

A peak at PK — an introduction to pharmacokinetics

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The aim of this paper is to give a high level introduction into pharmacokinetic (PK) data and analysis for programmers new to PK, or who require a refresher. Key theoretical concepts will be covered, such as: absorption, distribution, metabolism, and elimination (ADME), derived PK parameters, including area under the curve (AUC), C_{max} (maximum concentration observed), t_{max} (time of maximum concentration observed), and $t_{1/2}$ (half-life), and steady state. The data flow from CRF to tables, figures and listings (TFLs) will also be covered. We shall also briefly discuss the use of PK in the drug development process including types of clinical studies, e.g. single ascending dose (SAD), multiple ascending dose (MAD), bioavailability, mass balance, food effect, and drug–drug interaction (DDI) studies, and how this relates to the PK of the drug.

Keywords: Pharmacokinetics, PK, PK Parameters, PK Concentration, ADME

Introduction

PK analysis forms a major part of the understanding and development of the investigation medicinal product (IMP) and can also contribute heavily to the label once the drug has been approved.

Between 1964 and 1985, 318 new clinical entities were given to man for the first time by seven UK-owned pharmaceutical companies. By the end of 1985, 198 of these drugs had been discontinued. Of these, 78 (39.4%) had been discontinued due to serious pharmacokinetic (PK) issues.¹ Since the ability to predict the absorption, distribution, metabolism, and elimination (ADME) including toxicity earlier in the development of drugs, without having to dose either animals or humans, has been developed, this has reduced the number of drugs that fail due to PK to around 7%.² Even though the numbers have decreased considerably since 1985, they still show the importance of PK on the development of new clinical entities.

PK Theory

Pharmacokinetics literally means the ‘movement of drugs’ (from *Pharmaco* — drug, and *Kinetic* — movement). It is more commonly defined as ‘what the body does to the drug’. The study of pharmacokinetics is split into four processes:

1. Absorption — how the drug gets from its site of administration into the blood. Many factors affect the absorption phase, e.g. molecule size, degree

of lipid solubility, and route of administration. Absorption is not applicable for drugs given by intravenous (IV) injection since they pass directly into the blood and therefore do not need to be absorbed;

2. Distribution — how the drug moves from the blood to other parts of the body, for example, tissues and organs;
3. Metabolism — how the drug is broken down or transformed by the body into smaller molecules known as metabolites. Metabolites can be pharmacologically active, toxic or neither;
4. Excretion — how the drug is removed from the body.

Figure 1 shows a typical PK profile when measuring IMP after oral administration in plasma over time. Blood is composed of blood cells suspended in a liquid called blood plasma. Please note that we will continue to use blood plasma when talking about the drug concentrations, but the concentrations could also be measured in serum and also whole blood. Serum refers to blood plasma where the clotting proteins have been removed.

To be able to plot a PK profile such as this, blood samples are taken before and after dosing with IMP. Typically, sampling will be: pre-dose, post-dose every 30 minutes, every hour, and then at greater time points (see the section on ‘Data flow and TFLs’ for an example of this). The number of blood samples taken will be dependent upon:

1. How many doses of IMP are taken;
2. What is currently known about the IMP PK profile;
3. Safety and Regulatory/Ethical considerations.

Figure 1 shows what is happening in the blood plasma, but may not necessarily show what is

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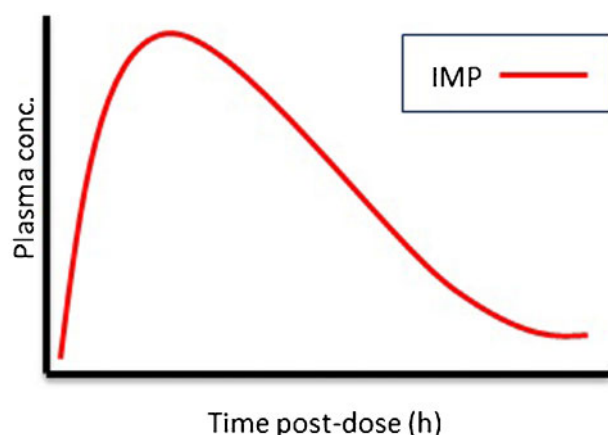


Figure 1 A typical linear PK profile for an IMP after oral administration

happening in the tissues and organs. At the point where plasma drug concentrations are measured, all four processes of PK are occurring in the body. In the beginning, absorption is occurring at the greatest rate and at the end, it is excretion. At the point where the plasma concentration is at its peak, the rate of drug entering the plasma is the same as the rate of drug being removed from the plasma.

Rates of absorption can be affected by the route of drug administration. Drugs given orally need to be absorbed into the blood via the gut, whereas those given intravenously do not. Figure 2 shows the difference in plasma concentrations between an oral and an IV dose.

PK measurements

From single plasma concentration profile, we can observe and measure certain PK parameters to describe the drug exposure in the body and the rate and extent of absorption. Figure 3 shows some of the more common measurements. C_{max} , is the maximum concentration recorded; t_{max} , the time taken to reach C_{max} ; AUC (area under the curve), a measure of the exposure to the drug and $t_{1/2}$ (elimination half-life), the time taken for the plasma concentration to fall by half its original value (as shown in Fig. 3 using a semi-logarithmic plot of the elimination phase only).

Bioavailability

Bioavailability is a term used to indicate the proportion of drug absorbed into the systemic circulation. In Fig. 2, you can see that the entire IV dose passes directly into the plasma and so it is said to be 100% bioavailable. The oral dose may not be fully absorbed (due to factors such as poor absorption or insufficient time at the absorption surfaces) and so the bioavailability is reduced compared to that of the IV dose.

Dose proportionality

Dose proportionality means that there is a constant ratio between the dose given and the PK profile. For

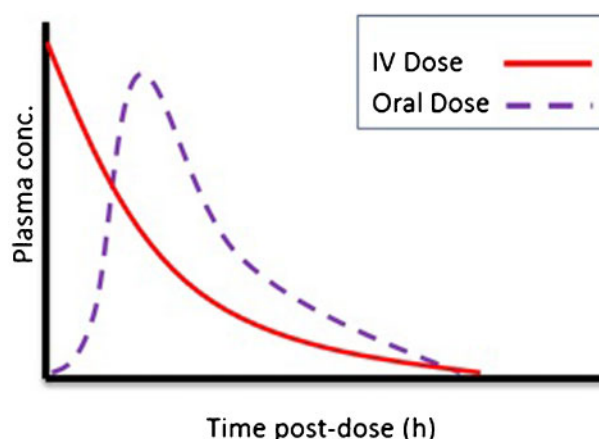


Figure 2 PK profile of an IV dose and an oral dose

example, if you double the dose, then you double AUC and C_{max} . T_{max} and $t_{1/2}$, however, would remain constant. If the IMP shows dose proportionality, then you can predict repeat-dose PK from a single dose and easily predict dose adjustments that may be needed for subpopulations.

Steady state

If a drug is administered continuously over a period of time, the drug will accumulate in the body. From Fig. 4, you can see the plasma concentrations increasing over multiple drug administrations. When the process of absorption is happening at exactly the same rate as elimination, this is called 'steady state'. The time taken to reach steady state, assuming that dosing intervals are kept constant, is dependent upon the half-life of the drug.

In Fig. 4, two doses are required for steady state to be reached. In practice, this would happen over a larger number of doses, though a loading dose may be given initially to get to steady state more quickly.

Types of Study

One of the main aims of phase I studies is to evaluate the PK of a drug, since this can impact the design of future studies, i.e. how often to dose or whether to dose fed or fasted, effect in specific populations, use of concomitant medication, and also when is the best time to collect PK samples. Not all the phase I studies are, however, performed when the drug is still in phase I some can occur in parallel to the drug being in phase II or III, for example, renal or hepatic studies. Below is a selection of PK studies with descriptions:

'Entry into human' studies are usually performed on healthy male volunteers, unless the appropriate pre-clinical tests have been done which would allow women to be dosed. If this is the case then in the USA, women must be included in the study. 'Entry into human' studies are usually **single ascending dose studies (SAD)**, where 6–8 different dose levels are tested in order to try and find the maximum tolerated dose. The aim of these studies with regards to PK is

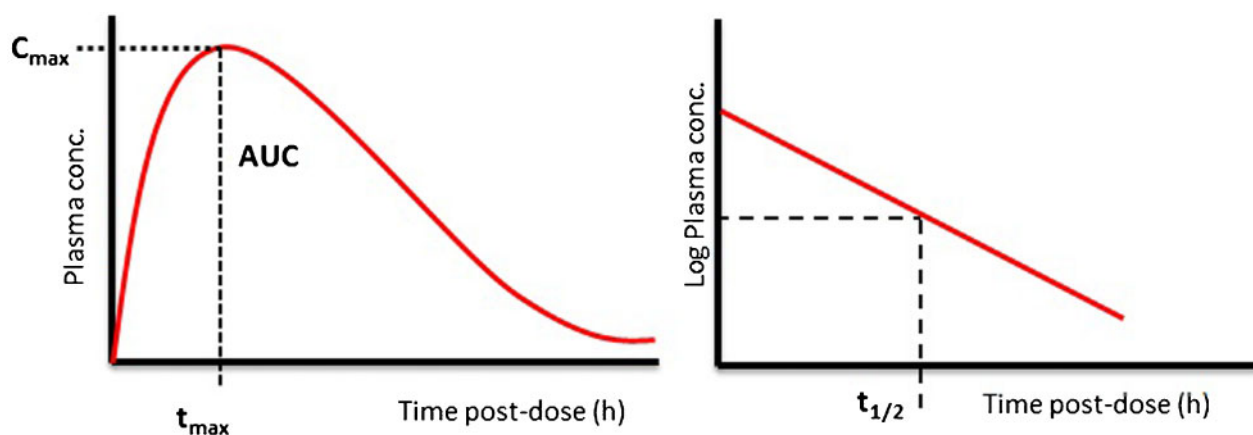


Figure 3 Common measurements used in PK analysis

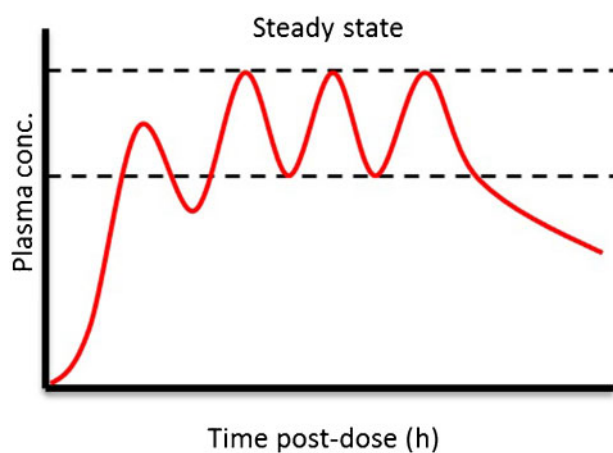


Figure 4 An IMP reaching steady state

to be able to start characterizing the PK profile of a single dose, across a range of doses which in turn allows you to begin to understand if the drug is dose proportional. A SAD study can also be used to calculate what the elimination half-life is, i.e. is it too long or too short. If the elimination half-life is short, then the drug may need to be dosed more often.

Multiple dose studies (MAD) are also usually performed in healthy volunteers, similar to 'Entry into human' studies. If the appropriate pre-clinical tests have been done, then women can be included. MAD studies also test a range of doses, but this time looking at multiple doses. The aim of these studies is to check whether the drug is safe and tolerable when a subject is dosed multiple times and also to reach steady state. To compare PK of multiple dose data against single-dose PK data to see if any changes over time, determine if there is any accumulation, and assess if steady state has been achieved.

A **Bioavailability** study is conducted to measure the amount of the drug that is actually absorbed from a given dose. There are two types of bioavailability study: relative and absolute. A relative bioavailability study tests the rate and extent of absorption of the drug when compared to another formulation or product of the same drug. The aim of a relative

bioavailability studies is to compare and contrast the difference in the PK profile. An absolute bioavailability study tests an IV infusion versus an oral dose. Since an IV infusion is 100% bioavailable, an oral dose is given to measure the bioavailability of the oral dose compared to the IV dose. The aim of an absolute bioavailability study is to know how much of the oral IMP reaches the systemic circulation compared to an IV dose since some of the oral drug may be lost during the absorption process.

Bioequivalence trials are the comparison of the bioavailability of two or more drug products. For example, the formulation of a drug may have changed slightly and you therefore want to confirm that this does not make a difference to the bioavailability of the drug and therefore, you can infer that the safety and efficacy will be the same for both formulations. Also when a drug is no longer patented, a bioequivalence study is run to confirm that the generic product is equivalent to the original marketed drug. A bioequivalence study is very similar to a bioavailability study; however, the aim is to show that the two formulations are statistically the same (within certain bound, generally between 0.8 and 1.25) in terms of PK parameters (C_{max} and AUC).

Drug–drug interaction (DDI) studies are conducted to test whether one drug enhances or reduces the effect of another drug. Does taking the second drug affect the absorption, metabolism or excretion of the first drug or *vice versa*? For example, certain antacids can prevent many medicines from being absorbed into the bloodstream. If this happens, the medicine may not work as well or may not work at all. DDI studies are normally either two- or three-period crossover studies. In a two-period crossover study, the subjects are dosed with the IMP and the other drug of concern in one period and the IMP on its own in the other period. If it is thought that the IMP could have an effect on the other drug, then a three-period crossover study is performed where the interacting drug may also be dosed on its own, in a period.

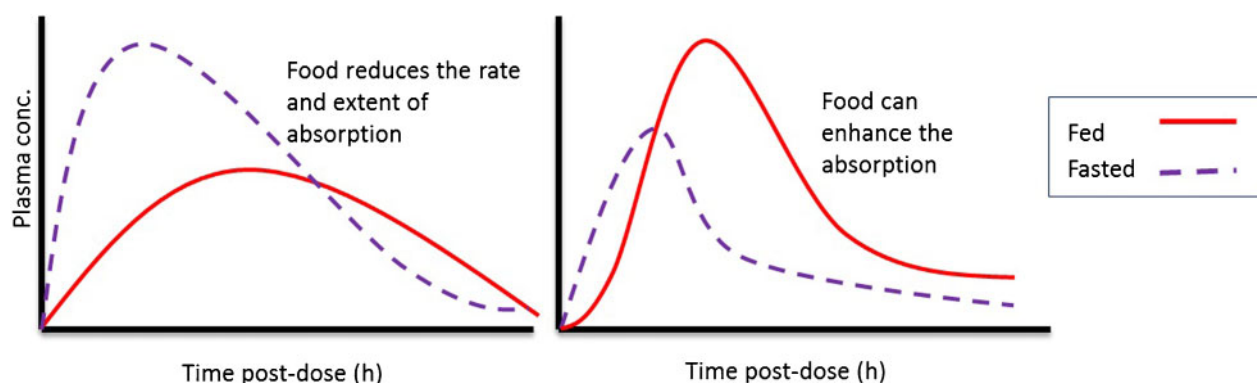


Figure 5 Effect of food on the plasma concentration curve following oral administration

Hepatic/renal impairment studies have a similar study design; subjects are classified into three groups: mild, moderate, or severe impairment. For renal impairment, the subjects are grouped by their creatinine clearance value and for hepatic impairment by their Child–Pugh score. Matched healthy volunteer controlled subjects are also included; these subjects are matched to renal or hepatic patients by weight, age, and gender. The PK profiles of the impaired patients are compared to their matched healthy volunteers. If the PK profile is considered to be statistically different, then impaired people may need to receive a dose adjustment.

Mass balance studies are conducted to determine the routes and rates of excretion and the metabolic profile of the IMP. These are done by administering a certain amount of radiolabelled drug. The radioactive marking is used to keep track of the drug and its metabolites by collecting the subject's blood, urine, and faeces, and assaying them for radioactive label. The aim of a mass balance study is to determine mass balance and the routes and rates of elimination of IMP, identify any circulatory and excretory metabolites.

Food effect studies are often conducted during the very early clinical trials in humans; they can be included as a subsection in the SAD or MAD studies. They test the effect of food on the rate and extent of absorption of a drug when given just after a meal

compared to when given under fasted conditions. The information on how food affects the absorption of the drug can affect the design of future trials, and therefore, its best to know this information as soon as possible. If there is a large food effect, then in future studies, the drug may need to be given when the subject/patient is in a fasted state. A food effect study is normally a two-period crossover study where subjects are dosed with drug under fasted conditions and then drug with food in the other period. Figure 5 shows an example of the effect that being fed or fasted can have on the absorption of the drug.

Data Flow and Tables, Figures and Listings (TFLs)

The role of the PK programmer in Roche starts with the review of the protocol. Following receipt of the CRF data, programming of the PK, study medication, and demographic data is then performed before transfer of the data to the pharmacologist. The pharmacologist analyses the data and then produces PK TFLs. They then send the derived PK parameters back to the PK programmer who can use these in the production of additional CSR PK TFLs. Figure 6 shows a generalised overview of this process.

Data flow

The patient should attend the visit at the clinic in accordance with the schedule of assessments in the

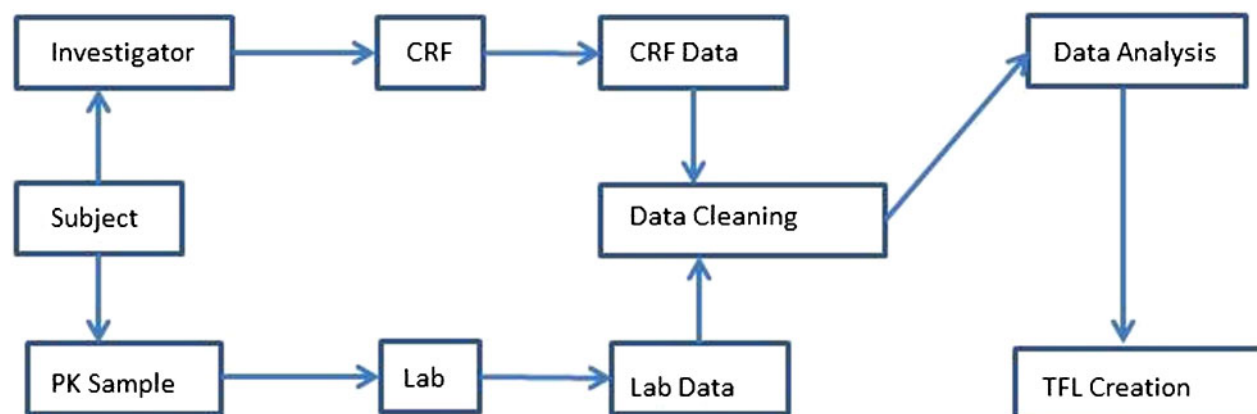


Figure 6 An overview of the data flow of the PK component in a clinical trial

Procedure	Day 1														Day 2
	Pre-dose	0 h	0.25 h	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6 h	8 h	12 h	15 h	24 h	
Drug Administration		x													
Pharmacokinetic Sampling	x		x	x	x	x	x	x	x	x	x	x	x	x	

Figure 7 An example of PK sampling in a protocol

#	Sample Number	Sample schedule	Date of Sample dd mon yyyy	Time of Sample (24 hr clock)	Sample comments
1	101	PREDOSE			
2	102	0.25 H			
3	103	0.5 H			
4	104	1 H			
5	105	1.5 H			
6	106	2 H			
7	107	3 H			
8	108	4 H			
9	109	6 H			
10	110	8 H			
11	111	12 H			
12	112	15 H			

Figure 8 An example of a CRF page used for capturing PK sampling information at site

protocol. PK sampling occurs before administration of the IMP (pre-dose) and at specified time points after administration of the IMP (post-dose). PK sampling may happen at multiple visits. Figure 7 shows a simple example of a PK sampling schedule from a protocol.

The investigator enters the sample information into the CRF. PK samples are sent to the lab for analysis. Figure 8 shows an example of a CRF page capturing this information (note that the 24-hour sample only occurs at the day 2 visit and so would not appear on the day 1 CRF page).

Data cleaning

The CRF data and electronic lab data are received in-house where they are combined to produce a single data file. Data checking is performed to ensure that:

1. CRF samples have a result, or a comment to explain why the test was not performed;
2. lab results have a matching CRF record;
3. dates and times are within the specified time windows detailed in the protocol.

Queries are sent out as appropriate if any data discrepancies are discovered. Once cleaned and reconciled, the data are sent for analysis. Data cleaning is crucial to the activities downstream.

Data analysis

In order for analysis to occur, the data need to be received, or transformed to an acceptable format, e.g. SDTM. Data manipulation may be required to do the following:

1. merge PK concentration data with IMP data;

2. create derived variables, e.g. time of PK sample from first IMP dose, and time of PK sample from last IMP dose.

Figure 9 shows concentration data for the IMP only, but concentrations of metabolites or biomarkers of interest may also have been provided from the lab for analysis.

Using a basic graph generator, the plotted data can be seen in Fig. 10. Note that the graph in Fig. 10 is very simple and that more sophisticated software would be used to generate the plot for purposes of analysing the data.

The dataset shown in Fig. 9 is used to create derived variables within WinNonlin[®], e.g. T_{max} , C_{max} , AUC, and $t_{1/2}$ for use with TFLs and to perform statistical analysis on if required.

TFLs and statistical analysis

The PK outputs produced for a clinical trial can vary greatly depending on the endpoints of the study; however, there are some outputs that are commonly produced for most trials. The individual concentration data and individual PK parameters data will all be listed. The actual sampling time will also be listed either on their own or with the individual concentration data. These data are important since they allow the team to see if the PK samples were collected at the correct time after dosing. If the PK sample was collected earlier or later than specified, this will affect the actual concentration level, for example, if the drug is still being absorbed into the blood and the sample is

Subject	Sample #	Visit	Scheduled sampling time	PK Analyte	Plasma Conc	Unit	Datetime of IMP dosing	Datetime of PK sample	Actual time from last dose
1001	101	Day 1	PREDOSE	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:07.21	-0.65
1001	102	Day 1	0.25 H	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:08.15	0.25
1001	103	Day 1	0.5 H	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:08.30	0.5
1001	104	Day 1	1 H	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:09.05	1.08
1001	105	Day 1	1.5 H	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:09.30	1.5
1001	106	Day 1	2 H	Drug A	BLQ	NG/ML	31OCT2011:08.00	31OCT2011:10.10	2.17
1001	107	Day 1	3 H	Drug A	11.1	NG/ML	31OCT2011:08.00	31OCT2011:11.00	3
1001	108	Day 1	4 H	Drug A	54	NG/ML	31OCT2011:08.00	31OCT2011:12.00	4
1001	109	Day 1	6 H	Drug A	534	NG/ML	31OCT2011:08.00	31OCT2011:14.04	6.07
1001	110	Day 1	8 H	Drug A	194	NG/ML	31OCT2011:08.00	31OCT2011:16.02	8.03
1001	111	Day 1	12 H	Drug A	105	NG/ML	31OCT2011:08.00	31OCT2011:20.00	12
1001	112	Day 1	15 H	Drug A	78.7	NG/ML	31OCT2011:08.00	31OCT2011:23.08	15.13
1001	113	Day 1	24 H	Drug A	57.9	NG/ML	31OCT2011:08.00	01NOV2011:08.00	24

Figure 9 An example of a PK dataset created by the programming group (only some variables are shown)

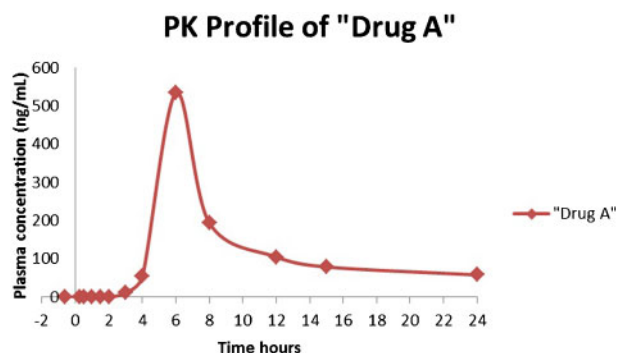


Figure 10 Plot of data from Fig. 9 (BLQ has been plotted as 0)

taken later than specified, this could result in a higher than expected concentration in the blood serum. Figure 11 shows a listing of the concentration data found in Fig. 9.

Summaries of PK concentration and PK parameters are also created. What data are summarized by will depend on the study; the data could be summarized by dose, drug, or hepatic/renal impairment group. Figure 12 shows an example of a summary of PK parameters for Drug A from a DDI study. A similar output could also be produced

Listing of Individual Concentrations by Visit

CRTN/Pt. No.	Sex	Baseline Age (years)	Visit	Study Day	Drug	Nominal time from first dose (h)	Actual time from first dose (h)	Drug Concentration (ng/mL)
123456/1001	Male	29	DAY 1	1	Drug A	-0.5	-0.65	BLQ
			DAY 1	1		0.25	0.25	BLQ
			DAY 1	1		0.5	0.5	BLQ
			DAY 1	1		1	1.08	BLQ
			DAY 1	1		1.5	1.5	BLQ
			DAY 1	1		2	2.17	BLQ
			DAY 1	1		3	3	11.1
			DAY 1	1		4	4	54
			DAY 1	1		6	6.07	534
			DAY 1	1		8	8.03	194
			DAY 1	1		12	12	105
			DAY 1	1		15	15.13	78.7
			DAY 1	2		24	24	57.0

CRTN = Clinical Research Task Number (center no.).

Figure 11 Listing of individual concentrations by visit

	Drug A	Drug A + Drug B
	N = 12	N = 11
AUC (ug*day/mL)		
Mean	1692.123	1750.269
SD	375.7369	333.9327
SEM	128.9302	101.9361
Median	1591.932	1630.024
Min-Max	1254.02 - 2333.62	1392.78 - 2151.12
n	12	11
Cmax (ug/mL)		
Mean	201.927	187.379
SD	35.5269	41.5363
SEM	21.8426	29.6357
Median	205.123	170.341
Min-Max	166.12 - 309.27	121.73 - 315.07
n	12	11

Figure 12 Summary of PK parameters by trial treatment in a DDI study

for the PK parameters for drug B if PK for Drug B was also collected.

Individual and mean plots will also be created on PK concentration data (see Fig. 10 for an example of an individual plot of PK concentration data). Each individual's data within a treatment/impairment/dose group could also be overlaid onto the same plot; however, if there are a large number of individuals within the treatment/impairment/dose group, the plot is easier to view when each individual's data are plotted separately. Box plots can also be created for PK parameters (see Fig. 13 which shows a box plots created from the AUC for one drug).

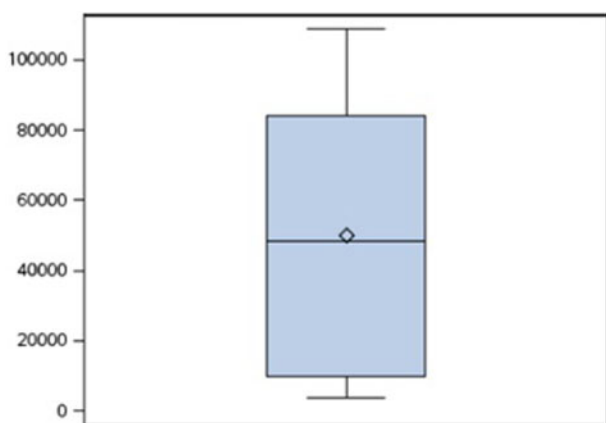


Figure 13 Example of a box plot for AUC in hour ng/mL

Statistical analysis can also be performed on PK data to calculate if two formulations are statistically the same in a bioequivalence study, or that the PK of a drug does not change significantly when given in combination with a second drug (DDI) and many more examples. Figure 14 shows an example where a linear mixed model was used to estimate 90% confidence intervals together with their corresponding geometric mean ratios, for the PK parameters obtained from the following comparisons:

- prototype B versus co-administered individual reference (B versus A);
- prototype C versus co-administered individual reference (C versus A).

The observed Drug X mean total exposures (AUC) from formulations B and C were 24% and 11% higher than that of A. For mean peak exposure (C_{max}) of Drug X, the formulations B and C were respectively 23% and 3 % higher than that of A. However, since the confidence interval for AUC and C_{max} are within the bounds of 0.8–1.25, then we can say that formulation C is bioequivalent to A. But we cannot claim that B and A are bioequivalent since the confidence interval upper limits are 1.40 and 1.45 for AUC and C_{max} respectively which is above 1.25.

Conclusion

In this paper we have given you a brief introduction into PK theory around absorption, distribution, metabolism and excretion, we have also looked into some of the PK parameters and calculations. Following this we moved

Pharmacokinetic Parameter	Comparison (*)	Unit	Geometric Mean Ratio	Confidence Interval		Confidence Level (Percent)
				Lower Limit	Upper Limit	
AUC	B vs A	hr*ng/mL	1.24	1.10	1.40	90
	C vs A	hr*ng/mL	1.11	0.97	1.25	90
C _{max}	B vs A	ng/mL	1.23	1.05	1.45	90
	C vs A	ng/mL	1.03	0.86	1.24	90

Figure 14 Estimated geometric mean ratios of PK parameters of Drug X

on to describe a selection of phase I studies and how they describe the PK of the drug. Then we discussed the PK data flow from collection through to TFLs and any statistical analysis, if performed.

Throughout this paper we have tried to highlight the importance of PK in the development on the drug. If the PK of a drug is undesirable then this can cause the development of the drug to be ceased. The PK of the drug will also have a big impact on the label of the drug, it will affect the frequency of dosing, whether the drug is given fed or fasted or if the drug should not be taken in conjunction with another drug. It will also state if specific populations should receive a dose adjustment (i.e. patients who are hepatically impairment).

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