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TUTORIAL

Tutorial: Statistical analysis and reporting of clinical pharmacokinetic studies

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Abstract

Pharmacokinetics is the cornerstone of understanding drug absorption, distribution, metabolism, and elimination. It is also the key to describing variability in drug response caused by drug-drug interactions (DDIs), pharmacogenetics, impaired kidney and liver function, etc. This tutorial aims to provide a guideline and step-by-step tutorial on essential considerations when designing clinical pharmacokinetic studies and reporting results. This includes a comprehensive guide on how to conduct the statistical analysis and a complete code for the statistical software R. As an example, we created a mock dataset simulating a clinical pharmacokinetic DDI study with 12 subjects who were administered 2 mg oral midazolam with and without an inducer of cytochrome P450 3A. We provide a step-by-step guide to the statistical analysis of this clinical pharmacokinetic study, including sample size/power calculation, descriptive statistics, noncompartmental analyses, and hypothesis testing. The different analyses and parameters are described in detail, and we provide a complete R code ready to use in supplementary files. Finally, we discuss important considerations when designing and reporting clinical pharmacokinetic studies. The scope of this tutorial is not limited to DDI studies, and with minor adjustments, it applies to all types of clinical pharmacokinetic studies. This work was done by early career researchers for early career researchers. We hope this tutorial may help early career researchers when getting started on their own pharmacokinetic studies. We encourage you to use this as an inspiration and starting point and continuously evolve your statistical skills.

INTRODUCTION

Pharmacokinetics is a quantitative discipline used to assess drug concentrations. Initial pharmacokinetic studies are conducted during drug development to understand the fate of the new drug in the organism (e.g., absorption, distribution, metabolism, and elimination). However, pharmacokinetics is also the cornerstone in understanding inter- and intra-individual variability in drug concentrations and drug response among individuals. Various

factors may affect the pharmacokinetics of a given drug, including drug-drug interactions (DDIs), drug-disease interactions, pharmacogenetics, impaired kidney- and liver function, etc. Even though the relationship between pharmacokinetics and efficacy (pharmacodynamics [PDs]) is not linear, pharmacokinetics is key to understand and mitigate variation in therapeutic efficacy and toxicity.

The European Medicines Agency (EMA; www.ema.europa.eu) and the US Food and Drug Administration (FDA; www.fda.gov) provide extensive guidance on pharmacokinetic investigations. This includes specific guidelines for the investigation of factors introducing drug variability. Moreover, several articles provide guidance on appropriate analysis and reporting of studies,[1,2] aiming to increase reproducibility. However, we still observe considerable diversity and inconsistency in the design, methodology, and reporting of clinical pharmacokinetic studies.

To improve this, we created a complete data analysis for a pharmacokinetic study exemplified by a mock clinical pharmacokinetic DDI study. We provide a step-by-step tutorial on important considerations when designing, analyzing, and reporting a clinical pharmacokinetic study. This includes a complete R code (Appendix S1, Sections I–VII) to compute noncompartmental analysis (NCA) and other relevant statistics. With this tutorial, we aimed to make an easily accessible tool and to inspire researchers at all career stages to get started on data analysis. This tutorial is created by a group of early career researchers and is ready for use in your own clinical pharmacokinetic study.

CONSIDERATIONS FOR METHODS

The method section of a clinical pharmacokinetic study aims to clearly and concisely report the methods used. It should contain sufficient detail to allow other scientists to design and conduct a similar clinical pharmacokinetic study. A short overview of important considerations when designing a clinical pharmacokinetic study is provided in Box 1 and Box 2. Additional considerations are available in the literature.[3,4]

Design, study criteria, and sampling

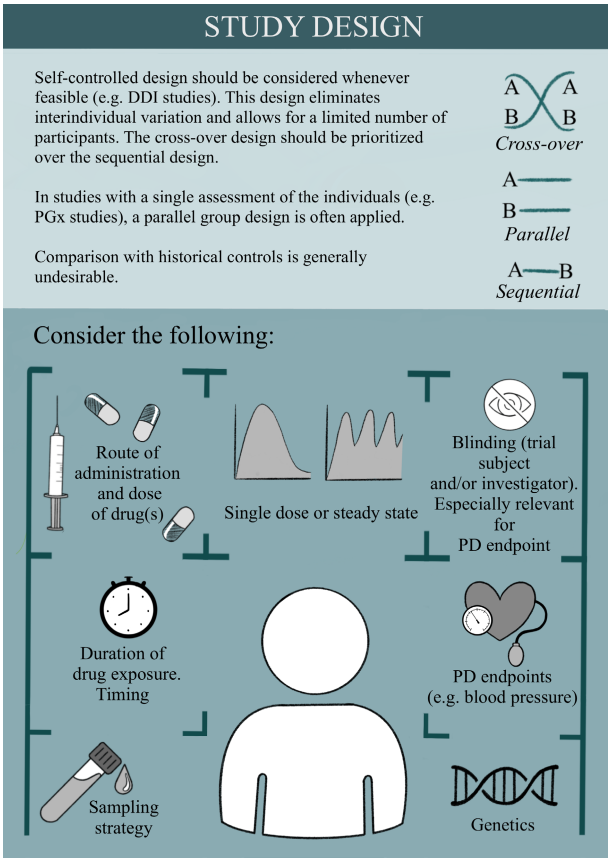
Define the overall study design at the beginning of the method section to help the reader understand the study's primary goal. Include a description of the choices you have made when designing the study and how the study was conducted, as described in Box 1. Provide a detailed description of the study timeline, preferably accompanied by a figure illustration (see section 4.2, Figure 1). This helps the reader understand the study design. If the study has multiple arms, a detailed description of the different arms is required.

List both in- and exclusion criteria used to select eligible trial subjects. This includes a description of excluded groups or populations (Box 2). Describe if baseline screening is conducted to account for safety considerations or alterations in drug effect, elimination, and metabolism. Include or consider special conditions or restrictions for trial subjects, such as dietary restrictions, alcohol and smoking restrictions, time for medication intake, sleeping habits, exercise, compliance assessment, or similar.

Study medication

Include a short description of the drugs used, including generic and product name, manufacturer, doses, administration routes and timing, duration, and if any special circumstances were followed (e.g., if medication should be taken with meals; Box 1). Cocktail studies are becoming popular as they combine several probe drugs (without

BOX 1 Important considerations when designing clinical pharmacokinetic studies.



Abbreviations: DDI, drug-drug interaction; PD, pharmacodynamic; PGx, pharmacogenetic.

BOX 2 Important considerations for study population and number of trial subjects for a clinical pharmacokinetic study.

STUDY POPULATION

Historically, many PK studies have been performed with healthy Caucasian male adults. Healthy trial subjects are often preferred in studies assessing DDIs, bioequivalence, PGx, etc.

We urge to include trial subjects regardless of sex, ethnicity, etc.

Patients are often included as trial subjects when ethical issues preclude the inclusion of healthy subjects e.g., chemotherapeutic agents. However, the inclusion of patients allows the study of pharmacodynamic endpoints that cannot be studied in healthy subjects. In some cases, such pharmacodynamic alterations might affect the substrate's pharmacokinetics.

NUMBER OF TRIAL SUBJECTS

Number of trial subjects should be sufficient to provide a reliable estimate of the magnitude and variability of the outcome.

The number of trial subjects should also be balanced according to ethical considerations.
- Too many trial subjects may cause unnecessary burden (or harm).
- Too few trial subjects may diminish the scientific value.

See sample size calculation in Supplementary, section VII.

Abbreviations: DDI, drug-drug interaction; PGx, pharmacogenetic.

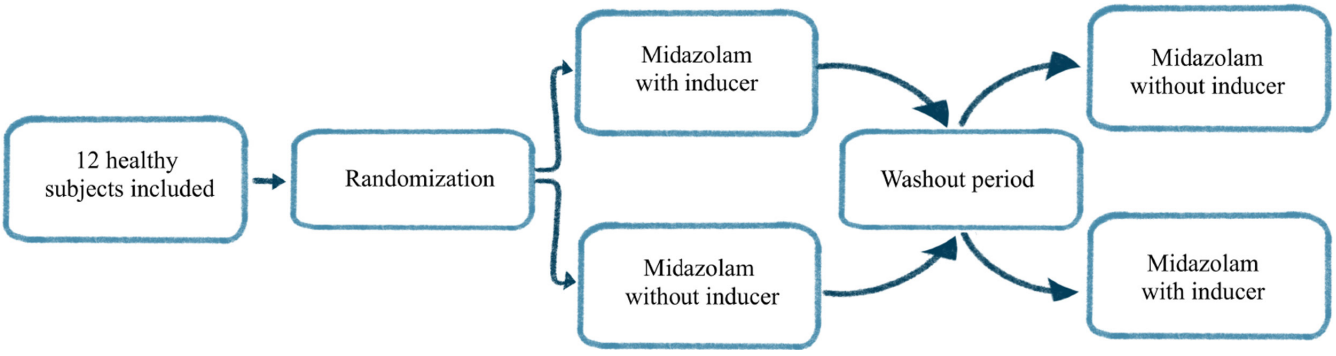


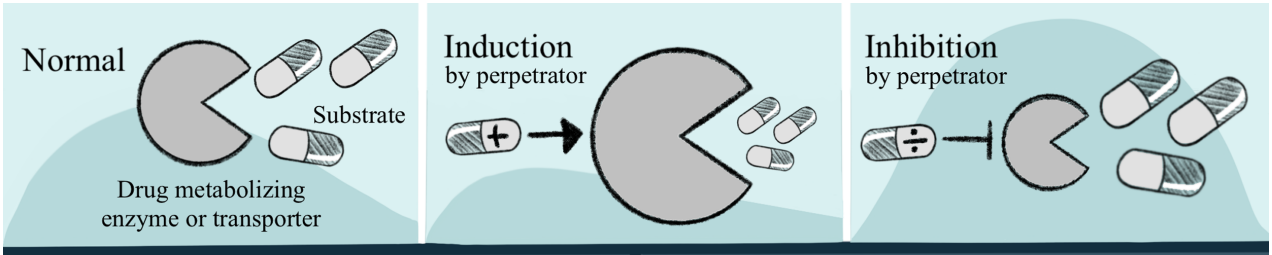
FIGURE 1 Example of how an overview of study design could be presented. The example is based on how the study used in this tutorial could potentially be designed and conducted as a crossover study.

mutual DDIs) to assess multiple metabolic pathways at the same time.[5,6] If the study uses cocktail drugs (see Box 3), refer to previous validation to ensure that the cocktail can be used for the desired purpose.

Study approval and ethical considerations

This section should include all approvals from regulatory authorities, including identifying numbers. The

BOX 3 Important considerations when designing clinical pharmacokinetic studies to investigate CYP enzyme or drug transporter-mediated DDIs. The individual substrate sizes reflect the plasma drug concentrations. Only examples of CYP enzyme substrates and perpetrators are presented. For information on transporter-mediated DDIs, please see the EMA and FDA guidelines (14, 15) and webpages (www.ema.europa.eu and www.fda.gov).



INVESTIGATIONAL DRUG IS SUBSTRATE/VICTIM

Perform DDI studies with index perpetrators, that predictably inhibit or induce drug transport or metabolism by a given pathway.

Index inducers
- Strong: decreases AUC by ≥ 80 %.
- Moderate: decreases AUC by ≥ 50 to < 80 %.
- Weak: decreases AUC by ≥ 20 to < 50 %.

Example: Rifampicin (CYP3A4)

Index inhibitors
- Strong: increases AUC by ≥ 5-fold.
- Moderate: increases AUC by ≥ 2- to < 5-fold.
- Weak: increases AUC by ≥ 1.25- to < 2-fold.

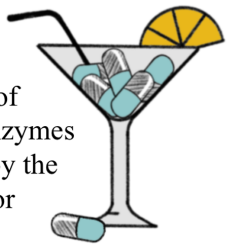
Examples: Enoxacine (CYP1A2), Ticlopidine (CYP2B6), Gemfibrozil (CYP2C8), Fluconazole (CYP2C9), Omeprazole (CYP2C19), Quinidine (CYP2D6), Itraconazole (CYP3A)

INVESTIGATIONAL DRUG IS PERPETRATOR

Perform DDI studies with index substrates, that predictably exhibit change in drug concentration when a given metabolic pathway is inhibited or induced.

Examples: caffeine (CYP1A2), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), midazolam (CYP3A4).

Cocktail study
Simultaneous administration of substrates of multiple CYP enzymes and/or transporters and thereby the evaluation of DDI potential for multiple CYP enzymes.



Endogenous substances as substrate
Some endogenous substances are metabolized or transported in the same manner as drugs, and may act as a biomarker of pharmacokinetics. However, it is difficult to extrapolate the effects and is generally not recommended for index studies.
Example: 4β-hydroxycholesterol (CYP3A4).
In recent years, circulating exosomes have also been investigated as biomarkers of CYP activity.

Abbreviations: AUC, area under the plasma concentration curve; CYP, cytochrome P450 enzymes; DDI, drug-drug interaction; EMA, European Medicines Agency; FDA, US Food and Drug Administration.

study should be conducted in accordance with the Helsinki Declaration and Good Clinical Practice (including International Conference on Harmonization [ICH] version) and provide the name of the monitoring unit. Most journals require the trial to be registered in a publicly available registry, such as the EudraCT database or

clinicaltrials.gov before initiation, and identifiers should be included in the manuscript. Finally, confirm that all subjects consented to participate in the clinical study.

Determination of drugs and metabolites in biological material

Describe how the biological material is: collected, prepared (tubes, centrifugation, etc.), stored (tubes, temperature, etc.), and analyzed. The drugs and their metabolites should be specified. A description of the analytical process could include (1) sample preparation (e.g., liquid–liquid or solid-phase extraction), use of internal or external standards, deconjugation procedures (e.g., enzymatic or chemical deconjugation), etc.; (2) separation technique for chromatographic methods (e.g., high-performance liquid chromatography); (3) compound detection/identification (e.g., UV or fluorescence detection, triple-quadrupole mass spectrometry or high-resolution mass spectrometry. Describe the exact equipment used for analysis and the providing company. If a previous study uses (and describes) a similar method, you might refer to this. If the analytical method has not previously been described, or the chromatographic method has been modified, the method description should also contain precise information on the chromatographic elution of the compound's analytical column (dimensions, particle size, and provider) and the elution profile (isocratic or gradient elution, eluent composition, column temperature, etc.). For mass spectrometric methods, detailed information on the detection settings should be given (e.g., *m/z* transitions or accurate masses, ionization techniques, spray voltages, and lens settings). Analytical methods not previously described should always contain detailed method validation data. Even if a previously published method is used, a section explaining the analytical procedure should still have essential information, such as accuracy/precision, within or between batch variation, the limit of quantification, and the limit of detection. The use of quality control material to control the method's accuracy should also be described.

Genotyping

Genotyping of trial subjects should be considered when clinically relevant single nucleotide polymorphisms (SNPs) may affect clinical pharmacokinetic outcomes in the target population. An example of this could be CYP2D6 and CYP2C9 poor metabolizers or similar. In the case of genotyping, describe which SNPs (including common nomenclature [e.g., star allele] SNP database identifier) are investigated, how the phenotype is assigned from genotype, and which equipment is used for the analysis.

Sample size calculation

In your study, you want to include the correct number of individuals (Box 2). A sample size calculation estimates how many trial subjects are required to make a valid conclusion on the primary end point, for example, a difference in midazolam 0–24-h area under the concentration-time curve ($AUC_{0-24\,h}$) with and without inducer. A sample size calculation is based on the following three variables: (1) level of significance, (2) power of the study, and (3) expected effect size and SD (as a measure of dispersion or variability). The third variable, effect size, and SD, is either based on previous studies within the same area or your best guess. Setting each variable affects the final sample size and should be described in the methods (including references). R code for sample size calculation is provided in Appendix S1, section VII, and the calculated sample size does not reflect the number of trial subjects included in the mock dataset. Additionally, the risk of dropout is mitigated by manually adding extra trial subjects to assure completion of the sample size in the event of dropouts. This is often set to 20% in pharmacokinetic studies.

In a publication, the sample size calculation could be described as: "A total of 5 subjects are required for this self-controlled study, to detect a ≥40% difference in midazolam $AUC_{0-24\,h}$ (with and without inducer) with 80% power and a two-sided significance level of 5%. This is based on mean midazolam $AUC_{0-24\,h}$ of 6.33 ng·h/ml (SD ± 1.12) without inducer and 4.47 ng·h/ml (SD ± 1.09) with inducer. To allow for a 20% drop-out rate, we aimed to include 6 subjects."

Statistics and pharmacokinetic analysis

Include overall methods for statistical and pharmacokinetic analysis. For more detailed discussions on performing the statistical analyses, see Section Statistical analyses for clinical pharmacokinetic drug-drug interactions studies. The appropriate description of the statistical methods used in this tutorial would be: "Demographic data are presented with medians and interquartile ranges (IQRs; 25th to 75th percentiles). Pharmacokinetic end points are calculated with NCA and presented as medians with IQRs and geometric mean ratios (GMRs) with 95% confidence intervals (CIs). We used a paired *t*-test to determine statistical significance ($p < 0.05$) as data followed a normal distribution. Time to maximum concentration (T_{max}) is presented as median and range and tested with Wilcoxon rank-sum

test. All data analyses were conducted using RStudio, version 1.3.1056, and the ncappc package[7] was used for NCA".

STATISTICAL ANALYSES FOR CLINICAL PHARMACOKINETIC DRUG-DRUG INTERACTIONS STUDIES

In the following, we discuss the statistical analysis in a clinical pharmacokinetic study, and the related R codes to conduct the analysis are available in Appendix S1, Sections I–VII. We performed all statistical analyses and plotting with RStudio, version 1.3.1056. The R script was tested with both Windows and macOS. We generated a mock dataset (Table S1) to simulate a clinical pharmacokinetic DDI study with 12 subjects. On two fictive trial days—with and without induction—the subjects were assigned a 2 mg oral dose of midazolam (index substrate, see Box 3). Sampling was conducted over the course of 12 h. A washout period would separate the two administrations in an actual trial to ensure the previous midazolam dose and the inducing effects are washed out. See Figure 1 for an example of study design. All analyses presented in this tutorial might be applied to other types of pharmacokinetic studies (and not only DDI) with minor adjustments.

The mock dataset was generated using Berkeley Madonna version 10.2.8. Baseline elimination half-lives ($t_{½}$) ranged from 1.8–3.1 h and elimination rate constant (k_e) was calculated using $k_e = \ln(2)/t_{½}$. Induction was simulated by increasing k_e by 19–72%. Plasma concentrations were obtained by building a model with an extravascular dose of 2 mg with a fixed absorption rate constant (k_a). The model was built using Runge–Kutta method four (RK4) and a single dose without any pre-existing drug in any compartment. The plasma concentrations were calculated by: $\mathrm{d}/\mathrm{d}t = k_a \cdot \mathrm{comp1} - k_e \cdot \mathrm{comp2}$. The full dataset is available in Table S1.

Considerations for descriptive statistics

Descriptive statistics provide a simple summary of a dataset. In clinical studies, it summarizes the demographics of the study population, such as age, sex, ethnicity, disease, etc. (see "4 Considerations for tables and figures," Table 1). It is also the first step of statistical analyses of the results when summarizing the outcome (e.g., mean plasma concentrations; see Figure 2 in "4 Considerations for tables and figures" and Appendix S1, Section I). Descriptive statistics include measures of distribution, central tendency (mean or median), and variability or dispersion (range, quartiles, coefficient of variance, or SD). The SD is sometimes confused with the standard error of the mean (SEM). SEM is a measure of precision and reflects the uncertainty to which the mean estimate could be generalized to the whole population. In contrast, SD is a measure of variation in the sample population. SEM is derived from SD and sample size and should not be used in pharmacokinetic analyses.

Mean and SD are most widely used. However, median is preferred in situations with small sample sizes, non-normal distribution, if there are outliers, and in case of missing or undetermined data that potentially distort the mean. In addition, T_{max} should be presented as median and range. T_{max} is the only categorical pharmacokinetic parameter because it only takes on one of the sampling time points and thus is not normally distributed.

Two often-used measures of mean in descriptive statistics are the arithmetic mean and the geometric mean. The arithmetic mean is the "standard" mean in which data values are summarized by addition and divided by the number of observations. This differs from the geometric mean in which data values are summarized by multiplication and calculated as the nth root of the product, where n is the number of observations. Both the arithmetic and geometric means are measures of a central tendency; the geometric mean is especially useful in smaller datasets where the dispersion of data is relatively large, and data might be highly skewed.[8] See Appendix S1, Section III.

In pharmacokinetic studies, the GMR is a valuable parameter of the observed effect size. The GMR is the ratio of a given parameter between the test group and the control group. This is often used for pharmacokinetic parameters, most importantly AUC and maximum plasma

TABLE 1 Demographic and clinical characteristics

	Group A	Group B
Number (%) of subjects	.. (..)	.. (..)
Sex, male number (%)	.. (..)	.. (..)
Age, years	.. ± .. *or* .. (..)	.. ± .. *or* .. (..)
Body weight, kg	.. ± .. *or* .. (..)	
BMI, kg/m^2		
...		
Biochemical parameters		
eGFR (ml/min/1.73 m^2)		
...		
Concomitant medication		
Drug A, number of subjects (%)		
...		

Note: Continuous variables are presented as "mean ± SD" *or* "median (IQR)."

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range.

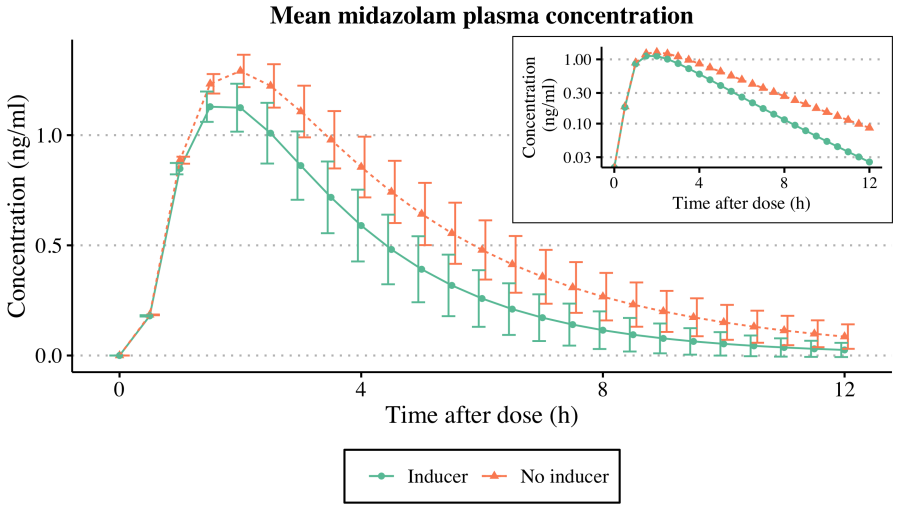


FIGURE 2 Plasma concentration-time curve. Midazolam plasma concentrations are presented as mean ± SD. Midazolam plasma concentrations are lower with inducer (green line, circled points) compared to without inducer (orange line, triangled points).

concentration (C_{max}). The observed effect size is interpreted based on no-effect boundaries, meaning that in a predefined range around one, there would be no clinically relevant effect of the observed effect size. The no-effect boundaries are often linked to drug response and require a good understanding of dose-concentration and/or concentration-response relationships for desirable and undesirable drug effects. In many instances, such a relationship is not established, and there is no clear correlation between (e.g., plasma concentration and adverse events). A more pragmatic solution is to use the bioequivalence boundaries of 0.8–1.25 as the no-effect boundaries.[9] If the CIs for systemic exposure fall within the predefined no-effect boundaries, there is likely no clinically significant difference in drug exposure. See Appendix S1, Section III.

Considerations for inferential statistics

In simple pharmacokinetic studies, the GMR and 95% CIs are used to interpret if there is a clinically significant difference in drug exposure between the two groups ("3.1 Considerations for descriptive statistics"). Other statistical methods exist to compare means between groups; these tests are generally not necessary to perform in DDI studies where their primary contribution to the results is a *p* value. The widespread use of *p* values as the determinant of statistical significance is an ongoing debate in the scientific community.[10] However, in some instances, *p* values are required by regulatory authorities, reviewers, etc., which is why we describe it here.

For simple pharmacokinetic studies, we recommend the Student's *t*-test, whereas the analysis of variance (ANOVA) is recommended in traditional bioequivalence studies.[9] Both the ANOVA and the -test are used to test the underlying null hypothesis of no difference between groups, and for a study like the one outlined here, they would provide similar results. The main difference between the two tests is ANOVA's ability to compute differences among three or more variables (or groups). At the same time, the Student's *t*-test only permits two variables (or groups). For these reasons, we apply the Student's *t*-test, and, more precisely, the paired *t*-test, to test the paired measurements from the same individual with and without a predefined exposure. The individuals must be independent of each other. The ANOVA and the *t*-test apply the same assumptions: normality and homogeneity of variance (see Appendix S1, Section VI). These assumptions are not always met in a pharmacokinetic study but may be mitigated by log-transforming the outcome variable before testing. This should only be done if necessary, as it complicates the interpretation of the results. Alternatively, other statistical methods that do not require normality could be used (e.g., Wilcoxon rank-sum test).

In Appendix S1, Section VI, we present how to compute descriptive statistics (mean and SD) and paired *t*-test of AUC from time of administration up to the time of the last quantifiable concentration (AUC_{last}). The computed output of a paired *t*-test in R includes a *p* value (if $p < 0.05$, the null hypothesis is rejected), a mean difference between pairs (sample estimates), and a 95% CI. We interpret this *p* value as a statistically significant result. However, we should still aim to determine whether the result is clinically relevant based on a clinical interpretation of the size of the difference. The summary statistics may not be applicable in the main manuscript of your article; however, the results should be included in the Data S1 as they may be valuable for future researchers (e.g., when doing power calculations, as described previously).

Considerations for noncompartmental analysis

The overall purpose of a clinical pharmacokinetic study is to assess drug pharmacokinetics (e.g., absorption,

distribution, metabolism, and elimination). The pharmacokinetic analysis relies on observed drug concentration measurements over time. In a controlled setting, where it is possible to draw multiple samples for every individual, NCA is a straightforward method to assess the basic pharmacokinetic properties. In cases of limited sampling or when a more profound understanding of drug pharmacokinetics is warranted, more advanced pharmacokinetic modeling may be required, but this is outside the scope of this tutorial. Based on measured plasma concentrations at observed timepoints (that do not always agree with the planned timepoints), the following parameters are calculated using NCA: AUC, clearance (CL), and $t_{½}$, whereas C_{max} is obtained directly from the observed data.

The NCA is a model-independent method that does not rely on assumptions about pharmacokinetic compartments. In contrast, the compartmental analysis considers the body to consist of interconnected, well-mixed, and kinetically homogenous compartments (e.g., blood, organs, and other tissues). In compartmental analysis, the pharmacokinetics of a drug is described by nonlinear models based on regression analysis made from certain assumptions. This means there is a potential for variability in the output of the analysis because assumptions may vary between models. The compartmental analysis is preferred for more advanced population pharmacokinetics. For pharmacokinetic analysis of dense datasets, NCA is sufficient and provides a reliable calculated output.

Historically, Phoenix WinNonlin has been the standard for performing NCA. However, numerous free alternatives have recently become available with the same overall properties. R offers at least four packages for NCA: NonCompart, ncar, PKNCA, and ncappc. The names of most output parameters in the packages are consistent with those used in Phoenix WinNonlin, and results from Phoenix WinNonlin have been used as a reference during the development of all packages to ensure accuracy. The output calculated by the NonCompart, ncar, and PKNCA packages can be formatted to the Study Data Tabulation Model format required for regulatory submission to, for example, the FDA. The output calculated by the ncappc package is suitable for NONMEM, a tool used for population pharmacokinetic/PD modeling.

The cornerstone of NCA is the calculation of AUC—all four packages in R use the trapezoidal method, which means that the AUC is calculated separately for each of the areas appearing between two sampling timepoints. The connections between the sampling timepoints are interpolated and defined as either linear or logarithmic. If sampling is conducted at appropriate times, the chosen method will not substantially impact the results. Often, especially when dealing with drugs displaying first-order kinetics, it is reasonable to combine the linear and logarithmic methods; use linear connections for the ascending curve (before C_{max}) and logarithmic connections for the descending part of the curve. This method is referred to as "linearup-logdown." The calculated output includes both $AUC_{0\text{-last}}$ and $AUC_{0\text{-}\infty}$. $AUC_{0\text{-last}}$ is calculated from the first observed to last measurable plasma concentration. $AUC_{0\text{-}\infty}$ is the AUC from the first sampled data point and extrapolated to infinity. The extrapolation of the terminal phase is based on the last observed concentration. In this case, the extrapolation contributes <10% of the $AUC_{0\text{-}\infty}$. The $AUC_{0\text{-}\infty}$ might be unreliable if the percentage extrapolated accounts for more than 20% of the total AUC.[11] Following NCA, it should be evaluated if the analysis's performance is acceptable by inspecting the individual regression coefficients. The coefficient describes the performance of the regression line in estimating the elimination rate.

In the R script, you must also define the variables, units, and relevant conditions (single dose or steady-state, oral dosing or intravenous, etc.). These parameters naturally influence the calculated output. The example of NCA included in Appendix S1, Section II is computed using the ncappc package.[7,11] The nomenclature is similar in the available packages, and an alternative package can be used with minor adjustments.

CONSIDERATIONS FOR TABLES AND FIGURES

Tables and figures are crucial elements when presenting data in a clinical study as they allow a quick look at data and should be interpretable on their own without having to read the main text. Tables are suitable for presenting complex data, whereas figures should allow the reader to overview the study findings quickly. The text supplements data presented in the figures and tables, and figures and tables supplement the data presented in the text. Important numbers and results should be prioritized in the main text, and data should not be repeated and reported multiple times. Remember, tables and figures should have a function for your article and improve the reader's ability to understand the study. Additional important considerations for reporting tables and figures are elaborated elsewhere.[12,13]

Tables

Table 1 traditionally contains the baseline characteristics and demographics of the trial subjects (Table 1).

The table is based on descriptive statistics and provides a quick overview of the similarities and differences between the trial groups. It might also be used to assess how well the trial group reflects on the general population. In a clinical pharmacokinetic study, Table 1 should briefly present baseline characteristics of the study population, such as age, sex, ethnicity, disease, biochemical assessment, and other factors that may affect drug pharmacokinetics (Table 1). In this setting with a self-controlled design, we prefer to present the baseline characteristics in the first paragraph of the results and leave room for more informative tables and figures.

Table 2 is suitable for presenting the outcome of the study. In a pharmacokinetic study, this includes a summary of pharmacokinetic parameters shown for the individual groups. Here, we present the relevant pharmacokinetic parameters (AUC, C_{max}, $t_{½}$, and CL) as medians and IQR, stratified by DDI (with and without induction; Table 2; also see "3.1 Considerations for descriptive statistics"). The GMR and 95% CI are included in Table 2. If the study analyzes multiple probe drugs or multiple metabolites, the table should include all of these. The *p* values computed in the paired *t*-test could be presented in Table 2 but is not necessary as GMR (95% CI) allows statistical interpretation. Thus, if the CI does not include 1.00, the inducer has a statistically significant effect. A complete R script is available in Appendix S1, Section II, computing all variables and an entire Table 2.

Additional tables might be relevant for presenting additional data and might be created according to the study design. For example, a table showing PD data obtained in the study could be appropriate. We do not include additional tables in the R script in this tutorial. However, in many cases, such computation would follow the same principles described in the remaining part of the R script (Appendix S1, Section I-VI).

Figures

Figure 1 is suitable for the presentation of the study design. Especially in studies where rather advanced designs are applied, the display in a figure often helps the reader understand the relationship among crossover, sampling, and other fixed events.

Figure 2 is appropriate for a visual presentation of an outcome summary. For example, the concentration-time curve in Figure 2 provides an accessible overview of the pharmacokinetics of the drug, both without and with induction, and clearly shows that a change in plasma concentrations is observed. This curve is based on the mean plasma concentration for all trial subjects at each of the planned sample timepoints (and not the actual timepoints). We created a y-log scale graph for the inset in the main graph. A y-log scale graph transforms the nonlinear terminal phase into a linear terminal phase. This is useful for evaluating drug elimination and allows visual interpretation of observed differences. It can be argued that connecting data points is inappropriate because the concentrations in between are unknown. However, we believe that the connections aid the understanding of the figure, which is the primary purpose of the figure. The complete R script for computing Figure 2 is available in Appendix S1, Section I. Appendix S1, Section V, provides a R script for computing individual graphs for each trial subject; such graphs might not be needed in the main manuscript but could be valuable in an appendix.

Figure 3 shows a spaghetti plot. A spaghetti plot visualizes data for each individual's specific pharmacokinetic parameters. In this case, the spaghetti plot displays changes in one of the pharmacokinetic parameters stratified by DDI. The preferred pharmacokinetic parameter for the spaghetti plot will be based on the mechanisms underlying the DDI and the observed changes in pharmacokinetic parameters. It might be C_{max} for absorption

TABLE 2 Noncompartmental pharmacokinetic analysis of midazolam in 12 healthy volunteers stratified by co-administration of an inducer

Drug	Parameter	Without inducer (median IQR)	With inducer (median IQR)	GMR (95% CI)
Midazolam	$AUC_{0\text{-last}}$, ng*h/ml	5.99 (5.57–6.97)	4.16 (3.88–5.08)	0.7 (0.65–0.75)
	C_{max}, ng/ml	1.28 (1.24–1.34)	1.12 (1.1–1.2)	0.88 (0.86–0.91)
	$t_{1/2}$, h	2.16 (2–2.59)	1.47 (1.37–1.81)	0.68 (0.63–0.73)
	CL/F, L/h	3236.67 (2716.94–3506.23)	4780.99 (3872.35–5131.13)	1.48 (1.38–1.59)
	T_{max}, h	2 (2–2)	1.5 (1.5–2)[a]	NA

This table is directly copied from the table computed in R, and the layout can be improved for publication.

Abbreviations: AUC_{last}, area under the curve from time of administration up to the time of the last quantifiable concentration; CI, confidence interval; CL/F, total body clearance; C_{max}, maximum concentration; GMR, geometric mean ratio; IQR, interquartile range; NA, not applicable; $t_{½}$, elimination half-life; T_{max}, time to maximum concentration.

[a]Tested with Wilcoxon rank test, $p < 0.05$.

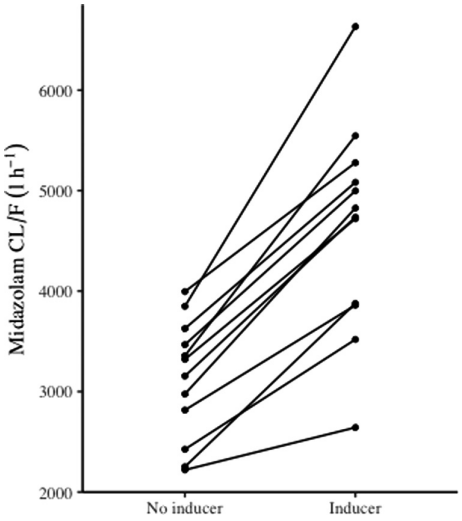


FIGURE 3 Spaghetti plot illustrating the changes in individual midazolam clearances with and without inducer

DDI, renal clearance, formation clearance of specific metabolites, $t_{½}$, or similar. With this plot, we can observe data trends for individual subjects and highlight any known variations in metabolism, such as genetic variants, which may be used to explain pharmacokinetic outliers. These plots are best used when the study population is small as it becomes overwhelming with large study populations. See Appendix S1, Section IV.

Additional figures might be relevant as described in "Additional tables."

DISCUSSION

This tutorial provides a short and easily accessible introduction to clinical pharmacokinetic studies to assist and improve the reporting and statistical analysis. This tutorial is developed by early career researchers for early career researchers and aims to ease the workflow during the design and analysis of clinical pharmacokinetic studies. The EMA and the FDA have extensive guidelines for the pharmaceutical industry,[14,15] supplemented by comprehensive considerations for postmarketing DDI studies.[3] Several journals have developed author guidelines addressing considerations on statistical methods and reporting.[1,2] It is of utmost importance that such guidelines be followed at all study planning and execution stages to ensure appropriate design and valid study outcomes. Poorly conducted or analyzed studies might result in conclusions that are interpreted incorrectly or clinically irrelevant. Journal guidelines are often kept on a general level, and performing this analysis may feel overwhelming when attempting to convert theory into practice. This tutorial showcases how to implement guidelines in practice and how to compute the statistical analysis using the widely available statistical program R.

Our experience is that the quality in the reporting of methods and results varies considerably in the literature, resulting in otherwise well-conducted studies having limited impact. In recent years, there has been increasing progress toward transparency in science. This means open access to raw data and statistical analysis (including complete scripts) should be provided in the Data S1 whenever feasible. When reporting clinical pharmacokinetic studies, we have observed two major lacks: reporting of individual data and reporting of mean and SD. The presentation of personal data is crucial but often limited by the General Data Protection Regulations (GDPR). However, graphical presentations, such as the spaghetti plot (Figure 3), are feasible within this framework. This also allows authors to highlight individuals that are pharmacokinetic outliers due to obvious reasons, such as genetic variants, concomitant medication, or similar. Sharing of individual data should always agree with current general data protection laws.

Additionally, we strongly emphasize that mean and SD is reported as a measure of variance. As described previously, the mean and SEM are essential variables for future studies when calculating sample size (based on your results). You might help increase the quality of future research by describing methods and results sufficiently.

For this tutorial, we created a mock dataset simulating a clinical pharmacokinetic study with the administration of a single dose of midazolam to 12 individuals with and without induction. In the real world, things are often much more complicated than presented here. When a drug like midazolam is metabolized, both the plasma concentrations of the parent compound and metabolites should be included in the pharmacokinetic assessment. The metabolites can be either active or inactive and might be further transported, metabolized, or have clinical efficacy. This influences how you must conduct your pharmacokinetic analyses and how you interpret your results. Other study design decisions might impact how you should address your data, such as steady-state dosing, specificity of the substrate, sampling strategy, etc. Beyond this point, it is not feasible to provide general advice within this framework. However, the methods described here apply to all such situations with minor adjustments.

CONCLUSION

Conducting clinical pharmacokinetic studies is essential, both during drug development and postmarketing, to better understand variability in drug efficacy and toxicity. To increase the quality of design and reporting, we provide a

tutorial with important considerations for designing and conducting statistical analyses in a clinical pharmacokinetic study. Additionally, we provide an easily accessible and ready-to-use R code that can be applied to your clinical pharmacokinetic study. We hope that you will find it helpful. Enjoy!

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The authors have put substantial effort into creating this tutorial, if you find it helpful when designing and performing your pharmacokinetic study, please consider citing it. The authors would like to thank Sissel Mogensen for the original drawings in Summary Boxes and Figure 1 and Flemming Nielsen for his valuable comments on and contributions to section Genotyping Determination of drugs and metabolites in biological material.

CONFLICT OF INTEREST

A.D. has given paid lectures for Astellas Pharma. T.S. has given paid lectures for Pfizer and Eisai and consulted for Pfizer. All unrelated to this work. All other authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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