FABIAN SYMPOSIUM

First administration to man

An Example

Clinical Pharmacology

SOLVAY

CLINICAL PHARMACOLOGY

PRESENTATION FABIAN SYMPOSIUM

- **Clinical part** — **Hans Bakker**
- **BioAnalytical part** — **Koos Drooger**

CLINICAL PHARMACOLOGY

- **(Theoretical) Drug Development:**
  - **Research / Discovery / Pharmaceutical development**
  - **Pre-clinical testing** — **(animal) pharmacology / toxicology**
  - **Phase I** — **Exploratory development; first administration to man (healthy volunteers)**
  - **Phase II** — **early patient studies**
  - **Phase III** — **full development**
  - **Registration**
  - **Phase IV** — **post marketing / line extensions**

CLINICAL PHARMACOLOGY

Exploratory Development

Aim:

- **Efficient selection of safe drugs with added value / market potential and / or**
- **to stop compounds as early as possible**

CLINICAL PHARMACOLOGY

Early (CNS) Phase I studies

Some examples:

- **Single Rising Dose Tolerability (SRDT) study (oral / iv)**
  NB: usually first administration to man !
- **Multiple Rising Dose Tolerability (MRDT) study**
- **Food interaction study**
- **Bioavailability / Metabolism study (radio-labelled drug)**
- **Pharmacodynamic study**
  - **e.g.: PET study / EEG study**

CLINICAL PHARMACOLOGY

Late Phase I studies

Some examples:

- Interaction studies
- elderly subjects / pediatric subjects
- Japanese subjects
- Hepatic - / renal impaired subjects
- Effect on QTc / cognition
- Bioequivalence studies (new formulations)

CLINICAL PHARMACOLOGY

Early Phase I studies

Aimed at:

- **Tolerability and Safety**
- **Pharmacokinetics**
- **Pharmacodynamics**

ASSESSMENTS

- **Tolerability / safety:**
  - **AE recording (MTD / dose limiting AE ?)**
  - **Physical examination**
  - **Neurological examination (EPS !)**
  - **Vital signs (orthostatic hypotension ?)**
  - **ECG (QTc prolongation ?)**
  - **Laboratory assessments**
    - **Hematology / Biochemistry / Urinalysis**

ASSESSMENTS

- **Pharmacokinetics:**
  - **Concentration of drug in plasma (/ urine)**
    - **Cmax**
    - **Tmax**
    - **AUC (exposure)**
    - **(inter subject) Variability**
    - **Elimination half-life time**
    - **Linearity of kinetics**
  - **Genotyping (e.g. Cyt P 450 2D6 polymorphism)**

PK Parameters

- **Relevance:**
  - **Absorption / Distribution / Metabolism / Excretion**
  - **Bio-availability**
  - **Relationship Dose - PK**
  - **Relationship PK - Tolerability**
  - **Relationship PK - Pharmacodynamics**

ASSESSMENTS

- **Pharmacodynamics:**
  (first indication of pharmacological activity ?)
  - **Effect on hormone concentrations in plasma**
    - **prolactin**
    - **cortisol**
    - **growth hormone**
  - **Body temperature**
  - **Cognition**
  - **Pharmaco-EEG**

Issues prior to first administration to man

- Clinical Research Organization (CRO)
- Evaluation pre-clinical data > Investigator Drug Brochure (IDB)
- Study design
  - Placebo controlled / double blind design ?
  - Subjects ?
    - Healthy volunteers vs Patients
    - Young vs Old
    - Male vs Female
  - Group size ?
  - Route of administration / formulation ?
  - Starting dose (fraction of NOAEL) / dose increments ?
- Medical Ethical Committee approval

DESIGN SRDT (first into man)

- Double blind, placebo controlled single rising dose tolerability study
- 27 healthy male volunteers
- Three sequential groups of 9 volunteers
  - each volunteer : 3 sessions (2 active, 1 placebo)
  - each session : 6 active, 3 placebo
  - wash-out period: (usually) 1 week
- Dose levels (mg) are studied in ascending order:

I	0.2	0.5	1
II	2	5	10
III	20	50	100

DESIGN SRDT

Example randomization schedule

Group I

Subject	Session I	Session II	Session III
1	0.2 mg	0.5 mg	Placebo
2	0.2 mg	0.5 mg	Placebo
3	0.2 mg	0.5 mg	Placebo
4	0.2 mg	Placebo	1.0 mg
5	0.2 mg	Placebo	1.0 mg
6	0.2 mg	Placebo	1.0 mg
7	Placebo	0.5 mg	1.0 mg
8	Placebo	0.5 mg	1.0 mg
9	Placebo	0.5 mg	1.0 mg

DESIGN SRDT

See : FLOW CHART

SRDT

Logistics of Safety / PK / PD Monitoring

- **Tuesday:**
  - dosing / sampling
- **Wednesday:**
  - shipment PK / Hormone / Safety samples (up to 18 / 24 hours post-dose) to analytical laboratory
- **Friday:**
  - interim safety report from phase I unit [Adverse events / vital signs / ECG parameters (QTc) / body temperature / results laboratory assessments]
- **Monday:**
  - results PK / Hormone samples
  - protocol amendments ? e.g. changes dose / sampling schedule
  - Solvay approval next dose level

BioAnalytical Part

Even voorstellen…

- **Koos Drooger**

werkzaam bij	**Solvay Pharmaceuticals sinds 1976**
1976 – 1979	**Analytisch Onderzoek (Adme groep)**
1979 – 1986	**Analytisch Onderzoek (Gaschromatografie)**
1986 – 2002	**BioAnalyse**
2002-	**Klinische Pharmacologie** **BioAnalytical Research Director**

FIRST IN MAN STUDY (SRDT)

AIM OF THE STUDY

- **Primary objective**
  - Explore the safety and tolerance of single oral doses of SLV 310 in healthy male volunteers.

- **Secondary objectives**
  - Explore the maximum tolerated single dose.
  - Explore the single dose pharmacological activity [establish the minimal single oral dose with pharmacological (D2 antagonistic and/or SSRI) activity].
  - Explore the basic single dose pharmacokinetic parameters

FIRST IN MAN STUDY (SRDT)

- **In order to achieve these objectives, the plasma and urine levels versus time curves of SLV310 have to be assessed in agreement with current guidelines.**

- **The analysis will be done with HPLC with MS/MS detection**

FIRST IN MAN STUDY (SRDT)

- **Dose levels will be studied in an ascending order. The proposed dose levels are: 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 mg.**

- **Plasma samples will be taken at: 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24 and 48 hour after dosing. Each dose session consists of 9 volunteers of which 6 will receive SLV310.**

FIRST IN MAN STUDY (SRDT)

- **After each dose session the plasma samples up to and including 18 hours were shipped to the BioAnalysis department of Solvay Pharmaceuticals. Samples from the SLV310 dosed volunteers (6 times 10 samples is 60 samples) were analyzed immediately**

FIRST IN MAN STUDY (SRDT)

◆ Tuesday	Dosing
◆ Tuesday/Wednesday	Sampling
◆ Wednesday	Transport
◆ Thursday/ Friday	Analysis (sample clean-up and concentration)
◆ Saturday/Sunday	Analysis (LCMSMS)
◆ Monday	Results, evaluation
◆ Tuesday	Dosing (new dose)

BioAnalytical Method

- Determination of SLV 310 in human plasma
- Range 0.02-20 ng/ml
- Validated at Solvay Pharmaceuticals

Sample preparation and clean-up

- Make standard solutions and dilutions of SLV 310 and ^{13}C SLV 310 (I.S)
- Sample volume: 0.5 ml of human plasma
- Add 0.5 ml of phosphate buffer and mix
- Add the appropriate amount of SLV 310 (calibrators)
- Add to all samples and calibration standards 10 ng internal standard (^{13}C SLV 310)
- Equilibrate

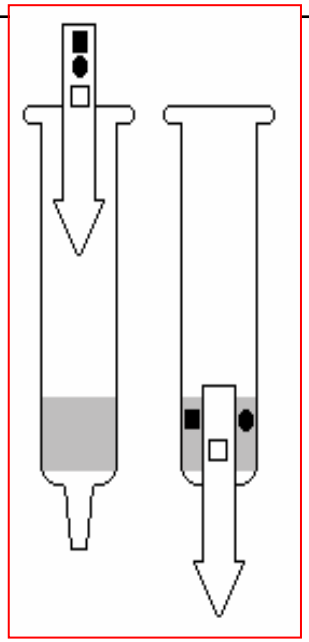
Clean-up and concentration

- Transfer sample onto Chem_elute extraction column.
- Wait for 4 minutes
- Wash the column with 3 ml of pentane.
- Extract column with two times 3 ml ethyl acetate/pentane (1:1 v/v) into a 10 ml vial.
- Add 100 ul of keeper solution (acetone/glycerol 99:1).
- Evaporate solvent using nitrogen at 60 °C.
- Dissolve residue into 100 ul of mobile phase.
- Vortex vial for 10 seconds.
- Transfer sample into LC-minivial
- Inject 45 ul of sample into HPLC system.

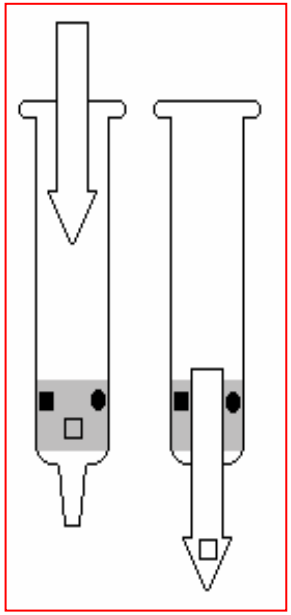
Chem-elute

- liquid-liquid extraction
- Diatomaceous Earth

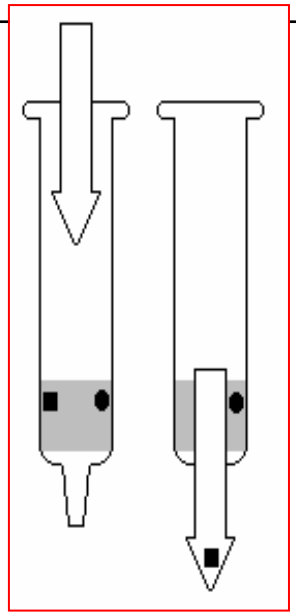
Chem-elute sample clean-up



introduction sample



wash (pentane)



elute (pentane/ethylacetate)

Apparatus

- Quartenery HPLC pump Perkin Elmer LC 200
- Degasser HP 1050
- Gilson autosampler, model 233XL
- PE Sciex API 365 tandem mass spectrometer equipped with Turbo Ionspray

LCMSMS conditions

- **Analytical column:**

  Spherisorb, phenyl 3 um 150 x 4.6 mm

- **Mobile phase:**

  Water/acetonitril/methanol 62.5:125:812.5 and

  10mMol Ammoniumacetate

LCMSMS conditions

Interface:

- **Turbo Ionspray**
- **Split ratio: approx 1:10**
- **Interface temperature: 300 ° C**

LCMSMS conditions

Ions:

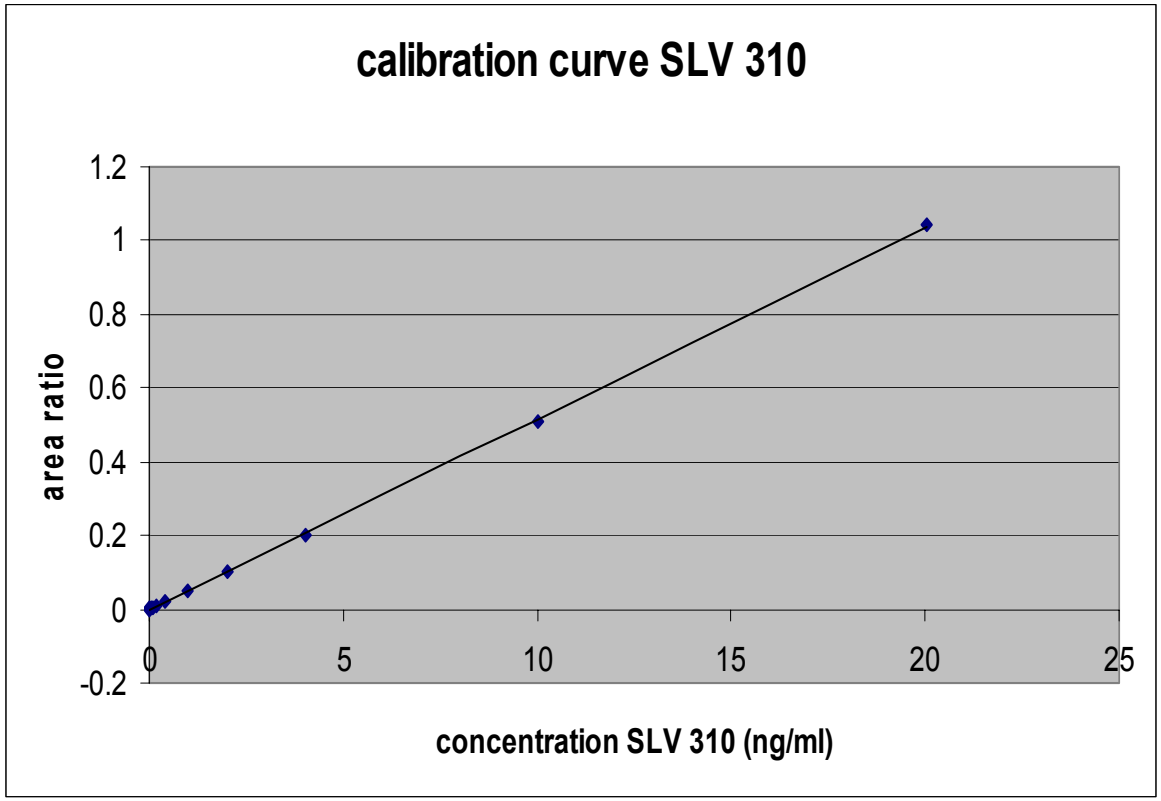
- **SLV 310**
  - **Precursor ion: m/z 418.2**
  - **product ion: 231.2**
- **^{13}C SLV 310:**
  - **Precursor ion: m/z 422.2**
  - **product ion 235.2**

LCMSMS

In study validation

Calibration standard	ST_00	ST_01	ST_02	ST_03	ST_04	ST_05	ST_06	ST_07	ST_08	ST_09	ST_10
Spiked level (ng/ml)	0.0000	0.0200	0.0400	0.100	0.200	0.400	1.00	2.00	4.00	10.0	20.0
Foud level Mean	0.0005	0.0190	0.0394	0.098	0.208	0.407	1.02	1.93	3.88	9.6	20.5
Bias (%)	-	-5.2	-1.7	-2.4	3.7	1.7	1.7	-3.5	-3.1	-3.7	2.6
inter-day s	0.002	0.001	0.003	0.007	0.011	0.018	0.037	0.071	0.193	0.360	0.618
inter-day C.V. (%)	428	7.5	7.5	7.0	5.2	4.4	3.6	3.7	5.0	3.7	3.0
n	10	10	10	10	10	10	10	10	10	10	10

Typical Calibration Curve

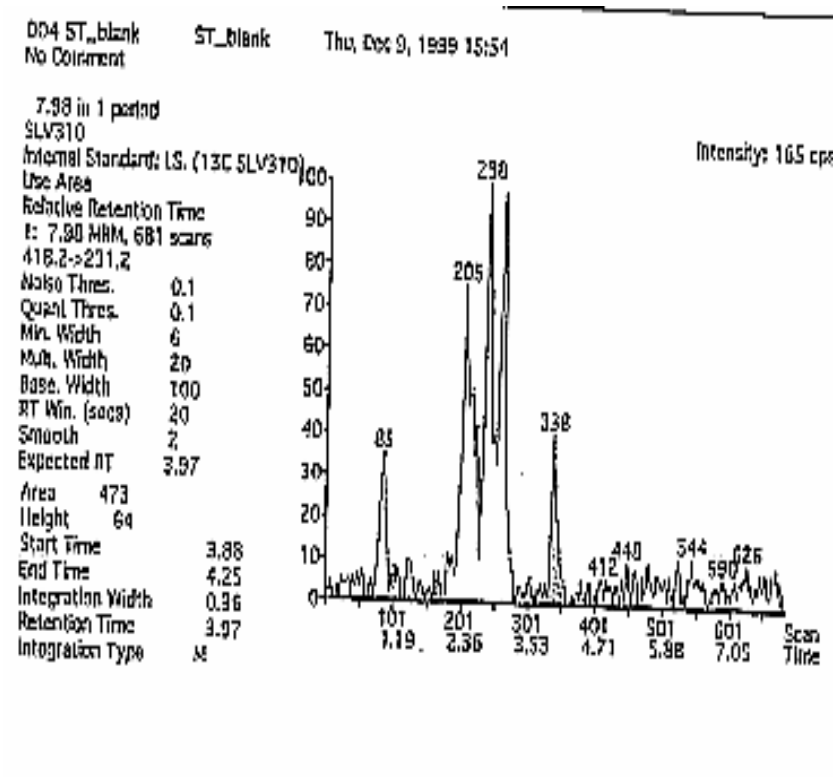


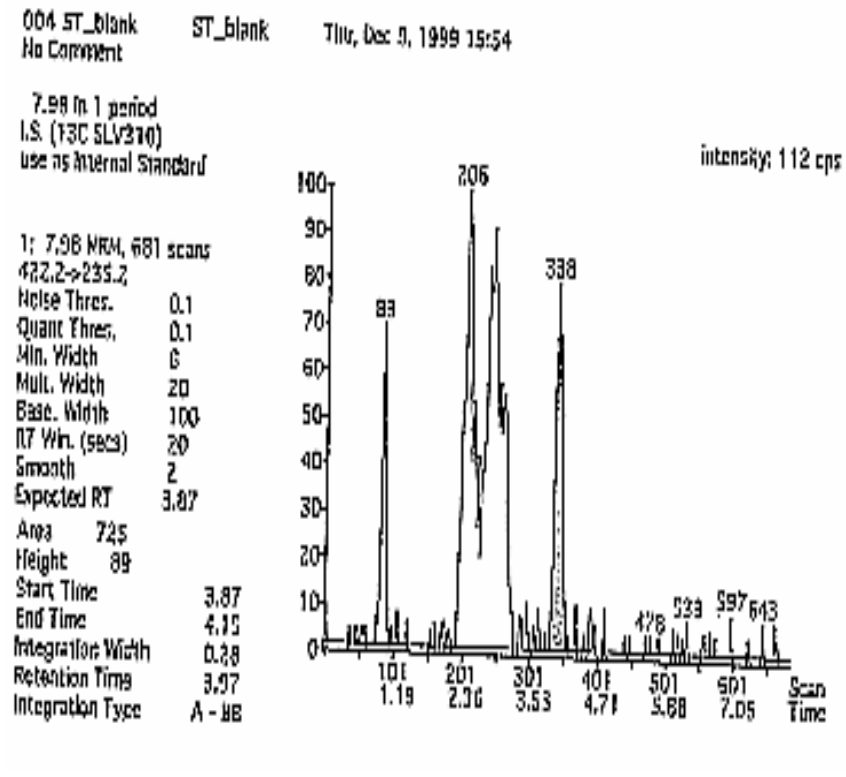
- Weighted Linear fit (1/x)
  y=a+bx
- r= 0.99991
- a= 0.00035
- b = 0.0514

In Study Validation

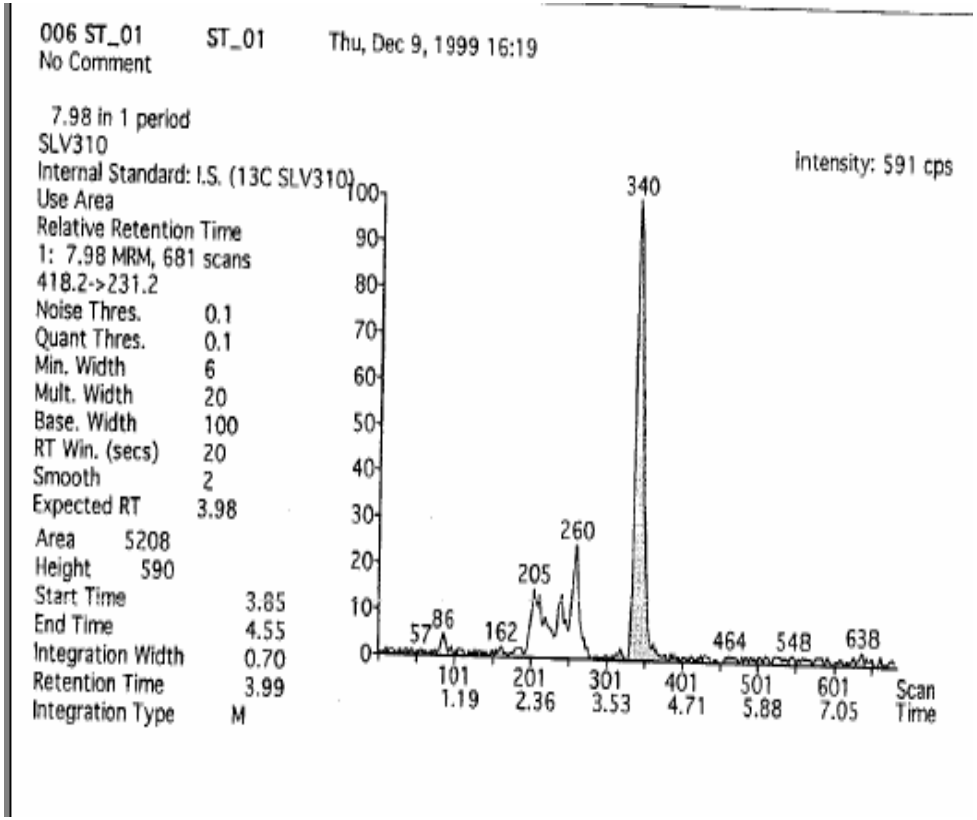
	QC No. P9054	QC No. P9055	QC No. P9056
Analysis batch no.	0.0503 ng/ml	0.503 ng/ml	5.03 ng/ml
Found level in ng/ml	0.0498	0.522	4.86
Bias in %	-0.96	3.8	-3.4
n	20	21	21
Intra-day s in ng/ml	0.0048	0.0450	0.1323
Intra-day CV in %	9.5	8.6	2.7
DF intra-day	10	11	11
Inter-day s in ng/ml	0.0044	0.0607	0.2143
Inter-day CV in %	8.9	12	4.4
DF inter-day	18	15	13

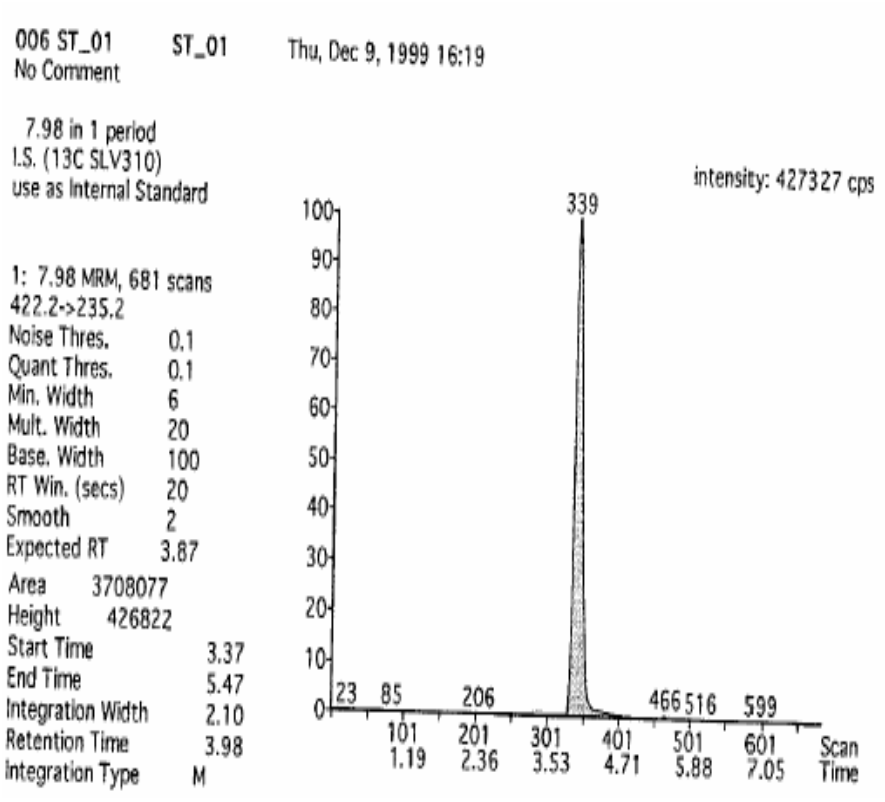
Typical LCMSMS chromatogram ST_blank (zero-calibrator without IS)



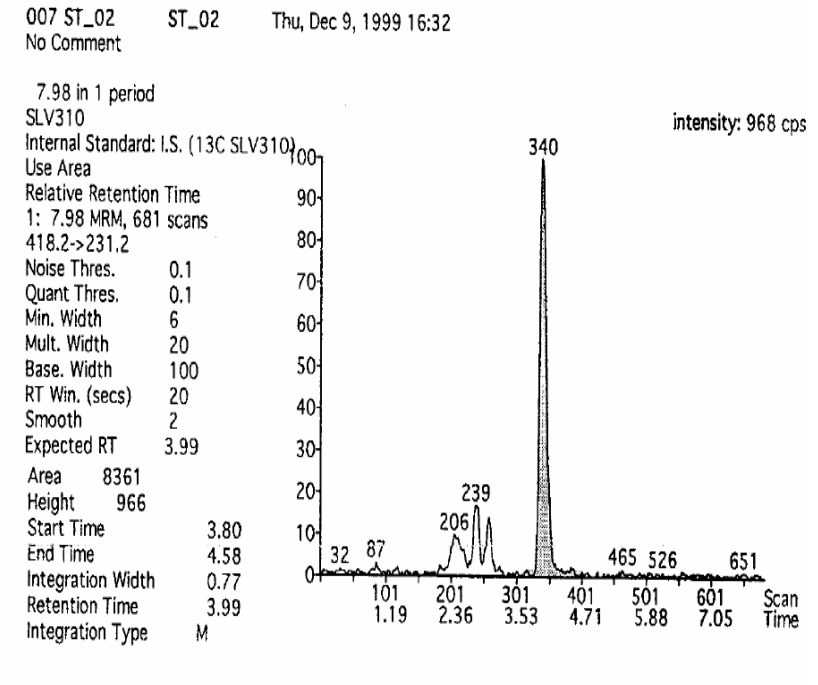


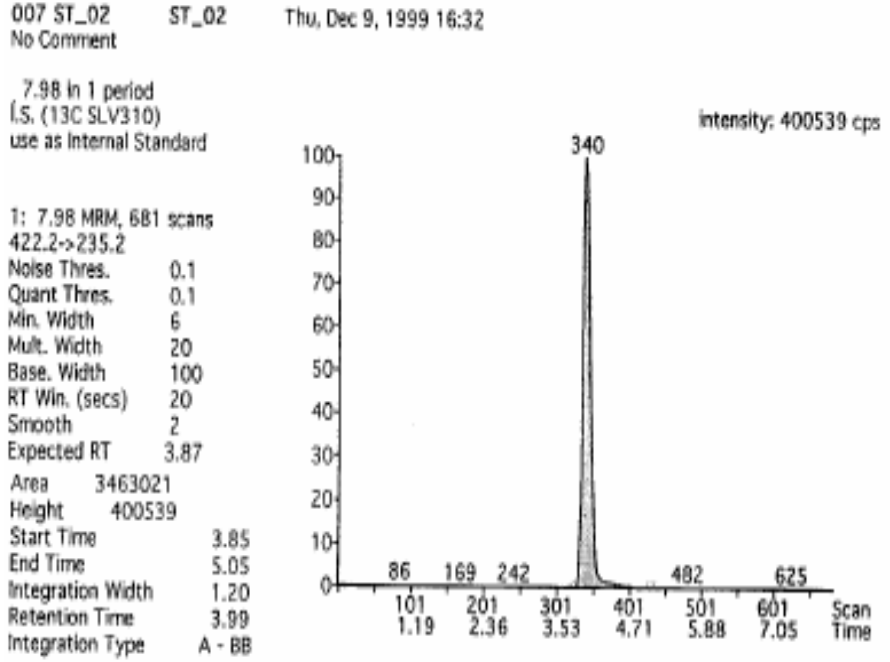
Typical LCMSMS chromatogram ST_1 (0.02 ng/ml)



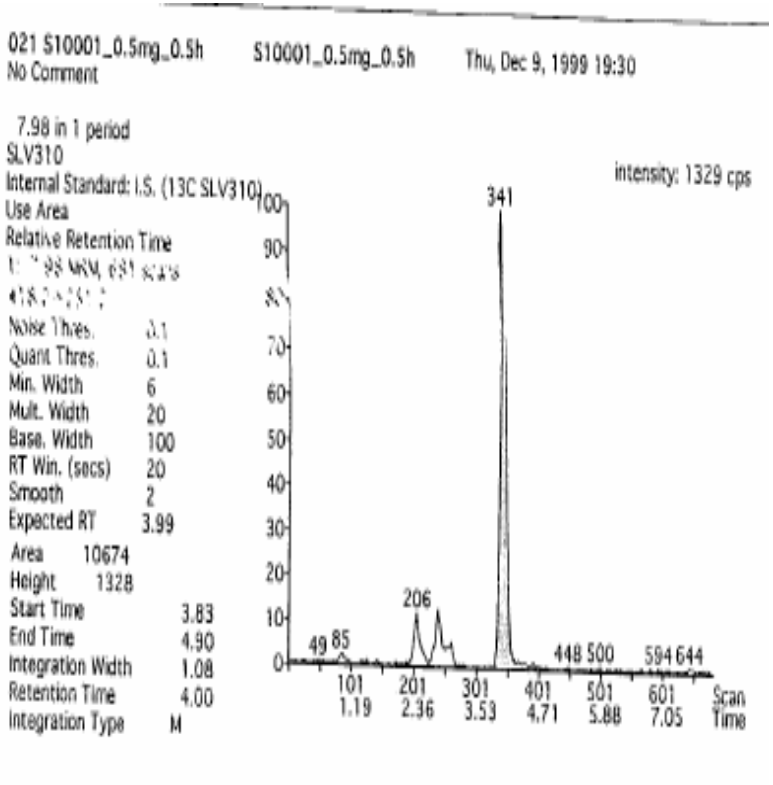


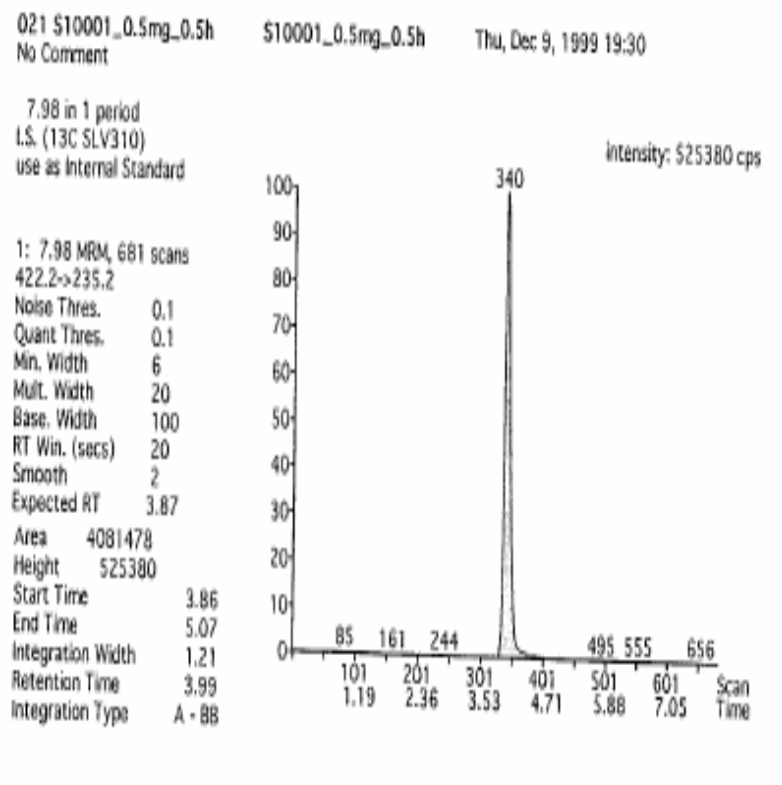
Typical LCMSMS chromatogram ST_2 (0.04 ng/ml)





Typical LCMSMS chromatogram real sample from SRDT study





Met dank aan:

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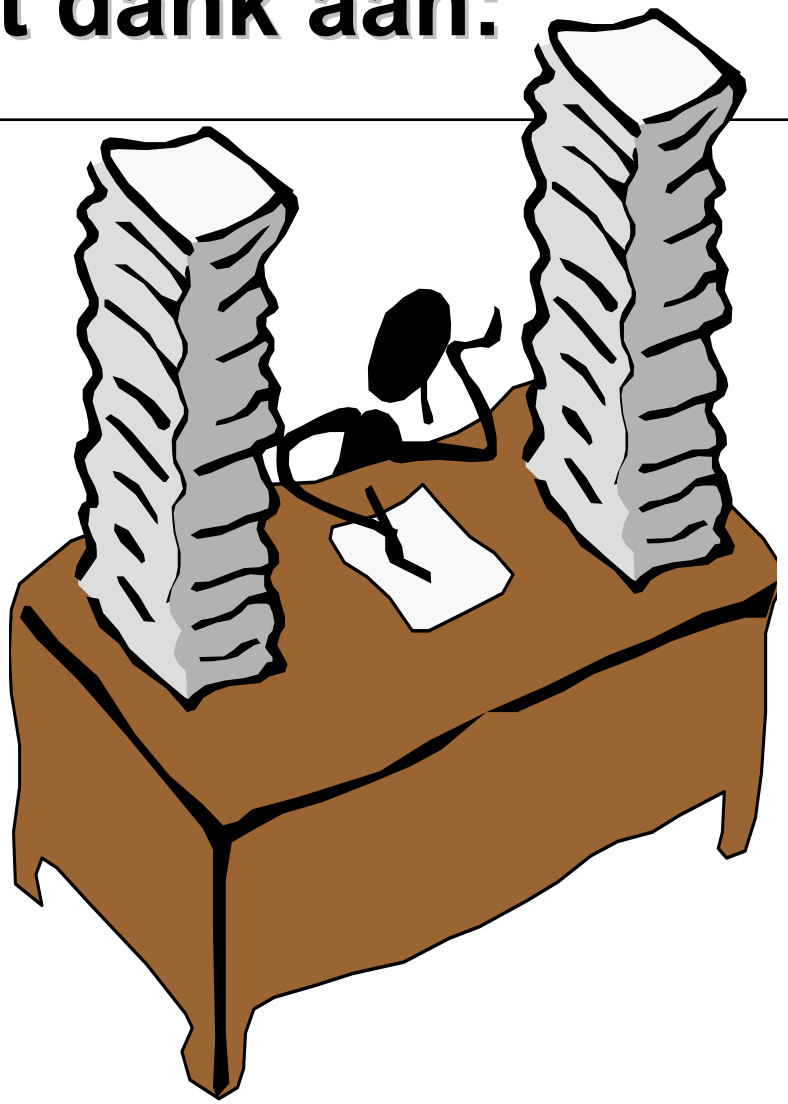
- Marcel Pistorius
- Ed Veenendaal
- Dick van der Stel



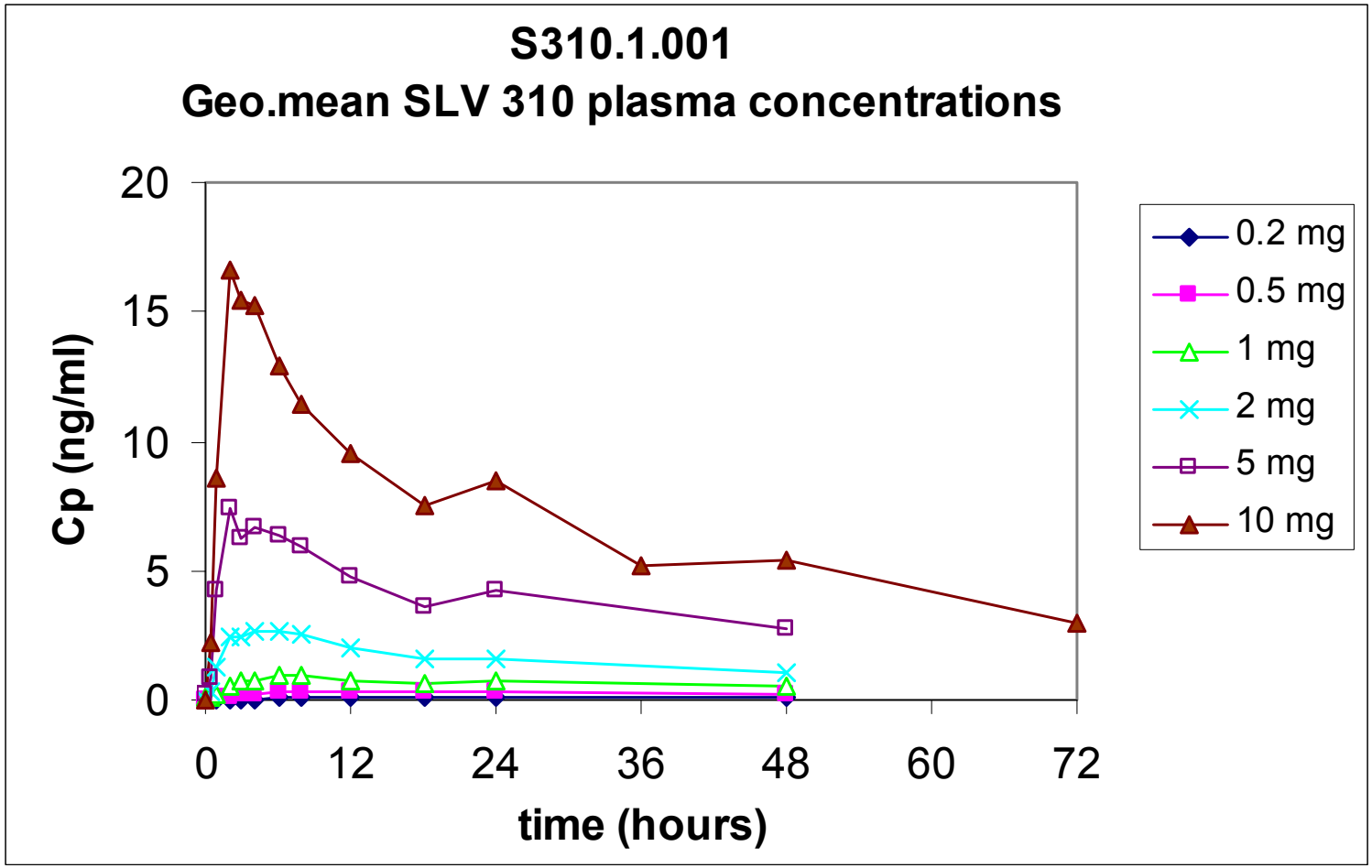
CLINICAL PART (Cont'd.)

SLV 310 THE COMPOUND

- D_2 antagonist / (Selective) Serotonin Reuptake Inhibitor
- Pre-clinical kinetics:
  - high first pass metabolism in rat, dog and monkey (low oral bio-availability)
  - elimination half-life: about 2 – 4 hours
  - non-linear kinetics in rat and dog; linear kinetics in monkey
  - in vitro Cyt P450 3A4, 2C9 and 2D6 involved in metabolism

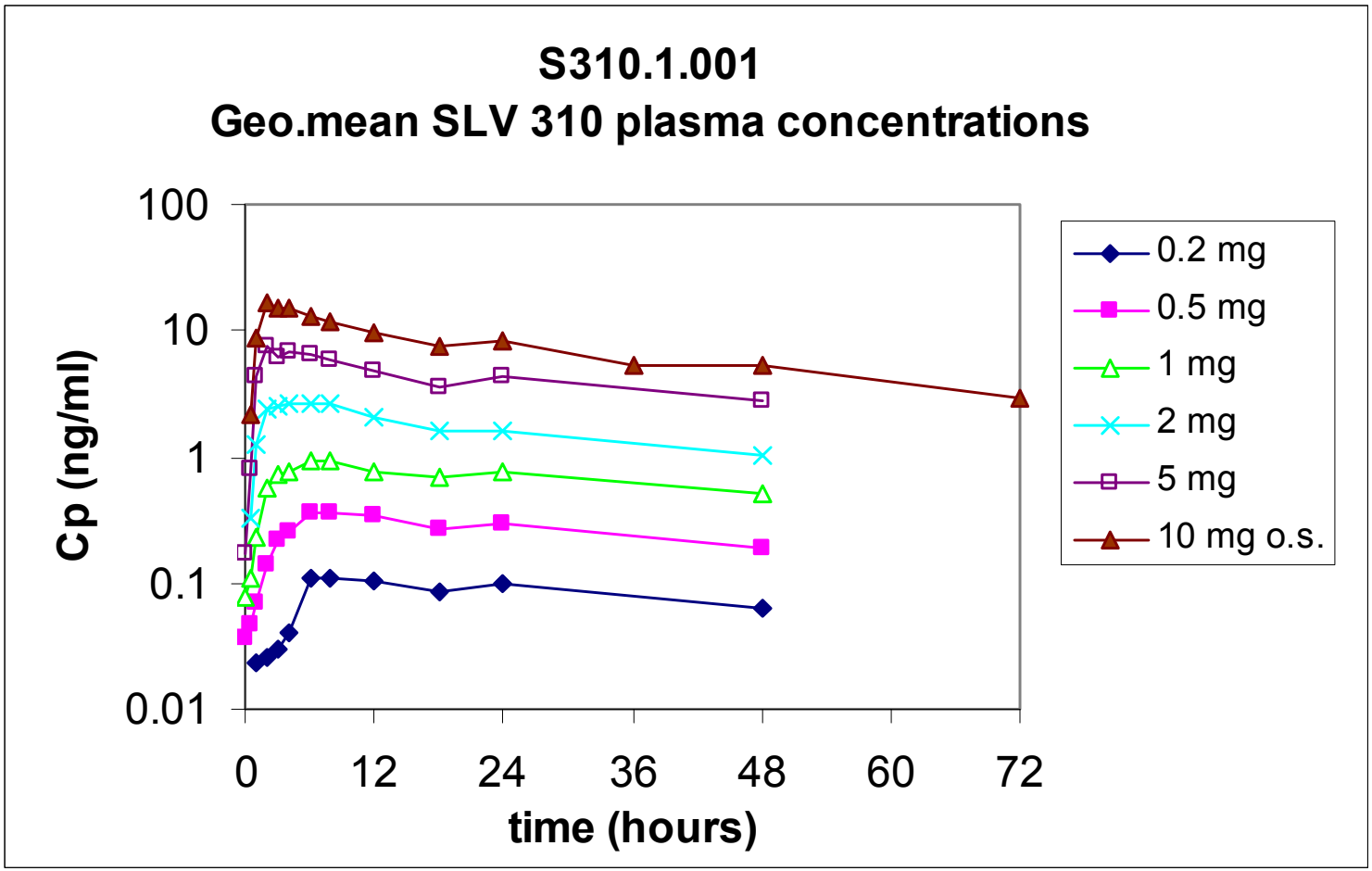
SLV 310 Plasma levels SRDT

(Oral Solution)



SLV 310 Plasma levels SRDT

(Cont'd.)



SLV 310 Plasma levels SRDT

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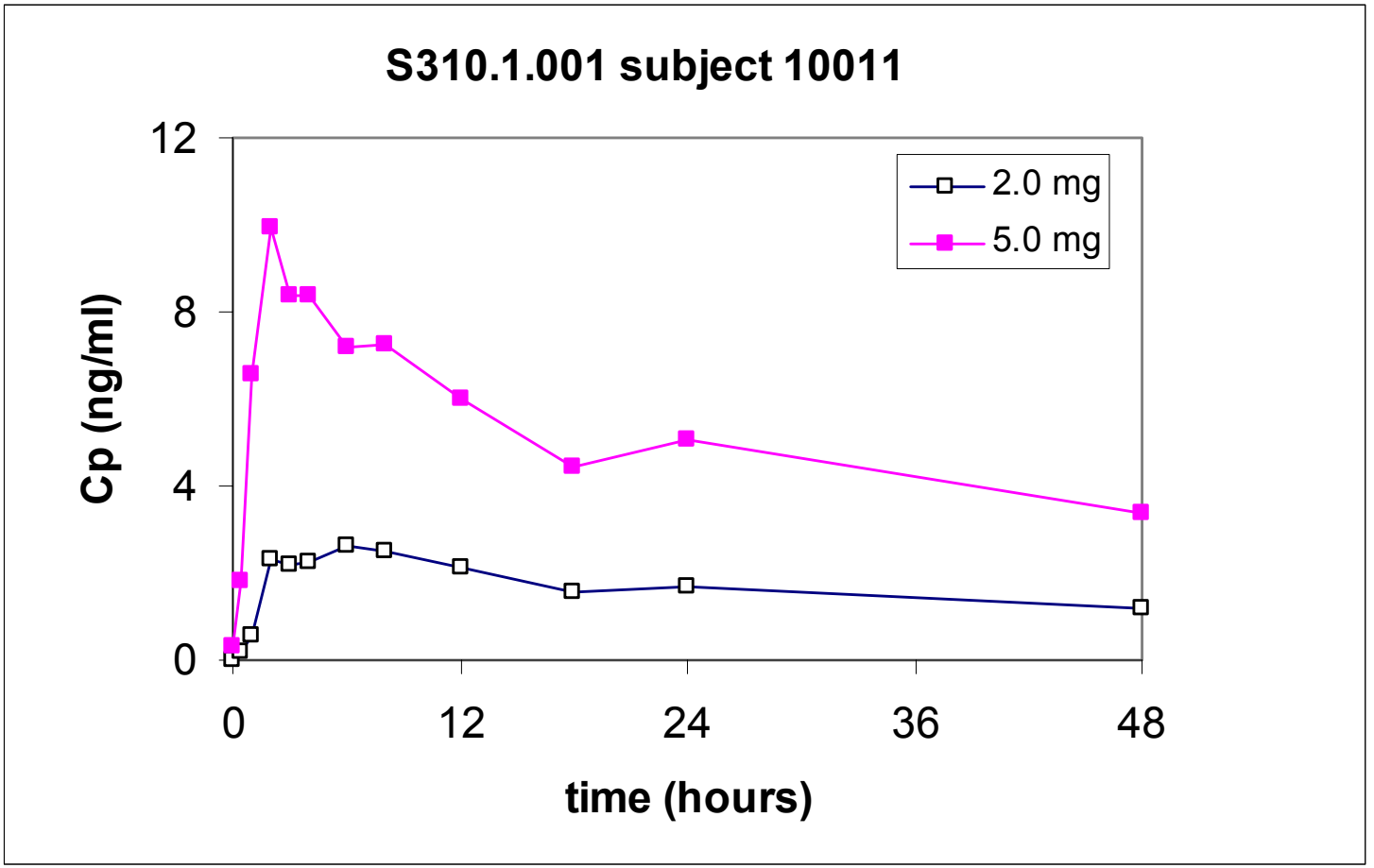

SLV 310 Plasma levels SRDT

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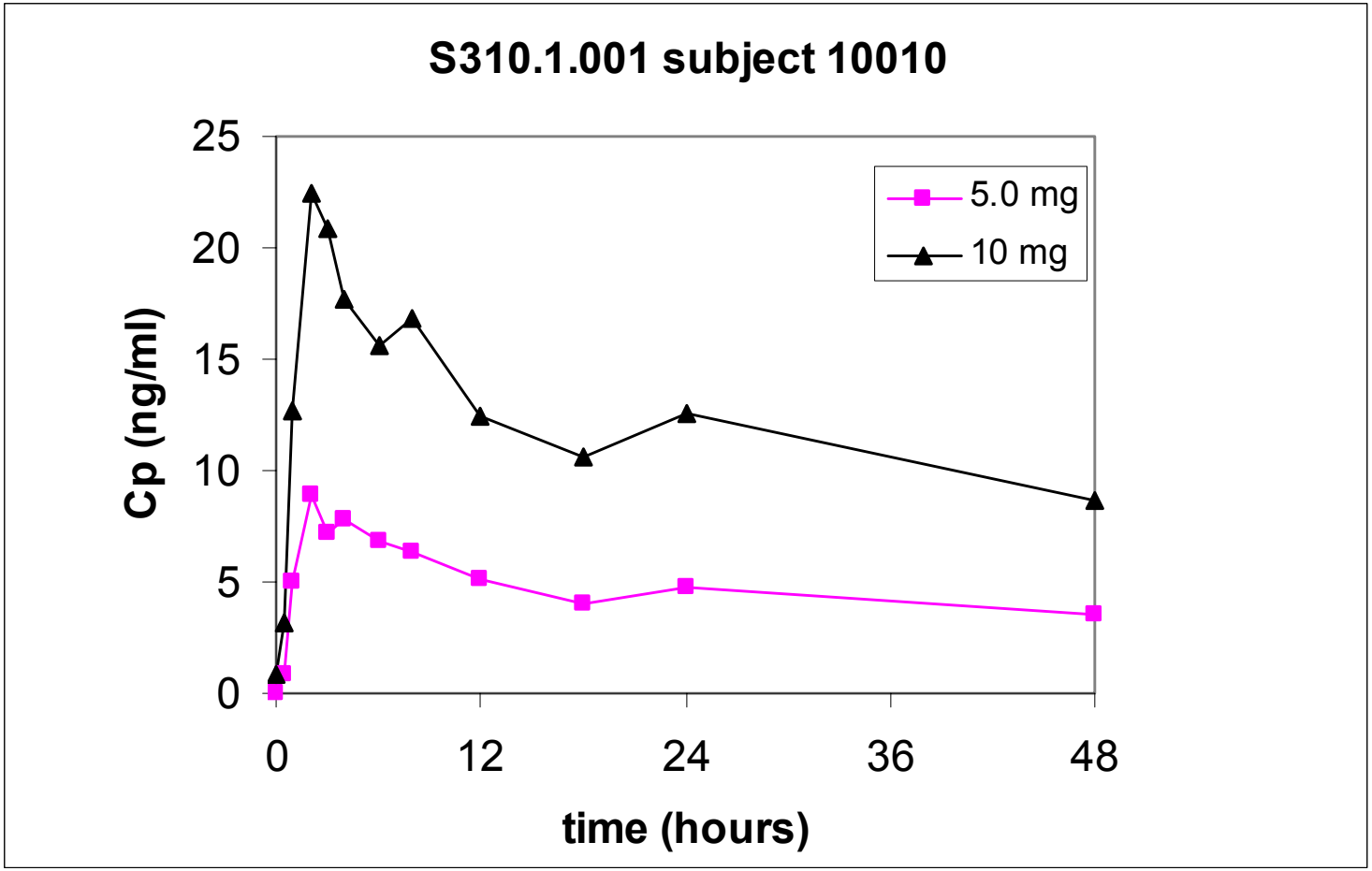
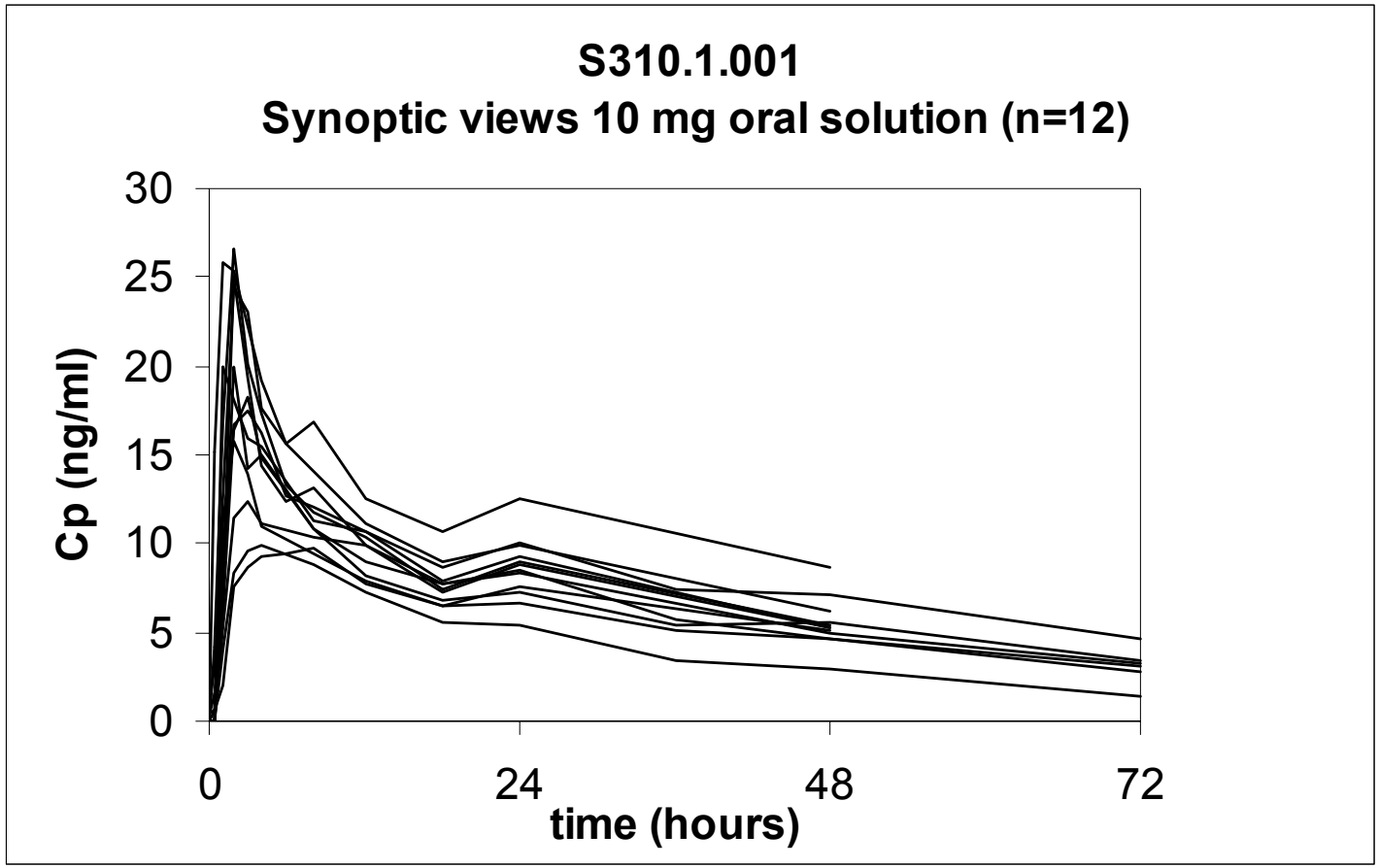
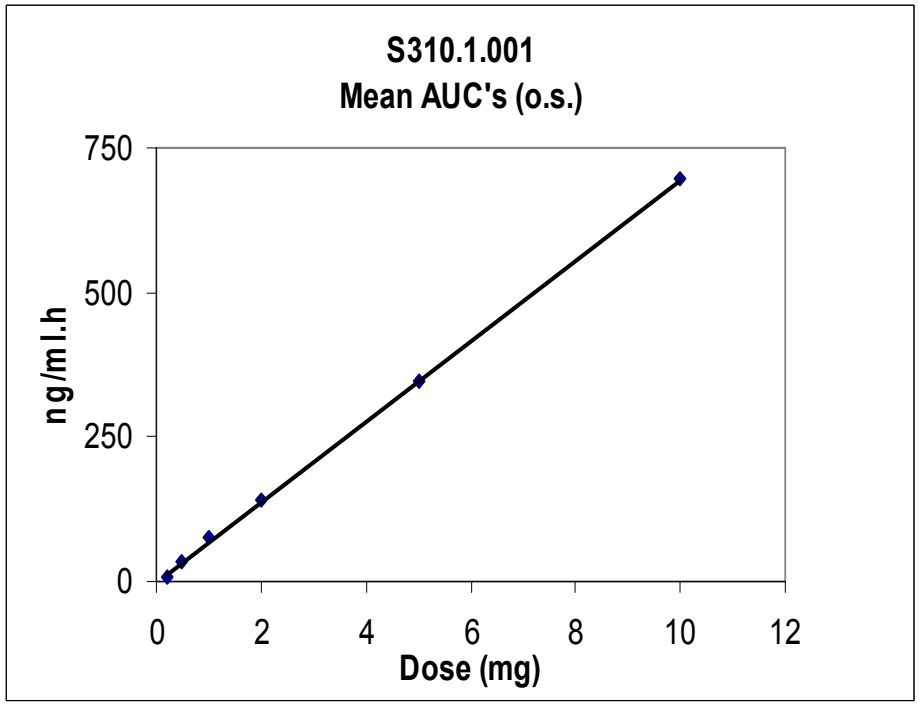


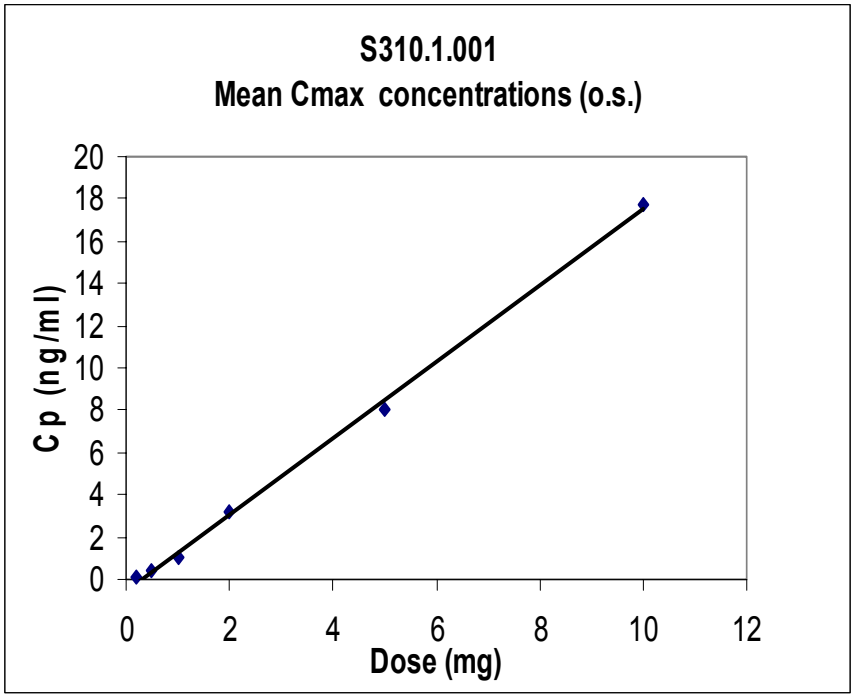
SLV 310 Plasma levels SRDT

(Cont'd.)

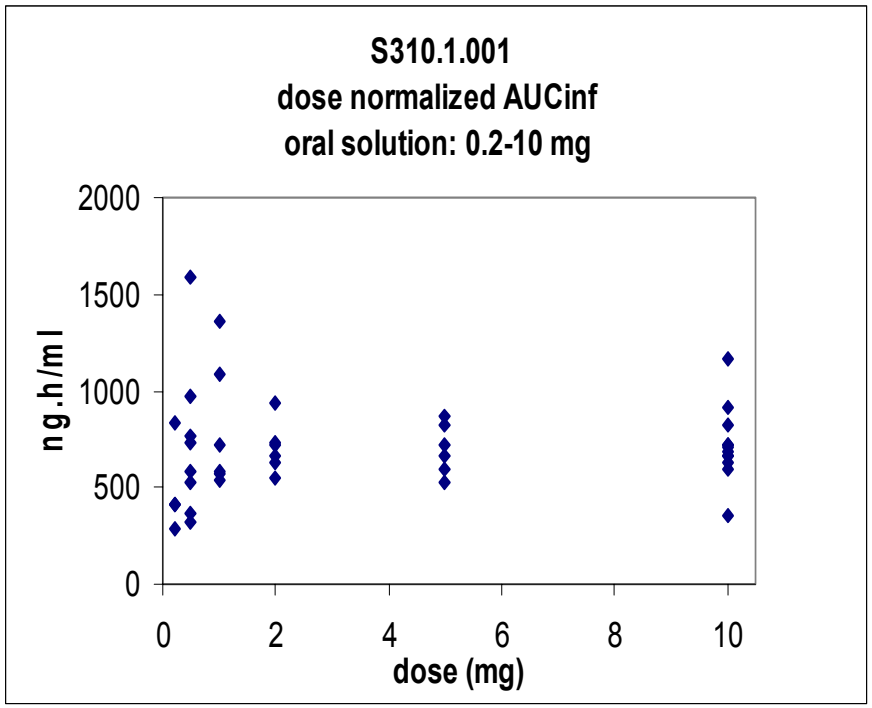


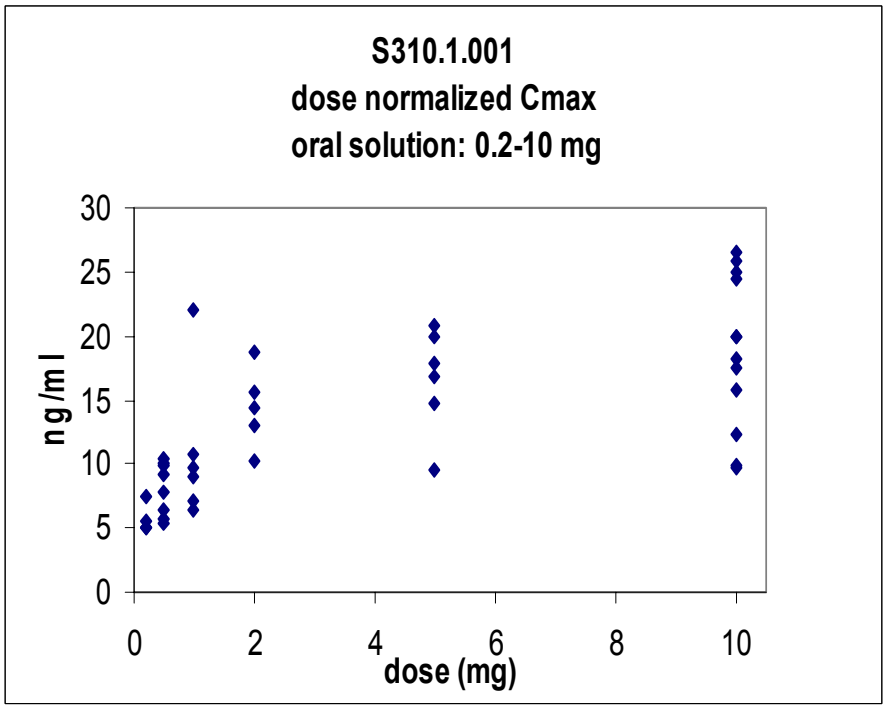
SLV 310 Linearity of single dose kinetics (Oral Solution)





SLV 310 Linearity of single dose kinetics (Cont'd.)





SLV 310 PHARMACOKINETIC RESULTS SRDT

	Cmax (ng/ml)	**AUC(inf)** (ng*h/ml)	**Tmax** (h)	**T1/2** (h)
	geo. mean	geo. mean	mean	mean
Dose level:				
0.2 mg	0.12	9.0	8.0	58.0
0.5 mg	0.39	32.4	7.8	70.6
1.0 mg	0.99	75.7	5.7	58.1
2.0 mg	3.20	139.0	4.5	39.8
5.0 mg	8.07	345	3.2	36.1
10.0 mg	17.7	695	2.8	39.5

SLV 310 PHARMACOKINETIC RESULTS SRDT

- No indications for high degree of first pass metabolism
- Decrease in Tmax with increasing doses
- Plasma concentration curves indicate recirculation of SLV 310
- Low inter-subject variability in plasma concentrations
- Half-life time 40-70 hrs
- Linear kinetics in investigated dose range
- No relation between PK and CYP2D6 status

SLV 310 PHARMACOKINETIC RESULTS SRDT

- **Long apparent elimination half – life time →**
  - **Cave: - exposure ↑**

    - duration tolerability / safety assessments

    - duration hospitalization

    - duration washout period between sessions

    pre – dose SLV 310 levels !

    - PK / PD sampling schedule

SLV 310 PHARMACOKINETIC RESULTS SRDT

Analytical results pre-dose (H0) samples Group II

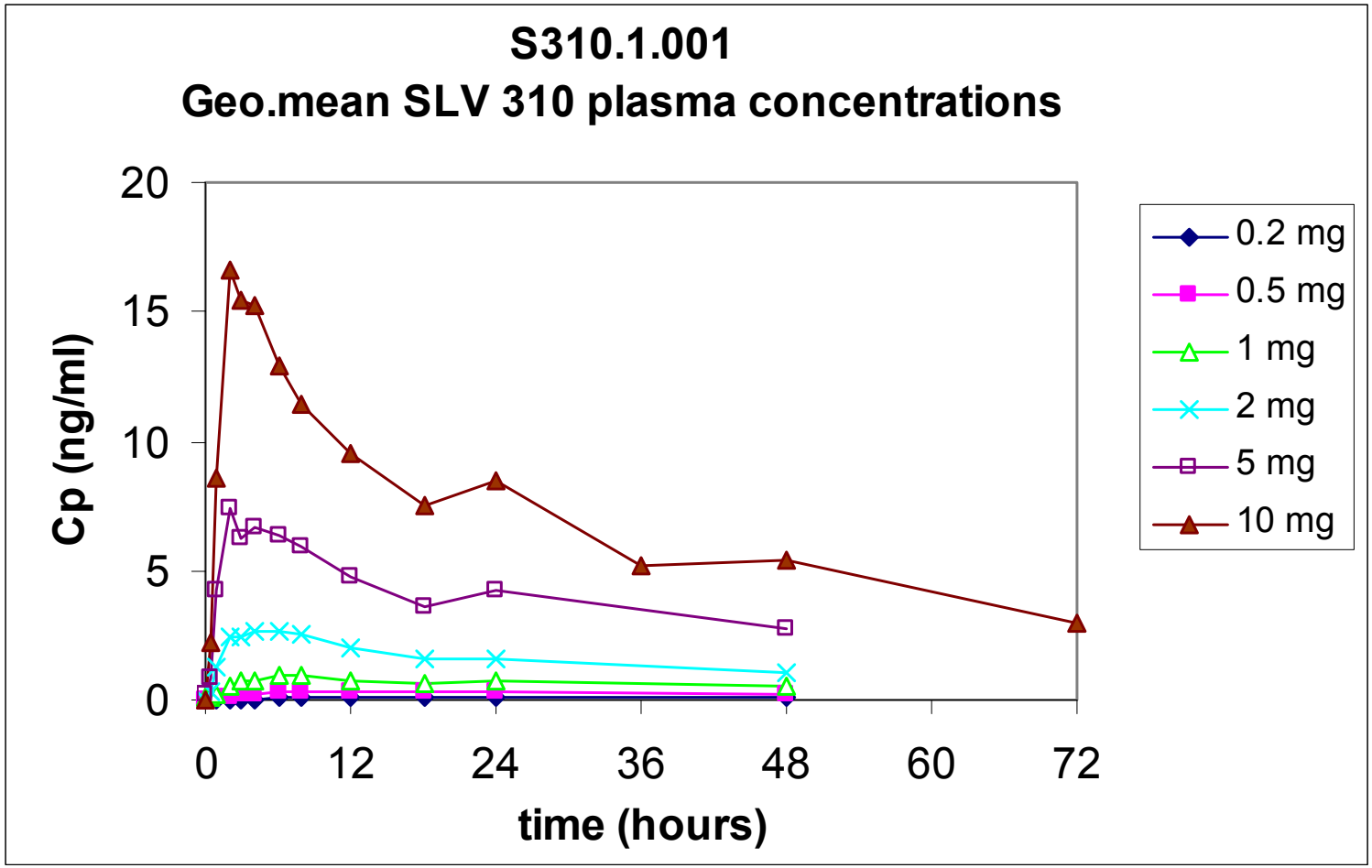
Subject	Session IV	Session V	Session VI
AN	< LLOQ	0.286	Placebo
AV	< LLOQ	Placebo	0.0184
AI	< LLOQ	0.171	Placebo
AU	< LLOQ	0.100	Placebo
AE	< LLOQ	Placebo	0.0235
AZ	< LLOQ	Placebo	0.0353
AQ	Placebo	< LLOQ	0.834
AJ	Placebo	< LLOQ	0.259
AM	Placebo	< LLOQ	0.425

ADAPTED DESIGN SRDT for Group III

- Hospitalization prolonged to two days
- Tolerability / safety assessments schedule adapted
- PK / PD sampling schedule adapted
- Washout period prolonged to two weeks
- 10 mg dose level repeated in session 7 (tolerability !)

SLV 310 Plasma levels SRDT

(Oral Solution)



SLV 310 Plasma levels SRDT

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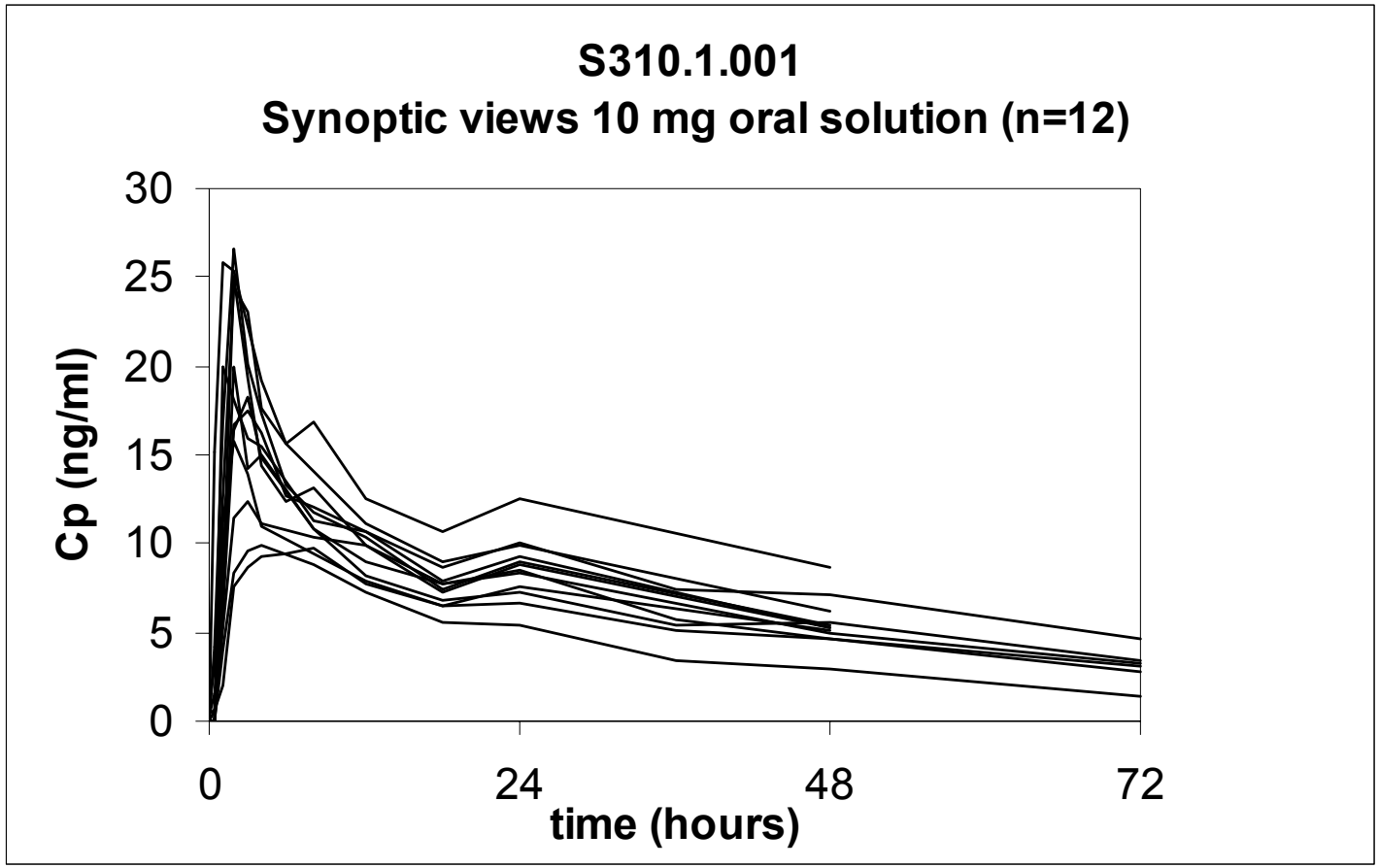


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