Microdosing with LC-MS Analysis: Variations on the Theme

A. Guenzi, G. Fischer, A. Gloge and B. Lausecker

Regulatory environment

- **The CHMP position paper (23 June 2004)**

"…less than 1/100th of the dose calculated to yield a pharmacological effect of the test substance based on primary pharmacodynamic data obtained *in vitro* and *in vivo* (typically doses in, or below, the low microgram range) and at a maximum dose of ≤ 100 microgram."

- **FDA Guidance for Industry, Investigators, and Reviewers Exploratory IND studies (January 2006)**

"…less than 1/100th of the dose of a test substance calculated (based on animal data) to yield a pharmacologic of the test substance with a maximum dose of ≤ 100 micrograms (for imaging agents, the latter criterion applies)."

Roche has a long-standing interest in microdosing

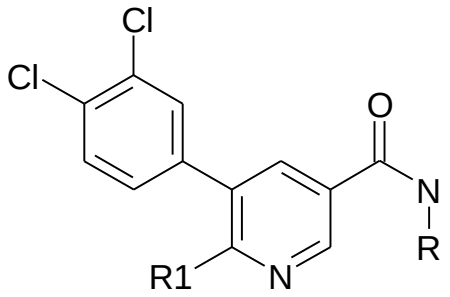
- **Member of CREAM (Consortium for Resourcing and Evaluating AMS Microdosing)**
- **Took part in the first published microdosing experiment[1] in 2005 that used commercially available AMS by providing 2 marketed drugs (diazepam and midazolam)**
- **Microdosing not used for decision making on developability or hypothesis testing, but rather to obtain the maximum information in special PK studies (mass balance, SAD) during normal development**
- **Only microdosing with cold material and LC-MS analysis used so far**

1. G. Lappin et al., *Clin. Pharmacol. Therap.* **2006**, 80, 203-215.

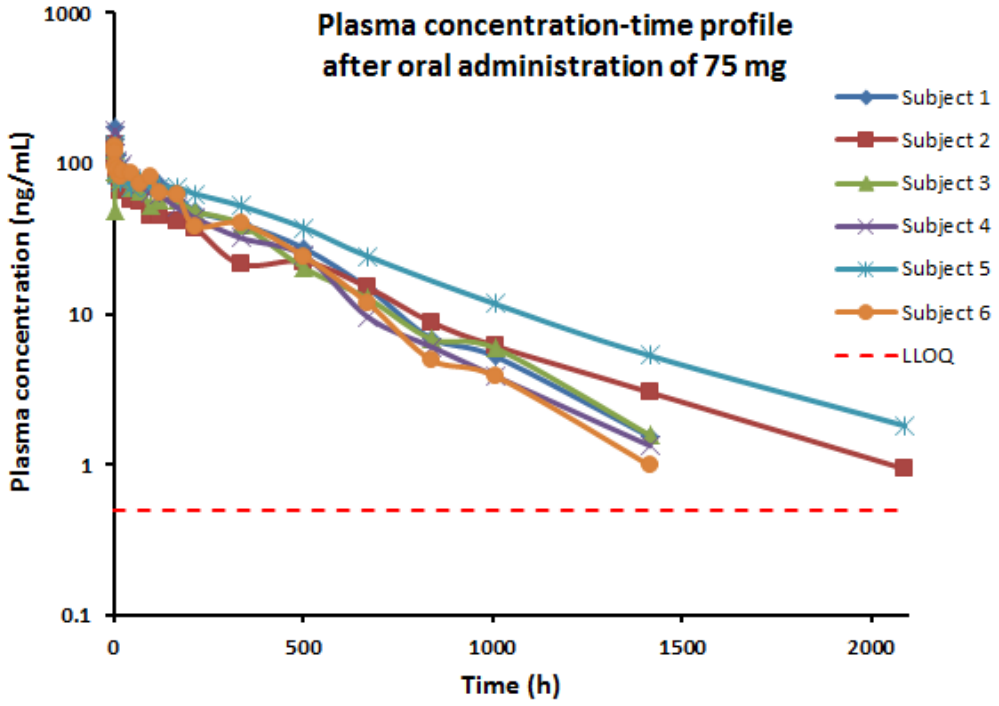
Case study 1

QUANTIFICATION OF AN I.V. MICRODOSE OF A VERY LIPOPHILIC DRUG WITH LONG TERMINAL HALF-LIFE

The drug and its p.o. PK profile



MW 463 amu
Chiral (at R)



Why microdosing?

Question: what is the absolute bioavailability of the compound?

- **The compound is highly insoluble in solvents suitable for i.v. administration**

  5 mL of concentrate solution (containing 2-hydroxypropyl-beta-cyclodextrin and PEG 400 in water) were diluted to 30 mL with 0.9% sodium chloride and infused over 30 minutes

- **The maximum achievable dose was 100 µg**

Consequences for the bioanalytical lab

- **The original method used to analyze the samples after p.o. administration had LLOQ 500 pg/mL**

  The new LLOQ must be 5 pg/mL or lower

- **The original method used protein precipitation followed by column-switching (injection of 0.3 µL plasma equivalents) for sample preparation**

  A more sophisticated sample preparation procedure is needed, and larger volume of plasma equivalent is injected

- **An additional validation is needed for just a few samples**

  Analysts are born to suffer… ☹

Modification of the assay

- **Increase volume of extracted plasma**

  From 10 µL to 250 µL

- **Introduce an off-line cleanup step**

  After addition of the internal standard in 50 µL water-ACN 1:1, apply all the sample to an SPE plate, wash with water and elute with ACN containing formic acid.

- **Increase the volume of plasma equivalents injected**

  From 0.3 to 167 µL (500x increase)

- **Introduce on-line dilution before on-line SPE (column switching)**

  100-µL of 100% organic SPE eluate injected with on-line dilution with water on the trapping column.
  Combined flow-rate to load the sample on the trapping column:
  3 mL/min

Consequences for the bioanalytical lab

- The original method used to anal… amples after p.o. administration had LLO…

  The new LLO… st be 5 pg/mL or lower

- The original method used protein precipit… wed by column-switching (injection of 0.… lents) for sample preparation

  A mor… preparation procedure is need… volume of plasma equivalent is inject…

- An additional validation is ne… r just a few samples

  Analysts are l… ier… ☹

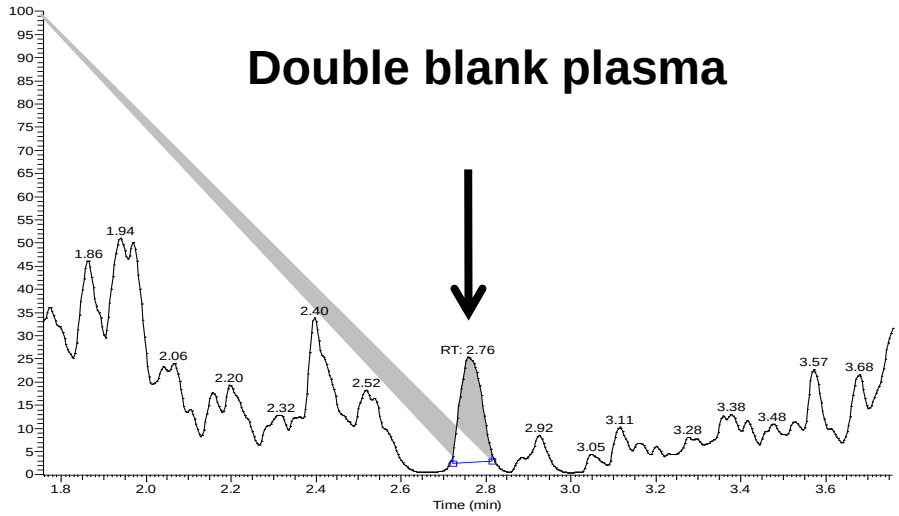
Achieved: 2 pg/mL

PPT+SPE, 500-fold increase in plasma volume equivalent injected

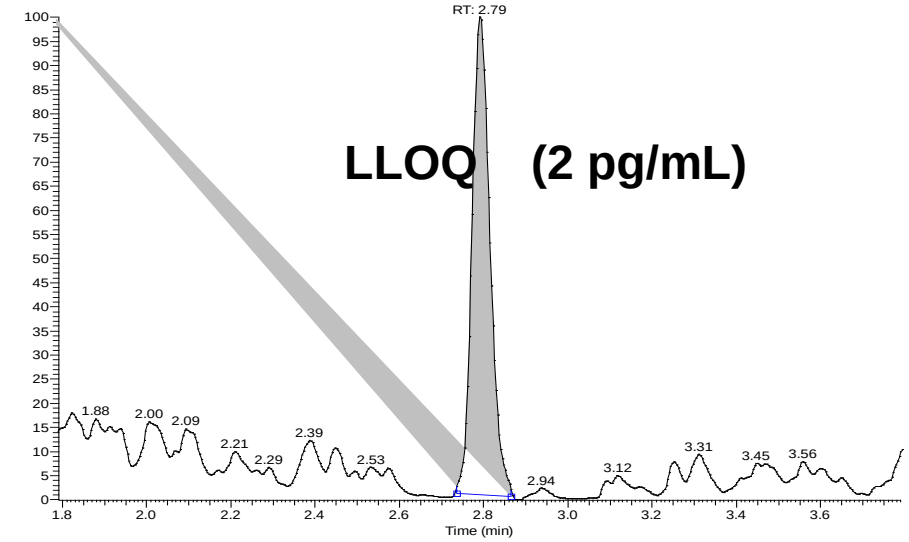
Too bad…

MS20-20110110_A005 - TIC - SM: 5 RT: 1.76 - 3.76 NL: 2.35E2
F: + c ESI SRM ms2 463.000 [347.899-347.901]

Double blank plasma

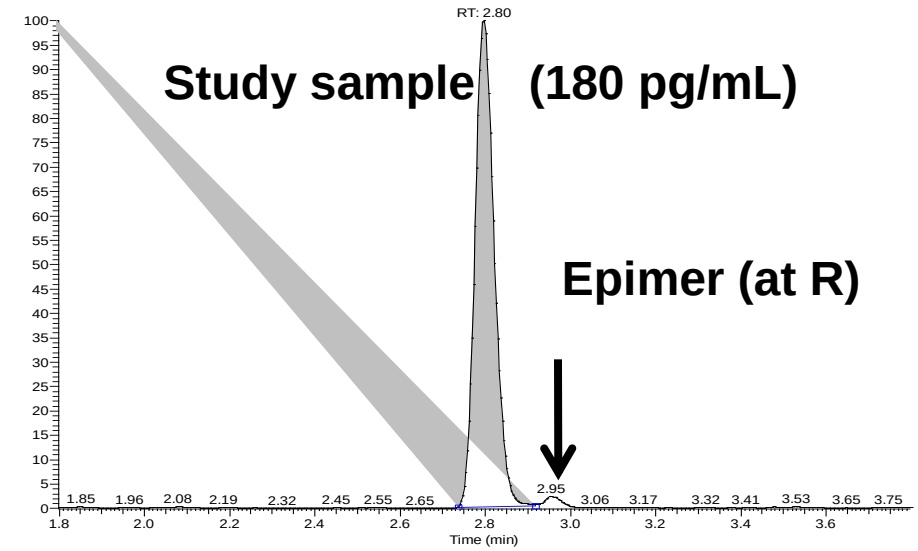


MS20-20110110_A021 - TIC - SM: 5 RT: 1.79 - 3.79 NL: 3.88E2
F: + c ESI SRM ms2 463.000 [347.899-347.901]



LLOQ (2 pg/mL)

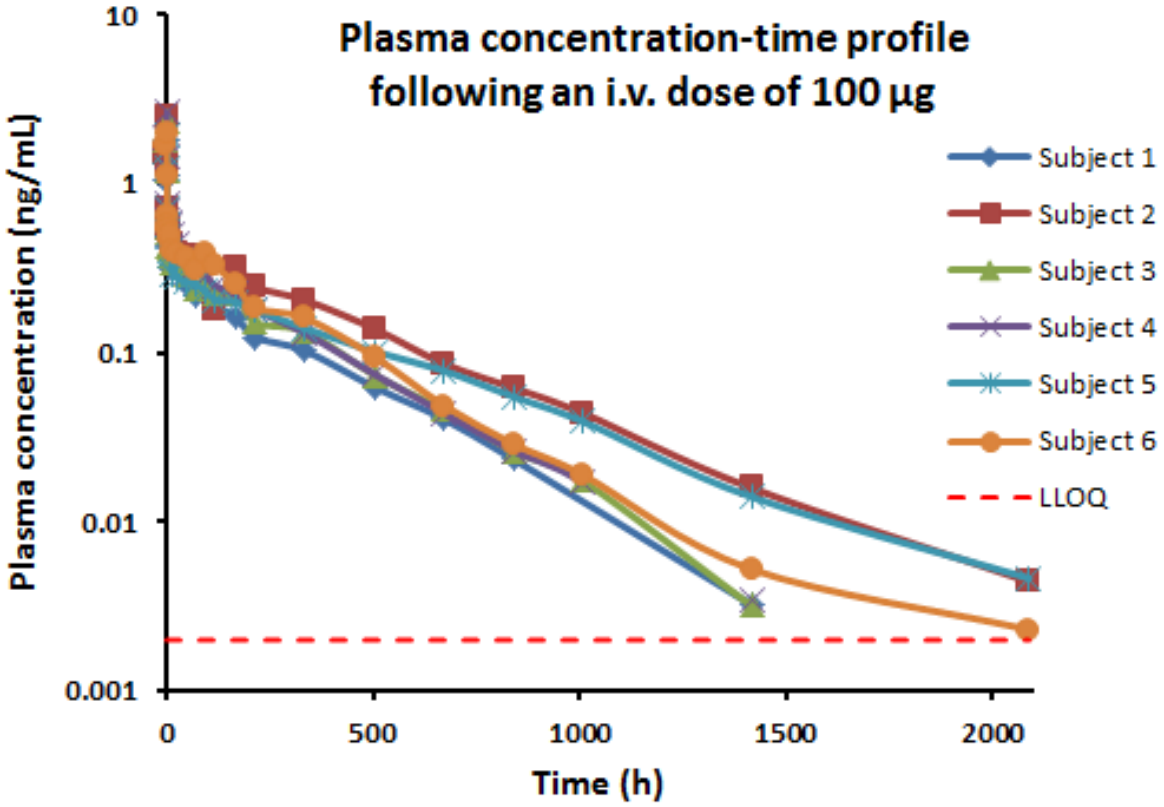
MS20-20110110_A023 - TIC - SM: 5 RT: 1.80 - 3.80 NL: 1.40E4
F: + c ESI SRM ms2 463.000 [347.899-347.901]



Study sample (180 pg/mL)

Epimer (at R)

Results of the microdosing study

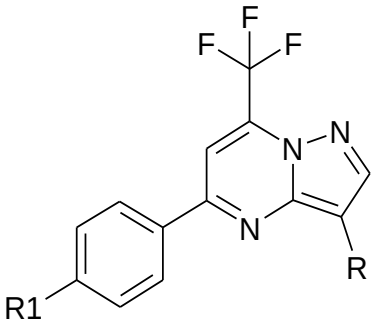


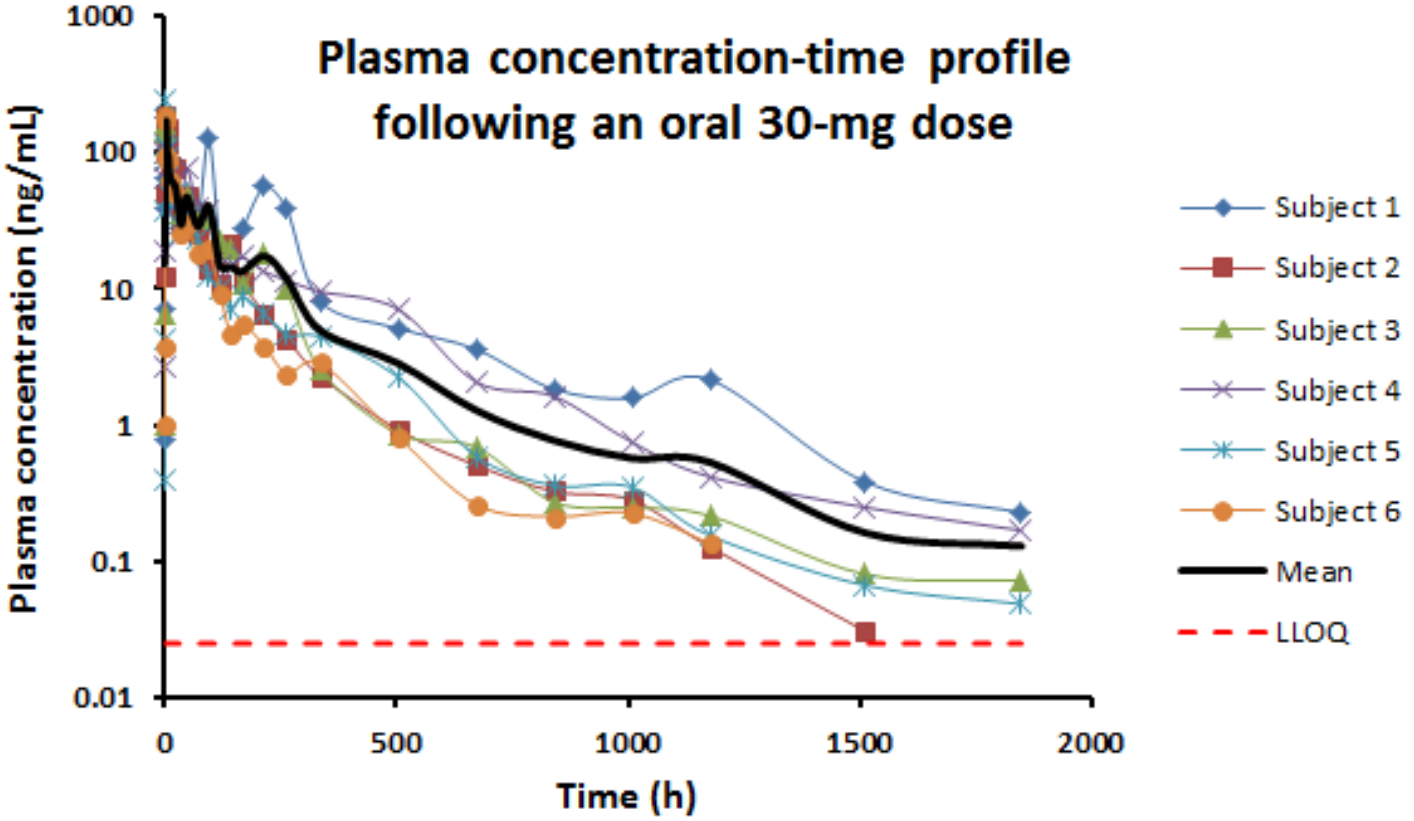
- **Fast initial decline**
- **Comparable elimination phase**
- **Adequate LLOQ**

Case study 2

QUANTIFICATION OF AN I.V. MICRODOSE OF A VERY LIPOPHILIC DRUG WITH LONG TERMINAL HALF-LIFE. CO-ADMINISTRATION OF ^{14}C-DRUG P.O. AND ^{13}C-DRUG I.V. IN THE HUMAN MASS BALANCE STUDY

The drug and its p.o. PK profile





Why microdosing?

Question: what is the absolute bioavailability of the compound?

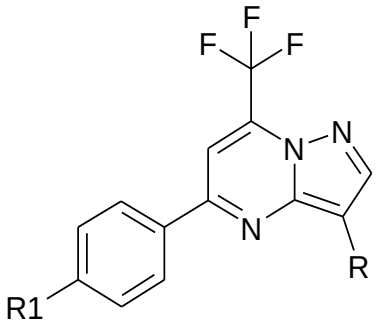
- The compound is highly insoluble in solvents suitable for i.v. administration
- The maximum achievable dose was 100 µg

Study design:

⇨ **p.o. administration of a 25-mg dose of ^{14}C-labeled compound (diluted with ^{12}C-material)**

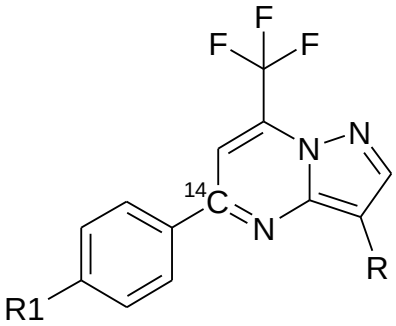
⇨ **i.v. administration of 100 µg ^{13}C-labeled (6 times) compound 4 hours later**

Analytes and ISTDs



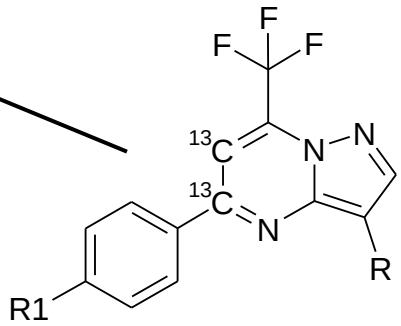
448 → 408 m/z
10 parts

+



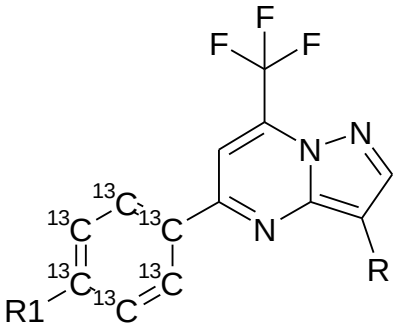
450 → 410 m/z
1 part

→ Given p.o.



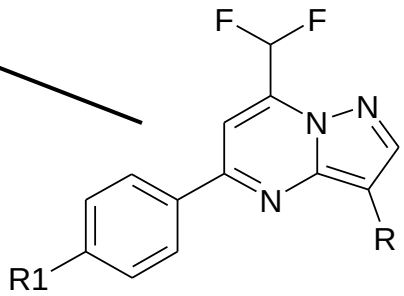
(contains 3 ^{2}H atoms)

ISTD for the p.o. assay
453→413 m/z



454 → 414 m/z

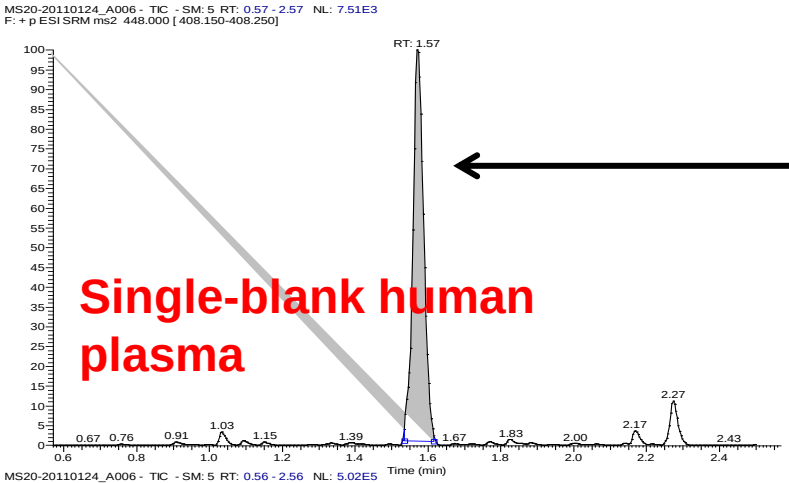
→ Given i.v.



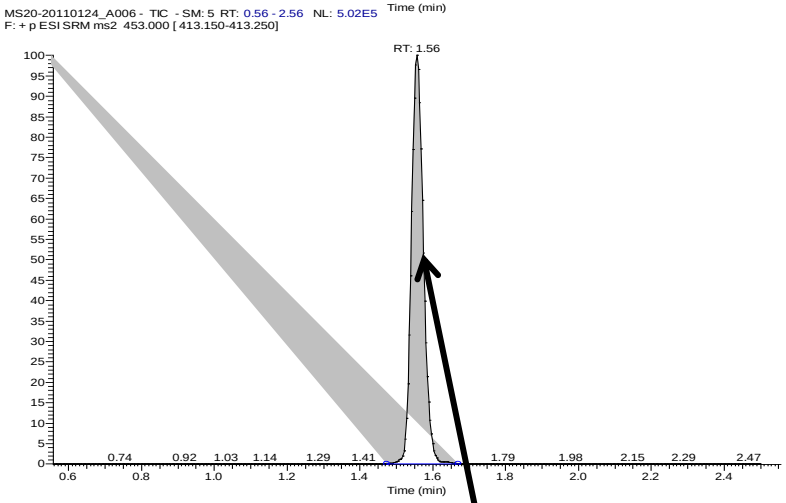
ISTD for the i.v. assay
m/z 444 → 171

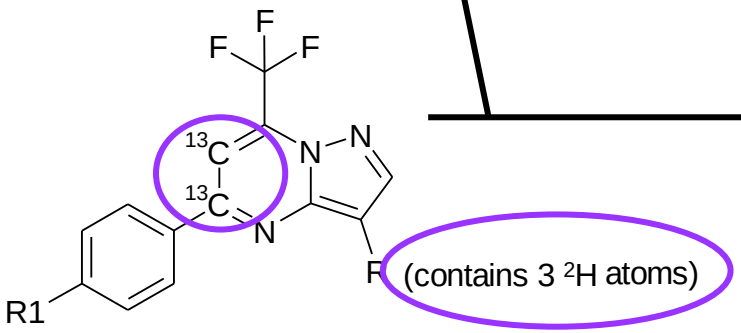
Consequences for the bioanalytical lab

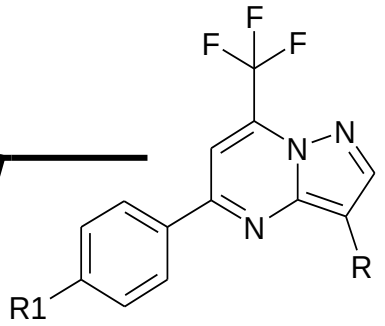
- **The original method is sufficiently sensitive (it was developed for the SAD study with a LLOQ of 25 pg/mL)**
- **An additional full validation is needed for the i.v. dosing (^{13}C-labeled material is the new analyte and a new ISTD is used) with a LLOQ of 10 pg/mL**

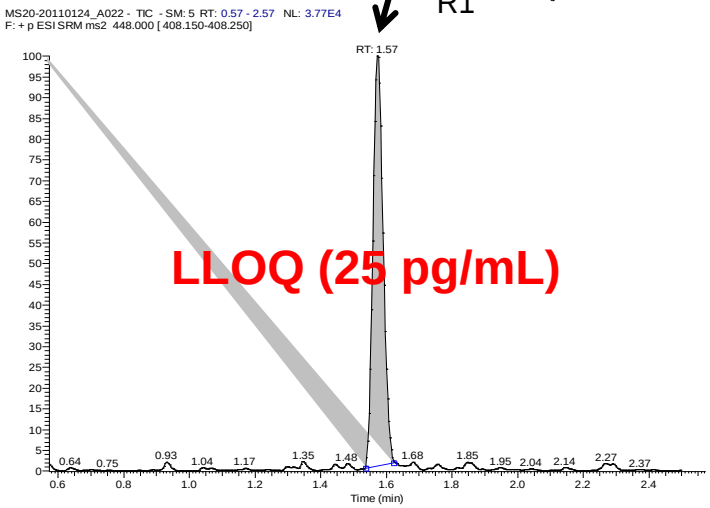


Single-blank human plasma

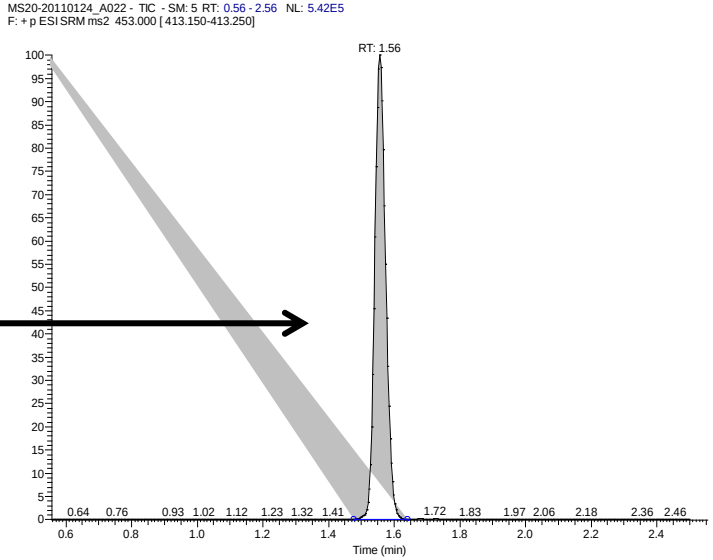




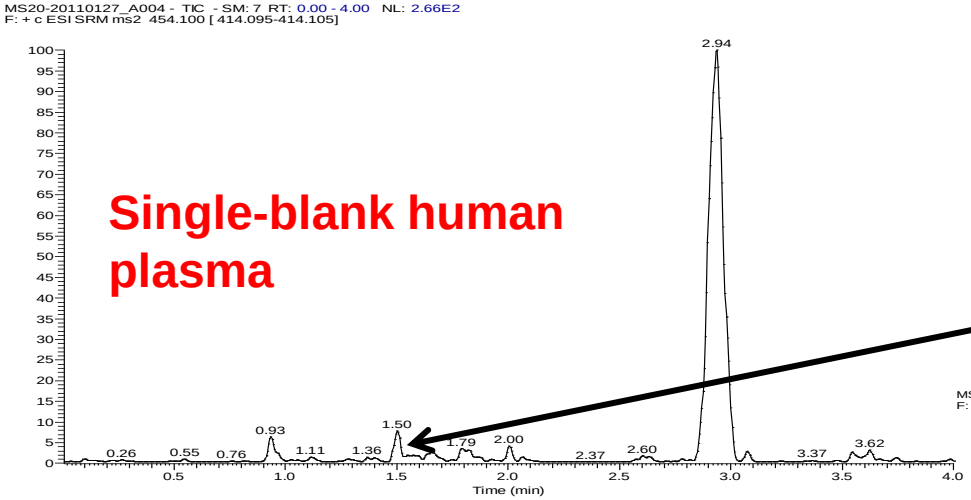




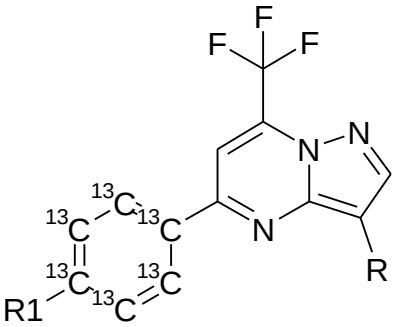
LLOQ (25 pg/mL)

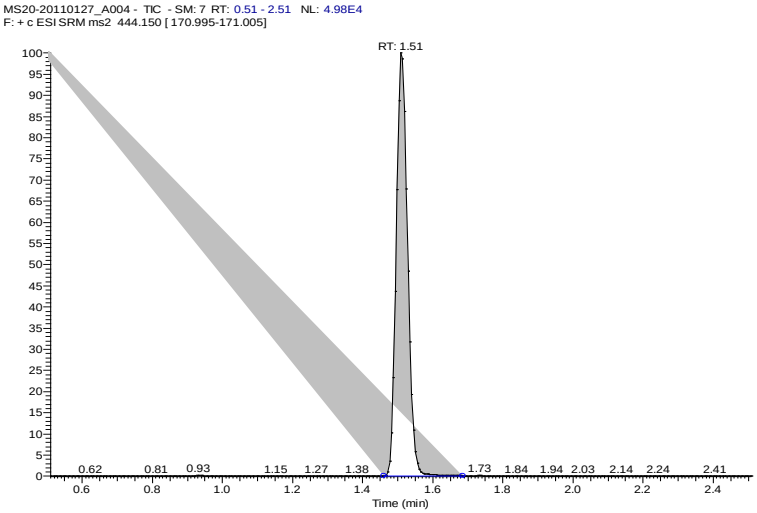


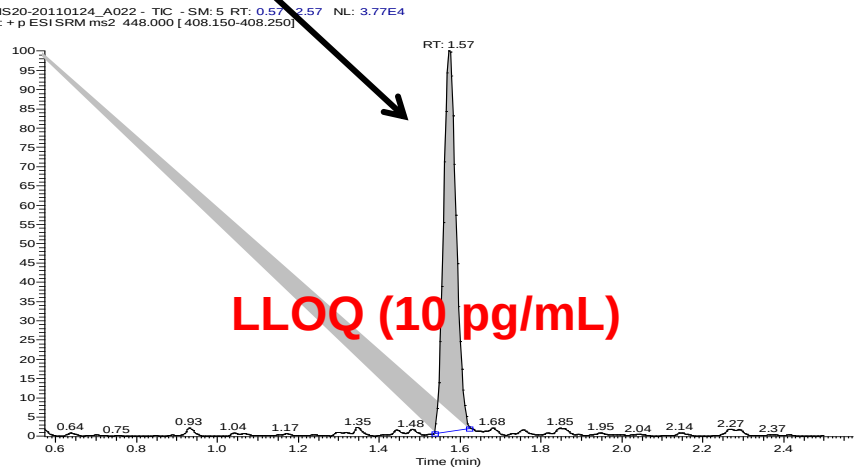
Assay for p.o. samples



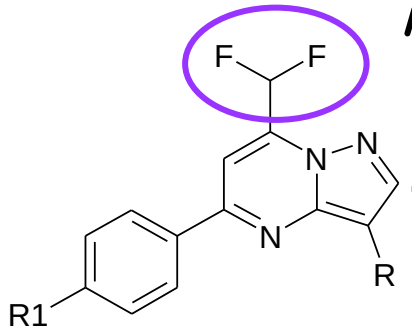
Single-blank human plasma

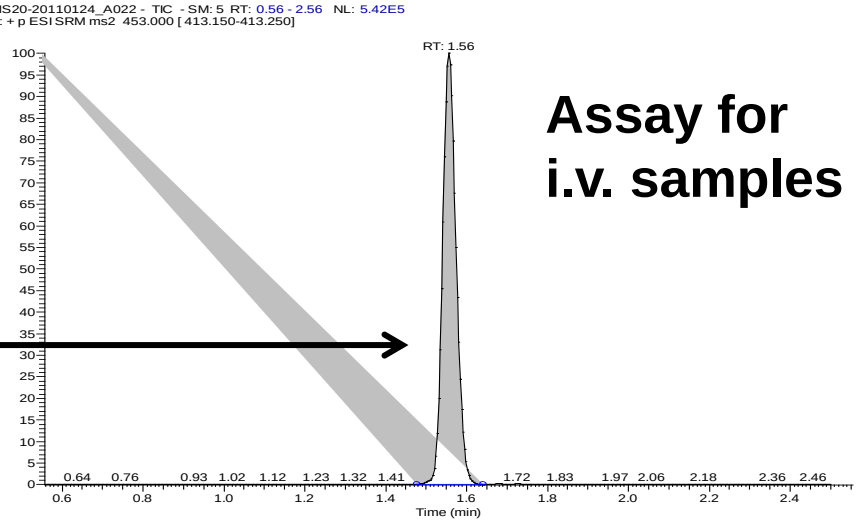






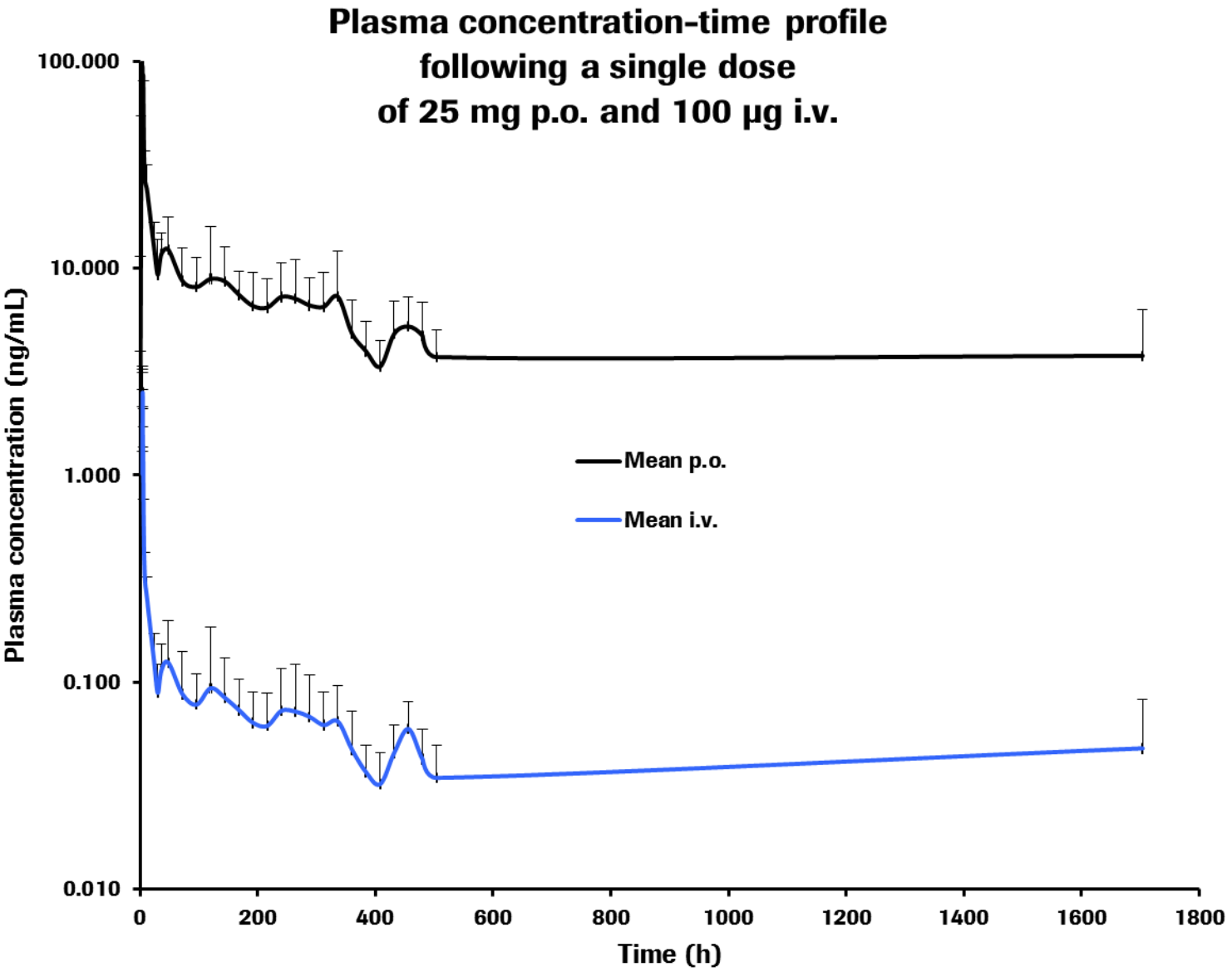
LLOQ (10 pg/mL)





Assay for i.v. samples

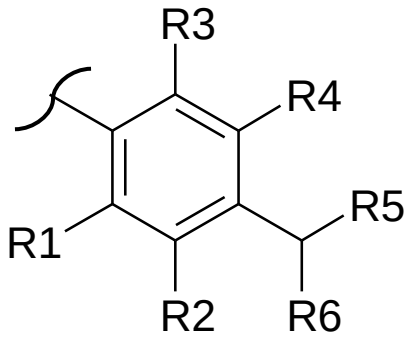
Results of the mass balance study



Case study 3

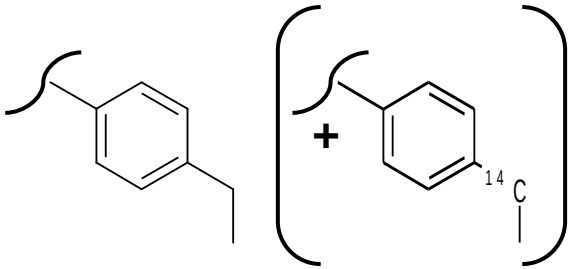
QUANTIFICATION OF AN I.V. MICRODOSE OF A DRUG (AND TWO METABOLITES) IN THE HUMAN MASS BALANCE STUDY

The drug and its metabolites

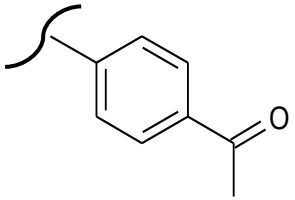


Compound	R1	R2	R3	R4	R5	R6	MW (amu)
Drug	H	H	H	H	H	CH_3	386.4
Drug ISTD	^{2}H	^{2}H	^{2}H	^{2}H	H	CH_3	390.4
M1	H	H	H	H	=O	CH_3	400.4
M1 ISTD	^{2}H	^{2}H	^{2}H	^{2}H	=O	CH_3	404.4
M2	H	H	H	H	H	COOH	416.4
M2 ISTD	^{2}H	^{2}H	^{2}H	^{2}H	H	COOH	420.4
$^{13}C_6$-Drug	H	H	H	H	H	CH_3	392.4
$^{13}C_6$-Drug	^{2}H	^{2}H	^{2}H	^{2}H	=O	CH_3	404.4

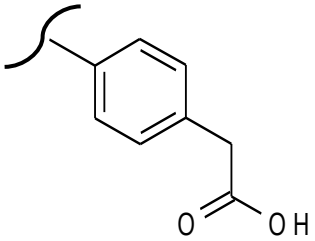
Analytes and ISTDs



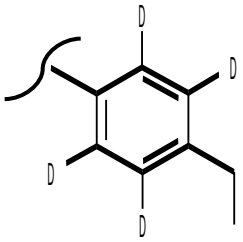
Drug



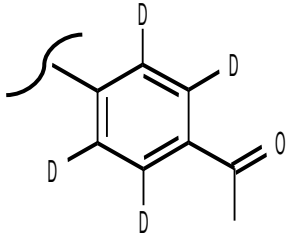
M1



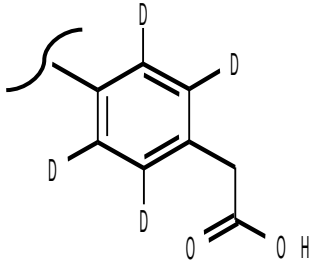
M2



ISTD for drug

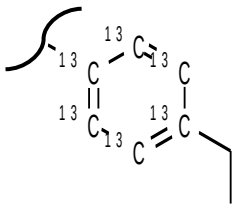


ISTD for M1

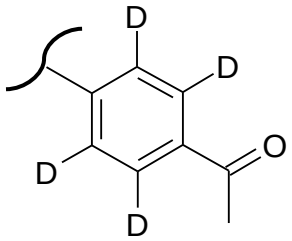


ISTD for M2

p.o. administration



Drug



ISTD for drug

concomitant i.v. administration

Why microdosing?

Question: what is the absolute bioavailability of the compound?

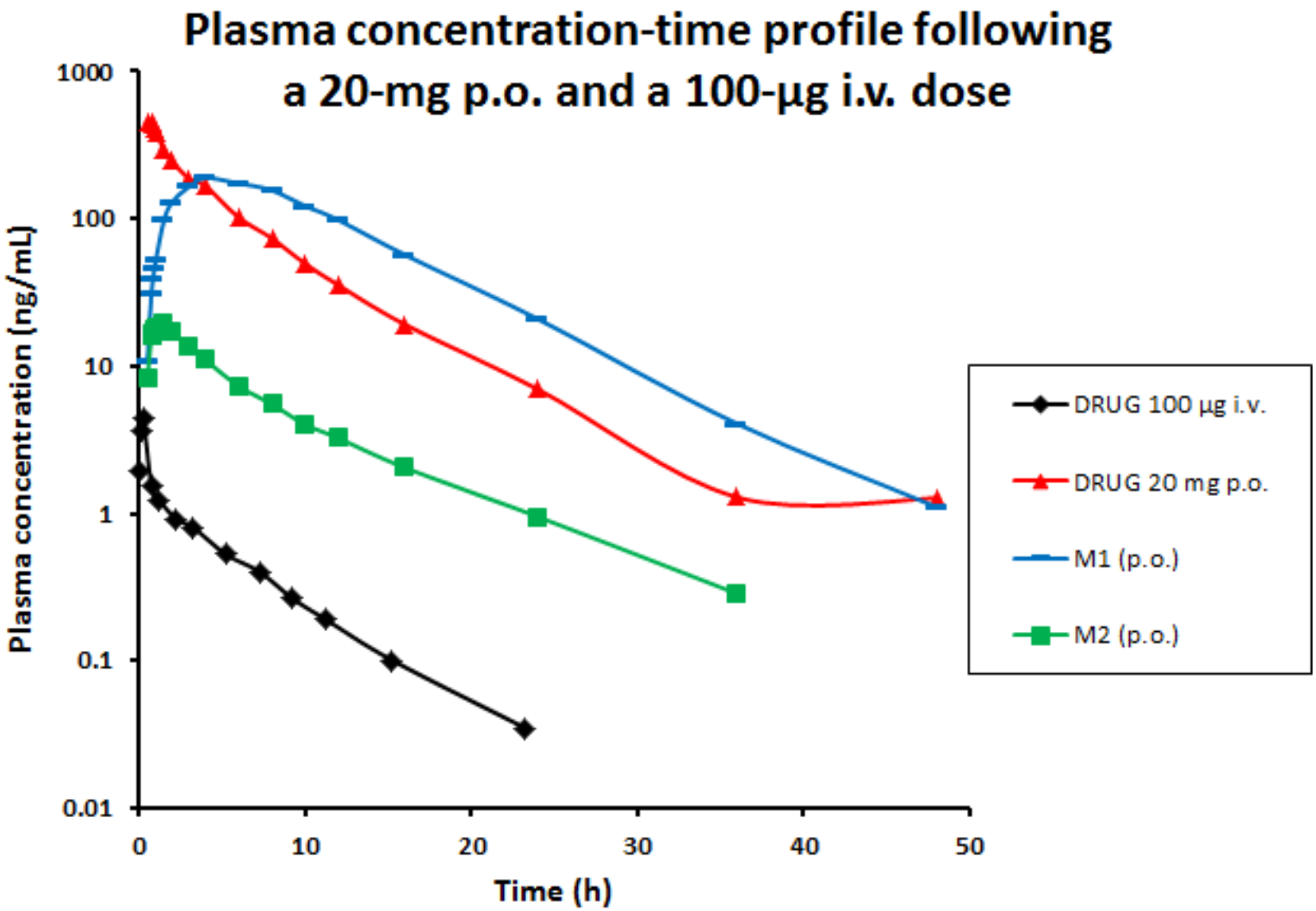
Study design:

- ⇨ **p.o. administration of a 20-mg dose of ^{14}C-labeled compound (diluted with ^{12}C-material)**
- ⇨ **i.v. infusion over 15 min of 100 µg ^{13}C-labeled (6 times) compound 45 min later**

Consequences for the bioanalytical lab

- **The original method for the parent (^{12}C) and metabolites is sufficiently sensitive (it was developed for the SAD study with a LLOQ of 200 pg/mL)**
- **An additional full validation is needed for the i.v. dosing ($^{13}C_6$-labeled material with a different ISTD); needed LLOQ 10 pg/mL**
- **Changes to the original method to achieve LLOQ of 10 pg/mL:**
  - **300 µL plasma instead of 100 µL**
  - **SPE used instead of protein precipitation**
  - **Injection of 200-µL equivalent of plasma instead of 25 µL**
  - **Use of a more sensitive instrument (Thermo TSQ Vantage instead of Sciex API4000)**

Results of the mass balance study



Validation approach

- **Ultra-sensitive LC-MS methods for application to microdosing are validated according to FDA guidance and Crystal City 3.**
- **The usual parameters (precision, accuracy, selectivity etc.) are all addressed**
- **The only shortcut taken is that long-term stability is not performed, since it is assumed that LTS for the ^{12}C drug could be extrapolated to the ^{13}C drug. All other stabilities are done.**

  Rationale:
  - Same chemical structure
  - Stable isotopes

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