

Pharmacokinetics and bioavailability  
derived from various body fluids

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Saliva samples instead of plasma samples

Willi Cawello, Schwarz BioSciences, Monheim am Rhein

- Introduction
- Sampling tissues/fluids to characterize PK
- Model of saliva sampling
- Examples
- Discussion, Conclusion / Perspectives

- Regulatory Definition (21 CFR 320.1(a)):

“**Bioavailability** means the **rate and extent** to which the active ingredient or active moiety is absorbed from a **drug** product and **becomes available** at the **site of action**.”

- Regulatory Definition (21 CFR 320.1(e)):

“**Bioequivalence** means the **absence** of a significant **difference in the rate and extent** to which the **active ingredient or active moiety** in pharmaceutical equivalents or pharmaceutical alternatives becomes **available at the site of drug action** when administered at the same molar dose under similar conditions in an appropriately designed study....”

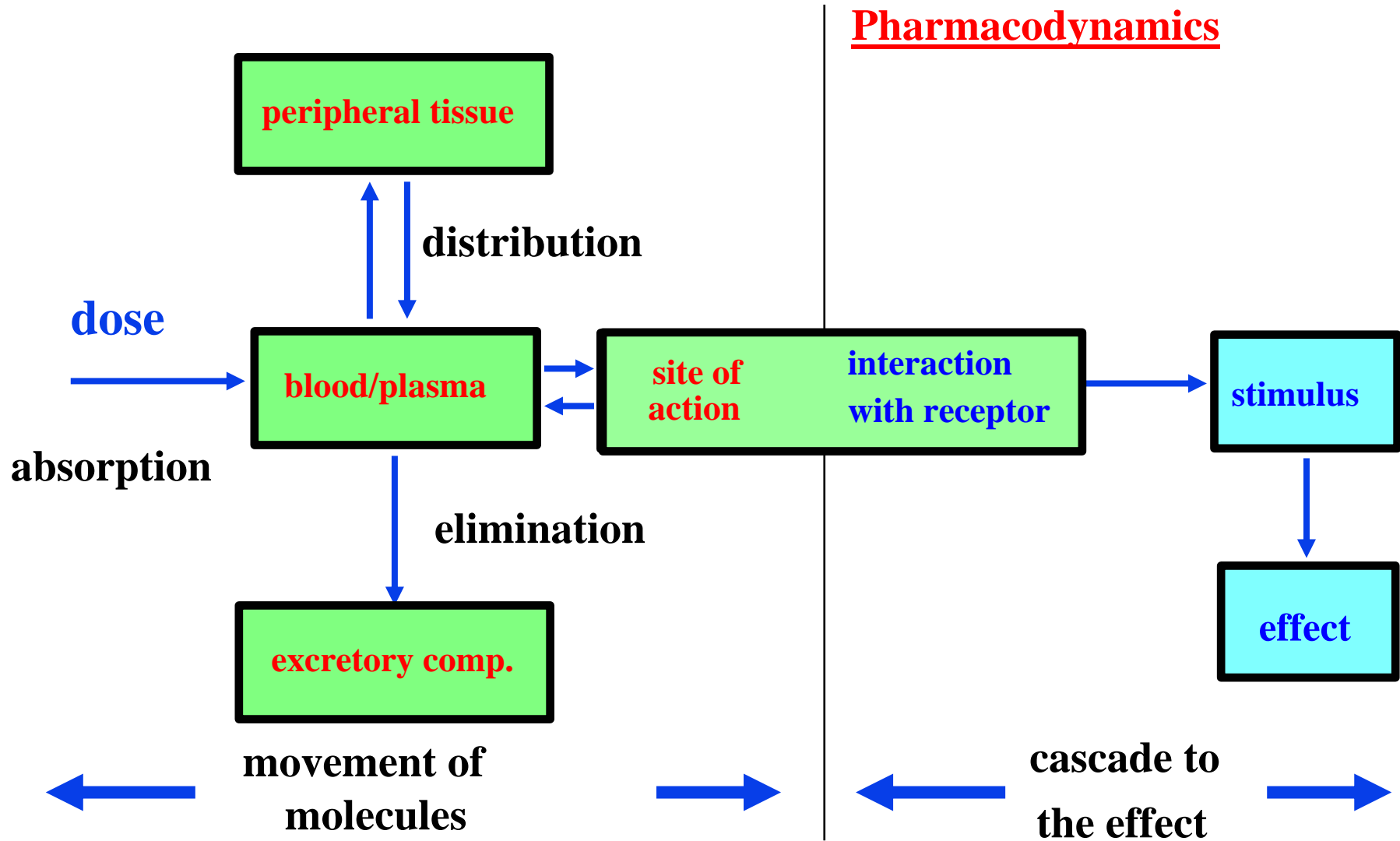
Collected from a presentation of Kofi A. Kumi, Office of Clinical Pharmacology and Biopharmaceutics  
Division of Pharmaceutical Evaluation III, ACPS 7/20/2001

### ■ **21 CFR 320.24**

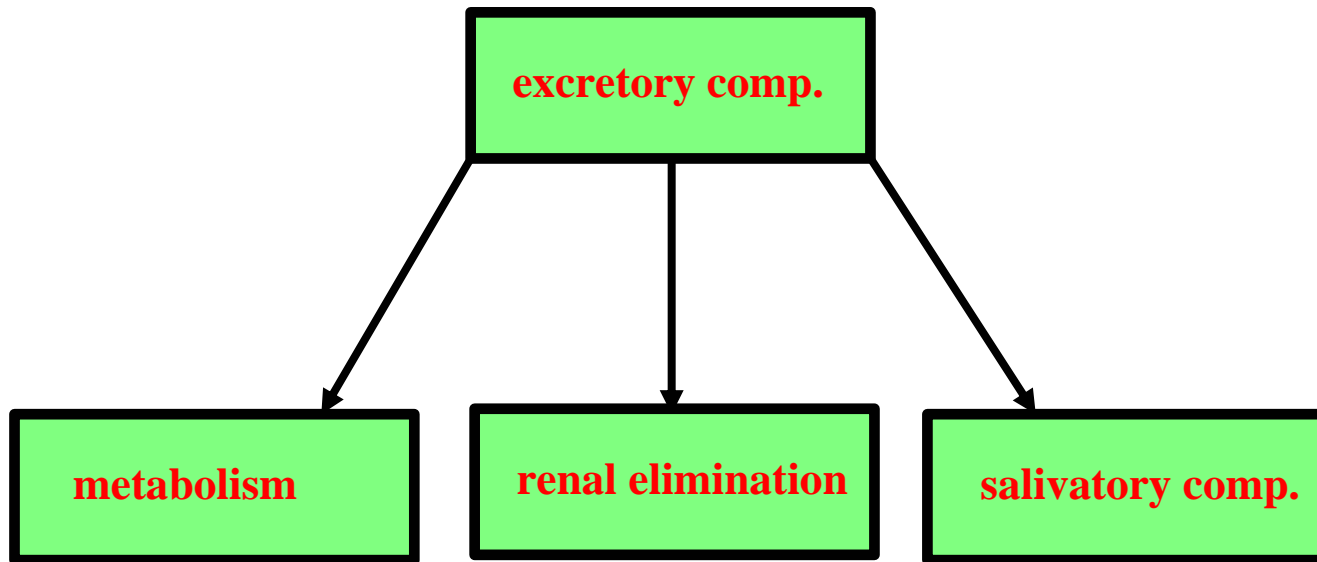
The following in vivo and in vitro approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the bioavailability or bioequivalence of a drug product:

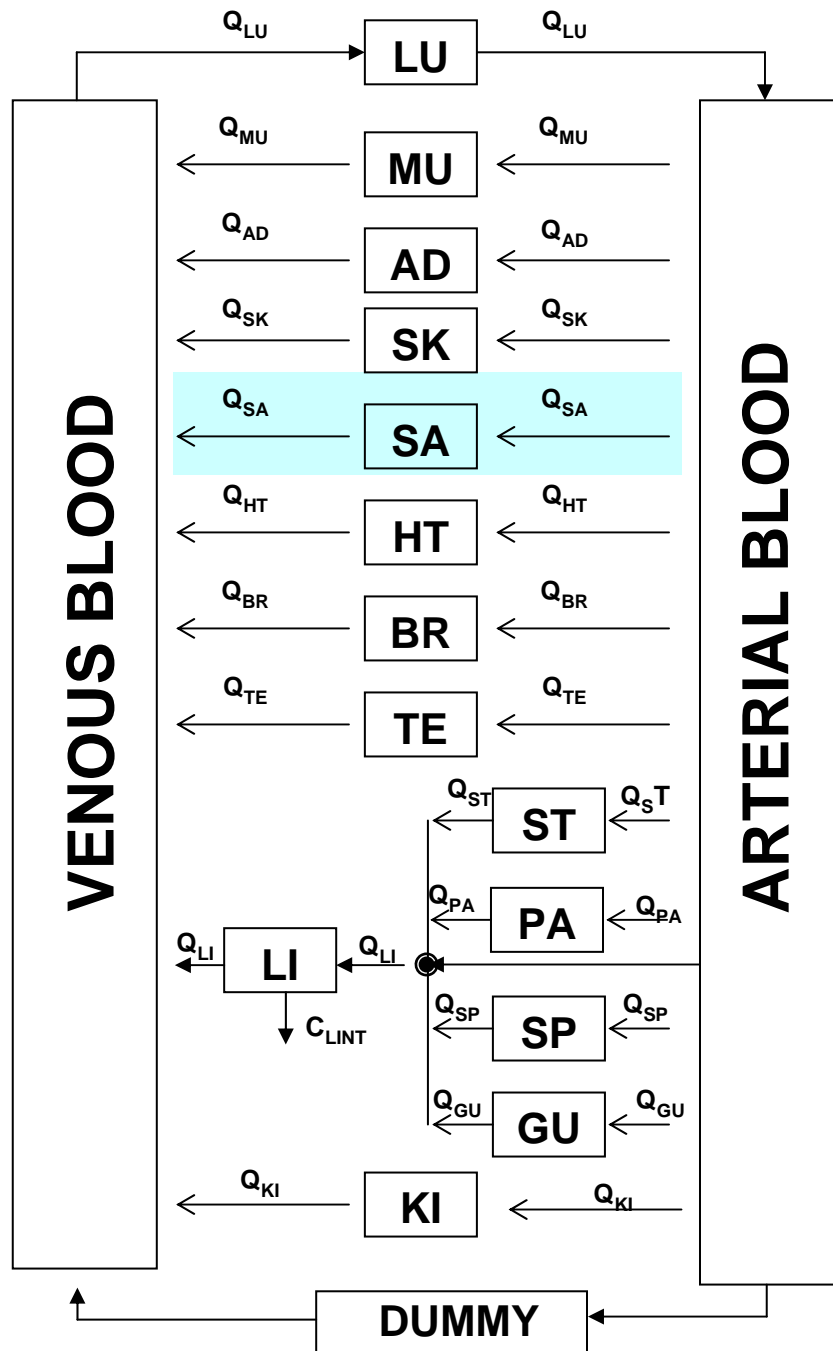
- Blood/plasma/serum drug conc. measurement in humans
- Urinary excretion in humans
- In vivo pharmacological effect
- Well-controlled clinical trials
- In-vitro test
- Any other approach deemed adequate by FDA

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# Excretion

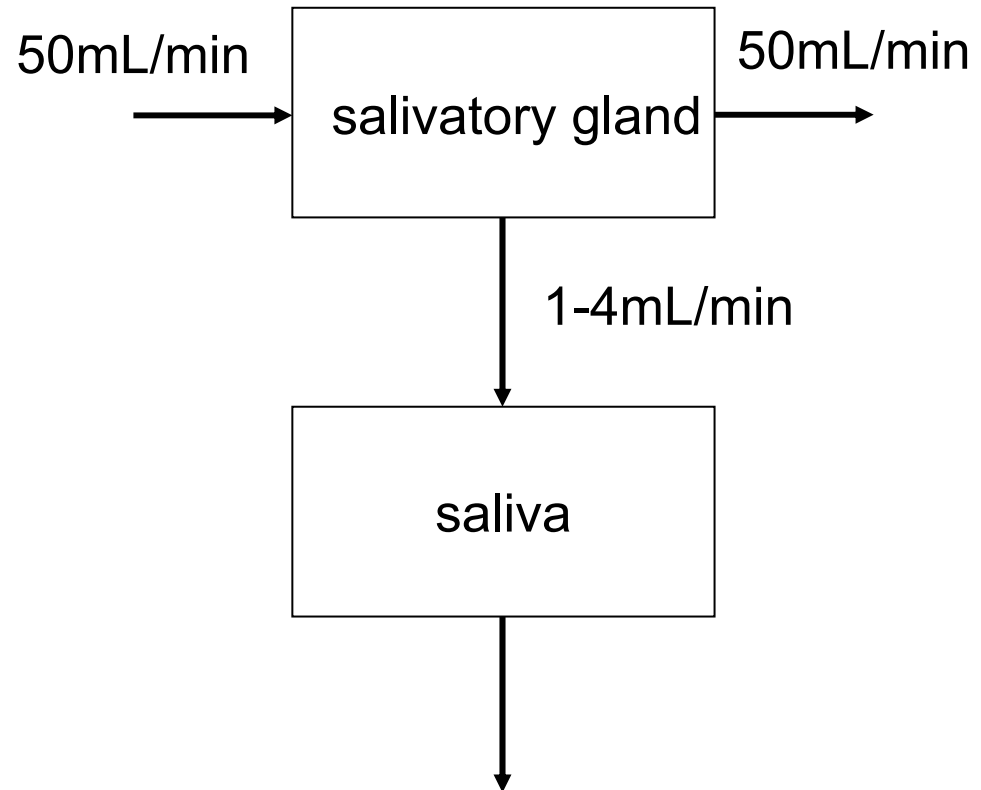
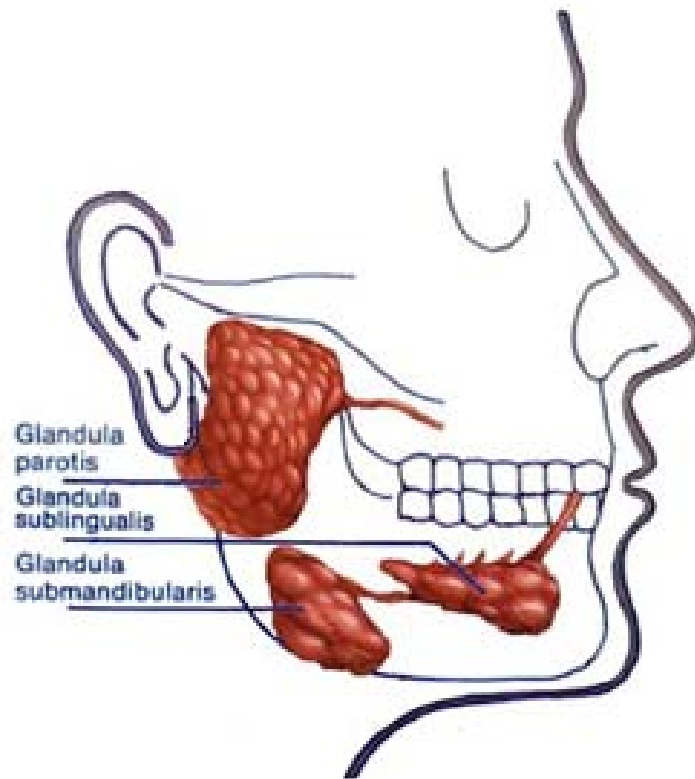




**Tissues**

MU	musculature
SA	salivatory gland
SK	skin
TE	testis
GU	thin intestine
ST	stomach
SP	spleen
LI	liver
PA	pancreas
LU	lung
HT	heart
BR	brain
AD	fat tissue
KI	kidney

# Pharmacokinetics in saliva



Mass of saliva glands: 30-50g  
Approximated Blood flow: 50-200mL/min

## What about mass balance during extraction to saliva?

Having a 1:1 extraction:

Concentration in plasma and in saliva is the same.

### Plasma

Before 'extraction'  $5\mu\text{g/mL}$

After 'extraction'  $4.902\mu\text{g/mL} = 5 * 50 / (50 + 1)$

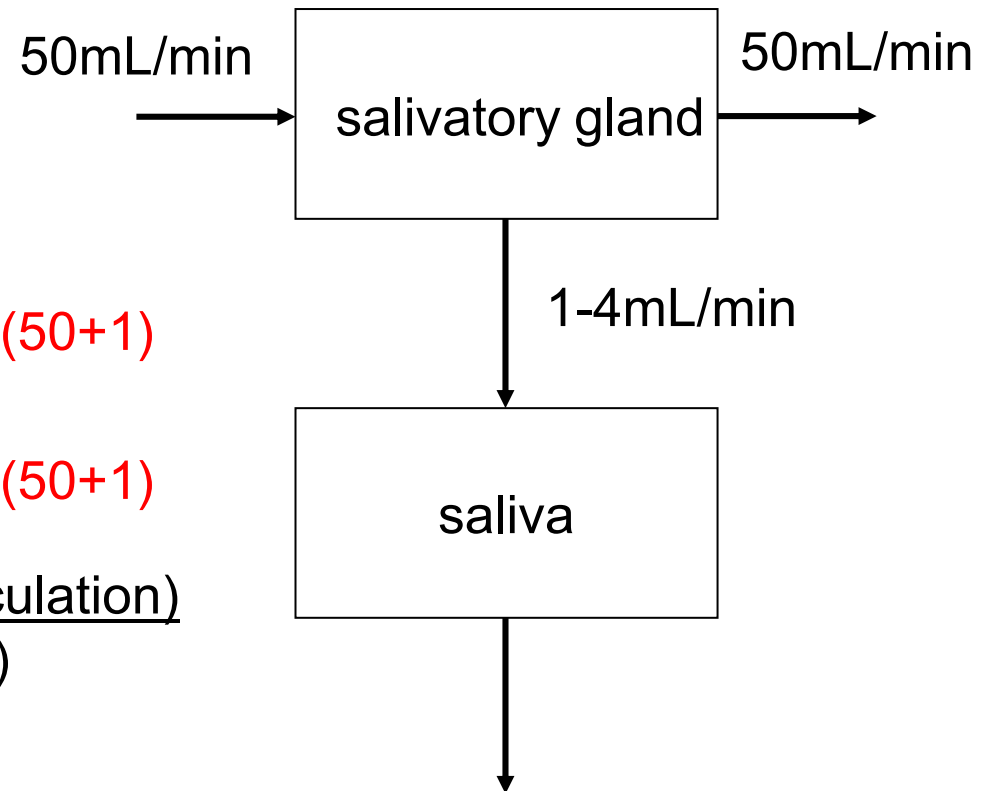
### Saliva

Concentration  $4.902\mu\text{g/mL} = 5 * 50 / (50 + 1)$

### Total Amount in Plasma (central circulation)

Before:  $12.5\text{mg}$  ( $2500\text{ml} * 5\mu\text{g/mL}$ )

After:  $12.495\text{mg} = 4.998\mu\text{g/mL}$



Ratio of saliva vs. plasma concentrations

$$R = \frac{1 + 10^{pH_{saliva} - pKa}}{1 + 10^{pH_{plasma} - pKa}} \cdot \frac{f_{u,plasma}}{f_{u,saliva}}$$

pH<sub>plasma</sub> is almost constant,  
f<sub>u</sub>=fraction unbound, f<sub>u,saliva</sub> is 1 for most drugs,  
pKa is the negative logarithm of acid dissociation constant

Equation derived from: a) Kozjek F, et al, Biopharm Drug Dispos 20: 183-191 (1999)  
b) Drobitch RK and Svensson CK, Clin Pharmacokinet 23: 365-379 (1992)

# Elimination by saliva

Examples of drug elimination by saliva (ratios collected from the literature)

<u>drug</u>	<u>ratio of saliva conc vs plasma conc</u>	<u>protein binding %</u>
Aminophenazon	0.79	>99
Amphetamin	2.76	
Antipyrin	0.89 - 1.00	
Carbamazepine	0.26-0.44	75
Coffein	0.55	<10
Diazepam	0.029	95-98
Digoxin	0.66 - 1.68	20-30
Isoniazid	1.02	10
Levetiracetam	1.3	<10
Lithium	2.86 - 3.43	
Paracetamol	1.40	20-50
Penicillin	0.015	>80
Phenacetin	0.60	35
Phenobarbital	0.30 - 0.43	59
Phenytoin	0.09 - 0.24	83-94
Sulfanilamid	0.87 - 1.08	20
Theophyllin	0.49 - 0.77	56

# Study of bioavailability and pharmacokinetics of theophylline following administration of two sustained release dosage forms as assessed by salivary data: Part II

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<sup>1</sup>*Department of Pharmaceutical Services, Kumamoto University Hospital and*

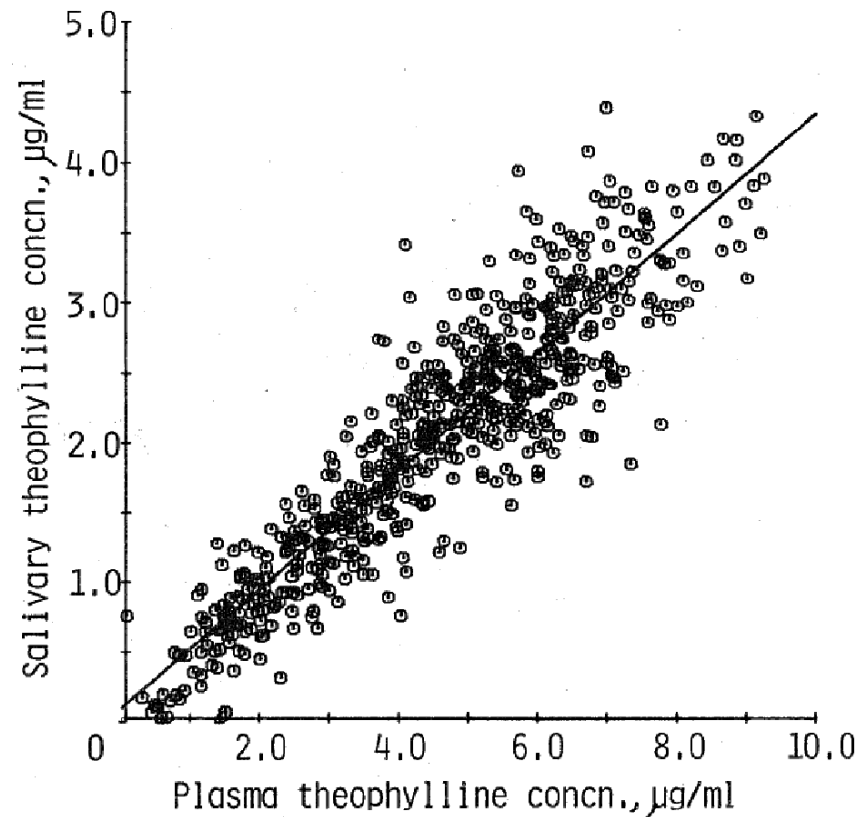
<sup>2</sup>*Department of Pediatrics, Kumamoto University Medical School, 1-1-1 Honjo,  
Kumamoto 860, Japan*

**Abstract.** Bioavailability and pharmacokinetics of theophylline following administration of a marketed sustained release tablet (Theo-Dur®) and a newly designed sustained release tablet (E-0686) have been studied in fifteen healthy volunteers by measuring plasma and salivary concentrations. The theophylline level in saliva was  $42.3 \pm 0.008$  (s.e.m.) % of the plasma level and its correlation coefficient was 0.908. This observation suggests that salivary levels are considered to be a good indicator of the plasma concentration of theophylline. Inter-

*International Journal of Clinical Pharmacology, Therapy and Toxicology, Vol. 24 No. 4 – 1986 (pp.196–201)*

# Examples of saliva sampling for PK evaluation

$$\text{Conc}_{\text{saliva}} \approx 0.423 * \text{conc}_{\text{plasma}}, r=0.908$$



*Fig.1* Relationship between theophylline concentrations in 600 plasma and salivary specimens.

## Examples of saliva sampling for PK evaluation

**SCHWARZ**  
P H A R M A

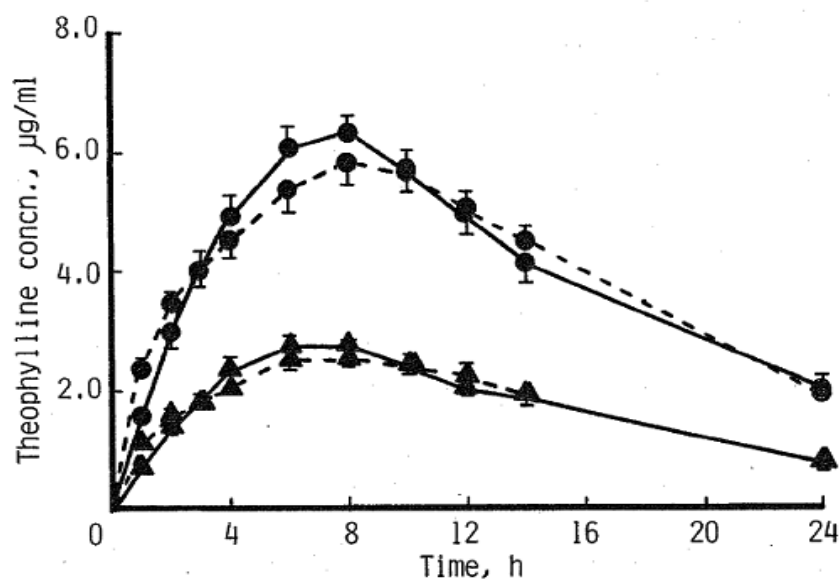


Fig. 2 Mean plasma (●) and salivary (▲) theophylline concentrations after administration of 300 mg Theo-Dur under fasting (---) and non-fasting (—) conditions in 15 healthy subjects.

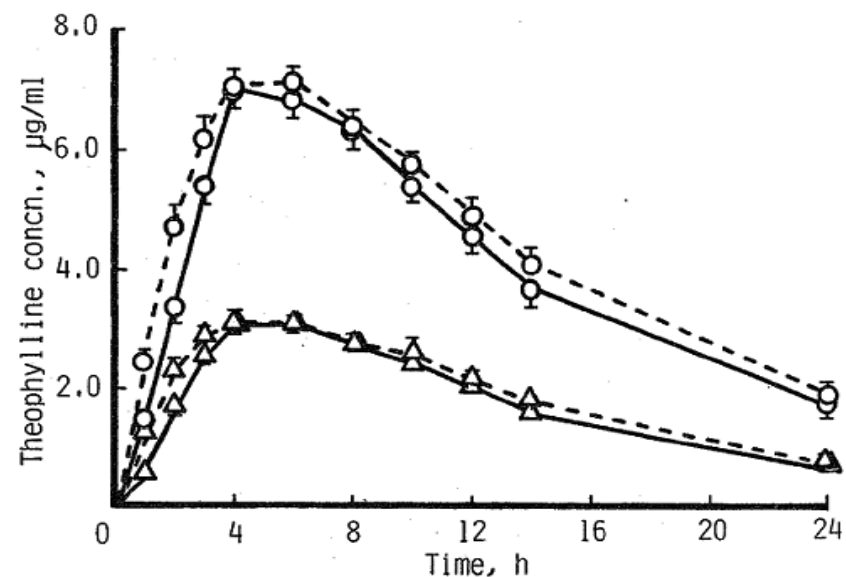


Fig. 3 Mean plasma (○) and salivary (△) theophylline concentrations after administration of 300 mg E-0686 under fasting (---) and non-fasting (—) conditions in 15 healthy subjects.

# Examples of saliva sampling for PK evaluation

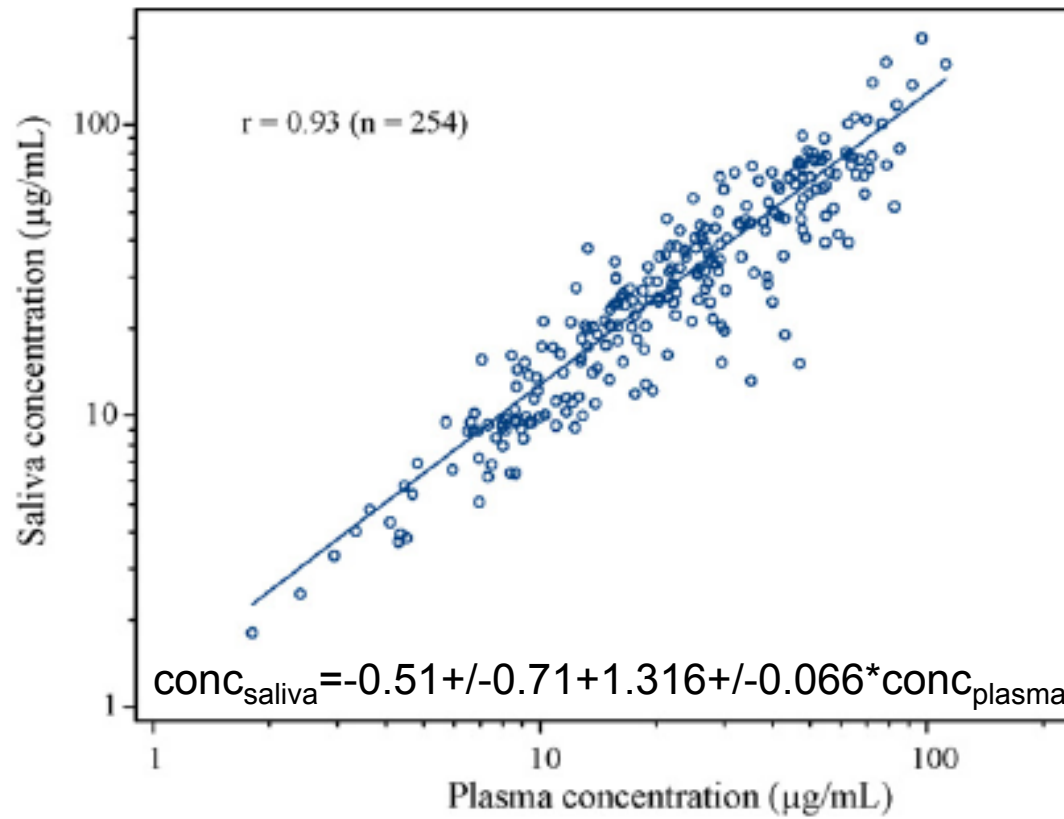
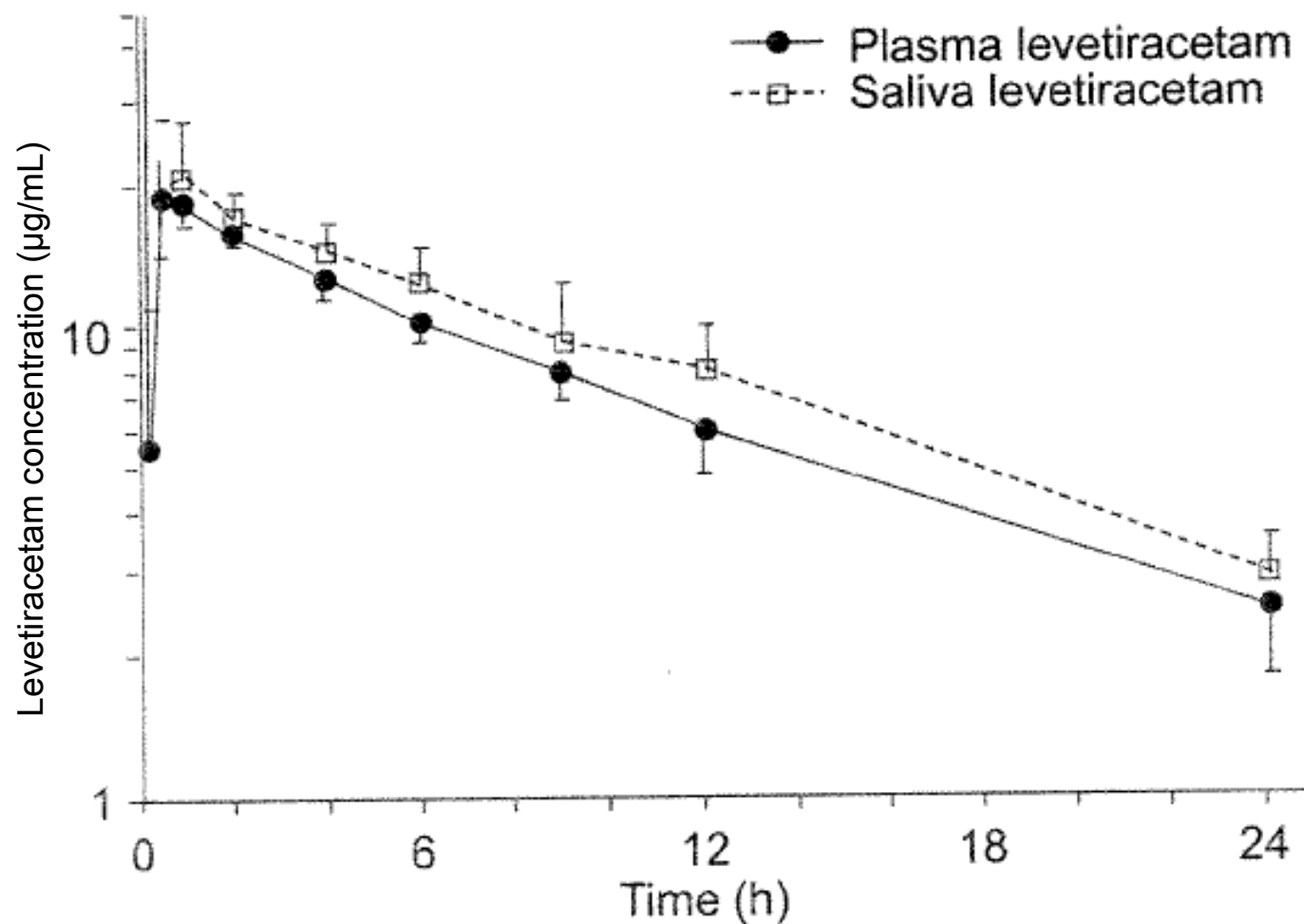


Figure 3 Levetiracetam saliva vs. plasma concentrations relationship. The line represents the least-squares regression line.

Published by: Fontain N.B., et al, Epilepsy Research 2007, in press

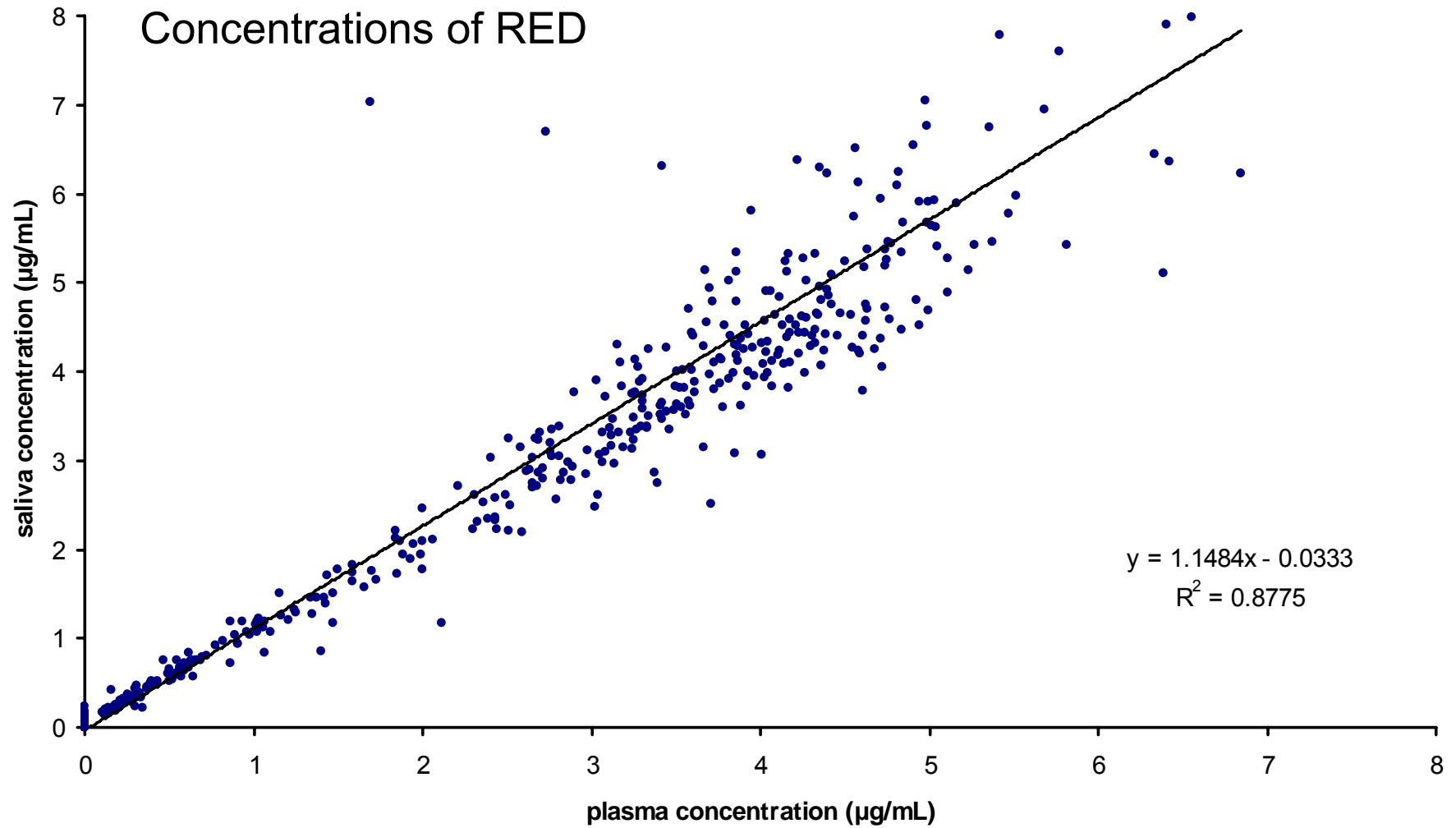
## Examples of saliva sampling for PK evaluation



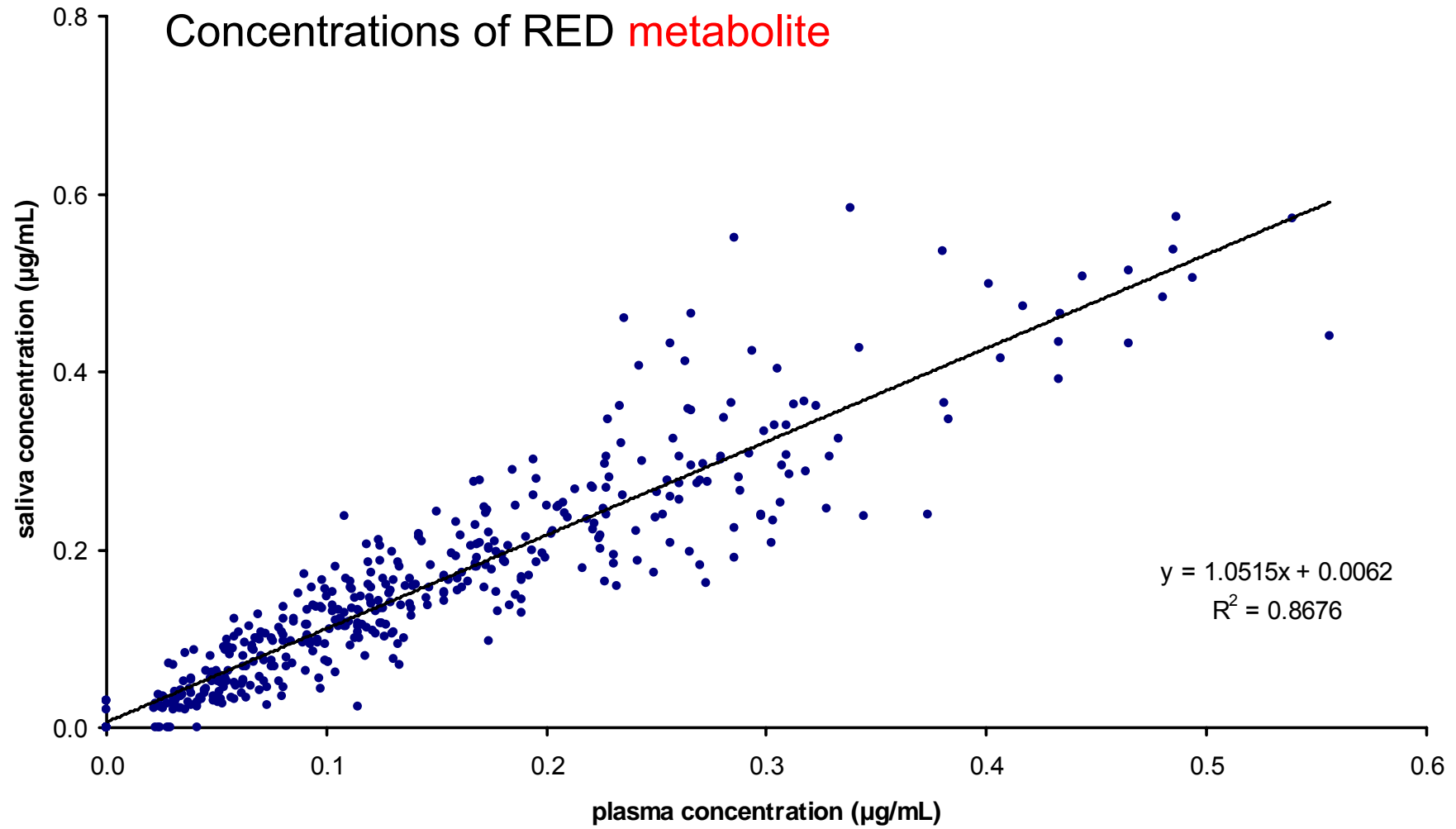
Concentrations after single oral dose of 750mg Levetiracetam (three 250mg tablets)  
Extract from: Lins RL, et al. *IntJClinPharmacol&Thera*; 45(1), 2007, 47-54

## Example: drug RED

# Comparison of plasma PK and saliva PK



# Comparison of plasma PK and saliva PK

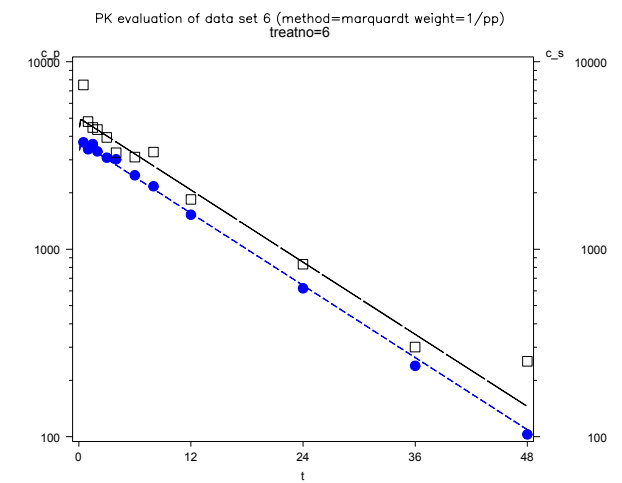
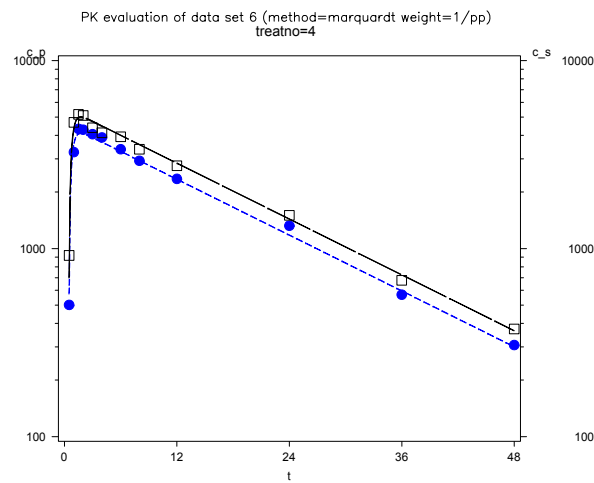
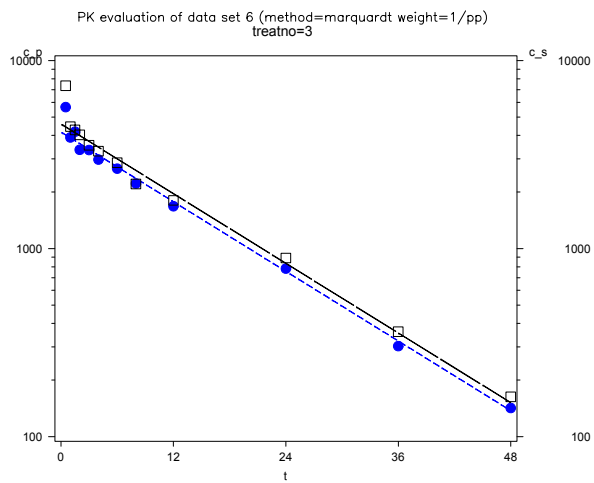
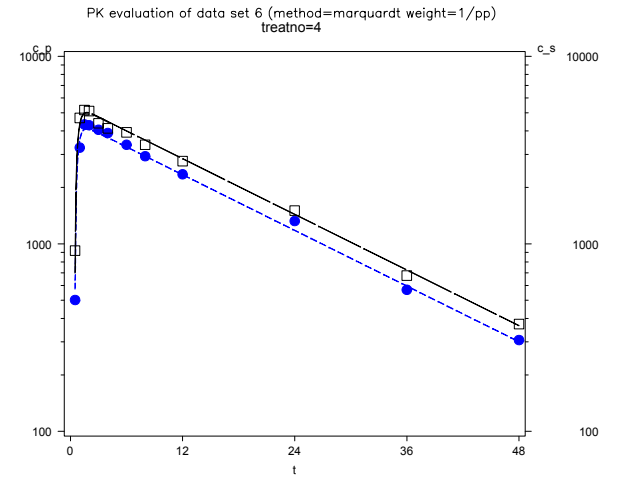
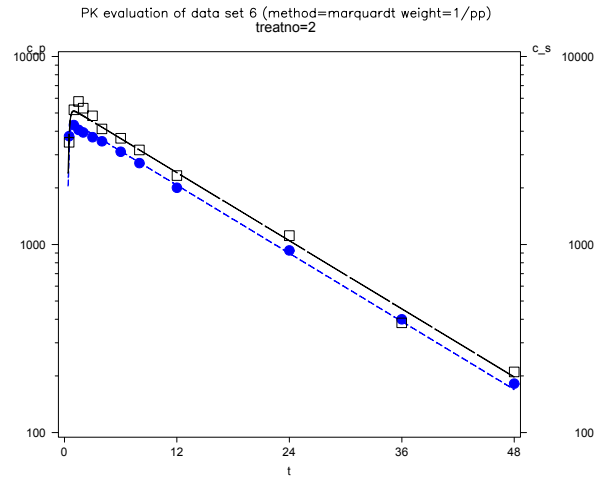
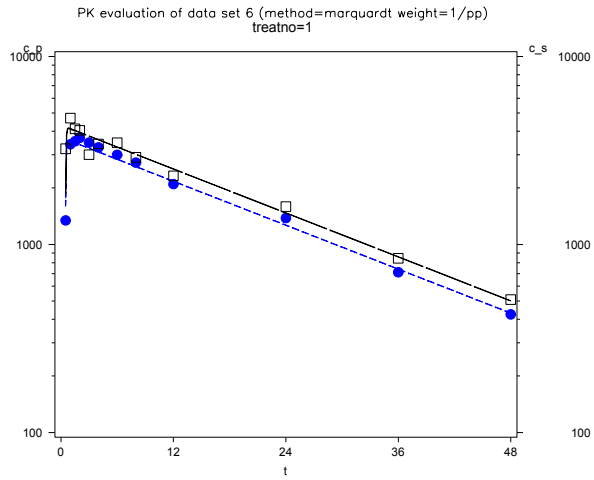


# Parameters of pharmacokinetics evaluation of bioequivalence

Treatment drug	AUC Plasma	AUC Saliva	BE % plasma BE % saliva	90%CI plasma 90%CI saliva
A RED	86.4/18.3	95.3/20.3	101.4	89.0-115.5
			99.9	88.1-113.3
B RED	85.1/17.6	94.9/18.6		
A metabolite	10.05/4.11	11.02/4.82	101.4	75.7-134.9
			102.6	74.1-142.1
B metabolite	9.98/4.11	10.73/4.45		

Arithmetic mean/sd, n=16, BE and 90% CI after log transformation

# Model dependent PK with plasma or saliva samples



One compartment model with first order absorption and first order elimination  
(Bateman function with parameters  $k_a$ ,  $k_e$ , dose, and  $V_d$ )

## Saliva samples

$$k_e = 0.0622 \text{ h}^{-1}$$

$$t_{1/2} = 11 \text{ h}$$

$$t_{\text{lag}} = 0.245 \text{ h}$$

$$V/f = 42.2 \text{ L}$$

## Plasma samples

$$k_e = 0.0635 \text{ h}^{-1}$$

$$t_{1/2} = 11 \text{ h}$$

$$t_{\text{lag}} = 0.187 \text{ h}$$

$$V/f = 49.6 \text{ L}$$

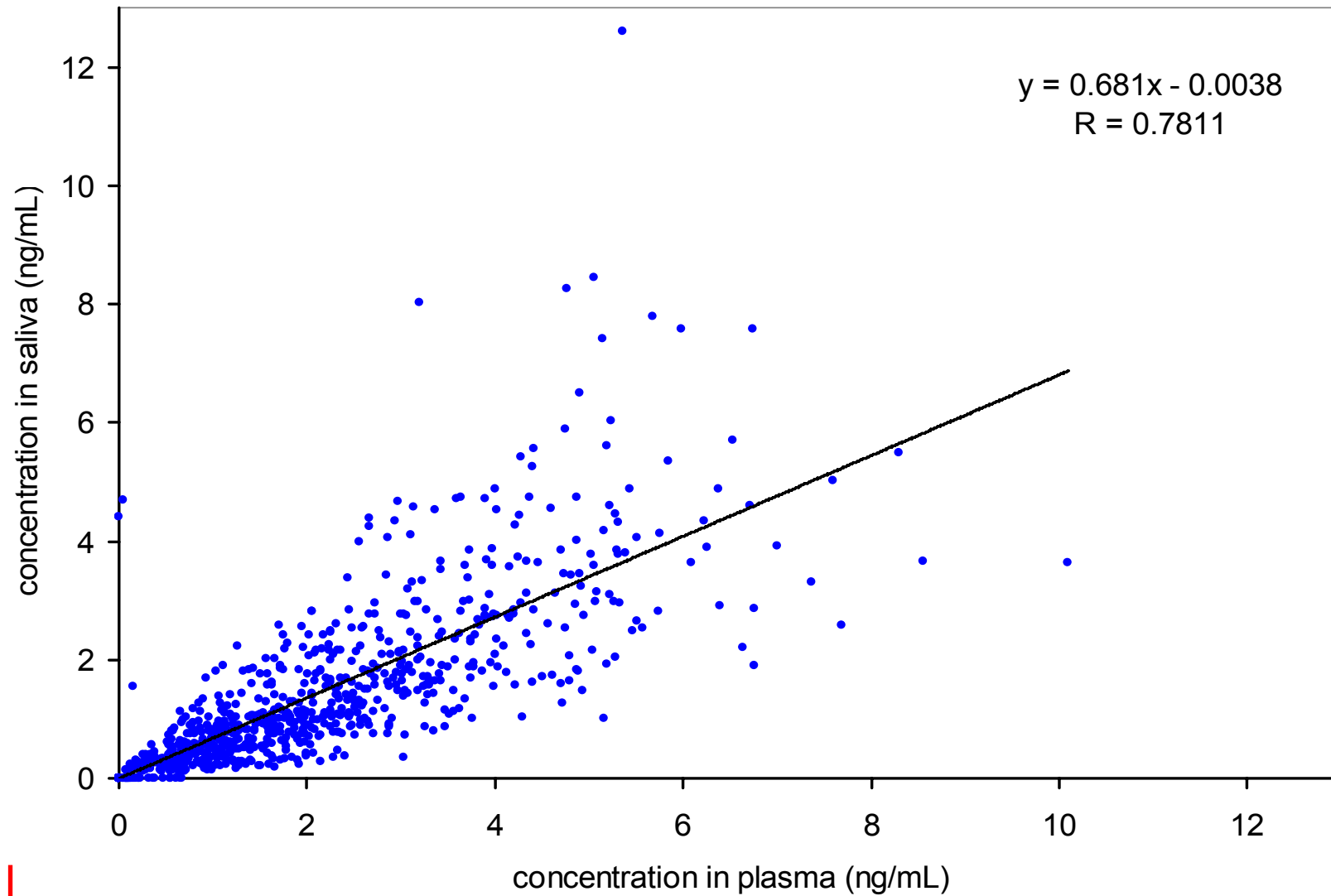
## Comparison of plasma PK and saliva PK

- Data from saliva samples are sufficient to characterize PK profile, model dependent PK and useful for BE evaluation.
- Rate constant of elimination resp.  $t_{1/2}$  is the same.
- Approximation of the volume of distribution using saliva samples results in about 15% lower values corresponding to 15% higher concentrations in saliva than in plasma.
- Similar results for the metabolite of RED.

Pharmacokinetics of RED in saliva is a good approximation (a surrogate) for the pharmacokinetics in plasma.

## Example: drug BLUE

# Correlation between BLUE concentrations in saliva and plasma



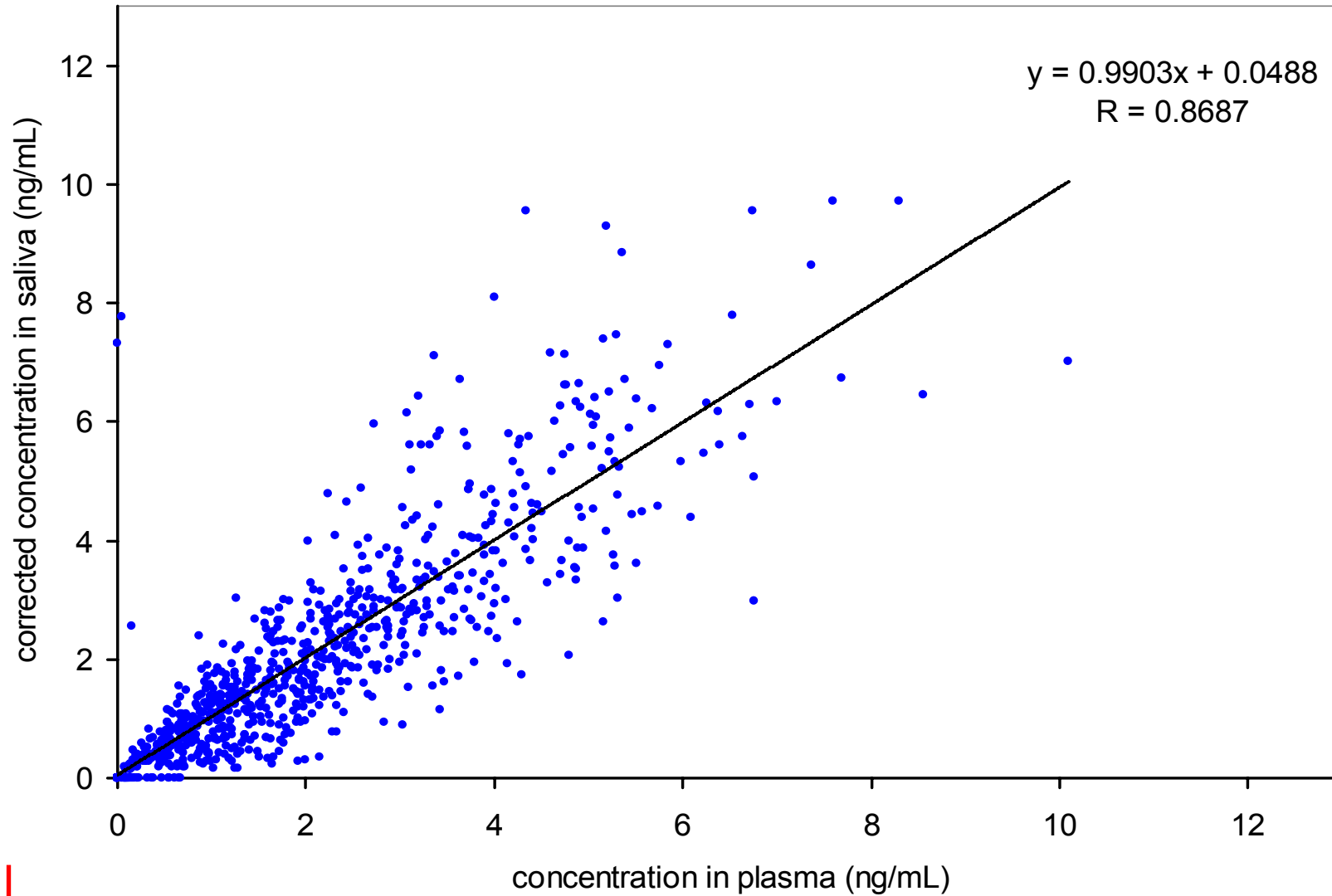
## Correlation between BLUE concentrations in saliva and plasma

Individual factor for correlation between plasma and saliva concentration

$$C_{\text{saliva}} = \text{intercept} + \text{factor} * C_{\text{plasma}}$$

parameter	intercept	factor
arithmetic mean	-0.018	0.671
SD	0.242	0.273
range	-1.04 - 0.586	0.297 – 1.424

# Correlation between BLUE concentrations in saliva and plasma (after individual correction)



## Results of „BE“ evaluation

Parameter	Medium	8mg BLUE - reference	8mg BLUE - test
$C_{\max}$	Plasma	3.69(45.1)	4.16(43.7)
	Saliva	3.17(63.5)	3.47(65.2)
$AUC_{(0-tz)}$	Plasma	44.9(43.8)	47.5(41.5)
	Saliva	33.3(65.2)	34.4(63.0)
$t_{\max}$	Plasma	5(3-6)	5(3-6)
	Saliva	5(3-8)	5(3-8)

$C_{\max}$  and  $AUC_{(0-tz)}$  : Mean (CV),  $t_{\max}$ : range

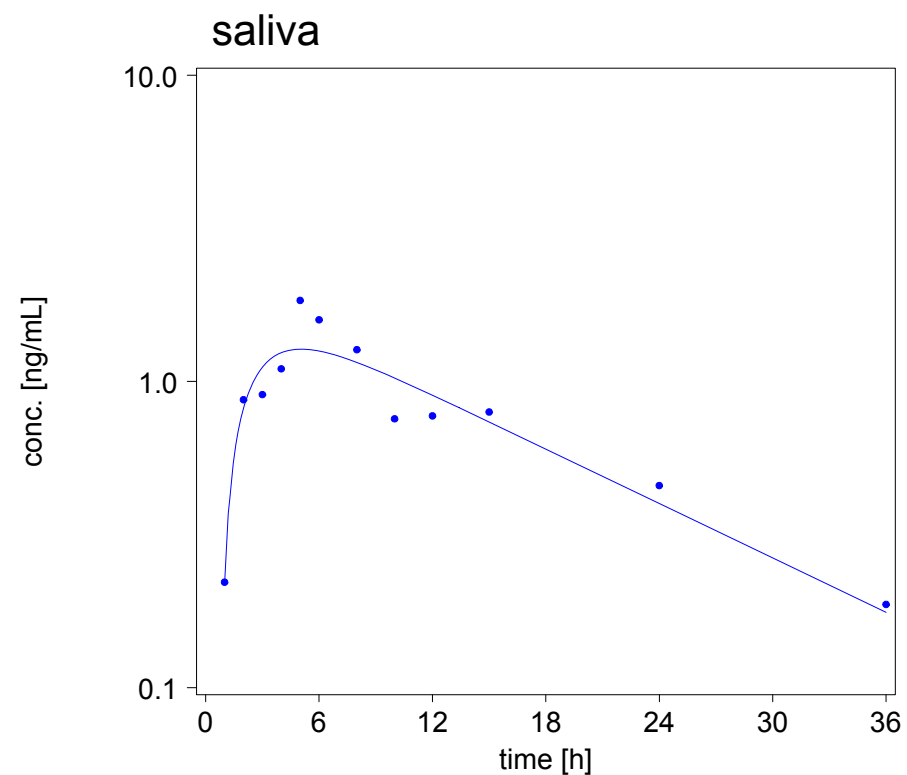
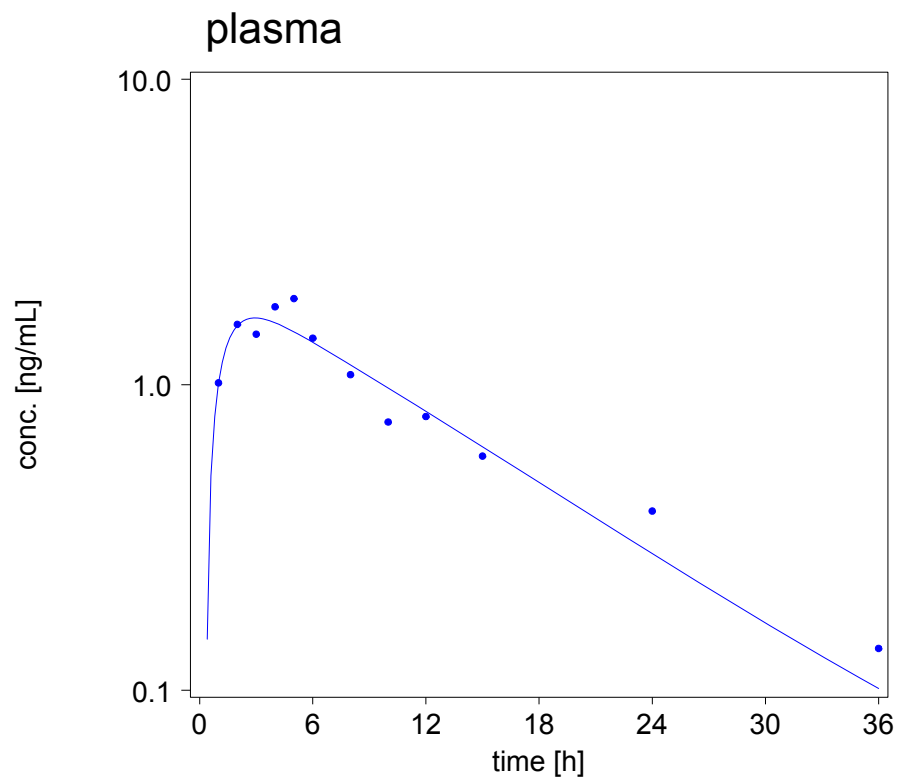
## Results of „BE“ evaluation

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Parameter	Saliva		Plasma	
	point estimate	90% CI	point estimate	90% CI
$C_{\max}$	112.0	98.4 – 127.5	112.3	101.8 – 123.9
AUC(0-tz)	105.7	94.9 – 117.8	106.6	99.3 – 114.5

ANOVA results relative bioavailability

# PK modelling



PK model: one compartment model with first order absorption and elimination (Bateman function)

$k_e$ (1/h)	treatment A (n=33)	treatment B (n=33)
plasma	0.1548-/+0.2084	0.1357+/-0.0596
saliva	0.1319+/-0.0792	0.1309+/-0.0595

- Saliva samples are a good surrogate for model independent PK and PK modelling (for drug BLUE in plasma).
- BE evaluation based on the saliva samples fit with the results of the evaluation based on plasma samples (especially after normalization using individual factors).

Use saliva samples instead of plasma samples for characterization of BLUE PK (after validation of the correlation and characterization of variability and maybe use of individual value of R estimated with 1 or 2 plasma samples).

- Using saliva samples instead of / in addition to plasma samples was shown to be a feasible tool to characterize the PK of a drug in plasma.
- Using a drug specific ratio for saliva over plasma concentrations can result in useful values for model dependent and model independent parameters of PK and BE testing.
- The BE evaluation based on saliva samples fits with the results based on plasma samples (especially after normalization using individual factors).

### Future perspectives

- Saliva samples not only for therapeutic drug monitoring but for PK / popPK evaluation
- Decreased costs for sample collection
- Increased explanatory power of Population PK with possible (more) sampling out of clinics (at more relevant time points after admin.)
- Increased acceptance for studies in children (by patients, parents, ethics committees, and authorities)
- Increased comfort for the patients/subjects by non-invasive sampling

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