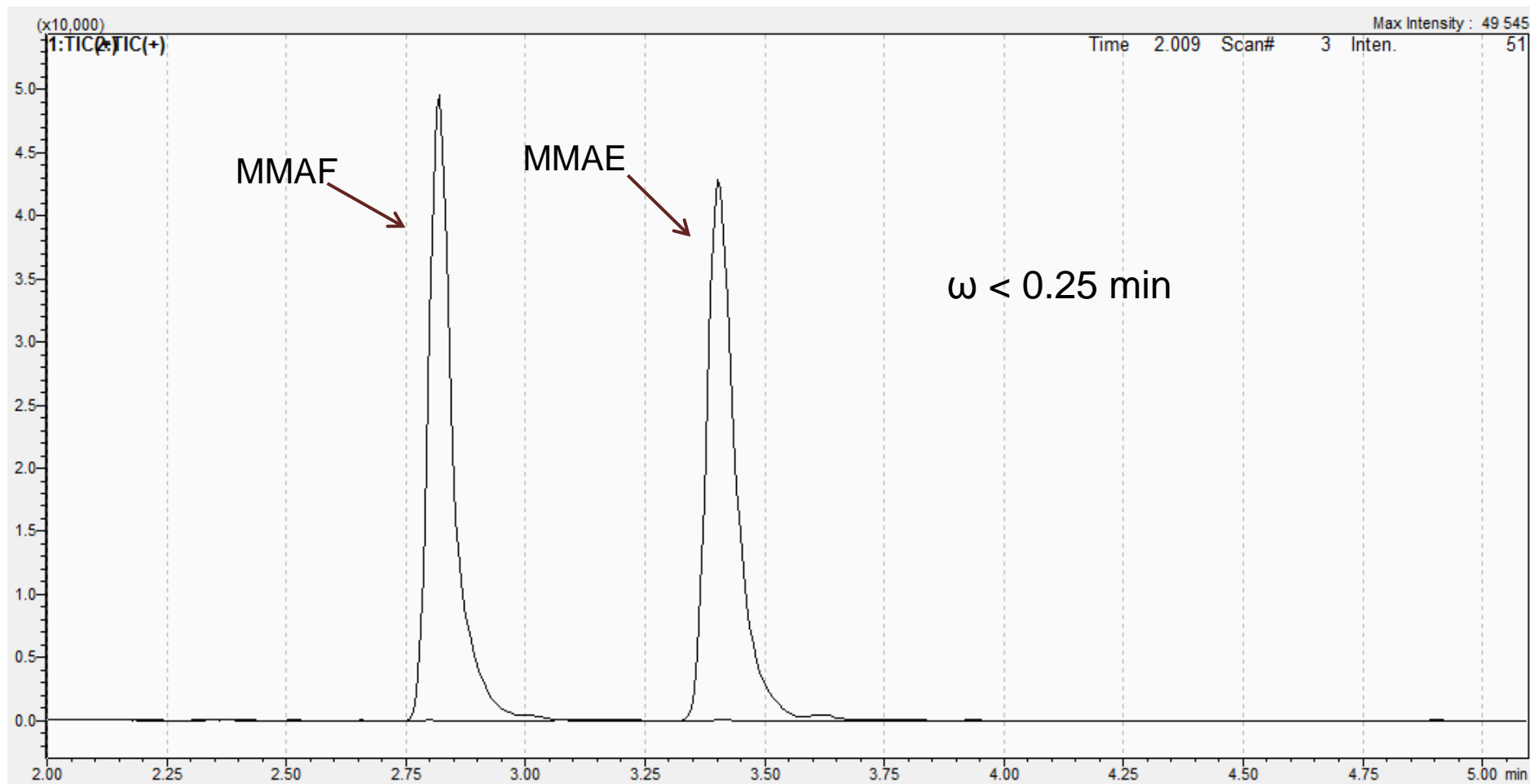
Optimization

- Interface

- Electrospray source parameters optimization (3 factors, 2 levels)
 - Gas flow nebulization (2L/min 3L/min)
 - Desolvation temperature line (250°C 300°C)
 - Heat Block temperature (400°C 500°C)
- Influence of nebulisation and Heat block temperature parameters
- Slight interaction between these two parameters



Results

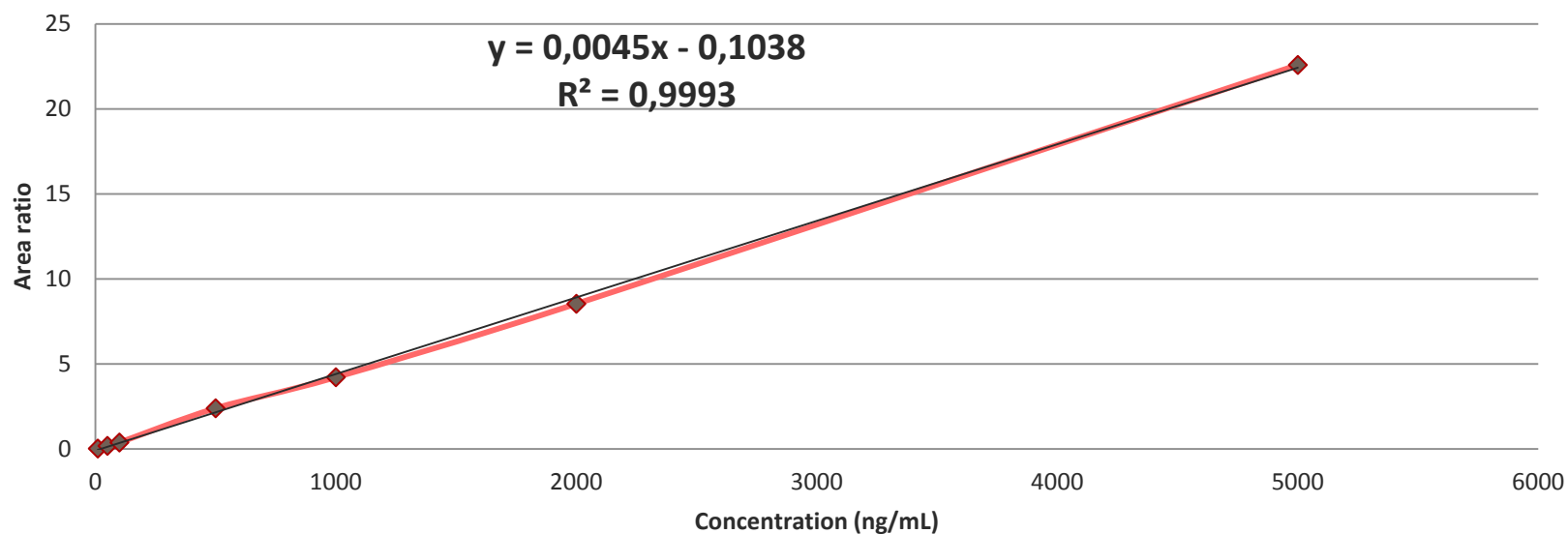


Constant elution time: $\sigma = 0.02$ min 50 successive injections in plasma

Calibration

- Matrix : Nude mice plasma
- Plasma volume: 20 μ L doping with MMAE at various concentration
- Internal standard (MMAF) : 5 μ L at 50ng/mL -> concentration in 25 μ L of sample : 10 ng/mL
- Linearity range:
 - **3 ng/ml to 5 μ g/mL**
 - LOD : 1 ng/mL 1.2 fmol on column LOQ : 3 ng/mL in plasma sample 3.6 fmol on column

MMAE Calibration internal standard Mice nude plasma



Results Ex vivo Free MMAE

Ex Vivo Free MMAE Assay



Incubation 37°C



T0h, T8h, T24h
T48h, T96h, T168h

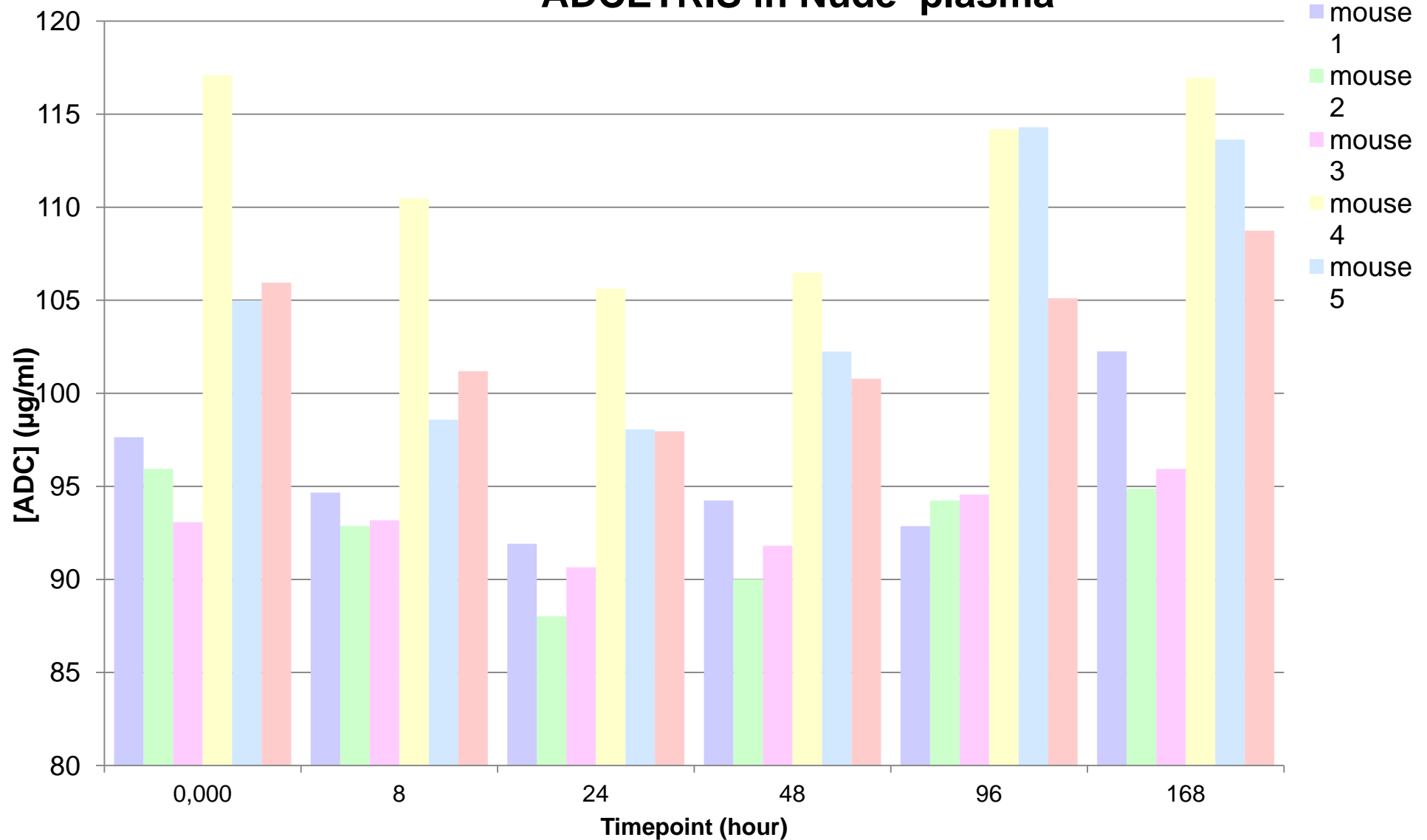
ADC stability
ELISA

Free MMAE TQ
Quantification

ADC spiking
in nude plasma mice
Final concentration 100 µg/ml

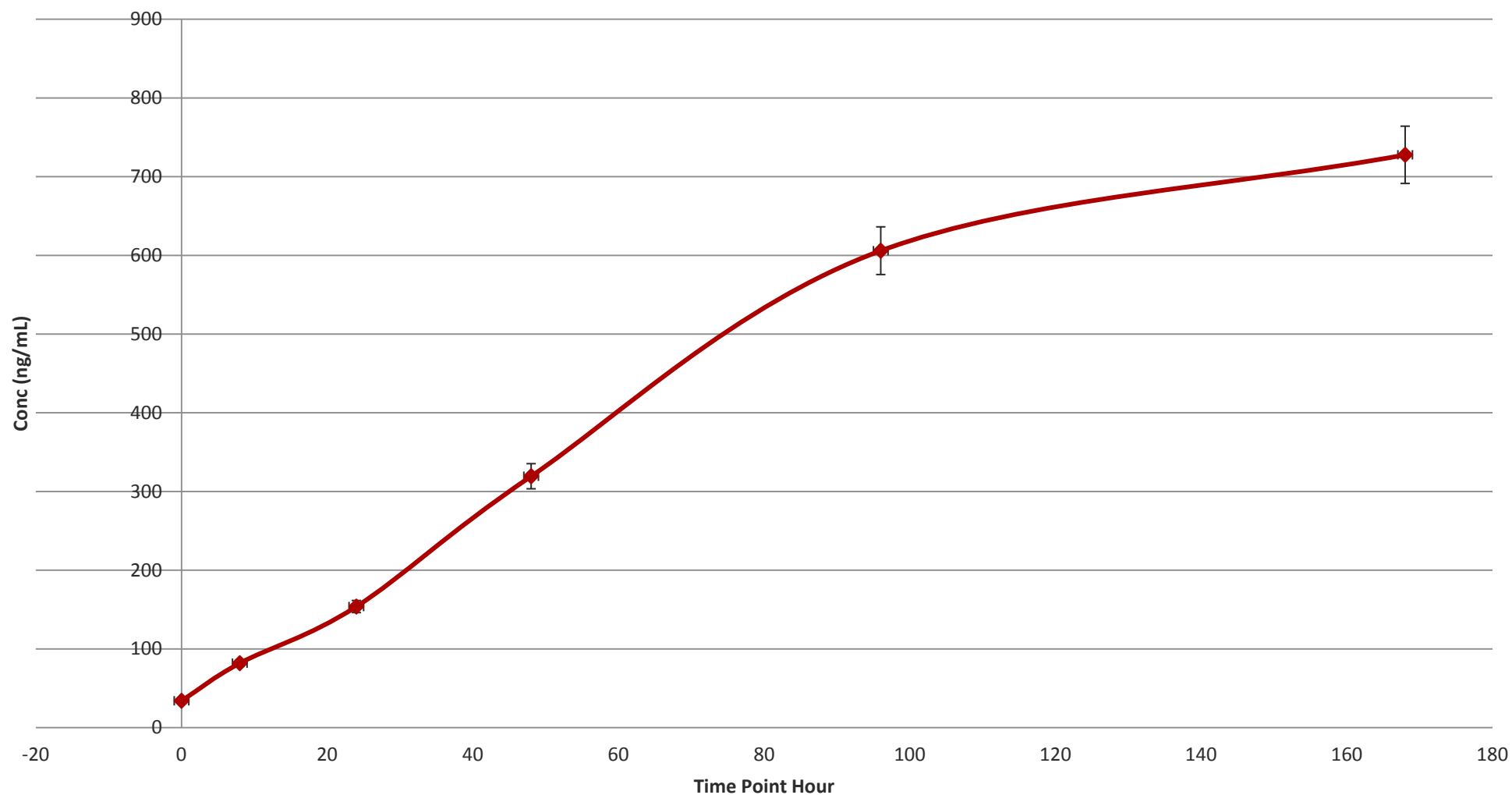
Results Ex vivo Free MMAE

ADCETRIS in Nude plasma

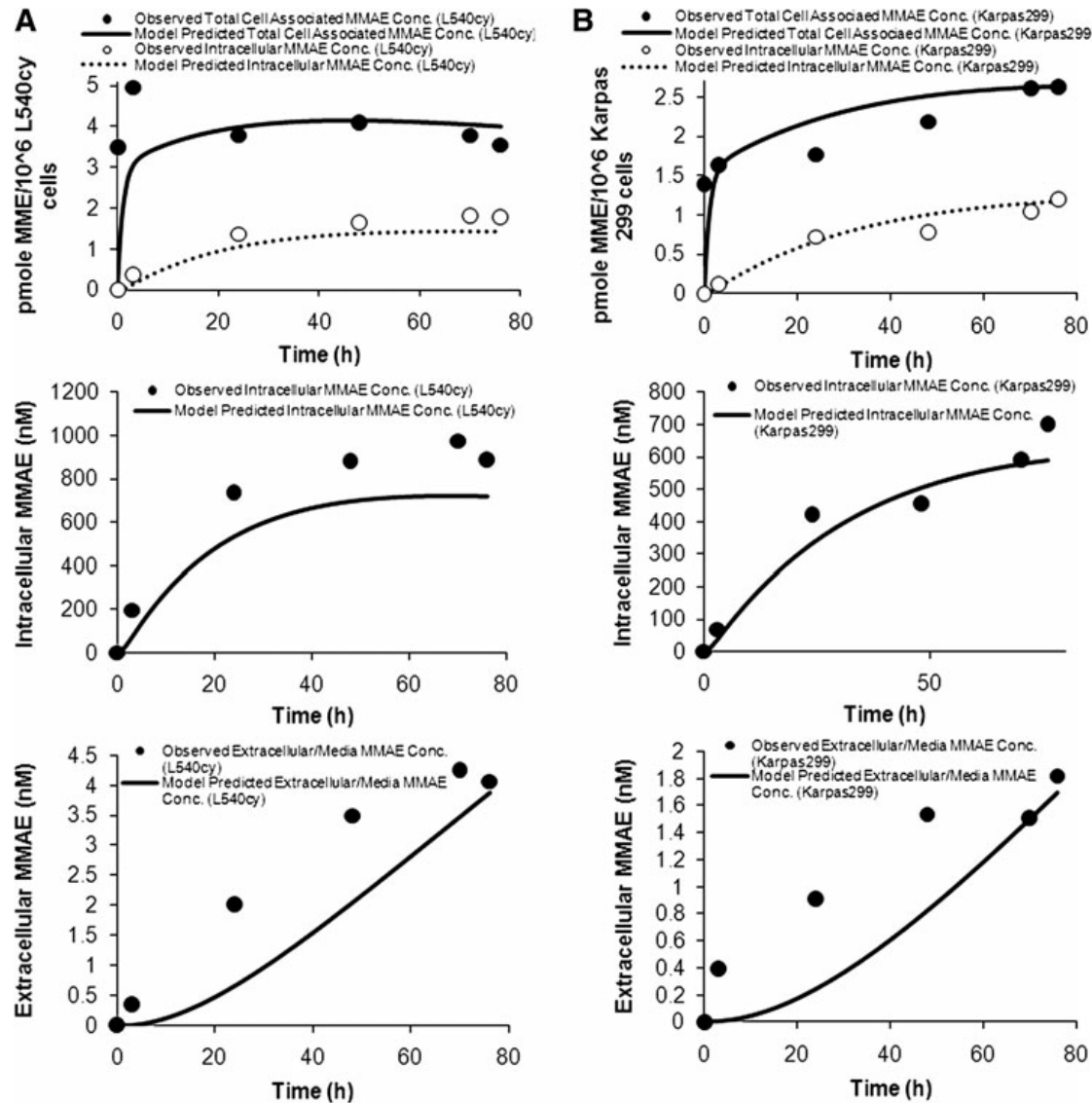


Results Ex vivo Free MMAE

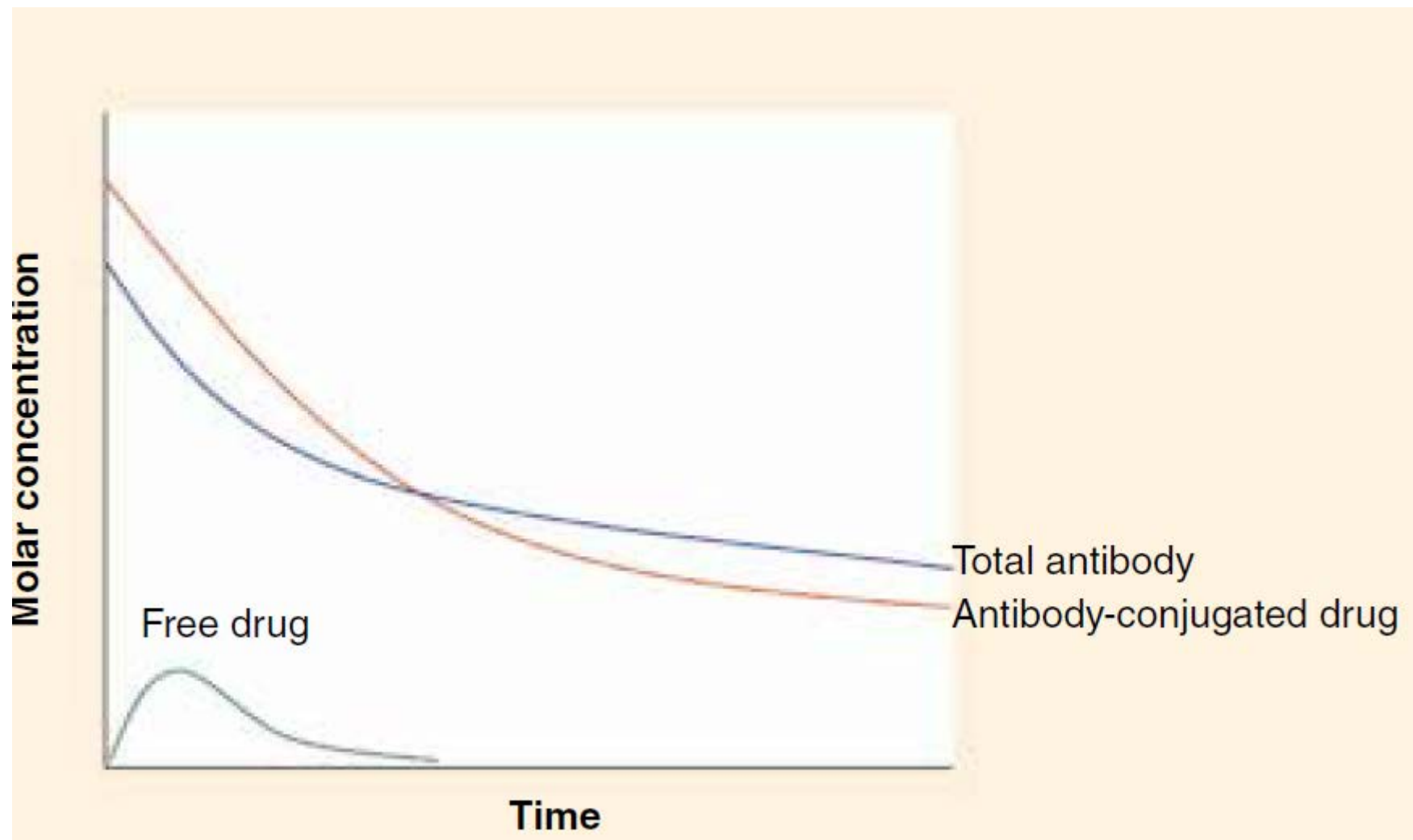
Free MMAE ADCETRIS *ex vivo*



Results In Vivo Free MMAE



Results In Vivo Free MMAE



Results In Vivo Free MMAE



ADCETRIS injection
2 conditions
100 μ g (5mg/kg)
300 μ g (15mg/kg)
Intravenous
100 μ l max injected/mouse

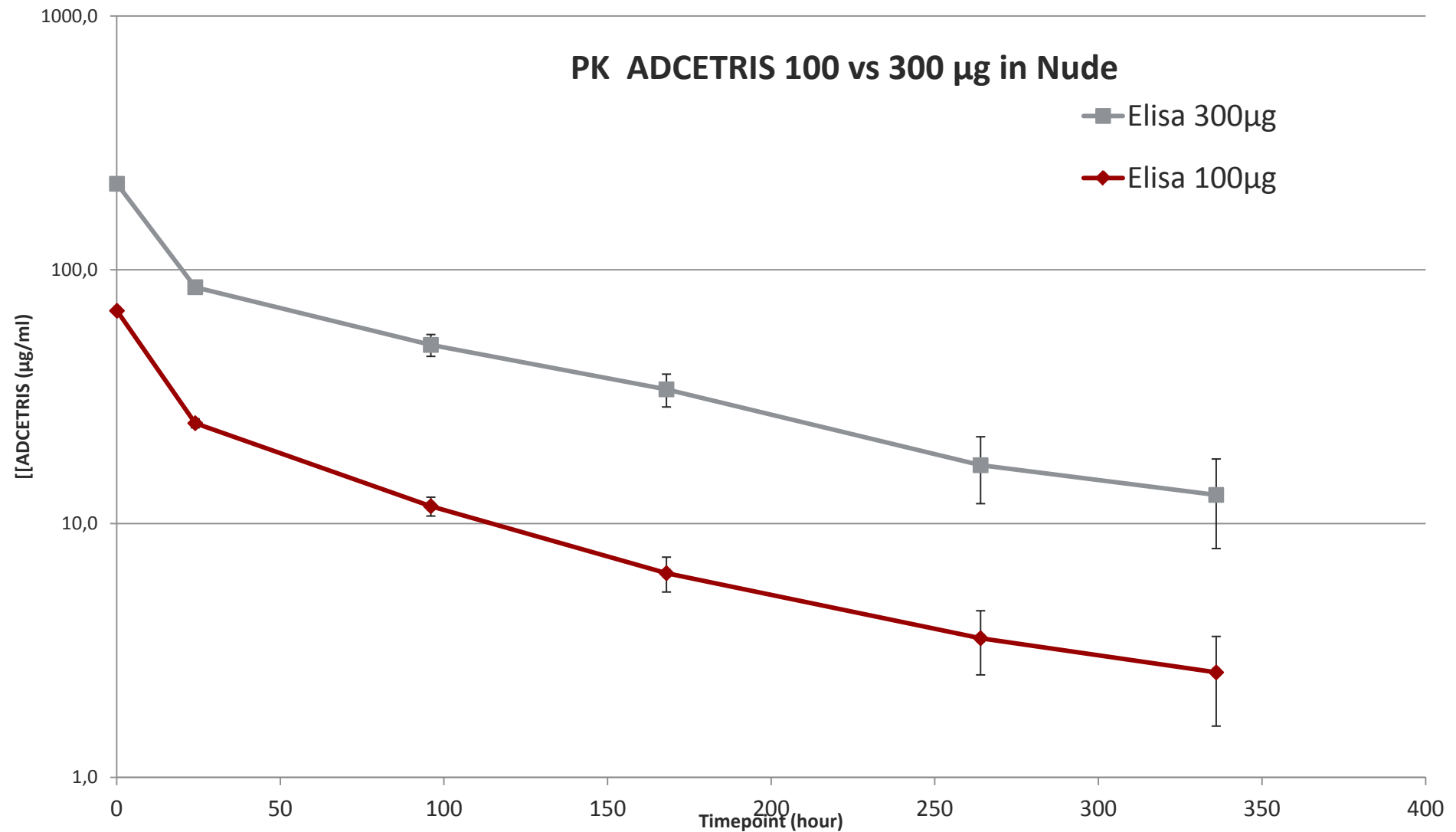


Different time point
5 minutes
24 hours
4 Days
7 Days
11 Days
14 Days

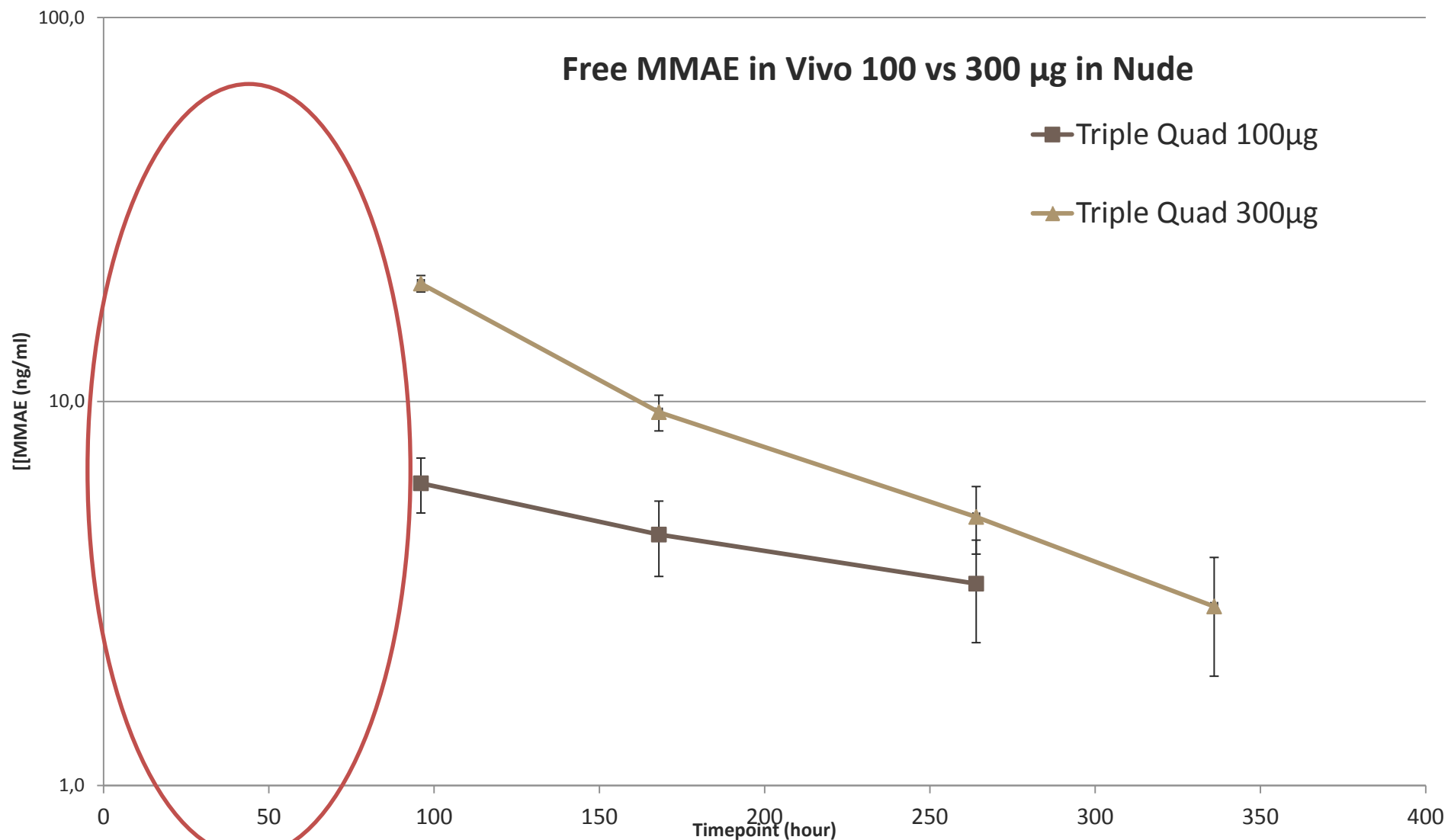
ELISA assay
5 minutes
24 hours
4 Days
7 Days
11 Days
14 Days

Free MMAE TQ
4 Days
7 Days
11 Days
14 Days

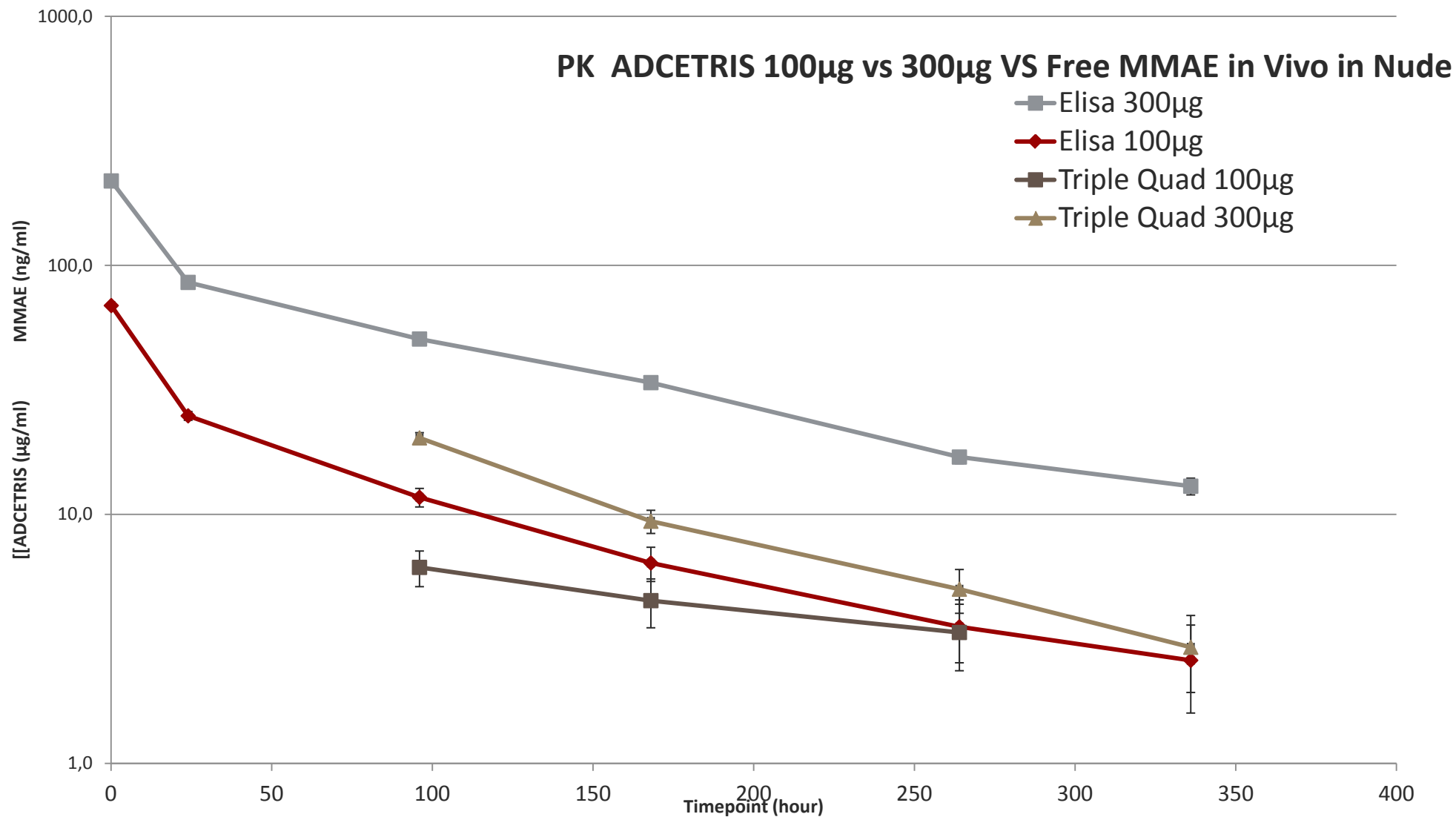
Results In Vivo Free MMAE



Results In Vivo Free MMAE



Results In Vivo Free MMAE



Conclusion

- LC-MS/MS is an advantageous technique for Free MMAE quantification
- Fast sample preparation
- Good Sensitivity PK analysis
- Alternative technique to determine DAR

Acknowledgements



- Daniel Lafitte
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