

Pharmacokinetic Profile of Sultiame in Healthy Volunteers with In Vitro Characterization of Its Uptake by Red Blood Cells



Kim Dao¹, Paul Thoueille¹, Laurent Arthur Decosterd¹, Thomas Mercier¹, Monia Guidi^{1,2}, Carine Bardinet¹, Eva Choong¹, Arnaud Castang³, Catherine Guittet³, Luc-André Granier³, Thierry Buclin¹



¹Service of Clinical Pharmacology, Lausanne University Hospital (CHUV), Lausanne, Switzerland; ²School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland; ³Advicenne Pharma SA, Nîmes, France

Introduction & Objectives

- Sultiame, an antiepileptic carbonic anhydrase inhibitor, is recommended in some countries as first-line treatment for benign childhood epilepsy with centro-temporal spikes¹

- Its pharmacokinetic profile has been scarcely studied^{2,3}

Objectives:

- Primary aim:** assess sultiame's PK profile, in order to design an appropriate paediatric formulation
- Secondary aim:** characterize sultiame's exchanges between plasma and erythrocytes through *in vitro* spiking experiments

Materials & Methods

Subjects

- 4 healthy male volunteers
- 25 ± 3 years [22 - 28 years]
- weight 69 ± 2 kg [65 - 71 kg]

Study design

- Single oral doses of 50, 100 and 200 mg of sultiame (Ospolot®) (fasting conditions)
- Open-label, during periods 1 (50 mg), 2 (100 mg) and 3 (200 mg) at 4-6 weeks interval

Analytical method

- Validated HPLC-MS/MS with protein precipitation by methanol
- Isotopically stable internal standard (d4-sultiame)
- LL0D/LL0Q: 0.001/0.05 µg/mL
- CV (imprecision): -1.9; +11.7 %
- No qualitative matrix effect

PK samples

- Plasma and whole blood samples: pre-dose, then at 15, 30 min, 1, 2, 3, 4, 5, 6, 8, 10, 24, 48, 72, 168, 336 and 504 h post-dose + haematocrit (Ht)
- Urine: pre-dose, from 0-2, 2-4, 4-8, 8-10 and 10 to 24 h post-dose

In vitro assays

Influx:

- whole blood (EDTA): sultiame spiked at 1, 5, 10, 20, 40, 80 mg/L

Efflux:

- whole blood (incubation 1h) at 10, 40 mg/L (37°C) + washing (NaCl 0.9%) + equilibrate with native plasma

Competition:

- zonisamide + topiramate (equimolar concentration⁴)

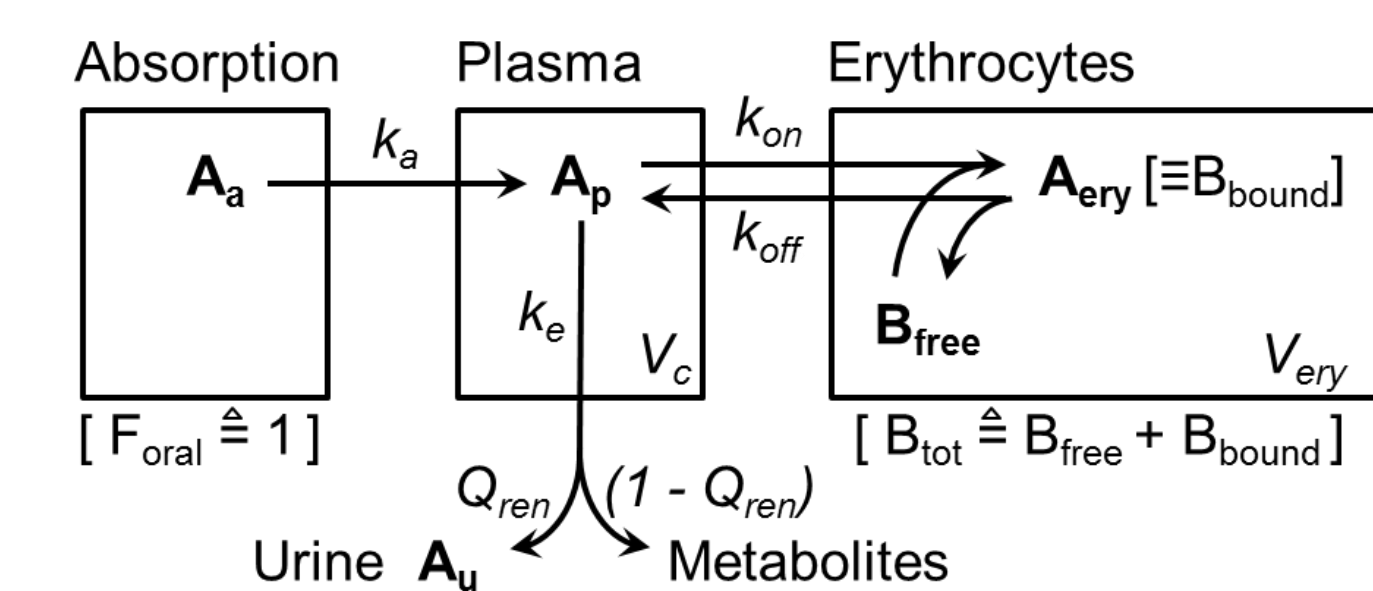
PK parameters

A) Noncompartmental analysis (STATA 13®)

- Erythrocytes concentrations deduced as:
$$C_{ery} = \frac{C_{whole\ blood} - C_{plasma} \cdot (1 - Ht)}{Ht}$$
- AUC_{0-inf}: log trapezoidal rule (extrapolation to infinity)
- λ_z : derived from log-linear regression
- CL/F = dose/AUC_{0-inf}
- $t_{1/2} = \ln(2)/\lambda_z$

B) Compartmental analysis (NONMEM®):

Model:



Abbreviations:
Ery: erythrocytes
Foral: bioavailability
CL_{plasma}: plasma clearance as $k_e = CL_{plasma}/V_c$
V_c: central volume of distribution
V_{ery}: cell volume of distribution
k_a: constant of absorption
k_{on}: association constant
k_{off}: dissociation constant
B_{tot}: ligand maximal binding capacity
B_{free}: free binding sites for sultiame
B_{bound}: amount of binding site occupied by sultiame
Q_{ren}: fraction eliminated by renal route;

Model development:

Differential equations to account for amounts of sultiame (mg):

1) In the absorption site (A_a): $dA_a/dt = -k_a \cdot A_a$ with A_a = Dose at t = 0
[k_a fixed to 1 h⁻¹ (data insufficient to describe absorption)]

2) In the central compartment (plasma A_p):
 $dA_p/dt = k_a \cdot A_a - k_e \cdot A_p - k_{on} \cdot A_p \cdot (B_{tot} - A_{ery}) + k_{off} \cdot A_{ery}$ with A_p = 0 at t = 0
[assumed to be in instantaneous equilibrium with other body fluids where free sultiame distributes]

3) In the cell compartment (A_{ery}):
 $dA_{ery}/dt = k_{on} \cdot A_p \cdot (B_{tot} - A_{ery}) - k_{off} \cdot A_{ery}$ with A_{ery} = 0 at t = 0
[assumed to encompass as well sultiame bound to receptors contained in other cells where A_{ery} expresses the amount of binding sites occupied by sultiame (B_{bound}). Amount of free binding sites corresponds to B_{free} = (B_{tot} - A_{ery})]

4) In urine (A_u): $dA_u/dt = k_e \cdot A_p \cdot Q_{ren}$ with A_u = 0 at t = 0

References

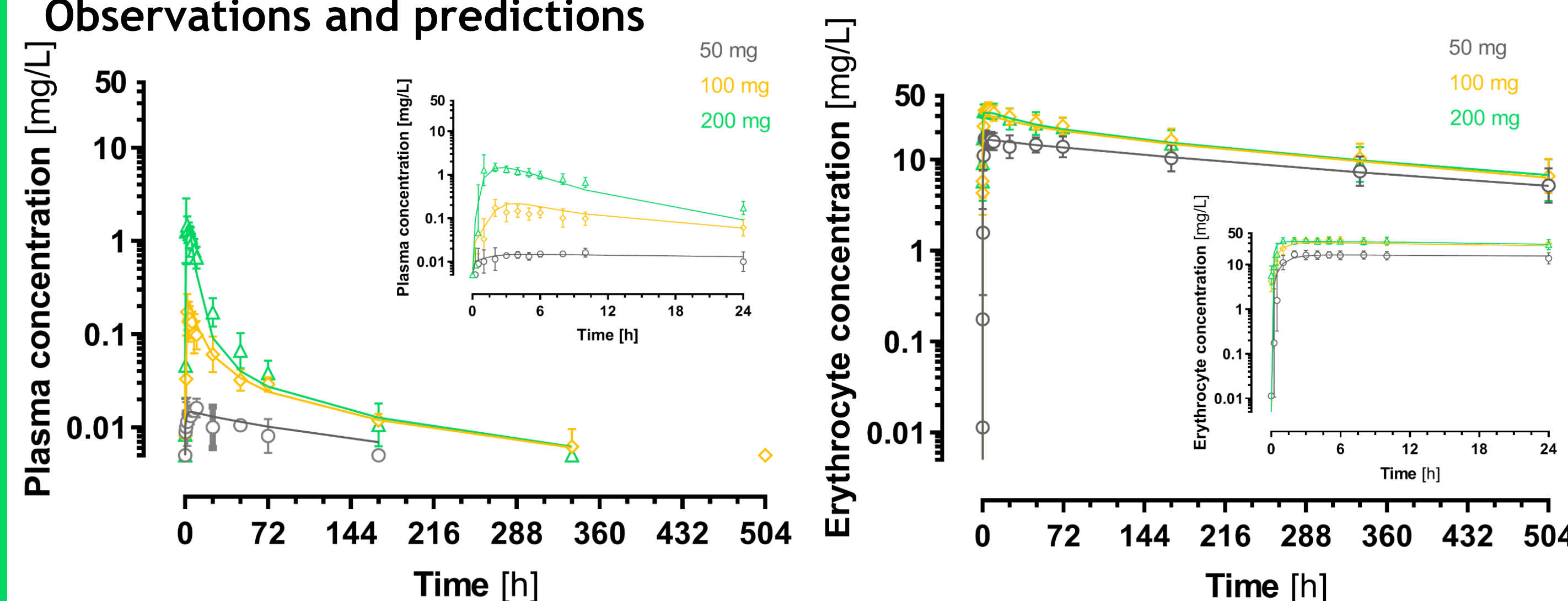
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Results

A) Non-compartmental pharmacokinetic parameters

	Sultiame 50 mg (n = 4)	Sultiame 100 mg (n = 4)	Sultiame 200 mg (n = 4)
Plasma parameters			
C _{max} (µg/mL)	0.02 (16)	0.18 (44)	1.88 (24)
median t _{max} (h) [range]	7.0 [2.0-10.0]	2.0 [2.0-6.1]	1.5 [1.0-2.0]
AUC _(0-inf) (µg·h/mL)	1.24 (63)	8.57 (33)	24.04 (27)
t _{1/2} (h)	50.8 (62)	90.9 (19)	40 (31)
CL/F (L/h)	40.4 (93)	11.7 (25)	8.3 (26)
V/F (L)	2964 (21)	1529 (21)	480 (26)
Whole blood parameters			
C _{max} (µg/mL)	7.91 (17)	15.87 (19)	17.19 (17)
median t _{max} (h) [range]	3.0 [2.0-8.0]	5.01 [5.0-6.0]	2.5 [1.0-3.0]
AUC _(0-inf) (µg·h/mL)	3323 (44)	4535 (39)	4527 (43)
t _{1/2} (h)	313 (23)	233 (16)	253 (21)
CL/F (L/h)	0.015 (37)	0.008 (34)	0.044 (41)
V/F (L)	6.8 (21)	7.4 (21)	16.2 (27)
Urine parameters			
Recovery unchanged (%)	1.61 (17)	5.24 (111)	16.73 (36)
Renal clearance (L/h)	0.17 (30)	0.14 (49)	0.13 (24)

B) Compartmental analysis: Observations and predictions



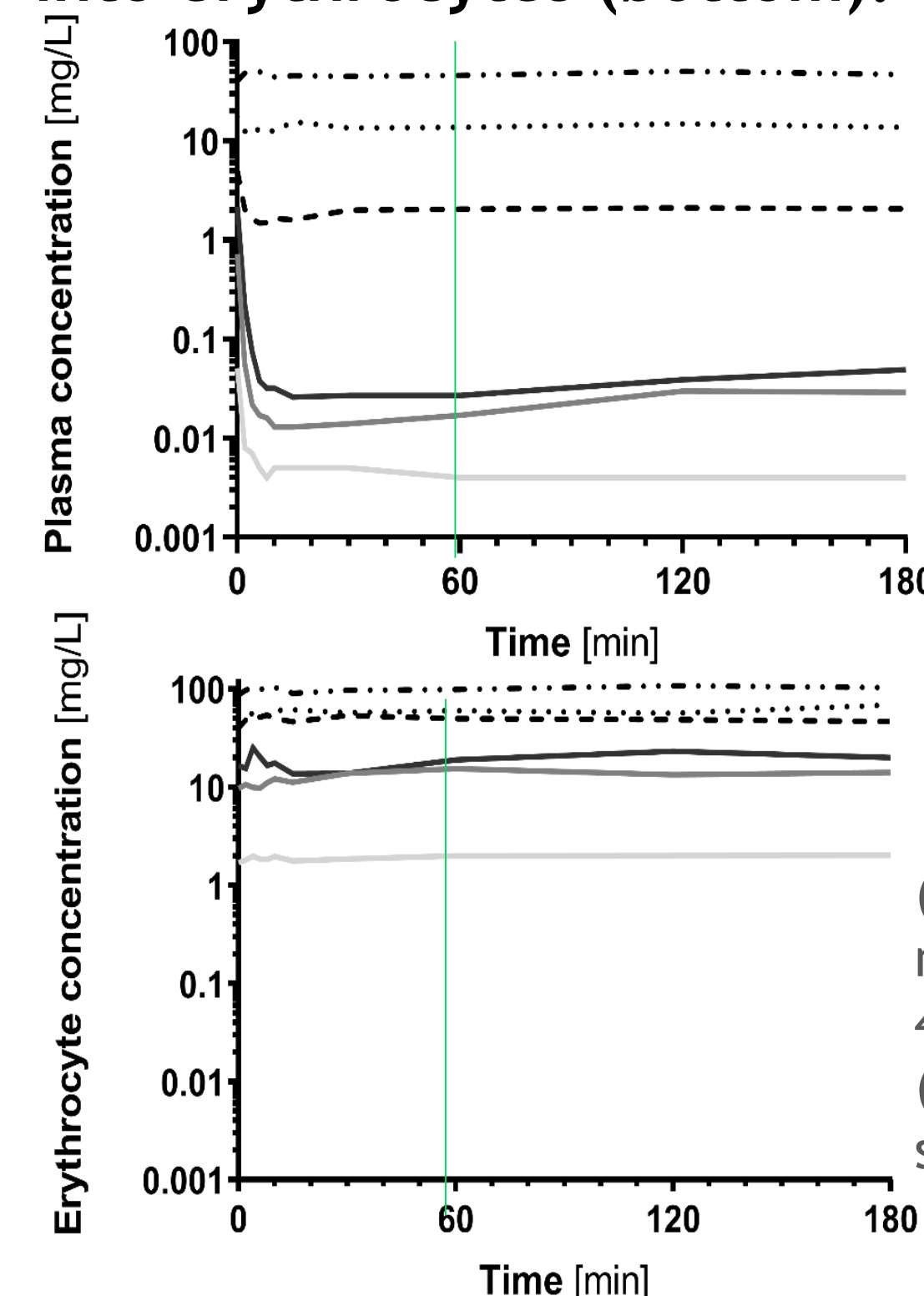
Pharmacokinetic parameters

Parameter	Estimate	RSE(%)	BSV(%)	RSE (%)
CL _{plasma} (L/h)	11	12	28.1	17
V _c (L)	56.3	24	9.3	117
V _{ery} (L)	2.93	6	-	-
k _a (h ⁻¹)	1	-	-	-
k _{on} (h ⁻¹ ·mg ⁻¹)	0.949	26	-	-
k _{off} (h ⁻¹)	0.796	31	-	-
B _{tot} (mg)	97.1	9	12.1	47
Q _{ren} (%)	24.7	15	32.3	132
σ _{prop,p} (CV%)	0.567	15	-	-
σ _{prop,ery} (CV%)	0.263	6	-	-
σ _{add,ery} (CV%)	0.012	6	-	-
σ _{prop,urine} (CV%)	0.433	16	-	-

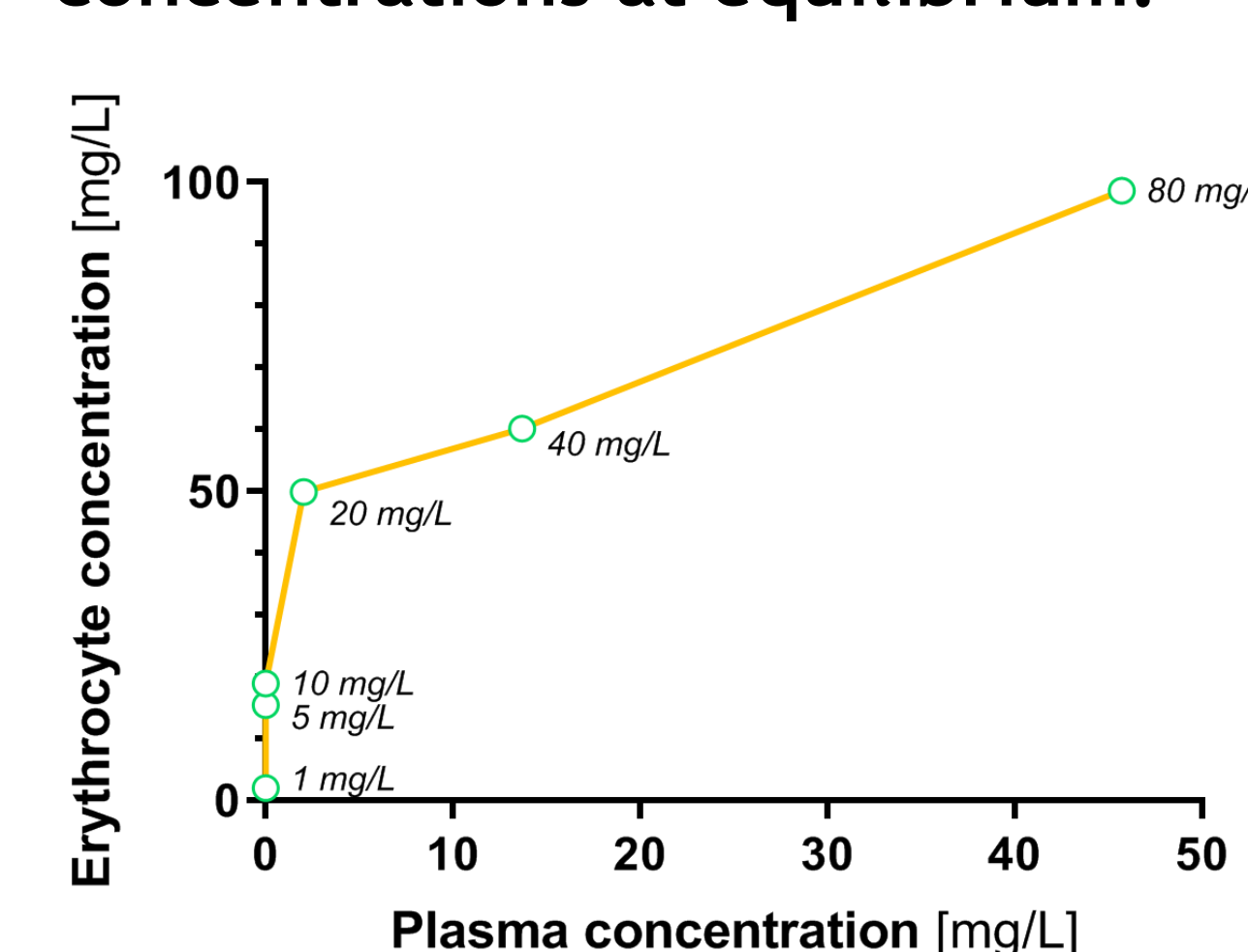
Abbreviations:
σ_{prop}: exponential residual error on plasma (p), erythrocytes (ery) and urine
σ_{add}: additive residual error
RSE: Relative standard error of the estimate, defined as SE(estimate)/estimate, expressed as a percentage, with SE(estimate) retrieved directly from the NONMEM output file
BSV: Between-subject variability

In vitro:

Influx of sultiame from plasma (top) into erythrocytes (bottom):



Correlation of erythrocyte/plasma concentrations at equilibrium:



(left side) at target concentrations of 1 mg/L (light grey), 5 mg/L (dark grey), 10 mg/L (black), 20 mg/L (dashed line), 40 mg/L (dotted line) and 80 mg/L (dashed-dotted line) (right side): correlation of concentrations according to the spiked concentrations at 60 min

Conclusions

- Sultiame displays non-linear distribution and readily concentrates into erythrocytes, probably due to a strong affinity for intracellular carbonic anhydrases
- In vitro* studies confirm saturable uptake by erythrocytes
- A three-compartment model with a ligand to receptor component adequately describes this non-linear distribution and allows estimating sultiame's clearance and volume of distribution
- Further studies and therapeutic monitoring interpretation should take this peculiarity into account