Muster: Englischer Prüfplan nach ICH 2

STUDY PROTOCOL

1 Titel page

Titel

Phase

Sponsor

Study Center

Principal Investigator

Project-No

Date

Version

2 Contents and Abbreviations

This protocol contains pages and appendices.

2.1 Content

2.2 Abbreviations

Abs.	Absatz
AMG	Gesetz über den Verkehr mit Arzneimitteln (Arzneimittelgesetz)
ANOVA	Analysis of Variance
AVB/P	Allgemeine Versicherungsbedingungen/Probandenversicherung
BAnz.	Bundesanzeiger
BGBl	Bundesgesetzblatt
CIOMS	
CPMP	Committee for Proprietary Medicinal Products
CRF	Case Report Form
EFPIA	
EC	European Community
ECG	Electrocardiogramm
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
QAU	Quality assurance Unit
AST	
ASL	
AE	Adverse Events
WHO	World Health Organization

3 Synopsis

Titel:

Sponsor:

Monitor:
Study Center:
Principal Investigator:
Project-No:
Objective:
Study Design:
Phase:
Substance in Trial:
Dose: **x mg**
Subjects:

4 Sponsor and Study Center

SponsorTel.: Fax:
Monitor: Tel.: Fax:
Principal Investigator
Study Center
Clinical Chemistry
Ethical Committee
Quality Assurance Institute of Quality-Systems in Medicine and Science (Hamburg)
The curricula vitae of the investigators and all involved scientists aregiven in appendix .

5 Introduction/Summary

Mindestens folgende Fragen müssen beantwortet werden:
Um was für einen Stoff handelt es sich (chemische und pharmakologische Klassifikation)?
Welche Vorstellungen gibt es über den Wirkungsmechanismus?
Welche Indikationen leiten sich daraus ab?
In welcher Formulierung wird er dabei regelmäßig eingesetzt?
In welcher(n) therapeutischen Dosierung(en) unter welchen Umständen wird er dabei regelmäßig eingesetzt?
Wie ist der Metabolismus?
Angaben zur Resorption aus Darm/Applikationsort in %
gemessene Wirkspiegel unter der therapeutischen Dosierung,
wenn vorhanden
Metaboliten und ob diese pharmakologisch wirksam sind
Ausscheidung über Leber, Niere
Proteinbindung
Zur Sicherheit und Verträglichkeit:
Sind besondere Vorsichtsmaßnahmen zu beachten?
Sind Interaktionen mit anderen Arzneimitteln bekannt?
Gibt es unerwünschte Arzneimittelnebenwirkungen?
Liegen Erfahrungen aus der Anwendung an Gesunden vor?
Risikoabschätzung
Literaturangaben

6 Study Objectives

6.1 Main Objective

This study is designed to assess the of the drugs after .

6.2 Variables

The parameters are described in detail in chapter .

7 Investigational Plan

7.1 Study Method

7.1.1 Study Design

7.1.2 **Regular Completion and Discontinuation of the Study**

The study will be completed, when subjects have passed the studyprotocol.

All subject data have to be documented and will be evaluated, if the study is discontinued. In this case, the sponsor will receive a summary report, and a notification will be given to the ethics committee.

The complete study will be discontinued by the principal investigator, if this seems to him necessary for medical and/or ethical reasons.

Protocol Modifications

Modifications of the protocol are permitted only if they are authorized by the sponsor and the principal investigator in writing.

Any modifications must be included in the study protocol as amendments and signed by the sponsor, the principal investigator and the Quality Assurance Unit.

All modifications of the study protocol will be reported to the ethics committee.

7.2 Ethical and Legal Aspects

7.2.1 Ethical Committee

Prior to the study, the study protocol, the information sheet and the informed consent for this study will be presented to an ethical comittee affiliated to the *Ärztekammer* for review and approval.A signed and dated statement of approval by the ethics committee will be included in the final report.

The members of the committee are listed in appendix .

7.2.2 Basic Guidelines and Documents

This study will be performed in accordance with the Note for Guidance "Good Clinical Practice for Trials on Medicinal Products in the European Community" III/3967/88-EN of July 11, 1990, with the Note for Guidance "Investigation of Bioavailability and Bioequivalence" (III/54/89-EN of December 1991) and the appropriate regulations of the AMG, the "Grundsätze zur ordnungsgemäßen Durchführung der klinischen Prüfung von Arzneimitteln" (BAnz. 39, December 30, 1989, p. 16617-16618) and the Declaration of Helsinki (revised version of Hong Kong, September 1989, latest German version published in BAnz. 108, June 1987, p. 1709).

7.2.3 Informed Consent

Before the clinical examination, the physician will explain the aim, scope and risks of the study to the subjects. He will explain the method of application, blood withdrawal as well as rules of conduct and any restrictions which may apply. Possible effects and side effects will be discussed. Subjects will be informed that they are free to withdraw from the study at any time, without giving any reason for doing so.

By judgement of the investigator they must be able to understand the full implications of their decision.

All participants must sign an informed consent form as evidence of consent.

The subjects information sheets and the consent forms are attached in appendix .

7.2.4 Subject Insurance

The study center will take out insurance cover for its subjects in accordance with "Allgemeine Versicherungsbedingungen/Probandenversicherung (AVB/P)". A copy of the policy is attached in appendix .

7.2.5 Qualification of the Principal Investigator

By his signature at the bottom of the study protocol, the principal investigator certifies that he has at least two years experience in conducting clinical drug trials, as required by German law (§ 40, Abs. 1, Nr. 4 AMG).

7.2.6 Notification of Health Authorities

The study center will notify this clinical investigation to the Behörde für
in accordance with § 67, 1 AMG. A copy of this notification will be included in the final report.

7.3 **Study Population**

Subjects will be recruited from the study center subject pool. A subject will qualify for the study, if he fulfills all inclusion criteria and none of the exclusion criteria.

7.3.1 Inclusion Criteria

- Ages:
- Sex:
- Physically and mentally healthy subjects as confirmed by an interview, medical history, clinical examination, laboratory tests and electrocardiogram. Negative result of HIV1 and hepatitis test (annual screening).
- Normal body weight in relation to height (Broca-Index -20% to +10%).
- Informed consent signed by the subject.
- Subjects must be cooperative and available for the entire study.
- Contraception.
- Gynecological examination/cancer screening.
- Additional examination (e.g., ultrasound scan).
- Abstinence from alcohol for 36 hours prior to commencement (as stated by subject).

7.3.2 Exclusion Criteria

- Evidence in the subject`s medical history or in the medical examination of any clinically significant hepatic, renal, gastrointestinal, cardiovascular, pulmonary, hematological or other significant acute or chronic abnormalities which might influence the absorption, distribution, metabolism or excretion of the active agent.
- Hypersensitivity to medicines, atopic eczema or allergic bronchial asthma.
- Laboratory test results outside the stated reference values of the laboratory and tolerance values as laid down by the study center, which may be an evidence of disease.
- Regular use of any medication within four weeks prior to commencement of the study (self-medication or prescription).
- Single use of any medication (including OTC) that are not expressively permitted within two weeks prior to start of the study.
- Alcohol, caffeine or tobacco abuse (more than 10 cigarettes a day) or drug addiction. *Positive drug screening in urine (limit of tolerance for cannabis 300µg/l).*
- Participation in a clinical investigation and/or blood donation (450 ml) within the past eight weeks.
- Following a diet which deviates from usual eating habits.
- Failure to attend to an examination appointment without prior agreement with the investigating physician.
- Pregnancy, breastfeeding period.
- Thrombophlebitis (for hormonal substances).

7.3.3 Withdrawals

The following circumstances may lead to discontinuation of the study by an individual subject who will then be recorded as a drop-out:

withdrawal for personal reasons

adverse events necessitating withdrawal from the study

sudden incidence of diseases

circumstances in which the health of the subject would be endangered upon continued participation in the study.

Subjects who discontinue the study for one of the above mentioned reasons will be documented in full in the final report.

All drop-outs will be replaced. In case of withdrawal for personal reasons this will be at the expense of the study center, in all other cases at the sponsor's expense whereby the study center is obliged to minimize such expenses. A backup subject will be assigned to the same application sequence as the drop-out and to an ID number following the last number assigned to a subject.

If a subject drops out because of an adverse event or a disease, the investigator will arrange a checkup, if necessary including laboratory tests. The actual health state will be documented. Laboratory tests which are not within tolerance ranges will be repeated until they are within the tolerance limits again, or no further change to the better can be expected. The sponsor will receive a summary report about these data separately.

7.3.4 Sample Size - Number of Subjects Planned

subjects will participate in the study.

7.4 Treatments

7.4.1 Treatments to be Compared

7.4.1.1 Test Drug a

Name:
Active substance:
Dosage Form:
Strength:
Dose: (.. units)
Batch/Lot No.:
Manufacturer:

7.4.1.2 Test Drug b

Name:
Active substance:
Dosage Form:
Strength:
Dose: (units)
Batch/Lot No.:
Manufacturer:

7.4.1.3 Reference Drug (c)

Name:
Active substance:
Dosage Form:
Strength:
Dose: (units)
Batch/Lot No.:
Manufacturer:

7.4.2 Identity of Test Materials

7.4.2.1 Labelling and Shipment

The investigational products will be provided by the sponsor in sufficient quantities, together with certificates of analysis and information on storage and stability. The address for delivery purposes is:

The investigational products must be furnished with a batch number and/or date of manufacture. Each container must be marked "*Zur klinischen Prüfung bestimmt*" and bear the name and address of the manufacturer, the designation of the drug, batch number, formulation, usage form and details of the contents such as weight, number of items or similar. The trial preparations must arrive at the study center at least three months before their expiry date.

7.4.2.2 Storage

The principal investigator will be responsible for proper storage of the investigational products.

All investigational products must be stored in a restricted area *at room temperature*/8 C. A drug dispensation log will be maintained throughout the study. In this log, all applications will be documented, with date and signature. A copy of this log, along with supplies not used, will be sent to the sponsor upon completion of the trial.

7.4.3 Dosage Regimen

7.4.3.1 Dosage

According to the administration plan the subjects will receive dosis, präparat

I. During the course of the study subjects will receive a total dose of (wirkstoff)

7.4.3.2 Wash Out

Wash out time between the two applications will be at least...

7.4.3.3 Administration

The trial drugs will be applied at approximately *8:00* with one minute time intervals between subjects to ensure correct blood sampling.

Special description?

7.4.3.4 Precautions, Antidotes

Adverse events are not expected to occur.

For the treatment of non-specific reactions (allergy, anaphylactic shock) an emergency equipment and a written standard operating procedure are available. A physician will be on call at all times.

7.4.4 Randomization

A consecutive number will be assigned to the names of subjects on a list. This number will be independent of any personal features.

The sequence of the applications (a - b or b - a) is assigned to the numbers by the randomization procedure PLAN of the SAS-Institute, Cary, NC, USA (see appendix).

7.4.5 Blinding

This is an open study, the treatments are not blinded. During the study the drug concentration as the main variable is neither to be recognized nor to be influenced by the investigators or the subjects.

7.4.6 Concomitant Drug Use

- Use of drugs, which are not explicitly permitted by the investigator is forbidden. Prior to each dosing, subjects will be asked about this point. The following drugs are permitted during this study:
- Homeopathics
- Paracetamol
- Acetylsalicylic Acid.

Any use of drugs will be documented in the case report forms (CRF), specifying the substance, dose, time and reason for use. If a subject has used a permitted or self-prescripted drug, the investigator has to decide about the exclusion of the subject. In this case and if the drug was applied in order to treat an adverse event, the physician has to justify his decision.

7.4.7 Compliance

Compliance with study conditions will be ensured by the presence of a medically trained person on the ward at all times. An investigator will supervise the study at all phases.

7.5 Criterion for Effectiveness

Testing the effectiveness by assessing the bioequivalence will be based on the following fundamental hypothesis:

Two formulations that do not differ significantly in the rate and extent to which they make the active ingredient available in the circulating blood will not differ much in their therapeutic efficacy [i].

The bioequivalence of the formulations will be confirmed as according to one of the stated procedures the calculated confidence interval of the AUC is xx% and thus will lie within the acceptance limits of 80% - 125% and that of the C_{max} is xxx% and thus will lie within the acceptence limits of 75% - 133%. The procedure for determining equivalence on the basis of logarithmic-parametric confidence intervals will have priority if (log-)normal distribution of the may be assumed. For this purpose the SAS UNIVARIATE procedure will be used to check whether the ANOVA residues deviate significantly ($p < 0.05$) from normal distribution.xxResultxx.Therefore, equivalence will be assumed on the basis of the non-parametric confidence intervals.

7.6 Effectiveness and Safety Variables

7.6.1 Effectiveness Variables

7.6.1.1 Primary Variable

AUC_{0-} : area under concentration time curve, extrapolated from 0 to infinity;

7.6.1.2 Secondary Variables

AUC_{0-tz}: area under concentration time curve, calculated by the trapezoidal rule (time 0 to last quantifiable sample $C(t_z)>0$); C_{max}: maximum concentration, stated as the larges[ii]t concentration measured; t_{max}: time to achieve C_{max};:terminal disposition rate constant, computed by log-linear regression (using at least three points on the curve with $C > 0$) as the amount of slope; the elimination constant with the optimal correlation coefficient significantly different from zero will be chosen; t½: terminal half-life ln(2)/ .

7.6.1.3 Primary Variable

AUC_{nx} : area under concentration time curve in the measuring interval (= n dosings intervals of = ## h) calcuated by the trapezoidal rule;

7.6.1.4 Secondary Variable

C_{max}: maximum concentration, stated as highest concentration measured;

t_{max}: time of the maximum concentration;

T_{cav}: the time interval in which the serum concentration exceeds the mean concentration. The mean concentration is $C_{av} = AUC_{nx} / nx$; PTF: Peak Trough Fluctuation in the measuring interval calculated as $(C_{max} - C_{min}) / C_{av}$.

7.6.2 Safety - Adverse Events

7.6.2.1 Severity

7.6.2.2 Causality

7.6.2.3 Documentation

7.6.2.4 Reporting

7.7 Study Procedures

Standardized case report forms (CRF) will be used to document all data concerning the course of the study. All entries will be completed in black ballpoint pen, they will be clearly legible and signed by the person who made the entry. All CRF will be checked, completed and signed on page 1 by the principal investigator. Corrections will be made in such a way that the original entry is not obscured. All corrections will be dated and initialled. A case report form is attached in appendix .

7.7.1 Screening

Within *three* weeks prior to the study, potential subjects must undergo screening in order to verify inclusion criteria and rule out exclusion criteria. The results of this screening will be used to document the state of health of the subjects prior to commencement of the study with the aim of comparing the pre-study health state with the post-study health state for insurance purposes.

7.7.1.1 Medical History

In a brief interview, medical and social histories will be evaluated by a physician in order to determine whether the subject fulfills the inclusion criteria.

7.7.1.2 Clinical Examination

The clinical examination includes measurement of blood pressure and heart rate as well as an examination of organ systems (ENT, CNS, cardiac, peripheral, vascular, pulmonary, musculoskeletal, abdominal, dermatologic and lymphatic). Blood pressure in sitting (according to Riva Rocci) and heart rate, body weight and height will be measured. An electrocardiogram (12 lead) will be recorded.

7.7.1.3 Laboratory Test

Hematology: erythrocytes, hemoglobin, hematocrit, white blood count, differential count, platelet estimate, reticulocytes.

Biochemistry: total protein, glucose, alkaline phosphatase, uric acid, creatinine, AST, ALT,G-GT, sodium, potassium,alpha-amylase, *drug screening (amphetamines, barbiturates, benzodiazepines, cannabis-metabolites, cocaine-metabolites, opiates (group reaction)), urinalysis, pregnancy test, additional tests.*

Negative results of HIV1-testing and hepatitis must be available (annual screening test).

Tolerance ranges are given in appendix . Subjects with values within these ranges may be enrolled in the study even if they exceed the reference ranges of the laboratory.

7.7.2 Study Conditions

7.7.2.1 Admission to the Study

Admission to the study will be effected upon the subject's arrival at the ward after his continued suitability has been determined in accordance with the inclusion and exclusion criteria. Should there be any doubts as to his state of health, the subject will not be admitted to the study. Subjects who fail to make themselves available upon commencement of the study, who are turned back due to non-compliance, or who cannot participate for personal reasons will be considered as not admitted to the study. These will be replaced by backup subjects until the required number is met. For compensation of the dropping-outs, subjects are recruited in surplus to meet the intended number of participants.

The calendar day of admission to the study will be designated day 1 in study documents.

7.7.2.2 Subject Identification

An identification number will be assigned to all subjects enrolled in the study. This ID, together with the subject's initials, must appear on all study documents. Subjects will have to wear a bracelet with this ID number as long as they stay on the ward. The bracelet cannot be removed without being destroyed.

7.7.2.3 Hospitalization

Subjects will arrive at the study center on the evening of the first day of the study at *19:00*. They will remain hospitalized until blood withdrawal *24* hours post application.

Subjects will return to the study center for blood withdrawals after discharge.

7.7.2.4 Diet

Fasting: The subjects must fast for a period of 12 hours prior to each application.

Meals:

Beverages: Beverages (e.g., mineral water, fruit juices (except citrus juices), fruit teas) will be offered on request, up to 3 liters per day. Alcohol, coffee, tea, cocoa or cola are strictly forbidden. No beverages will be allowed 30 minutes prior to and two hours after application.

For 24 hours prior to ambulant blood samples, consumption of alcohol and caffeine containing meals and beverages will be not permitted. During the course of the study, consumption of tobacco will be limited to 10 cigarettes per day.

7.7.2.5 Examinations

7.7.2.6 Pharmacokinetics

Prior to the first administration 40 ml blood will be withdrawn for bioanalytical calibration and quality control. This blood will be treated as described below.

Blood samples of 10 ml will be drawn at each of the following times by means of an indwelling catheter or venipuncture:

0 (directly prior to application), 0:10; 0:20, 0:30; 0:40; 0:50; 1:00; 1:20; 1:40; 2:00; 2:30; 3:00; 4:00; 6:00; 12:00; 16:00 and 24:00 hours after application.

//serum// Blood will be drawn from a vein into uncoated serum tubes (Monovette, Sarstedt). Between 25 and 40 minutes later, blood will be centrifuged for 5 minutes at 2750 g and 18 C. Serum will be decante into another tube (Serumröhrchen, Greiner Labortechnik), capped and stored at -20 C.

plasma// Blood will be drawn direct from a vein into ammonium-heparinate-coated tubes (Monovette, Sarstedt). The tubes will be stored on ice until they are centrifuged. They will be centrifuged for 10 minutes at 2750 g and 4 C. Plasma will be pipetted into another polypropylene tube, capped and stored at -20 C.

Both tubes, containing blood or serum/plasma will be labeled with the project number, name of substance, dose, subject's ID, number of study period and number of blood sample.

The sampling time will be documented in the CRF. Deviations from the scheduled times will be documented if the exceed the following ranges: BIOMETRIE !!!

7.7.2.7 Pharmakodynamic

7.7.2.8 Safety

A member of the clinical staff will ask the subjects 2, 6 and 12 hours post application if they have noticed adverse effects on their well-being. The question is: 'Did you notice changes in your well-being?'. If necessary, the answer will be verified without suggestive explanations.

7.7.2.9 Final Evaluation

Upon conclusion of the clinical part, a final evaluation will be performed in order to determine possible changes in the subject's state of health which will be reported as an adverse event or which might constitute grounds for an insurance claim. The results of final laboratory tests will only be reported if their results deviate from the tolerance ranges.

7.8 Bioanalytical Methods

7.8.1 Sample Transfer

The samples will be delivered deep-frozen and on delivery note to

complete address of the laboratory

They will be stored in controlled refrigerators and discarded three months after submission of the draft report.

For further processing the samples will be thawed in a water bath at room temperature, aliquoted and frozen again.

7.8.2 Analytical Method

Analytik will be determined using a validated method. The working range will be fixed to &.

7.8.3 Analytical Procedures

7.8.3.1 Equipment and Chemicals

Standard laboratory equipment will be used. Before starting the analytical works variable pipettes will be calibrated. The quality of the chemicals used referres to the method SOP. For calibration and quality control purposes the sponsor supplies standard substance of certified purity.

7.8.3.2 Validation

When starting the analytical works the method will be validated according to respective guidelines [iii]. Validation parameters will be selectivity, sensitivity, linearity, precision and accuracy and the stability of processed and unprocessed samples.

7.8.3.3 Revalidation

The method will be revalidated, if there are more than three weeks between the completion of the validation and the start of sample analytics. The parameters selectivity, linearity and intra-day precision will be revalidated.

7.8.3.4 Calibration and Quality Control Standards

Before starting the analytical works five pools for calibration standards and three pools for quality control standards will be prepared.

To assess the stability of the samples two additional pools will be prepared at the beginning of the clinical part of the study.

7.8.3.5 Sample Measurement

The samples will be analyzed in batches (sequences). An analytical sequence will consist of calibration and quality control standards and samples. The randomization plan will not be known to the laboratory staff.

7.8.4 Documentation und Archiving

The study procedures will be executed and documented in accordance with the principles of Good Laboratory Practice (GLP). All data entries to computers will be logged and all protocols will be stored together with raw data. The analytical documents, together with the raw data, will remain in the archive of the study center. Raw data copies of a representative number of samples will be part of the final report.

7.8.5 Analytical Report

Analytical methods und results will be presented in the final report. The validation, calibration and quality controls as well as reassays will be reported in detail.

7.9 Statistical Methods

7.9.1 Data Transfer

All valid analytical results will be transferred to the Biometrical Department on electronic data media for evaluation. The subject ID, the number of the study period and the sample number within the period, together with the concentration measured, will be recorded in accordance with the sample label.

7.9.2 Assigning and Treating Data

The analytical results will be assigned to the treatments according to the CRF entries.

All calculations will be based on the reported concentrations and the scheduled times. Only significant deviations from the scheduled times will be considered for the calculation of the pharmacokinetic parameters. A time shift of more than 10% of the sampling interval will be regarded as significant.

Pharmacokinetic parameters will be calculated without fitting to a compartment model. Estimates for missing values will be obtained by linear interpolation. Tabulated values will be rounded to the last digit. Statistical calculations will be performed with values, which had not been rounded.

All calculations - statistics and parameters - will be carried out using the software package SAS (SAS-Institute, Cary NC, USA).

7.9.3 Calculation of Variables

7.9.3.1 Areas under the Curve

The area under the curve from time 0 to the last measured concentration (AUC_{0-t}) will be calculated by trapezoidal integration (1). The area under the curve from time 0 to infinity (AUC_{0-}) results in

$AUC_{0-} = AUC_{0-t} + AUC_{t-}$

with $AUC_{t-} = /ß$

where is the estimator for the last measured concentration, resulting from the ß-regression (see 5.2.4.) and ß is the apparent elimination rate constant (2). The extrapolated fraction of the area:

$r_{AUC} = (AUC_{t-} / AUC_{0-})$,

will be tabulated by formulations and volunteers.

7.9.3.2 Peak Concentrations

The highest concentration determined in the measuring interval for a volunteer will be reported as C_{max}.

7.9.3.3 Peak Times

The time at which C_{max} occurred will be reported as t_{max}.

7.9.3.4 Elimination Rate Constant

The apparent elimination rate constant (beta, ß) will be estimated for each formulation and volunteer by log-linear regression from the linear portion of the logarithmic transformed concentration*time plot. The algorithm will start with the last three points with concentrations > 0 and will increment the number of involved points by 1 until C_{max} will be reached. The slope with the highest negative coefficient of correlation, which must be significantly different from zero ($p < 0.05$), will be reported as an estimate for the apparent elimination rate constant ß. The coefficient of correlation (R) and the first time point of the interval involved in the estimation of beta (t_{lin}) will be reported in order to trace the calculation of beta.

7.9.3.5 Elimination Half-Life

The (apparent) elimination half-life (t½) results in

$t½ = LN(2) /$

7.9.4 Statistics

All statistical calculations will be performed using the SAS-software. The analyses of variance will be calculated with the SAS-procedure GLM, which uses the method of least squares to fit general linear models (3).

7.9.4.1 Descriptive Statistics

The arithmetic mean (Mean), and for concentration related parameters also the geometric mean (GeoM), the standard deviation (SDev), coefficient of variation (CV), absolute minimum (Min) and maximum (Max), and median (Med) will be reported for each parameter and volunteer. The concentrations will be tabulated by formulation and time and the pharmacokinetic parameters by formulation. No Means, SDev and CV will be reported for parameter ratios, parameter differences wil be tabulated without CV. For t_{max} the individual subject differences and for all other parameters the subject ratios will be reported.

7.9.4.2 Analysis of Variance

An analysis of variance (ANOVA) will be performed on all parameters except tmax. The effects considered in the ANOVA model will be: formulation (TREATM), sequence (SEQ), study period (PERIOD), and volunteer within sequence (VOLN(SEQ)).

Based on fundamental pharmacokinetic relationships the multiplicative. model will be applied for all concentration related parameters such as AUC, Cmax, t1/2. This implies that these characteristics will be rather log-normal than normal distributed. The ANOVA, therefore, will be calculated after logarithmic transformation.

The ANOVA coefficient of variation for the multiplicative model will be calculated by means of the error mean square (MSE) from the ANOVA as

ANOVA-CV = 100 * [exp(MSE)-1]1/2.

The ANOVA-CV will be used for sample size evaluation (4).

The ANOVA tables will contain parameters such as R-Square (r^2 = square of the coefficient of correlation), C.V. (= coefficient of variation referring to the parameter mean), Root MSE (= standard deviation of the model), PARAM Mean (=general mean of the parameter) in addition to the analysis of variance. The probability for erroneously rejecting the hypothesis H0 of no significant effect will be presented in the column "Pr > F" with values < 0.05 regarded as significant. The error variance of the model will be taken as test variance for all effects but the sequence effect. The latter will be tested using the variance volunteer within a sequence "VOLN(SEQ)" as an error term.

Arithmetic and geometric means used for the calculation of point estimators such as differences or ratios between formulations will be derived from the ANOVA as least square means (LSMEANS) or exponential transformed LSMEANS, respectively. The LSMEAN from ANOVA may be different from the arithmetic or (transformed) geometric mean due to imbalances e.g., missing values or unequal numbers of formulation per sequence. In these cases the observed arithmetic or geometric mean, respectively, will not be an unbiased estimator for the class mean, whereas the least square mean is the best estimator for a class mean, which would be expected for a balanced design involving the class variable with all covariates at their mean value. Missing values may occur for AUC0- e.g., if it is not possible to calculate the apparent terminal elimination rate constant.

To demonstrate the appropriateness of the multiplicative ANOVA-model the residuals of the log-transformed AUC0-t, AUC0- and Cmax will be tested for normal distribution using the SAS-procedure UNIVARIATE, which produces a Shapiro-Wilk statistic for the null hypothesis that the residuals are normal distributed (5,6).

WILCOXON's matched pairs signed rank test will be used, in addition, to screen for differences between the formulations regarding tmax .

7.9.4.3 Confidence Intervals

For AUC_{0-t}, AUC_{0-}, C_{max}, , and t½ the geometric mean ratios as parametric point estimators and the corresponding shortest 90% confidence intervals will be calculated using the root of residual mean squares from the ANOVA of un-transformed data with subsequent exponential transformation. Nonparametric point estimators for the ratios of expected medians of test and reference and the corresponding nonparametric 90% confidence intervals will be calculated based on the MANN/WHITNEY/WILCOXON statistics using log-transformed data (7,8).

For t_{max} a nonparametric point estimator and the nonparametric 90% confidence intervals for the difference of expected medians will be calculated according to the MANN/WHITNEY/WILCOXON statistics using the untransformed data.

7.10 Quality Assurance System

7.10.1 Monitoring

7.10.2 Quality Assurance

Standard operating procedures will be available for all activities relevant to the quality of the study. At regular intervals, this study will be inspected by the Quality Assurance Unit (QAU). Results of these inspections as well as any objections will be reported directly to the management. The QAU will issue a certificate on their activities.

Quality assurance measures will also be taken for works which will be performed by external contractors. The procedures will be ruled by an SOP.

The clinical laboratory participates in quality assurance tests and fulfills the quality assurance guidelines laid down by the Bundesärztekammer.

7.10.3 Archival of Documents

If not transferred to the sponsor's archive, all study documents and raw data will be archived at the study center for 30 years. Copies on microfilm will be stored in a separate place. Data relating to subjects will only be transferred in coded form. All subjects can be identified at any time by means of a letter code in order to substantiate any insurance claims. Subject data are protected by law (Bundesdatenschutzgesetz of 20 December 1990 - BGBl I, p. 2954 ff.) and by professional confidentiality.

8 Reports

All reports to the sponsor will be written in English. The sponsor will receive the original final report. A copy of the report will be archived at the study center.

8.1 Final Report

All clinical, analytical and statistical results will be presented in a final report. The outline of this report will accord to the EFPIA/CPMP document "Structure and Content of Clinical Study Reports" of May 17, 1993.

The final report will contain:

- a description of the study course, a summary of the screening results and all adverse events; all original case report forms (CRF) and other recorded subject related raw data,
- a description of the analytical methods and results and a copy of the analytical raw data of at least 10% of all samples,
- a description of the statistical methods and a tabulated presentation of all data and parameters and ANOVA.

The following graphs will be created and included in the report:

Mean concentrations by time and treatment, with and without standard deviations;

individual concentrations as an overlay of all subjects by treatment,

individual concentrations of each subject by treatment.

8.2 Additional Reports

Upon completion of the study, a short report will be sent to the ethics committee, stating any undesired events and indicating whether study objectives have been attained. Reports to the authorities will be provided only if required by law.

9 Secrecy

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties unless such disclosure is required by law or regulations. Persons to whom this study protocol is disclosed must be informed that this information is confidential and may not be further disclosed. These restrictions will apply as well to all future communication if privileged or confidential. Confidentiality does not apply to information that was available or known to the study center prior to this contract.

The final report is the property of the sponsor. Publication of the report or of part of it may only be allowed when authorized by the sponsor in consultation with the study center.

10 References

11 Signatures

CRO (Director)	Location, Date
(Principal Investigator)	Location, Date
(Project Manager)	Date
(Head of Quality Assurance Unit)	Date
(Senior Statistician)	Date
(Senior Analyst)	Date
Sponsor	
(Monitor)	Date

12 Appendices

Appendix 1	Study Flow Graph
Appendix 2	Randomization Code
Appendix 3	Subject Information
	Informed Consent
	Insurance Policy
Appendix 4	Case Report Form
Appendix 5	Schedule
Appendix 6	Laboratory Screening - Reference and Tolerance Values
Appendix 7	Curricula Vitae of the Investigators
Appendix 8	Members of the Ethics Committee

ii

iii